PROGRAM

Sunday May 28th

Welcome reception at the National Board of Forensic Medicine in Linköping.

Monday May 29th
Registration from 0800 in the Klosterhotel.

		Moderators Carmen Jurado and Thomas Cairns
0900		Opening of the meeting
0920	T. Cairns, M. Schaffer, and V. Hill	Hair Analysis: Criteria for Forensic Acceptance
0935	C. Jurado	Quality control in hair analysis: The SoHT experience
0955	C. Stramesi, S. Pichini, A. Polettini, I. Palmi	Strategies of quality improvement of hair testing for drugs of abuse: The HAIRVEQ experience.
1015	M. Ventura, S. Pichini, R. Ventura, M. Pujadas ,R. Di Giovannandrea, P. Zuccaro, R. Pacifici, R. de la Torre	External proficiency testing program HAIRVEQ 2006. Evaluation of laboratories performance after providing guidelines on sample preparation, method validation and data evaluation.
1030		Coffee break
1100	D. Thieme, H.Sachs and G. Schwarz	Immunochemical Screening for Drugs of Abuse in Hair. Potential and Limitations
1115	E. Han, E. Miller, J. Lee, Y. Park, M. Lim, H. Chung, F.M. Wylie and J.S.Oliver	Detection of methamphetamine in hair by ELISA and GC-MS"
1130	G. Cooper and R. Kronstrand	ISO17025 Accreditation for Drug Testing in Hair – The UK and Swedish Experience
1200		Lunch
1300		Historical tour of the surroundings

Moderators
Robert Kronstrand and Fritz Pragst

Time	Authors	Title
1415	F. Sporkert, M. Augsburger, M. Bollmann, B. Schrag, M. Dovat, P. Mangin	Ethyl Glucuronides in hair – comparison with usual biological marker of chronic alcohol consumption in post-mortem and forensic cases
1430	B.M.R. Appenzeller, M. Schuman, M. Yegles and R. Wennig	Quantification of ethyl Glucuronides in white and pigmented hair
1445	R. Kronstrand, M. Roman, J. Ahlner, and N. Dizdar	Dose-hair concentration relationship and pigmentation effects in patients on low dose clozapine
1500	L. Martins, M. Yegles , N. Samyn, J. Ramaekes and R. Wennig	Segmental hair analysis of MDMA enantiomers by GC/MS-NCI.
1515	D. Thieme, B. Rolf, H. Sachs, D. Schmid	Correlation of metabolite ratios of amitriptyline in hair and genetic polymorphism
1530	W. Lechowicz, R. Stanaszek	Determination trazodone and its metabolite 1-(3-chlorophenyl)piperazine (mCPP) in Hair by High-Performance Liquid Chromatography–Electrospray Mass Spectrometry (HPLC–ESI-MS)
1545		Coffee break
1600		SoHT Buisiness meeting
1900		Concert in Klosterkyrkan
2000		Dinner in Kapitelsalen

Tuesday May 30th

Time	Authors	Title
		Moderators Michael Uhl and Johan Ahlner
0915	H. Druid and R. Kronstrand	Strategies in postmortem hair testing
0930	F. Scheufler and M. Uhl	Interpretation of forensic cases based on empirical data
0945	M. Moeller	Application of hair analysis in driving license regranting
1015	J. Strandberg, F. C. Kugelberg, K. Alkass, L. Bredberg, C. Oscarsson, R. Kronstrand, J. Franck, H. Druid	Evaluation of self-report, urinalysis and segmental hair analysis of drugs in samples from amphetamine users enrolled in a clinical trial
1030		Coffee break
1100	K. S. Scott.	Hair versus blood and vitreous humor in the interpretation of drug related deaths
1115	H. Druid, J. Strandberg, K. Alkass, I. Nyström, F. C. Kugelberg, R. Kronstrand	Segmental hair analysis as a tool for the evaluation of the role of abstinence in heroin overdose deaths
1130	F. Garcia-Bournissen, B. Rokach, T. Karaskov, G. Koren	Methamphetamine detection in maternal and neonatal hair; Implications for fetal safety.
1200		Lunch
		Moderators Christine Moore and Martin Josefsson
1315	C. Moore, M. Feldman, E. Harrison, S. Rana, C. Coulter, D. Kuntz, A. Agrawal, M. Vincent and J. Soares	Disposition of Hydrocodone in Hair
1330	Jin Young Kim, and Moon Kyo In	Determination of Ketamine and Norketamine in Hair by Gas Chromatography-Mass Spectrometry using Two-step Derivatization

1345	L. Gautam, K. S Scott, and M. D. Cole:	Extraction of hair melanin and binding study with amphetamine
1400	A Zucchella, C. Stramesi, L. Politi, L. Morini, and A. Polettini	Treatments against hair loss may hinder cocaine and metabolites detection
1415	T. Nadulski, S. Bleeck, J. Schräder and F. Pragst	Application of Headspace Solid Phase Microextraction for Analysis of Cannabinoids in Hair
1430	F. Garcia-Bournissen, B. Rokach, T. Karaskov, G. Koren	Cocaine detection in maternal and neonatal hair
1445		Closing of the meeting
1500		Coffee and farewell

Hair Analysis: Criteria for Forensic Acceptance

Thomas Cairns, Michael Schaffer, and Virginia Hill
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Both quality control and quality assurance are important elements that must be addressed in the event of litigation or some other legal proceeding resulting from a challenge to a positive hair drug test. While many analytical chemistry issues are adequately addressed by Good Laboratory Practices, workplace testing demands a higher level of accountability.
In general, any methods used in analysis must be rigorously validated against standards and controls illustrating a clear demonstration of capability not just at the chosen cutoff but at 25% above and below this critical cutoff level. Data will be presented to illustrate the application of such techniques in batch testing of hair samples for both screening and confirmation. Interjection of negatives and blind samples demonstrate the lack of carryover as well as consistent performance of the instruments during all sample analyses. Day to day instrument performance can be monitored by constructing a Levi-Jennings plot whereby deviations can be checked to permit appropriate remedies to be implemented prior to failure status.
In structural confirmation of drugs of abuse, the extensive use of isotope dilution mass spectrometry can provide quantitative results concurrent with identification or presence. Examples will be illustrated using liquid chromatography and gas chromatography mass spectrometry mass spectrometry for cocaine and metabolites, opiates and metabolites, amphetamines and carboxy-THC. Discussion of evolving criteria for confirmation will be presented.

