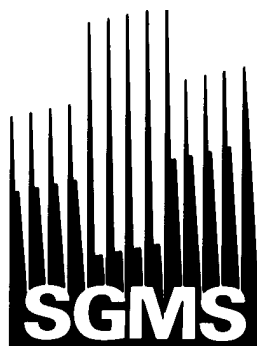


Swiss group for mass spectrometry
Schweizerische Gruppe für Massenspektrometrie



Groupe suisse de spectrométrie de masse
Gruppo svizzero di spettrometria di massa

Newsletter

Rigi Meeting 2003

and

General Assembly 2003

Dorint Hotel Blüemlisalp, Beatenberg

October 30 and 31, 2003

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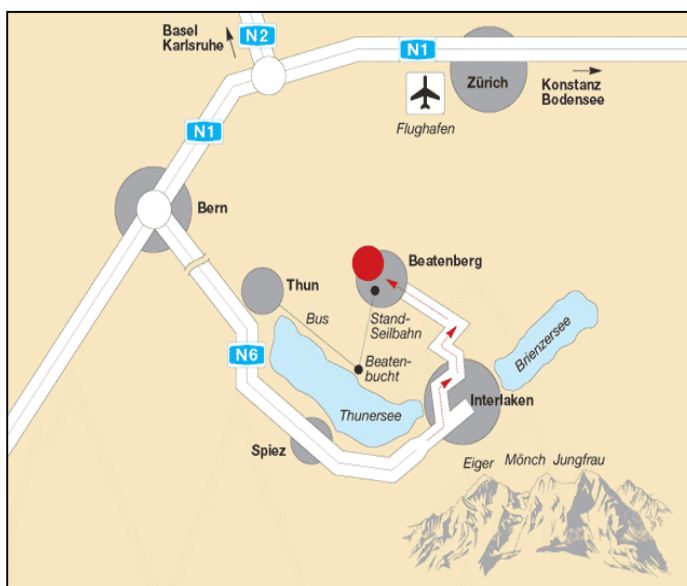
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Travel information

Please note that the Beatenbucht-Beatenberg mountain railway is running. No special prices for the SGMS Rigi Meeting!

By car: Take Highway no. 6 from Berne to Thun, then no. 8 direction Interlaken. Right before reaching Interlaken follow direction Gunten/Unterseen/**Beatenberg** at the first possible off ramp.

In Beatenberg: Don't be afraid of



having missed the Dorint Hotel Blüemlisalp. Beatenberg is a stretched mountain village. Our destination is located at the very far end. Parking lots of the Hotel are free of charge.

By train: Geneva , Lausanne and Fribourg take IC713 leaving Geneva at 07:30 platform 3, **Lausanne 08:06** platform 1 and **Fribourg at 08:51** platform 3 arriving in Berne at 09:13 platform 4

Basel take **IC965** leaving **Basel at 08:04** platform 8 arriving in Berne at 08:11. Please stay in the train. It will continue to Interlaken West.

Schaffhausen take D2559 leaving Schaffhausen at 07:07 platform 1.
Zurich take **IC810** leaving **Zürich at 08:04** platform 15 arriving in Berne at 09:13 platform 8

Berne take **IC965** leaving **Berne at 08:26** platform 6 arriving in Interlaken West at 10:14.

Interlaken take **Bus 109** leaving **in front of the main station at 10:25.**

The bus **will arrive at 10:53 in front of the Hotel Dorint Blüemlisalp.**

September 20, 2003

Dear member and sponsors of the SGMS

It's amazing, already 75 persons have registered for the Rigi meeting 2003*, 30 and 31 October at Dorint Hotel Bluemlisalp, Beatenberg. AND the closing date for registration has not yet passed. I remember the charts from Laurent Fay – former president of the SGMS – where he showed us the incredible amount of registrations through the very last days before the meeting. If this happens again, who knows how many persons we will meet at the Rigi meeting 2003 ? This is one reason for not finding a list of all the participants of the Rigi meeting 2003 in this newspaper. In addition we will again post this newsletter on the web and the committee does not want to offend the members of the SGMS by a list of the active members on the web open for public. Thanks for your understanding.

Due to the fact that we use for the meeting, the general assembly and later on for the dinner the same room, the management of the Dorint Hotel Bluemlisalp needs to change the arrangement of the tables. This certainly will take some time and will give us the pleasant possibility to meet for an apero in the lobby of the Dorint Hotel Bluemlisalp. This will be a perfect opportunity to meet and discuss. Even this year we will get the superb "Schweizer Buffet". Seating will be the same as last year on big round tables. So meet and mix till you find the persons you would like to dine with !

Andreas Staempfli

*Must be due to our invited speakers and of course due to the nice and perfect location !

Or could it be that there is another reason ?

October 30, 2003

11:15 – 11:30 Welcome Notes

<i>Starting Session: Small Molecules</i>	Chair: Andreas Staempfli
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11:30 - 12:15 Quantitative and Qualitative Analysis of Small Molecules by Mass Spectrometry: with or without Liquid Chromatography?
Gérard Hopfgartner

12:30 - 14h00 Lunch

<i>GC, LC Separations</i>	Chair: Raffaele Tabacchi
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14:00–14h45 A Time-of-Flight Mass Spectrometer: a New Horizon in GC Separations
René J.J. Vreuls

14:45 - 15:05 HRGC-EI-MS Method for Detection of In-house Moulds Attacks by Analysis of Microbial Volatile Organic Compounds (MVOCs) in indoor air
Sven Heekmann

15:05 – 15:25 A MS/MS Library of Natural Products on an Ion-Trap Instrument
Andreas Fredenhagen

