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16ème congrès annuel de la Société Française de Toxicologie Analytique (SFTA), 46ème congrès de The International Association of Forensic Scientists (TIAFT), & congrès annuel de la Society of Hair Testing (SoHT)

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Selective buprenorphine quantitation in blood and hair samples by LC-MS/MS detection of its N-methylpyridyl-derivatives

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Introduction: The potential of LC-MS in forensic toxicology is undisputed and includes substances of various pharmaceutical classes and chemical structures. Ionisation efficacy (e.g. electrospray ionisation) of toxicologically relevant compounds is mostly rather high due to the frequent presence of polar substituents. The comparatively low sensitivity of LC-MS detection of certain lipophilic compounds (e.g. steroids) lead to attempts to improve ionisation by chemical modification of the analytes. Derivatisation reactions using 2-hydrazino-1-methylpyridine (HMP), p-nitrobenzoyl chloride (NBC) and tris(2,4,6-trimethoxyphenyl)-phosphonium propylamine bromide (TMPP) may be applied to convert keto-, phenolic hydroxy- and carboxy- groups, respectively.
Methods: In the present study, the formation and LC-MS identification of buprenorphine-pyridyl-derivatives is described. Owing to several modifications of the morphinane structure (e.g. N-cyclopropylmethyl-substitution, ring closure by introduction of an 6-14 etheno-bridge) buprenorphine becomes rather unpolar and the molecule undergoes comparatively little fragmentation resulting in low product ion abundances in tandem mass spectrometry. In contrast, respective derivatives of buprenorphine form a series of selective and intense fragments.
Results: Detection limits and specificity of the buprenorphine derivatives were superior to those of unchanged buprenorphine under otherwise comparable LC–MS/MS conditions in serum and hair matrix. A detection limit of 0.1ng/mL in serum (based on a sample volume of 500µL) may be achieved. The stability of pyridyl derivatives was found to be sufficiently high in various solvents and permits to establish robust and reproducible buprenorphine quantitation procedures, e.g. in extracts from blood and hair samples.
Conclusion: Structural modification of substance properties by derivatisation –which is almost a default sample preparation in GC-MS– seem to have a considerable potential to enhance the sensitivity and specificity of LC-MS experiments in forensic toxicology.

Use of LC-MS/MS screening for hair analysis: application to detection of methadone in hair

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Introduction: Methadone is a widely used drug for the treatment of moderate to severe pain and for opioid dependency. This drug is widely abused largely by persons on heroin maintenance programs and is sometimes associated with administration to children for sedation. We present a series of cases in which methadone was detected in hair using LC-MS(MS).
Methods: For extraction of methadone, approximately 20-50 mg of washed hair was incubated with 2 mL methanol overnight at 60ºC. The methanol was transferred into a glass tube and evaporated to dryness. The extracts were reconstituted in mobile phase and injected into a LC-MS/MS. The extracts were separated on an Agilent Eclipse XBD C18 (4.6x150 mm, 5 μm particle size) using ammonium formate (pH 3.5, 50 mM) buffer and 1% formic acid in acetonitrile and gradient analysis. Methadone and other drugs of abuse were detected using an Applied Biosystems 3200 Q-Trap mass spectrometer coupled with a Turbo Ion Spray source and operated in MRM mode using 3 transitions per analyte. Methadone was detected using the following transitions: 310→265; 310→105, and 310→77; for detection of EDDP the transitions 278→234, 278→249, and 278→186 were used. Alternatively extracts were also analyzed conventionally by GC-MS using a model 5972 Agilent system on a BP-5 capillary column.
Results: The method gave reproducible and sensitive detection for methadone and 29 other common drugs or drug metabolites**.** The LC-MS/MS method was validated for all substances in hair**.** The limits of quantification for methadone and EDDP were 0.1 ng/mg of hair, based on the lowest calibrator. Limits of detection for methadone and its main metabolite were below 0.01 ng/mg hair, determined using spiked hair samples. The precision for methadone and 29 other common drugs or drug metabolites at 0.25 and 25 ng/mg was found to be within 10 % coefficient of variation, accuracy was >90%. Four cases involving children suspected of unprescribed exposure to methadone showed the presence of the drug in concentrations ranging from the LOQ to 3 ng/mg, with an average of 1.2 ng/mg. In two cases segmental analyses showed presence of methadone in a number of 1 or 2 cm segments. In another two cases multiple segments were also positive. In some segments the metabolite (EDDP) was also detected in much lower concentrations. Other drugs were also detected in some of the cases suggesting multiple drug exposure. This presentation will provide details of the cases together with analytical performance data to support the use of LC-MS(MS) for the detection of drugs in hair extracts.
Conclusion: Hair analyses for methadone can provide some useful information on a case, however interpretation can be complex.

Detection of benzylpiperazine (BZP) in hair as part of a quantitative multi analyte drug screen using LC-MS/MS

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Introduction: Benzylpiperazine (BZP) is an amphetamine type stimulant, currently legally available in New Zealand and widely used in 'Party Pills'. Following consultation with an expert scientific committee, the NZ government proposed a reclassification to Schedule C (the same as cannabis), due to come into force at the end of January 2008. Many methods have been published for the analysis of drugs of abuse in hair, for this method we have included BZP with amphetamine type stimulants, opiate type drugs and methadone in a single assay.
Method: Hair samples are segmented into the growth periods of interest and cut finely before weighing. Each analysis needs one 20 mg sample to test for: amphetamine type stimulants (BZP, amphetamine, methamphetamine, methylenedioxymethamphetamine and methylenedioxyamphetamine); opiate type drugs (codeine, morphine and 6 acetyl morphine); and methadone. Internal standard (deuterated analogue of each drug) was added to each sample. A five point calibration curve was prepared for each analyte. Following soaking in HCl overnight the extract was applied to a solid phase extraction cartridge (Bond Elut Certify) and eluted using dichloromethane: propan -2-ol: ammonium hydroxide (80:20:2). The sample is then dried before reconstitution (in 100 µL of mobile phase) for analysis by LC-MS/MS. A single injection of 10 µL is applied to a Phenomenx Luna SCX column (150 x 2.0 mm) using isocratic elution with acetonitrile (75 %) and ammonium formate buffer (100 mM) + 0.5 % formic acid (25 %) as mobile phase. This assay has been used in various scenarios, such as investigation in drug facilitated sexual assault or in child custody cases.
Results: Linear calibration (<0.999) was seen for the range 0.2 to 50 ng/mg of hair analysed (based on 20 mg). Spiked samples of 1.25 ng/mg gave intra- and inter-day precision CVs of 5 % and 8 % respectively (n=6 per day, 3 days). Extraction efficiency was calculated to be 68 % and accuracy was 108 %. Results for all analytes were acceptable.
Conclusion: This paper presents a fully validated assay for the analysis of multiple classes of drugs from a single hair extract using a single LC-MS/MS injection. The use of the assay will increase when the legislation changes and BZP becomes controlled under law.
Keywords: hair, benzylpiperazine, LC-MSMS

Monitoring methylphenidate treatment in children and adolescents by hair and saliva testing

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Introduction: Methylphenidate (MPH) is a phenethylamine derivative used in the treatment of attention-deficit hyperactivity disorder in children and adolescents. It is known that there is marked individual variability in the dose-response relationship for methylphenidate, and therefore dosage must be titrated for optimal effect and avoidance of toxicity in each child. Therapeutic monitoring for this drug is essentially lacking and alternative biological matrices should be investigated for non-invasive assessment of short and long term recond of drug use. We sought to monitor MPH treatment in children and adolescents by hair and saliva testing.
Methods: Hair samples were obtained from 35 subjects, diagnosed for ADHD and in treatment for at least the last six months with different oral doses of immediate and controlled-release MHP (from 5 mg to 36 mg/day). Whereas possible, (hair shaft length more than 3 cm) segmental hair analysis was performed of subsequent 3 cm hair strands, each representing hair growth in subsequent three-month periods. Saliva samples were obtained from 4 subjects starting treatment with 20 mg controlled release MHP. Samples were collected to investigate the 24 hour kinetics once a week. Using 3,4-methylenedioxypropylamphetamine as internal standard, hair samples were overnight digested with 0.1M HCl at 37°C and MHP extracted with Bond-Elut Certify columns while saliva samples (500 μl) were added with 500 μl of acetonitrile, mixed, centrifuged and organic phase evaporated to dryness. A procedure based on liquid chromatography-mass spectrometry (LC-MS) was applied, using a reverse phase column and a mobile phase of 80% 10 mM ammonium acetate – 20% acetonitrile with a 15 min gradient program and the mass spectrometer in positive electrospray ionization and selected ion monitoring acquisition mode. The method was validated in the range 0.15-50 ng MPH/mg hair and 0.15-50 ng MHP/mL saliva.
Results: Preliminary results on hair segments showed that, even in presence high inter-individual variability in hair segments concentration of MPH for children treated with the same dose, the mean values of MHP (from 0.48 to 1.29 ng MHP/mg hair) in different hair sections well correlated (r^2= 0.7)with drug dosage (from 10 to 36 mg/day). Analysis of salivary samples gave 24 kinetic profiles of 20 mg controlled release MHP with a steady state mean concentrations ranging from 10.61 ng/mL to 1.65 ng/mL one to 9 hours after drug administration, respectively. At 12 hours concentrations decreased to a mean value of 0.16 ng/mL saliva.
Conclusion: These data indicate the possible use of segmental hair analysis to monitor compliance of subjects under long-term treatment with MHP and the use of saliva as alternative to blood for MHP monitoring and consequent dosage titration once therapeutic and toxic concentration ranges will be established in this biological matrix.
Keywords: methylphenidate, hair testing, saliva testing

Development and application of reference materials for the determination of methamphetamine and amphetamine in hair

