

FABIAN SYMPOSIUM, 30 OKTOBER 2002, WEESP

-

THEMABIJEENKOMST "IS DE BIOANALYSE AF ?"

Woensdag 30 oktober 2002, Solvay Pharmaceuticals, C.J. van Houtenlaan 36, Weesp

Programma

- 09.30 Ontvangst, inschrijven, koffie
Opening, Jan Luteijn (Solvay, Weesp)
- 10.00 Howard Hill (Huntingdon, Cambridge, NL) – The future of bioanalytical technologies – lessons from the past
- 10.45 Koos Drooger en Hans Bakker (Solvay, Weesp) – Eerste toediening van kandidaat-geneesmiddelen aan de mens
- 11.30 Rainer Bischoff (RUG, Groningen) - Analysis of Disease Markers by HPLC – Mass Spectrometry
- 12.15 Lunch, posters
- 14.00 Fred Nagelkerke (LACDR, Leiden) – Visualisatie en quantificering van biochemische processen in levende cellen en weefsels.
- 14.30 Jacob Zeeman (Solvay, Weesp) – Bepaling van de eiwitbinding van potentiële nieuwe geneesmiddelen met behulp van HPLC
- 15.00 Marie-Paule Bouche (RUG, Gent, B) - GC-MS as a bioanalytical tool in the safety evaluation of sevoflurane inhalational anaesthesia
- 15.30 Albert Heck (UU, Utrecht) – Proteomics: from protein complex to organisms
- 16.00 Sluiting & Borrel

Abstracts van Lezingen

THE FUTURE OF BIOANALYTICAL TECHNOLOGIES –LESSONS FROM THE PAST

Howard Hill, Hintingdon Life Sciences Ltd, Woolley Road, Alconbury, Huntingdon, Cambridgeshire PE28 4HS

Are there strong enough drivers to justify a quantum leap in technologies available to the bioanalyst or are we doomed to enhance LC MS forever (forever = more than 15 years). Perhaps a look at history and the evolution of bioanalytical technologies (Spectrophotometric systems, TLC, GC, HPLC Column and detector technologies, SPE etc) their drivers and reasons why they were superseded can give us a look into the future. New technologies at the start of their life cycle fail to meet expectations (usually) –however after refinement they become the new standard and are subject to enhancement by both the manufacturer and user over their useful lifetime before being superseded by “BETTER” more user friendly technologies. We are currently well into the enhancement phase of LC MS MS; however many of these enhancements are high price high values innovations produced by the manufacturer- will their actions prolong the “useful” life of LC MS MS and thereby delay the implementation of innovation or only delay the implementation. Are we making the best use of what we have? Will nanotechnologies coupled with “highly” specific separation technologies and biosensors be the way forward. Are superblack boxes the future of bioanalysis as some of the automation systems become in clinical chemistry, indeed will bioanalysis become a branch of clinical chemistry.

EERSTE TOEDIENING VAN KANDIDAAT GENEESMIDDELEN AAN DE MENS

Koos Drooger & Hans Bakker, Afdeling Klinische Farmacologie, Solvay Pharmaceuticals, Weesp, Nederland

Rond de eerste toediening van een kandidaat geneesmiddel aan de mens worden een aantal aspecten belicht. Na een korte introductie over de plaats van de klinische farmacologie in de ontwikkeling van een nieuw geneesmiddel, wordt ingegaan op het ontwerp van een SRDT studie (eerste toediening aan de mens) met, in dit geval, de CNS stof SLV 310 bij Solvay Pharmaceuticals.

Aan de orde komen onder andere het doel van de studie, de gemeten parameters op het gebied van tolerabiliteit, veiligheid, farmacokinetiek en farmacodynamiek en de logistiek van een en ander.

Vervolgens wordt ingegaan op de bio-analytische aspecten van het analyseren van de farmacokinetiek monsters. In het kort wordt de analyse-methodiek weergegeven, alsmede een aantal kenmerkende aspecten voor dit type onderzoek

In het laatste deel van de presentatie wordt ingegaan op de interpretatie van de analyse resultaten en de consequenties van de bevindingen voor het verdere verloop van de studie.

