Česká
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Program konference

8. ročník konference Česká chromatografická škola – HPLC.cz 2021

19. - 22. 09. 2021
Vinařství U Kapličky, Zaječí

Organizační výbor

prof. PharmDr. Lucie Nováková, Ph.D.
Mgr. Michal Douša, Ph.D.
PharmDr. Kateřina Plachká, Ph.D.
Mgr. Taťána Gazárková
Jana Douša Hollová

pragolab
autorizovaný distributor
thermoscientific

Generální sponzor konference

Neděle		19. 09. 2021
11:00 – 18:30	Registrace účastníků	
	WORKSHOP	
14:00 – 18:00	Vývoj a validace chromatografické metody	L. Nováková, M. Douša
18:30	SLAVNOSTNÍ ZAHÁJENÍ KONFERENCE	

Pondělí		20. 09. 2021
	PLENÁRNÍ PŘEDNÁŠKA	
09:00 – 09:45	Vývoj HPLC v čase: od průlomových myšlenek k současným trendům	P. Česla
1.	UCTĚNÍ PAMÁTKY PROF. JANDERY	předseda sekce: P. Česla
09:45 – 09:55	Vzpomínka na prof. Pavla Janderu	P. Česla, J. Urban
09:55 – 10:25	Možnosti optimalizace gradientové eluce	J. Urban
10:25 – 10:55	Na co se do budoucna těšit v chromatografii?	F. Švec
10:55 – 11:25	Přestávka na kávu a diskuse u plakátových sdělení	
Přednáška generálního sponzora		
11:25 – 11:40	Demonstrace moderních separačních technik	L. Plaček, Pragolab
2.	ANALÝZA ROSTLINNÝCH MATERIÁLŮ	předseda sekce: L. Nováková
11:40 – 12:10	Moderní analýza rostlinných materiálů	P. Bednář
12:10 – 12:40	Mikroextrakce na bázi disperzní SPE - inovativní řešení pro přípravu vzorků	O. Novák
12:40 – 13:00	Separační metody jako nástroje rostlinné fyziologie, biochemie a hodnocení kvality rostlinné produkce	P. Tarkowski
13:00 – 14:30	Oběd	
Přednáška sponzora sekce		
14:30 – 14:40	Nové / unikátní LC kolony z dílny Chromservis	J. Merhaut, Chromservis
3.	KAPILÁRNÍ ELEKTROFORÉZA	předseda sekce: J. Urban
14:40 – 15:10	Analýza a charakterizace antimikrobiálních peptidů kapilární elektroforézou a izotachoforézou	V. Kašička
15:10 – 15:30	Kapilární elektroforéza ve spojení s hmotnostní spektrometrií s indukčně vázaným plazmatem pro analýzu derivátů oxaliplatiny	J. Petr
15:30 – 15:50	Kapilární elektroforéza jako všestranný nástroj pro analýzu chladicích kapalin	T. Křížek
15:50 – 16:20	Přestávka na kávu a diskuse u plakátových sdělení	

Přednáška sponzora sekce		**P. Šmejda, PE Systems**
16:20 – 16:30	**Nová LC 300 PerkinElmer**	
4.	**PRAKTICKÁ HPLC**	**předseda sekce: V. Kašička**
16:30 – 17:00	**Alternativní řešení problémů v HPLC**	**J. Hlaváč**
17:00 – 17:20	**In vino veritas – elektrochemicky detekovatelné antioxidanty ve víně, ovoci a lidské moči**	**A. Horna**
17:20 – 17:50	**KŘEST KNIHY „MODERNÍ HPLC SEPARACE V TEORII A PRAXI“**	**L. Nováková, M. Douša**
19:00	**Večeře, cimbálová muzika Primáš**	

Úterý 21. 09. 2021

Přednáška sponzora sekce		
9:00 – 9:10	**ACQUITY PREMIER komplexní řešení aneb jak minimalizovat nežádoucí adsorpci analytů v kapalinové chromatografii**	**L. Matulková, Waters**
5.	**SPOJENÍ SEPRAČNÍCH TECHNIK S MS**	**předseda sekce: O. Novák**
09:10 – 09:40	**Stanovení nízkomolekulárních alkylaminů metodou HILIC-MS**	**M. Douša**
09:40 – 10:00	**Moderní trendy v analýze fytohormonů**	**A. Pěnčík**
10:00 – 10:20	**Využití odlišných separačních potenciálů UHPLC a UHPSFC metod v analýze stereoisomerních steroidů**	**T. Gazárková**
10:20 – 10:40	**Strategie k vývoji metod pro stanovení genotoxických nečistot**	**J. Jireš**
10:40 – 11:10	**Přestávka na kávu a diskuse u plakátových sdělení**	
Přednáška sponzora sekce		
11:10 – 11:20	**Agilent LC/MSD – iQ pro Vaši laboratoř**	**J. Kovář, HPST**
6.	**SUPERKRITICKÁ FLUIDNÍ CHROMATOGRAFIE**	**předseda sekce: P. Bednář**
11:20 – 11:50	**Superkritická fluidní chromatografie polárních látek**	**K. Lemr**
11:50 – 12:10	**Chirální separace boronových klasterů pomocí superkritické fluidní chromatografie**	**O. Horáček**
12:10 – 12:30	**Enantioseparace deschlorketaminu a jeho metabolitů superkritickou fluidní chromatografií: lehký úkol?**	**N. Kolderová**
12:30 – 14:00	**Oběd**	
Přednáška sponzora workshopu		
14:00 – 14:10	**Využití vyšší účinnosti core shell částic ke zlepšení proteomických analýz**	**E. Kosovic, Phenomenex**
7.	**SOUTĚŽNÍ WORKSHOP**	
14:10 – 17:00	**Aneb, OTESTUJTE SI VAŠE VĚDOMOSTI V OBLASTI SEPARAČNÍCH METOD ☺**	**L. Nováková, M. Douša, F. Švec**
19:00	**Raut s živou hudbou**	

