

Cyclosporin A, tacrolimus, sirolimus and everolimus:

Immunosuppressants analysis by means of HPLC/tandem mass spectrometry

Immunosuppressive drugs is the term generally given to those pharmacological substances which are effective in suppressing the body's rejection of transplanted tissue or organs through the molecular manipulation of the human immune response, thus making a vital contribution to the success of such transplants. In most cases a combination of different immunosuppressive drugs is administered which interrupts the signal cascade from the antigen receptor to the cell nucleus by blocking the T-cell activation at various points in the reaction chain (1).

Cyclosporin A (CsA) and tacrolimus (FK 506) are used primarily after the transplant of complete organs and after bone-marrow transplants. CsA binds cyclophilin A and FK 506 binds the protein FKB12. In turn, each of the complexes binds to highly conserved domains of the cytosolic protein calcineurin A, and the activity of this serine/threonine phosphatase is thus blocked (8). This in turn means that there is no translocation of the transcription factors NF-AT, AP-3 and NF-KB to the cell nucleus (2,3). The activity of these nucleoproteins in the cell nucleus is imperative for the transcriptional activation of genes of the cytokines interleucin-2, -4 and -15; production of interleucins is run down. The cell cycle remains in the G0-phase, with no transition to the G1-phase. There is thus no T-cell activation (4).

Sirolimus (rapamycin) and everolimus are used after organ transplants. The molecule likewise binds the immunophilin FKBP12: afterwards, however, it forms a complex with a



serine/threonine kinesis which has not so far been described in any greater detail and is currently known as mTOR ("mammalian target of rapamycin"). This target molecule is in the signal transduction chain of the phosphatidylinositol-3'-kinase (4,5) and under physiological conditions it activates the 40S ribosomal protein S6 kinase (p70s6k) and the eukaryotic initiation factor 4E-binding protein-1. Because of the binding to mTOR and the resulting inhibition of activity, sirolimus interrupts the signal chain of the natural cell cycle of the T-lymphocyte and there is no progression from the G1-phase to the S-phase (6,7). Due to this function of preventing cell growth and destroying the cell cycle, sirolimus and analogous substances can be used in anti-tumour therapy.

Because of their strong pharmacological effect it is extremely important to keep the blood concentrations of immunosuppressive drugs within

therapeutic ranges. If concentrations are too low, this leads to the suppression of the rejection reaction being too weak and the transplanted tissue can suffer irreversible damage – which might lead to quick death. If, on the other hand, blood concentrations are too high, this increases the medicinal side-effects: CsA and FK506 are nephrotoxic, and all immunosuppressive drugs lead to hypercholesterolaemia. Strong intra- and inter-individual pharmacokinetic differences always require that quantities taken be set individually and that observation of the medication levels likewise be undertaken on an individual basis. Tandem mass spectrometry (LC/MS/MS) is a very specific analytical method with regard to the identification of individual components. Nevertheless, the potential risk of matrix interferences is high when extremely low concentrations are measured, as is the case with tacrolimus and sirolimus. Sample preparation procedures of varying effectiveness as

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well as chromatographic separations make it necessary to assess matrix blanks.

Chromsystems' quality controls make possible a reliable routine check of patients' measured values. Setting up a control measurement after every 20th patient sample is recommended, but at least after every 50th sample. Irrespective of the measuring method, the controls must be processed like regular patient samples. The controls are available in four concentrations (0082, 0083, 0084, 0085), of which three cover the normal lower levels and one is designed as a control of peak level directly after the medicine has been administered. Like CsA, tacrolimus, sirolimus and everolimus accumulate in erythrocytes, it only

makes sense to measure whole blood samples; accordingly, Chromsystems controls are based on a whole blood matrix. All controls are available lyophilised, in order to guarantee both long-term stability of the analytes and highest accuracy of the target values.

The blank control (0089) is designed for measuring matrix interferences (see above). If any "zero values" are ascertained, these can be used for adapting the measuring system. The blank control is also produced as a whole blood matrix and lyophilised. To achieve the highest possible accuracy in this case it is necessary that the zero value control be produced in exactly the same matrix as the quality controls 0082 to 0085. This is, of course, the case

with Chromsystems and all five controls are accordingly offered as a set with agreeing batch numbers.

The whole blood calibration standard 28003 completes the series of controls for immunosuppressive drugs. This standard, too, is supplied lyophilised and is processed like a patient sample.

All controls and the calibration standard are suitable for both HPLC methods and immunoassays.

