ABSTRACTS - POSTER

P1 Zum Nachweis von Alkylphosphaten im Urin bei Organophosphat-Intoxikationen

On the Detection of Urinary Alkylphosphates in Humans Poisoned with Organophosphate Pesticides

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Organophosphate compounds are widely used as insecticides. After uptake organophosphates decompose in humans into alkylphosphates in vivo and post mortem as well. A significant depression of unspecific serum cholinesterase activity is used as an indicator for organophosphate poisoning in vivo.

The detection of urinary alkylphosphates gives no verfication of a particular organophosphate compound but confirms an intoxication with organophosphates. We give a description of a method for the quantitative measurement of the following metabolites: O,O-dimethylphosphate (DMP), O,O-diethylphosphate (DEP), O,O-dimethylthiophosphate (DMTP), O,O-diethylthiophosphate (DETP), O,O-dimethyldithiophosphate (DMDTP), O,O-diethyldithiophosphate (DEDTP).

Outline of the method: Urin is dried under azeotropic conditions using isopropanol and nitrogen. The metabolites are treated successively with benzyl bromide and diazotoluene to form their corresponding benzyl esters. Benzyl bromide is commercially available and diazotoluene is prepared in two steps. First benzaldehyde and toluenesulphonic hydrazide give the appropriate hydrazone at ambient temperature, recrystallized in methanol. Afterwards alkaline hydrolysis of the hydrazone forms diazotoluene using benzyltriethylammonium chloride as phase-transfer-catalyst. The thio- and dithiophosphates are esterfied first using benzyl bromide followed by the esterfication of DMP and DEP with diazotoluene. This procedure obviously prevents isomerisation of DMTP and DETP which occurs when diazotoluene is used as sole reagent. The derivates are purified on SPE silica columns. The measurement is done using GC/MS.

Identification of all metabolites is possible by the parent molecular ions. The limit of detection is less than 150 ng/ml for all six metabolites in the full scan mode and thus the method is sufficient for detecting and quantifying these metabolites in cases of poisoning.

P2 Die schnelle Identifizierung von Arzneistoffen in biologischen Matrices mit LC-MS Iontrap und einer Spektrenbibliothek

Rapid identification of drugs in biological matrices with LC-MS Iontrap and a spectral library

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The identification of drugs and their metabolites in analytical toxicology is mostly done by GC and GC-MS with commercially available spectral libraries. LC-MS as a complementary technique has already shown its power in qualitative and quantitative analyses especially on polar drugs and drug conjugates. With the API techniques most of the drugs and their metabolites are detectable in biological matrices without derivatization step. Due to the fact that the fragmentation behaviour of quadrupol instruments, both in-source or in the collision cell, is dependent on the chromatographic conditions and the LC-MS system, the usage of spectral libraries with these systems is not very attractive.

We investigated the use of the Agilent LC-MSD Iontrap for a general unknown screening in serum and urine. The target compounds that we used were diazepam, midazolam, medazepam, desmethyldiazepam, clonazepam, trimipramine, thioridazine, LSD and haloperidol.

Therefore we developed a generic chromatographic gradient system with a ZORBAX C18 column and 0.1 % acetic acid and methanol as mobile phase. The ESI source in positive mode was used and the Iontrap mass analyzer acquired spectral data simultaneously in full scan mode and in data-dependent auto-MS mode. In the spectral library both MS and MS/MS spectra of each compound were stored. Serum and urine were spiked with several single compounds or a drug cocktail at concentrations of about 100 ng/mL, and for LSD of about 25 ng/mL. Sample work-up for serum was done by a single protein precipitation step and for urine by diluting with mobile phase.

With this approach all compounds in all matrices could be undoubtedly detected by the combined information of MS and MS/MS spectral data and the retention time. LC-MS Iontrap with a spectral library can be very powerful in the screening of unknowns in biological matrices. This concept has to be further investigated on a broader range of drugs and drug metabolites.