ANALYSIS OF DISEASE MARKERS BY HPLC - MASS SPECTROMETRY

Rainer Bischoff, University of Groningen, Centre for Pharmacy, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands [<http://www.farm.rug.nl/InterACT/btrs.html>]

Diagnosis of disease is often the first step to a successful therapy. Many diseases manifest themselves not only at the phenotypic but also at the biochemical level. In clinical analysis we diagnose and monitor disease by following biochemical parameters. With the development of new technologies and the completion of major gene sequencing projects, biomarker research has entered into a new phase. In particular, profiling of complex biological samples such as serum or urine for the discovery of novel peptide or protein markers promises to allow earlier detection of slowly progressing multi-factorial diseases such as cancer, chronic inflammation or metabolic disorders. At this early stage, it is often not possible to rely on one or a few markers and biomarker patterns may deliver more reliable prognostic values.

Discovery of biomarker patterns depends on analytical methods that can provide comparative, quantitative data. Well-controlled collections of samples need to be available that allow correlation of clinical history with the biomarker patterns. Next to giving an overview over various analytical approaches to biomarker studies, I will report about our work on cervical cancer and chronic obstructive pulmonary disease (COPD). Our initial data indicate that it is possible to detect specific tumor markers at the ng/ml level by LC-MS after proper sample pretreatment and that degradation products of extracellular matrix proteins may be developed into markers for ongoing tissue destruction during chronic inflammation.

Next to focusing on biomarkers themselves, we have an interest in profiling the active enzymes that are involved in tissue destruction. To this end activity-based affinity techniques have been developed to enrich matrix metalloproteases from biological fluids. Our initial data show that active enzymes can be successfully enriched using immobilised low-molecular weight inhibitors opening the field for profiling in diseased tissue or fluids such as bronchoalveolar lavage or synovial fluid. By focusing modern analytical techniques on medically relevant questions we hope to deliver new insights into disease mechanisms and provide access to better diagnostic tools.

Acknowledgements: The cervical cancer project is a collaboration with Prof. van der Zee (Gynecological Oncology, University Hospital, Groningen). Recombinant proteins were kindly provided by Prof. Silverman (Harvard Medical School, Boston; SCCA-1), Prof. Brömme (Mount Sinai School of Medicine, New York; Cathepsin K) and AstraZeneca R&D (Lund, Sweden; MMP-12). The COPD project is a collaboration with Prof. Postma (Pulmonary Research Division, University Hospital, Groningen).

VIDEO MICROSCOPY AND CONFOCAL LASER SCAN MICROSCOPY TO STUDY THE MECHANISMS OF CYTOTOXICITY IN INDIVIDUAL LIVING CELLS. APPLICATION AND NEW DEVELOPMENTS

J.Fred Nagelkerke, Division of Toxicology, Leiden/Amsterdam Center for Drug Research, Leiden University, P.O.Box 9503, 2300 RA, Leiden, The Netherlands.

In the past decade numerous studies have been performed using Video Microscopy (VM) and Confocal Laser Scan Microscopy (CLSM) to study in real time the effect of compounds on cells. An overview of the techniques themselves and the necessary hardware, the major types of calculations and how to handle cells and probes is presented in this paper.

Although VM and CLSM are very powerful techniques they have some drawbacks. Therefore, recently, technological innovations have emerged to overcome these drawbacks. The new techniques are Multi-Photon Excitation Microscopy (MPEM), Fluorescence Life Time Microscopy (FLIM) and determination of Fluorescence Resonance Energy Transfer (FRET). Also the discovery of the Green Fluorescent Protein (GFP) and subsequent development of derivatives thereof which all emit light upon excitation, without the need of a prosthetic group, has created new opportunities. Especially, transfection with vectors including GFP-DNA is very powerful to study protein localization and, in addition, is very suitable to perform FRET studies. Recently, also transgenic mice expressing GFP-tagged proteins at specific sites of the body have become available to perform in vivo studies.

BEPALING VAN DE EIWTBINDING VAN POTENTIËLE NIEUWE GENEESMIDDELEN MET BEHULP VAN HPLC

Jacob Zeeman

Solvay Pharmaceuticals BV, CJ van Houtenlaan 36, 1381 CP Weesp, The Netherlands

De mate van eiwitbinding is een belangrijke parameter bij de evaluatie van farmacologische en farmacokinetische eigenschappen van een potentiëel nieuw geneesmiddel. Sinds 1976 wordt de eiwitbinding bij Solvay bepaald met behulp van evenwichtsdialyse. Deze methode is bewerkelijk en tijdrovend en afhankelijk van de kwaliteit van het gebruikte bloedplasma. In 1984 publiceerde Allenmark een bepaling met behulp van HPLC op een covalent gebonden BSA kolom. Deze methode is gewijzigd en verbeterd en is aanzienlijk sneller dan die met behulp van evenwichtsdialyse. De resultaten komen vrij goed overeen met die van de evenwichtsdialyse. Door automatisering kunnen grote series verbindingen met bindingspercentages tussen 0 en 100% worden gemeten. De correlatie tussen beide methoden zal worden getoond.

