POSTER SESSION II

THURSDAY, MAY 31 th, 2012

CHAIRPERSONS: A. Malenović and Ł. Cieśla

15 .

Complex-numbers representation of the retention in two-dimensional TLC

Łukasz Komsta

Department of Medicinal Chemistry, Faculty of Pharmacy

Medical University of Lublin, Jaczewskiego 4, 20-090 Lublin, Poland

Main TLC coefficients such as spot position, R_F, k, R_M, ΔR_M and ΔR_F can be represented in 2D TLC as a single complex numbers, simplifying mathematical operations and their interpretation. When a spot position (x) is represented as a single complex number with one R_F as the real part and another as the imaginary part, the following generalizations can be done:

1. $\mathbf{R_F}$ can be then computed as $x/(1+1i)$, where $1+1i$ is complex position of the solvent front. The real part of R_F can be then interpreted as an average R_F value, where the imaginary part represents the difference between R_F values. The absolute value of it is the distance of the spot from starting point (origin). Module can be interpreted as the angle between diagonal and the trace of the spot.
2. ***k*** coefficient can be computed from complex R_F from default formula $(1-R_F)/R_F$. The real and imaginary parts are difficult to interpret, however absolute value is an average k value and the argument represents difference between the retention in two dimensions.
3. $\boldsymbol{R_M}$ coefficient is (as in the classic case) simply a decimal logarithm of complex k. Its real value is an average R_M and the imaginary value represents the difference.

Differences such as ΔR_M and ΔR_F have also meaningful interpretation when calculated in complex-way from the complex equivalents. The calculation can be also extended to some selectivity criteria, such as R_S coefficient.

When applying the linear regression to complex numbers with real and the same modifier concentrations, we obtain the complex extrapolated R_M with mean (averaged) R_M as the real part and extrapolated difference as the imaginary part. Analogous interpretation can be done in the case of slope. This can be useful for 2D TLC lipophilicity estimation with two modifiers of the same concentration.

16.

Assessment of β-lactams Retention in Hydrophilic Interaction Chromatography Applying Box – Behnken Design

Marko Jovanović, Tijana Rakić, Biljana Jančić Stojanović, Anđelija Malenović*
University of Belgrade, Faculty of Pharmacy, Department of Drug Analysis, Vojvode Stepe 450, Belgrade
*andja@pharmacy.bg.ac.rs

In this paper the retention prediction models for mixture of β-lactam antibiotics analyzed by hydrophilic interaction chromatography (HILIC) are presented. The aim of the study was to investigate the retention behavior of some organic acids including cefalosporines (cefotaxime, cefalexin, cefaclor, cefuroxime, cefuroxime axetil) and penicillines (ampicillin and amoxicillin). Retention of substances with acidic functional group in HILIC is considered to be interesting due to the lack of these investigations in literature. In the beginning of the study, classical silica columns were chosen for retention analysis. Than, preliminary study was carried out and factors with the most significant influence on retention factors were selected. The influence of these factors on retention factors were further investigated employing Box – Behnken design as a tool. On the basis of the obtained results the mathematical models were created and tested using ANOVA test and finally verified with four additional experiments. All the obtained models were adequate except of cefuroxime axetil, which showed non-retention behavior. This approach enables the presentation of chromatographic retention in many ways (three-D graphs and simple two dimensional graphical presentations). All of these gave the possibility to evaluate the impact of each factor and factor interaction on retention behavior and to predict the chromatographic retention under different conditions. The concentration of acetonitrile has shown the greatest impact on the retention factor of the analyzed compounds (directly proportional). Buffer concentration (directly proportional) and pH of the water phase (inversely proportional) had a similar but significantly less impact on the retention factor of the compounds. Furthermore, regarding the structure of the analyzed compounds, the potential retention mechanisms in HILIC were suggested.

17.

