Non-aqueous CE-MS of cinchona alkaloids - characterizationof a novel CE-ESI-MS interface

Hansen, Frederik André; Hansen, Steen Honoré; Petersen, Nickolaj J.

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Welcome

Welcome to the
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On behalf of the Scientific Committee and the Separation Sciences Foundation, it is our great pleasure to welcome you to CSSS2016 and the beautiful city of Copenhagen.

There is a long-standing tradition for research and development of separation techniques and methods at the University of Copenhagen, and their application in different aspects within the life sciences, in particular also the pharmaceutical sciences.

The motivation of this symposium is to bring together researchers from academia and industry as well as users of modern separation techniques, and provide a forum for discussions on challenges and benefits, exchange of ideas and experiences, and exploration of possible collaborations.

The symposium will showcase 18 invited talks by leading scientists, featuring both “old tigers” and “young lions”, thus providing perspective, fundamentals, insight and glimpses of how the field of separation sciences is developing. There will also be a poster session with submitted contributions, as well as a symposium dinner.

We wish everybody a great time in Copenhagen, a successful symposium and we look forward to your suggestions, feedback and comments on this symposium and the potential for future similar meetings.

We are very grateful to the Separation Sciences Foundation for the generous support of the symposium.

Jörg P. Kutter (Symposium Chair)

Steen Honoré Hansen (Symposium Co-chair)

Inga Bjørnsdottir

Babak Jamali

Nickolaj J. Petersen

CSSS 2016

Program
Day I - 8th June

8:30 – 8:45 *Registration*

8:45 – 9:00 *Welcome/Opening remarks*

Session Chair: Steen Honoré Hansen, University of Copenhagen

9:00 – 9:30 **Peter Schoenmakers**, University of Amsterdam
One- and two-dimensional LC strategies for separating very complex mixtures

9:30 – 10:00 **Jonas Bergquist**, Uppsala University
Lipid and steroid hormone screening in a clinical setting using high resolution separations and mass spectrometry

10:00 – 10:30 **Julie Schappler**, University of Geneva
Analysis of scarce biofluids: electromembrane extraction (EME) and capillary electrophoresis coupled to mass spectrometry (CE-MS) are a good match

10:30 – 11:00 *Coffee break*

Session Chair: Babak Jamali, AscendisPharma

11:00 – 11:30 **Gunda Koellensperger**, University of Vienna
LC-MS based metabolomics - strategies towards selectivity and high metabolite coverage

11:30 – 12:00 **Wim de Malsche**, Free University of Brussels
The influence of packing geometry on chromatographic peak capacity

12:00 – 12:30 **Rune Salbo**, Novo Nordisk A/S
Chromatographic separation under HDX-MS quench conditions

12:30 – 13:30 *Lunch*

13:30 – 15:00 **Poster session** *(with refreshments)*

Session Chair: Jörg P. Kutter, University of Copenhagen

15:00 – 15:30 **Stefan Lamotte**, BASF and **Klaus Unger**, University of Mainz
HPLC columns in 2016 - state-of-art-review and a critical appraisal

15:30 – 16:00 **Jan Christensen**, University of Copenhagen
Signal processing: The missing link in analysis of XC, XC-MS, and XC×XC data

16:00 – 16:30 *Coffee break*

16:30 – 17:00 **Govert Somsen**, Free University of Amsterdam
Intact protein glycoform profiling using CE-MS and HILIC-MS

17:00 – 17:30 **Thomas Welsch**, University of Ulm
A look back on the fascinating history of chromatography

19:30 *Symposium dinner*

CSSS 2016

Program
Day II - 9th June

8:45 – 9:00 *Housekeeping/Announcements*

Session Chair: Christian Janfelt, University of Copenhagen

9:00 – 9:30 **Nickolaj J. Petersen**, University of Copenhagen
Non-aqueous CE-MS of cinchona alkaloids - characterization of a novel CE-ESI-MS interface

9:30 – 10:00 **Attila Felinger**, Univerisity of Pécs
The performance of stationary phases for fast liquid chromatography

10:00 – 10:30 **Norman Dovichi**, University of Notre Dame
Capillary zone electrophoresis for deep bottom-up analysis of eukaryotic proteomes

10:30 – 11:00 *Coffee break*

Session Chair: Stefan Stürup, University of Copenhagen

11:00 – 11:30 **Susanne Wiedmer**, University of Helsinki
Distribution constants by liposome electrokinetic chromatography

11:30 – 12:00 **Staffan Nilsson**, Lund University
Pseudostationary phase CEC - Future & past and main challenges

12:00 – 13:00 *Lunch*

Session Chair: Inga Bjørnsdottir, Novo Nordisk A/S

13:00 – 13:30 **Wolfgang Lindner**, University of Vienna
Enantio-/Stereo-selective Liquid Chromatographic Separation: State of the Art, Quo Vadis

13:30 – 14:00 **Hans Maurer**, Saarland University
Opportunities and challenges of LC - high resolution – MS in clinical & forensic toxicology

14:00 – 14:30 **Ian Wilson**, Imperial College
Advances in metabolic profiling - miniaturisation and metabolic phenotyping

14:30 **Farewell**
Workshop adjourns

Symposium Chairs

Name	Affiliation	Country
Jörg P. Kutter	University of Copenhagen	Denmark
Steen Honoré Hansen	Steen Honoré Hansen	Denmark

Scientific Committee

Name	Affiliation	Country
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Stig Pedersen-Bjergaard	University of Copenhagen	Denmark
Alexander Jönsson	University of Copenhagen	Denmark
Olivia Dzwonkowski	University of Copenhagen	Denmark

Invited talks

One- and two-dimensional LC strategies for separating very complex mixtures

Peter Schoenmakers, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands

p.j.schoenmakers@uva.nl

Liquid chromatography (LC) has progressed a long way toward the separation of very complex mixtures of non-volatile analytes. Separations with a peak capacity exceeding 1,000 have been published, using one-dimensional column LC with (very) long columns and (very) long analysis times. Roughly, such high-resolution separations may provide one peak per minute.

Comprehensive two-dimensional liquid chromatography (LC×LC) is attractive, because it provides higher peak capacities in a reasonable time (e.g. 5000 peaks at about one peak per second) and the combination of two different retention mechanisms (selectivities). As a result, is increasingly used for the separation of complex samples. Examples of the separation of polymers, surfactants, dyes and peptides will be shown.

One significant bottleneck is the development of LC×LC methods, which even for experts tends to require a good deal of time and effort. Optimization software, such as the Program for Interpretive Optimization of Two-dimensional Resolution (PIOTR) may help reduce the time required to develop an optimal method from typically a few months to a few days.

Despite the progression of LC and LC×LC, many samples are too complex to be fully separated and even the most fantastic mass spectrometers struggle with the identification and quantitation of analytes that are introduced simultaneously in vastly different concentrations. Thus, there is a need for even better separations. Spatial three-dimensional LC promises peak capacities up to one million in the future.

Lipid and steroid hormone screening in a clinical setting using high resolution separations and mass spectrometry

Jonas Bergquist

Analytical Chemistry, Department of Chemistry Biomedical Centre and Science for Life Laboratory, Uppsala University, Box 599, 751 24, Uppsala, Sweden

Abstract

Steroids are natural lipid compounds with a perhydro-1, 2 cyclopentanophenanthrene ring system. Steroids can be divided into four major classes: progestagens, corticosteroids, androgens and oestrogens. Steroids and their metabolites play important roles in human physiology and are useful biomarkers for the diagnosis of several pathological conditions. Effectiveness of the diagnostic testing depends on the appropriateness of the exploited markers of diseases; hence they are controlling human body functions as a part of the endocrine, neuronal and immune systems. Therefore, the measurements of endogenous steroid levels in the body fluids are widely employed to evaluate the androgenic status of the pathological processes of the subject under investigation.

We have developed a routine ultra performance convergence chromatography (UPC^2)-MS/MS based diagnostic test for steroids/biomarkers of endocrine and metabolic diseases. The sample preparation procedure consisted of four steps: lipid extraction from plasma, enrichment of steroids by SPE, derivatization, and analysis of steroids by a UPC^2-MS/MS. A robust, sensitive and selective method for the determination of steroids in both plasma and tissues has been established and evaluated. The method showed excellent precision, accuracy, quantification limits, detection limits, and robustness.

In summary, this newly developed method is a valuable tool to evaluate action of steroids in human diseases.

Analysis of scarce biofluids: electromembrane extraction (EME) and capillary electrophoresis coupled to mass spectrometry (CE-MS) are a good match

Nicolas Drouin, Serge Rudaz, Julie Schappler*

Scarce biofluids are biological fluids, which are either difficult to obtain because the collection is invasive (*e.g.* cerebrospinal fluid) or available in minute volume (*e.g.* tissues, cell cultures, lachrymal fluid). These biofluids contain a large number of potential biomarkers such as amino acids, neurotransmitters, neuropeptides, and nucleobases involved in several pathologic processes.
In this context, electromembrane extraction (EME) appears an attractive sample preparation approach, featuring high selectivity towards low molecular weight (LMW) ionizable analytes with high recovery, fast extraction, and green chemistry. According to the low recovered volume, the nature of the extracted compounds, and the sensitivity goal, CE-MS/MS is particularly adapted to analyze the extracts obtained by EME.
In this study, a new parallel EME (Pa-EME) device was developed, based on a commercially PAMPA 96 well-plate and adapted in-house to be conductive and reusable. The design and the initial experimental parameters (applied voltage, extraction time, and agitation speed) were optimized with a second degree design of experiments (DoE) for the extraction of model basic LMW compounds. The device was further used for the extraction of numerous polar cationic metabolites (>50) from CSF and various supported liquid membranes composition (organic solvents and carriers) were evaluated. Due to the high number of experiments performed, the 96 well-plates format was perfectly adapted to the DoE and the screening studies. In order to improve the throughput of the analysis, a multisegment injection (MSI) approach enabling 4 analyses in one single run was performed. Results demonstrated that the combination of Pa-EME and CE-MS/MS is an efficient approach for the selective analysis of cationic metabolites from scarce biofluids.

LC-MS based metabolomics - strategies towards selectivity and high metabolite coverage

K. Ortmayr[1,2], M. Schwaiger[1], T. Causon[2], S. Hann[2], G. Koellensperger[1]

[1] Institute of Analytical Chemistry, Faculty of Chemistry, University of Vienna, Währingerstr. 38, 1090 Vienna
[2] Department of Chemistry, University of Natural Resources and Life Sciences-BOKU, Muthgasse 18, 1190 Vienna

The term untargeted metabolomics implies global and unbiased analysis. One key aspect is to find differential metabolic fingerprints related to different biological conditions. This task is followed by the complex and yet not routine task of annotating molecular formulas from mass spectrometric features (denotes unique mass and retention time). As a matter of fact, in practice the generality or comprehensiveness of the untargeted approach is compromised by the chemical diverse nature of the constituents of the metabolome, yet it is crucial to obtain selectivity for a high coverage of the metabolome, most desirable within one analytical run. It is commonly accepted that selectivity provided by high mass resolution solely is not sufficient, as identical masses are generated by structural isomers and/or in-source fragments. Hence, chromatographic selectivity is conceived as essential in untargeted metabolomics regarding both aspects, i.e. accurate differential quantification and feature annotation. Recently, in order to increase the throughput of analysis, novel on-line combinations of orthogonal chromatographic LC separations have been introduced and will be discussed regarding non-targeted metabolomic applications.

