POSTER SESSION I

WEDNESDAY, MAY 27th, 2015

CHAIRPERSONS: Josef Jampilek and Jim Van Durme

1.

Synthesis of 1,10-phenantroline derivatives; the GC-MS investigation

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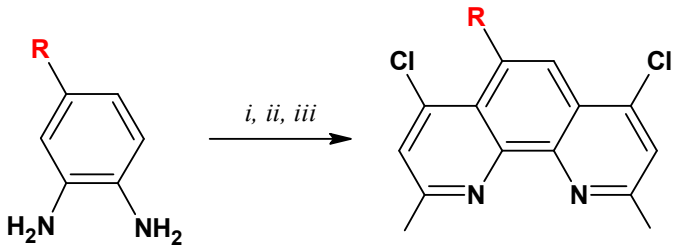
Phenantrolines (*o*-phenantrolines) is one of the most important class of compounds among *N,N*-heterocyclic organic compounds. 1,10-Phenantroline was first synthesized by F. Blau in 1898 [1]. This class of compound has been widely use as bidentate nitrogen donating ligands in coordination chemistry with numerous applications [2]. They found broad range of applications as ligands in transition metal catalyzed reactions, such as iron(II) and copper(I) [3]. They formed five-membered rings with metal cations [4].

This class of compounds can be received on various methodologies, including the classical Skraup, Friedlander, Doebner-Miller or Pavarov reactions [5]. Our research was based on a literature methodology [6] (Scheme 1).



R = F, Cl, Br, CH_3, COOEt

Scheme 1. Synthesis of selected 1,10-phenantroline derivatives. Reagents and conditions: *i* = Meldrum's acid, trimethyl orthoacetate, reflux; *ii* = diphenylether, reflux; *iii* = phosphoryl chloride.

The aforementioned novel 1,10-phenantroline derivatives were synthesized and analyzed by GC-MS techniques. On our oral presentation, we will present the GC chromatograms and MS spectra.

Literature:

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2.

Analysis of Vitamin C (Ascorbic Acid) in Raw, Pasteurized and Ultra High Temperature Cow's Milk Using HPLC – PDA – ESI (+) - MS as a highly sensitive and Highly Confirmatory Analytical Tool

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Here our research is related to quantification of vitamin C (Ascorbic Acid ,AA) content in raw , pasteurized and ultra high temperature (UHT)Cow's milk .For that purpose analytical procedure based on high performance liquid chromatography –photo diode array detector combined with a single quadruple mass analyzer interfaced with electro spray ionization operated in positive ion mode [HPLC- PDA – ESI (+)- MS] has been developed and validated for separation of AA , identification and quantification. AA was extracted from milk samples using 2.5 % solution of meta- phosphoric acid with extraction recoveries ranged 94 – 104 %. Separation of the analyte was carried out using C-18 column and isocratic elution of mobile phase consisting of 0.1 M acetic acid: acetonitrile (98: 2, v/v). Confirmatory identification for presence of AA in investigated milk samples were achieved using UV and MS data obtained from reference standard and sample at the same retention time .For quantification analysis ,HPLC – PDA – ESI (+) – MS (PDA mode) was used and the calibration curves for AA were constructed and was linear with R^2=0.9998 .Limit of detection(LOD) and limit of quantification(LOQ) were 0.052mg /L and 0.1 mg / L respectively .Obtained validation parameters showed that the method appears sensitive , accurate , precise ,specific and relatively simple in both sample preparation and equipment .The procedure provides a very useful tool for rapidly determination of AA in Cow's milk and has been successfully applied for study the effect of storage conditions on its content and the data will be in discussion .

3.

The preliminary studies of the selected aqueous mosses using HPLC and TLC fingerprint methods

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More than 14 000 *Bryophyta* (mosses) species are known, but only low percentage of them have been analyzed using chromatographic methods. It was ascertained based on the literature that mosses have complex chemical composition. The different compounds like monoterpenoids, sesquiterpenoids, diterpenoids, steroids, triterpenoids, bibenzyl derivatives, coumarins, flavonoids were found in their chemical composition.

In our work the extraction procedures were presented. The raw material was dried and it has been extracted by three days using dichloromethane and next the methanol as solvents using the Soxhlet apparatus.

The obtained extracts have been analyzed by HPLC and TLC methods to make the fingerprints of particular extracts. In HPLC method the selected gradient with the methanol-water as mobile phase and the phenyl-hexyl chromatographic column have been used.

The TLC analysis was performed on the silica gel in one-dimensional thin layer chromatographic systems with non-aqueous mobile phases. The methanolic extracts were also analyzed using two-dimensional chromatographic method with the RP-18 chromatographic plates in orthogonal systems with the application of aqueous and non-aqueous solvents.