THE STUDY OF AZOLE ANTIFUNGALS RETENTION BEHAVIOR BY EXPERIMETAL DESIGN AND ARTIFICIAL NEURAL NETWORKS

Ana Vemić, Tijana Rakić, Nađa Kostić, Biljana Jančić Stojanović, Anđelija Malenović*

University of Belgrade – Faculty of Pharmacy, Department of Drug Analysis, Vojvode Stepe 450, Belgrade, Serbia

* andja@pharmacy.bg.ac.rs

The most efficient way to provide data valuable for the evaluation of the analytes' retention behavior in liquid chromatography is the employment of certain chemometrical tools. In this study of azole antifungals' retention behavior the experimental design without and in the combination with artificial neural networks (ANNs) was applied. The investigated mixture consisted of the antifungals bifonazole, fluconazole, ketoconazole, clotrimazole, econazole and miconazole. These substances were selected based on their similar chemical structure. The experiments were performed on the chromatographic system Finnigan Surveyor Thermo Scientific. The analytical column was SunFire C18, 100 mm x 3.0 mm, 3.5 μm particle size. Flow rate was 0.75 mL min^{-1} and detection wavelength 265 nm. From the preliminary studies methanol content, pH of the mobile phase and column temperature were selected as the factors important for the further evaluation, while the content of triethylamine was set at 1%. The plan of experiments was determined by central composite design (CCD) comprised of full factorial 2^3 design, star design ($\alpha = \pm 1.7$) and four replications in central point. As the system outputs, the retention factors of all six investigated substances were chosen. The adequate models were built and from the corresponding coefficients the methanol content and pH value of the water phase were distinguished as the most influential factors. In the next step, the pattern for the analyzed system behavior was created employing ANNs. The network with highly predictive ability was obtained by network optimization. The final topology of network was 3–8–6, 12 experiments were used in a training set while the back propagation algorithm was optimal for the network training. High ability of defined network to predict the retention of the investigated azoles was confirmed by correlations higher than 0.9912 for all the analytes. Both presented approaches enabled the adequate prediction of azoles' retention behavior, as well as the extraction of the information important for the better understanding of the analyzed system.

18.

Determination of hyaluronidase activity by new HPCE method.

Jan Matysiak, Paweł Dereziński, Bartosz Urbaniak, Agnieszka Klupczyńska, Zenon J. Kokot,

Department of Inorganic & Analytical Chemistry
Poznan University of Medical Sciences
Grunwaldzka 6 Street, 60-780 Poznan, Poland
tel.: +48 61 854 66 11, fax: +48 61 854 66 09, e-mail: zjk@ump.edu.pl

Hyaluronic acid (HA), one of the most highly investigated substances in today's medicine, is a linear polysaccharide formed of disaccharide units containing N-acetylglucosamine and glucoronic acid. HA is found throughout the human body e.g. in the skin, vitreous humor and cartilage. Moreover it is a critical glucosaminoglycan in synovial joints because it is a main component of the synovial fluid. HA is used as a diagnostic factor for many diseases such as: rheumatoid arthritis, cancerous tumors and liver diseases. It is, therefore, important to quantify HA in the biological fluids and to study the profile of enzymatic digestion of this compound by hyaluronidase. There are many sources of this enzyme (including hyaluronidase from Hymenoptera venoms) and all of hyaluronidases have the ability to digest HA.

The aim of the study was to develop new capillary electrophoresis method for determination of enzymatic activity of hyaluronidase. The procedure was based on mixing of a known quantity of hyaluronic acid and an aliquot of hyaluronidase solution, followed by obtaining HPCE profiles after a known period of incubation. The activity of hyaluronidase was determined using multiple regression analysis in which sizes of the peaks of the main degradation products were used.

Studies were performed using HPCE instrument (Agilent Technologies) equipped with capillary of total length 64,5 cm, effective length 56 cm and inside diameter 75 μm. Separation was performed in the phosphate buffer (pH 8,10) in the electric field of 20 kV. Detection was performed at 220 nm.

The following steps and parameters were taken into account for the validation of the method: precision, accuracy, linearity, repeatability, LOD and LOQ. All steps of validation proved that the developed method is suitable for its intended purpose.

The developed HPCE method is characterized by short time of analysis, low volume of injected sample, a small amount of buffers used and as a result - a low cost of analysis.

The study confirmed that using HPCE it is possible to evaluate hyaluronidase activity, manifested in the creation of degradation products of hyaluronic acid. The amount and size of the peaks are dependent on time and the concentration of hyaluronidase.

