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Editor's note: ***The numbers before each title refer to the number assigned in the 1998 SOFT/TIAFT meeting program. The material has been reformatted for this printing. Every effort has been made to maintain the accuracy of the abstracts. Joseph R. Monforte, Ph.D., DABFT, ToxTalk Editor***

4: Safety Of Buprenorphine: Ceiling For Cardio-Respiratory And Subjective Effects At High IV Doses

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Buprenorphine (BUP), a partial μ agonist and κ antagonist, is under development for treatment of opioid dependence. The present study was conducted to test the safety and abuse potential of intravenous (IV) BUP in the range of doses used for maintenance treatment. Concerns have been raised about the potential acute health risk of buprenorphine if diverted and used by the IV route at doses equivalent to those used for maintenance. Subjective effects of BUP SL (12 mg) and IV/SL placebo (in random order) and BUP IV (2, 4, 8, 12, and 16 mg in increasing dose order) were evaluated in separate sessions in this single-blind, double-dummy study. Data were collected from 6 non-dependent male opioid abusers. Safety parameters included continuous monitoring of vital signs and oxygen saturation for 3 h after drug administration and additional collections over the next 72 h. Subjective measures included visual analog scales of global drug effects, ARCI subscales, and adjective rating scales. An increase in Systolic Blood Pressure for the 8 mg IV dose (+ 13.5 mm Hg) as compared to placebo was the only statistically significant change in blood pressure, heart rate or oxygen saturation among the 7 drug conditions. The mean maximum decrease in 0_2 saturation was greatest for the 8 mg IV dose (-7.3%). The main side effects were sedation, mild irritability, nausea, and itching. One subject did not receive the 16 mg IV dose because of severe nausea, which persisted for 24 hours (28 to 72 hr on some measures). Subjective measures included visual analog scales of global drug effects, ARCI subscales, and adjective rating scales. All active BUP conditions produced increases in positive subjective measures compared to placebo, including high, drug effect, good effects, drug liking, opioid agonist adjective rating scale, and MBG scale of the ARCI. Peak effects occurred 1 to 1.5 h after IV doses and 3 to 6 h after SL BUP. Duration of action was 24 hours or longer. Effects did not increase in an orderly dose-related manner; on many measures, the magnitude of effect was not different among all active doses. The effects of 16 mg IV tended to be less than those of 12 mg IV and varied in comparison to other active doses. Buprenorphine appears to have a ceiling for cardio-respiratory and subjective effects and a high safety margin when administered by the IV route in the absence of other drugs.

Key Words: Buprenorphine Intravenous Abuse Liability

5: Disposition of Heroin and Metabolites in Multiple Biological Matrices after Low Dose Oral Heroin Administration in Humans

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Clinical data regarding the disposition of heroin and metabolites after oral heroin administration is limited to case reports of overdoses by "body packers". We conducted a controlled clinical study during which nine healthy male volunteers with a history of heroin use were administered 10 mg heroin hydrochloride in the form of a gelatin capsule. Blood and saliva specimens were collected prior to drug administration and periodically thereafter up to 24 hours. All urine specimens were collected. Specimens were analyzed by solid phase extraction followed by gas chromatography/mass spectrometry for heroin, 6-acetylmorphine and morphine. The assay had a limit of quantitation (LOQ) of 1 ng/ml for each analyte. The total morphine concentration also was measured in the urine specimens.

Blood Heroin and 6-acetylmorphine were not detected in blood after ingestion. Morphine was first detected 7.5 to 30 minutes after drug administration with peak concentrations ranging from 2.2-15 ng/ml (mean=7.7 ng/ml, N=9), achieved 7.5 minutes to 4 hours later. Morphine concentrations declined thereafter, reaching the assay LOQ by 1-12 hours.

Saliva No analytes were detected in saliva after oral heroin administration.

Urine Heroin and 6-acetylmorphine were not detected in urine. Morphine was detected in the first void after drug administration for each subject (Mean time of void= 2.35 h, N=9). Peak free morphine concentrations ranging from 172-491 ng/ml were achieved at an average of 4.2 hours after drug administration. Peak levels typically occurred in the 1st-3rd voids. Peak total morphine concentrations ranging from 4317-12580 ng/ml were achieved at an average of 4.6 hours after drug administration. The time to the first negative sample for free morphine averaged 33.2 hours (Range= 21.5-54.7 h) with total morphine being detectable in 6/9 subjects at the last collected void at 68-75 hours. The time to the first total morphine negative for the remaining 3 subjects ranged between 53.1-77.9 hours.

These data indicate that after low dose administration of heroin by the oral route, heroin and 6-acetylmorphine are not detected in blood, saliva or urine. However, measurable concentrations of free and total morphine are excreted in the urine for approximately 30 and 53 hours, respectively.

Key Words: Heroin, Oral Administration, Blood, Saliva, Urine

6: The Role of Ethanol in the Etiology of Heroin-Related Deaths. Evidence for Pharmacokinetic Interactions between Heroin and Alcohol

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In order to evaluate the significance of pharmacokinetic interactions between heroin and alcohol in the etiology of heroin related deaths (HRD), the blood concentrations of ethanol (BAC), of free morphine (FM) and of total morphine (TM), as well as the total morphine concentrations in urine and bile were examined in a population of 39 lethal cases. The cases were included in the records of the Department of Legal Medicine and Public Health, University of Pavia in the period January 1997 - April 1998. Morphine was determined using the DPC Coat-A-Count radioimmunoassay with or without enzymatic hydrolysis. The cause of death was attributed to either heroin or associated heroin-ethanol intoxication. Cases were arbitrarily divided in two groups according to BAC (<1000 mg/l, LE, and >1000 mg/l, HE). The differences in the FM and TM concentrations in blood, bile, and urine, and in the FM/TM ratios between the two groups were statistically evaluated (Mann-Whitney U test).

A similar statistical evaluation was carried out on the data published by Goldberger et al. (*J. Anal. Toxicol.*, 18, 1994, 22-28) who studied the disposition of heroin and its metabolites (6-acetylmorphine, free morphine) in blood and urine in 23 lethal cases attributed to either heroin or heroin and alcohol intoxication. In this case the following variables in the LE and HE groups were compared: FM, TM, 6-acetyl-morphine concentration in blood (A), the FM/(FM+A) ratio, the FM/TM ratio, the urinary concentration of heroin (UH), 6-acetylmorphine (UA) and free morphine (UFM), and the UFM/(UFM+UH+UA) ratio.

Statistical analyses of data indicated that high BAC inhibits the hydrolysis of 6-acetymorphine to morphine (FM/(FM+A), $p = 0.0025$) and that the percentage of inhibition is correlated to BAC ($r = 0.67$). High BAC was also found to significantly decrease glucuronidation (FM/TM, $p = 0.0129$), and the excretion of free and conjugated heroin metabolites. According to these results, we advance the hypothesis that pharmacokinetic interactions between heroin and ethanol do occur in the organism of individuals exposed to high doses of these substances.

Keywords: heroin, ethanol, interaction

7: Heroin Metabolites in the Blood of Car Drivers.

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The purpose of the study was to test whether the determination of all possible heroin metabolites in blood may bring new information facilitating the correlation between the symptoms and analytical findings.

In the presented pilot phase 40 cases were analyzed from the period February 1997 to May 1998. On the basis of circumstantial information two groups were formed: "road traffic offenses" or "drivers" (n = 14) and "other offenses" (n = 26). As a sources of this information the police and court files and the results of medical examination before the blood sampling were used. Blood concentrations of morphine, M3G, M6G, 6MAM, codeine and C6G were determined by HPLC-APCI-MS. Other relevant drugs (cocaine, cannabinoids, amphetamines, benzodiazepines etc.) were analyzed by LC-MS or GC-MS after immunoassay screening. In both groups polydrug use was observed: only one "driver" and three "other offenders" had solely morphine and morphine metabolites in their blood. In all other cases, one to three additional drugs (cocaine, cannabinoids, methadone, amphetamines, benzodiazepines, ethanol) were detected. The concentrations of morphine and its active metabolite M6G in the group of "drivers" ranged from 3 to 80 µg/L and 8 to 248 µg/L, respectively. In the group of "other offenders" morphine concentrations were 1 to 95 µg/L and M6G 15 to 278 µg/L.

The determination of M6G as active heroin/morphine metabolite may bring new information concerning the exposition to opiates particularly in the view of new German law defining the mere presence of some drugs of abuse such as heroin or morphine in the blood of a driver as an offense.

Key Words: Heroin Metabolites, Car Drivers, Drugs of Abuse

8: Methadone Poisoning in Children

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Methadone is widely prescribed as a heroin substitute, often as a sweet green linctus (methadone concentration 1 mg/mL) which is attractive to children. We report 13 cases of methadone poisoning in children (6 males: 7 females) where serum or blood methadone concentrations were measured using capillary gas chromatography with NPD . With the exception of an 8.5 year old, all the children were below the age of 4 years.

Five children were found to be dead on arrival at hospital with serum or blood methadone concentrations of 0.23 to 0.63 mg/L (mean = 0.38). Methadone concentrations in the survivors on admission ranged from 0.06 to 0.40 mg/L (mean = 0.16) . In six of the survivors the parents implicated methadone and in the other two the toxicology results were diagnostic.

Although the children who died had a higher mean value, there was an overlap between the serum or blood methadone concentrations in the two groups. Consequently, methadone concentrations do not necessarily predict outcome. However the chances of survival are much greater if the child presents early, methadone poisoning is quickly diagnosed and treatment with an opioid antagonist is instituted.

Each child had been living with one or more opiate dependent parents who had been prescribed methadone in bottles fitted with reclosable child resistant lids. Poisoning occurred either because the child was capable of opening the bottle, the parents had not closed the lid or it was given to the child by a parent/sibling. Less than 10 mL of the mixture can cause serious poisoning in a small child and it is therefore particularly dangerous to children who may be living in a chaotic and uncontrolled home environment. Parents being treated with methadone should be made well aware of this and urged to store these preparations safely.

Key Words; methadone, blood concentrations, children.

9: Acetylcodeine, an Impurity of Illicit Heroin: Pharmacological Evaluation and Interaction with Diacetylmorphine.

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Acetylcodeine (AC) is one of the major impurities of manufacture in heroin. Data on its pharmacology and toxicology is limited and its ability to enhance the pharmacological activity of diacetylmorphine (DIAM) is not known. Acute toxicity, antinociceptive activity and spontaneous locomotor activity studies were conducted with AC and codeine alone and AC in combination with DIAM in male, ICR mice (s.c. injections). In the acute toxicity studies (assessed by convulsant activity) AC and DIAM were found to have similar CD_{50}s of 134 (120-153) and 116 (80-163) umol/Kg, respectively, but the spontaneous clonic-tonic convulsions observed in the AC treated mice were more violent and persisted longer than the DIAM-induced convulsions. Two mice treated with a high dose of AC died within 25 min. following

clonic-tonic convulsions whereas all the DIAM treated mice survived 24 hours. No spontaneous convulsions were observed in the codeine treated mice. The CD_{50} for codeine was 229 (189-278) umol/Kg. When AC was administered in combination with DIAM, the CD_{50} of DIAM was decreased to 40 (33-47) umol/Kg suggesting that the presence of AC in illicit heroin may increase the toxicity of the drug. AC was found to have a similar analgesic effect as codeine as determined by the tailflick test with ED_{50}s of 36 (31-43) and 52 (40-66) umol/Kg, respectively. In the spontaneous locomotor activity study, AC and codeine were found to have similar activities with ED_{50}s of 34 (26-45) and 30 (20-49) umol/Kg. DIAM was 10-fold more potent in increasing locomotor activity with an ED_{50} of 3.5 (1.9-6.8) umol/Kg. AC did not alter the locomotor effects of DIAM when given in combination. DIAM was found to have a therapeutic index that was 11-fold and 8-fold greater than the therapeutic index of AC in the tailflick and spontaneous locomotor activity studies, respectively.

Key Words: acetylcodeine, illicit heroin, impurity, interaction, toxicity

10: 6-Acetylcodeine as a Urine Marker to Differentiate the Use of Street Heroin and Pharmaceutical Heroin

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Pharmaceutically pure diacetylmorphine (heroin) is administered under strictly medicinally-controlled conditions to heavy heroin addicts, participating in the Swiss Heroin Maintenance Program. To check the feasibility of an analytical monitoring of concomitant consumption of street heroin, urines were collected unannounced and on the same day from randomly selected subjects. A previous chemical profiling of 170 street heroin samples has shown that >95% contained 1-5% of 6-acetylcodeine (AC). After solid-phase extraction of 10 ml-urine aliquots, resulting in a recovery of 84-92% AC, GC/MS in the SIM mode was used for quantitation. The identification of AC was based on the target ion m/z 341 and the ion ratios of the other characteristic ions m/z 282 and 229. The ions m/z 341 (AC) and 344 (AC-d_3, internal standard) were evaluated to quantitate AC. The limit of quantitation was 0.22 ng/mL, whereas the intra- and inter-day precision (n = 6, 10 ng/mL level) of the method was 3.2 and 7.4%, respectively. On that particular day 34% of the urine samples (n = 80) were ≥ the cutoff-concentration (0.22 ng/mL), with a content of 0.22 to 247 ng/mL AC. The percentage of positive urines was in correlation with that resulting from the self-declaration of interviewed program participants. Long-term studies including pharmacokinetics are necessary to check the reliability of the AC urine monitoring as a potential tool for following the socio-medical effects of the Heroin Maintenance Program.

Key Words: 6-Acetylcodeine, street heroin marker, GC/MS

11: A Prospective Analysis of Urine Specimens taken from Suspected Hospital Overdose Admission Patients

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Clinicians in major public hospitals in New South Wales generally do not rely on urine toxicology for acute patient management in potential overdose admissions. Blood is taken for paracetamol and for ethanol where clinically indicated; patients are treated symptomatically, and only if they fail to respond to management is comprehensive drug analysis performed.

In order to determine the usefulness of drug screening in the Accident and Emergency department, a prospective study was conducted of 93 urine drug screens. Royal North Shore is a major university teaching hospital in Sydney, serving a population of 750,000. Urine specimens were collected from overdose admission patients on admission, and stored for future analysis. Patients were treated symptomatically. Analysis of the specimens, using EMIT(, high performance thin layer chromatography and gas chromatography-mass spectrometry, revealed the presence of benzodiazepines, paracetamol, tricyclics and minor medications, none of which would have influenced acute patient management. Furthermore, many drugs were detected as a sequelae of treatment, for example, lignocaine, diazepam and thiopentone. In 47% of cases, drugs detected matched those stated to have been taken, 28% showed fewer drugs. Overall in 75% of cases no other drugs were identified.

The study demonstrated the need to use toxicology facilities judiciously, but justifies the practice of measuring blood paracetamol concentrations in all overdose patients.

Key Words: Urine Overdose Drugs

12: Detection Times of Morphine and 6-Acetylmorphine in Urine After Intravenous Heroin

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Detection times by GC-MS for total morphine and 6-acetylmorphine were determined following intravenous administration of 3, 6 and 12 mg doses of heroin·HCl to 8 male subjects. Subjects provided informed consent and the study was conducted on a closed clinical ward under carefully supervised medical conditions. All urine specimens were collected frozen and analyzed by GC-MS for total morphine and 6-acetyl morphine by DOD protocols. Mean detection times (hours to last positive) ± SEM at different cutoff concentrations are shown in the table.

Heroin Dose, mg	Morphine ≥4000 ng/mL	Morphine ≥2000 ng/mL	Morphine ≥300 ng/mL	6-AM ≥10 ng/mL
3	0.3 ± 0.3	2.7 ± 0.4	18.5 ± 2.5	2.3 ± 0.4
Range	0-2.7	1.6-4.5	10.2-29.6	1.2-4.5
6	3.8 ± 0.7	5.3 ± 1.0	24.8 ± 2.3	2.6 ± 0.4
Range	0-6.2	1-10.1	15.5-32.4	1.2-5.1
12	6.6 ± 0.6	10.1 ± 1.0	35.3 ± 3.7	4.5 ± 0.6
Range	4.1-9.3	5.9-13.5	20.2-53.3	2.3-7.5

The net effect of changing the confirmation cutoff concentration for total morphine, as proposed by DHHS from 300 ng/mL to 2000 ng/mL was to lower detection times to ranges similar to or slightly longer than those obtained for 6-AM. Use of the 4000 ng/mL DOD confirmation cutoff reduced detection times further. Overall, it appears that single doses of heroin will be detected for less than 12 hours at the 2000 ng/mL cutoff concentrations.