Středa		22. 09. 2021
Přednáška sponzora sekce		
09:00 – 09:10	**Kapalinová chromatografie Shimadzu**	**O. Hillmich, Shimadzu**
8.	**INOVATIVNÍ PŘÍSTUPY**	**předseda sekce: M. Douša**
09:10 – 09:30	**Aplikace nových reverzních a polárních reverzních stacionárních fází ve vysokoúčinné kapalinové chromatografii**	**M. Kohout**
09:30 – 09:50	**Syntéza a vyhodnocení separačních vlastností nových chirálních katexů**	**J. Herciková**
09:50 – 10:10	**Hydrofilní interakční chromatografie při separaci intaktních glykopeptidů**	**P. Kozlík**
10:10 – 10:30	**Degradační studie farmaceutických substancí a lékových forem**	**J. Heřt**
10:30 – 10:45	**Vyhlášení ceny za nejlepší poster a přednášku**	**L. Nováková**
10:45 – 11:00	**ZÁVĚREČNÁ PŘEDNÁŠKA**	**L. Nováková**

The Evolution of HPLC Over Time: From Breakthrough Ideas to the Current Trends

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Keywords: *keywords*

Almost seventy years have passed from the discovery of chromatography in 1903 to the introduction of the HPLC technique in 1967 [1,2], during which time the physicochemical basis of chromatographic separation of substances has largely been investigated. Already during this period, i.e. the nomenclature of the new analytical branch was formed, the mechanisms of retention of substances, and the kinetic processes counteracting separation were thoroughly described [3,4]. The subsequent spread of HPLC techniques to the analytical laboratories worldwide was related to the wider availability of commercial instrumentation, increasing column separation efficiency and improved detection capabilities of the analytes. Future trends can be traced in the literature published during the pioneering days of chromatography development [5], which are reflected in current advances in ultrahigh performance liquid chromatography, the use of superficially porous particles in the columns, and the multidimensional liquid chromatographic separations. These concepts are described and discussed with respect to the historical perspectives together with the advances *versus* limitations of its application.

References

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Optimization of gradient separation in liquid chromatography

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Keywords: *gradient elution, neurotransmitters, optimization, design of experiments*

Reversed-phase liquid chromatography is one of the most common retention mechanisms applied in separation sciences. On the other hand, it might show difficulties separating very polar compounds with limited retention that usually elute near the column hold up volume. Optimizing isocratic mobile phase composition for low-retention compounds causes unprecedented analysis time extension as less polar compounds provide significantly higher retention and elute later. Therefore, gradient elution liquid chromatography with a variable mobile phase composition through the analysis is a method of choice in the separation of samples with analytes differing in polarity.

Compared to isocratic elution, optimizing gradient elution is a more complex process focusing on selecting an overall gradient time and the initial and final concentration of organic fraction in the mobile phase. Various experimental protocols are used to find optimal elution conditions. Besides the try-and-error method that is the most time-consuming and less informative, retention modeling and design-of-experiments approaches are being used to optimize the gradient elution method.

Retention modeling allows a description of the retention behavior of analytes in the separation system and a prediction of retention times and peak bandwidths in experimental conditions that were not determined experimentally. Thus, a utilization of a proper retention model significantly decreases method development time, as usually only several gradient runs are necessary to describe separation space fully.

In the design-of-experiments approach, the experiments are planned to cover selected variables systematically. For example, for three experimental variables, each defined by the lowest, the middle, and the highest value, only fifteen experimental runs, including twelve design runs and three repetitive design center runs, are necessary. Then, a mathematical model is fitted through

individual experiments to fill in chromatographic information and to uncover variables that are statistically in/significant.

The presentation starts with the description of the retention behavior of polar dopamine-related compounds in isocratic liquid chromatography. Then, optimization of gradient shape is discussed utilizing input data from both isocratic and gradient elution. Finally, an optimization of a selected chromatographic variable by the design-of-experiments approach is discussed. The presentation's main aim is to provide the audience with simple and easy-to-use optimization protocols allowing optimization of gradient separation by using conventional spreadsheet software.