Moreover the biological activity with DPPH reagents has been studied.

Obtained results have been subjected the chemometric and the PCA analysis to find some differences or similarities between analyzed species of *Bryophyta*.

4.

The fingerprint analysis of selected *Cirsium* species using the HPLC and TLC methods.

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The *Cirsium* species from *Asteraceae* family have been analyzed of this work. The several chemical groups of biological active compounds like flavonoids, phenolic acids, sterols, triterpenes, sesquiterpenes occur in these plants. Based on the literature, it is known that the phenolic acids have various biological activities, especially bacteriostatic, fungistatic, antioxidant, anticancer, choleretic, potential sedative hypnotic, antianxiety and anticonvulsant, activity. The flavonoids display vasoprotective, hepatoprotective, anti-inflammatory, anticarcinogenic and free radical-scavenging properties.

Ten *Cirsium* species have been studied using two chromatographic methods. The powdered raw materials have been extracted in Soxhlet' apparatus and they have been extracted with dichloromethane and next methanol as solvents.

The obtained extracts have been analyzed using HPLC method with the selected methanol-water gradient using the phenyl-hexyl chromatographic column and the fingerprint chromatograms have been obtained. The selected standards have been analyzed in the same chromatographic conditions and the retention times of them were compared with obtained particular *Cirsium* chromatograms.

The TLC analysis was performed using various TLC plates. The silica gel chromatographic plates with selected mobile phase were used to analysis of particular *Cirsium* extracts using one dimensional method. The RP-18, cyanopropyl and diol bonded stationary phases were used in two-dimensional TLC method using aqueous and non-aqueous eluents.

For the better observation of the similarity of the chemical composition of particular *Cirsium* species the results have been subjected to the chemometric and PCA analysis.

5.

TLC isolation of biologically active compounds from extracts obtained from different *Potentilla* species

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Experience of many generations and study of plants growing around us, was a cause of medical and pharmaceutical sciences development. Now, natural folk medicine can be often good inspiration for studies of plant material. Plants used as drugs, contain the biologically active compounds or whole groups of compounds which can be isolated and used in various forms of the drug. Isolation of valuable fractions of extract eliminate unnecessary substances and reduce the dose of drug taken by the patient. Because of complicated matrix, preparative thin layer chromatography is a very good method for the raw plant extract separation. Application of biological detection combined with i.e. mass spectrometry should allows to find important bands on chromatogram.

During the works of comparing the chromatographic fingerprints of various *Potentilla* species rhizomes (A. JóŸwiak 2010-2014), one of examined methods was comparing the TLC bioautograms to find the differences among them. Bioautograms of four species: *Potentilla. erecta, P. collina, P. megalantha* and P. *crantzi* showed significant bacteriostatic/antibacterial properties.

The aim of our works was isolation of biologically active compounds/groups of compounds from extracts of examined plants. Extract was prepared from pulverized rhizomes in dichloromethane, evaporated and disolved again to needed volume. Chromatographic separation was performed on TLC systems: silica/mixture of organic solvents, under earlier found conditions. The samples were introduced as narrow bands (automatic applicator with evaporation of solvent) on silica and TLC plates were developed. Two methods: bioatugraphy and spraying with the special visualization agent, to localization bands of chomatogram were performed. The next step was the scraping the important bands and washing out it content from bed. Obtained fractions were rechromatographed in various system and after bioautographic confirmation of its biological properties, prepared to mass spectrometry analysis.

6.

Searching for urine biomarkers of prostate cancer using LC-MS and GC-MS metabolomic approach

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Prostate cancer (CaP) is one of the leading causes of cancer deaths in men worldwide. Development and application of new high-throughput and specific diagnostic methods is essential for early detection of CaP. In the present study urine metabolic fingerprinting was performed to determine potential biomarkers that could be useful for understanding and explanation of CaP pathomechanisms at molecular level. Urine samples from CaP patients (n=32) and healthy volunteers (n=32) were analyzed with the use of HPLC-TOF/MS in positive and negative ionization modes as well as GC-QqQ/MS in a scan mode. Afterwards, univariate statistical analysis (t-test or U Mann-Whitney test depending on data distribution) was applied for obtained data to select statistically significant metabolites between studied groups. Next, the advanced multivariate methods such as principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) were carried out in order to determine metabolites that were contributed the most into group classification. The identification of selected metabolites using NIST, HMDB, METLIN, KEGG and CEU Mass Mediator databases allowed for creation of a list of putative biomarkers and related biochemical pathways which they are involved in. As a result, 235, 248 and 28 statistically significant variables were selected for LC-TOF/MS analyses in positive ionization mode, negative ionization mode and for GC–MS analyses, respectively. Altered metabolites were found to be involved in amino acid, purine and glucose metabolism as well as urea and TCA cycles. The obtained results suggest that urine metabolic fingerprinting is a powerful tool which might be useful in research for CaP diagnosis and eventual further pathomechanisms explanation.