15:25 – 15:45 A Multi Residue HPLC/MS/MS Method for the Determination of 81 Pesticide Residues in Fruit and Vegetables
Sandra Rontree

15:45 - 16:05 Coffee Break

Clinical Chemistry and ...	Chair: Jean-Luc Wolfender
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16:05 – 16:25 Sensitive and Specific Determination of Vitamin K1 in Human Plasma by High-Performance Liquid Chromatography Tandem Mass Spectrometry
Katharina M. Rentsch

16:25 – 16:45 Application of Mass Spectrometry in Clinical Chemistry
Kornél Nagy

16:45 – 17:05 ACD/Labs MS Manager – Combine Heterogeneous Data to Homogenous Structured Knowledge
Matthias Weisser

17:05 – 17:25 Accurate Mass Analysis on a Chromatographic Timescale
Winfried Wagner-Redeker

17:30 General Assembly

19:00 Aperó

20:00 Blüemlisalp Dinner Buffet

October 31, 2003

<i>ICP Mass Spectrometry</i>	Chair: Marc Suter
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08:30 - 09:15 BioAnalytical Applications of ICP-MS

Scott D. Tanner

09:15 - 09:35 Elemental Speciation Capabilities of IC-ICP-MS in Environmental Water

Adrian A. Ammann

09:35 - 09:55 Magnetic Sector Field ICP-MS as Sensitive Detector for Speciation Analysis

Meike Hamester

09:55 - 10:15 Detection of Noncovalent Protein-Ligand Interactions Using Automated Nano electrospray Mass Spectrometry

Joerg Niebel

10:15 - 10:45 Coffee Break

<i>Protein Analysis</i>	Chair: Hansjörg Walther
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10:45 - 11:30 MS and Structural Studies of Proteins and Protein Interactions

 Agilent Technologies

Kenneth B. Tomer

11:30 - 11:50 Characterization of Native Proteins by ESI-MS Thanks to a Dual-Channel Microfabricated Electrospray Emitter

Niels Lion

11:50 - 12:10 A Practical Cryodetector Mass Spectrometer for High Sensitivity Detection and Energy Resolution at High Mass

Urs Matter

12:10 - 12:30 Quantitative and Data-Dependant MS acquisition

G.L. Corthals

12:30 - 12:50 Terminus-Specific Fragmentation, a Novel Tool for the Direct Characterization of Intact Proteins Using MALDI-MS/MS and MS/MS Techniques

Uwe Rapp

12:50 – 13:00 Closing Remarks**13:00 - Lunch at Dorint Beatenberg (voluntary)**

Quantitative and Qualitative Analysis of Small Molecules by Mass Spectrometry: with or without Liquid Chromatography ?

***Gérard Hopfgartner
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Life Sciences Mass Spectrometry
20, Bd d'Yvoy, 1211 Geneva, Switzerland***

For the analysis of pharmaceutical compounds in biological fluids liquid chromatography combined with mass spectrometric detection is the method of choice either as a concentration, purification or/and a separation step in the analytical procedure. High throughput quantitative analysis is achieved with high-flow or parallel LC using generic sample preparation approaches, such as direct plasma injection.

Triple quadrupole mass analysers are largely used for the quantification of small molecules, while ion trap mass spectrometers are more suited for drug metabolism studies. Both mass spectrometers are complementary and ideally one would like to have all features of both instruments in one system. The situation becomes even more complex with hybrid instruments such as quadrupole time of flight mass spectrometer, where medium resolution and accurate mass of the precursor ions and the fragment ions can be obtained with high sensitivity. Accurate mass MS/MS spectra can also be recorded on triple quadrupoles with enhanced resolution. Recently, hybrid instruments using linear ion traps have become available opening completely new possibilities in qualitative and quantitative analysis.

Quantitative bioanalysis on an disposable ESI Chip has been demonstrated without chromatographic separation. With the high selectivity of mass spectrometric detection how much chromatography do we really need ?

A chromatographic peak elutes within 10 and 30 seconds. For quantitative analysis this is sufficient while for qualitative analysis several injections of the

same sample are required to perform all relevant experiments. Fraction collection prior MS detection and infusion of the relevant peaks by ESI Chip is one approach to overcome the time constrain. With off-line analysis various MS experiments can be performed for structure elucidation by minimising analysis time and sample consumption.

Selected examples in drug metabolism and quantitative analysis will be presented with the emphasis fish out needles in a haystack and quantify them.

A Time-of-Flight Mass Spectrometer: a New Horizon in GC Separations

***René J.J. Vreuls
Vrije Universiteit***

***Department of Analytical Chemistry and Applied Spectroscopy
de Boelelaan 1083, 1081 HV Amsterdam, the Netherlands
(e-mail: Vreuls@chem.vu.nl)***

Time-of-flight mass spectrometry (TOF-MS) is a very powerful technique with limits of detection in the low pg range. Furthermore, the spectrum storage rate can be as high as 500 spectra per second. Both advantages have been exploited using a faster GC mode than is commonly used in the GC laboratory. In this mode columns are short and narrow, while temperature gradients are steep (complete volatility range in 3–10 min). In combination with TOF-MS, detection limits in the low pg-range have been obtained, viz. 1–6 pg for organophosphorus pesticides, 4–60 pg for triazine herbicides and 0.3–6 pg for polycyclic aromatic hydrocarbons. Linearity of response (2 pg–10 ng range) and RSD values at adequate levels were good. Due to the high acquisition rate, automated spectral peak deconvolution could be used. This highly powerful software option enabled calculation of spectra from overlapping peaks. In actual practice this meant that peaks had to be separated only by 3 spectra (e.g. 0.15 s at a spectrum storage rate of 20 Hz). This was especially valuable in case of complex samples. GC–TOF-MS was applied to the determination of the above compound classes in various aqueous and solid extracts (by means of miniaturised extraction procedures). An aliquot was injected into the GC system using splitless or large-volume injection. The technique has meanwhile been accepted and applied to various analyte groups, *e.g.* doping in urine, VOCs in drinking water, explosives in waste water and many more.