Key Words: Heroin, cutoff Concentrations, Detection times

13: Detection Times of 6-Acetylmorphine, Free Morphine and Total Morphine in Urine After Smoking Heroin

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Heroin was administered to four human subjects at doses of 3.5-13.9 mg by smoking. Urine samples were collected and tested by GC-MS procedures under blind conditions. The total amounts of 6-AM, free morphine and total morphine (M ± SEM) excreted in urine were 0.49 ± 0.14, 4.6 ± 1.0, and 53.5 ± 10.8 mole percent of the administered heroin, respectively. Detection times of the last specimen positive for the heroin metabolites at the cutoff concentrations indicated are presented in the table.

Heroin	6-AM	Free Morphine		Total Morphine	
Dose, mg	10 ng/mL	100 ng/mL	300 ng/mL	2000 ng/mL	4000 ng/mL
3.5 (n = 4)	0-4.9 hr	2.3-5.7 hr	7.9-27.5 hr	0-4.9 hr	0-2.3 hr
5.2 (n = 1)	2.3	2.9	7.5	2.3	0
7.0 (n = 2)	0-2.4	2.2-2.4	10.0-16.4	2.4-6.0	0
10.5 (n = 2)	2.3-5.9	2.3-8.4	10.7-28.9	2.3-8.4	0-5.9
13.9 (n = 1)	11.2	22.3	47.8	22.3	11.2

Free morphine and total morphine at cutoff concentrations of 100 ng/mL and 2000 ng/mL, respectively, correlated better with 6-AM at a cutoff concentration of 10 ng/mL and produced fewer false positives and false negatives than total morphine at the cutoff concentrations of 300 or 4000 ng/mL. Measurement of total morphine and 6-AM required two confirmation methods. Measurement of free morphine had the advantage that it could be assayed with 6-AM in one confirmation procedure.

Key Words: Heroin smoking, Cutoff concentrations, Detection times

14: Concordance of 6-Acetylmorphine with Free and Total Morphine in Urine After Intravenous, Intramuscular and Intranasal Heroin Administration

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Heroin is metabolized and excreted rapidly in urine as 6-acetylmorphine (6-AM), free morphine and conjugated morphine. Because other sources of opiates (codeine, poppy seeds) can produce morphine but not 6-AM, 6-AM is a useful marker for heroin exposure. We evaluated the relationship of 6-AM to free morphine and total morphine in urine following intravenous (3.0, 6.0 & 12.0 mg; N = 8 subjects, 624 specimens), intramuscular (6.0 mg; N = 6 subjects, 115 specimens), and intranasal (6.0 & 12.0 mg; N = 6 subjects, 337 specimens) heroin administration under controlled dosing conditions. Subjects provided informed consent and resided on a closed research ward under medical surveillance during dosing. All specimens were analyzed by GC-MS by Department of Defense protocols. The table compares the number of specimens identified in each cutoff category.

6-AM	#Specimens (Total Morphine)				#Specimens (Free Morphine)	
Cutoff, ng/mL	*<300*	*300-1999*	*2000-3999*	*≥4000*	*<100*	*≥100*
≥10	2	5	12	26	2	43
<10	761	230	27	13	983	48

Of the 45 6-AM positives (≥ 10 ng/mL), 84.4% had a total morphine concentration ≥2000 ng/mL and 95.6% had a free morphine concentration ≥100 ng/mL. Of the 78 samples of total morphine ≥2000 ng/mL and 91 samples of free morphine ≥100 ng/mL, 40 (51.3%) of the total morphine and 48 (52.7%) of the free morphine were negative for 6-AM (<10 ng/mL). Although 6-AM served as a reliable marker when present, there were many specimens containing ≥300 ng/mL of total morphine that tested negative (<10 ng/mL) for this marker.

Key Words: Heroin, Morphine, 6-Acetylmorphine, GC-MS, Cutoff Concentration

15: Identification and Measurement of Hydrocodone in Urine Following Codeine Administration

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Allegations of hydrocodone abuse have been made against individuals who were using physician prescribed codeine but claimed no illicit hydrocodone use. These allegations were based on the detection by gas chromatography-mass spectrometry (GC-MS) of low concentrations of hydrocodone (ca. 100 ng/mL) in urine samples containing high concentrations of codeine (>5000 ng/mL). We previously reported hydrocodone as a metabolite of codeine (Cone et. al., 1979) and recently had the opportunity to further investigate these findings. Five subjects in a controlled clinical study were orally administered 60 mg/70 kg/day and 120 mg/70 kg/day of codeine sulfate on separate days. Urine specimens were collected prior to and for 30 h following drug administration. In a separate study, a post-operative patient self-administered 960 mg of physician prescribed oral codeine phosphate/day, and urine specimens were collected on the third day of the dosing regimen. Unhydrolyzed urine specimens were analyzed by solid phase extraction followed by GC-MS. The two codeine formulations were also analyzed, and hydrocodone was not detected. In the controlled clinical study, codeine was detected in the first urine specimen following each drug administration; concentrations peaked rapidly (2-5 h) and ranged

from 1,475-30,848 ng/mL. Hydrocodone was initially detected at 6-11 h following codeine administration and peaked (32-135 ng/mL) at 10-18 h. In specimens collected from the post-operative subject, hydrocodone and codeine concentrations ranged from 47-129 and 2,099-4,020 ng/mL, respectively, and appeared to have reached steady-state. These data indicate that hydrocodone is a minor metabolite of codeine and may be excreted in urine at concentrations as high as 5% of parent codeine. Consequently, the detection of minor amounts of hydrocodone with high concentrations of codeine in urine should not be interpreted as evidence of hydrocodone abuse.

Key Words: Hydrocodone, Codeine, Urine Testing

16: A Practical Approach for Identifying Opiate Users in Workplace Drug Testing Programs with Simultaneous Detection of Codeine, Morphine, and 6-Acetylmorphine in Urine

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Proposed Department of Health and Human Services guidelines require opiates to be tested for total-codeine and total-morphine at cutoff concentrations of 2000 ng/mL, followed by 6-acetylmorphine (6-AM) at a cutoff concentration of 10 ng/mL. We recommend tests for free-codeine, free-morphine, and 6-AM at cutoff concentrations of 100, 100, and 10 ng/mL, respectively. For identifying heroin users, 6-AM at a cutoff concentration of 10 ng/mL correlates better with free morphine at a cutoff concentration of 100 ng/mL than with total-morphine at cutoff concentrations 300, 2000, or 4000 ng/mL (Table).

Drug	Cutoff ng/mL	Drug > cutoff 6-AM > 10 ng/mL	Drug < cutoff 6-AM > 10 ng/mL	Drug > cutoff 6-AM, ng/mL	
				1-9.9	0
Total-morphine	4000	7	9	1	0
Total-morphine	2000	10	6	3	3
Total-morphine	300	16	0	8	30
Free-morphine	100	16	0	6	3

Number of positive urine specimens from heroin users (n = 54).

Additional results indicated that the recommended cutoff concentrations for free-codeine, free-morphine, and 6-AM produce fewer false positive and false negative results than the other cutoff concentrations. The compounds can all be measured in one confirmation method using solid-phase extraction and pentafluoropropionic anhydride derivatization followed by GC-MS SIM analysis. Quantitations were linear from 6-1000 ng/mL for codeine, 5-5000 ng/mL for morphine, and 0.5-800 ng/mL for 6-AM. Recoveries were >90%.

Key Words: Workplace Opiate Cutoff, Free-codeine, Free-morphine, 6-Acetylmorphine

17: Urine Adulteration: Why is This Urine 'Klear' – How Much Klear is Required to Produce a Failed GC/MS Analysis for 9-Carboxy THC and Opiates?

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The adulteration of urine for the purpose of avoiding drug detection in the workplace urine drug-testing program, is an ongoing problem for forensic drug testing laboratories. Currently the most widely encountered chemical adulterant is Klear. Klear (500 mg potassium nitrite) is added to the urine after voiding and does not effect the color, temperature or smell of the urine. It has been reported that Klear when added to urine containing marihuana metabolites (and possibly opiate related compounds) causes the GC/MS confirmation test to fail. Klear does not appear to significantly affect either the initial immunoassay or the GC/MS confirmation of any of the other common drugs of abuse.

The objective of this study was to determine what concentration of nitrite was present in urine specimens adulterated with Klear and secondly to determine what concentrations of nitrite were required to prevent the GC/MS confirmation of 9-carboxy THC and opiates in urine. The concentration of nitrite in 50 urine specimens suspected of being adulterated was determined by ion chromatography (IC). To determine the effects of increasing nitrite concentrations on EMIT screening techniques and GC/MS confirmations, urine spiked with either 6 or 30 ng/mL 9-carboxy THC and 75 or 750 ng/mL of dihydrocodeine, codeine, hydrocodone, oxycodone, hydromorphone, morphine and oxymorphone were spiked with concentrations of potassium nitrite from 0 mcg/mL to 40,000 mcg/mL. These were then analyzed by both EMIT using Syva reagents for THC and opiates and by GC/MS. Nitrite concentrations were confirmed by ion-chromatography (IC).

In urine specimens presumed to have been adulterated the mean nitrite concentration was 3600 mcg/mL with a range of 0 mcg/mL to 11,300 mcg/mL. In all cases the EMIT screen for THC was positive and the GC/MS confirmation was unsuccessful. In the urine specimens spiked with various concentrations of nitrite, all were positive for THC and opiates by EMIT with no detectable inhibition of response. There was however a concentration dependant decrease in peak heights when analyzed by GC/MS which was found to be analyte specific. 9-carboxy THC and its deuterated internal standard were suppressed by 80% at 5000 mcg/mL with no recovery of either 9-carboxy THC or the internal standard at 10,000 mcg/mL. When analyzing the opiates, only hydromorphone, morphine and oxymorphone were effected by nitrite. The peak height of morphine was reduced by 70% at a nitrite concentration of 3000 mcg/mL, however no peaks were detected for either hydromorphone, morphine or oxymorphone when the nitrite concentration was 10,000 mcg/mL. This study shows that Klear, when added to urine prevents the confirmation of 9-carboxy THC but has no effect on the EMIT analysis and supports the findings of other authors. This study also shows that nitrite, at concentrations equivalent to those found in urine specimens adulterated with Klear, can not only prevent the confirmation of 9-carboxy THC, but also hydromorphone, morphine and oxymorphone.

Key Words: Klear, Opiates, THC

18: Lack of Correlation Between Performance Impairment and Pharmacokinetic Measures During the Distribution Phase Following Marijuana Smoking

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The relationship between pharmacokinetic measures and performance impairment is not well understood for smoked marijuana. This is especially true of the first 30 minutes after smoking when impairment is most reliably observed and Δ^9-tetrahydrocannabinol (THC) is being rapidly distributed from the plasma throughout the body. We have conducted two clinical studies to explore these relationships.

In the first study, 6 healthy research volunteers smoked on separate sessions one marijuana cigarette containing either 0, 1.75, or 3.55% THC. Plasma THC and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THCCOOH) were measured by GC-MS, and a battery of performance measures was assessed before and after marijuana smoking. At 20 min postsmoking, mean plasma THC levels were 27.5 and 48.8 ng/ml for 1.75 and 3.55% THC, respectively, and at 30 min postsmoking, mean THC levels were 17.3 and 29.7 for 1.75 and 3.55% THC, respectively. At these same times, accuracy on a test of grammatical reasoning was significantly impaired.

In the second study, 16 healthy research volunteers smoked on separate sessions two marijuana cigarettes that contained either 0, 1.75, or 3.55% THC. Plasma THC and THCCOOH were measured by GC-MS, and subjects performed four field sobriety tests (FST) 20 min after smoking ended. Mean plasma THC levels obtained immediately before FST testing were 15.4 and 28.3 ng/ml for 1.75 and 3.55% THC, respectively. Marijuana produced dose-related impairment on two of the FST, One Leg Stand and Finger to Nose.

In both studies, although significant marijuana-induced performance impairment was documented, there was no consistent correlation between any of the performance measures and plasma THC, THCCOOH, or the ratio of THC/THCCOOH across both marijuana doses. Thus, during the early distribution phase after marijuana smoking, there is no correlation between pharmacokinetic measures and performance impairment. Additionally, large inter-individual variation precluded identifying a threshold plasma THC level for impairment.

Key words: Marijuana, Performance, Pharmacokinetic-pharmacodynamic analysis

19: Quantitation of ^{9}-Tetrahyrdocannabinol In Commercially Available Hemp Seed Oil Products.

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There has been a recent and significant increase in the use and availability of hemp seed oil products. When taken orally these products are being marketed as a healthy source of essential omega fatty acids. While the health aspects of these oils is open to debate, the probability that oils derived from the hemp seed will contain ^{9}-tetrahyrdocannabinol (THC) is noteworthy. Recent additions to the literature site a number of studies illustrating that the ingestion of these products results in urinary levels of the THC metabolite, ^{9}-tetrahydro-cannabinol carboxylic acid (THCA), well above the administrative cut-off (50ng/ml) used during random drug screens. The purpose of this study is to quantitate the THC levels in commercially available hemp oils and to administer those oils tested to THC-free volunteers to determine urine metabolite levels after a 15g dose. Two extraction protocols were evaluated for removing THC from the oil matrix; a single step liquid/liquid extraction was compared to a two-phase process using both liquid/liquid and solid phase techniques. Gas chromatograph/mass spectrometry was used to determine THC levels in several products; four Spectrum Essentials (3 bottled oils and 1g capsules), two Health From the Sun (1g capsules and bottled oil) oils, along with single samples of both Hempstead and Hempola hemp oils. These hemp oil products contained THC concentrations of 36.0, 36.4 117.5, 79.5; 48.6, 45.7; 21.0 and 19.2 g/g respectively. FPIA and KIMS immunoassays were used to screen the urine samples and GC/MS was used to determine the amount of THC in each oil as well as confirm and quantitate THCA in the urine of study participants, immediately before and 6 hours after, each dose.

Key words: THCA, hemp oil, urine testing

20: Buprenorphine, Norbuprenorphine and Melanin in Human Hair.

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Hair analysis may be a useful tool for monitoring therapeutic compliance in drug treatment programs. The purpose of this study is to determine whether hair can be used as an adjunct specimen to plasma and urine for the monitoring of BUP treatment compliance. Subjects (n = 32) initiating buprenorphine (BUP) treatment were randomly assigned in a double-dummy, double-blind design to one of two treatment conditions: 8-mg oral liquid or 16-mg tablet once daily. Subjects were maintained on the assigned dose for 4 weeks; doses were then increased at 4 week intervals for up to 16 weeks. Hair and plasma were collected weekly and urine collected three times weekly. Questionnaires were administered weekly to document the occurrence of hair cuts and chemical treatments. BUP and norbuprenorphine (NBUP) concentrations in weekly hair and plasma were measured by LC/MS/MS. Urine specimens were analyzed by radioimmunoassay. Eumelanin (EU) and pheomelanin (PHEO) concentrations were measured in hair specimens from weeks 1 and 16 by UV spectro-photometry and HPLC. Preliminary data for the first four subjects who have completed the entire study are presented.

For the first subject, hair concentrations of BUP ranged from 4.5 (3 weeks) to 45.5 pg/mg (16 weeks). Hair concentrations of NBUP ranged from 4.8 (5 weeks) to 54.5 pg/mg (16 weeks). For the second subject, hair concentrations of BUP ranged from 5.3 (3 weeks) to 16.1 pg/mg (16 weeks); NBUP ranged from 20.8 (3 weeks) to 153.4 pg/mg (16 weeks). For the third subject, hair concentrations of BUP ranged from 17.8 (5 weeks) to 113.6 pg/mg (16 weeks); NBUP ranged from 67.7 (3 weeks) to 884.0 pg/mg (16 weeks). For the fourth subject, hair concentrations of BUP ranged from 8.1 (4 weeks) to 156.8 pg/mg (16 weeks); NBUP ranged from 43.7 (4 weeks) to 1438.5 pg/mg (16 weeks). Hair concentrations of BUP and NBUP were variable during a detoxification period (>16 weeks) in all subjects. EU concentrations ranged from 3.1 to 9.9 μg/mg for the four subjects and were consistent with histories of chemical treatments.