Acknowledgements

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7.

Identification of tributyltin in environmental water samples supported by means of discriminant models

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Tributyltin is a biocide agent which has been used as ingredient of antifouling paint with the aim to prevent the growth of organisms on coated with paint surface. However, proved that TBT is released into the environment and exceeds acute and chronic toxic levels. For this reason, different international regulations have been issued to effectively prohibit further usage of TBT, and thus to reduce progression of water contamination with TBT and its degradation products. Unfortunately, toxic effects are still observed [1]. It was a reason why, determination of TBT in different water bodies, is a subject of a strict on-going monitoring. The carried out research illustrate the usefulness of chemometric tools in an accredited laboratory in the context of the support process, the routine identification of the tributyltin cation (TBC) in environmental samples of water [1]. Water samples (1403) collected from 2011 to 2013 were analyzed according to European Norm PN-EN ISO 17353:2006 and described by means of chromatographic fingerprints using gas chromatography coupled with mass spectrometry. Then, for data sets after and before elimination of the baseline and peaks shifts removal [2], discriminant models were built using discriminant method - partial least squares - discriminant analysis [3]. Validated PLS-DA models for raw and preprocessed data sets allows correct discriminate 80.5% and 79.5% of the samples, respectively. Its sensitivity is 81.9% and 80.0% and specificity is 79.1% and 79.0%, respectively.

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8.

THE ENCOUNTER OF MACROFUNGHI RESEARCH AND DIRECT BIOAUTOGRAPHY

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Keywords: direct bioautography, mushrooms, HPLC-ESI-MS, TLC, antibacterial effect

Fungi are the most diversified group of organisms; nearly one million species are estimated. Their environmental and morphological diversity and interactions are remarkable. They produce compounds that also reflect this diversity. Among the basidiomycete mushrooms, large numbers of species produce different bioactive compounds (antioxidant, anti-microbial, anti-tumor, etc.). Many of these substances are derived from secondary metabolism. Our research focuses on those basidiomycete species that are suitable for human consumption, such as a variety of cultivated mushrooms (*Agaricus spp.*, *Pleurotus spp.*) as well as some wild mushrooms (*Flammulina velutipes*, *Agrocybe cylindracea syn. Agrocybe aegerita, Leccinum duriusculum*). The antibacterial activity of certain compounds of the fruiting body, capskin, hat meat, gills and stalk extracts was examined by thin-layer chromatography-direct bioautography using different Gram-negative and Gram-positive bacteria (X*anthomonas euvesicatoria, Pseudomonas syringae* pv. *maculicola*, *Aliivibrio fischeri, Bacillus subtilis,* etc.). During the test the developed dried chromatoplates were immersed in the bacterial cell suspension, and the inhibition zones of the antibacterial substances (chromatographic spots) were visualized with vital dyes or by bioluminescence detection. All examined mushrooms showed antibacterial effect against all tested bacteria. More characteristic inhibition zones were revealed with direct bioautography. Utilizing different reagents *in situ* in the adsorbent layer the antibacterial substances were characterized mainly as fatty acids (lipophilic) or phenolic substances. The major active component of all mushrooms has been identified by HPLC-DAD-ESI-MS as linoleic acid. The MS characterization of the minor active components is under process. Further investigations are planned such as classical bacteriological tests (eg. MIC), MS/MS and NMR examinations.

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9.

Optimization of the extraction of total heme from meat for determination by HPLC

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Myoglobin is the most important red pigment in meat, but also the content of hemoglobin and cytochrome C partially influence the meat colour [1]. Myoglobin is a compact globular protein consisting of globin and an iron containing heme group (Fe-protoporphyrin IX) as chromophore. The colour of myoglobin is determined by the redox state of heme and by the type of ligand bound at its sixth' coordination place, and is found in meat in three main forms: deoxymyoglobin (MbFe(II)), oxymyoglobin ($MbFe(II)O_2$) and metmyoglobin (MbFe(III)) [2]. For the production of meat products, traditionally nitrite or nitrate is added, whereby nitrosomyoglobin (MbFe(II)NO) is formed. As a result the typical red cured colour of meat products is attained [3]. Colour, a crucial factor for consumer's buying decision of meat and meat products, can be affected by several factors [1, 2]. In order to investigate the colour formation in meat and meat products in detail, it is of primary interest to have a reliable method for the determination of the total heme content.

