

Thursday 06 november 2008, University Medical Center Groningen



- 09:30 Registration, coffee
- 10:00 Jaap Wieling, FABIAN-board, Xendo Drug Development
Welcome & Opening
- 10:05 Vera Brinks, University of Utrecht
Biosimilars or why proteins cannot be copied
- 10:50 Reed Harris, Genentech Inc., South San Francisco, CA, USA
Comparability Assessment Strategies and Techniques for Post-Approval CMC Changes
- 11:35 Andreas Seidl, Novartis/Sandoz, Oberhaching, Germany
Development of biosimilars: Binocrit[®] - the worlds first biosimilar ESA as a case study
- 12:20 Lunch & Poster session
- 13:15 Robert van den Heuvel, Schering-Plough, Oss
Analytical assessment of biotech product quality
- 13:55 Claudia Berger, Solvay, Hannover, Germany
Oral recombinant enzyme replacement therapy: DMPK and Immunogenicity Assessment (A Case Study)
- 14:35 Joseph Marini, Centocor, Radnor, PA, USA
The challenges of "large molecule" ligand-binding assays - validation and bioanalysis
- 15:15 Meena Subramanyan, Biogen Idec, Boston, MA, USA
Immunogenicity considerations for biologics
- 16:00 Closure & Farewell drink

Directions

For directions:

<http://www.umcg.nl/azg/nl/bezoekers/5106/>

Maps



University Medical Center Groningen

Hanzeplein 1
9713 GZ Groningen
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In the hospital, please follow the 'FABIAN' signs starting from the main hospital entrance (Hanzeplein).
For attendees who come by car, a parking garage is underneath the hospital, close to the main entrance.

Abstracts of Lectures

L1

BIOSIMILARS OR WHY PROTEINS CANNOT BE COPIED

Vera Brinks, Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences (UIPS), Faculty of Science, Utrecht, the Netherlands

The expiry of patent protection of many biotechnological medicines has led to the development of biosimilars or follow-on biologics. These biosimilars are large complex molecules that are “similar” but not identical to the originator product. Due to their heterogeneity and complexity in structure and action, biological properties of both originator and biosimilar cannot be fully predicted in vitro. This makes the development of these (similar) biotechnological medicines difficult and stresses the importance for well defined production, characterization and purification in addition to in vivo testing. The European legislation on the development of biosimilars addresses these issues with EMEA guidelines on quality, non-clinical and clinical requirements, in particular immunogenicity. Some examples on factors influencing immunogenicity will be addressed.

L2

COMPARABILITY ASSESSMENT STRATEGIES AND TECHNIQUES FOR POST-APPROVAL CMC CHANGES

Reed Harris, Senior Director, Protein Analytical Chemistry, Genentech, Inc.

Comparability assessments require objective interpretation of a historical data set obtained using a comprehensive analytical approach. This presentation will describe Genentech’s current approach for demonstrating comparability for post-approval facility or process changes for therapeutic antibodies, and will highlight key limitations to our current analytical capabilities.

L3

DEVELOPMENT OF BIOSIMILARS: BINOCRIT® - THE WORLDS FIRST BIOSIMILAR ESA AS A CASE STUDY

Andreas Seidl, Uwe Demelbauer, Carsten Brockmeyer, Sandoz Biopharmaceutical Development, Hexal AG, Keltenring 1+3, 82041 Oberhaching, Germany

Biopharmaceutical medicines have great impact on the treatment of significant diseases and demand for such medicines continues to increase. The expiry of patent protection for major groups of biopharmaceutical medicines such as epoetin or growth hormone has been followed by the introduction of a regulatory pathway – the EMEA biosimilar-regulatory pathway - making it possible for assessment, approval and introduction of new versions of these existing biopharmaceutical medicines. Strict guidelines determine the development process including analytical, physicochemical and clinical studies. New versions of existing biopharmaceuticals must show comparability to a reference product and therapeutic equivalence based on clinical study data. The process of development for a medicine to be approved under the biosimilar regulatory pathway includes in-depth characterization of the originator product to define the development target, continuous comparison of the new version of the biopharmaceutical and the existing reference version by extensive physicochemical analysis and a final comparability exercise for confirmation of achieved similarity. Binocrit® (Epoetin alfa, Sandoz) was the first complex biopharmaceutical to be approved in Europe under EMEA biosimilar regulatory guidelines. For Binocrit® in comparison to the leading epoetin alfa (Eprex®, Johnson & Johnson), extensive analyses were completed. Results of the comparative analysis show comparability in primary, secondary and tertiary structures as well as receptor binding studies and bioactivity for both versions of epoetin predicting therapeutic equivalence. This was confirmed in several phase I and phase III clinical trials.