QUALITY CONTROL IN HAIR ANALYSIS. THE SOHT EXPERIENCE

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Instituto Nacional de Toxicología y Ciencias Forenses. Sevilla. SPAIN

Quality control must be the prime objective of any analytical laboratory in order to eliminate systematic errors and minimize the possibility of accidental errors. In hair analysis, the participation in external quality controls or proficiency testing programs is of paramount importance, since the only way to know the efficacy of our method is to compare our results with those obtained by other laboratories.

Since the Society of Hair Testing (SoHT) started in 1995, it has implemented and organized proficiency tests (PT) among its activities. At the beginning these PT were sporadically organized, the first in 1995, a second in 1997; but since 2001 a PT has been organized annually.

Sets of four or five samples were included in each exercise, at least one of them being a blank sample, in order to check the incidence of false positives. The positive specimens were obtained from drug abusers who consumed different types of drugs.

Participants must analyze the samples under routine conditions and they were requested to provide information about their experience and the analytical method applied in these analyses. The number of participants was more or less similar every year, around 20 (from 18 to 25), three of them being reference laboratories. Laboratories were asked to analyze opiates, cocainics, amphetamines and cannabis. But not all the labs analyzed all the compounds. For example: all the participants analyzed opiate and cocaine compounds in all the exercises; while the number of labs performing analysis of amphetamines and cannabis, as well, increased in each exercise, from 53% which analyzed amphetamines the first year to 100% the other years. The percentage of participants reporting results for cannabis increased from 47% in the first round to about 70% for the other years.

In general qualitative results were very good and similar for all the exercises: in the case of negative samples, only one false positive was reported for MAM over time; while in the case of positive samples about 5% of false negatives were reported every year. The majority of them came from the less experienced participants and they were reported for the compounds with the lowest concentrations, demonstrating that the establishment of adequate cut-off's is of paramount importance. Laboratories which have participated in several exercises have improved their results over time; thus emphasizing the need of enrolling in these PT programs.

Quantitative results were always very scattered, but improved each year. Laboratories applied different methods of extraction, including acidic extraction, enzymatic digestion and extractions with methanol and different types of buffer, but it is not possible to conclude that one approach is better than the other, since all the methods provided similar and very scattered recoveries. On the contrary, when taking into consideration the experience of the laboratory, participants performing more than 100 analyses per year reported similar and homogeneous results, independently of the applied method of extraction; while the data from the less experienced labs were very scattered.

The findings from the different exercises indicated that extensive experience in hair testing is very important, since it has been demonstrated that the longer the experience in performing hair analysis, the better the quality of the results.

STRATEGIES OF QUALITY IMPROVEMENT OF HAIR TESTING FOR DRUGS OF ABUSE. THE HAIRVEQ EXPERIENCE

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The Society of Hair Testing has tried since his foundation to give recommendations for hair analysis in order to assure the best performance and to guarantee reliable results. In Italy, the use of hair testing for clinical, administrative and forensic purposes has increased over the years and so is the number of laboratories performing this analysis. However, since no regulations have been issued to certify lab skills, the quality of results may vary considerably from lab to lab. Since 2002 the Istituto Superiore di Sanità in Rome, in cooperation with IMIM in Barcelona, has promoted an external quality control program (HAIRVEQ) to evaluate the reliability of Italian laboratories in hair testing for drug abuse. Unfortunately, exercise results are rather discouraging. In particular, the high percentage of false positives is the most warning outcome. Two different strategies were undertaken to try solving the problem. The first one has been to propose a Standardised Operative Procedure (SOP) to the participant labs. The defined SOP takes into account the different typologies and analytical potentials of labs involved. It consists of: incubation in diluted HCl (for basic drugs) followed by purification either by LLE or mixed-mode SPE; further incubation in basic medium (for cannabinoids) and purification by LLE. In both cases extracts are submitted to trimethylsilylation and the recommended instrumental analysis is GC-MS. The second strategy has been to scrutinize and discuss the critical steps of the whole procedure of poorly performing labs in order to single out possible sources of error. The improvements achieved by applying the two different strategies will be discussed.

External proficiency testing program HAIRVEQ 2006. Evaluation of laboratories performance after providing guidelines on sample preparation, method validation and data evaluation.

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Since 2002 the Istituto Superiore di Sanità of Rome, Italy, in cooperation with Institut Municipal d'Investigaciò Mèdica of Barcelona, Spain, has set up an external proficiency testing program (HAIRVEQ) to evaluate reliability in hair testing for drug abuse by laboratories from the Italian National Health Service. Results obtained in the last two rounds, performed in the first quarter of 2006, are presented. The aim of these two exercises was to evaluate the evolution of the laboratories' performance after providing guidelines on sample preparation, method validation and data evaluation in drugs of abuse testing in hair.
32 laboratories participated and 30 sent qualitative and/or quantitative results. Samples analysed in both exercises were real hair samples from drugs consumers, powdered or finely cut, mixed to ensure homogeneity and tested by GC/MS before sending to the laboratories. In the first round, a sample containing opiates and cocaine was sent to be analysed following routine methodology. In the second round, the same sample was resent together with an open hair sample, two samples containing cocaine, opiates, amphetamines derivatives and cannabinoids and finally a blank sample. Standard Operating Procedures on sample preparation, method validation and qualitative and quantitative data evaluation in the analysis of opiates, cocaine, amphetamine derivatives and cannabinoids in hair were also included. Qualitative and quantitative evaluation of results reported by laboratories in the two rounds and a comparison of results obtained between the two rounds are presented in order to discuss the influence of guidelines provided in the laboratory performance concerning drugs of abuse analysis in hair.

Immunochemical Screening for Drugs of Abuse in Hair Potential and Limitations

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Since extraction, solvent evaporation and subsequent reconstitution represent the first steps of any drug screening in hair, there is no principle technical difference between immunochemical analyses of drugs in various liquid specimens (hair extracts reconstituted in buffer, urine, sweat or saliva).
In routine hair analysis the particularity of an immunochemichal screening arises from the relatively low substance concentrations, the predominance of the parent drugs (which are usually not the preferred target analytes of the immunoassays) and the appearance of specific contaminations in high amounts (e.g. dyes, fatty acids).
Practical experiences with the screening of amphetamines, benzodiazepines, cannabinoids, methadone and opiates are presented. In particular, the potential of false positive and false negative response of the respective assays will be outlined, compared to the corresponding chromatographic-mass spectrometric confirmation analyses.