In this study, the method for extracting heme from meat samples, more specific dry fermented sausages, has been optimized and validated prior to further analysis by HPLC-UV. Chromatographic analysis was based on the method described by Wakamatsu et al. (2009) [4]. The separation was carried out by isocratic elution using methanol/ammonium acetate (80:20, v/v, pH = 5.16) at a flow rate of 1 mL/min. Forty microliters of each sample was injected. Detection of heme was carried out at the wavelength of 400 nm.

The influence of different factors was investigated to find suitable conditions for the heme extraction. The procedure of extraction was based on the method described by Lombardi-Boccia et al. (2002) using acidified acetone as extraction solvent. This ensures the extraction of heme from all heme proteins, in the form of acid hematin (hemin) [5]. In this study the effects of extraction solutions (combinations of water, HCl and acetone) and operational parameters, such as homogenization time (30 s versus 5 min using an ultra-turrax T25 homogenizer, IKA®, Staufen, Germany) and shaking (0 h versus 1 h using a nutating mixer, VWR International, West Chester, PA, USA) were studied. Also double and single extraction of the meat samples was examined.

The optimal extraction of heme was obtained with an acidified acetone solution of 78% acetone and 1.375% HCl, 5 min homogenization and no additional shaking. The recoveries obtained after double extraction were better than the values yielded after single extraction, indicating that a double extraction is more acceptable for the determination of heme in meat products. The optimized extraction method is a valuable tool for assessing the total heme concentration in salami samples by HPLC.

Acknowlegments

This investigation was done in the framework of an European Interreg IV a 2 seas program. The research project $MeCagrO_2$ involves safe products, sustainable processes and employment increased attractiveness for companies from the 2 Seas agro-food area. Note: "The document reflects the author's views. The interreg IVA 2 Seas Program Authorities are not liable for any use that may be made of the information contained therein." E. De Mey is a postdoctoral fellow supported by the Postdoctoral Mandate (PDM) from KU Leuven.

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10.

Detection of ovarian cancer based on metabolomic data obtained from liquid chromatography coupled with mass spectrometry

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Ovarian cancer remains as one of the major causes of deaths among different gynecological malignancies. Only in 2008, in US, it claimed 15,520 lives out of 21,650 new ovarian cancer cases mostly as a consequence of a late diagnosis. Over the last years, a rapid development of metabolomic approaches and advanced analytical platforms opened new possibilities of efficient and nearly non-invasive diagnosis based on tracing of low molecular compounds (metabolites). It is expected that certain metabolite(s) found in examined body fluids can be an indication of a developing disease. In order to enable discrimination between metabolite profiles obtained from healthy and diseased group of patients, different chemometric and machine learning data modeling approaches are used [1,2]. They take into account selected metabolites or even entire chemical fingerprints trying to discover unique pattern of metabolites differentiating healthy and diseased patients. In practice, multivariate modeling approaches are preferred over univariate ones because in most cases a single metabolite (a biomarker) has a little discrimination power.

We aim to build multivariate discriminant model that can assist in diagnosis of papillary serous ovarian cancer based on the chemical content of selected metabolites determined in serum samples of healthy and diseased women using the LC-MS technique. In contrast with the previous study [1], to discriminate the two groups of samples, a relatively simple linear multivariate model is considered - partial least squares discriminant analysis, PLS-DA [3]. Each sample has been described by 360 and 232 peak areas determined using the positive and negative detection mode. A detailed description of experiment (including cohort description), sample pretreatment, peak detection, etc. can be found in reference [1].

Due to a limited number of available samples, the PLS-DA model is validated in the course of the bootstrap procedure [4]. It consists of multiple drawing at random a predefined number of samples that serve as a model set used to build a model, whereas the remaining samples test model performance. For a given bootstrap sample, a number of PLS-DA models with increasing complexity are built and are characterized by the number of incorrectly recognized samples. When the number of bootstrap samples is reasonably large, it is possible to estimate prediction errors and associated uncertainty.

For the studied data set the best PLS-DA model, constructed for two groups of metabolites identified using positive and negative detection mode, leads to mean values of correct classification rates, estimated using the bootstrap procedure, approaching 80%.

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11.

Between batch differentiation of stamping inks from the same manufacturer by means of Thin Layer Chromatography

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Analysis of ink is an important part of forensic investigations which very often regards questioned documents with significant values, such as contracts or insurance claims. Even though writing inks are very common subject of research, greater attention should undoubtedly be paid on the stamping inks examination.

Hence, the aim of this study was to propose an approach allowing to compare black and red stamping inks and determine whether the developed method could discriminate between closely related formulas, that is inks from three different batches of the same manufacturer. Due to its importance in forensic ink analysis (despite destructive character), Thin Layer Chromatography (TLC) was applied.

