UNIVERSITY OF ZAGREB

FACULTY OF CHEMICAL ENGINEERING AND TECHNOLOGY

15th International Chromatography School

BOOK OF ABSTRACTS

12th– 13th June 2014

ZAGREB, CROATIA

INTERNATIONAL SCIENTIFIC COMMITTEE

Nebojša Avdalović (US)
Sandra Babić (HR)
Tomislav Bolanča (HR)
Mario Boras (AT)
Joachim Weiss (DE)

SYMPOSIUM ORGANIZING COMMITTEE

Danijela Ašperger (HR)
Sandra Babić (HR)
Tomislav Bolanča (HR)
Dragana Mutavdžić Pavlović (HR)
Mirjana Novak (HR)
Martina Periša (HR)
Šime Ukić (HR)
Mirta Zrnčić (HR)

GOLD SPONSORS

KemoLab d.o.o.
Kobis d.o.o.
Labena d.o.o.
LabEKO d.o.o.

SILVER SPONSORS

AlphaChrom d.o.o.
INSTRUMENTALIA Adria d.o.o
MERCK d.o.o.
PRIMALAB d.o.o.
Sartorius Croatia d.o.o.
Shimadzu d.o.o.

BRONZE SPONSORS

AnAs d.o.o.
i-TLC d.o.o.
Kefo d.o.o.
Vita Lab Nova d.o.o.

DONORS

Obrnuta faza d.o.o.
Ru-Ve d.o.o.

Editors:

D. Ašperger, T. Bolanča, D. Mutavdžić Pavlović, Š. Ukić

Published by:
University of Zagreb, Faculty of Chemical Engineering and Technology

For publisher:
B. Zelić

ISBN 978-953-6470-67-9

CONTENTS

FINAL PROGRAM

Thursday, 12 June 2014, morning

Opening of the 15th International Chromatography School, FCET, Marulićev trg 19

09.00-09.15 B. Zelić (Dean of FCET, HR), T. Bolanča (Vice Dean of FCET, HR):

OPENING REMARK

Session 1: Ion Chromatography

09.15-09.45 N. Avdalović (Thermo Fisher Scientific, US):

A NEW COLUMN TECHNOLOGY FOR THE ANALYSIS OF N-GLYCANS IN PROTEINS

09.45-10.15 J. Weiss (Thermo Fisher Scientific, DE):

HIGH RESOLUTION OR HIGH THROUGHPUT SEPARATIONS OF IONIC COMPOUNDS WITH 4 µm ION EXCHANGERS

10.15-10.45 A. Gelemanović (Primalab, SI):

CHROMATE–DETERMINATION BY ION CHROMATOGRAPHY USING MiPT

10.45-11.15 Coffee break

Session 1: Ion Chromatography

11:15-11:45 M. Novak (FCET, HR):

QSRR MODELING IN ION CHROMATOGRAPHY

11:45-12:15 V. Stankov (KemoLab, HR):

ION CHROMATOGRAPHY WITH INTEGRATED PULSED AMPEROMETRIC/ CONDUCTOMETRIC DETECTION AS SOLUTION FOR GLYPHOSATE ANALYSIS IN FOOD AND WATER SAMPLES

12.15-12.45 M. Sertić (FBF, HR):

CAPILLARY ELECTROPHORESIS - A FAST AND EFFECTIVE TOOL FOR VARIOUS ANALYTICAL CHALLENGES

12:45-14:15 Lunch break (organized by participants them self)

Thursday, 12 June 2014, afternoon

Session 2: Gas Chromatography

14.15-14.45 J. Zrostlíková (LECO, CZ):

DETAILED FOOD SAMPLE EXAMINATION: ONE STEP TARGET AND NON-TARGET ANALYSIS OF CONTAMINANTS BY GC-HR-TOF MS

14.45-15.15 J. Zrostlíková (LECO, CZ):

GC×GC-TOF MS ANALYSIS OF PETROCHEMICAL SAMPLES IN "REVERSED SETUP": FINE TUNING OF THE SEPARATION AND DATA PROCESSING STRATEGIES

15.15-15.45 I. Bačić (Forensic Science Centre "Ivan Vučetić", HR):

VALIDATION OF SPME-GC/MS METHOD FOR QUALITATIVE ANALYSIS OF GASOLINE AND DIESEL FUEL TRACES – FORENSIC APPROACH

15.45-16.15 Coffee break

16.15-16.45 G. Peček (HCPHS, HR):

DEVELOPMENT OF GAS CHROMATOGRAPHY MASS SPECTROMETRY (GC-MS) METHOD FOR DETERMINATION OF SELECTED PESTICIDES IN SURFACE WATER

16.45-17.15 B. Marošanović (Sojaprotein, RS):

DETERMINATION OF PESTICIDE RESIDUES IN FRUIT AND VEGETABLES BY GC/MS AND LC/MS/MS

17.15-17.45 A. Mornar (FBF, HR):

QUALITY ASSESSMENT OF DIETARY SUPPLEMENTS USED IN THE PEDIATRIC PATIENTS

Session 3: HPLC and UPLC

17.45-18.15 S. Bilsborough (Agilent Technologies, GB):

APPLICATION OF LIQUID CHROMATOGRAPHY MASS SPECTROMETRY IN TARGETED AND UNTARGETED ANALYSIS

Friday, 13 June 2014, morning

Session 3: HPLC and UPLC

09.00-09.30 J. Weiss (Thermo Fisher Scientific, DE):

APPLICATIONS OF ION-EXCHANGE/REVERSED-PHASE MIXED-MODE STATIONARY PHASES IN COMBINATION WITH CHARGED AEROSOL DETECTION FOR SEPARATING PHARMACEUTICALS

09.30-10.00 M. Boras (Waters, AT):

ACQUITY QDa DETECTOR – "FOR MORE INFORMATION AND CONFIDENCE"

10.00-10.30 V. Regvar (Shimadzu, HR):

EVERYTHING YOU NEED TO KNOW ABOUT LC/MS VS. LC/MSMS TECHNIQUES

10.30-11.00 Coffee break

Session 3: HPLC and UPLC

11.00-12.00 ***Instrument presentation session***

12:00-13:30 Lunch break (organized by participants them self)

Friday, 13 June 2014, afternoon

Session 3: HPLC and UPLC

13.30-14.00 M. Periša (FCET, HR):

FATE AND BEHAVIOUR OF SULFONAMIDES METABOLITES IN THE ENVIRONMENT

14.00-14.30 M. Bojić (FPB, HR):

APPLICATION OF LIQUID CHROMATOGRAPHY FOR CHARACTERIZATION OF CYTOCHROME P450 MECHANISM-BASED INACTIVATION

14.30-15.00 T. Kosjek (Jožef Stefan Institute, SLO):

TRANSFORMATION PRODUCTS: SAMPLE PREPARATION AND ANALYSIS

15.00-15.30 Coffee break

15.30-16.00 J. Ivanković (Merck, HR):

EFFECTS OF WATER CONTAMINATION ON HPLC AND LC-MS ANALYSES

16.00-16.30 D. Stipaničev (Hrvatske vode, HR):

NON TARGET SCREENING AT SELECTED LOCATIONS OF THE RIVER DRAVA

Session 4: Open session discussion

16.30 *Sample delivery for 13th proficiency test*

Award of the certificates

T. Bolanča (FCET, HR):

CLOSING OF 15TH INTERNATIONAL CHROMATOGRAPHY SCHOOL

PREFACE

Welcome to 15th ICS 2014

Thousands of scientists and engineers have worked on the development of chromatography over the last several decades. The result is one of the most versatile techniques that we have in chemical science today. The development is still going on with thousands of papers and many books being published every year. All this has been accomplished because there is an understanding of the physico-chemical principles of the chromatographic process. As an expert in chromatography each participant also needs to understand these principles and to learn how they are implemented into their daily practice.

International Chromatography School bridges the gap between the practitioner and chromatography science. It is intensive, focused on both theoretical and practical aspects of chromatography. More importantly, International Chromatography School is independent and provides an unbiased education based on scientific facts and long experience. Dissemination of knowledge plays one of the most important roles in the prosperity of particular expert, business economy of the particular company as well as for the global society. Holistically oriented policy of International Chromatography School (no registration fees) hopefully will contribute and make the difference.

President of the organization committee

Ph. D. Tomislav Bolanča, assoc. prof.

We would like to thank sincerely all the lecturers, sponsors and donors for their contributions at 15th ICS.

INVITED LECTURES

A NEW COLUMN TECHNOLOGY FOR THE ANALYSIS OF *N*-GLYCANS IN PROTEINS

N. Avdalović , J. Thayer, X. Liu

Thermo Fisher Scientific, 445 Lakeside Drive, Sunnyvale, CA 94086, USA
(Nebojsa.Avdalovic@thermofisher.com)

The *N*-Glycans bound to proteins, including antibodies, modulate many biological properties. Understanding glycoprotein glycosylation is an important step in unraveling the function of glycans in glycoproteins. Among the analytical technologies developed thus far, liquid chromatography, mass spectrometry, and LC-MS are most commonly used to analyze glycans in complex samples. Here we will describe some uniquely designed stationary phases including weak anion exchange-HILIC mixed-mode based phases enabling researchers to study diverse situations including quantitative, linkage, qualitative structural analysis in a single platform. In addition these stationary phases also provide a unique way to separate both native and fluorescently labeled *N*-Glycans based on charge, size and polarity. In addition, this analysis and characterization of *N*-Glycans will be using an optimized LC-MS workflow based on these stationary phases coupled online with the Thermo Fisher Scientific Q Excative benchtop Oribitrap mass spectrometer.

