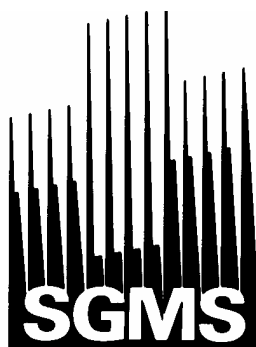


Swiss group for mass spectrometry
Schweizerische Gruppe für Massenspektrometrie



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

Newsletter

SGMS Rigi Meeting 2005

and

General Assembly 2005

Mercure Hotel, Beatenberg-Interlaken

October 27 and 28, 2005

11:15

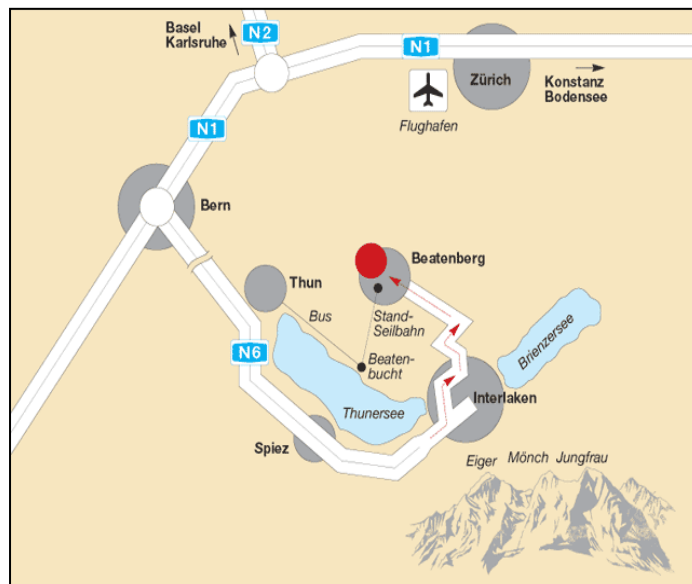
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Travel information

The Beatenbucht-Beatenberg mountain railway goes up every 20 minutes at :05, :25 and :45 till November 6, 2005.

By car: Take highway no. 6 from Berne to Thun, then no. 8 direction Interlaken. Right before reaching Interlaken follow directions to Gunten/Unterseen/Beatenberg.



In Beatenberg: Don't be afraid of having missed the Mercure Hotel Beatenberg-Interlaken. Beatenberg is a stretched-out village - probably the longest in Europe. Our destination is located at the very far end. Parking at the Hotel is free of charge. Please check-in first at the lobby.

By train (travel date October 27, 2005):

Genève: IC2517 at 07:10, Lausanne 07:45 and Fribourg 08:34 then Bern.

Basel: IC861 at 08:00 arriving in Bern at 08:56 then Bern.

Schaffhausen: IR2561 at 07:07 then Zürich.

Zürich: IC912 at 08:00 then Interlaken. Please stay in the train. It will continue to Interlaken West.

Bern: IC912 at 09:06 arriving in Interlaken West at 09:56.

Interlaken: Bus 107 in front of the main station at 10:10.

The bus will arrive at 10:38 in front of the Hotel Mercure (Dorint) Blüemlisalp.

Dear member and sponsors of the SGMS

It's already the 4th time that the annual Rigi Meeting of the SGMS takes place at Beatenberg. What has changed is just the name of the Hotel. During the past 3 meetings we met at the Hotel Dorint Bluemlisalp in Beatenberg. The new name is Mercure Hotel Beatenberg-Interlaken. The hotel management is still the same. Mr. Heinz Briner and his team will again give their best to meet our needs during our meeting on October 27 and 28, 2005.

Due to the fact that the same room is used for the meeting, the general assembly and later on for the dinner, the management of the Mercure Hotel Beatenberg - Interlaken needs to change the arrangement of the tables. This will take some time and will give us again the pleasant possibility to meet for an apéro in the lobby of the Mercure Hotel Beatenberg-Interlaken. Again this will be a perfect opportunity to meet and discuss. This year we will get an "Italian 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

P.S. I've got more than a dozen calls on behalf of not sending out the SGMS newsletter volume 23_1 in a printed version early this summer. I was told that many of our members would like the SGMS Newsletter printed as a booklet! So you get this time both volumes 23.

Thanks for understanding and please note that the committee will address the mailing of the newsletter during the general assembly.