Comprehensive GC (GCxGC) is a relatively new technique, which has proven to be very powerful for analysis of **very** complex samples. The compounds eluting from the first-dimension column are continuously trapped and reinjected into a very fast second-dimension column by means of a timed modulator. In the early stage of this technique, it has mainly been demonstrated in the field of oil industry, where various distillate fractions showed up containing thousands of ordered peaks. In the past few years many other applications have been described in the literature. Due to the narrow peaks in the second dimension, detection has to be in the 50–200 Hz range. Flame ionisation and electron capture detection have successfully been used. Identification is usually based on retention time determination in both dimensions using standards. Expertise knowledge and chemical intuition are also often used. The combination with TOF-MS resulted in a true three-dimensional separation technique. The high spectrum storage rate guaranteed full recording of the fast eluting peaks (150–250 ms at the base). Applications in the field of fruit (pesticides; volatile flavours), food (contaminants, flavours) and environmental analysis (pesticides; polycyclic aromatic hydrocarbons, PCBs and dioxins) will be demonstrated. Analytes of interest were isolated using newly developed miniaturised extraction procedures.

HRGC-EI-MS Method for Detection of In-house Moulds Attacks by Analysis of Microbial Volatile Organic Compounds (MVOCs) in Indoor Air

Sven Heekmann and Michael Oehme*
Organic Analytical Chemistry, University of Basel, Neuhausstr. 31
CH-4057 Basel, Switzerland

In the industrialized countries 80 - 90% of the time is spent indoors. The effect of indoor air exposure is therefore very important for people's health and well-being. Among other sources fungi are made responsible for affecting the air quality and therefore the indoor climate negatively by emitting odours and substances, which cause irritations.

Identification of indoor mould attacks is mainly done by sampling and counting spores and conidia. In contrast to spore emission which is highly dependent on seasonal and environmental parameters, mould generates continuously volatile organic compounds. These so-called microbial volatile organic compounds (MVOCs) are considered to be a very good indicator for mould attack, also when hidden. Most MVOCs suitable as mould tracers are rather polar C₆-C₈ compounds such as aliphatic and aromatic aldehydes, ketones and alcohols. A number of them are chiral and because of their natural origin non-racemic.

Because MVOCs might also arise from other sources such as building materials or paints, the expected non-racemic nature of the MVOCs produced by mould can be used to differentiate from technical products by enantio-selective GC-separation.

In this work first results are presented about a passive sampling technique using active charcoal adsorbents to trap MVOCs from mould. A sampling time of 28 days allow to collect 1,5 m³ of air. Solvent desorption by diethyl ether and separation by HRGC-EI-MS in the selected ion monitoring (SIM) mode

give detection limits of about 3-10 ng/m³ (absolute 5-15 pg) for most compounds and allow multi sampling analysis.

The separation and analysis of trace amounts of alcohols and other polar substances is still a demanding task. On-column injection was found most suitable giving excellent peak shapes as well as minimal thermal stress. Cross-linked polyethylene glycol phases were able to separate all relevant compounds at pg-levels from other compounds present in air. For the enantiomeric analysis a modified cyclodextrin phase was found where 12 of the selected chiral MVOCs could be separate in the same run.

A MS/MS Library of Natural Products on an Ion-Trap Instrument

Andreas Fredenhagen, Caroline Derrien & Ernst Gassmann
Syngenta Crop Protection, Basel, Switzerland
andreas.fredenhagen@syngenta.com

Natural products are a continuous and proven source of new lead compounds for the agrochemical and pharmaceutical industry. The screening for novel bioactive compounds is an increasingly challenging area due to successes in the past and thousands of compounds described in the literature. This is especially true in the agrochemical industry, where the bioassays for insecticides, herbicides and fungicides are essentially the same since the beginning of natural products research about 50 years ago. It is therefore important to detect known compounds directly in the extract at a very early stage of the discovery process. This process, called dereplication, allows prioritizing the work on strains which produce novel compounds.

A safe identification of compounds in a crude extract requires the use of several parameters, such as molecular ion, MS/MS spectra, retention time, and UV/VIS spectra. Additionally, a single method is needed for analyzing a wide range of substance classes. Hence, a LC/MS system consisting of a UV/VIS DAD detector and an ion trap mass spectrometer (ESI mode) was used for building up UV/VIS and MS/MS spectral reference libraries, respectively. An ion trap mass spectrometer was selected because it offers higher yields of daughter ions than many other types of mass spectrometers. MS/MS and UV/VIS spectral libraries were generated from commercially available and in-house stocked compounds. About 95% of the compounds were successfully recorded with positive ionization. Only those compounds, which gave no signal in positive ion mode, were analyzed in negative ion

mode. $[M + H]^+$ ion as precursor was the preferred choice. The resulting spectra are categorized statistically.

For some compounds **different MS/MS spectra** were observed by fragmenting $[M+H]^+$ or $[M+Na]^+$ as the precursor ion. The fragmentation mechanisms of the latter seem to be determined by the position of the chelated sodium ion rather than the stability of individual bonds. Therefore MS/MS of $[M+Na]^+$ ions exhibit more specific fragments than $[M+H]^+$ ions and thus allow a secure identification based on their MS/MS spectra.