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Control of Assays for Immunosuppressive Drugs

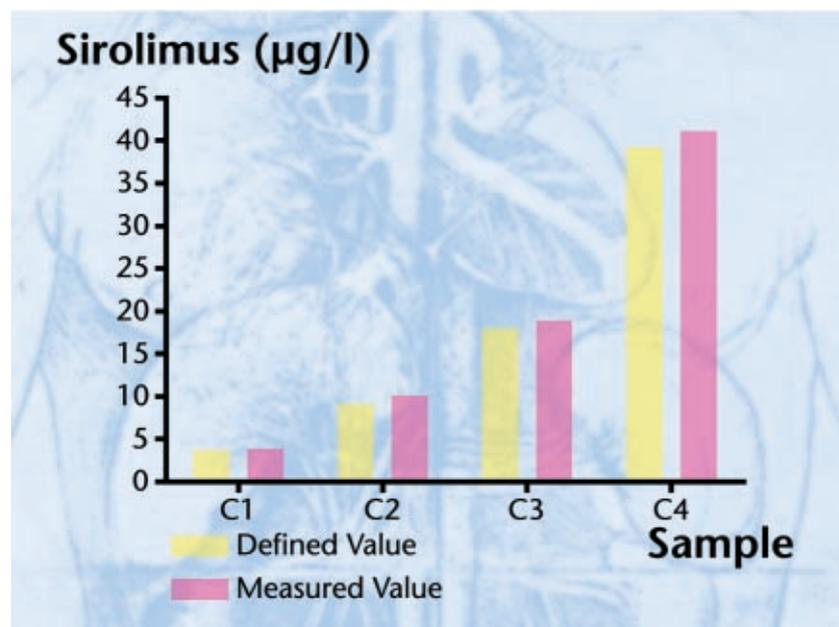
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The immunosuppressive drugs are characterised by substantial between-patient differences in their pharmacokinetics and a broad spectrum of unwanted effects. Cyclosporin and tacrolimus, still the mainstay of drug therapy for the avoidance of graft rejection, are routinely measured in blood to assist in their optimal prescription. Recently, attention has focused on a newer drug, sirolimus, for which drug monitoring in blood is a regulatory requirement in Europe.

There are no established reference methods for the measurement of the immunosuppressive drugs, nor are there certified reference materials for calibration. As a result, between-method assay performance varies substantially. This is due both to errors in calibration, and to differences in specificity of the antibodies used in the immunoassays. Data on method performance can be judged by external proficiency testing material, as presented on our web site (www.bioanalytics.co.uk), and by reference to consensus documents on immunosuppressive drug monitoring. A joint Working Group sponsored by the International Federation of Clinical Chemistry (IFCC) and the International Association of Therapeutic Drug Monitoring and Clinical Toxicology is reviewing current methods and monitoring strategies (1).

There are some interesting developments in immunosuppressive drug therapy, related to both the introduction of new drugs and the use of new drug combinations. These developments pose analytical challenges for the laboratory related to sensitivity

and has also underpinned all the clinical studies of the sirolimus analogue, everolimus, to be introduced in Europe early in 2004, and is being used by an increasing number of centres for the measurement of cyclosporin and tacrolimus.



and selectivity. A driving force for innovation in methodology has been the introduction of sirolimus since, currently, HPLC is the only technique available for its measurement. More than 100 centres world-wide are now measuring the drug by HPLC, about half of these using mass-spectrometric detection (LC/MS). This technique

The need for centres to prepare in-house calibrators for use in LC/MS assays has focused attention on the need for well-defined control material. This is particularly so for sirolimus, since there are no immunoassay results with which to compare locally prepared calibration material. Recently, we have tested a set of four freeze-

dried whole blood controls with defined-values for the measurement of sirolimus, using LC/MS (Chromsystems GmbH, München, Germany). The results are illustrated below and show good agreement between the mean of 5 replicates of each control, analysed in this laboratory, and the mean defined value. The average deviation from the expected value was 6.5%. A blank matrix and calibrator are also available from the same source. All these materials also contain defined values of cyclosporin and tacrolimus.

Even for immunoassays there is interest by manufacturers in defining their own calibrators against well sourced control material. Currently, we are supplying services to diagnostics manufacturers for the assessment of calibrator material using validated LC/MS assays, and by supplying proficiency testing material previously assayed on multiple sites.

Thus, in a continuously evolving field, there is a need for constant vigilance to ensure that assays for these critical dose drugs are both accurate and precise.

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Immunosuppressants

Whole Blood Controls Level I, Level II, Level III, Level IV Whole Blood Calibration Standard/Whole Blood Blank Control

Concentrations: Chromsystems Immunosuppressants Control			Level I		Level II		Level III		Level IV	
Substance	Method	Unit	Value	Range	Value	Range	Value	Range	Value	Range
Ciclosporin A	HPLC-UV	µg/l	96	77-115	242	193-290	476	381-572	1899	1519-2278
	Dade-Behring EMIT	µg/l	101	81-121	247	198-296	467	374-560	2051	1641-2461
	Abbott TDx	µg/l	110	83-138	274	206-343	528	396-659	1833	1375-2291
Rapamycin (Sirolimus)	LC-MS/MS	µg/l	3.7	2.6-4.8	9.1	7.3-10.9	18.0	14.4-21.6	39.2	31.4-47.0
Tacrolimus (FK 506)	Abbott IMx	µg/l	6.4	5.1-7.7	12.2	9.8-14.6	23.7	19.0-28.4	44.6	35.7-53.5
	LC-MS/MS	µg/l	3.4	2.7-4.1	8.2	6.6-9.8	18.1	14.5-21.7	36.0	28.8-43.2

Concentrations:			Whole Blood Calibration Standard		Whole Blood Blank Control	
Substance	Method	Unit	Value	Value	Value	Value
Ciclosporin A	HPLC-UV	µg/l	332	not detected		
	Dade-Behring EMIT	µg/l	338	not detected		
	Abbott TDx	µg/l	383	< 6,0		
Rapamycin (Sirolimus)	LC-MS/MS	µg/l	14.5	not detected		
Tacrolimus (FK 506)	Abbott IMx	µg/l	17.3	< 3,0		
	LC-MS/MS	µg/l	13.4	not detected		

Instructions Whole Blood Controls Level I, Level II, Level III, Level IV

These Chromsystems whole blood controls are designed to monitor the accuracy and precision of analytical procedures for the quantitative determination of Ciclosporin A, Rapamycin and Tacrolimus in whole blood. The lyophilised control is based on human whole blood and should be used routinely according to the instructions accompanying the assay procedure being used. While following these instructions the control is to be handled in the same manner as a patient specimen.

