Program Fall Meeting 2014

Dutch Society for Mass Spectrometry

Food and Environmental Mass Spectrometry

16 October 2014

Wageningen University and Research Centre

NVMS fall meeting

Food and Environmental Mass Spectrometry

16 oktober 2014 WUR, Zodiac room C0086

9:30-10:00	**Registration**	
10:00:10:15	**Opening fall meeting 2014, In Memoriam: Nico Nibbering**	
10:15-10:40	**Michel Nielen (RIKILT/WUR)** 'Macroscopic and microscopic spatially-resolved analysis of food contaminants and constituents using Laser Ablation Electrospray Ionization Mass Spectrometry Imaging'	Chair:
10:40-11:05	**Twan America (PRI)** 'Untargeted and targeted LC-MS proteomics in allergen research'	
11:05-11:30	**Melliana Jonathan (WUR)** 'Separation and identification of individual alginate oligosaccharides in the faeces of alginate-fed pigs'	
11:30:12:00	**Conference fund winner:** **Anne Bruinen (AMOLF)** 'Multimodal imaging for biological applications: X-ray microCT and mass spectrometry imaging combined'	
12:00-13:15	**Lunch**	
13:15-13:35	**Algemene ledenvergadering NVMS**	
13:35-14:00	**Ric de Vos (PRI)** 'MS-based metabolomics as tool in crop and food quality research'	Chair:
14:00-14:25	**William van Dongen (TNO Triskelion)** 'Improving food quality and safety using mass spectrometry'	
14:25-15:00	**Coffee/Tea break**	
15:00-15:25	**Sicco Brandsma (IvM VU)** 'Tracing POP-BDE routes through plastic waste streams in the Netherlands'	Chair:
15:25-15:50	**Ana Causanilles Llanes (KWR)** 'Mass spectrometric determination of erectile dysfunction pharmaceuticals in sewage water'	
15:50-16:00	**Closure**	
16:00-17:00	**Borrel/drinks**	

Macroscopic and microscopic spatially-resolved analysis of food contaminants and constituents using Laser Ablation Electrospray Ionization Mass Spectrometry Imaging

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Laser ablation electrospray ionization (LAESI) mass spectrometry imaging (MSI) does not require very flat surfaces, high precision sample preparation or the addition of matrix. Thanks to these features LAESI-MSI may be the method of choice for spatially-resolved food analysis. In this work, LAESI time-of-flight MSI has been explored for macroscopic and microscopic imaging of pesticides, mycotoxins and plant metabolites on rose leaves, orange and lemon fruits, ergot bodies, cherry tomatoes and maize kernels. Accurate mass ion map data were acquired at a sampling location x-y center-to-center distance of 0.2-1.0 mm and superimposed onto co-registered optical images. The spatially-resolved ion maps of pesticides on rose leaves suggest co-application of registered and banned pesticides. Ion maps of the fungicide imazalil show that this compound is only localized on the peel of citrus fruits. However, according to 3D LAESI-MSI the penetration depth of imazalil into the peel shows significant local variations. Ion maps of different plant alkaloids on ergot bodies from rye show co-localization in accordance with expectations. The feasibility of untargeted MSI in food analysis is demonstrated by ion maps of plant metabolites in cherry tomatoes and maize kernel slices. In the tomato case, traveling-wave ion mobility (TWIM) is applied to discriminate between different lycoperoside glycoalkaloid isomers; in the maize case quadrupole time-of-flight tandem mass spectrometry (MS/MS) is successfully used to elucidate the structure of a localized unknown. It is envisaged that LAESI-MSI will contribute to future research challenges in food science, agriforensics and plant metabolomics.

Untargeted and targeted LC-MS proteomics in allergen research

Twan America

Plant Research International, BU Bioscience, Wageningen UR, Droevendaalsesteeg 1, 6708 PB Wageningen

At Plant Research International (part of Wageningen UR) we operate a proteomics facility for high resolution characterisation of complex protein samples. Characterisation means

- identification of large number of proteins in the samples
- quantitative analysis (label-free) of differences between multiple extracts
- development and use of targeted quantitation methods for selected target proteins

In my presentation I will focus on applications in the characterisation of allergen samples, e.g. birch pollen allergen and gluten gliadin epitope quantitation.

Separation and identification of individual alginate oligosaccharides in the faeces of alginate-fed pigs

Melliana C. Jonathan[1], Guido Bosch[2], Henk A. Schols[1] and Harry Gruppen[1]

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The aim of the experiment was to develop a method for separation and identification of alginate oligosaccharides (AOS). To produce AOS, alginates with different composition of glucuronic acid (G) and mannuronic acid (M) were partially hydrolysed in oxalic acid, followed by isolation of G-rich and M-rich blocks. Further, the G-rich and M-rich blocks were treated with alginate lyase to obtain unsaturated AOS, or treated in acid to obtain saturated AOS. The resulting AOS were then analysed using UHPLC-MSn equipped with BEH Amide column. Saturated and unsaturated AOS from DP 2-10 could be separated and isomers were partially separated. Isomers of unsaturated AOS with DP 3 and 4 were annotated based on their fragmentation patterns.

