POSTER SESSION I

WEDNESDAY, MAY 24th, 2017

CHAIRPERSONS:

Marcin Chutkowski

and Grzegorz Jóźwiak

1.

Thin-layer chromatographic identification and quantification of anthocyanins in food products

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Anthocyanins constitute a group of flavonoids which is widely distributed in vascular plants. Anthocyanin dyes are classified as so-called natural non-food vegetable substances soluble in water. They give flowers and fruits intense colours, from orange through various shades of red and violet to black. Up to now, hundreds of natural anthocyanin dyes have been discovered and many of them are synthetically produced [1].

Anthocyanins have an antioxidant effects, they have the ability to catch free radicals. They counteract the fragility of blood vessels, mainly the capillaries and stimulate the production of rhodopsin - a substance important in the process of vision. Moreover, they lower the rate of oxidation reaction of LDL cholesterol, which is a component of atherosclerotic plaques [2]. The intense colour and health benefits of anthocyanins make them very popular in the food and cosmetics industries. In the food industry, anthocyanins are used to color beverages, juices, yoghurts, jams, sweets or wines [1, 3]. They are labeled as E163 on the list of food additives [4]. Anthocyanin dyes are used as indicators for assessment the quality of colour food. They also protect food products from spoilage, which is related to the antagonistic activity of these dyes against certain bacteria, viruses and fungi [1].

The purpose the studies was to develop a method for the identification and quantification of selected anthocyanins in food products. The standards were cyanin chloride, keracyanin chloride, pelargonidin chloride and delphinidin chloride. The presence of the above compounds was studied in juices (the commercial and home-made), syrups, nectars and non-carbonated drinks. The studies were conducted by thin - layer chromatography. The results of the TLC were densitometrically evaluated with use of the CD-60 model scanning densitometer. Spectrophotometric measurements were performed with use of a Varian Cary Eclipse Fluorescence Spectrophotometer.

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2.

Thin-layer chromatographic identification of amino acids in spider web

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Spider web is among the strongest biological materials and it is noteworthy that in proportion to the spider's weight, it is more durable than steel or Kevlar. The purpose of our study was the amino acid analysis of *Steatoda grossa* (Theridiidae) spider silk. Natural spider silk has unique properties such, as stretchability, durability or biocompatibility [1]. The main components of the thread are proteins, so it is important to know an exact chemical composition to better understand the properties of the thread. Moreover, due to its mechanical properties, spider silk seems a promising candidate material in technology and medicine. Knowledge of composition and structure of the spider web could help devise biocompatible coatings or microcapsules in which active substances of drugs could be contained.

Spider webs make an interesting research subject, also in the ecotoxicological context. Until now, it has not been specified whether and to what extent metals ingested with food alter the processes proceeding in the silk glands and if such changes could consequently influence chemical properties of the spun web threads. In this study, a simple food chain model: medium with cadmium → Drosophila hydei flies → females of the synanthropic *Steatoda grossa* spider, was used to investigate whether and to what extent metal, ingested with food, alters the amino acid composition of the spun web threads produced by the examined species. Three experimental groups were distinguished: control (C), spiders exposed to cadmium for four weeks (4-Cd), and spiders exposed to metal for one year (L-Cd).

In order to obtain the monomeric amino acids, the spider silk was preliminarily subjected to the acidic hydrolysis by means of hydrochloric acid at 110℃ for the period lasting 20 hours. The thin-layer chromatographic separation of amino acids was carried out on the silica gel pre-coated chromatographic plates, using the acetone + butanol + acetic acid + water mixture, 7: 7: 2: 4 (v/v/v/v). The development of the chromatograms lasted 4 h and the plates were visualized with the 0.5% ninhydrin solution.

The research was aimed at qualitative and quantitative determination of individual amino acids in spider web, depending on the presence (or otherwise) of Cd in the spider diet. The following amino acids were identified: alanine, glycine, glutamic acid, serine, cysteine, methionine, proline, threonine, isoleucine, arginine, leucine, phenylalanine, histidine and aspartic acid. Quantification of amino acid was performed with use of densitometry and the adequate results were presented.

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3.