GC-MS AS A BIOANALYTICAL TOOL IN THE SAFETY EVALUATION OF SEVOFLURANE INHALATIONAL ANAESTHESIA

Marie-Paule L. Bouche¹, Linda F. Versichelen², Michel M. Struys², Eric Mortier², and Jan F. Van Bocxlaer¹

¹Laboratory of Medical Biochemistry and Clinical Analysis, Ghent University, Harelbekestraat 72, B-9000 Ghent, Belgium

²Department of Anesthesia, University Hospital, Ghent University, De Pintelaan 185, B-9000 Ghent, Belgium

To date, sevoflurane or fluoromethyl-1,1,1,3,3,3-hexafluoro-2-propyl ether, has definitely found its place in clinical practice as a volatile general anaesthetic agent: it is one of the most widely used inhalational anaesthetics throughout the world, in both human and veterinary medical practice. However, during low-flow or closed circuit sevoflurane anaesthesia, compound A, an olefinic degradation product with a known nephrotoxicity in rats, is generated upon contact with alkaline CO₂ absorbents. The extent of its formation is dependent upon multiple factors such as fresh gas flow rate, absorbent temperature and water content. Whether compound A, formed during anesthetic use, produces similar toxicity in humans, is still subject of intense scientific debate, nevertheless its in-vivo uptake by patients has been demonstrated. In order to evaluate compound A formation in sevoflurane based low-flow and closed circuit anaesthesia and thus sevoflurane toxicity, a reliable and reproducible assay for the quantitative vapour phase compound A determination was developed.

Compound A determination was performed by capillary GC-MS (HP 6890-5973MSD). For injection (1 mL) we invoked an injection technique based upon automated headspace sampling and programmed temperature vaporization after cryogenic condensation (Tenax TA sorbent, liquid N₂, -80°C). Applying the latter, we successfully reconciled large gas volumes injected with small internal separation column diameter. As such, we created a simple and robust injection approach, readily applicable on a daily basis in clinical trial. Using the custom made thick film CP-Select 624 capillary column for chromatographic separation provided adequate retention as well as complete chromatographic resolution between compound A and sevoflurane, at the same time combined with an excellent peak shape and symmetry with respect to compound A, as well as an acceptable overall analysis runtime. Mass spectrometric detection proved vital in light of the intended sensitivity of the assay and, in addition, offered unsurpassed selectivity by means of the mass spectrum. Standards of compound A in the gas phase were prepared departing from liquid volumetric dilutions of stock solutions of compound A and sevoflurane in ethyl acetate. On account of its retention profile and ideal mass spectrum, in which all ionization is concentrated in the molecular ion, 1-iodo-2,2,2-trifluoroethane was chosen as an internal standard. After the development stage, our assay was thoroughly validated from a bioanalytical viewpoint. To establish adequate confidence in the developed methodology, several commonly investigated validation criteria, such as linearity, selectivity, precision, accuracy and sensitivity were studied. The results we obtained clearly indicate the suitability of the developed methodology for its intended use, namely the sound, reliable quantitative determination of compound A in vapour phase.

The developed method proved useful in various in-vitro and in-vivo clinical experiments, applying modern closed-circuit and minimal-flow anaesthesia techniques, of which the obtained results will be discussed. Generally speaking, we directed our research towards two main aspects in compound A generation. First of all, we investigated the influence of the anaesthesia set-up on compound A formation by comparing two closed-circuit set-ups, the recently introduced PhysioFlex® and a classical closed system with two unidirectional valves. On the second hand, we quantitatively evaluated how the nature, brand and chemical composition of the used carbon dioxide absorbent affects the generation of compound A. Here, a total of six different products were investigated, either during simulated anaesthesia conditions or in in vivo experiments.

MOVING STABLE ISOTOPES UP INTO THE FOOD CHAIN: QUANTITATIVE PROTEOMICS IN *C. ELEGANS* USING METABOLIC LABELING

Jeroen Krijgsveld¹; Janik Johansen¹; Rene Ketting²; Albert J.R. Heck¹

¹ **Biomolecular Mass Spectrometry, Utrecht University, Utrecht, The Netherlands;**

² **Netherlands Institute of Developmental Biology, Utrecht, The Netherlands**

Quantitative proteomics aims to examine the differential abundance of all proteins. Currently, one of the best approaches in quantitative proteomics uses the comparison of the ratio of labeled vs. unlabeled protein pairs by mass spectrometrical methods. Two approaches for stable-isotope labeling are *in vitro* derivatization of proteins by an isotope-containing reagent, and *in vivo* metabolic labeling. Although ICAT, an example of the first approach, can be applied to any sample, metabolic labeling offers distinct advantages since no derivatization is needed and all proteins are labeled universally. Because of their ability to grow in defined media, metabolic labeling has so far been limited to lower (single cellular) organisms. The present study aims to label higher organisms, such as *C. elegans*, and use this approach to identify proteins expressed in the germline, by comparing protein expression in the wild type and mutant organisms.