Detection of methamphetamine in hair by ELISA and GC-MS"

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This study was designed to compare presumptive enzyme linked immunosorbent assay (ELISA) results with gas chromatography-mass spectrometry (GC-MS) confirmatory results for methamphetamine and its metabolite, amphetamine in hair. Hair from 29 individuals suspected of methamphetamine use was collected. The hair was washed with 0.1 % sodium dodecyl sulphate, distilled water and dichloromethane. For ELISA, the samples were extracted by incubation in monobasic phosphate buffer for 1 hour and then neutralized with dibasic phosphate buffer to pH 6-8. For GC-MS, the samples were extracted for 20 hours in methanol containing 1 % hydrochloric acid. The methanol/acid solution was evaporated to dryness and the resulting residue was derivatized with trifluoroacetic anhydride. Methamphetamine and amphetamine were detected using GC-MS in selective ion monitoring (SIM) mode.
Overall, there was a good correlation between ELISA and GC-MS results. The Immunalysis Methamphetamine Microplate ELISA demonstrated a sensitivity and specificity of 97 % and 100 % respectively using a cut-off concentration of 0.5 ng/mg *d*-methamphetamine. The ELISA kit showed 63 % cross-reactivity with *d,l*-methamphetamine and did not cross-react to any significant extent with the licit *l*-methamphetamine isomer. Methamphetamine and amphetamine levels were found to range from 1.2-45.3 ng/mg and 0.1-2.5 ng/mg respectively using GC-MS.

ISO17025 Accreditation for Drug Testing in Hair – The UK and Swedish Experience

Gail Cooper[1] and Robert Kronstrand[2]

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Questions relating to the accuracy or reliability of test results are frequently asked during cross-examination in court or by customers assessing the suitability of a laboratory service. The international quality standard ISO17025, documents the criteria for testing laboratories wishing to demonstrate their technical competence in generating valid test results. As a consequence, laboratories worldwide are recognizing the importance of implementing Quality systems to ensure effective control of the testing services they provide.

The United Kingdom Accreditation Service (UKAS) and SWEDAC are the recognised national accreditation bodies in the UK and Sweden respectively. Both organisations are responsible for evaluating analytical testing laboratories for compliance to the ISO17025 standard including assessment of laboratory documentation, proficiency testing, measurement traceability, personnel competence, and measurement uncertainty calculations.

Accrediting methods for the analysis of drugs in hair represents a significant challenge to the analyst with respect to the lack of certified reference material, limited guidelines on best practice and reservations on the efficacy of hair testing. The experience of two laboratories accredited to ISO17025 in the UK and Sweden will be presented for both screening and confirmation methods.

Ethyl glucuronide in hair – comparison with usual biological marker of chronic alcohol consumption in post-mortem and forensic cases

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In forensic cases, the differentiation between alcohol abuse and normal/social drinking behavior is part of the daily work of forensic toxicologists. But often, adequate sample matrices for the interpretation of alcohol consumption are not available or had already been used for other toxicological routine analysis, especially when the request of the authorities was posed several weeks/months after the incidence.

For this reason, different biological marker of alcohol consumption in blood, urine and hair samples should be determined and compared for their suitability in the interpretation of drinking behavior with special focus to post-mortem cases. In a preliminary study, about 30 post-mortem cases with suspicion of an ethanol abuse "history" and one case of a hit-and-run-accident under the influence of alcohol were chosen.

Ethyl glucuronide was determined in post-mortem blood, urine and hair samples using a new developed solid-phase extraction method with Oasis-MAX columns, derivation with pentafluoropropionic anhydride and GC-MS-NCI determination in SIM-mode.

To measure CDT in blood, the Ceofix CDT reagent set (Analis) on a Agilent capillary electrophoresis with UV-detection (HP 3D-CE) was used. The other clinical markers like ASAT, ALAT, and GGT were analyzed by heterogeneous immunoassay on the Dimension® (Dade-Behring).

The determination of ethyl glucuronide in hair was possible in all cases, where hair was available, in a concentration range between 24 and 8170 pg/mg. EtG was also positive in 60% of the cases in urine. Our developed SPE method with Oasis MAX columns decrease sample preparation time by about 50% and eliminate derivation problems as a results of silica traces in the eluate of common extraction columns. The LOQ was estimated at 10 pg/mg.

Using the proposed cut-off of Yegles [1] for the interpretation of EtG concentration in hair, only one case could be considered as a social drinking behavior.

The detection of other long-term marker was only possible in 20% of all cases with limited interpretation value because of absence or degradation of plasma or serum samples.

Besides the limited interpretation value of CDT determined by CE-UV, only EtG in hair has proved suitable as marker of alcohol consumption in post-mortem cases. Our results underline the importance of hair as essential matrix in the evaluation of drinking behavior in post-mortem cases.

[1] Yegles M. et Pragst F. Annales de Toxicologies Analytique. Vol XVII, N°2 (2005), 121

Quantification of ethyl glucuronide in white and pigmented hair

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Determination of the concentration of ethyl glucuronide (EtG) in hair is a recent tool of growing interest for the diagnosis of alcohol abuse. However, little is known on the factors responsible for the incorporation/stability of EtG in hair.
Because of the mean age of patients suffering from alcohol abuse problems, subjects with white hair, grey hair, or dark hair streaked with grey, are frequently observed. Since melanin is reported to influence the incorporation and fixation in hair of many other drugs, the role of melanin was tested in this study.
EtG was measured in hair samples containing at the same time pigmented and white hair shafts. After washing with water and acetone, the hair samples were sorted out into pigmented and white hair shafts and pulverized. Following SPE extraction, EtG was measured using GC-MS in NCI mode. Analyses were performed in triplicate.
The EtG concentration observed ranged from 0 to 1240 pg/mg hair. Except for one sample, the mean EtG concentration was slightly higher in white hair than in the respective pigmented hair, but the difference was not statistically significant. When plotting EtG concentration in pigmented versus white hair, the correlation coefficient was 0.99 and the slope was 0.95.
In conclusion, this study demonstrates that unlike other drugs, the melanin content has not to be considered for the correct interpretation of EtG results in hair.

Dose-hair concentration relationship and pigmentation effects in patients on low dose clozapine

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Several hair components have been suggested as possible molecular sites for drug binding and interaction. Of these, keratin and melanin have been investigated in some detail in order to assess the mechanisms by which the binding occurs. Substances that are positively charged at physiological pH may interact by electrostatic forces between their cationic groups and the anionic carboxylic groups on the surface of the melanin polymer.