Different MS/MS fragmentation mechanisms were found for **oligomycin A** in the positive and negative ion mode. The first fragmentation step in ESI(+) is preferentially the ester bond followed by retro-aldol condensations at three sites, while in ESI(-) two independent pathways were observed by MS³.

A Multi Residue HPLC/MS/MS Method for the Determination of 81 Pesticide Residues in Fruit and Vegetables

***Gordon Kearney*¹, Lutz Alder², Anthony Newton¹, Jeannette Klein²
and Sandra Rontree³***

¹Waters Corporation, Manchester, UK

²Federal Institute for Risk Assessment, Berlin, Ger

³Waters BV, Almere, NL

The analysis of pesticide residues in fruit and vegetables in support of EU government monitoring programmes can be challenging in terms of both the reporting levels required and the number of target analytes. Given the large number of pesticide residues that may be found in foodstuffs, it is advantageous to determine as many as possible during a single analysis. 81 pesticides including carbamates, benzimidazoles, organophosphorus, oxime carbamate, sulfonylurea, triazines, cyclohexanedione oximes and ureas were examined. As the number and diversity of target analytes is increased the selectivity of the clean-up stage of sample preparation is compromised, resulting in a more complex sample matrix. Significant improvements in analytical selectivity may be achieved using the MRM method.

A MRM method was developed for the quantification of 81 pesticides and pesticide metabolites. A generic extraction procedure and cleanup was performed. The method was validated for 5 commodities: - raisin, avocado, tomato, wheat flour and lemon. For each of the five crop types matrix matched standards were generated at the 5, 20, 40, 60, 80 and 100 pg/ μ L levels for all analytes. LC separations were performed using a Waters Atlantis C₁₈ column 4.6 mm id x 100 mm. Experiments were performed on an Alliance 2795 HPLC coupled to a Waters MS Technologies Quattro Micro API triple quadrupole mass spectrometer.

A generic extraction and HPLC/MS/MS method, valid for a wide range of compound classes in a representative set of matrix types, was validated and shown to be suitable for the screening of 81 pesticide residue compounds in fruit and vegetables. The limits of determination achieved for the pesticides analysed are well below that required for surveillance monitoring in the EU. Therefore the method is clearly extendable to even greater numbers of pesticide targets within the compound classes examined.

Sensitive and Specific Determination of Vitamin K1 in Human Plasma by High-Performance Liquid Chromatography Tandem Mass Spectrometry

***Katharina M. Rentsch, Ursula Gutteck-Amsler, Arnold von Eckardstein
Institute for Clinical Chemistry, University Hospital Zürich, 8091 Zürich
katharina.rentsch@ikc.usz.ch***

Vitamin K1 (VK1) or phylloquinone is a cofactor in the posttranslational carboxylation reaction of glutamic acid residues in a number of clotting factors. Decrease of the activity of vitamin K (VK) dependent clotting factors can induce severe bleedings in humans. With the exception of intestinal malabsorption syndromes and oral anticoagulation, VK deficiency is rare in adults. Neonates, in contrast, would regularly develop VK deficiency due to small transfer via the placenta and low content of the breast milk and are therefore supplemented with VK for prophylaxis against VK deficiency bleeding. The determination of VK1 in blood is needed to differentiate bleeding in neonates and is useful to identify hypervitaminosis in orally anticoagulated patients who still have normal clotting factors despite a high dosage of VK antagonists.

Therefore, we developed a LC-MS/MS method for the determination of VK1 in human plasma.

The sample preparation consisted of protein precipitation, liquid-liquid extraction and further purification by solid-phase extraction. VK1 was analyzed by reversed-phase chromatography and detected by atmospheric pressure chemical ionization (APCI) tandem mass spectrometry on a triple stage quadrupole.

The calibration curves were linear in a range of 0.5–5.0 µg/l. The limit of quantification was 0.2 µg/l, the precision <6% and the accuracy 95.3–104%. Stability experiments with spiked plasma samples showed that the analyte was stable during 24 hours at room temperature and subdued light but degraded completely within 8 hours when exposed to daylight.

This highly specific method for the determination of VK1 allows the quantification of this compound in patients with unexplained bleeding problems and for clinical studies.

Application of Mass Spectrometry in Clinical Chemistry

Kornél Nagy
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Chemistry Institute, Mass Spectrometry Department
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e-mail: kory@chemres.hu

The main point of the presentation is to demonstrate the power mass spectrometry in the field of routine clinical chemistry and clinical research, emphasizing the importance of the life saving newborn screening.

The introduction of soft and atmospheric pressure mass spectrometric methods opened a new horizon for qualitative and quantitative analysis of clinically important diagnostic compounds, such as small molecular weight amino acids, acyl-carnitines, bile acids, fatty acids or even very high molecular mass biopolymers such as tumor marker glycoproteins. Thus, with the help of mass spectrometry (especially ES-MS/MS) early medical intervention, consequently avoidance of death, mental and physical retardation is possible. Applying mass spectrometry the sample requirements are very small and many illnesses can be screened simultaneously. Amino acid and acyl-carnitine profiles can be obtained from the same 5 μ l blood, besides the analysis times can be reduced down to 2 minutes. Such capabilities make mass spectrometry suitable for the screening of a whole population. Nevertheless, further research and developments are in progress to extend the list of screenable illnesses and to merge the existing methods in order to decrease cost and enhance performance. Also the characterization of high molecular weight components of blood receives more and more attention mainly via the analysis of tumor marker glycoproteins to find relationship between the micro heterogeneities of these molecules and the status of the malignant diseases.

