FABIAN SYMPOSIUM, 18 NOVEMBER 2005, OSS (NL)

-

THEMABIJEENKOMST "'A PHARMACOLOGIST'S DREAM … THE BIOANALYST'S CHALLENGE"

Locatie: Auditorium van NV Organon

www.bioanalyse.org

Programma

(lectures preferentially held in English)

09.15	Ontvangst, inschrijven, koffie
09.45	Opening, welkom door dr. Frans Maris, NV Organon, Oss
09.55	Terugblik op 1e lustrum FABIAN, dr. Wout van Bennekom, voorzitter FABIAN
10.05-10.50	Chris Kruse (Solvay, Weesp), The role of bioanalytics in future drug discovery and development
10.50-11.30	Marcel van Duin (Organon, Oss), Pharmacology in drug research; The translational challenge!
11.30-12.05	Nico Vermeulen (VU Amsterdam), In vitro and in silico screening of affinities to Cytochromes P450
12.05-12.40	Geny Groothuis (RU Groningen), Predictive value of in vitro ADME-Tox during drug development
	Pauze, lunch, postersessie
14.15-15.00	Graham Lappin (Xceleron), AMS: A Strategic Leap Forward in Human ADME
15.00-15.30	Jurgen Mensch (J&J, Beerse), UPLC/MS², Added value in ranking the permeability of drug candidates through artificial membranes
15.30-16.00	Philip Timmerman (J&J, Beerse), A tailored approach for discovery and early development bioanalysis : a scientific challange for the bioanalytical scientist
16.00	Sluiting & Borrel / Drink

Nadere informatie
De meest actuele informatie voor, over en van FABIAN vindt u op onze website
http://www.bioanalyse.org

Bijlagen
- abstracts van lezingen
- abstracts van posters
- locatie / reizen / overnachten
- last-minute posters

Abstracts van Lezingen

THE ROLE OF BIOANALYTICS IN FUTURE DRUG DISCOVERY AND DEVELOPMENT

Chris Kruse, Solvay Pharmaceuticals, Weesp, The Netherlands

In this lecture first the two keywords bioanalytics and drug discovery will be highlighted. Bioanalytics appears to be involved in all stages of the drug discovery and development process.
In the second part of the lecture the future role of bioanalytics will be addressed. The increasing demands for objective measurements of diseases and symptom pathophysiology and of efficacy of potential drugs will further emphasize the crucial role of bioanalytics in the future discovery of innovative drugs.

PHARMACOLOGY IN DRUG RESEARCH; THE TRANSLATIONAL CHALLENGE!

Marcel van Duin, NV Organon, Oss, The Netherlands

Geen abstract ontvangen

IN SILICO PREDICTION AND IN VITRO SCREENING OF DRUG BINDING AND METABOLISM: THE CASE OF CYTOCHROMES P450.

Nico P.E. Vermeulen. LACDR-Division of Molecular Toxicology, Department of Chemistry and Pharmacochemistry, Vrije Universiteit, De Boelelaan 1083, 1081 HV Amsterdam. E-mail: npe.vermeulen@few.vu.nl.

During the past two decades there has been an increased interest amongst scientists to explain the metabolism of drugs by Cytochromes P450 (P450s) in terms of molecular mechanisms and to predict this process on the basis of substrate- and protein structures and properties.
In this presentation, firstly a brief survey will be given on the general role of P450s in the disposition and (de-)toxication of drugs and other chemicals. P450 2D6, an important drug metabolizing enzyme and for us in this regard a model P450, for example, is genetically polymorphic and thus contributing to important inter-individual differences in drug response, in drug side-effects and in susceptibility to toxicity. Secondly, the construction of 3D-protein models of human and rat P450 2D-enzymes and, more specifically of their active site will be described. An improved protein model of the human P450 2D6 was recently generated, and critically evaluated, e.g. with molecular dynamics (MD)-simulations and substrate binding and docking experiments. Moreover, different docking algorithms in combination with different scoring functions have been evaluated to determine which approach is most reliable in predicting and screening of binding modes and binding affinities of Cyt P450-ligand complexes. Special emphasis was devoted to the role of active site water molecules.
Alternatively, in co-operation with a small biotech company (Kiadis, NL), we developed successfully a new on-line technology to screen individual components in metabolic mixtures or in mixtures (or libraries) of compounds for affinities to the Estrogen receptor (ER) and also to Cyt P450s. This so-called HRS-technology is based on (automated) gradient-HPLC, on-line connected to a newly developed P450-bioaffinity detection system. Interestingly, a novel P450-containing bioreactor unit could also be integrated on-line in the HRS-ER system. Several applications of this HRS-technology will be described.
Finally, the relevance of combining computational and bio-analytical technologies will be stressed for the prediction of drug binding and possible metabolism by Cyt P450s, e.g. for drug discovery and development purposes.

