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WORKSHOP on

Novel platform for multidisciplinary assessment of food and feed safety

Organized by:

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MultiCoop

Multidisciplinary approach to strengthen cooperation and establish novel platform for comprehensive assessment of food and feed safety

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TUESDAY, November 7, 2017

9:00–13:30 Leo hall

WORKSHOP on
Novel platform for multidisciplinary assessment of food and feed safety

Chairs:
Jana Hajslova, *University of Chemistry and Technology, Prague, Czech Republic*
Julie Meneely, *Queen's University, Belfast, United Kingdom*
Rudolf Krska*, University of Natural Resources and Life Sciences, Vienna (BOKU), Department of Agrobiotechnology (IFA-Tulln), Tulln, Austria*

8:30–9:00 **Registration for the workshop**

9:00–9:25 **EMERGING TOXINS IN CEREALS - FROM MULTI-TOXIN ANALYSIS TOWARDS METABOLOMICS**
Rudolf Krska, *University of Natural Resources and Life Sciences, Vienna (BOKU), Department of Agrobiotechnology (IFA-Tulln), Tulln, Austria*

9:25–9:50 **CHALLENGES AND LIMITATIONS OF MULTI-ANALYTE APPROACHES FOR MYCOTOXIN DETERMINATION**
Michael Sulyok, *University of Natural Resources and Life Sciences, Vienna (BOKU), Department of Agrobiotechnology (IFA-Tulln), Tulln, Austria*

9:50–10:15 **RELATIVE MATRIX EFFECTS AND MEASUREMENT UNCERTAINTY IN MYCOTOXIN ANALYSIS**
David Stadler, *University of Natural Resources and Life Sciences, Vienna (BOKU), Department of Agrobiotechnology (IFA-Tulln), Tulln, Austria*

10:15–10:40 **COCTAILS OF BIOACTIVE COMPOUNDS IN MILK THISTLE BASED FOOD SUPPLEMENTS**
Marie Fenclova, *University of Chemistry and Technology, Prague, Czech Republic*

10:40–11:05 **ASSESSMENT OF ANTIDIABETIC PHYTOCHEMICALS AND BIOACTIVITIES IN STEM JUICES FROM BANANA**
Jitka Viktorova, *University of Chemistry and Technology, Prague, Czech Republic*

11:05–11:30 **Coffee break**

11:30–11:55 **REIMS - FOOD FRAUD DETECTION IN REAL TIME**
Connor Black, *Queen's University, Belfast, United Kingdom*

11:55–12:20 **ALTERNATIVE ANALYTICAL STRATEGIES FOR DETECTION OF UNDECLARED MOISTURE BINDERS IN MEAT**
Vit Kosek, *University of Chemistry and Technology, Prague, Czech Republic*

12:20–12:45 **LC-MS BIOMARKER DISCOVERY AND APPLICATION IN THE FIELD OF FOOD ADULTERATION**
Olivier Chevallier, *Queen's University, Belfast, United Kingdom*

12:45–13:10 **LEAVING THE LABORATORY BEHIND: RAPID IN-FIELD FOOD AUTHENTICITY SCREENING USING HANDHELD SPECTROSCOPY**
Terry McGrath, *Queen's University, Belfast, United Kingdom*

13:10–13:30 **Questions & Discussion**

EMERGING TOXINS IN CEREALS – FROM MULTI-TOXIN ANALYSIS TOWARDS METABOLOMICS

R. Krska*, C. Büschl, M. Sulyok, F. Berthiller, R. Schuhmacher

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Mycotoxins are toxic fungal metabolites, occurring on a wide range of agricultural products. Several research projects, including the recently started European project "MyToolBox", aim for integrated approaches – combining pre- and post-harvest measures with efficient monitoring tools for control. The latter is crucial to provide food safety for the consumers and to determine the efficacy of mitigation measures. Analytical chemistry, in particular mass spectrometry, has evolved with a tremendous pace. While years ago, only single toxins could be measured, a clear trend is towards multi-toxin methods, providing a far more detailed picture [1]. One example is a multi-analyte LC-MS/MS method which has recently been developed by us and which is capable of determining more than 600 fungal, bacterial and plant metabolites, respectively, in cultures, cereals, food and feed products. LC-MS-based screening has also been playing a vital role in the discovery of novel mycotoxin conjugates - so called "masked" - forms of mycotoxins.
Metabolomics has emerged as the latest of the so-called –omics disciplines and shows great potential to determine hundreds to thousands of metabolites at once over a wide range of concentrations. After measurement of biological/food samples treated with a 1+1 mixture of labelled and non-labelled precursors, labelling-specific isotopic patterns can be reliably and automatically detected by means of the novel software tool ("MetExtract"). In a preliminary study, the great potential of the presented approach is further underlined by the successful and automated detection of novel plant-derived biotransformation products of the most prevalent Fusarium mycotoxin deoxynivalenol.
This paper will summarize trends and new findings in the area of multi-toxin screening of food and feed commodities and the possible implications of "emerging" mycotoxins on the safety and security of human food and animal feed.

