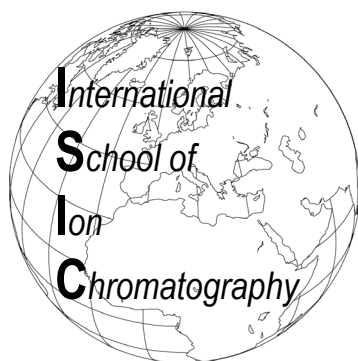


UNIVERSITY OF ZAGREB
FACULTY OF CHEMICAL ENGINEERING AND TECHNOLOGY

**12th International School of Ion
Chromatography**



BOOK OF ABSTRACTS

30 June – 01 July, 2011
ZAGREB, CROATIA

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CONTENTS

Final program	1
Preface	5
INVITED LECTURES	6
N. Avdalović, Y. Liu: CAPILLARY REAGENT-FREE ION CHROMATOGRAPHY SYSTEMS WITH SUPPRESSED CONDUCTIVITY AND ELECTROCHEMICAL DETECTION AND THEIR APPLICATIONS	7
T. Bolanča, Š. Ukić, M. Rogošić: „OptIC“: NEW IC METHOD DEVELOPMENT SOFTWARE	8
J. Weiss: STATIONARY PHASE DESIGN FOR ION-EXCHANGE CHROMATOGRAPHY – FROM STANDARD INORGANIC ANIONS AND CATIONS TO AMINO ACIDS AND PROTEINS	9
M. Divjak Gulin: POST-COLUMN CHEMISTRY FOR IMPROVED OPTICAL ABSORPTION DETECTION	11
K. Vinković: POSSIBILITIES OF PESTICIDE DETERMINATION BY ION CHROMATOGRAPHY	12
M. Luša, Š. Cerjan Stefanović, T. Bolanča, M. Rogošić, Š. Ukić: DETERMINATION OF INORGANIC IONS IN THE OILFIELD WATER BY ION CHROMATOGRAPHY	13
A. Jurišić, Ž. Zgorelec, M. Mesić: TEMPORAL AND SPATIAL NITRATE DISTRIBUTION IN AGRICULTURAL SOILS	14
V. Stankov: ION CHROMATOGRAPHIC DETERMINATION OF LACTOSE IN FOOD AND FEED	18
Lidija Pozaić Frketić: ESSENTIALS OF LABORATORY MANAGEMENT IN GLP AND TQM ENVIRONMENT	20
Š. Ukić, T. Bolanča: 10 TH PROFICIENCY TEST: CRITICAL REVIEW	21
M. Boras: CURRENT TRENDS IN LC – UNDERSTANDING THE SCIENCE OF ULTRA PERFORMANCE LC TECHNOLOGY	22

J. Weiss:		
	DETERMINATION OF ACTIVE PHARMACEUTICAL INGREDIENTS (APIs) AND COUNTERIONS – A COMPARISON BETWEEN HILIC AND MULTI-MODE CHROMATOGRAPHY	23
G. Maretić:		
	AGILENT 1290 INFINITY LC – INFINITELY MORE POWERFUL	24
P. Koselj, A. Čerin:		
	HPLC TROUBLESHOOTING – A PRACTICAL APPROACH	25
V. Gabelica Marković:		
	UPLC-MS IN DRUG DISCOVERY	26
T. Madić:		
	DETERMINATION OF IMPURITIES BY HPLC IN PHARMACEUTICAL APPLICATIONS	28
M. Zrnčić, A. Petrikovski, S. Babić, M. Kaštelan-Macan:		
	DEVELOPMENT OF MULTI – RESIDUE ANALYTICAL METHOD USING LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY FOR PHARMACEUTICALS DETERMINATION IN WATER SAMPLES	29
S. Pelko, M. Petrović, S. Pérez, M. Farré, A. Horvat, D. Barceló, M. Kaštelan-Macan:		
	APPLICATION OF LC-MS IN DETERMINATION OF AQUEOUS PHOTOTRANSFORMATION PRODUCTS OF VETERINARY PHARMACEUTICALS	31
POSTER PRESENTATIONS		33
D. Šamec, D. Kremer, J. Grúz, J. Piljac Žegarac:		
	FREE PHENOLIC ACIDS IN <i>MICROMERIA CROATICA</i> (PERS.) SCHOOTT MEASURED BY UPLC-MS/MS	34
I. Đapić, S. Kežić, A. Kammeyer, I. Jakaša:		
	DETERMINATION OF NATURAL MOISTURIZING FACTOR IN HUMAN STRATUM CORNEUM BY LIQUID CHROMATOGRAPHY	35
SPONSORS		36

Thursday, 30 June 2011 – Morning session

Opening of the 12th International School of Ion Chromatography, FCET,

Marulićev trg 20

9.00–9.15 S. Kurajica (Dean of FCET, CRO), T. Bolanča (FCET, CRO): *Opening remark*

Preface to 12th International School of Ion Chromatography, FCET, Marulićev trg 20

9.15–9.30 Š. Cerjan Stefanović (FCET, CRO): *Education in Chromatography*

Session 1: New Achievements in IC, FCET, Marulićev trg 20

9.30–10.30 N. Avdalović (ThermoFisher Scientific, USA): *Capillary Reagent-Free Ion Chromatography Systems with Suppressed Conductivity and Electrochemical Detection and Their Applications*

10.30–11.00 T. Bolanča (FCET, CRO): *“OptIC”: New IC Method Development Software*

11.00–11.15 Coffee break, FCET, Marulićev trg 20, Academic Club

Session 1: New Achievements in IC, FCET, Marulićev trg 20

11.15–11.45 J. Weiss (ThermoFisher Scientific, GER): *Stationary Phase Design for Ion-Exchange Chromatography – From Standard Inorganic Anions and Cations to Amino Acids and Proteins*

11.45–12.15 M. Divjak Gulin (Primalab, SLO): *Post-column Chemistry for Improved Optical Absorption Detection*

12.15–12.45 K. Vinković (Kemolab, CRO): *Possibilities of Pesticide Determination by Ion Chromatography*

12.45–14.00 Lunch break (organized by participants them self)

Thursday, 30 June 2011, Afternoon session

Session 2: Applications in IC, FCET, Marulićev trg 20

- 14.00–14.30** M. Luša (Pliva, CRO): *Determination of Inorganic Anions in Oilfield Water by Ion Chromatography*
- 15.00–15.30** A. Jurišić (Faculty of Agriculture, CRO): *Temporal and Spatial Nitrate Distribution in Agricultural Soils*
- 15.30–16.00** V. Stankov (SP Laboratory, SRB): *Ion Chromatographic Determination of Lactose in Food and Feed*

16.00–16.30 Coffee break, FCET, Marulićev trg 20, Academic Club

Session 3: Quality Assessment, FCET, Marulićev trg 20

- 16.30–17.00** L. Pozaić Frketić (Biotehnika, CRO): *Essentials of Laboratory Management in GLP and TQM Environment*
- 17.00–17.30** Š. Ukić (FCET, CRO): *10th Proficiency Test: Critical Review*

17.30–18.30 Poster Presentation Session; FCET, Marulićev trg 20

18.30 Welcome party, FCET, Marulićev trg 19, Faculty Hall

Friday, 01 July 2011, Morning session

Session 4: New Achievements in HPLC, FCET, Marulićev trg 20

- 9.00–9.45** M. Boras (Waters, AUS): *Current Trends in LC - Understanding the Science of Ultra Performance LC technology*
- 9.45–10.30** J. Weiss (ThermoFisher Scientific, GER): *Determination of Active Pharmaceutical Ingredients (APIs) and Counterions – A Comparison Between HILIC and Multi-mode Chromatography*
- 10.30–11.00** G. Maretić (AlphaChrom, CRO): *Agilent 1290 Infinity LC – Infinitely more powerful*