In summary, on the basis of the biosimilar regulatory pathway the first biosimilars have gained market approval in Europe. Biosimilars will increase choice and opportunity for patients, physicians and payors in this important area.

L4

ANALYTICAL ASSESSMENT OF BIOTECH PRODUCT QUALITY

Robert van den Heuvel, Schering-Plough, Oss, The Netherlands

In recent years, the technologies to analyze and characterize proteins have improved dramatically. Analytical tools now allow in-depth investigation of all relevant properties of a protein, and often orthogonal methods can be used. Regulatory requirements to ensure product quality and consistency of biopharmaceuticals have evolved in line with the improved analytical tools. During the development of biopharmaceuticals the quality guidelines of the International Conference on Harmonization (ICH) need to be followed.

In this lecture, I will discuss analytical approaches to ensure biopharmaceutical product quality and consistency. The overall quality strategy includes product characterization during development, adherence to GMP, validated manufacturing process, raw materials testing, in-process testing, stability testing, release testing and specifications. Several examples of release testing and product characterization will be given in the lecture.

L5

ORAL RECOMBINANT ENZYME REPLACEMENT THERAPY: DMPK AND IMMUNOGENICITY ASSESSMENT (A CASE STUDY)

Claudia Berger, Solvay, Hannover, Germany

No abstract received

L6

THE CHALLENGES OF "LARGE MOLECULE" LIGAND-BINDING ASSAYS - VALIDATION AND BIOANALYSIS

Joseph Marini, Centocor Inc., Radnor PA, USA

Ligand-binding assays (LBA) are the bioanalytical method of choice for measuring macromolecule protein therapeutics. Large molecule LBAs, as opposed to the small molecule bioanalytical method LC-MS/MS, do not directly measure the molecule itself. Instead, LBAs indirectly measure a binding reaction that is highly dependent on the quality of the reagents used. This talk will discuss the unique challenges encountered during the validation and bioanalysis of ligand-binding assays.

L7

IMMUNOGENICITY CONSIDERATIONS FOR BIOLOGICS

Meena Subramanyam, Ph.D., Clinical Science and Technology, Biogen Idec, Cambridge, MA

Development of anti-drug antibodies to protein therapeutics results in a wide range of clinical sequelae. Effect of anti-drug antibodies on pharmacokinetics and pharmacodynamics is more commonly seen. Less frequently, development of anti-drug antibodies may result in loss of efficacy due to neutralization of drug activity, thereby dampening the therapeutic effect. In some instances, development of anti-drug antibodies may also result in generalized immune effects, such as anaphylaxis, serum sickness, hypersensitivity etc. In rare cases, immunogenicity of a biotherapeutic can lead to neutralization of the endogenous human protein resulting in very serious outcomes. This talk will provide an overview of the immunogenicity experience with therapeutics approved for clinical use, including assay methods and discuss risk assessment approaches.

Abstracts of Posters

P01

CHARACTERIZATION OF IMPURITIES AND MODIFICATIONS IN PROTEIN PRODUCTS USING LIQUID CHROMATOGRAPHY AND DATA INDEPENDENT ACQUISITION TANDEM MASS SPECTROMETRY