References:
1) Vermeulen NPE (2003): Prediction of drug metabolism: The case of Cytochrome P450 2D6. Curr.Topics Med. Chem. 3, 1227 – 39.
2) De Graaf C, Vermeulen NPE, Feenstra KA. Cytochrome P450 in silico: an integrative modeling approach. J Med Chem. 2005 ; 48: 2725-55. Review.
3) Kool J, van Liempd SM, Ramautar R, Schenk T, Meerman JHM, Irth H, Commandeur JNM, Vermeulen NPE: Development of a novel Cytochrome P450 bio-affinity detection system coupled online to gradient reversed-phase HPLC. J Biomol Screen. 2005; 10: 427-36.
4) Van Liempd SM, Kool J, Reinen J, Schenk T, Meerman JHN, Irth H, Vermeulen NPE.
Development and validation of a microsomal online Cytochrome P450 bioreactor coupled to solid-phase extraction and reversed-phase HPLC. J Chromatogr A. 2005; 1075: 205-12

PREDICTIVE VALUE OF IN VITRO ADME-TOX DURING DRUG DEVELOPMENT

Geny Groothuis, Department of Pharmacokinetics and Drug Delivery, University of Groningen

Pharmacokinetic, metabolic and toxicity (ADME-T) screening during drug development is performed in laboratory animals, generally in two species a rodent (usually rats) and a non-rodent. However it has become evident that large interspecies differences in expression and substrate specificity of drug transporters and enzymes involved in drug metabolism exist. Moreover interspecies differences in toxification and detoxification potential are considered responsible for species-specific toxicity. Thus, for the prediction of ADME-T in man, in vitro testing in human tissues has gained growing interest in the past decades. A large scala of in vitro techniques were developed using animal and human tissues each with their specific benefits and disadvantages. They are serving not only the need for methods to investigate human specific processes, but also to reduce, refine or replace experimental animal testing, and allow higher throughput testing of the tremendously increasing number of new chemical entities that are nowadays produced in drug discovery. Moreover these in vitro preparations of human origin may serve to select the animal species which resembles the human situation best with respect to ADME-T for the particular compound, and which can be used for further safety testing.

AMS: A STRATEGIC LEAP FORWARD IN HUMAN ADME

Graham Lappin, Xceleron Ltd, York Biocentre, Innovation Way, Heslington, York, YO10 5NY, United Kingdom

The conduct of all clinical trials are subject to strong ethical considerations and regulatory constraints. None more so than the human metabolism studies, which almost invariably involve the use of drugs with a radiotracer incorporated into the structure. The amounts of radioactivity that can be administered to human volunteers is strictly controlled and these restrictions place severe constraints upon the experimental design. In the 1990s a technology was introduced, new to the pharmaceuticals industry; Accelerator Mass Spectrometry (AMS). Not to be confused with conventional LC-MS, AMS measures atoms of rare isotope and can detect 14C to the equivalent of 10-3 disintegrations per minute (dpm). Human metabolism studies designed around the use of AMS can be considered non-radioactive, which then removes the regulatory constraints on study design.
Studies designed around AMS have been conducted in susceptible populations, including patients in hospitals and women of childbearing age. [14C]-Drugs with very long plasma half-lives (10-20 days) can be safely administered and more than one radiolabelled dose can be administered (ie cross-over designs). This has enabled first pass metabolism to be studied in humans, as well as novel designs for the determination of absolute bioavailability. Only very small sample sizes are required which opens the possibilities of biopsy samples. Furthermore, it is possible to combine first in man or early Phase I clinical trials with human ADME experiments, thereby obtaining in vivo human data at the earliest possible time in the development cycle.
AMS has also opened the way for a new type of study known as microdosing, where very small amounts of drug are administered to humans as a prelude to full Phase I clinical trials. Microdosing studies, or human Phase 0 studies, allow the acquisition of metabolism data as the pre-development stage.

UPLC/MS²: ADDED VALUE IN RANKING THE PERMEABILITY OF DRUG CANDIDATES THROUGH ARTIFICIAL MEMBRANES

Jurgen Mensch, Mark Noppe and Marcus Brewster, Pharmaceutical Sciences, Johnson & Johnson Pharmaceutical Research and Development, a division of Janssen Pharmaceutica N.V., Beerse, Belgium

Rapid screening for gastro-intestinal permeation in early drug discovery can provide important information applicable to compound selection as well as help in guiding the chemical synthesis for compounds containing desirable bioavailability properties. As a fast and cost efficient screening tool, the Parallel Artificial Membrane Permeability Assay (PAMPA) has been introduced at J&JPRD to address permeability potential. It measures permeability through an immobilised lipid membrane and can be automated by robotics, allowing many compounds to be processed in parallel with high efficiencies. With the rapid development of combinatorial chemistry and increasing amount of compounds with poor solubility properties, analysis of samples from PAMPA studies has become the 'bottleneck' of this drug screening process. High throughput quantification has become an essential aspect of the drug discovery process.
This lecture describes the high throughput determination of the permeability ranking of compounds as assessed by PAMPA using Acquity Ultra Performance LC/MS² technology with automated optimization. MRM-transitions of all compounds were first determined by Quanoptimize, a Masslynx algorithm that automatically optimizes cone voltage and collision energy while running a short isocratic method and injecting the compounds directly into the mass spectrometer. Secondly, MRM methods were automatically created and coupled to a generic, 96-well plate compatible, UPLC method for use in the following quantitation experiments. The generic UPLC/MS² method resulted in an analysis time of 1.5min/sample and was shown to be suitable for >97% of the tested J&J compounds. From a test set of 744 J&J compounds that were processed in the PAMPA system, 294 compounds (40%) were UV undetectable and couldn't be ranked. These UV-undetectable compounds were analysed using the Acquity UPLC/MS² method and 92.2% (271 compounds) could be ranked after the UPLC/MS² analysis. Using the Acquity UPLC technology combined with the automated MS² analysis, a 4-fold increase of throughput was obtained compared to the traditional LC/MS system as well as a significant increase in sensitivity. It was