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Introduction: Methamphetamine (MA) has received the most attention as a drug of abuse in Korea. Thus, hair analysis for MA is critical because it is accepted by law enforcement agencies as one of important corroborative facts for MA abuse. As part of quality control, the need for a reference material (RM) for hair analysis has rapidly increased in our laboratory. In the present study, we developed two RMs, NISI RM 0711-01 and 0711-02 using authentic and drug-free hair samples, respectively, according to the recommendations of ISO Guide 35 for the determination of methamphetamine and its main metabolite, amphetamine (AP) in human hair. Moreover, the prepared RMs were distributed to eight participants in four laboratories under the National Institute of Scientific Investigation in Korea for the purpose of internal quality control.
Methods: For the preparation of NISI RM 0711-01 (103 vials, ca. 100 mg each), MA abusers' hair samples were collected, homogenized and finally bottled. For the preparation of NISI RM 0711-02 (97 vials, ca. 100 mg each), drug-free hair was soaked into the DMSO solution containing MA and AP until the concentrations of MA and AP were plateaued. The concentration of each bottle was determined using two extraction methods, agitation with 1% HCl in methanol at 38 °C and ultrasonication with methanol/5M HCl (20:1), followed by gas chromatography/mass spectrometry (GC/MS) after derivatization with trifluoroacetic anhydride (TFAA). The homogeneity of analytes was evaluated and their property values were determined with their uncertainties. Also, statistical analysis was conducted with the results of the internal proficiency test, where average, median, normalized inter quartile range (NIQR), Robust CV and Robust Z score were calculated.
Results: Satisfying homogeneity was reached for MA and AP in the prepared two RMs. Finally, the certified values of NISI RM 0711-01 were 7.64 ng/mg and 0.54 ng/mg and their expanded uncertainties were 1.05 ng/mg and 0.07 ng/mg for MA and AP, respectively. NISI RM 0711-02 was prepared at the level of 4.86 ± 0.55 ng/mg and 4.63 ± 0.44 ng/mg for MA and AP, for each. In the internal proficiency test, most participants showed satisfying performances except one with NISI RM 0711-01 and one with NISI RM 0711-02, where corrective action should be undertaken.
Conclusion: The preparation and/or use of RMs as well as the management and/or participation of proficiency tests are main areas in the quality assurance of analytical chemistry laboratories. Especially, the RM is indispensable to assess the trueness and precision of their measurement methods. The RMs we developed here can be useful in forensic laboratories for internal quality control and external quality assurance.
Keywords: hair analysis, reference material, proficiency test

Hair extraction recoveries from intact and powdered hair into methanol

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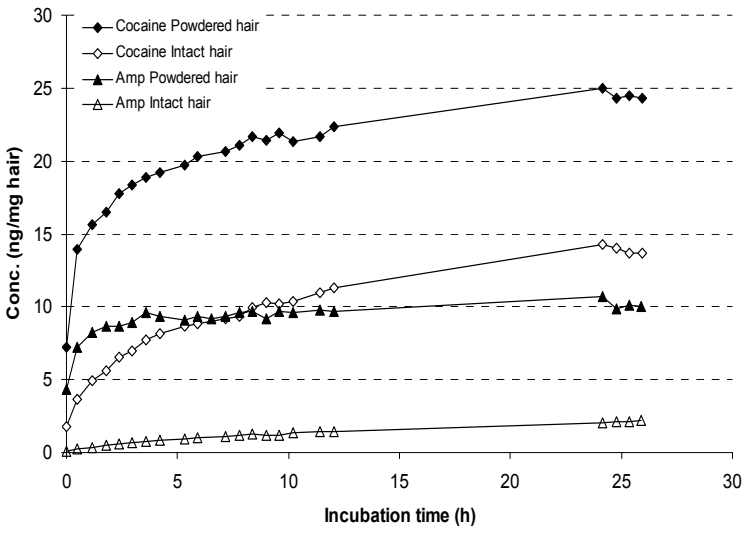
Introduction: Reports on drug detection in hair based on incubation of the hair in organic solvents or solvent buffer mixtures without the disintegration of hair are common. We have previously shown variations in extraction kinetics from hair into aqueous media. Obvious risks with such procedures are less than 100 % recovery owing to a short extraction time, morphological differences in hair samples or degradation of analytes. The aim of this study was to compare the extraction recoveries of cocaine, bensoylecgonine, codeine, amphetamine and MDMA into methanol from intact or powdered hair.

Methods: Two incubation procedures were compared, one using a shaking water bath and a procedure using sonication. The experiments were performed on a pool of authentic hair. Prior to the extraction, hair samples were washed thoroughly with our standard protocol including an initial wash with iso-propanol, and then three washes with phosphate buffer and a final iso-propanol wash. Five replicates of each experiment were performed. The stability of the analytes during incubation was verified in separate experiments. To 10 mg of hair was then added 2.0 mL of methanol and 50 µl of internal standard and the sample was incubated in a water bath (with orbital shaking) at 37 ºC for 26 hours or in an ultrasonic bath at 50 ºC for 12 hours. At 30 minute intervals a 20 µl aliquot was removed from the incubation mixture, diluted 1:2 with water and 1 µl was injected into the LC-MS-MS system. The LC-MS-MS analysis was performed on a SCIEX API 4000 MS-MS instrument equipped with an electrospray interface.

Results: Comparing the 12 h fractions, sonication did not show higher recoveries than incubation in a normal water bath. However, powdered hair generally showed much more rapid kinetics initially, followed by a slower increase or a plateau for the remaining incubation. For all analytes except bensoylecgonine, the powdering of hair markedly increased the recovery, however the impact was different depending on the drug as depicted in the figure. For amphetamine and codeine, the recovery was approximately five times higher using powdered hair whereas for cocaine and MDMA it was twice as high. Different extraction recoveries for cocaine and bensoylecgonine from powdered and intact hair resulted in different metabolite/parent compound ratios. Using powdered hair, the ratio became 2-3 times lower because of the higher recovery of cocaine.



Conclusion: We conclude that sonication did not improve recovery. The procedure with powdered hair gave higher recoveries but the increase was substance dependent.

Again, this emphasizes that each laboratory evaluate their methods and criteria for a positive result.

Extraction of drugs of abuse from hair. Can we determine realistic extraction recovery?

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Introduction: Drug analysis in hair has become very popular in recent years due to many advantages over 'traditional' matrices. Being able to test the hair for drug exposure long after the drug has been consumed is the principal reason for performing hair analysis. Drugs of abuse are incorporated into hair and many analytical applications have been published over the last decades. Quantitative methods for the detection of drugs of abuse in hair are usually validated using spiked hair samples. A complete digestion of the hair matrix is problematic as many drugs of abuse (e.g heroin, cocaine, benzodiazepines) are not stable under the conditions of digestion. Methanolic incubation has become the most common extraction method for drugs of abuse from hair. Extraction recovery determination using spiked hair samples may not give accurate results as the analytes in spiked hair samples are external of the hair, and not contained within the hair. Therefore, the aim of the study was to compare extraction recoveries of spiked hair samples, fortified hair samples and real hair samples using multiple extractions.
Methods: For extraction of drugs of abuse, 100 mg of hair was incubated with 2 mL methanol overnight at 60ºC. The methanol was transferred into a glass tube and evaporated to dryness. The extracts were reconstituted in mobile phase and injected into LC-MS/MS. To the hair samples, 2 mL of methanol was added, extracted and analyzed as described above. This extraction and analysis was repeated at least five times (exhaustive extraction). The following hair samples were used for analysis: Five different blank hair samples spiked with 30 common drugs of abuse, five different blank hair samples fortified with 30 common drugs of abuse and five different hair samples positive for common drugs of abuse. The extracts were separated on a Hypersil C18 column using gradient analysis. The drugs of abuse were detected using an Applied Biosystems 3200 Q-Trap mass spectrometer operated in MRM mode.
Results: Spiked hair showed for most drugs of abuse recoveries of ~90% following single extraction; by the third extraction only trace amounts of drugs could be detected in spiked hair samples. In fortified and real hair samples, extraction recoveries in the first extraction step varied significantly between 20 and 80 %, but most drugs were still detectable in the fourth and fifth extractions. For example the drug tramadol showed recoveries of 90%, 98%, 100% in spiked hair following 3 consecutive extractions. No drug was detected in further extractions. In contrast, the fortified hair sample, tramadol showed recoveries of 71%, 92%, 98%, 100% following consecutive extractions and in real hair; the recoveries were 72%, 90%, 96%, 100%. To further complicate the range of recoveries determined identical fortified hair samples also gave significantly different results. Amphetamine for example was extracted from a fortified hair sample with recoveries of 55%, 84%, 97%, 100% after consecutive extractions, whereas an identical hair sample under identical extraction conditions showed recoveries of 86%, 95%, 99%, 100%.
Conclusion: Spiked hair samples showed significant differences from fortified and real hair samples in terms of extraction recoveries after multiple extractions. Using spiked hair samples for calibration may lead to miscalculated concentrations in real hair samples. It is also clear that by performing 3 or more extractions, maximum recovery of drug can be achieved from either fortified or real hair specimens.

Testing for alcohol use in hair: is ethyl glucuronide (EtG) stable in hair?

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Introduction: The usefulness of biological markers of alcohol misuse, such as the measurement of ethyl glucuronide (EtG) in hair, is still under evaluation. There is a view that when EtG is detected above certain levels, it is strong evidence of moderate to excessive alcohol use; and that negative results are indicative of abstinence. However, there is also a consensus that positive results should not be regarded as definitive, but corroborative evidence. A negative result is also not definitive of abstinence because as it is not possible to rule out the effects of normal hygiene practices in hair such as shampooing, which will affect retrospective estimation of alcohol consumption over a period of many months. The main aim of this paper is to open the discussions on the usefulness of sectioning analysis of head hair samples in view of the potential limitations and to aid in the discussions of the subject.
Methods: 181 hair samples were tested for EtG, 102 of those were analysed as multiple sections of one month, covering three months, the first section being the most recent period and the third section the earliest period. The hair sections were washed with methanol dried and submitted to overnight sonication in water. The samples underwent SPE using anion exchange cartridges, followed by derivatisation with BSTFA before analysed by GC-MS/MS. The assay was linear over the calibration range 0.01 ng/mg – 0.5 ng/mg, and analytical cut-off was 0.010 ng/mg of hair assuming a 20 mg hair sample.
Results: The 95% percentile of the EtG levels detected in the first section for all the samples and for the group of samples analysed as multiple sections, were 0.21 ng/mg (N=181) and 0.22 ng/mg (N=102), respectively. The 95% percentiles of the levels detected in the second section and in the third section were 0.15 ng/mg (N=102) and 0.10 ng/mg (N=87), respectively. Of the samples where multiple sections were analysed, 67% (N=68) showed levels of EtG below the cut-off in all sections. Of the samples where EtG was detected, 65% of the second section samples (N=22) showed EtG levels on average 50% lower the levels detected in the first section. The levels detected of EtG in month three were on average 45% the levels of the previous month and 71% the levels detected in the first month. Analysis of variance showed the levels of the third section significantly lower ($p<0.05$) than the first section. The trend for the levels of the most recent hair growth being considerably higher than the preceding months could be due to normal hair hygiene, as EtG is soluble in water, and not due to any pattern of alcohol use.
Conclusions: The results of this study suggest that normal hair hygiene might wash out EtG from the hair producing the trend seen in the group of samples studied above. EtG in hair can be very useful diagnostic instrument for alcohol dependency, but in isolation could be misleading, even though the laboratory results are accurate. Unlike drug testing in hair of illicit drugs, where a single positive test can be sufficient to conclude that a person used drugs, a positive, EtG result can be only 'suggestive' of alcohol misuse or a negative result 'indicative' of abstinence and should not be regarded as definitive. We still need to be cautious regarding the interpretation of the results until further scientific evidence regarding the stability of EtG in hair becomes available. The recommendation is therefore that only the most recent month be tested for alcohol use using head hair and that data should be evaluated in conjunction with other biochemical tests and clinical evaluation.