HIGH RESOLUTION OR HIGH THROUGHPUT SEPARATIONS OF IONIC COMPOUNDS WITH 4 µm ION EXCHANGERS

J. Weiss

Thermo Fisher Scientific GmbH, Im Steingrund 4-6, 63303 Dreieich, Germany
(joachim.weiss@thermofisher.com)

One of the most topical subjects in conventional HPLC is the increase of sample throughput without sacrificing resolution by utilizing UHPLC techniques. This is typically achieved by packing separator columns of shorter length and smaller internal diameter with separation materials of smaller particle sizes. However, even at optimal flow rates the resulting back pressure often exceeds the pressure tolerance of traditional HPLC hardware. Therefore, we currently witness the development of HPLC instruments with significantly improved back pressure tolerance well above 80 MPa.

Since ion chromatography is part of liquid chromatography, it is not surprising that a similar solution for IC is demanded as well. The fundamental difference in instrument design, however, is the fact that the fluidic pathways in ion chromatography instruments are made of metal-free components with a significantly lower pressure tolerance which excludes the use of particle sizes of around 2 µm (or smaller) typically employed in UHPLC separations. While particle sizes of common ion-exchange materials used in analytical IC are typically around 8.5 µm, so-called fast ion-exchange columns do exist, featuring 5 µm particle sizes in smaller column formats (150 mm × 3 mm i.d.). Thus, the analysis times for anion and cation profiles could be decreased by 50 % in comparison with conventional ion exchangers. But even under these conditions, typical anion or cation profiles are characterized by a run time of around eight minutes.

One possibility for further decreasing analysis times in IC is a flow rate increase beyond the van Deemter optimum, which goes along with a loss of resolution due the relatively large particle size of the ion-exchange material. Thus, this approach is only feasible for samples with a simple analyte composition and little or no matrix contamination. Doubling the linear velocity of the mobile phase through the separator column cuts the analysis time in half, while keeping the back pressure of the separator column well below the maximum pressure tolerance of the system.

The latest development in ion chromatography hardware design is the expanded pressure tolerance of electrolytic eluent generation in capillary and analytical IC systems up to 34.5 MPa (5000 psi). This allows the use of higher linear velocities of the mobile phase in conventional ion exchangers or the use of separator columns packed with a resin of smaller particle size (4 µm). On the other hand, it also facilitates high resolution separations of complex samples through the use of 4 µm separator columns with standard length.

Besides the two major detection techniques for ion chromatography (conductivity and amperometry), a new type of detection mode based on charge measurements will be presented showing increased sensitivity and linear calibration behavior for weakly dissociated anions and cations.

CHROMATE–DETERMINATION BY ION CHROMATOGRAPHY USING MiPT

A. Gelemanović

Primalab d.o.o., Matije Gupca 12a, 49210 Zabok, Croatia
(andrea@primalab.hr, info@primalab.hr)

Chromium(VI) in particular represents a potential hazard to human health, as it is absorbed from food and drinking water, from the air that is breathed, from textiles, from utensils that contain metal and from toys. EU directive 2009/48/EC defines limits for chromate migrating from toys. A portion of the migration solution produced in accordance with regulation is placed on a preconcentration column where a rinsing solution is used to elute the matrix from the column. In the second step, chromatographic separation takes place on an anion exchanger. Detection is accomplished using a very specific post-column derivatization in combination with UV/VIS detection.

QSRR MODELING IN ION CHROMATOGRAPHY

M. Novak, P. Žuvela, Š. Ukić, T. Bolanča

Department of Analytical Chemistry, Faculty of Chemical Engineering and Technology, University of Zagreb, Marulićev trg 20, 10000 Zagreb, Croatia
(mirnovak@fkit.hr)

Finding a financially acceptable analytical method is an important goal of most modern analytical laboratories. Therefore, method optimization is not only necessary for successful analysis but also unavoidable from financial aspect.

Analytical method development and optimization is usually carried out using "trial and error" approach which can be time and resource consuming. Using any other approach is, accordingly, a better solution. In this work, Quantitative Structure-Retention Relationships (QSRR) methodology was applied in order to find a new, cheap and fast strategy for method development and optimization. The QSRR represents methodology which predicts retention time in chromatography on the basis of analyte's molecular structure. QSRR models were developed for prediction of retention for a set of carbohydrates in ion chromatography.

The models were developed for several isocratic elution conditions, using three different regression techniques: stepwise multiple linear regression (S-MLR), partial least square (PLS) and uninformative variable elimination - partial least squares regression (UV-PLS). The best prediction of retention parameter log *k* was obtained by using PLS.

The obtained predicted values were used for development of general isocratic retention model, *i.e.* model that is not limited to a specific isocratic run, but can predict retention for any isocratic elution.

In order to enable retention prediction for gradient elutions, a gradient model based on isocratic data was applied. By obtaining this model, it was possible to expand the prediction to practically any isocratic or gradient elution condition. Moreover, the use of QSRR methodology allowed application of the model for components which have not been used for modeling or, evermore, have never been analysed before.

The predicted retentions at gradient conditions showed good agreement with experimental values (*RMSEP*=18.34 %) proving great potential of the applied methodology in ion chromatographic method development and optimization.

ION CHROMATOGRAPHY WITH INTEGRATED PULSED AMPEROMETRIC/CONDUCTOMETRIC DETECTION AS SOLUTION FOR GLYPHOSATE ANALYSIS IN FOOD AND WATER SAMPLES

V. Stankov

Kemolab d.o.o., Nadinska 11, 10010 Zagreb
(vladimir.stankov@kemolab.hr)

Glyphosate (*N*-(phosphonomethyl)glycine) is the world's best-selling weed killer and one of the most widely used herbicides in Europe. It is used extensively in farming, in parks and public spaces, on railway lines and in gardens. It is also crucial for growing genetically modified (GM) crops, many of which are modified to withstand glyphosate.

An ion chromatography method for the determination of glyphosate in food (fruits, vegetables and cereals) and water was developed. Ion chromatography was carried out by suppressed conductivity detection on Thermo Scientific Dionex ICS-3000 for water samples and on Thermo Scientific Dionex ICS-5000 with integrated pulsed amperometric detection at gold electrode for food samples. The eluent contained 30 mM KOH flowing under isocratic elution for water samples on IonPac AS17 analytical column and 25 mM KOH for food samples under isocratic elution on IonPac AS11-HC analytical column. Limit of quantitation for water samples was 0.01 mg/L and for food samples (fruits, vegetables and cereals) 0.1 mg/L.

Water samples were directly injected and no sample pretreatment was required. Common inorganic ions didn't interfere. Food samples (fruits, vegetables and cereals) preparation included extraction of glyphosate with ultra-pure water.

The recoveries were in the range of 92.2-108.4 % for water samples and 70.3-86.7 % for food samples, the linearity as a coefficient of determination value was 0.9989 for water samples and 0.9991 for food samples.

US EPA set 0.7 mg/L as the maximum value for glyphosate in water. EU legislation has defined a maximum concentration of glyphosate in various matrices in the range from 0.05 mg/kg to 50 mg/kg.

CAPILLARY ELECTROPHORESIS – A FAST AND EFFECTIVE TOOL FOR VARIOUS ANALYTICAL CHALLENGES

M. Sertić, B. Nigović, A. Mornar

Department of Analytics and Control of Medicines
Faculty of Pharmacy and Biochemistry, University of Zagreb
Ante Kovačića 1, 10000 Zagreb, Croatia
(msertic@pharma.hr)

Capillary electrophoresis (CE) is a separation technique, usually described as complementary and/or supplementary to liquid chromatography. The separation of analytes is based on the difference in their electrophoretic mobility under the effect of an applied electric field, moving in a narrow capillary filled with an electrolyte solution towards one of the electrodes. It has several modes of separation which allow analysis of almost any type of analyte and/or matrix. Today capillary electrophoresis is applied for analysis inorganic anions, metal cations and small as well as large organic molecules, like proteins and nucleic acids.

During CE method development there are several parameters that can and/or have to be optimized: type, pH and concentration of the buffer, strength of the applied electric field, temperature, type and concentration of the organic modifier, type and concentration of surfactant, chemical modification of the inner capillary wall *etc.* It seems time consuming, but in fact in enables achievement of fast methods with good separation efficiency.

Capillary electrophoresis has many advantages over chromatographic techniques, such as short analysis time, high separation efficiency, simplicity of operation, low purchase and operation costs, small required sample volume, low solvent consumption and ecological friendliness. In spite of all advantages and its application possibilities, the technique still aspires to its full potential.