Measured hair concentrations of BUP and NBUP varied considerably between subjects. However, a gradual trend of increasing hair concentrations over time with increasing dose was observed for any single subject. These data demonstrate that hair may be a useful adjunct specimen to plasma and urine for the monitoring of BUP treatment compliance. This study was supported by NIDA Grant No. DA09096.

Key Words: Buprenorphine, Melanin, Hair

21: ^{3}H-Cocaine/Benzoylecgonine Uptake by Keratinocytes and Melanocytes in Culture

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The accumulation of certain xenobiotics in pigmented tissues such as hair has already been reported, and melanin drug interaction has been suggested to be involved in the incorporation mechanisms of various drug substances during the biosynthesis of hair fibers.

In the present experimental approach the uptake of ^{3}H-cocaine/benzoylecgonine (BZE) by keratinocytes was compared to that by melanocytes. The cultured cells were exposed to various ^{3}H-cocaine/BZE concentrations and incubation periods up to 168 hours. All test series were run in triplicate.

In both cell types the uptake of ^{3}H-cocaine/BZE was dependent on the drug concentration in the medium. After 72 hours an increased uptake of ^{3}H-cocaine/BZE was already found, ^{3}H-cocaine/BZE activity in 1 x 10^6 melanocytes was twice as much as in 1 x 10^6 keratinocytes. Following the time course over 168 hours, the uptake by melanocytes further increased leading to concentrations 5 times higher than in the keratinocytes.

These preliminary findings are discussed with respect to drug accumulation in the melanosomes due to melanin associated drug molecules.

Key words: Melanocytes, keratinocytes, drug analysis in hair

22: Segmental and Total Hair Analysis to Monitor Substance Abuse during Pregnancy

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Maternal exposure to cocaine (COC) and /or nicotine (NIC) has the potential to adversely affect the developing fetus. Drug self-reports are often unreliable and urine assays detect only recent use. In hair, drug use may be detected several months after the last dose, which provides an advantage over other biological samples. Therefore, maternal hair samples collected postpartum and assayed for COC and NIC in segments at measured distances from the root may provide an estimate of the quantity and timing of COC and/or NIC use during pregnancy.

Women participating in a study to identify risk factors for preterm rupture of membranes and preterm labor provided self-report of drug use, urine and hair specimens to identify those exposed to COC or NIC during pregnancy. Samples of urine and hair were collected postpartum, digested and screened by immunoassay (COC metabolite kit, and Cotinine (COT) urine kit, STC Diagnostics). Positive hair samples were evaluated further by two approaches: 1) analysis of the entire hair and 2) analysis of 1.5 cm segments. The entire hair and segments were digested, extracted and analyzed by GC/MS.

Of the initial 86 women studied, 1 reported the use of COC during pregnancy. Urine immunoassay identified 5 women (5.8%) as having used COC; 18 hair samples (20.9%) were positive for COC. Self-report identified 18 women (20.9%) who smoked cigarettes during pregnancy. Urine immunoassay identified 11 women (12.8%) who smoked during pregnancy; 30 hair samples (34.9%) were positive for NIC. Concentrations in hair by segmental analysis correlate well with concentrations in hair by intact analysis.

The STC immunoassays are sensitive screening techniques for the detection of COC and/or NIC in hair (limit of detection 0.1 ng/mg hair for both compounds). Hair analysis identifies more women who useor exposed to COC and/or NIC than does either self-report or urine analysis. Women who use COC or smoke before the pregnancy show a tendency to moderate or stop their use during pregnancy. Passive exposure to smoke may partially explain the large number of positive hair samples.

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Key Words: Nicotine, Cocaine, Segmentation

23: Quantitation of Cocaine, Benzoylecgonine, Cocaethylene, Methylecgonine and Norcocaine in Human Hair by Positive Ion Chemical Ionization (PICI) Gas Chromatography-Tandem Mass Spectrometry (GC/MS/MS)

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A total of 30 human head hair samples were analyzed for cocaine (COC), cocaethylene (CE), benzoylecgonine (BE), methylecgonine (EME), and norcocaine (NCOC) utilizing a sensitive positive ion chemical ionization (PICI) gas chromatography tandem mass spectrometry (GC/MS/MS) method. All 30 hair samples had been previously submitted to the laboratory and had confirmed positive for cocaine. Hair samples (20 mg each) were cut into small segments (2-5 mm) and incubated overnight at 45°C in 0.1 N HCL after the addition of 50 μL of an internal standard mix of COC-d_3 (1 ng/mg), BE-d_3 (0.5 ng/mg), EME-d_3 (0.25 ng/mg) and NCOC-d_3 (0.25 ng/mg). The samples were then extracted with a solid phase extraction procedure using World Wide Monitoring columns. The final extract was evaporated to dryness and derivatized with 50 mL of 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) and 50 mL of trifluoroacetic anhydride (TFAA) at 90°C for 15 min. The derivatized samples were allowed to cool to room temperature, evaporated to dryness and then reconstituted in 50 mL of ethyl acetate.

Parent set masses (bold ions) and product ions were m/z **304** and m/z <u>182</u> and 82 (COC), m/z **307** and m/z <u>185</u> and 85 (COC-d_3), m/z **318** and m/z <u>196</u> and 82 (CE), m/z **440** and m/z <u>318</u> and 105 (BE), m/z **443** and m/z <u>321</u> and 105 (BE-d_3), m/z **296** and m/z <u>182</u> and 82 (EME), m/z **299** and m/z <u>185</u> and 85 (EME-d_3), m/z **403** and m/z <u>386</u> and 105 (NCOC), m/z **406** and m/z <u>389</u> and 105 (NCOC-d_3).

Quantitation was accomplished by calculating the area ratio of the higher mass product ion (underlined ions) of analyte to the respective internal standard based on multilevel calibrations from 0.01 to 10 ng/mg. The GC/MS/MS method had an LOD of 0.01 ng/mg for all five analytes and an LOQ of 0.05 ng/mg.

COC, BE and EME were detected in all 30 samples and CE and NCOC were found in 19 and 28 samples, respectively. The average relative percentages of each metabolite normalized to the cocaine concentrations were 19.5%, 13.3%, 1.9%, and 3.4% for BE, CE, EME and NCOC, respectively.

Key Words: Cocaine, Hair, Tandem Mass Spectrometry

24: Determination of Dextromethorphan in drug abuser's hair by GC/MS

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Due to the easy availability, there has been a growing tendency to abuse the common medicines among young people in Korea. Zipeprol, dextromethorphan, nalbuphine and carisoprodol are among those non-controlled medicines most commonly abused. Among them, dextromethorphan has a long history of abuse. Dextromethorphan, which is an antitussive agent, produces little or no central nervous system depression, but manifestations of an acute overdose include hallucination and toxic psychosis. To obtain a hallucinogenic effect, young people usually take it in large amounts for recreational purpose.

In this study, to estimate the drug abuse history, the determination of dextromethorphan was established by GC/MS. Hair sample was washed with methanol, incubated with methanol (1% HCl) overnight at 37°C while stirring and extracted using solid phase extraction column on a vacuum manifold.

The calibration curve of dextromethorphan was linear from 5 to 1500 ng ($r = 0.9997$). The percentage of recovery from spiked hair for dextromethorphan was 107.3, 102.7, 105.6, 98.6 and 108.6% at 25, 50, 100, 500 and 1000 ng. Hair samples of 7 out of 13 dextromethorphan abusers showed positive results for dextromethorphan. The concentration of dextromethorphan was in the range 2.3 to 466.7 ng/mg in 7 specimens.

Key words: Abuse of non-controlled medicines, Dextromethorphan, Hair analysis.

25: Analytical Results Prior to and after Hair Treatment by Ultra Clean ™

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Ultra Clean ™, a commercially available hair care product, is recommended to remove medications as well as drug molecules from hair fibers. Its influence on the drug content of human hair was investigated.

Hair samples from persons (n = 14) with a known history of drug abuse were collected at autopsy. Each of the hair samples was divided into 4 strands and the proximal 4 cm segments were analysed prior to and after Ultra Clean ™ treatment. The shampoo was applied to two of the strands of each hair sample according to the instructions given by the manufacturer. The test series were run in duplicate. Hair analysis was performed by methanol extraction under ultrasonication, purification by solid phase extraction and GC/MS in SIM mode according to routine procedures for tetrahydrocannabinol (THC), cocaine, amphetamine, methylenedioxyamphetamine (MDA), methylenendioxy-meth-amphetamine (MDMA), methylene-dioxyethylamphethamine (MDE), heroin, 6-monoacetylmorphine (MAM), morphine, codeine, dihydrocodeine and methadone.

All drugs originally present in the hair fibers were still detected after a single application of Ultra Clean ™. In general the results varied within the ranges of the particular coefficients of variation, mostly showing a slight decrease as well as an increase in a few cases.

The findings clearly demonstrated that drug substances had not been sufficiently removed from human hair by a single Ultra Clean ™ treatment to drop their concentrations below the limit of detection of the analytical method applied.

Key words: Hair analysis, Ultra Clean ™, purifying treatment

26: Effects of Various Hair Stripping Treatments on Analytical Results

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Previously analyzed hair specimens were subjected to various hair stripping shampoos marketed locally and on the Internet to "cleanse the hair of drugs". These hair samples were prepared by a mechanical pulverization/aqueous extraction procedure and screened by a Kinetic Interaction of Microparticles in a Solution (KIMS) assay. Presumptive positive samples were washed with a buffered solution which was assayed to determine if successful decontamination had been accomplished. Confirmation was performed using established hair GC/MS extraction and derivatization procedures. Comparison of screening and confirmation values of methamphetamine, opiate and cocaine positive hair samples indicates that use of stripping agents generally reduces the amount of drug detected. The average reduction of drug detected by GC/MS confirmation was as follows: cocaine (BE) 5.45% ± 4.78, codeine 8.30% ± 7.88, and methamphetamine 8.00% ± 7.87%. Stripping agents containing lauryl sulfate showed no significant change in reduction of drug detected over agents not having this surfactant. Samples containing higher levels of drug detected were more sensitive to the drug reduction effects of stripping agents. Although these agents produced some statistically significant reductions in drug detected, no previously determined positive result was changed to negative.

Key Words: drugs, hair, stripping agents

27: Cannabinoids Detection in Human Hair Washed with Cannabio® Shampoo

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Today, cannabis plants are used in shampoo preparations, in foodstuffs (oils, nodles, crakers, etc.) or beverage (tea, etc.). These products are often Δ^9-tetrahydrocannabinol (THC)-free (< 1 %) in order to eliminate psychoactive effects, but some of them can include 1 to 3 % of THC. Gas chromatography-mas spectrometry (GC/MS) analysis of Cannabio® shampoo (Swihtco, 3205 Mauss, Switzerland) revealed the presence of THC (412 ng/ml) and two constituents of cannabis plants, cannabidiol (CBN : 4079 ng/ml) and cannabinol (CBD : 380 ng/ml).

In order to verify if normal hygiene practices with Cannabio® shampoo can cause positive hair results for cannabinoids, 3 subjects daily washed their hair with this shampoo during two weeks. After this period, hair specimens

were collected and extracted as described in our previously published procedure (Cirimele et al., *J. Anal. Tox.*, **20**, 1996, 13-16). In the 3 hair specimens, THC, CBD and CBN were never detected within their respective limits of detection 0.1, 0.02 and 0.01 ng/mg. We concluded that the use of Cannabio® shampoo during normal hygiene practices cannot be considered as a source of potential contamination of hair.

In a second experiment, drug-free hair specimens (200 mg) were incubated in 10 ml water/Cannabio® shampoo (20:1, v/v) for 30 minutes, 2 and 5 hours, respectively. After incubation, hair strands were washed with water and separated into two portions. One part was extracted directly, the second was decontaminated with methylene chloride and then extracted. After an incubation period of 30 minutes, the analysis of hair by GC/MS did not revealed the presence of THC, CBD and CBN either with or without decontamination. After an incubation period of 2 hours, specimens tested positive for CBD (0.113 ng/mg without decontamination and 0.100 ng/mg after decontamination) and CBN (0.019 ng/mg without decontamination and 0.018 ng/mg after decontamination). After an incubation period of 5 hours, specimens tested positive for CBD (0.247 ng/mg without decontamination and 0.135 ng/mg after decontamination) and CBN (0.021 ng/mg without decontamination and 0.020 ng/mg after decontamination). THC was never detected.

In conclusion, extensive but unrealistic use of Cannabio® shampoo can produce positive analyses in drug-free hair for CBD and CBN but not for the primary psychoactive drug THC.

Key Words: Cannabio® shampoo, hair analysis, cannabinoids contamination.

28: Detection of Cocaine and its Metabolites in Saliva and Urine by GC/MS during Clinical Drug Withdrawal

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There is an increasing interest in saliva as an alternative analytical body fluid. This study sought to determine the correlation of cocaine and its metabolites analysed in saliva and corresponding urine by GC/MS after reaction with MSTFA.

130 saliva samples (from 34 patients) were obtained from the oral cavity using a new developed collection device. Clin Rep® (Recipe, Munich GER) consists of a cotton roll treated with 1% citric acid and a centrifugation vial with a filter inset. Urine was extracted by liquid extraction using Toxilab A vials (DRG, Marburg GER). Saliva samples were prepared with chromabond drug® columns (Macherey-Nagel, Düren GER). Retention times were verified by deuterated standards.

From a total of 130 samples 9 urines were positive for cocaine metabolite above 100 ng/ml benzoylecgonine by FPIA. GC/MS identified benzoylecgonine in 7 samples, cocaine once and methylecgonine once in these samples. In saliva in all 9 corresponding specimens cocaine itself was detectable with a limit of detection of 10 ng/ml. By FPIA, however, no positive results were found. The other 121 samples were negative with urine and saliva as well. Cocaine was detectable in saliva for up to 4 days (maximal) after withdrawal of the drug.

Although analysis of cocaine in saliva requires sample extraction, derivatisation and analysis by GC/MS, saliva as sample material has many advantages: Saliva can easily be collected under supervision and therefore sample adulteration seems to be difficult. Patients do not feel disturbed in their privacy and the sample is obtainable at any time.

Key Words: Saliva, Cocaine, GC/MS

29: Saliva and Plasma Testing for Drugs of Abuse: Comparison of the Disposition and Pharmacological Effects of Cocaine.

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An inpatient clinical study was designed to characterize the pharmacokinetics and pharmacodynamics of cocaine, codeine, and methamphetamine in human subjects. This report describes the pharmacological effects and disposition of cocaine in plasma and saliva following subcutaneous (SQ) drug administrations. Three subjects received low doses (75 mg/70 kg) and high doses (150 mg/70 kg) of cocaine hydrochloride (HCl). Pharmacological effect data (pupil diameter, heart rate, blood pressure, subject-reported "High") and blood and saliva specimens were collected simultaneously.

Stimulated saliva was obtained by placing candy containing citric acid in subjects' mouths. Saliva was then collected in tubes. Biological specimens were analyzed by GC-MS for cocaine and metabolites.

Cocaine was detected in both saliva and plasma within 5-10 min following dosing, and cocaine concentrations in both matrices peaked within 30-60 min. Cmax measures for cocaine following low doses ranged from 749-1,682 ng/mL in saliva and from 258-370 ng/mL in plasma. Cocaine Cmax measures following high doses ranged from 2,342-7,249 ng/mL in saliva and from 406-655 ng/mL in plasma. Saliva/plasma cocaine ratios were generally greater than 2 in specimens collected for up to 24 hr following dosing. Benzoylecgonine (BZE) and ecgonine methyl ester (EME) were the primary cocaine metabolites detected in saliva and plasma. EME saliva/plasma ratios were generally greater than 1, and BZE saliva/plasma ratios were typically less than 1. Cocaine and metabolites were detected in plasma and saliva for approximately 24 hr following dosing.