11.00–11.30 Coffee break

Session 5: HPLC Troubleshooting, FCET, Marulićev trg 20

- 11.00–12.30** P. Koselj, A. Čerin, (Instrumentalia, SLO): *HPLC Troubleshooting – Practical Approach*

12.30–13.00 Commercial presentations, FCET, Marulićev trg 20

13.00–14.00 Lunch break (organized by participants them self)

Friday, 01 July 2011, Afternoon session

Session 6: HPLC Application, FCET, Marulićev trg 20

14.00–14.30 V. Gabelica Marković (Galapagos, CRO): *UPLC-MS in Drug Discovery*

14.30–15.00 T. Madić (PharmaS, CRO): *Determination of Impurities by HPLC in Pharmaceutical Applications*

15.00–15.30 **Coffee break, FCET, Marulićev trg 20, Academic Club**

Session 6: HPLC Application, FCET, Marulićev trg 20

15.30–16.00 M. Zrnčić (FCET, CRO): *Development of Multi - Residue Analytical Method Using Liquid Chromatography Tandem Mass Spectrometry for Pharmaceuticals Determination in Water Samples*

16.00–16.30 S. Pelko (Pliva, CRO): *Application of LC-MS in Determination of Aqueous Phototransformation Products of Veterinary Pharmaceuticals*

16.30 *Open discussion session*

Sample delivery for 11th proficiency test

Award of the certificates

T. Bolanča (FCET, CRO): *Closing of 12th International School of Ion Chromatography*

PREFACE

Welcome to 12th ISIC 2011

Ion chromatography can be classed as a mature analytical technique and it might be presupposed that it has reached a point where most new developments can be viewed as being more incremental than substantial. However, a close examination of recent developments and future trends suggests that ion chromatography is still undergoing rapid developments which involve some completely new directions. The major areas where ion chromatography development and prospects are strongest are new stationary phases, miniaturization, enhanced peak capacity (predominantly through the use of complex elution profiles and the chemometrics associated with optimization of separations), hyphenated ion chromatography systems, and the use of ion chromatography in bioanalysis. However, liquid chromatography methods for trace analysis of organic analytes are dominated by reversed-phase separations. Even analytes that seem less suited for reversed-phase liquid chromatography due to the presence of ionizable groups are often analyzed by this method. An alternative approach is the use of mixed-mode stationary phases (ion-exchange/reversed-phase), and such an approach has shown to be promising even further investigation are certainly welcomed. These trends are recognized by the organizing committee and in addition to conventional ion chromatography, this year for the first time ISIC will cover majority of liquid chromatography (reversed phase, normal phase, ion pair...).

The relationship between education, social enterprise, business and the economy is a rapidly growing one, the importance of which is widely recognized by governments, academics and practitioners. It is especially important in today's knowledge/learning society to make knowledge available. These aims are incorporated deeply in the ISIC organizing committee policy and making IC School totally free of charge we are hoping that our small contribution might make the difference.



President of the organization committee

Ph.D. Tomislav Bolanča, assoc. prof.

We would like to thank sincerely all the lecturers and sponsors for their contributions at 12th ISIC.

INVITED LECTURES

**CAPILLARY REAGENT-FREE ION CHROMATOGRAPHY SYSTEMS
WITH SUPPRESSED CONDUCTIVITY AND ELECTROCHEMICAL
DETECTION AND THEIR APPLICATIONS**

Nebojša Avdalović, Yan Liu

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As a result of increasing interest in the development of capillary ion chromatography (IC) systems and methods for determination of ionic species we have made significant efforts in designing a commercially viable capillary IC system with on line electrolytic eluent generation. The practice of IC in the capillary format (i.e. using capillary columns with internal diameters of 1 mm or smaller) has a number of advantages. Capillary IC Systems typically operate at 1 to 20 $\mu\text{l}/\text{min}$ and thus the amount of eluent consumed is very small. Capillary IC systems have improved capability for continuous operation with minimal intervention and thus minimize problems associated with system start-up and shutdown. The use of capillary columns can improve separation efficiency and/or speed. Separation processes in the capillary format require much smaller amounts of sample and thus capillary IC systems offer improved compatibility with a mass spectrometer. In addition, the practice of IC in the capillary format opens the door for the possibility of offering new selectivity for difficult applications using new columns packed with more exotic and difficult-to-make stationary phases.

In this presentation we will report our on-going efforts in the development of capillary Reagent-Free IC (RFICTM) systems with on-line electrolytic eluent generation and suppressed conductivity detection for determination of target ionic analytes. We will especially concentrate on the trace analysis application as well as on the use electrochemical detection in the analysis of carbohydrates on the same Capillary IC platform.

„OptIC“: NEW IC METHOD DEVELOPMENT SOFTWARE

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Ion chromatographic samples are often very complex. Extreme differences in peak area of neighboring components or similar retention times can cause unsatisfactory separation. Although isocratic elution provides the best resolution, it is obtained usually at very low eluent concentration. Consequently, the analysis time is large and chromatographic bands of strongly retained components are particularly broadened, which diminishes the accuracy and precision of the analysis. Therefore, gradient elution is becoming increasingly popular in modern IC. By varying the gradient profile, it is possible to obtain satisfactory separation in a much shorter analysis time. The process of finding the optimum IC separation conditions for any solution/stationary phase combination takes some time, even in the case of simply switching from an old to a new column of the same type. Initial experiments are needed in any case. Their number can be quite large in case of searching for an optimum gradient profile.

This lecture focuses on presenting different modeling and optimization approaches in gradient elution ion chromatography. These methodologies are based on complementary strategies including artificial neural networks, numerical integration approach using theoretical models, experimental data transferring from isocratic to gradient elution mode, signal processing and optimization based on multi criteria decision making. Each approach has advantages and disadvantages; however, extensive experimental testing of designed models resulted in developed software that contains all of the extracted information and conclusions.

STATIONARY PHASE DESIGN FOR ION-EXCHANGE CHROMATOGRAPHY – FROM STANDARD INORGANIC ANIONS AND CATIONS TO AMINO ACIDS AND PROTEINS

Joachim Weiss

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In the more than twenty years that encompass its birth and development, ion chromatography (IC) has undergone enormous changes. While in its earliest embodiments IC was focused primarily on the analysis of inorganic anions and cations, today IC has an important role in the analysis of organic ions as well. For the majority of applications, conductivity detection with suppression devices represents the most versatile detection system augmented today with a variety of other detection modes such as UV/Vis, various forms of amperometry as well as element-specific detection (IC-ICP) and mass-selective detection (IC-MS).

The presentation will focus on trends in stationary phase design as part of the development of IC over the last five years or so.

One of the trends is the design of hydroxide-selective ion exchangers for isocratic and gradient separations of inorganic and organic anions. Characterized by a high loading capacity and large-loop injection capability those new stationary phases are suitable for trace inorganic anion and cation analysis, especially in combination with electrolytically generated mobile phases of high purity. Based on ethylvinylbenzene/divinylbenzene, the resulting resins can either be surface-functionalized and agglomerated with micro-beads carrying the actual ion-exchange groups or grafted with an anion exchange polymer layer. Examples of these new types of stationary phases will be presented and compared with older generation columns in terms of applicability for the analysis of a variety of inorganic and organic anions. Other trends in IC stationary phase design include the use of high-capacity columns which allow the injection of more concentrated samples without overloading and peak broadening. High-capacity anion exchangers are developed for isocratic separations of inorganic anions and oxyhalides in drinking water and other sample matrices as well as for gradient separations of inorganic and organic anions improving the separation of monovalent carboxylic acids. Lately, the particle diameter of the support material has been scaled down to 5 μm . Together with smaller column dimensions (150 mm x 3 mm i. d.) significantly shorter analysis times are achieved without compromising on resolution and chromatographic efficiencies.