Identification and quantification of polyphenol compounds in lavender and thyme honeys using high-performance liquid chromatography with diode array detection and electrospray ionization mass spectrometry

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Honey, a natural product prepared from the nectar of various plants, is gathered, modified and stored in honey combs by honeybees (*Apis mellifera* L.). Honey is official in several pharmacopoeias (e.g. *Mel*, Ph. Eur. 5.1), valued for its antimicrobial and antioxidant activity, which can be largely attributed to its polyphenol content. Additional beneficial properties may characterize unifloral honeys that contain further specific compounds beside the usual sugar components and phenolic substances. Some compounds are unique to a certain type of unifloral honey, and may serve as markers that can be used for identification purposes and preventing honey adulteration.

The current study aimed at determining the major polyphenolic compounds in lavender and thyme honeys produced in Hungary, Croatia, France and Spain.

A sensitive method coupling high-performance liquid chromatography with diode-array detector and electrospray ionization mass spectrometry was optimized for the separation and identification of polyphenolic compounds. The novel method was successfully applied to quantify the polyphenols in lavender and thyme honeys after a simple sample preparation step. Separation was performed on a new generation of core-shell particle packed column (Sunshell C18 column; 30×2.1 mm, 2.6 μm, ChromaNik Technologies Inc, Japan). Fragmentation behavior of polyphenolic compounds was investigated using ion trap mass spectrometry in negative electrospray ionization. The MS, MS^n and UV data together with HPLC retention time of polyphenols allowed structural characterization of these compounds. Several validation parameters (repeatability and intermediate precision, LOD, LOQ, calibration range, and recovery) have been calculated in the developed method.

Our investigations confirmed that the above chromatographic techniques can be applied successfully for detecting marker compounds in honey samples and can be useful tools in checking the botanical origin of unifloral honeys.

4.

Chromatographic comparison of biological activity of essential oil and two *Acorus calamus* rhizome extracts using TLC with direct bioautography as detection method

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Acorus calamus, also called as sweet flag or calamus (in Poland - ajer or tatar herb) is a perennial, monocotyledon of the Acoraceae family in the genus Acorus. Herb grows in wetlands and marshlands - edges of small lakes, ponds and rivers, is native to India, central Asia, southern Russia and Siberia, and in Eastern Europe. Calamus was introduced into Europe and North America for its medicinal purposes, dried and shredded rhizoma is used as medical raw material. Biological properties of calamus rhizome extracts and essential oil: digestive - bitter substances (acorin), slightly sedative (azarone) are often reported. Preparations for external use has an antifungal and antimicrobal properties and more minor meaning activities.

Isolation of biological active compounds or groups of compounds from natural extracts allows using concentrated preparations, focused on their specific properties. Preparative TLC seems to be a good technique for isolation small quantities of interesting metabolites (groups of metabolites) from raw material as herbal extract.

Various biological methods of detection allow to fish out compounds of defined activity to find pharmacologically active drug candidates from its mixture in herbal extracts. The separation of active compounds (fractions) from *Acorus calamus* rhizoma is especially important because of content of acorin in the material, substance which is toxic for human and animals.

In our work aqueous and methanolic extracts and distilled essential oil from the plant were examined. Aqueous extract was obtained from shredded and dried rhizome by use of percolation method after prior ultrasonic accelerated maceration. To obtain methanolic extract the raw material was continuously extracted in Soxhlet apparatus. Essential oil was distilled with steam in Deryng apparatus. The TLC systems with silica layer and multicomponent eluent composed of organic solvents were used for separation of extracts and oil. Chromatographic systems were found in literature and used after small modification of solvent content and developing method.

The direct bioautography method were used for preliminary evaluation of antimicrobial properties of extract fractions. Separated extracts and oil were examined with *Bacillus subtilis* colony test. Test results will be used to TLC preparative isolation of fractions and for identification of compounds (groups of compounds) which will be responsible for bactericidal properties.

5.

Lipophilicity of selected terephthalamides

Violetta Kozik, Andrzej Bąk, Krystyna Jarzembek, Paulina Dybał, Danuta Pentak, Barbara Hachuła, Marcin Rojkiewicz, Aleksandra Świetlicka, Dominika Bożek, Joanna Kozłowska, Piotr Kuś

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The chromatographic data were determined for the investigated set of new amides derivatives by RP-TLC method and related with theoretical partition coefficient calculated by means of *in-silico* procedures. Statistically, significant correlation was found between experimental R_{MO} values and the quantitative descriptor of lipophilicity (logP) specified by OSIRIS and Sybyl predictors. The impact of the calculated physicochemical and structural descriptors on the retention parameters was elucidated by variable elimination procedure IVE-PLS, indicating the involvement of various factors on hydrophobic forces.