Ethylglucuronide as a biomarker of ethanol intake: Application to clinical and forensic cases

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Introduction: The ethyl glucuronide (EtG) is a specific biomarker for ethyl alcohol intake that has some advantages with respect to other biomarkers. In recent years there have been several papers published in relation to its forensic and clinical applications. The aim of this work has been to validate a LC-MS/MS analytical method in clinical and forensic samples (blood, urine, hair and vitreous humour), as well as its application to real cases in patients following Alcohol Dependence Programs (ADP), and corpses which have undergone legal autopsies.
Methods: Urine (n=568) and hair (n=23) samples were taken from patients following ADP. The autopsy samples (n=71) were taken from recently deceased bodies (postmortem interval< 24 hours) with no ocular damage. Ethanol levels were calculated by headspace gas chromatography (HS-GC), equipped with a Flame Ionization Detector (FID), with a limit of detection of 0,05 g/L. EtG levels were analyzed by Liquid Chromatography Mass Spectrometry in ESI conditions and using D5 EtG as Internal Standard. MS experiments were performed in Multiple Reaction Monitoring (MRM) following these transitions: EtG 221,2>74,7, 221,2>84,8, 221,2>221,.2; EtG d5: 226,2>74,7, 226,2 >226,2.
Results: The methods were fully validated in all samples with the following detection limits (LOD): 0,1ug/mL for urine, 0,025 µg/mL for blood and vitreous humour and 0,025ng/mg for hair. Quantitation limits (LOQ) were 0,25 µg/mL, 0,05 µg/mL and 0,05ng/mg, respectively. Inter day and intraday CV were lower than 20%, no interfering peaks in any blank sample**.** From the analysis of 568 urine samples, 124 were positive for EtG (range: 0,1-785 µg/mL, mean 46,5 µg/mL), while only 19 were positive for Ethanol. All the positive cases for ethanol were positive for EtG. Of the 23 hair samples only 8 were positive for EtG (range: 0,09-0,64 ng/mg). Of the 71 forensic samples 21 were positive for ethanol in vitreous humour and of these 16 were also positive for EtG (range =0,03-2,6 ug/mL).
Conclusions: The application of LC-MS/MS in the detection of EtG is an efficient analytical procedure for clinical and forensic purposes. In clinical cases, urine samples can provide immediate information about the patient's progress. EtG can be detected in vitreous humour but in very low concentrations and to understand the meaning of these levels, a detailed kinetic study of this compound is needed.

Determination of ethyl glucuronide in hair by derivative HS-SPME/GC-NCI -MS and methodical comparison with liquid injection-GC-NCI-MS and with LC-MS/MS

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Introduction: Alcohol markers in hair (ethyl glucuronide EtG and fatty acid ethyl esters FAEE) have found increasing interest for retrospective detection or exclusion of alcohol abuse. The concentrations of EtG found in cases of chronic excessive drinking vary over a large range from some pg/mg to 10 ng/mg and, according to the present state of experience, 7 pg/mg and 25 pg/mg are applied as the upper limits for abstinence and social drinking respectively. Sensitive routine techniques are required for accurate determination of EtG at these low concentrations. Therefore, a method based on derivative headspace solid phase microextraction (HS-SPME) and gas chromatography - negative chemical ionization mass spectrometry (GC-NCI/MS) was developed and applied to hair samples of teetotalers, social drinkers and alcohol abusers. The method is compared with liquid injection-GC-NCI/MS and with LC-MS/MS.
Methods: For all three methods, the hair samples were subsequently washed with water and acetone, and dried. About 30 mg were extracted with water and the aqueous extract was cleaned-up by solid phase extraction using Oasis® Max 3 ccm anion exchange columns (Waters Inc.). D_5-EtG was used as the internal standard. For determination by HS-SPME, the eluate (2 % HCOOH/methanol) was evaporated, derivatized with heptafluorobutyric anhydride (HBFA) and again evaporated to dryness. The dry residue was submitted to HS-SPME without any additional agents. The method was optimized with respect to the SPME fiber (75 µm Carboxen/PDMS) as well as temperature and time of derivatization (30 min at 80 °C) and extraction (22 min at 105 °C). The m/z 397, 399 and 596 for EtG and 402, 404 and 601 for D_5-EtG were used for GC-MS-NCI-SIM. For direct injection GC-NCI-MS the residue of the eluate was derivatized with pentafluoropropionic anhydride and measured as described previously. In case of LC-MS/MS, the residue was dissolved in 60 µl of the mobile phase (0.1% HCOOH in H_2O/Acetonitril 93:7 v/v) and 50 µl were injected with the use of a 10 x 2 mm 5 µm Hypercarb HPLC column. For comparison, all three methods were applied to a series of hair samples.
Results: In the evaluation of the automated HS-SPME method, a limit of detection (LOD) of 4 pg/mg and a limit of quantification (LOQ) of 9 pg/mg were obtained. In application to real samples the concentrations were reproducible. The results from the three methods, which were performed in different laboratories, were qualitatively in a good agreement, i.e., no false positive or false negative results were obtained with respect to the cut-offs given above. Quantitative differences can be explained by differences in sample preparation, extraction procedure and inhomogeneities of the samples.
Conclusion: It follows from this investigation that HS-SPME in combination with derivatization and GC-MS can be applied for polar substances such as EtG. The headspace extraction from the dry residue is an additional clean-up step and delivers reproducible results if deuterated standards are used. All three methods are suitable for practical application. It is seen from the methodical comparison that the most serious reason for uncertainty is the sample preparation and not the measurement.
Keywords: ethyl glucuronide, hair, HS-SPME/GC-NCI/MS

11-Nor-Δ⁹-tetrahydrocannabinol-9-carboxylic acid ethyl ester (THC-COOEt): Unsuccessful search for a marker of combined cannabis and alcohol consumption

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Introduction: In analogy to cocaethylene, 11-nor-Δ⁹-tetrahydrocannabinol-9-carboxylic acid ethyl ester (THC-COOEt) can be presumed to be a mixed metabolite formed during combined consumption of cannabinoids and alcohol. This hypothesis was studied by investigation of blood and hair samples of cases with known cannabis and alcohol use.
Methods: THC-COOEt and its deuterated analogue D_3-THC-COOEt were synthesized as reference substance and internal standard from the corresponding carboxylic acids and diazoethane and identified by GC-MS. For determination in blood (serum, plasma), solid phase extraction and subsequent derivatization with N-methyl-N-tert-butyldimethylsilyl-trifluoroacetamide (MBDSTFA) in case of GC-EI-MS and with pentafluoropropionic anhydride (PFPA) in case of GC-NCI-MS were used. Hair samples were analyzed by extraction with a two-phase mixture of aqueous buffer (pH 7.6) and iso-octane in ultrasonic bath for 15 h, separation and evaporation of the organic phase, derivative headspace solid-phase microextraction (HS-SPME) in presence of MBDSTFA and measurement by GC-EI-MS. For increase of the sensitivity, the derivatization was performed with PFPA followed by measurement using HS-SPME combined with GC-NCI-MS and GC-NCI-MS/MS.
The methods were applied to plasma samples from 18 drunk driving cases and four other volunteers which contained both ethanol (0.30 to 2.16 mg/mL in whole blood) and THC-COOH (7.6 to 252 ng/mL in plasma) as well as to 15 hair samples from drug fatalities or volunteers which were both positive for THC (0.05-2.04 ng/mg) and fatty acid ethyl esters as markers of chronic alcohol abuse (0.2-6.3 ng/mg).
Results: In none of these samples THC-COOEt could be found with limits of detection of 0.3 ng/mL in plasma and 0.01 pg/mg in hair.
Conclusion: Different from the formation of cocaethylene or fatty acid ethyl esters, there seems to be no efficient way of the metabolic formation of THC-COOEt. As a reason, the missing biochemical activation of the carboxylic group is discussed. Therefore, a use of this compound as a marker for combined cannabis and alcohol consumption appears not to be possible.
Keywords: hair analysis, marker of combined alcohol and cannabis consumption, 11-Nor-Δ⁹-tetrahydrocannabinol-9-carboxylic acid ethyl ester

High prevalence of positive FAEE hair tests among families involved with children's aid societies and associated concurrent drug use