Ordering information

Order no.	Product
0081	Immunosuppressants Whole Blood Control, Four-Level (I + II + III + IV), 4 x 2 x 2 ml
0082	Immunosuppressants Whole Blood Control, Level I, 5 x 2 ml
0083	Immunosuppressants Whole Blood Control, Level II, 5 x 2 ml
0084	Immunosuppressants Whole Blood Control, Level III, 5 x 2 ml
0085	Immunosuppressants Whole Blood Control, Level IV, 5 x 2 ml

Instructions Whole Blood Calibration Standard/Whole Blood Blank Control

This Chromsystems whole blood blank control is designed to monitor the influences of the matrix on analytical procedures for the quantitative determination of Ciclosporin A, Rapamycin and Tacrolimus in whole blood. The lyophilised control is based on human whole blood and should be used routinely according to the instructions accompanying the assay procedure being used. While following these instructions the control is to be handled in the same manner as a patient specimen. This blank control represents the original

matrix of the Chromsystems Immunosuppressants control and calibrator series of the identical Lot.

Ordering information

Order no.	Product
28003	Immunosuppressants Whole Blood Calibration Standard, 5 x 2 ml
0089	Immunosuppressants Whole Blood Blank Control, 5 x 2 ml

Organic solvents in the workplace

Impact of organic solvents in the workplace:

The Chromsystems kit for determining parameters in urine in the field of occupational medicine

People working in the chemical industry are exposed to numerous volatile substances which pose health risks. They are inhaled during breathing and absorbed through the lungs. Toluene and xylene - solvents used in the production of greases and oils, paints, glues, detergents and motor fuels - are very important in this respect. Styrene, an important source material for the synthesis of plastics, is also important.

Once in the body, all three substances are subject to metabolism. Toluene is converted into hippuric acid by means of oxidation and conjugation, and xylene is transformed into the corresponding o-, m- or p-methyl-hippuric acid. Styrene is oxidised to mandelic acid (85%) and phenyl-glyoxylic acid (10%). Toluene, xylene and styrene are lipophilic and



are distributed in the fatty tissue and nerve tissue. Their metabolites, on the other hand, are hydrophilic and can be secreted with urine. Acute poisoning with these substances leads to intoxication and hallucinations. Chronic poisoning causes damage to

the central and peripheral nervous systems. The TLV value (abbreviation for: Threshold Limit Value) was introduced to define the limits of non-harmful exposure. The TLV value defines the concentration at the workplace below which even long-term

exposure is not expected to lead to any damage to health. In addition, BEI values (abbreviation for Biological Exposure Index) were laid down which indicate the highest concentrations of a substance or its metabolite in biological material (e.g. urine, blood) at which, as a rule, employees' health is not affected.

The aim of medical examinations at work is to check the intake of pollutants so as to assess the impact on individual persons and to be able to minimise associated health risks. Most suitable for the analysis in question is the determination of metabolites in the urine, as - due to the high rate of metabolic degradation - more than 95% of substances absorbed are secreted out again within 24 hours and, as a result, the blood values are very low. In addition, urine values correlate best with the exposure to pollutants. →

→ Since November 2003 Chromsystems has been offering a new reagent kit for the specific monitoring of hippuric acid, methyl-hippuric acid, mandelic acid and phenyl-glyoxylic acid in urine in one HPLC run. The kit is distinguished by very simple preparation of samples and a high level of precision. Only 10ml of urine is needed. The urine is first stabilised and an internal standard added. After this the precipitate contained in the

urine is separated by centrifuging. The sample thus obtained can be analysed with an isocratic HPLC unit with ultra-violet detection. Inclusion of an internal standard means that analytical variations are minimised and a high level of precision and reliability are assured for the results.

The chromatogram demonstrates the faultless chromatographic separation. All metabolites can be analysed

within 20 minutes. Two different levels of controls and a urine calibration standard complete this kit.