19.

RPTLC DETERMINATION OF LIPOPHILICITY PARAMETERS OF POLYDENTATE SCHIFF BASES OBTAINED FROM *O*-HYDROXYARYL ALDEHIDES AND KETONES WITH AROMATIC DIAMINES

N. Stevanović[1], A.Blagus[2], A. Lolić[1], M.Natić, Ž.Tešić[1] and Rada Baošić[1]

[1]*Faculty of Chemistry, University of Belgrade, Studentski trg 12-16, Belgrade, Serbia*

[2]*Department of Chemistry, J. J. Strossmayer University, Franje Kuhača 20,Osijek, Croatia*

Lipophilicity is an important physicochemical property of bioactive compounds, because it is related to the tendency of molecule to be transported through biological membranes. The lipophilicity parameters of some polydentate Schiff bases obtained from *o*-hydroxyaryl aldehides and ketones with aromatic diamines (Table) have been determined by reverse-phase thin-layer chromatography on silica gel RP-18 plates (Merck, Darmstadt, Germany).

Name		Color
1,4–bis((2-hydroksybenzylden)amino)benzen	$C_{20}H_{16}N_2O_2$	orange
[1-(2 aminofenylimino)ethyl]–phenol	$C_{14}H_{14}N_2O$	yelow
N,*N*'-bis(2-hydroksy-1-naphthaldimin)-1,2-diaminobenzene	$C_{28}H_{20}N_2O_2$	black
1,4-bis(2-hydroksyphenyl-benzylideneamino)benzene	$C_{32}H_{24}N_2O_2$	red-brown
1,3-bis(2-hydroksy-1-naphthylmethylidenamino)benzene	$C_{28}H_{20}N_2O_2$	beige
1,4-bis(2-hydroksy-1-naphthylmethylidenamino)benzene	$C_{28}H_{20}N_2O_2$	brown
1,2-bis(2-hydroksy-3-metoksibenzylidenamino)benzene	$C_{22}H_{20}N_2O_4$	orange
1,3-bis(2-hydroksy-3-metoksibenzylideamino)benzene	$C_{22}H_{20}N_2O_4$	orange-red

The mobile phases were mixtures of methanol, acetonitrile and tetrahydrofurane (as organic modifier) with water. The concentration of organic modifier in the mobile phase ranged from 50 to 80% (v/v) in 5% increments. A linear relationship, with satisfactory correlation coefficient, was obtained between R_M values and the concentration of organic modifier in the mobile phase. Regular retention behaviour was observed, i.e. retention decreased regularly with increasing concentration of organic modifier in the mobile phase. R_M^o values were obtained by extrapolation to 100 % (v/v) water in the mobile phase. Lipophilicity, C_o, was calculated as the ratio of the intercept and slope values, for each binary system. The effect of structure on chromatographically obtained lipophilicity parameters, relation between these parameters and calculated ClogP as well as effect of mobile phase on retention behaviour of the investigated compounds were discussed.

20.

UTILIZATION OF CHARGE-TRANSFER GAS CHROMATOGRAPHY FOR ANALYSIS OF FORMATION WATER

Patryk Bielecki*, Daria Skraburska, Wiesław Wasiak

*Department of Analytical Chemistry, Faculty of Chemistry, Adam Mickiewicz University, Grunwaldzka 6, 60-780 Poznań, Poland, *e-mai: bielecki@amu.edu.pl*

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The implementation of new law on mining wastes, forces the changes in the rules dealing with formation water, which is extracted together with oil and natural gas. A condition that must be met by the mining company before the re-congestion of formation water into the orogenic belt, is to prove the constancy of the chemical composition of this water during the mining process. Therefore, it is necessary to develop a rapid and effective method for the comparative evaluation of the formation water composition before and after the mining process. Since, the formation water includes, inter alia, petroleum hydrocarbons (eg. BTEXs, olefins), it seems reasonable to apply complexation gas chromatography to their separation. In order to reduce time-consuming sample preparation process, it was decided to utilize head-space solid-phase microextraction technique with usage of the fibre coated with PDMS. This technique enables direct sampling of the headspace phase.