Mike van Oosterhout, Waters Chromatography Etten-leur, NL

Hongwei Xie, Martin Gilar, John C. Gebler Waters Corporation, Milford, MA USA

Sequence variants, heterogeneity and modifications such as oxidation and deamidation are common in recombinant protein products and have the potential to affect the safety and activity of therapeutic protein drugs. Effective control and monitoring these variations require a sensitive and reproducible strategy to identify and quantify such product and process related impurities. We have applied an online Ultra Performance Liquid Chromatography –Data Independent Acquisition Tandem Mass Spectrometry (UPLC-MSE) approach to map protein digests. Yeast enolase and alcohol dehydrogenase (ADH) samples were used to demonstrate the proof of concept for identification and quantification of low-level impurities and multiple modifications in protein products. Tryptic digests of enolase or ADH were separated on a 2.1x100mm, 1.7µm C18 Acquity PST column, eluted and fragmented in a QTOF instrument. Data were acquired in a parallel data independent acquisition mode (MSE). High sequence coverage was achieved for the target proteins. Modified peptides at sub-stoichiometric abundances and peptides from low-level impurities were successfully characterized. For enolase sample, 95.5% sequence coverage was obtained for enolase 1, and four minor protein contaminants were identified. The impurity proteins were enolase 2, Cu-Zn superoxide dismutase, glucose-6-phosphate isomerase and triosephate isomerase at level of 13.4%, 13.3%, 3.1% and 1.4% related to enolase 1. Three asparagine deamidation sites (N70, N109 and N156) in enolase 1 were characterized at varying levels of modification (6.1%, 27.0% and 32.8%). Interestingly, several major LC peaks were found to be partially/non-tryptic peptides resulted from unexpected proteolytic cleavage. Similarly, two impurity proteins were determined from ADH sample, and 4 deamidated asparagines, 2 oxidized methionines and N-terminal acetylation were identified and quantified in ADH1. These results support that UPLC-MSE is capable of characterizing impurities and modifications in protein products. We are using this approach for analysis of a monoclonal antibody.

P02

AN AUTOMATED LC/MS DATA ANALYSIS USING BIOPHARMALYNX: A CASE STUDY FOR CHARACTERISATION OF THERAPEUTIC INTERFERON PROTEIN

Mike van Oosterhout, Waters Chromatography Etten-leur, NL

Joomi Ahn, Ying Qing Yu, Martin Gilar, John Gebler Waters Corporation, Milford, MA USA

Liquid chromatography-Time of Flight Mass Spectrometry (LC/TOF-MS) has been extensively used in intact protein and peptide mapping analyses as an accurate analytical tool for protein characterization in biotherapeutic drug development. Conventionally, LC/TOF-MS data generated for these studies is interpreted either manually or being processed with a limited automation to confirm protein masses and peptide maps. They are time-consuming processes. Valuable protein information can be often missed due to the complexity of the samples during their data analyses. This poster describes the utilities of BiopharmaLynx, a new application manager designed for automated data processing and annotation. LC/TOF-MS analysis of recombinant interferon alpha was characterized using BiopharmaLynx. The results of automated data processing and peak annotation for intact protein and tryptic peptides are shown. Multiple batches of recombinant interferon expressed in two different cell conditions were compared qualitatively. Intact protein LC/MS data was deconvoluted to protein mass and its result was presented in user friendly browsers. Differences in protein modifications were assigned for control and analyte batches. Peptide mapping analysis in BiopharmaLynx also provides the abilities to identify and annotate peptide peaks based on their accurate molecular weight resulted from proteolytic digestion. 96 - 97 % coverage in Interferon peptide map was achieved. Modifications such as N-terminus acetylation, oxidation, and deamidation were identified for different batches. The comparison tools in the software allow direct differentiation of batches of samples. Detailed peptide information is displayed in tabulated format can be sorted and edited by user.

P03

CE-TOF-MS SYSTEMS USING NON-COVALENT MULTI-LAYER COATED CAPILLARIES FOR THE ANALYSIS OF PHARMACEUTICAL PROTEINS

Rob Haselberg*, Gerhardus J. de Jong and Govert W. Somsen

Department of Biomedical Analysis, Utrecht University, P.O. Box 80082, 3508 TB Utrecht, the Netherlands

Biopharmaceuticals play a more and more important role in current drug development, and already a considerable number of therapeutic proteins have been registered as regular drugs. Due to these advancements, one can observe a growing demand for separation-detection methodologies that allow analysis of intact proteins. The combination of capillary electrophoresis (CE) and electrospray ionization-mass spectrometry (ESI-MS) provides a powerful tool for the separation and characterization of intact proteins. Recently, we have shown that CE-MS analysis of intact acidic proteins can be performed efficiently by applying non-covalent multi-layer coated capillaries in combination with a sheath-liquid interface [1,2]. However, as the mass resolution provided by the applied ion trap mass analyzer was relatively low, the information obtained on protein identity was limited.