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6.

Determination of selected pollutants in fuel samples.

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The presence of sulfur in the fuel exerts an adverse effect on the physical state of the combustion engine, but is also undesirable for ecological reasons. Running the engine emits exhaust gases into the environment. In case of high sulfur content in the fuel, more toxic sulfur (IV) oxides are emitted into the atmosphere. Oxides contribute to smog, threatening the environment, irritating the respiratory tract. It can cause not only allergic reactions but also endangering human life and health. Due to serious environmental risks, the monitoring of sulfur content in fuels is very important. Determination of the sulfur content of liquid fuels for vehicles has been carried out in accordance with the applicable national standard, ISO / IEC 20846: 2011 based on international standard ISO 20846. In addition, the content of solid impurities in fuels was determined. More than 200 fuel samples were tested.

Literature:

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7.

Determination of the lipophilicity of selected furanosteroids

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Viridin and wortmannin belong to the small group of naturally occurring furanosteroids. The compounds have been widely known as potent inhibitors of the lipid kinase PI-3K. They show also anti-proliferative effects against a breast cancer line [1,2].
Different physicochemical parameters, such as lipophilicity are useful in the prediction of action, toxicity, metabolism, transport, pharmacokinetic and protein binding of various drug substances including described furanosteroids [3]. Because there is a lack in available literature the experimental value of lipophilicity parameter of both furanosteroids, the main aim of this work was to use thin-layer chromatographic technique in reversed phase system (RP-TLC) to predict the experimental value of lipophilicity parameter for viridin and wortmannin. Chromatographic lipophilicity parameter (R_{MW}) of examined furanosteroids have been studied under different conditions; various chromatographic plates for RP-TLC and different mobile phases like methanol-water, dioxane-water and acetonitrile-water. All chromatographic lipophilicity parameters (R_{MW}) obtained for two studied compounds accordance with Soczewiński-Wachtmeister equation were compared with the theoretical partition coefficients calculated by different computing programs: AlogPs, AClogP, AlogP, MlogP, xlogP2 and xlogP3 [4]. In addition to this, R_{MW} values determined for these drug substances were correlated with their binding energy with human proteins (i.e. human serum albumin HAS and also with sex hormone-binding globulin SHGB) obtained using docking software.

Our study confirms the usefulness of thin-layer chromatographic technique and calculation software in the predication of lipophilicity and binding energy with human proteins of examined furanosteroids.

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8.

Synthesis of crown thiaethers – potential complexing agents for transition metals

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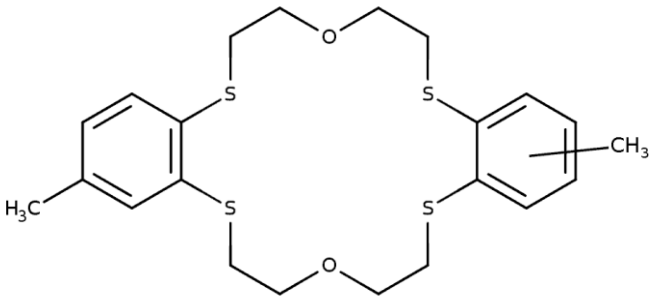
Thiacrown ethers are macrocylic compounds containing sulfur atoms. In opposite to standard crown ethers (which contains only oxygen atoms), thiacrown ethers are capable of forming complexes with transition metal cations, for example Co^{2+}, Ni^{2+}, Fe^{2+}, Rh^{3+}, Pd^{2+}, Ag^{+}, Cu^{2+}, Hg^{2+}, Cd^{2+} and many others [1]. Modification of macrocyclic ring structure, symmetry and positions of sulfur atoms leads to changes the metal complexation selectivity of crown thiaethers [2]. According to these properties they can be used in separation techniques as solid or liquid phase modifiers in chromatography (in stationary phase the macrocyclic ethers are chemically linked to polymer). Crown thiaethers can also be applied in waste water purification from heavy metals [3]. 2,3,11,12-Bis(4',4"(5")-methylbenzo)-1,4,10,13-tetrathia-7,16-dioxacyclo-octadecane was synthesized and isolated in pure form by column chromatography. Its structure was confirmed by ^{1}H, ^{13}C NMR spectroscopy and ESI-MS spectrometry. Further research are under way.