In this study, chemically modified silica (particle diam.: 5 μm, pore size: 300 nm) with cyclam-$CuCl_2$ complex, was used as a stationary phase for gas chromatography. Modified silica was applied to prepare a capillary PLOT (porous-layer open tubular) column (30 m × 0.32 mm). Prepared column was utilised directly to conduct the chromatographic analyses. Earlier studies [1] have shown that the properties of such complexes are suitable for the separation of volatile olefin mixtures. This work focuses on PLOT capillary column preparation and its application to qualitative analysis of the formation water by the HS-SPME-GC technique.

References

[1] P. Bielecki, W. Wasiak, *J. Chromatogr. A* 1217 (2010) 4648.

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21.

Rapid liquid chromatography-hybrid OrbiTrap mass spectrometry studies of polyphenols in Serbian honey

Silvio Kečkeš[1], Uroš M. Gašić[2], Dragana Č. Dabić[2], Dušanka M. Milojković-Opsenica[2], Maja M. Natić[2], and Živoslav Lj. Tešić[2]

[1]Analysis, Gandijeva 76A, 11070 Belgrade,Serbia

[2]Faculty of Chemistry, University of Belgrade, P. O. Box 51, 11158 Belgrade, Serbia

Polyphenolic profiles in honey samples collected from different regions of Serbia were analyzed using Ultra High Performance Liquid Chromatography (UHPLC) coupled with hybrid mass spectrometer which combines Linear Trap Quadropole (LTQ) and OrbiTrap mass analyzer. Detection was performed in the atmospheric pressure negative heated electron spray ionization (API-HESI) mode. Honey samples were of different botanical origin: Acacia (*Robinia pseudoacacia*), Sunflower (*Helianthus annuus*), Linden (*Tilia cordata*), Basil (*Ocimum basilicum*), Buckwheat (*Fagopyrum esculentum*), Oilseed rape (*Brassica napus*), Goldenrod (*Solidago virgaurea*). The existence of numerous metabolic markers, mainly flavonoids in all honey samples was proven based on their characteristic mass spectra and fragmentation pattern. Principal component analysis (PCA), as useful multivariate statistical technique for data evaluation, was utilized to select and establish floral markers of the botanical origin of Serbian honeys.

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22.

CONSEQUENCES OF CADAVERINE AND PIPERIDINE DURING THE PRODUCTION OF DRY FERMENTED SAUSAGES FOR THE FORMATION OF *N*-NITROSOPIPERIDINE

E. De Mey[a,b*], H. De Maere[a], L. Dewulf[a], T. Kowalska[b], M-C. Peeters[c], G. Derdelinckx[c], H. Paelinck[a],

[a]*Research Group for Technology and Quality of Animal Products, Catholic University College Ghent, Belgium;* [b]*Institute of Chemistry, University of Silesia, Katowice, Poland;* [c]*Department Microbial and Molecular Systems, Catholic University Leuven, Belgium*

The formation of the carcinogenic *N*-nitrosamines (NAs) in cured meat products is in general associated to the presence of biogenic amines in combination with the use of sodium nitrite. Cadaverine, a possible precursor of the NA *N*-nitrosopiperidine (NPIP), can accumulate in the sausages during the production process. Thereafter, piperidine, the direct precursors of NPIP, can be formed by the deamination and cyclisation of cadaverine. Although it is clear that during an intense heating of cured meat products, cadaverine and piperidine can be nitrosated [1], the exact conditions for NPIP formation in dry fermented sausage remains unclear. The aim of the study is to investigate the processing parameters, i.e. pH-decline and concentration $NaNO_2$,hich can influence the formation of NPIP in the presence of cadaverine or piperidine in a dry fermented sausage model.

