INSTITUTE OF CHEMISTRY, SILESIAN UNIVERSITY,
KATOWICE, POLAND

POLISH ACADEMY OF SCIENCES, THE KATOWICE BRANCH,
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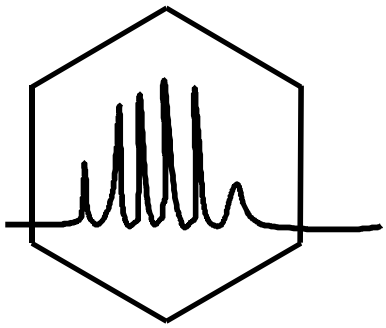

THE XXIXth SYMPOSIUM

'CHROMATOGRAPHIC METHODS OF INVESTIGATING THE ORGANIC COMPOUNDS'

JUNE 8th – 10th, 2005
KATOWICE – SZCZYRK

BOOK OF ABSTRACTS

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PLENARY SESSION I

GRADIENTS IN TLC

F. Geiss
Ispra, Italy

Gradient TLC is characterized by techniques with locally variable conditions on the separation bed. These gradients can remain stationary during development or move over the separation path. Since "gradus" in Latin means step, the term ‘gradient’ might have to be limited to discontinuous changes; however it is felt that purism would be out of place here.

Almost all TLC parameters can be varied in gradient fashion: layer thickness, layer composition, impregnation with liquid, solid, and gaseous substances, "activity", solvent composition, solvent concentration in the layer, solvent flow, pH value, temperature, and others.

In practical terms, the only really important gradient effect in TLC is solvent demixing when solvent mixtures are used. In this case, on/in the layer the selectivity is affected locally, it varies locally, and the "stationary phase" is no longer the homogeneous sorbent nor is the solvent composition, along the layer, homogeneous as it was in the tank. Hence, in the very frequent cases of multi-component solvents, the composition of the "mobile phase" also changes along the layer and with it selectivity (!). Unlike CLC, TLC is a frontal gradient development technique which, consequently, suffers from demixing of multicomponent solvents. In order not to confuse facts, the eluents in TLC done of multi-component solvents should absolutely NOT be called "mobile phases". For most users this is a hard-to-swallow fact. The lecture will, again, focus on the widely ignored situation which also explains why many attempts to transfer TLC conditions on columns frequently must fail, unless certain conditions are fulfilled.

PLENARY SESSION I

QUANTITATIVE APPLICATION OF TLC AND COMPUTATIONAL TECHNOLOGIES FOR THE ANALYSIS OF PHOSPHOLIPID PROFILES OF MAMMALIAN TISSUES AND THEIR PRODUCTS RESULTING FROM *IN VITRO* ACTIVATION OF THE RESPECTIVE ENDOGENOUS PHOSPHOLIPASES

F. M. Helmy, T. E. Mueller, A. Juracka

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Several detailed reviews on cellular phospholipase A_2 ($cPLA_2$) have been recently published, however, the role of $cPLA_2$ in cell function(s) remains uncertain. Continued, substantive efforts are required to develop necessary techniques to elucidate a broader understanding of the *in vitro* organ-specific functionality for all endogenous phospholipases. This understanding should help to provide the basic foundation for an explanation of the wide-range diversity of phosphoglycerides in various biological membrane systems.

We have developed a key protocol that utilizes multiple thin layer chromatographic (TLC) techniques and different mobile phases coupled with wavelength-specific densitometry and computational analysis to aid in this discovery. This combination permits the quantitative recognition of diverse phospholipids and their respective metabolic products resulting from the *in vitro* activation of their endogenous phospholipases.

Differential staining of the various phospholipids (one-dimensional chromatograms) was carried out by using the thionine reagent - selectively staining the acidic phospholipids (CL, PS, and PI) lavender and the plasmalogens green. Choline phospholipids (sphingomyelin and phosphatidyl choline, on aluminum oxide), required the Biebrich Scarlet reagent to be used as a visualizing reagent. Two dimensional TLC, selective hydrolysis (*e.g.,* 1% HCl cleaves the alkenyl ether bond of plasmalogens), and Leucofuscin staining is used for plasmalogen (*via* the released aldehyde) and their lyso-derivatives identification.

Automated densitometric measurements of the chromatograms were obtained at 600 nm (thionine λ_{max} at ~600 nm) or 560 nm (Leucofuscin λ_{max} at ~560 nm) or 520 nm (Biebrich λ_{max} at ~520 nm) using a CAMAG Scanner II and the CATS software package. Graphs of integrated intensities were digitized using UN-SCAN-IT software and imported into Microsoft Excel for graphical and numeric comparison.

Details pertaining to the above technologies will be discussed.

PLENARY SESSION I

LIQUID CHROMATOGRAPHIC DETERMINATION OF VANADIUM IN PETROLEUM OILS AND MINERAL ORE SAMPLES USING 2-ACETYLPYRIDNE -4-PHENYL-3-THIOSEMICARBAZONE AS DERIVATIZING REAGENT

M. Y. Khuhawar, G. M. Arain

Dr. M. A. Kazi Institute of Chemistry, University of Sindh, Jamshoro

Vanadium is a transition metal widely distributed in the environment and in biological samples. Vanadium is used widely in industrial process including the production of special steels, temperature resistant alloys, in glass industry, in the manufacture of pigments and paints, for lining arc welding electrodes and catalysts.

Liquid chromatographic method has been developed, based on precolumn derivatization of vanadium(V) with 2-acetylpyridine-4-phenyl-3-thiosemicarbazone (APPT). The complex is extracted in chloroform together with palladium(II), tin(II) and iron(III) and eluted and separated completely from Kromasil 100 C-18, 10µm (25cm x 4.6 mm id) column with methanol : water : acetonitrile (60 : 30 :10 v/v/v) with a flow rate of 1 ml /min. UV detection was at 260nm. Linear calibration curve was obtained with 2 – 12.5µg/ ml vanadium(V) with detection limit of 0.4µg/ml. A number of metal ions tested did not affect the determination of vanadium. The method was applied for the determination of vanadium in petroleum oils and mineral ore samples with vanadium contents of 0.32 – 2.3µg/g and 121.7 – 717.3µg/g with RSD of 1.5 – 4.5% and 0.38 – 4.7% respectively. The results correlated with reported values and by atomic absorption spectrophotometry.

PLENARY SESSION II

APPLICATION OF ELECTROKINETIC EFFECTS TO THE SEPARATION OF BIOLOGICALLY ACTIVE COMPOUNDS IN MINIATURIZED SYSTEMS

T. H. Dzido

Department of Physical Chemistry, Medical University of Lublin, Poland

Miniaturized separation systems designed as small planar devices (chips) are well known in contemporary biological and pharmaceutical analysis in spite of their relatively short development time. First publications regarding these devices appeared in the early nineties. Manufacturing of miniaturized separation systems is based on microelectronic technology. Electrophoretic and electroosmotic effects are applied for the separation of solutes of interest using these devices.

In this presentation, the following theoretical and practical aspects will be discussed:

- the construction of miniaturized devices,
- principle of action,
- mechanism of solute migration,
- separation efficiency,
- concept of total chemical analysis (μ-TAS).

It is significant that a very high efficiency of separation systems is provided by the chip devices. Some examples are listed as follows: plate number of 200 000 can be obtained using 1.5 cm long separation channel, some separations can take 1s or can be even shorter (1ms separations were also reported), the consumption of the solution of mobile phase is extremely low – only 1 μl (or less) of this solution is necessary to perform the complete separation, many separation channels can be combined in one set to enhance the throughput of analysis.

The examples of separation of different solutes (especially of biological and pharmaceutical origin) will be presented to illustrate the mentioned-above advantages based on the contemporary literature.

PLENARY SESSION II

MIXTURE ANALYSIS METHODS IN CHROMATOGRAPHY

B. Walczak[1,2], *Y. Vander Heyden*[2], *M. Sajewicz*[1], *T. Kowalska*[1]

[1]Institute of Chemistry, Silesian University, Katowice, Poland;
[2]ChemoAC, Fabi-VUB, Brussels, Belgium

The main goal of the Mixture Analysis (MA) methods is determination of a number of components in a studied mixture, and eventually their identification and quantification. The MA methods can be applied to any data matrix representing spectral profiles of the time evolving products. Typical examples of this type of data are the data produced by the so-called hyphenated chromatography (HPLC-DAD, GA-IR, GC-MS), or spectral data registered during monitoring of a given process or in the course of chemical reactions.

Modern multivariate chemometric methods of Mixture Analysis (MA) are usually considered as belonging to the two groups of approaches. The first group contains methods based on PCA such as, e.g., evolving factor analysis (EFA), heuristic evolving latent projection method (HELP), fixed-size window EFA (FSW EFA), and Spectra Factor Analysis (SPFAC). To the second group belong the methods based on Gram-Schmidt orthogonalization, such as SIMPLISMA and OPA. An excellent overview of the MA methods and their intercomparison can be found in [1].

All the above methods focus on identification of selective regions or selective features. If selective regions or features are properly identified, real spectra and time profiles can be retrieved, based on the Alternating Least Squares (ALS) method.

Selective regions in the time domain are identified by means of the PCA derived methods based on the data rank. For instance, FSW EFA applies singular value decomposition (SVD) to a 'window' of spectra that is moved along the data matrix. The inspection of the local rank of the matrix provides the number of co-eluting compounds at each analysis time and the concentration window (region of existence) of each compound in the mixture.

In SIMPLISMA and OPA instead of selective regions, selective features (variables or spectra) are looked for. The main concept explored in SIMPLISMA and OPA is based on the fact, that the 'pure variables' (components, features), are more distinct from each other, than from any remaining variables representing their mixture.

Outside chemistry, another mixture analysis technique (called Independent Component Analysis, ICA) evolved, gained high attention of the scientific community and found many attractive fields of application in science and technology. ICA can be considered an extension of the well known Principal Component Analysis (PCA).

Reference

[1] F. Cuesta Sanchez, Mixture Analysis by Multivariate Techniques: Peak Purity Assessment, PhD Thesis, VUB, Brussels 1999

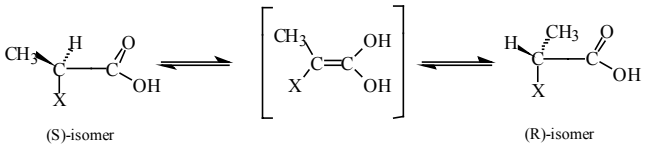
PLENARY SESSION II

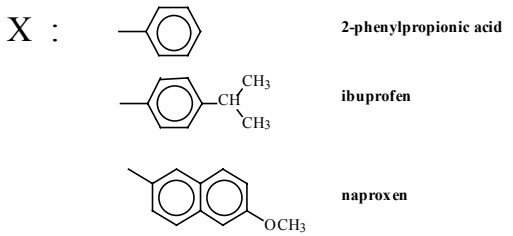
A CHEMOMETRIC STUDY OF THE OSCILLATORY TRANSENANTIOMERIZATION OF THE SELECTED PROFENS

R. Piętka, M. Sajewicz, T. Kowalska, B. Walczak

Institute of Chemistry, Silesian University, Katowice, Poland

In our earlier studies devoted to oscillatory transenantiomerization of the selected profens (i.e. ibuprofen, naproxen, and 2-phenylpropionic acid), when prolongedly stored both in the aqueous and the non-aqueous media [1-3], we discovered that this repeated change of configuration from the *S* to the *R* form and *vice versa* can be assessed not only with aid of polarimetry (which is the standard measuring tool with chiral compounds), but also by means of thin-layer chromatography (TLC) with densitometric detection. Developing of the stored profen solutions in the regular time intervals and then running the respective densitograms revealed an oscillatory change of the profens' concentration profiles and also of their position on the chromatograms. From an independent study [4] it comes out that the mechanism of transenantiomerization of profens most possibly involves keto-enol tautomerism, as schematically shown below:





Thus it seems very likely to expect that the above three types of the species (i.e. the *S*- and the *R*-isomer along with a respective tautomer) are simultaneously present in the oscillating samples, yet with their relative proportions steadily changing.

As TLC does not furnish a possibility to get a deep enough insight in the mechanism of transenantiomerization to prove coexistence of the two chiral antipodes plus an intermediary non-chiral tautomer, we decided to register a series of the profens' chromatograms run in the regular time intervals in the non-chiral chromatographic systems by means of HPLC/DAD and then to apply the chemometric approach, called Orthogonal Projection Analysis [5], in order to assess the number of chemical species (along with their respective UV spectra), which hide under concentration profiles of the respective profens. It was firmly established, that the aqueous environment (both as a solvent of the investigated profens and a chromatographic mobile phase)

is the major promoter of transenantiomerization, whereas the non-aqueous media basically hamper this process.

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PLENARY SESSION III

SEX SPECIFIC DIFFERENCES OF HUMAN FETAL CORTISOL PATHWAYS AND STEROID PROFILES

V. L. Clifton[1], V. E. Murphy[1], A. Osei-Kumah[1], N. Scott[1], W. B. Giles[1], R. Smith[1], P. G. Gibson[1], P. K. Zarzycki[2,3]

[1]Mothers and Babies Research Centre and Respiratory and Sleep Medicine of the Hunter Medical Research Institute, Newcastle, NSW, Australia; [2]Technical University of Koszalin, Koszalin, Poland; [3]Faculty of Pharmacy, Medical University of Gdańsk, Poland

We have recently identified that when asthma, regardless of its severity, is not treated during pregnancy with inhaled steroids female fetal growth is significantly reduced. When the mother uses inhaled steroids female fetal growth is comparable to the control population. The male fetus appears unaffected by asthma or its treatment. These findings suggested that factors that regulate fetal growth are gender specific. We then questioned whether placental cortisol metabolism and pathways regulated by cortisol differ between the male and female fetus of normal pregnancies. Placental cortisol metabolism, cytokine and glucocorticoid receptor (GR) expression was assessed in placentae collected from normal term pregnancies. Data was analysed based on fetal sex. Female fetuses had significantly increased placental 11β-HSD2 activity ($P=0.002$), higher basal cytokine mRNA expression and increased GR and MR expression ($P<0.05$) relative to placentae from male fetuses. These findings suggested that the female fetus may respond differently to an inflammatory challenge due to increased cortisol metabolism. Based on this information, we have questioned whether there may also be differences in other steroid pathways between male and female fetuses.

Cord blood plasma samples collected from male and female fetuses were processed using solid phase extraction technique (RP-SPE) and temperature-dependent inclusion chromatography to identify possible differences in the steroid profile between male and female fetus. Using principle component analysis there were significant differences in steroid levels expressed between male and female fetuses. These data indicate that male and female fetuses implement different strategies for growth which involve differences in steroid pathways.

PLENARY SESSION III

WHY MAY PLATE BE MORE EFFECTIVE THAN COLUMN?

P. K. Zarzycki[1,2]

[1]Technical University of Koszalin, Koszalin, Poland;
[2]Department of Pharmaceutical Chemistry, Medical University of Gdańsk, Gdańsk, Poland

Planar chromatography is still commonly used as the simple but efficient tool for the pre-purification, preliminary separation and quantification of complex chemical, biological and environmental samples. Unlike column chromatography, the planar counterpart can easily work as a two-dimensional method. For that reason, this technique has a great ability to separate a number of analytes that are components of complex biosamples including blood, urine, tissue, sewage water or sediments. It is noteworthy that from its nature the isocratic thin-layer chromatography has a great capability to separate the mixtures composed of particular solutes [1]. Moreover, using a TLC method the number of UV transparent metabolites can be easily located and quantified by applying a variety of sensitive, selective and non-expensive visualization mixtures [2]. Interestingly, in term of practical application the TLC systems are very suitable for the separation that is performed at different temperatures particularly, at low temperature region [3]. This is mainly because of the low flow rate and small amount of the mobile phase that is necessary to carry out the separation process. Moreover, there is no Joule's heat evolved due to electric current flow as in planar or column electrophoresis systems. Hence, to ensure proper plate temperature the cooling devices can work with great precision and consume much less energy.

This work does not attempt to compete column and planar systems against each other, rather to revisit the advantages of TLC and provide some experimental data to supports its efficient use in the research laboratory.

References

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PLENARY SESSION III

ANALYTICS OF NITROSOAMINES, NEW DISINFECTION BY-PRODUCTS AND SECONDARY AMINES, THEIR PRECURSORS, IN WATER SAMPLES

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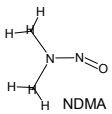
A. Mickiewicz University, Poznań, Poland

Keywords: nitrosoamines, secondary amines, drinking water disinfection, DBPs analysis



NDMA

Nitrosoamines, particularly, N-nitrosodimethylamine (NDMA), N-nitrosomethylethylamine (NMEA) and N-nitrosodiethylamine (NDEA) are highly mutagenic compounds that are thought to be carcinogenic to the human body. The US Environmental Protection Agency (US EPA) placed these compounds into the group B2, to which compounds of probable carcinogenicity to the human belong. According to the US EPA, the maximum admissible levels of concentration of these compounds in drinking water are 7 ng L^{-1} (NDMA), 20 ng L^{-1} (NMEA) and 2 ng L^{-1} (NDEA) with the risk estimation 10^{-5} [1]. Until now, knowledge of the topic of the formation of nitrosoamines has been based on the mechanism of secondary alkylamines reaction with nitrite. The problem became urgent, when, according to US data, the concentration of NDMA reached 10 ng L^{-1} in surface waters and was found at the concentration of 20 ng L^{-1} in drinking water production wells, which were under the influence of recharge water from the advanced wastewater system.

It was reported by Choi et al. and Mitch et al. in 2001 that N-nitrosodimethylamine is formed during the disinfection of water and sewage with chlorine. The results indicated the formation of NDMA as a result of chlorination with chlorine of water containing dimethylamine (DMA) and ammonia ions. In 2003 Gerecke et al. reported the formation of N-nitrosodimethylamine as a result of the reaction of NOM with chlorine. According to the authors NOM seems to be a more important precursor of NDMA than dimethylamine.

The results of the research carried out in the Department of Water Treatment Technology indicate that NDMA is formed not only as a result of chlorination with chlorine but also with chlorine dioxide. The results revealed that chlorination of water containing methylethylamine (MEA) and diethylamine (DEA) in the presence of ammonia ions results in the formation of N-nitrosomethylethylamine (NMEA) and N-nitrosodiethylamine (NDEA) respectively. Consequently, NMEA and NDEA joined the group of disinfection by-products which are thought to have an effect on human health.

In the light of the above, it is of key importance to propose new analytical techniques for the determination of N-nitrosoamines in water at the levels of nanograms per litre. Due to the fact that, except for NOM, secondary amines are the only precursors of N-nitrosoamines, it is also essential to introduce analytical methods for the determination of secondary amines in water at the level of a few hundreds of nanograms per litre.

The aim of the speech is to present the state-of-the-art and achievements of the authors in the field of analytical methods used for the analysis of N-nitrosoamines and secondary amines in water.

PLENARY SESSION IV

THE RECENT PROGRESS IN VACANT ION-EXCLUSION CHROMATOGRAPHY OF ORGANIC AND INORGANIC SUBSTANCES

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Recently, the vacancy ion-exclusion chromatography (vIEC) as the new approach of ion-exclusion chromatography (IEC) has been developed using a several ion-exchange resin columns. Basically, vIEC consists of analyte samples as a mobile phase and water as an injected sample. The peaks resulted with the conductivity detection were detected as negative "vacant" peaks (elution-dip peaks) corresponding to each of the analytes present in the sample appear. The peak areas and heights are dependent on the volume of sample water injected to the separation column and the degree of ion-exclusion/penetration of analytes retained on the resin phase. This system can be provided a sensitive detection and the asymmetric peaks, compared with the conventional IEC on the same separation column using water as a mobile phase. This is considered because the analytes in the mobile phase is adequately penetrated to the resin surface.

This lecture introduces the vIEC of several acids and bases, and its applications to several real samples. The outline is as following; (i) background, (ii) comparisons of IEC and vIEC, (iii) vIEC of aliphatic/aromatic carboxylic acids and inorganic acids on a weakly acidic cation-exchange resin in the H^+-form [1–4], (iv) VIEC of aliphatic amines on a weakly basic anion-exchange resin in the OH^--form [5], and (v) application to the real samples.

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PLENARY SESSION IV

MODELING OF THE ORGANIC ACID RETENTION ON A STRONGLY ACIDIC CATION-EXCHANGE RESIN IN THE H^+-FORM AND IN THE REVERSED-PHASE C-18 SYSTEM

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[4]Institute of Chemistry, Silesian University, Katowice, Poland

Ion Exclusion Chromatography (IEC) finds application mainly to separation of the weak acids. As mobile phase, just pure water is used in this technique. Then the characteristic leading (i.e. frontally tailing) peaks are obtained and retention depends on the concentration of solutes. Recently it was shown that this order could be reversed. Namely, in vacant Ion Exclusion Chromatography (vIEC), sample flows as mobile phase and pure water is injected as a sample. In this case, the symmetrical peaks are obtained.

A very similar peak pattern is observed in the reversed phase C-18 chromatography.

Despite of the fact that both techniques have been well known and applied for the years now, we still observe the lack of a model able to describe the functional dependency of the acid retention on the analyte concentration and to explain, why the vacancy peaks are symmetrical.

In this study, the two versions of the modified equilibrium-dispersive (ED) model for prediction of the retention for the organic acids was proposed. The first version was elaborated for description of the ion-exclusion chromatography and the vacancy ion-exclusion chromatography. The model was successfully tested with aid of a strongly acidic ion-exchange resin in the H^+-form, characterizing with the small pore diameter, equal to 60 Å. It turned out that the retention values and the peak shapes, predicted with use of the derived equations, are very well confirmed by the experimental data. This model successfully predicts the experimental observation also, that in vIEC – contrary to IEC – the symmetrical peaks are obtained.

The second version of the ED model was devised in order to scrutinize the adsorption of acids on the C-18 stationary phase in the reversed-phase chromatographic system. Also in this case, a good agreement between the experimental and the theoretical peak profiles was obtained in the classical, as well as in the vacancy chromatography mode.

PLENARY SESSION IV

GENERAL FUNCTION DESCRIBING THE VARIATIONS OF DIFFERENT PHYSICOCHEMICAL PROPERTIES OF ORGANIC COMPOUNDS WITHIN HOMOLOGOUS SERIES. CHROMATOGRAPHIC APPLICATIONS

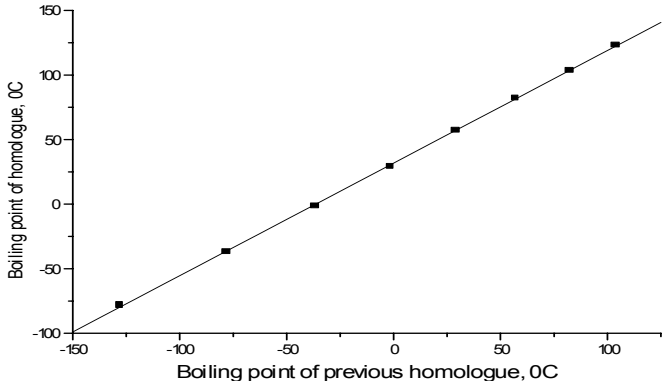
I. G. Zenkevich

Chemical Research Institute of St. Petersburg State University, St. Petersburg, Russia

Numerous physicochemical constants of organic compounds (boiling points, critical temperatures, critical pressures, refractive indices, relative densities, dynamics or kinematics viscosities, surface tensions, vapor pressures, dielectric constants, ionization potentials, partition coefficients in heterophaseous systems, etc.) are historically considered as principally different objects in chemical and mathematical senses. Hence, they require using various approaches for approximation and precalculation. Nevertheless, the general recurrent function describing the variations of all mentioned properties (A) within homologous series can be proposed. It connects the value of any constant for homologue with (n) carbon atoms with its value for previous homologue having (n-1) carbon atoms by the simplest linear dependence:

$$\mathbf{A(n+1) = \mathit{a}\, A(n) + \mathit{b}} \qquad (1)$$

The illustration of practically "ideal" linear dependence $T_b(n+1) = a\ T_b(n) + b$ for boiling points (T_b, ^{0}C) of perfluoro-*n*-alkanes C_1-C_9 is presented on the plot below ($a = 0.872 \pm 0.005$; $b = 31.8 \pm 0.4$, $\rho = 0.9999$; $S_0 = 1.1$):



The equation (1) is equivalent to another recurrent relationship:

$$\mathbf{A(n+1) = \{A(n-1)^2 + A(n) \times [A(n) - A(n-1) - A(n-2)]\} / [A(n-1) - A(n-2)]} \qquad (2)$$

and have the following mathematical solution:

$$\mathbf{A(n) = \mathit{c}\, \mathit{a}^n + \mathit{b}\, (\mathit{a}^n - 1) / (\mathit{a} - 1),} \quad \text{where } a, b, c \text{ – constants} \qquad (3)$$

At the hypothetical increasing the number of carbon atoms in the molecules ($n_C \to \infty$) the function $A(n_C)$ tends to infinity (at $a > 1$), or to a limits $\lim A(n_C \to \infty) = b / (1-a)$ (at $a < 1$).

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Various applications of this general function to the chromatographic parameters are considered. It permits us to predict retention indices (RI) of structurally related compounds by extrapolation, or reveal these compounds in complex mixtures at temperature programming (GC) or gradient (HPLC) regimes of analyses. For example, RIs of 2-alkanols on standard polydimethylsiloxanes are: 600±13 (2-BuOH), 691±11 (2-C_5OH), 793±10 (2-C_6OH), 891±10 (2-C_7OH), 991±10 (2-C_8OH), 1090±4 (2-C_9OH), 1192±6 (2-C_{10}OH), whilst there are no reliable data for higher homologues. Using the equation RI(n+1) = a RI(n) + b (a = 1.014 ± 0.009; b = 87.1 ± 7.5; ρ = 0.9998, S_0 = 3.6) one can predict RI values for 2-C_{11}OH (1296), 2-C_{12}OH (1401) and so on.

The unusual non-chromatographic applications of equation (1) are discussed also.

PLENARY SESSION V

DETERMINATION OF THE SWEETENER SUCRALOSE IN MILK-BASED CONFECTION BY PLANAR CHROMATOGRAPHY

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Sucralose is a relatively new sweetener with a sweetening intensity of 600 – 650 times compared to sucrose, having no calories and a taste profile very similar to sugar without any aftertaste. Because of its exceptional heat stability, excellent solubility characteristics, and high compatibility with commonly used food ingredients, it is employed especially in USA for many low-calorie products on the market. Since 1998 sucralose (1,6-dichloro-1,6-dideoxy-β-D-fructo-furanosyl-(2-1)-4-chloro-4-deoxy-α-D-galactopyranoside or 4,1´,6´-trichlorogalactosucrose) has been approved by more than 40 countries and it is going to be approved as an additive (E 955) for use in European countries in the near future.

Detection of sucralose is performed by derivatization, refractive index, pulsed amperometric detection or mass selective detector because of minor UV absorbance (< 200 nm). There is evidence that planar chromatography is advantageous in this case. By separation on amino phases the reagent for derivatization is already incorporated in the layer; it need not to be transferred in a separate step. By just heating the plate after chromatography sucralose reacts with the amino groups of the layer to fluorescent zones [1]. The derivatization step to fluorescent zones can rapidly and simultaneously be performed for all substance zones on the plate.

Sucralose was determined in Burfi, a popular Indian ethnic milk sweet, which is produced in all kinds of modes with various food ingredients. Sudies on storage stability of sucralose in food products like beverages and baked goods have been reported in literature, but no studies are cited in food systems even similar to milk based confections. Thus in this study stability of sucralose has been established and degradation products of sucralose were monitored in milk-based confection stored at various conditions.

Literature

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PLENARY SESSION V

STUDY OF OXIDATION PRODUCTS OF THE REACTION BETWEEN ORGANIC COMPOUND AND NO_2 AT GOLD SURFACE BY GC-MASS

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The oxidation of organic compounds by NO_2 has been studied by Scribner (1) and has been the basis of a chemiluminescence detector for gas chromatography and high performance liquid chromatography (2, 3). The nitrogen dioxide can oxidize different class of organic compounds at different temperatures of gold catalyst bed. The alcohols are easily oxidized to aldehydes at 200° C while hydrocarbons which are the main constituents of environmental and industrial samples do not react at temperatures below 400° C. The GC-Mass studies were performed to study the reaction products of some of this reactions. Gold-coated glass beads were packed on a glass tube with a side arm, which could be heated to desire temperature. A needle was connected to one end of this tube so that it could directly be connected to GC column. A stream of NO_2 was passed through gold-coated glass beads via the side arm of the tube. The sample was injected on the top of the glass beads. After the reaction of the compounds with NO_2 at gold surface, The products were swept to a GC-Mass column and the reaction products were identified.

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PLENARY SESSION V

PREPARATIVE LAYER CHROMATOGRAPHY OF PLANT EXTRACTS

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Therapeutic properties of herbs have been known for many years. Recently, herb therapy has become popular again. It is known that widely used herbal infusions or tinctures contain wide spectrum of compounds ranging from very important pharmacologically active ones to those of small or sometimes undesirable activity. Simultaneous evolution of chromatography as the method of analysis and separation enables the confirmation and development of chemotaxonomic investigations of new plant species as well as accomplishment of quality and quantitative determinations. Thin-layer chromatography especially proved to be very useful for analysis and isolation of small amounts of some compounds. Most significant and advantageous point of TLC technique are: its speed, cheapness and possibility to carry out the analysis of several solutes simultaneously, continuous development under equilibrated conditions, gradient and multiple development and scaling up the separation process.

In the chromatography of plant extracts on enlarged scale few main problems appear: "general elution problem" because of differentiated polarity of complex mixture components being separated, structural and chemical analogy of compounds and resolution decrease owing to band broadening.

The optimisation of preparative chromatography depends on choice of an appropriate chromatographic system (adsorbent and eluent), sample application and development mode to assure high purity and yield of desirable compounds isolated from the layer. For the so-called "difficult separations" it is necessary to perform re-chromatography by use system with different selectivity. But it should be taken into account that achievement of satisfactory results frequently depends on the compromise between yield and purity of mixture component being isolated. The aim of our presentation is the optimisation of preparative layer separations of plant extracts containing closely related compounds furanocoumarins, taxoids, alkaloids and other compounds – polar and non-polar impurities as a method of sample preparation and as method of isolation of pure components.

PLENARY SESSION V

EXAMINATION OF SURFACE AND BULK PROPERTIES. IS INVERSE GAS CHROMATOGRAPHY A USEFUL TOOL?

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Inverse gas chromatography has become an accurate, reliable and fast method for the physicochemical characterisation of polymers and their blends, fibres, modified silica and other surfaces, surfactants, as well as, commercial stationary phases and their mixtures and petroleum pitches, heavy residues of oil distillation. This technique has been also used to study of water sorption ability of different materials. The term "inverse" indicates that the examined material, e.g. polymer blend or modified silica, placed in the chromatographic column, are of interest in contrast to the common analytical gas chromatographic experiments. Carefully selected test solutes are injected into the flow of a carrier gas and transported over the surface of e.g. fiber. The retention time and the peak elution profiles of standard solutes, affected by interactions between the solute and stationary phase, are used to estimate those interactions.

The physicochemical properties of the polymers' surface examined below T_g of polymer were characterized by inverse gas chromatography (IGC). Dispersive component of surface free energy and specific interaction parameters describing surface ability to act as electron acceptor or electron donor are used to quantify the properties of the polymer surface.

The acid-base properties of the examined surface can be determined after calculating the specific component of adsorption energy ΔG^s. From its temperature dependence one may calculate the enthalpy of specific interactions ΔH^s which is used to calculate the acid-base properties of the examined surface, K_A and K_D reflecting the ability of the examined surface to act as electron acceptor and electron donor, respectively.

The estimated surface characteristics may be used to screen the changes occurring during their chemical modification, oxidation or plasma treatment.

For all materials being liquid in the temperature of IGC experiment Flory-Huggins interaction parameter ($\chi_{1,2}^{\infty}$), solubility parameter (δ_2) and/or Hansen three dimensional solubility parameter (including $\delta_d, \delta_p, \delta_h$ components) of corrected solubility parameter (δ_T). The use of such assumption allows the more sophisticated characterization of examined systems.

Acknowledgement

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PLENARY SESSION VI

CHROMATOGRAPHIC DETERMINATION OF METHYL GLYOXAL FROM HUMAN BLOOD OF DIABETIC PATIENTS

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Methyl glyoxal is widely distributed in biological materials and plays physiological important roles. It is a metabolite and is produced in the human body during glucose breakdown. It is also produced during glycolysis and sugar fermentation. Methyl glyoxal could completely inhabit the tumor development in mice.

Methyl glyoxal after pre-column derivatization with meso-stilbenediamine was eluted from reversed phase HPLC Column Kromasil, 100, C-18 , 5µm(150x 4.6 mm i.d) with methanol: water: acetonitrile (59: 40: 1 v/v/v) with a flow rate 1ml/min and UV detection at 254nm. Glyoxal and dimethyl glyoxal separated completely. The linear calibration curve for methyl glyoxal was obtained with 5 - 100µg/ml with detection limit of 1 µg/ml. Methyl glyoxal was determined from blood of the diabetic patients. The amounts found were within 15.8 – 35.54 µg/ml of blood with coefficient of variation 3.5 – 5.7%. The higher amounts of methyl glyoxal were observed from diabetic patients suffering from ketosis.

Similarly methyl glyoxal derivative of stilbenediamine also eluted from capillary gas chromatographic column HP-5, (30m x 320µm x 0.25µm) with column initial temperature 120 0C with heating rate 50 0C/min.up to 280 0C with nitrogen flow rate 1.5 ml/min, with split ratio 20: 1 and detection by FID. Analytical responses ware obtained. The method is being applied in blood samples. Gas chromatographic results are being compared with HPLC.

PLENARY SESSION VI

RESULTS FOR A MODIFIED APPARATUS FOR PPEC

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Pressurized Planar Electrochromatography (PPEC) is a separation technique in which the mobile phase is driven by electroosmotic flow, while the sorbent layer is pressurized in a manner that allows heat to flow from the layer through an electrically insulating, thermally conducting, sheet of aluminum nitride. PPEC has demonstrated the ability to produce fast and efficient separations, but our original apparatus gives poor reproducibility. Our presentation will discuss modifications that have been made to the apparatus and to the operating procedure. These changes result in better reproducibility and allow greater flexibility in designing separations.

In an early modification, an integral ball joint and socket joint was connected to the die block that applies pressure to the TLC layer. The internal design of the ball and socket joint was such that there was restricted alignment when the apparatus was stressed, and this resulted in pressure being unevenly applied. This effect caused an unacceptable variation in migration distances of the analytes. The ball joint and corresponding socket were separated, lapped for a good fit and realigned. This modification resulted in a much greater degree of self-alignment in the stressed apparatus. The final version was found to yield migration times of acceptable reproducibility.

Heat is generated during PPEC, and for this reason temperature control is necessary. This was achieved by installing channels in the two metal die blocks that pressurize the layer. Water is circulated through these channels from an external bath. Migration distance is shown to increase linearly with temperature between 3˚C and 40˚C.

Dzido has shown that presoaking TLC plates for sufficient time to achieve full saturation prior to electrochromatography separation results in increased migration distances and improved reproducibility. This has proven to be true in PPEC, and a pre-soak time of twenty minutes is used, after which the analyte is applied and the separation performed. The above improvements result in relative standard deviations of under two percent in migration distances.

PLENARY SESSION VI

EXTRACTIVE AND CHROMATOGRAPHIC TECHNIQUES AND METHODS OF GROUP – TYPE SEPARATION AND GROUP – CONTENT DETERMINATION OF PLANT, FUNGAL AND BACTERIAL METABOLITES

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Key words: plant, fungal and bacterial metabolites – primary and secondary, group – type separation and determination, extractive and chromatographic techniques and methods, review

Primary and secondary metabolites constitute characteristic components of each organism. They are especially important for biochemical homeostasis and bioactive properties of plants, fungi and bacteria. They are commonly represented by variety of chemical compounds with different structures. Separation, qualitative identification and quantitative determination of all of the compounds is always very difficult and often unnecessary. On contrary, group – type separation and group – type quantitative determination seems to be sufficient for most applications. Knowledge of qualitative and quantitative group – type data and the relationship between group content and culture or environmental conditions should facilitate understanding of organism's metabolism. Group – type separation can be also a very helpful first step for preparative isolation of particular substances using two-dimensional chromatography. Thus, it should facilitate the research of pharmacological function of secondary metabolites and search for new substances having therapeutic importance. The lecture presents: (i) Description of structure, polarity and hydrophobicity of the most important groups of chemical compounds belonging to plant, fungal and bacterial primary and secondary metabolites; (ii) Comparison of basic operations and the most important and the most effective separation techniques described in the literature and lately used for sample preparation and for group – type separation of biological material. Special attention will be given to liquid chromatography techniques and methods as the most important and the most selective; (iii) Specification of detection techniques used for identification of group affiliation and for quantitative calibration of group – type content.

Preliminary results of authors' research aimed to choose the most efficient separation techniques as well as detection and determination methods for group – type separation and group – type calibration of plant, fungal and bacterial primary and secondary metabolites will be also presented. The data suggest very high efficiency of following procedures: (i) accelerated solvent extraction (ASE), (ii) step elution by normal phase high performance liquid chromatography (NP-HPLC) with CN and DIOL bonded stationary phases and (iii) multicolumn chromatographic systems using column switching back-flashing of eluent in the column.

PLENARY SESSION VI

SELECTED CHEMICAL PROPERTIES OF ORGANIC LIGANDS AND IMPREGNANTS IN THE TLC-TYPE STATIONARY PHASE FOR DETERMINATION OF THEIR CONCENTRATION ON THE SILICA SURFACE

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At the end of the former decade, a new method of relative evaluation of the coverage density of the silica matrix with octyl, octadecyl, 3-cyanopropyl, and diol ligands was introduced [1]. That method bases on the *Raman* spectroscopy, and more specifically on a predominant, very broad, and finely structured *Raman* band in the frequency range from ca. 2285 to 1130 cm^{-1}. The obscure origin of that broad band proved a real challenge. A systematic study on the behaviour of the chemically bonded stationary phases of interest (i.e. of RP-8, RP-18, CN, RP-2 with paraffin impregnation and DIOL) at elevated temperatures, employing the methods of differential scanning calorimetry (DSC), *UV* spectrometry, *IR* spectoscopy and modified acquisition of the *Raman* spectra is described in [2]. In order to confirm the process of formation of the *UV*-active products in the chemically bonded stationary phases heated at 165°C, extracts obtained from washing these pretreated sorbents were further analyzed with aid of the HPLC/DAD method [3]. These investigations also furnished evidence that some of the thermal transformation products of medium polarity are cleaved from the silica matrix and can be extracted with such simple solvents as, e.g., *n*-hexane or methylene chloride. In the GC/MS study, some of the cleaved products of thermal modification of the investigated chemically bonded stationary phases were identified and generally they belonged to the alkenes and carbonyl compounds. Quantitative relationship between the changes of the stationary phase *UV* spectra (as a result of thermal modification) and the content of alkyl groups [4] enables evaluation of the content of aliphatic moiety capable to aromatize in the stationary phase exclusively by employing instrumentation typical of a TLC laboratory.

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HPLC-DIODE ARRAY (HPLC-DAD) AND GC-MS DETECTION OF PHENOLIC *trans*-AND *cis*-RESVERATROL (3,5,4'-TRIHYDROXYSTILBENE) IN PEANUTS AND PISTACHIONUTS

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Epidemiological and clinical researchs reported that phenolic compound resveratrol (3,5,4'-trihydroxystilbene) has been associated with reduced cardiovascular disease and reduced cancer risk. Resveratrol has been reported to act as an antimutagen and antioxidant and to possess cancer chemopreventive activity in mice and it has been confirmed that *trans*-resveratrol is an chemopreventive agent against to human breast cancer. *Trans*- and *cis*-resveratrol (3,5,4'-trihydroxystilbene) in edible peanut *(Arachis hypogaea* L.) and pistachionuts (*Pistacia vera* L.) was identified by High Performance Liquid Chromatographic Diode Array (HPLC-DAD) and Gas Chromatography-Mass Spectrometry (GC-MS). HPLC equipment consisted of the 5-μm Hypersil-ODS column (250 x 4.6 mm) [Phenomenex, CAL, USA] and mobile phase mixture was acetonitrile / bidistilled water (40/60 v/v) + TFA 0,1% (v/v). Diode-Array Detector (DAD) was set at 308 nm (UV detection). MS Data [m/e] and total ion chroma-tograms (TIC) of bis[trimethylsilyl]trifluoroacetamide (BSTFA) derivatives of resveratrol isomers was obtained by GC-MS. Gas chromatograph using a 30 m, 0.25 mm, 0.25 μm capillary column DB-5MS (J & W Scientific, Folsom, CA) was used for analyses and initial column temperature was 150 ^{o}C for 1 min. The column was heated to 300 ^{o}C at 10 ^{o}C min^{-1} and maintained at 300 ^{o}C for up to 20 min. Flow rate of carrier gas (He) was 1.0 mL min^{-1}, split ratio was 50:1. The proposed chromatographic methods for *trans*- and *cis*-resveratrol detection in peanut and pistachionut could be utilized for peanut-based industry.

PLENARY SESSION VII

UNSUPERVISED DECISION TREES TO SELECT ORTHOGONAL REVERSED-PHASE HPLC SYSTEMS FOR METHOD DEVELOPMENT

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Keywords*:* orthogonal chromatographic systems; decision trees; method development

Nowadays almost any pharmaceutical separation can be achieved in reversed-phase liquid chromatography, thanks to a wide range of columns available, containing different stationnary phases; which can be combined with different mobile phases. However, due to this large supply it is often not easy to select an appropriate starting point to separate a given mixture, e.g. a first choice of stationary and mobile phase, which is then optimized during further method development. In order to facilitate this choice and to enhance the chance of obtaining a good separation, it is interesting to select a set of systems with strongly different selectivities. In these so-called orthogonal systems differences in retention exist, since different retention mechanisms are present, and/or because of different charges of the solutes. The aim of this study was to explore the use of both univariate and multivariate decision trees for the selection of the most orthogonal systems from a given set. The methodologies used are unsupervised, since the normalized retention times of the solutes are the only data used. Two data sets were studied, containing retention data of 68 solutes on 32 and 38 chromatographic systems, respectively. Two approaches based on decision trees were explored. The first method used is univariate regression tree analysis. Each of the chromatographic systems is the response of one regression tree, which is built using the other systems (31 and 37, respectively) as explanatory variables. For each tree, the importance of each explanatory variable is computed and in the end all values are combined in the so-called "relative importance sum". Since an explanatory variable selected to define a split in a tree contains useful information of the described systems, one can understand that the most exlusive (and thus orthogonal) systems have low importance in the trees (and thus a low relative importance sum value). Secondly the so-called auto-associative multivariate regression tree (AAMRT) analysis is used. One decision tree is built describing all chromatographic systems as the multivariate response. The same chromatographic systems are also used as explanatory variables to define the splits of this tree (this means **X=Y** for the tree). Here, the systems with the lowest variable importance are the most orthogonal. Compared to previous studies based on different criteria (e.g. statistical tests, correlation coefficients color maps based on weighted average linkage dendrograms), analogue selections were obtained for both methods. The AAMRT approach may be preferred over the univariate methodology, since only one decision tree is needed.

PLENARY SESSION VII

DEVELOPMENT IN THE CHROMATOGRAPHIC SIGNAL PROCESSING

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In this work the recent results of the chromatographic signal processing elaborated in our institute are presented. The main fields were the followings:

- Fitting equations to chromatographic signals.

In this work mathematical functions for describing different spectroscopic signals were sought and then fitted to the digitized measuring points. The fitted curve is suitable for quick acquisition of spectroscopic information, noise filtering, and correction of baseline drift. The used chromatographic function for the data acquisition and fitting:

$$f(t)=\begin{cases} 0 \quad , if \quad t < M - \dfrac{D(4-a^2)}{2a} \\ H.\exp\left\{\left(\dfrac{4}{a^2}-1\right)\cdot\left[\ln\left(1+\dfrac{2a(t-M)}{D(4-a^2)}\right)-\dfrac{2a(t-M)}{D(4-a^2)}\right]\right\} \end{cases}$$

where M - peak maximum position, s
H - peak height, (H > 0),
a - asymmetry factor, (0 < a < 2),
D - standard deviation, (D > 0), s

- Determination peak shape asymmetry

A new method is presented for determination of peak shape asymmetry. We order mathematical function to the chromatographic peaks by fitting, and then a symmetrical Gauss curve is generated with the same height and width. The difference of the two functions is constituted and the asymmetry factor is calculated from this difference. Correlation between the peak shape and the calculated coefficient is established. The method was applied for different types of ion-chromatographic peak shapes and the results were interpreted.

- Curve fitting to chromatographic peaks in frequency domain

The Fourier transform of the peak shape function is suitable for describing the chromatographic peaks. A mathematical process was elaborated for the curve fitting in frequency domain, the curves are fitted separate to the real and imaginary parts of the Fourier transform. The process is presented using real HPLC chromatograms.

- Parameter estimation by Kalman filter in frequency domain.

The curve fitting has been done with the exponentially modified Gaussian function, obtained when convolving a Gaussian distribution function and an exponential decay function. The parameter estimation has been carried out by means of the two-dimensional extended Kalman filter.

PLENARY SESSION VII

MICELLAR LIQUID CHROMATOGRAPHIC DETERMINATION OF PRESERVATIVES IN COSMETICS AND FOOD SAMPLES

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An analytical procedure has been developed for the analysis of benzoic acid, p-hydroxybenzoic acid, methyl-, ethyl-, propyl-, isopropyl-, and butyl esters of p-hydroxy benzoic acid by micellar liquid chromatography. The sample after dilution in n-propanol was directly injected on Lichrosorb ODS, 5µm (250 x 4.6 mm i.d.) column and eluted with aqueous 2 % Brij-35 adjusted to pH 3.0 with phosphoric acid: propanol (80: 20 v/v) with a flow rate 1 ml min^{-1} and UV detection at 254 nm. Linear calibration curve was obtained simultaneously for each component within 50-500 µg ml^{-1} for benzoic acid and 5-150 µg ml^{-1} for rest of the components and detection limits were within 25 – 250 ng ml^{-1} corresponding to125- 1250 pg per injection (5 µL). The reproducibility in terms of average peak area and average retention time was obtained with coefficient of variation (CV) of 1.2 % and 0.5 %. The method was applied for the analyses of the components in cosmetics (shampoos, hand lotions, creams and bath foam) and food samples.

Key words: Micellar Liquid Chromatography, Parabens, Food samples

PLENARY SESSION VIII

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC (HPLC) AND GAS-CHROMATOGRAPHIC (GC) APPLICATIONS IN FOOD ANALYSIS

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Applications of High Performance Liquid Chromatography (HPLC) and Gas-Chromatography (GC) in food technology involve, on one hand, an analytical and quantitative testing of the product composition and, on the other hand, an assurance of product quality with increased productivity. HPLC and GC are fast techniques that, with high precision and specificity, separates mixtures into individuals ingredients. Used as routine methods, they have several advantages; they could be completely automated; sample cleanup and preparations are simple. Many kinds of column packings and solvents are available. For HPLC, retension behavior and resolution are affected by column properties as C-loading, chain length, porosity and by elution scheme properties as mobile phase, pH, organic modifier. The samples can be separated based on the solubility and polarity of the sample components. For GC, retension behavior and resolution are also affected by column properties and the samples can be identified and separated on the basis of volatile characteristics.

HPLC and GC are used in the food industry for the component analysis in both raw and processed food products. The alterations during food manufacturing or storage period can be sensitively monitored. Foods and beverages could be tested on components, additives and contaminants. In HPLC,food proteins, peptides, aminoacids, food lipids, phospholipids, food carbohydrates, aromatic and aliphatic alcohols, fat-soluble and water-soluble vitamins, organic acids, mycotoxins (aflatoxins, ochratoxins, fumonisins,trichothecenes,zearalenones) food preservatives (antimicrobials) and antioxidants, food colorants, and sweeteners, food pestisides (insectisides, herbisides, fungusides), food phenolic compounds (flavonoids, phenolic acids, complex phenols), food antibiotics as residues, food pigments (carotenoids, chlorophylls, anthocyanins, betalains, myoglobins, food hormones and residues of growth promoters, for N-nitroso compounds food and beverages, food organic bases (biogenic amines, various alkaloids, methylxanthines, heterocyclic aromatic amines), food anion and cations, polyhydroxy aromatic compounds (PAHs) could be determined whereas in GC, many macro and micro components could be identified. In GC analysis, mono-, oligo, and polymeric forms of carbohydrates, triglycerides, streols, and complex lipids, fatty acids, nitrogen-containing compounds, food volatile compounds and aroma compounds of food and beverages, food hydrocarbons, food additive analysis could be detected.

PLENARY SESSION VIII

THE PRESENCE OF SELECTED DRUGS AND PESTICIDES IN UPPER SILESIAN RIVERS BETWEEN APRIL 2002 AND APRIL 2005

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The research conducted over the last three years on the concentration of drugs and pesticides in the surface waters of the drainage areas of the upper Vistula and upper Oder rivers, forming the primary supply of water for the Silesia river, has allowed the following conclusions regarding these contaminants.

The following were selected for determination: selected drugs (ibuprofen, ketoprofen, diclofenac, carbamazepine); certain pesticides from the triazine and chlorinated phenoxyacids groups; and caffeine.

Caffeine concentration varies only slightly (by about 8 to 15%) in water dumped from treatment plants, which justifies the assumption that caffeine is supplied to rivers at a fairly steady rate.

Since real-time values of water flow at sampling points are not known, the determination of caffeine concentration for each point has been used as a reference, allowing the comparison of drug concentrations to maximum caffeine concentrations from different years.

It has been concluded that ibuprofen is now a permanent water pollutant, whereas the quantity of ketoprofen has grown dramatically over the last two years. In terms of pesticide concentrations, one may point to the high quantities of pyrethroids around large municipal areas.
It has also been determined that river water reservoirs act as filters for the passing water. In each case, the concentration of the determined substances was reduced at the reservoir outlet.

All analyses were conducted using the specially elaborated SPE extraction procedure on Bakerbond spe Polar Plus columns, with the extracts being analyzed with the GC/MS GCTrace equipped with the MS – MSTrace Finnigan detector and a DB-5ms 30m x 0.25mm x 0.25µm column.

APPLICATION OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY IN METABONOMIC STUDIES OF MOCLOBEMIDE

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In metabonomics which has been definited as "the quantitative measurement of multi-parametric response of living systems to pathophysiological stimuli or genetic modification" is required development of rapid and advanced analytical tools to comprehensively profile biofluid metabolites within consumers. One of method which can be used in metabonomic studies is HPLC.

The aim of this study was application of HPLC to metabonomic studies of moclobemide. Moclobemide (p-Chloro-N-(2-morpholinoethyl)-benzamide) is a short-acting and reversible inhibitor of monoamine oxidase A (MAO-A) which is widely used in the treatment of depression. This drug is eliminated by hepatic metabolism involving *C*-and *N*-oxidation of its morpholine ring to yield its two major metabolites in plasma, Ro 12-5637 and Ro 12-8095, which have little and no MAO-A inhibitory activity, respectively.

In metabonomic studies of moclobemide high-performance liquid chromatography has been applied to the measurement concentrations of moclobemide and its two metabolites in human plasma after administration single oral dose of a 150 mg of moclobemide to 20 healthy volunteers. The assay was performed after single extraction with dichloromethane in alkine pH using phenacetin as internal standard. Chromatographic separations were performed on the analytical column (125 mm x 4 mm) filled with Nucleosil100 C-18 of 5 µm particle size. The mobile phase was a binary mixture of acetonitrile and water (25:75 v/v) adjusted to pH 2.7 with 85% H_3PO_4. The flow-rate was 1 mL min^{-1}. Analytes were monitored by UV detector at 239 nm. Under these conditions the retention times of moclobemide, Ro 12-5637, Ro12-8095 and phenacetin were: 2.85 min, 3.45 min, 4.90 min and 5.85 min, respectively. The method has been validated for accuracy, precision, selectivity, linearity, recovery and stability, as well as used to the determination comprehensive profiles of concentrations of the parent substance and its major metabolites. On the basis of obtained concentrations pharmacokinetic parameters were calculated. The results confirm that HPLC is advanced analytical tool to metabonomic studies of moclobemide.

PLENARY SESSION VIII

ANALYSIS OF ORGANIC SULFIDES OXIDATION PRODUCTS BY OZONE WITH GAS CHROMATOGRAPHY AND ATOMIC EMISSION DETECTION

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Advanced Oxidation Technologies (AOT) are presently the most modern technologies of rendering organic sulfur compounds harmless in water and sewage. Among others, ozone, hydrogen peroxide and UV radiation are usaed in AOT reaction systems. Very reactive free radicals with dominating role of hydroxyl radical are formed in course of using the a/m factors in proper combinations. $OH^{\bullet}$ radical has one of the greatest oxidation-reduction potential of all, and the potential allows it to oxidize even very oxidation-resistant chemical compounds.

In this study a method of monitoring of several sulphide groups oxidation with ozone has been developed. To do this authors decided to use Gas Chromatography with Atomic Emission Detection (GC/AED). The device is especially useful to this type of tests as it makes possible identification of elements that are included in analysed chemical compounds. Both original compounds and products obtained in oxidation process include heteroatoms, which is a favourable circumstance for identification of those compounds with GC/AED. Quantitative analysis results are used in kinetic analysis of proceeding reactions.

Samples for analysis were prepared with liquid-liquid extraction with dichloromethane. The solvent allowed for quite high recoveries of analysed compounds and is characterised with low boiling point. That's why it is easily separable from analysed mixture during chromatography. The analysis was performed with temperature-programmed column. Temperature programs were individually developed for each analysed sulphide and its oxidation products. The analysis used capillary column HP-5 and HP-50+. Analysed sulphides oxidation products retention time comparisons with retention time of standards allowed their preliminary identification. Full identification of those compounds was performed with a computer program furnished with the GC/AED. Quantitative analysis of oxidation products was made using absolute calibration method.

Oxidation reaction rate for particular sulphides was determined with half-life period of a substrate – that is time during which concentration of a particular compound decreased from its original value to half of this value. Basing on chromatographic quantitative analysis percentage of the studied sulphide in reaction mixture was calculated comparing to its original quantity. As a result of chromatographic analysis relationship curves of oxidized compound quantity in reaction medium and its oxidation time were drawn and kinetics of oxidation products formation was determined.

DEVELOPMENT OF A GINKGO BILOBA FINGERPRINT CHROMATOGRAM WITH UV AND ELS DETECTION AND OPTIMIZATION OF THE ELSD OPERATING CONDITIONS

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A fingerprint chromatogram of a standardized Ginkgo biloba extract is developed on a monolithic silica column using a ternary gradient containing water, isopropyl alcohol and tetrahydrofuran. For the detection, a UV and ELSD (evaporative light scattering detector) are used. While the UV detector allows to detect volatile compounds or compounds with concentrations below the detection limit of the ELSD, the latter allows to detect non-UV absorbing compounds as the ginkgolides (A, B, C) and bilobalide in the extract. It was found that the ELS signal is rather noisy when the operating parameters proposed by the ELSD software are used. Therefore, these parameters are to be optimized.

The ELS detector can operate in an impactor 'on' or 'off' mode. For each mode, the operating parameters (the nebulizer gas flow rate, the drift tube temperature and the gain) are optimized by use of a well-balanced screening design to obtain the best signal-to-noise (S/N) ratio in the ELS fingerprint chromatogram. For both impactor modes, similar S/N ratios are obtained. However, a significant increase in S/N ratios is seen after optimization. For our separation, it was found that a temperature increase of 5°C (impactor off mode) and a gas flow increase of 1 l/min (impactor on mode) result in improved S/N ratios. When both the UV and ELS detector are used, as in this study, the ELSD can be used in the impactor off-position without the risk not detecting slightly volatile or low concentrated compounds. However, if only a ELS detector would be used, the impactor on-position is advised for a better detection of all components.