shown that UPLC/MS² could be a useful tool for the ranking of drug permeability through artificial membranes. Because of the improved throughput and sensitivity, all PAMPA samples are now routinely analysed using the Acquity UPLC/MS² technology.

A TAILORED APPROACH FOR DISCOVERY AND EARLY DEVELOPMENT BIOANALYSIS: A SCIENTIFIC CHALLENGE FOR THE BIOANALYTICAL SCIENTIST

Philip Timmerman, PRD-PSO, Johnson & Johnson, Beerse Belgium

Over the last years, ever increasing emphasis was put on the bioanalytical scientist, especially in the discovery and early development area. However, the focus has changed. Where wrestling with the instruments was a major headache in the eighties and nineties, the maturity of LC-MS/MS has paved the way for easy to obtain quantification limits and excelent selectivity/specificity.
However, LC-MS/MS has only partially kept it's promise. Indeed, throughput has increased by trimming down the sample prep. to the bare minimum or speeding op LC, 96-well technology has enabled faster throughput of notorious labour intensive sample prep techniques like SPE and L/L.
As a consequence of these advances in technology, the bioanalist's profile changed as well. IT has taken over from wet chemistry. In some cases even, the perception that MS will solve the problems has taken over from the biochemical or physicochemical mindset.
This presentation will reflect on the pitfalls of inadequately balancing quality of bioanalysis and speed and will provide insight on how early development can be supported, resulting in both PK or PK/PD data "fit for decision making". A few case studies will be discussed.

Abstracts van Posters

In vitro metabolite profiling in samples from metabolic stability screens to support lead optimization.

Harrie Peters, Suzanne de Goeij, Mike Holkenborg and Jim Thio
Department of Pharmacology, NV Organon, PO Box 20, 5342 BH, Oss, the Netherlands.

The high throughput assessment of in-vitro metabolic stability using liver microsomes and hepatocytes nowadays is routine practice in lead optimization. Drug discovery compounds have to meet certain metabolic stability criteria before being promoted to early drug development candidates.
A major cause for rejection of oral drug candidates in early drug discovery is (too) high hepatic clearance. An early indication of metabolic labile sites in pharmacologically potent compounds can guide the synthesis process in drug optimization towards both stable and active compounds.
Ideally metabolite profiling with LC-MS(/MS) should follow immediately after detection of metabolic stability screen to save time. Preferably also the remainder of the in-vitro incubation samples should be used to avoid re-incubation at higher concentration.
The proposed LC-MS approach requires both sensitive and selective mass spectrometric scanning techniques to fingerprint metabolites in low concentration in microsomal and hepatocyte samples (<100 nmol/l). A panel of MS/MS transitions, corresponding with observed mass changes for a range of expected phase 1 metabolites, has been employed in our metabolite profiling experiments.
In combination with MS precursor ion scans of characteristic fragment ions of the parent compound at hand, both the metabolic sites and biotransformation can be pinpointed.
A range of marketed drugs with elucidated metabolism patterns was analyzed in our human and rat liver microsomal stability screens in order to illustrate the merits of this approach.

Symbiosis Pharma. Vial-to-File™ concept - automated sample preparation *with CFR21 Part 11 compliancy*

Gerard Haak, Spark System Solutions B.V., P.O. Box 388, 7800 AJ Emmen, The Netherlands

The efficiency of the workflow in today's bioanalytical operation is determined by the quality of each step in the process. Optimizing more than one step leads to an overall workflow improvement. Such optimizations are: cleaner extract, minimal sample manipulation, shorter LC-cycle times and faster data analysis. Nevertheless, we also have come to accept smaller or larger flaws in the process steps, mainly caused by operational deadlines. Analyzing 25.000 samples per MS per year should be feasible. Most labs don't even come close and therefore continue investing in additional LC-MS systems. But that generally moves the bottleneck to elsewhere in the workflow.
A new Vial-to-File™ concept is presented that optimizes all the steps in the lab process, both on a hardware and software level. Recently the last hinder in the process, the laborious sample transfer from the cryogenic vial to an autosampler compatible format, was removed.
The Vial-to-File™ process allows us to put pierce able cryogenic vials with raw plasma into the Symbiosis Pharma system and collect a data file containing all information in a 21CFR-11 compliant environment. Furthermore Vial-to-File™ will drastically improve the overall workflow, improve quality of analysis, reduce operational costs and increases sample throughput. In short: more studies can be completed in less time.