In the present study, a time-of-flight (ToF) mass analyzer was applied for the CE-ESI-MS analysis of intact proteins. The ToF mass analyzer presents high mass resolution and accuracy. In order to prevent protein adsorption onto the capillary wall, CE-MS was performed using non-covalent multi-layer coated capillaries. For the analysis of acidic proteins a negatively charged double-layer coating, consisting of polybrene and poly(vinylsulfonic acid), was applied. For basic proteins, a positively charged triple-layer coating of successive layers of polybrene, dextran sulfate and polybrene, was used.

The performance of the developed CE-ToF-MS systems was evaluated. Optimal conditions for the analysis of proteins were determined. The influence of the concentration and pH of the background electrolyte on the separation and MS signal intensity was examined for a set of test proteins.

Furthermore, the composition and flow rate of the sheath liquid was optimized in order to find stable spray conditions and optimum MS responses for the proteins. The applicability of the CE-ToF-MS systems was studied by the analysis of (degraded) pharmaceutical proteins, with emphasis on the information gained by combining high-mass resolution mass spectra with CE separations. The developed CE systems allowed the separation and identification of several degradation products (deamidations, oxidations) and glycoforms of both acidic and basic biopharmaceuticals. Examples include recombinant human growth hormone, oxytocine, interferon- β , and recombinant humanized monoclonal antibodies.

1. J.R. Catai, J. Sastre Toraño, G.J. de Jong, G.W. Somsen, *Analyst*, 2007, 132, 75.
2. J.R. Catai, J. Sastre Toraño, P.M.J.M. Jongen, G.J. de Jong, G.W. Somsen, *J. Chromatogr. B*, 2007, 852, 160.

P04

In vivo microdialysis in rats: LC-MS/MS analysis of microdialysis samples

Brigitte Buscher, Ria Brust-van Schaik, Inge van Schöll, Astrid Capello and Florence Salmon
TNO Quality of life, Utrechtseweg 48, 3700 AJ Zeist, The Netherlands

Microdialysis can be used to monitor chemicals (drugs) in living tissues: microdialysis probes can be inserted in a blood vessel and various other tissues (muscle, adipose tissue, liver, brain, kidney, lung, etc) to obtain concentrations of the unbound drug. By doing so, the concentration of the drug can be determined in situ and continuously followed in time. Our hypothesis is that with the use of microdialysis the amount of animals can be reduced in Pharmacokinetic/Pharmacodynamic studies, since one animal can be continuously followed in time without sacrificing.

To investigate the feasibility of in vivo microdialysis, a pilot experiment was performed in rats. A microdialysis probe (CMA/Microdialysis) was placed in the vena jugularis and in the liver. Microdialysis was performed with a flow rate of 2 μ l/min (Ringer solution). Diclofenac, Dexamethasone, Methotrexate and Cyclosporine A were selected as model compounds. Microdialysis samples were collected before and after intravenous administration of each compound. The collected microdialysis samples (sample volume 10-40 μ l) were analysed with LC-MS/MS. The results will be presented on the poster.

P05

NEW APPLICATIONS OF QTRAP® AND TRIPLE QUADRUPOLE SYSTEMS IN PHARMACEUTICAL ANALYSIS

Applied Biosystems|MDS Analytical Technologies

Identification and quantitation of the metabolites of drugs and xenobiotics is a central challenge to understanding mechanisms of efficacy and toxicity. Productive MetID requires identification of as many individual metabolites of a compound in a single experiment as possible. The TripleTrap™ switching capabilities of Q TRAP® systems gives definitive identification of more metabolites than any other mass spectrometry system. This contribution will describe workflows designed to exploit the unique capabilities of QTRAP systems and their application to solve real world problems in metabolite ID. In order to support high throughput Bioanalysis, advanced new quantitative workflows for triple quadrupole technology will be presented as well.