Studies in human subjects with grey hair have shown that various drugs are detectable in both the colored (melanin rich) and white (melanin free) hair shafts of these individuals. Again this supports the proposition that keratin and hair proteins play an important role in the binding of drugs in hair. However, these studies have shown that chlorpromazine, cocaine, amitriptyline, and methamphetamine are found in significantly higher concentrations in pigmented hair strands than in senile white hair strands. This preference for binding to pigmented hair may be attributed to a strong ionic interaction between the positively charged drugs and the polyanionic melanin polymer that is absent in white hair. However, binding to hair protein may account for a significant part of the drug accumulation in hair.

Twelve grey haired patients treated with low-dose clozapine as an adjunct medication in their treatment against Parkinson disease were included in the study. Each patient's hair was divided into a pigmented and a non-pigmented portion and those were analyzed separately.
Clozapine and desmethylclozapine were analyzed with LC-MS-MS after extraction of the analytes from the dissolved hair.

Paired results from the analysis of pigmented and white hair confirmed the preference for binding to pigmented hair for both clozapine and its metabolite. Sixty to 82 % of the incorporated clozapine was found in the pigmented hair. As drugs could be detected in white hair binding to hair protein or association with other hair matrix may account for a significant part of drug accumulation in hair.
There was a correlation between dose and the measured concentration of clozapine and desmethylclozapine in pigmented hair as well as white hair and total clozapine with r^2=0.87 and r^2=0.74 for total clozapine and desmethylclozapine, respectively.

The results demonstrate that interpretation of hair drug concentrations is complicated by the extent of hair pigmentation. This effect of melanin on the incorporation of drugs into human hair must be considered when evaluating results of hair analysis quantitatively.

Segmental hair analysis of MDMA enantiomers by GC/MS-NCI.

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3,4-Methylenedioxymethamphetamine (MDMA) is one of the most frequently abused psychoactive additive substances known as "ecstasy". 3,4-Methylenedioxyamphetamine, (MDA), itself also a drug of abuse, is the N-demethylated metabolite of MDMA. These compounds are usually obtained as racemates and they present differences in the pharmacodynamics and pharmacokinetics properties. Several studies have evaluated the enantioselective disposition of MDMA and MDA in human matrices like saliva, urine or serum. However, to our knowledge, no study was done so far to determine the enantiomeric ratios (*R* versus *S*) of MDMA and MDA in hair. The aim of our study was to quantify by gas chromatography coupled to mass spectrometry (GC/MS) these enantiomers in hair segments.
Hair specimens were obtained from former ecstasy abusers following controlled MDMA administration. The MDMA self-administrated doses ranged from 75 to 100 mg. Hair specimens were washed and cut in 2 cm long segments. After digestion and solid phase extraction, the enantiomers were derivatised with a novel chiral agent, (2*S*,4*R*)-N-heptafluorobutyryl-4-heptafluorobutoyloxy-prolyl chloride, synthesized at the authors laboratory and quantified by GC/MS operating in the negative chemical ionization mode (NCI).
All specimens were positive for MDMA with a predominance of the (*R*)-enantiomer, the *R*/*S* ratios of MDMA varying between 1 and 2.8 and the concentrations ranged from 0.1 to 20.1 ng/mg. The enantiomers of its metabolite MDA were also quantified in most hair segments. The *R*/*S* ratios of MDA varied between 0.6 and 1.6, while the concentrations ranged from 20 to 1020 pg/mg hair. When segmental analysis was performed on single hair specimens, no inversion of the R/S ratios of MDMA and MDA was observed. The predominance of (*R*)-MDMA in hair was in accordance with those already published for other matrices and may be the consequence of the stereoselective metabolism.

Correlation of metabolite ratios of amitriptyline in hair and genetic polymorphism

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It is a common consensus in hair analysis, that absolute concentrations are influenced by a high number of parameters (e.g. hair colour) and barely reflect dosages or duration of drug administration. In contrast, metabolite ratios (e.g. benzoylecgonine/cocaine) are considered as significant parameters because the structural similarity of different metabolites and corresponding parent compounds often tend to level incorporation rates into hair.

In a case of an administration of amitriptyline to a group of >40 infants, significant variations of hair concentration ratios were observed. Each of the ratios

- Nortriptyline/amitriptyline,
- 10-hydroxynortriptyline/nortriptyline
- 10-hydroxyamitriptyline/amitriptyline
- E10-hydroxynortriptyline/Z10-hydroxynortriptyline

varied by factors of 5-10 between individuals. These variations are stable and reproducible over the time period covered by the hair samples.
The metabolism of amitriptyline is known to be influenced by polymorphism of the enzymes involved. CYP2C19 is mainly responsible for the demethylation of amitriptyline while hydroxylation is supposed to be predominantly controlled by CYP2D6.
Attempts to associate metabolite ratios in hair with genetic revealed that

- the amount of nortriptyline in hair (relative to amitriptyline) is highly correlated with the number of functional alleles encoding CYP2C19,
- the conformation of hydroxyl metabolites (i.e. E10/Z10-hydroxy isomers) is governed by CYP2C19 rather than CYP2D6
- there is no obvious correlation between CYP2D6 phenotype and hydroxylation of amitriptyline.

The study demonstrates that variations of metabolite ratios in hair samples represent significant individual differences of drug metabolism. This may partially be associated with genetic polymorphism.

Determination trazodone and its metabolite 1-(3-chlorophenyl)piperazine (mCPP) in Hair by High-Performance Liquid Chromatography–Electrospray Mass Spectrometry (HPLC–ESI-MS)

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The aim of this study was to develop, optimise and validate an analytical procedure to determine common antidepressant trazodone and its active metabolite 1-(3-chlorphenyl)piperazine (mCPP) in hair. Although mCPP, a piperazine-derived designer drug is still a legal substance in most countries it has been detected in seizures of ecstasy tablets on the drug scene. It shows stimulant and hallucinogenic effects similar to those of MDMA.
The target substances were extracted with 1-chlorobutane after alkaline digestion (0.5M NaOH, 1h, 70 °C) and analysed underivatised with high-performance liquid chromatography-electrospray ionisation mass spectrometry (LC–ESI-MS). ESI-MS parameters such as cone and capillary voltage, source tempera, desolvation temperature and gas flow were optimised with the application of direct inlet method. Quantification was performed using selected ion recording of positive molecular ions of the studied drugs, and deuterated ketamine used as internal standards.
The limits of detection were 0.1 ng/mg hair for mCPP and 0.02 ng/mg for trazodone. A linear response was observed for the two drugs from 0 to 5 ng/mg hair. Other validation parameters such as precision, accuracy, extraction efficiency were also determined and were satisfactory.
The method was applied to the determination of mCPP and trazodone in 7 authentic hair. A single administration of 80 mg of trazodone gave the following concentrations in hair sample collected one month after: 1st (1 cm) segment 5.93 ng/mg for trazodone and 0.1 ng/mg for mCPP; 2nd (1 cm) segment 0.04 ng/mg for trazodone and mCPP was not detected. Six remaining samples were negative. The method can be applied for differentiation an intake of mCPP from that of its precursor drug trazodone and others.