Sample processing and LC-MS/MS analysis of tedisamil plasma samples – a method comparison

B. Matthes[1], J. Dreymann[1], P. Struwe[1] and P.H. van Amsterdam[2]
[1] MDS Pharma Services Switzerland AG, 8320 Fehraltorf, Switzerland
[2] Solvay Pharmaceuticals, C.J. van Houtenlaan 36, 1381 CP Weesp, The Netherlands

Direct methods and extraction methods represent the most commonly used bioanalytical approaches for modern sample processing and quantitative drug analysis via LC-MS/MS.
For quantitative analysis of our example drug, the antiarrhythmic drug tedisamil, we validated both, a liquid-liquid extraction method and a direct method (protein precipitation). In any experiment, the resulting extracts were analysed using a 50mm RP column and a Sciex API 3000 MS instrument.
The extraction method, combined with a chemical internal standard, was expected to yield the purest extracts of the compound, represented a laborious method showing an average robustness in terms of performance paramaters and chromatography (k´>5). The protein precipitation, however, provided a very simple processing using a deuterated internal standard and a common C18 RP column for analysis. Both, sample processing and run time were thoroughly decreased enabling a bigger batch size without impact on chromatographic performance.
Our example shows, beyond giving evidence for progress in an in-house method development, that in presence of an deuterated internal standard, dilute-and-shoot can be the superior approach.

IMPROVED LC METHOD FOR THE DETERMINATION OF NA-VALPROATE IN BRAIN MICRODIALYSATES

Sophie Sarre, Carina De Rijck, Noud Grimberg en Yvette Michotte
Aurora Borealis Control, Caro van Eyckweg 9, 9408 CX Assen, The Netherlands

Na-valproate is a major anti-epileptic drug with a wide spectrum of anticonvulsant activity. For its clinical effects to occur, penetration into the central nervous system is required and the free drug concentration determines its effectiveness.

DETERMINATION OF P, CA AND MG IN FOOD AND FAECES FOR A MASS BALANCE STUDY USING MICROWAVE ASSISTED DIGESTION AND DETECTION BY INDUCTIVELY COUPLED PLASMA ATOMIC EMISSION SPECTROMETRY (ICP-AES) ELEMENTAL DETECTION.

Fred van Heuveln and Monique Veenstra
Xendo Drug Development Services, P.O. Box 137, 9700 AC, The Netherlands (www.xendo.nl)

Pharmaceutical products (phosphate binders) have been developed for the control of serum phosphorus in patients with chronic kidney disease (CKD) on hemodialysis. To measure the efficacy of a new phosphate binder, a mass balance study was set up, in which phosphorus (P), calcium (Ca) and magnesium (Mg) were measured in food and faeces.
The aim of this work was to investigate the use of microwave assisted digestion and ICP-AES as a method for the quantitation of P, Ca and Mg in food and faeces samples.
Validation procedures were conducted to determine the endogenous level variation, linearity and calibration curve, limits of quantitation, precision and accurcy and uniformity.
Microwave assisted digestion has been shown to be a fast, clean and reliable disclosure of elements from fibrous matrices such as food and faeces. ICP-AES has been proven to be a useful tool for the quantitative and qualitative determination of P, Ca and Mg in food and faeces with high precision and accuray.
With the validated method, several hundreds of food and faeces samples were homogenized and using microwave assisted digestion, P, Ca and Mg were determined in the clear solution using ICP-AES. The method was applied successfully in clinical studies with phosphate binders, in which the efficacy of the product was demontrated.

AN AUTOMATED LC/MS/MS PROTOCOL TO ENHANCE THROUGHPUT OF PHYSICO-CHEMICAL PROPERTY PROFILING IN DRUG DISCOVERY

Peter Alden, Darcy Shave, Kate Yu, Rob Plumb, and Warren Potts, Waters Corporation, Milford, MA

Introduction
Early screening of physicochemical properties ((PP) is an integral process for modern drug discovery. Typical PP profiling practice includes properties such as solubility, stability (pH and metabolic), permeability, integrity etc. The critical factor to consider in PP profiling is throughput. The bottlenecks that reduce throughput include MS method optimization for large variety of compounds and data management for large volume of data generated. We have developed an automatic LC/MS/MS protocol to not only allow automatic MS method development and data acquisition, but also allow data generated from mutilple tests to be processed by a single processing method, all in an automatic fashion. As a result, the physico-chemical profiling process was significantly simplified and throughput increased.

Methods
An HPLC system was used for separation. A generic HPLC method was used: columnwas a C18 reverse phase, 3.5 um, 2.1x50 mm. Mobile phases were ammonium acetate (pH 5.0) in Acetonitrile-Water, 10/90 for A, 95/5 for B. The flow rate was 1.0ml/minute with 0.4 mL/min. into MS via splitting. The total LC run time was 3.5 minutes. A tandem quadruple mass spectrometer was used for detection. Data acquisition was MRM. The Physico-chemical properties tested for each compound included solubility, pH stability, log P/log D, and microsome stability. The assay procedures were all based on methods previously published. A set of 48 commercially available compounds with wide chemical coverage were used in 96-well plate format for this study.