POSTER SESSION I

ANTIOXIDANT ACTIVITY OF GREEN TEA EXTRACTS ESTIMATED FROM CHROMATOGRAMS USING PARTIAL LEAST SQUARES REGRESSION AND A COLORIMETRIC REFERENCE METHOD

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In this study, a fast strategy for determining the total antioxidant capacity of Chinese green tea is developed. This strategy includes the use of experimental techniques, such as fast High-Performance Liquid Chromatography on monolithic columns and a spectrophotometric approach to determine the total antioxidant capacity of green tea. To extract the chemically relevant information from the obtained data, chemometrical approaches are used. Among them there are Correlation Optimized Warping to align the chromatograms, Robust Principal Component Analysis to detect outliers and Partial Least Squares and Uninformative Variable Elimination Partial Least Squares for modeling. These approaches allow to process the data and to construct a reliable multivariate regression model between the chromatographic and spectrophotometric data for the prediction of the total antioxidant capacity of green teas from fast chromatograms.

POLAR ORGANIC MODE-BASED CHROMATOGRAPHIC CHIRAL SCREENING STRATEGY ON POLYSACCHARIDE COLUMNS

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In this study, the performance of different polysaccharide-based columns (Chiralcel OD-RH, Chiralpak AD-RH, Chiralcel OJ-R and Chiralpak AS-RH) in polar organic solvent chromatography (POSC) is investigated. The aim of this study is to develop a chiral screening and optimisation strategy in POSC which then can be used as alternative for NPLC or SFC. Such alternative methods may be needed when for instance, the compound does not show a good solubility, is not separated using NPLC or when an SFC instrument is not available. In a first instance, different parameters, which can have an influence on the enantioselectivity are investigated using experimental design. The investigated factors are the type of mobile-phase basis solvent (methanol or acetonitrile), the kind of alcohol added to the mobile phase, % alcohol added to the mobile phase and the type of additive i.e. a basic (diethylamine (DEA)) or an acidic (trifluoro acetic acid (TFA)) additive. Afterwards, the concentration of additive is in the range of 0.01 % – 1 %.

Results showed that the concentration and type of additives (DEA and TFA) have an important influence on the separation. However the factors with the largest influence are the type of alcohol added and the basis solvent used to prepare the mobile phase (methanol or acetonitrile). Finally, a strategy to screen basic, acidic, neutral and bifunctional compounds within this technique, has been defined. Comparison of the screening results in POSC with results obtained in NPLC, using the same polysaccharide columns, shows that POSC can be used as alternative and even complimentary technique with NPLC. Furthermore, POSC allows as many baseline separations as NPLC, even in shorter analysis times.

DENSITOMETRY AS A DETECTION METHOD FOR SELECTED ALIPHATIC COMPOUNDS SEPARATED BY THE THIN-LAYER CHROMATOGRAPHY

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This work is continuation of study, conducted in Chemistry Institute of Silesian University, on detection of aliphatic compounds with the visualizing reagents and the indicators in a thin layer [1-6].

The majority of aliphatic, non-saturated compounds is characterized by the lack of a specific electronic spectra in the UV-VIS range. Therefore, the investigations have been undertaken to activate spectra of stearic acid, stearyl alcohol and stearamide by the detection in a thin layer. Densitometry in the range of the ultraviolet light has been employed to show the usability of systems developed in the thin layer.

The studied substances separated by adsorption thin-layer chromatography and then detected by aqueous solution of rhodamine B. The spots have been treated by the densitometric method. It has been shown that after the detection the chromatographic band is "activated" in the UV light. The limit of detection and the limit of determination of the studied substances were measured.

The proposed method of detection provides the opportunity for further chromatographic studies of compounds with poor UV spectra.

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CHROMATOGRAPHIC INVESTIGATIONS OF CHEMICAL DURABILITY OF PYROCATECHOL. PART II

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Research of chemical durability of drugs are led from many years in Department of Analytical Chemistry of Faculty of Pharmacy in Silesian Academy of Medicine. This work is continuation of the research of chemical durability of pyrocatechol [1].

The aim of the work was to investigate the chemical durability of pyrocatechol on silica gel as well as in solutions in relation to storage conditions. Investigations were made using NP-TLC method on silica gel and with chloroform – methanol, 1:9 (v/v) as mobile phase. Densitometric measurements were with λ=240 nm using Desaga densitometer.

Chromatographic plates were treated by temperature of 120^{o}C as well as UV radiation during 150 minutes before spotting, and after spotting of pyrocatechol solutions. The complete oxidation of pyrocatechol heated to 120 ^{o}C during 150 minutes after its spotting on chromatographic plate precoated with silica gel was stated. 88% of pyrocatechol spotting on silica gel decrease after 150 minutes of UV radiation.

Influence of water, physiological salt and ethanol on durability of pyrocatechol was also investigated. Pyrocatechol solutions mentioned above were subjected to exposition of visible light, UV radiation and temperature (40^{o}C).

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POSTER SESSION I

THE IMPACT OF HALOGENODERIVATIVES OF METHANE IN THE MOBILE PHASES ON THE R_F VALUESOF SUBSTANCES STUDIED BY TLC

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Hereby is the latest report on progress in porphyrin research carried out at the Institute of Chemistry, The University of Silesia in Katowice [1-7].

This work is a continuation of the study of comparison of separation effect of selected porphyrins and metalloporphyrins by adsorption thin-layer chromatography.

The aim of the presented investigation was to compare the retention parameter of investigated porphyrins and metalloporphyrins: porphine, mono-*tert*-butylporphyrin, tetra-*tert*-butylporphyrin and their nickel, copper and zinc derivatives in adsorption thin-layer chromatography. As mobile phases the following mixtures were used: n-hexane / dichloromethane, n-hexane / trichloromethane and n-hexane / tetrachloromethane in the volume proportions: 4:6, 5:5, 6:4, 7:3, 8:2.

The key issue in the study was to compare R_F and ΔR_F values of investigated substances obtained by using binary mobile phases.

From the above-described results of adsorption TLC studies it may be concluded that higher R_F values were obtained using n-hexane / dichloromethane as mobile phases and lower R_F values were obtained using n-hexane / tetrachloromethane as mobile phases.

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POSTER SESSION I

COMPARISON PROPERTIES OF LIPOPHILIC SELECTED PORPHYRIN. PART I

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Present work is continuation of lipophilic properties investigations of organic compounds about biological meaning. It was made in Institute of Chemistry Silesian University in Katowice [1-4]. Apply to estimation of lipophilic properties of selected alkyloxy porphyrin derivatives. To estimation lipophilic properties was used partition coefficient (log P). Log P was calculated by means of Rekker equation. Results were made comparison with other calculate methods, for example IAlogP, ClogP as well as on base experimental data. We were made an estimate of influence of binary mobile phase (CCl_4:MeOH, $CHCl_3$:MeOH, CH_2Cl_2:MeOH) on effects of chromatographic separation.

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POSTER SESSION I

NEW MODIFICATION OF STANDARD ADDITIVES METHOD FOR QUANTIFICATION OF COMPONENTS OF HETEROPHASEOUS MIXTURES

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Contemporary approaches in quantitative chromatographic analysis include methods of absolute calibration (I), external standard (II), internal standard (III), standard additives (IV), and internal normalization (V). However, this "rigid" classification cannot represent the whole variety of procedures really used in analytical practice.

Quantification by method (IV) for the determination of a total amount of target analyte in the sample (in general case of unknown volume) implies the application of a simplest relationship:

$$\mathbf{m_x = m_{add} P_x / (P_{x+add} - P_x)} \qquad (1)$$

where m_x is targeted amount, m_{add} – mass of added reference sample, P_x and P_{x+add} – parameters (areas or highs) of chromatographic peaks before and after its addition.

The methods I-III,V are traditionally recommended only for homogenous samples, whilst the single exception is (IV), which remains to be correct for *heterophaseous systems* of restrictedly mutually soluble liquids [1]. The artificial formation of these systems during sample preparation permits us to analyze the components of hydrophobic, viscous and thermally unstable matrices by the standard simple procedure, including the consecutive analyses of only one layer of heterophaseous systems before and after addition of reference sample.

However, if these matrices include the constituents being the active sorbents, the applicability of above mentioned procedure may be resulted by significant errors owing to the non-equal and non-controlled sorption of analytes and reference samples.

The new modification of method (IV) is proposed just for similar most complex mixtures. One of them is the experimental samples of anti-cold ointment containing olive oil extract of few medicinal herbs (90-95 %), essential oil of *Mentha piperita* (1.7 %) and fine-dispersed silica gel (specific surface 380 ± 40 m^2/g, 5-10 % by weight). The receiving of reproducible results in this case can be obtained by addition of reference sample of multi-component essential oil not *after*, but *before* transformation of initial sample into heterophaseous system. Simple modification of formula (1) is needed, because of the amounts of sample in two probes analyzed in parallel (m_{x1} and m_{x2}) should be close, but not absolutely equal one each other.

$$\mathbf{m_x = m_{add} P_x / [P_{x+add} - (m_{x2}/m_{x1})P_x]} \qquad (2)$$

It provides the high reproducibility of the results of duplicated measurements:

Sample	m_{x1}, g	m_{x2}, g	m_{add}, mg	Peak areas, mV× ms		$C_x = m_x / M_{sample}$, % by weight
				P_x	P_{x+add}	
1	1.099	1.083	52	145.0	602.7	1.60
				158.5	591.4	
	1.358	1.322	58	99.9	352.4	1.62
				91.6	338.3	
2	1.135	1.118	64	95.8	420.4	1.54
				88.2	434.8	
	1.133	1.149	63	87.4	393.5	1.60
				88.4	396.9	

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POSTER SESSION I

QUANTITATIVE CHROMATOGRAPHIC ANALYSIS AT HUGE LOSSES OF ANALYTES DURING SAMPLE PREPARATION. SPECIAL CHOICE OF TWO INTERNAL STANDARDS

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One of the important problems of quantitative chromatographic analysis is the loss of target analytes during various procedures of sample preparation from complex matrices (e.g., extraction, concentration, evaporation, etc.). The optimization of these stages to provide the appropriate recovery of analytes seems extremely time-consuming work providing any guaranties of success. The alternative way to reach the precise quantification in similar cases is the compensation of losses of analytes by the choice of appropriate methods of quantitative analysis, e.g., the method of standard additives using reference samples of compounds being determined. Another approach is the method of double internal standard (DIS) proposed in 1986 [1] in the "traditional" form having no objective advantages. As a result, its most "attractive" features remain unknown up to present.

Nevertheless, the special choice of two internal standards of the same series like that of target analyte (i.e., the previous and the next homologues), permits us to realize a new possibilities of quantification at huge losses of analytes during sample preparation, namely:

1. To simplify (or to eliminate at all) the procedure of the choice of separation conditions, because any normal linear homologues can be separated using all known types of chromatographic columns with any stationary phases or sorbents;
2. It is not necessary to evaluate the differences in the sensitivity of any chromatographic detectors to target analytes and standards, i.e. to use calibration coefficients;
3. Any distortions in the quantitative composition of probes during sample preparation indicate no influence on the precision of results (most important!).

The following general relationship can be used for quantification by DIS-method:

$$m_x = S_x [(m_{stand(x-1)} / (S_{stand(x-1)}) \times (m_{stand(x+1)} / (S_{stand(x+1)})]^{1/2} \quad (1)$$

where $m_{stand(x-1)}$ and $m_{stand(x+1)}$ – quantities of two internal standards added to the initial samples before any manipulations with them, S_x, $S_{stand(x-1)}$ and $S_{stand(x+1)}$ – areas of chromatographic peaks of target analyte and both standards in the finally prepared probes. The same relationship for concentrations C_x, $C_{stand(x-1)}$ and $C_{stand(x+1)}$ instead of masses is correct also.

The uncertainty of results (RSD, δm_x) can be evaluated by commonly used way:

$$\delta m_x = [\delta S_x^2 + (\delta S_{stand(x-1)}^2 + \delta S_{stand(x+1)}^2)/4]^{1/2} \quad (2)$$

Model experiment: The solution of 2-hexanone in ethanol (C_x = 8.12 mg/ml) has been concentrated before GC analysis by evaporation of the solvent (T_b 78.3 ^{0}C) approximately in 10 times (v/v), that was accompanied by losing of approx. 78 % (!) of analyte. Nevertheless, the DIS-method provides obtaining the precise results:

Object	Formula	T_b, ^0C	Loss, %	$C_{initial}$, mg/ml	S, mV×ms	δS, %
Standard (x-1)	$CH_3COC_3H_7$	101	90	8.09	268 ± 14	5.2
Analyte (x)	$CH_3COC_4H_9$	127.2	78	C_x = 8.12	452 ± 19	4.2
Standard (x+1)	$CH_3COC_5H_{11}$	150.4	64	8.16	751 ± 32	4.3

The determined concentration is C_x = 8.19 mg/ml (δC_x = 5.4 %, ΔC_x = ± 0.44 mg/ml).
Next problem to be solved is how to simplify the choice of two homologues required.

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POSTER SESSION I

RETENTION INDICES OF SOME FLUORINATED ARENES C_7-C_9 ON POLAR INORGANIC SORBENTS. UNUSUAL BEHAVIOR OF SILIPOR 600

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Gas chromatographic and GC-MS identification of fluorinated organic compounds is rather difficult owing to the numerous anomalies of their physicochemical properties, retention parameters and the existence of only one stable isotope ^{19}F. Searching the ways to provide the reliable identification of these compounds, GC retention indices (RI) of fluorinated arenes C_7-C_9 on standard non-polar polydimethyl siloxane OV-101 and inorganic polar sorbents of Silipor series (S600 and S075, LaChema, Czech Republic) have been compared.

Substituents in the molecule of benzene series	M_r	RI (OV-101)	RI (S600) (I)
-H (non-substituted benzene)	78	656 ± 8*	718 ± 2
-CH_2F	110	830 ± 2	906 ± 8
-CH_2CH_2F	124	924 ± 2	831 ± 6
1-F, 4-CH_2CH_2F	142	940 ± 3	851 ± 7
-CH_2CF_3	160	812 ± 3	812 ± 2
1-F, 3-CH_2CF_3	178	810 ± 2	813 ± 2
1-F, 4-CH_2CF_3	178	817 ± 2	812 ± 2
1-CH_2F, 3-CF_3	178	861 ± 2	908 ± 12
1-F, 3-$CHCl_2$	178	1106 ± 1	969 ± 5
1-CH_2CH_2F, 3-CF_3	192	961 ± 2	875 ± 8
-CHCl-CF_3	194	948 ± 4	849 ± 7
1-Cl, 3-CH_2CF_3	194	978 ± 1	850 ± 4
1-F, 4-CHCl-CF_3	212	939 ± 1	846 ± 5
1-CF_3, 3-CH_2CF_3	228	834 ± 1	836 ± 3
1-Cl, 3-CHCl-CF_3	228	1025 ± 3	857 ± 3
-CHBr-CF_3	238	1030 ± 2	879 ± 2
1-Cl, 4-CHBrCF_3	272	1206 ± 7	931 ± 6
Correlation coefficient of MWs with RIs		0.570	0.169

*) Interlaboratory averaged RI value.

Any groups of organic compounds can be characterized by relative variability of some properties (A), e.g., $s_A/\langle A \rangle$, %. The value of this parameter for molecular weights M_r is 22 %, whilst for RI (OV-101) and RI (S600) it is only 12 and 5 %, that means their insufficient information content for the unambiguous identification of fluoroarenes.

Moreover, surprisingly we have revealed unusual behavior of Silipor 600. RI data measured on column (I) in the period 1990-1992 are strongly different from RIs determined on the same column in 2005. At the same time, RI values of various compounds on the anew prepared columns with S600 (II), S600 after preliminary cleaning by EtOH/H_2O (III) and even with

additional drying of carrier gas (nitrogen) by molecular sieves (IV) indicate the high reproducibility. The possible reasons of these unusual properties of Silipor 600 are discussed.

Compound	Column I (1990-1992)	I(2005)	II	III	IV
cyclo-C_4F_8	375 ± 8	374 ± 2	374 ± 1	373 ± 2	374 ± 2
Benzene	849 ± 14	718 ± 2	721 ± 3	720 ± 2	719 ± 2
Tetrachloroethylene	760 ± 4	678 ± 2	682 ± 2	677 ± 2	680 ± 3

This work is partially supported by RFBR (grant № 04-03-32900).

POSTER SESSION I

THE POTENTIAL APPLICATION OF REFRACTIVE INDEX DETECTOR FOR DIRECT DETERMINATION OF HYDROCARBON GROUP – TYPE COMPOSITION BY HPLC – RID TECHNIQUE

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Keywords: NP-HPLC, refractive index detection, direct hydrocarbon group - type determination

The work presents theoretical principles and the analytical procedure enabling direct determination of the hydrocarbon group - type composition (HGTA) of crude oil products of different volatility using NP-HPLC in isocratic conditions with refractive index detector (RID). The procedure is based on the HPLC separation, using two eleuents with different known refraction coefficients and on the backflush of the most polar group of compounds. The average value of refraction coefficient of each individual group is calculated on the basis of the area of adequate peaks from both chromatograms and on known refraction coefficients of eluents. The peak area of all groups of compounds in the sample or alternatively only the area of the peaks representing compounds of known total concentrations should be used for the calculation. The hydrocarbon group - type composition (HGTA) of the sample is calculated using normalization by correction factors. The correction factors are calculated on the basis of refraction coefficient of each individual hydrocarbon group. Additionally, we address the problem of the incompatibility of the literature values of refraction coefficients determined under standard measuring conditions with the values of refraction coefficients characteristic for applied RI detector (e.g. temperature, detection wave length). The hydrocarbon group - type analysis and the calculation of exact refraction coefficients' values of particular groups would not be possible without consideration of these discrepancies. Presented results show usefulness the proposed method for determination of group - type composition of middle- and low-volatile crude oil products of different refining processes. The data are compared with the ones, obtained using different detection and calibration methods. The described methodology has more universal character. For example, it can also be used for the determination of group - type composition of plant, fungal and bacterial secondary metabolites. Preliminary results of such group - type analysis are also presented in the communication.

INVESTIGATION OF RETENTION FOR THE ADSORBING AND THE NOT ADSORBING ORGANIC ACIDS ON A STRONGLY ACIDIC CATION-EXCHANGE RESIN IN THE H^+-FORM

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Ion Exclusion Chromatography (IEC) is one of the methods that enable separation of acids, alcohols and other ionisable substances. Typically, the retention of ionisable species on the cation exchange resin falls within the interval of the retention times between completely dissociated analytes (such as, e.g., sulphuric acid) and practically non-dissociated and non-adsorbing analytes (such as, e.g., methanol). However, for the acids with long enough aliphatic chains and/or aromatic cycles, the retention time is much higher, than for methanol. It is connected with a relatively strong interaction between the resin and the hydrophobic functionalities of the analytes. Moreover, the retention time of the peak apex of the adsorbing acid is at the beginning growing with the concentration growth, and then it starts decreasing, whereas for the non-adsorbing acid the retention time of the peak apex is always increasing with the concentration growth. This observation is due to combination of the two retention mechanisms, i.e. ion exclusion and the non-dissociated acids adsorption.

The attempt of this study is to model separation mechanism by means of ion exclusion chromatography, coupled together with adsorption of the non-dissociated acid molecules.

Acknowledgment

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POSTER SESSION I

INVESTIGATION OF THE MASS TRANSFER KINETICS OF 5-PHENYL-1-PENTANOL, *tert*-BUTYLPHENOL AND AMYLBENZENE ON DIFFERENTLY PACKED COLUMNS

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In paper [1] we investigated the mass transfer kinetics of amylbenzene, 5-phenyl-1-pentanol and tert-butylphenol on differently packed columns, using the moment analysis method. Information on the mass transfer kinetics between the two phases is obtained from the first absolute moment and the second central moment, respectively. The values of the effective diffusivity were calculated from the first and the second moments. The results of these calculations suggest that the effective diffusivity decreases with the increasing sample concentration. It seems that the trend observed can be explained by assuming that the surface diffusion plays a predominant role in the mass transfer inside the adsorbent particles and the pore diffusivity can be neglected. The best results for 5-phenyl-1-pentanol were obtained, when assuming that the gradient of the surface concentration is the driving force. For the tert-butylphenol, the best results were obtained, when the gradient of the chemical potential was the driving force of the surface diffusion. In the case of amylbenzene, the internal mass transfer is controlled by the pore diffusivity.

The aim of this work was to validate the mass transfer kinetics, described previously [1]. Validation was made by comparing the shapes of experimental peak profiles with the theoretical simulations, using the Lumped Pore Model of chromatography.

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POSTER SESSION I

VACANT REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (VRP-HPLC)

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Traditionally, in elution HPLC, the analyzed sample is injected to the flowing mobile phase. As a mobile phase just pure solvent (like water) can be used, sometimes containing an extra additive like, e.g., buffer, the inclusion compounds, the ion-interaction reagents *etc*. In the case of IEC, this sequence was recently reversed and the so-called vacancy ion exclusion chromatography (vIEC) was proposed.

The characteristic feature of IEC is the same sign of the electric charge both of the dissociated functional groups of resin and of the analyzed ionic compounds. It follows that the negatively charged samples, *e.g.,* the dissociated acidic compounds, are repulsed (or excluded) from the resin, while the non-dissociated (i.e. neutral) ones can penetrate into the stationary phase. As a result, characteristic leading (frontal tailing) peaks are observed, when the pure water is used as eluent.

It is interesting to note that, in certain way, similar effect is observed in RP-HPLC, although this is due to a different retention mechanism. In this case, ions cannot be partitioned, involving the non-polar coverage of the silica gel.

In our presentation, the above statement will be confirmed and the retention mechanism described theoretically. It turns out, that – when just pure water is used as mobile phase – the acids' leading peaks are really observed in RP-HPLC. Additionally, it will be shown that the vacant peaks are also obtained by means of this technique. In this particular case, symmetrical peaks are observed, what can also be predicted by our theory.

POSTER SESSION I

STUDY OF THE LIPOPHILICITY OF SOME AZAPHENOTHIAZINES

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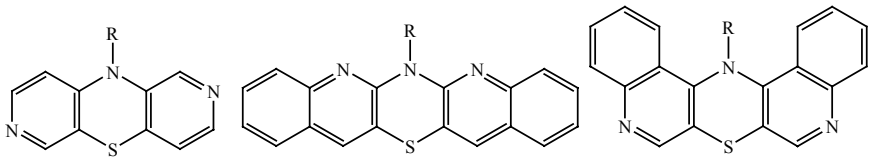
Phenotiazines attract attention because of their wide chemical properties and very interesting biological activities. Some modifications of the phenothiazine structures were directed into azaphenothiazines, where the benzene ring was substituted with an azine ring [1]. We modified the phenothiazine structure with the pyridine and quinoline rings to obtain dipyrido-1,4-thiazines 1 and quino-1,4-thiazines 2,3 in the cyclization reactions of disubstituted pyridine and quinoline derivatives and N-alkylations of the thiazine ring in diazino-1,4-thiazines [2-5].

The purpose of this work is to determine the lipophilicity as log P_{TLC} values of NH- and N-alkylsubstituted diazino-1,4-thiazines 1-3 by use of the RP TLC method (on RP-18 silica plates with acetone-aqueous TRIS (tris(hydroxymethyl)aminomethane) buffer as the mobile phase) and to compare with theoretical values (log P_{calcd}) obtained by the means of computer programs ClogP (CambridgeSoft. Com., Daylight Chemical Information Systems Inc., USA) and logKow (Syracuse Research Co., USA).

The differences between calculated and experimental values and among calculated values have been discussed. The influence of the azine ring and a shape of multicyclic ring system on the lipophilicity have been observed.



1 **2** **3**

R
a H
b CH_3
c C_2H_5
d $CH_2CH{=}CH_2$
e CH_2Ph

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POSTER SESSION I

STUDY OF THE LIPOPHILICITY OF SOME DIQUINOTHIAZINES

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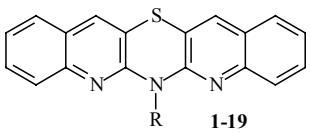
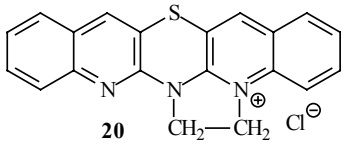
Phenotiazines attract attention because of their wide chemical properties and very interesting biological activities. Some modifications of the phenothiazine structures were directed into azaphenothiazines, where the benzene ring was substituted with an azine ring [1]. In continuation of our search for pharmacoactive quinoline derivatives we modified the phenothiazine structure with the quinoline ring to obtain diquino-1,4-thiazines being dibenzodiazaphenothiazines [2-4]. Lipophilicity is very important molecular property used in QSAR studies and plays crucial role in the design of new drugs with the required biological activity.

The purpose of this work is to determine the lipophilicity of substituted diquino[3,2-b;2',3'-e][1,4]thiazines 1-20, with the potential psychotropic and anticancer activity, by use of the RP TLC method (on RP-18 silica plates with acetone-aqueous TRIS (tris(hydroxymethyl) amino-methane) buffer as the mobile phase) and to compare with theoretical values obtained by the means of a computer program ClogP (CambridgeSoft. Com. USA).

The both parameters log P_{TLC} and log P_{calcd} show high lipophilicity of diquinothiazines 1-20, similar or in some cases higher than the phenothiazine drugs.

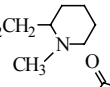
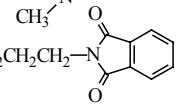


1-19



20

R

1 -H
2 $-CH_3$
3 $-CH_2CH_3$
4 $-(CH_2)_3CH_3$
5 $-CH_2CH{=}CH_2$
6 $-CH_2-C_6H_5$
7 $-C_6H_5$
8 p-CH_3-C_6H_4-
9 p-Cl-C_6H_4-
10 p-Br-C_6H_4-
11 p-NO_2-C_6H_4-
12 
13 $-CH_2CH_2NH_2$
14 $-CH_2CH_2N(CH_2CH_3)_2$
15 $-CH_2CH_2CH_2NH_2$
16 $-CH_2CH_2CH_2N(CH_3)_2$
17 
18 
19 $-CH_2CH_2CH_2NHCONHCH_2CH_2Cl$

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POSTER SESSION I

MODIFICATION OF STATIONARY PHASES USED IN LIQUID CHROMATOFRAPHY BY SOME TRANSITION METAL IONS

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Argentation Chromatography – the name of kind of chromatography derive from modification of surface of stationary phases by silver ions. This chromatography is widely used for separation unsaturated compounds [1]. But a different, than silver metal transition ions can change a properties of the given stationary phase. Phases modified of the transition metals ions can be improve separation of the given mixture. Modification of the silica adsorbent with metal salts was based on the following chemical reaction:

$$\mathbf{n\text{-}SiOH + Me^{+n} \leftrightarrow (SiO)\text{-}Me + nH^{+}}$$

Metal ions are linkd by ionic bonds to the surface of the silica stationary phase.

The literature contains a lot of examples of the modification procedures of different stationary phases by metal ions. The simplest, fast and cheep method is impregnation of the adsorbent surface by metal salts. So obtained stationary phase material is very attractive for separation of alkaloids [2], amino acids, amines, mercaptans and others[3-5].

SiO_2 60 TLC plates were dipping in 0.1 M water solution of nitrate salts of Ni^{+2}, Co^{+2} and Zn^{+2} and stayed in the solution during 24 h and excess of the salts was eliminated from the layer by prewashing of the plates by acetone (in acetone on SiO_2 all investigated ion salts stayed on the start line). So modified plates were hold at a temp. 100^0C during 1.5 h. On so prepared plates chromatograms of some PAH were developed in mobile phase n-heptane – carbon tetrachloride in all concentration range.

Modification of the silica gel layers by investigated ions salts influenced on the retention of the PAH and theirs peak shape and area. The influence depends on the composition of the mobile phase. For example: in pure hexane on SiO_2 layers modified by $Ni(NO_3)_2$ the investigated PAH stronger interaction with the layer than on the pure silica layer. In the system Ni^{+2} ions activated surface. Modification of silica surface R_F values of PAH of the Co^{+2} ions practically does not influence the retention of the PAH, but modification by Zn^{+2} ions deactivated silica surface (higher R_F values of the investigated PAH).

Addition of the carbon tetrachloride to mobile phase changes relation between retention data obtained on the modified surface and non-modified. In mobile phases with carbon tetrachloride so high differences in retention between surface modified by Ni^{+2} and Co^{+2} ions do not exist. Number of the theoretical plates is different on modified and non-modified surface. On SiO_2 modified by transition metal ions surface peak height and peak area are different. Modification of the silica surface of the transition metal ions can change retention, separation and efficiency of the chromatographic systems.

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SOME PROBLEMS OF THE USE OF THE UTLC PLATES

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In the last years the new kind of the stationary phase – so called monolithic stationary phase were used. Monolithic stationary phases now are often used in column liquid chromatography. In planar mode monolithic stationary phase are used in so called ultra thin-layer plates (UTLC). The UTLC plates are one of the great steps for miniaturization of the planar chromatography systems. The plates are size 60x36 mm and layer thickness is 10 μm. Adsorbent layer is fixed on the glass plates without any binder. To this time there are only few publication about chromatographic separation on UTLC plates.

In the presented paper the influence :

- migration distance,
- conditioning of the sorbent layer of mobile phase vapors

on separation results obtained on UTLC plates were demonstrated.

The separation results obtained in UTLC plates as:

- Number of the theoretical plates
- High of the theoretical plates
- Retardation factors
- Peak shape and area

were compared with the separation results on TLC, HPTLC plates.

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POSTER SESSION I

TWO DIFFERENT APPROACHES TO PREDICTION OF THE MOLAR ENTHALPY OF VAPORIZATION. A COMPARISON

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In our earlier papers [1-8] on derivation of thermodynamic magnitudes from gas chromatographic data, the relationships were presented for the non-reduced relative retention as a function of the analyte's boiling point and, respectively, of its molar volume or molar refraction. Obviously, it is the IUPAC's strong recommendation to employ chromatographic parameters, which make use of the reduced values (i.e. of those with the dead time subtracted). However, this does not explicitly, or quasi-automatically discriminate these chromatographic parameters, which are based on the non-reduced values.

In this paper an attempt was made to solve an important question, which parameters – those that incorporate the reduced, or the non-reduced measuring data – are better suited for determination of the selected thermodynamic values. This issue was scrutinized upon an example of the relative retention (r) and of the non-reduced relative retention (r_G), by a comparison of the results obtained with aid of the novel non-empirical equations:

$$r = A\exp\left(BX + C\frac{X}{T_B}\right) \quad [9]$$

and of those already published:

$$r_G = A\exp\left(BX + C\frac{X}{T_B}\right) + const. \quad [1]$$

where $X \equiv V_m$ (molar volume) or R_m (molar refraction), T_B – the analyte's boiling point, and A, B and C denote the physically meaningful fitting parameters of the equations (with B and C enabling determination of the thermodynamic parameter of interest and namely, of the analytes' molar enthalpy of vaporization, ΔH_{vap}).

Our relationships were tested upon the measured data, originating from the three classes of the test analytes (i.e. alkylbenzenes, aldehydes, and ketones), which differed in terms of polarity. The experimental data originate from the isothermal measurements, carried out on three different capillary columns, coated with the low- and medium-polarity stationary phases at five working temperatures from the 323–423K range.

After a statistical data processing, the obtained numerical values of the molar enthalpy of vaporization (ΔH_{vap}) were compared with the available data taken from literature, thus testing the fitting and predictive performance of the models compared.

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POSTER SESSION I

CAPILLARY GAS CHROMATOGRAPHIC DETERMINATION OF THE MOLAR ENTHALPY OF VAPORIZATION WITH THE SELECTED HOMOLOGOUS AND CONGENERIC ALCOHOLS

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In partition gas chromatography, the chromatographic process consists in an endlessly repeated transfer of an analyte from the stationary to the mobile phase and *vice versa* (which is in fact partition). This process can be compared to an unednding repetition of the vaporisation and condensation acts and for this particular reason, the thermodynamic parameter the best characterizing these transitions would be either the enthalpy of vaporization, or the enthalpy of condensation. Absolute numerical values of these two enthalpies are approximately the same, although they differ with the sign, due to the fact that the respective physical processes are mutually adverse (as it comes out from the Lavoisier – Laplace Law).

All these observations give rise to an idea, that instead of the traditional (i.e. direct) method of calorimetry (which in practice proves complicated and tedious), for measuring of the substances' molar enthlpies of vaporization or condensation, one might utilize the indirect technique of gas chromatography. How this goal can be achieved in an easy, rapid, and reliable enough manner, this will be discussed in present paper.

The idea itself is certainly not new and the approach benefits from the specially devised mathematical models, that couple together the chromatographic magnitudes with the physical ones (like, e.g., the analyte's boiling point, its molar volume, or molar refraction), thus making it possible to derive in a clever enough manner – as it was an example in our case – the molar enthalpy of vaporization, ΔH_{vap} [1-4].

This idea was put into practice in a wide variety of modifications (i.e., with use of ten different non-empirical equations) and the results attained were published, e.g., for the numerous alkylbenzenes, aldehydes, and ketones as the selected test analytes (the entity of the computed data were presented in [5]).

Presently, we have chosen a vast group of the test analytes from the class of alcohols for our study. The chromatographic data (i.e. the retention times of the test alcohols) were measured on the medium-polar stationary phase under isothermal conditions, at several working temperatures ranging from 323 to 423 K. After the preliminary mathematical and statistical data processing, the numerical values of the molar enthalpy of vaporization for individual alcohols were determined and finally compared with the available numerical data, found in literature.

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COMPARISON OF 8 AND 16 BITS SCANNING MODES FOR QUANTIFICATION OF CHROMATOGRAPHIC BANDS IN PLANAR TECHNIQUES

M. Przyjemska – Werner[1], E. Włodarczyk[2], M. Baran[2], P. K. Zarzycki[2,3]

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Most of the graphic programs are efficiently working with the 8-bits data set per reed-green-blue (RGB) channel or Gray Scale mode. For that purpose, densitometric scanning in UV-Vis region or even in fluorimetric mode can be done using simple and non-expensive computer driven office scanners or digital cameras [1,2]. Such hardware that is presently available on the market may capture the images with 8, 12 or 16-bits real color depth. From theoretical point of view, using 16-bit scanning mode it is possible to generate a data set linearly scaled to range from 0 to 65535 instead from 0 to 256 for 8-bits mode.

In our work we are testing the use of the office scanners with 8 and 16 bits image-processing technology for quantification of chromatographic spots generated by planar separation method. Selected dyes (methyl red and fluorescein) were separated on K-60WF_{254} silica TLC plates (Merck) using 100% methanol as the mobile phase. Chromatograms were developed at room temperature in horizontal TLC chamber (CHROMDES DS-L). The battery of spots obtained were scanned and analyzed using Scion Image software [3]. Our results suggest that the efficiency of 16 bits scanning mode for capturing of the quantitative information from the planar chromato-graphic bands can be strongly affected by the non-homogeneity of stationary phase surface.

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POSTER SESSION I

STUDIES OF BINARY MOBILE PHASES FREEZING POINTS AND SOLUBILITY OF SELECTED CYCLODEXTRINS IN DIFFERENT TEMPERATURES

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Binary methanol or acetonitrile-water mixtures are commonly used as the mobile phases for the number of separation and pre-purification techniques including capillary electrophoresis, planar and column chromatography as well as solid-phase extraction. In case of temperature optimisation the boiling and freezing points of liquid phase are limiting the "physical space" of the whole separation process. Moreover, the solubility of solid additives such buffers components or chiral additives is usually strongly affected by the temperature. In some cases additives solubility is changing significantly within relatively small temperature region. For that reason the effective use of host-guest modifiers such β-cyclodextrin at high concentration and in low temperatures is strongly limited and may even cause the column and detector failure or damage. On the other hand the separation of complex mixtures using such mobile phases may be very effective at sub-ambient temperatures, therefore, the solubility of chiral additives is one of the important factor for the designing of proper separation systems [1,2].

The objectives of this work were to measure the freezing point trajectories for selected binary mixtures as well as solubility of β-cyclodextrin and 2-hydroxypropyl-β-cyclodextrin at different temperatures and for given mobile phases compositions. The strong non-linear behaviour of acetonitrile/water mixtures observed on the freezing point/mobile phase concentration plot is discussed from practical point of view.

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POSTER SESSION I

ABILITY OF COMMERCIAL DS HORIZONTAL TLC CHAMBER FOR TEMPERATURE CONTROLLED PLANAR CHROMATOGRAPHY

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The main advantages of planar chromatography results from its simplicity and easy of operation. Therefore, the majority of TLC runs are usually performed at room temperatures using non-thermostated developing chambers [1]. Contrary to great progress in the construction of TLC chambers, the problem of high precision and reproducibility of the plates temperature in commercially available devices was not successfully resolved and this topic is still poorly recognised [2]. Because the selectivity and efficiency of separation process are temperature dependent and temperature may also significantly affect the precision and reproducibility of analysis, new devices for temperature controlled TLC are still constructed [3,4].

The aim of this work was to study the ability of commercially available DS horizontal chamber without internal thermostatic system for runs in elevated and sub-ambient temperatures. The constant and reproducible conditions were obtained using thermostatically controlled external oven. The times that are necessary for ensure proper chamber and TLC plates temperature equilibrium before beginning the chromatographic experiments were measured.

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POSTER SESSION I

OPTIMIZATION OF TLC DETECTION OF SELECTED UV-VIS TRANSPARENT STEROIDS USING THE PMA STAIN METHODOLOGY

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The main advantages of detection protocols that are involving phosphomolybdic acid (PMA) are simplicity, sensitivity as well as stability of the reaction products and reagent itself. Moreover, this process is usually quantitative and can be applied for visualization of number of UV-VIS transparent substances on the TLC plates. For that reason PMA is considered as a multipurpose stain, which is sensitive to low amount of components of interest and may be employed on silica gel, aluminum oxide, polyamide, RP-2, RP-18 and cellulose stationary phases [1].

In the present work we are reporting robust and sensitive detection protocol for selected UV transparent steroids like cholesterol and bile acids (cholic, lithocholic acids and taurodeoxycholic acid sodium salt) using PMA reagent. Visualization conditions were studied and optimized for steroids separated on TLC and HPTLC glass plates covered with silica gel ($K60WF_{254}S$) and octadecylsilane (RP-18W) stationary phases. Spot intensities on the plates after spraying them with the PMA in methanol (10%w/v) and heating in wide range of temperatures (from 40 to 120^{o}C) and times ranging from 2 to 40 minutes were quantified using Scion Image freeware. The best conditions for high signal intensity was determined using temperature(X)-time(Y)-analytical signal(Z) 3D-maps generated from the raw experimental data points. In contrary to the number described in literature "universal protocols" our study indicates that robust and sensitive quantification of our components of interest should be done at relatively low temperature regions below 100^{o}C and for heating times more than 10 minutes.

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RETENTION PROCESS IN RP-TLC SYSTEMS WITH POLAR BONDED STATIONARY PHASES

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The retention of a solute in RP – chromatography is a very complex process which depends on many factors. Therefore, the study of the influence of a mobile phase modifier concentration on the retention in different reversed phase chromatographic systems is very important for understanding the regularities of the retention and mechanisms of substance separation in a chromatography process. Composition changes and the nature of mobile phases enable tuning of the separated analytes' retention in a wide range of the retention parameters and optimization of the chromatographic process, as well. Optimization of the chromatographic process can be achieved by several different methods, one of them is so-called interpretative strategy. The key role in this strategy is the implementation of adequate retention models that couple the retention of solute with the composition of a mixed mobile phase. The employment of chemically bonded stationary phases composed of partially non – bonded silica matrix and organic ligands bonded to its surface in the everyday chromatography practice leads to the questions of the correct definition of retention model and dominant retention mechanism in such chromatographic systems. The retention model for an accurate prediction of retention factor as a function of modifier concentration, the heterogeneity of adsorbent surface should be taken into consideration. In this work the influence of mobile-phase composition on the retention of the sixteen model substances such as phenols, quinolines and anilines as test analytes in different RP-TLC systems with CN-, NH_2-, and Diol-silica polar bonded stationary phases has been studied. The aim of this study is to compare the performance of three valuable retention models assumed as the partition, adsorption/partition and adsorption mechanism of retention. All the models were verified for different RP-TLC systems by three statistical criteria. The results reported here show that heterogeneity of the adsorbent surface may be important in analysis of elution process in liquid chromatography.

POSTER SESSION I

BI-LANGMUIRIAN RETENTION MODEL IN ANALYSIS OF MODIFIER CONCENTRATION EFFECTS IN HPLC RETENTION PROCESS

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In this work, the influence of mobile-phase composition on the retention of selected test analytes in different normal- and reversed-phase chromatographic systems has been studied. The novel heterogeneous retention model for an accurate prediction of the analyte retention in the column chromatography with binary mobile phase has been proposed. This model has been formulated theoretically. The proposed model was experimentally examined by the author by use of three criteria: the sum of squared differences between the experimental and theoretical data, approximation of the standard deviation, and the Fisher test. From the presented results of the investigations it can be concluded that the four – parameter model proposed in this study provides an excellent agreement between the experimental and theoretical data for most NP and RP chromatographic systems studied. It performs equally well both at high and low levels of the organic modifiers.

POSTER SESSION I

STUDING OF COMPLEXATION PROCESS: AROMATIC COMPOUNDS CONTAINING –OH, –NH_2, –COOH GROUPS WITH 18-CROWN ETHERS OR ß-CYCLODEXTRIN BY MEANS OF GAS, LIQUID CHROMATOGRAPHY AND CAPILLARY ELECTROPHORESIS

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Quantitative treatment of complexation process during chromatographic and electrophoretic separation gives an advantage to predict retention mechanism of analysing objects and their elution oder.

It is considered physicochemical models which allow to get qualitative and quantatative evaluation of possible complexation process like *"macrocycle-analysing substance"* when 18-crown ethers or ß-cyclodextrin (ß-CD) are used as components of chromatographic phases (Gas and Liquid Chromatography, GC and LC) and running buffer (Capillary Zone Electrophoresis, CZE). Analysing substances are mostly aromatic compounds with –OH, –NH_2, –COOH groups, including mixtures of important neurotransmitters: adrenalin, noradrenaline, kynurenine, 3-hydroxykynurenine, dopamine, serotonin and others for LC and CZE.

McReynolds and Abraham's solvation parameter models were used for quantitative treatment of macrocycles' possibility to interact with analytes by various forces (dispersion, electrostatic, hydrogen bonds, n,π-electron pair interactions). Stationary GC phases (18-crown-6, DB-18-crown-6, Apiezon L+ß-CD, PEG+ß-CD, glycerin+ ß-CD) were prepared for this purpose and described by retention parametrs of different kind organic substances.

To evaluate meanings of complexation constants we varied macrocycles' concentrations in gas chromatographic stationary phases at different temperatures. We investigated the influence of running buffer properties like pH, electrolyte, organic modifier, macrocycle type and their concentrations on electrophoretic mobilities of ionogenic analytes by CZE method. It was ascertained the dependence of eluent composition and pH on analysing substances' retention by RP HPLC.

We showed that there is the same logic of stability constant's evaluating, which doesn't depend on the using method: GC, LC, CZE. It is based on consideration of chemical equilibrium settled in a chromatographic system. In conclusion we discussed limits of using physicochemical models and the quality of information they can provide for separation of complex mixtures containing bioactive substances.

POSTER SESSION I

OSCILLATORY TRANS-ENANTIOMERIZATION OF THE SELECTED PROFENS STORED IN THE ALCOHOL-AQUEOUS MEDIUM

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In the course of our original studies on the planar chromatographic separation of the two enantiomers, *S*-(+)- and *R*-(-)-ibuprofen, we discovered that a prolonged storage of this common profen drug in a pure enantiomeric form (e.g., as S-(+)-ibuprofen) in the ethanol-aqueous medium results in a rapid oscillatory (i.e. continuous) trans-enantiomerization of one stereoisomer into another. These quite unexpected oscillatory changes were traced with aid of thin-layer chromatography (TLC) and then confirmed with a classical method, i.e. by use of polarimetry. Application of TLC to successfully tracing configuration changes with the two enantiomeric antipodes is – to our best knowledge – the first reported case of using this well established separation technique to solving a purely physicochemical problem of such kind. We then selected two additional enantiomeric compounds from the same group of profens (i.e. *S*-(+)-naproxen and *S,R*-(±)-2-phenylpropionic acid), in order to check their susceptibility to trans-enantiomerization. It was clearly and convincingly demonstrated with aid of TLC and of polarimetric measurements that both, naproxen and 2-phenylpropionic acid, vigorously trans-enantiomerize in the ethanol-aqueous medium.

The molecular-level explanation of this absolutely striking behaviour with the three examined profens is partially derived from literature and it anticipates the self-catalyzed (with the electrolytically dissociated acidic proton) elementary step of the keto-enol tautomer formation. One way to confirm the anticipated molecular mechanism of the oscillatory trans-enantiomerization is to check the influence of a non-aqueous solvent, e.g., dichloromethane, on stability (or otherwise) of the three investigated profens.

Discovery of the oscillatory changes with the profens studied proved a real surprise to us, and especially in view of the multiple claims dispersed throughout pharmacological literature, that therapeutic activity of *S*-(+)-ibuprofen (and of the other *S*-(+)-profens as well) is incomparably higher than that of its *R*-(-)-antipode. Now it seems quite obvious, that in the course of pharmacokinetic transport of the *S*-(+)-profens through the aqueous environment of body fluids, their oscillatory trans-enantiomerization simply cannot be avoided, and hence the true curing potential of a pure enantiomer cannot reliably be assessed.

POSTER SESSION I

OSCILLATORY TRANS-ENANTIOMERIZATION OF THE SELECTED PROFENS STORED IN THE NON-AQUEOUS MEDIUM

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Oscillatory trans-enantiomerization of *S*-(+)-ibuprofen, *S*-(+)-naproxen, and *S,R*-(±)-2-phenylpropionic acid during a prolonged storage in the ethanol-aqueous (7:3, v/v) medium proved a striking and so far undescribed phenomenon, having an unknown molecular-level mechanism. In literature, a report was found [1] on the purposely induced and base-catalyzed keto-enol tautomerism of *S*-(+)-ibuprofen (finally leading to the trans-enantiomeric configuration change of this clearly defined steric structure), resulting in the respective racemate. From the general knowledge of organic chemistry it comes out, that keto-enol tautomerism can also be acid-catalyzed. As a relatively wide number of the known oscillatory reactions are self-catalyzed, it seems quite reasonable to await that oscillatory trans-enantiomerization of profens (all of them being the 2-phenylpropionic acids) embraces the self-catalyzed step of keto-enol tautomerism, with the acidic proton (H^+) split in the course of electrolytic dissociation from the acid's own carboxylic group.

In order to verify this hypothesis, which refers to the origin of the oscillatory trans-enantiomerization of profens, we decided to store the three aforementioned test compounds (i.e. *S*-(+)-ibuprofen, *S*-(+)-naproxen, and *S,R*-(±)-2-phenylpropionic acid) in the non-aqueous medium, which to a large extent hinders dissociation of weak acids. Thus we selected dichloromethane as a non-aqueous solvent and investigated the stability of steric configurations with the compounds of interest, stored as dichloromethane solutions. Several observations were made; the most important among them being that in the non-aqueous medium trans-enantiomerization of profens is substantially hindered, although not eliminated completely. This observation remains in full conformity with the know fact on a considerably hampered, although not entirely suppressed electrolytic dissociation of the compounds able to dissociate in the non-aqueous media.

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POSTER SESSION I

OSCILLATORY TRANS-ENANTIOMERIZATION OF THE SELECTED PROFENS STORED IN THE PHYSIOLOGICAL SALT MEDIUM

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Our earlier studies were devoted to investigating oscillatory trans-enantiomerization of the three selected profens (*S*-(+)-ibuprofen, *S*-(+)-naproxen, and *S,R*-(±)-2-phenylpropionic acid), when dissolved in the ethanol-water (7:3, *v*/*v*) mixture, or in the pure organic solvent (dichloromethane), and then stored for a prolonged period of time at the two measuring temperatures (6 and 22^{o}C). Upon the results obtained an assumption was made, that the elementary transition step from the *S* to the *R* configuration (and *vice versa*) consists in the proton (H^+) self-catalyzed keto-enol tautomerism of a given stereoisomer. This assumption was supported by a comparison of the efficiency of trans-enantiomerization in the aqueous and the non-aqueous medium. Aqueous medium undoubtedly favourizes electrolytic dissociation of the profens' carboxyl groups (and therefeore the keto-enol tautomerism also, which is a crucial transition step from one configuration to another), whereas electrolytic dissociation of profens in the non-aqueous medium is to a large extent hampered.

Our earlier experiments and an outcome thereof seem eloquent enough to suggest an onlooker that a pharmacological dispute on medical effects of the pure enantiomeric varieties of individual profens cannot be seriously taken in view of an evident steric instability of these compounds, due to a continuous oscillatory transition from one enantiomeric form to another.

In order to deeper scrutinize steric instability of the investigated profens in the natural environment of body liquids, in our current research we stored these compounds dissolved in physiological salt. Physiological salt normally consists of several inorganic salts (e.g., of NaCl, KCl, $NaHCO_3$, and $CaCl_2$) plus glucose, with sodium chloride as an absolutely predominant constituent thereof, and it is a known fact that it mimics an average liquid composition of a body cell cytoplasm. Using this very specific water-based solution to dissolve our profen samples, we attempted to store these compounds under the conditions roughly resembling those inside a living organism. It was experimentally confirmed that storage of the investigated profens in physiological salt also results in their continuous oscillatory trans-enantiomerization.

POSTER SESSION II

DETERMINATION OF TRACE AMOUNTS OF BISPHENOL-A IN WATER SAMPLES BY THE USE OF GAS-CHROMATOGRAPHY

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Even if the estrogene properties of Bisphenol-A (BPA, 2,2-bis-(4-hydroxyphenylo)-propane) have been reported since 1936 (C. Doods and W. Lawson [1]), only recently an attention has been paid to this compound [2,3]. Bisphenol-A is widely used in chemical industry to produce: polycarbonate resins, epoxy resins, unsaturated polyester and polysulphonate resins, and many more. Different products containing BPA are commonly used in many domains of our life, to mention lacquers covering cans for drinking water, food, and medicines. In addition, polycarbonate resins containing BPA are used to produce several internal elements of medical apparatus (e.g., for dialysis, blood oxidizing), baby bottles, and some plastic kitchen ware, as plates, cups, spoons, etc.

BPA, since 1996, has been classified by the European Commission as an external derivative having an adverse influence on human health and offspring. Bisphenol-A may be harmful because of its setting free from the plastics cans produced with the use of the above-mentioned resins and lacquers.

In the present work we proposed a method of isolation and chromatographic detection of Bisphenol-A in water samples having contact with polycarbonate plastic and cans internally covered by a polycarbonate. We also proved that BPA is migrating from the PC-made cans to the water they contain. The analysis of water samples towards contents of Bisphenol-A is performed by the following method: preconcentration of water samples using solid-phase extraction (SPE), drying the sorbent with an air stream, eluting the analyte by the use of methanol, evaporation of solvent excess, and finally GC analysis. For the identification and determination of BPA in the samples we used gas chromatography with flame ionization detection (FID) and low resolution mass spectrometry (LRMS). The water samples under study were collected from the PC-made commercial bottles, and from the canned food (vegetables).

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POSTER SESSION II

GC MS IN INVESTIGATION OF FRACTIONS OBTAINED ON THERMOCATALYTIC DEGRADATION OF POLYMER WASTE

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The EU countries have recommended different ways of polymer waste disposal. At present the plastics that are most often considered for raw material recycling are polystyrene (PS), polypropylene (PP) and polyethylene (PE). Their percent contribution in the total mass of waste plastics is the greatest. They are characterised by relatively simple structure, which suggests that the liquid products obtained on their degradation could be used for fuel composition. Recognition of the structure of the compounds obtained on degradation of these plastics is necessary for prediction of their effect on the new products in which they are to be used. The quantitative analysis of particular components of the degradation products is expected to permit such a modification of the process to ensure the highest possible yield of the most valuable ones.

Gas chromatography allows a separation of the mixture of products and the mass detector allows a determination of structures of particular components at a high probability. Moreover, on the basis of the chromatogram it is possible to establish the quantitative description of the mixture composition. The thermal degradation of PP, PS, PE was performed and studied by the GC-MS method. The reactor was a three-necked round-bottomed flask equipped with a thermocouple. The products were directed onto a column at the end of which a mercury thermometer was mounted to measure the temperature of vapours entering the cooler. The process of degradation was conducted for 60 minutes. The products of the thermal degradation were analysed separately for each polymer studied. The fractions were directed onto a GC MS spectrometer. The number of compounds separated in the mixture was the same for all polymers and equal 30. A large majority of these compounds were successfully identified, which permits making an attempt at proposing mechanisms of the reactions for each of the polymers studied.

DETERMINATION OF FATTY ACID METHYL ESTERS IN DIESEL OILS

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Although in Poland the regulations on obligatory addition of biocomponents (esters of plant-origin fatty acids) to liquid fuel have not been introduced yet, the EU directions require that the relevant laboratories would be prepared to determine the content of biocomponents in fuel. In Poland there is a standard referring to determination of biocomponents in petrol, however, no analogous solution has been recommended for Diesel oils. There is a Polish standard prEN 14103, however, it concerns analysis of pure biocomponents being fatty acid methyl esters (FAME) and permits determination of their composition prior to their addition to Diesel oil.

As the samples to be studied have a complex hydrocarbon matrix, gas chromatography was selected as the analytical method because it permits qualitative and quantitative characterisation of the fuel. In our study a standard gas chromatograph equipped with a flame-ionisation detector and a polar capillary column was applied. The method proposed permits determination of the biocomponents after their addition to Diesel oil. It enables analysis of fatty acids esters (of rapeseed oil origin) as well as discernment whether given esters are of rapeseed oil or other origin. The method was tested on Diesel oil samples containing biocomponents obtained as a result of transestrification of common plant-origin oils such as coconut, maize, flax, rice, sesame, sunflower, soybean, olive, grape stone, pumpkin stones and their mixtures.

The range of determined FAME concentrations is consistent with the recommendations of both Polish and European standards. The proposed procedure of determination of the content of biocomponents in liquid fuel was subjected to validation, determining such parameters as linearity, detection limit, trueness, intermediate precision and uncertainty.

The study was performed within the inter-university grant AE-UAM (PU-II/81)

POSTER SESSION II

COMPARISON OF GC/MS AND HPLC TECHNIQUES FOR EUGENOL DETERMINATION

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Essential oils and extracts obtained from many plants have recently gained popularity and scientific interest. Generally chromatographic methods are preferred for plant analysis.

In this work two chromatographic methods (GC/MS and HPLC) have been developed for separation and determination of eugenol from essential oils and alcoholic extracts.
Eugenol (4-Allyl-2-methoxyphenol) is a typical component many plants. Eugenol is an aromatic chemical, fragrance and a spice that is derived from Clove oil and Cinnamon leaf.

The results of the two chromatographic method were compared. The HPLC method was developed as an alternative for the GC method.

POSTER SESSION II

TLC MONITORING OF HYDROLYSIS OF 2,4-DITHIOPHENOBARBITAL

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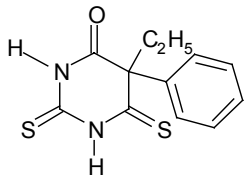
The term - stability of a compound is associated with the determination of the rate of its changes during the time of a storage under defined conditions and during appropriate time. The rate of degradation of the substance depends on the external factors (temperature, light, pH of the medium etc.) and on the chemical properties of the substance determined by its chemical structure.

Continuing investigations on the stability of thiobarbituric acid derivatives [1-3] the hydrolysis of 2,4-dithiophenobarbital (I) was investigated to determine its main degradation pathways.



2,4- dithiophenobarbial

The hydrolytic degradation of I was monitored by TLC method. A 1% solution of I was prepared in the borate buffer of pH = 10.0 and immersed in a water bath at 60 °C. At the appropriate time intervals 50 μl samples were withdrawn and spotted on the TLC plate. The plates were developed in the following systems: chloroform, chloroform:n-hexane 2:1 (v/v), and n-hexane:ethanol:triethylamine 7:1:1 (v/v/v). The visualization of spots was made under UV light (254 nm).