The influence of packing geometry on chromatographic peak capacity

During the past decade, the pillar array column format has come into consideration as an promising alternative for the packed bed and the monolithic column in the field of liquid chromatography. Where the flow resistance in the pillar column is comparable or even lower than that of monoliths, the ordered nature of the pillars allows for theoretical plate heights that are a factor of two smaller than (disordered) particulate columns with equally sized particles.

The use of lithographic and etching techniques offers the ability to conceive virtually any flow-through channel. In the present contribution, the impact of the support structures on dispersion and permeability will be discussed. The distance between the support structures and the concomitant optimal channel length will furthermore determine which sample complexity can be handled for a given pressure limitation.

Because concentration loadability of the non-porous silicon structures is for most applications insufficient we developed 2 strategies to render the pillars porous without losing the ordered nature of the array. The principle, layer properties and some separation results of a an additive sol-gel procedure will be presented, as well as for an electrochemical anodization approach.

Because of the 2D (or often referred to as 2.5D to emphasize that all structures have the same depth) nature of the pillar array one would intuitively expect that at large depths the performance becomes closer and closer to that of channel without channel walls. Unfortunately this is not the case. The situation is even more dramatic when non-verticality of the pillars is taken into account as well. Increasing volume loadability therefore occurs at the expense of inherent 2 D separation performance. For given micromachining limitations the depth should be chosen such that a compromise is reached between volume loadability and separation performance, which is dictated by the application. Also true 3D shapes are possible, which generally result in a similar trend as for a rectangular channel. One shape however (the I–shape) has the potential to optimize both features simultaneously. A number of bench marking results and applications will be shown using rectangular channels, revealing e.g. that a peak capacity of 800 can be reached by separating a phenone mixture on a 0.5 m long channel with a spacing between the structures of 2.5 µm and a depth of 18 µm.

Chromatographic separation under HDX-MS quench conditions

Rune Salbo, Ph.D., Senior Research Scientist
Protein Structure and Interaction, Novo Nordisk A/S, Denmark

Hydrogen-Deuterium Exchange (HDX) Mass Spectrometry (MS) is a technique for structural characterization of proteins in solution where the labelling of the backbone amides with deuterium is followed over time. It is essential for the experiment that the hydrogen-exchange reaction is quenched during the analysis in order to preserve the label. Therefore, disulphide bond reduction, digestion with pepsin, and chromatographic separation of the peptides must take place under suboptimal conditions at pH 2.5 and 0°C using very short gradients. The poor chromatographic separation can be counter-acted by implementation of ion mobility mass spectrometry, which adds another degree of separation. In this study the structural and dynamic changes of a protein upon binding of antibodies is probed and the antibody epitopes are identified.

HPLC column technology in 2016 – a state-of-art review and a critical appraisal

Stefan Lamotte, BASF SE, 670056 Ludwigshafen, Germany
Klaus K. Unger, Johannes Gutenberg-Universität, 55099 Mainz, Germany

Abstract

HPLC columns and stationary phases have reached a mature high-tech status. The most common of about four million annually worldwide sold columns are reversed phase (most of them n-octadecyl bonded silica) columns of 2 mm to 4.6 mm I.D. and 50 mm to 250 mm length packed with 2 µm to 5 µm size fully porous particles, 3 µm size core-shell particles or monolithic silica columns (1). When implemented in a suitable HPLC instrument and run at optimized conditions they fulfill the requirements in pharmaceutical and chemical analysis resolving the main product and its impurities at an abundance ratio of 1 : 1,000 in a short time at defined and certified conditions.

While the run to highly efficient columns and short analysis time was dominating the field for many years the chromatographic community has now discovered the huge potential of LC to adjust and to control the selectivity of separation for complex mixtures of hundreds constituents and for those containing more than thousand entities such as in bio fluids. In addition improved detection capabilities over the last decade enable us to detect more than the tip of the iceberg, making former easy separations much more complex. This will lead to multicolumn approaches, i.e. coupled selectivities in contrast to columns for higher plate counts. The concepts applied are: blending of adsorbents with different surface chemistries into so called mixed mode phases, coupling of columns with different surface chemistries in series, and column switching as a step into multidimensional LC. Next to separation in multidimensional separations generating quantitative results with the same precision as established for one dimensional HPLC will be the major task

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Signal processing - The missing link in analysis of XC, XC-MS and XC×XC data

Jan H Christensen[1,*], *Nikoline J. Nielsen*[1], *Søren Furbo*[1], *Giorgio Tomasi*[1]

[1]Analytical Chemistry group, Section of Environmental Chemistry and Physics, Department of Plant and Environment Sciences, University of Copenhagen Denmark

[*]Corresponding author e-mail: jch@plen.ku.dk

Keywords: Oil analysis, two-dimensional chromatography, curve resolution, feature detection and extraction, pixel-based analysis

Abstract

Non-targeted LC- and GC-High-Resolution-MS (XC-MS) experiments generate enormous amounts of data. Each data matrix comprises more than 10^7 data points, complicated by the presence of electronic- and chemical noise, artefacts and data redundancy.

We design analytical platforms that can be used for comprehensive analysis of petroleum samples. Sample preparation should therefore be non-selective or with complementary selectivity such that the entire range of compound properties can be covered by few analytical platforms. We aim to find column combinations that provide the most orthogonal separations (i.e., providing different relative retention times for the same compounds).

We construct custom-made signal processing procedures and develop generic functions for import and cropping of different types of analytical data and develop new strategies for feature detection and pixel-based analysis of multidimensional data. We work on solving the problems that arise for multidimensional separation systems (e.g., LC×LC and GC×GC) such as retention time shifts in multiple dimensions, complex baselines and the very large number of variables compared to samples.

We develop new scaling procedures using the analytical variation of each signal in replicate analyses to reduce the importance of non-chemical information and allow robust modelling of data with many more variables than samples. To do this, we also exploit the structure of data (sample × rt1 × rt2 × m/z) by employing multi-way models such as PARAFAC that can resolve and quantify even grossly overlapping peaks.

In this presentation, I will give an overview of different strategies for signal processing that we have developed and use in our group for analysis of petroleum samples.

Intact protein glycoform profiling using CE–MS and HILIC–MS

Govert W. Somsen[a], Elena Domínguez Vega[a], Jordy van Angeren[a], Klára Petrů[b], Sara Tengattini[c], Rob Haselberg[a]

[a]Division of BioAnalytical Chemistry, Vrije Universiteit Amsterdam, The Netherlands, g.w.somsen@vu.nl; [b]Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Kralove, Charles University Prague, Czech Republic; [c]Department of Drug Sciences and Italian Biocatalysis Center, University of Pavia, Italy

Mass spectrometry (MS)-based methodologies are implemented throughout all stages of biopharmaceutical production, providing information on molecular mass, primary sequences, post-translational modifications (PTMs), and higher order structures. Despite advances in MS resolution and accuracy, the attainable structural information on intact biopharmaceuticals – often mixtures of highly similar glycoforms – is limited by sample complexity and/or ionization suppression by major sample components. Therefore, separation prior to MS detection is mandatory to achieve reliable assignment of protein variants.

Capillary electrophoresis (CE) has the intrinsic capacity to produce narrow peaks for macromolecules as well as the selectivity to separate closely-related proteoforms under MS-compatible conditions. It will be shown that intact glycoprotein separation can also be achieved by hydrophilic interaction liquid chromatography (HILIC), with protein resolution being mainly governed by carbohydrate content. Comparison of CE–MS and HILIC–MS indicates a remarkable complementarity of the two techniques with respect to glycoform selectivity. Examples will highlight the performance of the developed CE–MS and HILIC–MS systems with focus on the analysis of glycoproteins of pharmaceutical interest, such as erythropoietin, antigen glycoconjugates, and monoclonal antibodies (mAbs).

A Look Back on the Fascinating History of Chromatography

Thomas Welsch

Institute for Analytical and Bioanalytical Chemistry, Ulm University,

Albert-Einstein-Allee 11, D-89081 Ulm, Germany

Chromatography influences our everyday life as no other analytical technique. In the second half of the last century, gas and liquid chromatography developed to the most important analytical tools for the separation and determination of components in simple and complex mixtures and matrices. This success story is based on the contributions of innumerable researchers and multifaceted progress in column technology and instrumentation.

The roots of chromatography date back to ancient Greece and Middle Ages, were filtration- and purification operations included frontal chromatographic processes. The investigation of “adsorption” and “capillarity” at the end of the 19th century using different materials e.g. paper and cotton were important for the discovery of elution chromatography by Tswett in 1903.

In my presentation I will show some examples for this early chromatography and I will highlight outstanding papers, which substantially contributed to the development of Gas Chromatography, High Performance Liquid Chromatography and Electrochromatography. Unfortunately, some of the pioneering papers were misread in order to become recognized by the chromatographic community.

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Non-aqueous CE-MS of cinchona alkaloids - characterization of a novel CE-ESI-MS interface

Frederik André Hansen, Steen Honoré Hansen and Nickolaj J. Petersen*
Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

We have recently in our group at the University of Copenhagen developed a robust and simple sheathless CE-ESI-MS interface (capillary electrophoresis – electrospray ionization-mass spectrometry). In this presentation the interface is characterized and compared with HPLC-MS for studying the composition of alkaloids in Cinchona bark.
One common problem for sheathless interfaces for CE-MS has been establishing a stable electric contact at the end of the separation capillary that does not induce band broadening or affect the spray stability. In our device the electric contact is generated through a submicron fracture in the capillary close the ESI tip. The fracture provides a zero dead volume and excellent conducting properties due to the large amount of ions in the electric double layer. Electric current exceeding the upper limit of CE instrumentation of up to 300 µA can easily be obtained. Furthermore, the increased conductivity of the buffer in the fracture generates field free pumping of the analytes towards the ESI spray tip.
In this study the device was used to analyze the four major alkaloids (diastereomeric pairs of quinine/quinidine and cinchonine/cinchonidine) in Cinchona bark samples, in a non-aqueous BGE system without using a chiral selector.
The separation was achieved in less than 15 min with a 75 cm capillary and a detection limit of 1 ng/mL, approximately 150-2000 times lower than the occurrence in the samples after standard sample preparation was achieved. The sheathless interface showed a 40-fold improvement of the detection limit compared to an Agilent® sheath liquid interface employing a similar non-aqueous BGE system.