Strategies in postmortem hair testing

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There are several situations when analysis of hair samples from decesed subjects may be valuable. In most instances, analysis of pharmaceutical and illicit drugs is of interest. In certain cases, the mere detection of a particular drug may be crucial for the investigation, e.g. to prove that a victim of a suspected criminal poisoning dying at a hospital several days after the event really had been exposed to a particular drug. The detection of certain drugs in hair may even sometimes assist in the identification of an unknown dead victim.

The value of more detailed quantitative analyses of drugs in hair may vary. It is well recognized that drug incorporation in hair depends on the physicochemical properties of the drug, and on the character of the hair, particular the melanin content. This means that comparisons of hair drug concentrations between individuals usually is risky. However, within the same individual, differences in drug concentration over time may be used to disclose changes in drug use pattern. Hence, careful segmental analysis for drugs may e.g. reveal periods of recent abstinence that could assist in the interpretation of a suspected intoxication.

There are certain logistic and practical aspects on postmortem hair sampling that should be considered. If hair is collected during or after an autopsy, the hair may be soaked with blood, that may contain high levels of certain drugs. Typically, it is suggested that hair should be collected from the posterior vertex. However, a dead person on an autopsy table is usually stiff, lying on its back and hence, the desired collection site is difficult to access. The appropriate way to resolve this practical problem is to turn the body around, or on the side. Good illumination is crucial. The hair should be cut exacly at the level of the scalp, and the hair strands then be carefully aligned, particularly if segmental analysis should be performed.

A rapid screening for drugs of abuse in hair may prove very valuable, since a negative result means that laborious verification analyses may be avoided. According to our experience, positive screening results are usually confirmed by the verification methods, let alone some detections fall short of the cut off levels. If an opiate overdose is suspected, analysis of short hair segments should be performed in order to allow for the detection of recent discontinuation of drug use, that may imply a reduced tolerance. In other cases, a drug exposure at a particular time point, sometimes long before the death, may be an issue; then the segmentation of hair should be adjusted accordingly.

The selection of cases for hair analysis may vary depending on the case-specific circumstances, but we suggest the indications for collection of hair samples be wide. Then, in some cases the samples could just be stored, and only analyzed when a particular question arises. Having stated that, our experience is that hair analysis often reveal drug exposure that was not expected, so a liberal attitude to performing various forms of screening analysis is recommended. In postmortem toxicology, most investigators have focused on the detection of illicit drugs in hair, e.g. to assess the degree of tolerance. However, if methods for the simultaneous determination of a large number of pharmaceutical drugs in hair were developed, such analyses would be extremely useful for the interpretation of postmortem blood drug levels, since most fatal poisonings involve pharmaceutical drugs rather than illegal drugs.

Interpretation of forensic cases based on empirical data

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Hair test results for drugs of abuse have been used more frequently in German criminal courts. Interpreting analytical findings is a difficult and challenging task for forensic toxicologists who must examine their work critically. Employing statistical data as a source of information could be a helpful tool.
Forensic reports on hair analyses submitted by the Bavarian State Criminal Police Office (Bayerisches Landeskriminalamt – BLKA) serve as the basis for the statistics. More than 1000 hair segments have been tested using gas chromatography/tandem mass spectrometry (GC/MS/MS) within the past two years. The quantity of analytical results and additional essential facts have been documented. We appreciate the selectivity and sensitivity of this technique, as we use it for conducting our daily routine detection of the most prevalently abused drugs and metabolites, such as cannabinoids (Δ^9-tetrahydrocannabinol = THC, cannabinol = CBN and 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid = 9-carboxy-THC), cocaine (benzoylecgonine, norcocaine, cocaethylene, anhydroecgonine, anhydroecgonine methylester), some synthetic drugs from the amphetamine group (amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine, 3,4-methylenedioxyamphetamine, 3,4-methylene-dioxyethylamphetamine), opiates (heroin, 6-acetylmorphine, morphine, acetylcodeine, codeine, dihydrocodeine, methadone, its metabolite 2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolidine). The limit of quantification (LOQ) for these analytes is 0.1 ng/mg hair, for the specific metabolite of THC 0.1 pg/mg hair.
Selected important outcomes of the statistics are as follows:

96% of the hair samples were taken from the head. A total of 86% tested positive for illicit drugs or their metabolites, among these 35% were cocaine positive, 21% were positive for opiates. A special topic is the detection of cannabinoids. The collection of data showed that 61% were positive for THC/CBN and 9-carboxy-THC. However, 10% of this group had a negative result for cannabis constituents but were found to be positive for 9-carboxy-THC. 11% of the hair segments had a positive result for THC/CBN, but the metabolite could not be confirmed. Additional specifics are median and maximum concentrations of analytes.

We consider the compilation of quantitative findings to be an important tool in the evaluation of results, even in cases of demanding enquiries in hair testing.

Application of hair analysis in driving license regranting

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Hair analysis is a powerful tool, to detect drug consumption in cases of regular and occasional use. Even single use can be detected under certain circumstances. However, it is obvious, from a (non systematic) survey that there are large differences in the legal approach between the European countries. Furthermore, it follows from these answers that there was not always made a clear difference between driving ability and driving liability or driver's aptitude. These terms have varying definitions in the different countries. There is no question, that the activities of the scientific societies are helpful to install useful procedures on national levels. This can be seen by the French example, where proposals of the SFTA have been adopted into legal regulations. In Italy and France, hair analysis for drugs in integrated as an option into the regranting process. However, on an international level, there is no harmonisation in sight.

