Dr. Max Lüthi Prize **5**

Remodelling, Optimization and Characterisation of Absorption Columns to Precipitate Hydrogen Chloride and Sulphur Dioxide

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The aim of the first part of this thesis is to create an extensive safety documentation for the operation of two absorption columns and a reactor, i.e. the drawing up of checklists, the revision of operating instructions as well as designing a reactions-, resistance- and a HAZOP-list. Besides, a comprehensive substance-data-collection for HCl and SO_2 has been made for the planned use in absorption.
A further aspect deals with the rebuilding of glass-raschig-ring-columns to modern high-capacity random and structured packed columns. For the description of these packings an overview of the applied measuring technique and analyses will be given.
The last part of this dissertation explores mass-transfer and hydraulics of the new baffles. For the mass transfer the investigation of the number of mass transfer units that the new packings provide for the system HCl/H_2O is of immediate importance.

Dr. Max Lüthi Prize **6**

Deposition of Fragrance Precursors on Fabrics

Pitt Allmendinger

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The purpose of this work was to consider the influence on deposition during a laundering. The deposition on cotton was measured under different washing conditions. A broad variety of molecules was investigated. An HPLC analytical method was developed to quantify the interesting substances in a washing emulsion. Adsorption of these substances to the vessel surfaces distorted the detection rate. Due to the addition of liquid detergents the problem could get overcome and adsorption was avoided. As a result, the recovery rate was between 90 to 100 % allowing for a reliable analysis. Since there are no interdependencies between the different substances, the deposition could be analyzed for several substances in the same experiment.
The following interrelations were found: An increasing deposition comes along with an increased substance concentration in the washing liquid. This was observed in the case of liquid detergent as well as with a fabric softener. If the liquid detergent concentration was increased, the deposition decreased. This behavior is caused by the formation of micelles, being formed if the liquid detergent concentration is high enough to allow for. The micelles are able to include the substances.
The ratio of the washing liquid to textile mass influences the deposition also. Using more liquid lowered the deposition. The influence of the molecular structure is not clear. Two different mechanisms have been determined. The first refers on solubility in the washing liquid, taking in account the polarity of the substances. The second refers on interaction between substance and substrate by conjugated pi-bond systems.

Analytical Chemistry **7**

Current Position of GC-MS and LC-MS in Clinical and Forensic Toxicology

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Reliable analytical data are a prerequisite for competent expertises in clinical and forensic toxicology. Nowadays, hyphenated mass spectrometric techniques, particularly gas chromatography/mass spectrometry (GC-MS) and liquid chromatography/mass spectrometry (LC-MS), are indispensable tools in clinical and forensic toxicology due to their high sensitivity and specificity. They are used for screening, library-assisted identification, and quantification of drugs, poisons and their metabolites, prerequisites for competent expertises in these fields. In addition, they allow studying metabolism of new drugs or poisons as a basis for developing screening procedures in biological matrices, most notably in urine, or toxicological risk assessment. Concepts and procedures using GC-MS and LC-MS techniques in these areas with special focus on multi-analyte procedures will be presented and discussed [1-7]. The presentation will close with a short discussion of the future position of GC-MS and LC-MS in these fields.

1. H.H. Maurer. Position of chromatographic techniques in screening for detection of drugs or poisons in clinical and forensic toxicology and/or doping control [review]. *Clin. Chem. Lab. Med.* 42, 1310-1324 (2004).
2. H.H. Maurer and F.T. Peters. Towards High-throughput Drug Screening Using Mass Spectrometry. *Ther. Drug Monit.* 27, 686-688 (2005).
3. H.H. Maurer. Advances in analytical toxicology: Current role of liquid chromatography-mass spectrometry for drug quantification in blood and oral fluid [review]. *Anal. Bioanal. Chem.* 381, 110-118 (2005).
4. H.H. Maurer. Hyphenated mass spectrometric techniques - indispensable tools in clinical and forensic toxicology and in doping control [review]. *J. Mass Spectrom.* 41, 1399-1413 (2006).
5. T. Kraemer and L.D. Paul. Bioanalytical procedures for determination of drugs of abuse in blood [review]. *Anal. Bioanal. Chem.* 388, 1415-1435 (2007).
6. H.H. Maurer. Current role of liquid chromatography-mass spectrometry in clinical and forensic toxicology [review]. *Anal. Bioanal. Chem.* 388, 1315-1325 (2007).
7. H.H. Maurer. Mass spectral approaches in impaired driving toxicology [review]. *Anal. Bioanal. Chem.* submitted(2008).

Analytical Chemistry **8**

Analytical Forum "SHARE and BENEFIT"
Exchange of Teaching and Teachers in Analyical Sciences

Urban Frey, Detlef Günther, Gerard Hopfgartner

Division of Analytical Chemistry, SCS, www.sach.ch

Analytical Chemistry is a field of research which is continuously expanding into a wide variety of interdisciplinary fields of science. The state of the art use of analytical instrumentation is a prerequisite for supporting other fields of research or further development of instrumentation. However, education of students is dominantly hosted in Chemistry and depends significantly on the existing infrastructure. In addition, research in this field becomes highly specialised and is so diverse that lab courses for students are lacking adequate instrumentation which makes "first-hand" education difficult to maintain excellence in education of Analytical Chemistry.
Reviewing the different Universities and Universities of applied sciences within Switzerland indicate however, that we have a large pool of resources for improving the teaching by sharing our expertise, but the interactions are missing. Based on this lack of interaction and the potential for improving the teaching, Division of Analytical Chemistry supports the formation of a platform where lectures for students can be "offered and booked". The lecture topics should be focused on techniques and instrumentation and should be combined with some "educational supportive" examples. Further details how to enter this platform, the benefit from participation will be presented.

Analytical Chemistry **9**

In-capillary enzymatic assays for automated drug metabolism studies on the nanoliter-scale.

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Cytochrome P450 enzymes (CYPs) play a central role in the oxidative metabolism of drugs and other xenobiotics. Considering the increasingly large number of chemical compounds in the pipeline of pharmaceutical companies, it is important during the drug discovery/development processes to have rapid, low-cost and automated CYP-based metabolism studies at hand.
We have developed a method for such studies and tested it for kinetics and inhibition experiments on CYP2D6. The O-demethylation of dextromethorphan into dextrorphan was thereby used as a probe of the enzyme's activity. Our approach uses the capillary electrophoresis technique requiring only 100-200 nl of enzyme and substrate/inhibitor solutions for the enzymatic assays. The produced metabolites are detected off-line by UPLC-MS allowing their identification and quantification.
Using this novel approach, CYP-based drug metabolism studies could be automatized and rapidly performed consuming very low amounts of enzyme compared to standard techniques. The proposed approach is thus suited for high throughput analysis, e.g. to screen drug candidates for CYP inhibition, rendering its application very attractive in the early stage of a drug discovery/development process.

Analytical Chemistry **10**

Applications of laser spectroscopy for CO_2 isotopologues: from the soil to the free troposphere

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A compact, mobile and cryogenic free quantum cascade laser based spectrometer was developed for field applications that require continuous and high precision CO_2 isotope ratio measurements at ambient air concentration. The analyzer simultaneously retrieves the concentration of all three main stable CO_2 isotopologues ($^{16}O^{12}C^{16}O$, $^{16}O^{13}C^{16}O$ and $^{18}O^{12}C^{16}O$), allowing the determination of both $^{13}C/^{12}C$ and $^{18}O/^{16}O$ ratios.
In the laboratory, a precision of the isotope ratios well below 0.1‰ is achieved for a 200 seconds averaging. Field studies included grassland ecosystem – atmosphere exchange (gradient and eddy-flux method) and forest soil C-dynamics studies. The derived isotope ratios were in excellent agreement with laboratory based isotope-ratio mass spectrometer measurements made on field-collected flask samples, and the CO_2 concentration values were consistent with standardized IRGA instrumentation. The instrument is currently being installed at Jungfraujoch (3580 meters above sea level) for continuous measurements of CO_2 isotopologues in the free troposphere. This analytical challenge is rewarded by the scientific potential of studies on the global CO_2 cycle, especially through the combination of continuous data with high-quality backward trajectories.

[1] E.W. Eugster, K. Zeyer, M. Zeeman, P. Michna, A. Zingg, N. Buchmann, L. Emmenegger, *Biogeosciences* **2007**, 4, 1.
[2] B. Tuzson, M.J. Zeeman, M.S. Zahniser, L. Emmenegger, *Infrared Physics & Technology* **2008**, 51, 198

Analytical Chemistry **11**

Reducing Matrix-Induced Mass Bias in MCICPMS

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Isotope ratios measured with a multi-collector-inductively coupled plasma-mass spectrometer (MCICPMS) always differ from the true isotopic composition of the sample and need to be corrected by internal and/or external standardizations even for interference-free samples. In a study to identify instrumental sources of mass bias, a significant dependence of the measured Nd isotope ratios on the ICP operating parameters was observed (Fig. 1).
For conventional nebulization, a range of carrier gas flow rates, sampling depths and acceleration voltages was found for which the absolute mass bias is increased, but more stable upon small variations of the respective parameter than under conditions where maximum signal intensity is measured.
Operating conditions that maximize sensitivity resulted in successively heavier isotope ratios in dependence on the concentration of a matrix (0.1-10 ppm Ho). If measurements were repeated at a higher carrier gas flow rate, the relative deviations between the matrix sample and bracketing standards could be reduced up to 6 times (Fig. 2).

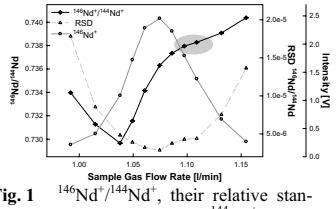

Fig. 1 $^{146}Nd^+/^{144}Nd^+$, their relative standard deviation (RSD) and $^{144}Nd^+$ signal intensity *vs.* carrier gas flow rate (CGF).

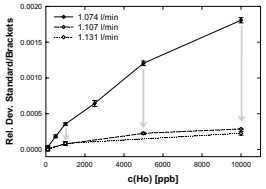

Fig. 2 Relative sample/bracket deviation of $^{146}Nd^+/^{144}Nd^+$ (R) for different Ho matrices at normal (1.074 l/min) and high CGF:

$$\frac{R_{sample} - (R_{bracket1} + R_{bracket2})/2}{(R_{bracket1} + R_{bracket2})/2}.$$

Analytical Chemistry **12**

LC-APPI-MS for the Analysis of 27-Hydroxycholesterol as Candidate Biomarker for Atherosclerosis

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Due to its role in maintaining whole-body cholesterol homeostasis, 27-Hydroxycholesterol is a potential biomarker for atherosclerosis and reverse cholesterol transport. Evaluation of this candidate biomarker in plasma samples of humans and animal models requires a sensitive and robust analytical method.
APPI (Atmospheric Pressure Photoionization) is the state-of-the-art of ionization techniques interfacing liquid chromatography and mass spectrometry (LC-MS) system. With the help of toluene as dopant and MeOH as LC eluent, APPI has improved the sensitivity of the analysis, in comparison to the published LC-APCI-MS method, allowing the quantification of 27-hydroxycholesterol from as little as 15 µL plasma with a limit of quantification (LOQ) of 40 ng/ml plasma. The method was validated also for the quantification of 27-hydroxycholesterol from 50 µL plasma, with LOQ of 10 ng/ml. A further advantage is that no prior derivatization is needed, unlike previously established LC-ESI-MS or GC-MS methods.
Preliminary results from analyses of plasmas from different knock-out mice show the potential of 27-hydroxycholesterol as a novel biomarker of atherosclerosis.