The results of TLC analysis indicate that in the course of hydrolysis of 2,4-dithiophenobarbital this compound is desulfurated to 2-thiophenobarbital and phenobarbital and these products undergo further degradation.

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POSTER SESSION II

INDIRECT DETECTION IN ION EXCLUSION CHROMATOGRAPHY

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Ion-exclusion chromatography (IEC) finds application in the analysis of weak and medium strength acids. The detection techniques include direct UV absorbance, refractive index (RI), potentiometric, conductivity as well as mass spectrometry. The direct UV absorption at 210 nm is the most frequently used detection method for volatile fatty acids. Its detection limit is rather high because of lacking a chromophore by solute. From the other side, due to its sensitivity to even trace amounts of contaminants, it cannot be used for complex samples without suitable sample pretreatment because of interference problems. The most commonly used in IEC conductometric detector also is characterized by high sensitivity because of small dissociations of acids, additionally depressed by the buffer.

Indirect photometric detection has been already successfully applied in ion chromatography as well as and capillary electrophoresis. In this case mobile phase contains an absorbing reagent of the same electric charge as analyzed solute, characterized by high molar absorption coefficient.

In the presentation it was confirmed the possibility of application of indirect detection in ion exclusion chromatography. In this case aromatic acids can be used as eluents. Two detection systems are discussed, photometric and conductometric. Derived equations as well as experimental data show indirect conductometric detection for solutes which ionic conductivities (diffusion coefficients) are smaller then background electrolyte. Direct detection (positive peaks) is observed in the opposite case. It was possible to obtain direct and indirect detection on one chromatogram, depending on the relative limit conductivity (diffusion coefficient) of solute and background electrolyte. Quantitative correlation between derived equation and experimental results was found. Phthalic acid, used as indirect detection probe, decreased retention of aliphatic fatty acids because of the competition on adsorption sites.

POSTER SESSION II

THE APPLICATION OF MACROCYCLIC REAGENTS FOR ELECTROPHORESIS SEPARATION OF ORGANIC COMPOUNDS WITH AMINO-, HYDROXYL- AND CARBOXYL GROUPS

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The addition of macrocycles (crown ethers, cyclodextrins) to a leading electrolyte is used for increasing of separation selectivity of organic compounds with different functional groups at capillary electrophoresis. It is carried out because macrocycles have an unique ability to form complexes with analytes by 'host-guest' type. Some macrocyclic reagents, particularly cyclodextrins may be chiral selectors.

Ratio of cavities dimensions of macrocycle and substrate, kind and concentration of macrocycle, pH of buffer electrolyte are significant factors.

By capillary zone electrophoresis next facts were obtained:

1. The influence of different crown ethers (18-crown-6; 15-crown-5; benzo-18-crown-6; 4,13-diaza-18-crown-6) to electrophoretic separation of dopamine and serotonin was studied. Best separation was achieved at using of 18-crown-6, because measure of ammonia ion (NH_4^+) completely corresponds to crown ether cavity dimension (2,66 –3,2 Å). Crown ether concentration was varied from 2 mM to 10 mM, leading electrolyte pH = 4,0.

2. The influence of β-cyclodextrin to electrophoretic separation of pairs of substances: tryptophan / kynurenine; vanilinmindalic acid / homovanilic acid; catehine / epicatehine was studied, last two substances is a stereoisomer of each other. Macrocycle concentration in buffer electrolyte is 3 – 30 mM, leading electrolyte pH = 8,14.

The possibilities of macrocycles use as solid-phase extraction sorbent modifiers were analyzed.

Systems of capillary electrophoresis "Kapel 103R" and "Kapel 103RE" (Lumex Ltd, St. Petersburg) equipped with photometric detectors (λ_{max} = 254 nm), "Agilent 1100" (HP, USA) with diode matrix array were employed in this investigation.

POSTER SESSION II

CHROMATOGRAPHIC DETERMINATION OF FERRO LACTATE AND FERRO GLUCONATE UTILIZED AS FOOD COLOR ADDITIVE IN TABLE OLIVE TECHNOLOGY

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Colour fixation in ripe olives (*Olea europaea* L.) is important process for table olive manufacturing technology. Ferrous lactate (E 585) or ferrous gluconate (E 579) are food color additives and added to olive-brine as % 0.05- 0.15. In manifactured final table olive product, max.permitted ferrous concentration should be ranged in 150 mg kg^{-1}. Both of these agents has color fixation and nutritive effect; especially lactate provides the color stabilization and has antioxidative effect. However these color agents are necessary for table olive brine process, the excess of the agents are not proper in food safety regulations. In this context, the need for regular and rapid chromatographic monitoring of ferrous lactate and/or gluconate in table olive technology and relevant food products will be important. Derivatized ferrous lactate (E 585) and/or ferrous gluconate (E 579) were performed by Gas-liquid chromatography (GC) by new developed method using a 60 m-capillary column (with 0.25 μm film tickness), 0.25-mm-inside-diameter WCOT fused-silica SGE (BP70X GC) capillary column installed on a Perkin Elmer (Auto System) Gas-liquid chromatograph with a Flame Ionization Detector (FID). Calibration study *(R^2*=0.9999); analytical method validation and recovery studies [y= 3.296 x + 0.025 (R^2=0.9999)] were performed and gave satisfactory results. The lowest ferrous lactate or ferrous gluconate values were observed in marketted table olives as % 0.058 and % 0.061 (n=6) (p<0.01), respectively depending upon the manufacturing company.

The proposed chromatographic method was appropriate for ferrous lactate (E 585) and/or ferrous gluconate (E 579) determination in table olive technology and could be utilized for quality control in table olive industry.

POSTER SESSION II

APPLICATION OF MICROWAVE IRRADIATION FOR SYNTHESIS OF SELECTED PORPHYRINS

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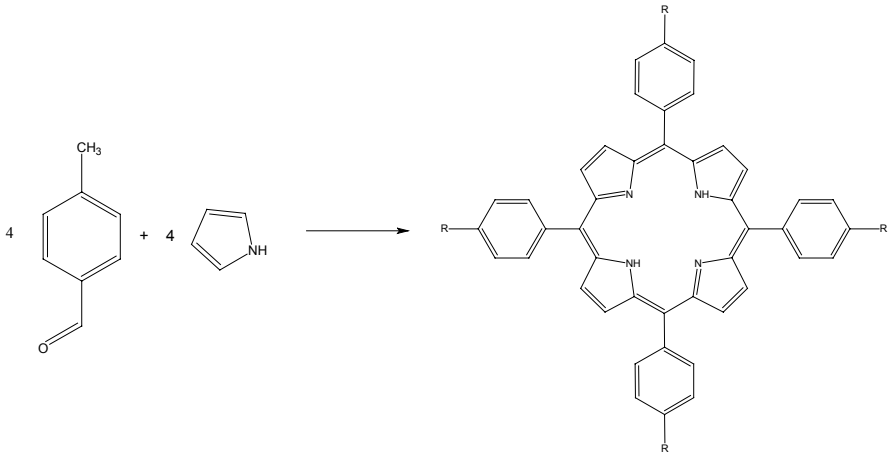
Recently a growth of microwave irradiation applications in chemical reactions is seen. There are a lot of advantages in the use of microwaves[1] in comparison with some generally used methods[2-3], like the need of less acid and no or less solvent what makes this an eco-friendly way of preparing, the reaction time is shorter, the yield is higher, no special equipment is needed, only commercially available microwave oven, …

To synthesize meso-substituted arylporphyrins 5 ml of propionic acid was added to equimolair amounts of arylaldehyde and pyrrole (0,1 mol). The mixture was irradiated in a domestic microwave oven for 3 minutes in 1-min periods at 300 W, followed by cooling to room temperature and homogenization[1]. The cooled reaction mixture is washed with water and afterwards extracted with chloroform. The reaction products were investigated by TLC-method.



The microwave method in our experiments has shown a higher yield and a lower use of solvent in comparison with other methods, what is good environmentally and economically.

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POSTER SESSION II

DENSITOMETRIC INVESTIGATION OF PROLINE TOLYLPORPHYRIN DERIVATIVE

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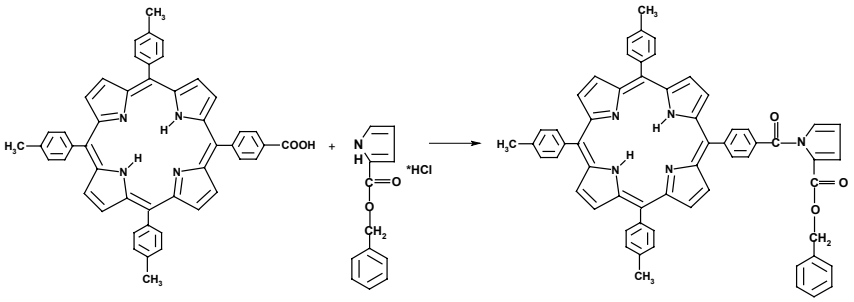
Proline (Pro) is the one of the most important amine acid because of its considerably role in biochemistry processes. Molecule of proline takes part in the structure of Haemoglobine molecule as an end of the side chain.

Densitometric method has once again become an important tool (along with the more sophisticated HPLC method) for qualitative and quantitative determination of the chemical reaction products [1,2]. We use densitometric method to investigate the products of condensation of tolylporphyrin with proline. The main product of this reaction is a potential photosensitizer in PDT diagnosis and therapy of cancers [3].



Condensation reaction of 5-(4`-carboxyphenyl)-10,15,20-tritolylporphyrin with L-Proline benzyl ester hydrochloride in presence of DCC (N, N`-dicyklohexylcarbodiimide) as a coupling agent yield the desired product [4]. Scheme of the reaction is shown above. Purification were conducted on chromatography column (30x2 cm), using as an eluent chloroform. The reaction mixture were spotted on silica gel plates as chloroform solution (2μL) and developed on 10cm way. Then developed plate was placed into densitometry chamber and scanned with 420nm light. From the densitogram we were able to determine percent content of main compounds.

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POSTER SESSION II

SYNTHESIS AND HPLC ANALYSIS OF ACYL ETHYL DEUTEROPORPHYRIN IX DICORBOXYLIC ACIDS

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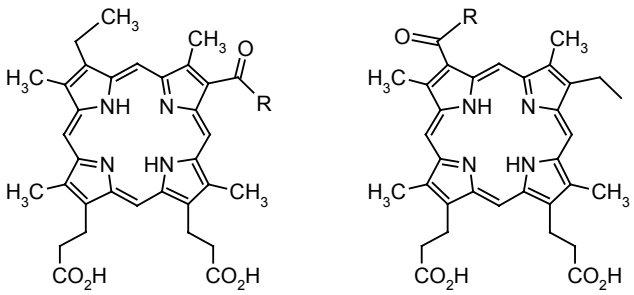
Porphyrins belong to a class of naturally compounds which are very important for various biological processes, like photosynthesis (chlorophylls), respiration, oxygen transport (hemes) and different rearrangement processes (vitamin B_{12}).

Photodynamic therapy (PDT) is a promising new treatment of small and superficial tumors [1-2]. In PDT, light, O_2, and a photosensitizing drug are combined to achieve a therapeutic effect. Porphyrins, chlorins, and bacteriochlorins are among the most useful photosensitizers for in vivo PDT, although other classes of porphyrinoids such as phtalocyanines and texaphyrines are also used [3].

To optimize amphiphilic a crucial property of sensitizers for tumour enrichment we aimed on combinatorial synthesis of porphyrin derivatives with lipophilic side chains of different length. The synthetic pathways began with Cu-3-ethyldeuteroporpyrin IX dimethyl ester or Cu-8-ethyldeuteroporpyrin IX dimethyl ester. Sets of amphiphilic compound mixtures were prepared by Friedel – Crafts acylation with carboxylic acid derivatives of different length (even or odd number of carbons atom in chains). After hydrolysis of the ester groups into acid functions, the mixtures were analyzed by HPLC on RP-18 columns with methanol : water (tetra butyl ammonium dihydrogenphosphate 2,5mM/dm^3) v/v 95:5.



R = C_2, C_4, … C_{18}, or R = C_3, C_5 … C_{19}.

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POSTER SESSION II

HIGH PERFORMANCE THIN-LAYER CHROMATOGRAPHY-DENSITOMETRY METHOD AS AN ALTERNATIVE WAY OF DRUG ASSAY

A. Skalska, M. Wójciak – Kosior, G. Matysik

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Barbituric acid (2,4,6 – pyrimidinetrion) is a very important compound widely used in many fields. It's derivatives are capable to produce all levels of central nervous system depression, in dependence of a dose, from mild sedation through hypnosis to deep coma and even death. Frequently barbiturates are the component of complex mixtures used in the therapy of pain of weaker genesis.

The HPTLC method combined with denstiometry has been successfully applied to the determination of barbituric acid derivates (e.g. phenobarbital) in the pharmaceutics. Chromatograms were developed with mixture of dichloromethane, ethyl acetate and formic acid (9.5:0.5:0.1). The proposed chromatographic system enables suitable separation of phenobarbital from the other components in drugs containing e.g.: caffeine, codeine or papawerin with acetylsalicylic acid. Obtained results were comparable with traditionally used HPLC method. In the proposed procedure, linearity, sensitivity, repeatability were found to be satisfactory for determination of phenobarbital.

The statistical data of quantitative HPLC and HPTLC-densitometry analysis were also calculated. The limits of detection were found to be 0.075 mg/mL for HPTLC-densitometry analysis and 0.06 mg/mL for the HPLC. The high recovery (97.81-102.94) and RDS values (2.1%) confirm the suitability of the HPLC and HPTLC-densitometry methods for the routine analysis of phenobarbital in pharmaceutical preparation.

The comparison traditionally used HPLC and HPTLC-densitometry method shows that HPTLC combined with densitometry can be used as an alternative way of barbiturates analysis in a serial laboratory practice. The presented chromatographic system enables quantitative determination barbituric acid derivatives in many samples e.g. containing the other synthetic drug or in biological material without any pretreatment.

POSTER SESSION II

COMPARISON OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) AND HIGH PERFORMANCE THIN-LAYER CHROMATOGRAPHY (HPTLC) COMBINED WITH DENSITOMETRY IN QUANTITATIVE ANALYSIS OF PHENOLIC ACIDS IN COMPLEX MIXTURES

M. Wójciak – Kosior, G. Matysik, A. Skalska

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In the present study HPLC and HPTLC method combined with densitometry were used to quantitative analysis of phenolic acids of similar chemical structure in plant extracts.

For quantitative analysis by HPLC and HPTLC methods two phenolic acids were chosen – caffeic and protocatechuic acids which frequently occur together in multicomponent plant extracts. They have similar moleculer structures – differ only by the –CH=CH- grouping which has only minor effect on the polarity. In RP HPLC there is some difference in retention, on the other hand, they are poorly separated in isocratic NP TLC. Satisfactory separation was obtained using the technique of multiple gradient development – MGD. There are several techniques of gradient elution in HPTLC. One of them is multiple gradient development (MGD). In this technique the plate is developed consecutively several times with solvents of the same or varying eluent strength to decreasing distances so that the zones separated in the former steps remain separated (do not merge).

Quantitative HPLC analysis was carried out in reversed phase system (chromatograph Waters 1525) with Zorbax SB C18 steel column 250x4.6 mm, filled with adsorbent with particle diammeter 5 μm. For the analysis of the compounds the following mobile phase was used: methanol/water/formic acid (25:75:0.05 v/v), at 1mL/min. flow- rate and at temperature 25^0C. Caffeic acid was determined at λ=320 nm and protocatechuic acid at λ=260 nm, 20 μL of each solution was injected.

Planar chromatography was carried out with Si60 10 x 20 cm precoated HPTLC F_{254} plates (Merck, Darmstadt, Germany). Chromatograms were developed "face down" in horizontal Teflon DS chambers (Chromdes, Lublin, Poland) adapted for gradient elution, using mixtures of heptane, diisopropyl ether, dichloromethane and formic acid as the eluents.

It is demonstrated that HPTLC in the MGD variant and HPLC are suitable for the quantitative analysis of phenolic acids in the standardization of plant preparations containing these compounds. Both methods are recommended by the European and the US Pharmacopeas. Combination of two instrumental methods are also a condition of Good Laboratory Practise (GLP). According to Food and Drug Administration in quantitative analysis of pharmacologically active substances the equivalence of two instrumental methods should be assessed.

POSTER SESSION II

DENSITOMETRIC DETERMINATION OF CAFFEINE IN YERBA MATE LEAVES AND HERBAL PREPARATIONS CONTAINING GUARANA (*Paullinia cupana*)

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Caffeine (1,3,7-trimethylxanthine) is a purine alkaloid that occurs in tea, coffee, cocoa beans, cola nuts, guarana seeds and mate leaves. In medicine, this alkaloid is used as a diuretic, a cardiotonic, an analeptic and an analgesic. Caffeine has attracted much scientific and public attention in recent years due to its stimulatory effects.

A TLC method with densitometric UV detection at $\lambda = 280$ nm has been developed for separation and quantification of caffeine from different herbal preparations containing guarana seeds and mate leaves.

Chromatographic separation on silica gel 60 F_{254} layers was performed with chloroform – ethyl acetate – formic acid, 5+4+1, (v/v/v), as a mobile phase. The method was validated for selectivity, stability of the analyte, linearity, precision, limit of detection and determination.

POSTER SESSION II

A COMPARATIVE STUDY OF EXTRACTION OF ISO-α-ACIDS FROM THE BREWERY SAMPLES AND QUANTITATIVE DETERMINATION BY MEANS OF HPLC

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Iso-α-acids and their chemically modified counterparts, which are contained in an enormous majority of beers, are a group of compounds, which originate from α-acids. They are called beer bitter acids and have antibacterial properties, enhance foam stability and give characteristic bitterness to beers.

The purpose of this research was to develop an improved extraction procedure for the analysis of the hop bitter compounds.

In the brewing process, iso-α-acids are generally quantified by means of HPLC with UV detection and it is believed that these particular results correlate the best with human perceptions of bitterness in beers.

The first step in the analysis of hop bitter acids is their separation from many interfering compounds present in beer. Liquid-liquid extraction has been the mainstay for separation of these acids (e.g., iso-α-acids, tetra-iso-α-acids, dihydro-iso-α-acids).

Iso-α-acids used to be extracted from the beer by liquid-liquid extraction, based on the EBC method 9.16. Each sample – i.e. each bottle – was analysed twice by means of liquid-liquid extraction, but one step was modified. The first sample was extracted by the liquid-liquid procedure with HCl and then with H_3PO_4. Indeed, using H_3PO_4 as an extractant, the foam which occurs in beer is smaller and the results are better than those obtained with aid HCl. Less foam in the H_3PO_4-extracted beers allows a better analysis afterwards.

Solid phase extraction (SPE) was shown to ensure satisfactory reproducibility also when using the UV absorption method (ASBC 9.6), which is a traditional way to measure bitterness in beers. After the reduction of iso-α-acids, specific absorptivity of active component changes and the liquid-liquid extraction procedures combined with HPLC show to be a reliable method for quantitative determination.

POSTER SESSION II

CHROMATOGRAPHIC ANALYSIS OF THE SELECTED AMINOACIDS ON THE CHEMICALLY BONDED STATIONARY PHASES IMPREGNATED WITH THE Cu(II), Co(II) AND Ni(II) CATIONS. PART I

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Aminoacids and their derivatives are commonly analyzed by means of thin-layer chromatography thanks to the simplicity of this method, its high sensitivity, and short time of the development of chromatograms. The most popular adsorbents used in these studies are: silica gel, aluminum oxide, and chemically bonded stationary phases. Although, satisfactory results can be obtained with the use of above mentioned phases, the studies aimed at their improvement are still carried out. Modification of stationary phases, including chemically bonded ones, is most frequently done by the process of impregnation. In case of analyses of aminoacids and their derivatives, the stationary phases, both traditional and chemically bonded, are usually impregnated with salts of transition metals or surfactants [1-5].

The main aim of this study is establishing of the influence of the cation and anion of the impregnating agents on the mechanism of retention of the selected aminoacids. The ready made TLC glassplates covered with the chemically bonded stationary phases of the RP-8 and RP-18 type were impregnated with solutions of salts of the selected transition metals. Six aminoacids were developed on the nonimpregnated and impregnated above mentioned stationary phases and then the respective R_F coefficient values were calculaced.

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POSTER SESSION II

IMPROVED GC-μECD ASSAY FOR THE METHYLMERCURY MEASUREMENTS: ITS ESTIMATION IN THE FISH MEATS

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Mercury is highly toxic radical, found, especially, in see organisms. The most easily adsorbed and the most toxic form of mercury is methylmercury. It affects the immune system, alters genetic and enzyme systems, and damages the nervous system, including coordination and the senses of touch, taste, and sight. Its toxic effect is caused by it interactions with thiols and amine groups.

Elaborated assay is based on the GC chromatograph equipped with the microECD. Separation was tested on the different polar as well as unpolar columns. The best results were obtained on the BP-10 (SGE), 25 m, 0.22 mm I.D., 0.25 μm stationary phase, column. It is worth to note that analyte strongly passivated the column therefore before use it was specially passivated by the mercury chloride.

Our improved sample preparation is based on preparing dissolved in water thiosulfate complex. In the next step thiosulfate is exchanged by chloride and cleaned solute is extracted to benzene. Such prepared sample was injected on the column by the pulsation splitless injection valve.

Elaborated assay is characterized by high linear dynamic range (nearly 4 orders of magnitude, $R^2 > 999$) up to the concentration 5 mg/kg b.w. Obtained limit of detection was below 2 ppb and precision of measurements for solute 2 and 8 % and for sample 2.5 and 18 % for concentrations 100 and 10 ppb, respectively. Recovery for reference material (CRM 463) was equal 95%.

As the practical result meat from 7 spaces of commercially available fishes was investigated. It turned out that concentration of the methylmercury varied in them from 10 to 500 ppb. Especially interesting is that its concentration was fifty times higher for European fishes in comparison to the African ones.

POSTER SESSION II

DETERMINATION OF BICYCLO[2.2.1]HEPT-2-ENE EPOXIDATION PRODUCTS BY MEANS OF GC/MS METHOD

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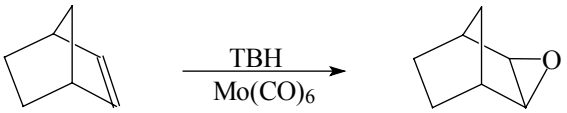
Institute of Organic Chemical Technology, Szczecin University of Technology, Szczecin, Poland

The production of oxygenates is currently one of the major directions of development of the petrochemistry. Exo-2-epoxybicyclo[2.2.1]heptane finding a wide application is many branches of industry.

The reaction course was shown in scheme.



The application of GC-MS technique enables the determination of substrate conversion and the evaluation of quantity of the product of this conversion, and in a further sequence the optimization of the process itself and its control.

The determinations of epoxidation products were carried out using the gas chromatograph Trace 2000 Thermo Quest coupled to a quadrupole mass detector MS Voyager (Finnigan) operating in electron impact ionization mode at 70 eV (TIC mode, range 30–400 da) on a DB-1 capillary column (length 30m, I.D. 0.32mm, film thickness 1μm) under the following conditions: programmable temperature of thermostat from 50 to 60°C with increasing temperature at the rate of 2°C·min^{-1}, and next from 60 to 200°C with increasing temperature at the rate of 5°C·min^{-1}, flow of carrier gas (helium) 1.5 ml·min^{-1}, sample injector temperature 250°C, split ratio 1:60, sample injection volume 0.1 μl. The quantitative analyses were performed using the method of internal standard with the application of multilevel calibration.

An analysis of mass spectrum of the products of epoxidation bicyclo[2.2.1]hept-2-ene was performed. The characteristic fragmentation ions were determined and the route of fragmentation of the molecules was presented, confirming their structure.

A statistical evaluation of developed method was performed. An accuracy and precision of this method was determined.

POSTER SESSION II

APPLICATION OF RP HPLC METHOD FOR INVESTIGATION OF ADSORPTION PROCESSES OF CHLOROPHENOLS

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The subject of performed studies involved the adsorption of chlorophenols occurring in wastewater from the production of pesticide 24-D on the carbon adsorbents and synthetic. This required the determination of these compounds concentrations in aqueous solutions at a level of 0.01–10mg/dm^3 with a high precision and accuracy.

The determinations were carried out using a Hewlett Packard HP 1090 liquid chromatograph equipped with Rheodyne injector (injection loop of 100 μl) and UV-DAD detector on a Zorbax SB C18 column (150 x 4.6mm I.D., 5μm) under isocratic conditions. A mixture acetonitryl-water (60:40 v/v) at flow rate 1 ml/min was used finally as a mobile phase. The calibration solutions were prepared directly before performing the determination. They were obtained from the stock solution by of appropriate chlorophenol in methyl alcohol by dilution with deionized water.

The statistical magnitudes characterizing the method such as precision, accuracy, confidence interval, linearity, range of method application were determined.

The kinetics curves were determined in the static system as well as the adsorption isotherms for chlorophenols on studied adsorbents. The course of the kinetics curves was described by a kinetic equation, whereas the adsorption isotherms by the Freundlich or Langmuir equation. The coefficients of these equations were calculated by the nonlinear method.

The equilibrium magnitudes of adsorption such as time of reaching the state of dynamic equilibrium, equilibrium concentration, capacity of monolayer and adsorptivity were determined. This enables to compare the studied adsorbents in relation to the respective chlorophenols.

POSTER SESSION II

THE INFLUENCE OF THE TYPE OF PHASE TRANSFER CATALYST ON THE *O*-ALKYLATION OF 1,4-DIHYDROXYBUTANE TO 1,4-BIS(ALLYLOXY)BUTANE CONTROLLED BY MEANS OF THE GC METHOD

E. Kaczmarczyk, E. Milchert, W. Paździoch, E. Janus

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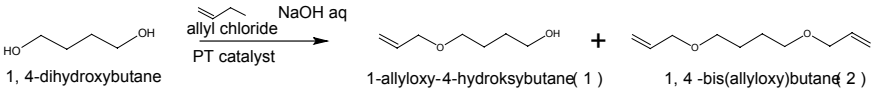
Allyl compounds comparise a large group of ethylenic compounds having unique reactivities and uses, often contrasting with those of the typical vinyl-type compounds. In allyl compounds the ethylenic group containing the double bond is not substituted by strong activating group to promote polymerization but is attached to a third carbon atom which generally bears one or more reactive hydrogen atoms. Allyl compounds have large commercial volumes and most of them are employed as intermediates in chemical processes or as polymerization monomers. Allylic chemistry also encompasses a much broader range of molecules than that of the simple CH_2-CH=CH_2 group.

Generally, the synthesis of allyl ethers of polyalcohols based on the Williamson's method.In our study we applied the method known as the phase transfer catalysis. A method of the selective synthesis of allyl ethers using allyl chloride and the quaternary ammonium salt as the PT catalyst.

The purpose of our work was to study the influence of the type of the PT catalyst on the *O*-alkylation process of 1,4-dihydroxybutane in a catalytic two-phase system. The reaction course was shown in scheme. The concentrations of product (2) were determined by gas chromatography method. SRI gas chromatograph with FID detector was used. Analysis conditions: DB-5 capillary column (length 30m, I.D. 0.53mm, film thickness 1.5μm), injector temperature 150ºC, flow of carrier gas (helium) 1 ml·min^{-1}. The column temperature was programmed: initial temperature 50ºC (hold for 1 min); temperature rate 10ºC·min^{-1} to 200°C (hold for 2 min), the next rate 20°C·min^{-1} to 250°C and the last temperature rate 25°·min^{-1} from 250°C to 280°C (hold for 5min). The structure of the product was determined by the mass spectrometry.



1, 4-dihydroxybutane — 1-allyloxy-4-hydroksybutane(1) — 1, 4 -bis(allyloxy)butane(2)

POSTER SESSION II

THE DIELS-ALDER REACTION IN THE IONIC LIQUIDS WITH LITHIUM AND SCANDIUM TRIFLUOROMETHANESULFONATE: A NEW AND RECYCLABLE CATALYTIC SYSTEM

A. Dramska, M. Antoszczyszyn, E. Janus, A. Sygit – Bajko

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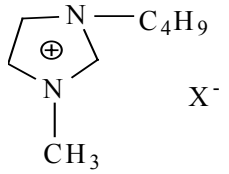
With regard to a widely undertaken environment protection, a particular emphasis is put on the alternative solvents or technologies in all the sectors of chemical industry. An ideal solvent should possess a low vapour pressure, as well as it should be stable in both chemical and physical respect. Very important is the possibility of it's recycling and the stability during the storage. The above requirements fulfill a new generation of solvents, such as the ionic liquids.

In the poster methods concerning the determination of the effect of ionic liquid – scheme (1) on the course of the Diels-Alder reaction between cyclopentadiene and dimethyl maleate have been presented.



[BMIM][X$^-$]; X$^-$ = OTf (1)

The reactions were carried out in the ionic liquids themselves or their mixture with the lithium and scandium trifluoromethanesulfonate: as new and recyclable catalytic system.

The GC analyses were performed using the Carlo Erba GC 8000: column DB 17; I.D. 0.53 mm, length 30 m, film thickness 1 μm). The sample injector temperature was 280ºC, flow of carrier gas (helium) 4 ml·min^{-1}, split ratio 1:40, sample injection volume 0.1 μl,. The oven temperature was programmed at 40^{o}C for 3 min., with temperature increase rate being 10^o/min. up to 120^{o}C for 5 min. and then temperature was increase 10^{o}C/min up to 200^{o}C, once the temperature was reached it was was kept that level for 50 minutes.

POSTER SESSION II

MONITORING AND FAST DETECTION OF PROGRESS OF THE O-ALKYLATION REACTION UNDER THE PTC CONDITIONS BY THE METHOD OF GC/MS

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Allyl ethers are a grup of compounds possesing very special properties. A main goal in our laboratories is to synthesis new allyl compounds. A new allyl etehrs are produced by O-alkylation polycyclic alcohols based on the rosin structure by the phase transfer catalysis (PTC) routes. PTC method has been considered to be one of the mostly effective tools in synthesizing organic chemicals from two immiscible reactants. Recently, it has been extensively applied to the reactions by alkylation, arylation, condensation, elimination, and polymerization, as well simple displacement. Quaternary salts are most commonly used as the phase transfer (PT) catalysts. The anions of the reacting salts transfer into the organic medium in the form of ion pairs. In our research we used Bu_4NHSO_4 as PT catalyst.

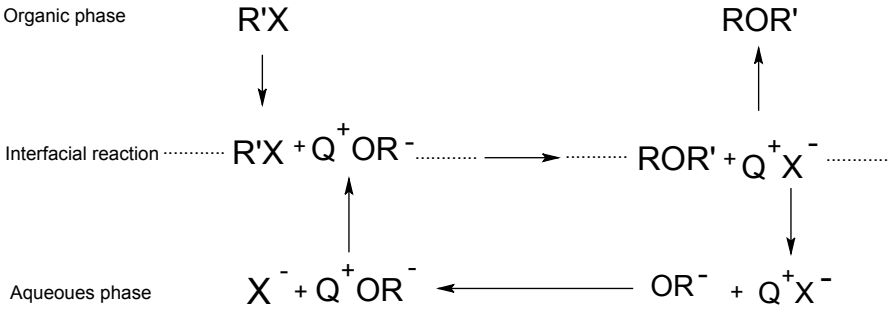

Fig. 1. The mechanism for O-alkylation of polycyclic alkohol under liquid-liquid PTC conditions.

In the poster, we investigated the potential of employing the GC/MS method for determination of reaction progress and mechanism. Samples were withdrawn periodically. The structure of products was confirmed by GC/MS.

The GC analyses were performed using the Trace 2000 ThermoFinnigan (capillary column DB 1; length 30 m, I.D. 0.32 mm, film thickness 1μm) equipped with quadrupole mass spectrometry detector MS Voyager (Finnigan). The mass spectra were obtained via electron impact ionization (EI) at 70 eV. The sample injector temperature was 250ºC, flow of carrier gas (helium) 4 ml·min^{-1}, sample injection volume 0.1 μl, split ratio 1:10. The column temperature was programmed: initial temperature 60ºC (hold for 5 min); temperature rate 15ºC·min^{-1}; final temperature 300ºC (hold for 14 min).

POSTER SESSION II

GAS CHROMATOGRAPHY/MASS SPECTROMETRY OF DITERPENE ALCOHOLS

A. Sygit – Bajko, W. Paździoch, M. Antoszczyszyn, E. Janus

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Abietic acid $C_{20}H_{32}O_2$ is an enantiomerically pure starting material which is cheap and easily avaible. One of the most attractive ways to convert abietic acid into derivatives with superior properties consists in its coupling with various dienophiles by the Diels-Alder mechanism. In our laboratory we obtained three different adducts, which have two or three carboxylic grups in their molecule. Diene adducts of abietic acid with maleic anhydride, fumaric acid and cyrylic acid were reduced with $LiAlH_4$ to polycyclic alcohols.

Gas Chromatography/Mass Spectrometry has been the analytical tool of choince for identification of abietic acid, and their structure is well know [1]. The aim of the present research is to apply GC/MS technique for the determination obtained new polycyclic alcohols based of the structure abietic acid. The mass spectra were analysed and fragmentation patterns were elucidated. Consequently, using trimetylsilyltrifluoroacetamide (BSTFA) as derivatisation agent, derivatives of alcohols were prepared for gas chromatography analysis. So far scientific literature has not noticed any research concerning determination synthsised alcohols by applied GC/MS method.

The GC analyses were performed using the Trace 2000 ThermoFinnigan (capillary column DB 1; length 30 m, I.D. 0.32 mm, film thickness 1μm) equipped with quadrupole mass spectrometry detector MS Voyager (Finnigan). The mass spectra were obtained via electron impact ionization (EI) at 70 eV. The sample injector temperature was 250ºC, flow of carrier gas (helium) 4 ml·min^{-1}, sample injection volume 0.1 μl, split ratio 1:10. The column temperature was programmed: initial temperature 60ºC (hold for 5 min); temperature rate 15ºC·min^{-1}; final temperature 300ºC (hold for 14 min).

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POSTER SESSION II

OPTIMISATION OF SEPARATION AND MEASUREMENT OF LIPOPHILICITY OF NEW DERIVATIVES OF BICYCLO[2.2.2]OCTANE-1,2-DICARBOXYLIC ACID IMIDE

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Compounds investigated are potential antagonists for 5-HT_{1A} (serotonergic) and D_2 (dopaiminergic) receptors. They exhibit anxiolytic or antidpressant activity. The lead structures for them are tandospirone, buspirone and ipsaspirone. The determination of compounds' lipofilicity is important in prediction of biological activity of them.

The determination of lipofilicity log k_w, log P) of these compounds as well as their chromatographic separation is difficult because their molecules pose basic centres. It is caused by strong ion-exchange interactions of heterocyclic nitrogen with residual silanols occurring on the RP sorbents based on silica matrix. The retention of bases is thus caused by ion-exchange with silanols and dispersive interaction with alkyl ligands which leads to band diffusion and peak assymmetry. In such cases there are difficulties in the determination of retention in chromatographic systems of type non-polar stationary phase / polar aqueous mobile phase. For the determination of retention coefficients of investigated compounds the suppression of their dissociation by the use of aqueous eluents at high pH or having basic agents such as aliphatic amines or ammonia. Amines or ammonia can suppress ionisation of bases and can also block surface silanols. It leads to the improvement of peak symmetry and increase of system efficiency. The formation of ion-pairs of bases with anions such as alkyl sulfonates or phosphates can also give improvement of peak shape. In systems with the best efficiency the relationships between retention and aqueous eluent composition were determined. The plots of log k (R_M) vs. mobile phase concentration were extrapolated to obtain log k in pure water - log k_w. For the optimisation of separation the lipophilicity measurement was performed on precoated C18 plates (Merck) in three eluent systems: water + polar modifier (methanol, acetone, dioxane) with addition of amines, ammonia or anionic ion-pair agents. The correlation of S and φ_0 values of lipophilicity parameters enables the choice of the best modifier and the adequate concentration of ammonia, amine or ion-association agent. The series of other lipophilicity parameters for the description the potential influence of side chain conformation as well as H-bond acceptor centres' distribution for the experimental lipophilicity value in various eluent systems was determined.

POSTER SESSION II

APPLICATION OF TLC AND DENSITOMETRY TO INVESTIGATION OF THE YIELDS OF *meso*-TETRAPHENYLPORPHYRINS

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Porphiryns and their derivatives play an important role in the biochemistry of all living systems. They form the backbone of pigments such as chlorophyll and heme, which have a universal bological application and were involted in the oldest metabolic phenomena on earth. Thus, knowledge of these systems and their excited states is essential in understanding a wide variety of biological processes, including oxygen binding, electron transfer, catalysis, and the initial photochemical step in photosynthesis [1].

In the present work, acetamidophenylporphyrins were synthesized by the conventional method of *Adler* using 4-methylbenzaldehyde A and 4-acetamidobenzaldehyde B with pyrrole C in the boiling propionic acid. The general equation for condensation with six possible porphyrins is:

$CH_3PhCHO + (CH_3CONH)PhCHO + Pyrrole \rightarrow$

$(2)Por_1 + (3)Por_2 + (4)Por_3 + (4)Por_4 + (5)Por_5 + (6)Por_6$

(2) 5,10,15,20-tetratolylporphyrin
(3) 5-(4-acetamidophenyl)-10,15,20-tritolylporphyrin
(4) 5,10-di(4-acetamidophenyl)-10,15-ditolylporphyrin and
5,15-di(4-acetamidophenyl)-10,20-ditolylporphyrin
(5) 5,10,15-tri(4-acetamidophenyl)-20-tritolylporphyrin
(6) 5,10,15,20-tetra(4-acetamidophenyl)porphyrin

Three reactions were conducted with different ratios of the aromatic aldehydes and pyrole: 3A+1B+4C, 2A+2B+4C and 1A+3B+4C [2,3].

The products formed from the condensation reactions were separated on silica gel column (2 x 40 cm) with chloroform-methanol v/v 9:1 as eluent. TLC was performed on silica gel type Kieselgel 60 F254, 0.2 mm layer thickness. Mobile phase was the mixture chloroform : methanol v/v 9.5:0.5. In order to confirm the qualitative TLC results densitometry was used. Densitograms were obtained by means of the Desaga CD 60 densitometer controlled by Pentium computer. The plates were scanned at $\lambda = 420$ nm.

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POSTER SESSION II

CHROMATOGRAPHIC (TLC) BEHAVIOUR OF SOME TETRAPHENYLPORPHYRIN DERIVATIVES AND CALIX[4]PYRROLES

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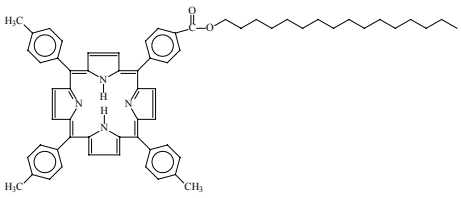
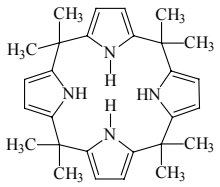
Two types of tetrapyrroles have been investigated by thin-layer chromatography. The first of the series are the tetraphenylporphyrin derivatives with long alkyl chain; the second: calix[4]pyrroles having octamethyl or tetracyclohexyl substituents in the 'meso' position.

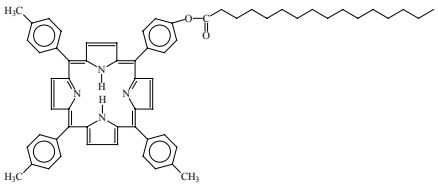
All of these compounds are interesting as potential ligands for different metal cations. They are used in a variety of areas of bioinorganic chemistry and are potential sensitizer for cancer therapy.

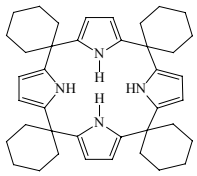
The porphyrins and calix[4]pyrroles were synthesized according to well known procedures described in chemical literature. All the compounds were characterized by physicochemical methods (UV-Vis, ^{1}H NMR, ^{13}C NMR, and ESI MS spectroscopy).

All of these tetrapyrroles were analyzed by means of thin-layer chromatography using precoated plates with silica gel and alumina as stationery phases. Several single- or two-component mobile phases were investigated. The spots of separated compounds were detected visually.









POSTER SESSION II

DETERMINATION OF HYDROQUINONE AFTER CHROMATOFRAPHIC SEPARATION

F. Buhl, B. Szpikowska – Sroka

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A simply and accurate analytical procedure for determination of hydroquinone after chromatographic separation was proposed. Optimum conditions for TLC separation of analyte were established. The UV (254 nm) detection of hydroquinone was used. Spectrophotometric quantification was by means of a sensitive and simply method based on oxidation of leuco crystal violet to the crystal violet.

The analyte was oxidized by IO_3^- iodate (V) anion. The colourless oxidation product was formed in the quantity equivalent to iodide ions. The I^- reacted with the excess of iodate (V) ions in acidic medium to form free iodine which oxidized leuco crystal violet (LCV) to the liberated crystal violet (CV^+) dye showing maximum absorption at 588 nm.

The suggested reactions are:

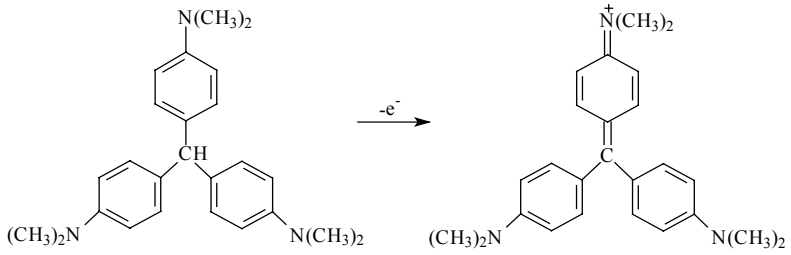
1. The oxidation of hydroquinone to the chinone by the added iodate (V) anion.

$$IO_3^- + \text{analyte} \rightarrow \text{analyte}_{oxid.} + I^-$$

2. The iodide ions reacted with the excess of iodate (V) ions in acidic medium (addition of HCl) to form free iodine.

$$5I^- + IO_3^- + 6H^+ \rightarrow 3I_2 + 3H_2O$$

3. The iodine oxidized leuco crystal violet (LCV tris(p-dimethylnitrophenyl)-metane) to the coloured crystal violet (CV^+).



LCV CV^+

The procedure was successfully applied to the determination of hydroquinone in cosmetics preparations and photographic developers.

POSTER SESSION II

DETERMINATION OF TETRACYCLINES IN HONEY

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The goal of our study was to determine the contents of tetracyclines in honey of a different origin.

Preparation of a sample of honey for chromatographic analysis was carried out with aid of Solid Phase Extraction (SPE). One examined usefulness of the different types of SPE columns for this particular analytical task. The obtained extracts were analyzed by means of high performance liquid chromatography (HPLC). Studies on tetracyclines contents in honey were carried out upon the different varieties of honey and upon the samples originating from different manufacturing and selling companies.

The results obtained enable qualitative and quantitative assessment of the contents of the investigated antibiotics in honey samples. Upon these results the quality of the honey samples can be evaluated, and also the sanitary regime in the course of honey production and conditioning for selling.

POSTER SESSION II

Py/GC/MS ANALYSIS OF 5,10,15,20-TETRAKIS(4-CARBOXY-PHENYL)-21H,23H-PORPHINE IN THE PRESENCE OF TMAH REAGENT

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In the last years, the pyrolysis – gas chromatography – mass spectrometry (Py/GC/MS) technique with the Curie Point pyrolyser was used for the analysis of some porphine derivatives [1-4] with good results.

The aim of this study was to determine the pyrolysis products of 5,10,15,20 – tetrakis(4-carboxyphenyl) – 21H,23H – porphine (TCPP). For this purpose, the thermally assisted hydrolysis and methylation (THM) reaction was done and tetramethylammonium hydroxide (TMAH) was used as the derivatization reagent. In this way, the characteristic components with substituted carboxyl group(s) of the pyrolysate of TCPP, especially even such components that were not detectable by conventional pyrolysis without the methylation reaction, could be with more ease identified.

The obtained Py/GC/MS results of TCPP provide more information about the chemical structures of the pyrolysate components and may be useful in the characterization of other carboxyporphyrins with unknown structures.

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POSTER SESSION II

Py/GC/MS ANALYSIS OF SOME 5,10,15,20-TETRAKIS(4-HYDROXYPHENYL)-21H,23H-PORPHINE DERIVATIVES

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In our earlier studies [1-4], the pyrolysis - gas chromatography – mass spectrometry (Py/GC/MS) technique was applied for the identification of the pyrolysis products of 5,10,15,20-tetraphenyl-21H, 23H-porphine (TPP) and some its derivatives having the same substituents at the aromatic rings, such as: $-CH_3$, -OH, $-OCH_3$, $-NHCOCH_3$, etc [1]. The thermal decomposition products of these compounds were obtained during 5 sec. at the temperature of 770 °C inside the Curie point pyrolyser directly attached to the split/splitless injector of the GC/MS instrument. The identification of these products was based on the interpretation of their low resolution mass spectra. In this way, it was found that during the pyrolysis of TPP, it forms more pyrolitic products in comparison with its other derivatives.

The aim of this work was the application of the Py/GC/MS method for the analysis of some 5,10,15,20-tetrakis(4-hydroxyphenyl)-21H,23H – porphine (THPP) derivatives with the hydrogen atoms of the hydroxyl groups substituted by $–CH_3$, $-COCH_3$ and $–Si(CH_3)_3$.

It was found that the main pyrolysis products of these compounds are easier to identify by the interpretation of their mass spectra. The knowledge about the chemical structures of these products permits us to get additional information about their pyrolysate composition and also it confirms the results of Py/GC/MS analysis of THPP.

The obtained results of the application of Py/GC/MS technique in the analyses of THPP derivatives maybe used for the determination of the chemical structures of different hydroxyporphyrins with unknown structures.

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POSTER SESSION II

ACRYLAMIDE DETERMINATION BY LIQUID CHROMATOGRAPHIC METHODS

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Acrylamide has recently been found in a range of heat treated food products. These findings caused considerable interest worldwide because acrylamide has also been classified as "probably carcinogenic to humans" by IARC in 1994 and it is a known neurotoxin.

A variety of different methods have been developed to determine acrylamide especially in water, biological fluids and recently in samples of heat treated food rich in starch. Most of them concern HPLC and GC-MS techniques of analysis combined with mass spectrometric detection. The assays employing GC-MS are usually based on derivatisation of a sample (in most cases it is bromination).

The derivatisation step is applied to increase the selectivity and sensitivity of GC analysis. The process of derivatisation is quite time-consuming but is still the most frequent and reliable in GC-MS analysis for the time being.

Although most LC methods do not apply the derivatisation procedure, more effort must be placed on clean-up steps.

Recently, new derivatisation methods have been proposed to improve the performance of single stage LC. One of them deals with 2-mercaptobenzoic acid as a derivatisation agent of acrylamide. During this reaction acrylamide is converted into the thioether.

In this study, derivatisation with 2-mercaptobenzoic acid was employed for the analysis of acrylamide performed on HPLC with UV detection.

The conducted experiment was aimed at optimization of analysis parameters and included the initial assays with standard derivatisation.

POSTER SESSION II

APPLICATION OF PLANAR CHROMATOGRAPHY ON SILICA STATIONARY PHASE IN BIOMEDICAL AND ENVIRONMENTAL ANALYSIS

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However the great variety of layers in planar chromatography is available, but still silica gel has been more popular. This application is possible due to the different particles size and physico-chemical properties suitable for many analysis.

Silica gels for chromatography have an exceptional value for laboratory separations, pilot and production-scale purifications.

Thin layer chromatography (using silica gel as a stationary phase) with densitometric detection has been used as fast and economical method for the identification and determination of biological active compounds in the extracts isolated from body fluids and environmental samples. This method was applied e.g. for the determination of:

- Oxycholesterols (7-ketocholesterol and 7-hydroxycholesterol) in plasma and meat samples (chloroform-acetone 9:1,v/v - as mobile phase),
- Phospholipids in amniotic fluids (chloroform/methanol/water 65:25:4),
- Nicotine and its metabolites in urine (ethyl acetate-methanol-acetic acid)
- Polycyclic aromatic sulfur compounds in sewage sludge (n-pentane-acetone 10:0.2 and di-chloromethane – n-pentane 4:6),
- Nitrogen containing polycyclic aromatic compounds (azaarenes and aminoarenes) in sewage sludge (dichloromethane-hexane-diethyl ether 12:8:1)

Moreover, TLC on silica gel has been applied in semi-preparative way to isolation of polynuclear aromatic hydrocarbons concentrates and their more polar (oxygen) derivatives from airborne particulate matter followed by GC-MS.

POSTER SESSION III

EXAMINATION OF DYE-PROTEIN INTERACTION BY GEL-PERMEATION CHROMATOGRAPHY

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Various chromatographic techniques are used for protein purification. Among these techniques affinity chromatography is of high selectivity because it is based on highly specific molecular recognition. A useful group of ligands is the textile dyes derived from symmetrical triazine, and Cibacron Blue F3G-A (CB) as the archetypal textile dye is widely used for this purpose.

The ability of therapeutic proteins human interferon-α2b (INF-α2b) and human growth hormone (hGH) to form complexes with CB was demonstrated by applying the absorption difference spectroscopy. Using the nonlinear least-squares method implemented in the Mathcad 5.0 program package, it was calculated that K_d of hGH – CB and INF-α2b – CB complexes at pH 6.9 were equal to 5.6 μM and 1.5 μM, respectively.

To confirm the formation of hGH – CB and INF-α2b – CB complexes an attempt was made to isolate these ones by gel-permeation chromatography. Dye-protein solutions were applied to a Sephadex G-50 column. The visible absorption spectra of the isolated hGH – CB and INF-α2b – CB complexes were registered in a comparison with free dye. The spectra of dye-protein complexes showed a shift to a long-wave side. It seems that hGH – CB and INF-α2b – CB interaction forces are sufficiently strong to withstand such nonequilibrium conditions. It follows that dye-protein complexes can be successfully isolated by gel-permeation chromatography if its stability is on the same level as of the ones mentioned above.

Moreover, the gel-permeation chromatography was used to investigate the influence of CB to the oligomeric state of proteins. It was found that CB induces the dimerization of INF-α2b at pH 5.0, at which INF-α2b is usually monomeric. It was demonstrated that CB does not cause the association of hGH.

POSTER SESSION III

OPTIMISATION OF PREPARATIVE LAYER CHROMATOGRAPHY OF ISOQUINOLINE ALKALOIDS FROM *Corydalis solida* EXTRACT

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Plants from *Papaveraceae* family are rich in organic bases, alkaloids which are substances having in most cases biological activity. In order to investigate their structure and properties as pharmacologically active compounds, their isolation from plant extracts is necessary. The first step it has to be the sample preparation from crude extract for preliminary elimination of ballast substances depending on the matrix. The use of solid phase extraction – classic column chromatography in optimised chromatographic system can be applied for the separation of crude extract, to isolation of fraction rich in alkaloids.

The main problem in chromatographic separation is the closely related structure and similar physicochemical properties of separated compounds. Closely related structure gives similar chromatographic properties and difficult task in separation. This situation required non only optimization of adequate chromatographic systems (adsorbents and mobile phases) but also the use of special modes of sample application, special techniques of development

The aim of the paper was selection of optimum conditions for the isolation of isoquinoline alkaloids from the *Corydalis solida* herb extract. Various sample application modes were compared: application from the edge of the layer, by set of capillaries and with the automatic applicator with the evaporation of sample solvent. The influence of the sample overloading (volume or mass) on the separation of neighbouring bands was also investigated to choice the maximum overloading. The separability of isolated bands on the layers of different thickness in aspect of maximum yield was also studied. The special techniques of development such as unidimensional multiple development (UMD) or incremental multiple development (IMD) were also applied in order to obtain the maximum resolution.

THE INFLUENCE OF STATIONARY PHASE AND ELUENT COMPOSITION ON THE SEPARATION OF SELECTED PSYCHOTROPIC DRUGS AND TRANQUILIZERS

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As the growth of the incidence of illnesses affecting central nervous system appears, it is essential psychotropic drugs should play a leading role in pharmacoteraphy. Simultaneously, the abuse of these drugs is observed. Therefore, constant improvement of identification methods and quantitative determination of the compounds is required.

The main problem connecting with the chromatographic separation of these drugs is their diverse chemical structure. Some of these compounds occur in form of non-ionized mole-cules. However, some of drugs have ionic structure and, depending on medium pH, occur in ionized or un-ionized form. In result, the various interactions with adsorbent surface appear which makes the find of uniform chromatographic systems for these compounds difficult.

The aim of this work is investigation of the following parameters on the separation process of these drugs:

- eluent's composition
- pH of mobile phase
- kind and concentration of ion-pair reagents adding to eluent
- kind and concentration of amines – blocking of free silanols and supressing bases' ionization

The following conclusions can be drawn from the obtained results:

- the necessity of group of substances according to their structure and differences in retention behaviour
- satisfied selectivity and efficiency in systems silica / methanol + diisopropyl ether
- the best selectivity and efficiency in systems C18 / aqueous eluents with addition of ammonia or diethylamine.

POSTER SESSION III

DETERMINATION OF HOMOCYSTEINE AND RELATED AMINOTHIOLS IN HUMAN PLASMA BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Homocysteine (Hcy), increasingly being recognized as a risk factor for cardiovascular disease that is the reason why we developed a new HPLC assay for rapid and sensitive measurement of total homcysteine. Total homocysteine is defined as the sum of all homocysteine species in plasma, including free and protein-bound forms.

Homocysteine present in human blood plasma is derivatized with 2-chloro-1-propylpyridinium iodide [1] and separated from other thiols derivatives by high-performance liquid chromatography (HPLC) with detection at 312 nm. The separation was performed by using LiChroCART, Purospher STAR RP-18, 4,6^{x}150 mm, 5 µm from Merck. The temperature was 25°C, the flow-rate 1.5 ml/min and the elution profile was as follows: 0-5 min 3% B, 5-7 min 20% B, 7-8 min 10 % B, 8-10 min 3 % B. Elution solvent was (A) 0,05 trichloroacetic acid buffer (pH=3,2 prepared from 0,05 M TCA and 0,05 M potassium hydroxide) [2] and (B) methanol.

Homocysteine is clearly separated from other thiols, the retetion time being 5,8 min, total analysis time is 10 min. To determine total homocysteine it is necessary to cleave disulphide bounds by the use of tris(2-carboxyethyl)phosphine hydrochloride [3] in order to form free sulfhydryl group.

The described method has several advantages: simple sample preparation procedure, simultaneous determination of low-molecular-mass aminothiols during the procedure, fast chromatography procedure, simple and prevalent mobile phases.

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POSTER SESSION III

TLC DATA IN STRUCTURE-ACTIVITY RELATIONSHIP STUDY OF COMPOUNDS WITH ANTIHISTAMINE ACTIVITY

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A structure-activity relationship (SAR) analysis of compounds with antihistamine activity was carried out and chromatographic data of 2-[2-(phenylamino)thiazol-4-yl]ethanamine; 2-(2-benzyl-4-thiazolyl)ethanamine; 2-(2-benzhydrylthiazol-4-yl)ethanamine; 2-(1-piperazinyl- and 2-(hexahydro-1*H*-1,4-diazepin-1-yl)benzothiazole; 2-[4-(1-alkyl)piperidinyl]benzothiazole; 2-(N,N',N'-dimethylalkyl-1,2-ethanediamino)benzothiazole; 2[1-(4-aminopiperidinyl)]benzothiazole were obtained. The NP TLC and RP2 TLC plates (silica gel NP $60F_{254}$ and silica gel RP2 $60F_{254}$ silanised precoated), impregnated with solution of aspartic acid (L-Asp) were used in two developing solvents as H_1- and H_3-antagonistic interaction models. The lipophilicity data of the examined compounds were obtained and used in the SAR assay. The logos P-values of particular compounds are extremely important for this kind of activity.