P3 Bestimmung von p-Phenylendiamin in postmortalem Blut und Mageninhalt

Determination of p-phenylenediamine in postmortem blood and gastric contents

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p-Phenylenediamine is mainly used for manufacturing azo dyes, drugs, and photochemical developers, for accelerating vulcanization, and dyeing furs. p-Phenylenediamine dihydrochloride is the major component of many hair dyes and is used in dairy industry as a reagent to control the effectiveness of pasteurization.

While handling the substance subacute poisoning can be caused by skin contact, inhalation or swallowing. Cases of acute poisoning are rare and exhibit symptoms of haemolysis, methaemoglobinaemia, and liver and kidney failure.

The analytical procedure for determination of p-phenylenediamine in biological material is presented. A liquid-liquid extraction method was used to isolate p-phenylenediamine from postmortem blood and gastric contents. For screening, identification and quantitative determination thin-layer chromatography (TLC), ultraviolet spectrophotometry, HPLC, and GC/MS were applied.

In a case of fatal poisoning with p-phenylenediamine the concentration in postmortem blood was 1.58 mg/mL, the absolute amount in gastric contents was 319 mg.

P4 Cocain-Screening im Haar durch Diagnostix Singlestep™ ELISA

Screening for Cocaine in Hair by Diagnostix Singlestep™ ELISA

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Elisa tests for hair examination on cocaine were already used by Segura (1999). The purpose of the study was to find out weather the Diagnostix Singlestep test on cocaine can be used as a pre-test for GC/MS examination of hair on cocaine. 50 hair were extracted with 4 ml methanol using an ultrasonic bath. After evaporating the solvent the residue was reconstituted in buffer (pH=7.4) of which 20 µL were transferred to the microtiter plates. 30 different negative hair samples were tested to determine the matrix corrected cut-off. Using this value 30 GC/MS positive hair samples with the lowest concentration of 0,1 ng/mg were tested. Even hair with such low concentrations showed a significantly positive result with the Diagnostix Singlestep test. After these experiments it can be stated that the test can be used for pre-testing hair in cases of driving ability in order to avoid unnecessary and time-consuming GC/MS procedures.

Segura J. (1999). J Chromatogr B 724: 9

P5 Quantitative Bestimmung von Sulpirid und Amisulprid im Blut mit HPLC und Fluoreszenz-Detektion.

Quantification of Sulpiride and Amisulpride in Blood by HPLC and Fluorescence Detection

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The method is based on a procedure published by Tokunaga et al. in 1997. It was found that the re-extraction could be left out for the routine extraction which is proved by the described cases. 1 ml serum, plasma or whole blood were diluted with 3 ml of 1 M sodium hydrogen carbonate and 16 ml of distilled water. After extraction on a Chromabond® C18 ec column (15 ml) the organic phase was evaporated to dryness, the residue was directly reconstituted with mobile phase (acetonitril/1 M potassium dihydrogen phosphate-buffer pH=3.0, 15/85) and injected. The LOQ was determined with 0.01 mg/L, the linearity was above r= 0.999 in a range from 0.05 to 0.6 mg/L (therapeutic range). Blood concentrations of drug impaired drivers ranged from 0.01 to 0.68 mg/L

sulpiride, amisulpride was found in a concentration of 0.16 mg/L in one case. Also 7 cases of sulpiride related death are presented, two of them with monointoxication of sulpiride and blood levels up to 1.8 mg/L.

Ref: Tokunaga H., et al. (1997) J Chromatogr B 691: 203-207

P6 Erhöhung des Injektionsvolumens bei der Kapillargaschromatographie durch Druckstoßinjektion

Increased Injection Volume in Capillary Gas Chromatography by Pressure Pulsed Injection

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Using capillary gas chromatography, various sample introduction techniques (e.g. PTV Large-volume injection, on-column large-volume injection [1]) are available for increasing the injection volume. Pressure pulsed splitless injection, while not being the most capable technique (the maximum injection volume is limited to about 5 µl), is based on the "standard" injection system of capillary gas chromatography. The aim of this study was to show the possibilities of this technique for toxicological analysis.