The performance of stationary phases for fast liquid chromatography

Attila Felinger

Department of Analytical and Environmental Chemistry, University of Pécs,

Ifjúság útja 6, H-7624 Pécs, Hungary, e-mail:felinger@ttk.pte.hu

During the recent years, a number of chromatographic columns with various types of packing materials dedicated for UHPLC instrumentation have been introduced. The most frequently used columns in this area are narrow and short (2.1 mm ID and 50 mm length), and are packed with sub-3-µm or sub-2-µm particles. Besides the use of the well-known and widely used fully-porous or core-shell packing materials, another potential is in the development of monolithic silica columns, where improved efficiency can be achieved and moderate column pressures are sufficient owing to the small skeleton size and large (through-pore size)/(skeleton size) ratios.

The kinetic performance of reversed phase columns packed with fully porous and core-shell particles with various particle diameters and the performance of a monolithic silica columns can be rather different. We also compared the kinetic performance of the same type monolithic column mounted by the producer or by the user. In addition to the results obtained with van Deemter plots and kinetic plots, we suggest a column-reversal method to examine the bed heterogeneity at the column ends and explain the reduction of column efficiency for early eluting solutes.

The monolithic column shows systematically better efficiency for early eluting compounds than the packed columns, therefore an additional band broadening effect is suspected for the packed columns. The effect of the presence of the frits and the bed heterogeneity of the columns near the frits can characterized by a column-reversal method. It has been shown that significant differences can exist between the two ends of the packed columns, while the monolithic column shows rather similar performance at either column end.

The flow reversal method is useful to characterize the sample band broadening in the immediate vicinity of the column ends. Although flow reversal has a peak compression effect, and the peaks observed with reversed flow are always narrower and more symmetrical than the peaks obtained without reversing the flow, flow reversal can be a useful tool to show the differences between the intrinsic plate heights of the columns and also for showing the difference between the two respective column ends. The local plate height was found much smaller in every case than the one we get for the unretained thiourea after a simple injection without arrested and reversed flow.

The local efficiency at the two respective column ends differ from each other, however the difference is negligible for the monolithic column. It cannot be guaranteed whether the inlet or the outlet of the columns perform better. That varies from one manufacturer to the other.

Title: Over 10 000 peptide identifications from the HeLa proteome using single-shot capillary zone electrophoresis tandem mass spectrometry

Authors: Liangliang Sun[1,] Guijie Zhu[1], Xiaojing Yan[1], Yimeng Zhao[1], Alexander Herbert[2], Michael Westphall[2], Matthew Rush[2], Matthew Champion[1], Josh Coon[2], Norman Dovichi[1]
1. Department of Chemistry & Biochemistry, University of Notre Dame, Notre Dame, IN, United States.
2. Department of Chemistry, University of Wisconsin, Madison, WI, United States.

Abstract: Capillary zone electrophoresis-tandem mass spectrometry (CZE-MS/MS) has recently attracted attention as a tool for shotgun proteomics. However, its performance for this analysis has fallen far below that of reversed phase liquid chromatography (RPLC)-MS/MS. Here, we report the use of a CZE method with a wide separation window (up to 90 min) and high peak capacity (~300).(1) This method is coupled to an Orbitrap Fusion mass spectrometer via an electro-kinetically pumped sheath flow interface for analysis of complex proteome digests. Single-shot CZE-MS/MS identified over 10,000 peptides and 2 100 proteins from a HeLa cell proteome digest in ~100 min. This performance is nearly an order of magnitude superior to earlier CZE studies and is within a factor of 2 to 4 of state-of-the-art nano ultrahigh pressure LC system.

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Distribution constants by liposome electrokinetic chromatography

Susanne K. Wiedmer

Department of Chemistry, University of Helsinki, POB 55, 00014 University of Helsinki, Finland

Different techniques have been developed to quantify molecular interactions and measure the interactions between drugs and lipid vesicles. Methods applied to interaction studies in pharmaceutical and biomedical sciences include equilibrium dialysis, ultrafiltration, ultracentrifugation, filtration, calorimetry, microdialysis, spectroscopic and chromatographic methods, and capillary electromigration (CE) techniques. Among the used methodologies, CE has proven to be an attractive tool because detailed information about the dynamics of molecular interactions under physiological conditions with minimal sample consumption can be obtained. Nowadays a common set up for investigating analyte-liposome interactions is liposome electrokinetic chromatography (LEKC).

In LEKC the capillary is filled with a liposome-containing background electrolyte (BGE) which serves as a pseudostationary phase. We have used LEKC to investigate interactions of local anesthetics or glaucoma drugs with liposomes comprising phosphatidylcholine and phosphatidylglycerol with and without cholesterol at different temperatures. In addition, lipids and sterols extracted from human red blood cells (RBC) and liposomes made from these were used as pseudostationary phase as well. The interactions between the compounds and the liposomes will cause a change in the electrophoretic mobilities of the compounds compared to conventional CE. By comparing these methods and measuring the electrophoretic mobilities of the liposomes, the retention factors can be calculated. Retention factors are used to elucidate the interactions between the drugs and a given composition of the pseudo-stationary phase. Distribution constants, on the other hand, are used to quantify the interactions between drugs and liposomes and these can be estimated from the retention factors and the phase ratio of the system. Comparison between theoretically determined partitioning coefficients and experimentally determined distribution constants were made.

PSP –CEC

Future & Past

Main Challenges

R. Blom, C. Nilsson, I. Harwigsson, S. Birnbaum, D. Chandrasekaren,

K. Shea & S. Nilsson

New insights in biomedicine and related areas require the parallel development of new analytical methods.
The development of nanoparticle-based capillary electrochromatography (CEC) in our lab will be reviewed. The nanoparticles, suspended in the electrolyte for use as pseudostationary phase (PSP) in CEC. In PSP-CEC, the stationary phase is used only once allowing fast column regeneration and circumventing carry-over effects.
Nanoparticles possess a favorable surface-to-volume ratio allowing highly efficient separations.

Used Nanoparticles including; Lucite-based, "Cubisomes", Gadolinium based, MIP-particles.
Mode; Partial- and Full-filling PSP.
Detection ?
Playmates; GFP, hGH, Lyz, Pepsin and small molecules
Why Future?

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Enantio-/Stereo-selective Liquid Chromatographic Separation: State of the Art, Quo Vadis.

Wolfgang Lindner
Institute of Analytical Chemistry, University of Vienna, Austria

Nowadays a large arsenal of chiral stationary phases (CSPs) and so-called "chiral columns" thereof is commercially available associated with the advertisement that for practically any "chiral" problem there will be a column available to solve the individual enantiomers of a chiral compound. However, there remain some dilemmas:

1. Which column will be suitable to solve the enantioselective separation task?
2. Is there a remaining group of chiral analytes (selectands, SAs) for which no adequate solution can be offered?

As the elucidation of the stereoselective molecular recognition principles of the CSPs for a given SA remains a difficult task and as enantioseparation can hardly be predicted a column screening remains still the first choice to be undertaken, externally or internally. In this context the polysaccharide type CSPs proved to be most often the first choice followed by the Pirkle type CSPs, macrocyclic CSPs and protein type CSPs. For very polar and chargeable SAs it turned out that the chiral ion exchangers developed by our group are most promising.

In this lecture special emphasis will be given also on the enantioselective LC-chromatographic resolution of free and N-protected amino acids and of short peptides as these SAs fall into the category of quite difficult analytical and preparative challenges. Related molecular recognition principles between the anionic, cationic and zwitterionic chiral selectors (SOs) and our CSPs and the given SAs will be discussed. It can serve as basis for further developments of highly dedicated CSPs in the frame of Quo Vadis aspects.

Opportunities and challenges of LC - high resolution – MS in clinical & forensic toxicology

Hans H. Maurer
Department of Experimental and Clinical Toxicology, Saarland University
D-66421 Homburg (Saar), Germany, hans.maurer@uks.eu

High-resolution mass spectrometry (HRMS) using double focusing mass analyzers was established in the 1960ies and revolutionized the elemental analysis of organic compounds. It became a powerful tool also in bioanalytical research and practice providing high selectivity and specificity. After developing modern LC-MS interfaces using electrospray ionization and its wide application in bioanalytical as well as in "omics" research and practice, the manufacturers realized a broad market for HRMS. Since the last few years, robust LC-MS apparatus mainly using time-of-flight or Orbitrap mass analyzers are getting more and more affordable to many laboratories. This is also represented by the remarkable increase of publications and review articles. Therefore, the aim of the presentation will be to give an overview on the current use of HRMS in general and in particular for drug screening and quantification, and drug metabolism studies. Limitations and further perspectives will close the presentation.

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"**Advances in metabolic profiling - miniaturisation and metabolic phenotyping"**

Ian D Wilson

Metabolic phenotyping (metabotyping) holds the promise of improving our understanding of the underlying biology of normal physiological processes such as growth and aging as well as defining mechanisms of disease, drug action, metabolism and toxicity. UPLC-MS, employing sub 2 micron particles and 2.1 mm i.d. columns of varying lengths from 5 to 15 cm, is now a standard platform for both global and targeted metabolite profiling. However, whilst this configuration has proved to be both robust and efficient, moving to UPLC-based microbore chromatography, with 1 mm i.d. columns, offers the possibility of making large gains via reduced sample and solvent consumption (thereby maximising the use of often limited samples and reducing consumable costs) and environmental impact. However, to be accepted, microbore systems have to be shown to be capable of providing efficient, repeatable and robust methods suitable for analysing large numbers of samples. An example of the successful transfer of a UPLC profiling method using a “conventional” 2.1 mm. i.d column-based to a 1 mm i.d. format will be provided with only minor modifications to an existing UPLC system will be provided. Further gains in productivity can be achieved via the modification of the gradient profile and compressing the separation into a shorter time. This approach using rapid microbore metabolic profiling (RAMMP) via UPLC-MS provides a high-throughput analytical platform for metabolic profiling and can enable that analysis of large numbers of samples in a single run, eliminating the need to analyse them in multiple batches. Reducing the 12 min analysis time of our standard method to 2.5 min lead to a reduction in peak capacity, from 150 to 50, and in the number of features detected, from ca. 19, 000 to ca. 6000. However, the RAMMP method achieved similar levels of group discrimination to that of “conventional” UPLC-MS when applied to rat urine obtained from studies on the effects of 2-bromophenol and acetaminophen. Despite the reduction in peak resolution the same features were responsible for discrimination of the different sample groups whether the conventional or RAMMP data was examined. The combination of reduced column diameter and length together with increased linear velocity and high pressures thus provided a five-fold reduction in analysis time, a ten-fold reduction in solvent consumption and the potential to also reduce sample consumption by ten-fold. Whilst we believe that such microbore methods could become the new routine we are also beginning to see the emergence of the next generation of capillary UPLC-MS and examples of the application of these new systems will be also shown, perhaps providing a glimpse of the future.