By now, the merits of hydroxide eluants in anion exchange chromatography are well understood. However, hydroxide-selective stationary phases have been prepared by using vinyl aromatic polymers. Such polymers are advantageous because they are compatible with highly alkaline eluants. While hydroxide-selective anion exchangers prepared in this way show good selectivity for common anions, the interaction of analytes such as bromate, chlorite, chlorate, and perchlorate with the π electrons of the aromatic backbone is an impediment to obtaining the proper selectivity in the presence of common anions. Consequently, search was undertaken for a suitable means of preparing hydroxide-selective stationary phases from entirely aliphatic components.

Ultimately, a novel electrostatic step-growth condensation polymerization method was developed using aliphatic epoxy monomers and amines. Unlike commonly employed grafting

methods, where the attachment of the graft polymer is via covalent bonds, the attachment of the graft polymer in this case is the result of strong electrostatic adhesion between the cationic condensation polymer grown directly off the substrate surface and anionic surface groups introduced through sulfonation of the substrate prior to the graft step. The resulting graft composite exhibits a high degree of hydroxide selectivity, good stability under alkaline conditions, high hydrophilicity, and excellent chromatographic properties.

By tailoring the reagents used in preparation of the step-growth polymer as well as the reaction conditions, a wide variety of different selectivities are achievable. In this presentation, several new stationary phases derived from this new synthetic method will be shown in the context of real-world application examples.

The latest trend in stationary phase design for ion chromatography is leading towards monolithic ion exchangers. Originally developed for the separation of large biomolecules such as peptides and proteins, a breakthrough has been made in the development of polymer-based monoliths for the separation of small molecular weight anions. The high permeability of a monolith resulting in a relatively low backpressure opens the door for high throughput anion analysis when column dimensions are miniaturized as well.

POST-COLUMN CHEMISTRY FOR IMPROVED OPTICAL ABSORPTION DETECTION

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UV/VIS detection is one of the most sensitive detection techniques in trace-level chromatography. Sometimes, however, spectrophotometric detection lacks sensitivity, selectivity or reproducibility and chemical derivatizations are required. By using Metrohm's rugged and versatile flow-through reactor, single- or multi-step derivatizations can be done fully automatically, in either pre- or post-column mode at any temperature between 25...120 °C. The variable reactor geometry allows to adjust the reactor residence time of the reactants according to derivatization kinetics. The flexibility of the reactor is demonstrated by optimizing four widespread post-column techniques: the relatively slow ninhydrin reaction with amino acids and the fast derivatizations of silicate, bromate and chromate(VI).

POSSIBILITIES OF PESTICIDE DETERMINATION BY ION CHROMATOGRAPHY

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More than 1055 active ingredients are currently registered as pesticide, which compose more than 16000 different pesticide products. Pesticide use raises a number of environmental and health concerns since they are toxic to living organisms. Over 98 % of sprayed insecticides and 95 % of herbicides reach a destination other than their target species, such as air, water and soil and it is necessary to monitor their presence in environment, food and water as well as their distribution, persistency and degradation path. Pesticides can cause a variety of acute and delayed adverse health effects from simple irritation of the skin and eyes to more severe effects such as affecting the nervous system, mimicking hormones causing reproductive problems, and causing cancer.

Currently, the most used techniques for pesticide analyses are GC, GC-MS and LC-MS, which allow simultaneous and sensitive determination of larger number of pesticides. However, these techniques are more suitable for non-polar pesticides than for smaller polar molecules, which are more difficult to handle regarding selectivity and detection.

Articles that describe pesticide analysis using ion chromatography are quite rare and mostly not recent. Usual application is pesticide separation on anion-exchange column with carbonate mobile phase followed by conductivity or eventually UV detection.

In the last few years new stationary phases were developed, detection techniques other than conductivity are commonly used and sensitivity is improved by involving new devices and miniaturization of ion chromatography. Thus, the aim of this work is to study the new possibilities of pesticide determination using combination of several stationary phases and detection techniques characteristic for ion chromatography.

Representatives of pesticides were selected according to chemical structure, usage and toxicity. Phenoxy-pesticides are represented by 2,4-D (2,4-dichlorophenoxyacetic acid), very common pesticide in use more than 50 years, dicamba (3,6-dichloro-2-methoxybenzoic acid) which replaced on market very dangerous 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) and moderately toxic chloramben (3-amino-2,5-dichlorobenzoic acid). Selection of pyridines are systematic pesticide picloram (4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid), triclopyr ((3,5,6-trichloro-2-pyridinyl)oxyacetic acid) which is banned in several countries and clopyralid (3,6-dichloro-2-pyridinecarboxylic acid) with characteristic accumulation in vegetables. Chosen phosphoamines are one of most used pesticides glyphosate (*N*-(phosphomethyl)glycine), its metabolite AMPA (aminomethylphosphonic acid) and similar glufosinate ammonium (2-amino-4-(hydroxy-methyl-phosphoryl)butanoic acid). Triazolic pesticides are represented by carcinogenic amitrole (3-amino-1,2,4-triazole) and taken from triazines are prometon, desmetrin and metabolite cyanuric acid.

Possibilities of pesticide determination will be studied first on capillary anion-exchange column with conductivity detection. Separation on microbore anion-exchanger with conductivity, UV and amperometric detection will be tested, as well as separation on cation-exchange column with at least conductivity detection. Finally, separation on ion-exclusion and mixed mode stationary phases with adequate detection techniques will be examined.

DETERMINATION OF INORGANIC IONS IN THE OILFIELD WATER BY ION CHROMATOGRAPHY

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Large amounts of oilfield water are produced during the production of crude oil and natural gas. This water has to be analyzed to realize the correct geological formation. Cations and anions are the main interest of this analysis and determination of anions are carried out using ion chromatography (IC). Determinations of anions were done in the past by using gravimetric, volumetric and photometric methods. These methods, unfortunately, analyze only one type of ion per operation. Ion chromatography offers an alternative, method to determine mentioned anions in short time and with lower effort. The fastest and the most accurate analysis could be performed if the instrumental parameters are carefully selected. For that case, full optimization process of ion chromatographic system should be done. Retention modeling and Multy Criteria Decision Making (MCDM) technique to select the IC optimum are mostly required.

In this work, artificial neural network for modeling of gradient retention is combined with several optimizations objective functions and 4 robustness functions in order to find the most appropriate combination to be applied in IC method development. The used multi-criteria were: maximal value of resolution function (objective function), minimal analysis time, maximal retention distance among two nearest neighboring components, and maximal robustness of the used resolution function. Optimal elution method was used and validated for determinations of fluoride, chloride, bromide, nitrate and sulfate in oilfield water analyses.

Keywords: ion chromatography, oilfield water, multy criteria decision making

TEMPORAL AND SPATIAL NITRATE DISTRIBUTION IN AGRICULTURAL SOILS

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Abstract

Excess application of nitrogen fertilizers in agricultural systems leads to nitrate accumulation in deeper soil layers which can result with groundwater contamination. These environmental issues impose the need for better understanding of the role and the fate of nitrogen in soils. Temporal and spatial nitrate distribution was determined at different nitrogen fertilization levels during maize and winter wheat vegetation period. Nitrates in soil extract were separated by Doinex ICS-1000 system. Results indicated that maize and winter wheat influenced soil vertical nitrate distribution differently. Ion chromatography method is a recommended method for nitrate detection in soil extracts because of many advantages that it offers, particularly high rapidity and simultaneous detection.