Structure of 2,3,11,12-bis(4',4''(5'')-methylbenzo)-1,4,10,13-tetrathia-7,16-dioxacyclooctadecane

Literature:

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9.

Voltammetric Determination of Heavy Metals in Green and Black Tea

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Tea is one of the most popular beverages that is important source of bioactive compounds that influence human health, especially acts as antioxidant in cancer diseases, cardiovascular diseases or in diabetes. On the other hand, the habitual tea drinking worldwide and its social and cultural roles, essential and non-essential metals present in black and green tea beverages can have health impacts or impose hazard to consumers and decline their well-being. Heavy metal contaminants might accumulate during tea growth, transportation, packaging or processing, and are harmful to human health.

Differential pulse techniques are extensively employed in electroanalysis due to their high sensitivity, good definition of signals and reduction of double layer and background currents. These advantageous properties arise from the subtractive nature of the signal and the rapid decay of the charging current in a constant potential pulse, which gives rise to well-defined peak-shaped responses. Differential pulse voltammetry was used for the direct simultaneous determination of Cd, Pb, Cu, Sb and Bi in 0.1 M HCl solution (pH 1) containing 1 M KCl. Zn was subsequently determined after raising the pH of the same solution to pH 4. Next, the pH of the medium was raised to pH 8.5 by adding NH_3/NH_4Cl buffer for the determination of Mn. Finally, Ni and Co were determined in the same solution after adding dimethylglyoxime.

The information on the total content of trace metals can be a criterion that makes the tea products admissible for consumption, hence, it is an important part of the quality control that assures purity, safety of black and green teas and evaluates the eventual intoxication risks.

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10.

Thin-layer chromatographic analysis of stanozolol

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Stanozolol is an synthetic steroid compound which has been approved for humane by FDA (Food and Drug Administration) in 1962. It will be used orally or intramuscularly in treating anaemia and hereditary angioedema [1]. Moreover, stanozolol like other anabolic steroids is commonly used in many different kinds sports by men and women to attain a competitive edge. For this reason it is classified as a controlled substance by WADA (World Anti-Doping Agency) [2]. The development of simple to use and economical thin-layer chromatographic method can be useful in the determination of stanozolol in counterfeit pharmaceutical products of anabolics as well as illegal drugs of anabolics available by black market. In this work a new, simple in use and economical TLC-densitometric method in normal phase system (NP-TLC) has been developed for the identification and quantitative determination of stanozolol in the samples containing stanozolol and its related compounds (impurities A and B). Different chromatographic conditions have been tested in this study. Of all applied chromatographic conditions, the mobile phase consisted of toluene-2-propanol in volume composition 45:2 or 43:5 and chromatographic plates precoated with the mixture of silica gel 60 and kieselguhr F_{254} (Art. 1.05567, E. Merck, Germany) are the best. Densitometric analysis was carried out at λ=228 nm. The proposed NP-TLC densitometric method was verified in terms of its specificity, linearity, precision, accuracy, robustness and sensitivity.

Developed TLC-densitometric method can be successfully used in routine quality control of stanozolol in bulk material as well as in available pharmaceutical formulations.

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11.