P06

EMPLOYING HIGHER RESOLUTION TO OBTAIN BETTER SELECTIVITY FOR QUANTITATION EXPERIMENTS ON A TRIPLE- QUADRUPOLE INSTRUMENT PLATFORM (API 5000™ LC/MS SYSTEM)

Anthony J. Romanelli¹, Jeffrey D. Miller¹, Xavier J. Misonne¹,
¹Applied Biosystems, Framingham, MA, USA,

Triple quadrupole mass spectrometers have proven to provide a high degree of selectivity and sensitivity by utilizing specific unit mass selection in the Q1 and Q3 quadrupoles. This scan type is referred to as SRM or MRM in the literature and is typically performed using “unit resolution” at approximately 0.6 – 0.8 AMU peak width at half maximum (FWHM). There may be cases however in which an isobaric metabolite or interference from the matrix can not be separated using chromatography or eliminated in unit resolution. In these situations it becomes important to be able to take advantage of advances in instrument designs in their ability to achieve higher mass resolutions (~0.1-0.2 AMU at FWHM). With enhanced resolution, the isobaric interference can be partially or completely resolved resulting in accurate quantitative data.

P07

A HIGHLY AUTOMATED WORKFLOW FOR FAST AND COMPREHENSIVE IDENTIFICATION OF IMPURITIES AND DEGRADATION PRODUCTS IN PHARMACEUTICAL PRODUCTS USING LC/MS/MS.

John Gibbons¹, Nicolas Rupcich² and David Duncan²,
¹Applied Biosystems / MDS Analytical Technologies, Concord, Ontario, Canada,
²Genpharm Inc., Etobicoke, Ontario, Canada

The analysis of forced degradation samples by LC-MS/MS is shown to accurately predict the major degradation product of a pharmaceutical drug product. A reduction in the incidences of failed stability studies and costs can be achieved by getting this information into the hands of development scientists earlier in the drug development process. A pharmacopeia related substances method was used with only minor modifications to the mobile phase buffer which eliminated costly method redevelopment. The modified method performed well with retention times for components matching those listed in the method. Software tools for compound optimization and method building simplified the study workflow reducing the dependence on highly trained and experienced operators to complete these types of studies.

P08

HARDWARE AND SOFTWARE DESIGN STRATEGIES FOR THE RAPID DETERMINATION OF OPTIMAL QUANTITATIVE MS/MS CONDITIONS

April L. Smith¹; Anthony J. Romanelli¹; Elliott Jones¹; John Janiszewski²; Hua-fen Liu¹; Steve Ainley³;
Richard Schneider²; Kevin Shirey³; Eva Duchoslav¹; Loren Olson¹
¹Applied Biosystems, Framingham, MA and Foster City, CA;
²Pfizer Inc., Westery, RI;
³Sound Analytics, East Lyme, CT

A new algorithm for high throughput optimization of compounds was tested against the old version of the same algorithm. The design is in hopes that the new algorithm will produce more sensitive, accurate tunes in a high-throughput environment. The new algorithm also incorporates saturation control, therefore the user does not need to worry about using too high of a concentration for optimization. The new algorithm gives the option to do a second injection to fine tune the method created with the first, whereas in the older version a second injection was required in order to get MS/MS data. The data generated from the new and old algorithms was compared to determine which workflow produced more sensitive MRM methods.

P09

ACCELERATED LC/MS/MS FOR THE QUANTITATION AND CONFIRMATION OF PESTICIDES IN FOOD AND WATER SAMPLES

André Schreiber¹, Doina Caraiman¹, Nadia Pace¹, Tim Hoffman¹, Byron Kieser¹, and CJ Baker²
¹ Applied Biosystems/MDS Analytical Technologies Concord, ON (Canada),
² University of Calgary, Calgary, AL (Canada)

Here we present a high-throughput LC/MS/MS method for pesticide screening that combines:

- Fast LC separation with small particle LC columns
- High selectivity and sensitivity of MRM detection
- Scheduled MRM to optimize cycle time for best sensitivity and reproducibility
- Fast and sensitive QTRAP® LC/MS/MS System Enhanced Product Ion scanning
- Confirmation based on mass spectral library search

P10

EFFICIENT PEPTIDE PURIFICATION BY HPLC, EFFECT OF PORE SIZE, PARTICLE SIZE AND CHEMISTRY.

Gezinus Grooten, Aurora Borealis Control BV, PO Box 2, NL-7760AA Schoonebeek, The Netherlands
No abstract received

P11

YMC-BIOPRO, POROUS AND NONPOROUS IEX COLUMNS.