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Introduction: The FAEE hair test is a novel biomarker used to assess excessive chronic alcohol consumption. The test holds significant potential for facilitating diagnosis of Fetal Alcohol Spectrum Disorders that are associated with heavy maternal alcohol consumption during pregnancy. Never before has the use of the FAEE hair test been reported in the context of assessing parental alcohol use. The current study aims to evaluate the prevalence of heavy alcohol use in a cohort of caregivers involved with children's aid societies, and its relation to concurrent drug use as measured by hair analysis.
Methods: A cohort study was performed and included all parental hair samples sent by children's aid organizations to the Motherisk Laboratory in Toronto, Canada, for FAEE analysis between October 2005 and December 2008. FAEE (ethyl myristate, ethyl palmitate, ethyl oleate, and ethyl stearate) were analyzed according to a previously established protocol. Briefly, approximately 20 mg of hair was weighed and chopped into 1-3 mm segments. FAEE were extracted using a liquid-liquid extraction involving n-heptane and dimethyl sulfoxide, and analyzed using head space solid phase microextraction (HS-SPME) coupled with gas-chromatography (GCMS). HS-SPME conditions were as follows: samples were preheated for 5 minutes at 90°C, 250 rpm agitation, then absorbed over 30 minutes at 90°C, 150 rpm agitation, then desorbed for 15 minutes at 260°C. The agitation mode was 60s right, 30s interval, 60 s left, 30 s interval, etc. GCMS injector, interface, ion source and quadrapole were at 260°C, 310°C, 230°C, and 70°C, respectively. The temperature program was 2 min at 70°C, then 20°C/min up to 300°C, hold 0.5 min at 300°C. The LOD and LOQ values were previously reported to be between 0.01 ng/mg for ethyl sterate and 0.04 ng/mg for ethyl oleate for LOD, and between 0.04 ng/mg and 0.12 ng/mg for corresponding LOQ values. Odds ratio analysis was conducted on all samples that were also concurrently tested for one or more drugs (amphetamine, methamphetamine, barbiturates, benzodiazepines, cannabinoids, cocaine, opiates, methadone, oxicodone), in order to evaluate associations between heavy alcohol use and drug use.
Results: A total of 346 samples were tested for FAEE and 33% yielded positive results above 0.5 ng FAEE/ mg hair, marking heavy chronic alcohol consumption. Significant odds ratios between testing positive for FAEE and methamphetamine (OR = 4.50, 1.10-18.34), and cocaine's metabolite benzoylecognine (OR = 1.84, 1.02-3.34), were found.
Conclusion: The current investigation reports for the first time ever the level of positivity for heavy alcohol use as measured by the FAEE hair test in a cohort of parents involved with children's aid services. The high prevalence of positive FAEE tests, one third of the cohort, demonstrates the utility of and need for this biomarker as a tool in the child welfare community as well as the hair tests' potential as a screening tool for maternal alcohol use associated with the pathogenesis of Fetal Alcohol Spectrum Disorders. The study also confirms the known association between alcohol and drug use, but for the first time ever measures this relationship using hair analysis, demonstrating the validity and usefulness of this technique.

Control of abstinence or proof of consumption: hair analysis as a tool within the process of re-granting the driving licence

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Introduction: Testing the driving ability for re-granting the driving licence is a major task of the department of traffic medicine of our institute. Driving ability can be defined as a general, not time restricted or case dependent, psychological and physical capability to drive a car safely. These examinations are required by the driving licence authorities in cases of driving under influence of drugs and/or alcohol. The driving licence is only re-granted if a drug or alcohol abuse can be excluded. Very often, drug abstinence or teetotalism is to complied with for a certain period of time after re-granting the licence.
Results: In cases of alcohol and/or drug abuse, hair analysis is a powerful tool in the examination to re-grant the driving licence. In our lab, the number of hair analyses of drugs of abuse increased from 60 cases in 2003 to about 850 cases in 2007. Since 2006 we also use Ethylglucuronid (EtG) determination in hair (about 300 hair analysis in 2007) to check for teetotalism or to detect repeated excessive alcohol consumption, respectively. During these last years, different studies were performed in our institute. Standards of the examination of the driving ability were expressed as outcome of a study with Cocain users: 65 % positive hair samples of test persons with negative tested urine samples (immunoassay test) during a six months period before taking the hair sample, and 66 % positive hair samples of subject who stated explicitly that they never have consumed Cocain. In a study with 154 cases EtG was analyzed in the hair samples and CDT-values were determined either by an immunoassay test or by HPLC. 84 cases were positive for EtG, thereof 39 cases with CDT-values above normal range and 45 with CDT in a normal range (< 2,6 %, immunoassay test), and 15 cases with CDT-values above normal range and 69 with CDT in a normal range (< 1,77 %, HPLC), respectively. Our guidelines and the quality management will be outlined.
Conclusions: Actually, there is no uniform examination procedure to re-grant the driving licence in the different cantons of Switzerland. In case of a licence withdrawal due to an abuse of drugs and/or alcohol, we strongly recommend a hair analysis as part of the examination. This tendency can also be observed in other European countries. At present, we are completing our protocol for the hair analysis procedure. Hair analysis will be used firstly to proof or exclude drug or alcohol abuse during the driving ability assessment and secondly - after the driving licence is re-granted - to supervise the drug abstinence and/or the teetotalism in the following 6 to 12 months. Our proposals have been adapted to the official recommendations. Different decisions of the federal court of Switzerland and of some cantonal courts have affirmed that a positive result of a hair analysis is a proof of consumption of alcohol or drugs within a certain period of time.
Keywords: hair analysis; driving ability; re-granting the driving licence, guidelines; quality management

Simultaneous determination of 18 benzodiazepines and their main metabolites in hair, blood and urine by LC-ESI-MS/MS. Application to the determination of triazolam in a drug-facilitated crime

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Introduction: We present an application of liquid chromatography- triple stage quadrupole-tandem mass spectrometry with electrospray probe (LC-ESI-MS/MS) for the determination of traces of 18 benzodiazepines (BZDs) and their main metabolites in biological specimens (hair, urine and blood). This technique is at this time one of the most powerful analytical methods to be used for qualitative and quantitative analysis of drugs traces in blood, urine and hair. Furthermore, segmental hair analysis provides retrospective information helpful to solve drug facilitated crimes in forensic expertise. A case report with triazolam is presented.
Methods: To 20 mg of decontaminated and cut hair, 100 pg/mg of clonazepam-d4 (Cerilliant provided by Promochem), was added as internal standard. Hair specimens were extracted with 2 mL dichloromethane/ether (80/20) after incubation one night at 56°C in Soerensen buffer pH 7.6. After centrifugation, the organic layer was filtered with PTFE 0.2 µm then evaporated to dryness at ambient temperature. Urine and blood were extracted with Toxi-tube A® (Varian) and with 5 ng/mL of clonazepam-d4. The residues were reconstituted by 100 µL of MeOH/ACN/ Formate buffer (25/25/50) and transferred in glass vials. Ten microliters were injected into the LC- MS/MS TSQ Quantum Ultra (ThermoFisher). Separation was achieved on a C_{18}-column (Uptisphere ODB 150 x 2 mm – 5 µm) at 30°C. Mobile phase (formate buffer 2mM pH 3 / ACN) was delivered in gradient mode for a total run time of 17 min. The mass spectrometer was operated in selected-ion monitoring mode with fragmentation of [M+H]+ ions. To each pseudo-molecular ion 2 to 3 product ions were acquired at a scan time of 0.1 s and a width of 1.0 a.m.u.
Results: Standard curves in hair (0.5-100 pg/mg) were prepared by spiking aliquots of blank hair and had $r^2 > 0.9877$ for all BZDs. LOD ranged from 0.5 - 2 pg/mg. Standard curves in blood and urine (0.5-100 ng/mL) were prepared by spiking aliquots of blank fluids and had $r^2 > 0.9816$ for all BZDs. LOD ranged from 0.5 - 1 ng/mL.
We applied this method to the determination of benzodiazepines and analogues in blood, urine and hair of a 58-year-old Japanese lady after she was robbed at home following the ingestion of a suspect coffee brought from a fast food by a compatriot. She awoke about twenty four hours after the drunk the coffee. Biological fluids were sampled 61 hours after the offence and hair was collected 15 days later. Analysis of blood showed no traces of triazolam (mean t1/2: 1.5-3h, therapeutic range: 2–20 ng/mL) while hydroxy-triazolam was determined in urine at a concentration of 2.6 ng/mL after hydrolysis. Three segments (2-cm) from the root of hair were analyzed. Triazolam was detected only in the proximal segment at a concentration of 1.3 pg/mg (LOQ: 1 pg/mg).
Conclusion: The low concentration of hydroxy-triazolam in urine 61 h after the offence and the low concentration of triazolam in hair, only in the proximal segment, were in accordance with a single intake. These results show the usefulness of LC-MS/MS as well as segmental hair analysis for the elucidation of drug facilitated offences or crimes. To our knowledge, it is the first report of the determination of triazolam in human hair after single intake.
Keywords: benzodiazepines, triazolam, drug-facilitated crime, LC-MS/MS

Midazolam drug-facilitated crimes: three recent observations in hair

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Introduction: We report 3 drug-facilitated crimes in which midazolam, an exclusively hospitable benzodiazepine in France was used. Cases 1 and 2: Two young women in their twenties became cocaine-addicted under the bad influence of the same man. They underwent rapes and mutilations. Those brutalities lasted months, or even years. These two women mentioned a ketamine intake without their knowing, the association cocaine/ketamine allowing them not to feel pain. This man is presently prosecuted for narcotics detention, breach of weakness and harmful substances administration. Case 3: A 73-years-old American tourist invited a male in his hotel room for a drink. He was found 3 days later in a comatose state and with a head wound. All his valuables (money, jewellery…) had been stolen. He has been hospitalised for 5 days after his aggression. The suspect is sued for robbery and rape.
Method: Each hair strand is divided in 1 cm-long segments corresponding at a period of about 1 month. A specific liquid-liquid extraction is realised for each molecules class (cannabinoids, opiates and cocaine, benzodiazepines, hypnotics, anaesthetics). Narcotics are detected by GC-MS and/or GC-MS/MS. 26 benzodiazepines and metabolites, and 12 hypnotics or sedatives are analysed by GC-MS, GC-MS/MS and/or HPLC-MS/MS. GHB is quantified by GC-MS. The whole hair strands of cases 1 and 2 are analysed (between 23 and 31 segments) because the facts lasted over a long period. Facts of case 3 happened only one month before the hair sampling, that's why only 3 segments are analysed.
Results: Case 1: Cocaine (9-178 ng/mg) and its metabolites, as well as MDMA (0.7-36.3 ng/mg) and its metabolite MDA were found overall the hair strand indicating a chronic intake of these drugs. The hypnotic zolpidem (20-280 pg/mg) was present over two 6-months long periods. The victim took regularly Stilnox®. Diazepam (80-90 ng/mg) and its metabolites were detected in three distinct segments and might have been prescribed to the victim to treat her depressive moods. Finally, midazolam (50-200 pg/mg) was identified in three different segments that might fit with the facts period. Its effects are similar to those of ketamine, anaesthetic absent of the victim's hair. Case 2: As previously, cocaine and its metabolites were detected overall the hair strand. The victim declared to have taken the following detected drugs: bromazepam (0.1-1.2 ng/mg), clonazepam (0.2-117.8 ng/mg), cyamemazine (0.4-14.6 ng/mg) and alimemazine (0.1-2.7 ng/mg). Zolpidem (0.1-2.4 ng/mg) was detected in all segments. Zopiclone (0.2-2.5 ng/mg) and lorazepam (2.2-33.3 ng/mg) were identified over a long period and nordazepam (270 pg/mg) in one segment; they can fit with treatments prescribed to the victim for drug addiction during hospitalisation. As in case 1, midazolam (130-170 pg/mg) was detected in three distinct segments whereas no ketamine was revealed. In conclusion, cases 1 and 2 put in light two chronic poly-intoxications: results are in agreement with the victims statements about the drugs they took. The other drugs are concordant with different cures the victims followed. Only presence of midazolam is surprising but this compound can have been mistaken with ketamine. Case 3: Hair analysis showed the presence of only one molecule in the segment corresponding to the facts: midazolam (150 pg/mg).
Conclusion: Although midazolam is not the most frequently found compound in drug-facilitated crimes and is quite difficult to get because of its exclusively hospitable use in France, it is nevertheless indispensable to include systematically its research in the analysis. The three cases described above took place in very different drugs contexts (drug naïve or excessive consumer) and give concentrations in hair that complete the few existing data.
Keywords: midazolam, drug-facilitated crimes, hair