Analytical Chemistry **13**

Label Free Detection of Single Proteins Using Deep-UV based Laser Fluorescence Lifetime Imaging Microscopy

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A large number of biological species have intrinsic fluorescence excited in the UV region of 260-280 nm, UV laser excitation is an attractive alternative to tag these compounds with fluorescence labels excited at visible region. In this contribution we present a deep UV fluorescence lifetime imaging microscopy system based on a mode-locked diode-pumped picosecond deep UV laser. The described setup is well-suited for biological applications for ultrasensitive detection of intrinsic fluorescence. (1) Label-free detection of single protein molecules.
We investigated the bursts of autofluorescence photons from tryptophan residues in β-Galactosidase molecules from *Escherichia coli* (*Ecβ Gal*) and fluorescence correlation spectroscopy of *Ecβ Gal*. The results demonstrate that deep UV laser-based fluorescence lifetime microscopy is useful for identification of biological macromolecules at the single molecule level using intrinsic fluorescence. (2) Label-free detection of antibody/antigen and protein/drugs interactions.
A label free method for detection of *Ecβ Gal*/anti- *Ecβ Gal* interactions and protein/drugs interactions have been demonstrated by means of steady-state and time-resolved fluorescence spectroscopy. The interaction can be monitored by fluorescence lifetime changes between free components in the interaction system and corresponding complex. Energy transfer between tryptophan and bound drug in protein-drugs complexes has been observed. (3) One-dimension miniaturized polyacrylamine gel electrophoresis with native fluorescence detection.
The mixture of three biological compounds (β-Galactosidase from *Escherichia coli*, apo-Transferrin and bovine serum albumin) have been separated using miniaturized gel electrophoresis and a staining free detection limit below 80 pg per band has been achieved.

[1] Q. Li, et al., *J. Phys. Chem. B*, **2004** ,108, 8324.
[2] Q. Li, S. Seeger, *Anal. Chem.*, **2006**, 8, 2732.
[3] Q. Li. S. Seeger, submitted.
[4] E. Riaplov, et al., submitted.

Analytical Chemistry **14**

Study of Small Combinatorial Dynamic Libraries using 2D NMR

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In a recent review Otto [1] pointed out that chemists used to neglect the study of complex mixtures. One of the reasons for this lack interest may be the absence of practical analytical tools and/or the fear to deal with very complex analytical data. But if some signals can be easily assigned to one molecule, the analysis of the data may become straightforward [2].

Herein we present an example of the kinetic and a thermodynamic study of a network of height interacting molecules using series of high-resolution 2D NMR spectra [3]. Computer-optimized spectral aliasing (COSA) [4] was used to optimize the carbon dimension of the HSQC reducing by a factor of 40, the experimental time.

[1] R. F. Ludlow, R. Frederick, S. Otto, *Chem. Soc. Rev.* **2008,** *37,* 101.
[2] E. A. Mahrous, R. B. Lee, R. E. Lee, *J. Lipid Res.* **2008,** *49,* 455.
[3] G. Gasparini, B. Vitorge, P. Scrimin, D. Jeannerat, L. J. Prins, *Chem. Comm.* **2008,** *in press.*
[4] D. Jeannerat, *J. Magn. Reson.* **2007,** *186,* 112.

Analytical Chemistry **15**

About the Electrospray Ionization Source in Mass Spectrometry: Electrochemistry and On-chip Reactions

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Electrospray ionization mass spectrometry (ESI-MS) has become a widely employed technique and great efforts have been made on its development. The inherent electrochemical properties of ESI induce several oxidations in positive ionization mode and are analytically important. For instance, sacrificial electrodes (such as copper or zinc), used to supply the electrospray current, produce cations that are able to react on-line with compounds of interest. Thus, the interactions between copper ions and ligands or peptides have been studied by using a sacrificial electrode.[1]

Another example is the *in-situ* electrogeneration of a dinuclear zinc compound for the tagging of phosphopeptides by using a zinc electrode. In order to perform this reaction, a microfabricated dual sprayer has been used. This device consists of a polyimide microchip with two microchannels connected only at the tip of the microchip. Thus, a phosphopeptides solution meets a tag solution within the Taylor cone. Such a combination between a sacrificial electrode and a chip allows to selectively tag target molecules.[2]

Finally, these dual-channel microsprayers were also used to study liquid-liquid interactions.[3] Two immiscible liquids were put in contact within the Taylor cone. Interfacial interactions such as those between lead and thioether crown or peptides and phospholipids will be shown.

[1] M. Prudent *et al.*, *Electrochem. Commun.* **2007**, *9*, 2067.
[2] M. Prudent *et al.*, *Anal. Chem.* **2008**, *80*, 2531.
[3] M. Prudent *et al.*, *Anal. Sci.* Submitted.

Analytical Chemistry **16**

Environmental proteomics for the analysis of stress response in *Chlamydomonas reinhardtii* (green algae)

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Multidimensional protein identification technology (mudPIT) has become a popular tool for analyzing complex protein extracts [1]. It is capable of detecting subtle changes in the proteome of an organism in response to (multiple) stressors, and is a promising tool for ecotoxicological risk assessment. In addition to identifying new protein biomarkers, it can also help to provide insights into modes of toxic action.
The green alga *Chlamydomonas reinhardtii* presents an attractive model for studying multiple stressor effects. Exposure to the herbicides diuron and paraquat in combination with UV radiation were analyzed in triplicates using X!Tandem and The Open Mass Spectrometry Search Algorithm (OMSSA). MudPIT analysis of the protein extracts from control samples showed significant variance in protein identification and reproducibility. This clearly improved when acquiring charge state +1 in addition to +2 and +3. Exposure to various herbicides and UV radiation showed significant changes in protein levels in *C. reinhardtii*. As expected from the underlying mode of action, paraquat exposure resulted in the induction of both chloroplastic (Fe) and mitochondrial (Mn) superoxide dismutases. Also, exposure with photosensitizer Rose Bengal, known to induce oxidative stress via singlet oxygen generation in *C. reinhardtii* [2], led to increased levels of the glutathione peroxidase GPX5, confirming gene expression results previously published. Other changes on the proteome level have been observed, but more careful investigation using pathway and cluster analysis is required in order to understand the underlying mechanisms of stress response.

[1] Washburn, M. B. et al., *Nat. Biotechnol.* **2001**, *19*, 242.
[2] Fischer, B. B. et al., *Plant Science* **2005**, *168*, 747.

Analytical Chemistry **17**

Investigation of Chemical Cross-Linking in Detail: Reactivities of Amino Acids

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Chemical cross-linking in combination with mass spectrometry has emerged as a powerful tool for the structure elucidation of tertiary or quaternary structures in proteins. Despite many applications, only few studies [1, 2] concerning the reactivity and selectivity of cross-linkers towards certain amino acids have been reported so far. The commonly applied N-hydroxy succinimide esters (NHS esters) are usually described to be selective for primary amines. We studied the reaction behavior of synthesized and commercial peptides with maximal chain lengths of 30 amino acids with the homobifunctional cross-linker disuccinimidyl suberate (DSS). During "decoration" of synthesized peptides with a protected N-terminus (Fmoc-EGGGXGGGE, where X = Lys; Arg; His; Tyr; Thr or Ser) a reaction was only observed for the lysine. For the peptides Fmoc-EGGXGZGGE with (X, Z) = (His, Tyr); (His, Ser), Tyr and Ser showed high reactivity due to the presence of His. For commercial peptides, tyrosine, serine, threonine and arginine were clearly identified by MS/MS measurements as reaction sites besides the N-terminus and lysine. In most cases, the reaction of Tyr, Ser, Thr and Arg was only observed as intra-link with a primary amine. Our data imply that the neighboring amino acids play an important role for the reactivity and selectivity of chemical cross-linkers.

[1] C.L. Swaim, J.B. Smith, D.L. Smith, *J Am Soc Mass Spectrom* **2004,** *15*, 736.
[2] M.D.N. Leavell, P. Novak, C.R. Behrens, J.S. Schoeniger, G.H. Kruppa, *J Am Soc Mass Spectrom* **2004,** *15*, 1604.

Analytical Chemistry **18**

Probing switch peptides by tandem mass spectrometry: on/off state quantification and applications

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Protein aggregation and formation of toxic oligomeric species is a fundamental biological problem related to many neurodegenerative diseases. Switch peptides that mimic the primary structure of amyloid β, a neuropeptide involved in Alzheimer's disease, allow triggering or inhibiting peptide oligomerization in a controlled manner [1]. We apply tandem mass spectrometry to monitor and improve characterization of switch peptide transition states and oligomerization kinetics.

Switch peptides containing molecular switches (pH and UV dependent) and derived from the aggregation promoting sequence of amyloid β were synthesized by solid phase FMOC biochemistry. "On" and "Off" states were monitored by solution phase circular dichroïsm measurements and gas phase tandem mass spectrometry (MS/MS) using an 11 T Fourier transform ion cyclotron resonance mass spectrometer with simultaneous electron capture dissociation (ECD) and infrared multiphoton dissociation (IRMPD).

MS data indicate that peptide oligomerization is a process involving the formation of dimers and trimers. MS/MS applied to monomers allows quantitating the amount of peptide in the "On" state relative to the "Off" state. The use of ECD is of particular importance in the current application due to high repeatability and reliability of the method towards relative product ion abundance quantitation. Further correlation between secondary structures and fragmentation patterns is under investigation. Advantages of adding MS and MS/MS data to classical aggregation/oligomerization kinetics studies will allow us to better understand the complexicity of low molecular aggregates and correlate peptide morphology with toxicity in neurodegenerative diseases.

[1] Mimna R., *et al. Angew Chem Int Ed Engl*, **2007**, 46, 2681-2684

Analytical Chemistry **19**

Determination of Perfluorinated Alkyl Compounds in Sewage Sludge

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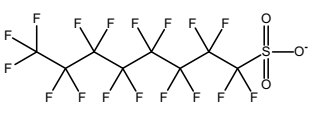
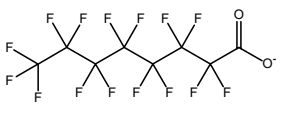
Perfluorinated chemicals (PFCs) are widely used and are being detected in the environment, wildlife and humans. Conventional wastewater treatment has limited effectiveness in removing PFCs from aqueous waste streams, and, thus, wastewater treatment plants (WWTPs) act as point sources to the aquatic environment. Some PFCs such as perfluorooctane sulfonate (PFOS) sorb partially onto sewage sludge and therefore the sewage sludge produced in a WWTP may be an important sink for PFCs.
A survey of anaerobically stabilized sewage sludges in the Canton of Zurich was performed to determine the levels of PFCs and to find possible hotspots. An analytical method was developed based on liquid solvent extraction and analysis by HPLC coupled to a tandem mass spectrometer using negative electrospray ionization. For total perfluorocarboxylates (e.g., PFOA), the concentrations ranged from 14 to 50 µg/kg. The concentrations for total perfluorosulfonates (mainly PFOS) ranged from 15 to 610 µg/kg. Among the twenty studied samples, the levels of six were above 100 µg/kg. These data indicate the widespread occurrence of PFCs in municipal WWTPs.



Perfluorooctane sulfonate (PFOS)



Perfluorooctanoate (PFOA)

Analytical Chemistry **20**

Toward error free *de novo* protein sequencing by combined liquid chromatography at critical conditions and tandem mass spectrometry

Julie Allegrand, Aleksey Vorobyev, Hisham Ben Hamidane, Hongqian Yang, Stanislav Shitov, Michael V. Gorshkov, Yury O. Tsybin

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INEPCP, Russian Academy of Sciences, 119334, Moscow, Russia

Despite of the recent progress in integrated LC-MS/MS based peptide and protein sequencing the challenge of reliable, reproducible and error-free analysis remains active. The approach of biomacromolecular liquid chromatography at critical conditions (BioLCCC) [1] allows distinguishing molecules according to unique functionalities, e.g. rare amino acids and post-translational modifications, present. However, the existing fundamental limitations of retention time predictions require integration of a complementary peptide and protein structure analysis technique, e.g. MS/MS in general and electron capture dissociation (ECD) in particular. Here, we present the advantages and further development of the BioLCCC-MS/MS combination leading to improved peptide and protein structural analysis.

Commercially available and synthesized in-house peptides, including a set of peptides with generic sequences H-RAAAAXAAAAK-OH with X being one of the 20 naturally occurring amino acids and 2 unnatural amino acids, were analyzed by the BioLCCC theoretical chromatograph, experimental analysis on a stand-alone HPLC, and coupled to an 11 T Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS) equipped with ECD and infrared multiphoton dissociation (IRMPD).

Preliminary data demonstrate a general correlation between theoretical and experimental retention times for the peptides studied. Currently undertaken detailed analysis is aimed to increase the accuracy of theoretical retention time prediction by optimization of specific functional groups binding energies. Product ion abundance in ECD will be used to improve the fundamental understanding of ECD and develop a quantitative model.

[1] Alexander V. Gorshkov, *et al. Anal. Chem.* **2006,** 78, 7770-7777.