Gezinus Grooten, Aurora Borealis Control BV, PO Box 2, NL-7760AA Schoonebeek, The Netherlands
No abstract received

P12

LAMP-BASED NATIVE FLUORESCENCE DETECTION OF PHARMACEUTICAL PROTEINS IN CAPILLARY ELECTROPHORESIS

B.J. de Kort*, D.Č. Radenović, G.J. De Jong, G.W. Somsen, Department of Biomedical Analysis, Utrecht University, P.O. Box 80082, 3508 TB Utrecht, the Netherlands

In the last decade, capillary electrophoresis (CE) has demonstrated its usefulness for the analysis of intact (i.e. non-digested, underivatized) pharmaceutical proteins. In protein CE, detection is typically carried out using UV absorbance detection at low wavelengths (200-220 nm), but unfortunately it may provide low selectivity and unstable baselines. More selective detection of proteins can be achieved by monitoring the native fluorescence of proteins. Fluorescence detection in CE is commonly carried out using laser-induced fluorescence detection (LIF). However, UV lasers present few spectral lines, are expensive and have limited lifetimes. Lamp sources would provide a much wider choice of excitation wavelengths.

Here we present a lamp-based fluorescence detection system for protein analysis in CE. Excitation was carried out at 280 nm whereas analyte emission was collected using a 335-nm cut-off filter. Wave-guiding principles inside the capillary are used to collect the protein emission light, while scattered excitation light is largely rejected. Detection limits obtained with this set-up appear to be comparable to LIF detection limits. We applied the fluorescence detector to the CE analysis of intact proteins and immunoglobulin G (IgG), and to a stability study of pharmaceutical preparations of recombinant human Growth Hormone (rhGH). CE with fluorescence detection revealed the presence of degradation products in formulations of rhGH that had been stored for a prolonged time. These results show that native fluorescence detection is a valuable analytical technique for the profiling of pharmaceutical proteins.

P13

METHOD VALIDATION APPROACH FOR THE DETERMINATION OF ANTIBODY RESPONSE TO BIOPHARMACEUTICALS

Marcel van der Linden, Ole Lagatie, Harry verdonk and Jan Dankers, Eurofins Medinet B.V., Department of Specialized Biomolecules, Bergschot 71, 4817 PA Breda, The Netherlands

Biopharmaceuticals are increasingly used as therapeutic agents but may induce humoral and cellular immune responses, with considerable consequences. It is therefore essential to select and/or develop assays for assessment of such immune responses. Initially, we developed and validated a specific electrochemoluminescence method for the determination of monoclonal antibody X (MAb-X) in human serum. Secondly, we developed and validated a specific method to determine antibody response to MAb-X in an ELISA based method using fluorescent detection. During method validation, we have performed several experiments, like determination of assay-specific cut-point and normalization factor, lower limit of detection (LOD), assay precision, selectivity and stability. The method was shown to be precise with an overall precision of less than 13.9 % over the whole control range, with an LOD of 14.8 ng/mL. An assay-specific cut-point for both IgG and IgM antibodies were determined. Furthermore, stability of anti-MAb-X antibodies in human serum has been shown at bench-top temperature for at least 3 days, at -70°C for up to 1 month and for at least 5 freeze/thaw cycles. Finally, it has been proven that the biopharmaceutical compound, MAb-X, itself has no effect on the immunogenicity method. In a second phase we validated a confirmation assay, used to eliminate false positive samples/patients following the initial screening of the samples. In conclusion, we set up and validated a specific method to investigate immunogenicity of MAb-X in human serum, performed in compliance with OECD-GLP.