ACD/Labs MS Manager – Combine Heterogeneous Data to Homogenous Structured Knowledge

***Dr. Matthias Weisser
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matthias@scienceserve.com***

Metabolite investigations impact both the discovery and development timelines of drug research. Mass spectrometry plays a key role in these investigations, since it provides both the sensitivity and selectivity required to promptly identify metabolites, degradants, and impurities. Current competitive pressures are challenging analytical groups to transform raw data into final reports ever faster. It would seem as though the bottleneck lies in the tedious analytical tasks that must be performed by the analysts. Since software has a proven track record in performing tedious tasks and handling large volumes of data, it is likely a good candidate for speeding up metabolic studies. ACD/Labs software has been used to accelerate metabolic studies in seven distinct ways relating to mass spectrometry:

Handling Instrumental Data from Multiple Sources

Support the predominant analytical data formats in one homogeneous platform for a wide variety of instrument and techniques.

Extracting Metabolite Samples from LC/MS

Reliably get rid of noise and eluent signal, and compare multiple LC/MS samples to find only those showing a different profile due to metabolic contributions.

Assigning and Elucidating Metabolites Using Tandem Mass Spectra

Get a head start on the analyses of observed fragmentation patterns with autoassignment algorithms.

Obtain insight into the possible fragmentation and rearrangement pathways, and the ionic forms of fragments, for a given organic chemical structure.

Linking Chemical Structures to Spectra and Chromatograms

Link an identified metabolite structure or structure-set representation of possible metabolites directly to the spectral or chromatographic parameters.

Storing and Retrieving Metabolite Structures and Analytical Data

Manage interconnected analytical data, chemical structures, and properties in an intuitive way.

Store and extract more knowledge through customizable data entry and viewing interfaces.

Provide shared access to information for authorized personnel and sites.

Generating Biotransformation Maps

Build biotransformation pathway diagrams that visually link observed metabolite structures and incorporate their accompanying properties, such as toxicology values and metabolism yields.

Reporting:

Create a table of metabolites with their chemical structures, relative tR, area percentage, dose subject information, etc.

Accurate Mass Analysis on a Chromatographic Timescale

***Stevan Horning, Thermo Bremen, Winfried Wagner-Redeker
Spectronex Basel***

The combination between a linear ion trap and an Fourier transform mass spectrometer offers a completely new range of experiments in research and routine analysis. The presentation describes key instrumental features of the new design and discusses recent applications in protein/peptide identification as well as in general analytical chemistry.

BioAnalytical Applications of ICP-MS

***Scott D. Tanner, Zoë A. Quinn, Vladimir I. Baranov
and Dimitry R. Bandura,
MDS-SCIEX, 71 Four Valley Drive, Concord,
Ontario L4K 4V8 Canada***

The particular characteristics of Inductively Coupled Plasma Mass Spectrometry (ICP-MS) that address perceived shortcomings of other mass spectrometric approaches to bioanalysis will be discussed. These benefits include: exceptional sensitivity (to elements) that is independent of the chemical form in the sample, relative insensitivity (tolerance) of concomitant materials in the sample, large (9 orders of magnitude) linear dynamic range, capability for absolute quantification. The deficiency of ICP-MS for this application is that the ionization source totally destroys molecular (speciation) information, other than that which is gained through prior sample manipulation (such as chromatography or immunoassay). This deficiency, however, is turned to advantage for the determination of the state-of-phosphorylation of proteins through measurement of the total P concentration, especially when normalized to another element that can be taken as characteristic of the total protein content of the sample. In many instances, S is a convenient normalizing element, especially where homologous proteins or samples are assayed. Recent phosphorylation assays will be presented, including the discrimination of cancerous whole cell lysates. A novel application of ICP-MS is to multiplex immunoassays where the various protein complexes may be distinguished and quantified by the use of distinguishable elemental tags. In many instances, the presence of an elemental tag (or multiple copies thereof) does not affect the efficiency of the affinity binding; in fact, many commercial antibodies specifically employ lanthanides as fluorescence enhancers. In the instance that many copies of

an isotopic tag (for example, Nanogold™) are incorporated, the sensitivity and detection limit are directly enhanced. Because there are more than 50 elements that are suitable to the purpose, and more than a hundred isotopes of those elements that can be obtained in enriched form, the potential for multiplex analysis is evident. Data that demonstrates the advantages anticipated will be given, and the conditions that allow for multiplex analysis will be discussed. Current results for the simultaneous determination of multiple biomarkers are presented.

Elemental Speciation Capabilities of IC-ICP-MS in Environmental Water

***Adrian A. Ammann
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In environmental research as well as in other research fields direct determination of metal species and other elemental species is becoming more and more important. Metals or elements released to the environment are neither degraded nor decaying. All they do is changing their binding which makes them more or less mobile or immobile in a cycling process.

Individual metal or elemental species play a decisive role which cannot be deduced from total elemental contents. Not all the species of an element or metal have the same toxicity and detailed knowledge is mandatory to say something about provenience, cause of metal mobilisation, bioavailability, toxic effects etc. Without this knowledge there is no base for a realistic risk assessment nor for a successful remedy.

For hydrophilic highly mobile metal species that are mostly negatively charged metal complexes or metal oxoanions a highly selective anion exchange separation was coupled to an ICP-MS. Samples can be preconcentrated on column and separated from the matrix which allows the detection of some species in concentrations below nano molar. For the first time several stabile metal complexes of synthetic and biogenic chelators as well as oxoanions could be detected in the same chromatographic run. An ICP-MS alone cannot provide structural information, however it will be exemplified how in combination with a structure sensitive ion chromatography such an elemental detector opens new possibilities to direct observe the mobility and reactivity of such species.