Age and chemical abuse. Evidence from hair analysis

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Introduction: The use of a drug to modify a person's behaviour for criminal gain is not a recent phenomenon. However, the recent increase in reports of drug-facilitated crimes (sexual assault, robbery) has caused some alarm in the general public. Drugs involved can be pharmaceuticals, such as benzodiazepines (flunitrazepam, lorazepam, clonazepam), hypnotics (zopiclone, zolpidem), sedatives (neuroleptics, some antihistamines) or anaesthetics (GHB, ketamine), drugs of abuse, such as cannabis, ecstasy or LSD or, more often, ethanol. Mistreatment of older people, whether it is abuse or neglect, can be classified as physical, psychological, or financial/material. Several types of mistreatment may occur simultaneously. Very few data are available in the international literature. It seems that mental abuse and neglect are more frequent, but physical abuses such as beating, pushing, kicking and possibly sexual abuses have also been reported.
Method: Drugs used to facilitate sexual assaults can be difficult to detect (active products at low dosages, chemical instability), can possess amnesic properties and can be rapidly cleared from the body (short half-life). In these situations, blood or even urine can be inadequate. This is the reason why some laboratories have developed an original approach based on hair testing.
Results: Hair was suggested as a valuable specimen in situations in which, as a result of a delay in reporting the crime, natural processes have eliminated the drug from typical biological specimens. Hair analysis may be a useful adjunct to conventional drug testing in sexual assault. It should not be considered as an alternative to blood and urine analyses, but as a complement. MS/MS technologies appear to be required for analyses in drug-facilitated cases. The experience of the authors will be presented in cases involving the elderly and chemical poisoning with alprazolam, diphenhydramine, doxylamine, clonazepam,flunitrazepam and promazine.
Keywords: hair, drug-facilitated crime, age, elder abuse

Identification of alprazolam in a suspected drug-facilitated sexual assault

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Introduction: A juvenile reported receiving small white pills prior to numerous sexual assaults over a four month period. Investigation uncovered alprazolam as a possible drug given to the juvenile. There was no history of alprazolam being prescribed to the juvenile. Hair was collected from the child and submitted to our laboratory for alprazolam testing.
Methods: Existing benzodiazepine immunoassay screens used in our laboratory for urine were evaluated for hair matrices. One kit demostrated superior results for alprazolam in hair, so it was fully validated using hair as the matrix. The limit of detection for alprazolam in hair was determined to be 40 picograms/milligram (pg/mg) of hair, with a 25 milligram sample size. Validation included analysis of five sources of blank hair, as well as hair spiked with alprazolam at known concentrations on three separate days. Confirmatory analysis of alprazolam in hair was also validated for this case. Our laboratory's liquid chromatography/tandem mass spectrometry (LC/MS/MS) procedure for benzodiazepines was altered to target alprazolam and its correlating deuterated internal standard. Hair samples were ground to a fine powder using a bead beater and incubated overnight in methanol after the addition of 2.5 nanograms of d_5-alprazolam (100 pg/mg). Methanol extracts were concentrated and analyzed by LC/MS/MS. Separation was performed on an Alltech Altima C18 analytical column (15 cm x 2.1 mm x 5 µ) using a mobile phase of methanol, water and ammonium hydroxide (60:40:0.03). Analysis by LC/MS/MS was performed by electrospray using an LTQ mass spectrometer by Thermofinnigan. A detection limit of 20 picograms alprazolam per milligram of hair was determined by analyzing hair spiked with alprazolam in triplicate on two days. Ten sources of blank hair were also analyzed to evaluate interferences.
Results: Using a combination of immunoassay and LC/MS/MS, alprazolam was qualitatively identified in hair from a juvenile victim in a suspected drug-facilitated sexual assault. Segmental analysis of 2-cm segments identified alprazolam in the hair correlating to growth during two of the four months that the alleged victim reported being drugged.
Conclusion: With minor modification to existing methods for benzodiazepines in urine, and several days of validation using spiked hair samples, suitable methods for alprazolam in hair were developed. The combination of immunoassay techniques and tandem mass spectrometry provided unequivocal identification of alprazolam in the hair sample of an alleged victim of drug-facilitated sexual assault.
Keywords: alprazolam, drug-facilitated sexual assault, hair analysis

Correlation of postmortem toxicology results with hair analysis in cases of suspected acute drug intoxication

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Introduction: The use of hair as an alternative matrix for drug testing is becoming more common. The principal advantage of hair over traditional biological specimens is that detection times are longer potentially allowing the identification of a wider spectrum of compounds and drug-use history. In postmortem toxicology the analysis of hair may be especially useful in cases where blood or other fluids are not be available. A disadvantage is that it may take up to a week for drugs to appear in hair after acute use. However, hair may be useful in determining the chronicity of drug use in acute drug deaths potentially affecting interpretation. This study examined postmortem toxicology results with hair in 14 medical examiner cases where acute drug intoxication was suspected based on scene investigation and case history.

Methods: Routine toxicology testing was performed at the Wayne County Medical Examiner's Office (WCMEO) using a combination of ELISA and/or GC-NP and GC-MS. Hair samples were blinded and sent to Immunalysis for analysis. Hair samples were screened by ELISA for carisoprodal, cocaine, benzodiazepines, fentanyl, methadone, opiates, oxycodone, tramadol, propoxyphene, amphetamine and methamphetamine. Confirmatory testing in hair was limited to the drug classes screened using either GC/MS, two-dimensional GC/MS or LC/MS/MS.

Results: The incidence of positive results (for the drugs that were tested in both traditional specimens and hair) for the 14 cases are presented below:

Confirmed Drugs	Trad. Samples	Cutoff*	Hair	Cutoff (IA)*
Cocaine, BZE, CE	3 / 4 / 0	100 (IA)	12 / 12 / 4	500
Morphine / 6-AM	8 / 5	50 (IA)	10 / 11	200
Codeine / Hydrocodone	7 / 4	50 (IA)	10 / 8	200
Oxycodone	2	25 (MS)	6	300
Benzodiazepines	5	100 (IA)	6	200
Methadone	1	12 (MS)	3	200
Fentanyl	4	2 (IA)	4	20
Tramadol	1	12 (MS)	1	1000
Propoxyphene	0	12 (MS)	1	200
Carisoprodal	0	1000 (MS)	1	1000

* Cutoff (biofluids (ng/mL), hair (pg/mg)) for initial test; IA = immunoassay, MS = GC/MS

Conclusions: With the exception of fentanyl in one case, all drugs detected in traditional specimens were also detected in hair, sometimes at very high concentrations suggesting, at least for these cases, that the majority of acute drug deaths are associated with chronic prior use of that drug. In theory, this could be useful information, especially for prescribed drugs subject to tolerance such as methadone. However, in the cases presented here, hair testing did not result in a change in the cause or manner of death. By virtue of longer detection times, the frequency of detection of drugs screened in hair was considerably greater than in traditional samples. This may provide a greater context to case history and impact the determination of cause and manner of death in natural and undetermined deaths.

Keywords: hair, postmortem, acute intoxication

Kinetics of disappearance of cocaine from hair after discontinuation of drug use

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Introduction: Methods that employ detection of various drugs in hair, in particular cocaine, have become popular in forensic medicine in recent years. An important use of these methods is monitoring of compliance with drug abstinence in clients of Children Protection agencies (usually parents of children). Nevertheless, the mechanisms and timeframe of drug disappearance from hair are not well characterized, but its understanding is crucial for clinical and forensic application of hair testing. Our aim was to evaluate the kinetics of disappearance of cocaine from hair after discontinuation of drug use

Methods: The Motherisk laboratory at the Hospital for Sick Children in Toronto routinely receives hair samples for toxicology analysis from various hospitals and children's aid societies (CAS) throughout Canada. Results of cocaine and benzoylecgonine (BE) hair analyses were obtained from the Motherisk Database for calculation of half-life of these compounds in hair. Patients were included in the study if they had gradually decreasing concentrations of cocaine and/or BE in sequential hair samples, with higher levels in the distal segments (i.e. earlier in time) and low or non-measurable levels in the segment, closest to the scalp (i.e. closer to the date of sampling). No information regarding actual patterns of drug use was stored in the database, but it is conceivable that patients referred by CAS would be under large pressure to discontinue drug use. The study was anonymous, and received ethics approval by the Ethics Review Board of the Hospital for Sick Children. Half life of cocaine and BE in hair was calculated using standard pharmacokinetics calculations. Regressions and comparisons were conducted by Mann Whitney U test and Spearman rank analysis, as appropriate.

Results: Results of 137 patients fulfilled the inclusion criteria for the study. The median half life of cocaine in hair was 1 – 1.2 months in females and 1.1 – 1.3 months in males. The median half life of BE was 0.9 – 1.2 in females and 1 – 1.3 in males. Half lives of cocaine and BE were not statistically different between males and females (Wilcoxon Rank test; P=0.93 for cocaine, P=0.99 for BE). Half lives of cocaine and BE were strongly correlated (Spearman Rank rho = 0.73; $p<0.001$).