Keywords: ion chromatography, nitrate distribution, soil

Introduction

Nitrogen (N) is essential for growth and reproduction of all plants. Most soils contain inorganic N in form of nitrate (NO_3^-), nitrite (NO_2^-) and ammonium (NH_4^+). Nitrate form is soluble in water and moves downward with soil moisture, whereas ammonium form is strongly held by the negative charges of clay and soil organic matter colloids. Excessive use of nitrogen fertilizers has a negative influence on soil (nitrogen accumulation) and water quality by nitrate leaching (Kadar and Nemeth, 1993; Zgorelec, 2006; Mesić et al., 2007).

Due to frequent soil sampling in agricultural fertilizers trails there is a need for selective and rapid analytical method for determination of nitrates in soil. Nitrates in soil extracts can be determined by: colorimetric methods, ion selective potentiometry (ISP), i.e. ion selective nitrate electrode (ISE) and ion chromatography (IC) methods. Most methods involve the preparation of extracts and extraction solution depends on the analytical method and the aim of investigation (Mulvaney, 1996). IC offers rapidity, sensitivity, selectivity and simultaneous detection (Weiss, 2000). Several studies involved IC as a method for nitrate determination in soils (Muo et al., 1993; Cruz and Loucao, 2002; Shand et al., 2008).

The aim of this research is to determine temporal and spatial nitrate distribution in agricultural soil under different rates of nitrogen fertilization by means of IC method.

Materials and Methods

This study was carried out in Central Croatia. This area is characterised by continental climate with mean annual temperature of 10.7 °C and annual average rainfall of 865 mm. The experiment had 10 treatments with different nitrogen fertilization levels, whence quantities of phosphorus (P) and potassium (K) were constant for all treatments (120 kg P ha⁻¹ and 180 kg K ha⁻¹). Only four treatments were taken into consideration in this investigation: 1. N₀+P+K,

2. N₁₀₀+P+K, 3. N₂₀₀+P+K, 4. N₃₀₀+P+K (kg N ha⁻¹). Soil sampling was conducted during maize vegetation period (June, 2007) and winter wheat vegetation period (June, 2008). Soil samples were taken from each treatment on the rectangle of 2 m³ in volume (1m x 1m x 2m), at 16 surface points (each 25 cm × 25 cm in size) and at 8 depths (0-25 cm, 25-50 cm, 50-75 cm, 75-100 cm, 100-125 cm, 125-150 cm, 150-175 cm and 175-200 cm). Soil sampling scheme is presented in Figure 1. In total, 1024 single soil samples were taken (128 soil samples per treatment x 4 treatments x 2 soil sampling).

NO₃⁻ content in wet soil samples was extracted in ultra pure water in 1:10 (w/v) ratio according to ÖNORM L 1092 norm. After the extraction, samples were centrifuged and filtrated in two steps: firstly through filter paper and then through cartridge (OnGuard IIP, Icc, Dionex, Sep-Pak Vac and OASIS HLM, Waters) to prevent contamination of the separation column with organic molecules. After that soil extract was decanted into vials and nitrate separation was performed on Doonex ICS-1000 system: separator column [Ion Pac AS 17 (4x250 mm) Dionex]; KOH eluent: (A)10 mmol L⁻¹ KOH, (B) 10 mmol L⁻¹ KOH - 35 mmol L⁻¹ KOH - 10 mmol L⁻¹ KOH in 12.5 min (C) 10 mmol L⁻¹ KOH (total separation time 30 min); flow rate: 1.0 mL min⁻¹; detection: suppressed conductivity.

Statistical analysis: Observed data were subjected to analysis of variance (ANOVA) using SAS Institute 9.1.3 and mean values were separated by Fisher's LSD test at P ≤ 0.05.

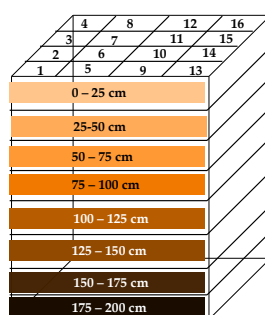


Figure 1. Soil sampling scheme

Results and Discussion

Average N-NO₃⁻ content per treatment and depth is presented in Figure 2. The highest significant differences between nitrate content according to different nitrogen fertilization levels were recorded at the first depth (0-25 cm) during maize vegetation period (Fig. 2.a) and they ranged from 11.8 mg kg⁻¹ in treatment with 0 kg N ha⁻¹ up to 87.8 mg kg⁻¹ in treatment with 300 kg N ha⁻¹. Similar results were observed during winter wheat vegetation period. At the first depth nitrate content varied between 7.1 mg kg⁻¹ in treatment with 0 kg N ha⁻¹ and 80.4 mg kg⁻¹ in treatment with 300 kg N ha⁻¹ (Fig. 2.b). In both investigated years nitrate content decreased with the increase of depth. Application of 100, 200 and 300 kg N ha⁻¹ significantly influenced nitrate status at the first two depths (0-25 cm and 25- 50 cm) during maize vegetation period.

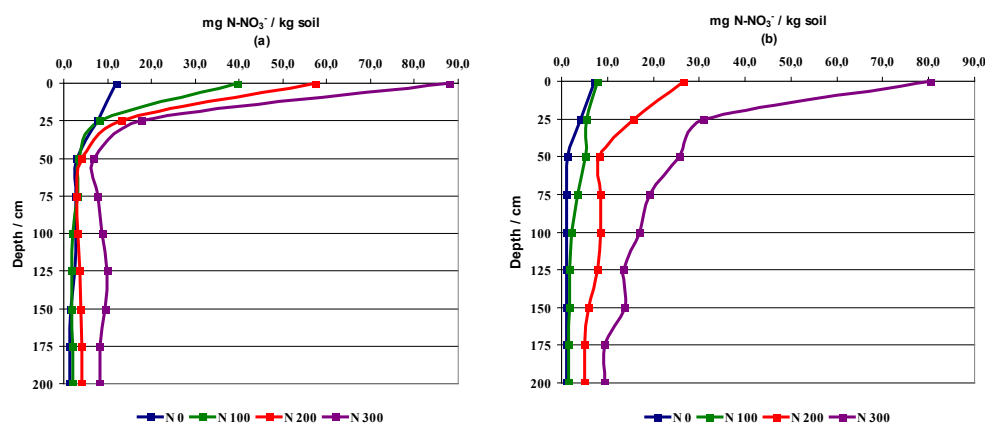


Figure 2. Average N-NO₃ content per N of treatments and depths (a) maize vegetation (2007) and (b) winter wheat vegetation (2008)

During winter wheat vegetation period, treatment with 300 kg N ha⁻¹ significantly influenced vertical nitrate distribution across the whole soil profile (0-200 cm). Many studies all over the world confirm these findings (Kadar and Nemeth, 1993; Alvarez et al., 2007; Guo et al., 2001; Nance et al., 2007).

Conclusion

Ion chromatography method can be applied for nitrate separation from soil extracts, especially because it is a very fast and selective method with simultaneous detection. In investigated soil samples due to simultaneous detection of Doinox ICS-1000 system, thirteen parameters (F⁻, Cl⁻, NO₂⁻, NO₃⁻, Br⁻, SO₄²⁻, PO₄³⁻, Li⁺, Na⁺, K⁺, NH₄⁺, Ca²⁺, Mg²⁺) were separated and quantified. Spatial and temporal distribution was also determined for ammonium and nitrite content. Application of different nitrogen fertilizers levels leads to nitrate accumulation in deeper soil layers. Maize and winter wheat influenced vertical nitrate distribution differently.

