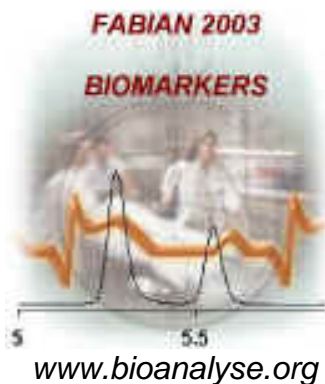


# FABIAN SYMPOSIUM, 12 NOVEMBER 2003, GRONINGEN

## THEMABIJEENKOMST "BIOMARKERS"

**Locatie: Rijksuniversiteit Groningen**



## Programma

- 09.30 Ontvangst, inschrijven, koffie
- 10.00 Opening door gastheer Prof. Rainer Bischoff, Universiteit van Groningen
- 10.10 Combilezing door Pfizer, Sandwich, UK:
  - David Muirhead: Biomarkers in exploratory clinical development, a Pfizer perspective
  - Ian James: Changes in collagen markers during scar formation and matrix remodelling
- 11.25 Ido Kema (AZ Groningen): Markers voor diagnose en follow up van carcinoid patienten
- 11.55 Uschi Rose (Organon, Oss): Het gebruik van cellulaire assays tijdens de klinische ontwikkeling van nieuwe drugs.
- 12.25 Lunch, posters
- 14.00 Ronald de Vries (Johnson & Johnson, Beerse, België): Intracerebrale kinetiek en dynamiek van galantamine (met acetylcholine als biomarker) in relatie tot de plasma kinetiek: een microdialyse studie
- 14.30 Filip De Keyser (UZ Gent, België): Nieuwe biomarkers in reumatoïde artritis
- 15.00 Marleen Simmelink (U-cytech, Utrecht): Cytokine ELISPOT analyses as a readout for T cell responses in vivo (vervangt Peter van der Meide als spreker)
- 15.30 Jos Thijssen (UU): Activity and reactivity: problems related to standardisation of assays
- 16.00 Sluiting & Borrel

### Bijlagen

- abstracts van lezingen
- abstracts van posters
- overzicht bereikbaarheid

## Abstracts van Lezingen

### BIOMARKERS IN EXPLORATORY CLINICAL DEVELOPMENT, A PFIZER PERSPECTIVE

**Dr David Muirhead, Clinical Assay Group, Pfizer Global Research and Development, Sandwich Laboratories, Kent CT13 9NJ**

In recent years large Pharma have dramatically increased their focus on biomarkers in an attempt to reduce drug development times and costs. This has presented the industry and its providers with many issues since there is no established approach or regulatory guidance relating to the implementation of biomarker data or the validation and operation of biomarker assays. This presentation is an overview of how Pfizer is approaching many of these issues and why we see it as essential to use biomarkers in our drug development programs. Consideration will be given to matters such as the classification and diversity of biomarkers, validation of biomarker assays, selection and identification of biomarkers and the impact of biomarkers on drug development.

### CHANGES IN COLLAGEN MARKERS DURING SCAR FORMATION AND MATRIX REMODELLING

**Dr Ian James, Clinical Assay Group, Pfizer Global Research and Development, Sandwich Laboratories, Kent CT13 9NJ**

#### ABSTRACT

The development of anti scarring agents has been hampered by the paucity of objective methodologies available to demonstrate efficacy. In an attempt to identify potential staging markers of effective healing biochemical changes in connective tissue properties and histological endpoints were measured in a human skin excisional wound healing model in which full-thickness, punch biopsies were re-excised at intervals up to 6 months after injury. The aim was to develop and evaluate these parameters as potential markers to delineate the stage of wound healing which could subsequently be used to monitor therapeutic interventions.

In addition to measuring collagen content, extractability by pepsin and the proportions of collagen III relative to collagen I, the concentrations of mature collagen cross-links were determined by HPLC, together with the elastin-derived, desmosine cross-links. Additional endpoints relating to collagen orientation and procollagen C-proteinase activity were assessed using histological sections. The results indicated, somewhat surprisingly, that none of the parameters measured was normalised within the 6-month follow up period, suggesting that disturbance of connective tissue metabolism following this type of skin wound lasts much longer than has previously been reported.

### PROFILING OF TRYPTOPHAN-RELATED PLASMA INDOLES IN CARCINOID PATIENTS BY AUTOMATED, ON-LINE, SOLID-PHASE EXTRACTION AND HPLC WITH FLUORESCENCE DETECTION

**Ido P. Kema<sup>1</sup>, Wim G. Meijer<sup>2</sup>, Gert Meiborg<sup>1</sup>, Bert Ooms<sup>3</sup>, Pax H.B. Willemse<sup>2</sup>, Elisabeth G.E. de Vries<sup>2</sup> Departments of Pathology and Laboratory Medicine<sup>1</sup>, and Medical Oncology<sup>2</sup>, University Hospital Groningen, and Spark Holland B.V.<sup>3</sup>, Emmen, the Netherlands.**

**BACKGROUND:** Profiling of the plasma indoles tryptophan (TRP), 5-hydroxytryptophan (5-HTP), serotonin and 5-hydroxyindoleacetic acid (5-HIAA) is useful in the diagnosis and follow-up of patients with carcinoid tumors. We describe an automated method for the profiling of these indoles in protein-containing matrices, as well as the plasma indole concentrations in healthy controls and patients with carcinoid tumors.