POSTER SESSION III

CAPILLARY ZONE ELECTROPHORESIS AND HIGH-PERFORMANCE LIGUID CHROMATOGRAPHY AS AN ALTERNATIVES FOR QUANTITATIVE ANALYSIS OF FLORFENICOL IN PLASMA

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Florfenicol (D-(threo)-1-(methylsulphonylphenyl)2-dichloroacetamide-3-fluoro-1-propanol) is a broad-spectrum, primarily bacteriostatic antibiotic with the spectrum of activity, including many Gram-negative and Gram-positive bacteria and is used exclusively in veterinary medicine. A capillary zone electrophoresis (CZE) and a reversed phase (RP-HPLC) method with UV detection have been developed for florfenicol analysis in plasma samples.

The suitabilities of both methods for quantitative determination of florfenicol were approved through validation specification such as linearity, precision, selectivity, accuracy, limit of detection and quantification. The capillary electrophoresis (CE) and high performance liquid chromatography (HPLC) assay were compared by analyzing a series of plasma samples containing florfenicol in different concentrations using these two methods. The extraction procedure is simple and no gradient elution or derivatization is required. Furthermore, the analysis time in the CE method is two times shorter than the respective parameter in HPLC and solvent consumptions is considerably lower. The calibration curve were linear to at least 0,05 – 10 μg mL^{-1} (r = 0.9998) and 0.1 – 10 μg mL^{-1} (r = 0.9998) for CE and HPLC, respectively. The separation efficiency are good for both methods. The detection limits for florfenicol were 0.015 μg mL^{-1} with CZE and 0.03 μg mL^{-1} with HPLC and there was considerably lower using CZE method, even thought UV detector was applied in the both cases. The both methods were selective, robust, and specific allowing reliable quantification of florfenicol and can be useful for clinical and biomedical investigations.

DETERMINATION OF ENROFLOXACIN IN ANIMAL PLASMA BY RP-HPLC METHOD

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Enrofloxacin is a fluoroquinolone synthetic antibacterial agent with a broad spectrum of activity. It is veterinary drug with highly active against a wide range of Gram-negative and Gram-positive bacteria, which bacterial action is based on their anti-dNA gyrase activity. This drug is suitable for the treatment of systematic infections in addition to urinary tract infections.

A rugged, simple and rapid high-performance liquid chromatographic method (RP-HPLC) for determination of enrofloxacin has been developed in pig plasma after ingestion single standard dose. The analytes were extracted from plasma using dichloromethane. Bupivacaine hydrochloride as internal standard was chosen. The compounds were separated on C-18 reversed-phase analytical column with dimensions of 125 x 4.6 mm as stationary phase. The mobile phase consisted of methanol-water (68:32 v/v) adjusted to pH 3.4 using 85 % ortophosphoric acid. A fluorescence detector was used with an excitation wavelength of 280 nm and an emission wavelength of 445 nm. The method has been validated for enrofloxacin in pig plasma. The quantification limit for enrofloxacin in plasma was 10 ng mL^{-1}, limit of detection was 5 ng mL^{-1}. Linearity of the method was confirmed in the range of 10 to 1000 ng mL^{-1}, correlation coefficient 0.999. Within-day relative standard deviations (RSDs) ranged from 1.84 to 13.52 % and between day RSDs from 2.95 to 14.47 %.

The reported RP-HPLC method could be a reliable alternative to other separation methods for the analysis of enrofloxacin. The assay is specific and can be reproducible and mean recoveries for enrofloxacin was 98 % for pig plasma over the range used. The suitability of the assay for pharmacokinetic studies was determined by measuring enrofloxacin concentrations in pig plasma after administration of a single intravenous 2.5 mg/kg dose of enrofloxacin.

POSTER SESSION III

IMPROVED RAPID GC ASSAY FOR THE CHOLESTEROL AND PLANT STEROLES ANALYSIS

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The amount of cholesterol and seteroles in food products can be used as an consumption food indicator. The high concentration of cholesterol is characteristic for animal products.

Elaborated method is based on (i) saponification of fats, (ii) their derivatisation to the methyl esters, (iii) extraction of the obtained esters and non-saponificated compounds and finally (iv) derivatisation of the cholesterol and plant sterols with BSTFA in pyridine. Described assay eliminate separation of the fatty acids from the non-saponificated compounds using column LC as well as separation of the sterols on TLC.

Verification of the method was obtained on three reference materials:

- CRM 164 – anhydrous milk fat containing, inter alia, cholesterol, β-sitosterol, campesterol and stigmasterol,
- CRM 162 – soya-maize oil,
- CRM 163 – pork/beef fat.

Measurements have been performed on the GC-FID (Agilent 6890) chromatograph equipped with the HP-1 capillary column (25 m length, 0.2 mm I.D. and 0.11 μm thick stationary phase). Temperature of the column: 250°C (4 min.) with gradient 5°C/min to 300°C (5 min.); injection valve (split – 25:1): 280°C and detector: 310°C. Carrier gas – helium, 15 psi.

It turned out that obtained concentrations of the analyzed cholesterol and sterols in the reference materials were obtained in the range of the producer uncertainty. The elaborated method have been used to identify plant fats in the butter, what was also confirmed by the fatty acids profile.

SELECTION RULES OF RP-HPLC COLUMNS IN ROUTINE METHODS OF ANALYSIS APPLIED IN MONOGRAPHS OF THE PHARMACOPOEIA

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Pharmacopean methods (European Pharmacopoeia, United States Pharmacopoeia) allow user to change chromatographic separation conditions to obtain the best result.

The kind of chromatographic column and, in the case of reversed phases columns, the nature of the stationary phase, have a direct impact on accurate result of analysis. In monographs of the Pharmacopoeia column and it's stationary phase are described in general way e.g. C8, C18, C18 BDS, CN etc. In the presence of such a variety commercially available columns with differences in chromatographic behaviour, especially with reverse-phase, the analytical chemist has a serious problem which column will be proper for actual analysis. The question is very important because of fact that manufacturers don't apply comparable methods of characteristic of stationary phases.

The review of performance tests of RP-HPLC columns, available in literature, and their helpfulness in column selection is a useful tool in the solution of this practical problem.

POSTER SESSION III

RAPID GAS CHROMATOGRAPHIC DETECTIONS OF CHOLESTEROL AND SATURATED-UNSATURATED FATTY ACIDS IN RAW AND BOILED QUAIL EGG YOLK

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Quail eggs contain many essential subtances for human growth and development and especially for child nutrition. Many studies and clinical investigations have made evident that *n*-3 polyunsaturated fatty acids, exert beneficial effects on human health. *N*-3 fatty acids are essential for normal growth and development and play an important role in the prevention of coronary artery disease, inflammatory, hypertension, autoimmune disorders and cancer. Cholesterol is a fat-like substance found in every living cell in the body and is made in necessary amounts by the body and is stored in the body. It is especially concentrated in the liver, kidney, adrenal glands and the brain. Cholesterol is required for cell wall structure,for producing vitamin D, for producing digestive juices, for insulating nerve fibers etc. The total cholesterol is the sum of the low-density lipoproteins (called LDL-cholesterol or bad cholesterol) and high-density lipoproteins (called HDL-cholesterol or good cholesterol). Dietary excesses, too much saturated fat and high intakes of cholesterol may increase the level in the blood. There is the need for regular and rapid chromatographic monitoring of cholesterol and fatty acids of alternative egg obtained from quail. Extracted lipids were performed by Gas-liquid chromatography (GC) by our new developed procedure using a 60 m-capillary column (with 0.25 μm film tickness), 0.25-mm-inside-diameter WCOT fused-silica SGE (BP70X GC) capillary column installed on a Perkin Elmer (Auto System) Gas-liquid chromatograph with a Flame Ionization Detector (FID). Analytical calibration study *(R^2*=0.9999); method validation and recovery studies [y= 4.355 x + 0.072 (R^2=0.9999)] were performed and obtained good results. Saturated and unsaturated fatty acids (FAs) as C14:0 , C14:1, C16:0, C16:1*n*-7, C18:0, C18:1*n*-9, C18:1*n*-7, C18:2*n*-6, C18:3*n*-3, C18:4*n*-3, C20:4*n*-3, C20:5*n*-3, C22:5*n*-3, C22:6*n*-3 were performed as simultaneously. Total polyunsatutared FAs , saturated FA contents, and total *n*-3/*n*-6 ratios were obtained. It was found 73.45 ± 1.07 mg cholesterol (n=6) (p<0.01) in one medium size of quail egg yolk. Boiling process does not affect the cholesterol content of quail egg yolks. The proposed chromatographic methods for fatty acids and cholesterol determination in quail egg yolks could be utilized for egg quality control industry.

RP-HPLC DETERMINATION OF VITAMIN E IN HUMAN BREAST MILK*

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The main biological function of vitamin E is as an antioxidant in the protection of PUFA of cell membranes from free radical damage. An adequate supply of vitamin E in milk to newborn and especially pre-term infants is vital for their normal development. A number of publications have used a variety of different methods to report levels of tocopherols in human milk at different stages of lactation. Here we compared two new hexane-based methods of extraction of vitamin E from pre-term human milk#. One of them is liquid-liquid extraction with hexane after saponification (1ml of 10 % $KOH_{aqueous}$ and 1ml of methanol containing 3 % of pyrogallol; 30 min at 70°C) and the other is liquid-liquid extraction with hexane directly after disruption of milk fat globules with methanol (10 min on ice, pyrogallol (2%) in methanol). A Waters HPLC system incorporating an Alliance 2690 separation module and a 996 photo-diode array detector was used in this study. Separation of tocopherols was achieved using a Waters Symmetry™ C_{18}-column (3.9x150 mm) and a C_{18}-guard column. Conditions of elution were the following: initially a linear gradient of acetonitrile in water (from 95 % to 100 % in 10 min) was applied, then 100 % of acetonitrile was held for 10min at a flow rate of 1 ml/min. The column temperature was set to 45°C. The range of wavelengths scanned was 275-350 nm. Peak areas of tocopherols were integrated at 295 nm. The identification of tocopherols in UV spectra was done by spiking the extracted samples with solutions of tocopherols in ethanol and their co-elution. To ensure a reproducible retention of the column, after each batch of 30 – 40 milk extracts the column was washed with propan-2ol (1 ml/min for 1 h at 45°C).

Direct extraction of tocopherols with hexane allowed 60.3±14.7 % recovery of the internal standard, δ-tocopherol. Application of the improved method that included a simple saponi-fication step gave 99.6±4.0 % recovery for the same internal standard. There was a significant relationship between tocopherol contents in human milk specimens measured using two new extraction procedures ($P < 0.01$). This implies that both simple methods of extraction can be used for assays of vitamin E in human milk, however the evidence presented herein suggests that extraction after saponification may significantly improve the reliability of such data.

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#Recruitment of mothers into the study was performed in accordance with the approval of The Local Research Ethics Committee

POSTER SESSION III

THE COMBINATION OF Ag^+-HPLC AND LONG CAPILLARY COLUMN FOR GLC TO STUDY THE CLA ISOMER PROFILE IN RAT LIVERS*

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Conjugated linoleic acid (CLA) isomers intake is know to produce changes in lipid metabo-lism in liver and adipose tissue. Really, CLA isomers consumption may modify hepatic fatty oxidation and synthesis. So, the aim of the present work was to develop the new chromatographic procedures to more selective and accurate characterization of a complex CLA isomer mixture in liver of rats fed diet enriched in 2% CLA isomer mixture (w/w) [1]. CLA isomers and other fatty acids containing conjugated double bonds [2] (CFAs) were fractionated using long capillary column (100m x 0.25mm i.d. x 0.2μm film thickness; Varian) for gas-liquid chromatography (GLC) equipped with an FID. The first step procedure of CLA isomers and CFAs analysis was mild saponification [3] of liver sample (~50 mg of DM), and then underivatized CFAs in a hydrolysate were preliminary chromatographically fractionated after reconstitu-tion of dried hydrolysate extract in 0.5ml of n-hexane [3]. No single method is able to resolve all ~400 fatty acids in the liver, even capillary columns of 100 m. Therefore, before GLC separation we used silver ion liquid chromatography (Ag^+-HPLC) with photodiode array detection [4] (Waters, USA) at 234 nm [5] for *pre*-fractionation of some classes of CFAs and CLA isomers like *trans-trans, trans-cis/cis -trans* and *cis-cis*. So, 100μl of processed sample was injected onto the silver-ion HPLC column. We collected seven fractions eluted between 23 and 85 min of isocratic chromatographic run (1.35ml/min). Obtained fractions were dried under argon; to a residue 2ml of 2M NaOH in methanol and 50μl of BHT in methanol (20mg/ml), and then reacted for 1h at 80°C. After cooling to reaction solutions 2ml of 25% BF_3 in methanol were added, and again heated for 60 min at 80°C. Then methylated fatty acids (FA-Me) were extracted with 5 ml of hexane. Obtained extracts were condensed to ~0.5 ml, and then methy-lated CLA isomers and CFA in condensed fractions were analyzed by an Agilent 6890N GC equipped with CP7489 capillary column (100 m) and FID. A satisfactory values of peak area to noise for assayed conjugated fatty acids in analyzed fractions were obtained using 1:1 split mode, injection volumes equal 5μl and the gradient temperature program as described previo-usly[3]. Combination of Ag^+-HPLC *pre*-fractionation, *pre*-concentration and injection of large amount of a sample onto a high-resolution capillary column appears to be the best analytical tool for more accurate and selective identification of CLA and CFA in assayed samples.

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[5] the maximum of the UV spectra of a fatty acids containing conjugated double bonds (like CFAs and CLA isomers)

POSTER SESSION III

THE COMBINATION OF GLC AND Ag^+-HPLC TO STUDY THE FATTY ACID AND CLA ISOMER PROFILE IN HEN EGG YOLKS*

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Feeding laying hens with CLA isomers should be a way to obtain functional *i.e.* CLA-enriched eggs (yolks) conferring beneficial health effects on consumers. Thus, it is essential to develop new highly efficient procedures for determination of fatty acid (FA), especially CLA isomers and other FAs containing conjugated double bonds (CFA) in hen egg yolks using the combination of gas liquid chromatography (GLC) and Ag^+-HPLC. CLA-enriched egg yolks were obtained by feeding 45 hens for 4 weeks with a diet containing 0.75% of CLA (Luta-CLA 60; BASF). Eggs from each hen were broken, yolks were separated from albumen, and then freeze-dried. The powdered yolks samples (± 50 mg) were placed into vials and treated with a mixture of 2 ml of 2M KOH in water, 2 ml 1M KOH and 50 µl of BHT (20 mg/ml) in methanol. The resulting mixture was then vigorously mixed and heated at 95°C for 5 min. The saponification was carried out at 25°C for 12 h. The hydrolysates were diluted with 3ml of water and then the obtained solution was acidified with HCl to pH ~1. Free FAs were extracted 4 times with portions of 3 ml of dichloromethane (DCM). The DCM layer was dried with Na_2SO_4 and extraction was repeated 4 times using 3 ml of n-hexane. Next, the n-hexane layer was combined with DCM layer and the organic solvents were removed under argon. 2ml of 2M NaOH and 50 µl of BHT in methanol were added to the residue and reacted for 1h at 80°C. After cooling the reaction mixture, 2ml of 25% BF_3 in methanol were added, and heated for 1h at 80°C. The cooled reaction mixture was diluted with 5 ml of water and then FA-MEs were extracted with 5 ml of n-hexane. Separation of FA-MEe was carried out using an Agilent 6890N GC equipped with CP7489 fused silica 100 m capillary column and FID, whereas CFA and CLA isomers were separated using Ag^+-HPLC with a photodiode detection (DAD)[#]. Since the saponification was carried out at 25°C, the proposed new method eliminates the risk of isomerization and degradation of PUFA. Long capillary GLA column and the proposed column temperature program appear to be the best compromise for satisfactory fractionation of physiologically important FAs of egg yolks and duration of GCL analysis. Excellent characterization of CLA and CFA was obtained using Ag^+-HPLC since DAD detected only FAs containing conjugated double bonds, whereas other FAs are undetectable.

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#M. Czauderna *et al., 2005. J. Anim. Feed Sci., 14, in press*

POSTER SESSION III

ACCUMULATION OF COUMARINS IN *Ruta graveolens* L. *in vitro* CULTURES – HPLC ANALYSIS

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Contents of coumarin compounds in callus cultures of *Ammi majus* L. and *Pastinaca sativa* L., maintained at our biotechnological laboratory, are markedly dependent on concentrations of growth regulators in the media [1, 2].

The aim of the present studies was to determine an effect of growth regulators on accumulation of this group of metabolites in *Ruta graveolens* L. shoot cultures. So far, we carried out only preliminary investigation of this problem [3].

Stationary liquid cultures of *R. graveolens* L. were maintained on 9 variants of Linsmaier-Skoog [4] medium under constant artificial light (900 lx), and a temperature of 25± 2°C. The variants differed in concentrations of growth regulators: α-naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BAP); the concentrations ranged between 1 - 10 mg/l of medium.

Ethanol extracts of biomass collected after 6-week growth cycles were used for determination of contents of 8 coumarin compounds with HPLC method (RP-18). Contents of individual metabolites were very diverse, clearly depending on concentrations of growth regulators in the medium variants. On some variants of the medium, considerable amounts of xanthotoxin (346.9 mg%), psoralen (248.5 mg%), scopoletin (239.6 mg%), isopimpinellin (172.3 mg%), coumarin (117.1 mg%) and umbelliferone (104.4 mg%) were obtained. Maximum contents of bergapten (46.6 mg%) and imperatorin (19.9 mg%) were much lower. Total content of the compounds under analysis showed also wide variations, ranging from 50.4 to 927.5 mg%, and depended upon NAA and BAP concentrations in the media.

The best "productive" medium was variant containing 1 mg/l NAA and 3 mg/l BAP.

The metabolites were successfully separated and quantified using RP-HPLC method.

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POSTER SESSION III

COUMARINS IN *in vitro* CULTURE OF *Ruta graveolens* Ssp. *Divaricata* (Tenore) GCMS – HPLC DETERMINATION

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Shoot–differentiating callus of *Ruta graveolens* ssp. *divaricata* (Tenore) Gams cultured under constant artificial light (900 lx) in stationary liquid phase on Linsmaier and Skoog (LS) [1] medium containing 2 mg/l NAA (α–naphthaleneacetic acid) and 2 mg/l BAP (6-benzylaminopurine) is a rich source of linear furanocoumarins and umbelliferone [2].

The aim of the present work was optimization of light conditions for production of coumarins in shoot–differentiating callus cultures*. These cultures were maintained in stationary liquid phase on LS medium containing growth regulators: NAA–1mg/l and BAP–1mg/l under light of different spectra: white (390-760 nm), far-red (770-800 nm), red (647-770 nm), blue (450-492 nm), UV-A irradiation (360-450 nm) and in darkness. Intensity of the photosynthetic active radiation (PAR) was 60 μmol $m^{-2}s^{-1}$ except for red and far-red light (20 μmol $m^{-2}s^{-1}$).

Contents of coumarin compounds (coumarin, scopoletin, umbelliferone, psoralen, xanthotoxin, bergapten, isopimpinellin, imperatorin) were determined (after 6-week growth cycles) in ethanol extracts of biomass. The metabolites were successfully separated and quantified using HPLC method (RP-18).

Maximal total content of metabolites was observed in the differentiating callus cultivated under blue and white light (94.37 mg/100 g and 94.14 mg/100 g of dry weight, respectively). The lowest contents of the metabolites were noted under UV-A irradiation (20.38 mg/100 g d.w.). These results confirm our earlier studies with *R. graveolens* shoot cultures [3]. Blue light facilitated accumulation of psoralen (17.49 mg/100 g d.w.), white light was beneficial for scopo-letin production (62.89 mg/100 g d.w.), whereas red light favored accumulation of isopimpinellin (14.97 mg/100 g d.w.) and umbelliferone (11.72 mg/100 g d.w.).

* *In vitro* culture of *R. graveolens* ssp. *divaricata* (Tenore) Gams was obtained from Institut für Biowissenschaften mit Botanischem Garten, Universität Würzburg, Germany within the framework of cooperation of this university with Collegium Medicum UJ

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POSTER SESSION III

SECONDARY METABOLITES IN *Lactarius deterrimus*

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Lactarius deterrimus, Lactariaceae, Basidiomycetes, belongs to higher fungi. It is edible fungus, commonly occuring in Poland. Fruit bodies of this species were used as a source for the isolation interesting secondary metabolites.

Fruit bodies of Lactarius deterrimus were collected in pine woods in the area of Krzyż village, Poznań Province, in September 2004 .

The investigated material were the fresh fruit bodies, extracted by benzine ether, methanol and water. This extract were purificated by TLC method on aluminium plates, covered by silica gel (DC- Alufolien Kieselgel F- 254, Merck). After purification extracts were analyzed with using kochromatographic TLC method, spectral methods (UV spectrum), HPLC method and by densytometric method. This procedure allowed to obtain numerous known fungal metabolites belonging to sterols (ergosterol, sitosterol, arachidonic acid);to indolic metabolites: tryptamine, melatonine, indoloacetamid, indoline, kynureine, 5OH kynureine and also aminoacids: waline, alanine, leucine. In ether extract was obtain dark red pigment which structure analized by TLC, UV, IR, ^{1}H NMR methods. This pigment has antibiotic activities, so it is important to agree their structure. Two polysaccharide fractions were isolated and purified from Lactarius deterrimus. Sugar content, determinet by Dubois method. The presence of monosaccharides was demonstrated after acid hydrolysis, using TLC on Silica Gel 60F-254.

The obtained results have showed that chromatografic method is appropriate for mushrooms metabolites.

POSTER SESSION III

CHROMATOGRAPHIC ANALYSIS OF INDOLE DERIVATIVES PRODUCED BY *Tricholoma equestre* (Basidiomycetes)

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From many years fungi are used in industrial processes to generate biologically active substances such as enzymes, vitamins, lipids, pigments and others. They are also a significant source of secondary metabolites, which are extremely important to human health. Special attention is given to the indole derivatives regarding their possible biological activity such as cytostatic, anti-tumor or anti-ageing properties. So, the aim of this study was to determine a content of above mentioned indole derivatives in fruit bodies of Tricholoma equestre (L. ex Fr., Quell).

Tricholoma equestre (commonly known as "Man-on-Horseback" mushroom and Gąska zielona in Poland) is a widespread mushroom belonging to Tricholomaceae family (Basidiomycetes).Growing in sandy soils in the pine forests. Fruit bodies of Tricholoma used in this study were collected in September 2004 in Krzyż Wielkopolski. The fruit bodies were dried at room temperature and its samples (weighted from 0,1g to 1g) were extracted for 12 hours with methanol. In the second step of performed analysis, these methanol extracts were analyzed on TLC silica gel plates (n-butanol - acetic acid - water 12:3:5 v/v/v) and indole derivatives were visualized with DAB reagent. The next step, there was HPTLC purification and identification of the indole compounds obtained by TLC method and then quantitative analysis of interesting substances with HPLC method. The following indole derivatives have been detected: 3-indol-3-acetamid, melatonine, tryptophane, tryptamine, 5-CH_3-tryptophane, 5-OH-tryptofane. The described chromatographic methods are appropriate for purification, isolation and analysis of indole metabolites from fungi.

The results of this study clearly showed that commonly growing mushrooms such as Tricholoma equestre can be valuable source not only nutrition elements but also and first of all of many interesting, bioactive substances, which can be utilized in pharmacology and medicine.

POSTER SESSION III

HPLC ANALYSIS OF FREE PHENOLIC ACID FRACTION IN SHOOTS OF *Ruta graveolens* L. CULTURED *in vitro* – PRELIMINARY RESULTS

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Different types of *R. graveolens* L. *in vitro* cultures are a rich source of alkaloids, coumarin compounds and volatile oil [1]. In our biotechnology laboratory, shoot cultures, that accumulated marked amounts of psoralen derivatives, approximating 1 g%, were established [2]. Accumulation of phenolic acids in *in vitro* cultures of this species has not been investigated yet. Native plant contains numerous phenolic acids [3]. This group of metabolites has interesting biological activities, including spasmolytic, antiinflammatory, cholagogic, hypolipidemic, anti-aggregative, immunostimulating activities and others properties.

The aim of this study was to determine qualitative composition and contents of free phenolic acids in *R. graveolens* shoots cultured in vitro. Overground parts of plants originating from the collections of 2 experimental gardens in Kraków and a commercial product were also analyzed for comparison.

Stationary liquid *R. graveolens* shoot culture was maintained on Linsmaier-Skoog medium containing α-naphthaleneacetic acid (NAA) - 2mg/l and 6-benzylaminopurine (BAP) - 2mg/l under different light conditions (blue, red, white light and UV-A irradiation).

Methanol extracts of shoots from *in vitro* cultures collected after 4 week growth cycles and of overground parts of plants growing in open air were subjected to HPLC analysis (RP-18) to determine contents of free phenolic acids. Eight compounds were analyzed: caffeic, chlorogenic, ferulic, p-hydroxybenzoic, p-coumaric, protocatechuic, syringic and vanillic acids.

The extracts from biomass obtained in *in vitro* cultures were shown to contain 3 compounds: p-hydroxybenzoic acid, protocatechuic acid and vanillic acid. Light conditions of *in vitro* cultures markedly influenced contents of the metabolites. Their total content was the highest in shoots growing under UV-A irradiation (59.0 mg%). Protocatechuic acid dominated in all extracts (8.1 - 41.7 mg%).

Material originating from natural stands had the same qualitative composition of free phenolic acids: chlorogenic acid, p-hydroxybenzoic acid, protocatechuic acid and vanillic acid. Chlorogenic acid (21.0 and 31.2 mg%) or protocatechuic acid (21.3 mg%) were the dominating components of this fraction.

The studies showed that contents of protocatechuic acid accumulated in *R. graveolens* shoots cultured *in vitro* were almost 2 times higher than in native plants.

Eight metabolites were successfully separated and quantified using RP-HPLC method.

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POSTER SESSION III

HPLC ANALYSIS OF FREE PHENOLIC ACIDS IN SHOOTS OF *Hypericum perforatum* L. FROM *in vitro* CULTURE – – PRELIMINARY RESULTS

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Phenolic acids are a group of metabolites possessing very valuable biological properties - spasmolytic, antiinflammatory, cholagogic, hypolipidemic, antiaggregative, immunostimulating activities and others. These compounds occur in a herb of *Hypericum perforatum* (St. John's wort) besides antraquinone derivatives, flavonoids, volatile oils and other constituents [1]. Accumulation of phenolic acids in *in vitro* cultures of this species has not been studied so far.

The aim of the present study was to analyze qualitative composition and contents of free phenolic acids in shoots of *H. perforatum* cultured *in vitro*. Overground parts of plants - commercial products of 2 polish manufactures were examined for comparison.

H. perforatum shoot cultures were maintained in stationary liquid phase on Linsmaier-Skoog medium [2], supplemented with α-naphthaleneacetic acid (NAA) at 0.1 mg/l and 6-benzylaminopurine (BAP) at 0.1 mg/l, at a temperature of 25 ± 2°C under different light conditions (blue, red white light and UV-A irradiation) for 4 weeks.

Free phenolic acids in methanol extracts of shoots from *in vitro* cultures and overground parts of native plants were determined by HPLC (RP-18) method.

The material from the *in vitro* cultures was shown to contain 6 metabolites: caffeic, chlorogenic, p-hydroxybenzoic, protocatechuic, syringic and vanillic acids. Light conditions of *in vitro* culture, markedly influenced qualitative composition and contents of the metabolites. Their total content ranged from 19.2 mg% (red light) to 34.9 mg% (blue light).

Chlorogenic acid quantitatively dominated in a majority of the extracts (17.2 - 21.4 mg%).

Commercial products under analysis differed considerably in qualitative composition and contents of free phenolic acids. Chlorogenic acid quantitatively dominated in both products, and only its contents were almost identical (33.7 and 33.1 mg%).

The studies have shown that *H. perforatum* shoots cultured *in vitro* are a good source of chlorogenic acid.

The procedure of HPLC analysis allowed for concomitant separation and quantification of 8 compounds.

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POSTER SESSION III

GC DETERMINATION OF OLEIC ACID CONTENT IN OLIVE OIL SAMPLES OXIDIZED WITH ADDITION OF TERTAPHENYLPORPHIRYNS AND ITS METAL DERIVATIVES

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There is a decrease in the biological and nutritional properties in edible fats due to the oxidative reactions. Factors precipitating the reaction are: temperature, ultraviolet light and selected metals. In recent years there has been a steadily growing interest in porphyrins as they play an increasingly important role in medicine and chemistry. Both porphyrin and its metallo-derivatives have been a subject of numerous previous experimental. This study was aimed because olive oil is becoming the most important oil in human nutrition.

The aim of the study was examining the influence of tetraphenylporphyrin and its copper (II), nickel (II) and cobalt (II) derivatives on the oxidation reaction of olive oil.

The oxidation reaction was conducted in temperature $60^0C \pm 1^0C$ using Schall Oven Test, in the period of six weeks. 30 g of olive oil was put on each of Petri's plates and then 20 mg or 50 mg of studied compounds were added to those samples.

Properties of tetraphenylporphyrin and its metalloderivatives were demonstrated through determining the peroxide value (PV) and iodine value (JV) - in conformity with recommendations of Polish Standard PN - ISO 3960/1996 and PN ISO 3961/1998. The peroxide values was assayed in one-week intervals untill the iodine value was assayed at the beginning and the end of the experiment. At the same time the content of oleic acid and pH in olive oil were determined.

It follows from the results obtained that the tetraphenylporphyrin and its copper, nickel and cobalt derivatives doesn't demonstrate any antioxidants properties, untill tetraphenyl-porphyrin cobalt demonstrate prooxidant properties during olive oil oxidation reaction. Obtained GC results shown decrease of determined fatty acid in studied samples particularly for cobalt derivative. This sample with cobalt derivativr addition, in comparison with remaining samples studied was characterized with transformation of liquid phase to solid one. This means that the derivative of cobalt caused polymerization of olive oil. Also it was observed the influence of pH value in olive oil samples particularly noted for teraphenylphorphiryns and its metal derivatives.

Further research is in progress.

POSTER SESSION III

DETERMINATION OF NAPROXEN AND IBUPROFEN BY PY-GC/MS METHOD

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Nonsteroidal antiinflammatory drugs (NSAIDs), e.g. naproxen and ibuprofen, are classified as „over the counter" (OTC) drugs, what facilitates self-treatment with the drugs by patients. Since the drugs are very easily available, there is a risk of drug abuse and for this reason control of drug concentration in the body seems to be useful.

The effects of NSAIDs is not so easy quantitatively determine like in case of e.g. antihypertensive drugs (blood pressure), or antidiabetic drugs (sugar level). Therefore, it is necessary to elaborate the method for determination of some analgesic drugs e.g. ibuprofen or naproxen, in biological samples. The elaborated method should be accurate, simple and rapid.

Using a pyrolyser as a thermochemical microreactor, the authors have developed the new technique of naproxen and ibuprofen determination by pyrolytic derivatization. The extract to be analyzed covered with a derivatizing mixture is placed directly on the surface of ferromagnetic wire. At high temperature, in the presence of a strong methylating agent - tetramethylammonium hydroxide (TMAH), the studied NSAIDs are quickly derivatized to methyl esters. Pyrolysis cell is coupled directly to GC capillary column inlet and the separated products are identified by mass spectrometry (Py-GC/MS). In the presented study all the parameters of Py-GC/MS analysis have been optimized. As compared to conventional techniques, the described method allows to reduce amount of the material, required for single analysis and to shorten the sample preparation time from hours to several minutes.

It was shown that the developed method of the pyrolytic derivatization to methyl esters can be applied for qualitative and quantitative analysis of naproxen and ibuprofen

POSTER SESSION III

THERMAL DEGRADATION PRODUCTS OF THE COMPACT PART OF THE HUMAN OSSEOUS TISSUE: A PYROLYSIS – GAS CHROMATOGRAPHY/MASS SPECTROMETRY STUDY

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The compact part of bone is a tissue of a lamellar structure, composed of collagen fibers net, which can be regarded as a natural polymer filled with hydroxyapatyte crystals.

In this study we have analyzed thermal degradation products of organic moiety of human femurs, which were collected post mortem for transplantology purposes from the healthy persons, who died at the age of 45-60 years as a result of accident or suicide. Bone transplants have been prepared (sterilization by 30 kGy gamma rays followed by freezing or lyophilization) by Blood Therapeutics and Blood Donation Centre in Katowice.

Bone compounds have been thermally degraded on the surface of pyrolytic wires (Curie temperature – 770°C) and the pyrolysis products formed were analyzed by gas chromatography – mass spectrometry (GC/MS).

Derivatives of benzene, pyridine, pyrrole, phenol, sulphur and nitryl compounds, aliphatic hydrocarbons (saturated and unsaturated) and fatty acid amides have been detected among the thermal degradation products of the analyzed material. The analyzed pyrolysates were dominated by pyrrole derivatives, which made up 30 – 50 % of the total pyrolysis products. High amounts of nitrogen-containing compounds confirm that proteins are significant components of bone. According to the literature data [1], type I collagen makes up to 85 – 95 % of the bone organic mass. Other proteins, such as: type V collagen, osteocalcin, osteonectin, osteopontin, bone syaloprotein and proteoglycans, are present in much less quantities.

The obtained profile of the thermal degradation products is consistent with amino acid composition of collagen proteins, which are predominant organic components of bones.

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3-HYDROXYTETRADECANOIC ACID IS AMIDE-BOUND COMPONENT OF LIPID A FROM *Desulfovibrio desulfuricans* Bacteria

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Lipid A is a biologically active component of endotoxins, which are the major pathogenic factor of Gram-negative bacteria. Endotoxins, lipid-polysaccharide heteropolimers, liberated from the outer membrane of microorganism may cause rapid decrease of blood pressure, tachycardia, inflammation and even death of infected macroorganism. Lipopolysaccharide (LPS) is a crucial factor contributing to the occurrence of sepsis and septic shock symptoms. However, LPS, administered in small doses, reveals antitumour and adjuvant activity and induces host immunity.

Concern in *Desulfovibrio desulfuricans* bacteria is related to their occurrence in a human alimentary tract and to suggestions that they may participate in the etiopathogenesis of gastrointestinal disorders associated with the inflammation of intestinal mucous, such as ulcerative colitis or Crohn's disease.

So far, little is known about the chemical structure of endotoxins derived from mentioned microorganisms. Nevertheless, it appears that lipid A from *D. desulfuricans* bacteria, as structurally conservative component of lipopolysaccharides, consists of a 1,6-linked glucosemine disaccharide esterified in positions 3,3' and substituted at positions 2,2' by amide-bound fatty acids (usually hydroxylated at 2 or 3 carbon atom).

Determination of amide-bound fatty acid from *D. desulfuricans* lipid A has been carried out on a standard soil strain La2226 and wild strain (DV/A), isolated from the faeces of patient with asiderotic anaemia and cholestasis. After bacteria had been cultured, LPS was isolated from the biomass, using the procedure described by Shnyra et al [1]. For selective separation of amide-linked fatty acids from a lipid A disaccharide core, Wollenweber and Rietschel's method has been used [2]. This method consists of the following steps:

- LPS de-O-acylation by methanolic solution of sodium methoxide;
- purification of the obtained structure by acidification (pH 2-4) with 2M acetic acid and precipitation of de-O-acylated LPS by cold ethanol, followed by centrifugation and precipitate rinsing by acetone;
- acid-catalyzed hydrolysis (4M HCl, 4h, 100°C) and esterification (2M HCl solution in metha-nol, 6h, 85°C) of fatty acids liberated from amide linkages followed by extraction of formed fatty acid methyl esters by hexane-diethyl ether mixture (1:1, v:v);
- GC/MS analysis.

Interpretation of obtained results shows that 3-hydroxytetradecanoic acid is amide linked with the saccharide core of *D. desulfuricans* lipid A.

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THERMOCHEMICAL METHYLATION AND PYROLYSIS-GAS CHROMATOGRAPHY/MASS SPECTROMETRY IN THE EVALUATION OF LIPID PROFILES OF MELANOSOMES FROM IRIS AND RETINAL PIGMENTED EPITHELIUM OF HUMAN EYES

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Melanosomes are subcellular structures responsible for *in vivo* synthesis of melanin biopolymers. Lipid membranes surround these specialized organelles of melanocytes and other lipid compounds, estrogens, are one of the melanogenesis stimulation factors. The presented study was performed is to verify pyrolytic profile of lipids in melanosomes isolated from iris and retinal pigmented epithelium (RPE) of human eyes, using thermochemical methylation in the presence of 10% methanolic solution of tetramethylammonium hydroxide (TMAH). TMAH-dependent transesterification or alkaline hydrolysis followed by esterification of the fatty acids released from compound lipids allows more selective analysis of the lipid components of melanosomes.

Melanosomes isolated from human iris or RPE were placed on the tips of ferromagnetic wires with Curie point of 770^{o}C and pyrolytic "on-line" derivatization to methyl esters was carried out in the presence of TMAH. The obtained thermal degradation products and their derivatives were analyzed by GC/MS.

It was found that the lipid profile resulting from thermochemical methylation differs significantly from this obtained by pyrolytic degradation of the analyzed melanosomes. The presence of TMAH resulted in considerably enhanced percentage of the products of lipid origin, in particular fatty acid methyl esters, as compared to classical pyrolysis without a methylating agent.

Comparative analysis of lipid constituents of melanosomes isolated from the studied biological materials have revealed that the percentage of such derivatives in the organelles from RPE outstrips that evaluated for iris melanosomes, which are predominated by the products of pigment and protein origin.

PYROLYSIS-GAS CHROMATOGRAPHY/MASS SPECTROMETRY IN THE ANALYSIS OF THERMALLY DEGRADED MELANOSOMES FROM HUMAN IRIS AND RETINAL PIGMENTED EPITHELIUM

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An iris and retinal pigmented epithelium (RPE) are especially pigmented parts of the human eyeball, what is crucial not only for esthetical and cosmetic reasons, but to preserve proper functions of a sight organ. Macromolecular pigments, melanins, are synthesized inside the specialized organelles of melanocytes, called melanosomes. These subcellular structures contain not only melanin biopolymers, but also some protein macromolecules, which, among other things, function as melanogenesis catalysts. Because of insolubility and insufficient volatility of discussed structures, their GC analysis should be preceded by thermal degradation of macromolecular compounds, which allows determination of pyrolytic profiles of the studied organelles.

To achieve the above-mentioned aim, melanosomes isolated from the human iris or RPE were placed on the tips of pyrolytic wires with Curie point of 770^{o}C and the thermal degradation products formed during pyrolysis were analyzed by GC/MS.

The derivatives of benzene, phenol, pyridine, pyrrole, indole, and nitriles were identified in the pyrolytic profiles of the analyzed structures of human eyeball. These compounds may be regarded as the thermal degradation products of both melanin and protein moiety. The main pyrolysis products of the melanosomes from iris are pyrrole- and phenol-derivatives, while phenol- and indole-derivatives dominate pyrolytic profile of RPE melanosomes. Thereby we have concluded that the analyzed organelles isolated from iris are substantially different than those from RPE, as regards the percentage of pyrrole-derivatives.

In the pyrolysates of the studied melanosomes, we identified also aliphatic hydrocarbons and some derivatives of sterols and fatty acids. The presence of alkanes and alkenes may be a consequence of both fatty acid decarboxylations and some chemical processes occurring in melanin structures under the influence of high temperature at the neutral gas atmosphere, such as generation of alkyl radicals followed by their recombination or disproportionation.

PYROLYSIS-GAS CHROMATOGRAPHY/MASS SPECTROMETRY AS THE ESTIMATION METHOD OF FATTY ACID PROFILE OF THE COMPACT PART OF HUMAN FEMORAL BONE

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Proteins, mainly these of collagen structure, are the major organic component of bones. However, fatty acids-containing lipid compounds also occur in a bone tissue, being for example important constituents of osteocyte membranes. The aim of this study was to determine the fatty acid profiles of human osseous tissue compact part and to estimate similarity between these profiles in the material prepared for transplantation.

Profile of fatty acids, components of human femur compact part, has been analyzed. Bones have been collected post mortem for transplantology purposes from the healthy persons, who died at the age of 45-60 years as a result of accident or suicide. Frozen or lyophilized bones, sterilized by 30 kGy gamma rays, have been delivered by Blood Therapeutics and Blood Donation Centre in Katowice. Powdered bone tissue has been thermally degraded in the presence of tetramethylammonium hydroxide (TMAH), and obtained products and their derivatives have been analyzed by GC/MS.

There are compounds with 14 to 20 carbon atoms among fatty acids building human bone. However, C16 and C18 fatty acids are predominant. Especially high percentage in analyzed compounds has octadecanoic acid (39% - 49%) and hexadecanoic acid (22% - 33%).

Storage manner of the investigated biological material (lyophilization or freezing) has no significant influence on the fatty acid profile of human bones.

POSTER SESSION III

FAST DENSITOMETRIC QUANTITATIVE SCREENING OF MYRICITRIN CONTENT IN CRUDE METHANOLIC EXTRACTS OF LEAVES FROM VARIOUS *Acer* SPECIES

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TLC is a simple but powerful and universal chromatographic method. It is widely used for the rapid phytochemical analysis, especially for qualitative parallel screening analysis with a large amount of samples and standards. No need to previous clean-up of sample, short time of analysis, easy way to controll major constituents separation and low solvent consumption are the main advantages.

Aceraceae family consists of about 150 species distributed mainly on North Hemisphere. So far those trees do not find special application in medicine, except of ethnological and clinical uses in Japan (particulary *Acer nikeonse*). It is interesting, that some *Acer* extracts posses activities which was also defined for myricetin and myricitrin.

TLC and HPTLC densitometric determination have been applied to the qualitative and quantitative analysis of myricitrin contentration in leaves extracts of 20 species from the *Aceraceae* family. The presence of myricitrin was affirmed by TLC and 2D TLC in five of them. The optimal mobile phase for myricitrin quantitative analysis without neccesity of extracts purification was selected. Spray or dipping reagents were not used during the densitometry to provide more rapid and simple analytical procedure. Myricitrin was for the first time identified in *A. velutinum*.

POSTER SESSION III

MACROCYCLIC AGENTS AND THEIR APPLICATION FOR CHROMATOGRAPHIC AND ELECTROPHORETIC SEPARATION OF COMPOUNDS FROM BIOLOGICAL LIQUIDS

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The complexation processes can be used for increasing of selectivity and efficiency of chromatographic separation. The application of chromatographic and electrophoretical methods employing macrocyclic agents (MA) allows not only the separation of drugs (MA influences on retention parameters) but the evaluation of enantiomeric purity of the sample. Analytical methods used for analysis of chiral compounds are high performance liquid chromatography (HPLC), capillary electrophoresis (CE), high performance thin layer chromatography (HPTLC) and gas chromatography (GC).

Lately, one of the most widely developing directions is the application of cyclodextrins (CD) (modified and native) as the compounds of mobile and stationary phases in HPLC and CE. Besides glycopeptide antibiotics, chiral crown ethers, chiral micelles, proteins are used as chiral selectors. Derivatives of polysaccharides (cellulose carbamates and ethers, amylose carbamates) are widely and successfully employed as chiral stationary phase modifier in HPLC.

Standard solutions (racematic in the case of enantiomeric separation) and biological liquids containing different drugs such as anesthetics, steroidal and non-steroidal anti-inflammatory drugs, doping, antihypertensive drugs and other are being investigated.

We perfomed the separation of exo- (drugs: prednisolone, cortisone acetate, dexamethasone) and endogenic corticosteroids (cortisol, cortisone, corticosterone, 11-dehydrocorticosterone) using reversed-phase HPLC with UV-detection. The results were obtained with application of β-cyclodextrin (concentration was varied from 0,3 to 10 mM), which was added to the mobile phase CH_3CN:H_2O (28:72, v/v). On the base of this experiment we can determine the remaind maintenance of drugs in serum. The constants of inclusion complexes were measured in the “cyclodextrin-steroid” system.

POSTER SESSION III

2D HPLC OF POLYPHENOLS PRESENT IN THE BARK OF *Salix purpurea* AND *S. Daphnoides*

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The bark of willow is a herbal remedy frequently used as antiphlogistic, antipyretic and antirheumatic [1]. The pharmacological activity is due to a presence of salicin and its derivatives [3]. According to the monograph of willow bark published in the European Pharmacopeia different species of *Salix* are a source of medicinal plant material contained not less than 1 % of salicin. However, some reports suggests that in addition to salicylic compounds also other constituents of bark, including polyphenols may be responsible for pharmacological effects [2, 4]. 2D HPLC system was developed to separate of the mixture of 54 standard compounds belonging to a group of flavonoids (22 compounds derivatives of flavanones, chalcones, flavones and biflavones), phenolic acids (30 compounds derivatives of cinnamic and benzoic acids), catechin and salicin. The above compounds were earlier isolated or chromatographically identified in different species of the genus *Salix* [3]. The HPLC separation in the first dimension was carried out on a Supelcosil LC-18 (150x3mm, I.D. 3µm) column under gradient elution in a mixture of methanol/0.1 % H_3PO_4 with increasing concentration of methanol from 3% to 70 % in 0.1 % H_3PO_4 (t_G 120 min.). The separation in the second dimension was performed on a Chromolith Performance RP18e (100x4.6mm) column with isocratic elution in a mixture of methanol/0.1 % H_3PO_4. In the HPLC optimized conditions the chemical composition of the bark of a number species and clones of *S. daphnoides* and *S. purpurea* originating from natural habitant and also cultivating for pharmaceutical purposes was compared.

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POSTER SESSION III

HPLC STUDY OF GUANINE QUADRUPLEX FORMATION IN THE PRESENCE OF METAL IONS

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Recently, an anticancer strategy was proposed that targeted a DNA enzyme, telomerase. Inhibition of telomerase activity in cancer cells can be achieved through formation of guanine quadruplex (G-4) structures on the telomeric DNA strand. The quadruplex is composed of guanine tetrads, which are square co-planar arrays of four guanine bases. Each base is both the donor and acceptor of two hydrogen bonds with its neighbors. Therefore it is important to study quadruplex properties and conditions of G-4 formation. Commonly used techniques for this purpose includes: NMR, X-ray structural analysis and circular dichroism spectroscopy. Here we try to evaluate HPLC method, which should enable the separation of different structures of G-4 present in solution. Our attention was initially focused on two chromatographic techniques: HPIEC (high performance ion-exchange chromatography) and IP RPHPLC (ion-pair reversed phase HPLC).

The purpose of this work is developing the separation conditions of guanine quadruplexes in the presence of metal ions using ion-exchange chromatography (IEC). The method should fulfil initial conditions: (i) separation should run in non-denaturating conditions, (ii) complexes G-4 with cations should be sufficiently stable in separation conditions, (iii) the mechanism of retention is expected to be sensitive to the complex formation. Taking these conditions into account, we designed analytical system. As a G-4 structure forming DNA, an oligonucleotide with human telomeric sequence d(GGGTTAGGGTTAGGGTTAGGG) was used. We studied eluting process of metal ion-G4 complex applying diffrent salts in mobile phase (NaCl, KCl, LiCl). LaChrom Merck/Hitachi HPLC system with DAD detector, was equipped with a TSKgel DEAE-NPR column (Japan). Initial investigations involved the influence of temperature and metal salts with isocratic and gradient eluting. Narrow peaks were obtained in isocratic conditions, in 0,4-0,8M concentration range of salt. However peaks were eluted in dead time. At salt concentrion less then 0,4M we received broader peak and results were unsatisfactory. The best retention (the peak shape and retention time) were observed in shallow gradient. The Li^+ and Na^+ ions gave one peak, which indicated probably quadruplex with antiparallel structure. In the chromatogram with K^+ 2 peaks appeared. The first, with short retention time we ascribed to the antiparallel tetraplex and the second, smaller one, to the quadruplex parallel structure. Concluding, we confirmed usefulness of IEC to separate and study the formation of four-stranded DNA structures and its complexes with metal ions. The further investigations will include application of the IP RPHPLC to separate quadruplexes.

POSTER SESSION III

ANALYSIS OF POLYPHENOLIC FRACTION OF *Crithmum maritimum* L. (*Apiaceae*)

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In this work the aerial parts of *Crithmum maritimum* L. (*Apiaceae*) were investigated on the presence of polyphenols. The material was collected in year 2002 from cultivation in botanical garden in Gdańsk from the plants control and fertilized with Tytanit formula. The methanolic extracts (exhaustive extraction in Soxhlet apparatus) of the plants were analyzed. Free phenolic acids and fractions obtained from the acid and alkaline hydrolyses were analyzed by 2D TLC on cellulose layer [1,2]. Next RP-HPLC analysis was performed using mobile phase methanol : water (20:80) with 1% acetic acid (v/v) [2]. Before HPLC samples were purified by SPE. In investigated extracts estimation of content of phenolic acids and flavonoids was done using for phenolic acids the Arnova method and for flavonoids Christ-Müller method [3]. The amount of phenolic acids in control plant extract was lower then in extract from fertilized plants, and were about 13 mg/g of dry mass and 21 mg/g dry mass respectively. Differences of flavonoid content in investigated extracts were not significant (about 0,76 - 0,68 mg/g of dry mass respectively).

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DETERMINATION OF METHIONINE AFTER CHROMATOGRAPHIC SEPARATION

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Methionine is an essential amino acid that is not synthesized in the body. It must be obtained through the diet or supplementation in adequate amounts in order to meet the body's needs. It plays an important role in biological methylations. It also enhances the synthesis of glutathione.

The aim of this work was the spectrophotometric determination of methionine after TLC separation. The optimization of the separation of methionine from other amino acids and pharmacologically active compounds was performed. The chromatographic process was carried out on TLC aluminium plates (silica gel 60 F_{254}) with n-propanol/water/chloroform (5:2:1) as the mobile phase. The spots were visualized under UV light and by use of ninhydrin reagent. The quantification of methionine was performed by the spectrophotometric method based on the redox reactions occurring in the system iodate ions, methionine and leuco xylene cyanol FF. The absorbance of xylene cyanol FF dye formed was measured at 613 nm.

The method was applied to the determination of methionine in complex pharmaceutical preparations and dietary supplement.

POSTER SESSION III

DETERMINATION OF DIAZEPAM AND ITS ACTIVE METABOLITE N-DESMETHYLDIAZEPAM IN HUMAN PLASMA BY GAS CHROMATOGRAPHY – NEGATIVE ION CHEMICAL IONIZATION MASS SPECTROMETRY

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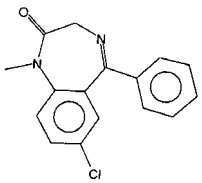
Diazepam is an anxiety agent. Used primarily for short-term relief of mild to moderate anxiety. It may also be used to treat symptoms of acute alcohol withdrawals, to help control epilepsy, or to relieve muscle spasms. It is metabolised by the liver cytochromes to N- desmethyldiazepam.



Diazepam

We have developed sensitive and selective method to determine the concentration of diazepam and its active metabolite – N-desmethyldiazepam. Samples were prepared using one step liquid-liquid extraction with tert-butyl methyl ether - dichloromethane (3:1) in presence of diazepam-d_5 as the internal standard. After derivatization with BSTFA + TMCS (99:1) samples were analyzed by gas chromatography – negative ion chemical ionization mass spectrometry with methane as reagent gas. The mass spectrometer was set in selected ion monitoring acquisition mode: monitoring ions at *m/z* 284 and 286 (diazepam), 234 and 324 (N-desmethyldiazepam-TMS), 289 and 291 (diazepam-d_5).

The recovery of diazepam and N-desmethyldiazepam were 98,3% and 96,3%, respectively. LOQ values for diazepam and N-desmethyldiazepam were estimated as 2 ng/ml and 1 ng/ml, respectively. The within-day and between-day coefficients of variation were less then 10% and the accuracy of the assay was in the range 97 – 107% for diazepam and 92 – 104% for N-desmethyldiazepam.

The method for the assay of diazepam and N-desmethyldiazepam in human plasma is sufficiently sensitive and selective to follow the pharmacokinetic studies as well as therapeutic drug monitoring.

POSTER SESSION III

DEVELOPMENT AND EVALUATION OF CAPILLARY ELECTROPHORESIS METHOD FOR CETIRIZINE IN PLASMA

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Capillary electrophoresis (CE) is a modern separation technique that has some distinct advantages for pharmaceutical analysis, such a high flexibility, efficiency, short time of analysis, and complementary separation mechanisms to chromatographic methods.

Cetirizine dihydrochloride is one of the second-generation, non-sedating H_1 receptor antagonist used for treatment of seasonal allergic rhinitis and chronic urticaria. Despite its widely use, does not offer any information on determination of cetirizine in biological fluids by CE and only a few chromatographic methods can be found in the literature.

A simple, sensitive and rapid capillary electrophoresis (CE) method for the measurement of cetirizine in human plasma has been successfully developed and validated. All CE experiments were performed on a P/ACE 2100 system (Beckman Instruments) equipment with automatic injector, UV detector (200 nm), and fluid cooled cartridge. Analyses were performed with an uncoated silica capillary with 75 μm I.D. and 57 cm length. The best results were obtained with 7s pneumatic injection using buffer solution composed of 20 mM sodium tetraborate decahydrate and 10 mM sodium dihydrophosphate. The sample preparation in this method is simple and rapid because of elimination of extraction and derivatization steps and requires only 0.5 mL of plasma. The plasma samples, prior to analysis, were deproteinized by acetonitrile, centrifuged, evaporated to dryness and redissolved in the running buffer.

The method was statistically validated for its linearity, accuracy, precision and selectivity. Linearity of the assay was demonstrated by running plasma standards in triplicate for five consecutive days over the range 20 - 2000 ng mL^{-1}, which spans the accepted therapeutic range. The mean equation (curve coefficients ± standard deviation) of the calibration curve obtained from seven points was $y = 1.37 (\pm 0.036) x + 0.033 (\pm 0.029)$; where correlation coefficient $r^2 = 0.998$. The described method is useful for the quantifying of cetirizine in real human plasma. Because of its high sensitivity and reproducibility, this electrophoretic method will be suitable for routine analysis of cetirizine in plasma samples and for research studies involving pharmacokinetics and bioavailability.

ON-LINE CONCENTRATION TECHNIQUES IN MICELLE ELECTROKINETIC CHROMATOGRAPHY (MEKC) AND CAPILLARY ZONE ELECTROPHORESIS (CZE) IN DETERMINATION OF THE TRACE QUANTITY OF BIO-ACTIVE SUBSTANCES

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One of the important problem in the diagnosis of different disease in their early stages is the determination of bio-active substances, like steroids and biogenic amines (adrenaline, noradrenaline, dopamine), in biological fluids (plasma, serum, urine). Low concentrations of steroids and catecholamines in these objects (at the level ng/ml) require a highly sensitive and selective method for their determination. Using capillary electrophoresis to accomplish this task in the biochemistry is limited by low sensitivity of UV-detection in CE that requires a special procedures of sample preparation including hydrolysis, separation from conjugates, extraction, concentration and derivatization.

The way to solve this problem is the use of different techniques of on-line concentration of probe in CE (stacking, sweeping, etc.). Stacking takes place when the ions of sample have reached the boundary of the zones of buffer electrolyte (with high conductivity) and sample matrix (with low conductivity). The sample is diluted by the buffer or water and within this zone analytes possess high local velocities that leads to their concentration of the boundary. At sweeping analytes are concentrated by pseudostationary phase (micelles). Conductivity the sample matrix is close to that of the electrolyte and in sample micelle concentration is zero.

The different methods of concentration: stacking with reversed migration micelles, stacking with high conductivity matrix (with use of additive NaCI), enhanced sample injection - reversed migration micelles and sweeping have been compared using model mixture of steroids.

By optimizing the stacking and sweeping conditions, about 15-30-fold and 100-fold (50 ng/ml) improvements, correspondently, in detection sensitivity were obtain for the steroids. That approach the detection limit in CE to that in HPLC.