Since *C. elegans* feeds on bacteria we reasoned that this organism could be metabolically labeled by growth on ¹⁵N labeled *E. coli*. Therefore, eggs were seeded on agar plates containing bacteria grown on media 98% enriched in ¹⁵N. Larvae were harvested in L4-stage and total protein extract was separated by 2D gel electrophoresis. Investigation, by MALDI-TOF analysis, of randomly picked spots revealed that of each protein 95% was labeled, while 5% remained in unlabeled form. To optimize this, nematodes were grown on labeled *E. coli* until the second generation L4. After this only completely labeled proteins were detected.

Since metabolic labeling does not require a derivatization step, like in the ICAT procedure, protein extraction of labeled and unlabeled nematodes can be performed in one tube directly after harvesting. Thus, differences in sample preparation are eliminated by definition. Furthermore, since labeled and unlabeled proteins co-migrate in 2D gels, both the identity and the relative quantity of a protein can be obtained from a single spot. We show this by mixing labeled and unlabeled worms in various ratios.

We apply the approach of metabolic labeling to the *glp-4* mutant, which is unable to generate a germline and is sterile because no germ cells are formed. Comparison of the labeled *glp-4* with the unlabeled wild-type strain (and *vice versa*) reveals differences in widely diverging ratios (1.5-15) in protein levels. These include many sperm (mitochondrial) proteins, structural germ line proteins as well as proteins not identified previously. Comparison of our data with an analysis of the *glp-4* mutant using cDNA-arrays points to many differences, and we will discuss implications of both methods. Furthermore, we will show that apart from 2D gel analysis, tryptic digests of mixed labeled and unlabeled proteins can be readily analyzed by multi-dimensional LC-MS.

Abstracts van Posters

PROTEOLYTIC DIGEST ANALYSIS USING AGILENT'S NANOLC AND MS/MS ION TRAP

Paul Goodley and Bernd Glatz; Palo Alto, CA and Waldbronn, Germany

Nanoscale 2D-LC in combination with nanoelectrospray ion trap MS/MS identifies proteins from complex digest mixtures at low femtomole levels. Skilled operators are typically required to monitor and maintain these powerful systems.

Problems such as a partially plugged sprayer needles, passive splitters or tubing may cause unstable flows which result in poor quality electrospray MS/MS results. Passive splitters which use exclusively pressure differences to control nanoflow can not compensate for the above uncontrolled events. However, active splitting using mass flow measurement can compensate for pressure differences and can continue to elute samples through the column even though some column or needle blockage may occur in the flow system. A system for a routine environment therefore needs:

- Reliable generation of shallow gradients with small delay volumes from 0-60% organic at 100-600 nl/min. Direct flow readouts for diagnostics
- Minimum operator skills to maintain trapping / analytical columns, electrospray needles, and a low dispersive system for maximum chromatographic fidelity.
- A stable software environment for reliable automation of LC/MS along with fully automatic post-processing protein identifications from digestion mixtures or gel spots. This poster describes a new, unique approach for accurate nanoflow solvent delivery designed for electrospray MS/MS.

THE USE OF CHEMOMETRY WITHIN LC-MS PROFILING STUDIES

Marko Ruijken¹, Karel H. Stegman¹, Diana Hijdra²

¹*Solvay Pharmaceuticals BV, CJ van Houtenlaan 36, 1381 CP Weesp, The Netherlands*

²*Hogeschool Leiden*

The amount of data from LC-MS experiments, coming from Impurity profiling, Degradation profiling and Metabolite profiling is due to automatization of sample preparation and acquisition extremely increased. The processing of data can be divided in two parts ; Significance en Relevancy. Solvay Pharmaceuticals has developed a Chemometrical module called MASSA (Mass Significance Selection Algorithm) which improves the search for significance in a LC-MS dataset. The functionality of this module is compared with a manual method for processing a dataset. The comparison is done with one Impurity profiling dataset and two Metabolite profiling datasets. The end result of the chemometrical module MASSA is a reduced digital dataset.