**METHODS:** Plasma, cerebrospinal fluid and tissue homogenates were prepurified by automated on-line solid-phase extraction (SPE) on Hysphere Resin SH SPE cartridges containing strong hydrophobic polystyrene resin. Analytes were eluted from the SPE cartridge by column-switching. Subsequent separation and detection were performed by reversed phase HPLC combined with fluorimetric detection in a total cycle time of 20 minutes. We obtained samples from 14 healthy controls and 17 patients with metastasized midgut carcinoid tumors for plasma indole analysis. In the patient group, urinary excretion of 5-HIAA and serotonin was compared with concentrations of plasma indoles.

**RESULTS:** Within and between series coefficients of variation for indoles in platelet-rich plasma were 0.6-6.2% and 3.7-12%, respectively. Results for platelet-rich plasma serotonin compared favorably with those by single component analysis. Plasma 5-HIAA, but not 5-HTP was detectable in 8 of 17 carcinoid patients. In the patient group platelet-rich plasma total tryptophan correlated negatively with platelet-rich plasma serotonin ( $p = 0.021$ ,  $r = -0.56$ ), urinary 5-HIAA ( $p = 0.003$ ,  $r = -0.68$ ) and urinary serotonin ( $p < 0.0001$ ,  $r = -0.80$ ).

**CONCLUSIONS:** The present chromatographic approach reduces analytical variation and time needed for analysis and gives more detailed information about metabolic deviations in indole metabolism than do manual single component analyses.

**Ursula M. Rose, Rick Brouwer and Frans Maris, N.V. Organon, Dept. of Drug Metabolism & Kinetics, P.O. Box 20, 5340 BH Oss, The Netherlands.**

Immunological and chromatographical bioanalytical methods are widely used for the pharmacokinetic evaluation of drugs and their metabolites in human serum or plasma during drug administration. Although these methods are reliable and can be performed under GLP regulations, their results can not always give answers to the questions one has. In the field of male Hormone Replacement Therapy or male Contraception, for instance, men are treated with androgens. These compounds exert a physiological effect through their biological activity and their interference with the endogenous androgen-estrogen balance. To get a clear insight in the androgenic and estrogenic effects of such a drug, not only compound levels have to be measured, but also metabolite levels and endogenous steroid levels. Evaluating the overall effect on the circulating androgen and estrogen milieu is difficult on the basis of biochemical measures. Such biochemical methods measure one androgen, i.e. the drug, and not the total of all androgenic factors, i.e. the drug, its metabolites and endogenous androgens. It would therefore be an improvement to have a method that measures total plasma androgenic bioactivity as a pharmacodynamic parameter. The same holds for the estrogenic activity. To this end, mammalian cell bioassays were developed that can measure androgen or estrogen bioactivity directly from a small amount (5 µl) of human serum. Chinese Hamster Ovary cells (CHO cells) were stably transfected with either the androgen (CHO-hAR) or the estrogen (CHO-hER) receptor, and with the luciferase reporter gene. In this assay, luciferase activity in cell lysates is a measure for the androgen or estrogen bioactivity in human serum. The maximal luciferase inductions reached were on average 10 and 20-fold for the hAR and hER, respectively. In the CHO-hAR assay, testosterone levels ranging from 12.5 to 360 nM could be measured in 2% human serum. The intra- and interassay coefficients of variation were <10% and 20%, respectively. In the CHO-hER assay, estradiol levels ranging from 0.0625 to 80 nM could be measured in 10% extracted human serum, and serum estrogen bioactivity correlated strongly with serum estradiol concentrations determined by autoDelfia method ( $r=0.874$ ,  $n=23$ ). The intra- and interassay coefficients of variation were <10% and <20%, respectively.

In conclusion, CHO-hAR and CHO-hER bioassays were developed that enable measurement of mammalian cells response to bioactive androgens and estrogens in circulation and provide a novel means to investigate patients receiving drugs acting through the AR or ER.

**Ronald de Vries, Johnson & Johnson Pharmaceutical Research and Development, Department of Bioanalysis, Turnhoutseweg 30, B-2340 Beerse, Belgium**

The decline in the function of the cholinergic cells is supposed to be responsible for the cognitive impairments in the early phase of Alzheimers disease. This has led to the development of drugs like Galantamine that selectively enhances cholinergic function by inhibiting acetylcholinesterase (AChE). As it is of interest to relate the plasma kinetics of Galantamine with its local kinetics and dynamics in the brain, a microdialysis study in the rat was initiated. In this talk, a short description of the mechanism of action of Galantamine will be given. Thereafter, the experimental in-life procedure of the microdialysis study will be discussed, followed by a discussion on the analytical aspects of the determination of the PK parameter (Galantamine) and the PD parameter (Acetylcholine). Finally, the PK and PD results of the study will be shown and discussed.