Acknowledgment

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Na co se do budoucna těšit v chromatografii? Kolonové technologie

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Klíčová slova: *kapalinová chromatografie, kolonové technologie, historie, výhled*

Od doby pionýrské práce M. Cvěta vynálezce chromatografie uplynulo zhruba 120 let. Za tuto dobu tato technika prošla neuvěřitelným vývojem a stala se třetí nejčastěji používanou metodou analytické chemie hned po vážení a měření pH. Přesto, nelze její vývoj považovat za skončený, neboť požadavky na tuto analytickou metodu neustále rostou. Čeho bychom chtěli v kapalinové chromatografii dosáhnout? Rychlých, vysoce účinných separací vyznačujících se velkou píkovou kapacitou. Vývoje kolon se značně zlepšenou selektivitou. Velikost kolon je třeba minimalizovat tak, aby umožnily separace velmi malých vzorků při omezené spotřebě, pokud možno „zelené“ mobilní fáze. Tato prezentace představí na konkrétních příkladech, jak se s těmito extrémními požadavky vypořádávají špičkoví světoví vědci ve výzkumu na jejich pracovištích. Na závěr budou uvedeny náměty pro další vývoj kapalinové chromatografie.

Modern analysis of plant materials

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Keywords: *liquid chromatography, mass spectrometry, ion mobility, plant, seed, multivariate statistical analysis, micromanipulation*

Mass spectrometry-based metabolomics is being increasingly utilized in various research fields including plant/natural products studies. Untargeted analysis of metabolites utilizing either direct mass spectrometry or its combination with chromatographic separation (first of all LC/MS) provides large amount of information when biomarker discovery is the main goal [1]. This communication deals with investigation of seed coat composition with respect to legume seed dormancy/domestication. Flow-injection electrospray ionization mass spectrometry in combination with multivariate statistical analysis allows to classify pea seeds according to their developmental stage and dormancy level and revealed compounds significantly changed during seed maturation and with respect to germination ability of studied genotypes. These biomarkers were studied in detail by a combination of liquid chromatography, ion mobility (using linear and cyclic IMS cells) and high resolution tandem mass spectrometry. Oligomers of gallocatechin belong among biomarkers of particular interest being tentatively put into context with polyphenoloxidase activity. Besides, combination of electronically driven micromanipulation with laser desorption-ionization imaging mass spectrometry allowed 3D metabolic profiling of hilum – a scar left on seed coat where seed was formerly attached to maternal plant [2].

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Microextraction based on dispersive SPE - an innovative approach to sample preparation

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Keywords: *miniaturization, dispersive solid phase extraction, plant hormones*

Cytokinins and auxins are naturally occurring plant growth regulators. They play an important role in controlling the growth and developmental processes in plants. Similarly to other phytohormones, their concentrations in plant tissues are usually very low (pmol per gram of fresh weight). Therefore, their identification and quantification are based on highly sensitive analytical approaches, such as an ultra-high performance liquid chromatography combined with a tandem mass spectrometry (LC-MS/MS). Moreover, sample preparation, especially removal of salts and isolation of analytes from a complex plant matrix, is the most critical procedure for achieving high-quality data. For many years, the purification of cytokinins and auxins has been based on a well-established solid phase extraction (SPE). However, dispersive SPE (dSPE) was introduced a few years ago as an effective and robust approach to isolating a wide range of analytes. In the contrast with conventional SPEs, this technique uses free solid phase particles dispersed in a liquid crude sample extract. We tested micro-dSPE as an innovative, fast and cheap approach to the isolation of selected phytohormonal groups (cytokinins and auxins). Our work was mainly focused on the investigation of the main parameters contributing to the extraction efficiency in comparison with conventional SPE technology.

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Separation methods as tools of plant physiology, biochemistry and evaluation of the quality of crop production

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Keywords: *chromatography; mass spectrometry; secondary metabolites; crops*

Separation methods are analytical tools indispensable in modern plant science research as well as quality assessment of food crops. Since plant tissue extracts represent complex multicomponent mixtures, sensitive, and sufficiently selective analytical methods are required for the determination of endogenous metabolites. Phytochemistry research groups at Palacky University and Crop Research Institute carry out chemical analyses of plants and plant products. They utilize various extraction and distillation methods to isolate primary and secondary metabolites, as well as elements. Molecular spectrometry, mass spectrometry with inductively coupled plasma, liquid chromatography, gas chromatography, capillary electrophoresis hyphenated with various detectors (UV/VIS, MS, FL, ELSD) are employed as methods of final analysis. These approaches allow us to obtain information on elements, small organic acid, amino acid, fatty acid, sugar, fructan, vitamin, terpenoid, phenolic, glucosinolate, cannabinoid, polyamine, cytokinin, and strigolactone content in relevant biological material. Data acquired might be used in fundamental research (plant biochemistry and physiology), quality assessments of genetic resources, and plant products in applied research or food production. The lecture is focused on HPLC/UHPLC separation of 2-MeS-cytokinins, quercetin and its conjugates, cannabinoids, amino acids, and sugars using Phenomenex chromatographic columns.

Capillary electrophoresis and isotachophoresis applied for analysis and characterization of insect antimicrobial peptides

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Keywords: *antimicrobial peptides; capillary electrophoresis; capillary isotachophoresis; acidity constant; ionic mobilities; effective charge*

Capillary zone electrophoresis (CZE) and capillary isotachophoresis (CITP) will be presented as high-performance separation methods for analysis and characterization of peptides [1]. In this study, CZE and CITP were used for qualitative and quantitative analysis and for investigation of acid-base and electromigration properties of polycationic antimicrobial peptides (AMPs) isolated from the venom of two species of Hymenoptera insects [2, 3]. These AMPs contain 10-20 amino acids (AAs), among others 3 to 7 basic AAs (His, Lys, and Arg).