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ION CHROMATOGRAPHIC DETERMINATION OF LACTOSE IN FOOD AND FEED

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Lactose is a disaccharide sugar that is found most notably in milk and is formed from galactose and glucose. Lactose or milk sugar occurs in the milk of mammals in range from 4 to 6% for cow's milk and from 5 to 8% in human milk. It is also a by-product in the manufacture of cheese.

Lactose intolerance is the inability to digest significant amounts of lactose, the predominant sugar of milk. This inability results from a shortage of the enzyme lactase, which is normally produced by the cells that line the small intestine. Lactase breaks down the lactose, milk sugar, into glucose and galactose that can then be absorbed into the bloodstream [1].

If there is not enough lactase to digest the amount of lactose consumed, some uncomfortable symptoms develop. Some adults have low levels of lactase. This leads to lactose intolerance. The ingested lactose is not absorbed in the small intestine, but instead is fermented by bacteria in the large intestine, producing uncomfortable volumes of carbon dioxide gas.

While not all persons deficient in lactase have symptoms, those who do are considered to be lactose intolerant [1].

With alkaline-tolerant polymeric columns, separation of carbohydrates is possible using anion-exchange chromatography.

The retention time of carbohydrates is inversely correlated with pKa value (carbohydrates are weak acids with pKa values ranging between 12 and 14) and increases significantly with molecular weight. The elution order of carbohydrates on such anion exchange columns is usually as follows: sugar alcohols elute first, followed by mono-, di-, tri-, and higher oligosaccharides.

The retention can be controlled by the concentration of sodium hydroxide. An increase of the sodium hydroxide concentration $[OH^-]$ has a dual effect on the retention of carbohydrates. The increase in ionic strength of the eluent causes a decrease in analyte retention, while the higher pH will increase the degree of dissociation resulting in an increase in analyte retention. If the $pH > pK_a$ (full dissociation) then the ionic strength will dominate the separation process and the retention decreases [2].

Determination of lactose in food and feed was done by IC DX500 system with pulsed amperometric detection (PAD). Separation and identification of lactose was done with analytical column CarboPac PA10 and guard column PA10. Mobile phase was 300mM NaOH flowing under isocratic elution. Sodium hydroxide gradients are not recommended because background current in PAD is sensitive towards changes in pH, resulting in a poor baseline stability. For the determination of lactose in food and feed, samples were diluted with ultra pure water, filtered through 0.2 μ m membrane filter, injected, and detected directly at a gold electrode following separation.

Validation of method was done in accordance with the European Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results.

For all of the samples, recoveries were from 85% to 101% and the relative standard deviations (RSDs) were smaller than 2.5%.

Limit of quantification of method is 0.01%.

SP Laboratory analyzed the samples of cheese, yoghurt, milk and other milk products, substitute milk for pigs and calves feeding, which were lactose-free and with higher content of lactose.

Determination of lactose was also performed in native whey, which is used in food for physically active individuals to strengthen and increase muscle mass, to prevent osteoporosis, strengthen immunity, reduce stress and it is very important because it regenerates liver, so it is essential to know the content of lactose for people who are lactose intolerant.

Content of lactose in lactose-free products in most cases was below 0,01% but some products contained up to 0,02% and in products with higher content of lactose concentrations were from 3% to 56%. EU and Serbian legislation haven't defined maximum concentration of lactose in free-lactose products.

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ESSENTIALS OF LABORATORY MANAGEMENT IN GLP AND TQM ENVIRONMENT

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The analytical laboratory is very dynamic and progressive environment. Goals and expectation are raised daily and independently whether it is about a university laboratory, privately owned research organization operating under TQM (Total Quality Management) regime or quality control laboratory in a pharmaceutical company under GMP (Good Manufacturing Practice) compliance.

There are many tools nowadays that are supporting laboratory management and facilitate modern laboratory operations. Mainly software based solutions, known as laboratory information systems, are set up to optimize laboratory operations. Composed of electronic lab notebooks, sample management, data acquisition, data processing, reporting and scientific data management; they include workflow and data tracking support, flexible architecture, and smart data exchange interfaces, which fully support its use in regulated environments.

The application of modern technologies in today's laboratories is required to achieve timely progress and remain competitive.

However, skills like effective planning, active participation, timely decision making, good knowledge of human resources and finally solid finance handling are main frame in managing laboratories. Together with information systems and laboratory automation they constitute complement system. Finally, laboratory managers who can create solutions to problems and effectively manage change and improvements through process optimization are still in high demand.

10TH PROFICIENCY TEST: CRITICAL REVIEW

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Proficiency testing is the use of inter-laboratory comparisons to determine the performance of individual laboratories for specific tests or measurements and to monitor a laboratory's performance. Participation in proficiency testing schemes provides laboratories with an objective means of assessing and demonstrating the reliability of the data they are producing. The most commonly used evaluation tool in proficiency testing is z- score. A z- score is the ratio of the bias estimate and the target value for the standard deviation of the results. Based on an adequate estimator for the standard deviation, the absolute values of the z-scores, which are standardized, allow a direct estimation of the performance of the laboratory by comparison with the following quality limits:

- $|z| < 2$: the performance of the laboratory is considered satisfactory.
- $2 < |z| < 3$: the performance of the laboratory is considered questionable.
- $|z| > 3$: the performance of the laboratory is considered unsatisfactory.

Seven laboratories provided the results of analysis of sample that has been delivered during 11th ISIC. The data were analyzed and the final report was sent back to the participants. It comprised a description of the preparation of the material and the test for homogeneity, an anonymous (coded) list of all valid data and the statistical results. The z- scores were expressed graphically.

**CURRENT TRENDS IN LC –
UNDERSTANDING THE SCIENCE OF ULTRA PERFORMANCE LC
TECHNOLOGY**

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In 2004, separation science was revolutionized with the introduction of Ultra Performance Liquid Chromatography UPLC[®]. Significant advances in instrumentation and column technology were made to achieve dramatic increases in resolution, speed and sensitivity in liquid chromatography.

For the first time, a holistic approach involving simultaneous innovation in particle technology and instrument design was endeavored to meet and overcome the challenges of the analytical laboratory. This was done to make analytical scientists more successful and businesses more profitable and productive.

There are a number of contributing factors achieving high-resolution UPLC separations. System design must be considered in order to achieve true UPLC performance. Although operating pressure and system volume are enablers of UPLC, the ultimate performance is only realized when system dispersion is optimized to match the resolving power of the chromatographic column.

DETERMINATION OF ACTIVE PHARMACEUTICAL INGREDIENTS (APIs) AND COUNTERIONS – A COMPARISON BETWEEN HILIC AND MULTI-MODE CHROMATOGRAPHY

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Determination of active pharmaceutical ingredients (APIs) and counterions are important assays in pharmaceutical drug development. Due to the wide variety of ionizable substituents and hydrophobicities of these pharmaceutical-related molecules, it is challenging to simultaneously separate APIs and respective counterions on reversed-phase columns. This is because many counterions such as sodium and chloride ions cannot be retained on these columns without the addition of ion-pair reagents.

HILIC is a technique that is complementary to RPLC with several benefits, including retention of polar analytes that cannot be retained on RPLC columns, improved compatibility with electrospray LC-MS for good sensitivity, and simplification of sample preparation by eliminating the need for evaporation and reconstitution of a sample when dissolved in a non-aqueous solvent. Consequently, HILIC has been used for analyzing APIs and counterions simultaneously. However, despite its suitability to separate highly polar molecules, HILIC fails to adequately retain more strongly hydrophobic APIs. Moreover, HILIC requires an organic solvent-rich mobile phase, which is undesirable from cost and environmental-sustainability standpoints. While HILIC is often useful for hydrophilicity-based class separation, its flexibility in method development is limited.