Magnetic Sector Field ICP-MS as Sensitive Detector for Speciation Analysis

Meike Hamester, Torsten Lindemann
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meike.hamester@thermo.com

This presentation describes the simultaneous detection of P and S containing compounds by micro-bore liquid chromatography interfaced to magnetic sector-field ICP-MS. This technique enables the determination of phosphorylated peptides and proteins which play an important role in proteomics and biochemistry.

The phosphorylation state can be determined by simultaneous P and S determination and by using S as a normalizing element for the total protein content. Sector-field ICP-MS enables the separation of the P and S signal from polyatomic interferences physically by their small difference in mass. Therefore, NO^+ , NOH^+ , COH^+ , O_2^+ , NO^+ and NOH^+ , which were created e.g. from acetonitrile or methanol containing mobile phases, do not interfere with the analyte determination and do not contribute to the background. Due to the low background and the high sensitivity, very low limits of detection were obtained.

Detection of Noncovalent Protein-Ligand Interactions Using Automated Nanoelectrospray Mass Spectrometry

***Sheng Zhang and Colleen K. Van Pelt
Advion BioSciences, Inc., Ithaca, New York, USA
Joerg Niebel
Advion BioSciences Ltd, Wachenheim, Germany***

The detection of non-covalent interactions for proteins and ligands by electrospray ionization mass spectrometry (ESI/MS) is an area of increasing interest as it directly provides the molecular mass and binding stoichiometry of the complex. The conventional ESI/MS means of analyzing these complexes is by using pulled-capillary nanoelectrospray which is time-consuming, tedious, and requires a skilled user. Recently an automated nanoelectrospray system, the NanoMate and ESI Chip, has been developed. This system offers a simple, one-time spray optimization for 100 samples, and removes the art form from nanoelectrospray. Other advantages of the system include low sample consumption, the ability to conserve sample not consumed in the analysis, enhanced spray stability, and no carry-over.

The automated system works by first aspirating a sample from a 96-well microtiter plate with a disposable, conductive pipette tip and then delivering the sample to the inlet-side of the ESI Chip forming a pressure seal around a through-wafer channel. The ESI Chips, which are manufactured by standard processes of the semiconductor industry, consist of a 10 x 10 array of inlets to a through-wafer channel on one side, and a corresponding 10 x 10 array of nozzles on the opposite side with each through-wafer channel leading to a nozzle. Nanoelectrospray is initiated by applying a 1.2 – 1.8 kV spray voltage and a 0.2 - 0.5 psi pressure to the sample in the pipette tip.

To demonstrate the automated nanoelectrospray system's ability to detect noncovalent protein-ligand interactions, A standard model system of the

binding of cytidine 2'-monophosphate to ribonuclease A was tested. Ribonuclease A was titrated with cytidine 2'-monophosphate in 10 mM ammonium acetate, pH6.8. Scatchard plot was used to determine the dissociation constants. The result is in good agreement with the previous report. Furthermore, the same approach is used to study the dissociation constants for an endocellulase system. An inactive mutant of endoglucanase catalytic domain from the *T. fusca* bacterium was titrated with ligands cellotetrose and cellopentose. This is the first report of using ESI/MS approach to study the interaction of cellulase and its oligosaccharides. The results reported here demonstrate that the automatic nanoelectrospray system can be used for the rapid screening of potential drug candidates in the drug discovery programs.

MS and Structural Studies of Proteins and

Protein Interactions

***Kenneth B. Tomer, Christine Hager-Braun, Elisabeth O. Hochleitner,
and Jenny M. Cutalo***

***National Institute of Environmental Health Sciences,
National Institutes of Health,
Department of Health and Human Services
Research Triangle Park, NC, USA***

Mass spectrometry has long been known for its utility in peptide and protein identification, especially in the field of proteomics. The biological activity of molecules is dependent on its structure, on modifications, such as phosphorylation and glycosylation, and on its interactions with other biomolecules. The utility of mass spectrometry for characterization of these more complex structural interactions has been less recognized.

Over the past several years, we have been developing and applying mass spectrometry-based techniques to probe protein structural problems and protein:protein interactions. In these studies, we have used protection assays, differential chemical modification studies of surface-accessible amino acids of complexed and non-complexed proteins, and cross-linking agents to obtain information about the interacting surface of the proteins. A variety of separation techniques, such as nanoscale capillary LC and affinity chromatography, combined with mass spectrometry have been used to determine the results of these experiments. Molecular modeling based on these results has enabled us to determine structural parameters.

The major biological focus of these studies has been the structural characterization of HIV proteins and their interactions with other proteins that are relevant to HIV infection. Specifically, we have been characterizing glycan heterogeneity at glycosylation sites on HIV gp120, mapping epitopes on HIV proteins recognized by antibodies and have been probing interactions between the HIV surface glycoprotein, gp120, and its cellular receptors. In this talk, our approaches to these problems will be outlined and several examples of their application will be presented.

Characterization of native proteins by ESI-MS thanks to a dual-channel microfabricated electrospray emitter

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Miniaturized analytical systems are more and more considered as promising tools for high-throughput protein analysis [1]. In the field of mass spectrometry, microfluidics enables the design of new miniaturized electrospray emitters [2-4] with functions that are difficult to implement with classical electrospray sources [5-7]. We present herein a planar dual-channel microchip made by plasma etching of polyimide foils. Two independent microchannels share the same outlet on the edge of the chip, where the electrospray is to be generated.