In the global data set, three subsets were considered: (1) blank dry fermented sausage samples, and samples fortified with (2) 500 $mg.kg^{-1}$ cadaverine, or (3) 10 $mg.kg^{-1}$ piperidine. Within each subset, two factors, i.e. concentration of $NaNO_2$ (0 or 150 $mg.kg^{-1}$) and pH-decline (to 4.8 or 5.5) were varied. During the production, samples were taken from the sausages prepared according the different recipes. Volatile *N*-nitrosamines were analyzed by gas chromatography coupled to a Thermal Energy Analyzer (GC-TEA) [2]. Biogenic amines were dabsylated and the derivatives were quantified by RP-HPLC-UV [3]. The results were statistically analyzed by means of ANOVA (PASW Statistics 18.0.0, SPSS Inc., Chicago, USA)

During the processing, the amount of cadaverine and piperidine increased in the blank samples, but no NPIP formation could be detected (< LOD=0.5 $\mu g.kg^{-1}$). When an excess of cadaverine (500 $mg.kg^{-1}$) was added, a slightly higher amount of piperidine was measured in the end product, but no *N*-nitrosamines were detected. Only in the models where 10 $mg.kg^{-1}$ of piperidine was added to the sausage dough, NPIP could be detected. but the concentrations remained under the limit of quantitation (< LOQ=1.5 $\mu g.kg^{-1}$). Alterations in pH and $NaNO_2$ gave no significant effect on the NPIP formation. The conclusion can be made that NPIP can be formed when an high concentration of piperidine is present but variation in pH or $NaNO_2$ had no influence. Finally, the addition of an excess of cadaverine could not provoke the formation of NPIP.

References

[1] Drabik-Markiewicz, G., Dejaegher, B., De Mey, E., Kowalska T., Paelinck H., Vander Heyden, Y. Food Chemistry, 126 (2011), 1539-1545.

[2] Gasarasi G., Troy P.D., Debeuckelaere W., Delvaux F., Derdelinckx G. J. Pharm. Belg., 56 (2001), 51-53.

[3] De Mey, E., Drabik-Markiewicz, G., De Maere, H., Peeters, M.-C., Derdelinckx, G., Paelinck, H., Kowalska, T. Food Chemistry 130 (2012), 1017-1023.

23.

TLC AND MAGNETO–TLC AS A METHOD FOR INVESTIGATION ON SELECTED d- AND f-ELECTRON ION ELEMENT COMPLEXES WITH ORGANIC LIGANDS

A. Wronka, W. Ferenc and I. Malinowska

Faculty of Chemistry, Maria Curie - Skłodowska University.

Pl. M. Curie-Skłodowskiej 3, 20-031 Lublin, POLAND.

The chemistry of coordination compounds is an important and challenging area of modern inorganic and bio–inorganic chemistry. Variety of metallurgical processes, analytical reagents and industrial catalysts involve the use of coordination compounds. They also play important role in biological systems and medicinal chemistry. Among complexes d and 4f electron metals compounds are one of the most interesting group.

In recent decades, progress in this area was significant. Exploring their physical properties such as solubility, structural and magnetic properties. Complex compounds have been investigated using various methods, such as: IR, Raman spectroscopy and X-ray analysis. In the present work, Thin Layer Chromatography combined with the magnetic field is proposed to use as complement method for determination of properties of investigated complex compounds. The retention analysis of investigated compounds may give us some information about their affinity to different stationary phase surfaces and about the influence of the central ion or organic ligand structure on the retention of these compounds.

In present research, the retention of 10 d- and 12 of 4f-electron complexes metals with different organic ligands in RP and NP chromatographic systems was studied. Taking into account the fact, that in present times magnetic fields are different than terrestrial origin in many places, it is interesting to examine, how the magnetic field influences on the properties of investigated compounds. Therefore, the chromatograms were developed simultaneously in two identical chromatographic chambers and one of them was placed in external magnetic field with two values of vector of inductivity(0.2 and 0.4 T).

In magnetic field, retention of some complexes have been changed, what means, that magnetic field influences the properties of the analyzed compounds and their interactions with surface of the stationary phase.

Chromatographic investigations in RP systems can give us a preliminary information about biological activity of the compounds. In mentioned systems the log k_w parameter was determined for investigated compounds inside and outside of magnetic filed. Presence of magnetic field changed the log k_w values of investigated substances. This is information is very important, because some of the analyzed compounds may be in future applied in medicine and beauty care.

24.