Here, the author presents an alternative to HILIC for API and counterion assays, which addresses aforementioned issues with HILIC. The proposed approach involves a mixed-mode chromatography column, the Acclaim Trinity, which provides cation exchange, anion exchange, and reversed-phase interactions. The new approach is suited to the simultaneous determination of APIs and counterions regardless of their hydrophobicity, provides better control of selectivity in method development, and does not require a high concentration of organic solvent in the mobile phase. In all pharmaceutical applications described here, a popular and heavily promoted HILIC column, the ZIC-HILIC column, was used for comparison purpose.

AGILENT 1290 INFINITY LC – INFINITELY MORE POWERFUL

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With more than enough power to handle any HPLC or UHPLC challenge, the advanced technology in the Agilent 1290 Infinity LC provides you with access to virtually limitless separation, detection, automation and throughput possibilities. The Intelligent System Emulation Technology (ISET) harnesses the wide power range and the superior accuracy and performance of the Agilent 1290 Infinity LC to emulate other systems for seamless transfer of methods between LCs, regardless of brand. It makes the 1290 Infinity LC the world's first truly universal LC system as it can execute other HPLC and UHPLC methods and deliver the same chromatographic results without any change of the instrument or the original method.

HPLC TROUBLESHOOTING – A PRACTICAL APPROACH

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HPLC is an analytical technique, which has now found its way in many research and control laboratories. Yet its operation is not as straightforward as some other analytical techniques. Proper care should be exercised when operating it, and, when unexpected results appear, problem sources should be located and removed in shortest possible time. Authors will present efficient methods of diagnosing and solving the most frequent problems in HPLC and suggest ways how to prevent those problems from happening again. Also, their “Seven Basic rules of Troubleshooting” will be presented.

Majority of problems in HPLC are related to pressure, leaking, analytical equipment (pump, autosampler), columns and problems in separation (bad separation, unsuitable peak shape) or quantification and quality of data.

In any troubleshooting, proper workflow should be: 1) Early identification of the problem, 2) Quick isolation 3) Quick correction and 4) Learn for future. Before trying to solve the problem, operator should try to locate the problem, document symptoms that describe it best, consider possible causes of the problem and select tests, which can help find the cause of the problem.

Some practical examples of HPLC troubleshooting will be presented, such as importance of instrument volumes and band spreading, proper connecting tubings and fittings, reasons why all gradients are not the same, causes of extraneous peaks, problems related to samples and sample vials and column-related problems.

Finally, authors will discuss how to prevent problems in HPLC from happening in the first place, how to make simple and effective rules for successful HPLC usage, with suggested steps and measures that could be taken on HPLC system, with personnel, laboratory organisation, laboratory cleanliness and by introducing both quality system and innovative climate into the laboratory.

UPLC-MS IN DRUG DISCOVERY

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Today's objective of drug discovery process is continuous seeking for new ways of increasing productivity and decreasing costs of developing new therapies. Therefore, the pharmaceutical industry is significantly driven by new technologies which could speed up process and decrease costs of drug discovery.

Each stage of the drug discovery is full of examples using many new technologies, but undoubtedly the most important and widely implemented analytical techniques is liquid chromatography - mass spectrometry (LC-MS). The revolution started in the mid 1980s with development of atmospheric pressure ionization techniques. However with recent advances in sensitivity, throughput and automation, liquid chromatography - mass spectrometry became the primary analytical platform throughout pharmaceutical research and development [1,2].

In early phase of drug discovery, there has been constant focus on high-speed chromatographic separations in order to reduce analysis time and maintain good efficiency. The commercially available technique of ultra performance liquid chromatography (UPLC) has proven to be of the most promising development in the area of fast chromatographic separations [3,4].

UPLC utilizes short, small diameter columns packed with sub-2 μm particles as stationary phase used in very high-pressure conditions (up to 1000 bar). The use of these very small particles allows a drastic improvement of the resolution per time unit, because chromatographic efficiency and optimal mobile phase velocity are both inversely proportional to the particle size. Due to high efficiency of sub-2 μm particles, the column length can be decreased to obtain equivalent resolution in a reduced analysis time.

Therefore, UPLC-MS separations have been implemented and almost substituted conventional HPLC-MS systems for the analysis of huge number of samples within all steps in drug discovery. The results obtained by UPLC-MS showed not only an improvement in sensitivity and resolution but also the improvement in total analysis time and solvent consumption. For that reason, the use of UPLC-MS in the early phase of drug discovery in our laboratories will be presented.

Macrolides are compounds which show excellent antibacterial activity and good bioavailability. However due to size and structure of macrolides the monitoring and identification of such molecules need an additional attention.

We have explored the differences in LC-MS performance by comparison of the UPLC method as an alternative to HPLC for the analysis of macrolide compounds and separation of isomers of macrolides. The identification of macrolide isomers with a significant improvement of the resolution and a significant reduction of the separation time will be discussed. Overall, the improvement in LC-MS method sensitivity, speed, and resolution provided by UPLC confirms that this technique is very valuable and suitable for the analysis of macrolides.

The additional advantage of using rapid UPLC-MS methods is in the analysis of compound libraries. Namely, the time of analysis of around 1.5 min/sample allows a throughput of samples obtained from a parallel synthesis and compound libraries.

We have established UPLC-MS systems in our laboratories providing a drastic increase in capacity for medicinal chemistry. That new implementation allows routine analysis of >400 samples/day per UPLC-MS open access system with gradient time of 1.5 min and cycle time of 2.0 min per analysis. High quality results, chromatograms, as well as spectra, are obtained by injection of 2 μ L of about 0.1 mg/mL sample concentration.

Further step forward was made with the implementation of open access UPLC-MS as a pre-QC analysis prior to preparative HPLC analysis. Depending on a retention time of a target compound on an open access UPLC-MS system, the focused gradient method can be selected on mass directed autopurification system without a need for a HPLC method development. Both systems operate in complementary conditions. Namely, the identical acidic and basic conditions on both systems allow medicinal chemists easy use of UPLC and prepHPLC systems, both operating in open access mode. We have proven that the transfer of UPLC-MS method to mass directed preparative HPLC method is simple and reliable.

Few years ago UPLC was used to determine compound lipophilicity [5]. This system emerges as a powerful method suitable for high-throughput physicochemical property measurements of therapeutic compounds. Recently, we have investigated the possibility of use RP-UPLC as a HT tool for determination of chromatographic hydrophobic index. This physicochemical parameter was determined for a set of coumarines. The results obtained on RP-UPLC-MS showed a great correlation with RP-HPLC measurements of hydrophobic index for the same set of coumarines. The application of this technique speeds up process of determination of physicochemical properties for about 4 times and also drastically decreases the solvent consumption.

The use of the open access facility has increased the overall sample throughput by 60% in above mentioned areas and even more opportunities are ahead...

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DETERMINATION OF IMPURITIES BY HPLC IN PHARMACEUTICAL APPLICATIONS

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Impurities in pharmaceuticals are the unwanted chemicals which even in small amounts may influence the efficacy and safety of the pharmaceutical products. As such their presence is strictly monitored in all stages of drug development and production which includes synthesis of active pharmaceutical substance, making of final formulation product, and as a part of stability testing for shelf life determination and confirmation.

ICH (International conference on harmonization) has issued detailed guidelines (Q3A(R2), Q3B(R2), Q3C(R5) and Q3D) regarding their classification, reporting threshold limits, control using appropriate analytical methods and qualification. This guidelines are in correlation with those of US and EU pharmacopoeia and are well adopted by their regulatory bodies (FDA, EMEA).

Impurities can be divided into three classes:

- Organic impurities (process and drug related)
- Inorganic impurities
- Residual solvents

Liquid chromatography is mainly used for determination of organic compounds and plays a critical role in analysis of pharmaceutical product.