Separation efficiency and speed of the analysis are the first focus while developing a new analytical method. A successful example is a CE method developed for simultaneous determination of six statin drugs. Base hydrolysis was used to open lactone rings of lovastatin and simvastatin in order to transform these compounds to the corresponding negatively charged hydroxyl acid forms. Charged analytes can be analyzed by Capillary Zone Electrophoresis (CZE) method instead of using Micellar Electrokinetic Chromatography (MEKC) approach, thus enabling the analytes to be determined in shorter analysis time. The first step during method development is the selection of the buffer pH, which determines the extent of ionization and thus the electrophoretic mobility of each analyte. With p*K*a values around 4.2-4.5, buffer pH above 6 would provide completely anionic forms of all six statin drugs. In order to find best separation conditions for six analytes, several running buffers were tested in CZE mode. In order to change method selectivity, by changing the zeta potential, dielectric constant and electrolyte viscosity, and separate all six statins, the influence of an organic modifier, *i.e.* acetonitrile and methanol, added in various concentrations (5-20 % *v/v*) was also tested. Still after choosing the appropriate buffer pH and type in addition with an organic modified, satisfying resolution and peak shape was not achieved. Therefore, although all analytes were ionized, a MEKC approach with an addition of sodium dodecyl suplhate (SDS) was tried. The crucial step actually was adding both SDS and 10 % *v/v* methanol in the electrolyte solution. In this way the analysis time was reduced and good resolution (Rs > 2) was maintained. At the end, fine tuning of method's parameters was conducted, by changing buffer concentration, temperature and separation voltage. Borate buffer concentration was

changed from 10 to 35 mM in order to optimize separation efficiency through the influence on the electroosmotic flow (EOF), the main driving force in CE. Temperature was tested in the range from 20-35 °C and throughout changes in electrolyte viscosity, both EOF speed and electroforetic mobility of the analytes were altered. Finally, in order to achieve the shortest possible analysis time and to further improve method efficiency, while remaining good resolution (Rs > 2.28), separation voltage was examined ranging from 20 to 30 kV. Under optimized conditions, using 25 mM borate buffer pH 9.5 with 25 mM SDS and 10 % *v/v* methanol, and 23 kV voltage and 30 °C, all six analytes were successfully separated in less than 5 min. Method was validated and applied for the analysis of six statin drugs in pharmaceutical samples.

For most analytical methods, especially in drugs purity determination, waste and drinking water analysis, a challenge lies in assuring method sensitivity. Therefore, low detection limits have to be accomplished. For example, to improve the sensitivity of the MEKC method developed for simultaneous determination of atorvastatin and its four impurities in bulk drug and formulated products, several approaches have been utilized. Injection time is related to peak width and height, therefore samples were injected hidrodynamically at 50 mbar pressure while the injection time was changed from 2 to 10 s. The increase of peak area was linear to injection time increment. However, since the peak width also increased, the resolution between closely migrating analytes reduced. Therefore, a compromise between peak area and the sensitivity of the method, and resolution and separation efficiency of the method was achieved at injection time of 4 s. Similar approach was used with altering separation voltage; decreasing the voltage results in higher peak areas, but the loss of resolution as well as an increase in migration time were used as limiting factor. Although atorvastatin has an absorbance maximum at a wavelength of 244 nm, determination was conducted at 214 nm because it proved to show higher absorbance for related substances and lower noise level, and thus providing lower detection limits (LOD) of atorvastatin's impurities. The key step that lowered limits of detection by a 3-fold time was using an extended light path capillary, which posses a bubble cell at the detection point. Using this simple CE method development steps, without the use of powerful and sensitive detector, detection of atorvastatin's related substances was able under the 0.1 % level.

Third main challenge for an analytical method is to overcome matrix effects which can cause interference or signal suppression/enhancement. An example of an uncomplicated sample preparation that achieved both elimination of interfering compounds and a pre-concentration, and thus enhancing sensitivity of the method, is a MEKC method developed for determination of lovastatin, the main active component in red fermented rice, and a toxic by-product citrinin expected in ppb levels. Complex matrix products were analyzed, for example containing red yeast rice, Commiphora mukul gum extract, Cynara scolymus leaf extract, L-arginine, Non-GMO soy bean concentrate, gamma oryzanol, black pepper extract, ginger root extract, rosemary leaf extract, turmeric root extract and cayenne extract in tablets. LOD for lovastatin of 0.2 μg/mL, and 0.03 μg/mL (ppb level) for citrinin was achieved, meaning that the complex matrix effects were avoided.

Therefore, CE is a versatile, effective, simple, fast and low-cost tool that can successfully be used to overcome various analytical challenges.

DETAILED FOOD SAMPLE EXAMINATION: ONE STEP TARGET AND NON-TARGET ANALYSIS OF CONTAMINANTS BY GC-HR-TOF MS

J. Zrostlíková[1], T. Kovalczuk[1], K. Kalachová[2], L. Drábová[2], J. Hajšlová[2]

[1] *LECO European Technical Centre Prague, Sokolovská 219, Prague 9, 190 00, Czech Republic (jitka.zrostlikova@leco.cz)*
[2] *Institute of Chemical Technology, Technická 3, Prague 6, 160 00, Czech Republic*

Today requirements in the analysis of food contaminants target at low ppb concentration levels and require confident confirmation of identity. At the same time, the methodics of sample preparation tend to be more time-efficient and universal, which results in less clean extracts and more potential matrix interference. Therefore new technologies of GC- and LC-MS analysis need to be implemented to meet the above requirements. New LECO multireflecting GC-TOF MS provides mass resolution 50,000 (at m/z 219) and mass accuracy 1 ppm at data acquisition rate up to 200 Hz. In this presentation the results from the analysis of pesticide residues and other contaminants in heavy food matrices will be demonstrated.

GC×GC-TOF MS ANALYSIS OF PETROCHEMICAL SAMPLES IN "REVERSED SETUP": FINE TUNING OF THE SEPARATION AND DATA PROCESSING STRATEGIES

J. Zrostlíková, T. Kovalczuk

LECO European Technical Centre, Sokolovská 219, Prague 9, 190 00, Czech Republic (jitka.zrostlikova@leco.cz)

Petrochemical samples have always been a challenge for GC separation. Because of their complexity, the separation by one separation mechanism (one column) does not bring sufficient resolution of the sample. GC×GC, on the other hand, utilizes two columns with different selectivity of stationary phase, and therefore the sample "dimensionality" can match with separation "dimensionality" which results in dramatically increased separation power.

The aim of this study was to optimize the GC×GC parameters for three different types of samples (i) crude oil, (ii) vacuum light gas oil (fraction: 370-430 °C) and (iii) vacuum residue (fraction +550 °C).

For the analysis of high boiling samples such as vacuum light gas oil and vacuum residue, the temperature stability of the selected columns is very critical. In GC×GC, second column is exposed to higher temperatures in the modulator and secondary oven than the first column. From this point of view, the use of polar column (which usually has less temperature stability) for the first dimension is more convenient. Besides this, the reversed column set-up has been shown to provide better separation of saturated from unsaturated and cyclic structures. In this study, the following column set has been used: 1st dimension**:** Rxi-17Sil, 15 m × 0.25 mm, 0.1 µm film, max. programmed temperature 360 °C (Restek, USA); 2nd dimension Rxi-5HT, 1 m × 0.25 mm × 0.1 µm, max. programmed temperature 400 °C (Restek, USA).

Using a relatively short first dimension column with thin film resulted in "reasonable" elution temperatures even for high boiling fraction. At the same time efficient separation of the structural groups has been achieved in the second dimension. Non-typically both columns were of same internal diameter, which is beneficial for the separation efficiency, as the same column flow is optimal for both first and second column.

In this presentation some aspects of fine tuning of some GC×GC parameters such as secondary oven offset and hot pulse for modulation will be discussed. Also the application of advanced software features such as classifications or data summary tables in this practical application will be demonstrated.

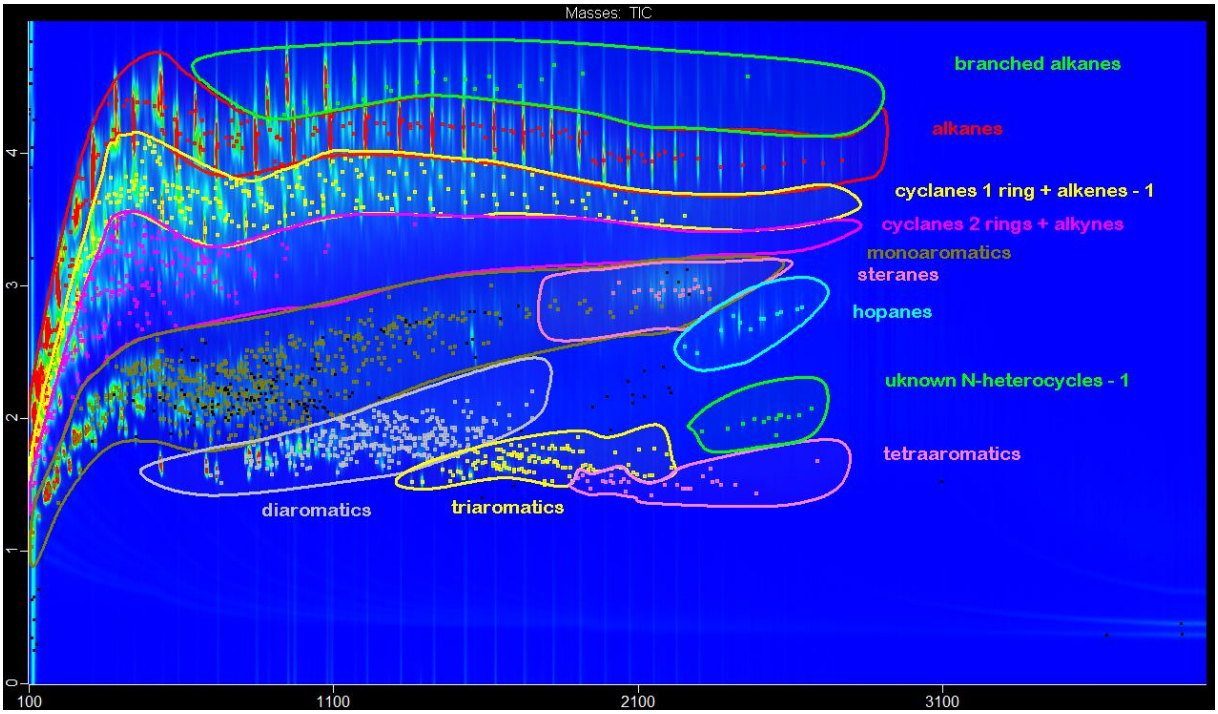


Figure 1. Contour plot of crude oil with classifications

VALIDATION OF SPME-GC/MS METHOD FOR QUALITATIVE ANALYSIS OF GASOLINE AND DIESEL FUEL TRACES – FORENSIC APPROACH

I. Bačić

Forensic Science Centre ''Ivan Vučetić'',
Ministry of the Interior, Zagreb, Croatia
(ivana.bacic@mup.hr)

Forensic Science Centre ''Ivan Vučetić'' has recently been accredited by Croatian Accreditation Agency by the HRN EN ISO/IEC 17025 standard in the field of fingerprints and identification, biological expertise, chemical-physical and toxicological expertise, ballistics and toolmarks.