The chromatographic separation of inks was performed on Merck TLC silica gel 60 plates (without fluorescent indicator) with use of following mobile phase: ethyl acetate/ ethanol/ water 70:35:30 (v/v/v). Development of the chromatograms was carried out to distance of 145 mm in normal CAMAG chamber. In order to investigate the influence of the substrate on the chromatographic separation of the dyes, the TLC analysis of ink extracted from the paper with 100 μl of methanol/ water 1:1 (v/v) was also conducted. The obtained chromatograms were inspected in UV light and densitometrically scanned (λ=418 nm for black and λ= 495 nm for red inks). On this basis, R_f values were calculated for each band, allowing to compare investigated inks more precisely.

The obtained results demonstrated the inability of differentiating examined stamping inks by means of Thin Layer Chromatography, when procedure described above was applied, as the products from different batches exhibit the same dye composition. A reliability of comparison process could be probably improved by introducing proper calibration standards and applying algorithms for the comparison [1], as the environmental and analytical factors (such as the influence of TLC plate) can impact ink chromatograms, resulting in misleading conclusions.

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12.

Optimization of chromatographic condition for separation of ziprasidone and its impurities by TLC

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Ziprasidone is a second generation antipsychotic drug used for the treatment of schizophrenia and the acute maniac or mixed episodes associated with bipolar disorder. Five recognized ziprasidone impurities originating from the synthesis as the precursor impurities (impurity I and IV) or representing degradation products of ziprasidone (impurities II, III, and V) are compounds significantly different in polarity and therefore represent a challenge for the simultaneous separation. The main objective of this wark was to optimize thin-layer chromatografic condition for simultaneous separation of ziprasidone and its five impurities.

According to preliminary study, central composite face centered design was chosen to examine the influence of four factors: the developing distance, the amount of toluene in the mobile phase, the amount of acetic acid in the mobile phase, and the spot band size, on the retention behaviour of examined compounds with special emphasis on critical pairs, i.e., impurities III and I as well as ziprasidone and impurity II. The optimal separation conditions were achieved on chromatographic plates precoated with silica gel 60 F_{254} and using toluene-methanol-acetic acid 7.5:0.5:0.5 (v/v/v) as mobile phase in ascending development mode to the distance of 110 mm. The reproducibility of separation by use of the selected TLC method was confirmed with low relative standard deviation of migration distances for all examined compounds (MD ± RSD): 22.47 mm ± 3.66 %, 30.70 mm ± 2.18 %, 40.87 mm ± 1.77 %, 46.37 mm ± 1.34 % , 76.72 mm ± 0.81 %, and 91.65 mm ± 1.39 %, for impurities III, I, II, ziprasidone, impurities V and IV, respectively. According to the obtained results the proposed TLC method can be used as reliable method for simultaneous separation of 6 compounds highly different in polarity with calculated logP values in the range 2.24 (impurity II) to 8.51 (impurity III) and can be further subjected to the validation process.

13.

Bioautography as a method of determination of antibacterial properties with selected thyme species

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Thyme is an aromatic evergreen plant with strongly pronounced curative properties, and with an increasing importance both for the economy and gardening in Europe. Its natural habitat includes vast territories of Europe, North Africa, and Asia. Thyme belongs to the family of *Lamiaceae* (formerly known as *Labiatae*), which embraces ca. 350 different species. The most common among these species is common thyme (*Thymus vulgaris* L.) [1,2]. Based on extensive phytochemical studies on chemical composition of this plant, the presence of several bioactive compound classes was revealed such, as phenolic acids, flavonoids, terpenoids, and essential oils [3]. Representatives of all these botanical classes are responsible for different positive pharmacological properties such, as antiseptic, antioxidant, antibacterial, and expectoral property [4,5].

Thin-layer chromatography combined with different biological and chemical detection methods is a particularly efficient and cheap analytical technique, well suited for studying herbal extracts. Coupling of thin-layer chromatography (TLC) with biological detection carried out by means of direct bioautography (DB), results in a novel TLC-DB technique, which enables a robust screening of herbal material in the search for those plants with strongly pronounced biological activity. Among pharmacologically important properties of herbal material is their antibacterial activity and this can easily be assessed with use of TLC-DB [6]. Characteristic feature of the TLC-DB method is that one observes the growth of bacteria directly on a chromatographic plate [7,8]. DB is the most frequently used one of all bioautographic methods.

Eighteen thyme (Thymus L.) species grown in Botanical Garden of the Maria Curie-Skłodowska University in Lublin underwent phytochemical analysis. All plant species were harvested in July, 2012, and dried under proper working conditions. Extracts from these plants were obtained, which were then analyzed by means of TLC-DB. The main goal of these investigations was to evaluate antibacterial properties of all the thyme species studied and to select those with the strongest pronounced antibacterial activity. The results obtained demonstrate considerable diversity of antibacterial properties of the eighteen investigated thyme species.