Preliminary results
After each assay was performed for the test compounds, the LC/MS/MS analysis was carried for each analyte. The LC/MS/MS protocol includes MS MRM parameter optimization, MS acquisition method creation, data acquisition, data processing and report generation. With the help of the software that was offered with the mass spectrometer, the whole analytical process was carried automatically, and the data generated from variety assays were all processed with the same software automatically. As a result, a single report was generated for the 48 test compounds with the results from all PP tests reported.
Examples of the results are: 1. Calibration dynamic range: Tolbutamide (0.1-10, 0.983), Protriptyline (0.1-10, 0.982), Norephedrine (0.1-10, 0.999), Doxylamine (0.1-10, 0.979), Sulconazole (0.1-10, 0.992), Alprenol (0.1-10, 0.983). 2.Solubility: 0.9 mg/mL for Tolbutamide, 1.2 mg/mL for Protriptyline, 0.8 mg/mL for Norephedrine, 1.1 mg/mL for Doxylamine, 1.9 mg/mL for Sulconazole and 1.8 mg/mL for Alprenol. 3. pH stability: samples at Time 0, Time 15 and Time 30 were measured. % Remaining is calculated by dividing the area at Time 30 by the area

at Time 0 and times 100. Half-life was derived from the plot of Time vs log(conc). Results are: at pH10, Tolbutamide (28.01% remaining, T1/2=18.04minutes), Protriptyline (51.85% remaining, T1/2=47.46minutes); at pH3, Norephedrine (73.87% remaining, T1/2=61.56minutes), Doxylamine (75.66% remaining, T1/2=79.44minutes); at pH7, Sulconazole (10.41% remaining, T1/2=6.50minutes), Alprenol (88.24% remaining, T1/2=641.03minutes). A complete report for all 48 analytes will be reported in detail on the poster.

AUTOMATED LC/MS/MS HIGH THROUGHPUT MULTI-MODE IONIZATION QUANTIFICATION PROTOCOL APPLIED FOR MICROSOME STABILITY TEST IN DRUG DISCOVERY AND DEVELOPMENT

Kate Yu1; Peter Alden1; Rob Plumb1; Li Di2; Susan Li2; Edward Kerns2; Paul Chilvers3; 1Waters Corporation, Milford, MA; 2Wyeth Research, Princeton, NJ; 3Waters Micromass , Manchester, UK;

Introduction
The application of LC/MS/MS technology in drug discovery demands throughput, sensitivity and wide coverage of diverse structures without compromising the data quality. The application of a combined ESI and APCI ionization source (ESCi) will increase the throughput and sample coverage by eliminating the need to physically change the ionization source and to repeat injections for failed samples.
Physical chemical information plays an important role in drug discovery. For example, the stability test screens compound stability in microsomes etc. providing important information about potential liabilities of drug candidates.We have developed an automatic method development protocol using the multi-modeionization coupled with UPLC-Tandem Mass Spectrometer, this protocol is applied to the routine microsome test.

Methods
The system was an UPLC-triple quadrupole MS. Microsome incubation was based on a published method. With the help of the MS software, the quantitative analysis was completely automated from the optimizations, to MRM method creation, to data acquisition and processing. Two strategies were used to ensure the quality of the analysis: First, this protocol was tested on 8 commercial compounds so that the instrument method was validated. Second, for the 96 well plate microsome stability test, a few standards with known metabolism were incorporated as QC checks. A 2.1x50 mm m particle size was used, the flow rate was 1.0 ml/minuteUPLC column with 1.7 L/mL flows into the mass spec via flow splitting.with about 400.

Preliminary results
Among the 8 test compounds, acetophenone can only be analyzed by APCI+, and Ibuprofen can only be analyzed by ESI-. Tolbutamide prefers ESI over APCI, and in APCI, it prefers positive over negative mode. For demonstration purpose, we have chosen the APCI- as the ionization mode for Tolbutamide to study the quantification limits. All other compounds showed preferences in ESI+ or APCI+.
The complete method evaluation example results are: Corticosterone (2-1000, r2 0.991, LOD 2.0 ng/mL), Daspone (0.1-1000, r2 0.996, LOD <<0.1 ng/mL), Hydroxyprogesterone (0.2-200, r2 0.990, LOD 0.2 ng/mL), Ibuprofen (0.1-500, r2 0.990, LOD 0.1 ng/mL), Sulfadimetnoxine (0.01-100, r2 0.995, LOD 0.01 ng/mL), Tolbutamide (0.1-1000, 0.991, LOD 0.1ng/mL). For the microsomal stability assay, samples at Time 0 and Time 15 were measured. % Remaining is calculated by divided the area at Time 15 over the area at Time 0 and times 100. From that, half-life is derived (2-3). As a part of the incubation routine, 3 additional drug standards with known microsomal activities were included in the assay for QC purpose. As previously measured by the HPLC/MS/MS, the ranges of percent remaining for these three compounds were 3-9% for Verapamil, 10-15% for Loperamide, and 44-59% for Zolpidem respectively. The example results of the ESCi UPLC/MS/MS microsomal stability assay are: Corticosterone(56.14% remaining, T1/2=18 years),Daspone(48.19% remaining, T1/2=14 years),17alpha-Hydroxyprogesterone (0.54% remaining, T1/2=2 years), Ibuprofen (74.73% remaining, T1/2>30 years),Loperamide (11.21%,T1/2=4 years), Sulfadimethoxine (87.65% remaining, T1/2>30),Tolbutamide(81.88% remaining, T1/2>30 years), Verapamil (3.79% remaining, T1/2=3 years), Zolpadem (47.12% remaining, T1/2=14 years). This protocol was then applied to 96 compounds in 96 well plate with detailed results to be reported on the poster.