Purity degree of synthetic AMPs was assessed by CZE in acidic background electrolytes (BGEs) using fused silica capillaries coated with cationic surfactants or polymers suppressing adsorption of AMPs on the capillary wall. Content of counterions of AMPs was determined by CITP.

Acid-base and electromigration properties of AMPs were evaluated using a combination of CZE and CITP methods [4, 5]. First, the dependences of the effective mobilities of AMPs on pH were measured in a series of BGEs within a wide pH range 1.80–12.1, at constant ionic strength of 25 mM, and at constant temperature of 25°C. From these dependences, the mixed acidity constants, $pK_{a,mix}$, and the actual ionic mobilities of AMPs were determined by the nonlinear regression analysis. Subsequently, the $pK_{a,mix}$ values were recalculated to the thermodynamic acidity constants, $pK_{a,th}$, using the Debye-Hückel theory as shown in [6]. The $pK_{a,th}$ of His was in the range 3.72-4.98, the $pK_{a,th}$ of α-amino group of N-terminal AAs was in the range 6.14-6.93, and the $pK_{a,th}$ of ε-amino group of Lys spanned the interval 7.26-9.84. The $pK_{a,th}$ of Arg was greater than 12 and could not be directly measured.

The effective charge of the AMPs was determined by CITP based on linear dependence of the CITP zone length of the analyte on its effective charge and injected substance amount. Cationic

CITP system with leading electrolyte composed of 10 mM ammonium leading ion, 40 mM acetate counterion, pH 4.1, and terminating electrolyte containing 40 mM acetic acid, pH 3.2, provided sharply separated AMPs zones with good linearity and repeatability of their lengths. The effective charges of the AMPs were found to be in the range 1.65-5.04, i.e. significantly smaller than the theoretical effective charges (2.86-6.99) calculated from the p$K_{a,mix}$ values.
CZE and CITP proved to be effective methods for analysis and physicochemical characterization of polycationic antimicrobial peptides in a microscale.

References

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The work was supported by the GAČR (grant no. 20-03899S) and by the CAS (project RVO 61388963).

Capillary electrophoresis connected with inductively coupled plasma mass spectrometry for analysis of oxaliplatin derivatives

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Keywords: Capillary electrophoresis; inductively coupled plasma mass spectrometry; chiral separation; online preconcentration; sweeping

Capillary electrophoresis (CE) hyphenated to inductively coupled plasma mass spectrometry (ICP-MS) represents an interesting and beneficial tool for many applications. Nowadays, CE-ICP-MS is used primarily for determination of metals, speciation analysis, and metal-ligand interaction studies, including metal-based nanoparticles, as recently reviewed [1]. However, CE-ICP-MS has a huge potential to affect also other fields by taking account of other advantages of CE, e.g., in chiral separations or by using online preconcentration methodologies. In this communication, our "in-house" CE-ICP-MS interface will be presented in two applications: (i) separation of oxaliplatin enantiomers [2], and (ii) determination of B and C pharmacopeia oxaliplatin impurities using sweeping online preconcentration [3]. The separation of (R,R)- and (S,S)-oxaliplatin was conducted in 40 mM borate buffer pH 9.5 with 60 mg/mL sulfated-β-cyclodextrin. LOD was 64 ng/mL determined from the ^{195}Pt isotope signal. The determination of oxaliplatin impurities was done at 25 mM phosphate buffer pH 2.15 with 175 mM SDS. With the injection time of 90 s, LODs ranged from 1 to 3 ng/mL. Hence, we believe CE-ICP-MS represents interesting avenues for the future research.

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Capillary electrophoresis as a versatile tool for the analysis of engine coolants

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Keywords: *capillary electrophoresis, engine coolants*

Coolants are routinely used across practically all fields of industry. Their lifetime is naturally limited, which leads to the production of immense amounts of waste that should be recycled. Several approaches to recycling spent coolants have been proposed and published [1, 2]. Despite that, only a negligible part of industrial coolants is recycled in reality. The main reason is the high energetic demand of most of the processes. Electrodialysis seems to be a promising technology [3] that could change the current situation as it fulfills both, environmental as well as economic criteria. Nevertheless, the introduction of a recycling line into practical use is a complex process that requires quality control of the spent coolant entering the recycling line as well as the recycled material.

Capillary electrophoresis offers fast separations and outstanding flexibility enabling simple and inexpensive exchange of whole separation environment as well as detection approach. Several analytical methods can thus be run on a single instrument in quick succession. This lecture shows the application of capillary electrophoresis in the analysis of engine coolants before and after their electrodialysis-based recycling. Aryltriazoles, ethanolamines, inorganic anions, organic acids, and denatonium benzoate are determined. As the individual groups of analytes differ in their properties, different approaches and techniques are used including direct UV detection for UV-absorbing analytes, indirect UV detection for non-absorbing ones, reversal of electroosmotic flow for anionic analytes, and field-amplified sample injection for enhanced detection of at low concentration levels. The resulting set of methods presents a fast and simple solution as all separations take up to 5 minutes and sample treatment consists of filtration and dilution only.