2-PHENYLPROPIONIC ACID AS MOLECULAR ROTOR IN THIN-LAYER CHROMATOGRAPHY SYSTEMS

Magdalena Knaś[1], Mieczysław Sajewicz[2], Teresa Kowalska[2], Jarosław Polański[1]

[1]Department of Organic Chemistry, [2]Department of General Chemistry and Chromatography, Institute of Chemistry, University of Silesia, 9 Szkolna Street, 40-006 Katowice, Poland

In our studies we have focused on several classes of compounds, that can act as molecular propellers. Molecular and chiral rotors are molecules able to produce a variety of special effects, due to their ability for the specific rotational motion, however, these effects are not well recognized [1].
In this work we present that in the thin-layer chromatography (TLC) systems, the chiral rotors can deviate their migration route from the expected straight-line direction. Profen drugs investigating by means of TLC show lateral relocation of the analyte spots in planar chromatograms. The investigated chiral 2-phenylpropionic acid and other profens, while migrating with the solvent on the TLC plates, deviated from the straight-line route [2]. We have also showed an influence of molecular chirality of impregnates (L- and DL-arginine) on RF values and chromatographic spots' deviation. TLC systems used in these experiments were composed of the three different stationary phases. We have obtained the enantioseparation of the 2-phenylpropionic acid, selected to these studies.
Researches of chiral propellers are an important part of nanotechnology, where the application of molecular mechanisms, mimicking the real macroscopic objects, such as aircraft propellers or windmills, plays a key role. Technology based on the proposed theory of molecular propellers can be used for the profens' separation. The identification of specific properties of various chemical compounds, in this case of rotation, may allow for implementation of new nanotechnology solutions in the pharmaceutical industry. It can be used in the manufacture of drugs, development of new technology and the economy.

[1]. G. S. Kottas, L. I. Clarke, D. Horinek, J. Michl, Chem. Rev. 2005, 105, 1281
[2]. M. Sajewicz, R. Piętka, G. Drabik, E. Namysło, T. Kowalska, J. Planar Chrom. 2006, 19, 273

25.

Polarimetric detection in high-performance liquid chromatography and its intrinsic weakness

Magdalena Knaś, Mieczysław Sajewicz, Jarosław Polański, and Teresa Kowalska

Institute of Chemistry, University of Silesia, 9 Szkolna Street, 40-006 Katowice, Poland

Polarimetric detection in HPLC might seem an of course matter, and especially in such areas as control of optical purity of drugs or fingerprinting of herbal extracts. However, relatively low popularity of polarimetric detection in HPLC is an admitted fact which certainly has the physically well founded reasons. Such reasons can be more profound than, e.g., an insufficient sensitivity of this type of detectors, when compared with PDA or ELSD.

In this paper, upon an experimental example of *R*(-)-naproxen we discuss physical phenomena (i.e., gelation of organic solvents by small organic molecules, the effect of molecular rotors, and oscillatory interconversion of chiral analytes) which might obstruct quantification of profen drugs with use of HPLC with polarimetric detection. We believe, however, that the discussed (or analogous) phenomena are of a far more general nature, which in fact hamper a widespread application of the polarimetric detection in HPLC.

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Achiral HPLC/DAD and HPLC/ELSD applied to investigation of the oscillatory peptidization with *L*-Pro, *L*-Hyp, and *L*-Pro–*L*-Hyp

M. Sajewicz, M. Sławacka, G. Sztafińska, T. Kowalska
Departament of General Chemistry and Chromatography, Institute of Chemistry, University of Silesia, Katowice, Poland

Non-linear reactions are relatively rarely encountered, to a large extent due to considerable analytical difficulties. If a non-linear reaction occurs in a colorless solution and moreover, if it is not accompanied by any spectacular heat or electric effect (like it happens, e.g., with redox reactions), then its discovery often is a matter of chance. The chromatographic techniques can be regarded as universal and effective tools serving the discovery and monitoring of the non-linear reactions.

Ivanov et al. [1,2] were the first ones to discover the spontaneous oscillatory polycondensation of the chiral silicon derivatives with aid of TLC. In our laboratory, we managed to demonstrate the spontaneous oscillatory chiral inversion of profen drugs and the other low-molecular-weight carboxylic acids with use of the chiral TLC [3,4], and the spontaneous oscillatory peptidization of certain single amino acids (e.g., phenylglycine, [5]) and the spontaneous oscillatory oligomerization of lactic acid [6] with aid of achiral HPLC/DAD and HPLC/ELSD.