[1] R. Krska, M. Sulyok, F. Berthiller, R. Schuhmacher. Mycotoxin testing: From Multi-toxin analysis to metabolomics. JSM Mycotoxins 67(1, 1-6 (2017).

CHALLENGES AND LIMITATIONS OF MULTI-ANALYTE APPROACHES FOR MYCOTOXIN DETERMINATION

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In the past decade, LC-MS/MS based multi-mycotoxin methods have become more and more popular as they are able to simultaneously cover all regulated toxins. As the application of sophisticated methods for sample clean-up is ruled out by the chemical diversity of this set of analytes, most of the related methods rely on some sort of unspecific clean-up (e.g. modified QuEChERS) or on isotopically labelled internal standards. However, none of these approaches is fully applicable if the range of analytes is further extended. Therefore, our method targeting several hundreds of fungal metabolites and a few plant toxins is based on the direct injection of diluted crude extracts.
Many authors have expressed their concern about the limited accuracy of this approach, as matrix effects might not be effectively under control. Indeed, the decrease (or more rarely the increase) of the analytical signal due to co-eluting matrix constituents is the most critical issue that needs to be minimized (by using a low injection volume in connection with a large flow rate) or thoroughly investigated during method validation. However, there is still a lack of related guidelines as matrix effects are sometimes not specifically mentioned in official documents (e.g. Commission Decision 2002/657/EC). Consequently, it remains unclear whether or not the term "recovery" includes matrix effects and how related criteria should be determined (external or matrix matched-calibration).
In relation to this, it is still to be discussed whether apparent recoveries that are low but consistent (i.e. demonstrating good precision) may be acceptable in multi-analyte analysis. In our view, the aspect that needs particular attention is the number of different individual samples per matrix that should be investigated to determine the variation of matrix effects within a given matrix. This is very often neglected by methods described in the literature that use matrix calibration matched to a single or a pooled blank sample.

RELATIVE MATRIX EFFECTS AND MEASUREMENT UNCERTAINTY IN MYCOTOXIN ANALYSIS

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In the recent years, the LC-MS/MS based multi-analyte approach has been demonstrated to be a powerful technique for the simultaneous determination of mycotoxins in food and feed [1]. Quantification of mycotoxins is increasingly based on the analysis of diluted crude extracts and external- or matrix-matched calibration. In essence, the response of the analyte is compared to a calibration curve of the analyte in neat solvent or matrix and, if necessary, corrected for the method bias. The method bias, expressed as apparent recovery (RA), can arise from incomplete recovery of the extraction (RE) or signal suppression/enhancement (SSE), also known as matrix effect. In everyday practice RA is evaluated based on replicate analysis of a single lot of a matrix for each analyte-matrix combination during initial method validation. Due to the heterogeneous nature of the matrix, RA may vary for different lots of the same matrix i.e. "lot-to-lot variation". Matuszewski et al. first found differences in SSE for a compound in plasma samples from different sources, which is referred to as relative matrix effect [2]. Also for mycotoxins, large differences in SSE have been observed for different varieties of sorghum and rice [3, 4]. However, most method validation studies neglect the lot-to-lot variation and validate the method based on a single lot of a matrix. We hypothesized that neglecting the lot-to-lot variation during method validation can lead to an underestimation of the measurement uncertainty (U). The objectives of this study were i) to find a realistic estimate of U for a LC-MS based multi-mycotoxin method and ii) to compare it to the performance criteria stated in official guidelines of the European Commission. This study presents the first calculation of i) the intra-laboratory U for 66 mycotoxins in figs and maize and ii) the inter-laboratory U for regulated mycotoxins in food and feed, both accounting for the lot-to-lot variation, and differs significantly from U calculated based on repeatability studies of a single lot of a matrix.