The method developed using standard AOS was then applied for analysing AOS present in the faeces of pigs which are a part of a study about alginate as a dietary fibre. Dietary fibres are not absorbed in the small intestine and they can be fermented in the colon by gut microbiota. The fermentation of dietary fibres was claimed to contribute in colon health. Alginate is a dietary fibre that was shown to be slowly fermented in vitro. The experiment in pigs provided more information on the speed and the mechanism of alginate degradation in vivo. AOS were detected in the water-soluble part of the faeces. The AOS were obviously degradation products of alginate due to the action of gut microbiota, because the pigs were fed alginate polymer. Unsaturated and saturated AOS were present. The results of AOS identification and the monosaccharide constituent composition of the faeces showed that the gut microbiota utilised M more than G.

Publication:

Jonathan, M. C.; Bosch, G.; Schols, H. A.; Gruppen, H., Separation and identification of individual alginate oligosaccharides in the feces of alginate-fed pigs. Journal of Agricultural and Food Chemistry 2013, 61, 553-560.

Multimodal imaging for biological applications: X-ray microCT and mass spectrometry imaging combined

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Mass spectrometry imaging (MSI) is a label-free molecular imaging technique designed to visualize the spatial distribution and identify molecules on a surface. X-ray micro computed tomography (X-ray microCT) uses the differences in absorption between different tissue types to generate tissue contrast. Internal features of biological samples can be readily visualized with this approach. Complete 3D CT models can be reconstructed from images acquired under different angles. The combination of MSI and X-ray microCT provides complementary information on both molecular and anatomical structures within the sample.

A zebrafish (*Danio rerio*) was used as a model for tuberculosis, a granulomatous inflammatory disease. The CT/MSI overlays correlate the structural and molecular content of the granulomas present in the organs of infected fish.

An active pixel detector assembly consisting of four TimePix chips was used for the microCT scans. This detector contains a total of 512x512 pixels of 55 µm in size. This results in high resolution and low noise X-ray images. The high dynamic range results in the ability to image both hard and soft tissue simultaneously. A 3D model was built from 100 projections under different angles. Tissue cryosections were prepared from the frozen and embedded fish after completion of the microCT experiment. Sections were employed for MALDI-MSI and SIMS imaging.

The distributions of endogenous lipids and specific lipids found in the tuberculosis granulomas were selected from the MSI data and their spatial distribution were visualized in a 2D image as well as projected on a 3D CT model.

MS-based metabolomics as a tool in crop and food quality research

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Comprehensive MS-based metabolomics profiling techniques are frequently applied nowadays in plant biology and plant-derived food research, in order to get a detailed view of the effects of, for instance, genetic variation, growth conditions, post-harvest effects, food processing, nutrition, etc. Especially the so-called untargeted approaches, in which all metabolites detected in the samples, both known and unknown, are taken into consideration, have provided novel insights into key metabolites and biochemical pathways related to economically important traits of (crop) plants and their products. During this presentation a few examples of using complementary untargeted metabolomics technologies to determine differences and similarities between samples in their global metabolite composition, in relation to specific differences between crop plant and their products, will be highlighted.

A major application of plant metabolomics is in research aimed to get insight into the genetical and biochemical mechanisms determining crop quality aspects such as taste, colour and health-related compounds. For instance, to identify metabolites that are key to tomato quality, ripe fruits from large series of tomato genotypes have been analyzed for both colour, sensory characteristics and phytochemical composition using various metabolomics platforms. Subsequent correlation analyses revealed novel compounds and biochemical pathways determining differential tomato quality, as well as genes controlling the accumulation of these quality-related compounds. These novel markers are now used in breeding programs aimed to develop new tomato varieties with improved quality characteristics.

Industrial processing for plant-derived food products is another important application area for metabolomics. An in depth metabolomics comparison of plant products analyzed at various steps of the industrial processing revealed those steps and activities that have major impact on the global metabolite composition and related consumption quality of the product. As examples, untargeted metabolomics has been used to determine metabolite alterations and losses occurring at each step of industrial processing of tomato fruit to paste. We also applied metabolomics to green coffee beans that were processed and dried after harvest at different conditions at a plantation in Brazil. Substantial differences in metabolite composition of coffees related to post-harvest practice were identified, including compounds determining final cup quality. By correlating untargeted metabolomics profiles to the sensory data of coffee brews, new marker compounds for cup quality were identified.