Poster presentations

Characterization of culture media containing non-ionic detergents by HILIC-ESI-TOF-MS

Eleni Lazaridi[1], Nikoline J. Nielsen[1], Lea Giørtz Johnsen[2]

[1] Analytical Chemistry Group, Department of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen, Thorvaldsensvej 40, Frederiksberg 1871, Denmark

[2]MS-Omics, Thorvaldsensvej 40, T644, Frederiksberg 1871, Denmark

Detergents are being widely used in cosmetics, drugs, food, and in production of microorganisms due to their amphipathic nature (a nonpolar "tail" and a polar "head") that allows them to act as solubilizers, emulsifiers and stabilizers [3]. Non-ionic detergents, such as Poloxamer 188 and Polysorbate 80, in culture media may interfere with the analyte separation and detection, and in particular with the analyte quantification when using liquid chromatography - mass spectrometry (LC-MS). It is very likely that the presence of detergents also gives rise to ion-suppression, and hereby interferes with quantification of co-eluting compounds. Moreover, Polysorbate 80 can cause challenges either to compound quantification due to its high ionisability or in case of lipid quantification fatty acids can be produced when Polysorbate 80 is methylated [2]. Poloxamer 188 can interfere with lipid extraction and recovery due to its emulsifier qualities [2].

Methods based on size-exclusion chromatography have been used for detergent removal, however these methods cannot be used for quantification of large molecules i.e. metabolites, or compounds that have similar molecular weight as the non-ionic detergent [1]. An alternative strategy was suggested by Jäpelt et al (2016) where Polysorbate 80 was at least partly removed by zinc-complexation followed by thiocyanate precipitation.

In this work it will be shown that this is also the case for another non-ionic detergent (Poloxamer 188), accompanied by a full characterization of the two non-ionic detergents as well as by testing the precipitation procedure in real samples. During the preliminary investigation the two non-ionic surfactants, as well as the precipitation solution: zinc nitrate and ammonium thiocyanate were characterized by HILIC-ESI-TOF-MS. Preliminary results showed that 1) both Poloxamer 188 and Polysorbate 80 give rise to chromatographic peaks originating from multiple homologues series, 2) detergents induce massive ion suppression, 3) the precipitation solution give rise to several chromatographic peaks and 4) the metal ions in combination with thiocyanate can precipitate both detergents tested.

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Two simple cleanup methods combined with LC-MS/MS for quantification of steroid hormones in in vivo and in vitro assays

Johan JuhlWeisser & Cecilie Hurup Hansen & Rikke Poulsen & LizetteWeber Larsen & Claus Cornett & Bjarne Styrishave

Analytical BioSciences, Department of
Pharmacy, Faculty of Health and Medical Science, University of
Copenhagen, Universitetsparken 2, 2100 Copenhagen, Denmark

Abstract Measuring both progestagens, androgens, corticosteroids as well as estrogens with a single method makes it possible to investigate the effects of endocrine-disrupting chemicals (EDCs) on the main pathways in the mammalian steroidogenesis. This paper presents two simple methods for the determination of the major steroid hormones in biological matrixes using liquid chromatography tandem mass spectrometry (LC-MS_2). A novel method was developed for the determination of 14 steroids in the H295R in vitro assay without the need for solid phase extraction (SPE) purification prior to LC-MS_2 analysis. The in vitro assay was validated by exposing H295R cells to prochloraz for inhibiting steroid hormone secretion and by exposing cells to forskolin for inducing steroid hormone secretion. The developed method fulfills the recommendations for the H295R assay suggested by the OECD. Furthermore, a simple off-line SPE methodology was developed for the necessary clean-up of in vivo assays. Samples, such as gonad tissue, plasma and serum, are complex biological matrixes, and the SPE methodology was optimized to remove salts and proteins prior to elution of target analytes. At the same time, lipophilic compounds were retained on the SPE cartridge during elution. This, combined with the multi-steroid LC-MS_2 method, made it possible to determine 10 steroids in male Sprague-Dawley rat gonad tissue. Furthermore, it was possible to quantify 6 steroids in the plasma. In general, the observed concentration of steroid hormones in plasma, testes, and H295R cell medium corresponded well with previous studies. The off-line SPE method was validated using spiked charcoal-stripped serum. Method recovery, accuracy, precision and robustness were all good. Instrument sensitivity was in the range of 55–530 pg/mL (LLOQ).

Selenium as an elemental detection label for quantification of Peptides

A comparison to fluorophore labelled peptide

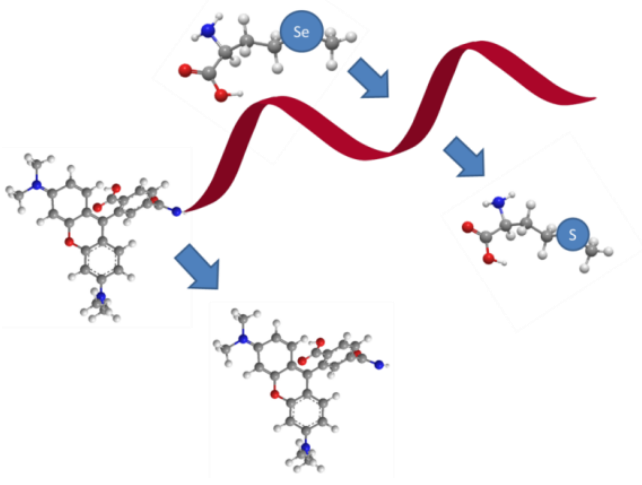
Møller, L. H.[a], Bahnsen, J. S.[a], Nielsen, H. M.[a], Østergaard, J.[a], Stürup, S.[a] & Gammelgaard, B.[a]

[a] University of Copenhagen, Department of Pharmacy

Peptides are involved in many physiological processes and are increasingly used as drug candidates. Peptide drugs most often offer high efficacy, selectivity and specificity and additionally low toxicity due to their similarity to natural occurring compounds in the body. In the development of new drugs, parameters as stability, pharmacokinetics and metabolism must be monitored and thus sensitive quantitative analytical methods are of great importance but can be a challenge in complex biological matrices.

In this study, selenium was introduced as an elemental detection label. By exchange of the naturally occurring amino acid Met with the selenium containing analogue, Se was introduced as an inherent label in the peptide structure and allowed for quantification by inductively coupled plasma mass spectrometry (ICP-MS). The impact on peptide properties of labelling peptides with the fluorophore TAMRA or the selenium (Se) containing amino acid SeMet was evaluated. The cell-penetrating peptide (CPP) penetratin (Pen) was used as a model peptide and three differently labelled variants of were synthesized; one labelled with Se (PenM^{Se}), one labelled with TAMRA (TAMRA-Pen) and one labelled with both Se and TAMRA (TAMRA-PenM^{Se}). The labelled peptides were characterized in terms of structural and physicochemical properties as well as uptake efficiency in HeLa cells. Furthermore, stability of the labelled peptides and identities of degradation products in cell media and cell lysates were evaluated by LC-ICP-MS, LC-FLD and LC-ESI-MS.

Selenium labelling caused minimal change in hydrodynamic radius and the physicochemical properties of the peptide and allowed for absolute quantitative determination of cellular uptake by ICP-MS. Selenium is thus proposed as a promising alternative label for quantification of peptides in general, altering the properties of the peptide to a minor extent as compared to commonly used peptide labels.



Comparison of three sample preparation procedures for the quantification of L-Arginine and asymmetric dimethylarginine in human plasma

Authors: A.M.V. Schou-Pedersen, J. Lykkesfeldt, Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen.

Background: Asymmetric dimethylarginine (ADMA), an L-Arginine derived metabolite, is an endogenous competitive inhibitor of endothelial Nitric Oxide Synthase (NOS), which generates the vasodilating NO. An increased concentration of ADMA has been correlated with the possible development of coronary heart diseases and ADMA is therefore considered to be an important early biomarker for endothelial dysfunction. The most widely used analytical method for the quantification of L-Arginine and ADMA rely on laborious solid-phase extraction (SPE), derivatization with a fluorescent tag followed by HPLC separation and fluorescence detection.

Aim: The aim of the current study was to investigate if simple and robust protein precipitation (PP) could replace SPE sample preparation. 2 types of PP procedures: (A) PP using cold TCA, 1 M, followed by neutralization and (B) PP using a mixture of cold ammonia, 25 %, and acetonitrile (10:90, v/v) were compared to procedure (C) consisting of Oasis MCX SPE clean up described in several publications. The three sample preparation procedures were evaluated with regard to recovery, precision, resolution, stability of samples and simplicity.

Methods: Separation of the ortho-phtaldialdehyde-mercaptoethanol derivatives of L-Arginine and ADMA was performed at 40 °C on a Phenomenex Gemini (150 x 4.6 mm, 5 µm) column. The mobile phase consisted of 50 mM potassium phosphate (pH 6.5) containing 14 % (v/v) acetonitrile. Detection was obtained at excitation wavelength of 340 nm and emission wavelength of 455 nm. Recovery of the three sample preparation procedures was determined by spiking human plasma with standard solutions of L-Arginine and ADMA (n=3). The precision was estimated from the recovery experiment. The stability of the samples was tested at 4 °C after 24 and 48 hours.

Results: The recovery (mean ± SEM) obtained with procedure (A) was found to 109 ± 1.75 % for L-Arginine and 98.0 ± 5.84 % for ADMA. In comparison, procedure (B) showed low recoveries of 68.4 ± 3.18 % for L-Arginine and 75.4 ± 7.59 % for ADMA. Regarding procedure (C), recovery for L-Arginine was 118 ± 2.02 % and for ADMA 94.7 ± 4.89 %. The precision was acceptable for all three procedures. Figure 1a and 1b shows examples of chromatograms obtained from either procedure (A) or procedure (C), respectively. Resolution was considered to be acceptable, although the resolution of procedure (A) was slightly better. The stability of the obtained supernatants was for all three procedures found to be acceptable (>90 % of parent) within 48 hours of storage at 4 °C.

Conclusion: Sample preparation procedure (A) and (C) performed similarly well regarding recovery, precision, resolution and stability of samples. Procedure (B) was disregarded due to low recoveries. Taking the simple and robust sample preparation of procedure (A) into account, this procedure was selected for further validation studies. The validated analytical method has proven useful in quantifying L-Arginine and ADMA in human plasma from clinical studies.