Each HPLC method used undergoes three stages; development, validation and QC practice. Development stage comprises of setting purpose and goal of method, selection of appropriate mobile phase, column, detection, sample preparation, definition of instrumental parameters and all other details considered necessary.

Validation covers wide range of different experiments which purpose is to demonstrate that the method is suitable for its intended use. This process should be conducted in line with ICH Q2(R1) guideline. Usually, during validation stage, slight modification of method occurs.

Finally new developments in LC as a technique, like columns with smaller particles, different kind of stationary phases, instruments with innovative and better capabilities offer and ask to further redesign present methods in order to achieve better insight in impurity profiles using less time and resources.

**DEVELOPMENT OF MULTI – RESIDUE ANALYTICAL METHOD
USING LIQUID CHROMATOGRAPHY TANDEM MASS
SPECTROMETRY FOR PHARMACEUTICALS DETERMINATION IN
WATER SAMPLES**

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Pharmaceuticals cover a wide range of different compounds and are used to cure disease and fight infection both in humans and in animals. Their occurrence in the environment has become an issue of great importance in the last decade. Because of their wide consumption they have been used in large quantities for years and for the last few decades they have been investigated as a new type of so called emerging contaminants. In the environment they can be found in low concentrations of $\mu\text{g/L}$ or ng/L but their continuous input can pose a potential long term risk for aquatic and terrestrial organisms as well as for human health. [1]

To detect and quantify pharmaceuticals in the environment is very complicated because the matrices in which they occur are complex, they have very diverse physicochemical properties and they are found in very low concentrations. Therefore it is of great importance to develop analytical methods that will be highly sensitive and selective in order to monitor pharmaceuticals in the environment. [2] Liquid chromatography coupled to mass spectrometry especially multidimensional mass spectrometry (LC-MSⁿ) is a powerful technique which can provide adequate specificity and sensitivity. [3]

The aim of this work was to develop a SPE-HPLC-ESI(+)-MS/MS analytical method for determination pharmaceuticals in wastewater. Investigated pharmaceuticals belong to different classes of antimicrobials: sulfonamides (sulfaguanidine, sulfadiazine, sulfamethazine and sulfametoxazole), a sulfonamide synergist from diaminopyrimidine class (trimethoprim), fluoroquinolones (ciprofloxacin, enrofloxacin and norfloxacin), macrolides (azithromycin, erythromycin, clarithromycin, roxythromycin, tylosin, azahomoerithromycin), tetracyclines (oxytetracycline, tetracycline), antihelmintics (febantel, levamisol, praziquantel), anesthetics (lidocain, procain), a steroid hormone (dexametazone) and antibiotic (tiamulin).

Sample preparation was done by solid phase extraction (SPE) and the pharmaceuticals were extracted from water using optimized SPE system. Chromatographic separation was done on HPLC – MS/MS Agilent 1200 and the detection on Agilent 6410 triple quadrupole mass spectrometer equipped with electrospray ionization (ESI). Mobile phase was 0.1% formic acid in water and 0.1% formic acid in acetonitrile in gradient mode and the chromatographic column was phenyl Synergi Polar 2,5 μm , 100 x 2.0 mm (Phenomenex)

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APPLICATION OF LC-MS IN DETERMINATION OF AQUEOUS PHOTOTRANSFORMATION PRODUCTS OF VETERINARY PHARMACEUTICALS

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One of the biggest goals of today's society is keeping the environment clean from so-called emerging contaminants. These contaminants do not necessarily need to be new, but their environmental fate and ecotoxicological study have not yet been evaluated which means they could be potential threat to environmental ecosystem. They comprise a wide range of compounds including human and veterinary pharmaceuticals, as well as their transformation products (TPs). Pharmaceuticals can reach environment as unchanged parent compound or a metabolite and once they are released into the environment are exposed to variety of surrounding conditions which can cause their transformation. One of the most important processes responsible for the transformation of such compounds is phototransformation by sunlight. This process is usually complex with more than one reaction often leading to multiple products and possible increase of toxic effect to organisms in environment. Therefore the identification of transformation products and the elucidation of photolytic reaction pathways in environment are of great importance.

Advanced instrumental methods for detecting traces of such compounds and identifying their structure are being developed and getting improved by each day. Liquid chromatography/mass spectrometry (LC-MS), because of its selectivity and sensitivity and its suitability for compounds with a wide range of masses and polarities, is one of the most powerful methods for performing the identification of the arisen products. One possible way of determination of the accurate mass and empirical formula is using orthogonal acceleration time-of-flight (oaTOF) analyzers.

The problem of the veterinary pharmaceuticals is much less elaborated than one of the human pharmaceuticals but is as the same importance. As the anthelmintics, according to The International Federation for Animal Health (IFAH), encompass the largest sector of the animal pharmaceutical industry by volume and value, they are the main topic of this work. The phototransformation of chosen anthelmintics were studied on aqueous samples in simulated environmental conditions. Analyses were performed on LC-MS² (QqQ) and for the structural identification of the formed products a quadrupole-time of flight (QqTOF) mass spectrometer was used providing an accurate mass. Combination of those two highly prompt instruments showed to be a powerful solution in the identification of the unknown compounds.

Keywords: anthelmintics, phototransformation products, LC-MS

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POSTER PRESENTATIONS

FREE PHENOLIC ACIDS IN *MICROMERIA CROATICA* (PERS.) SCHOTT MEASURED BY UPLC-MS/MS

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Croatia harbors about 500 endemic and subendemic species out of the total number of 5000 registered plants. The phytochemical profiles of most of these species have not yet been described. One of them is *Micromeria croatica* (Pers.) Schott., an endemic Illyric-Balkan species from genus *Micromeria* Benth. (family *Lamiaceae* Lindl.) known in Balkan ethnopharmacology for its multiple uses in traditional medicine. Traditional medicinal plants are especially rich sources of polyphenols and medicinal plant extracts are effective in treating various ailments caused by oxidative stress and free radicals, bacterial and/or viral infections. Phenolic acids are an important group of polyphenolic compounds which play important roles in plant resistance to pathogens and herbivores, allelopathy, oxidative stress and plant growth regulation. These compounds have also been shown to exert beneficial effects on human health. The research presented here was aimed at characterizing the free phenolic acid composition using UPLC-MS/MS as well as total phenol (TP) and total flavonoid (TF) contents using spectrophotometric methods in methanol extracts of *M. Croatica* flowers, stems and leaves from three mountainous localities in Croatia (Bačić kuk, Stupačinovo and Bojinac).

The highest TP content was determined for leaf extracts from Bačić kuk (29.74 ± 1.05 mg GAE/g dw) while the flower extract from Bačić kuk (27.67 ± 2.25 mg CE/g dw) showed the highest TF content followed by the leaf extracts from Bojinac (26.42 ± 1.93 mg CE/g dw) and Bačić kuk (26.19 ± 0.03 mg CE/g dw). Twelve free phenolic acids were quantified in flower, stem and leaf extracts of *M. croatica* collected at different localities. Free gallic, 3-hydroxybenzoic, chlorogenic, vanillic and sinapic acids were not detected in extracts. Caffeic acid was found only in the stem extract from Stupačinovo (103.3 ± 17.3 $\mu\text{mol/g dw}$), ferulic acid in the stem extracts from Stupačinovo (26.1 ± 3.9 $\mu\text{mol/g dw}$) and Bojinac (23.3 ± 3.1 $\mu\text{mol/g dw}$), and syringic acid in the stem extracts from Stupačinovo (83.8 ± 3.9 $\mu\text{mol/g dw}$), Bojinac (66.6 ± 6.3 $\mu\text{mol/g dw}$) and Bačić kuk (80.5 ± 7.1 $\mu\text{mol/g dw}$). Overall, the stem (166.33 ± 38.26 $\mu\text{mol/g dw}$) extracts contained significantly higher average content of free salicylic acid compared to the flower (86.40 ± 17.59 $\mu\text{mol/g dw}$) and leaf extracts (60.67 ± 6.89 $\mu\text{mol/g dw}$).