Relevancy in this digital dataset can be found by means of specific search modules, such as prediction or isotopic pattern or comparison with other chromatograms. From the results of the comparison experiment we conclude that the use of the chemometrical module MASSA makes it possible to process a LC-MS dataset for significance in a short time, reproducible and independent from the analyst. Furthermore the module gives a reduced digital dataset which easily can be used for further searches towards relevancy.

FLUORESCENT RECEPTOR ASSAYS BASED ON THE ESTROGEN RECEPTOR SUBTYPES ALPHA AND BETA

Theo de Boer, Debby Ojens and Kees Ensing

MERSKA BV, L.J. Zielstraweg 1, 9713 GX Groningen, The Netherlands

Detection and identification of compounds having estrogenic effects is of importance in drug discovery programmes within the pharmaceutical industry as well as in monitoring programmes for food products and environment.

Interactions of (xeno-)estrogens with the endogenous hormonal system of the exposed organism can affect embryos, gonads, and reproductive behavior. The estrogen receptor (ER) belongs to the nuclear receptor family, a class of soluble DNA binding proteins, mainly present in the cytoplasm of the cell, that act as ligand-activated enhancer factors. It consists of two different forms, expressed as ER- α (66 kDa) and ER- β (59 kDa). The ER- α is mainly located in the uterus and the ER- β can be found in vascular tissue.

Since radioactive receptor assays are not the preferred method of use, a fluorescent receptor assay (FRA) was developed for both receptor subtypes.

Method: In the FRA a fluorescent ligand (FL), calibrators and samples (Test-compounds, TC) are incubated with the recombinant human estrogen receptor (hER). A competition between the TC and the FL for binding to the hER will occur. When a steady state is accomplished, the FL/hER and TC/hER complexes are precipitated. The free fraction is separated from the bound fraction. The remaining bound (xeno-)estrogens (TC and FL) are dissociated from the receptor and collected into a standard 96-wells plate. Controlled volumes of the collected fractions are directly injected into an HPLC system with a fluorescence detector. The FL concentrations, expressed as peak areas, can be plotted on a semi-logarithmic scale against the calibrator concentrations.

Assay characteristics:

Assay time : < 4 hours (incubation: 2 x 1h) HPLC analysis: < 4 minutes; Eluent: 65% MeOH; 35% 50 mM

Phosphate buffer pH 6.9; Flow: 0.8 mL/min; T=30 °C; Inj = 100 μ L

[λ_{ex}] = 379 nm ; [λ_{em}] = 436 nm.

Selectivity differences for several ligands towards the hER- α and hER- β have been shown. Both assay types are capable of quantitation of (xeno-)estrogens below 1 nM (expressed as 17- β -estradiol equivalents).

METABOLIC FINGERPRINTING: A NEW TECHNIQUE TO IDENTIFY DISEASE RELATED AND NUTRIENT RELATED BIOMARKERS
A PILOT STUDY OF DIETARY VITAMIN C AND OSTEOARTHRITIS

R.A.N. Lamers¹, E.J. Faber¹, J. de Groot², R.H. Jellema¹, V.B. Kraus³, N. Verzijl², J.M. TeKoppele² and J.H.J. van Nesselrooij¹

¹TNO Netherlands Organisation for Applied Scientific Research, Nutrition and Food Research, P.O. Box 360, 3700 AJ, Zeist, The Netherlands

²TNO Netherlands Organisation for Applied Scientific Research, Prevention and Health, Gaubius Laboratory, P.O. Box 2215, 2301 CE, Leiden, The Netherlands

³Duke University Medical Center, Department of Medicine, Division of Rheumatology, Allergy and Clinical Immunology, Durham, NC 27110, USA

Objective In nutritional research an urgent need exists for biomarkers, since these are still lacking in the search for relationships between food and health. Metabolic fingerprinting, a combination of Nuclear Magnetic Resonance spectroscopy (NMR) and Multivariate Data Analysis Techniques (MVA), is a powerful technology to identify combinations of biomarkers in biological fluids. The main advantage of NMR compared to other analytical techniques is that a wide range of metabolites can be quantified simultaneously with no sample preparation and 'without prejudice' (1). Using MVA, a so-called metabolic fingerprint can be obtained from NMR spectra, consisting of metabolites that are specific for health or diseased state. Currently, at our institute a.o. studies are running in the field of osteoarthritis and osteoporosis in order to investigate the effects of nutrition on these diseases using metabolic fingerprints. In this poster presentation we will focus on osteoarthritis. A metabolic fingerprint for osteoarthritis will be presented, which will be used to study the effects of vitamin C on the development of the disease (2). The main objective of this study is to demonstrate the applicability of metabolic fingerprints in evidence-based nutritional and pharmaceutical studies.