**Prof Filip De Keyser, Afdeling Reumatologie, UZ Gent**

Biomerkers zijn biologische parameters, geassocieerd met diagnose en klinisch verloop van een aandoening. Op die manier zijn ze vaak een onmisbare hulp in het management van patiënten die aan de ziekte lijden. Dit geldt zeker binnen het vakgebied van chronische artritis. Gezien de initiële presentatie vaak atypisch is, en gezien heel wat klinische manifestaties kunnen overlappen tussen verschillende reumatische ziekte entiteiten, zijn biomerkers vaak doorslaggevend in de diagnose en de daaropvolgende therapeutische beslissing. De indrukwekkende evoluties inzake behandelingsopties, zoals deze zich hebben voorgedaan in de voorbije jaren, en de ontwikkelingen op dat vlak die ook in de komende jaren nog te voorzien zijn, hebben de interesse voor biomerkers heel sterk doen toenemen. Niet alleen zijn biomerkers van belang bij een diagnosestelling, voornamelijk in de vroege fase van de aandoening. Ze kunnen ook van bijzondere betekenis zijn bij de identificatie van subtypes van een aandoening waarvoor bijzondere therapeutische strategieën kunnen gelden; ze helpen beslissen of een therapie voldoende efficiënt is om te worden verder gezet en worden meer en meer ingebracht in de design van klinische studies. Dit laatste aspect is van uitermate groot belang voor de verdere ontwikkeling van het vakgebied.

De klassieke reumafactor is sedert meer dan een halve eeuw in gebruik. De predictieve waarde voor de diagnose reumatoïde artritis (RA) is eigenlijk beperkt, en dit vooral als gevolg van de beperkte specificiteit. Enkel hoge concentraties reumafactor hebben een specifieke associatie met RA.

Recent werd een groep antistoffen geïdentificeerd met interessantere diagnostische associatie. Het gaat om antistoffen tegenover gecitrullineerde eiwitten, verder kortweg 'anticitrulline' antistoffen genaamd. De historie van deze recent geïdentificeerde antilichamen begint bij de beschrijving van de antiperinucleaire factor-test (APF). Deze test detecteert antistoffen in het serum van patiënten met RA, gericht tegenover perinucleaire keratohyaline granules in humane mond mucosacellen. De aard van het antigeen in deze perinucleaire granules was lang onbekend. Het bleek uiteindelijk te gaan om een filaggrine eiwit, dat ook in de huid epidermis voorkomt. Verdere epitop-mapping van dit eiwit bracht aan het licht dat gewijzigde arginine residu's (citrulline) cruciale elementen waren in de reactiviteit van RA serum. Citrulline ontstaat als gevolg van een modificatie van arginine, via inwerking van het enzym deïminase. Sedert de identificatie van citrulline als centraal residu in de epitop herkend door deze RA geassocieerde antistoffen, zijn ook synthetische peptiden met dit motief beschikbaar gemaakt. Een van deze peptiden is het zogenaamde CCP (cyclisch gecitrullineerd peptide), waartegen de reactiviteit kan worden getest in een ELISA formaat.

Het is moeilijk op vandaag een precieze appreciatie te geven van deze nieuwe groep van antistoffen, zij het dat het buiten kijf staat dat ze specifiek en sensitiever zijn voor de diagnose RA dan de klassieke RF. De reden waarom finale validatie nog moeilijk ligt, is het feit dat vooral synthetische substraten voor anticitrulline antistoffen nog steeds evolueren, en dat gepubliceerde evidentie met de verschillende substraten nog beperkt is. Op basis van reeds beschikbare gegevens kan men toch stellen dat op een specificiteitsniveau van 98%, de test een sensitiviteit behaalt van minstens 50 tot 60%. Ter vergelijking: indien men voor de reumafactor een cut-off definieert die overeenkomt met een dergelijk specificiteitsniveau van 98%, dan bedraagt de overeenkomstige sensitiviteit nauwelijks 20%.

Artritis speelt zich in eerste instantie af ter hoogte van de synoviale membraan. En nochtans heeft het bijzonder lang geduurd vooraleer de reumatoloog de weg vond naar deze ontstoken membraan. Reden was vooral de moeilijke bereikbaarheid van dit weefsel, gezien het gewricht een gesloten ruimte is. De ontwikkeling van de reumatologische naaldarthroscopie heeft daarin verandering gebracht. Via lokale anesthesie en een minimale ingangspoort brengt de reumatoloog de scoop en de bioptietang in, en worden 10 tot 20 biopties afgenomen. De macroscopische evaluatie van de synoviale membraan geeft vooral een beeld van de vascularisatie. De bioptie en het pathologisch onderzoek dat daarop volgt, levert waardevolle informatie. Klassieke histologische parameters zoals de dikte van de synoviale lining-laag, de graad van vascularisatie en inflammatoire infiltratie kunnen reeds belangrijke aanwijzingen geven naar het type artritis. Meer ziekte-specifieke parameters kunnen worden opgezocht via immunohistochemie. We geven daarbij bijzondere aandacht aan intracellulaire gecitrullineerde eiwitten en aan het HLA-gp39 complex.

Tabel 1

Waarde van biomerkers voor diagnose, patiënten management en design van klinische studies

- Diagnosestelling in een vroege fase, waardoor vroege therapie mogelijk wordt
- Identificeren van specifieke subtypes van de aandoening, waarvoor specifieke therapeutische strategieën kunnen gelden
- Meten van de effectiviteit van een therapie, waardoor inefficiënte therapieën snel kunnen worden stopgezet
- Voorspellen van ongewenste nevenwerkingen
- Biomerkers die correleren met de klinische outcome of die een lange termijn outcome voorspellen, kunnen de design van klinische trial grondig reduceren (kleinere trials van kortere duur)

Tabel 2

Synoviale kenmerken in reumatoïde artritis versus spondylartropathie

Dikte synoviale lining-laag	RA>SpA
vascularisatie	SpA>RA
Neutrofielen infiltratie	SpA>RA