Acknowledgement

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14.

Qualitative evaluation of excise duty marker Solvent Yellow 124 in diesel oil samples

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Diesel oil is widely used for transport, heating and driving of agricultural machinery. According to its end use, the level of imposed excise duty on diesel oil is different. A rebated tax fuel (meant for heating purposes and driving agricultural machinery) is deliberately spiked with additives that can be visually recognized (for instance changing the color from yellow to red). In all EU countries two additives are introduced into diesel oil: a marker and a dye compound. Their choice and concentration levels may vary from country to country. Solvent Yellow 124 (SY124) is a fuel marker, and its concentration levels are found within 6.0 $mg \cdot L^{-1}$ and 9.0 $mg \cdot L^{-1}$. A dye compound changes fuel color to red (e.g. Solvent Red 164 and Solvent Red 19, etc.) [1,2]. Decreased concentration levels of excise duty components in diesel oil or their absence are a direct indication of a possible counterfeiting. Substantial differences in applied tax levels encourage illegal removal of excise duty components. Thus, by illegal changing designation of fuel, it gains artificially value on the market.

The reference method for the qualitative evaluation of SY124 in diesel oil is based on the HPLC analysis [3]. We have developed analytical procedure extended with the chemometric modeling to determine the content of SY124 as an alternative to reference method. Bearing in mind low levels of residual SY124 found after 'laundered' of samples a sensitive technique should be used. As illustrated in our study this objective can be achieved with excitation-emission fluorescence technique. To quantify SY124 fuel samples have been characterized by their excitation-emission spectra and further modeled using the three-way partial least squares [4]. Proposed procedure is compared with the reference method and its validation parameters are reported.

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15.

Determination of acrylamide in coffee samples using the fluorescence spectroscopy extended with chemometric modeling approaches as a simple and cost-effective alternative to separation-based methods

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Frying or baking of food products increases their taste, but on the other hand many dangerous for health compounds are formed – for instance acrylamide (AA). Its presence was found for the first time in 2002 in food products rich with carbohydrates [1]. AA is formed as a product of the Maillard reaction between amino acids (mainly asparagine) and reducing sugars (glucose or fructose) initiated during frying, roasting or grilling processes at temperature above 120°C [2].

It is proven that AA binds to hemoglobin and DNA [3], and thus it has neurotoxic and carcinogenic properties. For this reasons its content must be monitored. Usually it is done using different chromatographic techniques including high performance liquid chromatography, gas chromatography and liquid chromatography coupled with mass spectrometry or tandem mass spectrometry detection [4]. However, these techniques are in general expensive, time-consuming and laborious.

In this study the possibility of AA quantification in selected food products is examined using the fluorescence spectroscopy - a simple, cost-effective and very sensitive technique. It is known that presence of AA decreases fluorescence intensity of tryptophan (serving as a fluorescent probe). This property will be used for the construction of advanced chemometric calibration models [5] enabling quantification of analyte under the presence of additional fluorescence interferents. Ground coffee samples will be characterized by their fluorescence excitation-emission fingerprints and modeled with the second-order calibration methods [6].

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16.

Identification and separation of TNF-α after infection of three genospecies of *Borrelia burgdorferi* sensu lato by the use of chromatographic techniques. Preliminary results

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Lyme disease is caused by spirochetes of *Borrelia burgdorferi sensu lato* complex. *B. burgdoferi s.l.* is divided into 19 genospecies of which *B. afzelii, B. garinii* and *B. burgdorferi sensu stricto* are the most important human pathogens.

The spirochete *B.burgdoferi s.l.* stimulate the immune system to cytokine production like tumor necrosis factor TNF-α. It is a pro-inflammatory cytokine which cause plejotropic effects on various cell types. This cytokine is playing a key role in apoptosis, inflammation and immunity. Using Affymetrix oligonucleotide microarray (HG-U133A) analysis demonstrated that 17 ID mRNA from 93 ID mRNA is changing independently from genospecies *B. burgdorferi* s.l. 15 ID mRNA is dependent from the presence of infections only *B. garinii*, 13 ID mRNA exclusively for *B. burgdorferi* s.s., and 9 ID mRNA is specific for *B. afzelii*. This study is an attempt of quantitative and qualitative (QA/QC) identification of TNF-α, after infection of normal human dermal fibroblasts (NHDF) with *B. garinii, B. afzelii* and *B. burgdorferi s.s.* with microarray techniques supported by UHPLC and GPC.

17.