Analytical Chemistry **21**

Microfluidic Chip for Mass Spectrometric Detection of Metabolites in Single Cells

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The stochastic fluctuations in gene expression can lead to different phenotypes within isogenic cell populations. Thus, a single cell approach to '-omics' research is essential to integrate experimental data into reliable predictive models necessary to describe the cell at the system level.
Metabolomics is a valuable tool to study phenotype and its changes caused by environmental influences, disease or changes in genotype. However because of the difficulties to detect the wide chemical variety constituting the metabolome and due to the minute amount of sample available in each cell, the detection of metabolites in single cells is very challenging.
We exploit matrix-assisted laser desorption/ionization – mass spectrometry (MALDI-MS) to detect endogenous cell metabolites in single yeast cells. MS can cope with the diversity of the molecules under study and a specially adapted MALDI method can reach the sensitivity necessary to detect the small amount of sample contained in a single cell. Microfluidic devices are employed for cell sampling, handling and preparation. The coupling of microfluidics devices to MALDI-MS detection allows to perform a microscale preparation that can deal with the needs in terms of sensitivity, sample consumption, and high throughput for single yeast cell detection of metabolites. Using the method presented, MALDI-MS spectra of an extract of a single yeast cell lysate are obtained and detection of endogenous metabolites was achieved. The coupling of microfluidics and MALDI results in a powerful analytical tool for system biology that, by monitoring metabolome dynamics at the single cell level, allows to observe and analyze the stochasticity of biochemical processes giving a novel view into the cell behavior.

Analytical Chemistry **22**

Fe^{II} formation (pM) during Fe-redoxcycling driven by photoreduction of Fe^{III}-complexes - Implications for ROS production.

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Reactive oxygen species (ROS) can have devastating toxic effects and therefore have to be tightly controlled [1] by organisms extra- and intracellular. Under natural conditions a large part of extra cellular ROS are produced through iron redox cycling, generating and transforming toxic compounds. In order to know and control such extra cellular iron and ROS dependent adverse effects the photoreductive behavior of biogenic and anthropogenic Fe^{III}-complexes was investigated under basically natural resp. usual environmental test conditions. A light source imitating solar radiation for tanning purposes was applied and the composition of the matrix was chosen as close as possible to algal growth medium. In such quasi natural carbonate containing waters Fe^{II} is oxidized noticeably to Fe^{III} which forms insoluble oxo-hydroxy compounds. Hence, Fe^{II} appearing as an intermediate, [Fe^{II}] initial rates were measured in the 10^{-11} - 10^{-9} molar range by luminol based chemiluninescence detection [2]. Initial Fe^{II}-oxidation rates were measured as well in the same solution so that rate constants and net photochemical Fe^{II} formation rate constants could be calculated. Depending on structural features of the irradiated Fe^{III}-complexes marked differences in rate constants among Fe^{III}-complexes structures were found.

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Analytical Chemistry **23**

Fast analysis of doping agents by UPLC-QTOF-MS

Part I: Screening analysis

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More than 150'000 urine samples are analysed per year over the world for doping control. During major sporting events, results are required within 24 or 48 hours after samples reception meaning that the time delivery response must be as short as possible. Therefore, the entire analytical process, including sample preparation, analyte separation, selective detection and data mining needs to be optimized. The aim of this study is to analyze more than 100 doping agents excreted under their free form in urine, including diuretics, masking agents, β-blockers, stimulants, narcotics, and so on. Regarding sample preparation, the dilute and shoot was selected as the simplest and fastest sample preparation method. Because UPLC offers the opportunity to obtain short analysis time, while maintaining or even enhancing efficiency and sensitivity, it was used for separating analytes with a linear gradient of water/acetonitrile containing 0.1% formic acid in 6 minutes. Due to the peaks thinness (6 sec), high acquisition rates detector afforded by a quadrupole time of flight (QTOF) MS is required for selective detection by measuring exact mass of analytes.
Method sensitivity was evaluated by measuring the LOD of more than 100 substances in urine. An average value of 20 ng/mL was reached. The matrix effect was evaluated and the method validated.

Analytical Chemistry **24**

Improving Ion Transmission for Inductively Coupled Plasma Mass Spectrometry (ICPMS) – Back to Basics.

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Inductively coupled plasma mass spectrometry (ICPMS) is an established technique for trace and ultra-trace element determinations in a large range of applications. It offers very high sensitivity for almost the entire periodic table with a wide dynamic range and a flexible coupling to many existing sample introduction techniques. Nonetheless the detection efficiency of current instruments is severely limited and only one ion is detected from 5000 – 500000 atoms introduced into the ion source [1]. This is mainly a result of the design of the interface required to transfer the ions from the ICP to the high vacuum in the mass spectrometer in its classical sampler-skimmer configuration.
To improve ion transmission, a new interface configuration is currently under investigation. It involves a specially designed transfer stage incorporating a so called "ion funnel" [2]. This ion funnel is composed of a stack of ring electrodes with decreasing inner diameter, located downstream a sampler cone.
The transmission properties of such a device, when connected to a plasma ion source are studied in detail. Dependence of ion beam characteristics on pressure and applied fields will be presented.

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Analytical Chemistry **25**

DNA-nuclear receptor interaction studied by mass spectrometry

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Nuclear receptors, such as the retinoic acid receptor (RAR), interact not only with their ligands but also with other types of receptors. Previous biological analyses (gel-shift) have shown that two coactivators and a specific DNA sequence bind to the receptor but also induce a partial dimerization of RAR. Mass spectrometry (MS) has been shown to be a powerful technique to analyze changes such as these. Nondenaturing nanoelectrospray (nanoESI- MS), under soft conditions, and high-mass matrix-assisted laser desorption ionization MS (MALDI-MS) combined with chemical cross-linking were used to study these interactions. The RAR protein was incubated with either coactivators peptides (PF108 and PF124) or with various DNA sequences (DR5, A_9 and C_9) and then analyzed with MS. We were able to detect RAR alone as well as complexed with the two coactivators peptides. A complex between protein RAR and the double strand DR5 was detected with nanoESI. After cross-linking the high-mass MALDI showed that RAR binds the single strand DR5. Moreover, DNA induced the dimerization of RAR and this dimer could bind not only the single strand but also the double strand. The specificity of the binding was verified by incubating with a nonspecific control DNA (A_9 and C_9).

Analytical Chemistry **26**

Uncertainties in Kinetic Hard-Modelling of Multivariate Spectroscopic Data

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Kinetic hard-modelling of multivariate spectroscopic data [1, 2] is a well established method for the determination of reaction mechanisms and associated rate constants. Kinetic hard-modelling relies on a hard model defined by kinetic rate laws and requires the numerical integration of these rate laws nested into Beer's law in order to model the spectroscopic signal. The modelled absorbances are compared with the measured ones and the residuals are minimised in the least squares sense by optimising the corresponding rate constants. Uncertainties in experimental conditions, such as initial concentrations or dosing rates, are often neglected and thus errors in the fitted rate constants estimated from the analysis of one single experiment typically tend to be underestimated. In this contribution, we present a rigorous method for the propagation of uncertainties in initial concentrations and in dosing rates into the errors in the rate constants fitted by multivariate kinetic hard-modelling of spectroscopic data and non-linear optimisation [3].
First, the importance of the uncertainties in initial concentrations is shown for simulated spectroscopic data based on a second order rate law under batch conditions. Our method of error propagation leads to interesting and useful results for the optimum design of kinetic experiments used for the non-linear optimisation of second order rate constants by kinetic hard-modelling.
We then present an experimental validation of the method using the acid-catalysed reaction of benzophenone with phenylhydrazine in THF repeatedly investigated (seventeen times) by UV-vis and mid-IR spectroscopy under semi-batch conditions. By applying our method of error propagation to each single experiment, we have been able to cover an important proportion of the observed standard deviation in the rate constants obtained from all experiments. Differences between results obtained from UV-vis and mid-IR spectroscopy, as well as the importance of the dosing rate in the design of semi-batch experiments are discussed.

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Analytical Chemistry **27**

Comparison of non-matrix matched calibration using ns- and fs-LA-ICP-MS in geochronology

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Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) has become an important technique in the analysis of solid samples. As a spatial resolved, 'quasi' non-destructive and sensitive technique it is used for geochronology. The use of glass standards (NIST 610) for age determinations in Zircons ($ZrSiO_4$) has been discussed in the literature, but the reported results are contradictory to each other [1-3]. Therefore, we show the insights into that discrepancy. The ablation behavior of Si, Zr, Pb and U in the two matrices, glass and Zircon, was studied using ns- and fs laser ablation. For the comparison of the two laser systems, the detection efficiencies (detected ions/ablated atoms) were determined. For the estimation of the number of ablated atoms, the ablation rate and the material density were considered.
To broaden the scope of materials, the same procedure was carried out with minerals having different concentrations of Si.

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Analytical Chemistry **28**

Comparison of various chromatographic methods for the separation of saccharides

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The analysis of saccharides remains challenging in liquid chromatography because of their extreme polarity and the lack of chromophores. For this reason, several alternative methods were evaluated for the separation and detection of various saccharides. The reference method for this type of analysis is the anion exchange chromatography under basic conditions coupled with pulsed amperometric detector (HPAEC-PAD). This approach was successfully carried out for the separation of 7 standards (mono- to tetrasaccharides). In a second step, the method was applied for the purity profiling of maltotriose at 0.1% level of impurity. Limits of detection and quantitation were found acceptable for this purpose.
These results were compared with those obtained with a porous graphitic carbon (PGC) stationary phase. With the latter, it was also possible to separate anomers for all the tested saccharides. Finally, the HILIC (hydrophilic interaction liquid chromatography) mode was also explored. Chromatographic performance obtained was also satisfactory in terms of retention and selectivity for the mixture of 7 standard saccharides.
The last two methods were evaluated using evaporative light scattering detector (ELSD). For performing appropriately the impurity profiling of maltotriose using this detector, a heart cutting procedure was applied.

Analytical Chemistry **29**

Rapid Detection of Explosives on Human Skin by Neutral Desorption Extractive Electrospray Ionization (ND-EESI) Mass Spectrometry

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Low picograms of explosives 2,4,6-Trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), octahydro-1,3,5,7-tetranitro-1,3,5,7-tetraazocine (HMX), triaceton triperoxide (TATP) and nitroglycerin (NG) were successfully sampled from human skin in vivo, using a novel neutral desorption (ND) divce[1,2] with a nitrogen gas beam, for sensitive detection and rapid identification by extractive electrospray ionization (EESI) tandem mass spectrometry. Without any sample pre-treatment, the fragile metal complexes of explosives were gently sampled from skin. For most explosives on a skin surface, ND-EESI-MS provided a LOD (limit of detection) in the low picogram range. A linear dynamic range of 4 orders of magnitude (0.01 ng-100 ng) was found for RDX using the characteristic fragment (m/z 237), generated from $(RDX+ CH3COO)^-$ under CID conditions. The signal intensity for either RDX or TNT was maintained at the same level when the length of the sample transfer line varied from 2 cm to 200 cm. No serious sample carryover effect was found using a Teflon tube (200 cm length; 3 mm I.D.) as the sample transfer line. Less than 1 second was required to record a spectrum when a 200 cm-length tube was used to transport the sample plume generated by the ND process. Furthermore, the EESI source requires no optimization when changing samples, facilitating high throughput analysis of complex samples. The capability of ND-EESI-MS for remote analysis of explosives provides an alternative way for convenient fast screening of explosives under hazardous environment.

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Analytical Chemistry **30**

Rapid Classification of Perfumes by Neutral Desorption Extractive Electrospray Mass Spectrometry (ND-EESI-MS)

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We present a sample preparation-free approach for ultra rapid classification of perfumes by Extractive Electrospray Mass Spectrometry. The high throughput and simplicity of this method can be advantageous in the perfume industry, for example, for online quality control.
The experiments were run in positive ion mode on a commercial ESI quadrupole-time-of-flight mass spectrometer (QTOF UltimaTM, Micromass, Manchester, UK). The commercially available perfumes were deposited onto a paper strip by nebulizing from the original bottle. The smelling strip was then brought close to the capillary end of the ESI source (Z-spray, Micromass, UK) running a pure solvent mixture (methanol / water / acetic acid 40% / 40% / 20%). A stream of nitrogen gas at a flux of 0.3 - 3 l/min*mm^2 was directed at the probe strip surface to liberate analyte molecules.
Unique EESI-MS fingerprints of ten famous brands were obtained over the 50-800 m/z range. Distinctive sets of characteristic compounds unique for each sample were clearly observed. The feasibility of rapid forgery detection was shown on the example of an authentic and a counterfeit "Miss Dior" fragrance. In many countries a good part of the perfume market is occupied by fragrances "inspired" by famous brands. Producers of these inspired perfumes try to imitate the aroma of a famous brand. It can be extremely difficult to recognize such a product by a simple smell test. We show that such recognition can be achieved rapidly by EESI fingerprinting on the example of "Opium" (Yves Saint Laurent) and its inspired analog "Option" (Nova).