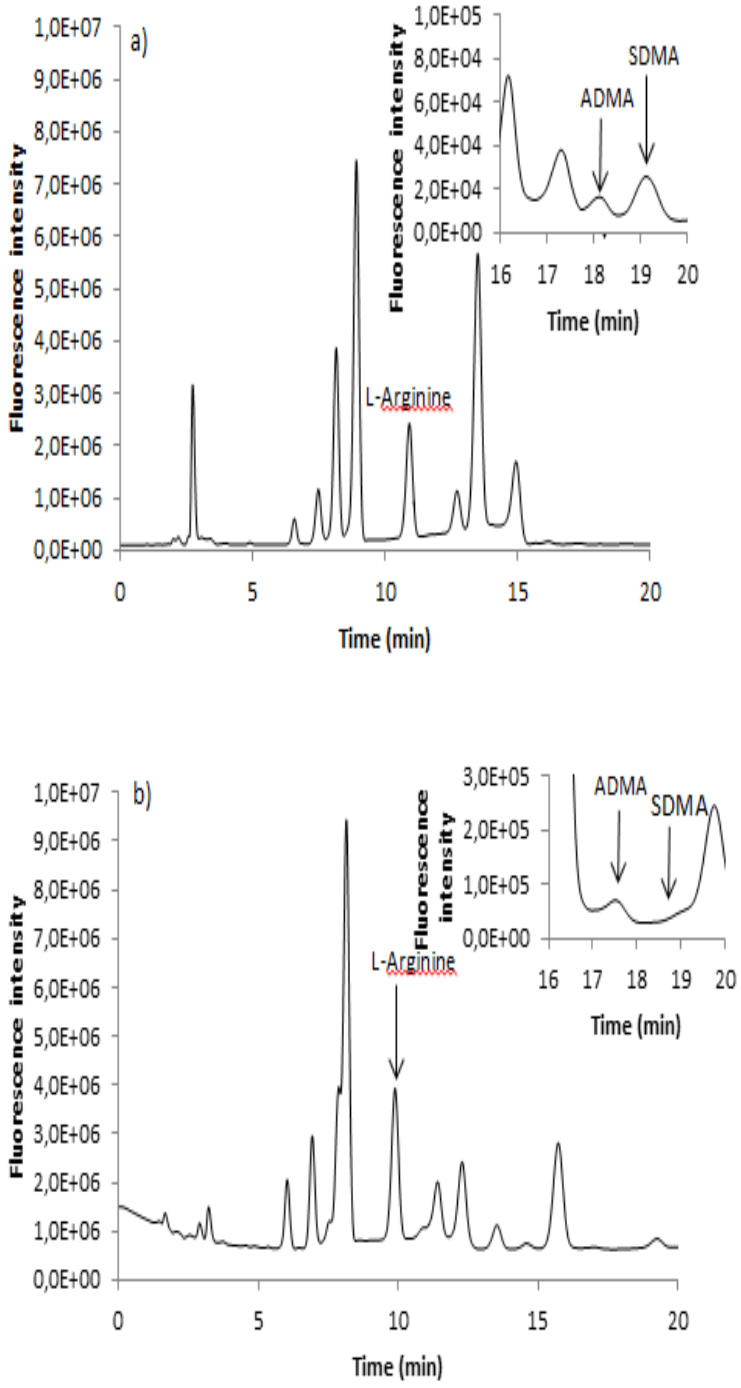


Fig. 1 Chromatograms of a human plasma sample extracted with either a) procedure (A) or b) procedure (C). The inserts show the resolution of ADMA to the similar, but inactive symmetric dimethylarginine (SDMA).

Analysis of pesticides and estrogens in wastewater by UPHL-ESI-TOF-MS

Nana W. Jørgensen[1*], Peter Christensen[1] , Jan H. Christensen[1]

[1] Analytical Chemistry Group, Department of Plant and Environmental sciences, University of Copenhagen, Thorvaldsensvej 40 1871 Frederiksberg, Denmark.

*Corresponding author mail: nanawolrath@gmail.com

Pesticides, estrogens and other pollutants are frequently detected in the influent and effluent of wastewater facilities[1][2]. Pesticides and estrogens are classified as health hazards for humans as well as for the natural ecosystems[3][4]. It is therefore of great importance that the effluent is cleaned and the concentrations of pesticides, estrogens and other pollutants are kept under the limits as specified by regulatory authorities. A significant parameter of importance for reliable identification and thus quantification of pollutants, especially for complex matrices like wastewater, is selectivity. The goal of selectivity is to *"distinguish and quantify the response of the target compounds from the responses of all other compounds"*[5]

In this study a sample preparation method will be optimized to increase selectivity for pesticides and/or estrogens in a wastewater matrix and a modified UPLC method will be validated with it.

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The use of metabonomics to detect cases of food fraud in chicken eggs

Amy Johnson and Dr David Thompson, Keele University

Food fraud is a centuries old problem that has been increasing in occurrence over recent years due in part to the globalisation of food supply chains. One particular aspect of food fraud that has recently experienced an increase in prevalence is the misrepresentation of conventional food products as organic, due to increasing consumer demand for organic produce. Chicken eggs are one of the more popular food products affected by this type of food fraud, with conventional eggs being misrepresented as organic, and thus are the focus of this research. This fraudulence of eggs, as well as being morally ambiguous, can also have an economic impact. To avoid these negative implications of food fraud, there are various laws and regulations in place to try to control the issue.

However, these laws and regulations rely on having suitable methods of detection for instances of food fraud. Many current methods used to authenticate food products are based on detecting single compounds in the samples, which makes it relatively easy for fraudsters to adulterate the products with these compounds. The main aim of this research is to use metabonomic technologies and chemical analysis methods, including the analysis of quality control samples, to develop metabolic profiles of both conventional and organic eggs. This will result in a highly robust method of discrimination between these eggs, using various different biomarkers and their concentrations, making it much more difficult for fraudsters to adulterate their products.

This research has thus far obtained some preliminary results showing the metabolic differences between cage and organic eggs, following HPLC-MS and statistical analysis.

The Electro Membrane Extraction Autosampler – Full automation of sample preparation and LC-MS analysis of large sample sequences

David Fuchs, Henrik Jensen, Stig Pedersen-Bjergaard and Nickolaj Petersen
Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen,

The introduced work describes an autosampler with integrated Electro Membrane Extraction (EME). The system integrates rapid sample extraction by EME and sample analysis by LC-MS. With the developed EME autosampler coupled to LC-MS, large sequences of complex samples can fully automated be analyzed without any manual sample handling step involved.

EME is a relatively new sample preparation technique using an electric field across a supported liquid membrane to extract charged analytes from complex sample solutions. Up to now, most EME have been accomplished as off-line processes in which extracted analytes were manually transferred to the analysis method of choice. The newly developed EME-autosampler eliminates this manual sample handling step and can be used for consecutive extraction and analysis of large sequences of samples without any manual sample handling.

For the setup a programmable CTC PAL autosampler was coupled to a LC-MS system via a 10-port-switching valve (figure 1). As electromembrane support a small hollow fiber (approx. 3 mm length, ID 330 µm, 150 µm wall thickness, 0.4 µm pore size) connected to fused silica capillaries inside a luer lock adapter was used. The fused silica capillaries were used for delivery of extracted analytes to the LC-MS system via the acceptor phase flow. The luer lock adapter with integrated electromembrane was coupled to a glass syringe. Electric contact close to the electromembrane was established by an electric wire coupled to the luer lock adapter at one end and to a programmable voltage sequencer at the other end. The glass syringe was mounted on the CTC PAL autosampler. As sample was drawn by the autosampler, the sample passed the electromembrane and analytes were extracted into the lumen of the hollow fiber electromembrane and transported to the LC-MS system via the acceptor phase flow.

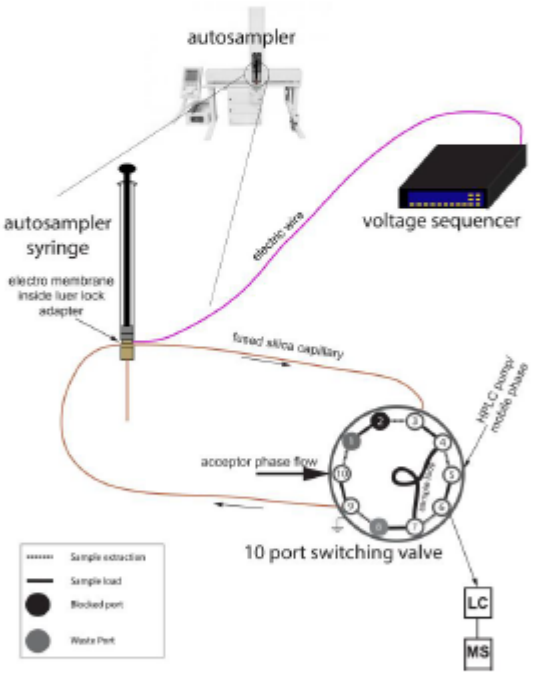

Figure 1: Schematic illustration of the EME-autosampler setup. The electromembrane was placed inside the autosampler's glass syringe. Autosampler and LC-MS were coupled via a 10-port-switching valve. Electric potential close to the membrane was applied by a voltage sequencer.

Both, switching of the 10-port valve between sample extraction and loading as well as switching of the applied electric potential were synchronized with the autosampler and were automatically controlled by the LC-MS software. The EME-autosampler was successfully applied for extraction and analysis of large sequences of the model drug methadone from blood plasma, rat liver microsomes or phosphate buffer.

Due to its high degree of automation – the system integrates a whole analytical workflow of sample extraction, separation and detection – the EME autosampler potentially can be used for all kinds of applications where fast and fully automated sample extraction and analysis is of interest.

Rapid Conformational Analysis of Protein Drugs in Formulation by Hydrogen/Deuterium Exchange Mass Spectrometry (HDX-MS)

Zeinab E. Nazari [1], Marco van de Weert [1], George Bou-Assaf [2], Damian Houde [2], Andrew Weiskopf [2], Kasper D. Rand *[1]

[1] Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, Co-penhagen 2100, Denmark

[2] Department of Protein Pharmaceutical Development, Biogen, 225 Binney St., Cambridge Massachusetts 02142, USA

ABSTRACT:

Higher order structure characterization of protein therapeutics is of considerable importance for validating function and safety, as well as in comparability studies of innovator biologics and their biosimilars. Hydrogen Deuterium Exchange coupled to Mass Spectrometry (HDX-MS) has become an established method for characterization of higher-order protein structure and dynamics. Here, we present a customized HDX-MS methodology that uses manual Solid-Phase Extraction (SPE) to allow fast and simplified conformational analysis of proteins under pharmaceutically relevant formulation conditions. Of significant practical utility, the methodology allows global HDX-MS analyses to be performed without refrigeration or external cooling of the setup. Real-world applicability was demonstrated by HDX-MS analysis of interferon-β-1a in formulation as well as the model compounds insulin and angiotensin II. Using the customized SPE-HDX-MS setup, extraction of labeled protein from the pharmaceutical formulation was optimized while monitoring the loss of deuterium during analysis (back exchange). Two modes of operation where identified. In Mode 1, we used DMSO-containing solvents for SPE, allowing the HDX-MS analysis to be performed at acceptable back exchange levels (<30%) without the need for cooling any components of the setup. In mode 2, SPE and chromatography was performed using a fast isocratic elution at 0 °C resulting in back exchange levels of 10-30%. Key advantages of the methodology include easy implementation, low sample use, possibility to use solvents of choice for optimized excipient removal from individual formulations, and fast data acquisition. Our results indicate that the SPE-HDX-MS system can be used as a reliable and robust method for fast conformation analysis of proteins in their intended formulations and thus could help to unleash the potential of HDX-MS methodology to address several current analytical challenges in pharmaceutical development research.