Conclusion: Cocaine and its metabolite BE could be detected in hair of former drug users for several months after abstinence was started. The calculated half life of over 1 month for cocaine implies that, depending on the initial concentration, at least 3 – 4 months have to pass for hair testing to become negative in the segment proximal to the scalp. This finding has important implications for monitoring of compliance with abstinence of former drug users, and suggests that caution has to be exerted when estimating breach of abstinence.

On the other hand, the nature of hair testing, which can only evaluate drug exposure of moderately large periods, would mean that abstinence may not be easy to differentiate from low level exposure in the first months after abstinence was started.

Keywords: Hair testing, cocaine, half life

Assessment of passive exposure to cocaine in preschool children from a Mediterranean city by hair testing

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Introduction: At present cocaine is the principal drug of abuse consumed in the Mediterranean basin. In domestic environments of cocaine users children, particularly the preschool ones, may be chronically and repetitively exposed to cocaine by second-hand drug smoking,, accidental drug ingestion, physical contact with the users or with contaminated house surfaces. Hair testing of eventually exposed children can provide information related to a retrospective wide time window. We determined the prevalence of passive exposure to cocaine and the association of exposure with objective physical findings in children presenting to the pediatric emergency department at Hospital del Mar in Barcelona, Spain without signs or symptoms suggestive of the exposure.
Methods: Hair samples were obtained by 181 children between more than 12 months and 5 years of age. Younger children were excluded since the eventual presence of cocaine in their hair could be partly due to in utero exposure to drug. Hair samples were analyzed for the detection of cocaine and benzoylecgonine (BZE), together with opiates and amphetamines by a standardized procedure based on gas chromatography-mass spectrometry. Parental sociodemographics and drug history and child birth weight were recorded. All the children were clinically examined.
Results: Preliminary results on 32 hair samples showed 8 cases positive to cocaine (concentration range:0.5-5.96 ng/mg hair) and/or BZE (concentration range:0.5- 2.45 ng/mg hair), one of which was also positive for MDMA (0.5 ng/mg) and MDA (0.3ng/mg). The parents of 5 out of the 8 exposed children tested positive for hair cocaine. In case of the other 3 children, parents were not present at the emergency room and hair collection was not possible. There was no association between parental sociodemographics and exposure to cocaine. None of the children presented with a complaint or was identified as having clinical problems currently associated with exposure to cocaine.
Conclusion: Among the inner-city children served by Hospital del Mar, it seems that significant numbers of infants and young children are being exposed to cocaine. Hair testing for cocaine and other drugs of abuse should be considered in case of children from suspected drug-abusing parents for medical follow-up, social intervention and eventual discontinuation of children from risky environments.
Keywords: cocaine, hair testing, preschool children

Simultaneous extraction of amphetamine, buprenorphine, cocaine, methadone, methylenedioxymethamphetamine, morphine and zolpidem from hair analyzed by LC-MS/MS

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Objective: A method by Kronstrand et al. [Forensic Sci Int. 2004;145:183-90] for simultaneous extraction of drugs from hair has been tested, expanded and then validated for seven drugs: amphetamine (AMP), buprenorphine (BUP), cocaine (COC), methadone (MET), methylenedioxymethamphetamine (MDMA), morphine (MOR) and zolpidem (ZOL). These seven drugs have been chosen for their differences in physically and chemically properties. Development of a simple and versatile method for detection of these different drugs in hair has been favoured, opening the possibility for the method to be expanded to other drugs. **Method:** The extraction procedure consists of the following steps; (1) Homogenization of hair; (2) Incubation of 10 mg hair with 500 µL methanol:acetonitrile:20mM formate buffer pH 3.0 (10:10:80) and 25 µL of internal standard (3.2 µg/mL of D_5-AMP, D_4-BUP, D_3-COC, D_3-MET, D_5-MDMA and D_6-MOR) at 37 °C for 18 h; (3) Centrifugation followed by direct injection of 5 µL of the supernatant onto LC-MS-MS. Analysis is done by LC-MS-MS with electrospray ionisation, using a C18-column and a gradient system consisting of mobile phase A (0.05 % formic acid in water:methanol (950:50)), and mobile phase B (0.05 % formic acid in methanol) separating the seven drugs successfully within 23 minutes. **Results:** Linearity is found for all seven drugs between 0.05 ng/mg – 50 ng/mg (R^2 > 0.999 for all seven drugs). Table 1 summarizes LOD, LOQ, recovery after extraction at 5.0 ng/mg, intermediate precision (n = 9 for analysis of Proficiency Test DHF 2/07 from GTFCH) and accuracy (n = 9, DHF 2/07) when the method validation is performed following ICH guidelines.

Drug	LOD (ng/mg)	LOQ (ng/mg)	Extraction recovery (%)	Intermediate precision (% CV)	Accuracy (%)
AMP	0.005	0.016	92.4	9.0	92.2
BUP	0.015	0.044	90.6	9.1 *	95.6 *
COC	0.002	0.007	92.0	15.0	78.4
MET	0.006	0.018	91.3	6.1 *	96.0 *
MDMA	0.002	0.006	91.7	10.7	95.8
MOR	0.009	0.027	92.8	8.8	90.4
ZOL	0.011	0.032	90.2	4.9 *	94.2 *

Table 1. Results of method validation
DHF 2/07 only contains AMP, COC, MDMA and MOR. Intermediate precision and accuracy for BUP, MET and ZOL is determined for blank hair spiked with these drugs (n = 12, spiked in the range 0.5-25 ng/mg)

Furthermore the newest Proficiency Test, DHF 3/07, from GTFCH has been analyzed using the validated method above. Quantification of AMP, COC, MDMA and MOR was successfully performed within acceptance criteria. Also, hair spiked with a solution of different basic drugs was analyzed using the validated method, resulting in successful extraction of 18 basic drugs from hair. **Conclusion:** In conclusion this method is useful for extracting AMP, BUP, COC, MET, MDMA, MOR and ZOL from hair, with a further possibility for expansion to other drugs in hair.

Keywords: hair, pharmaceuticals and drugs of abuse, LC-MS/MS

Screening of amphetamines in hair using a homogenous immunoassay procedure

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Introduction: Hair is a useful specimen to detect long-term use of drugs. Drug concentrations are usually low and sample volume is often limited, so sensitive screening methods are necessary. Following methamphetamine (METH) use, METH itself is generally found in hair, but amphetamine (AMP) may be present as a metabolite, or as an independent drug.
Methods: Hair (10 mg) from METH users (n = 20) as well as hair from drug free volunteers (n = 20) was cut; phosphate buffer was added (pH 2.7; 0.5 mL) and incubated (3 hrs/75^{o}C). The supernatant was analyzed using two enzyme linked immunosorbent assays (ELISA) and two homogeneous immunoassays (HEIA) on an Olympus 400 platform, one for METH, one for AMP. For ELISA, the supernatant was diluted 1:5 with PBS before plating; for HEIA, 10 µL was used, making the process compatible with most commercial chemistry analyzers.
Results: A screening cutoff of 500 pg/mg was used. The intra-assay precision at 250, 500, 1000 and 2500 pg/mg was determined to be 9.9%, 8.2%, 6.6% and 3.7% for AMP; 11.6%, 8.3%, 7.1% and 2.7% for METH respectively. All the negative specimens screened negatively using both ELISA and EIA. The results of the positive specimens are shown below.

Sample	***ELISA***	***HEIA Cutoff 500pg/mg***			***GC/MS (pg/mg)***	
	AMP	*METH*	*AMP*	*METH*	*AMP*	*METH*
1	P	P	P	P	930	>10,000
2	P	P	P	P	103	6121
3	P	P	P	P	283	2360
4	P	P	**N**	P	257	8671
5	**N**	P	**N**	P	233	9273
6	N	P	**N**	P	105	2046
7	P	P	**N**	P	2883	>10,000
8	P	P	P	P	311	7953
9	P	P	**N**	P	219	2707
10	P	P	P	P	108	6665
11	P	P	P	P	2390	>10,000
12	P	P	P	P	5944	>10,000
13	P	P	P	P	1089	9850
14	P	P	P	P	1825	>10,000
15	P	P	P	P	3689	>10,000
16	P	P	P	P	1130	>10,000
17	P	P	P	P	1135	>10,000
18	P	P	P	P	1115	9512
19	P	P	P	P	775	7088
20	P	P	P	P	645	5560

Cross-reactivity: The METH antibody cross-reacts with MDMA (65%); the AMP antibody cross-reacts with MDA (45%). The assay is precise, sensitive and conducive to rapid hair screening using commercial chemistry analyzers.
Keyords: homogeneous immunoassay; amphetamines; hair

Qualitative screening of cocaine in hair using a homogenous immunoassay procedure

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Introduction: Hair is a useful specimen to detect long-term use of drugs. Hair generally contains low drug concentrations and sample volume is often limited. Following cocaine use, the main drug detected in hair is the parent drug itself, so a screening immunoassay should be targeted to cocaine.

Methods: Hair (10 mg) from cocaine users (n = 19) as well as hair from drug free volunteers (n = 20) was cut; phosphate buffer was added (pH 2.7; 0.5 mL), and the hair was incubated (3 hrs/75^{o}C). The supernatant was analyzed using enzyme linked immunosorbent assay (ELISA) and by homogeneous immunoassay (HEIA) on an Olympus 400 platform. For ELISA, the supernatant was diluted 1:5 with PBS before plating; for homogenous EIA, 20 µL was used directly, making it conducive to commercial chemistry analyzers.

Results: An HEIA targeted at cocaine using a screening cutoff of 500 pg/mg has been developed. The intra-assay precision at 250, 500, 1000 and 2500 pg/mg of cocaine was determined to be 7.1%, 12%, 9.3% and 3.8% respectively. All the negative specimens screened negatively using ELISA and EIA. The results of the positive specimens are shown.