**Marleen Simmelink<sup>1</sup>, Peter van der Meide<sup>1</sup>, Erik Wischerhoff<sup>2</sup>, Wout van Bennekom<sup>2</sup>,**  
<sup>1</sup>*U-CyTech Biosciences, Bolognalaan 50, 3584 CJ Utrecht;* <sup>2</sup>*Dept. Biomedical Analysis, Faculty of Pharmaceutical Sciences, Utrecht University, Sorbonnelaan 16, 3584 CA Utrecht*

The enumeration of antigen specific T cells in the circulation of man is important for the evaluation of ongoing immune responses to and vaccination against a variety of infectious diseases, including HIV and malaria, in which T cells are an important component of protective immunity. T-helper (Th) cells, a notable T cell subpopulation, are involved in cellular (Th1) or humoral (Th2) immune responses. Stimulation of Th cells will result in secretion of different cytokines. Depending on the amount and type of secreted cytokine, a cellular or humoral immune response develops.

The Enzyme-Linked ImmunoSpot (ELISPOT) assay is a capture immunoassay, which is designed to enumerate cytokine secreting cells in single cell suspensions of lymphoid tissue, CNS tissue, bone marrow or preparations of peripheral blood mononuclear cells (PBMC). It is a highly sensitive technique detecting cytokine release at the single cell level, allowing direct determination of T cell frequencies. The high sensitivity and easy performance, makes the ELISPOT assay eminently well suited to monitor T cell responses.

As a first step, a cytokine-specific monoclonal antibody is immobilized on the surface of a 96-well polystyrene microtiter plate. The cells to be investigated are cultured in the wells and left to incubate for sufficient time to allow cytokine production. The secreted cytokine will bind in direct vicinity of the producing cells and, after removal of the cells by washing, detection antibodies reactive with the cytokine are added. These antibodies may be conjugated with an enzyme or fluorescent label. By employing a precipitating substrate for the enzyme, a spot is formed at the site where the producing cell was located. Spots can be visualized directly when a fluorescent label is used.

In the present study we have developed a dual color ELISPOT assay for the simultaneous detection of distinct types of cytokine-secreting cells. Th cells, which are classified as Th1 or Th2 are visualized as different coloured spots corresponding to respective types of cytokines.

#### ACTIVITY AND REACTIVITY: PROBLEMS RELATED TO STANDARDISATION OF ASSAYS.

**Jos H.H. Thijssen, Endocrinologie, Universitair Medisch Centrum Utrecht, KE 03.139.2, Lundlaan 6, Postbus 85090, 3508 AB Utrecht, J.Thijssen@AZU.NL**

In medicine measurements of compounds in biological fluids, like blood and urine, are being used to improve diagnostic approaches in patients but in practise a large proportion of the measurements is done in the follow-up of treatment. In particular for the determination of relatively complex glycoproteins immunoassays are being used. These assays are based on the reactivity of (small) parts of the molecule under consideration by specific antibodies. As a consequence differences in behaviour of the measurands in non-identical analytical systems have been observed.

Recently, the European Union (EU) has issued a directive on "Traceability of Standards to Higher Order Reference Materials" that requires standardisation of commercial reagents used for "in-vitro diagnostic reagents" towards a "higher order" reference preparation. The "International Federation for Clinical Chemistry and Laboratory Medicine, IFCC" has started a close cooperation with the "Bureau International des Poids et Mesures, BIPM" in order to try to develop guidelines in the very complex issue of standardisation of substances of biological origin.

Some examples of the background:

In endocrinology, measurement of the glycoprotein "Thyroid Stimulating Hormone, TSH" is a very important parameter to judge inappropriate functioning of the thyroid gland and to evaluate the success of the medical treatment in patients with pathological changes in thyroid function. In the physiological feed-back system between pituitary and thyroid, levels of TSH are very sensitive towards slight changes induced during treatment, basically reflecting the biological responses to non-optimal levels of the thyroid hormones thyroxine and/or triiodothyronin in blood of this individual. Recent discoveries point to the fact that the glycosylation of TSH shows specific variations, depending on the way the thyroid gland is functioning, also during treatment of a disorder. These specific glycoforms do not react in an identical way with all of the immunological reagents in clinical use. With support of the EU, a cooperating group in Europe is trying to improve the current situation.

Glycosylation also seems to play a role for "human Chorionic Gonadotrophin (hCG)", the so-called pregnancy hormone. In the early phase of pregnancy specific hyperglycosylated forms of hCG are present that are not always very reactive with the reagents that are being used to detect pregnancy. Determinations of hCG play an important role in the management of patients with specific tumours, they receive chemotherapy on basis of the detectability of hCG in their bodies. In this situation detection of all forms of the molecule is of utmost importance. These problems can be studied better on basis of very new preparations of six different hCG-related proteins, that have been prepared by IFCC and have been accepted by the World Health Organisation as "Reference Reagents".

The determination of the "Prostate Specific Antigen, PSA" shows different problems, caused by the fact that PSA is circulating in the body in different forms, because of its strong binding to several proteins. The sizes of these binding proteins are largely different causing changes in the reactivity of the PSA-molecule in the assays used. Because PSA is a sensitive marker for recurrence of tumours of the prostate, its use for that purpose requires knowledge on the forms present in the body.