FAST LC SEPARATION OF A PROTEIN DIGEST: A CASE STUDY USING A MONOLITHIC AND PARTICLE SILICA CAPILLARY COLUMN

J. Rozenbrand, W.P. van Bennekom, K.K. Unger, G.J. de Jong, Department of Biomedical Analysis, Faculty of Pharmaceutical Sciences, Utrecht University, Sorbonnelaan 16, 3584 CA Utrecht, The Netherlands; email: J.Rozenbrand@pharm.uu.nl

Monolithic columns, in which the chromatographic bed consists of a single piece of a rigid porous material, enhance mass transfer and permeability compared to particle columns. These highly permeable chromatographic columns enable high HPLC flow rates at low column back pressure without loss in column efficiency, resulting in fast analysis times.

The performance of a silica based capillary particle (160 x 0.1 mm i.d.) and monolithic (150 x 0.1 mm i.d.) column has been compared by using a nanoLC – MS system. As a model sample a myoglobin digest has been analyzed in a gradient system. In order to decrease the analysis time for this digest the flow rate could be increased up to 2.8 µl/min for the monolithic column. Almost all peaks could be separated at this relatively high flow rate. For the particle column it is not possible to work at this flow rate due to the high back pressure. The composition of the

gradient when starting the experiment has been varied. At optimal conditions a total analysis time less than 4 minutes has been achieved. Also the slope of the gradient has been varied. The peak capacity and resolution have been calculated. Finally the reproducibility of the retention time of both columns is tested. At a high flow rate the back pressure is larger causing in higher relative standard deviations. The interday reproducibility of the monolithic column is less than 0.9% for a flow rate of 1 µl/min.

Simultaneous Analysis of all Registered Anti-Hiv Drugs in Human Plasma by High Throughput HPLC-MSMS Analysis

Dick Schoutsen1), Hans Stieltjes1,2), Erno van Schaick 3), David Nauwelaers3) & Theo Noij1)
1) NOTOX BV, PO Box 3476, 5203 DL 's Hertogenbosch, The Netherlands; 2) Present affiliation: Johnson & Johnson, Pharmaceutical Research and Development, Turnhoutseweg 30, B-2340 Beerse, Belgium; 3) Virco BVBA, Gen. De Wittelaan L11 B4, B-2800 Mechelen, Belgium; correspondence: dick.schoutsen@notox.nl

AIDS and the associated infection with HIV (Human Immunodeficiency Virus) are considered to be the world's number one threat to human health. In the past 20 years much effort has been directed towards the development of new anti-HIV drugs and their effective administration to humans. Two key issues in the development of successful anti-HIV treatments are: 1) to optimize therapy in combination with other anti-HIV drugs and 2) to control the development of drug resistance.
In order to allow therapeutic drug monitoring (TDM) and the evaluation of drug resistance, an analytical method was required to determine 15 registered anti-HIV drugs. Although at first it was attempted to include all drugs in only one HPLC-MSMS method, during research it was decided to analyze them as two separate classes of compounds:
a. Non-nucleoside Reverse Transcriptase Inhibitors (NNRTI) and Protease Inhibitors (PI) (including one metabolite): 10 substances;
b. Nucleoside Reverse Transcriptase Inhibitors (NRTI): 6 substances.
Both methods were applied in combination with a third analytical method for the determination of a new anti-HIV drug in development; the focus of this paper however will be on the two multi-analyte methods.
In both methods SPE in a 96-well plate format is applied for sample concentration and sample clean up, followed by HPLC-MSMS analysis. The selected approach allows sample volumes of only 50-100 l plasma, while still maintaining an adequate sensitivity.
HPLC separation is established using a 50 mm x 2.1 mm C-18 column (dp = 3.5 m) under gradient elution conditions, enabling a high sample throughput with an HPLC cycle-time of 6.5 minutes. The HPLC conditions and the MSMS parameters are optimized in such a way that all 10 respectively 6 analytes are (mass) separated to allow a reliable analysis in human plasma.
Method validation, executed according to the FDA Guidance documents, revealed LOQ's in the range of 10-25 ng/ml for most substances. In addition to these characteristics the performance of the analytical method was also evaluated for accuracy, precision, sample stability, matrix effects, recoveries and response functions.
Apart from method development and validation, also the approach of quality assurance during sample analyses, i.c. QC evaluation, is discussed, as well as method evolution throughout a 24 months/15,000 samples research project.