[1] Malachová A, Sulyok M, Beltrán E, Berthiller F, Krska R (2014) Optimization and validation of a quantitative liquid chromatography-tandem mass spectrometric method covering 295 bacterial and fungal metabolites including all regulated mycotoxins in four model food matrices. J Chromatogr A 1362:145–156.
[2] Matuszewski BK, Constanzer ML, Chavez-Eng CM (2003) Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. Anal Chem 75:3019–3030.
[3] Njumbe Ediage E, Van Poucke C, De Saeger S (2015) A multi-analyte LC-MS/MS method for the analysis of 23 mycotoxins in different sorghum varieties: The forgotten sample matrix. Food Chem 177:397–404.
[4] Sulyok M, Krska R, Schuhmacher R (2007) Application of a liquid chromatography-tandem mass spectrometric method to multi-mycotoxin determination in raw cereals and evaluation of matrix effects. Food Addit Contam 24:1184–1195.

COCTAILS OF BIOACTIVE COMPOUNDS IN MILK THISTLE BASED FOOD SUPPLEMENTS

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Milk thistle (*Silybum marianum*) is a herb widely used for production of food supplements, expected to support the proper liver function and treat the liver diseases. The major health beneficial component is silymarin, a complex of structurally similar flavonolignans possessing a high antioxidant potency and hepatoprotective effects. However, our previous long-term research revealed also the presence of rather rich 'cocktail' of contaminants, especially mycotoxins from *Alternaria* and *Fusarium* fungi genera. Considering their toxic effects, especially the hepatotoxicity of HT-2 and T-2 toxins which were found to be present even at concentrations fulfilling the tolerably daily intake (TDI) for adults, the final health-beneficial effect of milk thistle-based food supplements is of a big question mark. Therefore, within our on-going interdisciplinary study, we focus on the full 'chemical' characterization of the milk thistle-based matrices that is required for planning and evaluation of the subsequent *in-vitro / in-vivo* experiments testing the milk thistle extract biological effects. As concerns the characterization of chemical pattern of present substances, in addition to analyses of contaminants (realized by the multi-detection method targeting mycotoxins, pesticides and plant alkaloids by the U-HPLC-HRM/MS approach), a great effort have been devoted to the characterization of chemical representation of particular silymarin flavonolignans, which can also significantly influence the final biological effects. For this purpose, we employed the advanced technology of Ion Mobility Q-TOF LC/MS instrumental platform (Agilent 6560), enabling the additional dimension in separation of isobaric ions. Additionally to the retention time and exact mass, also the drift time and collision cross section values are determined by the ion mobility analyser, reflecting the structural character of the molecule, thus allowing significant increase of potential to resolve as many structural isomers, as possible. In case of silymarin, the ion mobility technique enabled the clear separation of all of the isomeric flavonolignans and additionally revealed a presence of at least two more isomeric compounds (so far unknown), co-eluted and thus hidden under the condition of a common chromatographic setup. We hope that the project will bring new insights to the assessment of milk thistle-based food supplements safety.

Acknowledgements
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ASSESSMENT OF ANTIDIABETIC PHYTOCHEMICALS AND BIOACTIVITIES IN STEM JUICES FROM BANANA

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In this study, the stem juices from Musa × paradisiaca L banana plants cultivated in their original natural habitat in Vietnam and those cultivated in a greenhouse in the Czech Republic were investigated for the presence of phytochemicals with antidiabetic potency and respective bioactivities. The sample screening by UHPL-HRMS/MS method showed some differences in the pattern of bioactive compounds, both in terms of their number and concentration. p-hydroxybenzoic and gallic acids were dominating analytes screened in a stem juice from plant grown in Vietnam, while ferulic acid was the major compound found in juice obtained from greenhouse banana. Despite the differences in occurrence of potentially antidiabetic compounds, both extracts exhibited comparable inhibitory activity against α-glucosidase and α-amylase. The high throughput robotic platform was used for determination of bioactivities providing maximal efficiency, miniaturization and accommodation of many different assay protocols.

Acknowledgements
This work was also supported by the "Operational Programme Prague – Competitiveness" (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503) and the "National Programme of Sustainability I" - NPU I (LO1601 - No.: MSMT-43760/2015).