## Abstracts van Posters

(overzicht niet compleet; niet alle abstracts tijdig ontvangen)

### ANALYSE VAN ACETYLCHOLINE IN MICRODIALYSAAT, EEN VERGELIJK TUSSEN LC-ECD EN LC-MS-MS.

**Pieter A. Spaans, Biological Lead Optimization, WWA-A020, Solvay Pharmaceuticals, Weesp, Pieter.Spaans@Solvay.com**

Bij Solvay Pharmaceuticals worden farmaca gemaakt die actief zijn binnen het centrale zenuwstelsel. Microdialyse is een techniek om effecten van farmaca op neurotransmitter-niveaus in de hersenen te volgen. Voor de bepaling van de neurotransmitter acetylcholine wordt standaard gebruik gemaakt van een LC-ECD methode. Detectie van acetylcholine vindt plaats, na post-column enzymatische omzetting van acetylcholine naar H<sub>2</sub>O<sub>2</sub>, op een platina electrode. De zwakke schakel binnen de LC-ECD methode is de kolom, die niet bestand is tegen de combinatie fosfaatbuffer (pH8.4) en 100% waterige mobiele fase. Als alternatief is een LC-MS-MS methode ontwikkeld. Het vergelijking tussen de LC-ECD en LC-MS-MS methode wordt op de poster gepresenteert.

### A NEW CONCEPT FOR SAMPLE INTRODUCTION IN LC-MS

**Martijn Hilhorst<sup>1</sup>, Jaap Wieling<sup>2</sup> and Bert Ooms<sup>1</sup>**

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2) Xendo Laboratories, L.J. Zielstraweg 1, 9713 GX Groningen, The Netherlands, Email: jaap.wieling@xendo.nl**

Reducing the sample amount required for an analysis is an increasingly important aspect in most bio-analytical laboratories. Using less sample volume makes it possible to measure entire PK curves using a single lab-animal thereby improving the reliability of the data. Moreover, the number of lab-animals can be reduced. As state-of-the-art MS instrumentation is becoming more sensitive, smaller sample amounts are feasible. However, traditional autosamplers require relatively large sample volumes in order to inject samples quantitatively due to sample-loss. Traditional injection techniques requires injection of sample out of sample vial by means of a needle.

Consequences of this concept are the requirement of a preflush with sample before and a needle wash after injection. These steps are time consuming, cost sample, and involve the risk of carry-over.

A new injection concept is developed to overcome these problems. The sample is injected manually or by a pipetting robot directly onto a on-line solid-phase-extraction like cartridge that contains a sorbent that absorbs the sample but has no retention capabilities. Introduction in the LC system is achieved by simply placing the cartridge in the eluent flow-path. Or, if necessary, eluted to an on-line SPE LC-MS system. A system (Prospekt) that is normally used for on-line SPE extraction is ideally suited for this purpose. In the influence of geometry, type of sorbent, and elution conditions on recovery, carry-over, peak broadening and ease of use, was investigated. In collaboration with Xendo Laboratories and the University Hospital of Groningen, the applicability of sorbent sampling was studied by the determination of a PK curve of carbamazepine in rat plasma using on-line SPE-LC-MS-MS. The curves were compared with curves obtained by traditional autosamplers. Good correlation was found between the two injection techniques, indicating that sorbent sampling is a valuable alternative conventional techniques. Moreover, sorbent sampling enables the analyses of whole blood because no needle wash is required. Ongoing research in this area is performed to meet this challenging task.

### FEASIBILITY OF EMPLOYMENT OF <sup>13</sup>C-LABELS FOR MAGNETIC RESONANCE IMAGING, PET VERSUS MRI

**Geke Bosselaar, Tony Cijssouw, Paul ter Horst, Wouter Kuit, Wageningen University, The Netherlands**

A feasibility study was done on the use of <sup>13</sup>C-Magnetic Resonance Imaging (<sup>13</sup>C-MRI) in the process of drug discovery to specify the location of a <sup>13</sup>C-labelled drug in the brain. At this moment Positron Emission Tomography (PET) is used to test if the drugs reach their target. Due to the high costs of PET a demand for an alternative arose. Direct measurement of <sup>13</sup>C is not possible because of the low sensitivity of the <sup>13</sup>C nucleus. There are different techniques like Twin Spin Echo Double Resonance (T-SEDOR) and Cyclic J Cross Polarisation (CYCLCROP) in which a coupling is performed between the <sup>13</sup>C and <sup>1</sup>H spins (<sup>1</sup>H-<sup>13</sup>C) leading to a detection of the <sup>13</sup>C nucleus with the sensitivity of the <sup>1</sup>H nucleus. A number of calculations were done leading to the conclusion that even with these coupling techniques it is not feasible to detect the required concentration of 0.5 mM <sup>13</sup>C with a resolution of 1 mm<sup>3</sup>. Lately a new technique has arisen, Dynamic Nuclear Polarisation (DNP), which can increase the sensitivity of MRI. DNP has been used to enhance contrast between different tissues, but it might be possible to detect low concentrations of certain <sup>13</sup>C labelled compounds, however this technique is still under development for in vivo application. We finally concluded that <sup>1</sup>H-<sup>13</sup>C-MRI is not feasible at the moment to specify the location of drugs in the brain. Therefore we recommend continuing to use PET and keeping in touch with the developments of DNP and other developments in the MRI field.