Pharmacological effects observed after cocaine administration included increases in heart rate, elevations in blood pressure, dilation of pupils, and increases in subject-reported "High". The duration of pharmacological effects was consistently shorter than, or similar to the time course for detection of cocaine in saliva and plasma. These findings suggest that saliva testing may provide valuable insights into the onset and duration of cocaine-induced pharmacological effects. Overall, saliva testing appears to be a useful alternative to plasma testing for cocaine.

Keywords: Saliva, Cocaine, Plasma

30: Saliva and Plasma Testing for Drugs of Abuse II: A Comparison of Pharmacological Effects and Disposition of Codeine.

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This report describes the pharmacological effects and disposition of codeine (COD) and metabolites in plasma and saliva following oral administrations of low (60 mg/70 kg) and high (120 mg/70 kg) doses of COD sulfate gel capsules to three human volunteers. Pharmacological measures (pupil diameter, heart rate, "Liking," and "High") and blood specimens were collected concurrently. Saliva production was stimulated with hard candy containing citric acid. Plasma and saliva were analyzed by solid phase extraction followed by GC-MS for COD and metabolites. Mean COD concentrations ± S.E.M. are listed below:

Time post dose	COD sulfate (60 mg/70 kg)		COD sulfate (120 mg/70 kg)	
	ng/mL saliva	ng/mL plasma	ng/mL saliva	ng/mL plasma
5 min	0	0	0	0
10 min	3 ± 3	0	0	0
15 min	58 ± 59	2 ± 2	0	4 ± 4
30 min	251 ± 235	59 ± 22	148 ± 69	116 ± 67
1 h	432 ± 98	155 ± 71	608 ± 153	241 ± 133
2 h	312 ± 212	87 ± 21	604 ± 396	260 ± 55
4 h	129 ± 34	42 ± 18	424 ± 218	155 ± 53
8 h	61 ± 19	11 ± 9	127 ± 48	37 ± 8
23.75 h	5 ± 6	1 ± 1	4 ± 5	3 ± 1

Norcodeine was detected in plasma and saliva at concentrations generally less than 10% of COD, and morphine was not detected. Peak changes in pupil diameter, "Liking," and "High" typically occurred following peak codeine concentrations in saliva and plasma. These data indicate that saliva codeine concentrations are closely related to plasma concentrations. Consequently, saliva could serve as a useful non-invasive alternative matrix for drug testing.

Keywords: Codeine, Saliva, Plasma

31: Recruitment of Participants for Human Research Studies on Drugs of Abuse

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For the recruitment of human participants for an ongoing marijuana dosing protocol, the Intramural Research Program (IRP) of the National Institute on Drug Abuse (NIDA) has adapted multiple highly effective means of recruiting participants for research studies. Ethical guidelines require that for the protection of human participants, study candidates must have documented prior drug use and be of sufficient medical and psychological health to provide adequate informed consent. Participants for research studies have been recruited through word of mouth referral, advertisements in regional and local newspapers, daily cable television advertisements, and through the posting of informational flyers on local college campuses, in bars, coffee houses, and shops specializing in drug paraphernalia. These methods provide a wide pool of applicants, which is necessary due to the stringent medical and safety requirements of the study.

Out of 2991 telephone calls and walk-ins recruited for the marijuana study, 48% or 1425 candidates successfully completed the initial telephone drug and psychological history screening. These candidates were scheduled for further interviews. Thirty-nine percent or 559 participants received complete physical exams and extensive clinical laboratory and psychological evaluations. Seventeen percent or 99 participants were admitted to the pre-treatment phase of the study. Following further medical and psychological evaluations, 48% or 48 participants were randomized for experimental dosing. Most of the candidates initially screened were disqualified from participating in the study due to many factors, including: current active disease, clinical laboratory abnormalities, allergies, psychological or behavioral competence, current drug dependence or treatment, or an inability to comply with all study requirements due to legal or family social issues.

These factors represent a significant challenge to the recruitment of human research participants for drugs of abuse studies. The NIDA IRP has, over many years, developed procedures and personnel to address these issues to successfully recruit research participants, taking care to maintain the highest safeguards for the protection of human volunteers.

Key words: Human research, recruitment, marijuana

32: Evaluation of a On-Site Method for the Detection of Drugs of Abuse in Saliva

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Detection of drugs of abuse in saliva offers a convenient, non-invasive, method in place of or to complement conventional urine drug screening. A total of 72 saliva samples were volunteered by patients participating in a drug rehabilitation programme using the Salivette and swabs. Saliva samples are difficult to adulterate and samples containing visible blood were discarded. The saliva samples were analysed using of the COZART Rapiscan test system and screened for drugs of abuse using the laboratory-based method of microplate enzyme-immunoassay. Confirmational analysis was then carried out using solid-phase extraction followed by gas chromatography/mass spectrometry (31 cases for methadone and 24 cases for opiates) or high performance liquid chromatography (20 cases for benzodiazepines).

A swab was used to collect saliva from the subject. The swab was then inserted into a test cartridge housing the immunoassay. The test cartridge is designed to allow multiple drugs to be tested from a single specimen. This disposable testing cartridge was then inserted into the instrument for analysis. The COZART Rapiscan is a hand-held instrument which analyses, interprets and displays results of the test. Investigations of benzodiazepines, opiates, cannabinoids, amphetamines and cocaine metabolites were carried out with results for all drug groups displayed after five minutes. The instruments are factory set to give positive or negative results relative to a cut-off level but as part of this study these were qualitatively set.

All methods were able to detect the low levels of drugs present in saliva. The Rapiscan device was able to detect less than 1 ng/mL for the drugs tested. The Rapiscan response was linear for all drugs analysed. For methadone the calibration curve was linear over the concentration range of 0-250 ng/mL, $r^2 = 0.997$, with a CV of < 2.25% for each concentration measured (n = 7).

Spiked and case samples were analysed by the Rapiscan, by microplate and by GC/MS or HPLC. A linear response ($r^2 = 0.947$) was obtained when comparing the results of the Rapiscan with those by GC/MS for methadone (n =

17). Seven cases were positive for methadone by GC/MS (mean concentration = 0.40 ug/mL, range 0.15-0.73 ug/mL) with a corresponding Rapiscan response (mean = -399, range -167 to -733). The additional ten cases which were negative by GC/MS for methadone, were also negative by the Rapiscan and enzyme immunoassay. The results correlated well for all methods and drugs analyzed. Similar agreement was obtained with patient specimens for opiates and benzodiazepines.

Key words: saliva, drugs of abuse, on-site test

33: A Comparison of the STC Methamphetamine MICRO-PLATE EIA for Urine and Oral Fluid

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The traditional testing for determination of methamphetamine use has been through the collection and analysis of urine or blood plasma. Due to the invasiveness and potential violation of privacy for the donor with current techniques, oral fluid collection and testing has been investigated as an alternative method for the determination of methamphetamine use. One disadvantage of oral fluid is that the drug concentration is generally lower than what is found in urine.

The object of this study was to evaluate the STC Methamphetamine MICRO-PLATE EIA for oral fluid collected using the OraSure Oral Specimen Collection Device and compare it to the STC Methamphetamine MICRO-PLATE EIA for urine. The analytical sensitivity was evaluated by determining the LOD (Limit of Detection), which is defined as the average signal-noise ratio of the 0 ng/mL concentration (A_0) minus the noise times three (LOD = A_0 - 3SD). The oral fluid EIA LOD is 1.0 ng/mL, and the urine EIA LOD is 50 ng/mL. A study on the effects of sample pH determined that an oral fluid pH of 3, 4, or 5 caused false positives at a 10 ng/mL cutoff, but in the urine no effect of pH was found. Cross-reactivity studies involved 48 ubiquitous cross-reactants (10,000 ng/mL concentration), none of which produced a positive result in both assay formats, and 16 analogous cross-reactants. The following data for analogous cross-reactants is expressed in % cross-reactivity (oral fluid/urine, nd = not detected): L-methamphetamine (145/262), MDMA (358/827), MDA (1.1/3.4), B-phenethylamine (nd/0.22), D-amphetamine (nd/0.55), diphenhydramine (0.009/0.15), fenfluramine (5.7/1.0), isoxsuprine (nd/1.5), L-ephedrine (0.97/0.99), L-phenylalanine (nd/0.78), mephentermine (1.3/0.37), phentermine (0.07/0.1), phenylephrine (0.1/0.28), phenylpropanolamine (nd/0.19), procaine (0.07/0.16), and pseudoephedrine (1.3/1.5). The precision studies using 0, 5, 10, and 20 ng/mL oral fluid calibrators indicate intra-assay % CV of 8.3, 11.8, 10.5, and 11.4, and inter-assay % CV 4.2, 6.7, 10.6, and 8.1, respectively.

Eighty-one (81) matching saliva and urine clinical specimens (57 from self-reported, methamphetamine users and 24 from non-users) were tested with EIA and GC/MS (LOQ = 1 ng/mL). Thirty-nine (39) oral fluid specimens and 41 urine specimens were negative by GC/MS using a 10 ng/mL cutoff for oral fluid and a 500 ng/mL cutoff for urine. The oral fluid EIA detected 39 true negatives and 42 true positives; therefore, the percent sensitivity and specificity for the EIA oral fluid versus GC/MS were determined to be 100% and 100%, respectively.

Keywords: Methamphetamine oral fluid EIA/GC-M

34: Sweat Testing for Drugs of Abuse II: Comparison of Codeine Disposition in Sweat Collected with a Heated Sweat Patch Device and the PharmChek™ Sweat Patch.

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Opioids including heroin, morphine, and codeine have been detected in sweat collected from drug abusers. However, limited research has been performed to characterize the time course for secretion of drugs in sweat following controlled drug administration. This report describes the disposition of codeine in sweat collected from five human volunteers following oral administrations of low (60 mg/70 kg) and high doses (120 mg/70 kg) of codeine sulfate.

PharmChek sweat patches were applied to the torso to collect sweat during intervals ranging from 1 hr to 7 days. Sweat was also collected with a heated sweat patch device (Fast Patch) that was applied to the palm and torso for 5 to 30 min intervals. Patches were analyzed by GC-MS for codeine and metabolites.

Codeine was the primary analyte detected in Fast Patches and PharmChek patches; metabolites were not generally detected. Peak secretion of codeine usually occurred within 4-8 hr. Peak codeine concentrations ranged from 34-681

ng/Fast Patch and from 13-46 ng/PharmChek patch following the low dose. Peak codeine concentrations ranged from 54-1,123 ng/Fast Patch and from 10-13 ng/PharmChek patch following the high dose. Codeine was present in Fast Patches (worn for 30 min intervals) for 1-3 days following dosing. In comparison, codeine was only occasionally detected in PharmChek patches after dosing. No definitive relationship was evident between dose and the amount of codeine in patches. These data suggest that sweat testing with the PharmChek patch may be beneficial in monitoring drug use when the patch is worn for days to weeks. In comparison, the Fast Patch device provides a rapid method for sweat collection that may be highly effective in monitoring individuals for recent drug use.

Keywords: Sweat, Codeine, Fast Patch

35: Relationship of Pharmacological Effects to Codeine Concentrations in Plasma.

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An inpatient clinical study is being conducted to evaluate the pharmacological effects and disposition of cocaine, codeine, and methamphetamine in human volunteers. This report describes the relationship of pharmacological effects of codeine to plasma drug concentrations following oral codeine administration. Participants received low (60 mg/70 kg) and high doses (120 mg/70 kg) of codeine sulfate. Pharmacological measures (heart rate, pupil diameter, "High" and "Liking") were obtained concurrently with blood specimens. Plasma was analyzed by GC-MS for parent drug and metabolites.

Codeine was detected ca. 30 min after dosing with peak drug concentrations occurring typically at 1 to 2 hr. The mean peak codeine concentration (Cmax ± S.E.M.) for five subjects was 147 ± 50 ng/mL following the low dose and 378 ± 101 ng/mL after the high dose. The mean AUC (ng-min/mL) ± S.E.M. for the low and high dose was 28,326 ± 8,784 and 108,088 ± 18,346 respectively. Cmax and AUC measures for codeine were significantly greater ($p < 0.05$) following the high dose compared to the low dose.

Norcodeine was the primary codeine metabolite in plasma, and norcodeine concentrations were less than 10% of codeine. Normorphine was detected in some specimens, but concentrations were low relative to norcodeine. Morphine was not generally detected.

The primary pharmacological effect of codeine was miosis. Significant decreases ($p < 0.05$) in pupil size occurred at 15 min, 1 h, and 2 h after the high dose. Peak changes in heart rate, pupil diameter, "Liking" and "High" measures consistently occurred after peak plasma codeine concentrations resulting in counterclockwise hysteresis effects. The observed hysteresis may be related to metabolism of codeine to a pharmacologically active metabolite(s) or due to delayed distribution of codeine from blood to effector sites in the central nervous system.

Keywords: Plasma, Codeine, Pharmacology

36: Partitioning of Morphine and its Glucuronides in the Subcompartments of Blood

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Nowadays, special attention is paid to the ratios of morphine and its glucuronide conjugates which are recommended for evaluation of morphine or heroin medication and statements concerning last administration or the survival time. Blood samples in the forensic lab are often not well defined, and the subcompartments of blood cannot be considered as pharmacokinetically homogenous. Therefore, the dependence of morphine and glucuronide distribution between whole blood, plasma and red blood cells on hematocrit and water content was investigated.

Red blood cell-plasma-suspensions (hematocrit 10, 42, 44, 71%) were made up from a fresh blood sample and spiked with morphine, morphine-6- and morphine-3-glucuronide. The original samples as well as the corresponding parts of packed erythrocytes and plasma were analyzed by HPLC/fluorescence detection. Additionally, 10 blood specimens (hematocrit 38-54%) and decanted serum from heroin addicts were investigated.

The blood / plasma ratio of morphine concentrations was unaffected by variations in hematocrit and water content, while the corresponding ratios for morphine-3- and morphine-6-glucuronide were strongly influenced. Showing ratios of 0.53 to 0.65 and 0.52 to 0.62 in specimens with average hematocrit values (42, 44%) in blood samples with different

hematocrit values the ratios were 0.81 or 0.89 (hematocrit 10%) and 0.27 or 0.28 (hematocrit 71%). In contrast to the morphine conjugates, morphine was highly bound to / or partitioned into red blood cells ($ß_e$ = 55.9). The blood/serum ratios measured in authentic samples did not differ from the values established in red cell-plasma-suspensions within the precision of the analytical method.

Although preliminary data, the findings already demonstrate that metabolite ratios are highly dependent on the major constituents of the particular biological matrix under investigation.

Key words: Blood/plasma partitioning, morphine glucuronides, hematocrit

37: Post Mortem Findings of Foetal Drug Exposure in a Drug Abusing Mother

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In the long term following intrauterine drug exposure, infants reveal a wide spectrum of drug induced pathology, abnormal neurocognitive and behavioral development as well as an increased risk of sudden death syndrome. However, data on transplacental passage of foeto-placental metabolism of illicit drugs at a certain gestational age are rare.

In the case reported, a 17 year old girl was found dead at a public toilet with needle puncture marks. She had been known a heavy drug abuser for the last 2 years. Autopsy was performed 48 hours after death and revealed current pathomorphological findings such as massive brain and lung oedema due to an acute intoxication. Drug screening of blood and urine indicated recent heroin intake (4 ng 6-MAM/mL, 280 ng morphine/mL, 20 ng codeine/mL in femoral vein blood, 1170 ng morphine/mL urine. Chronic drug consumption was confirmed by hair analysis. The girl was found to be pregnant with a male foetus of a gestational age of 18-19 weeks. Amniotic fluid as well as foetal blood and organs were investigated and analysed by GC/MS and HPLC. In all foetal specimens morphine, 6-MAM, codeine as well as 3-morphine glucuronide were present, while 6-morphine glucuronide could not be detected. the drug concentrations (ng/mL or ng/g for amniotic fluid, brain and lung were:

	6-MAM	Morphine	M3G	Codeine
amniotic fluid	128	604	179	-
brain	140	710	615	60
lung	110	1030	1178	60

The findings of high foetal drug concentration at the second trimester of pregnancy are discussed under basic aspects of transplacental drug transfer mechanisms, organogenesis and possible drug accumulation in foetal circulation during pregnancy in drug abusing mothers.