Blood and urine samples were analysed using cannabinoides (THC, 11-OH-THC, THC-COOH) as model compounds. The sample preparation includes SPE (C18 cartridges) and a methylation procedure [2]. The analytes were separated on HP-1 (Hewlett Packard, 15 m x 0,25 mm i.d., 0,25 µm film thickness) and detected in SIM-Mode (HP 5890/II GC, MSD 5871). Using pressure pulsed injection, the inlet pressure was 250 kPa (Helium) during splitless time (1 min).

Standard solutions were injected to optimise the operating conditions (p, T, splitless time). By using pressure pulsed injection, the relationship between injection volume and peak area was linear up to 5 µl, so the detection limit can be reduced proportional to the injection volume. In the case of real samples, however, the influence of matrix components leads to an increased background level.

A further target was to reduce sample consumption. By increasing the injection volume from 1µl up to 5 µl it is possible to minimise the required material (blood or urine) from 0,5 ml to 0,1 ml. The same performance parameters were achieved using a slightly modified sample preparation procedure.

The results of this study demonstrate the potential of pressure pulsed injection. It is possible to reduce the sample volume required, if only low sample amounts are available or several target analyses are necessary. Lower detection limits can eventually be achieved.

[1] W. Engewald, J. Teske, J. Efer; Journal of Chromatography A 842 (1999)143 -161.

[2] Th. Daldrup, F.Mußhoff, in: Analytiker Taschenbuch Bd. 13, Editors: H. Günzler et al., Berlin, Heidelberg, New York: Springer 1995, p. 210

P7 Konzentrationen von Fettsäureethylestern in Haarsegmenten und Trinkverhalten

Fatty acid ethyl ester concentrations in hair segments and drinking behavior

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Fatty acid ethyl esters are products of the non-oxidative ethanol metabolism. They are deposited in hair and can be analyzed from this matrix by headspace solid-phase microextraction (HS-SPME) and GC-MS [1]. Segmental hair analysis for these compounds should be a suitable way of a retrospective and time-resolved control of the drinking behavior. Furthermore, from the distribution along the hair shaft some information about the deposition and elimination mechanism should be obtained.

Therefore, hair samples from alcoholics, who were in a hospital for withdrawal treatment, from abstinent volunteers and from occasional drinkers were divided into 3 to 12 segments. Each segment was twice washed with n-heptane and then 14 h extracted with an n-heptane/dimethyl sulfoxide mixture. The washing solutions as well as the extracts were analyzed for ethyl myristate, ethyl palmitate, ethyl oleate and ethyl stearate. The washing solutions should mainly contain esters from sebum excreted the last days before sampling whereas the esters from hair extracts should originate from earlier deposition. The concentrations were compared with the self-reported data about the drinking behavior during the last 12 months and the kind and frequency of hair care and hair cosmetics. Beside the total concentration also the concentration ratio of the four esters was interpreted.

From the results follows that the esters are mainly incorporated into hair from sebum. Caused by an accumulation effect, in cases with steady drinking behavior the concentrations increase generally from proximal to distal segments. A two weeks relapse of excessive drinking after two years abstinence leads to positive results over the whole hair lengths. On the other hand, the deposition continues several months after beginning of abstinence and only slowly decreases. In general excessive drinkers have much higher concentrations than occasional drinkers. However, also in hair of some strict teetotalers (children and Moslems) small concentrations of the esters were measured.

[1] F. Pragst, V. Auwärter, F. Sporkert and K. Spiegel: Analysis of fatty acid ethylesters in hair as possible markers of chronically elevated alcohol consumption by headspace solid-phase microextraction and gas chromatography-mass spectrometry. Forensic Sci. Int. (2001), in press.

P8 Bestimmung von Ethlyglucuronid in Haaren mittels GC-MS/NCI

Determination by GC-MS/NCI of ethylglucuronide in hair

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Ethylglucuronide (EG) is a minor non volatile ethanol metabolite which was first detected in serum, urine and hair by Aderjan and Skopp. EG was found to be a marker of alcohol consumption that can be detected after total elimination of ethanol from body. In these studies EG was determined after acetylation by GC/MS operating in EI mode.