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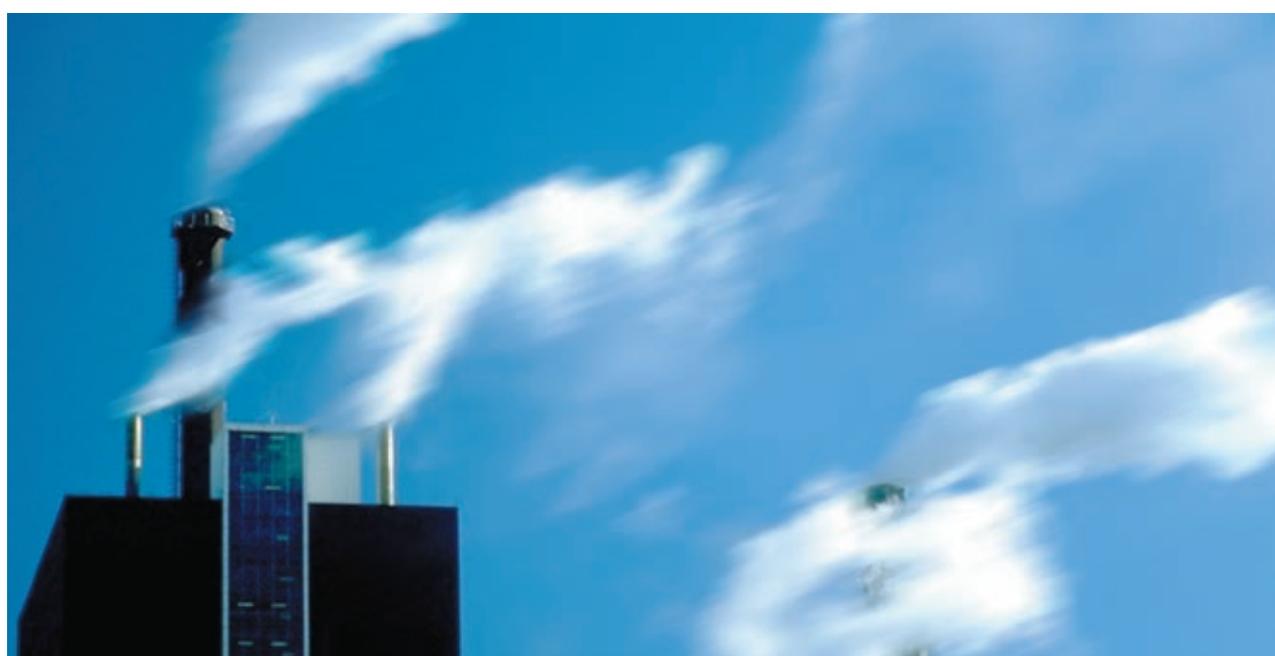
Determination of solvent Metabolites in Urine

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Aromatic solvents, such as toluene, xylene and styrene, are widely used in several applications, including industrial solvents and chemical intermediates. Since these compounds are often used in combination with each other, workers are usually exposed to a mixture of solvents, which can also be due to the presence of significant amounts of contaminants. Occupational exposure is commonly evaluated by biological monitoring with simultaneous measurement of the working environment. Since solvents are mainly absorbed by inhalation, air concentrations of these compounds are measured, and urinary metabolites are also determined. The results from both environmental and biological monitoring are then compared for example with the corresponding Threshold Limit Values (TLVs) and Biological Exposure Indexes (BEIs) developed by the American Conference of Governmental Industrial Hygienists (ACGIH).

Nevertheless, detectable concentrations of some of these metabolites, such as hippuric acid, can be found in the urine of non-exposed subjects. The Reference Values in the general population should be therefore taken into account, and the influence of different variables (diet, lifestyle, etc.) on the urinary levels of these substances should be evaluated.

The main end products of styrene metabolism are urinary mandelic acid (MA) and phenylglyoxylic acid (PGA). In the occupational studies, end-of-shift and next-morning MA + PGA are used as indicators of chronic styrene exposure. According to the ACGIH, the air level of styrene should not exceed 20 ppm (86 mg/m³). This value is strictly related to the next-morning urinary mandelic acid and



phenylglyoxylic acid, which should be less than 300 mg/g creatinine and 100 mg/g creatinine, respectively. As far as end-of-shift urinary levels are concerned, the mandelic and phenylglyoxylic acid concentrations should not be higher than 800 mg/g creatinine and 240 mg/g creatinine, respectively. In contrast, in the general population, average levels of 3.8 mg mandelic acid/g creatinine and 4.3 mg phenylglyoxylic/g creatinine are measured.

Toluene is commonly used as an industrial solvent for the manufacturing of paints, chemicals, pharmaceuticals, and rubber and a TLV-TWA of 50 ppm (188 mg/m³) has been established by the ACGIH. Toluene exposure levels can be determined from urinary hippuric acid levels, which is its major urinary metabolite. Nevertheless, when workers are exposed to low levels of airborne toluene (short exposures or TWA concentrations below 10 ppm), correlation coefficients between end-of-shift levels of hippuric acid and toluene concentrations in

air, are not statistically significant. Urinary hippuric acid levels are in this case very close to those measured in the general population (average levels 300–400 mg/g creatinine, ranging from 50 to 2000 mg/g creatinine). This means that there are a number of hippuric acid precursors in the environment, such as benzoic acid in food (e.g. plums, preserved food containing fish or eggs), which makes diet one of the major confounding factors.