P14

DEVELOPMENT OF AN AUTOMATED SPR-IMER-LC-MS/MS PLATFORM FOR ISOLATION, QUANTIFICATION AND IDENTIFICATION OF PROTEINS FROM PLASMA

E.C.A. Stigter*, G.J. de Jong, W.P. van Bennekom, Utrecht University, Faculty of Sciences, Department of Pharmaceutical Sciences (Biomedical Analysis), Sorbonnelaan 16, 3584 CA Utrecht, The Netherlands

Surface Plasmon Resonance (SPR) sensing is a well-known non-destructive optical technique capable of detecting minute amounts of protein, binding to or dissociating from receptor molecules immobilised on a sensor surface. The combination of SPR and Mass Spectrometry (MS) provides a means for selective binding, recovery and identification of specific proteins (i.e. based on their molecular mass) from complex matrices. This approach is often referred to as Biomolecular Interaction Analysis – Mass Spectrometry (BIA-MS) and facilitates ligand fishing. In spite of the fact that direct coupling of both techniques can be beneficial in terms of sample throughput, as a rule SPR and MS have been used separately (off-line) due to the bulky nature of most of the current SPR devices, their liquid handling and the use of chaotropic regeneration agents used to recover material from the sensor surface, which may cause compatibility problems with MS detection.

A study was initiated to investigate the coupling of SPR and LC-MS/MS for the efficient transfer of ligands isolated with the SPR sensor for further analysis. As model substances for such a system, mouse anti-interferon- γ antibody and the protein human interferon- γ are used. The interactions between these binding partners are studied both in buffer as well as in diluted plasma.

Dextran-modified sensor surfaces prove to enable reproducibly high protein binding and regeneration with minimal non-specific interaction of matrix proteins present in the sample.¹ A method for efficient on-line enzymatic digestion using either immobilized trypsin or pepsin is developed that allows direct coupling to the SPR.^{2,3} Separation and identification of the proteolytic peptides using LC-MS/MS show the presence of the model protein in the samples. Apart from presenting recent data on the investigations, the applicability of this approach in screening for (un-)wanted compounds and future research will be discussed.

[1] Stigter, E.C.A., De Jong, G.J., Van Bennekom, W.P., 2005. Biosens. Bioelectron. 21(3), 474-482

[2] Stigter, E.C.A., De Jong, G.J., Van Bennekom, W.P., 2007. Anal. Bioanal. Chem. 389(6), 1967-1977

[3] Stigter, E.C.A., De Jong, G.J., Van Bennekom, W.P., 2008. Anal. Chim. Acta 619(2), 231-238

P15

ELISA WITH ICP-MS DETECTION AS A SELECTIVE DETECTION TECHNIQUE FOR BIOMARKER ASSAYS

Simone Hof, Monique Putman and Jaap Wieling, Xendo Drug Development Services, P.O. Box 137, 9700 AC, The Netherlands

The aim of the study was to perform an ELISA in which a sensitivity enhancement was established and the matrix effect was reduced by using the biotin – streptavidin coupling and the ICP-MS as a detection system. In this study thyroid stimulating hormone (TSH) was determined in human serum samples using an sandwich ELISA with ICP-MS detection.

P16

STATISTICAL EVALUATION OF THE BENEFIT OF COMBINED USE OF ACCURATE MASS AND ISOTOPIC PATTERN

Marcus Macht¹; Petra Decker¹; Aiko Barsch¹; Ilmari Krebs¹ and Rob van der Heijden²

¹ Bruker Daltonik, Bremen, Germany

² Bruker Daltonics BV, Wormer, the Netherlands

The quality of sum formula generation depends on: a) the preciseness of the mass determination, b) the use of the isotopic pattern information and c) the accuracy of the isotopic pattern measurement. The accurate mass and the isotopic pattern provide two complementary dimensions, allowing a sum formula generation for compounds of masses up to ~500 Da. If the molecular mass is higher, additional information has to be used.

To achieve similar results based on the MS mass accuracy alone, a mass accuracy of 200 ppb would be required. While it is possible to achieve such a mass accuracy by using internal calibrations in current instrumentation once in a while, one has to achieve this on a constant reliable base in everyday routine to be able to apply it for such an application. This is currently not possible on any instrument and thus the combined use of the four dimensions (including MS/MS data) provides a higher selectivity than any instrument can routinely deliver today based on mass accuracy alone. For the statistical evaluation 125 known compounds of masses between 100 and 1000 Da were analyzed by ESI-TOF-MS using external calibration.