October 27, 2005

11:15 – 11:30 Welcome Notes**Starting Session:****Chair: Andreas Stämpfli****11:30 - 12:15 *Molecular Electronics: Devices of Tomorrow?*****Marcel Mayor****12:30 - 14h00 Lunch****Session 1****Chair: Laurent Bigler****14:00–14h45 *Use of Mass Spectrometry to Study the Structure and the Interactions of Proteins*****Eric Forest****14:45 - 15:05 *Mass Spectrometric Characterization of Infectious Disease Epitopes Recognized by Human Monoclonal Antibodies*****Kenneth Tomer****15:05 – 15:25 *Homology-driven Proteomics in Organisms with Unsequenced Genome by Automated LC-MS/MS de Novo Sequencing and MS Blast Search*****Patrice Waridel****15:25 – 15:45 *Combining Novel Fragmentation and Front-end Enrichment Techniques for Highly Increased Sensitivity and Selectivity of Phosphopeptide Detection*****Arnd Ingendoh****15:45 - 16:15 Coffee Break****Session 2****Chair: Jean-Luc Wolfender****16:15 – 16:35 *Looking Deeper into MALDI: Understanding and Predicting MALDI Phenomena using Numerical Models*****Richard Knochenmuss****16:35 – 16:55 *Investigating the Mechanism of Cd-binding by the Rainbow Trout Estrogen Receptor using ICP/MS and MALDI-TOF*****Victor J Nesatyy****16:55 – 17:15 *Gas Phase Dissociation of RNA*****Jan Tromp****17:20 General Assembly 2005****19:00 Apéro****20:00 Blüemlisalp Dinner Buffet**

October 28, 2005

Session 3
Chair: Stephan Brombacher

08:30 - 09:15 *The Quadrupole Ion Trap and Proteomics: New Developments in Top-Down and Bottom-Up Analysis*

Gary L. Glish

09:15 - 09:35 *Side Product Analysis of Minor Constituents in Perfumery Raw Materials Using Two-dimensional Gas Chromatography Coupled to Mass Spectrometry*

Fabian Kuhn

09:35 - 09:55 *Determination of Chloroparaffins in Sediments by High Resolution Gas Chromatography Coupled to Mass Spectrometry with Different Ionization Techniques*

Jana Hüttig

09:55 - 10:15 *Bile Acid Pattern – Quantification of Unconjugated, Glycine- and Taurine-conjugated Bile Acids*

Ines Burkard

10:15 - 10:45 Coffee Break

Session 4
Chair: Marc J.F. Suter

10:45 - 11:30 *Insight into Fulvic Acid Molecules by Electrospray Mass Spectrometry*

Thorsten Reemtsma

11:30 - 11:50 *Perfluorooctane Sulfonate Analysis and its close Relationship with Mass Spectrometry*

Ingrid Langlois

11:50 - 12:10 *Sub-2 μm Particulate HPLC Columns for LC-MS-(MS): Benefits and Limitations*

Anton Kaufmann

12:10 - 12:30 *LTQ Orbitrap – A Breakthrough Mass Spectrometer for Accurate Mass, High Resolution MS and MSⁿ Analysis on a LC Time Scale*

Winfried Wagner-Redeker

12:30 – 12:40 Closing Remarks

Molecular Electronics: Devices of Tomorrow?

Marcel Mayor

***University of Basel, Department of Chemistry
St. Johannisring 19, CH-4056 Basel, Switzerland
marcel.mayor@unibas.ch***

Silicon-based integrated circuits have continuously reduced the feature sizes of devices and therewith the cost per functional unit also. However, further decrease in feature size is nowadays becoming increasingly difficult (and expensive) due to physical limitations. Molecular electronics, understood as the integration of molecular structures to supplement specific functionalities on a semiconductor chip, is a promising alternative concept to further reduce both, the feature size and the costs per functional unit. However, currently this remains speculation as the technology to integrate and to address molecules and their assemblies on a chip has not been developed yet. But the strongly increased research activity has already led to some experimental breakthroughs.

Supramolecular chemistry plays a leading role in molecular electronics. Tailor-made molecular devices as potential functional units have already proven to perform specific functions like switching between two configurations triggered by external stimuli. In addition, the integration of molecular structures in electronic circuits crucially depends on the molecules' supramolecular assembly properties.

An introduction will present the historical background and worldwide current research activities in the field of molecular electronics. Subsequent, own contributions to molecular electronics will be discussed. In particular, investigations to integrate single molecules in electronic circuits and the resulting correlations between molecular structures and electronic transport

properties will be presented. The thereby gathered molecular structure vs. electronic conductivity information further allows designing molecular architectures with tailor-made properties, as will be shown by an example of a single molecule rectifier. Further research activities are geared towards molecules with electronic transport properties leading to particular physical properties, like persistent currents or hysteretic switching.

The lecture is focused on the amazing potential of molecular structures to be designed and synthesized to perform particular electronic functions, perhaps even in electronic circuits of tomorrow. However, the fact that this research is still in its infancy and that many innovative ideas and new concepts are still required for the parallel integration of large numbers of molecules or their assemblies will not be hidden.

Use of Mass Spectrometry to Study the Structure and the Interactions of Proteins

Eric Forest

***Institut de Biologie Structurale (CEA-CNRS-UJF)
Laboratoire de spectrométrie de masse des protéines
41 rue Jules Horowitz
38027 Grenoble Cedex 1 France
eric.forest@ibs.fr***

In the post-genomic era, the interest of proteins appears again as they execute and control the majority of cellular activities. Through their interactions they play a critical role in cell function or dysfunction.