During the accreditation process, validation of a method used for analyzing traces of gasoline and diesel fuel in fire debris or on the hands of a suspect was also performed. Gasoline and diesel fuel were chosen because they are the most common ignitable liquids that are used as fire accelerants. Method involves SPME fiber as a sample extraction technique and gas chromatography–mass spectrometry (GC/MS) system for analysis.

To carefully characterize the performance of this method, in the validation process several parameters were studied, such as limit of detection (LOD), split/splitless injection modes, selectivity, robustness and matrix influence. Finally, minimum requirements criteria for the identification of gasoline and diesel fuel are established.

The results of the validation process showed that SPME is a good alternative to traditional extraction techniques for chromatographic analysis. Validated method provides an easy sample preparation, good selectivity and sensitive detection of arson accelerants.

DEVELOPMENT OF GAS CHROMATOGRAPHY MASS SPECTROMETRY (GC-MS) METHOD FOR DETERMINATION OF SELECTED PESTICIDES IN SURFACE WATER

G. Peček[1], D. Mutavdžić Pavlović[2], S. Babić[2]

[1] *Croatian Centre for Agriculture, Food and Rural Affairs, Institute for Plant Protection, Rim 98, 10000 Zagreb, Croatia*
(gorana.hrlec@gmail.com)
[2] *Faculty of Chemical Engineering and Technology, Department of Analytical Chemistry, Marulićev trg 20, 10000 Zagreb, Croatia*

The use of pesticides is an inevitable part of food and crop production, which is the main source of their occurrence in the environment. Surface waters in close vicinity of agricultural fields are vulnerable to pesticide pollution, especially if located in areas with high agricultural production. Pollution of surface waters by chemicals presents a threat to the aquatic environment with effects such as acute and chronic toxicity to aquatic organisms, accumulation in the ecosystem and losses of habitats and biodiversity, as well as a threat to human health [1]. Surface run-off from the field is considered the most significant route of pollution of surface waters [2]. Thus it is important to monitor pesticides in such water bodies, where the input is expected.

There are a lot of different pesticides available and not all can be covered by a single analytical method owing to the differences in physico-chemical properties. In addition, introduction of new pesticides and banning others changes the use patterns in short or a long term. From these reasons there is a constant need for developing new analytical multiresidue methods or extending the scope of already existing ones.

Pesticide residues have been detected in water bodies in many research papers. Gas chromatography (GC) is often used in their determination in water [3-10]; it has high separation power, enables fast analysis and when coupled to mass spectrometer (MS) specificity and simultaneous identification and quantification can be achieved. Solid-phase extraction (SPE) is quick and efficient sample preparation technique for aqueous samples.

For the determination of selected pesticides in the surface water new SPE-GC-MS multiresidue analytical method was developed. Pesticides covered by this method were atrazine, terbuthylazine, dimethoate, acetochlor, dimethenamid, chlorothalonil, chlorpyrifos-methyl, metolachlor, metribuzin, chlorpyrifos, ethofumesate, pendimethalin, bentazone, oxyfluorfen, captan, kresoxim-methyl, fludioxonil, tebuconazole, metamitron and chloridazon. Samples were taken from surface water in close vicinity of agricultural fields. Sample preparation was done by SPE using Oasis HLB cartridges and the pesticides were extracted from water under optimized SPE conditions. Chromatographic separation was done on GC-MS Agilent 6890N using mass spectrometer 5975 inert as a detector. Pesticides were separated using the DB-17MS (30 m × 0.25 mm, 0.25 μm, J&W Scientific) chromatographic column and splitless injection.

References

1. European Parliament and Council, Directive 2008/105/EC, Off. J. Eur. Union **L 348**, 84 (2008).
2. D. D. Chiras, Environmental Science, 8th ed. (Jones & Bartlett Learning, Sudbury, 2010), p. 484.

3. J. J. B. Nevado, C. G. Cabanillas, M. J. V. Llerena, and V. R. Robledo, Microchem. J. **87**, 62 (2007).
4. T. A. Albanis, D. G. Hela, T. M. Sakellarides, and I. K. Konstantinou, J. Chromatogr. A **823**, 59 (1998).
5. C. Aguilar, F. Borrull, and R. M. Marcé, J. Chromatogr. A **771**, 221 (1997).
6. S. Wang, P. Zhao, G. Min, and G. Fang, J. Chromatogr. A **1165**, 166 (2007).
7. R.-S. Zhao, C.-P. Diao, X. Wang, T. Jiang, and J.-P. Juan, Anal. Bioanal. Chem. **391**, 2915 (2008).
8. J. Quintana, I. Martí, and F. Ventura, J. Chromatogr. A **938**, 3 (2001).
9. M. L. Hladik, K. L. Smalling, and K. M. Kuivila, Bull. Environ. Contam. Toxicol. **80**, 139 (2008).
10. S. Öllers, H. P. Singer, P. Fässler, and S. R. Müller, J. Chromatogr. A **911**, 225 (2001).

DETERMINATION OF PESTICIDE RESIDUES IN FRUIT AND VEGETABLES BY GC/MS AND LC/MS/MS

B. Marošanović, M. Pandurević-Todorović, D. Ujsasi, H. Farkaš, K. Sabo

SP Laboratorija, Industrijska 3, Bečej, Serbia
(splaboratorija@victoriagroup.rs)

Each of us dreams about healthy and safe food, without contaminants and pesticide residues. Organic farming is very popular, but the products are more expensive than commercially produced. On the other hand, a commercial production requires the use many of different pesticides. Pesticides are toxic chemical compounds that are used to suppress and destroy harmful microorganisms, insects, fungi, rodents, weeds and other undesirable pests in agricultural production. Modern agricultural practice has large number of pesticide produced based on organophosphorus, organochlorine, organonitrogen and other compounds. In the world, industrial production of pesticides are in million tons for different effects, such as insecticides, herbicides, fungicides, rodenticides, nematocides, acaricides and others.

SP Laboratory is accredited for method determination of pesticide residues in fruit and vegetables. This is the review of residual active substances of pesticides in fruit and vegetables during period from 2012 to April 2014.

Determination of multi-pesticide residues based on method EN 15662 [1]. Sample preparation has been done by QuEChERS method following acetonitrile extraction/partitioning and clean-up by dispersive SPE. Different techniques were used for determination of pesticide residues such as gas chromatography with mass spectrometer (GC6890N/MS5975, RTL PEST3 by Agilent, USA) and liquid chromatography with MS/MS detector (Ultimate 3000/TSQ by Thermo scientific, USA).

Analytical quality control and method validation procedures for pesticide residues analysis in fruit and vegetables were done by SANCO document [2]. Recovery for all pesticide residues were in range of 70 to 120 %. Precision (RSD) was less than 20 %. LOQ of the method is 0.01 mg/kg. Also, the total number of pesticide residues that we can determine is 402. Validation of method has done on 32 different samples of fruit and vegetables, like as high water content, high acid content, high sugar content and other.

Processed results are related to the 948 samples of fruit and vegetables, on presence of 423 pesticide residues. Overview of the analyzed samples of fruit and vegetables is given in Table 1.

Pesticide residues contained about 55% of the analyzed samples of fruit and vegetables.

About 20 % samples of fruit and vegetables, which were determined presence of pesticide residues, had only one active substance of pesticides, while all other samples contained at least two or more active substances of pesticides. The concentration of active substances of pesticides, that were determined in samples of fruits and vegetables, were in line with our and EU legislation except for 27 samples. In 903 samples of fruit and vegetables, analyzed on 423 pesticide residues, we found 67 different active sup stances of pesticides. Reason for so many different active substances in samples is probably due to the production of fruit and vegetables in very small gardens. Also, in Serbia, we don't have production of pesticides, only packing, and we import a huge number of the pesticides, with same active substances, but from different production companies.

Based on the processed results, it can be concluded that more than 95 % of the analyzed fruit and vegetables are in accordance with Serbian legislation and EU Regulation (EC) No. 396/2005.

Table 1. Overview of the analyzed samples of fruit and vegetables.