Key words: Foetal drug exposure, transplacental drug transfer, heroin metabolite concentrations

38: Endogenous Morphine - A Protagonist in Pain Mechanism?

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The presence of endogenous morphine has been clearly demonstrated by gas chromatography/ mass spectrometry in the brain and in other tissue or fluids of mammals. Brain morphine concentration become significantly higher after prolonged food deprivation. Five-fold higher morphine levels were detected by gas chromatography/ mass spectrometry in rats fasted for four days rising from 0.23 ± 0.013 ng/g (normally fed rats) to 1.06 ± 0.13 ng/g in rats after 96 hours of fasting with free access to water.

The release of endogenous morphine from rat brain slices was studied in vitro using different incubation media.

All samples were hydrolyzed, extracted by solid phase and derivatized before GC/MS analysis which was performed in selected ion mode (SIM). GC/MS analysis of perfusate samples demonstrated that depolarization due to high potassium concentration increased the release of endogenous morphine from rat brain slices and this effect was calcium dependent.

After the replacement of the normal incubation medium (Krebs bicarbonate) with another medium containing high concentration of potassium (KCL), the amount of morphine in the perfusate rose from 0.11 0.021 ng/g/min (basal value) to 0.48 0.06 ng/g/min. Morphine levels at the end of the depolarization were higher than initial brain content. This may be due to the continuous morphine synthesis in the brain in response to stimulation, as it happens for other neurotransmitters.

These findings indicate that endogenous morphine might function as a neuromodulator/neurotransmitter agent in the CNS of mammals.

Key words : endogenous morphine, GC/MS, solid phase extraction

39: Mortality Associated with Methadone Programs

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Methadone is an orally active opioid used to treat persons with a history of physiological dependence to opioids. Methadone maintenance reduces the risk of death for a heroin addict, but only after stabilisation on a methadone dose. This study reviews the toxicological factors associated with methadone-related deaths.

Methods We have reviewed all methadone deaths that occurred in NSW during 1994 and reviewed and reassessed their manner and causes of death with information on their source of methadone, dose, duration of usage and clinical histories. All cases involved a full toxicological examination and autopsy. Risk analyses were conducted for death with adjustment for age and gender and comparison with a long-term cohort study of heroin addicts.

Results There were 89 deaths involving methadone in this period. Of these 38 were maintenance patients on a NSW program, 29 used diverted methadone syrup and 18 cases consumed the tablet form (Physeptone). Of the 13 NSW methadone patients who died in the first two weeks of maintenance treatment, at least 10 died from iatrogenic methadone toxicity. The mean (±s.d.) blood level in this group was 0.84 ± 1.4 mg/L. The risk of dying from drug toxicity in the first 2 weeks of a MP was 7-times the risk prior to admission and 84 times the risk later in maintenance at which blood concentration of methadone 0.69 ± 0.43 mg/L. Starting doses range from 30 mg. Another 26 deaths from accidental toxicity were caused by diverted take-home doses of methadone syrup. This group had mean methadone levels of 0.33 ± 0.18 mg/L. There was however a weak correlation between methadone dose and blood concentration.

Conclusions These data show that toxic methadone concentrations show little difference in the types of methadone associated deaths and that iatrogenic toxicity to methadone is a significant risk for death in the early stages of methadone maintenance programs as is unsupervised use of methadone in opiate-naive persons.

KEYWORDS: methadone, toxicity, heroin treatment programs

40: Urinary Excretion of 11-Nor-9-Carboxy-Δ^9 -THC and Cannabinoids in Drug Users.

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The present study was undertaken to obtain detailed information concerning the urinary excretion profiles of cannabinoids and the main urinary tetrahydrocannabinol (THC) metabolite, 11-nor-9-carboxy-Δ^9 -tetrahydrocannabinol (THCCOOH), in drug users. Informed consent was obtained from subjects starting to serve their prison sentence.

Drug habits were assessed by means of a questionnaire. Subjects were classified as infrequent users (cannabis use < once a week), or frequent users (cannabis use ≥ once a week). The subjects agreed to give up to 5 urinary specimens daily until negative. The specimens were analysed semi-quantitatively by EMIT® (cut-off 20 ng/ml, representing cannbinoids). Positive specimens and several negative ones were analysed for THCCOOH by a gas chromatography using EC-detector with a limit of detection 1.4 ng/ml, considerably lower than cut-offs according to NIDA (15 ng/ml), and our institute (10.3 ng/ml). Creatinine was measured in all specimens.

All 21 participants (1 participated twice) admitted to having used cannabis, but not all went through the whole follow-up period. A total of 78% of the EMIT positive specimens were confirmed by GC analysis (THCCOOH cut-off 10.3 ng/ml). Relating THCCOOH concentration to creatinine generally gave a smoother time curve than the THCCOOH concentration itself.

Infrequent users had a mean apparent THCCOOH urinary elimination $t_{1/2}$ of about 1.3 days (pooled data). Frequent users had urinary elimination $t_{1/2}$ from 1 day up to about 10 days. In infrequent users the median time from last reported intake until the last positive observed was 12 days (cannabinoids), 5 days (THCCOOH 10.3 ng/ml) and 4 days (THCCOOH 15 ng/ml), respectively. In frequent users the median time from last reported intake until last positive observed was 27 days (cannabinoids), 22 days (THCCOOH 10.3 ng/ml) and 17 days (THCCOOH 15 ng/ml). In one subject 16 negative gaps (THCCOOH positive specimen followed by a negative and again a positive specimen) were observed.

The study of the urinary excretion profiles of illegal drugs in prison inmates is a method of obtaining data that cannot ethically be obtained from healthy volunteers. The present results may aid in the interpretation of urinary THCCOOH and cannabinoid drug testing results.

Key-words: Cannabinoids, 11-Nor-9-Carboxy-Δ^9 -Tetrahydrocannabinol, Urinary Excretion

41: Simultaneous Determination of Δ^9-Tetrahydrocannabinol and 11-nor-9-Carboxy-Δ^9-Tetrahydrocannabinol in Human Plasma by Solid Phase Extraction and Gas Chromatography Negative Ion Chemical Ionization Mass Spectrometry.

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In our previously described method for sensitive determination of Δ^9-tetrahydro-cannabinol (THC) and its main metabolite 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THCA) in plasma (Foltz et al. *Biomed Mass. Spectrom.* **10**: 316, 1983), it was necessary to split the extracts for separate derivatization and injection. We have now modified this method to permit simultaneous extraction, derivatization and analysis of both analytes using solid phase extraction (SPE) with gas chromatography and negative ion chemical ionization mass spectrometry.

THC-d_3 and THCA-d_3 are added to each 1-mL plasma specimen as internal standards; protein is precipitated with 1 mL of acetonitrile and the resulting supernatants diluted with 4 mL of 0.1 M sodium acetate (pH 7.0) prior to application to Clean Screen (2ST4C020) SPE columns. THC and THCA were eluted separately and then pooled, dried under air and then derivatized with trifluoroacetic anhydride and hexafluoroisopropanol. Under negative chemical ionization conditions the derivatized THC-d_0 gives abundant molecular anions (m/z 410) and the derivatized THCA-d_0 gives abundant fragment ions (m/z 422) formed by loss of $(CF_3)_2CHO$ from its molecular anion.

The recoveries of THC and THCA were 74 and 17%, respectively. Intra- and inter-run accuracy and precision at 1 ng/mL (% target ± % CV) for THC were 98.0 ± 4.1% and 95.7 ± 2.8%, respectively. Even with low recovery of THCA, the method had acceptable intra- and inter-run accuracy and precision of 102.0 ± 2.9% and 104.3 ± 13.8%, respectively, at 1 ng/mL. Similar results were achieved at 10 and 75 ng/mL. A lower limit of quantitation of 0.5 and 1.0 ng/mL was established for THC and THCA, respectively. The upper limit of quantitation was 100 ng/mL. This method is currently being used to establish stability parameters for THC and THCA in plasma and will be employed in a pharmacokinetic study of human subjects exposed to cannabis.

Keywords: THC, THCA, NICI MS

42: Effect of the Detoxifying Carbohydrate Drink The Blend™ on Urine Screening for Cannabinoids

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The significant legal and economic consequences of a positive drug test produce an incentive among users of illicit drugs to escape detection. Various strategies including detoxifying or cleansing remedies are recommended to test clean, especially adressing cannabis users.

The effect of the cleansing carbohydrate drink The Ultimate Blend™ was investigated in 8 heavy cannabis users. Urine samples collected prior to and at last 2 hours after fluid intake were assayed by 3 manually (Triage®, Toxi Quick®, Quick Screen 6) and 4 automatically (Emit-st™, Abuscreen Online®, Abbott ADx™, Cedia® DAU) processed

immunoassays. Changes in 11-nor-delta-9-carboxytetrahydrocannabinol concentration were confirmed by GC/MS, and, additionally, apperance, pH-value, creatinine concentration as well as [Na^+], [K^+], [Ca^{2+}] and [Cl^-] were determined.

The decrease in concentrations of THCOOH, creatinine, [Na^+] + [K^+], [Ca^{2+}] and [Cl^-] in final samples, expressed as a percentage of the initial values are shon in the following table. pH values decreased from 7.0 to 5.5.

	THCCOOH	Creatinine	[Na^+]+[K^+]	[Ca^{2+}]	[Cl^-]
range (%)	6-37	8-33	5-22	8-40	5-25
mean (%)	24.6	17.0	13.8	19.3	14.2
SD (%)	7.5	9.1	6.5	12.6	6.6

The ingestion of the drink could give rise to false negative results in immunological testing for cannabinoids depending on the starting concentration of drug metabolites and on the immunoassay or cut-off applied. There was a rather uniform decrease for creatinine and electrolytes in all samples collected afterwards, and dilution seemed to be the predominant effect of the carbohydrate drink. The measurement of "dilution" parameters can help to recognize urine samples that are less suited for urine screening and to take appropriate precautions against unreliable results from watery specimens.

Key words: Urine analysis, The Ultimate Blend, „dilution" parameters

43: The Effect of Consumption of Hempenale on Urine Cannabinoid Screens

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The Division of Forensic Toxicology, Armed Forces Institute of Pathology, conducted a study to determine if the consumption of Hempenale caused positive cannabinoid urinalysis results using 4 immunoassay screens. Hempenale is a beer brewed with hemp seeds and, although these seeds contain no THC, they do contain a number of other cannabinoids that are found in marijuana. It is possible that military members whose random urinalysis yields a positive cannabinoid result will claim that they simply drank Hempenale and, for this reason, were positive on the marijuana screen. The objective of this study was to determine the validity of this contention in order that proper defense and prosecution of positive cannabinoid urinalysis cases may proceed without undue burden on either the defendant or on the government.

Ten subjects were included in the study 9 of whom drank Hempenale ad libitum within a single day (total number of ales consumed ranged from 5 to 14). Urine specimens were collected throughout the drinking period. The 10th individual drank two Hempenale each day over an 8-day period, collecting urine specimens before and after drinking.

In total, 146 urine specimens were collected and assayed using the Abbott AxSYM (FPIA), STC Micro-Plate (EIA), Roche Online (KIMS), and Immunalysis (RIA) immunoassay kits. Positive immunoassay results were subsequently analyzed for 11-nor-delta-9-tetrahydro-cannabinol-9-carboxylic acid using a gas chromatography/mass spectrometry method. All of the immunoassay results were negative except for 10 specimens analyzed by RIA; all of these specimens were negative by the GC/MS method. These results suggest that Hempenale, when consumed in a "normal" pattern of social drinking, will not cause a positive cannabinoid urinalysis result, thereby effectively invalidating the "Hempenale defense".

Keywords: Hemp, Cannabinoids, Urine drug screens

44: Cannabinoid Urine Positives after Ingestion of Hemp Seed Oil

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Low dose, fifteen milliliters, of Cold-Pressed Hemp Seed Oil was given to three subjects, two males and one female for 5-7 days. No psychotropic effects were reported. Urine samples were collected on day zero, prior to dosing, and on a daily basis for 10-14 days. Samples were screened for delta-9-tetrahydrocannabinol (THC) metabolites by immunoassay,

and confirmed for the major metabolite of THC in urine 11-nor-delta-nine-tetrahydrocannabinol carboxylic acid (9-THCA) by GC/MS. Urine positives occurred 1-2 days after dosing, with the peak occurring at 5-7 days after the first dose. Urine became negative for THC metabolites 5-7 days after the last dose. In two individuals total metabolite concentration did not exceed the 50 ng/mL cutoff. GC/MS confirmation showed concentrations of 9-THCA from 1-11 ng/mL. In one individual urines were positive for THC metabolites in excess of 50 ng/mL on days 1-8 after initial dose. GC/MS confirmation gave concentrations for 9-THCA of 13-30 ng/mL. Low doses of Cold Pressed Hemp Seed Oil may result in positive urines for THC (marijuana) metabolites using the 50 ng/mL screening and 15 ng/mL confirmation cutoff.

Keywords: Hemp Seed Oil, Marijuana, Urine drug screens

45: Comparison of Immunochromatographic Rapid Tests for Screening of Benzodiazepines, Amphetamine and its Derivatives (Ecstasy) in Urine

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Immunochromatographic rapid tests for drugs-of-abuse testing in urine have been developed by many pharmaceutical manufacturers.

Materials: Nine different commercially available rapid tests for detecting benzodiazepines, amphetamines and methamphetamines in urine were compared (Boehringer Frontline, Syva Rapid Test, Biosite Triage, Roche Ontrak Teststik (amphetamines only), Forefront Instacheck , D.S.S.I. microline Screen, Bionike/Biomar ToxiQuick, Syntron/von Minden, PBM/Mahsan).

Method: The following commonly prescribed benzodiazepines or their forensically relevant metabolites (Alprazolam, Bromazepam, 7-Amino-Clonazepam, Desalcylflurazepam, 7-Amino-Flunitrazepam, 7-Amino-Nitrazepam, Nordazepam, Triazolam) were added to drug-free urine to obtain samples containing benzodiazepines in different concentrations above and below the cut-off concentration of 300 ng/mL indicated by the manufacturers. The same procedure was used to test for amphetamine, methamphetamine, MDMA, MDEA, MDA, MBDB, ephedrine, phenylethyl-amine, tyramine and phenylpropanolamine. Finally 40 samples of real material were tested by Boehringer and Bionike with confirmation by HPLC.

Results: The limits of detection (LOD) of the compared rapid tests vary considerably, but Boehringer Frontline and Bionike/Biomar ToxiQuick reached the lowest LOD with an average benzodiazepine concentration of 25 ng/mL. The Boehringer Frontline had no false positive results and the Bionike had just one false positive out of 20 samples confirmed negative by the highly sensitive HPLC method. The Roche Ontrak Teststik was positive only for amphetamine. Biosite Triage showed the highest rate of interference with phenylethylamine and tyramine, the metabolites of putrefaction.

Key Words: Rapid Tests, Benzodiazepines, Ecstasy

46: Amphetamines Detection with Laboratory and On-Site Immunoassays: Effect of Antibody Specificity

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Workplace drug testing in the US typically includes screening for amphetamines: amphetamine (A) and methamphetamine (M). US DHHS and DOT regulations specify initial screening for amphetamine at a cutoff of 1,000 ng/mL. Immunoassay manufacturers have utilized A-specific antibodies, M-specific antibodies, a combination of both and less specific antibodies as they attempt to optimize A and M detection while minimizing false positive screens due to cross-reacting substances. Immunoassays used in laboratories, such as Date Behring Syva EMIT II and Roche Diagnostics Systems OnLine, are designed to detect both A and M. Non-instrumental immunoassays designed for on-site drug testing use antibodies that are more specific and more specific for either A or M. The purpose of this study was to compare A and M detection by EMIT II, OnLine, the M-specific Princeton BioMeditech AcuSign-5 and the A-specific Roche Diagnostics Systems TesTcup-5 in a random set of specimens.