It was the aim of this study to trace the spontaneous peptidization of the two biologically important amino acids, i.e., *L*-proline (*L*-Pro) and *L*-hydroxyproline (*L*-Hyp), largely responsible for the architecture of the mammalian and human muscles, and also peptidization thereof in a binary *L*-Pro–*L*-Hyp system. The investigations were carried out with the 70% aqueous solutions of these amino acids in MeOH with aid of the non-chiral HPLC/DAD and HPLC/ELSD. In the two cases of single amino acids, the non-linear concentration changes were observed both with *L*-Pro and *L*-Hyp, although they considerably differed in terms of dynamics. Pattern of the *L*-Pro and *L*-Hyp concentration changes in the binary *L*-Pro–*L*-Hyp system allows an assumption as to the formation of the *L*-Pro- and *L*-Hyp-derived homooligopeptides, and also of the mixed *L*-Pro–*L*-Hyp heterooligopeptides.

References

1. P.V. Ivanov, V.I. Maslova, N.G. Bondareva, O.A. Yur'eva, N.V. Kozlova, E.A. Chernyshev, K.Yu. Odintsov, E.A. Zykunova, Rus. Chem. Bull. 46 (1997) 2138.
2. E.A. Chernyshev, P.V. Ivanov, D.N. Golubykh, Rus. Chem. Bull., 50 (2001) 1998
3. M. Sajewicz, R. Piętka, A. Pieniak, T. Kowalska, Acta Chromatogr. 15 (2005) 131.
4. M. Sajewicz, R. Piętka, G. Drabik, T. Kowalska, J. Liq. Chromatogr. Relat. Technol. 29 (2006) 2071.
5. M. Sajewicz, M. Matlengiewicz, M. Leda, M. Gontarska, D. Kronenbach, T. Kowalska, I.R. Epstein, J. Phys. Org. Chem. 23 (2010) 1066.
6. M. Sajewicz, M. Dolnik, D. Kronenbach, M. Gontarska, T. Kowalska, I.R. Epstein, J. Phys. Chem. 115 (2011) 14331.

27.

Application of the chiral TLC to enantioseparation of *DL*-proline

M. Sajewicz, M. Matlengiewicz, M. Juziuk, M. Penkala, M. Weloe, T. Kowalska
Departament of General Chemistry and Chromatography, Institute of Chemistry, University of Silesia, Katowice, Poland

Chromatographic enantioseparation of the racemic and scalemic mixtures is a difficult task, both analytically, and on a preparative and technological scale. So far, the majority of analytical enantioseparations have been carried out by means of column chromatography (HPLC and GC), with a far less pronounced contribution from the side of TLC. Such disparity cannot be fully excused though, because (i) separation performance of planar chromatographic techniques is just enough for direct enantioseparation of a single pair of compounds; (ii) direct enantioseparation by means of planar chromatography usually can be obtained with use of relatively simple stationary phases; and (iii) such enantioseparation is usually easier to obtain and moreover, it is far less expensive than by means of instrumental techniques. In monograph [1], an overview is presented of applicability of the chiral TLC technique to direct enantioseparation with the different compound classes. It is rather obvious that for practical reasons, the most important enantioseparations are those of therapeutically and biologically active enantiomers (e.g., drugs, amino acids, and hydroxy acids). Considerable contribution to the direct enantioseparation of amino acids by means of the chiral TLC was made by Bhushan et al. [2].

In the Department of General Chemistry and Chromatography, University of Silesia, methods of direct enantioseparation by means of the chiral TLC have been developed for a longer period now. Target compounds belonged to the three classes, i.e., profen drugs (e.g., [3]), hydroxy acids (e.g., [4]), and amino acids (e.g., [5]). One approach is the complexation TLC, based on impregnation of the commercial silica gel layer with the transition metal cations (e.g., Cu(II), Co(II), Ni(II), Mn(II), or Fe(II)). Enantioseparation is obtained due to the different chelating constants of a given cation with the two antimers of a given compound. So far, this particular approach was the most widely tested upon the example of the enantioseparation of *DL*-lactic acid [6,7].