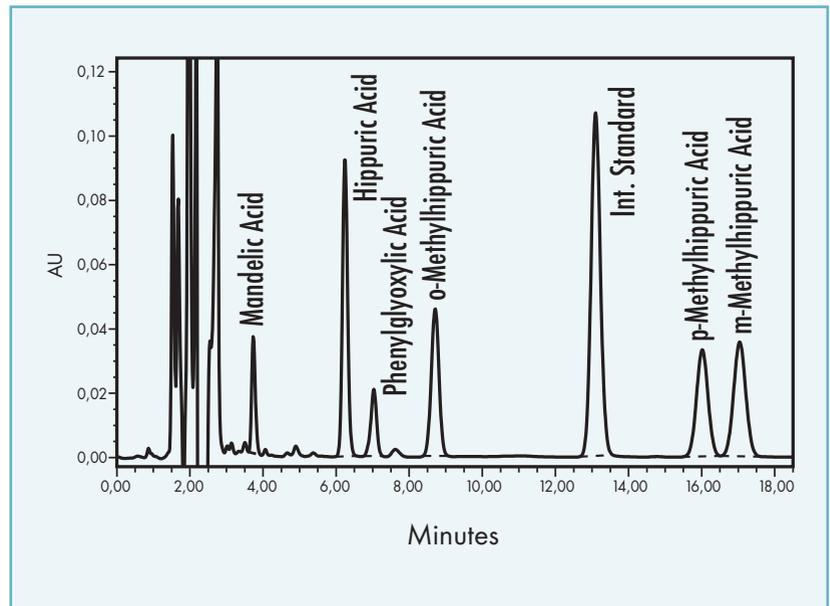
Xylene is a colourless liquid that catches on fire easily. Commercial or mixed xylenes are composed of three isomers (meta-, ortho- and para-xylene) and are extensively used in the chemical industry. Occupational exposure to xylene can be assessed by determining urinary concentrations of its metabolites, m-, o- and p-methylhippuric acids. The ACGIH has established a TLV-TWA for airborne xylene of 100 ppm (350 mg/m³), whereas end-of-shift level of the three acids should be less than 1500 mg/g creatinine. The reference values in

the general population are 0.59, 18.9, and 0.43 mg/g creatinine for meta-, ortho-, and para-methylhippuric acid, respectively. Since, as mentioned above, both general population and workers are usually exposed to mixtures of solvents, a simple and sensitive method for the quantification of even low urinary levels of their major metabolites is needed.

A reagent kit for the simultaneous determination of mandelic, hippuric, phenylglyoxylic, ortho-, meta-, para-methylhippuric acids has been developed by Chromsystems – Diagnostic by HPLC. The sample preparation is very simple and just 10 ml of urine sample are required. A chromatographic run of 18 minutes allows the separation of six metabolites which can be quantified by the inclusion of the internal standard. The results can be then compared with the BEIs established by the ACGIH.

This reagent kit is a time- and cost-saving tool for risk assessment related to the exposure to toluene, xylene, and styrene.

Chromsystems Reagent kit for the HPLC analysis of Hippuric Acid, Methylhippuric Acids, Mandelic Acid and Phenylglyoxylic Acid in urine



- > **Only 10 μ l sample volume**
- > **Internal standard included**
- > **Simple sample preparation**

Specifications

Limit of quantification: 15 mg/l
 Intraassay: CV < 2.5 %
 Recovery: 100 %
 Run time: 20 min
 Specimens: urine
 Stability of samples: up to 64 h (ambient temp.)

	Limit of quantification:	Linearity:
Hippuric Acid:	15	up to 18000 mg/l
Methylhippuric Acid:	15	up to 7000 mg/l
Mandelic Acid:	15	up to 4000 mg/l
Phenylglyoxylic Acid:	15	up to 1700 mg/l

BEI Indices
 Hippuric Acid: 1500 mg/l
 Σ Methylhippuric Acids: 2000 mg/l
 Mandelic Acid: 400 mg/l
 Phenylglyoxylic Acid: 100 mg/l

For the Chromsystems HPLC analysis of occupational medicine parameters in urine any isocratic HPLC system with UV detection is suitable.

Sample Preparation

- > Place 1000 μ l Internal Standard into a reaction vial.
- > Add 10 μ l urine and mix briefly (vortex).
- > Centrifuge 5 minutes at 13 000 rpm.
- > Inject 20 μ l of supernatant into the HPLC system.

Ordering information

Order no. Product

43000 Reagent Kit for the HPLC Analysis of Hippuric Acid, Methylhippuric Acids, Mandelic Acid and Phenylglyoxylic Acid in urine. For 100 analyses.

Chromsystems Controls (lyoph.):

0141 Occupational Medicine Urine Control, Bi-level (I + II), 2 x 5 x 0.5 ml
 0142 Occupational Medicine Urine Control, Level I, 5 x 0.5 ml
 0143 Occupational Medicine Urine Control, Level II, 5 x 0.5 ml

Olanzapine, quetiapine and promazine: HPLC analyses of neuroleptic drugs

Neuroleptic drugs are psychotropics used mainly in the treatment of schizophrenia and mania. It is not, however, a therapy in the real sense but rather an alleviation of the symptoms of psychoses. The aim is also to enable people affected to be reintegrated into society. This group of substances first appeared in the world of psychotropics in 1952 with the discovery of chlor-promazine and has been substantially enriched as a result of intensive research. The effect of neuroleptic drugs is attained by manipulating synaptic impulse transmissions (1,2,3).