Urine specimens that tested positive for amphetamines using the Dade Behring Syva EMIT II monoclonal immunoassay using a 1,000 ng/mL cutoff were used in the study. Fifty (50) specimens were provided by PoisonLab, Inc. (PL, San Diego, CA) and fifty-three specimens were provided by American Medical Laboratories, Inc. (AML, Chantilly, VA). All specimens were quantified for A and M by GC/MS at the two labs. Of the 103 specimens, 28 or 27% were

negative for both A and M. 25 had only A above the GC/MS cutoff of 500 ng/mL, 6 had only M above the 500 ng/mL GC/MS cutoff and 44 had both A and M above 500 ng/mL. Specimens from PL were mostly A and M, while specimens from AML contained mostly A alone. This, M predominated in the West Coast lab and A predominated in the East Coast lab. 26 of 28 false positives by EMIT II came from AML. OnLine had four false positives (3.9% of 103 specimens) and one false negative that contained 580 ng/mL of A. The M-specific AcuSign-5 had 5 (4.9%) false positives and 28 false negatives (37.3% of 75 true positives). The A-specific TesTcup had one (1.0%) false positive and 12 false negatives (16.0%) of 75 true positives). For detection of amphetamines overall, the A-specific TesTcup detected 84% of the true positives compared to 62.7% for the M-specific AcuSign-5. OnLine had much fewer false positives than EMIT II, 3.9% versus 27.2%.

Key Words: Amphetamine, Methamphetamine, Immunoassays

47: Evaluation of Three Immunochromatographic Rapid Tests for Screening of Amphetamines/Methamphetamines, Benzodiazepines and Cocaine in Urine.

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In a number of situations a rapid analysis of drugs in urine is required. For this purpose reliable and validated tests are needed that are easy to perform without special laboratory equipment. Recently the evaluation results of three rapid assays of the Frontline® product line (Roche Diagnostics Boehringer Mannheim GmbH) for screening of drugs of abuse in urine for cannabis, opiates and cocaine have been published. This system has now been extended by a combined amphetamine/methamphetamine and by a benzodiazepine test. Furthermore the existing cocaine test has been replaced by an improved product.

In this multicenter study the rapid assays were evaluated with authentic clinical and forensic urine samples. The rapid assays were compared with immunological laboratory screening methods; in case of discrepancy between Frontline® and the comparison method a confirmation method was employed (GC-MS or HPLC).

With 309 urine samples Frontline® Amph/Methamph was compared to FPIA/GC-MS resulting in 97% sensitivity and 98% specificity at a cutoff 300 ng/ml which is mostly used in Europe and 96% sensitivity / 99% specificity at a cutoff 1000 ng/ml which is recommended by NIDA (with separate color scales for each cutoff). Synthetic drugs such as MDA, MDMA, MDE(A) and MBDB were also detected with very high sensitivity. The Frontline® test was developed for selective detection of the d-enantiomers of amphetamines/methamphetamines. This enantioselectivity resulted in lower sensitivity when compared with analytical methods that cannot distinguish between d- and l-isomers.

Preliminary results of Frontline® Benzodiazepines indicate high sensitivity with reactivities towards a broad spectrum of benzodiazepine derivatives.

Frontline® Cocaine was evaluated with altogether 921 urine samples detecting 402 of 403 positive samples (sensitivity > 99%) and finding 513 of 518 correctly negative (specificity = 99%). The 5 false positive samples contained cocaine/benzoylecgonine at a concentration only slightly below the cutoff of 300 ng/ml.

The evaluation showed that the new Frontline® rapid assays gave highly reliable results.

Key Words: Rapid Test, Immunoassay, Urine Screening

48: Methadone Conversion to Methadone Metabolite during GC/MS Analysis of Urine Samples

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During validation of a GC/MS method for the methadone metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), it was noted that detectable levels of EDDP were found during analysis of drug-free urine samples spiked with methadone. To verify this observation, extracts of methadone-spiked urine samples were analyzed by GC/MS and reverse-phase HPLC. While the HPLC method indicated no EDDP in the extracts, GC/MS data clearly

identified EDDP by retention time and selected ion mass (SIM) fragmentation data. Quantitative analysis of methadone conversion to EDDP by GC/MS was performed using EDDP-D_3 as internal standard and measuring the resulting GC/MS-SIM signal data. The amount of EDDP observed was variable, but was consistently > 50 ng/mL at methadone concentrations > 10,000 ng/mL. In one case, reducing the GC injector port temperature from 260° C to 180° C reduced the observed EDDP concentration from 201 ng/mL to 53 ng/mL at an initial methadone sample concentration of 10,000 ng/mL.

These findings were valuable in verifying the accuracy of a recently-developed immunoassay specific for EDDP [CEDIA® DAU EDDP (Methadone Metabolite) Assay, Boehringer Mannheim Corp.]. Urine samples spiked with high concentrations of methadone were negative by the immunoassay method, but positive by GC/MS. The HPLC method was used to confirm the absence of EDDP in spiked samples, as well as in a small percentage of samples from methadone treatment clinics which, by the CEDIA® immunoassay method, tested strongly positive for methadone but negative for EDDP. These samples were suspected to result from surreptitious addition of methadone to urine samples by patients who divert and sell the majority of their methadone dosage. In conclusion, alternative methods (i.e., HPLC or LC/MS) should be considered when determining the concentration of EDDP in samples containing high concentrations of methadone.

Key Words: EDDP, GC/MS, thermal conversion

49a: Performance of the CEDIA® EDDP (Methadone Metabolite) Assay

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Methadone, a synthetic diphenylheptane-derivative opiate agonist, is primarily used as a substitute for heroin and other opiates during withdrawal to eliminate symptoms and to temporarily maintain chronic relapsing heroin addicts. Screening methadone is an important clinical tool for evaluating compliance. However, a small subset of patients metabolize methadone to 2-ethylidene-1,5-dimethyl3,3-diphenylpyrrolidine (EDDP) so quickly that they appear negative upon conventional methadone immunoassay screening. The purpose of this study was to evaluate the performance of the new CEDIA® DAU assay for EDDP. Assay performance was assessed as part of a multi-center clinical evaluation of EDDP utility as a screening tool. The evaluation included three Boehringer Mannheim Corporation Hitachi Model 717 analyzers, one Hitachi Model 747 analyzer and one Hitachi Model 917 analyzer.

Within-assay reproducibility over the five analyzers range from 2.1 to 5% at a mean EDDP concentration of 75.5 ng/mL, and 2.4 to 4.7% at a mean concentration of 128.8 ng/mL. Between assay reproducibility was assessed on one Hitachi 717 analyzer, the Hitachi 747 analyzer and the Hitachi 917 analyzer. Coefficients of variation over a 14 day period (n = 30) were from 3.8 to 6.3% at 72.3 ng/mL and 3.5 to 4.5% at 126.9 ng/mL. Cross-reactivity to methadone was tested and was <0.02%. Method comparisons gave correlation coefficients of 0.981 (n = 80) compared to GC/MS and 0.964 compared to HPLC (n = 79).

Key Words: Methadone, EDDP, Immunoassay

49b: Clinical Application of the CEDIA® EDDP (Methadone Metabolite) Assay

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Screening urine samples from patients being treated with methadone is used to evaluate compliance. Because patients taking methadone often produce urine screens which are negative for methadone, we evaluated the clinical use of an assay specific for the methadone metabolite, 2ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), and compared the results obtained with results from the CEDIA®DAU methadone assay. The cut-off concentrations were 300 ng/mL for the methadone assay and 100 ng/mL for the EDDP assay.

Consecutive patient urine samples (480) submitted for methadone screening were obtained from 106 male subjects (mean age of 48.6 years- range 31-69). The average length of opiate use was 255 months (range 8-516). The average length of methadone treatment was a 165 weeks (range 1-1 361) and the mean daily dosage of methadone was 43 mg per day (range 20-90).

Of the 480 samples, 397 were positive for methadone and EDDP, 45 were positive for EDDP but negative for methadone while 19 specimens were positive for methadone but negative for EDDP. Nineteen urine samples were also negative for both methadone and EDDP. Of most interest in this study were the 45 samples with EDDP levels greater than 1 00 ng/mL but which were negative for methadone. These specimens, representing 9.4% of the samples, are most likely from patients who exhibit either genetically determined or drug induced rapid metabolism of methadone and for whom a methadone screen used alone, would return a false negative and an incorrect clinical conclusion. Longitudinal data on the ability to detect methadone use using both methadone and EDDP assays will be presented.

Key Words: Methadone, EDDP, Immunoassay

50: Use Of The IMMULITE® Chemiluminescence Immunoassay System For The Screening Of Post-Mortem And DUID Blood Specimens

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The Diagnostic Products Corporation IMMULITE benchtop random-access immunoassay analyzer was evaluated in our laboratory. This system uses enzyme-amplified chemiluminescence and centrifugal separation of reactants. Untreated post-mortem blood specimens or Driving Under The Influence Of Drugs (DUID) blood specimens were placed directly into the instrument sampling cup. The performance of kits designated as opiates (OPI), benzoylecgonine (BE), and tetrahydrocannabinol carboxylic acid (THCA) were compared to the corresponding Abbott TDx kit methodology. Specimens testing positive by IMMULITE and/or TDx were submitted for confirmation and quantitation by gas chromatography/mass spectrometry (GC/MS).

IMMULITE methodology required no sample preparation while the TDx required a buffer dilution for OPI and BE, and an acetonitrile extraction for THCA. Both methodologies produced the same results for COC with 15 positive and 44 negative specimens (n = 59). GC/MS confirmed the accuracy of all positives. OPI comparisons (n = 61) produced 14 positives and 47 negatives for both techniques, however, GC/MS confirmed 2 false positives (FP) and 1 false negative (FN) by TDx vs. 1 FP by IMMULITE. THCA testing produced 23 and 22 positives and 29 and 32 negatives by IMMULITE and TDx, respectively. GC/MS confirmed 2 FN by TDx and 1 FN by IMMULITE.

Precision studies using three varying concentrations of unaltered postmortem blood for each analyte were conducted (n = 10 or 11), and morphine precision was found to range from 8.1% to 10.8%, BE from 5.5% to 33.7%, and THCA from 3.2% to 13.2%. Cross-reactivity studies were performed with approximately 70 drugs at blood concentrations ranging from 5000 to 10,000 ng/mL and showed less than 2% cross-reactivity.

The IMMULITE was found to produce reliable screning results with less inaccuracies than TDx. Furthermore, postmortem blood and DUID blood specimens required no specimen pretreatment.

Key Words: Immulite, Postmortem, DUID

51: Urine Concentrations of Benzodiazepines, Including Flunitrazepam, After a Single Oral Dose, Using the Cassette COBAS® INTEGRA Serum Benzodiazepines Assay

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A new reagent, cassette COBAS® INTEGRA Serum Benzodiazepines (SBENZ) for analysis of benzodiazepines in serum and/or plasma on the COBAS INTEGRA 700 analyzer using fluorescence polarization (FPIA) technology, was further evaluated for analysis of benzodiazepines in urine. Urine from 10 different donors was spiked with nordiazepam; recovery was within 95.2% at 130.6 ng/mL and within 91.7% at 41.2 ng/mL. Precision in urine was evaluated according to NCCLS Guideline EP5-T2 where the total precision at 7.4 ng/mL nordiazepam was 19.4%, 10 ng/mL was 11.1%, 19.6 ng/mL was 6.34%, 27.24 ng/mL was 4.98%, 41.22 ng/mL was 3.24%, 51.66 ng/mL was 2.71%, 83.72 ng/mL was 1.74%, and 104.6 ng/mL was 1.8%.

Clinical samples, N = 66, were evaluated by GC/MS for nordiazepam and oxazepam, LOD 1 ng/mL, a commercially available FPIA, LOD 40 ng/mL and the SBENZ urine application, LOD 7 ng/mL. The diagnostic sensitivity of the two immunoassays vs GC/MS was 100% and the diagnostic selectivity was 87.5%.

A clinical study was conducted to assess the ability to detect flunitrazepam (FNP) by the different methods. The clinical study consisted of four individuals (2 male, 2 female) who had taken a single 2 mg dose of FNP. Urine was collected over a 72-hour period. The urine samples were evaluated by GC/MS with a 1 ng/mL LOD for 7-aminoflunitrazepam, a commercially available FPIA and COBAS INTEGRA SBENZ. The GC/MS, SBENZ, and a commercial FPIA picked up 45, 41, and 35 positive FNP urines respectively. Three samples were negative by all methods.

The improved detection of FNP use by the SBENZ assay as compared to the other commercially available FPIA may be explained by increased cross-reactivity of the major metabolite 7-aminoFNP which cross-reacts 60.8% compared to 15% (*Clin. Chem.*, Vol. 38, No. 2, 271-275 (1992)) for the other commercially available FPIA. SBENZ cross-reactivity to the other metabolites of FNP, were 0.47% to 7-acetamido-FNP, non detectable to 7-acetamido-3-OH-desmethyl-FNP, 12% to 7-amino3-OHFNP, 47.6% to desmethyl-FNP, and 13.8% to 3-hydroxyFNP. The cassette COBAS INTEGRA SBENZ assay may be used for the analysis of serum, plasma and urine.

Key Words: Flunitrazepam, Serum, Urine

52: Application of the Cassette COBAS ® INTEGRA Serum Barbiturates Assay to the Analysis of Barbiturates in Urine and Saliva

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Editor's note: We regret that this abstract did not appear on the disk provided.

53: A New Abuscreen® OnLine II Immunoassay for the Detection of Opiates 300/2000

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On September 30, 1997, the proposal for raising the opiate screening cutoff from 300 ng/mL to 2000 ng/mL was finalized in the Federal Register. In order to comply with the SAMHSA guidelines a new configuration of the OnLine reagents has been developed. The Abuscreen OnLine II continues to use the KIMS technology and is designed for the qualitative (300 and 2000 ng/mL cutoffs) and semi-quantitative 2000 ng/mL cutoff for the detection of morphine, codeine and their metabolites in urine. This new version of OnLine uses soluble drug-conjugate derivative and microparticle-bound antibody. This assay is a two reagent system for the non-Cobas instruments and a three reagent system for the Integra 700. This assay also offers broad dynamic range (0-8000 ng/mL) and serum applications without pretreatment.

Applications are currently available on the Hitachi 717, 747, 917, Olympus AU800, AU5200, and the Cobas Integra 700. The intra- and inter-assay precision for the entire range of the curve is less than 5% on all instruments tested.

Forty GC/MS positive samples tested positive relative to 300 ng/ml and 2000 ng/ml cutoffs for all applications. Complete agreement with 1000 negative samples (including 10 GC/MS tested negatives) was shown with OnLine I on all analyzers. Cross-reactivity studies demonstrated similar results when compared to the generation I OnLine Opiate assay.

In addition, this assay was also compared to the new EMIT II opiates assay on the Hitachi 717. Assay precision as CV% was 1.9% at 300 ng/ml (300 ng/ml cutoff) and 3.3 at 2000 ng/ml (2000 ng/ml cutoff) versus 1.1 % at 300 ng/ml and 3.1% at 2000 ng/ml for the EMIT II assay.

Clinical accuracy of these assays at a 300 ng/mL cutoff was tested by using fourteen morphine-positive urines containing no codeine. These samples were diluted to ±20% of the cutoff based on GC/MS values. The OnLine II demonstrated a mean recovery of 104% and a mean recovery of 160% with EMIT II. At a 2000 ng/mL cutoff, six undiluted clinical samples containing morphine and/or codeine with GC/MS values ranging from 1228 to 1984 ng/mL were tested. All samples were identified as negative in the OnLine II assay and three of the six were positive in the EMIT II assay. Cross-reactivity studies revealed higher values for hydrocodone and hydromorphone in the EMIT II assay.

Key Words: Opiates, OnLine, Urine

54: Abuscreen OnLine® Immunoassay for the Detection of Drugs of Abuse on the Integrated Hitachi 917 Analyzer

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Applications for the Abuscreen OnLine DAT tests were developed on a new integrated Hitachi 917 analyzer. These assays include Amphetamines 1000 cutoff, Barbiturates 200 cutoff, Benzodiaze-pines 100 cutoff, Benzodiazepines 200 cutoff, Benzodiazepines 300 cutoff, Cocaine 300 cutoff, Methadone 300 cutoff, Opiates 300 cutoff, Opiates 2000 cutoff, PCP 25 cutoff, Propoxyphene 300 cutoff, THC 20 cutoff, THC 50 cutoff, and THC 100 cutoff. This random access analyzer has the capacity to consolidate the testing of clinical chemistry, therapeutic drugs, drugs of abuse, specific proteins, thyroids, and electrolytes on one system. The throughput of the instrument is 800 tests per hour and the maximum throughput is 1200 tests per hour with ISE.