Analytical Chemistry **31**

Electrochemical Detection of Tagged Proteins by SECM

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Electrochemical detection of proteins by scanning electrochemical microscopy (SECM) has been performed mainly by coupling it with common protein detection techniques like immunodetection[1] or metal staining.[2, 3] Thus, general or specific protein detection can be achieved in separately experiments. Here in, we propose a new protein detection method based on the protein tagging with benzoquinone. This new methodology is less time consuming than immunodetection or metal staining and can be used for general or specific protein detection, thanks to the pH dependant specificity of the tagging reaction.[4] Thus, by using this method relevant information for protein identification and sensitive protein quantification (5 ng per band) can be obtained at the same time.
The electrochemical detection principle of tagged proteins is based on the electrochemical reduction of a redox mediator (ferricyanide) on a scanning electrode surface and its chemically recycling by the quinone-protein adducts on the polyvinylidene fluoride (PVDF) membrane (see Figure 1).

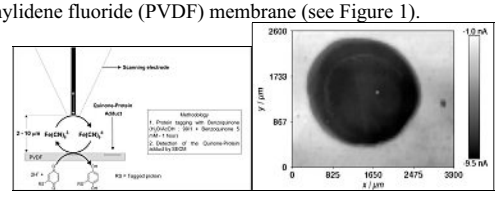

Figure 1. a) Electrochemical detection principle of tagged proteins with benzoquinone by mediated reduction of quinone-protein adducts. b) Constant height SECM image of a tagged protein spot (BSA, 500 ng) over PVDF with benzoquinone in a solution of $K_3[Fe(CN)_6]$ 2.9 mM with KNO_3 0.11 M. Working electrode Pt (20 µm diameter). Counter electrode Pt, quasireference electrode (QRE) Ag. E_{tip} = -0.1 V Vs QRE. Probe-substrate distance = 4 µm, step size = 50 µm and translation rate = 50 µm/s.

Analytical Chemistry **32**

Stable isotope labeling-based protein quantitation probed by top down mass spectrometry

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The stable isotope-based protein quantitation method, ANIBAL, implemented in bottom-up mass spectrometry, is based on protein chemical derivatization with two chemical tags, aniline and benzoic acid, targeting carboxyl- and amino side chains, respectively, at protein level [1]. Here, we extend the application of the ANIBAL method to top-down mass spectrometry to distinguish protein isoforms and deliver more complete protein characterization.

Chemical derivatization with carbodiimide chemistry was employed to label proteins, e.g. ubiquitin, first in 1M pyridine pH 5.0 containing either light or heavy aniline, followed by the addition of an EDC solution and second in HEPES 200 mM pH 8.0 containing light or heavy benzoic acid, respectively. The extent of derivatization was monitored by MALDI TOF MS. In-depth sample analysis was performed on an 11 T LTQ FT-ICR MS/MS.

Top-down high-resolution mass spectrometry of ANIBAL stable-isotope labelled proteins revealed deep insights into the obtained protein population and provided information complementary to bottom-up mass spectrometry. Preliminary analysis of derivatized ubiquitin reveals a population of 10- to 12-fold aniline labelled proteins, which corresponds to 83% to 100% yield of theoretically possible derivatization. Complementary to bottom-up MS, the top-down MS approach allows for localization of ANIBAL labels on the protein sequence in heterogenous mixtures of labeled proteins thereby indicating protein structural preferences for chemical derivatization. The two main challenges are the reduced solubility of the intact protein after derivatization and the observed adducts.

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Analytical Chemistry **33**

Continuous nitrous oxide isotopomer measurements based on quantum cascade lasers

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The intramolecular distribution of ^{15}N in N_2O can be used to obtain important information on the geochemical cycle of N_2O because isotopic fractionation is characteristic for different processes. The isotopomers $^{14}N^{15}N^{16}O$ and $^{15}N^{14}N^{16}O$ (or $^{15}N^{\alpha}$ and $^{15}N^{\beta}$) have the same mass and can only be determined by mass spectrometry (IRMS) through the complex analysis of NO^+ and N_2O^+. In contrast, laser spectroscopy offers the inherent advantage of site selectivity combined with high sensitivity and time resolution.

We present a laser spectrometer consisting of a thermoelectrically (TE) cooled, pulsed quantum cascade laser (QCL) at 4.6 μm, a multipass cell with a path length of 56 m and a TE cooled IR detector, allowing continuous, liquid nitrogen-free operation. With this instrument, the isotope mixing ratios of $^{15}N^{\alpha}$, $^{15}N^{\beta}$ and $^{14}N_2{}^{16}O$ can be analyzed simultaneously at 1 Hz time resolution. Using a prototype laser, a precision of 3 ‰ was achieved for $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$ with an averaging time of 300 s at 9 ppm N_2O. This creates new possibilities for numerous environmentally relevant studies based on relative isotope abundance.

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Analytical Chemistry **34**

Online Control of Ethanol Fermentation by a MFC-type Biosensor

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A microbial fuel cell type biosensor integrated in a 500 mL fermenter was constructed and employed for ampero- (μA) and potentiometric (mV) measurements. The aim was to follow microbial activity during a wine fermentation by saccharomyces cerevisiae and to detect the end of sugar consumption. Three different sensor setups were tested to register online electrochemical signals produced by the ethanolic metabolism of glucose and fructose (artificial wine). First set-up: A reference electrode was used to record potentiometric values, which rose from 0.26 to 0.5 Volt in about 10 hours during the growth phase. In a second set-up a combination of ampero- and pseudo potentiometric measurements delivered a maximum voltage of 35 mV. In a third type of arrangement a reference electrode was added to the anodic fermentation compartment to record separate ampero- and potentiometric measurements. In this case the reference potential rose to 0.44 Volt while the current maximum recorded by the working electrodes, reached 27 μA. To compare the electrochemical signals with standard values, the fermentation was also monitored by optical density (600 nm) analysing biomass production. HPLC with an Aminex column and RI detector was used for fructose and glucose conversion, and ethanol production was analyzed by GC with methanol as internal standard.

Analytical Chemistry **35**

Extractive Electrospray Ionization Mass Spectrometry of Breath for Monitoring Intake of Pharmaceuticals in Real-Time: Valproic Acid

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Monitoring the levels of pharmaceuticals and their metabolites is of utmost importance. This is especially true when there are changes in dosage, clinical condition or concomitant medications. For example, if the antiepileptic drug valproic acid is below therapeutic levels it will not manage seizures efficiently and above a certain threshold adverse effects become more frequent. Thus, frequent blood tests are required to supervise the plasma concentration. However, blood sampling is painful, invasive, requires specialized personnel, and produces hazardous waste. An alternative that overcomes these disadvantages is breath analysis. Recently, extractive electrospray ionization (EESI) mass spectrometry was successfully applied in our laboratory for analysis of breath. Basically, the volunteers exhale through a tube that guides the breath to the area where a pure solution is electrosprayed onto a MS sampling interface. Here, the compounds in the breath can be ionized and later analyzed. This technique requires no sample storage or pre-treatment, thus analysis can be performed in real-time. We have found that unique features in the EESI mass spectral fingerprints of individuals under valproic acid can be used for monitoring its intake and perform pharmacokinetic studies. Current work towards identifying these biomarkers and how they relate to the valproic acid plasma concentration is underway. However, it is evident that EESI MS of exhaled breath allows the real-time measurement of drugs intake in a pain-free and non-invasive manner, which will ultimately permit better patient therapy management in the clinical setting.

Analytical Chemistry **36**

Development of an optimized LC-MS system for the rapid isolation of minor bioactive compounds from crude plant extracts

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In phytochemical investigations, the isolation of pure compounds is often mandatory for complete structural elucidation and/or bioactivity assessment. In this respect, semi-preparative HPLC offers interesting features such as rapidity, selectivity, efficiency, and automation possibility. Numerous applications have demonstrated its potential for the isolation of major plant metabolites. However, in the case of minor compounds, which can be responsible for the main bioactivity of a given plant, the task becomes tricky since it is essential to avoid any coelution with other constituents from the vegetal matrix and the optimization of the chromatographic separation is thus essential. Another issue is their problematic detection within complex extracts, due to their very low concentration. Coupling semi-preparative HPLC to mass spectrometry (semi-prep LC-MS) provides an efficient solution since monitoring of selected ion traces is possible with high sensitivity. In this study, semi-prep LC-MS was used for the micro-isolation of biomarkers which are implied in plant defence signaling, directly from crude extracts. Two potentially new compounds were detected using ultra-performance liquid chromatography coupled to time-of-flight mass spectrometry (UPLC-TOF-MS), followed by a multivariate data treatment. Their isolation was found to be challenging since numerous coelutions occurred with constitutive metabolites. The column geometry, mobile phase flow rate, sample loading and every sources of extra-column contributions were taken into account to maximize efficiency. Experimentally, an effective plate number equal to 35'000 was achieved with conventional semi-preparative pumps by coupling columns in series. The selectivity was optimized at the analytical scale (UPLC) thanks to modeling chromatographic software and optimal conditions were then geometrically transferred to semi-preparative scale for isolation, enabling a significant gain of time for method development. Thanks to the high purity of the isolated compounds, micro-flow NMR data of good quality were obtained and the two new biomarkers characterized as polar derivatives of jasmonic acid conjugated with isoleucine.

Analytical Chemistry **37**

Fast analysis of doping agents by UPLC-QTOF-MS/MS

Part II: Confirmatory analysis

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For doping control, the analysis of samples is achieved in two steps: a rapid screening and, in a case of a positive result, a confirmatory analysis. The latter is dedicated to unambiguously determine the presence of a forbidden analyte. To definitively incriminate an athlete, following the World Anti-Doping Agency guidelines, different samples have to be simultaneously analyzed: urine spiked with the corresponding standard, blank urine and the suspect sample. All materials should be submitted to the entire analytical process. After a specific sample preparation, the putative compound is analysed on a short UPLC column (50 mm) and detected by mass spectrometry to obtain accurate mass of parent and fragment ions. Following the WADA expectations, spectra obtained for the spiked and suspect urine must fit in the number of fragments and intensity ratio. Whereas, in the blank urine, no specific fragment ions had to be found.
A highly selective method was developed for each doping agent (more than 100 substances) by setting the cone voltage and the collision energy. Thanks to the QTOF analyzer, it was possible to obtain in the same chromatographic run, a TOF-MS and a QTOF-MS/MS information. At the expected retention time an acquisition of parallel scanning at two voltages allows to obtain molecular ions (precursor) and fragment pattern with high mass precision (< 5 ppm).

Analytical Chemistry **38**

Evaluation of the Ultra Performance Liquid Chromatography for rapid chiral separations.

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Three strategies are usually applied to analyse chiral drugs by LC: 1. Use of chiral stationary phases (CSP), 2. Addition of chiral selectors (i.e. cyclodextrins) to the mobile phase, 3. Precolumn formation of diastereoisomers with an optically pure reagent. These last years, there has been a strong development of columns packed with sub-2µm particles in ultra high pressure conditions (UHPLC) to achieve faster and more efficient separations. The UHPLC technology has to be evaluated for stereoselective separation since the analysis time can be strongly reduced. To the best of our knowledge, CSP packed with sub-2µm particles are not yet commercially available. Thus, the approaches 2 and 3 were evaluated for rapid chiral analysis, using amphetamine derivatives and beta-blockers as model compounds.
In approach number 2, cyclodextrins were selected as mobile phase additive and chiral separations of amphetamines derivatives carried out in less than 5 minutes. This strategy was compared with the addition of chiral selectors in capillary zone electrophoresis (CZE) and several drawbacks were highlighted. In approach number 3, the analysis of diastereoisomers after derivatization procedure was evaluated using two different derivatization reagents. Complex separations of several amphetamine derivatives were achieved in 2 to 5 minutes, with high efficiency. The developed method was applied to the enantiomeric purity determination of amphetamine and methamphetamine. Similar results were obtained with beta-blockers and a separation of 5 couple of enantiomers was carried out in less than 3 minutes.