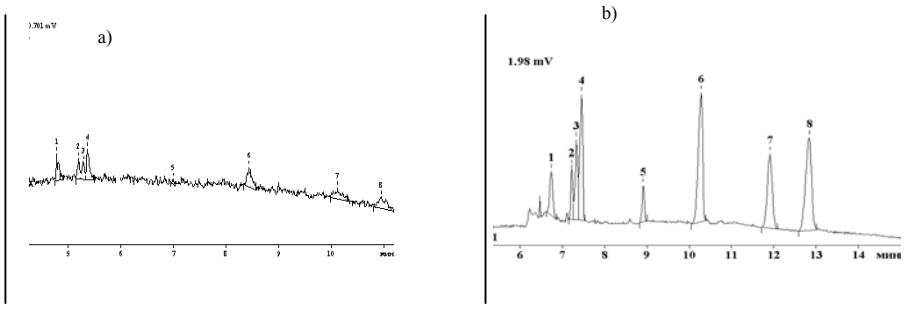

Figure 1**.** Optimisation of sweeping on the mixture of standarts of steroids. Electrophoregram for a mixture containing 2,5 μg/ml (1) progesterone, (2) 11-dexoxyprogesterone, (3) 17-hydroxyprogesterone, (4) 11-dexoxycorticosterone, (5) corticosterone, (6) 11-dehydrocorticosterone, (7) cortisone, (8) cortisole. Sample matrix, electrophoresis buffer (without SDS) with the same conductivity and with addition 5mM β-CD. Separation voltage: -25kV. Electrophoresis buffer, 25 mM H_3PO_4 (pH = 2,5), 10 mM SDS, 4,5M uria. Injection: (a) 60 mbar·s, (b) 3000 mbar·s.

We have developed the method of the determination of steroids in biological fluids (serum and urine) by MEKC with on-line concentration (sweeping) with detection limit for about 3 ng/ml (S/N=3). The results of quantitative analysis of steroids in these objects of patients with endocrine disorders indicate good correlation with those obtained by RP-HPLC.

POSTER SESSION IV

FORMATION AND OCCURRENCE OF N-NITROSAMINES IN MEAT PRODUCTS

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N-nitrosamines are potent carcinogenic compounds that occur in numerous foodstuffs, also in meat products. They are formed out of decomposition products of proteins, especially biogenic amines and a nitrosylating agent (NO^+) derived from nitrite or nitrate ions. This latter compound can be present in smoke also.

The N-nitrosamines detected in meat products are nitrosodimethylamine (NDMA), nitrosodiethylamine (NDEA), nitrosodibutylamine (NDBA), nitrosopiperidine (NPIP) en nitrosopyrrolidine (NPYR). These volatile nitrosamines are detected by gas chromatography coupled with a thermal energy analyzer (GC-TEA). The components are vacuum distillated in alkaline environment. The distillate is extracted with dichloromethane. After an acid and alkaline washing step, the extract is evaporated and injected in the GC-TEA. The different components are quantified in the order of ppb values.

Besides the screening of the meat products available on the Belgian market, research is done on the possible pathways of the formation of N-nitrosamines in the different groups of meat products. The relation between the status of the raw materials, the used ingredients additives, the process parameters and the occurrence of the N-nitrosamines mentioned above are investigated in model preparations. So the influence of the following parameters is analysed : doses of nitrite and nitrate, pH of the environment, the bacteriological status of the raw materials, kind of spices, presence of oxygen, kind and doses of anti-oxidants, intensity of the heating process, smoking, circumstances of fermentation.

From these findings recommendation will be formulated to reduce the amounts of nitrite, nitrate, biogenic amines and N-nitrosamines in meat products.

POSTER SESSION IV

THE BEHAVIOUR OF THE PIGMENT IN THE FERMENTED RED BEET JUICE

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Epidemiological studies demonstrated that food can have beneficial effects on human health in addition to its nutritional value. The reported exceptional antioxidant activities of beet extracts has increased the interest in red beetroot compounds. Vinson et al. [1] ranked beets among the ten most potent vegetables with respect to antioxidant activity.

Red garden beet is one of the four most important vegetables in Poland together with white cabbage, carrot and onion. Among 16 cultivars of red garden beet "Chrobry" was the one with the highest betanin content (about 0,3% of juice).

Lactic acid fermentation can have preservative effect on beet juice. It is a result of a combination of several factors and the production of organic acids (lactic, acetic) is the most important [2].

The behaviour of the pigment in the fermented juice was investigated and the results were compared with those of non-fermented pigment extracts from "Chrobry" variety conducted in our laboratory.

A liquid chromatograph ThermoSeparation Product consisting of a Spectra System P2000 pump, photodiode detector UV6000LP and SN 4000 integrator was used. The separation of pigments was performed on Ace 5 C18 column (250x4,6 mm id),fitted with a C18 ODS guard column. All samples were filtered thorough a 0,45 μm Millipore filter prior to chromatography. HPLC method described by Stintzing at all, [3] with a little changes was used. Betalains were monitored at 470 and 538 for betaxantins and betacyanins, respectively.

The dominant red violet pigment components were betanin, isobetanin, while vulgaxantin was the major yellow pigment. Betanin was the main red violet component in juices before fermentation. Lactic acid fermentation influenced both red and yellow pigments.

After fermentation huge amounts of betanidin were found. Also isobetanidin was observed after this process.

It is observed in this study that fermented juices from Chrobry cultivar contained higher betanidin than betanin levels.

The research is financially supported by the PBZ-KBN-094/P06/2003 grant.

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POSTER SESSION IV

DETERMINATION OF POLIHYDROXY ALCOHOLS ACCUMULATED IN YEAST CELLS

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Technical University of Łódź, Łódź; Poland

Water activity is one of the important environmental factors affecting microbial growth. Hypertonic stress results in outflow of water from the cell resulting in desiccation. After a transfer to a hiperosmotic medium, adaptation is required. The adaptive response to this change is intracellular accumulation of organic compounds which act as compatible solutes which are not toxic to the cells, even at the high concentrations required for stabilization of the osmotic equilibrium. In yeasts, compatible solutes are polihydroxy alcohols, such as glycerol, arabitol, mannitol, erythritol and xylitol.

A liquid chromatograph ThermoSeparation Product consisting of a Spectra System P2000 pump, refractive index detector RI-150 and photodiode detector UV6000LP and SN 4000 integrator was used. Analyses were performed using Aminex HPX $87H^+$ column, fitted with guard column. Eluent consisted of 0,005 M H_2SO_4 in water.

Complete analysis was achieved within 30 min at 60° C and at a flow rate of 0,6 ml/min.

In all our tested yeast strains we observed glycerol. Presence of arabitol or erythritol depended on media used.

The research was financially supported by KBN-3P06T6122 grant.

COMPARISON OF PBDe DETECTION LIMITS AND LINEARITY MEASURED BY GC/MS/MS AND GC/ECD

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The most widely used detectors for polybrominated diphenyl ethers (PBDE) determinations are mass spectrometers (MS) and electron capture detectors (ECD).

The determination of PBDE was carried out by GC coupled with tandem mass spectrometric detection, using electrone ionisation (EI) and by GC coupled with ECD. The aim of these studies was a comparison of two methods of PBDE determination for detection limits and linearity.

The standard solution of PBDE contained 6 natural congeners as follows: BDE-28, BDE-47, BDE-99, BDE-153, BDE-154 and BDE-183. The limits of detection (LD) were estimated on the ground of standard deviation values. The standard solution in the concentration of 0.5 ng/ml of each natural congener was ten times analyzed. Afterwards, the average area and standard deviation (σ) values were estimated. The limits of detection were defined as 3σ and limits of quantification (LQ) as 3LD. The ranges of LD for all 6 congeners of PBDE in GC/MS/MS and in GC/ECD analysis were respectively 0.27 – 0.71 [pg/g] and 0.11 – 0.19 [pg/g]. The estimated range of LQ for GC/MS/MS were 0.83 -2.16 [pg/g] and for GC/ECD were 0.32 – 0.58 [pg/g]. In both developed methods the highest LD and LQ values were for the higher brominated congener BDE-183.

Next examined parameter of both methods was linear range. For GC/MS/MS and GC/ECD methods the linear range were estimated for 6 PBDE congeners standard solutions. The GC/MS/MS method showed to be linear in all examined concentration range from 1 to 2000 [pg/µl]. In the case of GC/ECD the linear range was examined in the range from 0.5 to 100 [pg/µl], but the method showed to be linear only up to 80 [pg/µl]. In fact, ECD is known for being linear only over a limited concentration range.

POSTER SESSION IV

CONCENTRATION MEASUREMENTS OF HYDROGEN IN THE URBAN AREA OF KRAKÓW

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Significant increases of the hydrogen concentration in the air can be expected with a broad introduction of new hydrogen based energy sources into the global economy. Consequently, increased hydrogen emissions may have profound atmospheric impact. Elevated hydrogen concentration in the troposphere may result in major reduction of hydroxyl radicals, leading to higher concentration of methane, one of the major greenhouse effect gasses [1]. Since photochemical reactions with hydrogen in troposphere are not well known, it's impossibly at this time to establish a solid, scientific connection between a green house effect and hydrogen concentration increase in the atmosphere.

In the poster a developed continuous method for hydrogen measurements in air is presented. The air sample is analyzed on the two chromatographic columns filed with molecular sieve type 5A working in the back flush mode. A Pulse Discharge Helium Ionisation Detector is used for hydrogen detection (PDPID) [2], [3]. For ambient air sample volume 5 cm^3 the hydrogen delectability level is equal of 0,05 ppm (detection limit in air is about $6{,}24\times10^{-12}$g H_2/cm^3)

The presented method will be used for hydrogen monitoring in air.

This work is partly supported by grand no. 3 P04D 060 24 and no. 4 T12B 004 28 from the Polish State Committee for Scientific Research (KBN)

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CHROMATOGRAPHIC SYSTEM OF THE MEASUREMENT OF HELIUM CONCENTRATION IN GROUNDWATER USING HEAD-SPACE METHOD

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Helium concentration in groundwater can be used as an indicator of groundwater age, usually in the range of $10^2 - 10^7$ years [1, 2, 3]. Surface water in equilibrium with atmosphere and at temperature of 10°C contains $4{,}8\times10^{-8}$ cm^3STP He/g water. Total helium in water consist of ^{4}He and ^{3}He. The rate of ^{3}He/^{4}He in atmosphere is about 2×10^{-8}. Concentration of ^{4}He is usually measured using mass spectrometric method. Gas chromatographic method allows to measure concentration of total helium.

Within this work chromatographic system of the measurement of helium concentration in groundwater using head-space method is presented. The analyses are performed with the aid of the Shimadzu gas chromatograph equipped with mikro TCD detector with detection limit in air of about 2×10^{-10}g He/cm^3. Helium extracted from 1-litre water sample is enriched in a trap immersed in liquid nitrogen. The trap is made of stainless steel and is filled with active charcoal. Volume of 5 cm^3 of helium accumulated at the top of the trap is injected to two chromatographic columns working in the "back flush" mode. Obtained signal for helium is up to about 2 mV which corresponds to the concentration of 4×10^{-11}g He/cm^3 in water. Detection limit of method is at the level of 2×10^{-12} gHe/cm^3 water.

This work was partly supported by grant no. 3 P04D 060 24 and no. 4 T12B 004 28 from the Polish State Committee for Scientific Research (KBN).

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POSTER SESSION IV

SEASONAL VARIATION OF PAHs AND *n*-ALKANES DISTRIBUTION IN ATMOSPHERIC AEROSOL IN KRAKÓW

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The results of monitoring for 16 polycyclic aromatic hydrocarbons (PAHs) and 28 *n*-alkanes associated with atmospheric aerosols in Kraków, Poland during the period from January 2004 to August 2004 are presented. The seasonal variations of the characteristic, abundance and dominant sources of pollutants are reported. Twenty-four hour aerosol samples were collected in three different urban places. After a high-volume air sampling, ultrasonication extraction with dichloromethane, preconcentration in an evaporator and further concentration under a gently stream of high purity nitrogen, the extracts were separated by column chromatography into aliphatic, PAHs and polar fractions. Finally, *n*-alkanes and PAHs fractions were analyzed by a capillary gas chromatograph coupled with a mass spectrometry detector (GC-MS) operated in selected-ion monitoring (SIM) mode.

Moreover, the dominant sources of investigated air pollutants were estimated using indices and molecular diagnostic ratios. The obtained data showed the importance of anthropogenic emission from the traffic-source.

CHROMATOGRAPHIC ANALYSIS OF SOME 1,4-DITHIIN DERIVATIVES

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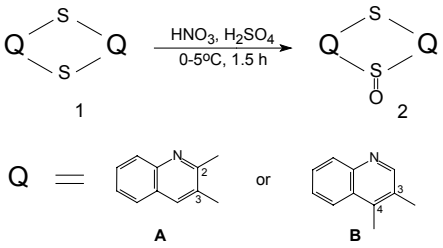
Sulfurization of quinoline with elemental sulfur may serve as a source of 1,4-dithiins of type 1 [1]. The main products: thioquinanthrene 1a and isothioquinanthrene 1b were composed from two 3,4-quinolinediyl units. They were isolated from quinoline sulfurization products by several recrystallizations. Mother liquors were evaporated to dryness and a solid residue, representing a complicated mixture of compounds, was obtained.

Application of column chromatography technique to this mixture allowed to separate further isomeric dithiins 1c-f. For spectroscopic analysis small amounts of each compound were purified by thin-layer chromatography and recrystallized from methanol. All investigated dithiins 1 underwent oxidation to monosulfoxides 2 when treated with nitrating mixture.



Compound	Q units	S bridges
1a	B + B	C_3-S-C_4 C_4-S-C_3,
1b	B + B	C_3-S-C_3 C_4-S-C_4,
1c	A + B	C_2-S-C_3 C_3-S-C_4,
1d	A + B	C_3-S-C_3 C_2-S-C_4,
1e	A + A	C_2-S-C_2 C_3-S-C_3,
1d	A + A	C_2-S-C_3 C_3-S-C_2,

Thin-layer chromatographic behavior of the compounds 1 and 2 has been studied, detection was performed with UV light.

Reference

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POSTER SESSION IV

APPLICATION OF THIN-LAYER CHROMATOGRAPHY FOR INVESTIGATION OF REACTION OF SOME β,γ-QUINOLINYL SULFIDES WITH A NITRATING MIXTURE

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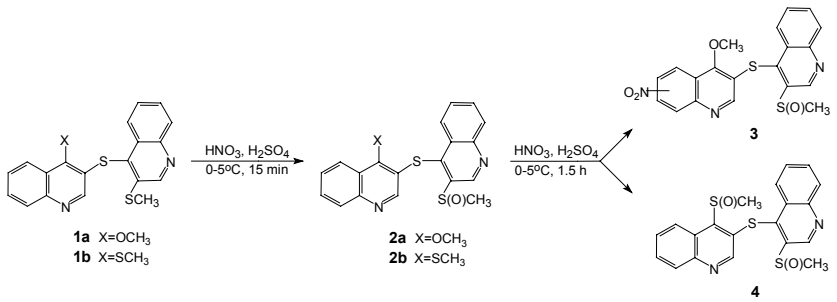
The mixture of nitric and acetic acids, depending on its composition and type of reagent can operate as oxidation or nitration agent.

As it was described before [1,2], the reaction of 4-substituted diquinolinyl sulfides type 1 with mixtures of fuming nitric acid and concentrated sulfuric acid started with 3'-methylthio group monooxidation and yielded the 3'-alkylsulfinyl diquinolinyl sulfides 2 as the primary product. Further treatment of compound 2a with nitrating mixture results in nitration at benzene ring of 4-metoxy quinoline unit [1], but in case of compound 2b proceeds as 4-methylthio group oxidation, giving the mixture of isomeric disulfoxides 4 [2]. Small amount of nitroquinolones were detected among products of both reactions

Use of thin-layer technique proved very successful in studing the progress of the reactions and in determination of path leading to quinolone derivatives. Two chromatographic systems were applied, detection was performed with UV light.



1a X=OCH_3
1b X=SCH_3
2a X=OCH_3
2b X=SCH_3

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APPLICATION OF ASE TO THE ISOLATION, AND GC/MS TO THE DETERMINATION OF ACRYLAMIDE IN FOOD SAMPLES

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In recent years, acrylamide has been the subject of intensive medical and analytical research due to its highly carcenogenic properties. Acrylamide can arise in high-temperature food processing and its content levels exceed those specified in WHO norms.

Polyacrylamide is also often utilized as a polyelectrolite in water purification processes. The concentration of its monomere acrylamide in drinking water should not exceed 0.05 ug/l. The aim of the present study has been to determine the conditions of acrylamide extraction from foodstuffs and to assess the effectiveness of the whole process of sample purification.

Crushed samples (chips, fries, peanuts, etc.) were extracted with water on ASE 200 at 70 degrees Celsius at 30 atmospheres. Extracts were drained, centrifuged and extracted with SPE. Best results were obtained using Discovery DSC-MCAX and Bakerbond spe Octadecyl colums. Chromatographic GC/MS analysis was used.

Acrylamide was also determined from water samples collected from a newly opened installation.

Acrylamide contained in water was brominated, extracted with ethyl acetate and determined by chromatography using the DB-WAX capillary and an ECD detector.

POSTER SESSION IV

DETERMINATION OF SELECTED PESTICIDES IN WATER BY GC/MS FROM SILESIAN WATER UTILITY PLANTS

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Surface waters are the primary source of potable water in the Upper Silesian region.

In the present analysis, water samples were collected from the inflows of water utility plants located in Strumień, Goczałkowice, Maczki, Dziećkowice and Kobiernice.

Further, installation rinsing waters and sediments were analyzed, and the pesticide contents was monitored in the Vistula river at the inflow to Goczałkowickie lake.

The isolation procedure employed allowed for the determination of the following acidic and neutral pesticides: atrazine, simazine, metolachlor, 2,4-D, cypermethrin and lambda-cyhalothrin, identified in earlier analyses.

The pesticides were isolated from water through SPE extraction on using Bakerbond spe Polar Plus columns and determined with the GC/MS technique using SIM.

The results obtained allow the assessment of the effectiveness of the processes used in the production of potable water.

APPLICATION OF GC/MS TO THE DETERMINATION OF SELECTED DRUGS FROM WATER UTILITY PLANTS

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The growing use of drugs in medicine (including veterinary medicine) has led to their increasing and now measurable concentrations in surface waters as well as drinking water installations.

The aim of the study was the determination of acidic and neutral drugs isolated from surface waters used in the production of potable water.

Further, installation rinsing waters and some sediments generated in water production were subjected to analysis.

Based on the screening studies on surface water and purified waste water samples, the following drugs were identified as most common in the Silesia region, and selected for determination: diclofenac, ibuprofen, ketoprofen, carbamazepine and their metabolites, as well as caffeine.

In the condensation and isolation of assays, the solid-phase extraction technique was used, employing the Bakerbond spe Polar Plus columns conditioned with hexane, acetone and water at pH = 4. Water samples were also acidified to a pH of 4. Condensed assays were eluted with acetone.

Chromatographic analysis was conducted on the GC/MS GCTrace with the MS – MSTrace Finnigan detector on a DB-5ms 30m x 0.25mm x 0.25μm column.

The results obtained allow the assessment of the effectiveness of the used for the water treatment processes in the plants investigated.

POSTER SESSION IV

CHROMATOGRAPHIC METHOD OF DETERMINATION OF ETHYLENE THIOUREA IN WORKPLACE AIR

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Ethylene thiourea (CAS Registry no. 96-45-7) is used extensively as an accelerator in the curing of polychloroprene and other elastomers. Exposure to ethylene thiourea may results from the very widely used ethylene bisdithiocarbamate fungicides. Ethylene thiourea may be present as a contaminant in these fungicides and can also be formed when food containing the fungicides is cooked.

Ethylene thiourea is an antithyroid substance and animal carcinogen. It is a skin, eye, and mucous membrane irritant to laboratory animals. Human toxicity is limited. The IARC has determined that there is sufficient evidence for carcinogenicity of ethylene thiourea to animals but inadequante evidence for humans.

The Interdepartmental Commission for Maximum Admissible Concentrations and Intensities in Poland established only NDS-MAC (TWA) value of 0,1 mg/m^3 for ethylene thiourea.

Ethylene thiourea could be determined in workplace air in the concentration range from 0.01 to 0.2 mg m^{-3} by liquid chromatography with UV detection. The use of an Nucleosil C_{18}, length 25 cm, internal diameter 4,6 makes it possible to separate ethylene thiourea from any interference.

Specimens of air for the determination of ethylene thiourea can be taken by a four – piece sampling cassette containing two glass fiber filters. This way of collecting ensures full separation of the compound tested from the air. Water was used for desorption of ethylene thiourea with an efficiency of about 100%.

[1)] Maximum Admissible Concentrations
[2)] Maximum Admissible Short-Term Concentrations

2-METHYLNAPHTHALENE – DETERMINATION METHOD IN WORKPLACE AIR

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2-Methylnaphthalene (CAS: 91-57-6) is used as dye carrier. Pure 2-methylnaphthalene is primarily used in vitamin K production and as a chemical intermediate.

Exposure to 2-methylnaphthalene can occur through inhalation, ingestion, and skin contact.

The Interdepartmental Commission for Maximum Admissible Concentrations and Intensities in Poland established NDS-MAC (TWA)[1)] value of 25 mg/m^3, and NDS-MAC (STEL)[2)] value of 50 mg/m^3 for 2-methylnaphthalene.

Determination of a worker's exposure to airborne 2- methylnaphthalene is made using a XAD-2 resin tube (100/50 mg sections). Samples are collected at a maximum flow rate of 6 litres/hour until a maximum collection volume of 12 litres is reached. 1 ml carbon disulfide is used for desorption. Analysis is conducted by gas chromatography using a flame ionisation detector (GC-FID). The column used in this study is a 60 meter Rtx-5MS capillary column 0,25 μm d.f., and 0,32 mm I.D. This column makes it possible to separate 2- methylnaphthalene from any potential interferences.

The determination limit of the method is 2,5 mg/m^3. Desorption efficiency is 98%. The evaluation of precision of the developed method defined a total uncertainty of measurement is about 11,82% for 2-methylnaphthalene.

[1)] Maximum Admissible Concentrations
[2)] Maximum Admissible Short-Term Concentrations

POSTER SESSION IV

DETERMINATION OF PCDDs AND PCDFs IN BOTTOM ASH FROM INCINERATION OF HOSPITAL WASTES

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Keywords: dioxins, furans, hospital wastes , environmental analysis, ashes

Polychlorinated dibenzo-p-dioxins and dibenzofurans are two series of tricyclic aromatic compounds with exhibit similar physical, chemical and biological properties. This group of compounds comprises 75 dioxins (PCDDs) and 135 related compounds called furans (PCDFs). All differ from one another by the location and number of chlorine atoms attached to the molecule, and their varying degree of toxicity. The most often referred to is the 'Seveso dioxin' or 2, 3, 7, 8 tetrachloro-dibenzodioxin (2378 TCDD), which is also the most toxic one [1].

Dioxins are not manufactured as commercial products or ingredients. They occur as unintended by-products of incomplete combustion and certain chemical processes. These combustion processes include: municipal and hospital waste incinerators; motor vehicles; wood burning, smoking; forest fires; compost heaps, etc. The metal industry is another source of dioxins, which appear during melting and refining operations or scrap recovery. A few chemical manufacturing processes also emit trace quantities of dioxins [1].

The results from the determination of PCDD and PCDF in bottom ash from incineration of hospital wastes are presented. Ash samples were collected from two installations of incineration plants for hospital wastes. For the determination of PCDDs and PCDFs in bottom ash a portion of 5-10 g of samples was taken from about 0.5 – 1 kg of raw material or raw material after separation out of metal and glass parts and after homogenization.

Samples of bottom ash were extracted with toluene in Soxhlet apparatus. Afterwards extracts were purified following standard analytical procedures based on acid/base silica, basic alumina and carbon/celit columns and the Power-Prep system to automate the cleanup of dioxins from environmental samples. The eluates were evaporated up to about 50 μl and analyzed by GC-MS technique. Both High Resolution Gas Chromatography (HRGC) coupled with High Resolution Mass Spectrometry (HRMS) and capillary column gas chromatography (GC) with ion trap tandem mass spectrometer (MS/MS) were used for PCDDs and PCDFs quantitative analysis and confirmation. The recoveries of the present internal standards by the cleanup method with Power-Prep were between 54,4 and 100 %. The ranges of dioxins in the ashes were between 0.65 and 363.77 pg TEQ/g. The absence of 2378 TCDD was also observed.

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DETERMINATION OF ALLYL CHLORIDE IN WORKPLACE AIR BY CHROMATOGRAPHIC METHOD

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Allyl chloride (3-chloropropene) (CAS Registry No. 107-05-1) is a colourless to pale yellow liquid with a pungent, unpleasant odour. Allyl chloride has not been reported to occur in nature. It may be released to the atmosphere during its manufacture or in its use as a chemical intermediate in the manufacture of pharmaceuticals, varnishes, epoxy resins, adhesives, glycerol and insecticides.

This toxic, highly flammable liquid, forms explosive mixtures with air at ambient temperatures. Individuals may be exposed to allyl chloride through breathing of contaminated air or through a skin contact.

Based on the toxicity of allyl chloride the exposure and limit values: NDS-MAC (TWA) – 2 mg/m^3 were proposed by the Interdepartmental Commission for Maximum Admissible Concentrations Intensities.

The determination method is based on the adsorption of allyl chloride vapours on activated charcoal (200/50 mg sections), desorption with NN-dimethylformamide and gas chromatographic with flame ionization detection (GC-FID) analysis of the resulting solution. This method makes it possible to separate the allyl chloride in the presence of 2,2-dichloropropane, 1,1-dichloropropane, 1,2-dichloropropane, 1,3-dichloropropane, epichlorohydrin and allyl alcohol. Calibration was carried out with standard solutions of allyl chloride in NN-dimethylformamide, using the following conditions: measurement range from 0,5 to 5 mg m^{-3}, 1 ml volume of sorbent for desorption, for determination 30 l air volume, 2µl injected sample. This method was fully validated.

POSTER SESSION IV

GC/MS ANALYSES OF THE DEGRADATION PRODUCTS OF REACTIVE ORANGE DYE

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Reactive Orange 113 is one of azo derivatives belonging to the overwhelming majority of synthetic dyes currently used in the industry. Commercial importance, the impact and toxicity of dyes that are released in the environment have been extensively studied.

Our knowledge concerning dyes behavior in the environment and health hazards involved in their use is still incomplete.

The purpose of this work was to investigate the degradation mechanism of the azo dye. Reactive Orange 113 aqueous solution was neutralization by advanced oxidation process (AOP) involving ozonation (O_3) and Fenton (H_2O_2/Fe^{2+}) processes.

On the basis of GC/MS analysis of Reactive Orange 113 dye decomposition of aqueous solution is postulated.

APPLICATION OF PLANAR CHROMATOGRAPHY ON DETERMINATION OF POTATO GLYCOALKALOIDS

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Glycoalkaloids are a group of natural toxicants which are present in all parts of Solanum species (potato, tomato, sweet pepper, eggplant). Potatoes contain two major glycoalkaloids, α-solanine and α-chaconine, which together account for 95% of the total glycoalkaloid content. They are at high levels in leaves, stems and sprouts of the potato plant and are normally at very low levels in potato tubers. However, on exposure to light the potato tuber will produce elevated levels of these glycoalkaloids, with the highest levels being in the sprouts as they emerge from the tuber. Potatoes will also produce high levels of glycoalkaloids in response to bruising, cutting and other forms of physical damage, as well as to rotting caused by fungi or bacteria. In these instances high levels of glycoalkaloids are present in the potato. However in non-damaged potatoes, greening is a warning sign. Glycoalkaloids are toxic to humans; the lethal dose is considered to be 3-6 mg per kg body mass. There are no reports of ill effects from consuming potatoes that have normal levels of these potentially toxic compounds. Potatoes with a content of glycoalkaloids higher then 200 mg/kg are considered to have a toxic effect. Therefore this level was determined by hygienic regulations in the Czech Republic as permitted amount of glycoalkaloids in potatoes.

A number of analytical techniques have been employed to assess the distribution and level of. glycoalkaloids in potatoes (TLC, HPLC, capillary electrophoresis etc.).

The objective of our study was to elaborate a simple and rapid procedure for the determination of glycoalkaloids in potatoes from the Czech retail. An HPTLC method for the quantification of glycoalkaloids from potatoes was used. Glycoalkaloids were extracted from grated potatoes with 1% acetic acid in water containing ion-pairing reagent sodium salt of 1-pentanesulfonic acid. The extracts were purified on silica gel columns and analyzed by instrumental high-performance thin-layer chromatography. HPTLC plate silica gel 60 (Merck) were used for separation with chloroform-methanol-2% aqueous NH_4OH (70+30+5) as the mobile phase. Because of the weak absorption in the UV spectral range glycoalkaloids were detected by dipping in methanol diluted Ce(IV) sulfate reagent and heating at 120 ^{0}C for 2 min. The chromatogram was scanned in absorbance mode (tungsten source, λ = 540 nm) SENS and SPAN parameters were 135 and 10, respectively. The R_F of α-solanine under these conditions was 0.12, of α-chaconine 0.20. The detection limit was 3 mg/kg, the limit of quantification was 10 mg/kg of potato.

POSTER SESSION IV

DEVELOPMENT OF HPTLC METHOD FOR DETERMINATION OF OTA IN MUST AND WINE

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Ochratoxin A (OTA) is a mycotoxin with nephrotoxic, nephrocarcinogenic, teratogenic and immunosuppresive properties, produced above all by species *Aspergillus* and *Penicillium* as well as other moulds. It has been detected in various foodstuffs, including grape must and wine. A number of survays have been conducted to monitor OTA levels in wine and must. Most of them have been concerned with quantification of OTA levels in wine, without information available on the origin of the contamination. The project aimed at assessing the risk of OTA in wine in Europe was initiated within the V Framework Programme of the EU. It is designed to answer the questions not only about OTA levels in wine, but also about conditions of OTA production in grapes. In the relationship to this project was prepared a pilot study in the Czech republic. Vineyards of the Southern Moravia were selected for the study. The OTA levels were determined in fresh juice pressed from grapes, in must and in young wine.

A sensitive HPTLC method for quantification of ochratoxin A in wine and must has been developed for requirement of this study. Wine samples were centrifuged, purified on commercial immunoaffinity columns (VICAM) and analyzed by instrumental HPTLC on silica gel layers with fluorescence detection. Chromatography was performed on 20 cm x 10 cm silica gel 60 HPTLC plates (Merck No. 5641). Diluted calibration standard (1, 5, and 10 μL corresponding to 100, 500, and 1000 pg OTA), and purified samples (20 μL) were applied to the plates by spot technique with a Camag model III automatic TLC sampler 1 cm from the edge of plate, distance between samples was 6 mm. The plates were developed with benzene-methanol-acetic acid, 18 + 1 + 1 (v/v), in the dark, in a saturated 20 cm x 10 cm vertical development chamber. After drying in a stream of cold air ochratoxin A was measured by fluorescence densitometry by means of a Camag TLC Scanner II with mercury lamp and K 400 secondary filter. The excitation wavelength was 333 nm, the emission wavelength 420 nm, and the SENS and SPAN parameters were 195 and 60, respectively. The R_f of ochratoxin A under these conditions was 0.24.

The detection limit was 5 ng L^{-1}, the limit of quantification was 15 ng L^{-1} of wine. Average recovery of ochratoxin A from spiked samples of wine was 95 % in the range 25 – 100 ng L^{-1} wine. The average relative standard deviation of repeatability (RSDr) was 6.7 %. Validation of the method was performed according to the principles used for HPTLC methods.

THE RAPID METHOD FOR THE COMPLEX ANALYSIS OF FERMENTATION BY-PRODUCTS IN DISTILLERY SPIRITS WITH APPLICATION OF GC

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The aim of our studies was to develop a quick method for determination of by-products contained in raw spirits. For this purpose, the capillary GC allowing to identify and determine the concentrations of substances contained in such groups of impurities as: carbonyl compounds, acetals, esters, higher alcohols, methanol and glycols was applied. Some of the substances from the groups mentioned above have a negative effect on the organoleptic properties of spirits even if their content is very law. Therefore, determination of the by-products composition in spirits is an important element of scientific and research works in the modernisation of alcoholic fermentation technology.

The methods of calibrating the standards required for qualitative identification on the basis of absolute referential retention times of standard peaks have been developed. Calibration of detector for the individual compounds was carried out by the method of multilevel external calibration ESTD. From the various columns offered, PC-WAX 57-BC (Chrompack, length - 50 m, ID - 0.32 mm) was selected on the basis of comparative tests. The conditions of chromatographic separation, parameters of signal integration and data reporting by a computer analytical station with software Chem-Station cooperating with a gas chromatograph (Hewlett Packard 6890 with EPC system and FID detector optimized for capillary columns) were optimized.

The analytical procedure of the method is characterized by high selectivity and a good precision determined on the basis of parameters of statistical evaluation within the wide range of the determined compound concentrations including determination of minute amounts. The relative error within the concentration range from 0.1 to 10 mg/100 ml A_{100} was within the limits from 5.8 to 0.45%, which can be accepted as correct values. Coefficient variation within the range of low concentrations from 1.0 - 0.1 mg/100 ml A_{100} was within the limits of 2.4 to 6.2%.

The method, because of its good selectivity, can be suitable for investigations of by-product compositions in spirits and it can help to find the reasons causing their bad organoleptic quality. Moreover, the short time of determinations allows it to be applied for routine control of the impurity concentrations in the spirits for industry.

POSTER SESSION IV

ATMOSPHERIC VOLATILE ORGANIC COMPOUNDS (VOCs) MEASUREMENTS IN FYODOROVSKOJE AND NEAR ZOTINO VILLAGE

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Biogenic non-methane volatile organic compounds (NMVOC) such as isoprene and terpenes, alkanes, alkens, alcohols, esters, carbonyls and acids play an important role in the oxidative capacity of the atmosphere. Over 90% of the total VOCs entering the atmosphere are biogenic. The biogenic VOCs are frequently classified into three categories - isoprene, monoterpenes, and others which contribute about 45%, 10% and 45%, respectively, of the total annual global biogenic VOC emission. VOCs can react in the lower atmosphere in the presence of oxides of nitrogen (NO_x) to form ozone (in the presence of sunlight) and other chemical compounds in photochemical smog. Some VOCs may represent a potential threat to human health. Our knowledge about VOC emission from Siberian forests is still insufficient. As a part of Terrestrial Carbon Observation System project (TCOS) we performed a measu-rement campaign during summer 2004. The aim of our research was to understand the biogenic fluxes, vertical exchange and atmospheric transport of VOC over the forests.

We measured the atmospheric mixing ratio of different VOC species above the forest canopy at a tower near to Zotino (central Siberia, 60°45'N, 89°23'E) and in Fyodorov-skoje (near Moscow, (56˚ 27'N, 32˚ 55'E). The most dominant VOC species above the forest canopy near Zotino were isoprene and monoterpenes like α-pinene and *p*-cymene. *P*-cymene was the main monoterpene on every height and every time. In Fyodorovskoje isoprene and α-and β-pinene were the main components above the forest canopy. Isoprene and α-pinene were the dominant compounds during the flights.

The data obtained will make a contribution to modelling and knowledge about the atmospheric distribution of VOC species over the rarely investigated Siberian forest.

RP-HPLC EVALUATION OF PESTICIDES CONTENT IN COTTON AFTER MECHANICAL AND CHEMICAL TREATMENT

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Widespread use of pesticides has resulted in their presence and persistence in crops and their occurrence in final products. It is striking that the key beneficial groups in cotton are similar in many parts of the world, but their impact and value have often proven difficult to demonstrate. Information on pesticide residue levels in cotton fabrics has a great importance for human consumption in particular for marketing and human safety. Such information is scanty in underdeveloped countries. A few papers show occurrence of many pesticides in cotton and its oil after treatment of cotton field. Currently regulations are increasingly more strict in terms of the residual level of chemicals used. According to European criteria, 100 ÖKO TEX Standard the content of pesticides in cotton seeds was regulated.

The purpose of this work was to explore a liquid chromatography methods for determination the level of contamination 20 pesticides in cotton of various origins. Liquid-liquid and liquid-solid extraction were applied to isolate pesticides from cotton samples. The liquid-solid procedure was performed on graphitized carbon black (Carbopac B) material in solution. The components were separated and quantified by reversed-phase HPLC on Purospher C-18e column with ultraviolet detection. The detection limits by this method of all the pesticides considered were between 0.005 and 0.03 ng/g. Changes of hazardous substances in cotton after mechanical and chemical treatment during the production cotton bend-cloth, starting from the raw materials in bales and ending with the finished product were monitored.

The content of pesticides was compared with the criteria of the 100 ÖKO TEX Standard.

POSTER SESSION IV

APPLICATION OF REVERSED-PHASE CHROMATOGRAPHY IN INVESTIGATION OF OXIDATION OF POLYETHOXYLATED OCTYLPHENOLS BY PERMANGANATE

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Polyethoxylated octylphenols (OPEOs) are well known synthetic detergents, which have been extensively used in pulp, paper, and textile industries. The popularity of these surfactants are attributed to the combination of the hydrophilic ethylene oxide chain, of variable length, with the hydrophobic alkyl chain, thereby making them versatile enough for an infinite number of applications. However, recent reports of estrogenicity as well as the toxicity and persistence of their degradation products such as octylphenol, mono- or di-ethoxylated octylphenol ethers, and some oxidized derivatives indicate to use the requirement of a method for waste-water treatment from these compounds.

This study was conducted to assess the viability of permanganate oxidative treatment as a method to reduce OPEOs concentration in raw water. The kinetics, reaction pathways and product distribution of oxidation of OPEOs by potassium permanganate were studied in phosphate buffered solutions under constant pH and isothermal. The reaction products were separated by RP-HPLC on ZORBAX SB C18 column with UV detection under isocratic condition using 70% methanol/water as mobile phase.

Experimental results indicate that the reaction is first order with respect to both OPEOs and $KMnO_4$. The OPEOs-$KMnO_4$ reaction may proceed through further oxidation and/or hydrolysis reaction pathways, greatly influenced by the acidity of the solution, to yield CO_2, oxalic acid, formic acid, glycolic acid and phenol derivatives.

POSTER SESSION IV

GAS CHROMATOGRAPHY-MASS SPECTROMETRY IN INVESTIGATION OF COMPOUND GROUPS PRESENT IN THE UPPER SILESIA COAL EXTRACTS

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Several coal samples from the Upper Silesia Coal Basin were investigated by chromatographic methods. Coals were ultrasonificated in DCM/MeOH and the extracts fractionated into aliphatic, aromatic and polar compounds by preparative thin layer chromatography. Gas chromatography-mass spectrometry was applied to analyse aliphatic and aromatic hydrocarbon distribution using the Agilent Techn. gas chromatograph equipped with HP-17 column (60m x 0.25mm), coated by 0.25 μm stationary phase film. The gas chromatograph was coupled to a mass spectrometer operating in EI mode at 70 eV (full scan, 50 - 650 da). Data were processed with the Hewlett Packard Chemstation software. All compounds were identified by their mass spectra, comparison of retention times to these of standard compounds, literature data and interpretation of MS fragmentation patterns [1-2]. Distribution of several groups of biomarker compounds were investigated against the influence of biological origin, conditions of organic matter deposition and thermal maturity.

Generally three types of *n*-alkane and acyclic isoprenoids distribution related to coal rank and weathering can be distinguished in the coal extracts: 1) monomodal with dominating *n*-C_{18} or *n*-C_{19} and high pristane concentrations (moderate to very high values of Pr/*n*-C_{17} ratio), CPI values about 1,5, 2) monomodal with short-chain *n*-alkanes predominating, CPI values near 1,0 and acyclic isoprenoids (pristane and phytane) in much lower concentration with Pr/*n*-C_{17} values 0,5-1,1; 3) monomodal or slightly bimodal forming a wide cluster of *n*-C_{20}-*n*-C_{27} peaks with high odd-over-even carbon-atom-number predomination (CPI ~ 1,6). Pristane concentrations are very high with values of Pr/*n*-C_{17} in the range of 3,0-7,1.

A diterpenoid group contained only low amounts of phylocladane and isopimaranes. Pentacyclic triterpanes are abundant in all extracts, except two, probably highly weathered coals. The distribution is similar in all samples and typical for moderate mature organic matter. Polycyclic aromatic hydrocarbons with 2-5 rings such as naphthalene, phenanthrene, anthracene, fluorene, fluoranthene, chrysene, triphenylene, pyrene, benzopyrenes, benzofluoranthenes and perylene were found together with their alkyl C_1-C_5 derivatives. Naphthalene and phenanthrene derivatives show the highest concentrations.

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POSTER SESSION IV

GC-MS IN ASSESSMENT OF A DEPOSITIONAL ENVIRONMENT INFLUENCE ON PROPERTIES OF YOUNGER SEDIMENTARY ORGANIC MATTER

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Differences in the facies of Polish brown coals were investigated applying chromatographic methods such as preparative thin layer chromatography, gas chromatography mass-spectrometry, and flash pyrolysis/GC-MS. Brown coals and loose sediments accompanying them were solvent extracted and the obtained extracts fractionated by TLC into fractions of aliphatic hydrocarbon, aromatic compounds and polar (NSO) compounds. Aliphatic and aromatic hydrocarbons were directly analyzed with GC-MS while polar fractions were methylated with BF_3 to obtain methyl esters of carboxylic acids. These compounds distribution was investigated with GC-MS. An Agilent Techn. gas chromatograph was applied equipped with HP-17 column (60m x 0.25mm), coated by 0.25 μm stationary phase film. The gas chromatograph was coupled to a mass spectrometer operating in EI mode at 70 eV (full scan, 50 - 650 da). Data were processed with the Hewlett Packard Chemstation software. All compounds were identified by their mass spectra, comparison of retention times to these of standard compounds and literature data and interpretation of MS fragmentation patterns [1-2].

Aliphatic hydrocarbons and function polar compounds occurring in so called "aromatic fractions" of the extracts show variable distribution related mainly to the biological source of organic matter and, only in the lower extend, to thermal maturity of organic matter. In the case of brown coals lithotype influence seems to be dominating since such compounds as diterpenoids are related chiefly to wood conifer trees (xylithic lithotype) while amyrine products such as olenenenes are related to reed facies giving rise to detritic brown coal lithotypes. However, oxicity of depositional environment also influences chemical composition of extracts. The wide range of oxygen compounds deriving from their biogenic precursors such as ketones, aldehydes and carboxylic acids of ursane, oleanene or abietane were found in detritic brown coal samples. Sulphur derivatives (thiophenes and thioles) are present in higher concentrations in the samples deposited in anoxic conditions.

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POSTER SESSION IV

CHEMICAL PROPERTIES OF ORGANIC MATTER DISPERSED IN SEDIMENTS ACCOMPANYING BROWN COAL BEDS

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Sedimentary organic matter dispersed in rocks and sediments accompanying brown coal seams of several Polish mines was the object of chromatographic analyses. Host rock extracts were TLC fractionated into fractions of aliphatic hydrocarbon, aromatic compounds and polar (NSO) compounds. Aliphatic and aromatic hydrocarbons were directly analyzed with GC-MS while polar fractions were methylated with BF_3 to obtain methyl esters of carboxylic acids. These compounds distribution was investigated with GC-MS. An Agilent Techn. gas chromatograph was applied equipped with HP-17 column (60m x 0.25mm), coated by 0.25 μm stationary phase film. The gas chromatograph was coupled to a mass spectrometer operating in EI mode at 70 eV (full scan, 50 - 650 da). Data were processed with the Hewlett Packard Chemstation software. All compounds were identified by their mass spectra, comparison of retention times to these of standard compounds and literature data and interpretation of MS fragmentation patterns [1-2]. Results were compared with similar results received for brown coals of the same mines. Distributions of several biomarker types were investigated belonging to *n*-alkanes, diterpenoids, hopanoids, fatty acids, and aromatic hydrocarbons such as alkyl derivatives of naphthalenes and phenanthrenes together with their partly hydrogenated and functionalised precursors.

It was found that organic matter dispersed in sediments of the Lubstów and Kazimierz open cast mines (the Konin Brown Coal Basin) shows geochemical properties similar to the surrounding brown coals while the Turów and Bełchatów sediments contained organic matter significantly different to organic matter of brown coals. It is caused by differences in sedimentation related to depositional environment and the type of primary biogenic organic matter. Shales and silts of these mines contain organic matter enriched in bacterial and algal remains influencing composition of the extracts. The vascular plants organic matter derived mainly from deciduous trees while the main coal-forming matter derived from conifers and reeds and grasses. Slightly higher thermal maturity calculated from biomarker parameters may be attributed to floor location of the sampling site in the case of some samples.

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POSTER SESSION IV

GC-MS IN CHARACTERISATION OF THE JAWORZNO MINE COALS

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The exploiting area of the Jaworzno mine is situated in the eastern part of the Upper Silesia Coal Basin and belongs to the Carboniferous Łaziska Beds. The investigated coal seam profile was sampled and samples solvent extracted. The extracts were separated into aliphatic and aromatic hydrocarbon fractions by preparative thin layer chromatography. Their chemical composition was investigated by GC-MS with Agilent Techn. gas chromatograph equipped with HP-17 column (60m x 0.25mm), coated by 0.25 μm stationary phase film. The gas chromatograph was coupled to a mass spectrometer operating in EI mode at 70 eV (full scan, 50 - 650 da). Data were processed with the Hewlett Packard Chemstation software. All compounds were identified by their mass spectra, comparison of retention times to these of standard compounds, literature data and interpretation of MS fragmentation patterns [1-2]. Distribution of several groups of biomarker compounds were investigated against the influence of biological origin, conditions of organic matter deposition and thermal maturity.

Group composition of coal extracts is variable with aliphatic hydrocarbons content being in the range of 1.1-11.2 % (wt.: wt.) and aromatic hydrocarbons in the range 6.4-30.4%. The main groups of biomarkers related to biological origin of organic matter are *n*-alkanes, diterpenoids, steranes and pentacyclic triterpanes together with their substituted derivatives and 2-3 ring aromatic hydrocarbons such as retene and cadalene. Aliphatic fractions were dominated by *n*-alkanes showing slight odd-over-even carbon atom number predominance (CPI values from 1.6-3.6), related to the input of vascular plants into primary biogenic matter. The presence of tetracyclic diterpanes, beyerane, phyllcladane and kaurane, indicates a possible input of evolutionary ancestors of *Araucariaceae* and *Podocarpaceae* families [3]. Values of biomarker environment parameters indicate suboxic conditions.

Several maturity parameters were calculated using geochemical biomarker parameters based upon hopanes and moretanes, kaurane and phyllocladane isomers, alkylnaphthalenes, and alkylphenanthrenes. There is a significant variability in thermal maturity of coal organic matter of the "Jaworzno" mine, probably related geological events occurring in nearby such as volcanic eruptions, however in most cases it approaches the end of diagenesis.

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APPLICATION OF THE GC ASSAY TO THE ANALYSIS OF THE COPLANAR CONGENERS OF POLYCHORINATED BIPHENYLS

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Coplanar congeners of PCBs (cPCBs) are characterized by nearly plane structure caused by two aromatic rings. Such conformation is characteristic for the so-called *non-ortho* substituted PCBs (chlorines are not in *ortho* position in the relation to phenyl) especially biphenyls substituted by chlorines in the position *para* (C4). Four such congeners can subsist, indicated by IUPAC nomenclature as PCB 77, PCB 81, PCB 126 and PCB 169. These compounds are analogs of the extraordinary toxic 2,3,7,8-tetrachlorodibenzo-*p*-dioxine. Three of them (PCB 77, PCB 126 and PCB 169) are strongly toxic.

Analysis of these compounds is laborious and time consuming. Therefore we have decided to apply elaborated earlier by us, and accredited by PCA (Polish Centre for Accreditations), method for the seven means PCB congeners. The assay is based on the extraction PCBs from fat using SPE. Silica-gel column was used with 10% w/w water. Eluate was evaporated and analyzed using GC-μECD.

The assay was applied to the analysis of PCBs in edible oils. It turned out that their concentrations were below our detection limit (about 0.15 ppb). Therefore oil was spiked with the analyzed PCBs: PCB 77 and PCB 81 – 0.05, 0.5 and 5 μg/g and PCB 126 and PCB 169 – 0.01, 0.1 and 1 μg/g. Obtained detection limits are equal: PCB 77 – 4 μg/g, PCB 81 – 4 μg/g, PCB 126 – 1 μg/g and PCB 169 1 μg/g. Linear dynamic was about 3 orders of magnitude (from 5 to 5000 μg/g for PCB 77 and PCB 81 and from 1 to 1000 μg/g for PCB 126 i PCB 169) with the correlation coefficient R^2 = 0,99. Recovery, 70 ÷ 110%, for all congeners agreed with the Polish Norm PN-EN 1528-1 what means that elaborated assay can be recognized as a quantitative one.

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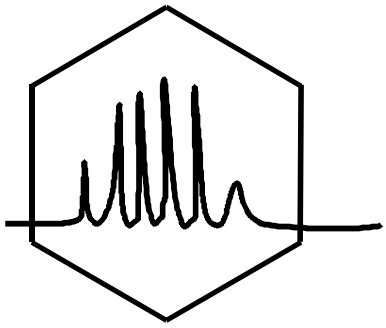

THE XXIXth SYMPOSIUM

'CHROMATOGRAPHIC METHODS OF INVESTIGATING THE ORGANIC COMPOUNDS'

JUNE 8th – 10th, 2005

KATOWICE – SZCZYRK

ANNEX

CONTENTS

GENERAL FUNCTION DESCRIBING THE VARIATIONS OF DIFFERENT PHYSICOCHEMICAL PROPERTIES OF ORGANIC COMPOUNDS WITHIN HOMOLOGOUS SERIES. CHROMATOGRAPHIC APPLICATIONS

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Numerous physicochemical constants of organic compounds (boiling points, critical temperatures, critical pressures, refractive indices, relative densities, dynamics or kinematics viscosities, surface tensions, vapor pressures, dielectric constants, ionization potentials, partition coefficients in heterophaseous systems, etc.) are historically considered as principally different objects in chemical and mathematical senses. Hence, they require using various approaches for approximation and precalculation (a lot of methods are known at present).

Nevertheless, the general recurrent function describing the variations of all mentioned properties (A) within homologous series can be proposed. It connects the value of any constant for homologue with (n) carbon atoms with its value for previous homologue having (n-1) carbon atoms by simplest linear dependence:

$$\mathbf{A(n+1)} = \boldsymbol{a}\,\mathbf{A(n)} + \boldsymbol{b} \qquad (1)$$

This recurrent function possesses the very interesting mathematical properties (to be considered in the special publications) and extremely high “extrapolation capability”. It means that if anybody has a set of data, and the problem is to predict unknown value of the next member of this series, it is not necessary to search the special approximation function. This task can be solved as simply and quickly as possible using the general regularity (1). It can be applied to any arithmetical or geometrical progressions, exponential functions, etc., and provides appropriate precision of approximation even for logarithmic and trigonometrical functions, as well as factorials.

It is remarkable to note that Fibonacci numbers (1, 1, 2, 3, 5, 8, 13, 21, 34, 55, etc.) can be successfully approximated by recurrent functions (1). The coefficient *a* in this equation tends to the value of ***golden section***, i.e. 1.618…. (!!!).

Principal mathematical rpoperties of function A(n+1) = a A(n) + *b*

The equation (1) is equivalent to another recurrent relationship which permits us to precalculate the value of any property for homologue using the values for two previous homologues:

$$\mathbf{A(n+1)} = \{\mathbf{A(n-1)}^2 + \mathbf{A(n)} \times [\mathbf{A(n)} - \mathbf{A(n-1)} - \mathbf{A(n-2)}]\} / [\mathbf{A(n-1)} - \mathbf{A(n-2)}] \qquad (2)$$

The mathematical solution of equation (1) is:

$$\mathbf{A(n)} = \boldsymbol{c}\,\boldsymbol{a}^{\mathbf{n}} + \boldsymbol{b}\,(\boldsymbol{a}^{\mathbf{n}} - 1) / (\boldsymbol{a} - 1), \quad \text{where } a, b, c \text{ – constants} \qquad (3)$$

At the hypothetical increasing the number of carbon atoms in the molecules ($n_C \to \infty$) the function $A(n_C)$ for various physicochemical properties of organic compounds tends to infinity (at $a > 1$), or to a limits at $a < 1$:

$$\lim A(n_C \to \infty) = b / (1-a) \qquad \text{(only at } a < 1\text{)}.$$

How to calculate using new "universal" approximation function?

Answer: Very simply, e.g., using standard **ORIGIN** software

Example 1: Processing the boiling points (T_b, ^0C) for *n*-alkyl fluorides $C_nH_{2n+1}F$

n_C	T_b, ^0C	Difference, $\Delta T_b = T_b(i+1) - T_b(i)$
1	**-78,5**	
2	**-37,7**	**40.8**
3	**-2,4**	**35.3**
4	**32**	**34.4**
5	**62,8**	**30.8**
6	**93,2**	**30.4**
7	**120,6**	**27.4**
8	**142,2**	**21.6**
9	**166-169**	**(?) 23.8-26.8 (exceeds the previous one)**

The task: Check T_b values for *n*-nonyl fluoride (seems like unreliable)

Step 1. Enter the target data set into the column A[X]:

Microcal Origin – D:\ORIGIN\UNTITLED

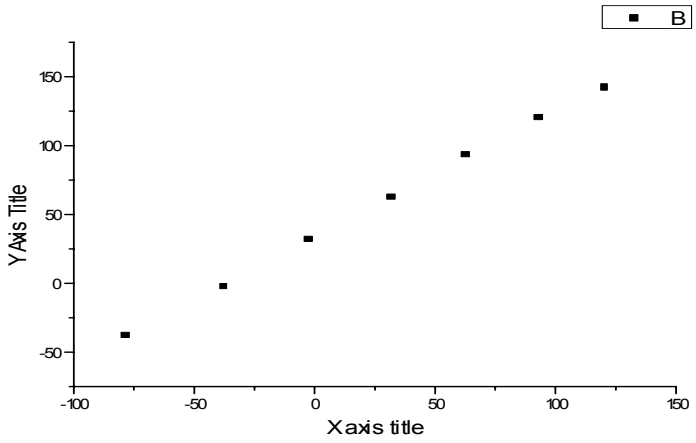
Data1		
	A[X]	**B[Y]**
1	**-78,5**	
2	**-37,7**	
3	**-2,4**	
4	**32**	
5	**62,8**	
6	**93,2**	
7	**120,6**	
8	**142,2**	

Step 2. Select the data excluding the first one (-78.5) and copy them into the column B[Y] with shifting by one row up:

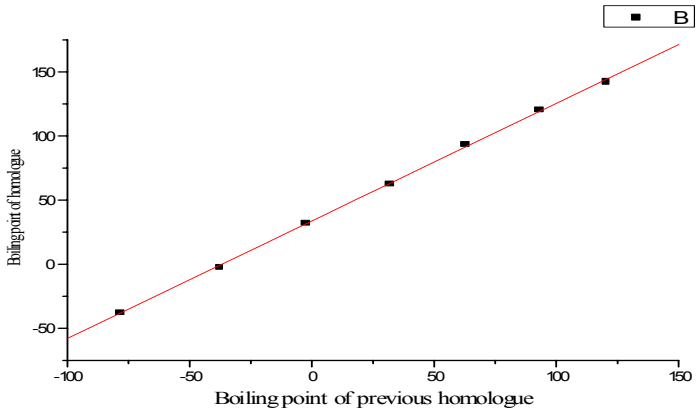
Microcal Origin – D:\ORIGIN\UNTITLED

Data1		
	A[X]	**B[Y]**
1	**-78,5**	**-37,7**
2	**-37,7**	**-2,4**
3	**-2,4**	**32**
4	**32**	**62,8**
5	**62,8**	**93,2**
6	**93,2**	**120,6**
7	**120,6**	**142,2**
8	**142,2**	

Step 3. Choose "Scatter" in the option "Plot" for these set of data:



Step 4. Choose "Linear Regression" in the option "Fit" for this plot:



Linear Regression for Data 1__B:

Y = A + B * X

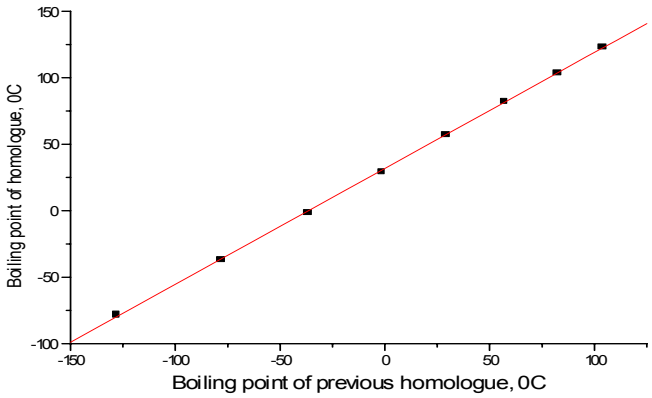
Param	Value	sd
A	33,79109	0,65944
B	0,91664	0,00922

R = 0,99975
SD = 1,61412, N = 7
P = 1,9549E-9

Final step: Calculate T_b values for *n*-nonyl fluoride using resulting equation:

$T_b = 0.917 \times 142.2 + 33.8 = 164.2\ ^0C$
The reliability of this result seems to be approximately equal to SD, i.e. $\pm$ 1.6 ^{0}C
This T_b can be independently precalculated using ACD software:
The result $T_b = 164.9 \pm 3.0\ ^0C$, i.e. it has the less precision than that for new approach.