Identification of instability products of the cathinone derivatives

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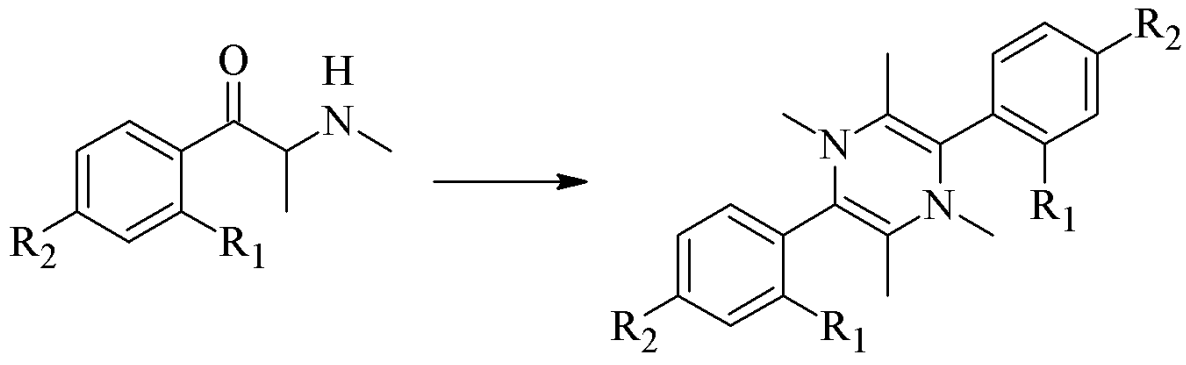
In this poster we presented the identified by GC–MS techniques instability products of 2-(methylamino)-1-(2-methylphenyl)-1-propanone (**1a**) (2-MMC) and 1-(4-chlorophenyl)-2-(methylamino)propan-1-one (**1b**) (4-CMC) in common organic solvent, chloroform. These products were formed by intramolecular condensation leading to appropriate cyclic dimers or trimers. Additionally the use of amination reaction with 2-(pyrrolidin-1-yl)-1-(5,6,7,8-tetrahydronaphthalen-2-yl)pentan-1-one (TH-PVP) for identification of instability product will be presented. The presented findings can be used in future to identify and separate new cathinone derivatives and their decomposition products or metabolites.



1a	**1b**
$R_1 = CH_3$	$R_1 = H$
$R_2 = H$	$R_2 = Cl$

Scheme 1. Dimerization of **1a** and **1b**

12.

Separation and quantitation of selected water-soluble vitamins using different HILIC stationary phases

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Hydrophilic interaction chromatography (HILIC) was first described by Alpert who used the method for separation of proteins, peptides, amino acids, oligonucleotides and carbohydrates. The HILIC is based on polar stationary phases combined with partly aqueous eluents (around 2–40% water) containing acetonitrile or other solvents, e.g., alcohols. Hydrophilic interaction chromatography has been proved to be a useful technique for the retention and separation of polar compounds offering selectivity complementary to RP chromatography.

The group of water-soluble vitamins consists of vitamin C (L-ascorbic acid) and eight vitamins of group B including thiamine (B1), riboflavin (B2), nicotinic acid (B3), pantothenic acid (B5), pyridoxine (B6), biotin (B7), folic acid (B9) and cyanocobalamin (B12). These substances are a group of compounds with hydrophilic character. This suggests they should be retained under HILIC conditions. However, B vitamins are characterized by structural complexity and have diverse chemical and physicochemical properties. For these reasons their chromatographic separation is rather challenging task.

The objective of this study is to develop and validate a HILIC method for the separation of selected water-soluble vitamins with a variety of functional groups, on a diol (Acclaim Mixed-Mode HILIC-1) and zwitterionic (Kanuer Eurospher II 100-5 HILIC) columns. Chromatographic conditions including type and percentage of organic solvent in the mobile phase, pH and concentration of buffer salt have been investigated.

It has been found that the mobile phase composition plays an important role in the separation of B vitamins in each of the columns. Besides, both pH and concentration of applied buffer salt effects on the separation selectivity of the analyzed compounds have been discovered. Finally, the optimal conditions for selective separation of B-group vitamins mixture were proposed.

13.

Comparison of isocratic retention models for hydrophilic interaction liquid chromatography

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Quantitative retention versus eluent composition relationships are of fundamental importance for method development in chromatography. Therefore, in this work the applicability and accuracy of six different retention models used for retention prediction in hydrophilic interaction chromatography (HILIC) have been studied.

Four of the tested models assume additivity of the reversed phase liquid chromatography (RPLC) and of the HILIC contributions to the energy of retention in chromatographic systems, i.e. the mixed mode retention mechanism. The two other non-linear models were adopted from RPLC.

The models have been compared and verified on the basis of various and numerous experimental data exhibiting strongly nonlinear and/or U-shaped retention dependencies versus mobile phase composition. The accuracy of all the models has been statistically verified by means of different statistical criteria and the optimal solution has been proposed.

14.