Flow-Induced Dispersion Analysis for Probing Anti-dsDNA Antibody Binding Heterogeneity in Systemic Lupus Erythematosus Patients

Nicklas N. Poulsen[1], Morten E. Pedersen[1], Jesper Østergaard[1], Nickolaj J. Petersen[1], Christoffer T. Nielsen[2], Niels H. H. Heegaard[2,3] and Henrik Jensen[1]

[1]Department of Pharmacy, University of Copenhagen, Universitetsparken 2, 2100 Copenhagen, Denmark;
[2]Department of Autoimmunology & Biomarkers, Statens Serum Institut, Artillerivej 5, 2300 Copenhagen, Denmark;
[3]Department of Clinicial Biochemistry, Odense University Hospital, University of Southern Denmark, Sdr. Boulevard 29, 5000 Odense C.

Detection of immune responses is important in the diagnosis of many diseases. For example, the detection of circulating autoantibodies against double-stranded DNA (dsDNA) is used in the diagnosis of Systemic Lupus Erythematosus (SLE). It is, however, difficult to reach satisfactory sensitivity, specificity and accuracy with established assays. Also, existing methodologies for quantification of autoantibodies are challenging to transfer to a point-of-care setting.

Here we present the use of Flow-Induced Dispersion Analysis (FIDA)[1] for rapid (minutes) measurement of autoantibodies against dsDNA. A schematic presentation of the assay is given in figure 1.

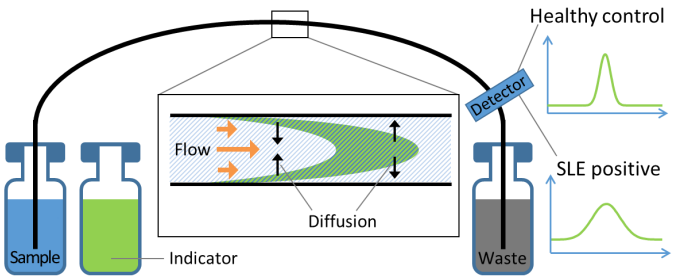

Figure 1. The automated FIDA protocol is performed using commercial equipment for capillary electrophoresis. The inlet vials contain sample, indicator solution and washing solutions. A hydrodynamic flow is employed giving rise to a parabolic flow profile (insert). The dispersion of the indicator measured at the detector increases if antibodies in the sample have bound the indicator

Short fluorescently marked dsDNA sequences of approximately 30 base pairs were used as indicators (ligands). The diffusivity of the indicator is measured by Taylor Dispersion Analysis (TDA). A narrow indicator zone is injected in a capillary prefilled with sample and the zone is eluted with sample. If the sample contains antibodies against double-stranded DNA the indicator (dsDNA) will be part of a larger complex and the apparent diffusivity decreases. The assay is fully automated with the use of standard CE based equipment employing fluorescence detection.

FIDA is robust towards matrix effects as demonstrated by the binding curves obtained in up to 85% human blood plasma. The binding curves were obtained with a model monoclonal antibody against dsDNA. FIDA allows for flexible exchange of the DNA sequences used to probe for the autoantibodies. Plasma samples from SLE positive patients were analyzed using the new FIDA methodology as well as by standard indirect immunofluorescence and solid-phase immunoassays. Interestingly, the patient antibodies binds DNA sequences with different affinities, suggesting pronounced heterogeneity amongst autoantibodies produced in SLE.

In conclusion, the FIDA based methodology is a new approach for autoantibody detection, and holds promise for being used for patient stratification and monitoring of disease activity.

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Simple and versatile thiol-ene - based microfluidic devices for chromatographic separations

Alexander Jönsson, Unai Orruño, Jörg P. Kutter and Josiane P. Lafleur
Department of Pharmacy, University of Copenhagen, DENMARK

Porous polymer monoliths are a new category of materials which have attracted lots of interest as alternatives to packed beds in the last 20 years. They take advantage of the ease of processability associated with polymers to generate monoliths, films, and beads, often with well-defined porosities and high specific surface areas. These materials are prepared using a very simple molding process carried out within the confines of a closed mold, such as the microfluidic chip channel. We have recently reported a novel, rapid and simple method for the preparation of emulsion-templated monoliths in microfluidic channels based on thiol–ene chemistry. The method allows monolith synthesis and anchoring inside thiol–ene microchannels in a single photoinitiated step. These monoliths offer promise as stationary phases for on-chip separations thanks to their narrow size distribution and the possibility to easily modify their surfaces with chemical groups for various retention modes. The cross-linked beads of the monolithic structure closely resembles a packed column but with a higher degree of packing. This makes the on-chip microcolumn a convenient replacement for the, often difficult, packing of columns in microchannels. For reverse-phase separations, alkyl mercaptans or 1-alkenes can be covalently bonded to the surface of the monolith via thiol-ene click chemistry. We will present preliminary results demonstrating on-chip solid phase extraction (SPE) of model analytes using these novel high surface area materials.

Additionally, the thiol-ene microfluidic channels support electroosmotic flow, allowing for separation via Microchip Capillary Electrophoresis (MCE) or ElectroChromatography (MCEC). For example, chiral molecules can be separated within a thiol-ene based microfluidic device by the addition of cyclodextrins to the running buffer or by immobilizing the cyclodextrins directly to the channel walls via thiol-ene chemistry. We will present preliminary data on the separation of chiral amino acids in thiol-ene microfluidic chips using these principles.

Detecting the differences between dead on arrival and normally slaughtered chickens using metabonomic profiling

Kate Sidwick and David Thompson
Keele University

Food fraud is a challenge within the global food industry that affects the public on an everyday basis. This was apparent in 2013 when horse meat was discovered in processed beef products. The confidence in the meat industry has subsequently decreased, causing a need for reliable methods for meat authentication to be developed.

Methods for detecting food fraud usually involve searching for the presence or absence of a certain substance within the product to confirm its authenticity, but this can be easily adulterated. This project aims to use metabonomic methods to create a metabolite fingerprint of the product, leading to a more reliable technique using a combination of markers, and their concentrations, to authenticate the product, which is much more difficult to falsify. This will involve using liquid chromatography-mass spectrometry based protocols, including established quality assurance methodology.

Specifically, animals that have deceased before slaughter are not allowed to be sold as food produce, so this is an area that can be targeted by food fraud. The Animal By-Products (Enforcement) Regulations 2013 states that all animals found to be dead on arrival to the slaughterhouse must be removed and stained with a colouring agent in order to distinguish it as a product not fit for human consumption. Large portions of poultry are dead on arrival to the slaughterhouse, which decreases the amount of sellable products for that company. This gives an economic reason for fraudulent activity to occur where the dead on arrival poultry is processed normally and allowed to continue into the human food chain. At present, there are no methods to detect this specific food fraud. With the use of metabonomic methods, markers could be found to determine whether the product was in fact dead on arrival and should not be in the food chain.

Analysis of pyrolysis oils on GC-qTOF and GCxGC-qTOF

Mette Kristensen[1*], *Peter Christensen*[1], *Jan H. Christensen*[1]

[1]University of Copenhagen, Department of Plant and Environmental Sciences, Frederiksberg, Denmark

[*]Corresponding author e-mail: mkri@plen.ku.dk

Keywords: PetroPhase 2016, Denmark, Comprehensive Gas Chromatography, Pyrolysis Oils.

Abstract

Gas chromatography coupled to mass spectrometry (GC-MS) has for a long time been the preferred analytical tool in the area of petroleomics, but not all petroleum components are easy to measure with this analytical platform and focus has mainly been on hydrocarbons and PAHs. However, the most surface active compounds are expected to be some of the heavier oxygen- and nitrogen containing compounds, and especially the interest in analysing carboxylic acids has increased. This compound class is also suspected to be responsible for wettability in oil reservoirs [1] and for being potentially toxic to aquatic organisms [2]. In this study the focus will be on analysing pyrolysis oil. Pyrolysis oil is made by an anaerobic distillation of biomass and contains a higher fraction of carboxylic acids, alcohol, ketones, aldehydes and other water-soluble compounds compared to crude oils. To analyse some of the oxygen- and nitrogen containing compounds in pyrolysis oil, derivatization is necessary to make them GC compatible. Four derivatization agents are tested in this study: BSTFA + 1% TMCS, BF3•MeOH, MCF and HCl:MeOH. With the use of online derivatization, gas chromatography and high resolution mass spectrometry (GC-qTOF), we aim to describe a comprehensive method for fingerprinting of oxygen- and nitrogen-containing compounds in pyrolysis oils. Different emission current and acquisition rates of the GC-qTOF system are tested and the optimal settings are found. For further separation of this complex mixture, comprehensive two dimensional gas chromatography coupled to high resolution mass spectrometry is applied (GCxGC-qTOF). The final methods (GC-qTOF and GCxGC-qTOF) are applied to characterize oxygen- and nitrogen-containing compounds in pyrolysis oil.

[1] Standal et al., Journal of Petroleum Science and Engineering 24, 131–144 (1999)

[2] Aeppli et al., Environ. Sci. Technol. 46, 8799−8807 (2012)

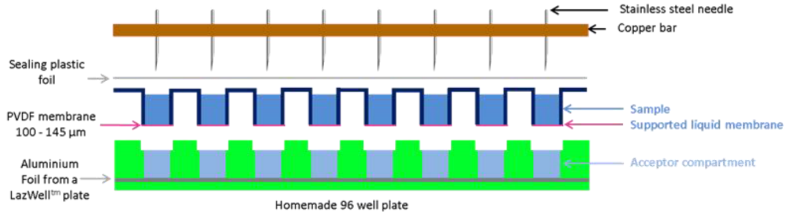
Tittle
Electromembrane extraction for cationic metabolome analysis

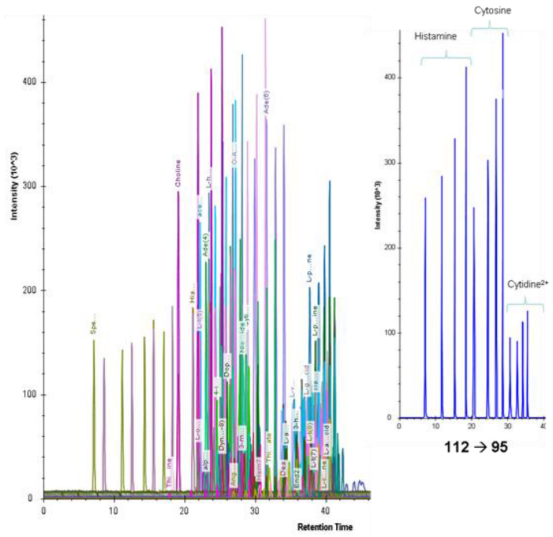
Background :
Electromembrane extraction (EME) consists in the migration of charged compounds under an electrical field, through a supported liquid membrane (SLM) impregnated on a microporous hollow-fiber. In this study, a new set-up of parallel EME (Pa-EME) device was developed to extract a set of 50 basic compounds, representative of different chemical and physiological families (e.g. amino acids, peptides, nucleobases, etc.) from cerebrospinal fluid (CSF).