REIMS – FOOD FRAUD DETECTION IN REAL TIME

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Fish fraud detection is mainly carried out using a genomic profiling approach requiring long and complex sample preparations and assay running times. Rapid evaporative ionisation mass spectrometry (REIMS) can circumvent these issues without sacrificing a loss in the quality of results. Our study investigated various aspects of fish fraud (speciation, catch method and geographic origin) to assess how the REIMS technology could aid the detection of fish fraud. 478 samples of five different white fish species were subjected to REIMS analysis using an electrosurgical knife. Each sample was cut 8-12 times with each one lasting 3-5 seconds and chemometric models were generated based on the mass range m/z 600-950 of each sample. The identification of 99 validation samples provided a 98.99% correct classification in which species identification was obtained near-instantaneously (≈2s) unlike any other form of food fraud analysis. Significant time comparisons between REIMS and polymerase chain reaction (PCR) were observed when analysing 6 mislabelled samples demonstrating how REIMS can be used as a complimentary technique to detect fish fraud. Additionally, the geographic origin of different shrimp samples and the catch method of haddock samples were found to be capable of detection using REIMS, the latter being a concept never previously scientifically reported. Overall REIMS has been proven to be an innovative technique to aid the detection of fish fraud and has the potential to be utilised by fisheries to conduct their own quality control (QC) checks for fast accurate results.

ALTERNATIVE ANALYTICAL STRATEGIES FOR DETECTION OF UNDECLARED MOISTURE BINDERS IN MEAT

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Replacing the portion of high value net muscle proteins by addition of various moisture binders into meat and products thereof is one of relatively common adulteration practices which is rather difficult to identify. The total meat protein content in meat products is in Czech Republic regulated by the Decree No. 69/2016 and for its control Kjedahl method (determination of nitrogen bound in proteins after mineralization) is commonly used. To compensate the decrease of protein content determined by Kjeldahl method, low grade protein such as collagen can be added to increase nitrogen content to common values. To provide an unambiguous evidence on such adulteration, reliable analytical strategies have to be available. Worth to notice that those methods recommended by the above mentioned Decree are rather complicated and time consuming, moreover the results might be questionable under some conditions.

For a pilot experiment, a sample set of pork and/or chicken meat homogenate adulterated with various collagen protein powders or, alternatively, with plasma powder, chicken separated meat and carrageenan was prepared for testing by two alternative analytical strategies. The first of them was represented by Rapid Evaporative Ionization Mass Spectrometry (REIMS). This recently introduced ambient mass spectrometric technique was used to spot possible unusual profiles in the obtained mass spectra of tested samples. The second strategy we employed was the detection of specific 3-methylhistidine dipeptides, carnosine, balenine and anserine, which are contained only in meat protein and their content is specific for particular meat species. The additional markers on which authentication of net protein content can be the amount of specific amino acids with 1- and 3-methylhistidine, i. e. amino acid profile as determined by HPLC-MS after sample hydrolysis has to be evaluated. Critical assessment of the feasibility of the above analytical strategies for fraud detection will be presented.

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LC-MS BIOMARKER DISCOVERY AND APPLICATION IN THE FIELD OF FOOD ADULTERATION

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Due to increasing number of food fraud incidents, there is an inherent need for the development and implementation of analytical platforms enabling detection and quantitation of adulteration.

A methodology based on marker discovery employing LC-HRMS data with subsequent transfer of these markers to LC-MS/MS with rigorous in-house validation proved to be an efficient strategy in the development of simplified MS based approach to address the problem of food adulteration. Two different application/case studies on oregano adulteration and shrimp speciation will be presented to demonstrate the applicability and the performance of the developed methodology.

LEAVING THE LABORATORY BEHIND: RAPID IN-FIELD FOOD AUTHENTICITY SCREENING USING HANDHELD SPECTROSCOPY

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Food fraud is an extremely topical and important issue. It is estimated to cost the world economy $US49b per year. The National Food Crime Unit estimates that it costs British families £1.17b per year. The British Retail Consortium suggest 1 in 10 consignments of imported basmati rice has been adulterated, whilst our recent work on oregano fraud showed 25% of retail product, on UK shelves, was adulterated and this pattern is similar globally.

Economic gain is often the goal. Authentic products are substituted with (or diluted by) inferior/ cheaper products, as was exemplified by the 2013 European horsemeat scandal. However, there are also health implications, for example the 2008 adulteration of Chinese infant formula, with melamine, which saw over 300,000 babies hospitalised with multiple mortalities.

Conventional methods used to determine food authenticity are laboratory based, require skilled operators, are expensive or time consuming and can take days or even weeks to complete. Meanwhile the product passes along the supply chain and in many cases reaches the supermarket shelf or consumer tables before results are reported. New and better ways of checking the authenticity of foods and their ingredients are required. Methods capable of rapid detection anywhere, anytime, in the food supply chain are what industry are demanding. This can be achieved through the use of handheld spectroscopic analysis in conjunction with chemometric modelling.

This presentation discusses our approach to addressing these issues using our latest work on rice authenticity as an example.