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This work has been supported by the Technology Agency of the Czech Republic, projects TJ01000170 and TJ04000540.

Alternativní řešení problémů v HPLC

Alternativní analytická chemie je oblast vědy, která hledá netradiční, jednoduchá řešení problémů tam, kde je klasická analytická chemie limitována konvencemi zavedenými v průběhu jejího používání. Díky systematickému vyvracení mýtů o nutnosti dodržování zavedených dogmat v oblasti separačních metod se v minulosti podařilo vyvinout např. extrakritickou fluidní chromatografii jako účinnější alternativu původně tolik nadějné superkritické fluidní chromatografie.

V této přednášce bude pozornost věnována některým problémům, které tradiční analytická chemie dosud vyřešit neumí. Mezi ně patří např. zvýšení účinnosti chromatografické kolony na nekonečno či povýšení životnosti kolony nad životnost operátora. Tyto a některé další problémy spojené s používáním chromatografických metod budou v přednášce diskutovány a posluchači budou seznámeni s výsledky hledání alternativních způsobů jejich řešení.

In vino veritas – electroactive antioxidant in wine, fruit and human urine

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Keywords: *Coulometric detection, HPLC, FIA/ ECD, wine, fruit, urine*

We had been dealing with application of RP- HPLC with electrochemical CoulArray detection to the analysis of 32 natural antioxidants in beer, wine, tea and plant extracts [1] . Recently, because of the need to analyse hundreds of samples of various fruit we started to use 4 channel CoulArray as a detector in flow injection analysis. The time of the analysis was reduced to 66 s. FIA/ CoulArray seems to very useful tool in the evaluation of antioxidant activity of wine, fruit, plant extracts and also human plasma and urine for better understanding of targeted nutritional therapy in human health.

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Acknowledgements

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ACQUITY PREMIER komplexní řešení aneb jak minimalizovat nežádoucí adsorpci analytů v kapalinové chromatografii

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Keywords: *Acquity Premier system, MaxPeak High Performance Surfaces (HPS)*

Waters Premier systémy obsahují novou inovativní technologii MaxPeak High Performance Surfaces (HPS), která účinně snižuje nespecifickou adsorpci v důsledku interakcí analytů s kovy, vše bez složitých mobilních fází a pracných metod.

Waters Premier systémy, ve spojení s Waters Premier kolonami a QuanRecovery vialkami a platíčky, jsou zárukou lepší citlivosti a reprodukovatelnosti pro analyty typu:

- Organické kyseliny
- Organofosfáty
- Oligonukleotidy
- Fosfopeptidy
- Kyselé glykany
- Fosfolipidy
- Další analyty typu Lewisových bází

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Low Adsorption UPLC Systems and Columns Based on MaxPeak High Performance Surfaces: The ACQUITY PREMIER Solution,

Determination of low molecular weight alkylamines by HILIC-MS

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Keywords: *HILIC, mass detection, alkylamines*

A volatile organic compounds (VOC) any organic compound having an initial boiling point less than or equal to 250° C measured at a standard atmospheric pressure of 101.3 kPa. In organic chemistry, amines are compounds and functional groups that contain a basic nitrogen atom. Amines are formally derivatives of ammonia, wherein one or more hydrogen atoms have been replaced by a substituent such as an alkyl or aryl group. The usual synthesis of metformin (MET) involves the one-pot reaction of dimethylamine (DMA) hydrochloride and 2-cyanoguanidine over heat. DMA present in MET is susceptible to oxidation yielding unsymmetrical dimethylhydrazine, which further oxidizes in contact with air or other oxidation agents to N-nitrosodimethylamine (NDMA). DMA together with other compounds is accepted to play its role as a precursor of the formation of NDMA in water and sewage. NDMA was classified by U.S. Environmental Protection Agency as a probable human carcinogen. In December 2019, the U.S. Food and Drug Administration began testing samples of MET for the content of NDMA. A sensitive and specific hydrophilic interaction chromatography (HILIC) method for the separation and determination of dimethylamine (DMA) in active pharmaceutical ingredients (APIs) and in dosage forms of metformin (MET) has been developed and validated. A feasible analytical method based on HILIC coupled with mass spectrometry detection (HILIC-MS) was established using a simple sample preparation. The separation of MET was achieved on a Cortecs HILIC column using a mixture of 10 mmol/L ammonium formate adjusted to pH 4.8 and acetonitrile (25:75, v/v) at 0.8 mL/min flow rate. The a single-quadrupole mass detector was operated in positive ion mode. Quadrupole mass analyser was employed in selected ion monitoring mode using a target ion at m/z = 46 as [M+H]+. The HILIC-MS method was validated as per International Council on Harmonization (ICH) guidelines in terms of linearity, limit of detection, limit of quantification, selectivity, accuracy, precision and intermediate precision. The method was demonstrated to be applicable for the determination of DMA in routine quality control evaluation of commercial samples of metformin of both API and dosage forms. The HILIC-MS method was developed as a simpler and faster alternative to compendial method for determination of DMA (as specific impurity F) in MET described in European Pharmacopoeia.