In this study, we present the results of our efforts aiming to elaborate a simple yet efficient method of the enantioseparation of *DL*-proline by means of the complexation TLC, testing a selection of the transition metal cations. This approach is particularly important due to the fact that amino acids (proline included), when dissolved in various different (aqueous and non-aqueous) solvents, tend to spontaneously peptidize and in the column techniques, one has to be skillful enough to separate the monomeric amino acid antimerss from the oligopeptides (which can be regarded as an evident drawback). In TLC, similar problems with the interference of the oligopeptides can be encounterd.

References

7. *Thin Layer Chromatography in Chiral Separations and Analysis*, praca zbiorowa pod red. T. Kowalskiej i J. Shermy, CRC Press Taylor & Francis Group, Boca Raton, Florida, USA, 2007.
8. R. Bhushan i J. Martens, *Amino Acids: Chromatographic Separation and Enantioresolution*, HNB Publishing, New York, 2010 (rozdziały 8 i 9).
9. R. Piętka, *Zastosowanie chromatografii cienkowarstwowej oraz innych instrumentalnych technik analitycznych do badania reakcji oscylacyjnych wybranych kwasów 2-arylopropionowych*, Rozprawa doktorska, Uniwersytet Śląski, Katowice, 2008.
10. D. Kronenbach, *Zastosowanie chromatografii cienkowarstwowej z detekcją densytometryczną oraz innych instrumentalnych technik analitycznych do badania reakcji oscylacyjnych wybranych aminokwasów i hydroksykwasów pochodnych kwasu propionowego i kwasu masłowego*, Rozprawa doktorska, Uniwersytet Śląski, Katowice, 2012.
11. M. Gontarska, *Zastosowannie chromatografii cienkowarstwowej z detekcją densytometrryczną oraz innych instrumentalnych technik analitycznych do badania reakcji oscylacyjnych wybranych pochodnych kwasu octowego i kwasu propionowego*, Rozprawa doktorska, Uniwersytet Śląski, Katowice, 2011.
12. M. Sajewicz, M. Gontarska, D. Kronenbach, T. Kowalska, Acta Chromatogr. 20 (2008) 209.
13. M. Sajewicz, E. John, D. Kronenbach, M. Gontarska, T. Kowalska, Acta Chromatogr. 20 (2008) 367.

28.

BETTER OR FASTER? THAT IS A QUESTION!

ANALYSIS OF PAHS (POLYCYCLIC AROMATIC HYDROCARBONS) BY UHPLC UTILIZING MULTIPLE DETECTION METHODS

S. Golba[1], A.S. Swinarew[1], J. Gabor[1]
Z. Grobelny[1], B. Swinarew[2], M. Szklarska[1]
[1]*Institute of Materials Science, University of Silesia, 40-007 Katowice, Poland*
[2]*Institute for Engineering of Polymer Materials and Dyes, Paint and Plastics Department, 44-100 Gliwice, Poland*

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Polycyclic Aromatic Hydrocarbons (PAHs) are known to be carcinogenic compounds containing several benzene rings. Government agencies, including the European Union's Scientific Committee on Food, identified PAHs as critical pollutants harmful to human health and mandated exposure limits to them. The wide variety of matrices in which these compounds are found requires various detection levels. The method commonly utilized to analyze PAHs is GC-MS, where for GC column 30 m long with an inner diameter of 0.25 mm and 0.25 μm film thickness the analysis takes about 60 minutes. An alternative method is UHPLC utilizing fluorescence detection.

In this announcement we present a method for analysis of PAHs using the Nexera UHPLC equipped with a Pinnacle DB PAH 1.9 μm, 50 x 2.1 mm column and the multiple DAD and FLD detectors enabling detection of trace-level components. Resolution was investigated using a standard mixture of 16 PAH (1 to 20 $mg \cdot L^{-1}$, acetonitrile solution). The described method allows for both accurate and fast determination of 15 + 1 PAHs in less than 5 minutes.