Analytical Chemistry **39**

Ultra-fast separations with 10mm columns packed with sub-2µm particles: Some qualitative and quantitative perspectives in pharmaceutical analysis

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Nowadays, one of the main objectives of analytical laboratories is to develop rapid and efficient procedures for performing qualitative and quantitative analyses. The pharmaceutical industry is interested to cope with a large number of samples and to reduce the time response delivery, particularly for quality control of pharmaceutical formulation. A simple strategy consists in decreasing column length since analysis time is directly proportional to the latter. Because efficiency is also affected, a simultaneous particle size reduction is mandatory to limit resolution decrease. Therefore, ultra-short columns (i.e. 10 mm) filled with 1.9 µm particles were packed and evaluated.
Basic chromatographic performance (efficiency, backpressure, etc) were evaluated through Van Deemter curves and compared with 150 mm, 5 µm and 50 mm, 1.9 µm columns packed with identical stationary phase chemistry. Several simple pharmaceutical formulations (e.g. local anaesthetic, spasmolytic, anti-hypertension drugs) were selected and a method was developed and validated for each of these substances following ICH guidelines. This study demonstrates that 10 mm columns packed with 1.9 µm particles represents a good alternative to conventional columns for simple pharmaceutical formulations analysis. Equivalent quantitative performance with a significant analysis time reduction (up to 60-fold) was obtained with the use of an optimized chromatographic system, to avoid extra-column dispersion.

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Analytical Chemistry **40**

Very efficient separations in isocratic and gradient modes using UHPLC technology at ambient and high temperature.

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The UHPLC strategy which combines sub-2µm porous particles and ultra high pressure (>400 bar) was investigated to generate very high resolution in both isocratic and gradient modes, for mobile phase temperatures between 30 and 90°C.
In isocratic mode, experimental conditions to reach the highest possible efficiency were determined using the kinetic plot representation (ΔP_{max}=1000 bar). By limiting the column length to 450 mm, the maximal plate count (around 100'000) with the lowest analysis time was achieved using a mobile phase temperature of 90°C. An excellent agreement was found between experimental efficiency values and predicted values from kinetic plots, for many different set of conditions.
For a given gradient length in UHPLC, the longest column does not necessarily provide the maximal peak capacity. Therefore, a compromise should be found between column length and efficiency to reach the highest peak capacity. We used the (N, t_0) data from the kinetic plot method to demonstrate that a 150 mm column should ideally be selected for gradient lengths up to 100 min, while the 3x150 mm columns coupled in series was attractive only for t_{grad}> 350 min. At higher temperature (90°C), peak capacities were increased by about 30% vs. 30°C, for a constant gradient length. Finally, some separations of standardized complex plant extracts produced at the industrial level were carried out in gradient mode using a UPLC-TOF-MS instrument. These separations clearly demonstrated the benefits of working in optimal conditions to obtain the best resolution per unit of time.

Analytical Chemistry **41**

Contactless Conductivity Detection for Microseparation Techniques

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Capacitively coupled contactless conductivity detection (C4D) for capillary electrophoresis has gained considerable popularity over the last few years. The method generally allows the facile detection of all charged analytes with good sensitivity, including those species which cannot be quantified with optical means. The principles have been thoroughly investigated and a comprehensive understanding of the fundamental properties has been reached.

In our laboratory, a range of projects based on C4D are carried out. In clinical analysis the method is suitable for the determination of inorganic electrolytes as well as of organic species of interest which are not UV-active. Further applications are in therapeutic drug monitoring and also possible is the use of the method in enzymatic assays for neutral species such as urea and ethanol.

A new portable and all-battery powered CE-instrument has been designed and tested in the Tasmanian wilderness. A further instrumental advance has been made by coupling CE with sequential injection analysis (SIA) for automated injection and capillary flushing. The approach should be useful in process analysis. Highly rapid separations in short conventional capillaries are also feasible with this arrangement.

Contactless conductivity detection was furthermore shown to be suitable for detection in HPLC for the quantification of non-UV-absorbing species. The method is, within limits, compatible with gradient elution and particularly suitable for detection when using micro-scale monolithic columns.

Analytical Chemistry **42**

Monitoring of Enzymatic Reactions with Capillary Electrophoresis Using Contactless Conductivity Detection

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The use of capillary electrophoresis with contactless conductivity detection was evaluated for the monitoring of enzymatic reactions. The non-ionic species ethanol, glucose, ethyl acetate and ethyl butyrate were made accessible for analysis by capillary electrophoresis via charged products or byproducts obtained in enzymatic conversions using hexokinase, glucose oxidase, alcohol dehydrogenase and esterase [1]. Two of the reactions, namely the conversion of glucose with glucose oxidase and that of ethylacetate with esterase, were also successfully demonstrated on a microchip-device. The determination of urea in human blood as clinical application of this method was investigated. The results were compared with the established methods and were found to be very close [2].

The digestion of proteins with pepsin and trypsin, important in food chemistry and in proteomics, can also be successfully monitored by capillary electropherosis with contactless conductivity detection. The method was furthermore applied to monitor the enantioselective hydrolysis of esters of amino acids with lipases. The enantiomeric excess (e.e.) as well as the product yield were studied. Lipases from porcine pancreas and wheat germ were compared with regard to their efficiency for the hydrolysis and enantioselectivity. Studies are also in progress on the application of the method for the investigation of acetylcholinesterase inhibitors such as the drug galantamine.

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Analytical Chemistry **43**

Applications of Capillary Electrophoresis with Contactless Conductivity Detection in Clinical Analysis

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Clinical analysis of inorganic and small organic species in biological fluids has been successfully carried out with capillary electrophoresis using contactless conductivity detection. The inorganic cations (potassium, ammonium, sodium, calcium and magnesium) and anions (nitrate and sulfate), which play an important role in the composition of human body fluids, were successfully determined in human serum and urine [1]. The determination of valproic acid, a small organic molecule which is an anticonvulsant and mood-stabilizing agent, was also achieved [2], and the method can be used in therapeutic drug monitoring (TDM). Similarly the antibiotic tobramycin was determined in plasma samples [3]. Currently being investigated is the determination of small native organic ions of clinical interest such as uric acid, lactate and pyruvate.

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Analytical Chemistry **44**

Separating Stereoisomers Using Capillary Electrophoresis with Contactless Conductivity Detection

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The separation and quantification of enantiomers is an important application of capillary electrophoresis as it is possible to use even expensive chiral reagents due to the low buffer volumes employed. However, non-UV-active or non-fluorescent compounds usually have to be chemically derivatized in order to allow detection. Conductivity detection makes such compounds directly accessible. It was found possible to determine many small amines in their protonated form by using a combination of a non-charged cyclodextrin and a chiral crown-ether as selectors in the separation buffer [1-3]. Also successful with this approach was the separation of the stereoisomers of di-, tri- and tetrapeptides which are otherwise difficult to distinguish [4]. Currently investigated is the separation of the enantiomers of small organic acids of biological importance, such as lactic acid.

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Analytical Chemistry **45**

Rapid Affinity Classification of Kinase Inhibitors by Mass Spectrometry

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Protein kinases have emerged as a major drug target in the last years and therefore tools are required for a rapid classification of inhibitors according to their affinity for a certain target. We are comparing different nanoelectrospray mass spectrometry (nanoESI-MS) based methods to quantify binding affinities and qualitatively determine, by competition experiments, the relative affinity of several clinical inhibitors. Method 1 is based on monitoring the noncovalent complex signals of two different inhibitors competing for binding to protein [1]. The binding affinity order obtained for p38 was: BIRB796 > VX-745 > SB202190 > BIRBanalogue5 > PD-173074, and for Lck: CGP076030 > BIRB796 > PP1 ≈ PP2 > CGP062464.
Method 2 is based on ligand depletion [2]. The signal of two inhibitors is monitored for different concentrations of protein. The binding affinity order obtained for p38 was: VX-745 > SB202190 > BIRBanalogue5 ≈ Bay43-9006 > BIRBanalogue4 > PD-173074, and for Lck: Bay43-9006 > CGP062464 ≈ CGP076030 > PP2 > PP1 > Tarceva.
With few exceptions, the results of both methods agree well. The advantages and disadvantages of the used methods will be discussed. The qualitative binding orders obtained are compared to standard IC_{50} measurement. Sample consumption, speed of the measurements and ease-to-use of the nanoESI-MS based methods versus IC_{50} measurements will also be compared.

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Analytical Chemistry **46**

Application of NMR spectroscopy and multivariate analysis in the characterization of the "Golden root", *Rhodiola rosea* (Crassulaceae)

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The number of studies applying chemometry to the analysis of NMR spectra is rapidly increasing. However, the combined use of NMR spectroscopy with multivariate statistics remained an important and recent research field in phytochemistry [1]. The examination of complex different metabolites in a plant system requires powerful analytical tools to compare, classify and discriminate a large set of samples [1].
Rhodiola rosea (Crassulaceae) known as "Golden root" or "Arctic root" is widely distributed at high altitudes throughout Europe and Asia. The root has been used in the traditional medicine to stimulate performance, eliminate fatigue, and prevent high sickness.
^{1}H-NMR spectra of *R. rosea* crude extracts were acquired and submitted to statistical analysis. The composition of the four wild populations collected in Mattmark (Switzerland) at 5 different times over a one year period were compared. The presence of the two markers (Salidroside and Rosavin) was detected in the extracts.
In order to minimize metadata related to statistical analyses associated with NMR experiments, emphasis was put on sample preparation, instrument configuration, data acquisition, data pre-processing and multivariate analysis.

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Analytical Chemistry **47**

Direct Determination of Native Proteins in Miniaturized Capillary Electrophoresis System

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Lysozyme (14.4 kDa), BSA (66.4 kDa) and beta-galactosidase (116 kDa) were successfully separated and determined directly within 80 seconds using a laboratory-made miniaturized capillary electrophoresis apparatus provided with a confocal fluorescence spectrometry. Several parameters controlling on the detection limits, including focusing effect, laser power and buffer composition were tested and optimized. Separation buffer was 10 mM phosphate containing 4 mM CTAB at pH 2.5. The LOD values for lysozyme, beta-galactosidase and BSA were found as 9.0, 13.0 and 55 fg/µl, respectively. This miniaturized CE system offers a lot of advantages for protein analysis. First; it provides reproducible separation reducing wall-adsorption effects at low pH. Second, the analysis time observed from our system is in the range of chip electrophoresis applications. And finally, LOD values for standard proteins are much more lower than that of obtained from traditional gel electrophoresis method.

Analytical Chemistry **48**

Investigation of new solid gold reference materials and their application for gold analysis using LA-ICP-MS

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The potential of laser sampling has been continuously described in numerous studies [1]. The high spatial resolution sampling and high sensitivity makes LA-ICP-MS an attractive technique for determining major, minor and trace elements in solid samples. The capabilities of the technique has been successfully applied in the analysis of precious, gold-matrix-based samples [2-3].
New gold reference materials were characterized (element distribution and composition) and tested for investigation of Pre-Columbian gold artifacts. The reference materials (NA1, NA2) were produced by Norddeutsche Affinarie AG., Germany. The composition (1 % Ag, 99 % Au for NA1 and 5.5 % Ag and 94.5 % Au for NA2) allowed matrix-matched solid calibration for the analysis of the gold objects. The evaluation of the reference materials were performed using nano- and femtosecond laser ablation systems in combination with liquid and gold standard-based solid calibration (FAU7 – NIST 8053, 8054, 8055, FAU10 – NIST 8062, 8063, 8064). The obtained results were compared to the element concentration values determined by the manufacturer and were in agreement for most of the elements. Liquid calibration and gold standard-based solid calibration was found to be applicable for the analysis of gold artifacts. The differences between the two calibration strategies were in the order of 10 % for most of the elements. Figures of merit and the results will be discussed in this presentation.

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Analytical Chemistry **49**

Tip-Enhanced Raman Spectroscopy of Membranes in Liquids

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The ability to perform tip-enhanced Raman spectroscopy (TERS) in liquids is of great importance, for example for the in-vivo visualization of membrane proteins in a cell. Knowledge of the movements and activities of these proteins would provide much-awaited answers to the mechanisms of cell growth, transformation and transport. However, TERS experiments have been carried out only in air or vacuum so far. These are unrealistic environmental conditions for most biological systems.