Method A longitudinal intervention study was carried out with guinea pigs developing osteoarthritis during ageing, randomly divided in treatment groups with normal and varying vitamin C doses. Clinical and histo-pathological parameters were determined and assessed. In addition to this, NMR and MVA of guinea pig urine were carried out, yielding a metabolic fingerprint for osteoarthritis. Thereupon, the metabolic fingerprint has been used to visualise the effects of vitamin C on osteoarthritis.

Results A metabolic fingerprint for osteoarthritis has been found, consisting of combinations of exclusive NMR signals specific for osteoarthritis. Vitamin C had no effect on cartilage degeneration but could affect osteoarthritic inflammation.

Conclusions Metabolic fingerprinting can be of significant benefit in nutritional (and pharmaceutical) research, as there is an urgent need for evidence of significant effects of nutrition on health. The suitability of metabolic fingerprinting for this purpose has been shown with this study.

APPLICATION OF A HYBRID QUADRUPOLE-LINEAR ION TRAP MASS SPECTROMETER FOR THE QUANTITATION OF METABOLITES IN BIOLOGICAL FLUIDS

Tanya N. Gamble, J.C. Yves Leblanc, and Nadia Pace, Applied Biosystems/MDS Sciex, 71 Four Valley Drive, Concord, ON, Canada L4K 4V8

LCMS has emerged as a sensitive, rapid and highly automated technique for identification and quantitation of drug metabolites in biological samples. However, sensitivity and linearity requirements for both identification and quantitation are rarely found on a single MS platform, thus significantly reducing the level of automation and throughput. The novel hybrid RF/DC quadrupole-linear ion trap MS (Q TRAP™) marries the sensitivity of ion trap full scan mode with the traditional quadrupole scan modes (such as MRM). The combined capabilities on a single MS platform increases the efficiency and throughput achievable in screening and quantitation of metabolites. The Q TRAP™ system was used for the quantitation of alprazolam, bromocriptine and buprenorphine in urine samples in order to assess its performance in MRM and full scan mode.

APPLICATION OF A PROTOTYPE QUADRUPOLE-LINEAR ION TRAP MASS SPECTROMETER IN THE IDENTIFICATION AND CHARACTERIZATION OF GLUCURONIDE

Nadia Pace, Tanya Gamble, J.C. Yves Le Blanc, Applied Biosystems/MDS Sciex, 71 Four Valley Drive, Concord, Ontario, Canada, L4K 4V8

Identification and characterization of metabolites from biological matrices has benefited from LC/MS in the last decade. Although this method can be extremely sensitive and selective, proper identification of metabolites at low levels in the presence of interferent ions can still be challenging. One approach frequently used for the identification of phase II metabolites is to use a neutral loss scan on triple quadrupole MS instruments. However, structural information is frequently obtained in MS instruments that provide more sensitivity in full scan product ion mode such as a 3D-trap or a QqTOF instrument, thus increasing the analysis time. The advent of a hybrid RF/DC quadrupole-linear ion trap mass spectrometer (Q TRAP™) which provides an enhanced sensitivity product ion scan at high scan speeds, while maintaining all traditional triple quadrupole scans such as neutral loss, leads to a simplification in the analysis step and opens the door to automation.

THE USE OF MS3 CAPABILITIES OF AN RF/DC HYBRID QUADRUPOLE-LINEAR ION TRAP MASS SPECTROMETER IN STRUCTURAL ELUCIDATION

Io Ari Gritsas(1), Themis Flarakos(1) and J.C. Yves Le Blanc(2);
(1) MDS Pharma Services, 2350 Cohen Street, Ville St-Laurent (Quebec) H1M 1R3, Canada;
(2) Applied Biosystems|MDS Sciex, 71 Four Valley Drive, Concord (Ontario) L4K 4V8, Canada

Structural Elucidation

In a triple quadrupole instrument, the fragmentation of molecules is known to result in a “fragment rich” MSMS spectral pattern. However, little information can be obtained on the pathway from these information rich spectra. Hence, ion traps with their MS_n capabilities are more frequently used to get a better understanding of the fragmentation pathway and are viewed as the instrument of choice in structural elucidation. A hybrid RF/DC quadrupolelinear ion trap mass spectrometer with MS3 capabilities was used in the investigation of the fragmentation pathway of dextromethorphan and results are compared to other instruments conventionally used for this type of application.