Sample name	No. of samples/2012	No. of samples/2013	No. of samples/I-IV 2014
Aronia	0	0	1
Pineapple	1	0	0
Banana	1	8	0
Blueberry	3	2	0
Peach	2	21	0
Broccoli	1	1	0
Onion	1	24	0
Apple	23	84	5
Strawberry	5	16	1
Cauliflower	1	14	0
Cucumber	1	20	1
Potato	3	49	0
Pear	4	20	1
Blackberry	31	29	2
Raspberry	44	134	8
Pepper	11	27	1
Tomato	5	41	1
Salad	1	8	0
Carrot	1	0	0
Plum	13	45	3
Cherry	29	71	13
Garlic	0	1	0
Cranberry	0	1	0
Black currant	0	2	0
Pea	0	5	0
Grape	0	26	0
Cabbage	0	22	0
Orange	0	7	0
Leek	0	1	0
Spinach	0	5	0
Beans	0	0	1
Total/year	181	684	38
TOTAL	Number		903

References

1. EN 15662: 2008 *Foods of plant origin – Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE-QuEChERS-method*
2. *Analytical Quality Control and Method Validation Procedures for Pesticide Residues Anaylsis in Food and Feed* – Document No.SANCO/12571/2013

QUALITY ASSESSMENT OF DIETARY SUPPLEMENTS USED IN THE PEDIATRIC PATIENTS

A. Mornar[1], M. Sertić[1], B. Nigović[1], D. Amidžić Klarić[2], I. Klarić[3]

[1] *Department of Analytics and Control of Medicines, Faculty of Pharmacy and Biochemistry, University of Zagreb, Ante Kovačića 1, 10000 Zagreb, Croatia (amornar@pharma.hr)*
[2] *Department of Clinical Pharmacy, University Hospital Dubrava, Avenija Gojka Šuška 6, 10000 Zagreb, Croatia*
[3] *Department of Public Health, R. Dž. Čauševića 1, 76000 Brčko DC, Bosnia and Herzegovina*

There is a sharp upward trend in the use of dietary supplements since 1990s, which resulted in widespread interest of health practitioners and patients in these products as well as awareness of their safety and quality. They are readily available to the public in pharmacies, drugstores, grocery stores and health food stores as well as by mail and via the Internet. These products are widely promoted, often with unsubstantiated claims of benefit and rarely with any mention of potential toxicity, interactions with drugs or quality control. Manufactures are not required to get approval by U.S. Food and Drug Administration (FDA) or to register their products with the FDA before producing or selling dietary supplements; they just have to make sure that product label information is truthful and not misleading. Manufactures are responsible for establishing their own manufacturing practice guidelines, because there are no FDA regulations to ensure the identity, quality and purity of dietary supplements. Commonly, dietary supplements are not required to be standardized. As the term "standardization" may mean many different things, some manufacturers use the term standardization incorrectly to refer to uniform manufacturing process. Following a recipe is not sufficient for a product to be called standardized, thus the presence of the word "standardized" on a supplement label does not necessarily indicate the quality of the product and correct amounts of active ingredients or excipients.

Liquid herbal dietary supplements may contain significant levels of ethanol arising from its use as an extraction solvent. Ethanol is metabolically active; therefore formulation without ethanol or with the lowest achievable level should be selected to avoid systematic exposure when the target population is children. Unfortunately, the safety evaluation of the ethanol content in dietary supplements for pediatric population is, at the moment, not harmonized between different European Union Member States.

In 2014 a survey of young Croatian parents was performed. Results of the national survey examined opinion of parents on dietary supplements intended for pediatric population indicated that parents consider dietary supplements a natural way of healing, safe but not effective as they expected. Research has shown that many children use dietary supplements that contain herb or propolis extracts.

Therefore, the aim of our work was to propose a novel headspace gas chromatographic (HSS-GC) method for determination of eight volatile constituents of liquid dietary supplements used in the pediatric patients.

23 different dietary supplement products were taken randomly from the local community pharmacies and health food stores. These products were produced by 9 manufactures from 5 different countries and were collected during 2012 and 2013. Two batches of each product were analysed. The proportion of claimed amount of ethanol was drastically different among the investigated products. Labeled specification on only one product stated ethanol content 2.1 %. On three products alcohol was mentioned only as an ingredient without any further information. One product was labeled as ″alcohol-free″. Finally, labeled specifications on 18 products did not state anything about alcohol content.

Sample preparation was performed with a model G1888 headspace sampler from Agilent Technologies (Santa Clara, CA, USA). The headspace oven temperature was set at 90 °C, while the loop and transfer line temperatures were set at 115 °C and 120 °C, respectively. Vial equilibration time was set at 20 min.

The chromatographic analysis was performed using an Agilent 6850 series gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with a flame ionization detector. Elution was achieved using a DB-624 column (25 m × 0.33 mm, 0.5 μm) (Agilent Technologies, Santa Clara, CA, USA). The injector and detector temperatures were 250 °C and 300 °C, respectively. The injection port was operated at a 1:15 split. Flow rate of carrier gas nitrogen was set at 5.0 mL/min. The gradient temperature program was involved in order to obtain a good resolution (Rs ≥ 2) between all 8 analytes and internal standard, acetone, in 10 min.

Method validation was performed following the recommendation of International Conference on Harmonization. The method was validated for selectivity, linearity, precision and accuracy. The limits of determination and quantitation as well as extraction efficiency were also tested.

The developed and validated method was used for quality assessment of various dietary supplements used in the pediatric patients. Ethanol was found in all investigated products. Still, it should be pointed out that 14 products contained less than 1 % of ethanol. In the product labeled as ″alcohol-free″ ethanol was found to be present at low level (4 ‰). The concentration of ethanol found in eight samples was between 1 and 5 %. Finally, one of the preparations analyzed contained a considerable amount of ethanol, more than 10 %. As it was expected other alcohols were present in levels lower than the limit of quantitation.

Finally, the concentration of As, Ag, Cd, Co, Cr, Ni, Pb and Sn in samples were determined by Graphite Furnance Atomic Absorption Spectrometry (GFAAS). All metals were found in low levels.

APPLICATION OF LIQUID CHROMATOGRAPHY MASS SPECTROMETRY IN TARGETED AND UNTARGETED ANALYSIS

S. Bilsborough

Agilent Technologies

The hyphenation of mass spectrometry with liquid chromatography provides increased analytical power in terms of sensitivity, selectivity and confirmation of identity of compounds in relatively complex matrices. When screening for compounds using LC/MS there are two principal workflows that may be used, those of targeted and untargeted analysis. Targeted analysis is typically performed using triple quadrupole mass spectrometers, which provide the lowest limits of quantitation combined with high selectivity when working with complex matrices. However, with such targeted techniques, the method must contain the list of compounds that are to be targeted and any compounds present in the sample that do not form part of this target list are not detected. For more general screening where the detection of unexpected compounds present in the sample is required, untargeted analysis may be performed using quadrupole time of flight mass spectrometers. This instrument generates full spectral data allowing post-analysis interrogation of the data file using data mining tools. An additional advantage of this technique is the ability to generate empirical formula of compounds using the accurate mass measurement capability of the instrument, providing confirmation of identity of detected compounds and also the ability to determine the identity of true unknowns, such as emerging contaminants. With this presentation, we provide an overview of the two workflows together with example applications where the techniques are routinely used.

APPLICATIONS OF ION-EXCHANGE/REVERSED-PHASE MIXED-MODE STATIONARY PHASES IN COMBINATION WITH CHARGED AEROSOL DETECTION FOR SEPARATING PHARMACEUTICALS

J. Weiss

Thermo Fisher Scientific GmbH, Im Steingrund 4-6, 63303 Dreieich, Germany
(joachim.weiss@thermofisher.com)

There are many challenges in HPLC method development for pharmaceutical compounds. Although reversed-phase liquid chromatography (RPLC) is the most widely used analytical separation technique for analyzing non-ionic pharmaceutical drugs, reversed-phase columns often fail to provide satisfactory results when separating ionic drugs or counter ions of anionic or basic drugs. Anionic counter ions are highly hydrophilic and elute close to the void, so that the analysis is often compromised by sample matrix. Hydrophilic basic drugs often interfere with the counter ion and hydrophobic basic drugs exhibit excessively long analysis times. Although ion-pair chromatography can be used to overcome these difficulties, it often requires long equilibration time and a complex mobile phase that is often incompatible with subsequent mass spectrometric detection. On the other hand, anionic counter ions and highly basic compounds can be separated by ion-exchange chromatography. However, for hydrophobic compounds commonly used silica-based ion- exchangers provide inadequate hydrophobicity, while polymer based ion-exchangers suffer from lower chromatographic efficiency.

Silica-based mixed-mode stationary phases combine both anion and/or cation exchange with reversed-phase properties. The packing material of the trimodal stationary phase is based on Nanopolymer Silica Hybrid (NSH) technology consisting of high-purity porous spherical silica particles coated with charged nanopolymer particles. The inner pores of the silica substrate are modified with an organic layer that provides both reversed-phase and anion exchange properties. The outer surface is modified with cation exchange functionality. This arrangement ensures distinctive spatial separation of the anion exchange and cation exchange regions, which allows both retention mechanisms to function simultaneously and be controlled independently. Thus, the selectivity of this stationary phase is orthogonal to reversed-phase columns. Multi-mode stationary phases are ideal for the separation of anionic and basic compounds with different hydrophilicities as well as for simultaneous separation of basic, acidic and neutral pharmaceuticals. However, the simultaneous analysis of pharmaceutical drugs and their counter-ions requires alternate means of detection, because non-chromophoric counter-ions cannot be detected with traditional absorbance techniques. Charged aerosol detection (CAD) has been introduced to overcome traditional non-specific detection shortfalls. It can be utilized to characterize active pharmaceutical ingredients (APIs) together with their counter-ions.

In this presentation, I will discuss column chemistries, chromatographic properties, the principles of charged aerosol detection, and some exemplary pharmaceutical applications.

ACQUITY QDa DETECTOR – "FOR MORE INFORMATION AND CONFIDENCE"

M. Boras

Waters GesmbH, Hietzinger Hauptstraße 145, 1130 Vienna, Austria
(Mario_Boras@waters.com)

LC-MS analyses are needed more and more in analytical laboratories today. Regardless is it for method development, sample profiling, synthetic chemistry or purification.