Evaluation of self-report, urinalysis and segmental hair analysis of drugs in amphetamine users enrolled in a clinical trial

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Monitoring prescribed and non-prescribed drug use, and conversely, drug abstinence, during drug treatment programs is important for the evaluation of compliance and success of the treatment. Usually, repeated urine testing is used to monitor drug abstinence. However, the drug abuse panorama has changed over the years, and today many drug users are working, implying that they might find it difficult to appear twice a week at an addiction treatment centre to provide urine samples. Hair testing offers some advantages over urine testing; the collection of hair is less embarrassing for the patients, and one hair sample can cover a long time period.

We collected hair samples from 19 amphetamine abusers participating in a clinical trial with naltrexon. The hair samples were cut into segments and analyzed for drugs of abuse. The subjects also provided self-reports of their drug use over time. The aim of this study was to find out if the drug use pattern as determined by segmental hair analysis correlated with the drug use reported by each subject. During the treatment program, urine samples were collected two times a week and analyzed for the most frequent drugs of abuse. Three hair samples were cut from the posterior vertex of the scalp at the beginning of the enrolment. The hair samples were cut into 1 cm segments and washed with 2-propanol, three phosphate buffer washes and a final wash with 2-propanol. After extraction with a mixture of acetonitrile, methanol and formate buffer, an aliquot was analyzed with an LC/MS/MS method, covering amphetamines, cocaine, benzoylecgonine, flunitrazepam, diazepam, and virtually all opiates abused in Sweden.

Urine collection was incomplete before the start of the enrolment, and since most hair samples were collected at this time, urine test results were only available for comparison in a few cases. Similar detections were made in hair and urine, but for a more firm assessment, several more cases need to be studied. Self-reports were, on the other hand, filled out by all but four subjects, and most of them had provided very detailed information of the drug use over a period of a few up to several months. In total, 81 hair segments were analyzed. Amphetamine was detected in all but one of these. In eleven cases the self-report and the concentrations of amphetamine in hair matched fairly well. In four cases the results were difficult to interpret. In ten cases additional drugs were found in hair. The second most frequent drug detected was methamphetamine, followed by cocaine, benzoylecgonine and tramadol.

This study indicates that segmental hair analysis with LC/MS/MS may be used to map drug use pattern over time, but further studies are needed to evaluate its possible use to replace urine testing for control of abstinence in drug treatment programs.

Hair versus blood and vitreous humor in the interpretation of drug related deaths

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Information obtained from medical records and police reports in drug related deaths is often misleading and does not give a true indication of past drug use of the individual concerned. Regarding the interpretation of toxicological findings, it is often important to obtain information on past drugs use, particularly for drugs where tolerance can develop. In this study, 20 postmortem hair samples were obtained from a variety of suspected drug related death cases. In addition to hair, blood and vitreous humor were also collected. The samples were analysed for the presence of selected benzodiazepines, methadone, morphine and 6-acetyl morphine. Drug history was obtained from police reports and medical histories where possible and the results of the analysis of all three matrices compared with the histories to determine how good a match to the known history each matrix was.

In 7 of 20 of the cases blood and vitreous humor sample results were found to match with information gained by the police reports and medical histories. In 9 of 20 cases the hair sample results were found to match with information gained by the police reports and medical histories.

The study indicates that hair analysis is important if a full interpretation of post-mortem toxicology findings is to be carried out even when a full drug use history is thought to have been obtained.

Segmental hair analysis as a tool for the evaluation of the role of abstinence in heroin overdose deaths

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Victims of lethal heroin overdose often present with fairly low blood concentrations of morphine. Reduced tolerance due to abstinence has been proposed to account for this finding. The aim of the present study was to examine the role of abstinence in drug-related deaths by comparing recent and past exposure to opioids using segmental hair analysis with the postmortem blood morphine concentrations in deceased heroin users.

The study included 60 deceased drug addicts in the Stockholm area, Sweden. In all cases a full autopsy, extensive toxicological testing and microscopic examination were performed. Information about the circumstances and history of the deceased was compiled from the police investigation and medical records. Three portions of hair were collected from the posterior vertex. One portion was subjected to a drug screen with a sensitive LC-MS-MS method, modified to also include the μ-opioid receptor agonists fentanyl, buprenorphine, methadone, propoxyphene, tramadol and ketobemidone. Positive results were confirmed and quantified with a second analysis (GC-MS for opiates and amphetamines or LC-MS-MS for benzodiazepines) preceded by segmentation and a standardized washing procedure. To this end, a new set of hair samples from the same subjects was carefully aligned and sectioned into short segments; 5 mm long for the most recent segments (S1-S3), and 10 mm long for the outer segments (S4-S5).

In 32 cases, death was not related to heroin intake. In 18 of the 28 heroin fatalities, opioids were absent in the most recent hair segment (S1), suggesting a reduced tolerance to opioids. However, the blood morphine levels were similar to those found in the 10 subjects that showed continuous opioid use. Among continuous users, some subjects showed a gradual decrease in opioid levels over time, others an increase, and yet other cases had fairly uniform levels in all segments. There was no correlation between the concentrations of opioid drugs in hair and the blood morphine concentrations. Hair and blood analysis disclosed an extensive use of additional drugs that directly or indirectly may influence the opioid system.

In conclusion, we found that more than half of the heroin overdose victims had discontinued opiate drug use for variable periods of time before death, a finding that *per se* may link abstinence to propensity for overdose death. However, these subjects did not show lower blood morphine levels than obviously tolerant subjects. The finding of a large number of other drugs in blood at the time of death, and presence of contributing pathology rather suggests that polydrug use and other factors, are more causally important for these deaths than abstinence.

Methamphetamine detection in maternal and neonatal hair; Implications for fetal safety.

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Methamphetamine (MA) abuse is a serious health problem of epidemic proportions. Exposure to this drug, particularly during pregnancy, is difficult to ascertain. Presently, there is sparse information on gestational exposure to this potentially toxic drug.

To quantify MA accumulation in hair, identify co-exposure of MA with other drugs of abuse and characterize correlations between concentrations of MA in maternal and neonatal hair.

The Motherisk laboratory at the Hospital for Sick Children routinely carries out analysis of MA in hair. Mothers and infants with positive results for MA in hair were identified from the laboratory database.