FEASIBILITY OF TOXICOLOGICAL GENERAL UNKNOWN SCREENING USING A HYBRID RF/DC QUADRUPOLE-LINEAR ION-TRAP MASS SPECTROMETER

Pierre Marquet(1), Franck Saint-Marcou(1), Tanya N. Gamble(2) and J.C. Yves LeBlanc(2)
(1) Department of Pharmacology and Toxicology, University Hospital, Limoges, France;
(2) Applied Biosystems/MDS Sciex, Concord, ON, Canada

Single mass spectrometry with in-source collision induced dissociation, tandem-mass spectrometry (MS/MS) and MS/MS with information-dependent acquisition (IDA) have been investigated for general unknown screening (GUS) of drugs and toxic compounds.

Single-MS techniques are repeatable and reproducible on same but not on different types of instruments. A simple MS/MS strategy is not really compatible with GUS, as a limited number of pre-defined ions must be selected before fragmentation. Preliminary studies showed the potential of IDA, an auto-adaptive MS/MS product-ion scan mode where, at each unit time, the m/z ratios above a given intensity threshold are selected for fragmentation. The aim of this study was to evaluate the feasibility of a GUS procedure with IDA, using a hybrid quadrupole-linear ion-trap mass spectrometer (QqQlinear ion trap).

IDENTIFICATION OF PHOSPHORYLATION SITES BY MEANS OF ESI HYBRID RF/DC QUADRUPOLE-LINEAR ION TRAP MASS SPECTROMETRY

Ivan K Chu*, Feng Zhong, Yves Leblanc and Nic Bloomfield, Applied Biosystems/MDS Sciex, 71 Four Valley Drive, Concord, Ontario, Canada, L4K 4V8

Liquid chromatography (LC) coupled to tandem mass spectrometry (MS) has previously been applied for the determination of phosphorylated site in peptides using techniques such as in-source fragmentation, constant neutral loss and precursor ions scan. Though the later two approaches have proven to be highly specific, they can only be ‘truly’ and effectively applied to triple quadrupole mass spectrometer (QqQ) instrument. However, it is difficult to obtain high sensitivity product ion information on these same QqQ MS platforms. Using an hybrid RF/DC quadrupole-linear ion trap instrument, which combines the ‘true’ neutral loss scan or ‘true’ precursor ion scan capabilities of a QqQ and the improved sensitivity in product ion scan, phosphorylated peptides could be identified more selectively while obtaining high quality fragmentation spectrum on the same LC-MS experiment. Further more, when combined to functionality such as Information Dependant Acquisition (IDA), it is possible to identify specifically the phosphorylated peptide in negative mode via precursor ion scan (PI) or in positive mode via neutral loss scan (NL) and obtain positive ion mode fragmentation in an automated fashion.

IMPLEMENTING INFORMATION DEPENDENT ACQUISITION TO AN HYBRID RF/DC QUADRUPOLE- LINEAR ION TRAP MASS SPECTROMETER

Alina Dindyal, Jane Y. Zhao, Nic Bloomfield, J.C. Yves Le Blanc, Applied Biosystems|MDS Sciex, 71 Four Valley Drive, Concord (Ontario) L4K 4V8, Canada

The increasing importance of LC/MS in areas of drug discovery and proteomics has created a higher demand on the analytical capabilities of instruments in terms of sensitivity, throughput and automation. The advent of tools like Information Dependent Analysis (IDA) enables user to improve their throughput by generating MS and MSMS data in a single analysis. However, in order to ensure that the information generated will be useful, one has to either 1) design a high level of logic into the decision making process or 2) use instrument scans that will provide the desired information. The benefit of the former approach lies into the gathering of useful information based on the specificity of the analytical data generated. This can be achieved efficiently if the mass spectrometer instrument can combine scan modes that provide specific MS information such as a triple quadrupole instrument with full scan MSMS sensitivity. The TRAP™ hybrid quadrupole linear ion trap system brings together the power of instrument specificity and sensitivity with the automation level of IDA to maximize the collection of useful data to a plethora of applications – the range of analyses performed spanned from discovery and toxicology to proteomics applications. This poster presents the

implementation and application possibilities of IDA on a Q TRAP™ system to maximize data collection from single MS to MS3.