Traditionally, mass spectrometry is seen as a costly technique, consuming bench space or floor space and requiring careful sample-specific instrument adjustment. Nowadays cheaper mass spectrometers are available, but handling the technique still remains complex.

At the same time users are more interested in results interpretation and reporting, less time and focus is available to explore all possible MS instrument capabilities in depth.

To facilitate affordable and easy-to-use LC-MS analyses, new Waters Acquity QDa Detector combines familiar operation (like a traditional optical detector) with the power of mass information, for both HPLC and UPLC methods.

With its novel approach to instrument design, Acquity QDa has same footprint, size and ease of use as an optical PDA detector. It can be ready in matter of minutes and can be safely shut down when not in use. Its source requires no physical optimization (e.g. probe position) and all parameters are software controlled.

Simplified operation offers same quality of results as with bigger quadrupole MS, but with significantly lower investment, both in funds and in training and start-up time and should bring LC-MS closer to the masses, being occasional users or students.

EVERYTHING YOU NEED TO KNOW ABOUT LC/MS *VS*. LC/MSMS TECHNIQUES

V. Regvar

Shimadzu d.o.o.

Considering drastic increase in environmental awareness on a global scale, to meet the rigorous demands of environmental and health organizations, analysis became more delicate than ever. Liquid chromatography combined with mass spectrometry has become one of the leading techniques that fulfills these demands, especially in the field of pesticide analysis. In recent years, environmental and health legislation and regulation became more and more complex, at the same time increasing the number of pesticides and residue levels to be analyzed and monitored. In addition, another idea was to exclude false negative and false positive results, which were often present in LC/MS analysis. All these arguments led to the development and prevalence of use of LC/MSMS technique - liquid chromatography combined with tandem mass spectrometer. Besides offering more accurate and reliable results, LC/MSMS also provides better sensitivity and higher throughput. With all this in mind, it is no surprise that LC/MSMS today is one of leading approaches in the field of environmental analysis.

FATE AND BEHAVIOUR OF SULFONAMIDES METABOLITES IN THE ENVIRONMENT

M. Periša, M. Mitrevski, S. Babić

Department of Analytical Chemistry, Faculty of Chemical Engineering and Technology, University of Zagreb, Marulićev trg 19, Zagreb, Croatia
(mperisa@fkit.hr)

The worldwide consumption of pharmaceuticals provides their continuous release together with their metabolites in the environment. Pharmaceuticals concentrations are increasing in the environment since many pharmaceuticals are incompletely removed during their passage through wastewater treatment plants (WWTPs) which are considered as the main point of their release into the environment. Due to their potential impacts on the aqueous ecosystems and human health there is a rising concern with their occurrence and persistence in the aquatic environment. Also, it is very difficult to predict pharmaceuticals behaviour in the environment since they have different physico-chemical properties.

Among different classes of pharmaceuticals, antibiotics are of the biggest concern since they can affect bacteria in the environment and thus disturb natural elemental cycles. Hence, it is necessary to take a closer look at the fate and effects of these compounds in the environment. Sulfonamides represent one of the most commonly used families of antibiotics in human and veterinary medicine. Once in the body of man or animal sulfonamides are metabolized to more polar compounds that are more likely to pass through the WWTPs. As a result pharmaceuticals are found together with their metabolites in different environmental samples.

Once pharmaceuticals and their metabolites reach an environmental system, there are multiple routes (biotic and non-biotic) for their possible elimination which are very important for limiting their presence in the environment. Biodegradation is biotic process, while in non-biotic processes sorption, hydrolysis, photolysis are included. Results of all these processes (except sorption) are compounds (degradation products) which can be even more toxic than the parent compounds in some cases. Therefore, it is very important to consider metabolites and their degradation products when studying pharmaceuticals presence in the environment.

According to the all mentioned facts, the aim of this study is to investigate fate and behaviour of N4-acetylated metabolites in the aquatic environment.

APPLICATION OF LIQUID CHROMATOGRAPHY FOR CHARACTERIZATION OF CYTOCHROME P450 MECHANISM-BASED INACTIVATION

M. Bojić[1,2]

[1] *Department of Biochemistry, Vanderbilt University School of Medicine, 642 RRB, 2200 Pierce Avenue, Nashville, Tennessee, USA*
[2] *Department of Medicinal Chemistry, Faculty of Pharmacy and Biochemistry, University of Zagreb, A. Kovačića 1, 10000 Zagreb, Croatia*
(mbojic@pharma.hr)

Introduction

Drugs represent foreign substances to an organism (xenobiotics). When ingested the main objective organism is to eliminate a xenobiotic. For this to occur drug has to be modified and turned into more polar metabolite that is susceptible to elimination. Usually this starts at an early stage, after absorption of a drug in intestine and most predominantly in liver (first pass metabolism).

Cytochromes P450 present most significant superfamily of enzymes for the metabolism of xenobiotics in humans being involved in metabolism of over 50 % of drugs on the market. Structurally P450s are hemoproteins with iron in the active site bound to the porphyrin ring and conserved cysteine residue. Functionally P450s are monooxygenases that utilizes oxygen by incorporate hydroxyl group to the substrate while other oxygen atom goes into byproduct – water:

$$RH + O_2 + NADPH + H^+ \rightarrow ROH + H_2O + NADP^+$$

As cytochromes P450 metabolize significant number of drugs on the market, special precaution in drug development is devoted to the drugs that could potentially interfere with a metabolism of other drugs (drug-drug interactions, DDI). If a drug (perpetrator) is reversible inhibitor of P450 3A4 it can cause the accumulation of another drug that is metabolized through the same enzyme. This leads to possible side effects/toxic effects that can be overcome by discontinuing the perpetrator.

In case of irreversible inhibition, enzyme has completely lost activity. To regain normal levels of activity, enzyme has to be expressed de novo. As such this type of inhibition has potential for significant drug-drug interactions. In pharmaceutical industry potential drug candidates that can cause irreversible inhibition of P450 2D6 and P450 2C19 tend to be discontinued from further research.

Screening of potential cytochrome P450 inhibitors

Microsomes from a pool of human livers are usually used for in vitro screening. To determine if a potential drug inhibits any of the liver cytochrome P450 enzymes incubations are performed with a marker substrate of the individual cytochrome P450 (Table 1).

Table 1. Marker substrates of individual cytochromes P450.

Cytochrome P450	Substrate	Reaction	Detection
1A2	caffeine	N^3-demetylation	UVD (273 nm)
	phenacetin	*O*-deethylation	UVD (254 nm)
2A6	coumarin	7-hydroxylation	FLD (338 nm → 458 nm)
2B6	*S*-mephenytoin	*N*-demethylation	UVD (204 nm)
2C8	paclitaxel	6α-hydroxylation	UVD (230 nm)
2C9	tolbutamide	methyl hydroxylation	UVD (229 nm)
2C19	*S*-mephenytoin	4'-hydroxylation	UVD (204 nm)
2D6	dextrometorphan	*O*-demethylation	UVD (280 nm) FLD (280 nm → 330 nm)
2E1	chlorazoxazone	6-hydroxylation	UVD (290 nm)
3A4/5	nifedipine	oxidation	UVD (254 nm)
	testosterone	6β-hydroxylation	UVD (240 nm)
	midazolam	1'-hydroxylation	UVD (220 nm)
4A	lauric acid	hydroxylation	radio-detection (^{14}C, ^{3}H)

Mechanism based inactivators are metabolism based inhibitors thus they require NADPH and time for the inactivation effect to be observed (preincubation). Incubations with marker substrate are usually performed under physiological conditions (50 mM K-phosphate, pH 7.4, 37 °C), after which product is isolated by liquid-liquid extraction and reconstituted in mobile phase. Typical isocratic HPLC analysis is performed on reverse phase column and hydrophilic product elutes prior substrate. Majority of marker substrates contain aromatic ring that absorbs UV light and enables easy detection (lauric – fatty chain acid is detected by radiography).

Determining the type of P450 inhibition

If metabolism (time and NADPH dependent) inhibition is observed next step is determining type of inhibition using recombinant cytochromes P450 (Figure 1). Inactivation can be the result of covalent binding of inhibitor to and/or to apoprotein. If the inhibitor binds to the heme iron, quasi-irreversible inhibition is observed, that can be reversed *in vitro*.

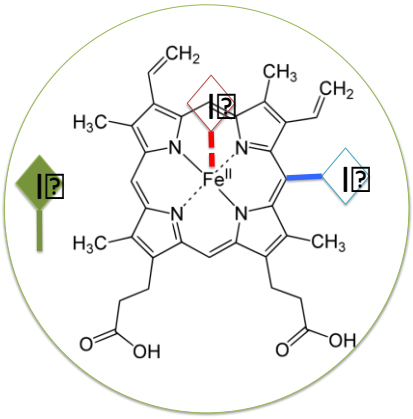


Figure 1. Possible sites of cytochrome P450 inactivation.

Independently of the type of inactivation, ultimate techniques used for analysis are HPLC and LC-MS. In case of quasi-irreversible inhibition procedure is simple as it uses residual activity measurement with probe substrate by HPLC, after dialysis of incubation with and without ferricyanide.

Heme loss assay is usually performed on C4 column using gradient method with water and acetonitrile acidified with trifluoracetic acid (0.05-0.1 %) as mobile phases. This eluent enables direct injection of incubation onto a column. Advantage of the method is robust, but requires up to an hour long runs.

Before relatively demanding protein MS analysis covalent binding has to be confirmed by autoradiography or scintillation counting of an inhibitor marked with radioisotope. Samples are usually digested prior to LC analysis.