Characterizing the structure of a protein and the interaction with its partners is a key step in the knowledge of its function and in the use of this protein either as a drug or as a drug target.

Beside the well known role of mass spectrometry (MS) in proteomics, MS can provide useful information in structural biology when associated with other techniques.

For more than ten years the combination of hydrogen exchange and MS has been widely used, providing views on protein structure and protein dynamics. Associating proteolysis with proteases working at low temperature and low pH, local information can be obtained. It enables the identification of the solvent accessible regions of a protein, the location of conformational changes induced by the binding of a partner and the following of the folding or unfolding of proteins. With the help of directed mutagenesis, it is also possible to finely define the interaction regions with ligands or other proteins. This method gives structure information in complement to the classical techniques such as NMR or X-ray crystallography. Compared to them, MS offers the advantage of requiring small amounts of sample in conditions close to the physiological ones. Furthermore MS can work much easier on large proteins or complexes.

Several examples illustrating the power of this approach will be given.

Mass Spectrometric Characterization of Infectious Disease Epitopes Recognized by Human Monoclonal Antibodies

Kenneth Tomer

***National Institute of Environmental Health Sciences
National Institutes of Health
Research Triangle Park, NC 27709 USA
tomer@niehs.nih.gov***

As a response to an infection, the immune system produces antibodies. The determination of the antigenic structure recognized by the antibody through epitope mapping provides information about the interaction between antigen and antibody for the diagnosis of a disease on a molecular level, for characterizing the pathogenesis of the infectious material, and the development of interfering drugs or preventative vaccines. We have been using mass spectrometry to characterize the epitopes recognized by human monoclonal antibodies produced against infectious agents including the human immunodeficiency virus, HIV, anthrax bacillus, and the hepatitis C virus, HCV.

To determine the antigenic region of the protein, we first bind the antigens to immobilized antibodies. Then, a combination of proteolytic enzymes with analysis of the products by direct analysis using MALDI/MS and MALDI/MS/MS is used to characterize the fine structure of the effective epitope. In these studies, we initially determined antigens on HIV proteins, e.g., p24 and gp120, recognized by monoclonal antibodies(1). The epitopes in our initial studies were continuous and linear in nature. Subsequent epitopes that we studied, however, appeared to be discontinuous or conformational in nature, as is the case with most B-cell epitopes. To adequately describe these epitopes, we incorporated differential surface modification reactions into our experimental repertoire. In differential

surface modification, amino acid residues on the surface of bound antigens are chemically modified with reagents, such as acetic anhydride for lysines or hydroxyphenylglyoxal for arginines. The antigen in the absence of the antibody is chemically modified under identical conditions. The antigens are proteolyzed and the extent of modification of specific residues between the bound and unbound antigens are compared using tandem mass spectrometry. Residues within the epitope will be protected by the presence of the antibody. Based on our results, we observed that this approach, combined with crystallographic structural data or molecular model-based structures, enabled us to provide information about discontinuous epitopes as well as linear epitopes (2).

Specific examples from human neutralizing anti-HIV antibodies will be presented.

1. Parker, C.E., D.I. Papac, S.K. Trojak, K.B. Tomer. 1996. *J. Immunol.* 157:198-206.
2. Parker, C.E., L.J. Deterding, C. Hager-Braun, J.M. Binley, N. Schulke, H. Katinger, J.P. Moore, K.B. Tomer. 2001. *J. Virol.* 75:10906-10911.

Homology-driven Proteomics in Organisms with Unsequenced Genome by Automated LC-MS/MS de Novo Sequencing and MS Blast Search

Patrice Waridel¹, V. Surendranath¹, H. Thomas¹, A. Frank², P. Pevzner² and A. Shevchenko¹

¹ Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstrasse 108, 01307 Dresden, Germany, ² Department of Computer Science and Engineering, UC San Diego, CA 92093-0114, USA waridel@mpi-cbg.de

The characterization of proteomes of many important model organisms, especially within plant and insect kingdoms, is hampered by the paucity of genome sequences and remarkable phylogenetic diversity of proteins in wild-bred species. Here we report a strategy for automated identification of proteins from organisms with unknown genome by a combination of nanoLC-MS/MS, automated de novo sequencing and Mass Spectrometry driven BLAST (MS BLAST) sequence similarity search.

Tryptic digests of proteins separated by 1D or 2D electrophoresis are analyzed by a linear trap instrument LTQ (ThermoElectron) coupled to a nanoLC system (Dionex). The entire pool of 5,000 to 10'000 MS/MS spectra is filtered by a pattern recognition algorithm to remove spectra of trypsin and keratins peptides, as well as non-peptide background. The remaining spectra are interpreted de novo by PepNovo software, which takes about 0.5 sec per spectrum. The resulting redundant, degenerate and partially inaccurate sequence candidates are submitted to the web-accessible MS BLAST tool for protein identification. As protein identification relies on the similarity of peptide sequences (rather than on their identity), sequence polymorphism that commonly occurs in wild-bred species is tolerated.