**Markus Kostrzewa, Bruker Daltonik GMBH, Bremen (D)**

Investigation of differences in the protein composition of cells and tissues, so called Expression Proteomics, has become one of the most important scientific fields in the recent years. Powerful but elaborate technologies like 2-D gel electrophoresis or multidimensional HPLC are used to separate proteins and identify differences in samples by mass spectrometry, i.e. to identify proteins or protein modifications involved in disease progression or developmental processes.

More recently, a novel discipline arose which is using proteomic technologies. Goal is the identification of biomarker signatures with a prognostic or diagnostic value in clinical studies. Peptides and small proteins from crude samples, body fluids or cell culture supernatants are captured through specific surface functionalities, washed to get rid of interfering contaminants and analysed by MALDI-TOF mass spectrometry. The measurement results in a multi-marker profile which is compared between e.g. healthy and diseased individuals to find a set of peptides/proteins with diagnostic value. The accuracy of mass spectrometric measurement in combination with multimarker analysis using sophisticated software tools is estimated to enable disease diagnostics, prediction and prognosis in near future.

Here, we present a set of superparamagnetic microparticles with a variety of surface chemistries to bind biomolecules through specific chemical interactions. Different coatings like reversed phase, ion exchange, or metal-affinity functionalities can be used to enrich peptide and protein classes from samples and to purify those prior to mass spectrometric measurement. Bead handling is facile and scalable, multidimensional separation procedures are possible. The whole sample preparation procedure has been transferred to an 8-needle pipetting robot. Thereby, reproducible profile spectra of high quality and information content can be acquired for clinical proteomic profiling studies and biomarker discovery.

## ANALYSIS OF ASSYMMETRIC DIMETHYL ARGININE (ADMA): A NOVEL RISK FACTOR FOR ENDOTHELIAL DYSFUNCTION.

**Jan Dankers, George Wouters, Arnold Verbeek, Francisca de Jong, Analytico Medinet B.V. Bergschot 71, P.O. Box 5510, 4801 DM Breda, The Netherlands**

Asymmetric dimethylarginine (ADMA) is an endogenous and competitive inhibitor of nitric oxide synthase, therefore ADMA may be a novel risk factor for vascular disease. Because of its biological importance, the determination of ADMA in human plasma have become more important. ADMA was validated using HPLC with fluorescence detection, a SPE clean-up and derivatisation with orthophthaldialdehyde (OPA).

## NOREPINEPHRINE DETECTION IN "TREE SHREWS" URINE USING LC-TANDEM MS TO VERIFY THAT STRESS-INDUCED ALTERATIONS ARE PREVENTED BY THE NK1 RECEPTOR ANTAGONIST SLV 323

J.A.M. Berk<sup>1</sup>; A. van der Laan<sup>2</sup>; A.Wolthuis<sup>1</sup>; L. J. Oppenheimer<sup>1</sup>; G. Hommema<sup>1</sup>, P.H. van Amsterdam<sup>2</sup>, M.G.C. van der Hart<sup>3</sup>, M.B. Hesselink<sup>2</sup> and E.Fuchs<sup>3</sup>

<sup>1</sup>KCL BioAnalysis, Leeuwarden, The Netherlands; <sup>2</sup>Solvay Pharmaceuticals, Weesp, The Netherlands; <sup>3</sup>Clinical Neurobiology Laboratory, German Primate Center, Goettingen, Germany.

**Introduction**

Pharmacological: Substance P and its receptor, the neurokinin 1 (NK1R) have been discussed as possible targets for new antidepressant therapies [1]. Here we investigated the therapeutic potentials of the NK1R antagonist SLV 323 in the chronic psychosocial stress paradigm of adult male tree shrews [2]. Animals were subjected to a 7-day period of stress before the onset of daily oral administration of SLV 323 (20 mg/kg/day). The stress continued throughout the treatment period of 28 days. Urine samples were collected daily for determination (among others) of noradrenaline and creatinine.

Analytical: Analytical methods were developed and "in study" validated. The Jaffe's colorimetric creatinin analysis method is considered as a well known assay and not described. The isolation [3] of catecholamines with liquid liquid based extraction using complex formation in combination with ion pairing is more or less unknown probably due to the relative complexity of the composition of reagents and/or extraction procedure. In this study urine samples were extracted and measured using the LC-MS/MS (API 3000). Established key figures are presenting the analytical method. The analytical part of the study was conducted in accordance with GLP.

**References**

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APPLICATION OF CIM™ DISK AND TUBE MONOLITHIC COLUMNS FOR FAST AND EFFICIENT DOWNSTREAM PROCESSING OF HUMAN PLASMA PROTEINS

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The problems that arise in down-stream processing of therapeutic proteins from complex mixtures are, above all, losses caused by the purification procedures, resulting in lower yield. The introduction of modern filtration and chromatographic techniques, especially the sterile filtration, ion-exchange chromatography and affinity chromatography allows the production of highly purified concentrates of therapeutic proteins and also of single plasma proteins.

However, the chromatographic techniques in particular still suffer from serious flaws, especially in terms of speed and capacity. Therefore, the risk of unwelcome changes or loss of activity in the sensitive biopolymers can not be ruled out and is only prevented by careful investigation of the production process.

This work will present the application of CIM Convective Interaction Media™ novel polymeric monolithic columns, for in-process analyses and fast analytical and preparative separations of pharmaceutically relevant biopolymers. The questions like the optimisation of the chromatographic parameters for an efficient separation and the effect of the operational parameters on the binding capacity and yield will be addressed. Finally, real applications using ion-exchange purification of a clotting factor IX (FIX) will be discussed.