Example 2: The illustration of practically "ideal" linear dependence $T_b(n+1) = a\ T_b(n) + b$ for boiling points (T_b, ^{0}C) of perfluoro-*n*-alkanes C_nF_{2n+2} (C_1-C_9) is presented by the plot below ($a = 0.872 \pm 0.005$; $b = 31.8 \pm 0.4$, $\rho = 0.9999$; $S_0 = 1.1$):



It permits us to precalculate boiling points of perfluoro-*n*-alkanes by the same way as that used in Example 1.

Similar linear dependencies characterize all other physicochemical properties of organic compounds (boiling points, critical temperatures, critical pressures, refractive indices, relative densities, dynamics or kinematics viscosities, surface tensions, vapor pressures, dielectric constants, ionization potentials, partition coefficients in heterophaseous systems, etc.)

Application in chromatography

The application of recurrent relationships (1 and 2) in chromatography seems in accordance with their general predestination:

Prediction of any physicochemical or chromatographic constants of organic compounds within any sequences of homologues is possible by simplest way without special choice of approximation function

Example 3. Confirmation the non-constancy of H-factor.

Homomorphous factor (H) is one of the historically first parameters recommended for prediction of GC retention indices (RI) for substituted organic compounds (RX) using RIs for their simpler structural analogs (non-substituted compounds RH):

H = RI (compound R-X) – RI (compound R-H)

Within large parts of various homologous series H ≈ const. However, for some higher homologues the growth of H-values is observed, e.g., for 1-alkanols relative to the corresponding *n*-alkanes:

n_C in 1-ROH	$RI_{non\text{-}polar}$	H (relative to *n*-alkanes)	H(i+1) – H(i)
6	856 ± 7	256	-
8	1066 ± 5	266	10
10	1264 ± 9	264	-2
12	1466 ± 11	266	2
14	1664 ± 12	264	-2
16	1868 ± 12	268	4
18	2075 ± 11	**275**	**7**
20	2289 ± 5	**289**	**14**
22	2488 ± 11	**288**	**-1**

Keeping in mind that H ≠ const, how to predict RIs for worse characterized sub-groups of organic compounds, namely 2-alkanols:

n_C in 2-ROH	$RI_{non\text{-}polar}$	H (relative to *n*-alkanes)
4	600 ± 13	200
5	691 ± 11	191
6	793 ± 10	193
7	891 ± 10	191
8	991 ± 10	191
9	1090 ± 4	190
10	1192 ± 6	192
≥ 11	No reliable data	For the first view there are no reasons to suspect non-constancy of H-values

Using the equation RI(n+1) = *a* RI(n) + *b* (*a* = 1.014 ± 0.009; *b* = 87.1 ± 7.5; ρ = 0.9998, S_0 = 3.6) one can predict RI values for higher homologues of 1-alkanol series, namely 2-C_{11}OH, 2-C_{12}OH and so on.

n_C in 2-ROH	Predicted RI value
4	-
5	695
6	791
7	891
8	990
9	1092
10	1192
11	**1296**
12	**1401**

Hence, the non-constancy of H-factors can be interpreted as general regularity, reflecting objective RI variations within homologous series.

The "universal" character of recurrent equation (1) stimulates its unusual non-chromatographic applications (discussed).

THE GC/AED STUDIES ON THE REACTIONS OF ORGANIC SULFIDES WITH OZONE

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Industrial waste composition depends, among others, on kind of production process. Petrochemical, paper and textile industry are main source of organic sulfur compounds in wastewater. Those compounds are also present in wastewater produced by food industry and in municipal wastewater. Concentrations of organic sulfur compounds in industrial waste can be quite high. Water pollution with those compounds is especially noxious because bacteria occurring in this environment cause their degradation to compounds like hydrogen sulfide that is dangerously toxic and has unpleasant odour. Thus, decomposition of organic sulfur compounds present in wastewater may lead to incidental intoxications. Volatile organic sulfur compounds present in wastewater should be processed, so to prevent their release into atmosphere. Those compounds present toxic and odour hazard, especially when they are present in aquatic environments of low oxygen content [1]. Reduction of sulfates in oxygen-depleted environments leads usually to hydrogen sulfide that may further react giving simple, volatile organic sulfur compounds, such as: methanethiol or dimethylsulfide. Pollutions of this kind are created during desulfurization of crude oil, during regeneration of oils and in coke engineering during coal processing [2, 3].

Organic sulfur compounds are frequently mutagenous, teratogenous and carcinogenous. Many organic sulfur compounds may accumulate in human, animal and plant tissues and in bottoms of water reservoirs [4].

Very intensive studies have been undertaken during the last decade on development of toxic water and wastewater pollution efficient removal methods. New group of organic sulfur removal methods from wastewater is called the Advanced Oxidation Technologies (AOT), and processes taking place during AOT are called the Advanced Oxidation Processes (AOP).

Reviews describe many aspects of wastewater treatment with AOT but problem of organic sulfur compounds removal is described only marginally [5, 6].

During studies on water or wastewater polluted with organic sulfur compounds purification only slight interest was shown so far in identification of products formed as a result of those processes. Knowledge about kinds of products formed as a result of organic sulfur compounds oxidation are fragmentary, incomplete, and frequently treated marginally. While kinetics of oxidation reactions of some organic sulfur compounds with classic oxidizers is fairly well known, identification studies on products created as a result of advanced oxidation are, for unclear reasons, largely neglected.

AOP have advantage over classic oxidation methods for their usage do not introduce pollutions into natural environment [7-9]. Advanced oxidation processes use simultaneously several factors, such as ozone, hydrogen peroxide, and UV radiation. Various combinations of

those factors are used: UV/O_3, UV/H_2O_2, O_3/H_2O_2, O_3/H_2O_2/UV. This group of methods includes also processes carried with Fenton's reagent composed of hydrogen peroxide and iron (II) cation . Method of advanced oxidation including photocatalytic degradation of organic compounds in aqueous suspensions of semiconductors (UV/TiO_2) is known [10]. AOT include also methods consisting of electrochemical generation of highly reactive ozone [11-14] or free radicals [15-22] that cause degradation of toxic organic compounds into very simple ones, including gaseous. Common feature of all mentioned AOT is that in course of influence of proper agents reactive oxygen species (ROS) are created. ROS formed due to usage of AOP are characterised with high reactivity, and majority of them – with low selectivity of action towards organic compounds [23].

Aim of this study is to examine reactions of selected organic sulfides with ozone. The examination uses gas chromatography with atomic emission detection for quantitative and qualitative assessment of oxidation products.

Preparing samples for chromatographic analysis

Samples were prepared with liquid-liquid extraction method. The method allows relatively fast separation of individual constituents of reaction mixture. After trials of solvents for extraction it was found that dichloromethane provides the best properties. The solvent allows high recovery of analysed substances and has low boiling point, so it is easily separable from analysed mixture during chromatography. From a reaction vessel 2 cm^3 of reaction mixture was pipetted on selected time. Collected sample and 2 cm^3 of methylene chloride were placed in capped tube, shaken for 15 seconds and left until phases are separated. Next, lower – dichloromethane phase was transferred to another tube and dried with anhydrous $MgSO_4$. Dried dichloromethane solution was filtered and analysed.

Chromatographic analysis

Parameters of operation of gas chromatograph coupled with atomic emission spectrometer were: plasma cavity and transmission line temperature were 270^oC; injector temperature: 260^oC, stream splitter: 20:1; solvent clearance time from detector from 0,9 to 2,2 min; carrier gas was helium with flow of 2 ml/min. The following reaction gases were used: hydrogen, oxygen and methane-nitrogen (10% : 90%) mixture. Chromatography of individual samples was performed with programming of temperature. The analysis was performed with temperature-programmed column. Temperature programs were individually developed for each analysed sulfide and its oxidation products. The analysis used capillary column HP-5 and HP-50+. Analysed sulfides oxidation products retention time comparisons with retention time of standards allowed their preliminary identification. Full identification of those compounds was performed with a computer program furnished with the GC/AED. Retention times and elemental composition of analysed sulfide oxidation products were compared with standards. This allowed their identification. Quantitative analysis of oxidation products was done with absolute calibration method.

Reaction speed determination

Selected sulfides oxidation reaction speed in various oxidizing solutions were determined with aid of substrate decay halflife time, that is time for decrease of a compound concentration to the level equal to half of initial concentration. Basing on chromatographic

quantitative analysis percentage of a substrate in reaction mixture, in relation to its initial level, was calculated and curves drawn in relation to quantity of a given substance in reaction medium during its reaction with ozone. Compound halflife time was determined using graph equation obtained basing on measurement points. Results are shown in tables and graphs.

Conclusions:

- Symmetrical dialkyl sulfides in series: diethyl, dipropyl and dibutyl are oxidized with ozone to corresponding sulfoxides, and with almost equal speed. No relationship between sulfide oxidization speed and length of alkyl chains was found.
- 1,4–thioxane oxidation with ozone speed is approximately 4-times lower than speed of tetrahydrothiophene oxidation. Probable cause of this phenomenon is large electronegativity of oxygen atom. Being present in 1,4–thioxane it pulls electrons off sulfur atoms and decreases its negative charge, making oxidization of this sulfide more difficult.
- Sulfides possessing two electronoacceptor substituents linked to sulfur atom undergo oxidization with ozone much more slowly than sulfides possessing two electronodonor substituents.
- Presence of one electronoacceptor substituent and one electronodonor substituent on one sulfur atom does not significantly influence sulfide oxidation speed in comparison to speed of oxidation of sulfides possessing both electronodonor substituents.
- It is a rule that in case when a sulfide slowly oxidizes into sulfoxide the resulting sulfoxide relatively slowly oxidizes into sulfone. The only exception from this rule is 2-chloroethyl-methyl sulfide that oxidizes fast into sulfoxide, and very slowly into sulfone.
- For experiments aimed on comparison of effectiveness of sulfides oxidation with ozone dibutyl sulfide was chosen as a model organic sulfur compound. Determined halflife times of this sulfide allow ranking the used AOT system according to increasing halflife times:

$$O_3/H_2O_2/UV \; < \; O_3/UV \; < \; H_2O_2/UV \; < \; O_3 \; << \; H_2O_2$$

Low $t_{1/2}$-value (fast oxidation) — high $t_{1/2}$-value (slow oxidation)

- In all processes which used UV radiation dibutyl sulfide was undergoing decomposition to gaseous products.

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INDIRECT DETECTION IN ION EXCLUSION CHROMATOGRAPHY

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Ion Exclusion Chromatography

Ion-exclusion chromatography (IEC) finds application in the analysis of weak and medium strength acids [1]. The detection techniques include direct UV absorbance, refractive index (RI), potentiometric, conductivity as well as mass spectrometry. The direct UV absorption at 210 nm is the most frequently used detection method for volatile fatty acids. Its detection limit is rather high because of lacking a chromophore by solute. From the other side, due to its sensitivity to even trace amounts of contaminants, it cannot be used for complex samples without suitable sample pretreatment because of interference problems. The most commonly used in IEC conductometric detector also is characterized by high sensitivity because of small dissociations of acids, additionally depressed by the buffer.

Indirect Detection

Indirect photometric detection has been already successfully applied in ion chromatography as well as and capillary electrophoresis [2]. In this case mobile phase contains an absorbing reagent of the same electric charge as analyzed solute, characterized by high molar absorption coefficient.

Objective

In the presentation it was confirmed the possibility of application of indirect detection in ion exclusion chromatography. In this case aromatic acids can be used as eluents. Two detection systems are discussed, photometric and conductometric. Derived equations as well as experimental data show indirect conductometric detection for solutes which ionic conductivities (diffusion coefficients) are smaller then background electrolyte. Direct detection (positive peaks) is observed in the opposite case. It was possible to obtain direct and indirect detection on one chromatogram, depending on the relative limit conductivity (diffusion coefficient) of solute and background electrolyte. Quantitative correlation between derived equation and experimental results was found. Phthalic acid, used as indirect detection probe, decreased retention of aliphatic fatty acids because of the competition on adsorption sites.

Materials and Methods

Measurements were performed on a chromatograph consisting of Dionex (USA) GP40 gradient pump, AD20 Absorbance detector, ED40 electrochemical Detector, LC30 chromatography oven, PeakNet 5.1 chromatographic data acquisition and analysis, TosoHaas (Japan) TSKGel SCX(H^+) 300 x 7.8 mm I.D. column. Manual injections were performed using a 100 µl syringe (Scientific Glass Engineering, Ringwood, Australia).

Changes of the background electrolyte concentration

Indirect detection is based on the measurements of changes of the concentration of background (probe) electrolyte. Its electric charge should be the same as a solute. It means that

in the case of the acid analysis also background electrolyte should be acid. Usually it is used diluted solution of strong, completely dissociated acid. However, optimal conditions from the detection point of view are sometimes not optimal for separation. Changes of the background electrolyte concentration can be calculated from the definition of dissociation constant and mass conservation equation. For diluted solutes we can assume that the concentration of hydrogen ions is constant along the column: $[H^+] = const.$ In this case, outside solute region (chromatographic peak) electroneutrality condition can be expressed as:

(1) $$[H^+] = [B^-].$$

Tested acidic solute, *HR*, injected onto the chromatographic column decrease concentration of the dissociated form of background electrolyte. According to electroneutrality condition in the peak maximum, in the mobile phase:

(2) $$[H^+] = [B^-]^{max} + [R^-],$$

where: B^- and R^- denote dissociated forms of the background electrolyte and solute, respectively.

Equations (1) and (2) can be easily transformed to:

(3) $$[R^-] = [B^-] - [B^-]^{max}.$$

Conductometric detection

Conductivity of the diluted electrolyte, *G*, is a function of its limiting ionic conductivities, λ_i, stechiometric coefficients, ν_i, valence, z_i, surface area of the electrodes, *A*, and distance between them, *l*:

(4) $$G = (\nu_+ c_+ z_+ \lambda_+ + \nu_- c_- z_- \lambda_-)A/l.$$

For mono-mono valent acid above equation can rewritten as:

(5) $$G_b = (\lambda_{B^-}[B^-] + \lambda_{H^+}[H^+])A/l.$$

In the peak maximum this conductivity depends also on the solute concentration:

(6) $$G^{max} = (\lambda_{B^-}[B^-]^{max} + \lambda_{H^+}[H^+] + \lambda_{R^-}[R^-])A/l.$$

Chromatographic peak height can be calculated as a difference of the conductivities obtained from the equation (6) and (5):

(7) $$\Delta G = (\lambda_{B^-}[B^-]^{max} + \lambda_{R^-}[R^-] - \lambda_{B^-}[B^-])A/l = (\lambda_{R^-}[R^-] - \lambda_{B^-}[R^-])A/l.$$

From equations (3) and (7) finally we can obtain:

(8) $$\Delta G = (\lambda_{R^-} - \lambda_{B^-})\,[R^-]A/l.$$

From the equation (8) we can read out that height of the chromatographic peak should be directly proportional to the concentration of dissociated form of analyzed acid. Additionally, it should be possible to obtain both, direct and indirect, conductometric detection on one chromatogram. Peak direction depends then on the relative limiting ionic conductivity of solute versus background electrolyte.

Photometric detection

According to Lambert-Beer law, the background absorbency, A_b, of the acid, *HB*, used as a buffer can be expressed as:

(9) $$A_b = l\varepsilon_{B^-}[B^-] + l\varepsilon_{HB}[HB],$$

where: *l* – denotes length of detector cell, ε_{B^-} and ε_{HB} – molar absorption coefficients of dissociated and undissociated forms of acid, respectively.

Absorption in the peak maximum based on equations (1) and (9) is described by:

(10) $A^{max} = l\varepsilon_{B^-}[B^-]^{max} + l\,\varepsilon_{HB}[HB]^{max}$.

Height of the chromatographic peak can be obtained from the equations (9) and (10):

(11) $\Delta A = l\varepsilon_{B^-}[B^-]^{max} + l\,\varepsilon_{HB}[HB]^{max} - l\varepsilon_{B^-}[B^-] - l\,\varepsilon_{HB}[HB]$.

After combining equation (11) with equation (3), using definition of the dissociation constant and assumption that the concentration of undissociated form of solute acid is constant along the chromatographic peak ($[HB]^{max} = [HB]$), we can obtain:

(12) $\Delta A = -l\varepsilon_{B^-}[R^-]$.

Estimation of [R$^-$]

The mass conservation equation of the solute acid can be given as:

(13) $c_i V_i = ([R^-] + [HR])V_P$,

where V_P ($V_P = c_i V_i / c^{max}$) denotes volume of the peak maximum.

From equation (13) and definition of dissociation constant we can obtain:

(14) $[R^-] = c_i V_i / V_P(1 + [H^+]/K_a) = c_i V_i K_a / V_P(K_a + [H^+])$.

After column equilibration the mass balance of the background electrolyte can be presented in the form:

(15) $c_b = [B^-] + [HB]$.

Because concentration of the solute is usually small and because buffer suppresses its dissociation, it can be assumed that concentration of hydrogen ions is constant along the column. Outside of the chromatographic peak concentration of the dissociated form of background electrolyte equals to the concentration of hydrogen ions, as it is described by equation (1).

After resolving quadratic equation obtained from the equations (14) – (16) finally we obtain:

(16) $$[R^-] = \frac{c_i V_i K_a}{V_P(2K_a + \sqrt{K_b^2 + 4K_b c_b} - K_b)}$$

where peak volume is described by:

(17) $V_P = V_R(2\pi/N)^{1/2}$.

Equations (8), (12) and (17) can be used to predict indirect conductometric as well as photometric detection. Chromatographic peak height should be directly proportional to the amount of the solute acid and increased asymptotically with the dissociation constant. The highest peak is obtained for completely dissociated acids (anions). On the other hand, peak height decreases with the increase buffered acid dissociation constant and its concentration.

Experimental verification

Aliphatic acids, including oxalic, malonic, formic and acetic, were selected as the test compounds. The IEC separation of the tested acids is presented on Fig. 1. As a mobile phase 1 mM solution of phthalic acid was used. Two detectors, conductometric (A) and UV-300 nm (B) were serially connected to the column. It turned out that in all cases indirect photometric detection was obtained, whereas indirect conductometric detection was obtained only for acetic acid. For other analyzed acids direct conductometric response was observed. Finally, it should be also mentioned that phthalic acid influences retention of tested acids by the competition with hydrophobic adsorption sites.

Conclusions

It was confirmed the possibility of application of indirect detection in ion exclusion chromatography. In this case aromatic acids can be used as eluents. Derived equations as well as experimental data show indirect conductometric detection for solutes which ionic conductivities (diffusion coefficients) are smaller then background electrolyte. Direct detection (positive peaks) is observed in the opposite case. Quantitative correlation between derived equation and experimental results was found. Phthalic acid, used as indirect detection probe, decreased retention of aliphatic fatty acids because of the competition on adsorption sites.

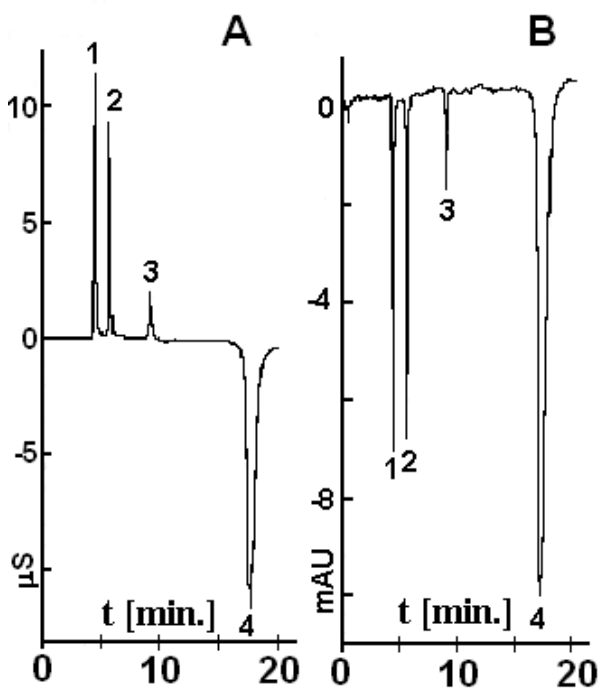

Fig.1. Ion-exclusion chromatograms of: (1) oxalic, (2) malonic, (3) formic and (7) acetic acids. Chromatographic conditions: mobile phase - 1 mM phthalic acid, flow rate - 0.5 ml/min; injected volume - 20 µl, conductometric (A) and UV-300 nm (B) detectors.

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IMPROVED GC-μECD ASSAY FOR THE METHYLMERCURY MEASUREMENTS: ITS ESTIMATION IN THE FISH MEATS

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Mercury toxicity

Mercury is highly toxic radical found, especially, in see organisms. The most easily adsorbed and the most toxic form of mercury is methyl-mercury. It is easily adsorbed by the fishes and other aqueous organisms alimentary tracts, in this way introducing the food chain. Inorganic mercury compounds are changed to the most reactive alkyl connections by some aerobic or anaerobic bacterial from the oceanic sediments. It affects the immune system, alters genetic and enzyme systems, and damages the nervous system, including coordination and the senses of touch, taste, and sight. Its toxic effect is caused by it interactions with thiols and amine groups.

Because of its highly toxicity maximal concentration of the total mercury is limited, in Poland by *Rozporządzeniem Ministra Zdrowia z dnia 28 maja 2004r* (1,0 mg/kg for the predatory fishes, 0,5 mg/kg for others). In some countries (Japan, Greece and USA) also concentration of methylmercury is limited (in Japan - 0,3 mg/kg, for non-predatory fishes in Greece and USA 0,7 mg/kg and 1,0 mg/kg for all fishes, respectively).

Objective

The aim of the presentation was to improve GCμECD assay for the methyl-mercury. Different, polar as well as unpolar, columns were tested to obtain the performance of the separation.

Experimental

Apparatus

Experiments were performed on the Gas Chromatograph model 6890 (Palo Alto, Agilent, USA) equipped with μECD detector. It was used medium polar column BP-10 (25 m x 0.22 mm I.D.) (SGE, Australia) covered by 0.25 μm 14% cyanopropylyhenyl polysiloxane.

Chromatographic conditions: temperature gradient - from 70°C (1 min.) with the speed 35°C/min. up to 160°C; injector temperature - 260°C; detector temperature - 250°C; gas (He) pressure before injection - 70 psi during 2 min; gas pressure during the analysis - 25 psi.

Procedure

Elaborated assay was tested by analyzing methyl-mercury in different fishes (norwegian salmon, baltic salmon, european sheat-fish, tuna, eel, pike perch and african sheat-fish) obtained from the nearest super-market. Methyl-mercury, connected with the proteins by –SH group, was uncoupled by the acidification with hydrochloric acid. Obtained methyl-mercury chloride was extracted to the benzene. To remove fats from the sample methyl-mercury chloride was re-extracted to water using sodium thiosulfate. Finally, obtained complex was again

transformed to chloride by copper chloride and extracted to benzene. Such obtained sample was injected (5 µl) on the column using splitless pulsation method.

It turned out that before every analytical series (about 6 h) column, to improve performance and detection limit, should be passivated. It was obtained by 10 *on-column* injections of 10 µl saturated solutions of mercury chloride in toluene. Comparisons of the GC chromatograms of 0,005 mg/kg standards obtained on the column *before* and *after* passivation are presented on Fig. 1. It was found that in the both cases the same peaks area are obtained. However, peak broadening decreased performance and increased detection limit of the assay.

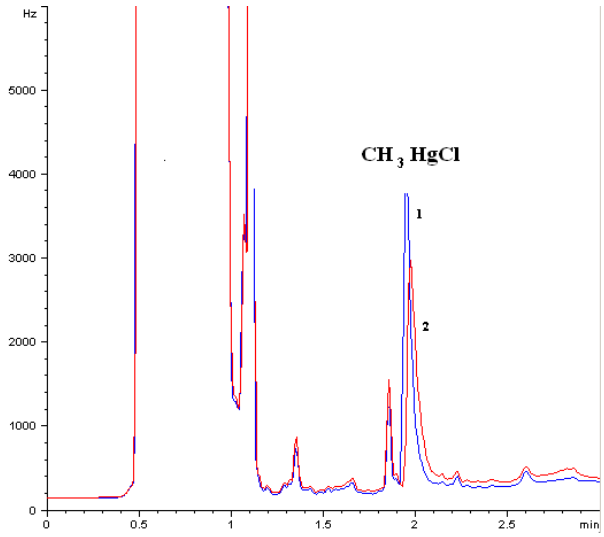

Fig. 1. GC chromatograms of the CH_3HgCl standards obtained on the column (1) – passivated, (2) – 6 hours after passivation.

Elaborated assay has been tested on the reference material CRM 463 Tuna-fish (3.04 ± 0.16 mg/kg CH_3HgCl). Obtained results are presented on the Table I.

Table I. Obtained results of the analysis of reference material (n=3).

Sample	Certificated concentration of CH_3HgCl [mg/kg]	Obtained concentration of CH_3HgCl [mg/kg]
CRM 463 Tuna-fish	3.04 ± 0.16	2.87 ± 0.13

Results

Elaborated assay is characterized by high linear dynamic range (nearly 4 orders of magnitude, $R^2 > 999$) up to the concentration 5 mg/kg b.w. Obtained limit of detection was below 0,002 mg/kg and precision of measurements for solute 2 and 8 % and for sample 3 and 18

% for concentrations 0,1 and 0,01 mg/kg, respectively. Recovery for reference material (CRM 463) was equal 94%.

As the practical result meat from 7 spaces of commercially available fishes was investigated. It turned out that concentration of the methyl-mercury varied in them from 0,01 to 0,50 mg/kg. Especially interesting is that its concentration was fifty times higher for European fishes in comparison to the African ones.

It turned out that average concentration of the methyl-mercury changed from 0,010 mg/kg up to 0,453 mg/kg (Fig. 2), for different fishes. In the literature [3] it was found that more than 90% of accumulated in the fishes mercury is in the form of the methyl-mercury. Therefore we can assume that in all samples its concentration was below permissible Polish norms (*Rozporządzenie Ministra Zdrowia z dnia 28 maja 2004r*.) However, it is worth to note that in the case of european sheatfish concentration of the methyl-mercury was above the Japanese norms.

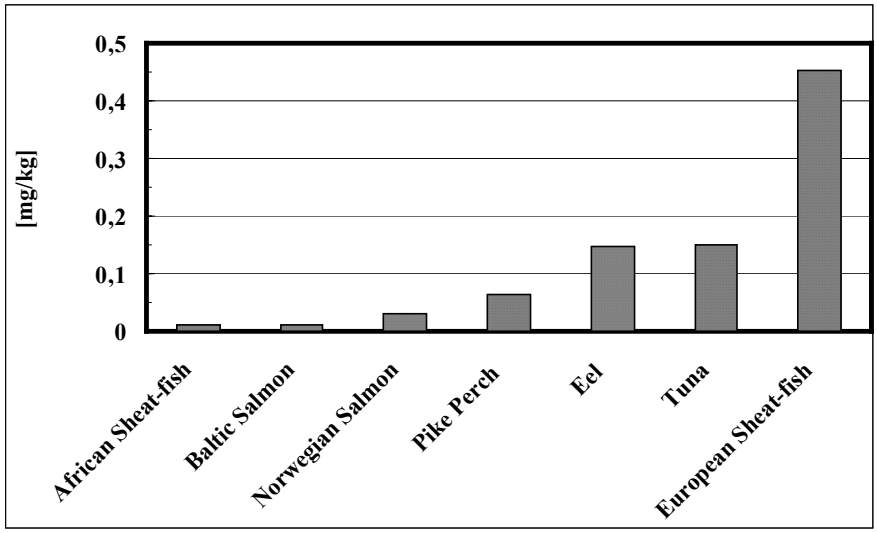

Fig. 2. Concentration of methyl-mercury [mg/kg] in different fishes.

Conclusions

1. From the tested column the best results were obtained on the BP-10 (SGE), 25 m, 0.22 mm I.D., 0.25 μm stationary phase.
2. It turned out that our improved assay, based on the gas chromatography, of methyl-mercury analysis is characterized by the repeatability RSD below 15% and recovery (94%) and detection limit (0,002 mg/kg) enabling its analysis in the meat of fishes.
3. It was found that before GC measurements column should be passivated.

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MEASUREMENT OF VITAMIN E IN HUMAN MILK USING HPLC WITH UV DETECTION[#]

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Abstract

The extraction of vitamin E from human milk using hexane with and without prior saponifi-cation were compared. Analyses of milk extracts were carried out by HPLC incorporating UV detection and a C_{18} column. A significant relationship between the measurement of α– and γ–tocopherol in human milk was obtained with either method ($P<0.01$), though saponification resulted in higher and more consistent recoveries for the internal standard, δ–tocopherol, (99.6±4.0%), and significantly improved the reliability of the data. The detection limit of the method (including saponification, extraction and HPLC analysis) was 0.65 μg/ml of δ-toco-pherol in milk. The proposed saponification step was a simple addition to the method as it was performed in the same tube as the extraction, it only took 30 min and did not involve the use of an inert gas. The improved method was used to measure vitamin E in a number of breast milk samples.

1. Introduction

The main biological function of vitamin E is as an antioxidant in the protection of polyun-saturated fatty acids of cell membranes from free radical damage. An adequate supply of vitamin E in milk to newborn and especially preterm babies is vital for their normal development. A number of publications have used a variety of different methods (1-3) to report levels of tocopherols in human milk at different stages of lactation. In these papers tocopherols were measured after direct extraction with hexane following the disruption of milk fat globules with ethanol or methanol without saponification. This method was originally developed for extraction of tocopherols from human plasma and serum (1-3) and was used for human milk without additional validation (4-5). However, the milk matrix is very different from plasma or serum and the efficiency of liquid-liquid extraction may be different. Additionally, there will be the possibility that some vitamin E through its interaction with other lipid and non-lipid constituents of milk, will not be easily available for extraction using hexane. The official method of measurement of vitamin E in foods, oils and various lacteal matrices involves alkaline saponification and has been widely used (6-8). Saponification is carried out prior to extraction as a separate step and is designed to overcome interactions between lipids, lipid soluble vitamins and the matrix. It is considered essential for perturbing both hydrophobic and polar interactions. Saponification is often regarded as a lengthy and tedious procedure that potentially can cause degradation of vitamin E and this seems to be a possible reason why it has

[#] The research was supported by funds of North Staffordshire Medical Institute, The Institute for Science & Technology in Medicine and The Royal Society.

been avoided (9,10). Sometimes, as a precaution against damage to vitamin E, it is performed under an inert gas (6) and always in the presence of antioxidants such as like pyrogallol, BHT and ascorbic acid (6-8). Use of an internal standard is very important to ensure that this protection is adequate. Here we compared two hexane-based methods of extraction of vitamin E from human milk. One of them is liquid-liquid extraction with hexane after simple saponification and the other is liquid-liquid extraction with hexane directly after disruption of milk fat globules with alcohol.

2. Experimental

All organic solvents were of HPLC grade (HiPerSolv), KOH and HCl (ARISTAR grade) were purchased from VWR International Ltd., Poole, UK. α-, γ-tocopherol and tocopherol acetate, were obtained from Sigma, Dorset, UK. δ-tocopherol was purchased from Supelco, Dorset, UK. Ultra-pure water was used throughout the study.

2.1. Collection of milk samples and preparation of standards

Recruitment of mothers into the study was performed in accordance with the approval of The Local Research Ethics Committee. An informed consent was obtained. Twenty mothers with a plentiful milk supply who have given birth prematurely were recruited into the study (Table1). Milk was expressed into sterile plastic containers using an electrical pump that is used routinely on the neonatal unit. Each milk specimen represented a complete expression of one breast. After collection milk was immediately frozen and kept in the freezer at –40^{o}C until analysis. Analyses were performed within 10 days of collection.

Table 1. Clinical data of milk donors (n=20)

Parameter	Average ± STD	Range
Age, years	28 ± 6	20 – 41
Gestation, weeks	32 ± 4.5	24 – 38
Milk volume per breast, ml	86 ± 32	35 – 170
Days since delivery	9 ± 2.5	3 – 13
Days expressing	8 ± 2.3	3 – 12

Stock solutions of δ-tocopherol standards (10 mg/ml) were prepared in ethanol and kept for one week at –20^{o}C. Spiking solutions of δ-tocopherol standards were prepared on the day of analysis by appropriate dilution of stock solutions. Other tocopherol standards were prepared in the same way.

2.2. Extraction of tocopherols from human milk

After defrosting milk specimens were warmed to 38^{o}C and briefly sonicated to achieve homogeneity of the specimen. For the same reason the sampling of milk was performed at 38^{o}C and continuous stirring. All milk specimens were extracted using both methods in triplicate. To evaluate the content of native δ-tocopherol, all milk specimens were also extracted without δ-tocopherol spike using both methods.

Method I

1 ml of milk was spiked with 10 µl of δ-tocopherol standard (7-10 µg) then 1 ml of metha-nol containing pyrogallol (3%, w/v) followed by 1 ml of aqueous KOH (10%, w/v) were added. Samples were vortexed and placed in the water bath. Saponification was performed at 70°C for 30 min. After the first 15 min of saponification the tubes were briefly vortexed. After cooling the tubes on ice, samples were acidified to pH ~2 with 6M HCl. Then 4 ml of hexane was added. Tubes were vigorously vortexed for 20 sec three times and kept on ice in between mixing. To separate the emulsion the tubes were centrifuged at room temperature at 1300 *g* for 10 min. The top organic layer was carefully transferred to a clean Pyrex container and evaporated on the warm plate set to 40°C under a gentle stream of nitrogen. The fatty residue was reconstituted in 0.5 ml of methanol/propan-2ol (50%/50%, v/v) with warming to 30°C.

Method II

1 ml of milk was spiked with 10 µl of δ-tocopherol standard. To disrupt milk fat globules 1 ml of methanol containing pyrogallol (2%, w/v) was added. Tubes were kept on ice for 10 min. Then 3 ml of hexane was added. After this step the extraction with hexane, separation, evaporation and reconstitution of the samples was identical to Method I described above.

To assess the linearity of the recovery of both methods we used a pooled milk sample (n = 18). Pooled milk was sampled as described above, spiked with different amounts of δ-tocopherol (0.7, 1.4, 2.8, 7, 14 and 28 µg/ml for Method I and 1.49, 4.66, 7.46, 18.5 and 37.3 for Method II, n = 4 for each concentration) and extracted accordingly.

To protect vitamin E from possible degradation during extraction and saponification we have used pyrogallol (1% final concentration).

2.3. Chromatographic system and conditions

A Waters HPLC system (Waters Corp., USA), incorporating an Alliance 2690 separations module and a 996 photodiode array detector and operated by Millennium^{32} software, was used in this study. Separation of tocopherols was achieved using a Waters Symmetry™ C_{18} column (3.9x150 mm) and a guard column, Waters Symmetry™ C_{18} (3.9x 0 mm). Conditions of elu-tion were the following: initially a linear gradient of acetonitrile in water (from 95 to 100% in 10 min) was applied, then 100% of acetonitrile was held for 10 min at a flow rate of 1 ml/min. The column temperature was set to 45°C. The range of wavelengths scanned was 275-350 nm. Samples were kept in an autosampler at 30°C. Injection volume was 25 µl. Peak areas of toco-pherols were integrated at 295 nm. The identification of tocopherols in the HPLC spectra was done by spiking the extracted samples with solutions of tocopherols in ethanol and their co-elution. To ensure a reproducible retention of the column, after each batch of 30 – 40 milk extracts the column was washed with propan-2ol (1 ml/min for 60 min at 45°C). To evaluate the recovery of the δ-tocopherol spike after extraction, 10 µl of the spiking solution was di-luted in 0.5 ml of methanol/propan-2ol (50%/50%, v/v) and 25 µl of this solution was injected on the column. The peak areas of δ-tocopherol spike in milk extracts were expressed as per-centages of the peak areas of δ-tocopherol solutions prepared as described above. The amounts of native α- and γ-tocopherols were calculated on the basis of their peak areas and the percent of recovery. The latter was calculated for each sample using the peak area of δ-

tocopherol spike. Regression analysis was performed using Minitab software. The difference was considered significant for $P<0.05$. Means and standard deviations are presented.

3. Results and discussion

Typical chromatograms at λ= 295 nm showing the elution profile of δ, γ and α–tocopherol in spiked and non spiked human milk are shown in Fig.1. In agreement with previous reports (4-5) δ-tocopherol in milk was not present or its levels were below the limits of UV detection. So, δ-tocopherol was used in the study as an internal standard for quantification of re-covery and levels of other tocopherols. The major forms of vitamin E found

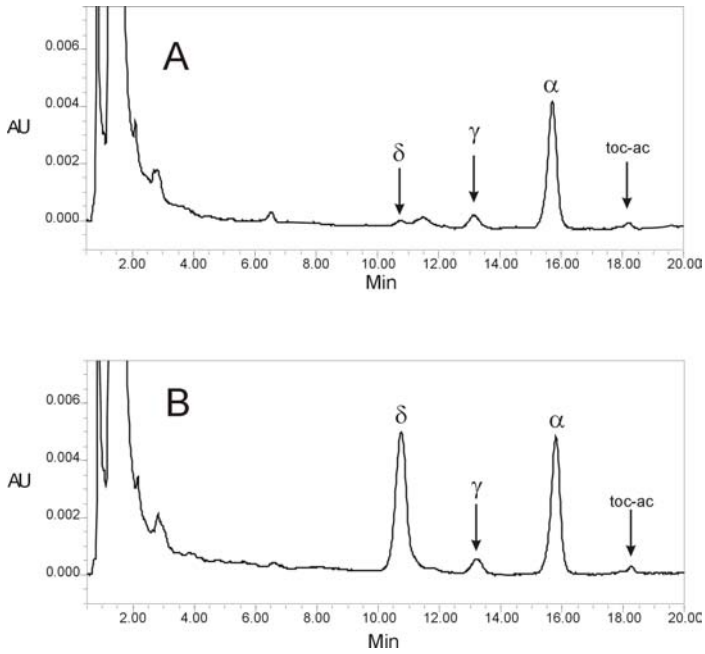

Figure 1 Chromatograms at 295 nm of different forms of vitamin E extracted from human milk using *Method I*. Conditions of extraction are as described in Experimental. A – non-spiked milk specimen, B – the same milk specimen spiked with 7 μg/ml δ–tocopherol. Arrows indicate the elution times for δ–, γ–, α–tocopherols and tocopherol acetate

in milk were α–, γ–tocopherol while tocopherol acetate was present in minor amounts in some milks. Recovery of the δ-tocopherol spike from pooled milk sample was linear within a physiological range of concentrations for both extraction procedures (Fig. 2). For *S/N*>3 the overall detection limit of Method I (including saponification, extraction and HPLC analysis) was 0.65 μg/ml of δ-toco-pherol in milk. Percent of recovery of the internal standard for the two methods of extraction are shown in Fig. 2. When extraction was performed directly, without saponification, reco-very was approximately 60% and variable (range 19–82%) depending on milk specimen. On the other hand, recovery after saponification was nearly 100% within a more

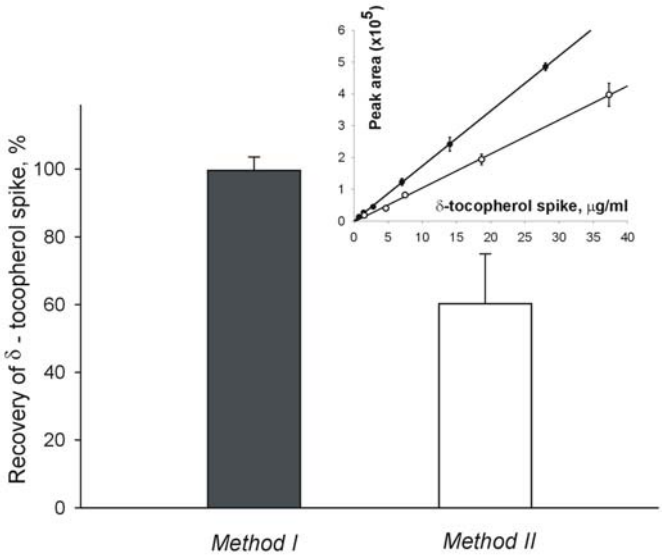

Figure 2. Recovery of the internal standard (δ–tocopherol spike) from human milk using two methods of extraction. Conditions of extraction are as described in Experimental. Means and standard deviations are shown, n = 20. Insert: recovery of δ–tocopherol spike from pooled milk sample using two methods of extraction. Conditions of extraction are as described in Experimental. Regression analysis (Method I: *y(x) = 255 + 17311 x;* $r^2 = 0.999$*;* Method II: *y(x) = 10593 x;* $r^2 = 0.998$) was performed by plotting the integrated peak areas of δ-tocopherol against concentration of spike. Key: closed circles Method I; open circles Method II. Means and standard deviations are shown, n = 4.

reproducible range 94–107%. There are two possibilities such an improvement. First, after saponification the milk matrix does not influence the recovery. Second, the conditions of liquid-liquid extraction with hexane from saponified milk are better optimised than in direct extraction. Decreased recovery of the internal standard leads to less accuracy of measurement. The correlation of α– and γ–tocopherol in milk specimens measured after direct extraction and extrac-tion after saponification is shown on Fig. 3. There was a significant relationship between the two methods of extraction of both, α-tocopherol ($r^2 = 0.868$, $P<0.001$) and γ–tocopherol ($r^2= 0.851$, $P< 0.001$). The levels of the major form of vitamin E, α-tocopherol, in milk specimens were variable within the range of 2.8–15 μg/ml (7.2±4.2 μg/ml (Method I) versus 7.0±4.1 μg/ml (Method II)). Although we were able to demonstrate a significant relationship between measurements obtained with the two methods, the method including saponification gave higher and more consistent recoveries for the internal spike and therefore, more reliable data. The proposed saponification step was a simple addition to the method as it was performed in the same tube as the extraction, it only took 30 min and did not involve the use of an inert gas.

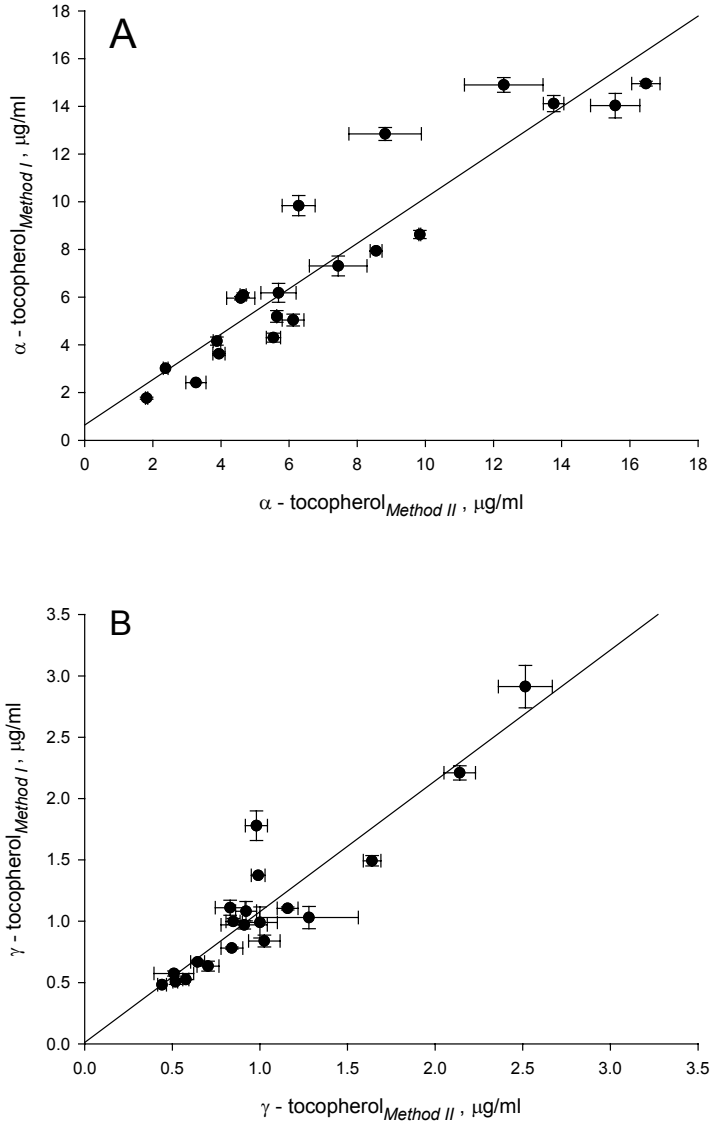

Figure 3. Correlation of the levels of native α– and γ–tocopherol in human milk specimens measured as a result of two methods of extraction. A – Regression analysis (*y(x) = 0.063 + 0.952 x*; $r^2 = 0.868$; $P < 0.001$) was performed by plotting the integrated peak areas of native α-tocopherol in 20 milk specimens measured after extraction using Method I against the integrated peak areas of native α-tocopherol in the same specimens measured after extraction using Method II. B. Regression analysis for γ – tocopherol (*y(x) = 0.01 + 1.07 x;* $r^2 = 0.851$, $P < 0.01$) was performed in the same way as for α-tocopherol. Average and standard deviation of three measurements for the same milk specimen with two methods are shown.

Conclusions

Two extraction methods for the evaluation of vitamin E in human breast milk were com-pared. Direct extraction of tocopherols with hexane allowed 60±15% recovery of the internal standard, δ-tocopherol. Application of the improved method which included a simple saponi-fication step gave 99.6±4.0% recovery for the same internal standard. There was a significant relationship between tocopherol concentrations in milk specimens measured using the two extraction procedures (P<0.01). This implies that both methods of extraction can be used for determination of vitamin E in human milk, however the evidence presented herein suggests that extraction after saponification may significantly improve the reliability of such data.

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GC MS IN INVESTIGATION OF FRACTIONS OBTAINED ON THERMOCATALYTIC DEGRADATION OF POLYMER WASTE

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Introduction

Since the middle of the 20th century polymers have become inherently present in our everyday life. Increasing production and use of such polymers as polyethylene, polypropylene or polystyrene raised the problem of dealing with their waste. Even today the production of polymers increases as it is evidenced by the appearance of new production lines, e.g. in Poland the Polski Koncern Naftowy "Orlen" has invested in new installations for production of polyolefin. The only installation of catalytic cracking in Poland, localised in Płock, aims at producing maximum amounts of ethylene and propylene being initial components for production of polyethylene and polypropylene. These two and polystyrene give the greatest contribution in plastic waste. There are two main methods of disposal with plastic waste: the first is the energy recycling in which plastic waste is used as fuel and the second is the materialrecycling, which is more difficult but also gives greater possibilities. It is known that thermal decomposition of polymers may give multicomponent liquid products, and as a consequence it provokes some troubles in using the fractions obtained [1]. If the fractions are to be used for composition of fuels, and in particular Diesel oil or heating oil, the number of components in a given liquid fraction must be considerably reduced. GC-MS seems to be the best method for investigation of complex multicomponent mixtures [2]. It permits determination of the structure of each component and hence estimation of the effect of a given component on the final mixture to be used as fuel. The GC-MS method also allows an estimation of a percent composition of the liquid fractions obtained from the thermocatalytic decomposition of waste polymers, which is sufficient when the processes are conducted on industrial scale and the components are mixed in hundreds of tons. It has been shown that the use of such catalysts as aluminosilicates can effectively reduce the number of components of the liquid fraction obtained from decomposition of plastic waste [1-3]. Also such substances as TiO_2, Fe_2O_3 or ZrO_2 have been found to be effective catalysts of polyolefin degradation [2,4]. Thus it is interesting to check if the petrocoke, obtained during joint distillation of used oil and polymers and rich in d-group metals, can also work as catalysts leading to collection of fractions of desired parameters. The types of petrocoke studied included the coke obtained after distillation of used oil and that obtained after distillation of used oil with addition of 50%wt of polymers mixture containing 45% wt of polyethylene, 35% wt polypropylene and 20% wt of polystyrene. The petrocoke samples were obtained in the laboratory in a steel reactor.

Experimental

The processes of polymer degradation were conducted in the glass apparatus schematically shown in Fig. 1. The reactor was the three-necked flask equipped in a thermocouple. The reaction was performed for a mixture of polymers without petrocoke (model), for a mixture of

polymers with 10% wt of the petrocoke obtained from distillation of used oil (petro 0%) and for a mixture of polymers with 10% wt of the coke obtained from distillation of used oil and 50% admixture of polymers (petro 50%). There was a mercury thermometer at the top of the column. The polymer load was: PE – 18g, PP – 14g, PS – 8g, which gives 45% PE, 35% PP and 20% PS. The catalyst addition was 10% thus 4g.

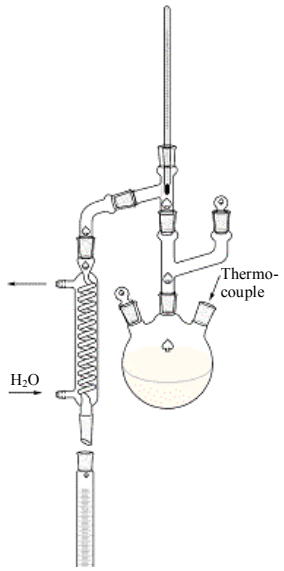

Figure 1. Apparatus for processes of polymer degradation

Each process was performed in the same conditions. The flow of the water in the cooler and the power of the heating cup were fixed. Parameters of the reaction: temperature of the reaction mixture, temperature at the top of the column (vapours blown into the cooling system) and increase in the volume of the liquid phase, were read out and noted every two minutes. Changes in these parameters are illustrated in Figs. 2,3,4. The liquid phases obtained were collected and characterised by different methods. Among others these phases were subjected to GC – MS measurements in the system of the gas chromatograph 5890 Series II and the analyser of the mass spectrometer AMD 402. The chromatograph was equipped with a 30 metres long capillary column RTX – 5MS.

The stationary phase was a mixture of 95% of dimethylosiloxane and 5% diphenylo-siloxane. The carrier gas (helium) flow rate was 1 ml/min. The temperature programme was: 2 minutes at 80ºC followed by heating to 300ºC in 10 minutes. The latter temperature was maintained for 15 minutes. The electron impact ionisation method was applied. The chromatograms obtained (Figures 5,6,7) permit determination of the qualitative composition of the mixture and estimation of its quantitative composition.

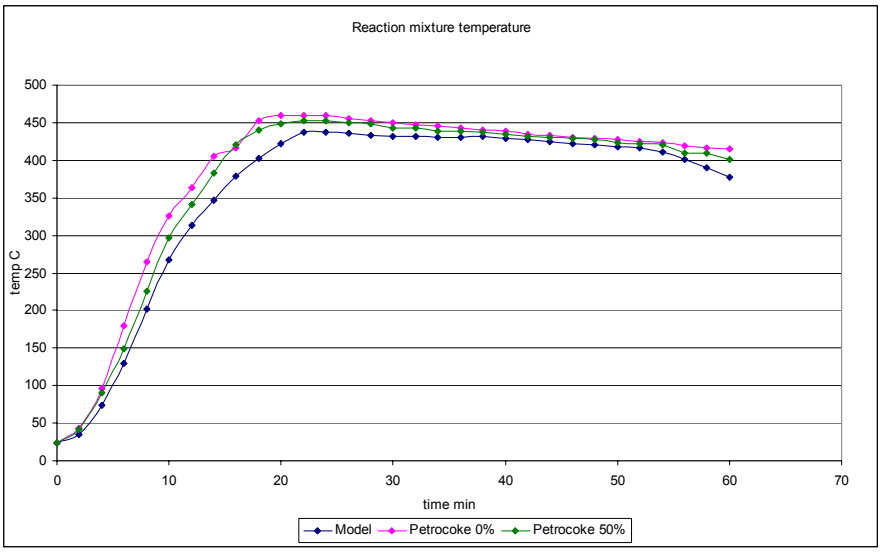

Figure 2. Changes of mixture temperature over time.

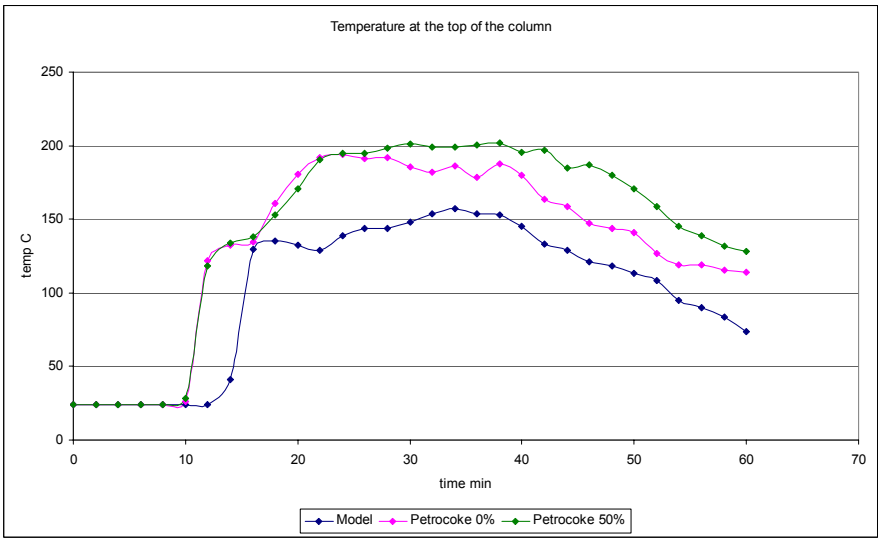

Figure 3. Changes of the temperature at the top of the column over time.

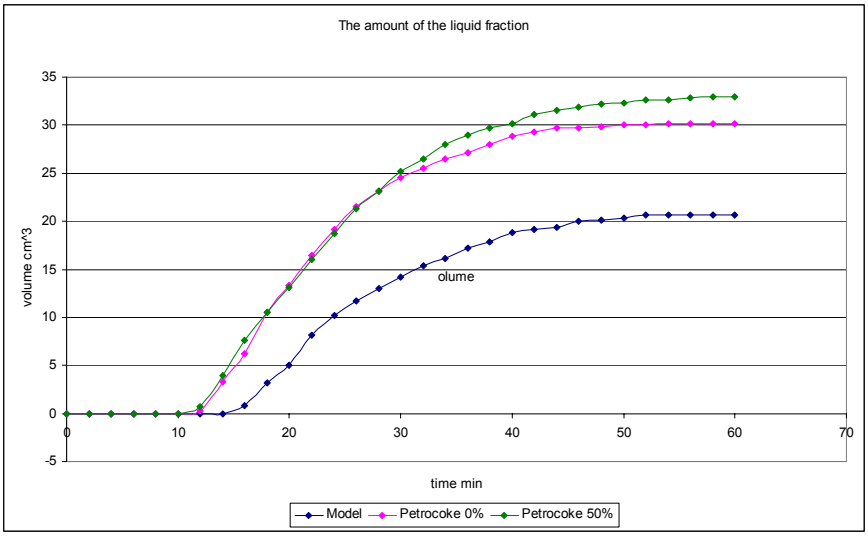

Figure 4. The amount of the liquid fraction obtained as a function of time.