The present study reports for the first time the free phenolic acid content of different plant parts of endemic plant *Micromeria croatica* (Pers.) Schott. According to these results *M. croatica* is a rich source of potent phenolic antioxidants including flavonoids and phenolic acids. Significantly higher TP and TF contents were found in leaves and flowers in comparison to stems of *Micromeria croatica* (Pers.) Schott, while significantly higher content of salicylic acid - phenolic acid used in treatment of pain and inflammation - were found in stem extracts.

DETERMINATION OF NATURAL MOISTURIZING FACTOR IN HUMAN *STRATUM CORNEUM* BY LIQUID CHROMATOGRAPHY

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Skin is the largest human organ and its vital role is to protect body from the environmental effects. *Stratum corneum*, the outermost layer of the skin, is the primary barrier preventing body from water loss and ingress of pathogens and allergens. Filaggrin (filament-aggregating protein) is a key structural protein required for proper epidermal barrier function responsible for the mechanical strength and integrity of the *stratum corneum*. Filaggrin is proteolytically degraded into pool of free amino acids including histidine and glutamine which are further degraded into 2-pyrrolidone-5-carboxylic acid and urocanic acid. Approximately 50% of natural moisturizing factor (NMF), retained in matured corneocytes of the *stratum corneum*, is made of free amino acids and their derivatives. NMF is found to have substantial part in regulating hydration of the *stratum corneum*, pH of the skin, lipid synthesis, diffusion permeability etc.

We developed a simple RP-HPLC method using UV detection for determination of free amino acids and their metabolites in the *stratum corneum* which comprise more than half of total NMF including 2-pyrrolidone-5-carboxylic acid, histidine, tyrosine, trans- and cis-urocanic acid, phenylalanine and biogenic amine, histamine. The method is based on non-invasive the *stratum corneum* detachment by tape stripping and subsequent extraction of tape strips in concentrated ammonia. After evaporation of ammonia, the residue is reconstituted in deionized water, filtered and injected into HPLC. The advantage of this method is omitting the use of non-volatile solvent for extraction and derivatization procedure. The method showed to be reproducible and sensitive enough to determine above mentioned NMF components in the *stratum corneum* of normal skin and skin of atopic dermatitis patients.

Keywords: natural moisturizing factor, atopic dermatitis, filaggrin, histamine, liquid chromatography

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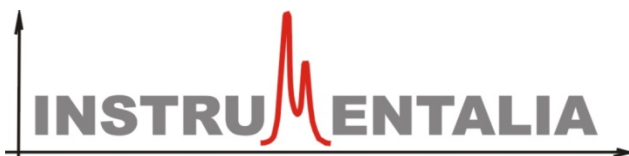


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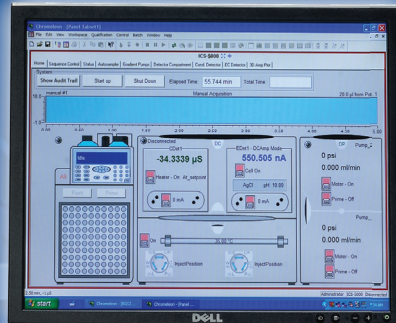
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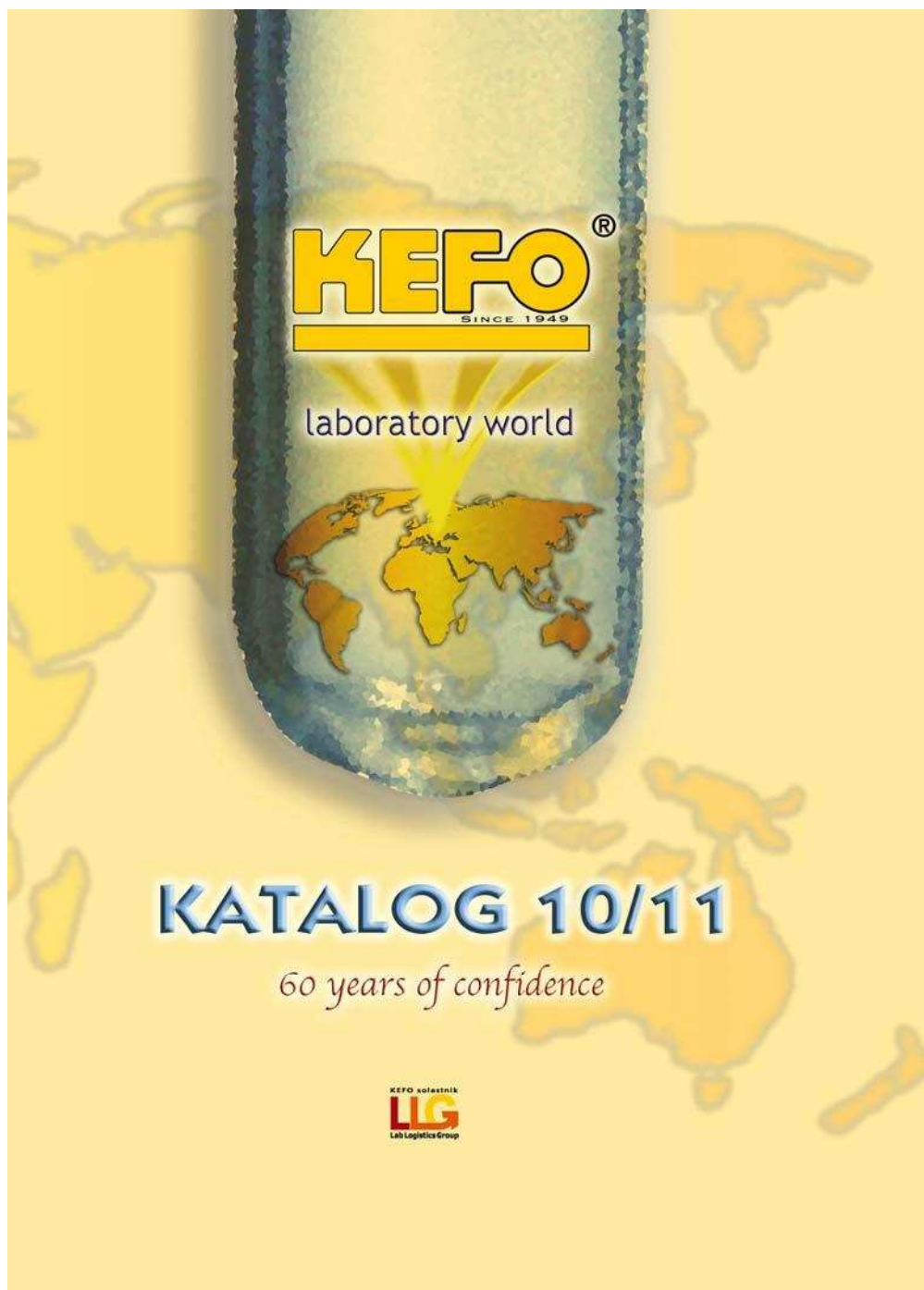


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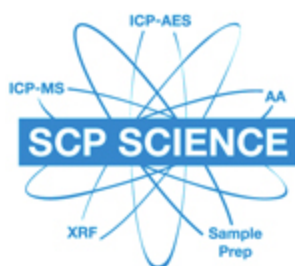


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