Multivariate analysis of variance of designed chromatographic data

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[2]*Plant Bioactives Group, Post-Harvest and Wine Technology Division, Agricultural Research Council (ARC), Infruitec-Nietvoorbij, Stellenbosch, South Africa*

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The main goal of our project is a study of the kinetics of the rooibos tea fermentation. Realization of this goal requires, among others, identification of the main components of the tea extracts involved in the fermentation process. In order to estimate an influence of the semi-fermentation and fermentation process on the concentrations of these components, ten plants were studied, and three subsamples of each plant underwent three different treatments (i.e., extracts of the raw, semi-fermented and fermented materials were investigated). The resulting chromatographic data has the dimensionality of 120 samples x 56 compounds. It is the multivariate data which requires application of the multivariate analysis methods. At this stage of our study, we would like to estimate a significance of the treatment effect and to identify these compounds, which significantly contribute to it. As a well-suited method of analysis of variance of multivariate correlated data, the ASCA method was applied for the log transformed data. However, the results of ASCA were inconsistent with the assumption of the PQN normalization method. To solve the normalization problem, we propose application of the pair-wise log ratios.

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Simple and rapid screening procedure for 66 synthetic cannabinoids by liquid chromatography-tandem mass spectrometry

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In recent years many synthetic cannabinoids (SC) have appeared on the drug market. These substances sold as 'herbal highs' or 'research chemicals' belong to different chemical classes. According to reports of the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), more than 160 SC were introduced to the European market up to 2015. Despite the increasing number of SC, there are few comprehensive screening methods for their detection in biological specimens. The variety of SC, their low active doses, low concentrations in biosamples, as well as rapid and numerous metabolic changes create great analytical problems. The analytical method should be sensitive, selective and screening procedures should be open, i.e. ensuring the possibility of continuous inclusion of new compounds.

The purpose of this study was to develop a fast and simple liquid chromatography-tandem mass spectrometry (LC-MS/MS) screening procedure for detection and identification of 66 SC in blood.

Blood samples (0.2 mL) were precipitated with acetonitrile (0.6 mL). Analyses were performed on an Agilent Technologies 1200 series liquid chromatograph connected to a 6460 Triple Quad mass spectrometer. The separation was achieved a Kinetex C18 2.6u 100Å (100×4.6 mm) column (Phenomenex). The mobile phase consisted of a mixture of 0.1% formic acid in acetonitrile (v/v) and 0.1% formic acid in water (v/v) was delivered under the following flow rate conditions: 0 min – 0.5 mL/min, 1 min – 0.5 mL/min, 3.5 min – 0.8 mL/min, 10 min – 0.8 mL/min, 10.5 min – 0.5 mL/min, 16 min – 0.5 mL/min, and the following mobile phase gradient conditions (shown in relation to acetonitrile content): 0 min – 40%, 1 min – 40%, 3.5 min – 60%, 4.5 min – 90%, 10 min – 90%, 10.5 min – 40%, 16 min – 40%. Dynamic multiple reaction monitoring (dMRM) with positive ion detection was applied (retention time window was set at 1 min). The total number of transitions monitored was 199, and the total analytical run time was 16 min.

Despite differences in chemical structures, the method allowed the simultaneous detection and identification of 66 SC from different groups (naphtoylindoles, phenylacetylindoles, naphtoylindazoles, naphtoylpyrroles, dibenzopyranes and other). The application of the gradient flow rate and gradient mobile phase conditions made that all of the compounds were well differentiated by their retention times and/or transitions. The retention times of compounds were from 2.53 to 9.15 min. Prepared blood calibration curves (number of replicates for each level, n = 3) were linear in the range of from 0.1-5 to 100 ng/mL with correlation r in the range of 0.9958-0.9993. The limits of detection (LODs) established for 49 compounds (for the signal-to-noise ratio equalling 3 (S/N=3) for the transition with the lowest intensity) were in the range 0.001-0.48 ng/mL making this assay suitable for the analysis of biological material

We developed a sensitive LC-MS/MS method for simultaneous identification of 66 SC in blood. The developed procedure allows performing rapid screening analysis and requires only 0.2 mL of blood. The procedure can be easily expanded for more substances. Such

methods are needed for forensic and clinical laboratories due to the ever-increasing spectrum of new SC.

Keywords: LC-MS/MS, synthetic cannabinoids, blood screening analysis