This design allows the integration of a sheath liquid (typically acidified methanol) directly delivered within the Taylor cone generated at the outlet of the main channel. Efficient mixing in the Taylor cone allows the analysis of compounds directly from aqueous solutions. The proper control of high voltages applied in the aqueous and organic microchannels respectively allows differential pumping of the sample solution and of the sheath liquid. We present here the use of such a dual-channel microchip with the study of protein conformational charge states: it is now established that the protonation state of a protein is directly related to its conformational state [8]. Mass spectra from model proteins show that no further denaturation due to the organic sheath liquid itself occurs in the Taylor cone. Moreover, preliminary results about the analysis of intact biological complexes demonstrate the potential of the approach in protein structural and functional studies.

A Practical Cryodetector Mass Spectrometer for High Sensitivity Detection and Energy Resolution at High Mass

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Conventional micro-channel plate (MCP) detectors work by ejection of electrons following the impact of high velocity ions to initiate an electron cascade. It follows that this process is much less efficient for large, low velocity ions, and thus MCP detector efficiency decreases exponentially with increasing mass. This fact can be readily observed during the analysis of intact proteins, nucleic acids and polymers, where the usefulness of mass spectrometry has so far been severely limited above 10 kDa. Superconducting tunneling junction (STJ) detectors have no such limitation however, because the kinetic energy of a particle is measured directly, and detection efficiency is 100% for all masses.

Here we describe the first commercial STJ mass spectrometer intended for routine laboratory use. Known as Macromizer, this is a MALDI ToF instrument with a 16-pixel STJ detector array. The detector is held at 0.3 K within a closed system ^4He - ^3He cryostat, requiring no user intervention.

Very high mass proteins are detected without difficulty. Human immunoglobulin A, molecular weight MW approximately 400 kDa at 30 fmol and Bovine immunoglobulin M, MW approximately 1000 kDa at 1 pmol, is seen in its singly protonated state at 1 pmol. Singly charged BSA, MW 66.4 kDa, can be detected at 1 fmol. Because detector efficiency is mass independent, the relative intensity of these peaks represents the true abundance of these ions and semi-quantitative conclusions can be drawn. All detector events result from particle impacts, hence Macromizer spectra are free from noise, and sensitivity is predictably high. Human immunoglobulin G,

molecular weight approximately 150 kDa, is seen as singly protonated monomer, dimer, trimer and tetramer ions. The latter ion has a mass approximating 600 kDa.

Because detector efficiency is mass independent, the relative intensity of these peaks represents the true abundance of these ions. All detector events result from particle impacts, hence Macromizer spectra are free from noise, and sensitivity is predictably high. At 7.5 pmol, monomer, dimer and trimer ions of IgG can be observed with a single laser shot. Singly charged BSA, MW 66.4 kDa, can be detected at 500 amol.

Macromizer generates an energy vs mass scatterplot in addition to intensity vs mass spectra. A doubly charged ion possesses twice the energy of a singly charged ion of the same mass, and these species can be energy resolved. An energy filtering tool allows de-convolution of mass spectra by subtraction of multiple charge states. We have used Macromizer for the analysis of complex protein mixtures such as serum and cell lysates. Using simple prefractionation, a wide range of proteins with masses from 3 –150 kDa are observed. As a Proteome profiling and biomarker discovery platform, we demonstrate that Macromizer out-performs alternative technologies in terms of mass range and sensitivity at high mass.

Quantitative and Data-Dependant MS Acquisition

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Two topics will be presented. Firstly, the synthesis and application of two new alkylating reagents, N-t-butyliodoacetamide and iodoacetanilide, and secondly, a result dependent MS workflow. The ability to join these two projects is an exciting development underway in our laboratory that will finally be discussed.

N-t-butyliodoacetamide and iodoacetanilide have been synthesised to purity in their d0-light and in their respective d9 and d5 heavy forms. The mass differences of 5 and 9 Da avoid possible problems of overlapping isotope distribution and the peptide mass increments after alkylation are 113 and 133 Da allowing for efficient labelling, ionisation and peptide fragmentation. The compounds are simple to use and derivatisation is based on widely applied alkylating procedures, covalently binding to peptides containing cysteines. Consequently no additional steps are required to existing MS-identification procedures. The preliminary results show that these reagents can be applied for both protein quantitation and identification by PMF and/or MS/MS techniques. Importantly all the peptides, including non-cysteine containing peptides, are kept with the sample which enables supplemental and simultaneous biochemical analysis of the sample and discovery of potential post-translational modifications.

Protein identification using automated data-dependent MS/MS is now a standard procedure in proteomics. For the purpose of unambiguous protein identification data-dependent acquisition is some times redundant acquisition,

as (too) many peptides from the same protein are fragmented. To increase the quality of information but decrease the amount of information, a non-redundant MS (nrMS) strategy has been developed where data-analysis is an integral part of the overall MS acquisition and analysis, and not an endpoint as traditionally performed. In the nrMS workflow a MALDI-MS/MS instrument is used. MS and restricted MS/MS data are searched and identified proteins are used to generate an "exclusion list", after in silico digestion. Peptide fragmentation is then restricted to only the most intense ions not present in the exclusion list.

Terminus-Specific Fragmentation, a Novel Tool for the Direct Characterization of Intact Proteins Using MALDI-ISD and MS/MS Techniques

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The selective characterization of protein termini is a classical protein chemical problem, which is relevant, in particular in the case of recombinant protein characterization. It is particularly difficult to solve with classical techniques, such as Edman sequencing, in the case of N-terminal modification. The specific fragmentation of protein termini was achieved using a new method that is solely based on mass spectrometry. Terminal *peptides* were generated in the mass spectrometer in a first step and structural information was obtained in a second step. This *terminus-specific fragmentation* (TSF) represents a combination of 1) the pseudo-MS/MS technique reflector-in-source decay (reISD) on a MALDI-TOF mass spectrometer 2) with the additional MS/MS capabilities provided by MALDI-TOF/ TOF instrumentation. The N-terminal c-ions and C-terminal y-ions from intact proteins were selected in the timed ion gate of the TOF/TOF mass spectrometer and further fragmented using either the unimolecular laser-induced decomposition (LID) or decomposition induced by high-energy collisions with an inert gas (CID).