Several examples or irreversible inhibition discovery

Lapatinib, an oral breast cancer drug, can cause idiosyncratic hepatotoxicity that has been related to mechanism-based inactivation of cytochrome P450 3A. Chan *et al*. (2012) used glutathione as trapping agent of a reactive metabolite and confirmed quinoneimine metabolite of O-dealkylated lapatinib as mechanism-based inactivator.

Similar approach using GSH related trapping agent was used to demonstrate protein adduct formation of quinone methides by P450 2B6 bioactivation of tamoxifen that was further confirmed by ESI-LC-MS of whole protein.

Ritonavir, a protease inhibitor, causes mechanism-based inactivation of P450 3A4 through heme destruction that was attributed to isocyanate intermediate.

Conclusion

High performance liquid chromatography represents basis of metabolism studies. Accompanied with other techniques like ion exchange chromatography (used for His-tagged cytochrome P450 purification), thin layer chromatography (determining metabolites of radioactive substrates), 16O2/18O2 incubations with subsequent high resolution MS analysis (mechanism of P450 catalysis, determining substrates of orphan P450s) *etc*., it represents the most powerful tool for studying of all aspects of cytochromes P450.

References

1. Chan ECY, New LS, Chua TB, Yap CW, Ho HK, Nelson SD. Drug Metab Dispos 40 (2012): 1414-22.
2. Guengerich FP. Experimental Approaches to Analysis of Reactions of Cytochrome P450 Enzymes. In Drug Metabolism Prediction, Wiley-VCH Verlag GmbH & Co. KGaA, 2014, pp199-219.
3. Lin H-I, D'Agostino J, Kenaan C, Calinski D, Hollenberg PF. Drug Metab Dispos 41 (2013): 1813-24.
4. Sridar C, D'Agostino J, Hollenberg PF. Drug Metab Dispos 40 (2012): 2280-2288.

TRANSFORMATION PRODUCTS: SAMPLE PREPARATION AND ANALYSIS

T. Kosjek, E. Heath

Jožef Stefan Institute, Ljubljana, Slovenia
(*tina.kosjek@ijs.si*)

Conventionally, trace-level analysis of micropollutants in the environment deals with quantitative determination of target analytes that were preselected during analytical method development. Yet, such approach, known as "targeted analysis, is often insufficient to assess the environmental relevance of emerging contaminants. To better understand the risk that they pose to the environment, it is essential to consider the occurrence of parent microcontaminants, their metabolites and their transformation products (TPs). In contrast with the first two groups, the identity of the TPs, their origin, fate and consequently the effects on human and other living organisms are mostly unknown.

There are several reasons for this but the most important is their challenging identification that requires application of advanced instrumental methods. Among these methods, liquid chromatography with mass spectrometry (LC-MS) has experienced an immense progress, in terms of both technology development and application.

Among sample preparation methods for determination of TPs, and in the environmental analysis in general, solid phase extraction (SPE) is of utmost importance. SPE had used silica based C18 and related reversed phase sorbents, which have been to a high degree replaced by polymeric sorbents, enabling increased method flexibility and greater specific surface area. Further development lead towards increasing SPE selectivity, which in our case has to compromise the ability to avoid interferences and concurrently not lose the unknown compounds of interest such as TPs.

The lecture will present the principals of sample preparation and analysis for determination of TPs by using examples from our past research.

EFFECTS OF WATER CONTAMINATION ON HPLC AND LC-MS ANALYSES

J. Ivanković

Merck d.o.o. Andrije Hebranga 32, 10000 Zagreb, Croatia
(*jasenka.ivankovic@merckgroup.com*)

The performance of LC-MS instruments regarding resolution and sensitivity has tremendously improved in recent years. Also the regulatory requirements to reach the limits of detection of various analytes have become increasingly challenging. These prerequisites raise the purity requirements for solvents.

Ultrapure water is the most frequently used solvent in an analytical laboratory and its purity plays a critical role in LC-MS analyses. In parallel with higher demands to ultrapure water quality, its handling is also important because ultrapure water is highly prone to leach of contaminants out of container surfaces (e.g. stabilizers or plasticizers from plastic or silica and alkali from glass) and easily absorbs contamination from the laboratory environment (e.g. air).

Any impurity present in reagent water will cause ghost peaks and elevated baselines, signal suppression and/or adduct formation with target molecules and will therefore decrease sensitivity (signal-to-noise ratio), increase the limit of detection (LOD) and/or increase the complexity of a mass spectrum. These effects not only compromise analyses, complicate data interpretation and add the risk of repeating experiments, but also decrease column life time and maintenance intervals of analytical instruments. Thus, to perform reliable LC-MS measurements, it is critical to pay attention on the organic and ionic level in high purity water, to avoid particulates and bacteria and prevent ultrapure water quality from degradation by its careful handling in the laboratory.

During the presentation, the effect of contamination in ultrapure water on LC-MS analyses will be discussed as well as a number of easily avoidable ultrapure water handling pitfalls will be presented. Specifically the effect of organics, ions, particulates, bacteria present in ultrapure water will be discussed in details. In addition, the effect of high purity water storage, the effect of equipment cleaning and the effect of the laboratory environment and air contamination on ultrapure water quality degradation will be demonstrated. Finally, optimal combination of water purification technologies to obtain ultrapure water of sufficient quality for LC-MS analyses will be introduced.

The data presented in the seminar should help analysts to critically evaluate the potential risks involved in using ultrapure water of poor quality in an LC-MS laboratory.

NON TARGET SCREENING AT SELECTED LOCATIONS OF THE RIVER DRAVA

D. Stipaničev[1], S. Repec[1], S. Širac[2]

[1] *Hrvatske vode, Central Water Management Laboratory, Ulica grada Vukovara 220, 10000 Zagreb, Croatia (drazenka.stipanicev@voda.hr, sinisa.repec@voda.hr)*
[2] *Hrvatske vode, Department of Development, Ulica Grada Vukovara 220, 10000 Zagreb, Croatia (sinisa.sirac@voda.hr)*

Investigations of the occurrence of pesticides, pharmaceuticals, household chemicals, personal care products and emerging contaminants are the most intensively studied areas of environmental analytical chemistry because of the growing threat of water pollution by chemicals which effect every constituent of ecosystem from microorganisms to humans. In order to meet European requirements defined within European Union Water Framework Directive and to improve quality of waters there is a need to prioritize chemicals within river basins for monitoring, reduction, or for improving scientific knowledge.

Screening of surface water is challenging task for environmental researchers because organic compounds are present in water in complex mixtures at very low concentrations and for their determinations specific analytical method and high resolution instruments are needed. High resolution instruments are able to identify unexpected compounds and also to quantify known contaminants with little sample manipulation. For screening of organic pollutants in this work direct injection of large volume surface water samples on ultra high performance liquid chromatography (UHPLC) coupled to time-of-flight spectrometry (Q-TOF/MS) was used and this approach will be detailed and illustrated.

AUTHOR INDEX

SPONSORS

KemLab

Prodaja i servis laboratorijske opreme

Thermo Scientific -Dionex

Ionski kromatografi
kompletan asortiman kromatografskih rješenja za sve korisnike.

Izvrsne performanse za vrhunske rezultate.
Analizirajte analite koje do sada niste mogli detektirati

Thermo Scientific TKA

Sustavi za ultračistu vodu
sa i bez reverzne osmoze za ASTM.
TIP I, II i III, kapaciteta od 3 do 300 L.

Gilson

Pipetman - najlakši, najpreciznija i najkvalitetniji prijenos Vaših tekućinskih uzoraka

Labconco

Vaše povjerenje nagrađuje sigurnošću u radu,uštedom, dugoviječnošću i izuzetno točnim rezultatima.

NUVE

Kvaliteta, sigurnost, robusnost.
Široka paleta proizvoda.
Najbolji izbor za svaki labaratorij.

ThermoFisher SCIENTIFIC

Protecting your laboratory environment LABCONCO®

GILSON®

nüve

biosan Medical-Biological Research & Technologies

LABOGENE

BOECO Germany

ADAM THE RIGHT BALANCE Speed, Performance, Value

KOBIS

Thermo SCIENTIFIC

Kobis d.o.o. ovlašteni je distributer tvrtke Thermo Scientific, USA. Kao zastupnici, svjetskog lidera u području instrumentalne analitičke opreme i programskih rješenja, nudimo individualni pristup svakom korisniku. Rezultat ovakvog rada je inovativno rješenje za svaki zahtjev laboratorija.