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Modern trends in phytohormone analysis

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Keywords: *phytohormones, liquid chromatography, supercritical fluid chromatography, tandem mass spectrometry*

Plant hormones (phytohormones) are signaling molecules that control physiological processes during plant growth and development as well as responses to biotic and abiotic stresses. The spatial distribution of phytohormones within organs, tissues and individual cells is essential for many developmental processes. Currently, our main interest is the organelle-specific analysis of phytohormones, which reveals their intracellular distribution and helps us to understand their homeostasis maintenance at the cellular and organelle levels. The analysis of phytohormones is a great challenge due to their various physicochemical properties and the very low concentrations in which they are present in plant tissues. Moreover, many of them form the structural isomers, and ultra-high performance liquid chromatography (UHPLC) coupled with tandem mass spectrometry (MS/MS) is crucial to achieve our challenging analytical goals. We have recently developed several UHPLC-MS/MS methods for targeted analysis of phytohormones, e.g. auxins [1], as well as a method for simultaneous profiling of major phytohormonal classes [2]. In the last decade, instruments based on the supercritical fluid chromatography (SFC) combined with MS/MS (SFC-MS/MS) have been improved and become the additional approach to analyze low abundant compounds in complex biological matrices [3]. This method utilizes supercritical carbon dioxide as a major component of the mobile phase combining the advantages of gas chromatography and liquid chromatography. It results in an excellent separation efficiency of chiral and structurally related isomers in a short time. We have developed a method for analysis of phytohormones with 1260 Infinity II SFC/HPLC Hybrid System coupled with 6495 Triple Quadrupole utilizing a unique combination of LC and SFC equipped with MS/MS detection.

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Utilization of different separation potentials of UHPLC and UHPSFC methods in the analysis of stereoisomeric steroids

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Keywords: *steroid analysis; UHPSFC; UHPLC; mass spectrometry*

The analysis of isomeric and isobaric steroids remains a troublesome analytical challenge despite many published studies using traditional gas chromatography and liquid chromatography techniques. Structural similarity resulting from a typical steroid skeleton and minor structural modifications, such as hydroxyl group position, is often multiplied even further by the common loss of one to three water molecules in MS and MS/MS spectra, leading to the creation of additional isobars. Thus, the achievement of full chromatographic separation is crucial to eliminate the observed interferences and correctly quantify the targeted analytes.

The present study aims to develop high-throughput UHPLC and UHPSFC chromatographic methods for the quantification of biogenic and synthetic steroids in mouse plasma samples. A set of 37 steroids from the C19 (androstanes), C21 (pregnanes), and synthetic steroid groups generated a total of 11 critical pairs/groups, resulting in a challenging separation of 28 analytes. The development of the UHPSFC method included an extensive screening of 19 stationary phases across the polarity range as no generic stationary phase has been discovered yet. The columns with the best separation score of isomers were selected for further optimization of the gradient program using CO_2 and various organic modifiers with additives as a mobile phase. The UHPLC screening was carried out using ten C18, aromatic, and fluorinated stationary phases, followed by detailed gradient optimization on selected C18 columns using acetonitrile and 0.1% formic acid in water as a mobile phase. The protein precipitation method was selected over supported liquid extraction for sample pretreatment of mouse plasma samples.

The study was supported by the Project of Specific Research, SVV No. 260548, GA UK project No. 348221, and the STARSS project (Reg. No. CZ.02.1.01/0.0/0.0/15_003/0000465) co-funded by ERDF.

A METHOD DEVELOPMENT STRATEGY FOR THE DETERMINATION OF PHARMACEUTICAL GENOTOXIC IMPURITIES

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Keywords: LC–MS, Genotoxic impurity, Azide, *N*-Nitrosamines, Metformin, Sartans

Abstract

Availability of safe human drugs is, without any doubt, a key component in the public healthcare of any developed country. One of the main reasons for drug recalls by Food and Drug Administration (FDA) and European Medicines Agency (EMA) was the content of one or more genotoxic impurities in the final product. Recently, organic azides and *N*-nitrosamines were triggering the most genotoxicity alerts. Limiting the content of such genotoxic impurities in active pharmaceutical ingredients (APIs) and drugs forms (DFs) is crucial for the safety profile of the product and the solution always requires a multidisciplinary approach.

Such an approach was demonstrated during the process optimization study with the goal of decreasing the *N*-Nitrosodimethylamine (NDMA) content in the final pharmaceutical products containing metformin. The entire study was conducted in the environment of the pharmaceutical company Zentiva, k.s. and in compliance with the guideline M7(R1) of International Council for Harmonisation. The analytical method utilises an instrument consisting of high-performance liquid chromatograph coupled with a tandem mass spectrometer. The mass spectrometer was operated with atmospherical pressure chemical ionization (APCI) in positive ion mode. The method was calibrated using an isotopically labelled internal standard. Validation of the method was performed according to the guideline Q2(R1) ICH. Furthermore, the performance of the method was evaluated during an interlaboratory comparison using a regression analysis. Key factors influencing the formation of NDMA in metformin containing products were formulated according to the results of the performed process optimization study. NDMA content was determined in 469 samples of metformin API and FCT during the study. A hypothesis explaining the mechanism of NDMA formation in the studied products was formulated.