P17

SOFTWARE-SUPPORTED TOP-DOWN PROTEIN SEQUENCE CHARACTERIZATION AND ASSIGNMENT OF TERMINI

Detlev Suckau¹, Lars Vorweg¹, Anja Resemann¹ and Rob van der Heijden²
¹ Bruker Daltonik GmbH, Bremen, Germany
² Bruker Daltonics BV, Wormer, The Netherlands

In a mass spectrometric Top-Down analysis using MALDI-MS/MS or ESI-MS/MS, the undigested protein is subjected to fragmentation. This allows to partially sequence the protein, to detect signal peptides, modifications, sequence variations and mutations. As such data analysis can be time consuming, we developed software tools to provide advanced Top-Down assignment of protein ID and N- and C-terminal. The Protein ID is based on sequence tags via Mascot sequence query or MS Blast search. The software comprises automatic detection of signal peptides or C-terminal sequence extension, automatic matching of mass offsets to Unimod modifications based on the selected ion series and the creation of Sequence Tags is by a flexible, configurable algorithm. This approach is demonstrated for a 33.5 kDa protein.

P18

ADVANCES IN THE OPTIMIZATION OF A MICRO-FLOW HPLC (LC-MS) SYSTEM

Rein Reitsma, Separations Analytical Instruments, The Netherlands

Current trends in HPLC system development have embraced higher pressures, higher temperatures and lower flow rates to increase separation efficiency and analytical throughput. By combining recent advances in microscale fluid delivery, small particle (~ 3µm) stationary phases, high temperature separations and chip-based UV absorbance detection to produce a fully integrated microflow gradient HPLC system, the overall chromatographic performance has been optimized for short cycle times, high resolution and better detection sensitivity.

Results of tests using several pharmaceutical formulations indicate that the system design optimization enables separation efficiencies approaching that of U-HPLC and interesting savings in organic solvents.

P19

INTEGRATED BIOPHARMACEUTICAL DEVELOPMENT: NEW CHALLENGES TO EXPEDITE THE PROOF-OF-CONCEPT STAGE AND TIME TO MARKET

Izaak den Daas, Johan Wemer, Ineke Jonker, Thijs van Iersel, Ard Tijsterman and Jaap Wieling, Xendo Integrated Biopharmaceutical Development, Hanzeplein 1, Entrance 53, 9713 GZ Groningen, The Netherlands

Biologics have been the major building blocks within Xendo's life cycle of 18 years and still today are among the major drivers behind their success - if not becoming the most important one. With the establishment of a brand new cGMP compliant small-scale manufacturing facility next to the three early clinical development units and the bioanalytical laboratories, a full package of biologics research and development services has been accomplished. The entire package is suitable to provide major biotech companies as well as small virtual biotech start-ups highly integrated and high-level development programs.

P20

(PRE)CLINICAL IMMUNOGENICITY ASSAY DEVELOPMENT

Hans Mocking and Cees Koopal, TNO Quality of Life, Dept. of Analytical Research, Utrechtseweg 48, 3704 HE Zeist, The Netherlands.

The importance of evaluating the immunogenicity of biopharmaceuticals is generally accepted these days. One approach to measure immunogenicity is using Biacore technology. A versatile instrument for this task is the Biacore T100, especially due to its GLP/Part 11 compliancy. Immunogenicity analysis of animal and human samples imposes specific challenges on the Biacore assay development and the analysis of various sample matrices, e.g. serum, plasma, feces, requires a customized approach. Some specific issues relating to assay development for these complex matrices are highlighted, with emphasis on the biochemistry and immunochemistry at the sensor surface.

cIEF–MALDI-TOF-MS FOR THE ANALYSIS OF (BIOPHARMACEUTICAL) PROTEINS

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Complex protein mixtures can efficiently be separated by capillary isoelectric focusing (cIEF) (1). Hyphenation of cIEF to MALDI–TOF MS via a spotting device offers a powerful tool for the repeatable analysis of proteins in different application fields. However, the coupling of these two techniques is a matter of compromises; compounds that are necessary in cIEF like ampholytes, detergents and polymers cause signal suppression and are less suitable for MS detection (2). This presentation describes the use of a cIEF–UV–MALDI–TOF system for the analysis of a mixture of proteins and the applicability in degradation studies of biopharmaceutical compounds (3).

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- 3) L.H.H. Silvertand, J. Sastre Toraño, G.J. de Jong, W.P. van Bennekom, 'Development and characterization of capillary IEF-MALDI-TOF MS for protein analysis', submitted for publication.