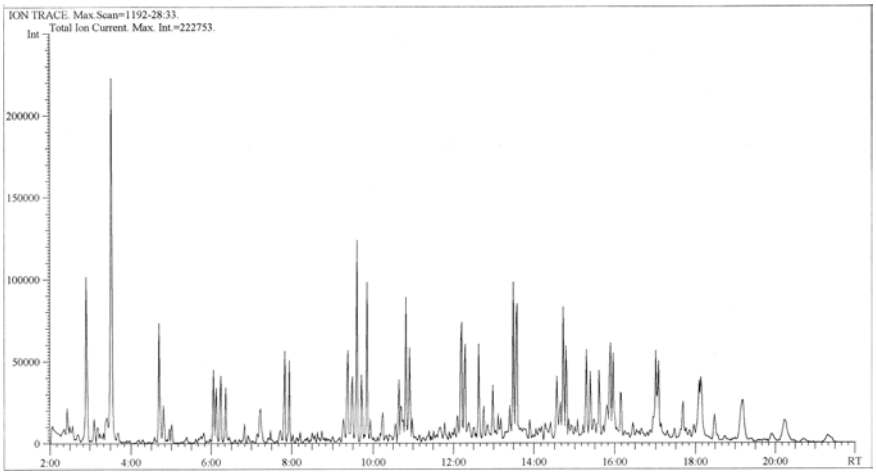

Figure 5. Products of the process without a catalyst - "Model".

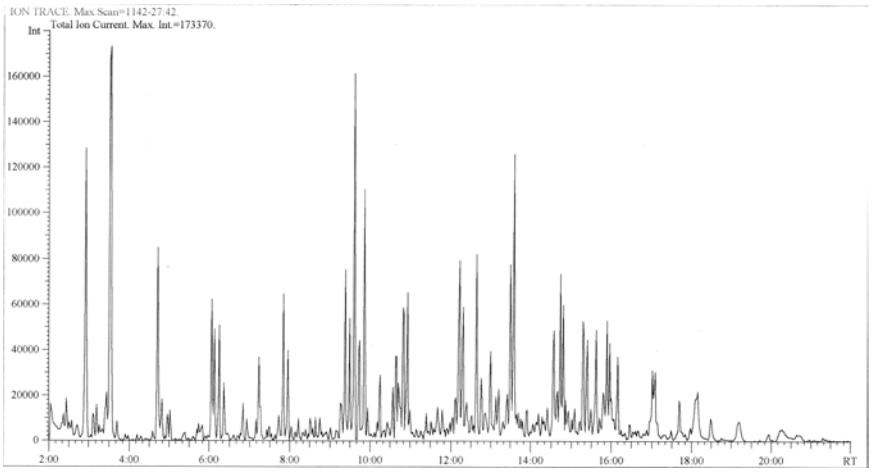

Figure 6. Products of the process with petrocoke "0%" as a catalyst.

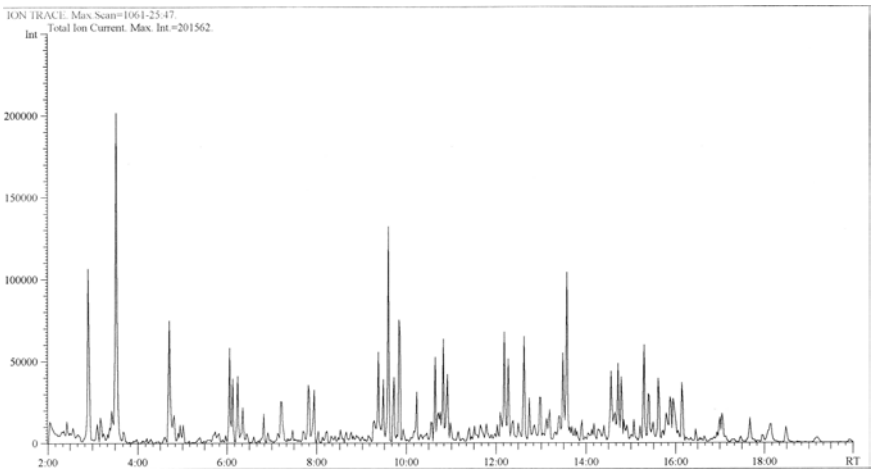

Figure 7. Products of the process with petrocoke "50%" as a catalyst.

The chromatograms testify to the presence of a whole gamut of compounds formed in the process. As only large amounts of the compounds can affect the quality of the fuel to which a given fraction would be added, an analysis was performed of the most intensive signals. Table 1 gives the list of compounds that were identified. In the chromatograms of chain hydrocarbons present as vapours there are two peaks typical of polyolefin decomposition, one assigned to alkene with one double bond, and the other to alkane. Table 1 does not give the twin alkanes for higher polyolefins, but their presence is confirmed in the chromatograms.

Table 1. Destilation products/retention time.

Compound	Retention time
2,4-dimethylo-1-heptene	2:54
styrene	3:31
α-methylo-styrene	4:42
3,5-dimethylo-heptane	4:48
1-dodecen	7:50
dodecan	7:56
1-tetradecen	10:49
tetradecan	10:55
1-pentadecen	12:12
1-hexadecen	13:30
1-heptadecen	14:43
1-octadecen	15:53
1-nanodecen	17:00

The above-presented results proved the expectations for a usefulness of GC-MS method and further experience in catalysis of polymer decomposition. Our research permits such a design of the process that the fraction obtained could be an effective fuel component. It has already been established that polyolefin decomposition involves mainly formation of chain unbranched hydrocarbons. It would perfect to be able to obtain the fraction C_5-C_{10} with the highest possible number of structural isomers. Polystyrene decomposition gives mainly two products: styrene and ά-methyl-styrene, which would help control the content of aromatic compounds in the fraction obtained. The ideal solution would be to get only one product from each polymer, however, it seems hardly achievable in the near future.

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DETERMINATION OF FATTY ACID METHYL ESTERS IN DIESEL OILS

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Key words: FAME (*Fatty Acid Methyl Esters*)**,** biocomponents, plant oils, diesel oil, GC

Abstract

Although in Poland the regulations on obligatory addition of biocomponents (esters of plant-origin fatty acids) to liquid fuel have not been introduced yet, the EU directions require that the relevant laboratories would be prepared to determine the content of biocomponents in fuel. In Poland there is a standard referring to determination of biocomponents in petrol, however, no analogous solution has been recommended for Diesel oils. There is a Polish standard EN 14103, however, it concerns analysis of pure biocomponents being fatty acid methyl esters (FAME) and permits determination of their composition prior to their addition to Diesel oil.

As the samples to be studied have a complex hydrocarbon matrix, gas chromatography was selected as the analytical method because it permits qualitative and quantitative characterisation of the fuel. In our study a standard gas chromatograph equipped with a flame-ionisation detector and a polar capillary column was applied. The method proposed permits determination of the biocomponents after their addition to Diesel oil. It enables analysis of fatty acids esters (of rapeseed oil origin) as well as discernment whether given esters are of rapeseed oil or other origin. The method was tested on Diesel oil samples containing biocomponents obtained as a result of transestrification of common plant-origin oils such as coconut, maize, flax, rice, sesame, sunflower, soybean, olive, grape stone, pumpkin stones and their mixtures.

The range of determined FAME concentrations is consistent with the recommendations of both Polish and European standards. The proposed procedure of determination of the content of biocomponents in liquid fuel was subjected to validation, determining such parameters as linearity, detection limit, trueness, intermediate precision and uncertainty.

Introduction

The methods most often used in analysis of fatty acids methyl esters (FAME) are gas chromatography (GC) and high-performance liquid chromatography (HPLC). Of these two GC has the widest application in analysis of biodiesel oil. First of all GC with capillary columns is used for determination of FAME as pure biofuel or a bioaddition to diesel oil. The first paper on analysis of esters by GC by Friedman et al. z 1986, concerned methyl and butyl esters from soybean oil, however, not all components of the biofuel were identified then.

American Oil Chemist's Society (AOCS) officially recognised the GLC with capillary columns as the fundamental in FAME analysis [1]. In Europe the recommended FAME determination is described in the standard EN 14103 [2]. GC is characterised by high precision and accuracy, however, the results are affected by the peak overlapping or drifting baseline [3].

GC analysis of FAME is performed mainly with polar columns. The majority of GC analyses are made with a flame ionisation detection (FID), but equally good results are obtained with mass spectrometry detection (MSD). The less common use of the latter follows from economical reasons. Hitherto studies on improvement of the method have shown that the results of analysis are influenced e.g. by the length and diameter of the column, thickness of the film, type of the film or the split used [4].

The most often used FAME dosage technique is the classical split [5,6], which eliminates the risk related to reloading the column when the analyte contains large amounts of FAME. The typical split used in analysis of methyl esters varies from 1:10 to 1:200, however, it did not permit detection of all esters, which is related to the similarities in their structures and small differences in mass. This problem was solved by applying the cold sample injection, e.g. on-column injection [4] or programmed-temperature vaporizing (PTV) injection [2,4].

The simplest method of quantitative determination of FAME is based on the use of internal standard. An alternative is the method of the calibration curve but it is more time-consuming and requires a good repeatability of results of analyses.

Replacement of GC by LC ensures a shorter time of analysis [3] and permits analysis of a wider range of the biodiesel components (methyl esters, mono-, di- and triglycerides, glycerine) without the need of preliminary transformations. The method permits only total determination of the content of particular classes of compounds.

HPLC can be used with different detectors: UV [7,8], density detection [9], flame – ionisation (FID) [10], refractive index detection [11,12], evaporative light scattering detection (ELSD) [13,14], mass spectrometry (MSD) [15] or atmospheric pressure chemical ionization mass spectrometry (APCI – MS).

Holcapek, 1999, has shown that for ELSD and APCI – MS detection the method's sensitivity decreases with increasing number of double bonds in the ester chain, while the UV detection does not quantify saturates. Refractive Index detection is mainly used in the isocratic HPLC for determinations of fats. The density detection is not used because of the need of using the mobile phase and the solvent showing toxic properties and the FID detection shows limitations in response factors [16].

The other chromatographic methods used for analysis of FAME include the gel chromatography ensuring a short time of FAME analysis. In this method the separation is based on the hydrodynamic volumes or effective molecular size in solution and it gives only total contribution of particular groups of compounds from the biodiesel [17]. This method often works with the refractive index detection [17].

The first method used for control of the process of biodiesel production was (TLC/FID) [18]. It is a good and simple analytical method, nevertheless it was abandoned because of low accuracy and relatively high cost of equipment needed [3].

Analysis of biodiesel was also performed with a combination of two LC and GC methods in the hope of elimination of the problems encountered in GC method alone and improvement of compound separation [19].

Besides the chromatographic methods, also spectroscopic ones have been used in analysis of biodiesel. Some test studies were performed with ^{1}H and ^{13}C-NMR. Gelbard et al., [7] reported using ^{1}H-NMR to monitor the number of protons bonded to mono-, di- and

triglycerides and the proton from the methyl group in the ester. Dimmig [8] proposed the use of ^{13}C-NMR for monitoring of the methyl group from the esters and the glyceride carbon from glycerides. Another technique useful for monitoring of the reaction of transestrification is the near infrared spectroscopy NIR [20], because the ester methyl groups give strong absorption bands at 6005cm^{-1} and 4425-4430cm^{-1} , while triglycerides give only broadened bands.

The NIR method [20] is less time-consuming, easier and faster than GC. It has been suggested that GC should be a supplementary method of NIR and not the main recommended one. The first attempts at fat analysis by NIR were reported over 40 years ago [21].

Analytical part

1. The range of application

The method proposed in this work can be applied for determination of biocomponents that is FAME, obtained by transestrification of plant oils, in the diesel fuel. The method permits determination of the FAME concentrations in the range covering those recommended by the Polish and European standards, i.e. up to 5% (w/w).

2. Equipment

a) Gas chromatograph equipped with a flame ionisation detector (FID) and split-splitless injector (HP 5890 series II)
b) Capillary column with a polar phase of the polyethylene glycol type (we used a J&W INNOWAX column of 30m in length, internal diameter of 0.32mm, coated with a stationary film phase of 0.5μm in thickness).
c) Microliter syringes of 5 mm^3 in capacity, with scale interval of 0.05mm^3 and of 100mm^3 in capacity with scale interval unit of 1mm^3.
d) Measuring flasks of 10cm^3 or 25cm^3 in capacity, pipettes and micropipettes.

3. Standards

The standards used were chromatographically pure, purchased from Fluka, Sigma and Aldrich. The diluents can be hydrocarbons with no impurities interfering in the analysis of the components determined, e.g. heptane.

4. Obtaining of standard mixtures of FAME of plant oil origin [22]

The reaction of transestrification, i.e. the reaction of methanol solution of potassium hydroxide with glycerides (soybean oil), was carried out at ambient temperature. A portion of 2.5g of potassium hydroxide was dissolved in 23cm^3 of methyl alcohol containing at the most 0.5% of water. To this solution 150cm^3 of soybean oil was added. The mixture was stirred as long as glycerine stopped evolving, that was for about 1 hour. The bottom layer of glycerine was removed and the rest of the mixture containing traces of KOH and methyl alcohol (the majority was in the glycerine layer) was neutralised by orthophosphoric acid of a concentration of about 70%. Trace amounts of methyl alcohol, K_3PO_4 and H_3PO_4 were removed by washing the ester layer a few times with water till neutralisation (disappearance of acidic pH). The ester layer was dried with anhydrous sodium sulphate (VI) to prevent hydrolysis of the esters obtained. The reaction gave a mixture of methylene esters of the following acids: 9.99% palmitic, 3.20% stearic, 24.89% oleic, 48.55% linoleic, 4.85% linolenic, and 8.48% of other acids. The esters were used for preparation of mixtures used for determination of FAME in Diesel oil.

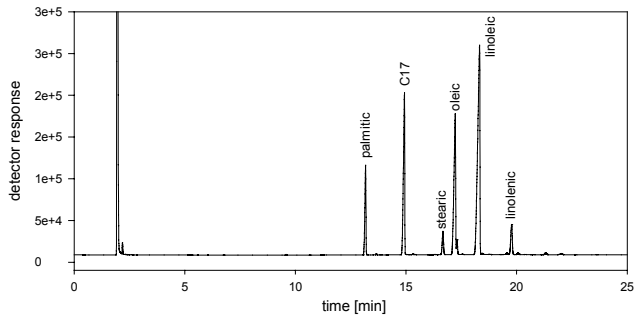

Fig. 1 Chromatogram illustrating the sequence of elution of fatty acid methyl esters obtained in transestrification of soybean oil (0.5% concentration in heptane).

5. Preparation of standard solutions and analyses in the Interpolative Internal Standard Method (IISM) of FAME determination in diesel fuel

Preparation of internal standard solution. The internal standard solution was prepared in a concentration of 10mg/cm^3 in heptane. After each operation (internal standard addition, supplementing of the measuring flask to the mark with heptane) the solution was weighted to the accuracy of 0.0001g.

Preparation of standard mixtures. Standard mixtures were prepared of methyl esters weighted to the accuracy of 0.0001g. The standard mixtures contained:
Standard A – 0.7% (w/w) of each of the methyl esters of the following acids: lauric, myristic, palmitic, stearic, oleic, linoleic, linolenic in diesel oil.

Standard B – contains 0.2% of each of the methyl esters of the following acids: lauric, myristic, palmitic and stearic and 1.2% of each of the methyl esters of oleic, linoleic, linolenic acids in diesel oil.

Standard C – contains esters of the same acids as in standard B but in the reverse concentrations, so those in high concentrations in standard B are in low ones and vice versa.
1cm^3 potions of the standard solutions were placed in a 10cm^3 flask. Then to each of the flasks 1cm^3 of the internal standard solution was added and the flask volume to the mark was supplemented with heptane. After each operation the flask was weighted.

Preparation of diesel oil to be analysed for FAME determination. To a 10cm^3 flask a portion of 1cm^3 of the oil studied and 1cm^3 of the internal standard solution were placed then the flask was supplemented with heptane. After each operation the flask was weighted to the accuracy of 0.0001g.

Retention time determination. On the basis of the chromatograms of the standard mixtures, the mean retention times are calculated relative to that of methyl heptadecanoate. The retention times obtained in the chromatographic conditions described below are given in Table 1.

Chromatographic analysis. In both above described methods, the chromatographic analysis was performed on a J&W INNOWAX column at the carrier gas flow rate of 2.7cm^3·min^{-1} and the temperature program 170^{o}C (5min) 6^{o}C·min^{-1} 230^{o}C. On the basis of the retention times obtained from the chromatograms recorded, the compounds occurring in a sample analysed can

Table 1. Relative retention times

Chemical compound	Relative retention time
lauric acid methyl ester C12	0.42
myristic acid methyl ester C14	0.64
palmitic acid methyl ester C16	0.88
methyl heptadecanoate C17	1.00
stearic acid methyl ester C18	1.12
oleic acid methyl ester C18:1	1.15
linoleic acid methyl ester C18:2	1.22
linolenic acid methyl ester C18:3	1.33

be identified. The chromatograms presented in Fig. 2 may be helpful for identification of the esters. Analogous analysis should be performed on a column of a different polarity (e.g. CP-Sil 5CB), in order to make sure that there are no other compounds of the same retention times.

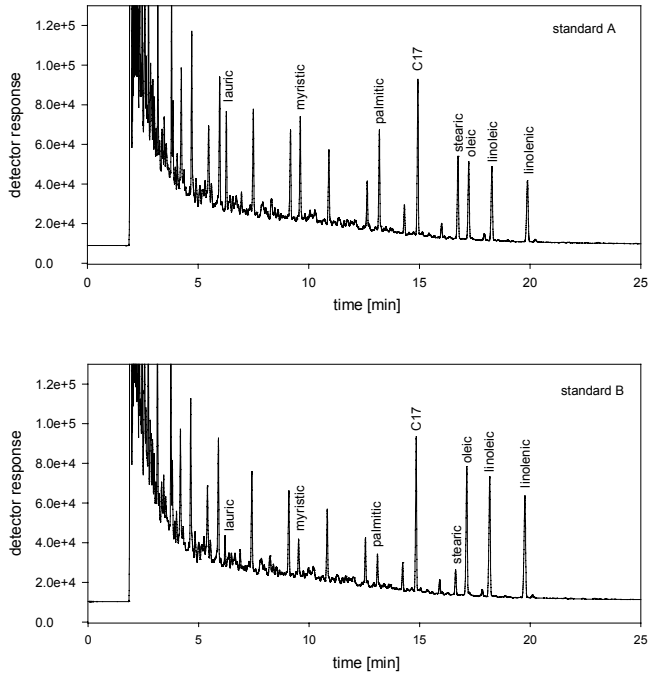

Fig. 2. Chromatograms obtained by the method of internal standard (standards A and B)

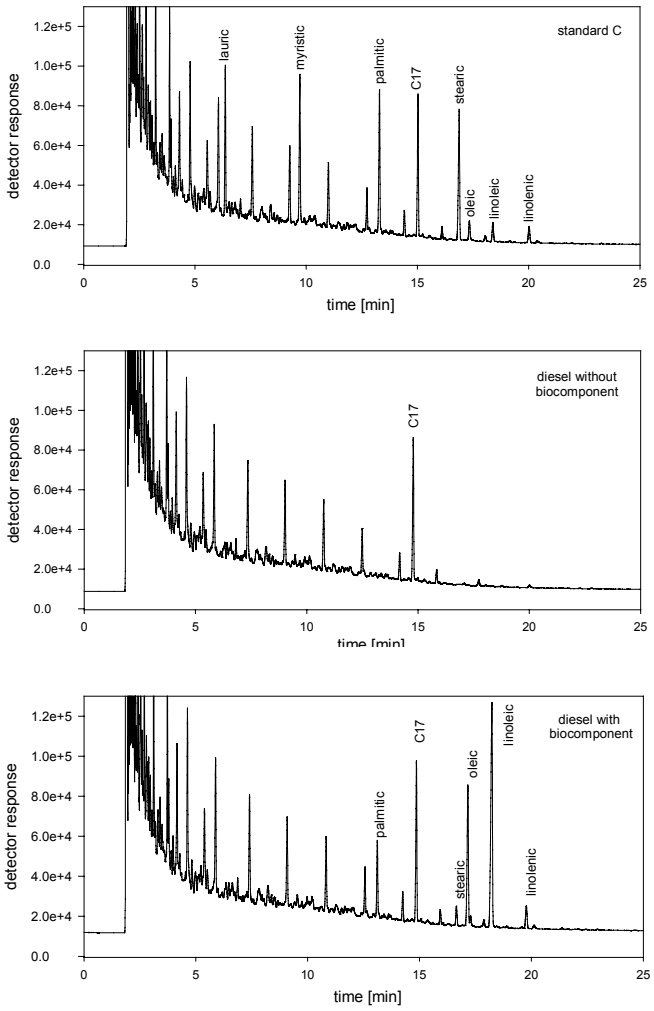

Fig. 2. (continued) Chromatograms obtained by the method of internal standard (standard C and the diesel with and without biocomponent)

Calculation of correction factor. Three chromatograms of each of the above standard mixtures should be taken and for each of them the values of the correction factor are calculated. The final correction factor is calculated as the arithmetic mean of the correction factors calculated from eq. (1) for different chromatograms, when their values do not differ from each other by more than 0.02. The correction factor (f_i) are calculated from the equation (1)

$$f_i = \frac{G_i A_w}{G_w A_i} \tag{1}$$

where:
G_i – content of the component to be determined
G_w – content of the standard
A_i – the area of the peak corresponding to the component to be determined
A_w – the area of the peak corresponding to the standard.

Calculation of the content of methyl esters. Identification of the components can be performed on the basis of the relative retention times or by comparing the chromatogram of the sample with that of the standard mixtures. The percent content of a given component in a sample is calculated from equation (2):

$$\%(m/m)_i = \frac{G_w A_i f_i}{G_i A_w} \cdot 100 \quad (2)$$

where:
G_i – mass of the sample
G_w – mass of the standard added to the sample
A_i – the area of the peak of the component to be determined
A_w – the area of the peak of the standard
f_i – the correction factor of the component to be determined.

The result is converted into % (v/v) according to equation (3):

$$\%(V/V)_i = \frac{\%(m/m)_i \cdot d_D^{20}}{d_E^{20}} \quad (3)$$

where:
d_D^{20} - density of the diesel analysed, g·cm^{-3}
d_E^{20} - density of the ester to be determined, g·cm^{-3}

The amount of biocomponents added to the diesel oil was calculated by summing up the amounts of particular esters. Analysis of the percent contribution of particular esters in the biocomponent brings the information on the type of oil used for production of the esters added. The fatty acids compositions characterising biocomponents obtained from particular plant sources are given in Table 2.

6. Determination of the validation parameters [24, 25]

Linearity. In the method based on the internal standard application the accuracy of the results was improved by the correction factors. They were determined for different concentrations so the ratio of the area of the peak corresponding to a given ester to the area of the peak corresponding to the internal standard can be used for verification of the linearity of the detector indications.

Table 2. Fatty acid composition in % in oils coming from different plant sources [23]

Oil or Fat	Fatty Acid Composition (Wt. -%)							
	lauric C12	myristic C14	palmitic C16	stearic C18	oleic C18:1	linoleic C18:2	linolenic C18:3	erucic C22:1
Babassu	44-45	15-17	5.8-9	2.5-5.5	12-16	1.4-3		
Canola			4-5	1-2	55-63	20-31	9-10	1-2
Coconut	44-51	13-18.5	7.5-10.5	1-3	5-8.2	1.0-2.6		
Corn			7-13	2.5-3	30.5-43	39-52	1	
Cottonseed		0.8-1.5	22-24	2.6-5	19	50-52.5		
Linseed			6	3.2-4	13-37	5-23	26-60	
Olive		1.3	7-18.3	1.4-3.3	55.5-84.5	4-19		
Palm		0.6-2.4	32-46.3	4-6.3	37-53	6-12		
Peanut		0.5	6-12.5	2.5-6	37-61	13-41		1
Rapeseed		1.5	1-4.7	1-3.5	13-38	9.5-22	1-10	40-64
Safflower			6.4-7.0	2.4-29	9.7-13.8	75.3-80.5		
Safflower. high-oleic			4-8	2.3-8	73.6-79	11-19		
Sesame			7.2-9.2	5.8-7.7	35-46	35-48		
Soybean			2.3-11	2.4-6	22-30.8	49-53	2-10.5	
Sunflower			3.5-6.5	1.3-5.6	14-43	44-68.7		
Tallow (beef)		3-6	25-37	14-29	26-50	1-2.5		

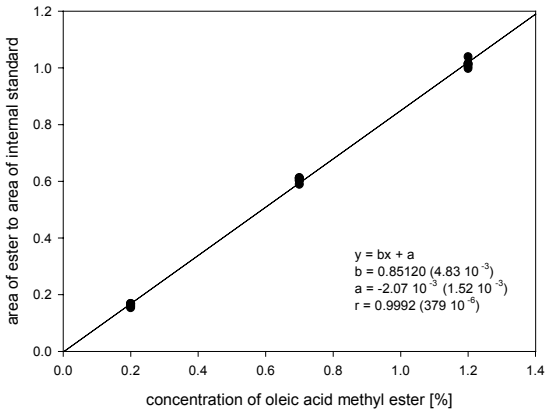

Fig. 3. The ratio of the area of the peak corresponding to oleic acid methyl ester to that of the peak corresponding to the internal standard versus the ester concentration.

Table 3. The regression parameters calculated from the ratio of the area of the peak corresponding to the ester under determination to that of the peak corresponding to the internal standard.

Ester under determination	Regression parameters of the equation y = bx + a		
	b	a	r
palmitic	0.79827 (7.87 10^{-3})	0.03061 (0.0110)	0.9993 (612 10^{-6})
stearic	0.94042 (0.0171)	-0.01915 (0.0131)	0.9989 (1.01 10^{-3})
oleic	0.85120 (4.83 10^{-3})	- 2.07 10^{-3} (1.52 10^{-3})	0.9992 (379 10^{-6})
linoleic	0.85959 (0.0155)	0.01237 (5.31 10^{-3})	0.9977 (1.56 10^{-3})
linolenic	0.84418 (0.0193)	1.59 10^{-3} (7.99 10^{-3})	0.9998 (316 10^{-6})

Limit of detection. The limit of detection for particular esters was estimated as three times the signal to noise ratio on the basis of a chromatogram recorded for blank sample. The values are comparable for all esters and equal 5· 10^{-4} %(w/w).

Repeatability. The repeatability of the method proposed was assessed on the basis of the calculated standard deviation values of content determinations of particular methyl esters in the diesel oil containing the biocomponent obtained from transestrification of soybean oil. The measurements were performed for four series of six independent analyses. Measurements for each series were made on the same day. The results are given in Table 4.

The repeatability estimated as a relative standard deviation varies between 1 and 5%.

Table 4. Concentrations of particular methyl esters obtained from transestrification of soybean oil in a sample of diesel oil – estimation of the method repeatability.

Ester determined	Series number	Ester concentration % [w/w]						Mean	Standard deviation	RDS
palmitic	1	0.5596	0.5640	0.5997	0.5673	0.5694	0.5604	0.5701	0.0150	0.0263
	2	0.5686	0.5497	0.5625	0.5646	0.5594	0.5821	0.5645	0.0107	0.0190
	3	0.5760	0.5643	0.5661	0.5406	0.5565	0.6173	0.5701	0.0260	0.0455
	4	0.6389	0.6069	0.6036	0.6142	0.5963	0.6522	0.6187	0.0220	0.0356
stearic	1	0.1734	0.1770	0.1679	0.1711	0.1771	0.1678	0.1724	0.0042	0.0242
	2	0.1618	0.1668	0.1837	0.1668	0.1819	0.1768	0.1730	0.0091	0.0524
	3	0.1677	0.1645	0.1634	0.1656	0.1631	0.1646	0.1648	0.0017	0.0102
	4	0.1700	0.1643	0.1784	0.1704	0.1654	0.1623	0.1685	0.0058	0.0346
oleic	1	1.3631	1.3319	1.3180	1.3424	1.3388	1.3608	1.3425	0.0172	0.0128
	2	1.3260	1.3148	1.3221	1.3383	1.3175	1.3410	1.3266	0.0108	0.0082
	3	1.3198	1.3430	1.3005	1.3164	1.2960	1.3191	1.3158	0.0167	0.0127
	4	1.3269	1.3482	1.3536	1.3325	1.3380	1.3418	1.3402	0.0099	0.0074
linoleic	1	2.5400	2.5739	2.5373	2.5265	2.5208	2.5672	2.5442	0.0216	0.0085
	2	2.5221	2.5107	2.5094	2.5610	2.5152	2.5388	2.5262	0.0202	0.0080
	3	2.5370	2.5311	2.5345	2.5531	2.5540	2.5141	2.5373	0.0149	0.0059
	4	2.5253	2.5548	2.5601	2.5348	2.5321	2.5300	2.5395	0.0143	0.0056
linolenic	1	0.2706	0.2598	0.2704	0.2565	0.2624	0.2567	0.2627	0.0064	0.0244
	2	0.2616	0.2586	0.2622	0.2604	0.2469	0.2649	0.2591	0.0063	0.0244
	3	0.2583	0.2661	0.2587	0.2627	0.2556	0.2697	0.2619	0.0053	0.0204
	4	0.2486	0.2474	0.2600	0.2599	0.2498	0.2440	0.2517	0.0067	0.0268

Indirect precision. The indirect precision was estimated as the relative standard deviation calculated for all results, that is for the 4 series of 6 results. The calculations were made for the same data as those used for the assessment of the method's repeatability (Table 4). The values obtained are presented in Table 5.

Table 5. Indirect precision calculated for particular esters obtained from transestrification of soybean oil in the diesel oil, together with the values of extended uncertainty (U) and the coefficient of extension (k).

Ester determined	Mean	Standard deviation	RSD	U	k
palmitic	0.5808	0.0288	0.0496	0.064	2.7
stearic	0.1697	0.0064	0.0377	$4.8\ 10^{-3}$	2.2
oleic	1.3313	0.0171	0.0129	0.023	2.1
linoleic	2.5368	0.0181	0.0072	0.036	2.0
linolenic	0.2588	0.0073	0.0283	$4.3\ 10^{-3}$	2.0

As expected, the indirect precision is higher than the repeatability as the former is affected by a greater number of variables (e.g. the time of measurements).

Uncertainty. The extended uncertainty does not belong to the basic validation parameters, however, it is often used for assessment of suitability of a given analytical procedure. It describes the quality of the results. The uncertainty was calculated according to the GUM recommendations [26]. He calculations were performed by the software recommended by GUM, that is by GUM Workbrench provided by Metrodata GmbH [27]. The concentrations of particular esters in diesel oil were calculated by the earlier described relation eq.(2).

The calculations were performed for the same results as those used for estimation of the method's repeatability.

The values of total and extended uncertainties were estimated taking into regard the effect of the following parameters.

a) Standard uncertainties related to determination of the correction factor:
   - Standard's purity
   - Standard's mass
   - Mass of the standard containing solution
   - Internal standard purity
   - Mass of the internal standard
   - Mass of the internal standard containing solution
   - Peak areas assigned to the standard and the internal standard.

b) Standard uncertainties related to the sample:
   - Internal standard's purity
   - Internal standard's mass
   - Mass of the solution containing internal standard
   - Mass of the diesel oil sample with biocomponents
   - Peak areas assigned to the ester under determination and the internal standard.

The values of the extended uncertainty and extension coefficient calculated by the above-mentioned software are given in Table 5.

Accuracy. The method's accuracy was estimated on the basis of a comparison of the results obtained with the values determined by the gravimetric method in the procedure of preparation of the reference standards of the known content of methyl esters in diesel oil. Prior to biocomponents addition to the diesel oil sample the composition of the biocomponents was determined by the chromatographic method.

The uncertainty was also assessed for the reference standard.

The consistence of the results was verified using the following relation:

$$| x - x_{ref} | < 2\sqrt{u(x)^2 + u(x_{ref})^2} \quad (4)$$

When the relation holds, the result is assumed consistent with the reference value.

Table 6. Comparison of the results of measurements with the reference values taking into account the uncertainties.

Ester	Reference value x_{ref}	Uncertainty for reference material U	$\| x - x_{ref} \|$	$2\sqrt{u(x)^2 + u(x_{ref})^2}$
palmitic	0.5570	0.0110	0.0238	0.0649
stearic	0.1691	0.0026	0.0006	0.0055
oleic	1.3420	0.0160	0.0107	0.0280
linoleic	2.5090	0.0280	0.0278	0.0456
linolenic	0.2607	0.0031	0.0019	0.0106

Conclusions

1. The procedure proposed permits determination of FAME in Diesel oil. It does not require special initial sample preparation and can be realised on a standard gas chromatograph with a polar capillary column.
2. The procedure is characterised by high accuracy and precision.
3. The procedure permits determination of fatty acid esters in the ranges recommended by the European and Polish standards.

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DETERMINATION OF TRACE AMOUNTS OF BISPHENOL-A IN WATER SAMPLES BY THE USE OF GAS-CHROMATOGRAPHY

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Abstract

In the presented work, some results of determination of BPA (bisphenol A) are given, obtained by an application of SPE (solid phase extraction) method for the extraction of the liquid-solid type, and further analysis of such obtained extract by the gas chromatography. It was proven that BPA is set free from the polycarbonate containers and migrates to the food and the water these containers contain.

Introduction

Even if the estrogene properties of bisphenol A (BPA, 2,2-bis-(4-hydroxyphenylo)-propane) have been reported since 1936 (C. Doods and W. Lawson [1]), only recently an attention has been paid to this compound [2,3]. Bisphenol A is widely used in chemical industry to produce: polycarbonate resins (PC), epoxy resins, unsaturated polyester and polysulphonate resins, and many more. Various products containing BPA are commonly used in many domains of our life, to mention lacquers covering cans for drinking water, food, and medicines. In addition, polycarbonate resins containing BPA are used to produce several internal elements of medical apparatus (e.g., for dialysis, blood oxidizing), baby bottles, and some plastic kitchen ware, as plates, cups, spoons, etc.

BPA, since 1996, has been classified by the European Commission as an external derivative having an adverse influence on human health and offspring. Bisphenol A may be harmful because of its setting free from the plastics cans produced with the use of the above-mentioned resins and lacquers.

In the presented work we proposed a method of isolation and chromatographic detection of bisphenol A in water samples having contact with polycarbonate plastic and cans internally covered by a polycarbonate. We also proved that BPA is migrating from the PC-made cans to the water they contain. The analysis of water samples towards contents of bisphenol A is performed by the following method: preconcentration of water samples using solid-phase extraction (SPE), drying the sorbent with an air stream, eluting the analyte by the use of methanol, evaporation of solvent excess, and finally GC analysis. For the identification and determination of BPA in the samples we used gas chromatography with flame ionization detection (FID) and low resolution mass spectrometry (LRMS). The water samples were collected from the PC-made commercial bottles, and from the canned food (vegetables).

Experimental

Apparatus and Reagents

The chromatographic separation was performed using the following hardware.

- Gas chromatograph VARIAN CP-3380 equipped with flame ionization detector (FID). A CP-SIL 5 CB (30 m x 0,32 mm; DF=1,0 μm) capillary column was used (temperature 250ºC, helium flow rate at 3.5 ml min^{-1}).
- Gas chromatograph PERKIN ELMER (AUTOSYSTEM XL-TURBOMASS). A DB-5 (30 m x 0,25 mm; DF=0,25 μm) capillary column was used. Temperature programme: 4 min at 40ºC, then programmed at 10ºC min^{-1} to 300ºC and held for 10 min; injector temperature: 250ºC; detector temperature: 280ºC.
  A Bakerbond spe vacuum manifold was used for the elution of BPA from SPE columns.

Sample preparation / Solid phase extraction

Enrichment preconcentration of the analyte from the water samples was performed on little SPE columns with constant sorbent C18. The columns were conditioned by passing in turn: 5 ml of a mixture of the methyl chloride and methanol (1:1 v/v), 5 ml of methanol, and 15 ml of deionized water. Such prepared columns were injected by certain amount of the water analyzed. Once the total amount of a sample was put, the sorbent was dried for 10 minutes under vacuum, and the preconcentrated compounds were washed away by the use of 3 ml of methanol. The extract was dried up and further dissolved in 0.25 ml of methanol. The obtained samples were measured by GC/FID and GC/MS systems. BPA identity was proven by the use of low-resolution mass spectroscopy (GC-MS), taking into account distinctive ion with atomic mass of 228.

Results and discussion

Calibration graph. Recovery

Some dependencies between the peak height and the analyte concentrations were determined as a result of a chromatographic analysis of samples containing increasing amounts of BPA. Based on these dependencies, the calibration graphs were prepared. To this goal, some pattern solutions were used with the BPA concentration ranging from 1 to 100 μg ml^{-3}. The final peak area was taken as an average of three experiments in turn. Calibration plot was described with the use of the general equation: $y = ax + b$, where y is the peak area, and x – the amount of determination compound in μg ml^{-1}. For the observed range of concentration values, a linear route is observed of the calibration curves for calibration coefficients greater than 0.9995. The obtained parameters of the calibration curve for the analyzed compound are reported in Table 1.

Table 1. Parameters of calibration curve, recovery rate, and detection limit for the analyzed compound

Determined values	a	b	r^2	Recovery [%]	LOD [μg L^{-1}]
BPA	29.05	-60.45	0.9995	92.6	0.30

Recovery test were performed for the deionized water, with significant, known amount of BPA added (0.5 μg /500 ml^{-3}). These tests were performed using the above described method, and the following result was obtained for C18 sorbent – 92,6%.

Determination of BPA in drinking water having contact with polycarbonate plastic

The drinking water for the analysis of BPA concentration was collected from a 19-

liter can made of PC plastic. Such cans are widely used by several companies for distributing large amount of drinking water for the company's staff. Six parallel determinations of BPA concentration were performed for three water samples. The results are presented in Table 2, as arithmetical-average results for the interval confidence 95%.

Table 2. Average concentrations of BPA in mineral water stored in PC bottles (n=6)

Water sample	Concentration [μg L^{-1}]
water A	0.55 ± 0.07
water B	0.61 ± 0.07
water C	0.72 ± 0.06

Determination of BPA in the water previously stored in steel cans internally covered by thin coat made of polycarbonate lacquers

During this research, we analyzed the water previously stored, together with corn cobs and olives, in steel cans. The cans under study were collected from Polish supermarkets. All these cans were internally covered by a thin coat made of a polycarbonate plastic. As already mentioned, BPA is one of the compounds that may be liberated from such plastic materials. The main goal of our research was to prove that BPA is migrating from the polycarbonate layer of a can to the food this can contains.

The analyzed water was originally stored, since the production moment, in four different kind of steel cans. The water from the cans was collected in a separatory funnel and shook for a half of an hour, together with methyl chloride (1:1). Once a sample was separated into two layers, the lower layer was removed, while the upper layer was passed by a column with the C18 sorbent. The sample analysis was performed according to previously described procedure of sample preparation.

In Table 3 some results of the determination of BPA in water samples from the steel cans under study are presented.

Table 3. Concentrations of BPA in water from steel cans for storing corn cobs (samples 1-3) and olives (sample 4).

Sample number	Concentration [μg L^{-1}]
1	3.3
2	4.3
3	3.9
4	2.6

In Figure 1, four chromatograms are shown, concerning: (a) standard solution of BPA in methanol (GC/FID), (b) extract of the water from steel cans for storing corn cobs (GC/FID), (c) standard solution of BPA (GC/MS), and (d) extract of the water from steel cans for storing corn cobs (GC/MS).

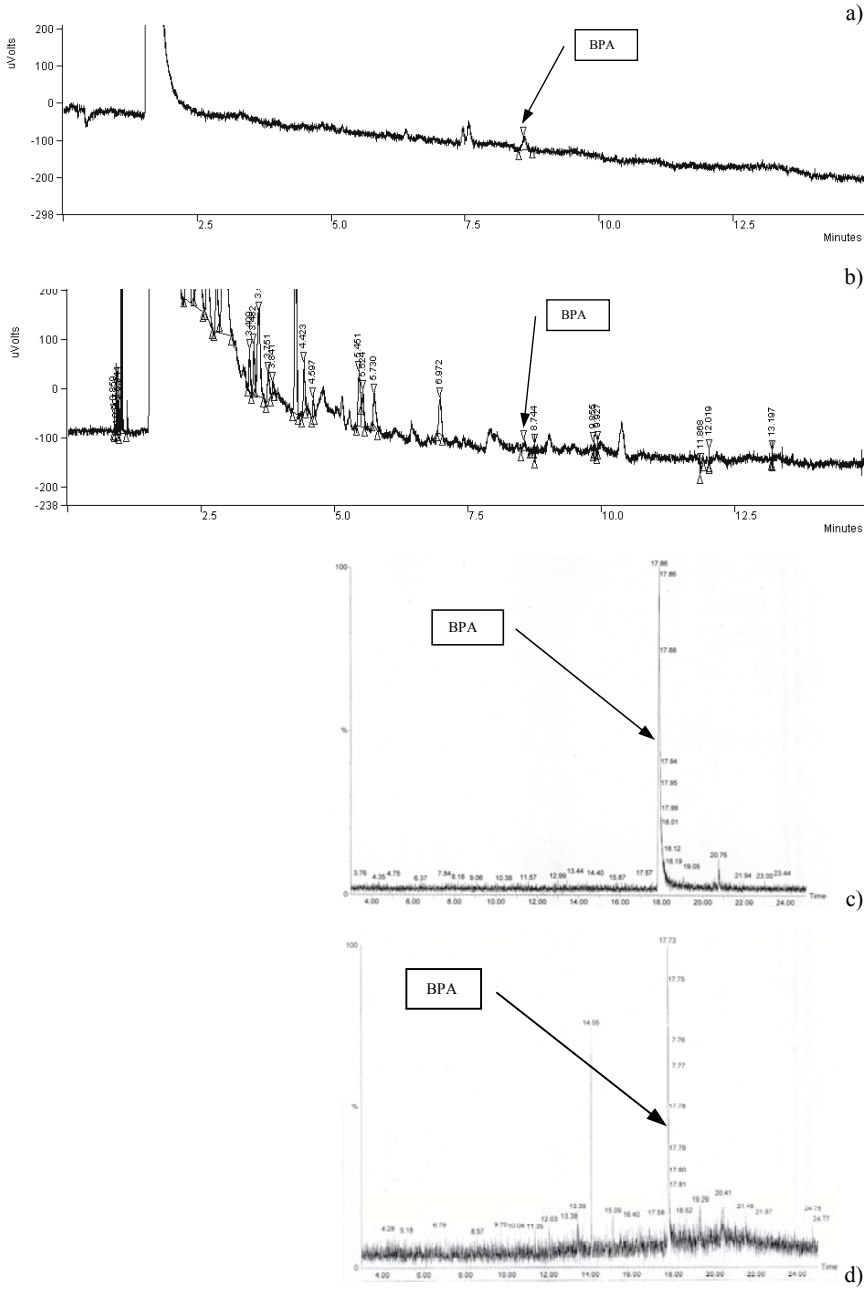

Figure 1. Sample chromatograms: (a) standard solution of BPA (GC/FID), b) extract of the water from steel cans for storing corn cobs (GC/FID), c) standard solution of BPA (GC/MS), d) extract of the water from steel cans for storing corn cobs (GC/MS)

Conclusion

A method based on solid–phase extraction followed by gas chromatography with FID detector has been established for determination of BPA in the water with a detection limit of 0.3 μg l^{-1}. The proposed method of extraction and preconcentration of the analyte is characterized by high amount of recovery – 92%. Based on the obtained results of our experiments, it was proven that BPA is migrating from the PC-made cans to the drinking water they contain.

There is no similar research mentioned in the literature. It's worth noting that drinking water from the Polish market was put into investigation. The measured concentration of BPA in drinking water is contained in the range of 0.55÷0.72 μg L^{-1}. Such concentration is below the limits of the European Commission – provisional tolerable daily intake TDI for BPA – 0.01 mg • kg^{-1} body weight • day^{-1} [4]).

Our further research proved that BPA is also migrating from the plastic coverage of steel cans to the food that is stored in these cans. Even though the measured concentration levels of BPA in the food are quite low, one should pay an attention to the fact that BPA and its derivatives are very harmful to consumer health. Thus, monitoring BPA concentration in the food is a necessity.

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VACANT REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (vRP-HPLC)

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Elution HPLC

Traditionally, in elution HPLC, the analyzed sample is injected to the flowing mobile phase. As mobile phase, just a pure solvent (e.g., water) can be used, occasionally containing an extra amount of a buffer, inclusion compounds, ion-interaction reagents *etc*. This order was, recently, reversed in the so-called vacancy ion exclusion chromatography (vIEC) [1].

Ion Exclusion Chromatography

The characteristic feature of IEC is the same sign of the electric charge of the resin's dissociated functional groups and of the analyzed ions. It follows that the negatively charged samples (e.g., the dissociated acidic compounds) are repulsed (or better said, excluded) from the resin, while the non-dissociated (i.e. neutral) ones can penetrate into the stationary phase. As a result, characteristic peaks with the leading (frontal) tailing are observed, when pure water is used as eluent [2].

Objective

It is interesting to note that – in certain way – a similar effect is observed in RP-HPLC, although caused by a different mechanism. In that case, ions cannot be partitioned with participation of the hydrophobic (non-polar) coverage of the silica gel matrix.

In our paper, the above statement will be experimentally confirmed and the retention mechanism is going to be described in a theoretical way. In RP-HPLC – with pure water used as mobile phase – the leading peaks of the acids are really observed. Moreover, it was shown that vacant peaks can also be obtained by means of this particular technique, however in this technique the vacant peaks are much less symmetrical then in vIEC method.

RP-HPLC with water used as mobile phase

Ions are generally not separated with aid of HPLC and they are eluted in the dead column volume [3]. In pure water used as mobile phase, the leading peaks of moderately strong acids are obtained, as it is shown on Fig. 1. This is due to the fact that the more diluted acid zones of the peaks are also more dissociated and therefore less retained on the column. Thus the peak maxima migrate slower along the column than their front parts It is interesting to note, that the leading peak was obtained for the completely dissociated nitric acid as well. Probably this is due to the specific solvation of the nitrite ions. The biggest agglomerates cannot penetrate into the sorbent and are therefore eluted earlier.

Vacant RP-HPLC

It has turned out that in v-RP-HPLC the asymmetrical (tailing) peaks are obtained and the retention time is proportional to the sample concentration (Fig. 2).

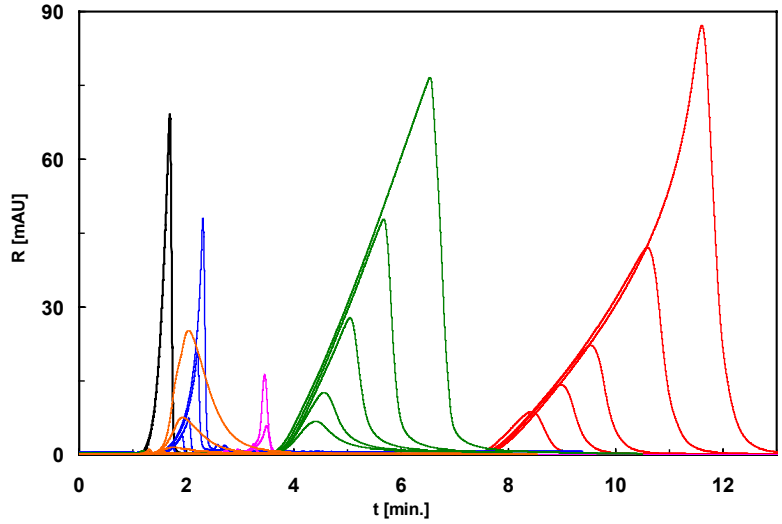

Fig.1. Experimentally obtained peaks of different acids in water used as mobile phase. Chromatographic conditions: column with precolumn - Hibar RP-18 5 μm, 250x4 mm ID (Knauer), temperature 30°C, flow rate 1 ml min^{-1}, mobile phase water, volume injected 20μL, detector UV 210/254 nm. The analyzed acids: nitric – black, oxalic – orange, formic – blue, acetic – pink, salicylic – green, and benzoic – red.

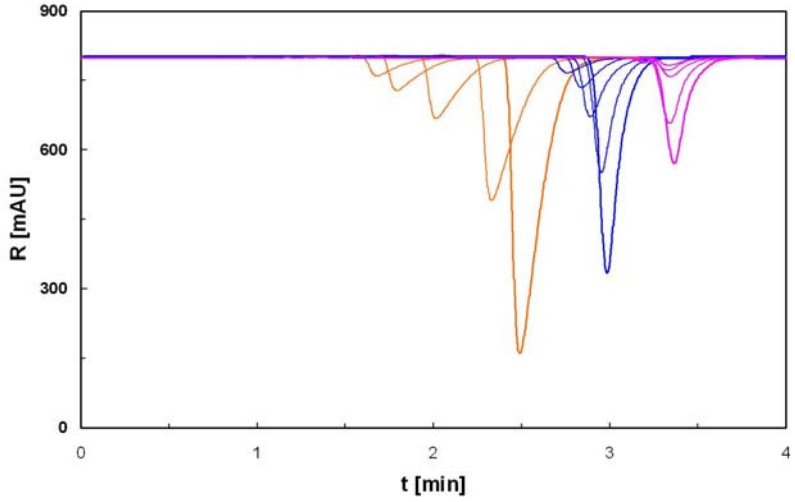

Fig.2. Experimentally obtained peaks of different acids in water used as mobile phase in vacant RP-HPLC. Chromatographic conditions: column with precolumn - Hibar RP-18 5 μm, 250x4 mm ID (Knauer), temperature 30°C, flow rate 1 ml min^{-1}, mobile phase water, volume injected 20μL, detector UV 210/254 nm. The analyzed acids: oxalic – orange, formic – blue and acetic – pink.

Theoretical approach

The proposed model is based on the assumption, that the non-dissociated acid as well as its dissociated form can penetrate into the water occluded in the adsorbent pores. However, adsorption of the non-dissociated acid strongly prevails over the adsorption of the R^- ions. The proposed theoretical approach correctly modeled the dependency of the retention time on the analyte concentration in both the classical and the vacancy mode of RP-HPLC. The comparison between theoretical and experimental peak profiles will be presented on a poster.

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HPLC STUDY OF GUANINE QUADRUPLEX FORMATION IN THE PRESENCE OF METAL IONS

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Inhibition of telomerase activity in cancer cells can be achieved through formation of guanine quadruplex (G-4) structures on the telomeric DNA strand. Such structures are involved in a variety of important cellular processes and are emerging as a new class of therapeutic targets for cancers. As shown in Fig. 1 the quadruplex is composed of guanine tetrads, which are square co-planar arrays of four guanine bases. Each base is both the donor and acceptor of two hydrogen bonds with its neighbors.

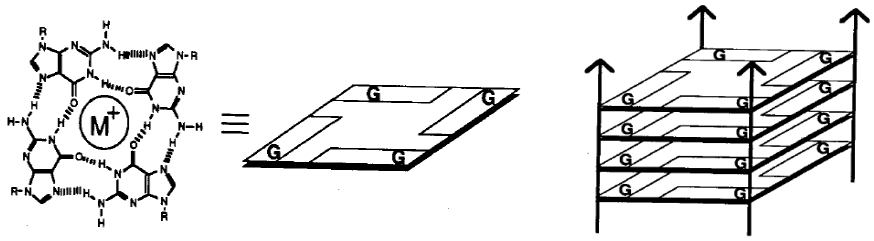

Fig1. The structure of G-4 – metal ion complex.

Therefore it is important to study quadruplex properties and conditions of G-4 formation. Commonly used techniques for this purpose include: NMR, X-ray structural analysis, and circular dichroism spectroscopy. Here we try to evaluate HPLC method, which should enable the separation of different structures of G-4 present in solution and their characterization with UV-Vis spectra. Our attention was focused on two chromatographic techniques: HPIEC (high performance ion-exchange chromatography) and IP RPHPLC (ion-pair reversed phase HPLC). Both of these methods (related to dsDNA) use short columns (2-5 cm) with stationary phases containing micropellicular particles (1-3 μm). These chromatographic packings assure fast mass transport and enable isocratic or shallow gradient elutions of high molecular weigth analytes.

The purpose of this work is developing the separation conditions of guanine quadruplexes in the presence of metal ions using ion-exchange chromatography (IEC). The method should fulfil initial conditions: (i) separation should run in non-denaturating conditions, (ii) complexes G-4 with cations should be sufficiently stable in separation conditions, (iii) the mechanism of retention is expected to be sensitive to the complex formation. This means, that retention time of free DNA and its complex with metal cation should be different. We designed an analytical system taking these requirements into account. As a G-4 structure forming DNA, an oligonucleotide with human telomeric sequence d(GGGTTAGGGTTAGGGTTAGGG) was used. We studied retention of metal ion-G4 complexes applying different salts in mobile phase

(NaCl, KCl, LiCl). LaChrom Merck/Hitachi HPLC system with DAD detector, was equipped with a TSKgel DEAE-NPR column (Japan).

Initial investigations involved the influence of temperature and metal salts on isocratic and gradient elutions. Narrow peaks were obtained in isocratic conditions at metal concentration exceeding 0.5 M. Unfortunately, peaks were eluted in dead time. Probably, these salt concentrations (above 0.5 M) are too high and quadruplexes of particular cations do not retain on the column, irrespective of their structures. The best chromatograms were obtained with isocratic elution in 0.35-0.40 M concentrations range of salts.

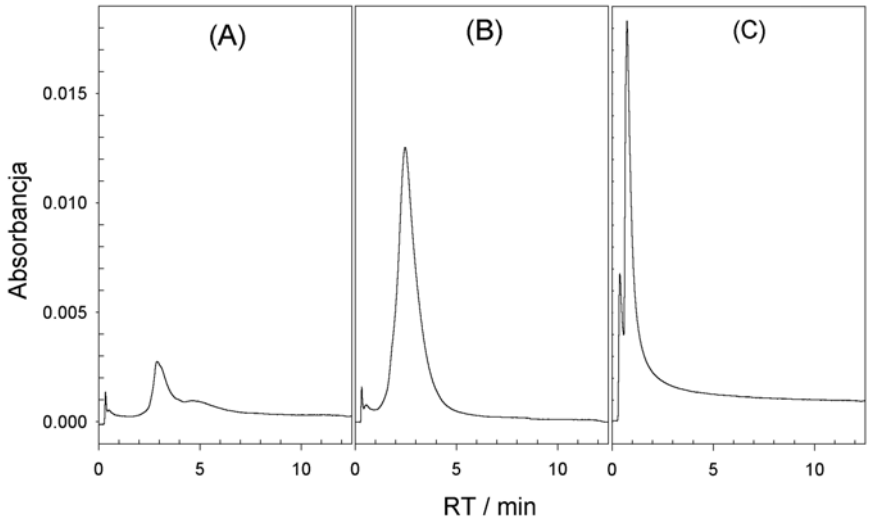

Fig 2. Chromatograms of G-4 quadruplexes eluted isocratically with 0.36 M KCl (A), 0.36 M NaCl (B), 0.36 M LiCl (C). Conditions: column TSKgel DEAE-NPR (4.6mm ID x 7.5cm; d_p = 2.5μm), flow rate 1 ml/min, 20 mM Tris buffer pH = 8.7. $\lambda_{monitoring}$=260 nm.

In the presence of Li^+ (Fig. 2C) only one peak was observed (RT = 0.64 min). In the case of Na^+ (Fig. 2B) two peaks appeared: the first one, similary as for Li^+, in dead time (0.35 min) and the second at RT = 2.46 min. More complex chromatogram was obtained with K^+ (Fig. 2A). Three peaks can be recognized: the first one, in dead retention time (0.32 min), the second at RT = 2.91 min and the last (the smaller) at RT = 4.24 min. Single peak in Fig. 2C suggests, that Li^+ probably destabilizes structure of quadruplex [1]. One can assume, that the random coil structure exists in the presence of lithium ion. Similarly, first peak for Na^+ elution (Fig. 2B) may be identified as a random coil structure, whereas the second peak sould be ascribed to the tetraplex structure (probably antiparallel – Fig. 3) [2,3]. Agreement of first two retention times for K^+ with those for Li^+ and Na^+ suggest in this case also the presence of random coil structure and antiparallel quadruplex structure. Additional peak at retention time of 4.24 min represents probably parallel quadruplex structure [4,5].