The method was validated by automated analysis of proteins from the alga *Dunaliella salina*, the bug *Triatoma infestans* and the moth *Cerodirphia speciosa*, from which a number of proteins were identified by cross-species matching to known protein homologues from other model organisms.

Combining Novel Fragmentation and Front-end Enrichment Techniques for Highly Increased Sensitivity and Selectivity of Phosphopeptide Detection

Arnd Ingendoh

Bruker Daltonik GmbH, D-28659 Bremen

The characterization of serine and threonine phosphorylation is usually challenging. The commonly applied collision-induced dissociation (CID) results in neutral loss of the labile phosphate group with often insufficient further fragmentation of the peptide chain itself, and subsequently very limited sequence information. We will present a novel concept of MS/MS in the ion trap by combining CID and electron transfer dissociation (ETD), which is particularly suitable for phosphorylation identification due to its non-ergodic nature: the prompt fragmentation along the amid backbone following the electron capture leaves the amino acid-phosphate bond intact..

Since phosphopeptides are often present at very low concentrations and more difficult to ionize, they are prone to suppression by other peptides in nanoESI, in particular in highly complex mixtures. Therefore, a front-end enrichment is highly recommended. Here, various methods are presented and discussed, ranging from functional surfaces on magnetic beads to TiO₂ columns.

Digests of standard proteins spiked with sub-stoichiometric phosphopeptide amounts and phosphopeptide enriched digests from Arabidopsis were analyzed using nanoLC-MS/MS.

The comparison of ETD and CID spectra shows the benefits of ETD, where dephosphorylation of the parent ion was not observed. CID in contrast shows generally the phosphate loss as the most abundant signal, indicating the phosphorylation presence. The combination of both fragmentation methods within one acquisition cycle provided improved fragmentation data and enabled the unambiguous determination of sequence and phosphorylation site.

Looking Deeper into MALDI: Understanding and Predicting MALDI Phenomena using Numerical Models

Richard Knochenmuss

***Novartis Institutes of Biomedical Research
Basel, Switzerland***

Recently developed quantitative models of UV-MALDI will be presented. Molecular dynamics (MD) gives microscopic insight into MALDI and demonstrates the interaction between ablation/desorption and ionization processes. The MD approach is especially helpful for visualizing the MALDI plume, and understanding the characteristics which are important for ion-molecule reactions that determine ion intensity distributions. Both desorption and ablation regimes generate free ions, and yields are in accordance with experiment. The first molecular ions are emitted at high velocities shortly before neutral desorption begins, due to surface charging caused by electron escape from the top of the sample. Later ions are entrained and thermalized in the plume of neutral molecules and clusters. Clusters are found to be stable on a nanosecond time scale, so the ions in them will be released only slowly, if at all.

The continuum model for MALDI is more computationally tractable than MD, and includes the entire event, not only a limited spatial and temporal region. It is based on thermodynamic and kinetic principles that have been validated by the MD model and experiment. Primary matrix ionization occurs by excitation pooling. Analyte ions are generated by reaction with the matrix ions. This model is capable of quantitative or semi-quantitative reproduction of numerous MALDI characteristics. It predicts such remarkable MALDI phenomena as the matrix and analyte suppression effects.

These models can help users to understand, interpret and plan MALDI experiments. For example, the predicted generality of the matrix suppression effect has recently been demonstrated in an industrial setting, and taken advantage of for routine analysis of low MW compounds by MALDI.

Investigating the Mechanism of Cd-binding by the Rainbow Trout Estrogen Receptor using ICP/MS and MALDI-TOF

Victor J Nesatyy, Adrian A Ammann, Marc J-F Suter

***Swiss Federal Institute of Aquatic Science and Technology (EAWAG)
CH-8600 Dübendorf
victor.nesati@eawag.ch***

Recent studies have shown that certain heavy metals can mimic the effect of the endogenous estrogen receptor (ER) agonist 17 β -estradiol (E2) and lead to estrogen receptor activation. Possible interaction sites of the human ER have been identified using molecular biology tools (Stoica et al. *Endocrinology*, 2003, 144, 2425). We have used a combination of ICP/MS and MALDI to evaluate interactions of the ER with Cd on the macro- and micro levels.

MALDI analysis of the peptide mass profiles following proteolytic digestion was used to identify the sites of the rainbow trout ligand-binding domain involved in cadmium-binding. The results from these experiments indicated that Cd preferentially shields Cys groups against various chemical modifications, underlining their involvement in Cd-binding. This agrees well with known strong metal-binding properties of thiol groups in e.g. phytochelatins.