In this work, TERS has been performed in liquid for the first time. TERS of a self-assembled monolayer of thiophenol on a gold surface was successfully carried out in water as the proof-of-principle study. TERS was then used to investigate erythrocyte membranes and the membrane proteins were identified with high spatial resolution. Our results paves the way for detailed nanoscale chemical analysis of lipid bilayers and other biologically important molecules in their natural physiological environment, which will give valuable insight into the working mechanisms of a cell.

Analytical Chemistry **50**

Mass Spectrometry Analysis of Phospholipids Complexation Reactions in Biphasic Systems

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Electrospray ionization mass spectrometry analysis of species formed in a biphasic system is proposed. This was achieved by using a dual-channel microsprayer [1], where channels filled with different immiscible phases meet at the Taylor cone. As a first approach, the interfacial complexation of aqueous lead (II) ions by 1,4,7,10-tetrathia-cyclododecane (TTCD) was studied [2]. In complete agreement with previously reported electrochemical data, the formation of the 2:1 complex between the ligand and the metallic ion was observed. In the next stage, complexes formation between copper, calcium and small peptides (Phe-Phe, Angiotensin III and Leu-Enkephalin) and L-α-dipalmitoyl phosphatidylcholine (DPPC) were also observed and correlated with electrochemical experiments. In this way, the association between metallic cations, small peptides and phospholipids could not only be assessed from electrochemical measurements at the liquid-liquid interface, but also from biphasic mass spectrometry experiments. Moreover, complementary and highly valuable information, like phospholipid oligomers formation and association stoichiometry values, were obtained from this rather complex system. Indeed, using this methodology, the interaction between membrane disrupting peptides and phospholipids will be addressed in the near future.

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Analytical Chemistry **51**

How to Generate Peak Capacity in HPLC

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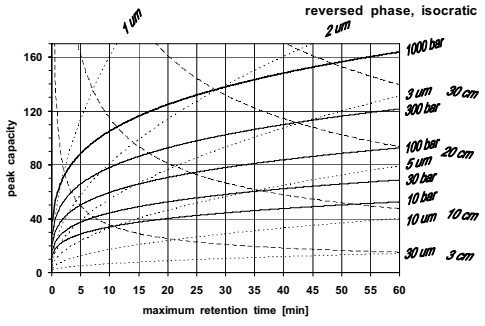
Complex samples need to be run on a chromatographic set-up with high peak capacity (PC). The concept of PC is broader than the one of theoretical plate number because it covers both the column characteristics and the run time of the separation. A desired PC can be obtained with many combinations of particle diameter, column length, pressure, and analysis time: either with a short column, small-diameter packing, short analysis time, and high pressure; or with less pressure at the expense of a longer column, larger-diameter packing, and longer analysis time. These combinations can be presented in the form of nomograms.[1] The practical limits of PC are given by the maximum pressure delivered by the pump in use.
These considerations are based on an old paper by Halász and Görlitz[2] and apply the concept of HPLC columns used at their van Deemter optimum.



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Analytical Chemistry **52**

A comprehensive metabolite profiling of *Isatis tinctoria* leaf extracts

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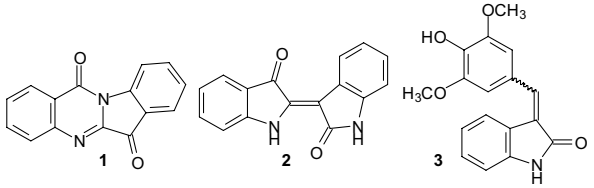
Woad (*Isatis tinctoria* L., Brassicaceae) is an ancient indigo dye and anti-inflammatory medicinal plant, which has been used and cultivated in Europe since antiquity. The anti-inflammatory potential of lipophilic leaf extracts was confirmed in a broad-based pharmacological profiling, in various animal models, and in a clinical pilot study [1]. Tryptanthrin (**1**), indirubin (**2**), indolin-2-one (**3**), and γ-linolenic acid were identified as active principles inhibiting COX-2, 5-LOX, the expression of the inducible nitric oxide synthase, human neutrophil elastase, and the release of histamine from mast cells. To further characterize the pharmacologically active extracts, we carried out a comprehensive metabolite profiling with the aid of online spectroscopic measurements (HPLC coupled to PDA, ELSD, APCI- and ESI-MS, and HRESI-MS). Off-line semi-preparative HPLC-NMR analysis was used for structure elucidation of some constituents. So far, more than 60 compounds belonging to various structural classes such as alkaloids, flavonoids, fatty acids, porphyrins, lignans, carotenoids, glucosinolates and cyclohexenones have been unambiguously identified, and tentative structures proposed for additional compounds.



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Analytical Chemistry **53**

Qualitative and quantitative analysis of *Indigo naturalis* samples by LC-PDA-MS and qNMR

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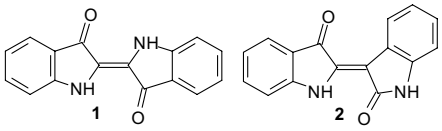
Indigo naturalis (Quingdai) is used in the Traditional Chinese Medicine (TCM) to treat chronic diseases such as psoriasis, and various cancers. The drug is obtained from indigoferous plants such *Baphicacanthus cusia* (Acanthaceae), *Isatis indigotica* (Brassicaceae) or *Polygonum tinctorium* (Polygonaceae) via a fermentative extraction process. *Indigo naturalis* contains indigo (**1**) and indirubin (**2**). Indirubin is a kinase inhibitor, mainly of CDK5/GSK2 [1]. A proposal for a European Pharmacopeia monograph for *Indigo naturalis* has been recently published, whereby **1** (minimum content 2.0%) and **2** (minimum content 0.13%) should be determined by HPLC [2]. The remaining 97% are undefined. We determined the indigo content of eight different *Indigo naturalis* samples via quantitative ^{1}H-NMR. A comparison with the results of the proposed pharmacopoeia method clearly revealed, that the HPLC assay consistently gave much lower indigo concentrations due to poor solubility of indigo. NMR spectra showed that one *Indigo naturalis* sample contained significant amounts of sucrose as formulating agent. All *Indigo naturalis* samples contained large amount of inorganic material (mainly Ca^{2+} and carbonate). Minor organic compounds in *Indigo naturalis* were identified by HPLC-PDA-MS.



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Analytical Chemistry **54**

Tandem Mass Spectrometry of Oligonucleotide-Cisplatin Adducts

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Cis-diamminedichloroplatinum(II) (cisplatin, *cis*-DDP) is a cornerstone of anticancer therapy and became one of the most widely used drugs for the treatment of various epithelial malignancies. The cytotoxicity of cisplatin is mainly based upon its affinity to adjacent guanines in nucleic acids, resulting in the formation of 1,2-intrastrand adducts. In this study the gas-phase dissociation of DNA- and RNA-cisplatin adducts is investigated by electrospray ionization (ESI) tandem mass spectrometry (MS/MS). The fundamental mechanistic aspects of fragmentation are elucidated in order to provide the basis for the tandem mass spectrometric determination of binding motifs and binding sites of this important anticancer drug. It is shown that the binding of cisplatin to vicinal guanines drastically alters the gas phase fragmentation behavior of oligonucleotides. The 3′-C-O bond adjacent to the GG sequence is preferably cleaved, leading to an extensive formation of the corresponding w-ion. This observation was even made for oligoribonucleotides, which usually tend to form c- and y-ions under CID conditions. The absence of counter ions of equal abundance indicates that oligonucleotide-cisplatin adducts are following more than one dissociation pathway in the gas-phase. Several mechanisms that explain the increased cleavage of the 3′-C-O bond and the lack of a complementary a-ion are proposed. Results of additional MS/MS experiments on methylphosphonate-oligodeoxynucleotides confirmed the proposed mechanisms.

Analytical Chemistry **55**

High-mass MALDI MS: Characterization of large molecular size Hemoglobin-Based Oxygen Carriers

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Hemoglobin-based oxygen carriers (HBOCs) are blood substitutes based on chemically modified hemoglobin (Hb) of either bovine or human origin. Unprotected Hb quickly dissociates into its constituent dimers that are filtered by the kidney, resulting in nephrotoxicity. Extracellular Hb is bound and detoxified by the plasma protein haptoglobin (Hp) which also promotes clearance by the Hb scavenger receptor pathway [1]. Investigation of the complex formation between Hp and differently modified Hbs contributes to the overall understanding of specific toxicity and clearance of HBOCs. Here we report on the first use of high-mass MALDI-TOF MS to study large hemoglobin/haptoglobin complexes. From the data obtained, it is clearly seen that HbA_0 and chemically modified Hbs bind Hp with different affinity. Further, multimeric state of HBOCs with covalent modifications involving their α–globin subunits was characterized. Structural similarities of these HBOCs and oxidized Hb were found. The obtained results provide valuable information on mass and stoichiometry of haptoglobin binding, which might help in rational design of HBOCs with limited Hb toxicity.

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Analytical Chemistry **56**

Coupling UPLC with MS : possibilities and issues. Application to the analysis of CYP450 substrates for drug metabolism.

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In liquid chromatography (LC), there is a need for high throughput separations, particularly in the pharmaceutical analysis. The decrease in the average particle size of chromatographic supports represents an attractive approach to achieve faster analysis time without reduction of overall performance. Recently, several companies have commercialized columns packed with sub-2 µm particles and chromatographic systems able to withstand ultra high pressures (UPLC, up to 1000 bar). With the recent development of separation technologies, there has been a strong development of universal, sensitive and selective detection techniques, such as mass spectrometry (MS). The new generation of MS should be fully compatible with fast and even ultra-fast separations (*i.e.* duty cycles lower than 5 and 1 minute, respectively). For this purpose, full scan acquisition rates have been significantly improved (up to 10'000 uma/s) and dwell time drastically reduced (5 ms).
In this work, the coupling of UPLC with a new generation of single quadrupole instrument was investigated for the analysis of several CYP450 probe substrates and their metabolites, detected in both positive and negative modes. The effect of numerous operating parameters (*e.g.* mobile phase flow rate, pH, gradient length, Scan/SIM mode, dwell time, polarity switching, *etc*) on sensitivity and acquisition rate was studied. Limits of quantitation (LOQ) were determined for the drug mixture in optimal conditions.

Analytical Chemistry **57**

Biological Applications of Combined Atomic Force Microscopy-Raman Spectroscopy and Tip-Enhanced Raman Spectroscopy

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Biological systems can be highly heterogeneous on the nanometer scale. Our combined setup allows the investigation of the exactly same part of such samples by confocal laser scanning microscopy (CLSM, ca. 500 nm resolution), Raman spectroscopy (provides chemical information without staining), and AFM (imaging with nanoscale resolution). Additionally, tip-enhanced Raman spectroscopy (TERS) with laser-illuminated, metal-coated AFM tips provides Raman spectroscopic information with 20–50 nm lateral resolution. An AFM-CLSM study of river-water biofilms has demonstrated their heterogeneity at the nanometer scale [1]. The polysaccharide alginate was used as a first model system for TERS experiments on biofilm matrix constituents [2]. Spectroscopic features of these weakly Raman scattering polymers and specific marker bands for their identification in biological systems will be discussed. Additionally, the protein cytochrome c was investigated, whose Raman spectra when excited by visible-light lasers are usually dominated by strong heme bands overwhelming the weak amino acid signature. Only TERS was able to display both, heme and apoprotein bands in one spectrum [3]. Our combined setup is and will be applied to the investigation of biofilms, biomineralization, and artificial lipid membranes.

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Analytical Chemistry **58**

Towards nanoscale molecular analysis and chemical imaging at atmospheric pressure by near field laser ablation mass spectrometry

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Techniques for spatially resolved chemical analysis with high resolution have become of great relevance in the past few years. Methods that allow samples to be investigated at ambient conditions are especially suitable for the chemical characterization of biological samples such as tissues or even live cells.

In previous work [1], our group already demonstrated for the first time that near-field laser ablation at *atmospheric pressure* can in principle be coupled to mass spectrometry (SNOM-MS). We now present an improved setup that combines near-field laser ablation at atmospheric pressure with an ion-trap/time-of-flight mass spectrometer, which was developed for this application. [2]

With this instrument, spatially resolved molecular analysis yielding full mass spectral information for samples at atmospheric pressure could be shown for the first time with a lateral resolution on the low µm scale. [3]

By further improvements in sensitivity, this setup will ultimately allow chemical imaging on the nanoscale at atmospheric pressure to be performed for various applications in material and life sciences.