Determination of resveratrol in fruit juices and herbal infusions

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Fruit juices and infusions prepared from dried herbs are widely used in pharmaceutical industry and as traditionally home-made curative preparations. The most common are those which are intensely coloured, i.e., juices and infusions prepared from blueberries, chokeberries, raspberries, and pomegranates. All these intensely coloured raw materials are rich in polyphenols, and resveratrol is one of their constituents. To one of the resveratrol isomers, *trans*-resveratrol (*trans*-3,5,4'-trihydroxystilbene), a broad spectrum of therapeutic properties are ascribed. The most significant are antioxidant, anti-inflammatory, detoxificating, antibacterial, and antifungal properties. Based on the results of clinical investigations, *trans*-resveratrol is considered as responsible for suppressing of lipid levels in the blood serum and it is renowned for an anticancer activity.[1]

Currently, herbal raw materials rich in *trans*-resveratrol have attracted wide attention from the side of manufacturers of dietary supplements and cosmetics. An intense search has started for different botanical materials which might serve as a rich source of resveratrol and an alternative to the grape vine.

In this study, fruit juices (obtained from raspberries, chokeberries and pomegranates) and infusions made of dried fruits (blueberries and chokeberries) were investigated. Infusions made of dried fruits and aqueous solutions of fruit juices underwent the solid phase extraction (SPE) treatment. Then polyphenols (including resveratrol) were extracted with ethyl acetate from the SPE cartridge sorbents and analyzed by means of HPLC. Qualitative analysis of resveratrol with use of an inner standard (IS) was performed.[2,3]

It was demonstrated that the contents of the phenolics (inclusing resveratrol) strongly differ and depend on the plant species and an anatomical part of the plant.

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18.

Determination of hexachlorocyclohexane (HCH) isomers and their biodegradation products in environment samples by GC

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The majority of organochlorine pesticides (OCP), including hexachlorocyclohexane isomers (HCH), have been banned in many countries due to their mutagenic and carcinogenic properties [1]. However, due to their persistency and lipophilicity, these compounds and their degradation products are still present in the environment [2]. Therefore, it is necessary to monitor the soils which are contaminated with OCP, as well as to apply and refine the methods which enable soil remediation. Among the methods used to purify the polluted soils we can distinguish also the bioremediation technics. A crucial element favouring these methods is their natural character. As non-invasive methods which do not destroy the structure of soil, they offer a possibility of ground recultivation without its significant and costly transformation.

An important element of our research was chromatographic estimation of an effectiveness of biodegradation of the HCH isomers by the bacteria strains which have been preliminarily isolated from the grounds polluted by these pesticides. In these studies, the methods of preparation and analysis of samples contaminated with HCH isomers and their biodegradation products have been developed. For isolation of target compounds, solid-phase extraction (SPE) was used. The analysis of the obtained extracts was carried out by means of capillary gas chromatography with mass spectrometric (MS), as well as electron capture detector (ECD). Usage of the GC/MS technique enabled identification of the products acummulated in soil through biodegradation of organochlorine pesticides. These products were identified on the basis of the acquired mass spectra.

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19.

Application of smoke condensates in smoking process

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Food smoking processes are an important source of Volatile Organic Compound (VOC) emissions of which some are hazardous pollutants. An alternative to traditional wood smoking, is to use purified smoke condensates. The goal of this study is to compare the emissions to the air between both a traditional and innovative smoking process using mackerel as a case.

Two batches of identical amounts of salted mackerel were each subjected to a specific optimized smoking processes: beech wood smouldering and by smoke condensate atomization. After smoking, homogenized mackerel samples obtained from both processes were compared using sensory and chemical-analytical method (HS–SPME–GC–MS). Sensory analyses revealed no significant organoleptical differences ($p = 0.05$) between two types of fish products. However, aroma profiling revealed significant differences in aroma properties, indicating that also VOC emissions might be different. Therefore, two experiments were done to evaluate VOC emissions to the environment using identical smoking programs, but different smoke generating methods as discussed earlier (at 60 □C). After the smoking cycle valves were opened and 4 sequent air samples were collected in the exhaust during first 3 minutes. The VOCs composition of the emitted gases was objectively evaluated by means of chemical-analytical (TD-GC-MS).

The chemical compositions of residual smoke emissions proved to be different.The overall VOC concentrations for different compound classes proved to be mostly higher in the smoke condensates exhausts. Moreover, despite high initial concentrations, a strong descending trend was observed for VOCs emissions within the first 3 minutes of smoking processes. Interestingly, several hazardous compounds from the BTEX (Benzene, Toluene, Ethylbenzene, Xylene) and polycyclic aromatic hydrocarbon groups were measured only in the wood smoke: benzene (0.03 mg/m^3), ethylbenzene (0.01 mg/m^3), toluene (0.05 mg/m^3), xylene isomers (0.03 mg/m^3), styrene (0.01 mg/m^3) and naphthalene (0.01 mg/m^3).