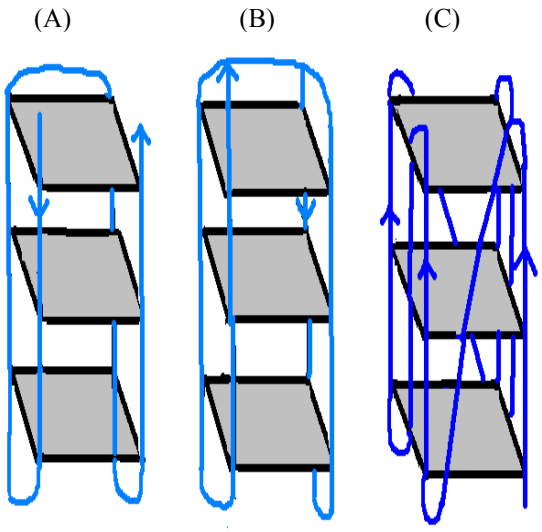

Fig 3. Possible intramolecular guanine quadruplexes for telomeric DNA. (A) An antiparallel chair-type - all three loops run edgewise and connect " adjacent-adjacent-adjacent ", (RT = 2.46 min). (B) An antiparrallel basket-type - one diagonal and two edgewise loops that connect "adjacent-diagonal - adjacent", (RT = 2.46 min). (C) A parallel structure - (RT = 4.24 min).

In experiments with isocratic elution many problems were encountered including poor repeatability of retention times and broadening of peaks. For these reasons we tried to apply shallow gradient elution to improve results of separation. Better reproducibility of retention times and sharper peaks were observed in gradient conditions. Tab.1 summarizes results for different gradient conditions.

Tab. 1 Retention times (min) of the chromatographic peaks observed for G-quadruplexes eluted with gradient of NaCl, KCl, and LiCl. Other conditions as in Fig. 2.

Gradient[a)]	**KCl**			**NaCl**			**LiCl**		
	RT[1]	RT[2]	RT[3]	RT[1]	RT[2]	RT[3]	RT[1]	RT[2]	RT[3]
0.34-0.36	0.35	5.40	8.02	0.35	5.23	-	0.37	-	-
0.35-0.37	0.32	3.28	4.59	0.35	3.28	-	0.43	-	-
0.35-0.39	0.32	3.40	4.98	0.30	3.25	-	0.99	-	-
0.36-0.38	0.32	2.59	4.06	0.35	2.53	-	0.88	-	-
0.36-0.40	0.32	2.69	4.01	0.32	2.43	-	0.35	-	-

a) $(M\ Cl^-)_{start} \rightarrow (M\ Cl^-)_{end}$

Careful inspection of data presented in Tab.1 enables formulation of the following conclusions: (i) number of peak for particular cations is the same as for isocratic elution (Fig. 2), (ii) small changes in gradient conditions affect seriously separation, and (iii) the same assignment of peaks to particular quadruplex structures is valid as that discussed above.

Finally we studied impact of sample preparation on retention of oligonucleotide G-4 containing 0.36 M KCl. We checked the influence of heating and denaturation of quadruplexes. Three samples were prepared: (A) - directly injected on the column after the preparation, (B) – sample (A) was heated at 80^0C for 10 minutes and quickly cooled down in ice (denaturated), (C) - heated at 80^0C for 10 minutes, and slowly cooled down for 2 hours (annealed). Samples were separated with KCl gradient (0.36 M to 0.38 M in 10min, 0.38 M to 0.8 M in 5 min and back to 0.36 M in 5 min). The find steep gradient was applied to wash column and to elute high molecular weight aggregates.

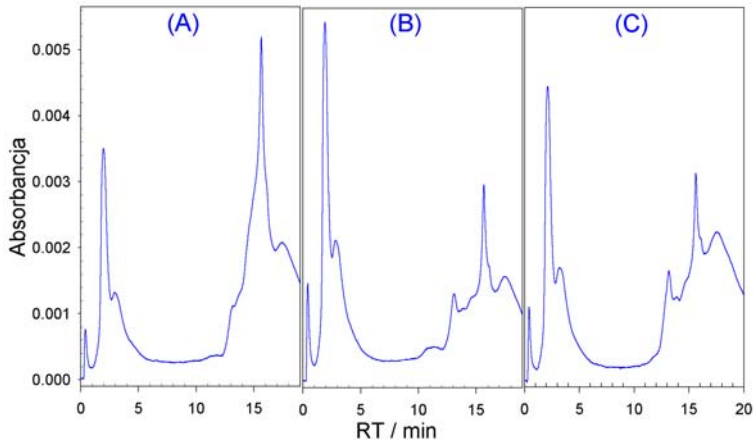

Fig. 4 HPLC separation of G-4 – K^+ quadruplexes. Samples containig: 1x 10^{-5} M G4, 0.36 M KCl, and 20 mM Tris pH =8.7 were treated as described in text.

In all cases three peaks are present in the range of 0 - 10 min, but their relative intensities vary. Higher peaks are observed for heat-treated samples (B) and (C). Unexpectedly, samples (B) and (C) give similar separations that indicates poor efficiency of the denaturation procedure. It should be pointed out that additional peaks are present in the high gradient region (12-15 min), suggesting formation of high molecular weight products.

Concluding, we confirmed the usefulness of IEC to separate and study the formation of four-stranded DNA structures and their complexes with metal ions. The further investigations will include trials with the IP RPHPLC technique.

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CHEMICAL PROPERTIES OF ORGANIC MATTER DISPERSED IN SEDIMENTS ACCOMPANYING BROWN COAL BEDS

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1. Introduction

Sedimentary organic matter dispersed in rocks and loose sediments accompanying brown coal seams is a geochemically important indicator of changes occurring in depositional environment. Investigation of its chemical composition can enable to appraise an input of the particular groups of organisms and conditions of deposition of primary biological material, such as the oxygen content or the salinity of depositional environment. This is achieved by recognising compounds derived from organisms whose remains participated in sedimentary organic matter formation and inhabiting the given environment type (e.g. gammacerane, oleananes, diterpanes, dinosterane) or generated from biological material after its deposition (e.g. phytane or diasteranes) in appropriate conditions [1-2]. These compounds, called biomarkers, have molecular structures directly inherited from primary biological material without or only with minor chemical alteration during their deposition in sediments (mainly defunctionalisation and hydrogenation of unsaturated bonds). Since they are usually found in low concentrations compared with the total organic matter, their distribution is commonly investigated by gas chromatography-mass spectrometry.

2. Experimental

2.1 Rock extraction and extracts fractionation

Several samples of rocks and loose sediments accompanying brown coal seams of the Lubstów, Kazimierz (the Konin Brown Coal Basin), Bełchatów and Turów open cast mines were the subject of the following analyses. Rocks were solvent extracted with a mixture of DCM:EtOH (4:1) in a ultrasonic bath. The extracts were TLC fractionated into fractions of aliphatic hydrocarbons, aromatic compounds and polar (NSO) compounds. The 1% solutions of extracts (20-30 mg in CH_2Cl_2) were applied onto the 20 x 20 cm glass plates pre-coated with silica gel (Kieselgel 60 F_{254}, Merck) previously activated at 105^{o}C (35 min). Plates were developed in a TLC tank with *n*-hexane as a developer in saturated vapour conditions (development time about 40 min). The fractions were distinguished by the UV long-wave fluorescence of aliphatic (lack of fluorescence), aromatic (blue-violet and yellow), and polar compounds (brown) and by comparing R_f values of reference compounds (*n*-eicosane, phenanthrene and quinoline representing three respective compound fractions) developed on the same TLC plate.

2.2 Gas chromatography-mass spectrometry

Aliphatic and aromatic hydrocarbons were directly analyzed with GC-MS while polar fractions were methylated with BF_3 to obtain methyl esters of carboxylic acids and their distribution was investigated with GC-MS. An Agilent Techn. gas chromatograph was applied equipped with HP-17 column (60m x 0.25mm), coated by 0.25 μm stationary phase film. The experimental conditions were as follows: carrier gas - He; temperature program: 50^{o}C (isothermal

for 2 min), heating rate to 175 °C at 10 °C/min, to 225 °C at 6 °C/min, and to 300 °C at 4 °C/min, final isothermal temperature: 300 °C was held for 20 min. The gas chromatograph was coupled to a mass spectrometer operating in EI mode at 70 eV (full scan, 50 - 650 da). Data were acquired in a full scan mode and processed with the Hewlett Packard Chemstation software. All compounds were identified by their mass spectra, and comparison of retention times of their peaks to these of standard compounds and literature data and interpretation of MS fragmentation patterns [1-3].

3. Results

Results obtained for organic matter dispersed in the host rock were compared with the similar results received previously for brown coals of the same mines [4-5]. Distributions of several biomarker types were investigated belonging to *n*-alkanes, diterpenoids, hopanoids, fatty acids, and aromatic hydrocarbons such as alkyl derivatives of naphthalene and phenanthrene together with their partly hydrogenated and functionalised precursors.

Organic matter of the samples from the first exploitation level of the Turów mine is characterised with the increased content of short-chain *n*-alkanes comparing to the brown coal of this mine (Fig. 1). Significant odd-over-even carbon number atoms predomination was found in their distribution for organic matter containing numerous xylithic fragments. This predomination was absent in the case of OM from spherosiderites and silts underlying the first brown

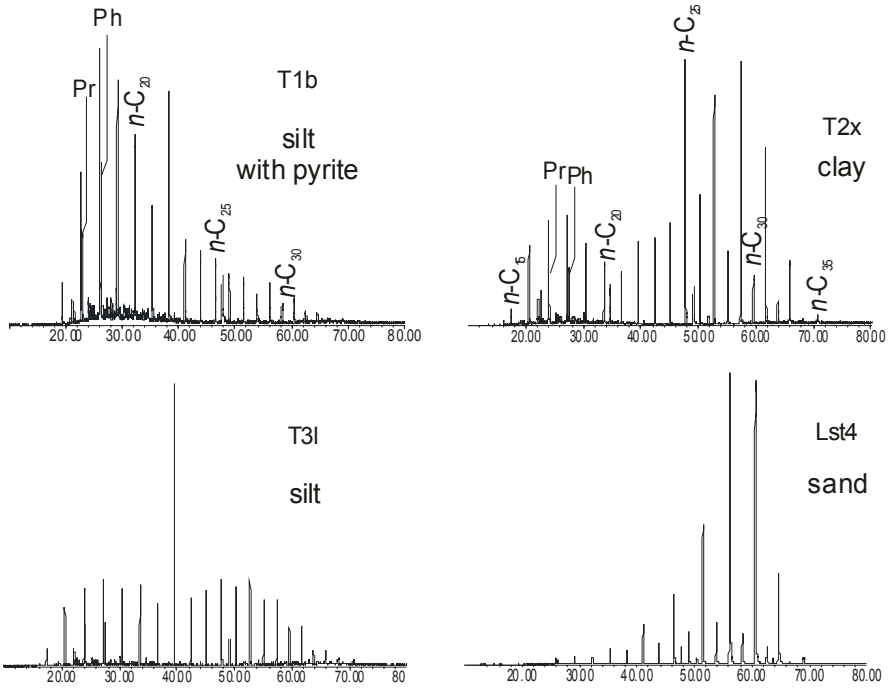

Fig. 1. Distribution of *n*-alkanes in the extracts of organic matter dispersed in sediments accompanying brown coal seams

coal seam. The type of distribution found for aliphatic ketones and fatty acids closely follows those found for *n*-alkanes. Hydroxyl and ketone derivatives of oleanene and ursene coming from angiosperm tissues were commonly identified in the extract fractions of medium polarity.

In the case of samples from the Lubstów mine, chemical composition of organic matter dispersed in arenaceous sediments is similar to chemical composition of detritic brown coals forming the lower seam of the mine. It may be assumed that both its biological source and depositional conditions were similar to these of brown coals deriving from detritic material of angiosperm plants, especially of the reed facies.

Properties of organic matter occurring in the Bełchatów tuffites were similar to organic matter of silts underlying the Turów brown coal seam, while organic matter of the so-called "leafy" shale shows features of a typical detritic brown coal formed from biogenic matter of variable biological sources such as conifers, reeds/grasses and bacteria.

Comparative study of humic (macromolecular) fraction of organic matter dispersed in sediments enabled to identify types of lignin products present in them. Organic matter content in the rocks was sufficiently high to investigate by flash pyrolysis/gas chromatography-mass spectrometry it without previous preconcentration. Since chemical composition of lignin depends on the type of vascular plants deposited, it may be applied to find primary biological source of organic matter [6]. It was found that all samples show a significant input of herbaceous lignin deriving from grasses and reeds. However, the Turów silts and clays contained also deciduous trees lignin while organic matter of such biological source is generally absent in the brown coals and main coal-forming matter derived from conifers and reeds and grasses.

Slightly higher thermal maturity of organic matter present in the Turów mine sediments accompanying brown coals, calculated from biomarker parameters, may be attributed to floor location of the sampling site in the case of some samples.

4. Conclusions

Significant differences found in the geochemical characteristics of organic matter dispersed in rocks accompanying brown coals seams may be attributed to significant changes in depositional environment. In the case of the Bełchatów and Turów deposits they are related to different biogenic source of organic matter and depositional conditions. However, the features of the Lubstów organic matter dispersed in sediments show the large similarity of brown coal building the lower seam of the mine, sufficient to assume that the most of environmental changes were related to increased input of sands. There is no an obvious relationship between host sediment lithology and a type of organic matter.

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GC-MS IN ASSESSMENT OF A DEPOSITIONAL ENVIRONMENT INFLUENCE ON PROPERTIES OF YOUNGER SEDIMENTARY ORGANIC MATTER

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1. Introduction

The analysis of geochemical liquids such as crude oils, hydrothermal bitumens, extracts of coals and host rocks containing dispersed organic matter or pyrolysates of organic matter of various origins presents an interest in several fields: investigation of organic matter evolution in deposits, geological prospecting, quality fuel assessment, and environmental protection. Biomarkers, compounds deriving from their biological precursors synthesized by living organisms in biosphere, are widely applied in geochemistry for the assessment of source biological organic matter type, its depositional environment and thermal maturity of organic matter. Their detection, identification and quantification are commonly performed by means of gas chromatography (GC) or gas chromatography-mass spectrometry (GC-MS).

It is possible to identify biological source of organic matter by biomarkers due to several groups of compounds related to the particular genera or families of plants. One of them are diterpenoids. However, in the most cases biochemistry of all living organisms is similar, and such compounds are not numerous and seldom preserved well in sediments. As a result, the biogenic source identification is the most often performed using relatively higher concentrations of the particular compounds synthesized predominantly by a given group of organisms. For example, cholestane content (geochemical derivative of cholesterol) is lower than stigmastane content in organic matter with high vascular plant input [1-2].

The object of the investigation were differences in the chemical composition of various facies of Polish brown coals, such as detritic, detroxylithic, xylodetritic and xylithic lithotypes, which are related to different conditions of the depostitional environment. To this aim chromatographic methods such as preparative thin layer chromatography, gas chromatography mass-spectrometry, and flash pyrolysis/GC-MS were applied.

2. Experimental

2.1 Brown coal extraction and extracts fractionation

Brown coals and loose sediments accompanying them were solvent extracted in DCM/MeOH (4:1, vol.:vol.) in an ultrasonic bath. The extracts were fractionated into aliphatic, aromatic and polar (NSO) compound fraction by preparative thin layer chromatography in the following conditions. The 1% solutions of extracts (20-30 mg in CH_2Cl_2) were applied onto the 20 x 20 cm glass plates pre-coated with silica gel (Kieselgel 60 F_{254}, Merck) previously activated at 105 ^{o}C (35 min). Plates were developed in a TLC tank with *n*-hexane as a developer in a saturated vapour (development time about 40 min). The fractions were distinguished by the UV long-wave fluorescence of aliphatic (lack of fluorescence), aromatic (blue-violet and yellow), and polar compounds (brown) and by comparing R_f values of reference compounds (*n*-

eicosane, phenanthrene and quinoline representing three respective compound fractions) developed on the same TLC plate.

2.2 Gas chromatography-mass spectrometry

Chemical composition of aliphatic and aromatic hydrocarbon fractions was investigated by GC-MS An Agilent Techn. gas chromatograph was applied equipped with HP-17 column (60m x 0.25mm), coated by 0.25 μm stationary phase film. The experimental conditions were as follows: carrier gas - He; temperature program: 50 °C (isothermal for 2 min), heating rate to 175 °C at 10 °C/min, to 225 °C at 6 °C/min, and to 300 °C at 4 °C/min, final isothermal temperature: 300 °C was held for 20 min. The gas chromatograph was coupled to a mass spectrometer operating in EI mode at 70 eV (full scan, 50 - 650 da). Data were acquired in a full scan mode and processed with the Hewlett Packard Chemstation software. All compounds were identified by their mass spectra, and comparison of retention times of their peaks to these of standard compounds and literature data and interpretation of MS fragmentation patterns [1-2]. Distribution of several groups of biomarker compounds were investigated against the influence of biological origin, conditions of organic matter deposition chemical changes at diagenetic stage of brown coal evolution.

3. Results

Comparing with organic matter being at higher stages of thermal evolution, both brown coal extracts and their macromolecular fraction show complex composition inherited directly from their biological precursors. The following compound groups, related to biological origin/depositional environment were identified in the extracts:

- *n*-alkanes showing generally three main types of distribution (Fig. 1): bimodal with well seen odd-over-even carbon number predominance, monomodal dominated by short chain *n*-alkanes, and monomodal dominated by long-chain *n*-alkanes;
- tricyclic (pimarane, isopimarane, abietane structures) and tetracyclic diterpenoids;
- pentacyclic triterpanes, among them derivatives of oleanane and ursane formed from amyrine (angiosperm indicator);
- partially or completety aromatized derivatives of diterpenoids and pentacyclic triterpanes;
- derivatives of diterpenoids and pentacyclic triterpenoids with methoxy, hydroxyl, carbonyl and carboxylic functional groups;
- heterocyclic sulphur compounds.

In Py/GC-MS products of flash pyrolysis of the extracted macromolecular fraction of brown coals phenol and benzene derivatives dominated. Aromatic hydrocarbons with more than to aromatic rings were absent.

4. Conclusions

Aliphatic hydrocarbons and function polar compounds occurring in so-called “aromatic fractions” (fractions of medium polarity) of the extracts show a very variable distribution related mainly to the biological source of organic matter and, only in the lower extend, to thermal maturity of organic matter. In the case of brown coals lithotype influence seems to be dominating since such compounds as diterpenoids are related chiefly to wood

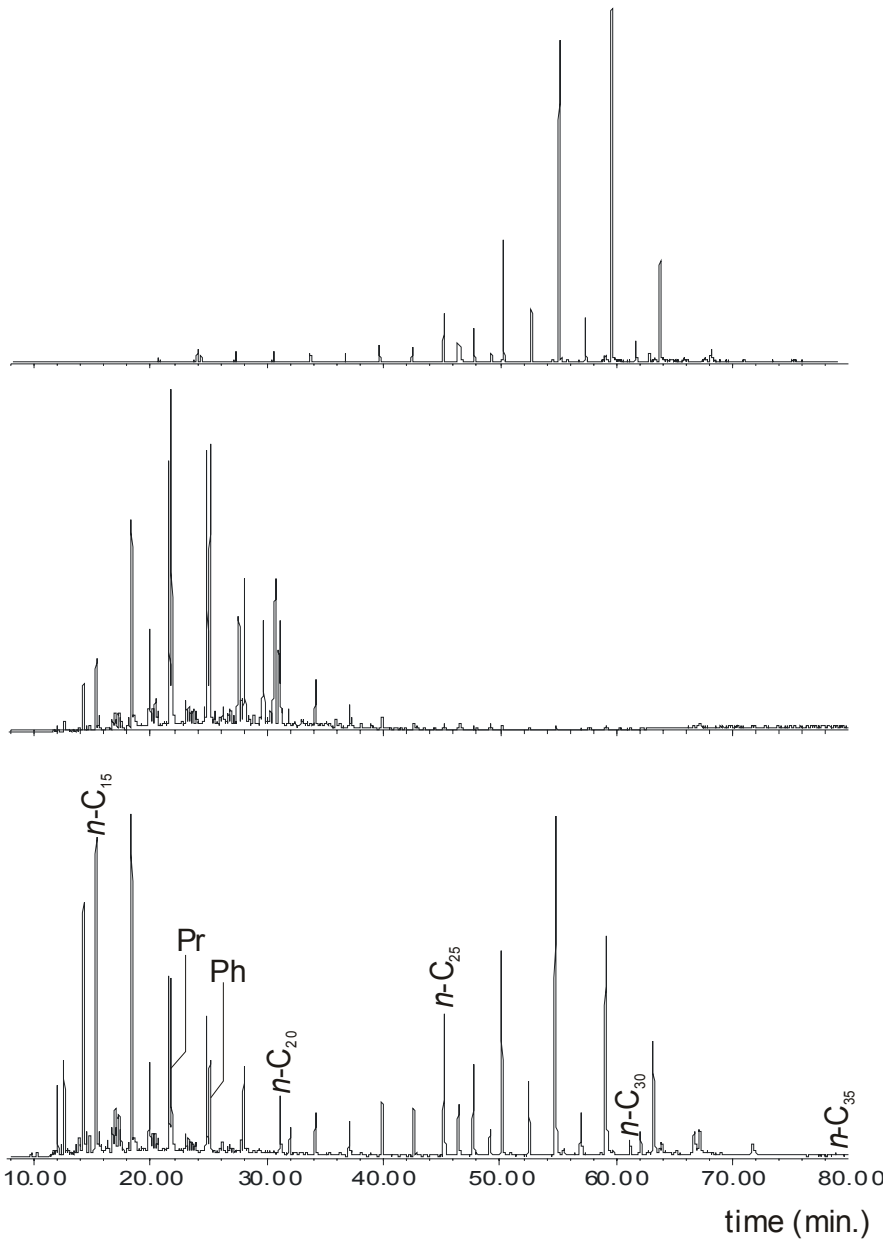

Fig. 1. Types of *n*-alkane distribution found in the brown coal extracts

conifer trees (xylithic lithotype) while amyrine products such as olenenenes are related to reed facies giving rise to detritic brown coal lithotypes (Fig. 2). However, oxicity of depositional

environment also influences chemical composition of extracts. The wide range of oxygen compounds deriving from their biogenic precursors such as ketones, aldehydes and carboxylic acids of ursane, oleanene or abietane were found in detritic brown coal samples. Sulphur derivatives (thiophenes and thioles) are present in higher concentrations in the samples deposited in anoxic conditions.

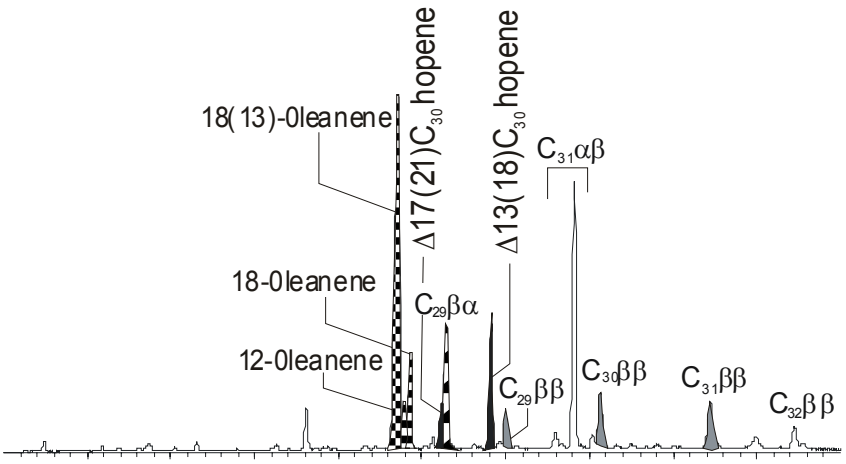

Fig. 2. Distribution of pentacyclic triterpenoids in the Lubstów brown coals

The dominating phenolic compounds in Py/GC-MS pyrolysis products come from thermal destruction of lignins and their distribution differs depending on different biogenic source. Guiacol and its aliphatic derivatives dominate in conifer-origin organic matter while phenol and cresols in herbaceous lignins.

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GC-MS IN CHARACTERISATION OF THE JAWORZNO MINE COALS

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1. Introduction

The exploiting area of the Jaworzno coal mine is situated in the eastern part of the Upper Silesia Coal Basin. The 207 seam belongs to the Carboniferous Łaziska Beds of the Cracow sandstone series (Westfalian C) [1-2]. They show lithology different to that found for the most of Carboniferous sediments occurring in the central and western parts of the Upper Silesia Coal Basin. They comprise only a few thick coal seams with coarse-grained sandstones or even conglomerates. In the profile of the 207 seam, there is a tonstein bed [3].

The aim of the project was to investigate organic geochemistry of the Jaworzno mine coals in detail. Since they show variable thermal maturity (rank) and petrographic composition their biomarker analysis may give an important insight into geological history of the area.

2. Experimental

2.1 Coal extraction and extracts fractionation

The investigated coal seam profile was sampled and samples were solvent extracted DCM:EtOH (4:1) in a ultrasonic bath. The extracts were separated into aliphatic and aromatic hydrocarbon fractions by preparative thin layer chromatography in the following conditions. The 1% solutions of extracts (20-30 mg in CH_2Cl_2) were applied onto the 20 x 20 cm glass plates pre-coated with silica gel (Kieselgel 60 F_{254}, Merck) previously activated at 105^{o}C (35 min). Plates were developed in a TLC tank with *n*-hexane as a developer in a saturated vapour (development time about 40 min). The fractions were distinguished by the UV long-wave fluorescence of aliphatic (lack of fluorescence), aromatic (blue-violet and yellow), and polar compounds (brown) and by comparing R_f values of reference compounds (*n*-eicosane, phenanthrene and quinoline representing three respective compound fractions) developed on the same TLC plate.

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3. Results

The Jaworzno mine coals show a wide variability in the extract yields from 0,2-10,1% (wt.:wt.). The group composition of coal extracts is also variable with aliphatic hydrocarbons content being in the range of 1.1-11.2 % (wt.: wt.) and aromatic hydrocarbons in the range 6.4-30.4%.

The main groups of biomarkers identified in the coal extracts are *n*-alkanes, acyclic isoprenoids such as pristane and phytane, diterpenoids, steranes and pentacyclic triterpanes. Aliphatic fractions were dominated by *n*-alkanes (Fig. 1) while in aromatic fractions the highest concentrations were found for 2-3 ring aromatic hydrocarbons such as aliphatic derivatives of naphthalene and phenanthrene, mainly retene and cadalene. The substituted derivatives of diterpanes, steranes and pentacyclic triterpanes were also found, however in lower amounts. Their partially aromatised products are common in the aromatic hydrocarbon fractions.

3.1 Biogenic sources of organic matter of the Jaworzno mine coals

n-Alkane distributions show a slight odd-over-even carbon atom number predominance related to the input of vascular plants into primary biogenic matter (plant waxes). The presence of tetracyclic diterpanes, beyerane, phyllcladane and kaurane, indicates a possible input of evolutionary ancestors of *Araucariaceae* and *Podocarpaceae* families [6].

Values of biomarker parameters used to assess environment type indicate suboxic conditions of coal depositional environment.

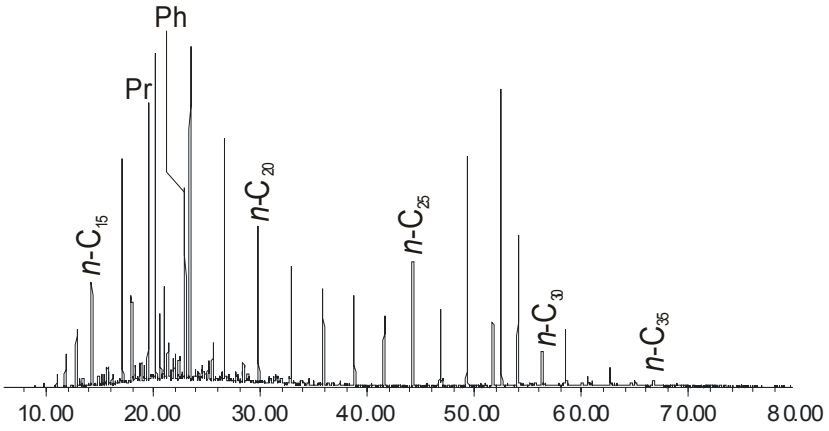

Fig. 1. *n*-Alkane distribution in the selected sample 20 of the "Jaworzno" mine coal

3.2 Thermal maturity (rank) of the investigated coals

Several maturity parameters were calculated using geochemical biomarker parameters based upon hopanes and moretanes, kaurane and phyllocladane isomers, alkylnaphthalenes, and alkylphenanthrenes (Fig. 2) [5, 7-8].

There is a significant variability in thermal maturity of coal organic matter of the "Jaworzno" mine. Carbon Preference Index values are in the range of 1.6-3.6 with slight decreasing tendency with the depth. Values of $C_{31}\alpha\beta$ 20 S/(20S+20R) ratio are in the range of 21-41%. The trends in values of parameters based on aromatic hydrocarbons agree with those found for

aliphatic biomarker parameters. Variability in the values of maturity parameters is probably related geological events occurring in nearby such as volcanic eruptions; however, in most cases thermal maturity of organic matter indicated by biomarker parameters approaches the end of diagenesis.

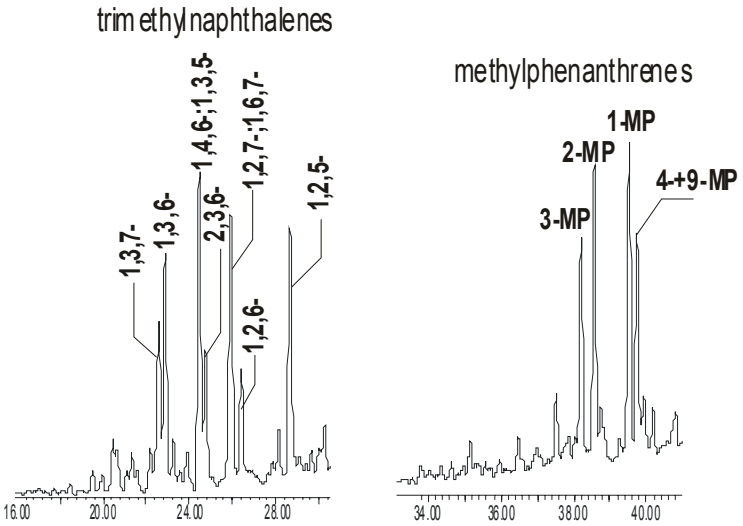

Fig. 2. Trimethylnaphthalene and methylphenanthrene distributions used for thermal maturity assessment (the sample 20)

4. Conclusions

Organic matter of the investigated coals of the Jaworzno mine were deposited in the suboxic conditions. Vascular plants organic matter is the dominant organic matter source. Thermal maturity, assessed by commonly applied geochemical biomarker parameters, shows a significant variability, with most of the parameter values slightly increasing with the sampling depth.

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GAS CHROMATOGRAPHY-MASS SPECTROMETRY IN INVESTIGATION OF COMPOUND GROUPS PRESENT IN THE UPPER SILESIA COAL EXTRACTS

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1. Introduction

Due to a high chemical reactivity of coal its weathering leads to its major alterations. The resulting physical and chemical transformations of coals have been widely studied since they negatively affect coal coking properties, decrease the calorific value, decrease the yield of products of hydrogenation and liquefaction together with the product quality and adversely influence froth flotation [1-2]. Weathering also causes coal to lose its cohesiveness and to disintegrate into progressively finer fragments. Such changes can occur both in coal storage and in natural weathering in coal seam.

Moreover, such weathered coals show altered geochemical properties reflected by such parameters as vitrinite reflectance (Ro), results of Rock-Eval Analysis (T_{max}, hydrogen index - HI, oxygen index - OI) and biomarker parameters, for example ratios based on aromatic hydrocarbons [2-3]. Due to both technological and research reasons much effort has been expended to find techniques which could reliably indicate coal weathering and estimate changes caused by its impact. The large body of data concerns laboratory simulations of coal weathering or its oxidation thought to play its most important part [4-5]. However, the weathering of coal takes place both below and above water table under combined aerated and aqueous conditions or, in some cases, as a result of circulation of oxidising fluids. Laboratory simulations of coal oxidation generally are performed in air, which leads to ignoring the role of water in oxidation processes [6]. Temperatures in such simulation, often elevated above 50^{o}C do not correspond to more moderate temperatures of natural weathering. Thus, it is essential to study in detail natural cases of coal weathering, compare them with unaltered samples to estimate the extent of changes in coal matter.

2. Experimental

2.1 Coal extraction and extracts fractionation

Several coal samples from the Upper Silesia Coal Basin were investigated by chromatographic methods. Coals were ultrasonificated in DCM/MeOH (4:1, vol.:vol.) and the extracts fractionated into aliphatic, aromatic and polar compounds by preparative thin layer chromatography in the following conditions. The 1% solutions of extracts (20-30 mg in CH_2Cl_2) were applied onto the 20 x 20 cm glass plates pre-coated with silica gel (Kieselgel 60 F_{254}, Merck) previously activated at 105^{o}C (35 min). Plates were developed in a TLC tank with *n*-hexane as a developer in a saturated vapour (development time about 40 min). The fractions were distinguished by the UV long-wave fluorescence of aliphatic (lack of fluorescence), aromatic (blue-violet and yellow), and polar compounds (brown) and by comparing R_f values of reference compounds (*n*-eicosane, phenanthrene and quinoline representing three respective compound fractions) developed on the same TLC plate.

2.2 Gas chromatography-mass spectrometry

Chemical composition of aliphatic and aromatic hydrocarbon fractions was investigated by GC-MS An Agilent Techn. gas chromatograph was applied equipped with HP-17 column (60m x 0.25mm), coated by 0.25 μm stationary phase film. The experimental conditions were as follows: carrier gas - He; temperature program: 50 °C (isothermal for 2 min), heating rate to 175 °C at 10 °C/min, to 225 °C at 6 °C/min, and to 300 °C at 4 °C/min, final isothermal temperature: 300 °C was held for 20 min. The gas chromatograph was coupled to a mass spectrometer operating in EI mode at 70 eV (full scan, 50 - 650 da). Data were acquired in a full scan mode and processed with the Hewlett Packard Chemstation software. All compounds were identified by their mass spectra, and comparison of retention times of their peaks to these of standard compounds and literature data and interpretation of MS fragmentation patterns [7-8]. Distribution of several groups of biomarker compounds were investigated against the influence of biological origin, conditions of organic matter deposition and thermal maturity.

3. Results

Generally, three main types of *n*-alkane and acyclic isoprenoids distribution can be distinguished in the coal extracts (Fig. 1):

1) monomodal with dominating *n*-C_{18} or *n*-C_{19} and high pristane concentrations reflected by moderate to very high values of Pr/*n*-C_{17} ratio (1,3-4,2), CPI values are about 1,5 for these samples;
2) monomodal with short-chain *n*-alkanes predominating, CPI values near 1,0 and acyclic isoprenoids (pristane and phytane) occur in much lower concentration than in the previous group with Pr/*n*-C_{17} values 0,5-1,1;
3) monomodal or slightly bimodal forming a wide cluster of *n*-C_{20}-*n*-C_{27} peaks with high odd-over-even carbon-atom-number predomination (CPI ~ 1,6). Pristane concentrations are very high with values of Pr/*n*-C_{17} in the range of 3,0-7,1.

The type of *n*-alkane distribution is related both to coal rank and its weathering.

A diterpenoid group comprised only phylocladane and isopimaranes present in low amounts. Tricyclic triterpenes were not found in the samples.

Pentacyclic triterpanes are abundant in all extracts, except two, probably highly weathered coals. The distribution comprised hopanes in the range from C_{29} to C_{34} (from 17α,21β(H)-30-norhopane to 17α,21β(H)-29-tetrakishomohopane) and moretanes in the range from C_{29} to C_{32} (from 17β,21α(H)-30-normoretane to 17β,21α(H)-29-bishomohopane). 17α(H),21β(H)-hopane (C_{30} αβ) occurs in the highest concentration in all extracts (Fig. 2). The type of distribution is similar in all samples and typical for moderate mature organic matter. Hopanes of the ββ series were not found in the extracts, except the 3 sample (Ro=0,67%, one of the lowest values).

Polycyclic aromatic hydrocarbons with 2-5 rings such as naphthalene, phenanthrene, anthracene, fluorene, fluoranthene, chrysene, triphenylene, pyrene, benzopyrenes, benzofluoranthenes and perylene were found together with their alkyl C_1-C_5 derivatives. Naphthalene and phenanthrene derivatives show the highest concentrations. They were applied to calculate thermal maturity parameters, for all the samples except the 7 and 13 sample where naphthalene and phenanthrene derivatives are absent.

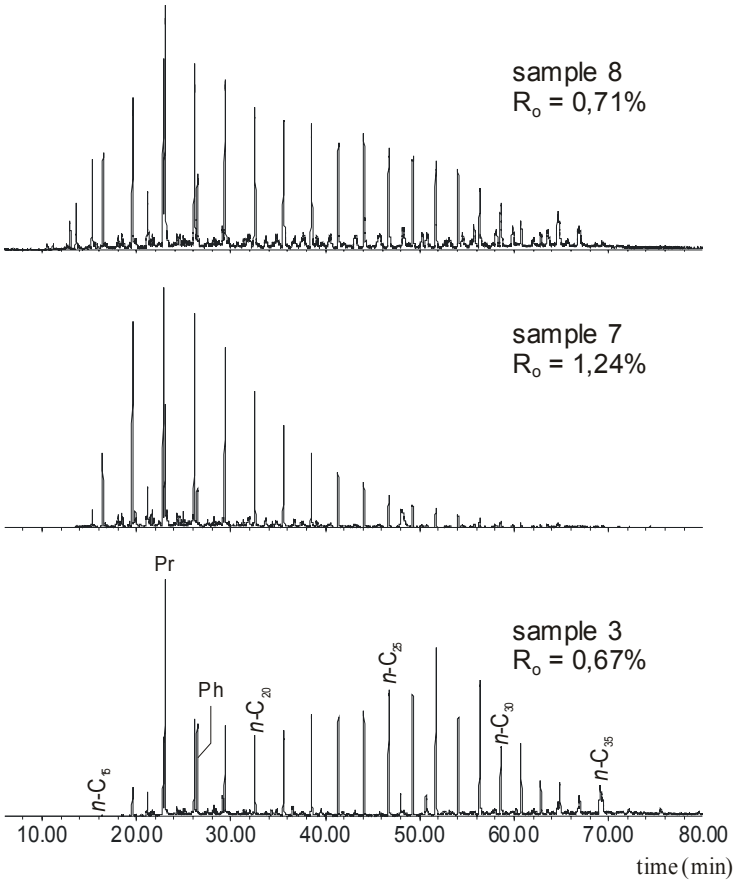

Fig. 1. Three types of *n*-alkane distribution in the investigated coals of the Upper Silesia Coal Basin

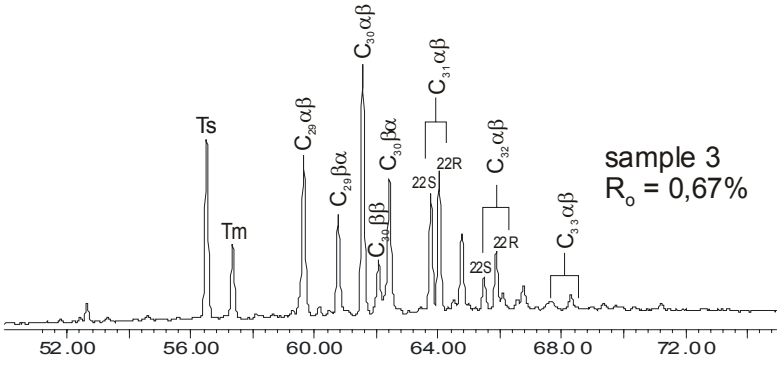

Fig. 2. Distribution of pentacyclic triterpanes in the coals of the Upper Silesia Coal Basin

4. Conclusions

The influence of thermal maturity is well pronounced in the investigated coals showing good relationship with calculated biomarker parameters. In the case of the 7 and 23 coals (samples with the highest Ro > 1,1%) concentrations of *n*-alkanes also decreased significantly.

However, two of the coals sampled closest to the fault plane (1 and 3, distances from the fault 0,2 and 0,3 m, respectively) show an aberrations in all their maturity parameters based on aromatic hydrocarbons (alkyl naphthalenes and alkylphenanthrenes), alkyl dibenzofuranes and alkyl biphenyls. Their values are 2-3 times higher than in neighbouring coal samples. Such alteration may result from thermal influence of the fault movement together with oxidising influence of hydrothermal fluids.

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CHROMATOGRAPHIC INVESTIGATIONS OF CHEMICAL DURABILITY OF PYROCATECHOL. PART II

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1. Introduction

Durability of drug is one of its basic property. It is understood as tolerance to physical, chemical and biological factors activity [1]. Each change in structure and properties of form of drug can lead to loss, weakness of pharmacological activity and sometimes even to increase of toxicity.

From many years in Department of Analytical Chemistry in Faculty of Pharmacy of Silesian Academy of Medicine are led investigations of chemical durability of drugs. This work is continuation of research concerning chemical durability of pyrocatechol [2]. Pyrocatechol has antiseptic activity [3].

The aim of this work was estimation of chemical durability of pyrocatechol on silica gel as well as in solutions in relation to storage conditions.

2. Experimental

2.1. Chemicals

Ethanol, sodium chloride, methanol (POCh, Poland), chloroform (Chempur, Poland), and pyrocatechol (PS PARK, UK) were analytically pure.

2.2. Basic solutions

50 mg of pyrocatechol was dissolved in 25 mL of distilled water, solution of physiological salt and ethanol, relatively, obtaining solutions with concentration of 2 mg/mL.

2.3. Exposure of basic solutions

From basic solutions mentioned above was taken 5 mL of individual solutions into the flasks and they were subjected to exposure of visible light, UV radiation (λ=254 nm) as well as temperature (40^{o}C).

2.4. Conditions of research made by thin layer chromatography

2.4.1 Investigation of pyrocatechol durability on silica gel

Standard ethanol solution of pyrocatechol in amount of 5 μL was spotted on chromatographic plates precoated by silica gel 60 F_{254} (E. Merck, #1.05554). Plates were subjected to activity of:

a) temperature of 120°C
b) UV radiation (λ = 254 nm) in room temperature

during 150 minutes. After that 5 μL of pyrocatechol as standard was spotted.

2.4.2 Investigaion of pyrocatechol solutions subjected to exposure

Aluminium plates precoated by silica gel 60F_{254} (E. Merck, #1.05554) were activated during 30 minutes in temperature of 120°C. Pyrocatechol solutions were spotted using micropipette (CAMAG, Switzerland) in volume of 2 μL on chromatographic plates.

2.4.3. Chromatographic plates development

Chromatograms were developed in classical chromatographic chamber made by CAMAG to height of 14 cm from start line, using 50 mL mixture: chloroform+methanol (9:1, v/v) as mobile phase. Chromatographic chamber were saturated with mobile phase used during 15 minutes.

2.5. Densitometric investigation

2.5.1 Densitometric investigation of pyrocatechol

Densitometric investigation were made using densitometer Desaga. Densitograms of pyrocatechol were made with λ = 240 nm. Wavelenght mentioned above was chosen experimentally (from 220 to 360 nm) as optimum.

2.5.2 Densitometric investigation of solvents

5 μL of ethanol, distilled water and solution of physiological salt were spotted on activated chromatographic plate and the plate were developed using chloroform+methanol (9:1, v/v) as mobile phase. After drying plate was subjected to densitometric analysis with λ = 240 nm on paths adequate to solvents spotted.

3 Results and Discussion

3.1 Densitometric investigation of chromatographic plate

Chromatographic plate precoated with silica gel (without pyrocatechol spotted) developed earlier using chloroform+methanol (9:1, v/v) as mobile phase was subjected to densitometric analysis. In the place of front of mobile phase was stated peak with high intensity (fig.1).

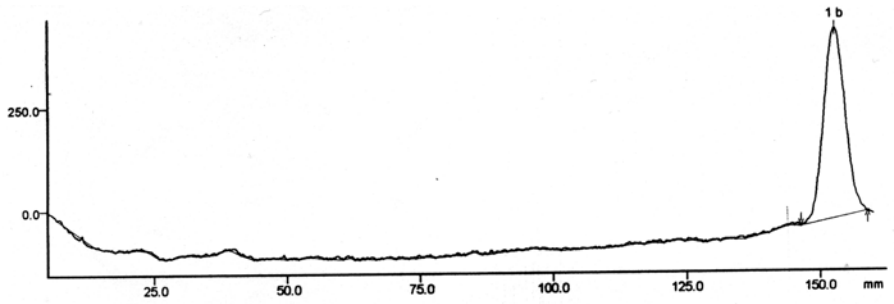

Fig. 1. Densitogram of plate precoated with silica gel developed using benzene as mobile phase.

It shows that mobile phase used eluates the impurities from chromatographic plate. Such a peak is observed on all densitograms of both pyrocatechol and solvents.

3.2. Densitometric investigation of solvents of pyrocatechol.

Ethanol, distilled water and solution of physiological salt, earlier developed on plate precoated with silica gel and with chloroform+methanol (9:1, v/v) as mobile phase, were subjected to densitometric investigation. There is only one peak origin from impurities evaluated with front of mobile phase on densitograms of ethanol, distilled water and physiological salt. It

was stated that solvents used (ethanol, distilled water and solution of physiological salt) do not contain any impurities possible to densitometric detection with λ = 240 nm. Those solvents could serve to investigation of pyrocatechol durability.

3.3. Pyrocatechol durability on silica gel

Densitogram of pyrocatechol, spotted on plate after earlier its activation in temperature of 120°C during 150 minutes in presented in fig. 2.

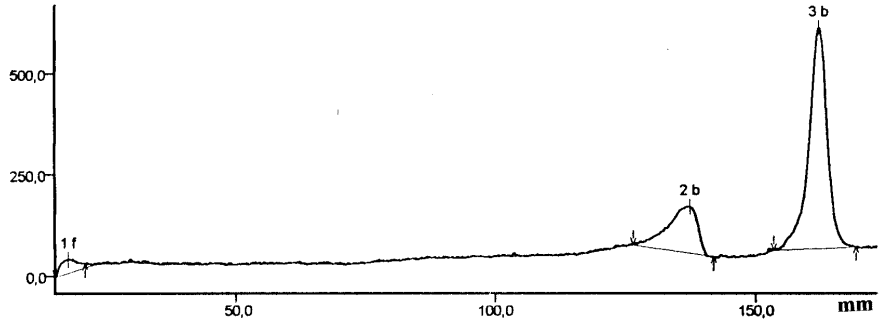

Fig. 2. Densitogram of pyrocatechol standard (silica gel activated in temperature of 120°C during 150 min.)

The following chromatographic bands are seen on densitograms (fig. 2): at the start (1f) with area 117.7 mm^2 as well as second with $R_F = 0.84$ from pyrocatechol (2b) with area 735 mm^2. Peak „3b" comes from impurities of stationary phase, which are eluated by mobile phase (that peak was seen on all densitograms of pyrocatechol, figs. 1-5). Area of pyrocatechol spot makes 86.2%, and substances at the start - 13.8% of total peaks area.

Densitogram of pyrocatechol, which after spotting on chromatographic plate was heated during 150 minutes in temperature of 120°C is presented in fig. 3.

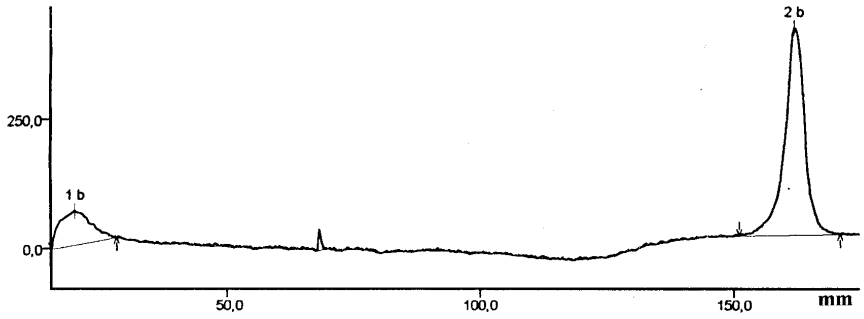

Fig 3. Densitogram of pyrocatechol, which after spotting on silica gel was heated during 150 minutes in temperature of 120°C.

In densitogram presented in fig. 3 band placed at the start (1b) with area 471.2 mm^2 is seen. One more peak with small area and R_F=0.24 is observed as well. That peak was not specified by densitometer.

Therefrom that heating of pyrocatechol after its spotting on silica gel causes complete pyrocatechol oxidation.

Densitogram of standard of pyrocatechol spotted on silica gel (silica gel before spotting of pyrocatechol was subjected to UV radiation with wavelenght λ = 254 nm during 150 minutes) is presented in fig. 4.

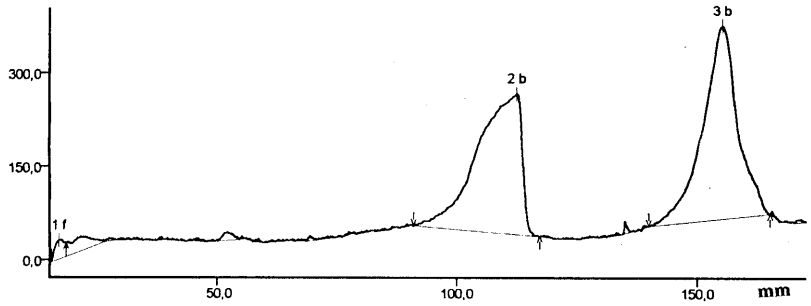

Fig. 4. Densitogram of pyrocatechol standard form plate precoated with silica ge (silica gel before spotting of pyrocatechol was subjected to UV radiation with wavelenght λ = 254 nm during 150 minutes).

Densitogram presented in fig. 4 shows at the start peak „1f" with area 65.8 mm^2 as well as peak „2b" origin from pyrocatechol (R_F=0.65) with area 2392.7 mm^2. Area of spot at the start makes 2.7%, and pyrocatechol - 97.3% of total area.

Densitogram of pyrocatechol which after spotting was subjected to UV radiation with wavelenght λ = 254 nm during 150 minutes is presented in fig. 5.

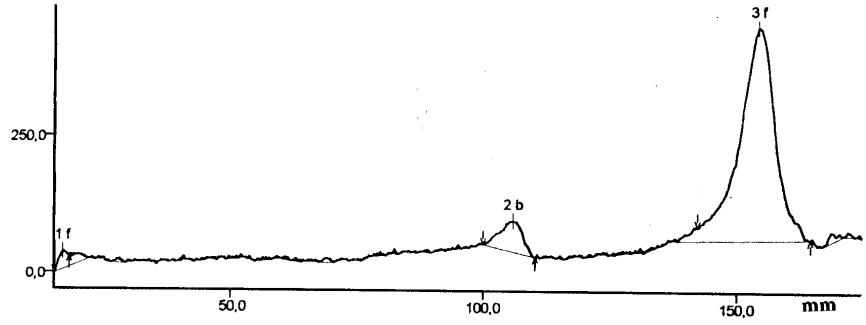

Fig. 5. Densitogram of pyrocatechol which after spotting was subjected to UV radiation with wavelenght λ = 254 nm during 150 minutes.

There is peak (1f) at the start with area 59.2mm^2 (17%) as well as pyrocatechol peak with R_F=0.61 and area 289 mm^2 (83%) on that densitogram. The area of pyrocatechol (289 mm^2) in these conditions makes 12% af area of pyrocatechol (2392.7 mm^2) investigated as standard. Exposure of pyrocatechol, spotted on silica gel, with UV radiation causes pyrocatechol oxidation as well as change of adsorption in relation to pyrocatechol spotted as standard. It was stated that after 150 minutes of exposure of pyrocatechol spotted on silica gel with UV radiation and with wavelenght λ=254 nm, decrease as many as 88% of pyrocatechol.

Moreover the way of preparing of chromatographic plate decidedly influence on pyrocatechol adsorption. On chromatographic plate precoated with silica gel, which was activated during 150 minutes in temperature of 120^{o}C the R_F value of pyrocatechol is 0.84 (fig. 2). However on chromatographic plate, which was exposured during 150 minutes with UV radiation the R_F value of pyrocatechol is 0.65 (fig. 4).

3.4. Influence of ethanol, distilled water as well as solution of physiological salt on pyrocatechol durability.

Changes of colour of pyrocatechol solutions in ethanol, distilled water and solution of physiological salt, exposured to visible light, UV radiation (λ = 254 nm) and temperature of 40°C are presented in Table 1.

Table 1. Change of colour of pyrocatechol solutions because of the influence visible light, UV radiation and increased temperature (40°C).

Solution investigated	**Visible light**		**UV light**		**Increased temperature (40°C)**	
	After 70 h	After 160 h	After 35 h	After 80 h	After 165 h	After 380 h
Pyrocatechol in distilled water	Light yellow	Brownish	Light brown	Brown	Light brown	Brown
Pyrocatechol in solution of physiological salt	Light yellow	Brown	Yellow-brown	Light brown	Rusty-brown	Dark brown
Pyrocatechol in ethanol	Yellow	Yellow	Light yellow	Yellow	Yellow	Yellow-brown

Basic solutions were colourless and transparent. Changes of colour can testified about pyrocatechol distribution in those solutions. The most sensitive solution of pyrocatechol in solution of physiological salt, already after first 24 h in incubator showed changes of colour. Smaller changes were in water solution of pyrocatechol, whilst the smallest sensitive was ethanol solution of pyrocatechol kept in the same conditions.

Investigated solution were undergone to chromatogrpahic analysis using NP-TLC on the day of preparing of standard solutions of pyrocatechol as well as after finishing their exposure to visible light (160 h), UV radiation (80 h) and temperature of 40^{o}C (380h).

Chromatograms obtained were undergone to densitometric analysis. The results obtained are presented in Table 2.

Table 2. R_F values and areas (%) of chromatographic bands of solution of pyrocatechol in ethanol, distilled water and solution of physiological salt exposured to visible light (160h), UV radiation λ=254 nm (80h) as well as temperature of 40°C (380h).

	Pyrocatecho in ethanol		**Pyrocatechol in distilled water**		**Pyrocatechol in solution of physiological salt**	
Standard	R_F	area [%]	R_F	area [%]	R_F	area [%]
	0.65	**100%**	0.05 **0.79**	6.4% **93.6%**	0.04 **0.75**	8.0% **92.0%**
Visible light (160h)	R_F	area [%]	R_F	area [%]	R_F	area [%]
	0.66	**100%**	0.05 **0.75**	9.5% **90.5%**	0.05 **0.72**	9.5% **90.5%**
UV radiation (λ=254nm) (80h)	R_F	area [%]	R_F	area [%]	R_F	area [%]
	0.70	**100.0%**	0.04 **0.74**	12.8% **87.2%**	0.04 **0.72**	15.0% **85.0%**
Incubator (380h)	R_F	area [%]	R_F	area [%]	R_F	area [%]
	0.04 **0.68**	7.8% **92.2%**	0.05 **0.75**	14.0% **86.0%**	0.04 **0.71**	20.8% **79.2%**

Results presented point that ethanol has the biggest stabilizing properties in relation to pyrocatechol, whilst the smallest stabilizing properties in relation to pyrocatechol has physiological salt.

4 Conclusion

1. The complete of pyrocatechol heated during 150 minutes in temperature of 120°C after its spotting on chromatographic plate precoated with silica gel was stated.
2. After 150 minutes of UV irradiation with λ = 254 nm – about 80% of pyrocatechol on chromatographic plate decrease.
3. It was stated that ethanol has the biggest stabilizing properties in relation to pyrocatechol.
4. Pyrocatechol dissolved in solution of physiological salt as well as heated in temperature of 40°C and irradiate wit UV light undergo a lightly bigger changes in relation to water solution.

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