Towards a paperless bioanalytical GLP lab; the implementation of a Scientific Datamanagement System

Jan Dankers, Jean-Paul Boon, Toos de Mooy, Jos vd Elshout & Henrieke de Bruin

In our bioanalytical lab we quantify candidate drugs and biomarkers in order to investigate the pharmacokinetics, pharmacodynamics and efficacy parameters of these compounds. All this work is performed in compliance with the OECD guidelines for GLP. During the actual analysis we generate electronic output from several data systems. The output from all of these information sources will feed a final report. As paper is the medium of choice for archiving procedures, all electronic output is printed on paper, dated and initialed. These papers will be archived together with all the other relevant study documentation for more than 15 years. Two years ago we decided to move from the 100% paper archive to an (partial) electronic solution by investing in a dedicated Scientific Data Management System. In this poster we will present the results of this process.

A new approach for the analysis of 16 Anti Epileptic Drugs (AEDs) in human plasma

Renata Kamsteeg, Toos de Mooy, Jos vd Elshout, Robert vd Wegen, Jean-Paul Boon & Jan Dankers

Decennia long epileptic patients are treated with the first generation Anti Epileptic Drugs. The second generation drugs are developed which are generally better tolerated, cause less sedation, and have fewer central nerve side effects. Lots of these drugs are still being used in combination therapy. It is therefore that in the research of new antiepileptics there is a need for reliable assays on almost all of these compounds. In order to serve the pharmaceutical industry for pharmacokinetic research and or therapeutic drug monitoring purposes we have developed and validated 3 methods for a combined, fast and reliable analysis of 16 antiepileptic drugs.

Bioanalytical Support for excretion studies in LO: a case study

Dillen, Lieve; Verhemeldonck, Marc; Roelant, Dirk; Timmerman, Philip
Johnson & Johnson Pharmaceutical Research and Development, Department of Bioanalysis, Beerse – BELGIUM

For one of our drug discovery projects, a lead compound was considered for further development. However, the compound showed low oral bioavailability in rat and dog (10-20%). Also rapid plasma clearance, while no extensive tissue distribution was observed. Bioanalytical studies revealed glucuronidation as one of the major metabolic pathways. Therefore, to understand the rapid clearance of the drug from plasma, an excretion study was started already in the lead optimization phase of the program. A strategy to support this excretion study, essential for quality results, will be presented. Stability aspects and quantitation of the glucuronide metabolite through enzymatic deconjugation to the unchanged drug will be adressed.
The results of the excretion study showed that phase II metabolism in conjunction with bile excretion are an important factor explaining the observed PK effects.

Challenges in determining the protein binding of two in all aspects very different analytes in one plasma sample: bifeprunox and valproate.

Monique Putman[1], Geertina Katuin[1], Lisbeth Hjort Christensen[2], Gregg Pratt[3]
1) Xendo Laboratories, Groningen, Netherlands
2) Lundbeck A/S, Copenhagen, Denmark
3) Solvay Pharmaceuticals Inc., Marietta, GA, USA

To determine the protein binding of drugs the bioanalist has two well established methods to choose from: ultrafiltration (UF) and equilibrium dialysis (EQD). The first being a relatively fast technique would be the preferred method of choice in a modern high throughput bioanalytical laboratory. Moreover as equilibrium dialysis is not only quite slow but also rather laborious and has a serious impact on the total sample load for the lab. Unfortunately the choice between the two methods is not free but governed by the chemical-physical properties of the analytes.
In the case study we tested both methods extensively for two molecules which had to be measured in one plasma sample; the limited total available sample volume put an extra challenge to our work. Bifeprunox is an apolar basic compound with a relative high protein binding and has plasma concentrations in lower and sub ng/ml range. Valproic acid is polar acidic compound showing a moderate protein binding and having plasma levels in the 10 – 100 µg/ml range. As we were faced with hundreds of samples to be analyzed, our goal was to set-up the fastest but accurate and precise method.
For both compounds we investigated:
- comparison of UF to EQD
- adsorption to materials
- influence of drug concentration
- reproducibility
- influence of freeze-thaw cycles
- time to reach equilibrium in the dialysis set-up

Further extra efforts were needed to be able to reliably measure low pg/ml bifeprunox concentrations in protein free plasma. The poster presents an explanation and the pro's and con's of the techniques, the experiments performed, their outcome and the validated quantification methods used.

Trypsin immobilised in dextran-modified fused silica capillaries for on-line peptide mapping

E. C. A. Stigter, C. Steenbeek, G. J. de Jong and W. P. van Bennekom
Department of Biomedical Analysis, Faculty of Pharmaceutical Sciences, Utrecht University, Sorbonnelaan 16, 3584 CA Utrecht, The Netherlands; email : E.C.A.Stigter@pharm.uu.nl