Another method for the determination of genotoxic azide impurities in sartans was developed. The method utilises a very efficient liquid chromatograph Waters Acquity I-Class coupled with a highly sensitive tandem mass spectrometer Xevo TQ-XS. The method allows for accurate quantification of both impurities GTI-azide-1 and GTI-azide-2 at levels below 1/10th of the specification limit, which is

crucial in the context of pharmaceutical analysis. The limit of quantification was determined to be 0.033 ppm and 0.025 ppm for GTI-azide-1 and GTI-azide-2, respectively.

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Supercritical fluid chromatography of polar compounds

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Keywords: *supercritical fluid chromatography, polarity range, cathinones, dyes, plant gums*

Modern ultra-high performance supercritical fluid chromatography (UHPSFC) attracts attention as a useful alternative to gas chromatography and high-performance liquid chromatography. Although SFC was considered to be useful for apolar and mildly polar compounds, UHPSFC is also successfully applied for the separation of polar and even ionic analytes [1]. To elute strongly retained polar compounds in an appropriate time and to reach a reasonable peak shape, the mobile phase is modified using mixtures of alcohols with water and additives such as ammonium salts. Although the mobile phase is not in a supercritical state after some modifications, the term SFC is still used. The proper setting of a gradient and wide range of stationary phase chemistries are also useful for the separation of polar analytes. Many examples of polar compound separation can be found in the literature [1], some examples are discussed in details [2-4].

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CHIRAL SEPARATIONS OF CARBORANES IN SUPERCRITICAL FLUID CHROMATOGRAPHY

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Keywords: *Carboranes, chiral separations, supercritical fluid chromatography*

Boron clusters are artificial three-dimensional structures, which exhibit unique physicochemical properties. Carboranes as a subgroup of boron cluster compounds were derived by substituting BH units for CH units. The similar steric volume occupied by a rotating phenyl ring and an icosahedral carborane cage has led to the extensive research of carborane moieties as new pharmacophores. The carborane moiety has already substituted phenyl groups of some conventional pharmaceuticals, e.g., aspirin (Figure 1), tamoxifen, penicillin, lidocaine, celecoxib, etc., and their activity has been tested. [1]

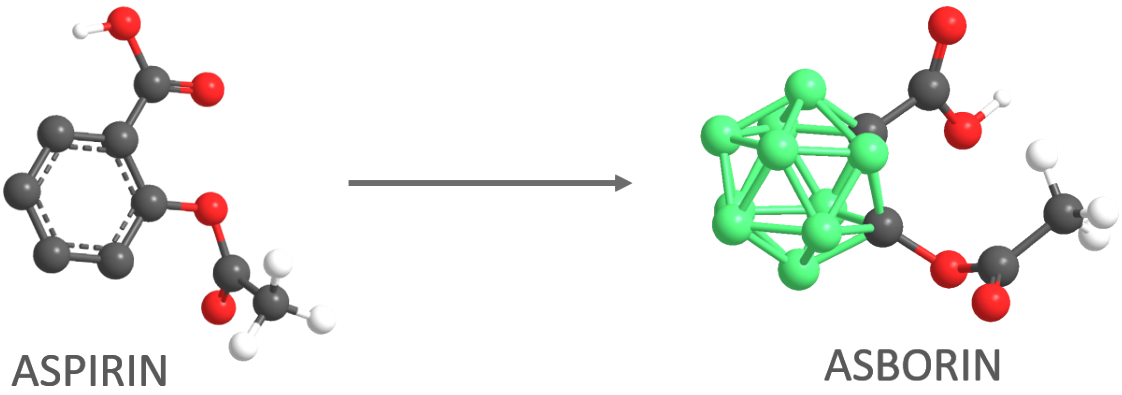

Figure 1. Asborin. The carborane analogue of aspirin.

Chirality of carboranes is caused by introducing endo-/exoskeletal substituents, which impair the symmetry of the cage. Hence, it is vital to evaluate analytical methods for chiral separation of boron clusters concerning their potential use in pharmacy. Although the successful chiral separations of neutral, zwitterionic, and recently anionic carboranes were achieved by HPLC [2], no chiral separations of these species have been carried out in supercritical fluid chromatography so far.

Supercritical fluid chromatography is generally accepted as the most widely used and the most versatile technique for chiral separations in the pharmaceutical industry, thanks to the more straightforward and faster chiral method development and faster chiral separation methods than in HPLC. [3] Hence, supercritical fluid chromatography was employed to assess the chiral separations of the carboranes. Firstly, the chiral screening method in gradient elution was performed on nine polysaccharide-based columns. Secondly, the successful enantioseparations obtained in chiral screening were subsequently optimized in isocratic elution to achieve the baseline separation of the carboranes in the shortest possible time.

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Supercritical Fluid Chromatography For Enantioseparation Of Deschloroketamine And Its Metabolites: An Easy Thing To Do?

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Keywords: *supercritical fluid chromatography, enantioseparation, deschloroketamine*

Deschloroketamine (DCK) is classified as a new psychoactive substance that belongs among dissociative anesthetics. It is being abused instead of ketamine with which it shares many attributes. Unlike ketamine, there is lack of information about metabolism, toxicity or the difference in the effects of the DCK enantiomers [1].