Seven synaptic messengers play a major role in the central nervous system. These are dopamine, nor-adrenaline, serotonin, acetylcholine, g-aminobutanoic acid, glutamic acid and glycine. On the basis of the inhibiting effect on various messengers and various side-effects, neuroleptic drugs are divided into two categories (4): On the one hand the older, "classic" neuroleptic drugs which unfold their effect primarily by blocking pre- and post-synaptic dopamine receptors; and, on the other hand, the newer, "atypical" drugs of which the first, clozapine, was discovered in the early 1970s as a consequence of the search for substances with fewer side-effects. For these atypical neuroleptic drugs the receptors for serotonin (also for noradrenaline and histamine) play a more important role than the dopamine receptors (4).

to movement which appear gradually, similar to Morbus Parkinson, are also described. What was noticeable in the tests with the prototype of atypical neuroleptic drugs, clozapine, was that these extrapyramidal disorders were minimal, compared with the placebo level. Moreover, the so-called negative symptom of acute psychoses such as a lack of drive or communication seemed to be reduced more effectively with clozapine. Against this background the atypical neuroleptic drugs seemed, in the initial years, to be an ideal medication with no appreciable side-effects (5,6).

However, in the course of time it became apparent that atypical neuroleptic drugs, too, could trigger serious side-effects. Depending on the substance, there was a significant increase in weight while the medication was

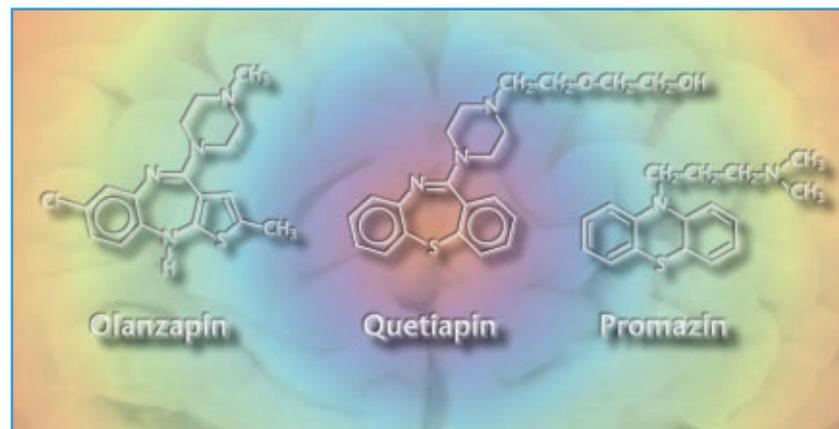


Illustration: structural formulas of olanzapine, quetiapine and promazine

Structurally, olanzapine and quetiapine are derived from clozapine, which means that they belong to the group of tricyclic neuroleptic drugs. Quetiapine has a dibenzothiazepine ring instead of the dibenzodiazepine, and olanzapine has a thienobenzodiazepine ring as its basis. Promazine is also a neuroleptic drug from the tricyclic group and is included among the phenothiazines.

efficiency of psychotropics therapy for the patient, and at the same time increase its safety, Therapeutic Drug Monitoring of atypical neuroleptic drugs is necessary. In addition, the patient's compliance is checked, thus assuring optimum success for the therapy.

The Chromsystems reagent kit for HPLC analysis of olanzapine, quetiapine and promazine in serum/plasma is distinguished by sample preparation by means of highly specific solid phase extraction. In this case, and in contrast to online sample preparation, a highly purified eluate gets on to the pre-column and HPLC column. As a result, not only is the risk of interferences and underlying peaks reduced, but the column lifetimes are also substantially increased. In addition, the solid phase extraction offers optimum preconditions for the automation of sample preparation by means of the Gilson® ASPEC™, which makes a high daily throughput of samples possible. The Chromsystems product is so far the only commercially available reagent kit which also allows reliable measurement of the olanzapine metabolite desmethylolanzapine.

Chromsystems offers not only the calibrator contained in the kit but also two groups of controls for quality assurance. The robust ultraviolet method offers determination limits which are significantly below the therapeutically relevant concentration range. The product has been on the market since May 2004.

Classic neuroleptic drugs

Haloperidol (Haldol®)
Flupentixol (Fluanxol®)
Fluspirilene (Imap®)
Chlorprotixene (Truxal®)
Levomoprazine (Neurocil®)
Sulpiride (Dogmatil®)
Promazine (Sparine®)

Atypical neuroleptic drugs

Clozapine (Leponex®)
Olanzapine (Zyprexa®)
Risperidone (Risperdal®)
Ziprasidone (Zeldox®)
Amisulpiride (Solian®)
Quetiapine (Seroquel®)

Antibiotics frequently administered

Psychiatrists distinguish between the fast effects of neuroleptic drugs, reducing anxiety and acting as a sedative, and those effects which take days or weeks to unfold and deal with psychotic symptoms such as hallucinations, delusions and disruptions to thinking.