Competitive binding experiments with radio-labelled E2 indicated a ten times higher affinity of E2. However, ICP/MS showed that despite this higher affinity, increasing E2 concentrations were unable to release pre-equilibrated Cd from the ligand-binding domain. Increasing Cd concentrations on the other hand resulted in a release of pre-equilibrated E2 or prevented binding of E2 possibly due to conformational changes induced by Cd-binding. Additional investigation of this phenomenon using MALDI in combination with hydrogen/deuterium exchange experiments is currently underway.

Gas Phase Dissociation of RNA

Jan Tromp, Selina Monn and Stefan Schürch

***Mass Spectrometry Group
Department of Chemistry and Biochemistry
University of Bern, Switzerland***

Antisense oligonucleotides are nucleic acids on the order of 12 to 20 nucleobases, which are designed to hybridize to a complementary mRNA sequence, thus, inhibiting gene expression. Therefore, they are of great interest in human cancer therapy and for diagnostic applications. In order to improve affinity, bioavailability, biostability and the binding specificity, oligonucleotide analogs exhibiting chemical modifications are evaluated. Due to the presence of unnatural structural elements, the standard sequencing techniques are likely to fail and alternative approaches for rapid and accurate sequencing of chemically modified oligonucleotides have to be developed. Tandem mass spectrometry is a highly attractive candidate for this task, as it is capable of providing the high degree of structural information required.

The dissociation of DNA has been investigated by several groups over the past decade. However, there is hardly any data available on gas phase dissociation of oligoribonucleotides (RNA) and their analogs. Thus, the fundamental mechanistic aspects still need to be defined in order to develop mass spectrometry based protocols for sequence identification.

Oligoribonucleotides and modified oligonucleotides were subjected to low-energy collision-induced dissociation in a hybrid quadrupole time-of-flight mass spectrometer. In contrast to the dissociation of DNA, dissociation of RNA was found to be independent of nucleobase loss and it is characterized by cleavage of the 5'-P-O bond. To evaluate the influence of different 2'-substituents and nucleobases on the dissociation of RNA a number of modified oligoribonucleotides were analyzed. Experiments demonstrated that

the dissociation mechanism of RNA is not influenced by the nucleobase, thus, supporting a mechanism where dissociation is initiated by formation of an intramolecular cyclic transition state with the 2'-hydroxyl proton bridged to the 5'-phosphate oxygen.

The Quadrupole Ion Trap and Proteomics: New Developments in Top-Down and Bottom-Up Analysis

Gary L. Glish

***University of North Carolina, Department of Chemistry
CB# 3290
Chapel Hill, NC 27599***

While the quadrupole ion trap is already a very powerful and commonly used tool for bottom-up proteomics analysis, performance improvements can be made to increase the information available in these experiments. New methods for CID in the quadrupole ion trap that provide increased sequence coverage, and, in some cases, generate immonium product ions will be presented. Also, a method to increase throughput by analyzing multiple peptide ions simultaneously will be discussed. As a complement to the bottom-up approach, the analysis of intact proteins (top-down) using infrared multiphoton photodissociation will be presented.

Side product analysis of minor constituents in perfumery raw materials using two-dimensional gas chromatography coupled to mass spectrometry

Fabian Kuhn¹, Joachim Schmid¹ and Philip Marriott²

¹Fragrance Research, Givaudan Schweiz AG, CH-8600 Dübendorf,

***²Chrom and Mol Separations Group, RMIT University, Melbourne, Aus
Fabian.Kuhn@givaudan.com***

Large volume perfumery raw materials have to be investigated more and more thoroughly and registered with traces down to 0.1%. Mainly for terpene derived chemicals this can represent a tremendous analytical challenge, due to the complexity of the mixture at this concentration level. Comprehensive two-dimensional gas chromatography (GCxGC) provides an increased separation power compared to one-dimensional GC and has proved useful for essential oil analysis [1]. Its capability for the side product analysis of Javanol® [2], a derivative of campholenaldehyde with a natural sandalwood-oil scent, was tested. GCxGC separations were conducted on apolar x polar and polar x apolar column sets using a longitudinally modulated cryogenic system (LMCS, Chromatography Concepts). Mass spectra were recorded using a quadrupole mass spectrometry (qMS, Agilent) system with an electronic upgrade for faster scanning (up to 10000 amu/s) allowing an acquisition rate of 20 Hz. By comparing GCxGC-qMS with GC-qMS data (slower scanning), improved spectral quality due to reduction or absence of interferences with coeluting compounds or column bleed was observed but with reduced sensitivity. Nevertheless, identification of known constituents by library searches was possible. Structure elucidation of unknowns by mass spectral interpretation, however, was further facilitated by the 'structured separations' of components in the 2-D plane compared to the 1-D retention of single column GC analysis. At the moment significant effort is required to

interpret 2-D data because of the lack of an integrated GCxGC-qMS system with dedicated MS software. Nevertheless, GCxGC-MS proved to be a powerful technique for this kind of analysis.

[1] R. Shellie and P. Marriott, *Flavour Fragr. J.* 2003, 18, 179.