A study has been started to investigate the use of immobilised bovine pancreas trypsin for on-line peptide mapping. For this reason the enzyme was covalently immobilised in dextran- modified fused silica capillaries with internal diameters of 50 and 75 µm. By introducing the dextran layer into the capillary, the surface density of the immobilised enzyme increased from 2.9 ng mm-2 (when the enzyme was directly coupled to the capillary wall) to 11.0 ng mm-2 (when the enzyme was coupled to the dextran). The enzyme activity was tested with the model substrate N-Benzoyl-L-Arginine Ethyl Ester (BAEE) and the apparent activity of the immobilised enzyme proved to be flow-dependent. The immobilised enzyme retained its full activity during two weeks in operation.
On-line protein digestion with nano-LC separation was conducted with horse cytochrome C. Flow-dependent protein digestion was observed with an increase in the number of fragments for a decrease in flow. A sample of 10 pmol non-denaturated Cytochrome C was digested using a 2.75 µl microreactor of 50 µm i.d. operated at a flow of 1 µl min-1. Peptide fragments due to autolysis of the immobilised trypsin were not observed.
On-line peptide mapping using the described reactors is competitive as for the time necessary for digestion and the number of fragments formed compared to off-line digestion methods. The reactors can be produced easily and reproducibly and are attractive for coupling with nano-LC for on-line peptide mapping.

Locatie / reizen / overnachten

Lokatie: Auditorium van NV Organon, graag melden bij de receptie, Molenstraat 110, Oss

Routebeschrijving - Organon Oss

Vanuit richting Utrecht / Eindhoven / ‘s-Hertogenbosch:
Volg de A2 (E25) richting ‘s-Hertogenbosch tot het knooppunt Hintham
Neem de afslag A59 - Oss/Nijmegen
Volg de A59 tot de afslag Oss/Heesch
Einde afslag rechtsaf (Cereslaan)
Ga bij de stoplichten rechtsaf (Ruwaardsingel)
Ga bij de 2e stoplichten (u ziet een Shell benzinepomp aan uw linker hand) linksaf de Nieuwe Hescheweg in
(1) Bij het 3e stoplicht ziet u de Organon-hoofdingang (1) aan de rechterkant. Ga hier naar rec:hts en meteen weer naar rechts om bij de hoofdingang van Organon te komen.
(3) Bij het 3e stoplicht ziet u het AP-gebouw aan de linkerkant (3). Ga naar Links en meteen naar rechts. Voor en achter het AP-gebouw is voor gasten mogelijkheid tot
parkeren.
Zie voor verdere informatie het plattegrondje

Vanuit richting Arnhem / Nijmegen:
Volg de A59 richting s-Hertogenbosch
Neem de afslag “Oss-Oost”
Einde van de afslag rechtsaf (Graafsebaan -N329)
Ga bij de stoplichten linksaf de Julianasingel in
Ga bij de stoplichten rechtsaf de Nieuwe Hescheweg in
(1) Bij het 3e stopticht ziet u de Organon-hoofdingang (1) aan de rechterkant. Ga hier naar rechts en meteen weer naar rechts om bij de hoofdingang van Organon te komen.
(3) Bij het 3e stoplicht ziet u het AP-gebouw aan de linkerkant (3). Ga naar links en meteen naar rechts. Voor en achter het gebouw is voor gasten mogelijkheid tot parkeren.
Zie voor verdere informatie het plattegrondje

N.V. Organon
Molenstraat 110
Postbus 20
5340 BH Oss
Tel. (0412) 661222
Telefax (0412) 662617 en
662156

Route description by car - Organon Oss

From direction Utrecht / Eindhoven / 's-Hertogenbosch
Follow the A2 (E25) direction 's-Hertogenbosch until junction Hintham
Take the exit A59 - Oss/Nijmegen
Follow the A59 tilt exit Oss
End of exit turn right (Cereslaan)
Turn right at the lights (Ruwaardsingel)
Go straight on till the second lights (there is a Shell service station on your left) and turn left (Nieuwe Hescheweg)
(1) At the third traffic light you will see the Organon main entrance (1) on your right. Take two consecutive right turns to reach the Organon main entrance.
(3) At the third traffic light you will see the AP-building (3) on your left. Turn left and then right. In front of and behind the AP-building is a parking lot for guests.
For further information see map

From direction Arnhem / Nijmegen
Folow the A59 direction s-Hertogenbosch
Take the exit 'Oss-oost'
At the end of the exit turn right (Graafsebaan - N329)
Turn lft at the Lights (Julianasingel)
Turn right at the next lights (Nieuwe Hescheweg)
(1) At the third traffic lght you will see the Organon main entrance (1) on your right. Take two consecutive right turns to reach the Organon main entrance.
(3) At the third traffic tight you wiLL see the AP-buitding (3) on your left. Turn left and then right. In front of and behind the AP-building is a parking lot for' guests.
For further information see map on previous page

N.V.Organon
Molenstraat 110
P0 Box 20
5340 BH Oss
Tel. +31 (412)661222
Telefax +31 (412) 662617 and 662156

Oss Hotels

City Hotel
Raadhuislaan 43
5341 GL Oss
Tel. 00 31 (0)412 633375
(City Centre)

Hotel de Weverij
Oostwal 175
5341 KM Oss
Tel. 00 31 (0)412 694646
Fax 00 31 (0)412 694647
(City Centre)

Best Western Hotel De Naaldhof
Docfalaan 22
5342 LD Oss
Tel. 0412 662129
(Not in the City Centre)