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Analytical Chemistry **59**

Assessment of Diesel exhaust particulate exposure and surface characteristics in association with levels of oxidative stress biomarker

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Exposure to PM_{10} and $PM_{2.5}$ (particulate matter with aerodynamic diameter smaller than 10 µm and 2.5 µm, respectively) is associated with a range of adverse health effects. Surface characteristics (chemical reactivity, surface area) are considered of prime importance to understand the mechanisms which lead to harmful effects. A hypothetical mechanism to explain these adverse effects is the ability of components (organics, metal ions) adsorbed on these particles to generate Reactive Oxygen Species (ROS), and thereby to cause oxidative stress in biological systems.

The aim of the present research project is to test whether there is a correlation between the exposure to Diesel Exhaust Particulate (DEP) and the oxidative stress status. For that purpose, a survey has been conducted in real occupational situations where workers were exposed to DEP (mechanical yards in bus depots).

Different exposure variables have been considered: particulate number, size distribution and surface area; particulate mass ($PM_{2.5}$ and PM_4); elemental and organic carbon; total adsorbed heavy metals (iron, copper, manganese); surface functional groups present on aerosols.

An oxidative stress biomarker (8-hydroxy-2'-deoxyguanosine) has been determined in urine of volunteers, and urinary levels of this biomarker will be compared to exposure variables in order to gain a better understanding of the relation between the particulate characteristics and the formation of ROS by-products.

Analytical Chemistry **60**

**An automatic titration device for the determination of chemical constants using 2D NMR.
Application to the determination of pKa's in complex mixtures**

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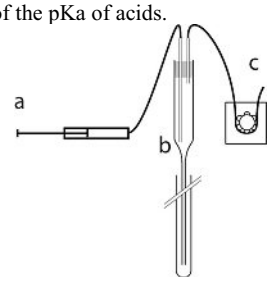
We have developed an automatic titration system allowing chemists to study changes in chemical shifts upon addition. Depending on the application, these changes can be translated into pKa's, binding constants, etc. In order to illustrate how complex mixtures can be studied using this method, we follow signals in 2D HSQC spectra instead of simple 1D ^{1}H spectra so that signal overlap can be resolved in the carbon dimension. The HSQC spectra were acquired using 10.00 ppm window in the carbon dimension in order to reach high resolution in 20 times less time than normal full spectra would require. This allows to measure carbon chemical shifts changes with high precision and accuracy in 30-minute experiments making it possible to run a full titration overnight. In this poster, we demonstrate the use of the device for the determination of the pKa of acids.



The titrant is added using a computer-controled push-syringe (a) into the mixing chamber (b). A peristaltic pump (c) insures the proper mixing of the solution.

Analytical Chemistry **61**

Screening for New Scaffolds for IMAC Purification

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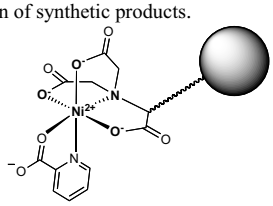
For immobilized metal ion affinity chromatography (IMAC), Ni^{2+} immobilized on nitrilotriacetic acid (NTA) is most often used for the purification of proteins carrying either a C- or N-terminal histidine (His)-tag [1, 2]. More recently, 1,10-phenanthroline was successfully implied as a tag suitable for the purification of peptides synthesized on solid-phases [3].
In our approach, we searched for new tags based on picolinic acid to be used in IMAC for the purification of synthetic products.



Structural, thermodynamic and electronic properties of different picolinic acid derivatives alone and complexed with Ni-NTA were investigated using computational methods based on the Density Functional Theory in both gas and solvent (water) phases. The results were then correlated with the binding data (binding affinity K_D and kinetic constants k_{on} and k_{off}) determined by surface plasmon resonance experiments.

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[3] H.-G. Frank, M. Casaretto, K. Knorr, Lonza AG, International Patent, **2006**.

Analytical Chemistry **62**

High Spatial Resolution Chemical Investigation of Inorganic Nanostructures

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The challenge to gather information with nanometer spatial resolution from different types of samples gained importance due to the ongoing miniaturization of production processes. Several techniques can be applied for high resolution information such as SEM, STM, AFM. Yet all these techniques mostly show topographic information. The structural information is very important to make statements about chemical composition and distribution of different substances within heterogeneous materials. Titanium dioxide can form three different cristalline phases namely Brookite, Anatase and Rutile. During the formation of TiO_2, mono- and polycristalline particles can appear. These phases differ in the occupation of different cristalline sites as well as in bulk properties. The aim of designing particles and their bulk properties make it necessary to determine the structure of the nanoparticles formed in a reaction.Using topographic methods only like high resolution AFM (down to around 10 nm lateral resolution) modifications to the particles cannot be distinguish. With additional data from confocal Raman microscopy, we are able to determine the chemical compostition of the particles as well as their structure on a submicrometer scale. Similar experiments have been done using tip-enhanced Raman spectroscopy on organic substances, dyes [1] and other mostly resonant model systems [2]. We have now extended the use of these methods to non-resonant inorganic samples allowing us to discern and locate inorganic nanoparticles.

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Analytical Chemistry **63**

Development of an HPLC-MS method for the differentiated quantification of the 15 major human bile acids in serum

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Background and hypothesis: Bile acids are the major degradation products of cholesterol and they undergo considerable structural modification through hepatic and intestinal metabolism. They are biologically important as mediators of dietary lipid absorption as well as ligands of the nuclear receptor Farnesoid X Receptor (FXR) and hence regulators of lipid and carbohydrate metabolism. The aim of our research is to develop a method based on liquid chromatography coupled to mass spectrometry (HPLC-MS) for the differentiated quantification of bile acids in serum.
Methods: The quantification of these compounds requires a highly sensitive method since bile acids are present at micromolar concentrations in serum. An HPLC-MS method was developed using reverse-phase chromatography and methanol/ammonium acetate buffer 10mM, pH 6.8 as a mobile phase. Analysis of the compounds is performed using electrospray ionization (ESI) in the negative mode.
Results: By selecting ESI as the ionization technique and optimizing the chromatographic conditions our newly developed method allows the separation of the 15 major human bile acids in less than 20 minutes and the quantification of 14 bile acids in serum samples of healthy volunteers. In addition, we found that bile acid concentrations show considerable intraindividual variation which, depending on whether they are conjugated or not, is related to food intake or circadian rhythm.
Conclusions: We developed and validated a novel sensitive and fast method for the quantification of bile acids in serum. The high degree of intraindividual variation in serum concentrations render the preanalytical phase difficult to be controlled.

Analytical Chemistry **64**

The Laser Ablation Glow Discharge Time Of Flight Mass Spectrometry (LA-MS-TOF-MS) and its capability for high spatial resolution analysis)

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Laser Ablation (LA) – Glow Discharge (GD) – Time Of Flight (TOF) Mass Spectrometry (LA-GD-TOF-MS) will be presented as a new combination of two analytical techniques along with its design and preliminary experimental results. The LA was used for direct solid sampling into a GD plasma for the ionization of ablated material. The measurements were performed by using a pulsed GD coupled to a TOF-MS. Various ablation parameters were selected to change the mass load of the GD. Furthermore, the material was introduced within different temporal GD regions. The results indicate, that the direct ionization of the laser-generated microplasma can be significantly enhanced (factor 7) when ablating the material into the afterpeak GD regime. Furthermore, laser energy, sampling position and gas atmosphere were studied in detail and figures of merit will be discussed.

Analytical Chemistry **65**

Towards Determination of Affinities Between Adenovirus Fiber Knobs of Different Serotypes and Soluble CD46

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Adenoviruses (Ads) are among the best-characterized viruses. They were discovered in the early 1950s as "adenoid-degenerative agent" [1]. More recently, research on Ad-based vector technologies has increased, and Ads have become one of the most widely used vectors for gene therapy of genetic diseases, cancer treatment and vaccination [2,3]. To date, 52 human Ad serotypes have been identified and classified into six distinct species A-F. The use of species C-derived Ad vectors in clinical applications is limited due to side effects and low efficiencies of transfection. Ad vectors derived from B species promise to overcome some of the clinical drawbacks of species C Ads [4]. Recently, several groups including ours identified membrane cofactor CD46 as an attachment receptor for species B Ad serotypes 3, 11 and 35 [5, 6]. We further characterized the binding sites of CD46 involved in binding of the four different species B serotypes 3, 7, 11 and 35. To explore if CD46 is the sole receptor for these viruses, we are determining the affinities of recombinant fiber knobs (FKs) of different Ad serotypes to CD46. We have produced five different histidine-tagged Ad-FKs using Baculovirus mediated expression in insect cells, and affinity purification on Ni-agarose. These soluble FKs are currently characterized by virus blocking experiments. In the next step, the affinities are measured by surface plasmon resonance analyses. The results will be discussed at the meeting.

Keywords: Adenoviruses: Ads; fiber knobs: FKs

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Analytical Chemistry **66**

Quantitation of oxycodone and its metabolites in human plasma using on-line SPE combined with LC-MS/MS

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Oxycodone (OXC) is an analgesic opioid largely prescribed in pain management, extensively metabolized in noroxycodone (NOXC), oxymorphone (OXM) and noroxymorphone (NOXM). To our knowledge, no validated analytical method has been published for their simultaneous quantitation in biological matrices. We developed a sensitive, specific assay for the determination of OXC, OXM, NOXC and NOXM in human plasma, using liquid chromatography tandem mass spectrometry (LC-MS/MS), ranging from 100 to 50'000 pg/ml for each compound.

Two important aspects in the assay method development will be addressed in this poster. Firstly, OXM and NOXC are isobaric analytes sharing common fragment ions in MS/MS spectra. This involves a baseline separation of these two analytes for their correct quantification. Additionally, the relatively high polarity of these analytes challenges their retention on classical reversed-phase LC media. Secondly, sample preparation consists in plasma protein precipitation followed by automated SPE with the Prospekt 2 system, which normally uses one new cartridge for each sample. Here we propose to use a single cartridge for several analyses (typically 30 or more) in order to reduce significantly the cost per sample without scarifying the efficiency of sample clean-up.

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Analytical Chemistry **67**

Bulk and In-Depth Analyses of CMR Materials (PLD Coatings) by fs-LA-ICP-MS

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The design of colossal magnetoresistive materials (CMR) for the production of next generation computer storage devices has attracted increasing attention. In this study, the feasibilities of femtosecond laser ablation inductively coupled plasma mass spectrometry (fs-LA-ICP-MS) to determine the elemental composition of thin (~ 100 nm) CMR layers grown by pulsed laser deposition (PLD) were investigated.

For this purpose, protocols for single spot and rastering mode fs-LA were conceived allowing for in-depth and "bulk" analyses of layers produced by PLD of $La_{0.6}Ca_{0.4}Mn_{0.8}Fe_{0.2}O_3$ precipitated on a crystalline $LaAlO_3$ substrate. It could, for instance, be shown that the La-, Ca-, and Fe-specific layer composition deviated by 11, 26, and 3 %, respectively, from the expected ones. Furthermore, the composition significantly altered towards the substrate interface. Changes of up to 15 % (La) were observed. These data were found to be highly consistent with the composition measured by Rutherford backscattering (RBS) indicating non-stoichiometric PLD. Depth profiles were measured using a "flat-top" laser beam profile formed by aperture imaging which enabled to improve the depth resolution as recently reported by Pisonero et al. [1].

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Analytical Chemistry **68**

Studying the Distribution of Dyes in an Electrospray Plume

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Electrospray ionization (ESI)-Mass spectrometry (MS) is one of the most widely used analytical methods in biochemistry, especially in the investigation of noncovalent complexes [1]. The study of the distribution of compounds in the plume is important for developing ESI-MS into a reliable method for the quantitive analysis of non-convalent interactions. A better understanding whether the ion peaks obtained by ESI-MS offer an accurate 'snapshot' of the bulk solution is important in this context. In this project, we investigated the distribution of two compounds in the plume using different methods, i.e. laser induced fluorescence spectroscopy [2] and confocal Raman microscopy. Mixtures of dyes, (i.e. nile red, nile blue, rhodamine 6G and coumarin 307) with ionic or nonionic character were studied. The fluorescence measurements showed that nile red, a nonionic compound, can only be observed in the center part of the plume while ionic compounds, eg. rhodamine 6G, can be observed everywhere. Similar results were also obtained in the spatially resolved Raman measurements. All these results imply that the segregation between ionic and nonionic compounds is apparent in the plume, eg. nonionic compounds are more likely to stay on the axis of the plume. The ionic or nonionic character of an analyte can thus siginificantly affect its distribution in the plume and influence the selectivity and sensitivity of ESI-MS measurements.