[2] J.A. Bajgrowicz, et al., *Helv. Chim. Acta* 1998, 81, 1349.

Determination of Chloroparaffins in Sediments by High Resolution Gas Chromatography Coupled to Mass Spectrometry with Different Ionization Techniques

Jana Hüttig and Michael Oehme

***University of Basel, Organic and Analytical Chemistry
CH-4057 Basel***

Chloroparaffins (CPs or polychlorinated n-alkanes) are the most complex halogenated contaminant mixtures. The total number of congeners is unknown but far exceeds 10` 000 single compounds.(1) The separation of individual compounds cannot be accomplished by capillary gas chromatography (GC). CP chromatograms normally show a big hump with more or less unresolved peaks. Most quantitative methods are based on GC combined with high and low resolution mass spectrometry (MS) despite some limitations (2). Detection of CPs by MS often involves electron-capture negative ionisation (ECNI) due to its high selectivity and sensitivity. The applicability of HPLC-APCI(-)-LRMS and GC × GC-ECNI-TOF-MS was also recently demonstrated (3, 4).

Additionally, two further GC-MS methods were developed in our research group in the past 2 years (HRGC-EI-MS/MS and HRGC-NICI-MS). Their comparability to HRGC-ECNI-HRMS was shown⁵. CP concentrations were determined in sediments from the North and Baltic Sea by HRGC-LRMS with the three different ionization techniques. The benefits and drawbacks of each technique will be discussed. Total CP concentrations ranged between 5-377 ng/g dry weight. The Baltic Sea seemed to be higher contaminated than the North Sea⁶.

(1) Shojania, S. *Chemosphere* 1999, 38, 2125-2141.

(2) Reth, M.; Oehme, M. *Anal. Bioanal. Chem.* 2004, 378, 1741-1747.

(3) Zencak, Z.; Oehme, M. *Rapid Commun. Mass Spectrom.* 2004, 18, 2235-2240.

(4) Korytar, P.; Parera, J.; Leonards, P. E. G.; Sanots, F. J.; de Boer, J.; Brinkmann, U. A. *T. J. Chromatogr. A* 2005, in press.

(5) Zencak, Z.; Borgen, A.; Reth, M.; Oehme, M. *J. Chromatogr. A* 2005, 1067, 295-301.

(6) Hüttig, J.; Oehme, M. *Arch. Environ. Contam. Toxicol.* 2005, accepted.

Bile Acid Pattern – Quantification of Unconjugated, Glycine- and Taurine-conjugated Bile Acids

I. Burkard, A. von Eckardstein and K.M. Rentsch

***Institute of Clinical Chemistry, University Hospital Zurich
ines.burkard@usz.ch***

Purpose: Differentiated quantification of the five most common bile acids as well as their glycine- and taurine-conjugated derivatives can be of clinical benefit for the diagnosis of selected cholestatic disorders and other diseases affecting bile acid metabolism. Furthermore, the bile acid pattern can also be useful in experimental and clinical research.

Methods: A HPLC-(tandem) mass spectrometry method was developed for the quantification of unconjugated as well as glycine- and taurine-conjugated cholic, chenodeoxycholic, deoxycholic, ursodeoxycholic and lithocholic acid, respectively. Preliminary normal ranges were determined in 21 healthy volunteers (10 men and 11 women; mean age, 34 years) with serum cholesterol concentration < 6.2 mmol/l (mean, 4.5 mmol/l) and 84 healthy individuals from an epidemiological cohort.

Results: The following serum concentrations (range; $\mu\text{mol/l}$) of unconjugated, glycine- and taurine-conjugated bile acids were found in 21 healthy volunteers:

	unconj. bile acids ($\mu\text{mol/l}$)	glycine-conj. bile acids ($\mu\text{mol/l}$)	taurine-conj. bile acids ($\mu\text{mol/l}$)
cholic acid	0.009-0.497	0.058-0.971	n.d.a-0.433
chenodeoxycholic acid	0.028-1.258	0.142-3.421	0.022-0.620
deoxycholic acid	0.013-1.596	n.d.a-0.909	n.d.a-0.177
ursodeoxycholic acid	n.d.a-0.371	n.d.a-0.796	n.d.a-0.023
lithocholic acid	n.d.a-0.034	n.d.a-0.060	n.d.a-0.003

n.d.a., not detected

Conclusions: Our sensitive and selective HPLC-(tandem) mass spectrometry method allows the quantification of unconjugated and conjugated bile acids in serum samples of healthy and diseased people. This bile acid pattern can provide useful diagnostic information when incorporated in the battery of standard liver function tests and be helpful for clinical research.

Insight into fulvic acid molecules by electrospray mass spectrometry

Thorsten Reemtsma

***Technical University of Berlin
Department of Water Quality Control
10623 Berlin, Germany***

Decades of research on humic material seemed to confirm that this kind of natural organic matter was chemically ill-defined and consisted of a seemingly infinite number of unique molecules. It is the merit of electrospray ionization-mass spectrometry that this picture has changes so rapidly in the past years, at least for the very polar fulvic acid fraction of humic material. High resolution mass spectrometry provided the first information on single intact molecules of fulvic acids and offered the potential to investigate these molecules further. Meanwhile molecular formulas of hundreds to thousands of fulvic acid molecules could be determined.