In our study in order to improve the limit of detection (LOD) we developed a method to detect EG by GC/MS operating in negative ionisation (NCI) mode. We analysed hair specimens of 23 persons, 17 of these specimens were taken from persons at autopsy where alcohol was found positive in blood, the other 6 specimens were provided from occasional alcohol consumers. Hair was washed by water and acetone, and then pulverized. After several assays varying the incubation time and the incubation solution, the best extraction was found to be an overnight incubation in a mixture of methanol/water (1/1). After centrifugation, the supernatant was evaporated and after derivatisation with pentafluoropropionic anhydride the residue was injected into a GC/MS operating in a NCI mode. EG (m/z=496, 342) was determined in selected ion monitoring using the EG-d_4 as internal standard. The recovery was about 60 % and the LOD was 31 pg/mg hair.

In the control hair specimens of occasional users no EG was detected. In the hair specimens taken from persons at an autopsy, 9 samples were found positive with EG concentrations ranging from 0.062 to 5.8 ng/mg hair, in 3 samples only traces were found (concentration < LOQ), whereas in 6 samples no EG could be detected in hair in contrary to the alcohol findings in blood. Our results point out that the use of the NCI mode in GC-MS significantly improves LOD for EG in hair and confirm that the determination of this ethanol metabolite in hair provide complementary information concerning excessive chronic alcohol consumption.

P9 Pilotstudie zur Kreatinin-bezogenen Nomierung von Ethylglucuronid (EtG)-Konzentrationen im Urin

A Pilot Study on Creatinine-corrected Ethyl Glucuronide (EtG) Concentrations in Urine

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The determination of EtG in urine becomes increasingly important for alcohol abstinence and relapse monitoring during treatment of alcohol disease. Because of the possibly extreme water uptake and a rapid change in drinking behaviour during clinical treatment, it seems necessary to relate the actual EtG concentration to the individual creatinine excretion. Therefore, in two experiments with healthy volunteers, we investigated the creatinine dependence of the renal EtG elimination. In addition, this experiment seemed important for the determination of the individual ratio between alcohol dose and the total EtG amount excreted in urine.

One of the volunteers consumed 0.12 L of wine within 0.5 hours (ethanol dose: 11 g = 0,19 g/kg body weight). In the elimination phase of EtG, after 1.25 hours, she took up 0.5 L of water within 30 min. The other volunteer consumed 0.5 L of wine (ethanol dose: 45 g = 0,75 g/kg body weight) within 1.5 hours and after 9.5 hours she took up 0.7 L of water within 30 Minutes. From each of the two volunteers, within 11 and 20 hours from the start of drinking, we collected seven and twenty-two urine samples, respectively. The analytical measurements

were performed using in-house procedures, using the Jaffe´-method for creatinine determination, LC/MS/MS for EtG determination and the ADH-method for ethanol determination.

Due to taking up water, the EtG concentration in urine markedly decreased within thirty minutes, however, the EtG elimination re-normalised within the following 30 Minutes. The standardisation the EtG concentrations to an average value of 100 mg/dL creatinine showed, that the water intake had no influence on the absolute EtG excretion. At the end of monitoring period for this experiment, the EtG elimination was still on going.

For the investigation of individual EtG kinetics, it is important to know, whether or not, the EtG elimination is influenced by forced diuresis and extensive drinking of beverages or water. The EtG determination can be used as an alcohol abstinence marker. However, it is necessary to additionally analyse the creatinine excretion, to exclude "false negative" concentrations due to internal dilution. Experiments with 10 test persons in which the ratio between ethanol dose and total EtG excretion in urine is determined under distinct circumstances are in progress.

P10 Die Geschichte von "Smarnica"
The story of "smarnica"

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It is a widespread belief among people in Slovenia that wine made from free-growing vines - the so called "smarnica" – contains large amounts of methanol, and that drinking it brings on a kind of psychotic state. Even in chemistry textbooks, "smarnica" is mentioned as a drink rich in methanol. This should be the main reason why Article 31 of the Wine and other Grape and Wine Products Act (Official Gazette RS, no. 70/97) prohibits trade in wine made from free-growing grapes and other alcohol-containing products made from such grapes. Many square meters of vineyards were planted with it and are now substituted with vintage vines. However "smarnica" can grow and give a high agricultural produce without treatment with pesticides. Methanol is not a by-product of alcoholic fermentation. It is formed by demethylation of pectin with the help of esterases. Fruits-different juices, also freshly pressed, often contain methanol. Higher methanol concentration can be expected when during the fermentation fruits remain in the fermenting vat.