INTRODUCING NEW VYDAC DENALI 120Å SILICA-BASED COLUMNS AND MEDIA FOR SMALL MOLECULE SEPARATIONS.

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A HIGHLY SENSITIVE ASSAY FOR SLV308, N-DESMETHYL-SLV308 AND SLV308-N-OXIDE IN PLASMA AND URINE USING HPLC WITH TANDEM MASS SPECTROMETRY

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SLV308 is a potential psychotropic drug currently in development at Solvay Pharmaceuticals for the indication of Parkinson's disease. It is unique on the basis of its mechanism of action; a combination of partial dopamine D2 receptor agonistic activity with full serotonin 5-HT1A receptor agonistic activity and also a receptor agonistic activity on the noradrenalina1 and a2 receptors.

Clinical pharmacology studies with SLV308 required measurement of the parent compound as well as of two active metabolites. The original aim was to develop a method capable of measuring all three compounds at low pg/ml levels in one run. Unfortunately this approach appeared not to be feasible. Consequently two different methods had to be developed:

1. A bioanalytical method to measure human plasma levels of SLV308 and its N-desmethyl metabolite was developed. This method, based upon HPLC with MS/MS detection, made use of 13C4 labeled SLV308 internal standard and was proven to be suitable for the measurement of SLV308 and its metabolite for plasma levels ranging from 0.02 – 20 ng/ml and 0.100 – 20 ng/ml respectively. Method validation on an MDS- SCIEX 4000 was done with respect to system performance, absolute recovery, response function, accuracy, precision, sensitivity and specificity.

2. A second method was developed to measure SLV308-N-oxide in samples from clinical kinetic studies. This method, also LC-MS/MS made use of a 13C4 labeled SLV308-N-oxide internal standard was validated for plasma levels ranging from 0.100 – 20 ng/ml.

The values for the validation parameters were established using data from reference solutions, calibration standards, quality control samples at four levels and blank plasma samples analyzed in five accepted validation batches. Data was processed using standard univariate statistics, ANOVA and regression analysis.

Both methods fulfilled the current international criteria for bioanalytical method validation.

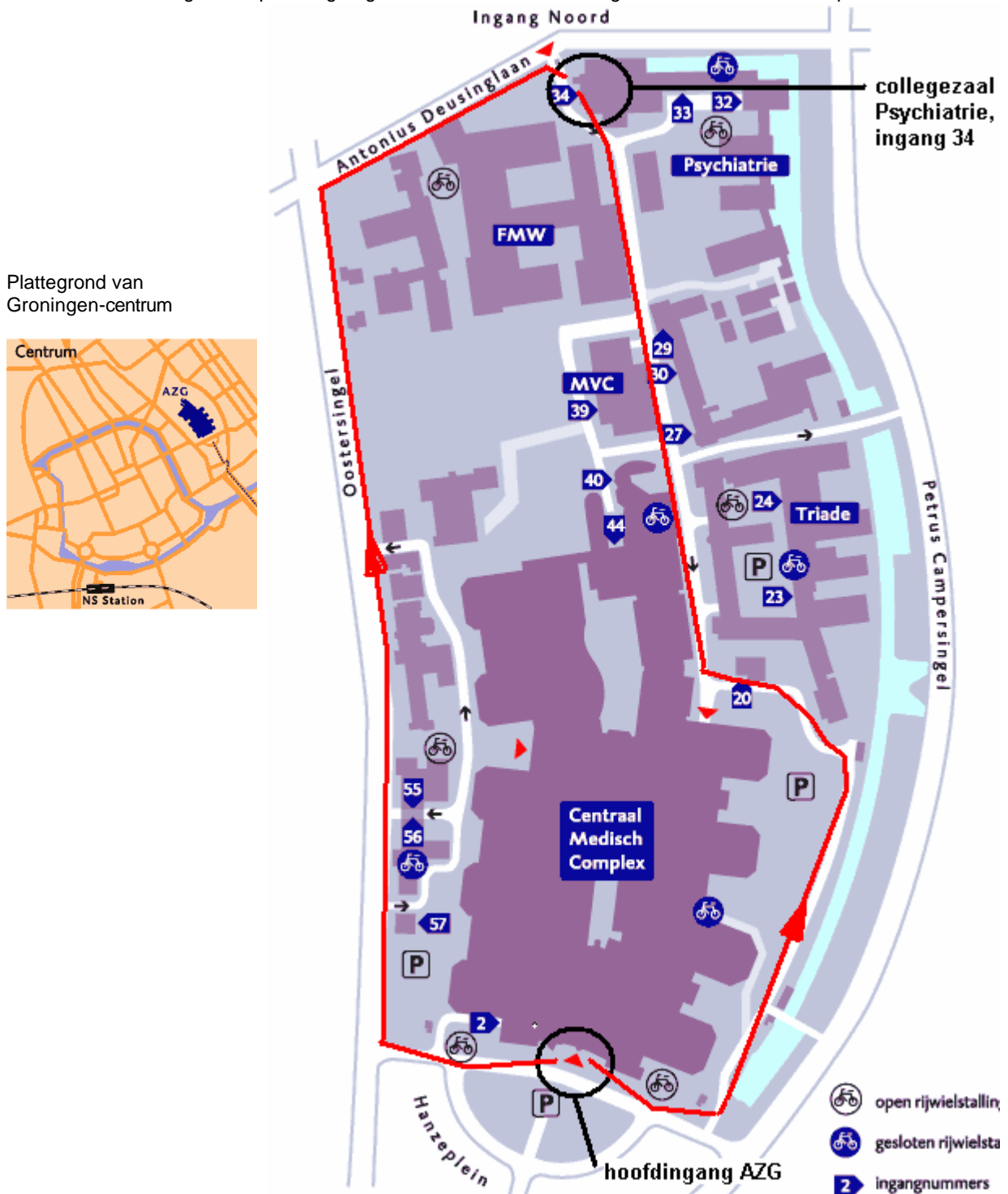
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In life science research an urgent need exists for biomarkers that reflect prognosis, diagnosis or progression. Biomarkers have considerable potential in aiding the understanding of the relationship between disease or health and environment. Metabolites play an important role as biomarkers. Biological fluids contain thousands of metabolites and thus form a wealth of information about an organism's genotype and its exposure to the environment. In the past three years, headway is made in the field of biomarker research at TNO with the study of biological fluids using <sup>1</sup>H Nuclear Magnetic Resonance spectroscopy (NMR) and multivariate data analysis. This technique enables classification of samples according to fine distinctions in NMR spectra of biological fluids. Metabolites that contribute mostly to these differences, potential biomarkers, can be selected and identified when clinical endpoints correlate with the classification according to NMR and multivariate statistics. The applicability of our biomarker selection and identification approach ranges from health, safety/efficacy, diagnosis/prognosis to monitoring. For example, we found a novel diagnostic biomarker for osteoarthritis, which was used in an intervention study to investigate possible effects of vitamin C on the course of disease (results shown). This study aimed at the finding of a diagnostic biomarker, with samples taken from healthy versus severely diseased subjects. Our current research is targeting now the discovery of early and prognostic biomarkers. For this purpose, NMR and multivariate data analysis are applied on clinical end points with additionally a number of intervening time points. Time-course NMR data of biological fluids contains valuable information about biorhythms. Diseases as well as environment disturb biorhythms. Such perturbations affect the biological system's metabolism and are supposed to show up in time-course biological NMR data upon which early biomarkers can be selected and identified. These early biomarkers are of major importance in the understanding of the origins of diseases or effects of environment. Current medicine is mainly oriented at relieving symptoms of diseases. Early biomarkers will bring the prevention of diseases nearer. Our approach has the ability to select and identify biomarkers that reflect early detection, prognosis, diagnosis and progression. NMR and multivariate data analysis thus provides a sensitive tool in the evaluation of environmental effects, such as intervention studies with nutrients or drugs, on the course of a disease for instance. The technique is extremely useful in areas like food and nutrition, pharmacy, health care, toxicology and chemistry.