The most well-known side-effects of classic neuroleptic drugs are problems in movements in the muscle system, the so-called extrapyramidal disorders. These involuntary movements (e.g. acathisia/"inability to sit still"), extending to spasmodic tension in muscles and muscle groups are a result of a blocking of the dopaminergic receptors. Besides fast side-effects such as tongue cramps, hindrances

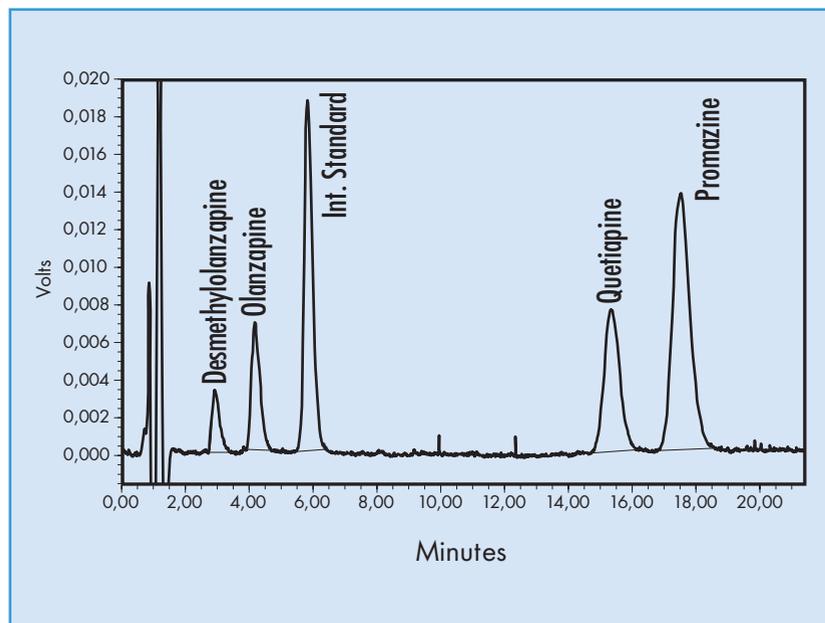
being taken, changes to the haemogram (agranulocytosis) and a heightened risk of breast cancer associated with the rise in the prolactin level. It is also known that, after atypical neuroleptic drugs have been administered for a longer period of time, the danger of psychotic illness may increase significantly (7,8,9,10).

The plasma level of neuroleptic drugs can vary greatly from person to person and may be influenced by co-medication or other circumstances. If, for example, carbamazepine is administered at the same time this causes, just as smoking does, a lowering of the olanzapine level by an induction of the metabolising enzyme CYP1A2. In order to improve the

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Chromsystems Reagent kit for the HPLC analysis of Olanzapine, Quetiapine, and Promazine in serum/plasma



> **Low limit of quantification**

> **Robust UV-VIS detection**

> **Metabolites included**

Specifications

Olanzapine/Desmethylolanzapine
 Limit of quantific.: 5 µg/l
 Linearity: up to 1000 µg/l (therapeutic range: 20–80 µg/l)
 Intraassay: CV = 3.5 %
 Recovery: 95 %

Quetiapine

Limit of quantific.: 10 µg/l
 Linearity: up to 3000 µg/l (therapeutic range: 40–400 µg/l)
 Intraassay: CV = 3.0 %
 Recovery: 90 %

Promazine

Limit of quantific.: 5 µg/l
 Linearity: up to 3000 µg/l (therapeutic range: 100–500 µg/l)
 Intraassay: CV = 4.0 %
 Recovery: 80 %
 Runtime: < 20 min

For the Chromsystems HPLC analysis of Olanzapine in serum/plasma any isocratic HPLC system with UV-VIS detector is suitable.

tion column. Draw complete sample through column by centrifugation or suction, discard effluent.

> Draw 1 ml Wash Buffer 1 followed by 1 ml Wash Buffer 2 through the column by centrifugation or suction, discard effluent.

> Elute with 400 µl Elution Buffer.
 > Inject 50 µl of the eluate into the HPLC system.

Ordering information

Order no. Product

26000 Reagent Kit for the HPLC Analysis of Olanzapine, Quetiapine, and Promazine in serum/plasma For 100 analyses

Chromsystems Controls (lyoph.):

0147 Olanzapine Serum Control, Bi-Level (I + II), 2 x 5 x 5 ml
 0148 Olanzapine Serum Control, Level I, 10 x 5 ml
 0149 Olanzapine Serum Control, Level II, 10 x 5 ml

Sample Preparation

> Precondition the solid phase extraction column with 1 ml Equilibration Buffer 1 followed by 1 ml Equilibration Buffer 2.
 > Apply 100 µl Internal Standard and 1 ml serum/plasma to the extrac-

Porphyrias

The necessity of porphyrin profiling

The heme molecule is an iron-containing prosthetic group of many proteins such as haemoglobin, myoglobin and the cytochromes. Its importance lies in its capacity to bind oxygen. Heme is formed from succinyl coenzyme A and glycine in an eight-step reaction chain. The two principal places where heme biosynthesis takes place are erythroid cells (preliminary stages of the erythrocytes) and the hepacytes of the liver. Eight enzymes are involved in the actual synthesis. These enzymes successively transfer the heme precursor molecules – which are termed porphyrins – into heme. The synthesis of these enzymes can be impaired by genetic defects, such mutations being in fact hereditary. A misfunction of one of these enzymes leads to an accumulation of porphyrins. This pathological accumulation is termed porphyria and has different forms (see table).