Our measurements over the past years have confirmed that the content of methanol in samples of wine from free-growing grapes is medium high and comparable to the methanol content in vines from cultivated varieties. The general progress in methods of wine production (cold pressing, lowering of mashing time) may have contributed to this.

The kinds of psychotic state reported after drinking "smarnica" may have had the roots in alcoholic psychoses which are usually developed after several years of heavy drinking. The question if "smarnica" contains any other psychotropic substances is still open.

P11 Trunkenheit am Steuer nach Einführung der Atemalkoholanalyse
Drunk Driving after the introduction of breath alcohol analysis

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The effect of preventive provisions by introduction of Breath Alcohol Analysis (BAA) was studied. In order to investigate the effect of BAA introduction, the Blood Alcohol Concentration (BAC) determined for two periods (before and after of introduction) were collected, Average Values, Median, Frequency Profile were calculated and compared.

BAC investigations were carried out by means of gas chromatography simultaneously on two packed columns (6.6% Carbowax 20M, 80/120 Carbopack BAW; 0.3 % Carbowax 20M, 80/100 Carbopack C) with use of headspace technique. The result of the study shows that the introduction of BAA in Middle-Transdanubian brought no relevant change in the level of intoxication of people driving under the influence of alcohol.

P12 Methodenoptimierung zur Detektion von THC-COOH und dessen Glucuronid mittels LC/MS/MS mit APCI und ESI

Optimisation of methods for the detection of THC-COOH and its glucuronide by LC/MS/MS using APCI and ESI

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For „Workplace Drug Testing", „Driving under the influence of Drug" and for forensic cases the confirmation of immuno-assay tests results on cannabinoids in urine is necessary. This is commonly performed by GC/MS after liquid/liquid or solid phase extraction (SPE) and derivatization - with a NIDA cut-off of 15 ng/mL, but can also be performed with a rapid method by LC/MS/MS using negative APCI. This method has been recently developed in our laboratory [1]. Furthermore, a rather time-consuming LC/MS/MS method has been developed for the differentiation of THC-COOH and its glucuronide-conjugate [2] using positive ESI with a turboionspray-source, with the disadvantage of lower sensitivity than APCI for THC-COOH. However, with APCI the glucuronide is fragmented to its aglycon; this was the reason for using ESI instead of APCI. Aim of this work was to give a comparison of the APCI and ESI technique for further method development, with respect to sensitivity for both, THC-COOH and its glucuronide.

LC/MS/MS was performed using a triple-quadrupole-MS (Sciex API 365) with APCI and turboionspray sources. Post-column addition of solvent was used to increase the compatibility of HPLC-eluents and flow-rates with the ionisation-process (addition of methanol for APCI).

A sensitive and rapid method for the simultaneous detection of THC-COOH and THC-COOH-glucuronide in urine samples is presented. Although the differentiation of THC-COOH and THC-COOH-glucuronide is not of forensic importance in urine samples, it may play a role for the interpretation of cannabinoid-concentrations in serum.

[1] Weinmann W., Goerner M, Goerke R., Vogt S., Svoboda M., Schreiber A.: Rapid confirmation of 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) in urine by LC/MS/MS using negative APCI. Proc 48th Conf Mass Spectrom Allied Topics (ASMS) Long-Beach, 2000, Log-No. 877.

[2] Weinmann, S. Vogt, R. Goerke, C. Müller, A. Bromberger, Simultaneous determination of THC-COOH and THC-COOH-glucuronide in urine samples by LC/MS/MS. For. Sci. Int. (2000) 113, 381-387.