The OnLine DAT assay format, based on the KIMS technology, has been successfully applied to the 917 in either a dedicated DAT analyzer format or in an integrated format. A calibration curve for each assay was obtained using a Logit Log 4 parameter or a Logit Log 5 parameter with either four or five calibrators. The precision at 0.75x, 1.0x, and 1.25x the cutoff was measured. The average intra-assay CV was 1.7% and the average inter-assay CV was 2.8% at these concentrations for all assays.

		Intraassay Precision			Interassay Precision		
Assay	Cutoff	0.75X	X	1.25X	0.75X	X	1.25X
Amphetamines	1000	2.0	1.4	1.5	2.0	1.4	1.5
Barbiturates	200	3.0	2.8	1.5	3.3	2.8	2.6
Benzodiazepines	100	1.9	1.0	1.3	2.1	1.7	1.6
Benzidiazepines	200	2.7	1.9	1.5	3.3	2.1	2.2
Benzodiazepines	300	1.2	1.4	1.1	1.8	2.0	1.7
Cocaine	300	1.1	0.9	0.6	1.4	1.0	0.7
Methadone	300	3.2	1.5	1.0	3.7	2.2	2.0
Opiates	300	1.2	1.0	0.8	1.6	1.2	1.9
PCP	25	4.3	2.0	2.1	6.6	5.0	4.0
Propoxyphene	300	1.3	1.6	1.0	3.1	2.4	2.4
THC	20	3.2	2.9	1.5	8.5	4.6	3.3
THC	50	1.1	1.5	2.2	4.0	3.3	5.8
THC	100	1.7	2.0	1.9	2.6	3.8	3.2

In a clinical correlation study, OnLine results were in 100% agreement with 50 GC-MS confirmed clinical positive samples per assay. The cross-reactivities for all the assays were similar to those reported for OnLine applications on other analyzers. These reagents will be offered in prepacked 20 mL or 70 mL Hitachi reagent pies, ready for use on the 917 analyzer.

Keywords: Immunoassay, Drugs of Abuse, KIMS

55: Comparison of Derivatizing Reagents and Internal Standards For the Analysis of Opiates

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There are a number of problems associated with confirmation analysis of opiates. Some issues that have plagued laboratories are incomplete derivatization, "dirty" derivatives, enolization of the ketones in hydrocodone and hydromorphone, and labeled internal standards interfering with the ions of the target drug. Now with the higher cut-off mandated by SAMHSA, an additional problem has arisen—at higher concentrations target drug ions contribute significantly more to internal standard ions resulting in failed ratios.

This study explores differences in derivatizing reagents and internal standards in opiate analysis. Compounds included were morphine (-D3, -D6), codeine (-D3, -D6), hydromorphone (-D3), hydrocodone (-D3, -D6), and oxycodone (-D3, -D6). In our study we evaluated three derivatives (TFA, TMS, and PPA) and nine internal standards. All samples were analyzed by GC/MS/SIM unextracted for the amount of derivatization as well as for enolization of the ketones.

Although extracted samples have shown otherwise, the results suggested that the derivative with least amount of enolization was TMS using BSTFA with 1% TMCS. The derivative with the most complete derivatization was also TMS. Unfortunately, TMS derivatives of opiates also have more ion interferences. These coincident ions can cause problems on the low and high ends of calibration curves especially with the new cut-off. The derivative that appears to have the fewest interferences is the TFA using TFAA. Overall the internal standards that are more highly labeled provided less contribution to the target drug ions. The unlabeled drug ions also contributed less to higher labeled internal standard ions. This means fewer problems on both ends of the calibration curves.

Key Words: Opiates, Internal Standards, Derivatizing Reagents

56: Whizzies - A Urine Drug Screen Adulterant

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The internet and drug culture magazines tout nitrite containing products as a means of beating a urine drug test. Our study focuses on a product called "Whizzies", supplied in two vials containing sodium nitrite. The directions provided with the Whizzies state to "Add one vial to 45 - 60 mLs of urine." This results in a 16 to 21 mg/mL concentration of Whizzies in a urine sample. We performed studies to evaluate the effectiveness of Whizzies on urine containing free Δ^9-carboxy-THC (THCA).

We added Whizzies in concentrations ranging from 2 mg/mL to 25 mg/mL to a series of human urine samples containing 75 ng/mL of THCA. The effect of Whizzies on Roche ONLINE and Diagnostic Reagents Incorporated (DRI) marijuana immunoassay reagents at 20 and 50 ng/mL cutoffs was determined. After four hours, all samples tested negative at the 50 ng/mL cutoff. The Roche Online reagents tested negative at 20 ng/mL when the Whizzies concentration exceeded 12.5 mg/mL. The DRI reagents tested negative at 20 ng/mL when the Whizzies concentration exceeded 4 mg/mL. All samples were analyzed by GC/MS. There was no recovery of free drug or internal standard in any of the samples.

Human urine samples containing Whizzies at 40 mg/mL and free THCA at concentrations ranging from 2 ng/mL to 500 ng/mL were analyzed with the Roche and DRI marijuana immunoassays using 20 and 50 ng/mL cutoffs. After two hours, the Roche ONLINE reagents tested positive at 50 ng/mL when the concentration of THCA was 250 ng/mL or greater. The Roche ONLINE reagents tested positive at 20 ng/mL when the concentration of THCA was 50 ng/mL or greater. The DRI reagents tested positive at 20 ng/mL when the concentration of THCA was 250 ng/mL or greater. All samples were analyzed by GC/MS. There was no recovery of free drug or internal standard in any of the samples.

This data demonstrates that Whizzies is effective at reducing the apparent free THCA concentration as measured by immunoassay and GC/MS. The effects of Whizzies on conjugated THCA will be covered.

Key Words: Adulterant, Nitrite, Whizzies

57: Measurement of Nitrite in Adulterated Urine Samples by High Performance Ion Chromatography

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Although nitrite is present as a normal constituent of urine, it has been shown recently that samples adulterated with nitrite clearly have nitrite concentrations that are orders of magnitude greater than nitrite from natural sources. With the increased availability of commercial products like Klear that can help the donor mask the presence of drugs in the urine it has become necessary for laboratories that are involved in the workplace drug testing be able to detect and confirm the presence of adulterants like Klear.

We have developed a method that uses high performance ion chromatography to quantify the amount of nitrite present in adulterated urine samples. The method uses the IonPac AS 14 analytical column with the Dionex Bio LC AI 450

equipped with an anion self-regenerating suppressor and a conductivity detector. Urine samples are spiked with potassium bromide which serves as an internal standard and diluted with mobile phase. The diluted urine samples are injected into the HPLC system where the anions are resolved isocratically using a mobile phase consisting of 3.5mM sodium carbonate and 1 mM sodium bicarbonate. The nitrite is quantified using a single point calibrator (1000ug/ml).

Using this method we have shown that samples from patients whose urine is infected with bacteria and have a positive reaction on a clinical reagent strip for nitrite have very low to unmeasureable concentration of nitrite. This observation confirms earlier published data. This high performance ion chromatography method has excellent day to day precision with a CV of 6% at concentrations of 1200 ug/ml and 3000 ug/ml. The recovery of nitrite spiked into urine samples at a concentration of 12,000 ug/ml was at 95%. The method is capable of accurately quantifying nitrite up to a concentration of 40,000 ug/ml. Analysis by HPLC of urine samples from Workplace drug testing programs that screened positive by the nT Perfect adulteration detection reagent (Chimera Research & Chemical) showed that the concentration of nitrite in these samples was greater than 1000ug/ml. This method provides definitive confirmation for nitrite.

Key Words: Nitrite, Ion Chromatography, Adulterated

58: Determination of Five Abused Drugs in Nitrite Adulterated Urine by Immunoassays and Gas Chromatography-Mass Spectrometry.

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The objective of this study was to investigate the effect of nitrite adulteration on the detection of five commonly abused drugs by immunoassay screening and GC-MS analysis. The drugs tested are cocaine metabolite (benzoylecgonine), morphine, 11-nor-^{9}-tetrahydrocan-nabinol-9-carboxylic acid (THC-COOH), amphetamine and phencyclidine. The immunoassays evaluated included the instrument-based Abuscreen ONLINE assays, the on-site Abuscreen ONTRAK assays, and the one-step ONTRAK TESTCUP-5 assay. Multianalyte standards containing various levels of drugs were used to test the influence of nitrite.

In the ONLINE immunoassays, the presence of up to 1.0 M of nitrite in the multianalyte standards had no significant effect on benzoylecgonine, morpnine and phencyclidine assays. With high concentrations of nitrite, ONLINE became more sensitive for amphetamine (detected more drug than what was expected) and less sensitive for THC-COOH (detected less drug than what was expected).

No effects of nitrite were observed on the results of the Abuscreen ONTRAK assays.

Similarly, no effects were observed on the absolute qualitative results of the TESTCUP-5 when testing with the nitrite adulterated standards. However, the intensities of the signals produced which indicate the negative test results were slightly lowered in the THC and phencyclidine assays.

The presence of 1.0 M of nitrite did not show dramatic interference with the GC-MS analysis of benzoylecgonine, morphine, amphetamine and phencyclidine. In contrast, the presence of as little as 0.03M of nitrite ion resulted in significant loss in the recovery of THC-COOH and its internal standard by GC-MS. The problem of nitrite adulteration could be alleviated by sodium bisulfite treatment even when the specimens were spiked with 1.0 M of nitrite ion. Although bisulfite treatment of nitrite containing samples resulted in the recovery of undegraded THC-COOH by GC-MS, the net recovery of THC-COOH depended on urinary pH and time and conditions of sample storage. The presence of nitrite that might arise from all possible natural sources did not interfere with the GC/MS analysis of THC-COOH.

Key words: nitrite adulteration, immunoassay screening, GC-MS analysis.

59: Gravity-Detect® and pH-Detect® Assays for Urine Adulteration Testing

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Urine specific gravity and pH have been used to assess urine adulteration in drug of abuse testing. We have developed Gravity-Detect® and pH-Detect® assays for the determination of urine specific gravity and pH. Both are liquid, ready-to-use reagents and are applicable to automated clinical analyzers.

The Gravity-Detect assay is based on a linear relationship between the urine chloride concentration and the specific gravity. The chloride concentration is determined colorimetrically and the absorbance measured is directly proportional to the urine specific gravity. The pH-Detect assay is based on a specifically engineered linear relationship of enzyme (G6PDH) activity with the pH of the reaction. A higher enzyme activity indicates a higher pH value of the sample. A linear calibration curve is established for both methods. The unknown sample specific gravity/pH values are calculated with the corresponding absorbance from the calibration curve.

Clinical urine samples were exposed to various commonly encountered adulterating agents (i.e. bleach, water, ammonia, hydrogen peroxide, liquid soap, sodium nitrate, table salt, vinegar and gluteraldehyde) to simulate urine adulteration.

The pH of these treated (adultered) samples were determined by a pH meter and subsequently assayed with the pH-Detect test. An average recovery of 103% was observed. A correlation with a regression equation of pH-Detect (y) = 2.62 + (0.56)pH meter (x) and a correlation coefficient (r) of 0.90 was obtained using 120 samples.

The specific gravity of the above samples was determined by a digital urine specific gravity refractometer and with the Gravity-Detect test. An average recovery of 99.9% was observed. A correlation with a regression equation of Gravity-Detect (y) = 0.10 + (1.10)refractometer (x) and a correlation coefficient (r) of 0.90 was obtained using 120 samples.

Used together, both pH-Detect and Gravity-Detect are effective and convenient in determining urine adulteration.

Key Words: Urine Adulteration, Specific Gravity, pH.

60: In vitro Assessment of the Toxicity of Cocaine and its Metabolites in the Human Umbilical Artery.

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Cocaine toxicity has generally been attributed to its vasoconstrictive and sympathomimetic effects. Placental abruption, preterm birth, and low birth weight associated with cocaine abuse has been correlated with increased systolic/diastolic ratios in human umbilical artery blood flow. We used an in vitro model to assess the effect of cocaine and its metabolites on the umbilical artery. Our objectives were to pharmacologically confirm the presence of adrenergic innervation using tyramine, to evaluate the ability of cocaine, norcocaine and cocaethylene to potentiate vasoconstriction by 5-hydroxytrytamine (5HT) and norepinephrine (NE), and to examine the ability of ketanserin, a $5HT_2$ antagonist, to block the vasoconstrictive toxicity produced by cocaine.

Rings of umbilical arteries (3mm) were collected and perfused in isolated tissue baths under physiological conditions. Isometric contractions were measured in paired tissues following exposure to NE, 5HT, or tyramine in the presence and absence of cocaine, norcocaine, or cocaethylene (10μM). The rings were exposed to ketanserin (0.03μM) and the dose response curves for 5HT and NE were reconstucted in the presence of cocaine.

The vasoconstrictive effect of tyramine was enhanced in the presence of cocaine, suggesting that tyramine produces a direct postsynaptic effect on the tissue. The vasoconstrictive effects of 5HT and norepinephrine were significantly enhanced by cocaine. Norcocaine significantly augmented the maximum response to NE in our preparation. Ketanserin completely attenuated the potentiation of NE and 5HT by cocaine. These data suggest that enhanced vasoconstriction of NE and 5HT by cocaine and potentiation of the maximum response to NE by norcocaine in the human umbilical artery may be important components of perinatal cocaine toxicity. Furthermore, ketanserin was able to suppress the umbilical artery constriction produced by cocaine, demonstrating antidotal potential for this compound in acute perinatal cocaine toxicity.

Key Words: Cocaine, Human Umbilical Arteries, Vasoconstriction

61: Urinary Detection Times Following Chronic Oral Cocaine Administration

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Chronic drug administration can result in accumulation and longer elimination times upon cessation of use. It has been suggested that cocaine metabolites can be detected in urine for several weeks following long-term, high-dose administration (Weiss and Gawin, 1988). We are currently conducting a clinical study of chronic oral cocaine

administration. Cocaine abusing volunteers were administered oral cocaine in up to 16 sessions. The study was conducted on a closed clinical ward. In each session, volunteers received 5 equal doses of oral cocaine with 1 hour between doses. Across sessions, cocaine was administered in ascending doses with an initial dose of 100 mg (500 mg/day) up to 400 mg (2g/day), increasing by 25mg/dose/session (125 mg/session). Participation in the study was terminated if cardiovascular safety parameters were exceeded. For the 11 volunteers included in this report, the final completed daily doses ranged from 750 mg to 2000 mg. Urine specimens were collected throughout the study and collection continued during the withdrawal phase (range 4.85-12.82 days). Specimens were analyzed with the EMIT® d.a.u.™ Cocaine Metabolite Assay. Three cutoff concentrations of 300, 150 and 75 ng/mL were employed. The mean (N=11) urinary detection times (days±SEM) at each cutoff concentration were as follows:

Mean Detection Times (range)
Cutoff Concentration (ng/mL)

Last Consecutive Positive			Last Positive		
300	150	75 (N=9)	300	150	75 (N=9)
3.1±0.1	4.2±0.1	5.2±0.2	4.1±0.1	5.1±0.1	6.4±0.1
(1.3-5.0)	(2.3-5.9)	(3.7-7.2)	(3.0-5.1)	(3.2-6.7)	(4.2-8.1)

Four subjects continued to test positive for cocaine metabolite at the time of their discharge (one at 300 ng/mL; three at 75 ng/mL). All other subjects produced at least one negative urine specimen before discharge. These detection times are longer than the typically reported detection time of approximately 2-3 days following acute dosing, but are consistent with the elimination half-life for benzoylecgonine, approximately 7.5 hours. Overall, these data indicate that chronic cocaine administration results in urine detection times of 3-5 days at the 300 ng/mL DHHS cutoff concentration. However, the use of lower cutoff concentrations would provide detection times of 3-7 days.

Key Words: Chronic cocaine, Oral cocaine, Urinary detection time.

62: Oral Creatine Supplementation and Its Effect on Urinary Creatinine, pH, and Specific Gravity Measurements.