The investigation of fulvic acid isolates by ESI-MS revealed an astonishing degree of regularity at very different levels: (a) periodic intensity distributions were visible in scan spectra recorded by quadrupole MS, (b) the oligomeric character of fulvic acids of higher molecular weight was detected by size-exclusion chromatography (SEC) -MS analyses and (c) regularities were determined also on the level of elemental composition of fulvic acid molecules by TOF- and FTICR-MS. And finally (d) Q-TOF-MS investigations suggest that also the structure of fulvic acid molecules is very regular. Based on the results obtained by these mass spectrometric methods structure proposals have been developed for low molecular weight fulvic acids.

With SEC coupled to high resolution MS it is now possible to compare NOM isolates of different origin, to study the reactivity of these molecules and to take up the question of their source materials and formation processes,

again. Mass spectrometry may finally enable us to replace the yet only operational definition of fulvic acids by a well defined chemical definition, based on elemental composition and molecular structures.

Perfluorooctane Sulfonate Analysis and its close Relationship with Mass Spectrometry

Ingrid Langlois and Michael Oehme

***University of Basel, Organic and Analytical Chemistry
CH-4057 Basel***

Since the 1950ies, polyfluorinated compounds (PFCs) are industrially produced mainly as paint and adhesive additives, as insecticide, as fire fighting foam, as well as coating for textile, leather and food paper. Perfluorooctane sulfonate (PFOS, $C_8F_{17}SO_3^-$), a possible final biodegradation compound of some PFCs received a lot of attention due to its ubiquity in the environment¹ and also due to its detection in the human blood in the ppb range².

Coupled to high performance liquid chromatography, mass spectrometry is the unique detection technique for PFOS analysis in biota. Limits of detections in the pg range can be reached³. The possibility of MS will be discussed to distinguish the structural PFOS isomers present in standard solutions and biota. Additionally, a new derivatisation procedure was developed for GC-MS to widen the applicability in environmental analysis. Results obtained by electron and negative chemical ionisation as well as by high resolution MS will be presented.

(1) Giesy, J. P.; Kannan, K. *Environ. Sci. Technol.* 2001, *35*, 1339-1342.

(2) Olsen, G. W.; Church, T. R.; Miller, J. P.; M, B. J.; Hansen, K. J.; Lundberg, J. K.; Armitage, J. B.; Herron, R. M.; Medhdizadehkashi, Z.; Nobiletti, J. B.; O'Neil, E. M.; Mandel, J. H.; Zobel, L. R. *Environ. Health Perspect.* 2003, *111*, 1892-1901.

(3) Berger, U.; Langlois, I.; Oehme, M.; Kallenborn, R. *Eur.J.Mass Spectrom.* 2004, *10*, 579-588.

Sub-2 μm Particulate HPLC Columns for LC-MS-(MS): Benefits and Limitations

Anton Kaufmann

Kantonales Labor, Zürich

The introduction of pressure stable sub-2 μm spherical HPLC particles and the extension of the upper pressure limit for HPLC equipments (e.g. Ultra Performance Liquid Chromatography: UPLC) opened new frontiers in terms of LC resolution, sensitivity and speed. Peak widths produced by such analytical systems can approach 1 second, requiring more detection data points per time unit. This requirement presents no significant problems for analytical methods designed to quantify only a few analytes. Yet, multiresidue methods – as commonly used in the field of pesticides or veterinary drugs in food – require new concepts. Depending on the available quadrupole instrumentation, shorter dwell times and/or time programming of MS/MS transitions can be used. Such approaches are feasible, yet drifting analyte retention times require a careful and time consuming definition of time windows. Furthermore, the column flow presents another potential conflicting requirement. Depending on the given column diameter, to optimum linear velocity for the mobile phase has to be selected, to produce the best chromatographical resolution. The sensitivity of ESI or APCI interfaces are flow-dependent as well. The user has to therefore manage these two possibly diverging flow requirements.

Some applications utilizing such principles are presented. e.g. The sub-ppb quantification of a banned drug (chloramphenicol) in honey and the high-throughput screening of some 40 antibiotics in bovine urine from slaughtering houses. Benefits and limitations relevant to practical analytical work are critically discussed.

While modern tandem-mass spectrometry is suited for sub-2 μm particles stationary phases, the full potential can only be released by using high speed MS techniques like TOF. The high resolution of sub-2 μm chromatograms in combination with high speed, high resolution MS – as obtained by ESI-TOF – are expected to approach the selectivity of LC-MS-MS techniques. The much shorter chromatographical run times produced by sub 2- μm particulate HPLC columns and the full scan sensitivity of TOF is defining new benchmarks in terms of resolution, speed and sensitivity.