Improving food quality and safety using mass spectrometry

W.D. van Dongen

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Mass spectrometric based methods enable to describe and predict properties of food products and processes. It concerns identifying so-called quality markers which correlate with quality, safety, taste or fragrance of foodstuffs. Examples of this approach are the identification of compounds that render the identification of substances that play an important role in flavor development in cognac. Moreover, food safety assessment is currently based on known individual food components. More and more questions concern the assessment of complex chemical mixtures or matrices with a high percentage of unknown compounds. For these complex food products it is unrealistic to identify and quantify the complete forest of peaks and perform a safety assessment for all peaks observed. A pragmatic protocol for safety assessment of complex food products is currently under development, based on an integrated assessment of exposure, toxicology and chemical analysis. A cost-effective analytical strategy has been developed aiming to assign high-risk substances in complex mixtures present at toxicological relevant concentrations, rather than time consuming peak-by-peak identification and quantification. For this purpose the threshold of toxicological concern (TTC) principle is used. The TTC principle was defined assuming threshold values for classes of chemicals based on their chemical structures and known toxicity of chemicals that share similar structural characteristics. TNO Triskelion is setting up (bio-)analytical strategies that allow the use of the TTC principle for safety testing of complex food matrices. In this presentation these (bio-) analytical tools involving mass spectrometry are explained and demonstrated for several food matrices.

Tracing POP-BDE routes through plastic waste streams in the Netherlands

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There is currently only limited understanding of the dynamics of the distribution and levels of tetra-, penta-, hexa- and heptabrominated BDE congeners (POP-BDE) in waste streams in Europe and worldwide. This study investigated how plastic waste materials that may contain POP-BDE are sorted, separated, disposed of, recycled, landfilled, incinerated and/or exported in the Netherlands. This was coupled to measured concentrations of POP-BDEs in samples taken from four points in the waste streams to study the mass flow of these regulated POPs through the Dutch plastic cycle.

A cost-effective, fast 'direct probe' screening method developed at IVM was applied to quickly determine the presence or absence of POP-BDEs. The method makes use of a direct probe coupled to atmospheric pressure chemical ionization-high resolution time-of-flight-mass spectrometry (APCI-HRTOF-MS). The method can be used to screen samples for POP-BDEs in plastic, so that the more laborious solvent extraction procedures are only done when quantifiable amounts are present. In total 90 samples were selected for determination of the POP-BDE concentrations, using gas chromatography with the electron capture negative ionization technique and mass spectrometry detection (GC/ENCI-MS), using a highly sensitive method[3,4].

This study provides a unique POP-BDE dataset for the relevant plastic waste streams in the Netherlands. POP-BDEs were rarely found in single automotive parts (when detected, it was only in car parts from the USA) or WEEE items. Shredder material (consisting of a large number of shredded items), frequently did contain POP-BDE. The samples in which POP-BDEs were detected confirmed that c-PentaBDE can be found in automotive, whereas the c-OctaBDE pattern is found in WEEE. DecaBDE (not a Stockholm POP) is frequently found in plastic fractions from shredded automotive and WEEE material and recycled plastic pellets.

Based on the mass flow analysis, 22% of the POP-BDE in WEEE is expected to end up in recycled plastics. In the automotive sector, 14% of the POP-BDE is expected to end up in plastics recycling, while an additional 19% is expected to end up in second-hand parts (reuse). POP-BDEs were detected at low concentrations in some new products made from recycled plastic (imported products). This also indicates that the legacy of POP-BDEs will be with us for quite some years to come, until the plastics are downcycled to the point that they are sent for incineration.

Mass spectrometric determination of erectile dysfunction pharmaceuticals in sewage water

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Erectile dysfunction pharmaceuticals were released into the market in the 90's (Viagra®) and 00's (Levitra® and Cialis®), only available under medical prescription. The difficulty to access to them seem to induce consumers to use rogue online pharmacies or counterfeit products, sometimes containing the active compound (patent protected until 2013) or designer analogues.

Sewage-based epidemiology is a powerful tool for assessing population lifestyle of a studied area, as everything that human beings consume is excreted via urine and/or feces either unchanged or transformed into metabolites. The occurrence of all three erectile dysfunction treatment drugs has been reported in sewage water at very low concentrations (5-30 ng/L) mainly as the parent compounds. However, most of the literature on pharmacokinetics in humans shows that after a single dose 90% of the drug was excreted as metabolites with no detectable parent in either feces or urine.

Consequently, the identification of metabolic transformations and analogues is particularly important to assess environmental distributions. This work presents an analytical method based on liquid chromatography coupled to tandem mass spectrometry for the quantification of the three active pharmaceutical ingredients and their transformation products and analogues.

The method was applied to real influent samples collected during the week-monitoring campaigns of 2013 and 2014 in the cities of Amsterdam, Eindhoven and Utrecht, and a festivity in Amsterdam in 2012 and 2014. For the case of Viagra®, the estimated use from the load in sewage water was compared to the expected "modeled" load from official sales data. The difference was considered as illegal use.

AGENDA GENERAL ASSEMBLY OF THE DUTCH SOCIETY FOR MASS SPECTROMETRY

Date: 16 October 2014

Time: 13:15

Place: WUR, Zodiac room C0086, Wageningen

Agenda:

1. Board changes
2. Recap NVMS Spring Meeting @ Rolduc
3. IMSC 2018
4. NVMS Conference Fund
5. A.o.b.