P13 Systematische Analyse von Muskelrelaxantien mit quarternärem Stickstoff im Vollblut mit Ion Trap LC-(ESI)MS. Zwei Fälle

Screening for neuromuscular blocking drugs (quaternary amines) in whole blood by ion trap LC-(ESI)MS-method. Two case reports

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To develop a method for the unequivocal identification and quantitation of quaternary nitrogen muscle relaxants in biological matrices. We studied the applicability LC-(ESI)MS (ion trap) to screening whole blood for the presence of rocuronium, pancuronium, vecuronium, mivacurium, atracurium and suxamethonium.

Blood samples were extracted by using SPE on BondElut C18 HF columns. Elution was accomplished by methanol, containing 0.1 M acetic acid. The HPLC system consisted of a C18 Inertsil[?] column and a mobile phase of ammonium acetate buffer/acetonitril (gradient 5-60% acetonitril). The MS detection was optimized for each compound by auto tuning the ion optics and tuning the ESI source settings manually. Where MS/MS was used to identify a compound, the collision energy was optimized.

Extraction by SPE was simple and efficient. Extraction recoveries were over 75% for all compounds. HPLC was able to separate all compounds, except mivacurium and atracurium. Final identification of the compounds was achieved by using the MS or MS/MS data. MS settings were changed twice during the run. Application of universal settings for all compounds led to a poor detection limit for suxamethonium. The eventual limits of detection in whole blood varied between 0,01 and 0,1 mg/l.

The method was applied in two cases. These were a case of homicide or suicide with Pavulon[?] (pancuronium; detected in blood and tissues) and a case of a possible mix-up of injections in a hospital (blood, urine and injection fluids analyzed).

The system described is suited to identify therapeutic concentrations of quaternary neuromuscular blockers in whole blood. Full-scan mass spectra of the compounds showed molecular ions and MS/MS spectra showed fragments typical for of the particular compounds. LC-ESI-MS allowed an unequivocal differentiation of all muscle relaxants involved.

P14 Trisialo-Fe_2-transferrin verbessert nicht die diagnostische Aussagekraft des Kohlenhydrat-defizienten Transferrins (CDT) als Marker chronisch excessiver Alkoholaufnahme

Trisialo-Fe_2-transferrin does not improve the diagnostic accuracy of carbohydrate-deficient transferrin (CDT) as a marker of chronic excessive alcohol intake

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We wanted to assess whether (a) including trisialo-Fe_2-transferrin in carbohydrate-deficient transferrin (CDT) and (b) using absolute or relative CDT concentrations affects the diagnostic accuracy of CDT as a marker of chronic excessive alcohol intake.

Criterion standards for diagnosis of alcoholism and alcohol intake: Composite International Diagnostic Interview (CIDI) and Time Line Follow Back (TLFB). Study groups (alcohol intake in the last 4 weeks before blood sampling): 24 controls (≤280 g/week, no alcoholism), 17 hazardous drinkers (>280 g/week, no alcoholism), 53 alcoholics (>280 g/week, alcoholism diagnosis). CDT analysis: %CDTri-TIA, including about 50% of trisialo-Fe_2-transferrin in and ChronAlcoI.D. excluding this transferrin isoform from CDT.

Results: Depending on the cut-offs for the CDT/transferrin ratio (upper or lower limit of the test-specific borderlines) and on the patient group: *Diagnostic sensitivity*: 18-70% (%CDTri-TIA), 41-82% (ChronAlcoI.D.). *Diagnostic accuracy*: 63-75% (%CDTri-TIA), 70-84% (ChronAlcoI.D.). Compared with %CDTri-TIA, ChronAlcoI.D. showed higher diagnostic sensitivities and accuracies (significantly increased only in alcoholics). *Diagnostic specificity* (88-96% for both tests) and *areas under the ROC curve* (0.611-0.980) were not significantly different between the two tests. Absolute CDT concentrations (ChronAlcoI.D.) showed a similar diagnostic specificity, but significantly reduced sensitivity and accuracy compared with CDT/transferrin ratios (ChronAlcoI.D).