Sample	**ELISA**	**HEIA**	**GC/MS results (pg/mg)**			
Cut-off	*500 pg/mg cocaine*	*500 pg/mg cocaine*	***BZE***	***Cocaine***	***NC***	***CE***
1	P	P	3492	>10,000	2746	ND
2	P	P	9531	>10,000	2419	ND
3	P	P	375	4501	62	389
4	P	P	9614	>10,000	2847	9521
5	P	P	>10,000	>10,000	4797	>10,000
6	P	P	5779	>10,000	662	ND
7	P	P	3978	>10,000	ND	8282
8	P	P	2492	>10,000	644	1589
9	P	P	6564	>10,000	722	840
10	P	P	>10,000	>10,000	3556	9868
11	P	P	7672	>10,000	2347	ND
12	P	P	>10,000	>10,000	2287	756
13	P	P	>10,000	>10,000	7927	214
14	P	P	132	1181	ND	ND
15	**N**	P	231	728	ND	ND
16	P	P	>10,000	>10,000	ND	7113
17	P	P	>10,000	>10,000	ND	833
18	P	P	>10,000	>10,000	ND	ND
19	**N**	P	107	573	ND	ND

Cross-reactivity: The cocaine antibody cross-reacts 100% with CE, but has lower cross-reactivity to BZE. The assay is precise, specific and sensitive, and is suitable for the rapid screening of hair specimens at a cut-off concentration of 500 pg/mg of cocaine.

Keywords: homogeneous immunoassay; cocaine; hair

Development and validation of a single LC-MS/MS assay following SPE for simultaneous hair analysis of amphetamines, opiates, cocaine and metabolites

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Introduction: The two major problems in hair analysis are the sample size and low targeted concentrations. To overcome these limitations, a liquid chromatography-electrospray-tandem mass spectrometry method (LC-MS/MS) allowing the simultaneous analysis of 27 amphetamines, opiates, cocaine and metabolites has been developed.
Method: This LC-MS/MS method allows the identification and quantification of 17 amphetamines (amphetamine*, BDB, m-CPP, dexfenfluramine, DOB, DOM, ephedrine*, MBDB, MDA*, MDEA*, MDMA*, methamphetamine*, methylphenidate, 4-MTA, norephedrine, norfenfluramine and PMA), 4 opiates (morphine*, codeine*, ethylmorphine, 6-MAM*), cocaine* and 5 metabolites (ecgoninemethylester (EME)*, benzoylecgonine (BZE)*, anhydroecgoninemethylester (AE), cocaethylene*, norcocaine*). After decontamination and cutting steps, internal standards (*deuterated equivalents) were added to 50 mg of hair sample. Analytes were extracted for 18 h at 45 °C using phosphate buffer (pH 5), followed by SPE clean-up using MCX® extraction cartridges (Oasis®, Waters). Analytes were separated on an Atlantis T3 column (Waters, 150 mm x 2.1 mm, 3 µm) by a gradient of acetonitrile and formate buffer (2 mM, pH 3) and detected in Multiple Reaction Monitoring mode (API 2000, Applied Biosystems®). For each analyte, two transitions were monitored: one for the quantification and one for the confirmation. Our validation procedure consisted of the study of linearity, intra-day and inter-day variability and accuracy for 5 days (5 replicates at 3 concentration levels). External quality controls were also analysed to check the accuracy of the method. Using this whole procedure, the method was routinely applied to hair samples.
Results: 17 amphetamines, 4 opiates and 6 cocaine derivatives are satisfactory identified by MRM in 14 minutes. Calibration curves are performed either by "linear through zero" or "quadratic 1/X weighting" regression. The method is linear from 0.05 to 10 ng/mg. The limits of detection (lod) range between 0.001 and 0.02 ng/mg. Precision has been checked by intra-day and inter-day CVs and associated relative bias, which are lower than 25 %, 21 % and 22 %, respectively. The results of 3 GTFCh's external quality controls (spiked and drug addicts' hair) confirm the accuracy of the method. The following table presents 3 positive results of adult drug addicts (patient A: global analysis; patients B and C: segmental analysis; nd = not detected: < lod):

		Concentration (ng/mg)									
S	Segment	EME	AE	Coc.	Norcoc.	BZE	Codeine	Morph.	6-MAM	MDA	MDMA
A	global	0.5	0.5	11.0	0.6	2.4	1.0	4.84	8.89	nd	<0.05
B	0-1.5cm	<0.05	<0.05	0.17	<0.05	<0.05	0.07	nd	nd	0.11	0.87
	1.5-3cm	<0.05	<0.05	0.15	<0.05	<0.05	<0.05	nd	<0.05	0.08	0.78
C	0-1cm	nd	nd	nd	nd	nd	<0.05	0.20	0.07	nd	nd
	1-2cm	nd	nd	nd	nd	nd	<0.05	0.15	0.13	nd	nd
	2-3cm	nd	nd	nd	nd	nd	<0.05	0.25	0.39	nd	nd
	3-4cm	nd	nd	nd	nd	nd	<0.05	0.52	0.81	nd	nd
	4cm-end	nd	nd	nd	nd	nd	0.07	0.56	1.57	nd	nd

Conclusion: This sensitive LC-MS/MS method allows the simultaneous identification and quantification of amphetamines, opiates, cocaine and metabolites in a 50mg hair sample and could be considered as useful for clinical and forensic toxicology diagnostic purposes.
Keywords: drugs of abuse, LC-MS/MS, hair

Niaprazine in hair. Children under influence

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Introduction: Niaprazine, under the trade name Nopron, is largely used in France as an hypnotic agent for occasional insomnia of children. This compound is available without medical prescription. The first case involving repetitive administration of niaprazine as a drug-facilitated sexual assault is reported. Three children (2 girls and 1 boy) were repetitively sedated and assaulted by their father-in-law between 2002 and 2006. Niaprazine's liquid formulation represents a good potential access to surreptitiously administer it in beverages. According to the request of the judge in charge of this case, hairs of victims were collected, segmented and screened for sedatives by LC-MS/MS.
Method: After decontamination and cutting in small pieces, 20 mg of hair was incubated overnight in a phosphate buffer (pH 8.4). The aqueous phase was extracted by 5 ml of a mixture of diethyl ether/methylene chloride (80/20), in presence of diazepam-d_5, used as internal standard (IS). Hair extract was separated on a XTerra MS C18 column using a gradient of acetonitrile and formate buffer. Drugs were identified by 2 transitions (m/z 357>106 and 177 and 290>154 and 198 for niaprazine and IS, respectively). LOQ of niaprazine was 10 pg/mg.
Results: Niaprazine was detected in the range 21 to 382, <LOQ to 315, 2642 to 3431 pg/mg for the three children, respectively. These concentrations could not be compared with previous results, due to a lack of literature. In particular, it was not possible to put any quantitative interpretation on the dosage that was administered to the children. It is however obvious that repetitive administrations have occurred but it is not possible to determine the number of exposures. Given the length of the hair, exposure to niaprazine should have occurred at least during the previous months.
Conclusion: The surreptitious administration of niaprazine to obtain sedation was considered as a drug-facilitated crime, even in an intra-familial situation. According to the French law, the drug can be considered as a chemical weapon.
Keywords: hair, children, niaprazine, drug-facilitated crime

Determination of nicotine in hair samples of 1000-year old mummies

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Introduction: In the literature there exist some reports about cocaine, nicotine and cannabinoids found in ancient hair of mummies from South America or Egypt. Most of the results were critically discussed for the use of improper techniques or contamination of the sample material. Recently, an exhibition of 70 mummies from around the globe was organized at a German museum. The display presents exhibits assembled by the world famous mummy project, involving various specialists for anthropology, pathology, radiology, molecular biology and toxicology.
Methods: Hair samples of seven ancient Peruvian mummies were analyzed for drugs of abuse (cannabinoids, opiates, cocaine-like substances) using modern routine GC-MS techniques. Additionally a GC-MS screening procedure was performed following methanolic ultrasonication of the samples. For the analysis of nicotine and cotinine a GC/MS procedure employing deuterated internal standards was performed in selected ion monitoring mode (3 characteristic ions).
Results: The tests revealed negative results except for nicotine in the hair of 3 mummies. Nicotine was measured in concentrations of 57.5 ng/mg in the hair of a woman, 14.1 ng/mg in a child and 11.4 ng/mg in a further female mummy, but all cases revealed negative results for cotinine. The washing solutions yielded negative results for both analytes, i.e. nicotine as well as cotinine.
Discussion: In 3 out of 7 hair samples of ancient Peruvian mummies nicotine was found in concentrations which were reported in cases of active smokers. It has to be considered however that the nicotine metabolite cotinine was not detected in any case. This typical nicotine metabolite would indicate an active ingestion (body passage), due to lower concentrations the metabolite is however not even detectable in every active smoker. In our opinion, even with respect to negative results in the washing solutions, the present results cannot definitely confirm an active consumption in the life time of the analyzed mummies: an external contamination, e.g. by transfer from smoking visitors or employees of the museum as well as in their respective lifetime, cannot be excluded. Computer tomography (CT) scans revealed symptoms of tuberculosis in one female mummy. It is thus also plausible, that an external contamination of this mummy's hair resulted from a ritual use of the 'sacred' tobacco plant, e.g. during a ceremony in which a shaman would treat the patient with tobacco smoke.
Key words: nicotine, hair analysis, mummy

Detection of amitriptyline and nortriptyline in various organs and hair by LC-MS/MS in the homicidal fatality of a one-month-old girl

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Case report : A four-year-old girl was admitted to an intensive care unit in a desperate state. She died a few minutes later. Toxicological analyses revealed a *post-mortem* blood concentration of the antipsychotic drug cyamemazine at a concentration of 3,4 µg/mL, which was the direct cause of death. Informed of the facts, the prosecutor then ordered the exhumation of a one-month-old baby, deceased 6 months beforehand in circumstances which at the time had been considered normal. The body, locked in a zinc coffin, was rather well preserved. Toxicological analyses were carried out of the liver, the intracerebral liquid, and a 3-cm lock of hair.
Method : After crushing the viscera, a pre-treatment by subtistilline-A and a liquid-liquid extraction, analyses were realised by HPLC-MS/MS in positive ionisation mode on a C_{18} analytical column using a gradient of acetonitrile and 2 mM formate buffer at pH = 3. The hair was pulverized using a ball mill, then sonicated for 2 hours in methanol, and subsequently purified by solid phase extraction on OASIS HLB® cartridges. Quantification is based on the main ion m/z = 233 common daughter of m/z = 264 for nortriptyline, m/z = 278 for amitriptyline and m/z = 267 for nortriptyline D3 used as internal standard.
Results : The massive presence of amitriptyline and nortriptyline in the liver was measured at a concentration of 29,8 and 3,6 µg/g. According to the scientific data, these concentrations are high enough to cause death. Hair analyses revealed the presence of amitriptyline and nortriptyline at concentrations of 1811 and 53 pg/mg respectively, while complementary analyses showed the presence of bromazepam in the hair at a concentration of 740 pg/mg, thus documenting previous administrations. The mother would confess later to having used the drinkable form of the pharmaceutical LAROXYL® by pouring the content of a 20 ml bottle (at 40 mg/mL) into the feeding-bottle of her child. The milk was sweet but still bitter and the whole family helped to feed the crying baby.
Conclusions : We reported on an unusual homicide by poisoning. This method is the first to describe LC-MS/MS analysis of amitriptyline and nortriptyline in hair.
Keywords : amitriptyline, death, hair.