To achieve facile monitoring of enantiomers of DCK and its metabolites in biological samples, we decided to develop a chiral separation method using mass spectrometry (MS) detection. As an MS compatible mobile phase, supercritical CO_2 with various organic modifiers has been chosen. However, enantioseparation achieved with an optimized method was diminished by excessive peak distortion of DCK, which rendered its quantitation impossible. We found that the peak distortion is caused by a chemical reaction producing a stable carbamic acid salt. To suppress its formation, ethansulfonic acid can be used, however, then enantioseparation of metabolites is not achieved. Thus, currently, two methods of chiral separation shall be used to fully determine the composition of a DCK-containing biological sample.

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Applications of novel reversed phase and polar reversed phase stationary phases in high-performance liquid chromatography analysis

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Keywords: *high-performance liquid chromatography, reversed phase chromatography, novel stationary phases, clonazolam*

High-performance liquid chromatography (HPLC) is the most popular analytical technique used for research and development as well as routine analysis purposes. From the number of analyses, more than 90% is performed in reversed phase HPLC mode. This prevalence triggered an immense development of such sorbents leading to more than 600 reversed phase columns, which are nowadays commercially available.[1] Despite this variety, there are still many separation challenges, which are difficult to tackle by the most used "classics", a reversed phase bearing octadecyl alkyl chains. Therefore, the current development is directed to so-called polar reversed phases that possess an additional unit in the structure of the immobilized organic selector. In this contribution, we present our effort to develop a new type of a polar reversed phase for applications in research and development as well as in industry.

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Synthesis and evaluation of separation properties of naphthalene-based chiral cation-exchangers

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Keywords: *Chiral cation exchanger; liquid chromatography; chiral separation; basic pharmaceuticals; chiral stationary phase*

Currently, high-performance liquid chromatography (HPLC) enables routine chiral separation in many areas of scientific research and industrial production of enantiomerically pure materials and drugs from their racemic mixtures. The most common first choice for enantiomer separation is the use of chiral stationary phases (CSPs). Nowadays, hundreds of chiral columns are commercially available, however, the demand for novel, highly specific CSPs remains strong. To address this need, chiral ion exchangers, which can separate a broad variety of charged or chargeable substances, are being developed.

In this work, we focused on the synthesis and evaluation of strong cation-exchangers (SCX) CSPs based on a naphthalene core, featuring 2-aminocyclohexane-1-sulfonic acid as a chiral unit. We synthesized four different chiral selectors and immobilized them by two different ways on modified silica. The target chiral stationary phases were slurry packed into chromatographic columns and evaluated in polar organic mode HPLC. We show that the way of immobilization plays an important role in chiral separation of the model basic substances. [1]

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Hydrophilic interaction liquid chromatography in the separation of intact glycopeptides

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Keywords: *glycoproteomics; glycopeptides; hydrophilic interaction liquid chromatography*

Glycosylation is one of the most frequent and complex post-translation modification of peptides [1]. It plays an important role in many biological processes such as protein folding and secretion or cell-cell adhesion. Furthermore, alterations of glycosylation are related to several inflammatory diseases, various cancer types or to Alzheimer's disease [2]. Despite the recent innovations of bioanalytical techniques, analysis of glycoproteins is still a challenge mainly because of their micro- and macro- heterogeneity [2]. Reversed-phase chromatography (RP-LC) is typically used for the analysis of protein digests but typically does not adequately resolve glycoforms of peptides. An alternative chromatographic mode for separation of glycopeptides is hydrophilic interaction liquid chromatography (HILIC) which is able to separate glycopeptides that are inadequately resolved in RP-LC [3]. In this work, we show the separation potential of HILIC in the analysis of hemopexin glycopeptides. Under the optimized conditions, our HILIC method was able to efficiently separate the glycoforms of the same peptide backbone including separation of the isobaric glycoforms. We achieved efficient separation of core and outer arm-linked fucose of bi-antennary and tri-antennary glycoforms of the SWPAVGNCSSALR peptide and bi-antennary glycoform of the ALPQPQNVTSLLGCTH peptide, respectively. Moreover, we demonstrated the separation of antennary position of sialic acid linked via α2-6 linkage of the monosialylated glycopeptides. Furthermore, we compared different HILIC stationary phases, i.e., HALO® penta-HILIC, Glycan BEH Amide, and ZIC-HILIC in the separation of complex N-glycopeptides of hemopexin and IgG glycoproteins, because choosing the right HILIC stationary phase is a crucial step when optimizing the HILIC method. The HALO® penta-HILIC column provided the best separation results and the ZIC-HILIC column the worst.

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Degradation study of the pharmaceutic substances and drug products

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Keywords: *Forced Degradation Study, Chemical stability, Pharmaceutic substance, HPLC*

In the chromatographic community, the term Forced degradation study is often understood only as a test that is part of the validation of a chromatographic method to demonstrate that the developed method is stability-indicating. The lecture aims to introduce this concept in terms of focusing on the study of the intrinsic stability of pharmaceutic substances and the prediction of stability problems during the development of drug products.. [1].

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Partneři a sponzoři konference

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