LTQ Orbitrap – A Breakthrough Mass Spectrometer for Accurate Mass, High Resolution MS and MSⁿ Analysis on a LC Time Scale

***Winfried Wagner-Redeker, Spectronex AG, CH-4002 Basel
Stevan Horning, Thermo Electron GmbH, D-28199 Bremen***

Identifying modifications or structural changes of molecules is of increasing importance in most all fields, including drug discovery, metabolomics, and proteomics. Since these species are often present in minor concentration, the requirements for more powerful mass spectrometers are demanding. There is a clear need for accurate mass determination combined with MS/MS capabilities and highest possible dynamic range as a standard detection tool for on-line chromatography. The knowledge of the accurate mass of a precursor ion and its product ions allows for significantly higher confidence and reliability of identification. The combination of high dynamic range with accurate mass and low detection limit in an instrument is a key to success.

The LTQ Orbitrap is a newly developed mass spectrometer which is a hybrid system with two analyzers, a linear ion trap and an Orbitrap analyzer. The linear ion trap and Orbitrap are characterized by a high ion storage capacity combined with a high scan rate and high MS/MS sensitivity; while the Orbitrap achieves high mass resolution and excellent mass accuracy with external calibration in full MS and MS/MS modes of operation.

The resolution in an Orbitrap analysis is proportional to the time the transient signal is acquired. For a resolution of 7,500 (at mass 400) the transient signal is detected for about 90 ms, where as a resolution of 60,000 requires a detection time of about 750 ms. The LTQ Orbitrap is able to make use of this time productively by operating the linear ion trap fully in parallel to the Orbitrap detector acquisition. This allows, for example, the acquisition of several MS/MS scans concurrent to the acquisition of a high resolution MS

spectrum - fully automated through *data dependent* scanning. If high resolution and accurate mass information of the product ions is required, the Orbitrap analyzer is operated in the 'sequential mode', with MS and MS/MS scans performed in the Orbitrap. This yields accurate mass information on the precursor and product ions in a fully data dependent manner, without the need of an internal calibrant.

This presentation gives an overview of the new LTQ Orbitrap mass spectrometer and the Orbitrap technique. Application examples will demonstrate the power of accurate mass for full MS and MSⁿ scans and its full compatibility with the LC time scale.

Please join us for the

General Assembly of the SGMS 2005

Thursday, October 27, 2005

~17:30 h

Dorint Hotel Blüemlisalp, Beatenberg

Agenda (Draft)

1. Nomination of the scrutinizers.
2. Approval of the minutes of the 2004 general assembly.
3. President's 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 2006 membership fee.
7. Admission of new members.
8. Election of the Auditors.
9. News from the SCS - HJ. Walther.
10. News from ESMS - L. Bigler.
11. SGMS homepage - MJF. Suter.
12. Individual proposals.
13. Miscellaneous
 - Mailing the Newsletter: by email?

Individual proposals must be **sent by email before October 12, 2005** to andreas.staempfli@roche.com.

(Please mention "SGMS General Assembly 2005: " in the subject line!)

The President
Andreas A. Staempfli

- President* **Andreas A. Stämpfli**
F. Hoffmann-La Roche AG
PRBT-S, Bau 65 / 109
CH-4070 Basel
andreas.staempfli@Roche.com
Phone +41-61-688 3131 Fax +41-61-688 7408
- Vice President* **Jean-Luc Wolfender**
Institute de Pharmacognise et Phytochimie
Université de Lausanne, BEP
Dorigny
CH-1015 Lausanne
jean-luc.wolfender@ipp.unil.ch
Phone +41-21-692 4541 Fax +41-21-692 4505
- Secretary* **Thomas Läubli**
Brechbühler AG
Steinwiesenstrasse 3
CH-8952 Schlieren
thomaslaeubli@brechbuehler.ch
Phone +41-44-732 3126 Fax +41-44-730-6141
- Treasurer* **Stephan Brombacher**
Novartis Pharma AG
WKL-127.4.06
PO Box
CH-4002 Basel
stephan.brombacher@novartis.com
Phone +41-61-696 1863 Fax +41-61-696 3123
- Internet* **Marc J.-F. Suter**
EAWAG
Ueberlandstr. 133
CH-8600 Dübendorf
suter@eawag.ch
Phone +41-44-823 5479 Fax +41-44-823 5311
- EMS representative* **Laurent Bigler**
OCI, Universität Zürich
Winterthurerstr. 190
CH-8057 Zürich
lbigler@oci.unizh.ch
Phone +41-44-635 4286 Fax +41-44-635 6812
- NSCG representative* **Hansjörg Walther**
c/o Solvias AG
WKL-127.5.58
Postfach
CH-4002 Basel
hansjoerg.walther@solvias.com
Phone +41-61-686 6165 Fax +41-61-686 6100
- Newsletter* **Andreas A. Stämpfli**

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