The present study and data from the literature indicate that including (parts of) trisialo-Fe_2-transferrin (by the %CDTri-TIA) reduces the diagnostic sensitivity and thus accuracy of CDT as a marker of chronic excessive alcohol use. Compared with absolute CDT concentrations, CDT/Tf ratios show an overall improved diagnostic accuracy.

P15 Bestimmung von 27 Benzodiazepinen in Serum mittels HPLC/DAD und automatisierter SPE

Determination of 27 benzodiazepines in serum with HPLC/DAD and automated SPE

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The aime of the study was to develop a method for extraction and determination of 27 Benzodiazepines and their metabilites for several purposes like TDM (antiepileptika), clarification in case of intoxication or pharmakokinetic studies.The work was performed on a Varian HPLC equipment. Evaluation was done with Varian Star PolyChrom / PolyView Software. Serum containing Lorazepam glucuronides was hydrolysed before extraction.

Solid phase extraction was performed with C2-Prospekt cartridges followed by conditioning with methanol and phosphatebuffersolution. After injection of the sample (200µl) a washing step was performed with phosphatebuffersolution and elution by a gradient containing phosphatebuffer and acetonitril on the analytical column (Spheri 5 RP-8 from Brownlee-Column, saved by a RP-2 Newguard precolumn). The chromatograms were detected at 254 nm, spectras with a wavelenght range from 210 to 350 nm.

The quantification and identification of the Benzodiazepines were made by comparison with library registred spectras of the drugs in defined concentrations. This HPLC / DAD method allows separation and quantification of almost all benzodiazepines and their metabolites, which are available in Europe. The detection limit of all analysed drugs is low enough to measure in the therapeutic range for each drug and perform TDM. Following, the detection limit of some drugs: Clonazepam 0.005mg/l, 7-Amino-Clonazepam 0.05 mg/l, 7-Acetamido-Clonazepam 0.01 mg/l; Diazepam 0.01 mg/l, Desmethyl-Diazepam 0.01 mg/l, Oxazepam 0.025 mg/l, Flunitrazepam 0.01 mg/l, Desmethyl-Flunitrazepam 0.005 mg/l, 7-Amino-Flunitrazepam 0.025 mg/l, 7-Acetamido-Flunitrazepam 0.0025 mg/l und 3-OH-Flunitrazepam 0.01 mg/l. The interassay reproducibility with 19 injections

of the same mix of 5 drugs over a period of 4 months, presented recoveries between 66 and 124%, with standard deviations between 0.031 and 0.156 and variation coefficients of 11.5-17.3%. After more than 1000 injections with the same analytical column, the results are still satisfactory.

P16 Drogenscreening in Plasma und Urin mit abselut™ NEXUS

Drug Screening in Plasma and Urine with abselut™ NEXUS - The SPE column involved in the drug discovery market and racing industry

Elisabeth Korte
Varian Deutschland GmbH

Broad-spectrum drug screening is frequently required in forensic and clinical toxicology, and doping control in racing industry. Solid phase extraction (SPE) has become the technique of choice for sample work-up, even for difficult samples like horse urine, which is often limited by the tendency to block the sorbent bed. The aim of this work was to test the use of a single SPE sorbent, abselut™ Nexus, as a universal sorbent for the extraction of drugs from plasma, human and horse urine.

The abselut ™ Nexus sorbent was designed to extract a wide range of pharmaceuticals from biological fluids while providing high, reproducíble recoveries. It is a highly crosslinked spherical polymer with a unique combination of hydrophilic and lipophilic moieties. Elimination of the conditioning step and optimisation of elution means that one method can be applied to a wide range of analytes. In addition, the high binding capacity of abselut ™ Nexus allows small bed volumes of 30 mg and 60 mg to be used.

The data indicate good recoveries for basic, neutral, and weakly acid drugs from urine and plasma. Recoveries for a range of anabolic steroids from horse urine were generally equal or better than those obtained on a C18 column. The result is a major improvement compared to the C18 sorbent, where blocking is frequent.

Abselut ™ Nexus can be used without conditioning, which will save time and solvent. The performance was not affected when the sorbent run dry.