Analysis of carbofuran and 3-hydroxycarbofuran in hair: interpretation difficulties

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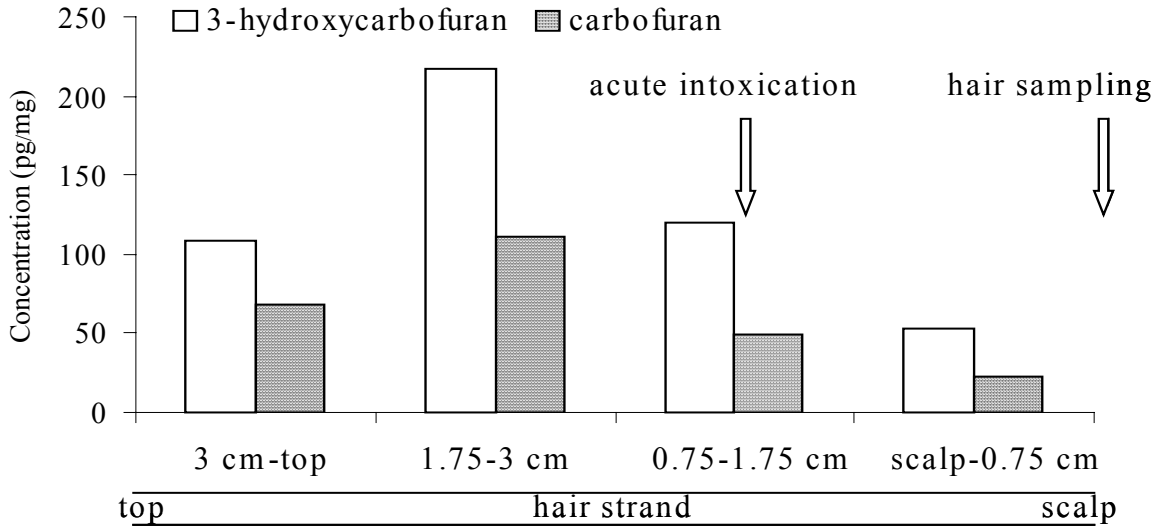
Introduction: Carbofuran is a carbamate insecticide used for the treatment of soil, exhibiting systemic properties known to be highly hazardous for humans. We report here two cases of non fatal intoxication with carbofuran for which concentration values in hair have been measured. The first patient history presented numerous acute intoxication episodes (attempted homicide) and the second patient had only had one exposition (a single attempted suicide).
Methods: Detection and quantitation of carbofuran and its metabolite, 3-hydroxycarbofuran, were performed using a Sorensen buffer incubation followed by a liquid-liquid extraction and a liquid chromatography-electrospray-tandem mass spectrometry (LC-ES-MS/MS) analysis according to the method described by Dulaurent et al. [Forensic Sci Int. In Press].
Results: For the first patient, three hair strands were sampled at three different periods at 1.5 and 8.5 months after the first sampling. Carbofuran and 3-hydroxycarbofuran were found in all segments of the three strands with concentrations ranging from 23 to 557 pg/mg and 50 to 645 pg/mg respectively. Nevertheless, in the last hair strand (6.5 cm length) sampled 20 days after the last exposure, carbofuran and its metabolite were found in all segments whereas the previous acute intoxication had taken place 8.5 months before. For the second patient, a single hair strand was sampled 1.5 month after the single contact. The following figure presents concentrations profile of his hair strand:



Conclusion: Carbofuran and its metabolite could be detected in hair of victims of acute intoxication. Nevertheless, we found also these products in hair segments, which correspond to a period before exposure. What could be the explanation(s): a) migration of these compounds in the hair? b) sweating effect (i.e.: when a carbamate intoxication occurs, there is an enhance of sweating which can contaminate the whole length of the hair)? c) accumulation of compounds in lipid tissues during previous contacts and spreading of these compounds from lipid tissues to hair by way of blood? d) pollution of all segments of the hair strand during dichloromethane decontamination?
Key words: pesticide, hair, LC-MS/MS

Characterization of addictives practices by a combining general unknown screening and targeted screening by UPLC-MS/MS in hair: A case report

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Introduction: A 24-year old man, known for abuse of drugs, was arrested for torture and acts of cruelty against his 4-year old child. Two weeks later, the judge orders a hair analysis of father. His hair was cut from the root (3 cm) in order to estimate his full drug use history during the last three months.

Materials and Methods: The hair sample was twice decontaminated using 5 ml methylene chloride and cut into small pieces (≈ 1mm). About 85 mg were incubated overnight in phosphate buffer at pH 8.4, in the presence of a internal standard. After liquid – liquid extraction with a mixture of methylene chloride/diethyl ether (90/10) and evaporation to dryness, the residue was reconstituted in 100 µL of mobile phase. 15 µL were injected into the ACQUITY UPLC-MS/MS system (Waters). Separation was achieved on ACQUITY UPLC™ HSS C18, 1.8 µm (2.1 x 150 mm). Mobile phase (ammonium formate buffer 5mM pH=3/ACN) was delivered in gradient mode for a total run time of 15 min. The detection was performed by ACQUITY TQ Detector (Waters) in single MS mode and MRM mode. Single MS scan mode from 80 to 650 m/z in positive ESI (4 values of cone voltage) and in negative ESI (2 values of cone voltage). The MRM mode used 2 transitions and their ratio to confirm the presence of each compound. Each molecule was matched against a MS library of 527 compounds and a MRM library of 138 compounds.

Results: After 2 screenings, several drugs were matched:

	UPLC/MS/MS	UPLC/MS
Codeine	+	+
Morphine	+	+
6 MAM	+	+
MDA	+	
MDMA	+	
Amphetamine	+	
Benzoylecgonine	+	+
Cocaine	+	+
Cocaethylene	+	
Paracetamol	+	
Hydroxyzine	+	+
Cetirizine	+	
Propoxyphene	+	+
Cyamemazine	+	
Meprobamate	+	
Diazepam	+	
Papaverine		+
Noscapine		+
Phenacetine		+
Pheniramine		+
Nicotine		+
Cotinine		+

Conclusion: Once more, this case demonstrates that hair analysis can be used to detect drugs which were taken several months ago. The power of Acquity UPLC-MS/(MS) contributes widely to this result.

Keywords: hair analysis, drug use history, UPLC-MS/MS

A liquid-chromatography-electrospray ionization-tandem mass spectrometry method following SPE extraction dedicated to the determination of ethyl-glucuronide in hair

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Introduction: Ethyl-β-D-6-glucuronide (EtG) is a stable Phase II metabolite of ethanol. Its detection in hair is of interest in both a clinical and forensic context with the aim of monitoring alcohol abuse. We present a validated liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) method dedicated to EtG analysis in hair.

Methods: To 30 mg of a washed and cut (1-2 mm length) hair sample, 2 mL of water and 100 µL of internal standard solution (EtG-D5, 100 µg/L in methanol) were added. After ultrasonication (2h, 50 °C), the extracts were cleaned-up with graphite SPE cartridges (Clean Screen, UCT, USA). Chromatography was then performed using an Uptisphere-3SI Column, 100 x 2 mm, 3 µm particle size (Interchim, France) and the mass spectrometer was operated in the negative ion mode for detection. The MRM transitions monitored were: m/z 221→ 75 and 221→ 85 for EtG, 226→ 75 for EtG-D5. Validation procedure was performed according to the international proposed experiments, evaluations, and acceptance criteria for validation of new analytical methods in this context. The intra- and inter-assay precision and accuracy were assessed at four concentration levels relative to calibration range. In addition, the developed method was applied to several hair samples taken from: 4 fatalities (F) with documented excessive alcohol consumption, 12 known alcoholics (HD: heavy drinkers) according to physicians of the emergency unit and 7 social drinkers (SD).

Results: The method exhibits a detection limit of 4 pg/mg, a quantification limit of 10 pg/mg and the calibration curves were linear from 10 to 3000 pg/mg. Intra- and inter assay precision standard deviation and relative bias were less than 20% over the calibrating range. In addition, no influence of interfering compounds on the signal was observed for both EtG and EtG-D5. For all SD patients EtG hair concentrations were <10 pg/mg and for every HD as well as F patients, EtG hair concentrations were >50 pg/mg. The following table presents the results for HD patients:

	heavy drinkers (n = 12)											
Blood ethanol[a]	3.8	<0.1	3.2	0.4	3.9	3.6	5.1	<0.1	1.9	3.0	4.9	4.6
MCV[b]	98	99	106	102	107	90	95	94	83	97	99	100
GGT[c]	444	509	140	185	341	46	422	51	57	104	42	40
EtG[d]	180	54	370	341	303	60	252	60	92	66	497	365

[a]g/L at time of hair sampling ; [b]Mean corpuscular volume in fL ; [c]gamma-glutamyltransferase (U/L) ; [d]pg/mg of hair

Conclusion: This method is fully validated. The choice of the cut-off value remains a real problem as values between 30 and 100 pg/mg can be found in the literature. Our preliminary results suggest that a EtG concentration ≥ 50 pg/mg can be related to an effectively heavy alcohol consumption. A lower cut-off value (30 pg/mg ?) could perhaps be proposed. However, in order to avoid the risk of false positive results due to the high sensitivity of such a LC-MS/MS method, further studies are needed to clarify cut-off values of EtG concentrations in hair for diagnostic purposes.

Key words: hair, ethyl-glucuronide, LC-MS/MS