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Analytical Chemistry **69**

Quantitative Analysis of Compounds in Breath by Extractive Electrospray Ionization Mass Spectrometry

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Extractive Electrospray Ionization (EESI) Mass Spectrometry is a new technique for sample analysis without pretreatment. The sample is directed into an electrospray where the analyte is ionized and then sampled into the mass spectrometer. This method holds great potential for a broad set of applications due to its simplicity and ability to yield information in real-time. Some of the applications explored so far include breath analysis, food spoilage screening, perfume recognition, as well as chemical reaction monitoring. However, most of these previous studies have been limited to qualitative analysis. Thus, the goal of this work was to explore the quantitative capabilities of EESI, especially for breath analysis.
One approach was to generate an aerosol from a solution to simulate breath containing the analyte of interest. This simulated "breath" was analyzed directly with EESI. In this fashion, quantification of nicotine in the breath of a regular smoker and a occasional smoker could be performed.
Another substance which was investigated was limonene which is a flavor ingredient in chewing gum. The signal of limonene in breath after chewing different gums was quantified by a calibration via an exponential dilution chamber.
Further investigations will include quantification of additional compounds such as pharmaceuticals or metabolites. Additionally, the EESI Interface will be coupled to a spirometer to control the flow rate and volume of the breath to standardize the analysis.

Analytical Chemistry **70**

NMR Based Search for Monoterpene Aldehydes from *Amomum tsao-ko*

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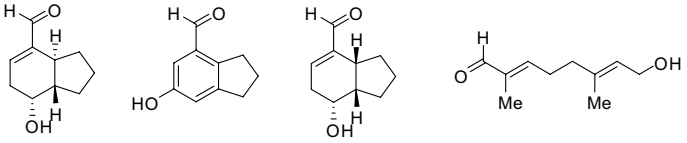
Amomum tsao-ko Crevost et Lemaire, a member of the family Zingiberaceae, has been used for centuries as food, spice and perfume in China, Japan and Korea. Its fruits have also found applications in Traditional Chinese Medicine (TCM) for the treatment of stomach illness, digestive disorders and throat infections. In previous phytochemical investiagtions of the species, bicyclic nonane aldehydes, tsaokoin, isotsaokoin [1], trans-2,3,3a,7a-tetrahydro-1H-indene-4-carbaldehyde, cis-2,3,3a,7a-tetrahydro-1H-4-carbaldehyde, 4-indanecarbaldehyde and 5-indanecarbaldehyde [2], had been reported.



1 **2** **3** **4**

We carried out a systematic search for monoterpene aldehydes from the fruits of this species, whereby the isolation process was guided by 1H-NMR of fractions (1 mm microprobe, aldehyde resonance at δ_H 9-10 ppm) spectra. Two new monoterpenes, rel-(3aS,7R,7aS)-7-hydroxy-2,3,3a,6,7,7a-hexahydro-1H-indene-4-carbaldehyde (**1**) and 6-hydroxy-4-indancarboxaldehyde (**2**), were isolated along with known aldehydes such as **3** and **4**, and other compounds. Cytotoxity of the isolates against some human cancer cell lines was determined.

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Analytical Chemistry **71**

Profiling of two Chinese medicinal plants, *Sophora flavescens* and *Ligusticum chuangxiong*, by off-line LC-NMR and LC-MS

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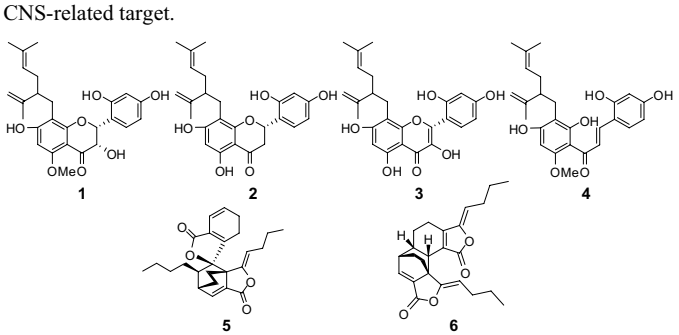
For the identification of new natural product based lead compounds, we combine initial screening of extract libraries in a range of functional assays with HPLC-based micro-fractionation for activity profiling and chemical profiling by LC-MS and off-line NMR [1]. A 1-mm microprobe with z-gradient was used to measure one and two dimensional NMR spectra [2], and fractions were obtained by peak-based fractionation of a single injection of 40 mg of extract on a semipreparative (10 x 250 mm i.d.) HPLC column. The protocol was applied to two plants used in Traditional Chinese Medicine, *Sophora flavescens* and *Ligusticum chuanxiong*, to identify 32 compounds including **1**-**4**, and **5**-**6**, respectively, as structures with promising activity on a CNS-related target.



1 **2** **3** **4**

5 **6**

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Analytical Chemistry **72**

Can Analyte Dissociation and Fluctuating Carbon Contamination Signals in Surface-Enhanced Raman Spectroscopy Be Prevented?

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Signal losses and fluctuating carbon contamination bands are 'bottlenecks' in the application of surface-enhanced Raman spectroscopy (SERS) for reliable chemical analysis. They originate mainly from prolonged laser irradiation of the sample during data collection that causes analyte decomposition and/or loss of the enhancing capabilities of the adsorption site. In this work, a laser illumination/signal collection technique, the 'multiple points collection method' (MPC) is introduced to circumvent these problems. The MPC is based on the use of a pair of galvanic mirrors to scan the laser beam rapidly and steadily across the sample surface. Each position is irradiated for <10 μs, at a rate of ~0.5 Hz. The SER spectrum is obtained by summing the signals collected from a large array of non-overlapping sample points. The MPC is compared with the conventional 'single point collection' method, where the laser beam is statically focused onto a particular spot and the scattered signals acquired. The MPC has the following advantages: (i) illumination and collection efficiencies are not compromised, (ii) Signal losses originating from analyte decomposition and/or alteration of the enhancing capabilities of the adsorption site, are avoided, (iii) high quality SER spectra for analytes such as biomolecules and dipicolinic acid (a common marker for bacteria spores) can be easily obtained, and (iv) the occurrence of broad amorphous carbon bands and the commonly observed temporal fluctuations in SERS are prevented. The success of the MPC is attributed to the reduction of local sample heating, as the time interval between the laser irradiations of a spot is much longer than the actual irradiation time itself.

Analytical Chemistry **73**

Indicator Displacement Assays as Molecular Timers

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Indicator displacements assays (IDAs) have emerged as powerful analytical tools. They are based on dyes which compete with analytes for binding to synthetic receptors. So far, IDAs have primarily been used to determine the identity and/or the quantity of certain analytes.
We show that a multicomponent IDA can also be employed to obtain information about the history of chemical inputs. A simple mixture of three commercially available dyes and the organometallic complex $[(Cp^*RhCl_2)_2]$ is employed to time the addition of ADP and ATP with good resolution [1].

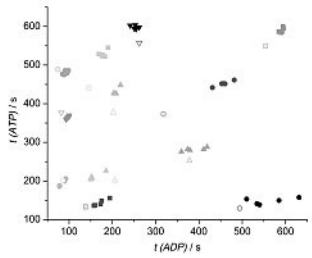

Fig.1.: The ADP and ATP addition times determined by the molecular timer in comparison with the real addition times for 12 test samples. The predictions are shown as filled symbols (5 measurements each) and the real addition timed are indicated by empty symbols.

The signal of the timer is read by UV/Vis spectroscopy and the data is analyzed via a multivariate analysis.

[1] A. Buryak, F. Zaubitzer, A. Pozdnoukhov, K. Severin, submitted.

Analytical Chemistry **74**

Real-time, on-line monitoring of organic chemical reactions using extractive electrospray ionization tandem mass spectroscopy

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Extractive electrospray ionization mass spectrometry (EESI-MS)[1] for real-time, on-line monitoring of organic chemical reactions was demonstrated for a well established pharmaceutical process reaction (one-step Michael addition reaction with phenylethylamine (PEA) and acrylonitrile in ethanol)[2] and a widely used acetylation reaction in the presence of a nucleophilic catalyst, 4-DMAP[3] (multiple-step acetylation reaction of benzyl alcohol with acetic anhydride catalyzed by 4-DMAP in dichloromethane). EESI-MS, with a commerical quadrupole-time-of-flight (Q-TOF) mass spectrometer, provides real-time information that allows determining the optimum time for terminating the reaction based on the relative intensities of the precursors and products. In addition, analysis via EESI-MS permits on-line validation of proposed reaction transients, which appears during the catalytic pathway of 4-DMAP, relying on tandem MS. The relatively simple setup allows this method to be implemented on any type of MS instrument with ESI / APCI interface. The EESI-MS features an instant response (<0.2s) and does not require sample pre-treatment, making it a powerful and convenient tool for on-line characterization and full control of chemical and pharmaceutical reactions, resulting in maximized product yield and minimized environmental costs.

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Analytical Chemistry **75**

Calcilytics – A New Treatment for Established Osteoporosis

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Osteoporosis is characterized by low bone mass and micro-architectural deterioration of bone tissue that leads to fragility and increased risk of fractures. Traditional therapies for osteoporosis inhibit bone resorption and prevent further bone loss. However, as many osteoporosis patients have already lost a substantial amount of bone at the time of diagnosis, there is a need for agents that stimulate new bone formation. The only anabolic treatment for osteoporosis, approved for the US and EU markets, is Forteo®/Forsteo® (Teriparatide, the 1-34 fragment of parathyroid hormone (PTH)) which causes a significant increase in bone mass and reduces vertebral fracture risk substantially. This peptide must be administered by daily subcutaneous injection and the therapy is costly. An orally active, low molecular weight compound with the same efficacy would be a highly attractive alternative for the patient.
Instead of applying exogenous PTH, mobilization of endogenous stores of the hormone can be envisaged. PTH is stored in relatively large amounts in parathyroid cells and its secretion is controlled by a calcium-sensing receptor (PCaR) located on the cell surface. Antagonists of PCaR (calcilytics) mimic a state of hypocalcemia and stimulate PTH release to the blood stream.
The starting point for the Novartis calcilytics project was a proprietary structure found in a HTS screen using a functional assay in the FLIPR format based on recombinant human PCaR. Optimization of the series resulted in an increase in *in vitro* potency by a factor of >100. First oral applications in rats with these highly potent calcilytics were rather disappointing with regard to PK/PD parameters. The presentation will focus in the second part on how these limitations were overcome. PK/PD data in rats and dogs will be shown that mark the best of our derivatives attractive for development as oral calcilytics. There is excellent correlation of drug exposure and PTH release. High levels of PTH are reached in plasma within minutes in both species after p.o.. application which revert to baseline in about 1-2 hours. This profile is a prerequisite for bone anabolic action, since it is well known that persistently elevated levels of PTH stimulate not only osteoblasts (bone forming cells) but also osteoclasts, the bone resorbing cells. The net result is increased bone turnover rather than the desired gain in bone mineral density (BMD).

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Total Synthesis of the Resorcylic Lactone-based Kinase Inhibitor L-783277

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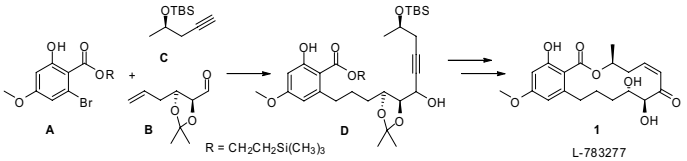
Kinases have emerged as important drug targets in cancer and inflammatory disease and several low-molecular-weight kinase inhibitors have now been introduced into clinical practice.[1] The natural product L-783277 (**1**) belongs to the family of resorcylic acid lactones (RALs), which includes compounds such as zearalenone, C292 (LL-Z1640-2), hypothemycin, or radicicol, and which exhibit a diverse range of biological activities.[2] L-783277 (**1**) is a potent inhibitor of the Ser/Thr kinase MEK.[3] We have accomplished the first total synthesis of macrolactone **1**, which is based on the consecutive assembly of the key fragments **A**, **B**, and **C**.[4] The development of an efficient enantioselective synthesis of **1** and a more detailed characterization of its biological effects are the primary goals of this research project. This presentation will discuss the details of the synthesis of **1** and the preparation of a number of analogs. Preliminary data on the in vitro biologically activity of these compounds will also be presented.



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