Methods
This original Pa-EME device allowed the simultaneous extraction in a 96 well-plate format from a commercial filtration well-plate and a homemade conductive well-plate. Polymeric filter of the filtration well-plate was impregnated with various organic solvents and carriers, which were screened for SLM selectivity.
Capillary electrophoresis coupled to tandem mass spectrometry (CE-MS/MS) is an effective approach for metabolite analysis. In order to follow the high throughput of Pa-EME and minimize ionization variation in MS, multisegment injection (MSI) was used, reducing analysis time from 50 min *per* sample to 15 minutes.

Results
As expected, analyte selectivity depended on the SLM composition: higher extraction of low molecular weight (LMW) compounds was obtained with N-nitrophenyloctyl-ether compared to nonanol. The latter was preferable for HMW molecules such as peptides. For the most polar compounds, a carrier was added to the SLM to enhance mass transfer.

Conclusion
Pa-EME is a very selective approach, with high recovery for most basic compounds in a reduced extraction time. The combination with a high throughput methods such as MSI-CE-MS/MS, have demonstrated to be a promising approach for multitargeted metabolome analysis.





LC-MS/MS-based monitoring of in vivo protein biotransformation: quantitative determination of trastuzumab and its deamidation products in human plasma

Peter Bults[1,2], Rainer Bischoff[2], Hilde Bakker[1], Jourik Gietema[3] and Nico van de Merbel[1,2]

[1)]PRA Health Sciences, Assen, the Netherlands
[2)]Department of Analytical Biochemistry, University of Groningen, the Netherlands
[3)]Department of Medical Oncology, University Medical Center Groningen, the Netherlands

With this poster we present an LC-MS/MS-based method for quantitatively monitoring the *in vivo* deamidation of the biopharmaceutical monoclonal antibody trastuzumab at a crucial position in one of its complementarity determining regions (CDR) [1].

The Asn55 residue in the heavy chain of trastuzumab is susceptible to deamidation *in vitro* and *in vivo.* As this amino acid is located at the top of the CDR2 loop of trastuzumab, which is involved in the binding of trastuzumab to the HER2/neu receptor, it is not unlikely that deamidation of Asn55 may result in a conformational change in the CDR2, thus leading to changes in binding affinity of trastuzumab to its receptor and, potentially, to an altered pharmacological effect.

The multiplexed LC-MS/MS assay using selected reaction monitoring (SRM) allows simultaneous quantitation of five molecular species derived from trastuzumab after tryptic digestion: a stable signature peptide (FTISADTSK), a deamidation-sensitive signature peptide (IYPTNGYTR), its deamidated products (IYPTDGYTR and IYPTisoDGYTR), and a succinimide intermediate (IYPTsuccGYTR). Digestion of a 50 µL plasma sample is performed at pH 7 for 3 h at 37 °C, which combines a reasonable (>80%) digestion efficiency with a minimal (<1%) formation of deamidation products during digestion.

The LC-MS/MS method was validated in accordance with international bioanalytical guidelines over the clinically relevant range of 0.5 to 500 µg/mL with bias and CV values well below 15%. Deamidation of trastuzumab was observed in plasma both in a 56 day *in vitro* forced degradation study (up to 37% of the total drug concentration) and in samples (*in vivo*) obtained from breast cancer patients after treatment with the drug for several months (up to 25%). Comparison with a validated ELISA method for trastuzumab showed that deamidation of the drug at the CDR leads to a loss of recognition by the antibodies used in the ELISA assay.

[1] Bults et al, Anal. Chem., 88 (2016) 1871-1877.

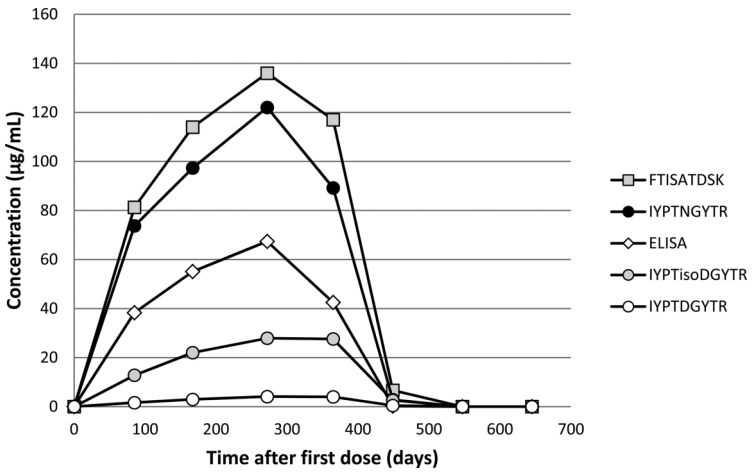


Figure: LC-MS/MS and ELISA results in plasma obtained from a breast cancer patient on long-term treatment with trastuzumab. LC-MS/MS concentrations obtained for peptides FTISADTSK, IYPTNGYTR, IYPTDGYTR, and IYPTisoDGYTR.

Title:

Efficient, Effective, and Proven Approach to Chiral Method Development for Purification Scale Up

Authors:

J Preston, Michael McCoy, Sean Orlowicz and Ramkumar Dhandapani

Locations:

Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

Presenter at the symposium:

Theis Kirkhoff Guldbech

Abstract:

With the majority of today's pharmaceuticals being chiral compounds, and a renewed focus on the differences in biological activity of isomers, chiral separations and isolations have continued to grow in importance. Our lab screened hundreds of compounds on various Chiral Stationary Phases (CSPs), and over the years has developed an efficient experimental strategy for identifying the appropriate CSP for a unique chiral separation. Due to the success of this chiral screening service, many customers have returned asking for the isolation of pure enantiomers. As a result, we have explored the additional challenges of chiral separations as we scale up to preparative HPLC. In this poster, we demonstrate through case studies, a unique and effective approach to both CSP screening and subsequent scale up for chiral small molecules. When screening compounds for the appropriate CSP, the analyte's physical properties often define the mode of chromatography most likely to be successful for any given CSP. Screening Normal Phase, Reversed Phase , Polar Organic, and SFC modes of chromatography on a selection of six orthogonal polysaccharide-type phases has proven to be successful for more than 80 % of the unique submissions. Mobile phase composition in each mode is optimized to achieve resolution. When isolating enantiomers, we must further consider the physical and chemical properties of the analytes. Preparative isolations on various CSPs can be drastically affected by scale up phenomena such as; loading capacity of the CSP itself, stability of the sorbent, and nonlinear chromatography. In summary, through real world examples we have developed an effective strategy for identifying chromatographic conditions for chiral separation of small molecules with moderate chiral complexity. We have successfully demonstrated the ability to transition those analytical separations to preparative isolations.

List of participants

Participants

Name	Affiliation	Country
Aas, Hoai	University of Oslo	Norway
Andersen, Kirsten	University of Copenhagen	Denmark
Bergquist, Jonas	Uppsala University	Sweden
Bjørnsdottir, Inga	Novo Nordisk	Denmark
Brønnum-Hansen, Kirsten	Leo Pharma	Denmark
Bults, Peter	PRA Health Sciences	Netherlands
Callewaert, Manly	Free University Brussel	Belgium
Chen, Fuxin	University of Copenhagen	Denmark
Christensen, Jan H.	University of Copenhagen	Denmark
Cornett, Claus	University of Copenhagen	Denmark
Cozzi, Federico	University of Copenhagen	Denmark
Cuesta, Unai Ortiz de Orruno	University of Copenhagen	Denmark
Dovichi, Norman	University of Notre Dame	USA
Drouin, Nicolas	University of Geneva	Switzerland
Dzwonkowski, Olivia	University of Copenhagen	Denmark
Felinger, Attila	University of Pécs	Hungary
Fitzpatrick, Carmel	Eurofins	Ireland
Fuchs, David	University of Copenhagen	Denmark
Gabel-Jensen, Charlotte	Novo Nordisk	Denmark
Gammelgaard, Bente	University of Copenhagen	Denmark
Gjelstad, Astrid	University of Oslo	Norway
Goodall, David	Paraytec	UK
Guldbech, Theis Kirkhoff	Phenomenex	USA
Halvorsen, Trine G.	University of Oslo	Norway
Hansen, Cecilie Hurup	University of Copenhagen	Denmark
Hansen, Frederik André	University of Copenhagen	Denmark
Hansen, Steen Honoré	University of Copenhagen	Denmark
Hermansen, Susanne	University of Copenhagen	Denmark
Horsefield, Rob	Agilent Technologies	Sweden
Hussein, Thara	University of Copenhagen	Denmark

Participants

Name	Affiliation	Country
Jamali, Babak	Ascendis Pharma	Denmark
Janfelt, Christian	University of Copenhagen	Denmark
Jehrbo, Jon	Novo Nordisk	Denmark
Jensen, Klaus	Ferring	Denmark
Jensen, Henrik	University of Copenhagen	Denmark
Johnson, Amy	Keele University	UK
Jönsson, Alexander	University of Copenhagen	Denmark
Jørgensen, Nana Wolrath	University of Copenhagen	Denmark
Fabricius , Niels	Egalet Danmark	Denmark
Koellensperger, Gunda	University of Vienna	Austria
Kretschmann, Andreas	University of Copenhagen	Denmark
Kutter, Jörg P.	University of Copenhagen	Denmark
Lamotte, Stefan	BASF	Germany
Lang, Caroline	University of Copenhagen	Denmark
Larsen, Bente	University of Copenhagen	Denmark
Lazaridi, Eleni	University of Copenhagen	Denmark
Lindner, Wolfgang	University of Vienna	Austria
Malsche, Wim de	Free University of Brussels	Belgium
Matthes, Annemarie	University of Copenhagen	Denmark
Maurer, Hans	Saarland University	Germany
Mittag, Tina	Novo Nordisk	Denmark
Møller, Laura Hyrup	University of Copenhagen	Denmark
Nazari, Zeinab Esmail	University of Copenhagen	Denmark
Nielsen, Ines Maria	Agilent	Denmark
Nielsen, Nikoline Juul	University of Copenhagen	Denmark
Nielsen, Helle Lundberg	University of Copenhagen	Denmark
Nilsson, Staffan	Lund University	Sweden
Pedersen, Kenneth Munk	University of Copenhagen	Denmark
Pedersen-Bjergaard, Stig	University of Copenhagen	Denmark
Nickolaj J. Petersen	University of Copenhagen	Denmark

Participants

Name	Affiliation	Country
Petersson, Patrik	Novo Nordisk	Denmark
Porse, Karen	LEO Pharma	Denmark
Poulsen, Nicklas Nørgård	University of Copenhagen	Denmark
Salbo, Rune	Novo Nordisk	Denmark
Schappler, Julie	University of Geneva	Switzerland
Schoenmakers, Peter	University of Amsterdam	Netherlands
Nickolaj J. Petersen	University of Copenhagen	Denmark
Sidwick, Kate	Keele University	UK
Sjögren, Jörgen	Ferring	Denmark
Sjölander, Annika Lindgren	Novo Nordisk	Denmark
Skonberg, Christian	Novo Nordisk	Denmark
Somsen, Govert	VU University Amsterdam	Netherlands
Soor, Anke	Novo Nordisk	Denmark
Stürup, Stefan	University of Copenhagen	Denmark
Styrishave, Bjarne	University of Copenhagen	Denmark
Thompson, David	Keele University	UK
Unger, Klaus	University of Mainz	Germany
Vogensen, Stine Byskov	Novo Nordisk	Denmark
Voigt Schou-Pedersen, Anne Marie	University of Copenhagen	Denmark
Weisser, Johan Juhl	University of Copenhagen	Denmark
Welsch, Thomas	University of Ulm	Germany
Wiedmer, Susanne	University of Helsinki	Finland
Wilson, Ian	Imperial College	UK

To and from the airport

Taxi

Taxis can pick up passengers at terminal 1 and 3 and will get you to downtown Copenhagen in about 20 minutes depending on traffic.