Almost 85% of persons positive for MA in hair were positive for at least one other drug of abuse, mostly cocaine.
Eleven mother–baby pairs with positive hair for MA were identified. One neonate (9%) had a negative MA result even though the mother was positive. There was not any positive neonatal hair with negative maternal hair for MA. Methamphetamine levels in mothers and neonates correlated significantly.
MA concentrations in babies' hair were not significantly different from those of mothers.

To our knowledge, this is the first report on fetal exposure to MA during pregnancy showing transplacental transfer of the drug, with accumulation in fetal hair.
Hair measurement of MA in neonates is a useful screening method to detect intra-uterine exposure to the drug.
Our data indicate that positive exposure to MA strongly suggests that the person is a polydrug user, which may have important implications for fetal safety.

Disposition of Hydrocodone in Hair

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The use of prescription drugs, including synthetic opiates, is increasing in the USA with emergency room reports showing a dramatic rise in prescription opiate abuse. As part of an on-going study, the hair of admitted opiate users was analyzed for the synthetic opiates, hydrocodone, hydromorphone, oxycodone and oxymorphone, in order to determine if there was any correlation between self-reported frequency of opiate intake and the concentration of drug detected in hair. Twenty-four hair specimens collected from volunteers were found to contain synthetic opiates. All 24 contained hydrocodone (130 – 15933 pg/mg); and four showed the presence of hydromorphone (59 – 504 pg/mg). The specimens were also analyzed for morphine, codeine and 6-acetylmorphine. Hair specimens from five self-reported codeine users showed high concentrations of hydrocodone (592 – 15933 pg/mg) in addition to codeine (575 - 20543 pg/mg), but neither morphine nor hydromorphone was present in any of those hair specimens. While the analysis of some opiates in hair has been previously published, this is the first study where the hydrocodone and hydromorphone concentrations have been measured following self-reported opiate intake.

Determination of Ketamine and Norketamine in Hair by Gas Chromatography-Mass Spectrometry using Two-step Derivatization

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Ketamine (KET) is a central nervous system depressant that produces a rapid-acting dissociative effect. This drug possesses also potent analgesic properties at subanesthetic doses and has recently been a growing tendency for abuse of KET in Korea. Several chromatographic methods have been reported for the determination of KET and/or its metabolite norketamine (NKT) in biological matrices. However, the methods described require negative chemical ionization to improve selectivity or lack sensitivity for an accurate determination of KET and NKT, because of the low concentrations of target compounds or complex hair matrix.

The method procedure involves decontamination of hair with distilled water and acetone, acidic hydrolysis and extraction in the presence of the internal standard with 0.25 mol/L methanolic hydrochloric acid, a two-step derivatization by the successive treatment of trifluoroacetic anhydride (TFAA) and *N*-methyl-bis-(trifluoroacetamide) (MBTFA), and GC/MS analysis.

The method was linear over the range of 0.15-25.0 ng/mg for KET and 0.1-40.0 ng/mg for NKT with good correlation coefficients ($r^2 > 0.9997$). Analytical recoveries were above 95%. The within-run and between-run imprecision (CV) was 0.5-5.6% and 2.2-11.5%, respectively. The limits of detection (LODs) were 0.032 ng/mg for KET and 0.013 for NKT. By using two consecutive derivatization procedures, the sensitivity of the analytes could be improved. The LODs for KET and NKT were nearly 10-25 times lower than those reported by previous studies based on GC/MS method.

The GC/MS method described is highly sensitive, reliable, and suitable for the determination of KET and NKT in hair. The applicability of the method was proven by analyzing an authentic hair sample.

EXTRACTION OF HAIR MELANIN AND BINDING STUDY WITH AMPHETAMINE

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Drug-melanin interactions have been studied in order to determine the role of melanin in the incorporation of drugs into hair. To fully understand the implications of a positive hair test result, it is important to understand how drugs are incorporated into hair and how they bind to melanin. Previous research into drug-hair binding has shown that different amounts of drug bind in hair of different colours. There are no structural differences in hair of different colours other than in the type and content of melanin present. For this reason, this investigation focuses on amphetamine binding to hair melanin in order to find out the binding capacity and the binding sites present. Previous studies in the drug melanin interaction have used commercially available melanin such as sepia melanin and tyrosine melanin. This is the first time that hair melanin has been used for the binding study. The different polar groups present in melanin could serve as attachment points for the interaction of amphetamine.

In this regard, black Asian human hair was used to extract hair melanin following an using the already published procedure. Scanning electron microscopy, UV/Vis and the IR spectroscopy were used to determine the similarities and the differences of hair melanin with commercially available melanin. Hair melanin was used for the binding study with amphetamine using indirect spectroscopic studies measuring the decrease in absorbance of amphetamine due to the interaction of melanin. Phosphate buffer (0.1M) at the physiological pH was chosen for the binding medium. Samples were incubated for 1hr 30 min with constant shaking. Background correction was done at each step using the same amount of melanin as present in the sample for matrix matching. The interaction was monitored using a Genesys 6v, UV/Vis spectrophotometer in scanning mode from 250-264 nm focussing on changes in the amphetamine spectral fingerprint at 257.2 nm. Positive controls were prepared using the same amount of AP as in the sample with the addition of buffer instead of melanin at each step. Buffer was used for the background correction to run positive controls.

To determine the classes of binding sites, association/dissociation constants and the binding capacity of melanin, number of μmoles of drug bound and the dry weight of melanin in μg was plotted versus the free drug concentration The non linear regression curve fit was used from the graph pad prism version 4.03 This study has shown the difference of hair melanin with the sepia and tyrosine melanin and proved that AP binds to hair melanin in-vitro.