Pokrivamo područja:

- Tekućinske kromatografije
- Plinske kromatografije
- Masene spektrometrije
- Elementne analize
- Molekulske spektroskopije
- Reologije
- Ekstrudera
- Potrošnog materijala
- Laboratorijske opreme
- Laboratorijske programske podrške
- Tehničke i edukacijske podrške stručnih i certificiranih inženjera

Thermo SCIENTIFIC

PEAK SCIENTIFIC

TOSOH

La-Pha-Pack Werner Reifferscheidt GmbH

PSS POLYMER STANDARDS SERVICE

Više informacija na: **www.kobis.hr**

Kontakt: **kobis@kobis.hr, tel. 01 6545 742**

Labena

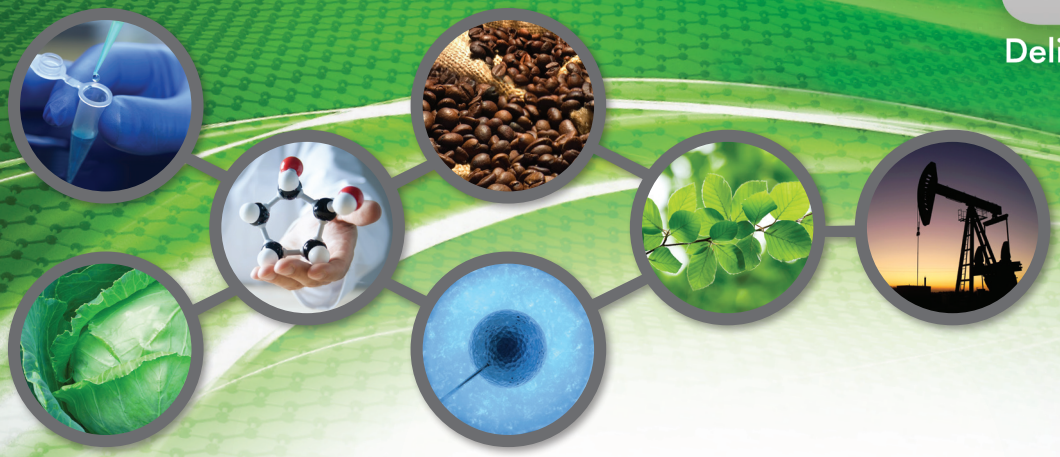
Potpuna rješenja u laboratorijskoj i procesnoj analitici

- Kromatografski viali
- Sistemi za ultra čistu vodu
- RAMAN analizatori
- Centrifuge
- Spektrofotometri
- PH metri
- Filteri za šprice
- Membranska filtracija
- Vage
- Elektroforeza
- Pipete
- Opća laboratorijska oprema

Labena
www.labena.hr

Kontakt na telefon: 01/ 655 01 06 **ili na e-mail:** info@labena.hr

Delivering the Right Results



LECO changed the analytical world by defining a new era of Mass Spectrometry

For more than 75 years, industries around the world have trusted the family-owned LECO Corporation to deliver technologically advanced products and solutions

Pegasus® 4D GCxGC-TOFMS

Industry Standard TOFMS

- Power of separation – GCxGC
- Precision of identification – TOFMS
- Performance of Pegasus 4D GCxGC-TOFMS

Pegasus® GC-HRT

Discovery with Confidence

- Accuracy – mass measurement < 1 ppm
- Ultra High Resolution – 50000 FWHM
- Maximum Confidence – High Resolution TOFMS

www.leco-europe.com

Contact us for a quote or any additional information

LECO Corporation

LabEKO d.o.o. zastupnik za LECO
Baštijanova 9a, 10000 Zagreb, Hrvatska
Tel: +385 1 3634 433
Fax: +385 1 3634 798
Email: **labeko@labeko.hr**
Web: **www.leco-europe.com**

instrumentalia

prodajni program

HRVATSKA
INSTRUMENTALIA Adria d.o.o.
T (01) 662 3883
F (01) 662 3884
E sales@instrumentalia.hr

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Liquid Chromatography, Convergence Chromatography, Supercritical Fluid Chromatography, Mass Spectrometry, Lab Informatics, Sample preparation, LC Columns and Accessories
www.waters.com

TA

Thermal Analysis, (DSC, TGA, EGA – MS, DMA, DVS), Rheology, Microcalorimetry, Flash Diffusivity, Dilatometry, Thermal Conductivity,
Rotational High Temperature Viscometer
www.tainstruments.com

Horizon technology

Automated Extraction System for trace organic contaminants in aqueous samples, SPE discs, Automated Solvent Drying, Solvent Evaporation and Sample Concentration,
Automated Extraction System for SPE cartridges
www.horizontechinc.com

PRIMAlab
a member of the Metrohm group

PRIMALAB d.o.o., Matije Gupca 12a, HR-49210 Zabok
Tel.: +385 (49) 221-679, 221-957 OIB:83028109264
Fax: +385 (49) 222-116 info@primalab.hr

Metrohm

Metrohm Applikon

Metrohm Autolab B.V.

Kambič Laboratorijska oprema

hielscher

PHARMA TEST

LAUDA

HAMILTON

Fungilab

H·W·S Labortechnik

KINEMATICA

RUDOLPH RESEARCH ANALYTICAL

HEL better chemistry - faster

DROPSENS

protherm FURNACES

KERN

ATTAIN SUPERIOR MASS SPEC PERFORMANCE

Confidence means attaining superior analytical performance, 24/7 reliability, maximum uptime and productivity—everything you expect from the leader in mass spectrometry. Across your MS platforms, Agilent provides one single, consistent user interface. After 40+ years of innovation and experience with the industry's largest installed base, Agilent's best-in-class portfolio continues to push the boundaries of MS technology, delivering market-leading solutions.

Learn more about Agilent mass spec technology at:
www.agilent.com/chem/MS

The Measure of Confidence

Scan the QR code for more information about Agilent's LC/MS, GC/MS, ICP-MS solutions and MassHunter Software

© Agilent Technologies, Inc. 2014

Agilent Technologies
Authorised Distributor

ALPHA CHROM

MERCK MILLIPORE

Direct Q UV

Excellent water purification system for Academy labs at best price

From Tap to pure and ultrapure water – with easy and convenient dispense!

- High quality water to meet the requirements of your most critical applications
- Water with low TOC provides important benefits to HPLC users: higher sensitivity and longer column lifetime
- Application Pak - fine-tune your ultrapure water quality to match your research needs
- Compact all-in-one system for the most efficient use of your lab space
- User friendly maintenance

Merck Millipore is a division of MERCK

sartorius
preko znanosti do rješenja

sartorius croatia

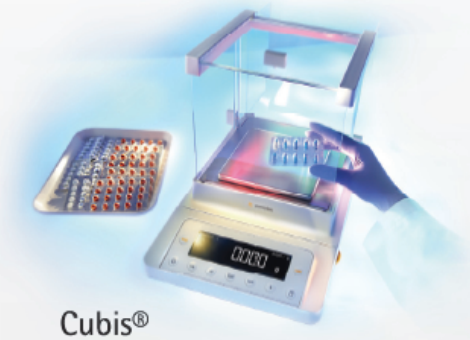
Unaprijedite
svoje pipetiranje!

Nova Sartorius Picus
elektronska pipeta.
Najmanja, najlakša,
najpreciznija!

reddot design award
winner 2012

Arium® Pro
Sustav pročišćavanja vode

Jednostavnost uporabe s maksimalnom
fleksibilnošću. Visoki standard kvalitete,
cijenovno prihvatljiv i ekološki prihvatljiv!



Cubis®
Prva modularna serija
laboratorijskih vaga

Definicija vage nove klase.
Najveća preciznost za
najmanje veličine uzoraka!
Prva lab. vaga s potpuno
automatskim niveliranjem.

Sartorius Croatia - Libra Elektronik d.o.o. - Ovlašteno zastupništvo i servis Sartorius RH
Savska 45a, 10 290 Zaprešić tel: 01 / 3340 - 290, fax: 01 / 3340 - 299
mail: sartorius@sartorius.hr web: www.sartorius.hr

SHIMADZU
Excellence in Science

Quality

Sustainability

Innovation

www.shimadzu.hr

AnAs
ANALYTICAL ASSISTANCE

T. Maretića 6
HR-10010 Zagreb
Tel: 01 6625606, 6625607
Fax: 01 6625608
www.anas.com.hr

Tvrtka **AnAs d.o.o.** osnovana je 1991. godine. Temeljna djelatnost tvrtke je snabdijevanje analitičkom opremom i pripadajućim priborom brojnih znanstvenih i sveučilišnih institucija, zavoda za javno zdravstvo i industrijskih pogona u Hrvatskoj.

Tijekom godina postali smo ekskluzivni zastupnici renomiranih proizvođača opreme za analitičku kemiju iz područja kromatografije i spektroskopije **(Bruker, Analytik Jena, CEM, DBS, Teledyne ISCO, Teledyne Tekmar, …)**.

Da bismo korisnicima pružili što kompletniju uslugu, ponudu smo proširili priborom za pripremu uzoraka, kromatografskim i spektroskopskim potrošnim materijalom te certificiranim referentnim materijalima (**Restek, Kinesis, Meinhard, Inorganic Ventures, CPAchem, Specac, Starna, …**).

Široka paleta proizvoda koje nudimo, u kombinaciji s našim znanjem i iskustvom stečenim radom u laboratoriju, omogućuje nam da ponudimo najbolje rješenje za sve vaše analize.

Za svu prodanu opremu osiguran je servis u garantnom roku i izvan njega. Serviser je kvalificiran i treniran kod principala, te ima ovlaštenja kao ekskluzivni i certificirani servisni inženjer, a na poziv dolazi promptno.

Jedno od temeljnih načela jest etikom vođeno poslovanje, jer se tako zadovoljavaju neke ključne vrijednosti: vjerodostojnost, pouzdanost, kompetentnost i čestitost.

Na zadovoljstvo naših korisnika kontinuirano unaprjeđujemo i prilagođavamo poslovanje njihovim potrebama, ostvarujući time svoj cilj.

i-TLC

CAMAG

THIN LAYER CHROMATOGRAPHY

KEFO®
SINCE 1949

VITA LAB NOVA

phenomenex®
…breaking with tradition℠

KNAUER

Vita Lab Nova d.o.o.
Vrančićeva 1A
HR-10 000 Zagreb
Tel : 00385-1-466-75 13
Tel/Fax: 00385-1-466-75 14
e-mail: vita.lab@zg.t-com.hr

Vita Lab Nova d.o.o. je tvrtka sa dugogodišnjim iskustvom u prodaji, servisu i korisničkoj podršci vrhunskih proizvoda za područje kromatografije – potrošnog materijala, SPE, GC i HPLC kolona te HPLC i UHPLC uređaja.