The most frequent acute porphyria is due to uroporphyrinogen synthase

ILLNESS	ENZYME DISORDER	COURSE
Porphobilinogen synthase defect porphyria	Porphobilinogen synthase	acute
Acute intermittent porphyria	Uroporphyrinogen synthase	acute
Congenital erythropoetic porphyria (Morbus Günther)	Uroporphyrinogen cosynthase	
Porphyria cutanea tarda	Uroporphyrinogen decarboxylase	chronic
Hereditary coproporphyria	Coproporphyrinogen oxidase	acute
Porphyria variegata	Protoporphyrinogen oxidase	acute
Protoporphyria	Ferrochelatase	

The primary hereditary porphyrias

deficiency. The symptoms of this illness are attacks of colic and neurological dysfunctions. Other porphyrias affect the skin (e.g. photodermatitis) or produce neurovisceral symptoms (e.g. abdominal cramp, heart/circulation disorders, neuropathies). As the clinical pictures are very similar an exact diagnosis is required. The identification of the type of porphyria is possible by means of a differential

diagnosis which establishes which porphyrin is being accumulated. This makes it possible to ensure that correct therapeutic measures are taken, particularly in acute situations.

The Chromsystems HPLC reagent kit for the analysis of porphyrins in urine serves to draw up a complete profile of all urinary porphyrins required for a differential diagnosis.

For this, a binary gradient HPLC system with fluorescence detection is necessary. The kit has an analysis time of 18 minutes, making it possible to carry out highly specific and rapid examinations. The unique internal standard of this product elutes after approx. 9 minutes, in the same range of retention times as the analytes. Thus runtime as well as analytical fluctuations are minimised.

Oxidation of the urine sample is not necessary as the porphyrinogens will already have been oxidised spontaneously, through the oxygen in the air, into the fluorescent porphyrins. Two different levels of controls for quality assurance complete the product.

Ordering information

Order no. Product
 44000 Reagent kit
 44100 HPLC column
 0145 Control Level I
 0146 Control, Level II

Behind the Scenes at Chromsystems

Part 1: The dispatch/warehousing department



Dear Reader, our new series of articles entitled "Behind the Scenes at Chromsystems" is designed to tell you more about Chromsystems and what happens in our company. Every issue of DIALOG will focus on one particular department – ranging from order processing, dispatch/warehousing and research and development to production, quality management and sales and marketing. In short, we invite you to take a walk round our company.

Welcome to Chromsystems!

The dispatch/warehousing department

In the basement of Heimbürgstrasse 3 is the Chromsystems warehouse and dispatch department. Dispatch, where all the company processes finally come together, is responsible for the proper packing of any product and for its correct delivery - thus making its own particular contribution to the company's image. Chromsystems delivers its products to 60 countries, and in 13 years the dispatch department has built up a great deal of experience, enabling it to take special account of various details relating to a range of different countries. We believe that correct, punctual deliveries are of central importance: a wide variety

of import regulations have to be observed, and it is here that organisation plays an important part. 95% of all incoming orders are processed - i.e. packed and dispatched - on the day they arrive, and that is something our dispatch department is proud of.

Ursula Kuc has been managing our warehousing activities for seven years now. She knows every product and every packing unit – and the organisational skills which she and her colleagues have can usually be found behind every Chromsystems promise to dispatch special sizes and out-of-the-ordinary filling quantities to addresses in Europe or overseas. Routines and time schedules are tight, which means that everybody has to have a strong sense of responsibility – but the working atmosphere is unfailingly good, with the staff in the warehouse dealing with enquiries (always welcome!) in a friendly way, even when they have to work beyond normal daily hours. Together with her colleagues *Ursula Kuc* is responsible not only for packing and dispatching kits, but also for a range of other tasks. These include receiving incoming deliveries, distributing goods

within the company, purchasing, raw materials management, equipment maintenance and part of the production.

The move to new premises in July 2002 brought a number of benefits for the warehouse. Storage capacity for finished products was quadrupled, and the raw materials store now has more space. There is now twice as much space for packing kits, and staff have better connections to the company's IT network. The rooms are bright and friendly and all staff have their own lockers. In future, too, there will be innovations for the department, with further automation and an expansion of the IT system.

So the next time you get a package from Chromsystems with your latest delivery - you'll know who's behind the dispatch/warehousing department.

Best wishes for the summer!



Dates

In the second half of 2004 Chromsystems will be represented at the following international and national fairs:

8–11 June 2004
MedLab 2004,
Padova, Italy

27–29 July 2004
AACC Clinical Lab Expo 2004,
Los Angeles, USA

1–3 September 2004
TDM and Pharmacogenetics of
Psychotropic Drugs,
Lausanne, Switzerland

13–15 September 2004
International Conference on Vita-
mins 2004, Pardubice, Czech Rep.

7–9 October 2004
II. National HPLC Symposium,
Ankara, Turkey

4–6 November 2004
Journées International de Biologie,
Paris La Défense, France

24–27 November 2004
Medica 2004, Messe Düsseldorf,
Germany

News

Chromsystems will be certificated by August 2004 according to DIN EN ISO 9001: 2000 and DIN EN ISO 13485: 2003.

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