Innovative smoking technique proved to be an effective method for producing smoked food product with organoleptic properties equal to those of traditionally smoked one. However, the post – smoking emissions during processing require further studies to verify the risk of carcinogenic compound accumulation and human respiratory system damage when exposed for prolongued periods (e.g. workplace environment).

20.

LC-MS and GC-MS based untargeted metabolomics to study urogenital tract cancer heterogeneity

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Metabolomics is a complex study of small molecules that represent the end point of biological processes in cells, tissues or biofluids such as blood or urine. A comparative analysis of the metabolic profile of urine from 30 patients with cancer of the genitourinary system (bladder (n=10), kidney (n=10) and prostate (n=10)) and 30 healthy volunteers as a control group was provided by LC-TOF-MS and GC-QqQ-MS. The LC-MS analysis were provided with a gradient elution of mobile phase consisted of 0.1 % formic acid in water and 0.1% formic acid in methanol on an 150 mm x 4.6 mm x 2.7 µm Ascentis Express C-18 column (Supelco analytical, USA). For GC-MS analysis, helium was used as a mobile phase in a gradient elution on an 30 m × 0.25 mm × 0.25 µm ZB-5MS column (Phenomenex, USA). The data analysis was provided by the use of U-Mann Whitney test or student's t-test, principal component analysis (PCA) and partial least-squares discriminant analysis (PLS-DA). 247 compounds from LC-MS analysis (dataset A) and 27 compounds from GC-MS analysis (dataset B) was found to be statistically significant different in healthy group compared to diseased patients. The PLS-DA was used to form two models (A and B) from dataset A and B, respectively. A relatively high sensitivity which means correctly classified patient (81.81 % for model A and 100 % for model B) and specificity meaning correctly classified healthy volunteers (71.43 % for model A and 100 % for model B) were obtained. The overall classifications to a specific group were 88.1 % for model A and 89.2 % for model B. The combination of chromatographic, spectrometric analyses and chemometric techniques can allow the identification of potential biomarkers based on the differences in metabolites level.

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21.

ANALYSIS OF INSECT SIGNALLING USING ION-TRAP MASS SPECTROMETRY A CASE OF DENDROLIMUS PINI

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Understanding of insect signalling provides effective tools for pest control. For instance, artificial sexual attractants can be used to lure male insects to traps rather than let them find the calling females. Ion-trap mass spectrometry coupled with a capillary gas chromatography is an efficient tool for analysing blends of volatile substances such as sex pheromones. This work shows the analysis of sex pheromone of pine-tree lappet moth (*Dendrolimus pini*), a serious pest of pine forests in Europe and Asia. This species belongs to a large family of Lepidoptera that comprise the second largest insect pest of coniferous trees.

(a) (b)

Fig. 1. (a) Male and (b) female of pine-tree lappet moth (*D. pini*).

Caterpillars of pine-tree lappet moth are the most dangerous defoliators of pine trees in Poland. On average, each caterpillar consumes 900-1000 needles, which destroys the assimilation apparatus and weakens the trees making them vulnerable to secondary pests. The straightforward consequence of this damage is the death of pine forests. One of the efficient and environment-friendly methods for the forest protection against *Dendrolimus pini* pest takes advantage of disturbing the mating flight with synthetic sex pheromone lures. The pheromone lures available so far are based on substances discovered in the early 1980s. They were tested in many countries and appeared rather inefficient in the forest protection.

Thus, a project was started to discover the full composition of the sexual pheromone of pine lappet moth (*Dendrolimus pini*) and to provide an improved analogue of this pheromone for better forest protection. After a challenging search for an effective method of sample collection (see a companion poster by Rudziński et al.), a variety of SPME samplers was used in a stationary sampling system. Namely, the following SMPE fibres were evaluated: polidimethylsiloxane (PDMS), carboxen-polidimethylsiloxane (CAR/PDMS), divinylbenzene-polidimethylsiloxane (DVB/PDMS), polyethylene glycol (PEG) and polyacrylate (PA). In the first phase of the study, the fibres were evaluated in a static headspace mode against the authentic standards of 5E, 7Z-C12-OH and 5E, 7Z-C12-CHO that are the recognized components of the sex pheromone of *D. pini*. Then, the pheromone samples were collected from living calling females of *D. pini*, with SPME fibers placed not more than 4 mm from the extruded ovipositor of each insect. The GC/MS-IT analyses of adsorbed analytes were carried out using a Thermo 1300 GC gas chromatograph coupled with ITQ 700 ion-trap mass spectrometer with 70eV EI ion source. Our results show which of the SPME cartridges used are appropriate for identifying the individual components of sex pheromone blend that the female pine-tree lappet moth emits.