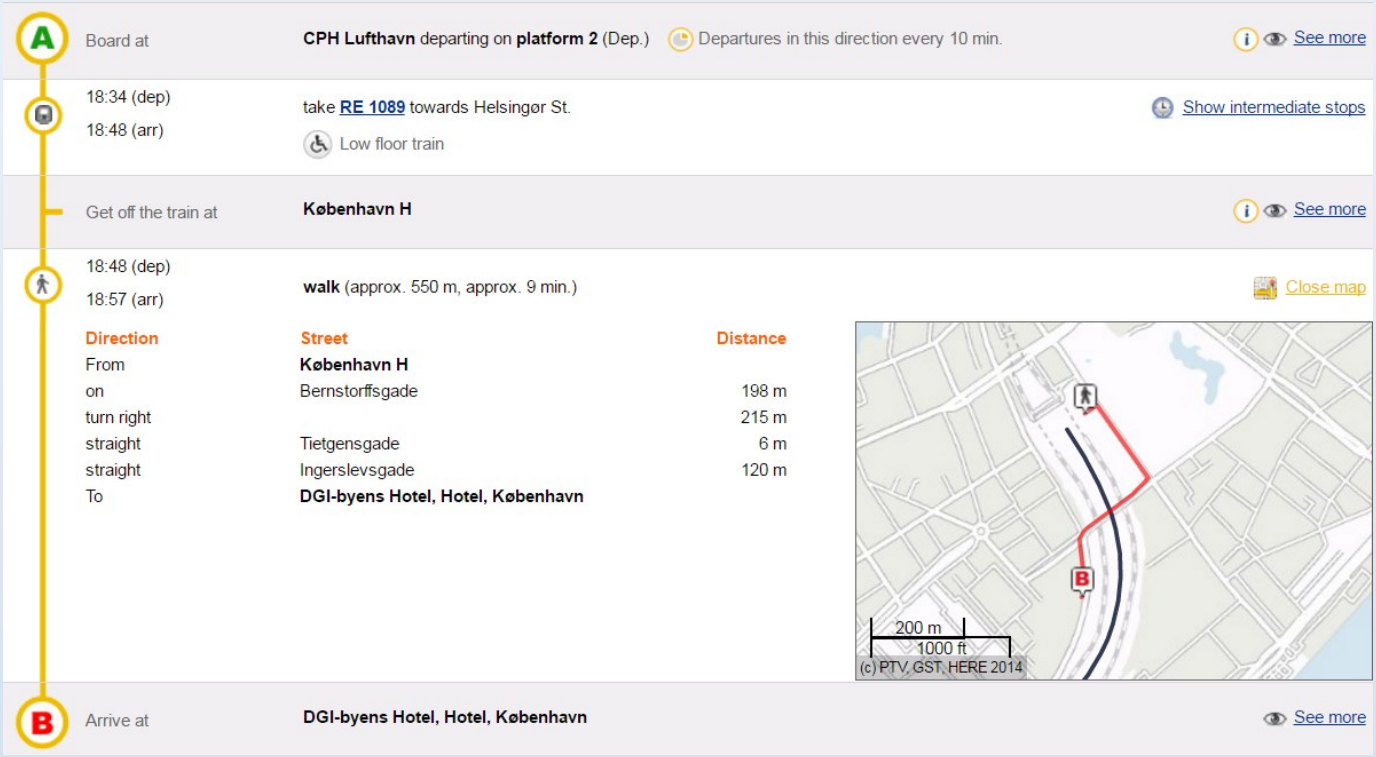
Taxis are usually waiting outside each terminal. It will cost approximately DKK 250-300.

Dan Taxi: +45 70252525 TaxiNord: +45 48484848

Train

The train station is located by terminal 3. You can take a free shuttle bus from terminal 1 to terminal 3, which will take 5 minutes.

According to date and time of your arrival you can coordinate your individual transportation via **www.rejseplanen.dk** (the image below shows transportation from the airport to DGI Byen on June 8 at 18:34).



The trains run every 10 minutes during the day and will get you to Copenhagen Central Station in about 13 minutes. During the night the trains run 1-3 times an hour.

Citypass tickets can be bought at the ticket machines in terminal 3 just above the railway, and at the DSB ticket sales counter, also located in terminal 3. Please note that the machines only accept coins and credit cards, not notes.

Metro

The metro is located right above terminal 3.

All the trains go in the same direction from the airport (M2 to Vanløse Station), so you do not have to worry about getting on the wrong train.

The trains run with 4-6 minutes intervals during the day and evening. During the night the train runs every 15-20 minutes. It will take you 13 minutes to get to Nørreport Station (hub in city centre) from the airport.

Tickets can be bought at the metro station and at the DSB ticket sales counter in terminal 3. Please note that the machines only accept coins and credit cards, not notes.

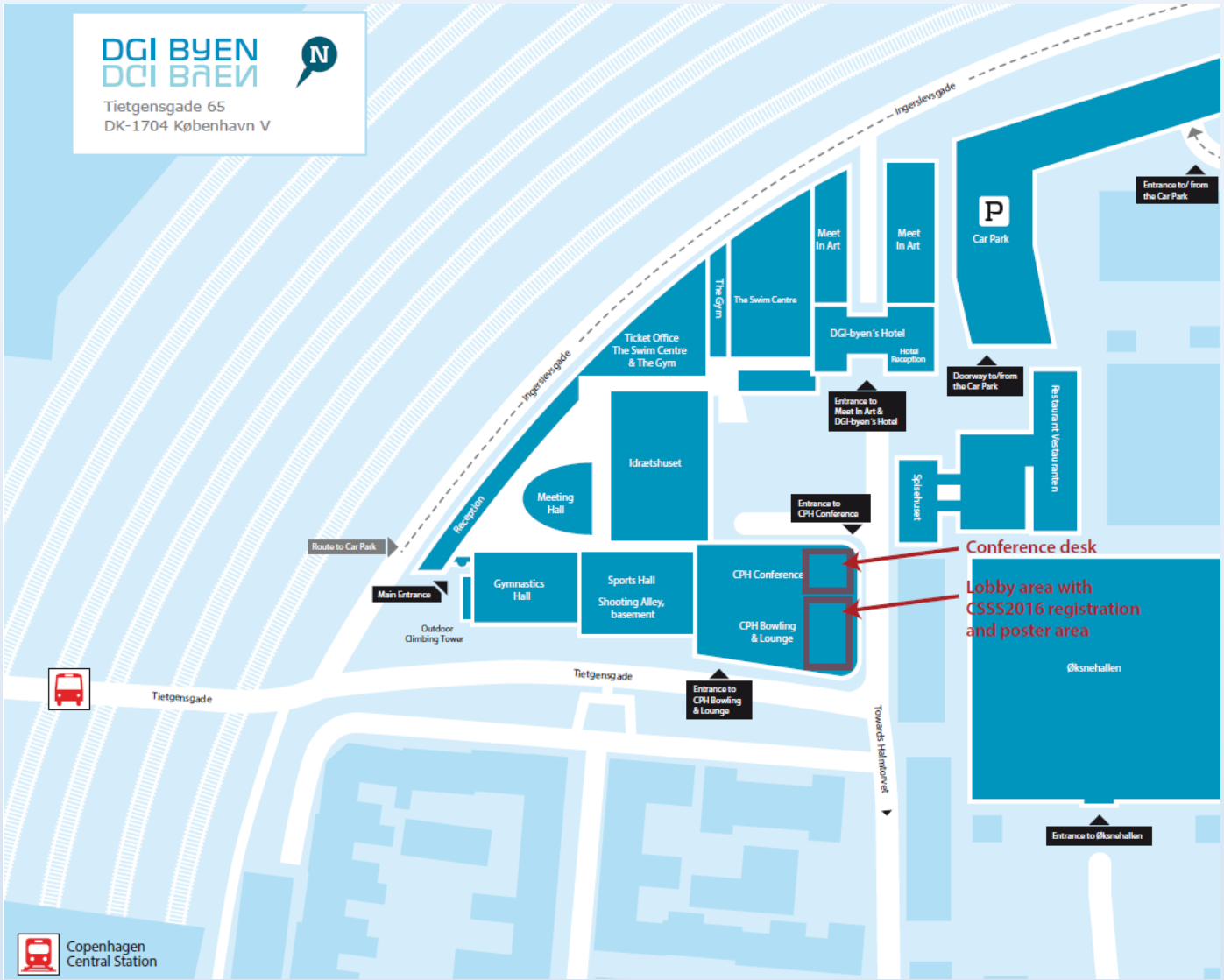
Bus

Bus 5A will take you directly to Copenhagen Central Station, City Hall Square, Nørreport and other stations. It takes about 30-35 minutes from the airport to the Central Station.

The bus runs every 10 minutes at day. The bus runs all night as well, but not as often.

Tickets can be bought at the ticket machines in terminal 3, or you can buy a ticket on the bus. Please note that the bus drivers only accept coins.

Map of DGI Byen



Emergency numbers in Denmark

Numbers for police, ambulance and fire services: who to call and what to say in an emergency…

Fire, Police, Ambulance - Tel: 112

The European SOS number 112 can be dialled to reach emergency services - medical, fire and police - from anywhere in Europe. This Pan-European emergency number 112 can be called from any telephone (landline, pay phone or mobile cellular phone). Calls are free. It can be used for any life-threatening situation, including:

Serious medical problems (accident, unconscious person, severe injuries, chest pain, seizure)

Any type of fire (house, car)

Life-threatening situations (crimes)

Copenhagen & Hovedstaden Emergency Medical Number

The out-of-hours emergency medical service number (lægevagten) in Copenhagen and the Hovedstaden region is **38 69 38 69**. It operates between 16:00 and 08:00 on weekdays, and throughout the day on weekends and public holidays. Callers will speak to a doctor, and may be referred to an emergency consultation. Callers should have their health cards to hand.

CSSS2016 related urgent cases

Jörg P. Kutter: +45 2671 0519

Notes

Notes