Treatments against hair loss may hinder cocaine and metabolites detection

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It is known that cosmetic treatments such as bleaching, dyeing, and perming may decrease the original drug concentration in hair. However, to our knowledge, there are no published reports on the influence of treatments against hair loss on drugs of abuse detection in hair. Our attention on the matter was drawn by some real cases where the analysis of hair (overnight incubation in 0.1 M HCl, mixed-mode solid phase extraction, trimethylsililation, and GC-MS analysis in SIM mode) did not produce valid results for cocaine and metabolites. A series of very intense interfering peaks with ion fragments common to cocaine (CO), benzoylecgonine (BE), and cocaethylene (CE) were found to cover up the "cocaine" region of the chromatogram (temperature program: 100°C for 2.25 min; 100-180°C at 40°C/min; 180°C-290°C at 10°C/min). In one of these cases the subject declared he had used a lotion containing minoxidil in order to prevent hair loss. Starting from this observation we analysed a minoxidil water solution with our GC-MS procedure and found that the interfering peaks belonged to four different TMS derivatives of minoxidil. Minoxidil interference was further investigated by applying Tricoxidil® (a minoxidil solution, 2% w/v) to the hair of cocaine-free volunteers (at the recommended dosage and mode of use) and to a CO-positive hair strand dipped into Tricoxidil. Hair were analysed before and after treatment. In both cases interfering peaks were absent in the chromatograms of untreated hair and appeared in treated hair. In the CO-positive hair detection of CO, BE, CE, and internal standard (either deuterated cocaine or scopolamine) was completely hindered after treatment with minoxidil. Attempts to separate interfering peaks from cocaine and metabolites by modifying the temperature program failed. None of the hair washing methods tested (methanol; dichloromethane; sodium dodecyl sulphate water solution, 1% w/v followed by methanol; phosphate buffer 0.1 M, pH 6 followed by methanol) succeeded in removing minoxidil interference. However, a simple solution to partially overcome the problem is to dry up the derivatised extract, reconstitute it in methanol (in order to switch back minoxidil derivatives to the native molecule), and re-inject it: owing to the higher polarity, underivatised minoxidil does not interfere any more with the chromatography of CO and CE (not undergoing trimethylsilylation), at the expense of the disappearance of BE and ecgonine methyl ester (both producing TMS derivatives), though. This strategy was applied to 4 real cases where minoxidil interference was recognised: in 2 of these cases cocaine was detected. It is also recommended in such cases to analyse an alternative hair sample (pubic or axillary). The problem of minoxidil interference on cocaine detection may be limited to procedures involving trimethylsilylation, which is probably the most commonly adopted derivatisation in laboratories performing hair analysis for drugs of abuse. However, we did not carry out investigations with other derivatising methods (e.g. acylation).

Application of Headspace Solid Phase Microextraction for Analysis of Cannabinoids in Hair

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In general, headspace solid phase microextraction (HS-SPME) in combination with GC-MS, with a suitable method of hair digestion or hair extraction and, if necessary, with a suitable derivatization has become an efficient alternative to the conventional analytical methods for several drugs in hair.

In the present study, a new, relatively simple and sensitive method for determination of tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN) was developed, based on alkaline hair hydrolysis, liquid-liquid extraction, combined derivatization and headspace solid-phase microextraction (HS-SPME), and GC-MS-EI. The method was optimised with respect to the extraction solvent, temperature and time of SPME preincubation and extraction as well as type and volume of the derivatization reagent. After addition of D_3-THC as internal standard, about 15 mg hair were dissolved in 0.5 mL 1 N NaOH and the analytes were extracted twice with 2 mL *iso*octane. The solvent of the organic phase was evaporated in a 5 mL headspace-vial and 10 µL of the derivatization reagent BSTFA were added. THC, CBD and CBN were detected directly from this mixture by HS-SPME and EI-GC-MS in SIM-mode with LOD's of 0.02 ng/mg.

The method was applied to a larger number of hair samples collected in context of driving ability investigation. Besides the sensitive detection of THC, it is possible to determine CBD and CBN. The results were evaluated with respect to the ratio of the three cannabinoids.

Furthermore, first experiments were carried out to detect also 11-nor-9-carboxy-^{9}-tetrahydrocannabinol (THC-COOH) by derivative HS-SPME and GC-MS.

Finally, with the aim to detect combined abuse of cannabinoids and alcohol, the ethyl ester THC-COOEt and the corresponding deuterated standard d3-THC-COOEt were prepared and a method for their determination in hair based on derivative HS-SPME and EI-GC-MS (LOD 0.02 ng/mg) as well as on neutral hair extraction and direct NCI-GC-MS-MS (LOD 0.05 pg/mg) were developed. Application of the method to hair samples of subjects with known combined alcohol and cannabis use gave no evidence for the presence of this compound. In the same way THC-COOEt was not detected in blood samples which were both positive for THC-COOH and alcohol. From this, it can be concluded that THC-COOEt is not formed to a substantial extent during combined alcohol and cannabis use.

COCAINE DETECTION IN MATERNAL AND NEONATAL HAIR

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Cocaine exposure during pregnancy in a cocaine-abusing mother is not uncommon. Fetal exposure to cocaine is potentially damaging, although the actual extent of the risk is controversial. Cocaine use during pregnancy is difficult to ascertain, and maternal reports are likely to be inaccurate due to a variety of reasons.
Taking advantage of the fact that cocaine and its metabolite benzoylecgonine accumulate and can be detected months after exposure in maternal and neonatal hair, a hair immunoassay for cocaine and benzoylecgonine was developed at Motherisk laboratory in Toronto.
The aim of this study was to find evidence of cocaine exposure during pregnancy using hair measurements, and the relationship between maternal and fetal exposures.

We identified 102 mother-child pairs who had cocaine and/or benzoylecgonine positive hair.
Median cocaine concentration was 3.02 ng/mg of hair in the mothers (range 0.11 – 208.7) and 0.34 ng/mg of hair in the neonates (range 0 – 24.21) ($p<0.001$). Median BE concentrations were 0.43 ng/mg of hair in the mothers (range 0 – 89.2) and 0 ng/mg of hair in the babies (range 0 – 23.25) ($p<0.03$).

Thirty-eight (37.2%) babies had negative cocaine and benzoylecgonine results even though their mothers were positive. None of the pairs identified included mothers with negative results. Sensitivity of the test for maternal use of cocaine using neonatal hair was 63.37% (95% CI 53.1% – 72.7%).

Babies' cocaine in hair was positively correlated with maternal cocaine and BE in hair ($r^2=0.23$ and $r^2=0.12$ respectively, $p<0.001$ for all correlations). Babies BE was also correlated with maternal cocaine and BE concentrations in hair ($r^2=0.47$ and $r^2=0.29$, $p<0.001$ for both correlations). Not surprisingly, maternal cocaine and BE concentrations in hair were strongly correlated ($r^2=0.81$, $p<0.001$).

Fetal hair grows in the last trimester. A positive result in the neonate's hair may indicate maternal use after pregnancy became known and is therefore a strong indicator of maternal addiction.
Transplacental exposure to cocaine of babies of addicted mothers seems extensive. The dose response relationship of both cocaine and benzoylecgonine between maternal and neonatal hair suggests that neonatal hair may be used to disclose maternal exposure of cocaine.