ICAT LABELED PROTEIN ANALYSIS VIA AUTOMATED LIQUID CHROMATOGRAPHY/ORTHOGONAL MALDI QqTOF

Chris Lock†, Iryna Chervetsova†, Tim Griffin*, Hookeun Lee* and Ruedi Aebersold*, †Applied Biosystems/MDS SCIEX, 71 Four Valley Drive, Concord, Ontario, Canada, L4K 4V8. *Institute for Systems Biology, 4225 Roosevelt Way N. Suite 200, Seattle, WA 98105

The coupling of the Applied Biosystems/MDS SCIEX oMALDI ion source to QSTAR mass spectrometer has enabled high quality sequence information to be obtained from unknown MALDI generated peptides. The unique geometry results in the laser induced ionisation process being effectively decoupled from the TOF analyzer enabling both high resolution and mass accuracy to be achieved in both MS and MSMS type experiments. The application of the technique to automatically analyse biotin-linked isotope labelled affinity tag (ICAT) labelled peptides, previously separated by liquid chromatography and spotted onto a 96 well plate is demonstrated. Automatic peak selection and collision energy adjustment to generate high quality MSMS spectra is shown.

THE ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) BY LC/MS/MS USING A NEW ATMOSPHERIC PRESSURE PHOTOIONIZATION SOURCE

Gary Impey, Byron Kieser, Jean-François Alary, Applied Biosystems/MDS Sciex, 71 Four Valley Drive, Concord, Ontario, Canada, L4K 4V8

A new atmospheric pressure photoionization source (Robb, Covey and Bruins¹) uses photons to ionize large quantities of a dopant molecule added along with the vaporized mobile phase. Analyte molecules are efficiently ionized through secondary reactions initiated by the charged dopant. This new technique was used to develop an analytical method for polycyclic aromatic hydrocarbons (PAHs), comparing the difference under reverse phase and normal phase chromatographic conditions. A total of 16 PAHs were analyzed including the determination of unknown quantities in atmospheric samples from both rural and urban areas.

¹Robb, D.B., Covey, T. R., Bruins, A. P., Anal.Chem. 2000, 72, 3653-3659

EXPERIENCES WITH MONOLITHIC LC PHASES IN QUANTITATIVE BIOANALYSIS

**N.C. van de Merbel, H. Poelman and E.W.J. Hooijschuur
Pharma Bio-Research Group B.V., P.O. Box 200, 9470 AE Zuidlaren**

Recently, monolithic silica, a new type of silica-based LC phase, has become commercially available, which offers the possibility of performing high-resolution separations in very short chromatographic run times. Due to the relatively large (typically 2 µm) macropores, this stationary phase has a low flow resistance and can thus be operated at high mobile phase flow rates with only limited back-pressure, while mesopores of typically 12 nm in the silica skeleton provide the surface area needed to achieve a proper chromatographic performance also at these high flow rates. Typically, by simply increasing the mobile phase velocity, chromatographic run times can be reduced by a factor of 5 with no loss in resolution.

While the potential of these monolithic LC phases has been demonstrated in several cases, mostly with standard solutions, its use for quantitative bioanalysis has not been reported so far. It is the aim of this presentation to give an overview of some bioanalytical applications of monolithic columns. The transfer of existing methods from conventional LC phases to monolithic silica as well as the development of new methods will be covered. The use of several detection modes, both tandem mass spectrometry and conventional techniques such as UV and fluorescence, will be addressed. The suitability of the approach will be illustrated by presenting (cross-)validation results.

Routebeschrijving

- Vanaf A1 U neemt afslag 3 Muiden/Weesp. U volgt de borden Weesp. Vervolgens volgt u de borden doorgaand verkeer/Ind. Noord. Eenmaal onder het spoorviaduct volgt u de borden richting Bussum. Bij de T-splitsing gaat u linksaf (Amstellandlaan) en bij de tweede verkeerslichten rechtsaf (Casparuslaan). Over de brug bent u op de Pr. Irenelaan, deze volgt u met de bocht naar rechts waarna u vanzelf op de C.J. van Houtenlaan uitkomt. U gaat rechtsaf het terrein van Solvay Pharmaceuticals op.
- Vanaf A9 U neemt afslag Gaasperplas/Weesp. Beneden aan de afslag gaat u linksaf. Bij de stoplichten rechtsaf. U blijft de N236 richting Weesp volgen. Eenmaal over de boogbrug gaat u bij de tweede verkeerslichten linksaf (afslag Weesp). U gaat direct weer linksaf het terrein van Solvay Pharmaceuticals op.
- Openbaar vervoer Vanuit het NS-station Weesp neemt u bus 130, 139 of 177. U stapt uit op de halte C.J. van Houtenlaan.