## Routebeschrijving

FABIAN wordt gehouden in de collegezaalzaal Psychiatrie (ingang 34, zie plattegrond hieronder) van het Academisch Ziekenhuis, vlakbij de Noordingang van het complex aan de Antonius Deusinglaan. Als u via de hoofdingang binnenkomt kunt u bij de receptie van het ziekenhuis vragen naar de interne route, maar het eenvoudigst is het om buiten linksom of rechtsom het complex te lopen (ca. 5 minuten, zie plattegrond hieronder: volg de rood aangegeven routes op de kaart).

Het Academisch Ziekenhuis Groningen ligt aan de oostkant van het Groninger stadscentrum, aan het Hanzeplein. Het ziekenhuis is op meerdere manieren goed te bereiken. U kunt met de fiets komen of met de auto en gebruik maken van de ondergrondse parkeergarage. Verder is het ziekenhuis goed bereikbaar met het openbaar vervoer.



### Openbaar vervoer

U kunt het AZG goed bereiken met het openbaar vervoer. Zowel stads - als streekbussen rijden vanaf het Centraal Station in ongeveer tien minuten naar het AZG.

Als u naar de afdeling Psychiatrie van het AZG wilt, kunt u het beste gebruik maken van de volgende buslijnen:

- Stadsbuslijnen 3 (Lewenborg) en 6 (Beijum);
- Streekbuslijn 507.

De volgende buslijnen stoppen bij de hoofdingang van het ziekenhuis:

- Stadsbuslijnen 4 (Oosterpark) en 7 (Station Noord);
- Streekbuslijnen 55, 63, 65 en 165;
- Q-liner 139.

### Taxi

Komt u met een taxi naar het AZG, dan kan de chauffeur u afzetten bij de Noord-ingang van het AZG, op de Antonius Deusinglaan. Wilt u het ziekenhuis per taxi verlaten, dan kunt u bij een van medewerkers bij de balie in de ontvangsthuis vragen om een taxi voor u te bellen.

### Auto

Als u met de auto naar het ziekenhuis komt, volg dan de ANWB-wegwijzers. U komt automatisch in de parkeergarage die onder de ontvangsthuis ligt. Bezoekers kunnen hier betaald parkeren. Het eerste halfuur is kosteloos. Daarna wordt een oplopend tarief gehanteerd, afhankelijk van de tijd dat u geparkeerd staat. De tarieven worden op de betaalautomaat in de ontvangsthuis vermeld. De parkeergarage is vierentwintig uur per dag geopend. Er zijn ook verschillende fietsenrekken om uw fiets te stallen.