The reISD spectrum as a first step provided near-terminal information such as sequence tags from the undigested, isolated protein, which allowed identifying the protein by database searching and determining the proper processing state of the terminus with respect to that reference sequence. In the reISD spectra, typically c-, a-, y- and z-ions were observed and diagnostic mass differences between such ion series were found to be 45 Da for c-a ions

and 15 Da for y-z ions, which allowed to assign the ion types based on these diagnostic mass differences. The alignment of the determined sequence tags relative to the corresponding terminus allowed to confirm the expected N-terminal structure of the protein or to indicate an aberration. In the latter case, the second step, i.e., the selection of ISD fragments as precursor ions for downstream MS/MS analysis using the TOF/TOF, TSF provided fragment ion information from the isolated N-terminus from any precursor ion of the c-ion series. These spectra were directly being used for standard MS/MS-based database searching and protein identification. They allowed to unequivocally confirming the nature of blocked N-termini and even C-termini of intact proteins without the need of protein digestion and chromatographic isolation of terminal peptides. Various proteins containing N-terminal modifications were analyzed and a protein molecular weight range from 6 to 66 kDa has been used in this study, including recombinant proteins.

Minutes of the 2002 General Assembly of the Swiss Group for Mass Spectrometry (corrected version; corrections in bold)

Hotel Dorint Beatenberg – 14.11.2002

The assembly starts at 14:20. It is the first time that the meeting takes place at the Hotel Dorint, Beatenberg. The president, L. Fay opens the meeting, following the approved agenda, sent out previously with the newsletter Vol 20_2.

1. Nomination of the scrutineers:
Jean Luc Wolfender and Fabrizio Sabini are nominated as the scrutineers.
2. Approval of the minutes of the 2001 General Assembly:
The assembly approves the 2001 minutes to 100% without any further questions.
3. President's report and its approval:
The president reads his 2002 report (see Newsletter 21_1). The report is approved without any further questions.
4. Treasurers report:
Our Treasurer H.P. Moser informs the assembly about the normal expenses on our two accounts. The bank account has a balance of Fr. 13'417.00 per 30.9.02 and the Post Account has a balance of Fr. 22'181.00 per 1.10.02. The total balance is at + Fr. 3.5'598.00 compared to Fr. 36'777.00 (2001). The financial situation is very good and well under control.
5. Auditors report and approval of the treasurer's report and auditor's report:
Peter Hirter and **Urs Ranalder** (not Kurt Schellenberg) confirm the perfect bookkeeping and ask for acceptance of the treasurer's report. Both, the treasurer's report and the Auditor's report are approved to 100% by the members.
6. Decision of the 2003 membership fee:
The assembly decides with no opposition that the annual fee for the regular membership remains unchanged at Fr. 25.-/Year for individual members and Fr. 100.-/Year for Collective members.

7. Admission of new members:
15 new members are admitted to the SGMS:
Figueiredo Joachim Neves, Schlichtherle-Cerny Dr. Hedwig, Göbel Anke, Tzouros Manuel, Hornberger Dr. Martin, Stolz Siegfried, Matter Urs, Kaufmann A., Knochenmuss Dr. Richard, Giger Mathias, Laue Grit, Schnider Christia, Tromp Jan, Niebel Jörg, Kölliker Stephan
8. Election of the President and the new SGMS committee
100% agree to elect Andreas Stämpfli as the new president of the SGMS. Andreas accepts the election and thanks the members for the trust they have in him. 100% agree to the new committee including Jean Luc Wolfender as new member of the committee.
9. SCS (Swiss Chemical Society) – Information
HJ. Walther has some points to mention:
 - Markus Straub becomes Head of the NSC
 - Promotion for young talents (Chemistry) will be supported
 - Promotion for contacts between potential students and universities
 - Support for recruiting activities
 - Contact with international organisations (FECS; IUPAC)
10. News from the ESMS
R. Tabacchi has no news to report. For an update go to the homepage (Link on the SGMS Homepage). 6th IMSS Conference from 31.9.-5.10.03
11. Individual Proposals
none
12. Miscellaneous:
 - Marc Suter: Internet Info: 22 job offerings last year: Site works fine.
 - The Assembly decides to stay in the DORINT for the next meeting in 2003.

Thomas Läubli

Secretary of SGMS

Swiss group for mass spectrometry
Schweizerische Gruppe für Massenspektrometrie



Groupe suisse de spectrométrie de masse
Gruppo svizzero di spettrometria di massa

Please join us for the

General Assembly of the SGMS 2003

Thursday, October 30, 2003

17:30 h

Dorint Hotel Blüemlisalp, Beatenberg

Agenda

1. Nomination of the scrutineers.
2. Approval of the minutes of the 2002 general assembly.
3. Presidents report and its approval.
4. Treasurer's report.
5. Auditor's report and approval of treasurer's and auditor's report.
6. Decision on the 2003 membership fee.
7. Admission of new members.
8. Election of two Auditors
9. News from the NSCG - HJ. Walther.
10. News from ESMS - R.Tabacchi.
11. News from the IMSS - A. Staempfli
12. Individual proposals.
13. Miscellaneous.

Individual proposals must be **sent by (e)- mail before October 15, 2003** to the president: andreas.staempfli@roche.com

The President

Andreas A. Staempfli



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