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Oral creatine supplementation has become a popular performance and strength enhancer among amateur and professional athletes. Creatine is spontaneously converted to creatinine. The objective of this study was to determine the effect of oral creatine supplementation on urinary creatinine, pH, and specific gravity measurements.

Four subjects were administered a loading dose of creatine monohydrate (5 g, 4 times daily for 5 days), followed by maintenance dosing (5 g, 1 time daily for 5 days). All discrete urine specimens were collected during the course of the study that included the collection of urine specimens 2 days prior to and following creatine administration. A total of 307 discrete urine specimens were obtained during the course of the study and the urinary creatinine, pH, and specific gravity values were determined. The data including mean (SD) are summarized below:

Subject	Time of Urine Collection Relative to Creatine Administration	N	Creatinine (mg/dL)	S.G.	pH
1	Before	16	64.0 42.6	1.010 0.006	5.8 0.6
	During/After	71	78.9 51.5	1.012 0.007	6.2 0.6
2	Before	10	327.8 64.7	1.035 0.002	5.7 0.3
	During/After	60	219.0 46.7	1.027 0.003	6.1 0.4
3	Before	8	182.4 45.3	1.024 0.003	6.3 0.4
	During/After	70	104.7 62.2	1.017 0.007	6.3 0.6
4	Before	10	131.3 26.8	1.016 0.004	6.1 0.2
	During/After	62	123.2 65.6	1.012 0.005	5.8 0.3

The majority of discrete creatinine and specific gravity values and all discrete pH values were within normal limits. Regression analysis of the discrete specific gravity and creatinine concentration values revealed the following range of correlation coefficient (r^2) values: 0.6063 - 0.8274. Individual plots of all discrete urine specimen values (creatinine,

specific gravity, pH) versus 'time' did not reveal any apparent effect of creatine administration on these measurements. These data suggest that oral creatine supplementation, given at recommended doses, does not appear to influence the discrete urinary creatinine, specific gravity, or pH values.

Key Words: Creatine Supplementation, Creatinine, Urine

63: Determination of Cannabinoids in Water and Human Saliva by Solid-Phase Microextraction and Quadrupole Ion Trap Gas Chromatography-Mass Spectrometry

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Solid-phase microextraction (SPME) was applied to the determination of cannabidiol, Δ^8-tetrahydrocannabinol, (Δ^8-THC), Δ^9-tetrahydrocannabinol (Δ^9-THC) , and cannabinol in pure water and human saliva. The inherent extraction behavior of the cannabinoids in pure water was evaluated along with optimization of the method in human saliva. The commercially available polydimethylsiloxane (PDMS) SPME fibers were found to be the best class for the cannabinoid analysis. Partition coefficients were found to be extremely large for all of the cannabinoids (log K > 4.0). Equilibrium times for the 7- and 30 μm PDMS fibers were 50 and 240 minutes, respectively. A shorter extraction time of 10 minutes with the 30 or 100 μm PDMS fiber may be used for multiple extractions from the same vial, thus conserving the sample necessary for analysis and speeding up the total analysis time.

Recoveries for the cannabinoids in saliva, relative to pure water, were dramatically improved by a method developed in our lab involving addition of glacial acetic acid to the sample vial prior to performing SPME. Using this method, recoveries relative to SPME in pure water ranged from 21 - 47 % depending on the cannabinoid. The linear range for spiked saliva samples was established at 5 - 500 ng/mL (r^2 > 0.994) with precisions between 11 - 20 % RSD. The ultimate level of detection by SPME for the cannabinoids in saliva was 1.0 ng/mL, with signal-to-noise values > 12. A saliva sample collected 30 minutes after marijuana smoking was subjected to SPME and traditional liquid-liquid extraction analysis. Internal standard quantitation results for Δ^9-THC by both methods yielded comparable results. In conclusion, the SPME method was found to be less laborious and eliminated the use of organic solvents used in traditional methods of extraction.

Key Words: Solid-Phase Microextraction (SPME), Cannabinoids, Saliva

64: Sweat Testing for Drugs of Abuse I: Comparison of Cocaine Disposition in Sweat Collected with a Heated Sweat Patch Device and the PharmChek™ Sweat Patch.

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An inpatient clinical study is currently in progress to evaluate cocaine, codeine, and methamphetamine disposition in different biological matrices. This report describes the disposition of cocaine and metabolites in sweat collected from five human volunteers after subcutaneous administrations of low (75 mg/70 kg) and high doses (150 mg/70 kg) of cocaine HCl.

Sweat was collected from the palm and torso with a heated sweat patch device (Fast Patch) for 5 to 30 min intervals. The Fast Patch device contains a heating unit to stimulate sweat production. Sweat was also collected with PharmChek sweat patches worn on the torso for intervals ranging from 1 hr to 7 days. Patches were analyzed by GC-MS for cocaine and metabolites.

Cocaine secretion in sweat typically peaked within 2 hr after dosing. Peak cocaine concentrations ranged from 237-2,085 ng/Fast Patch and from 44-124 ng/PharmChek patch for the low dose and from 120-3,579 ng/Fast Patch and from 52-87 ng/PharmChek patch for the high dose. Cocaine was present in Fast Patches (worn for 30 min intervals) for up to 3 days after dosing. In comparison, cocaine was detected for approximately 1 day in PharmChek patches worn for 1 to 16 hr intervals after dosing. A clear relationship was not evident between dose and drug concentration in patches.

The primary cocaine metabolites in patches were benzoylecgonine and ecgonine methyl ester. Concentrations of these metabolites were generally less than 10% of cocaine. These data suggest that sweat testing with the PharmChek patch is beneficial in monitoring drug use when the patch is worn for days to weeks. Sweat testing with the Fast Patch device

may be a practical alternative to urine drug testing since the Fast Patch device allows for rapid, noninvasive specimen collections, and drug can be detected for up to 3 days after use.

Keywords: Sweat, Cocaine, Fast Patch

65: Detection of Urinary Ecgonidine as an Indicator of Active Smoking of Cocaine

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When cocaine is smoked, methyl ecgonidine is formed and also consumed as a pyrolytic product. Methyl ecgonidine is then metabolized to a stable compound, ecgonidine, and excreted in urine. Ecgonidine is a zwitterion and highly water-soluble. A method was developed to quantitatively identify ecgonidine in urine. After initial separation of benzoylecgonine and methyl ecgonidine from urine at pH 5.5 ± 0.5 using a solid phase extraction (SPE) technique, the pH of the solution was readjusted to 2.0-3.0. The acidic solution reduced the dissociation of the carboxylic acid and improved the lipophilic and cationic character of ecgonidine. The compound was extracted from the solution with the SPE technique with a 89-99% yield. Ecgonidine was then detected as the *tert*-butyldimethylsilyl derivative by GC-MS. Quantitation was linear over the concentration range of 7-2000 ng/mL. Concentrations as low as 7 ng/mL can be detected by this procedure.

Ecgonidine was detected in 96% of benzoylecgonine positive urine specimens (n = 23) from a random drug testing program indicating smoking as the major route of cocaine administration. In all specimens, the amount of methyl ecgonidine, the parent pyrolytic compound, was extremely small compared to the amounts of ecgonidine suggesting that ecgonidine is a better marker for identifying ingestion of cocaine by smoking. Detection of ecgonidine in urine is an indication of active or knowing use of cocaine and can be used to refute innocent ingestion defenses.

Key Words: Cocaine Smoking, Pyrolytic Product, Ecgonidine Detection

66: Cocaine Half Lives in Drug Abusing Patients Presenting to the Emergency Department

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Cocaine half life has been previously reported to be 30-90 minutes in human volunteers and has been postulated to be accelerated by the presence of elevated temperatures. We report the half life of cocaine in patients presenting to an inner city emergency department (ED) for treatment of acute cocaine intoxication.

Blood for cocaine and metabolite concentrations was drawn as soon as feasible after arrival and at intervals thereafter and frozen immediately. Concentrations were determined by an extractive alkylation/GCMS procedure. The mean age of the patients was 24.4 ± 7.3 years; 2 were female, 8 were male. Two patients ingested crack, 2 were IV users, 1 insuflated, 2 smoked crack cocaine, one both smoked and ingested crack. Two patients were admitted to the ICU, two were admitted to floor beds and 6 patients were discharged from the ED. One patient died.

Pharmacokinetic modeling revealed that the half life for cocaine ranged between 64 and 93 minutes for all patients and the mean initial cocaine concentration was 0.606 ± 0.478 mg/L. Metabolite concentrations (mg/L) were as follows: Ecgonine methylester 0.471 ± 0.382, ecgonine 0.454 ± 0.387, and bezoylecgonine 2.047 ± 2.986. Cocaethylene was not detected in any patient and norcocaine was detected in 3 patients with a mean concentration of 0.261 ± 0.436. Route of administration, body temperature, initial concentration, degree of hydration and presence of other drugs did not have a clinically significant effect on cocaine clearance. In addition, cocaine concentrations varied widely and were not predictive of the severity of clinical findings or outcome.

Key words: cocaine, half-life, hyperthermia

67: Cocaine Related Myocardial Ischemia

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Cocaine has been shown to produce myocardial ischemia through various mechanisms. However, the true incidence of myocardial ischemia and its characteristics is poorly characterized. Clinical findings in 157 patients presenting to an emergency department after acute cocaine use with and without chest pain were compared.

Recent cocaine use was determined by patient history (n = 14), a urine drug screen (n = 35) or by quantification of blood cocaine concentrations (n = 108). Blood cocaine concentrations were measured by an alkylation extraction/GC/MS procedure.

Most patients, regardless of the presence of chest pain had EKG changes that mimicked chronic hypertension including left ventricular hypertrophy, left atrial enlargement and aberrant conduction abnormalities. Rarely did those patients who were chronic abusers have normal EKG findings. The mean age of patients with cocaine related chest pain (n = 60) was 37.5 ±7.5 yr. Three patients ingested crack, 23 smoked crack and 3 patients insuflated cocaine.

The mean blood cocaine concentration (mg/L) in patients with cocaine related chest pain was 0.2 ± 0.5 and for those without chest pain, was 0.25 ± 0.4. Mean presenting vial signs in patients with chest pain were as follows: blood pressure 127.6 ± 19.0/81.9 ± 13.3 mmHg, heart rate 86.8 ± 17.1 bpm, respiratory rate 18.3 ± 6/minute and temperature 98.3 ± 1.15°F. In patients with chest pain, 2 had slight elevations of cardiac isoenzymes. Eight patients were admitted to the hospital for noncardiac reasons such as pneumonia. No patient had a myocardial infarction or demonstrable ischemia. Contrary to popular opinion, cardiac ischemia from acute cocaine use seems to be an uncommon event in the drug abusing population

Key words: cardiac ischemia, cocaine, electrocardiogram

68: Lessons Learned from an Unexplained Hospital Death

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A 35 year old man was brought to the emergency department at an inner city hospital because he was throwing objects from a balcony and was eating dirt and glass. His medical history was significant for cocaine abuse and a psychiatric illness. On arrival he was noted to be combative and was placed in an isolation room. At this time, his vital signs were: blood pressure 120/60 mmHg, heart rate: 60 bpm, respiratory rate 18/minute, temperature 98.0°F. The patient was found to be pulseless and without respiratory effort one hour after his last evaluation by a nurse. Resuscitative efforts were unsuccessful. The cause of death was listed as cardiopulmonary arrest from an unknown cause. Postmortem findings were significant for the presence of cocaine, petichae, an enlarged heart and areas of myocardial fibrosis consistent with chronic cocaine use. Review of the medical record revealed several discrepancies that make it difficult to determine a cause of death. The patient had presented with two prior episodes of acute cocaine intoxication that had similar features except on the two previous occasions the patient was hypertensive and tachycardiac. Sudden death within 48 hours of cocaine use is usually attributed to cardiac arrhythmia. It is most likely that this patients death was related to cocaine use. The etiology of sudden, unexplained death in the clinical and forensic setting, the importance of chart interpretation and the role of postmortem analysis will be discussed in light of other cases and animal studies.

Key words: Sudden death, cocaine, cardiopulmonary arrest

69: Postmortem Redistribution of Cocaine in Rats (C Jurado) - WITHDRAWN

69: Surface Ionization Mass Spectrometry W. Bernhard - ADDED

70: Postmortem Diagnosis of Cocaine Toxicity: the Utility of Brain Concentration Measurements

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In 1985, Spiehler and Reed first suggested that brain is a better sample for postmortem cocaine quantification than either blood or liver, because (1) cocaine distributes evenly in the brain, eliminating problems of site dependency, (2) brain concentrations "...are representative of the drug at the site of action at the time of death," and (3) "information on the recent or remote use of cocaine can be obtained from the relative concentration of cocaine and benzoylecgonine (BE) in blood and brain tissue." PET studies have shown that cocaine is not as evenly distributed as once thought, but the fundamental correctness of their conclusions has been repeatedly confirmed. Yet brain cocaine concentrations are rarely determined. We present here data from 15 decedents where both blood and brain concentrations were determined.

Decedents were 73% male, 80% white, with a mean age of 38.5 years, and a mean postmortem interval of 14.9 hours. Mean values for blood cocaine and benzoylecgonine were 3.1 ± 4.2 and 3.0 ± 3.1 mg/L, respectively. In the brain the concentrations were 8.7 ± 11.4 and 1.7 ± 1.3 mg/Kg. Five of the decedents died with classic symptoms of excited delirium, the remainder with a variety of medical conditions (hemorrhagic pulmonary edema, cardiomegaly, hemorrhagic pancreatitis, and seizures). In each instance, when the absolute drug concentrations and ratios for cocaine:benzoylecgonine in the brain were considered in relation to the autopsy findings and scene reports, a plausible mechanism for the cause of death could be constructed. Similar attempts based on blood concentration measurements, could not always distinguish the cause of death. Autopsy and scene findings must be related to measured drug concentrations at the time of death, and the only way to estimate those is to quantitate the amount of drug and metabolite in the brain.

Key Words: Cocaine Brain Concentrations

71a: An Improved Solid-Phase Extraction Procedure for the Recovery and Quantitation of Amphetamine and Related Compounds from Blood and Hair

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Solid-phase extraction (SPE) has become the standard approach to sample preparation for the GC or GC/MS analysis of drugs in biological fluids. While this technology has proven to be applicable to a wide variety of compounds, the determination of amphetamines has been especially challenging because of their volatility. Significant loss of analyte occurs both when the elution solvent is evaporated and under the thermal conditions employed during the derivatisation procedure. A SPE method, which addresses both these limitations, is described for the improved recovery and quantitation of amphetamine (AP), methamphetamine (MA), 3, 4-methylenedioxyamphetamine (MDA), 3, 4-methylenedioxymethamphetamine (MDMA) and 3, 4-methylene-dioxyethylamphetamine (MDEA) in blood and hair.

The initial extraction was achieved using ISOLUTE CONFIRM HCX mixed-mode SPE columns. The drugs were determined using GC/MS with tri-deuterated amphetamine as the internal standard. The problems with volatility were addressed by simple addition of 100L of tartaric acid (1mg/mL in ethyl acetate) to each standard and analytical sample prior to evaporating to dryness. All of the amphetamine compounds demonstrated significant increases in recovery.

Data is also presented on the kinetics of the derivatisation of these compounds using pentafluoropropionic anhydride. In contrast to many reports in the literature which call for elevated temperatures (> 40C) for extended times (> 20 minutes) our data shows that the reaction is complete for all species in less than 2 minutes at room temperature. This

represents a significant reduction in the total time of analysis. Recoveries were greater than 87% for all five amphetamines from both blood and hair and were linear over the concentration ranges studied ($r^2 > 0.993$).

Keywords: Extraction, Amphetamines, Derivatisation

71: Unusually High Blood Benzoylecgonine Concentrations in Cases with Renal Failure

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Over a two year period 23 out of 75 decedents (31%) where chronic renal failure or end stage renal disease was considered either a primary or secondary cause of death were found to be positive for cocaine and/or metabolites by GC/MS in appropriately preserved blood specimens. The average blood benzoylecgonine concentration in all cases was 5.1 mg/L (N = 23, cutoff = 0.050 mg/L). Only 8 cases contained ≤2.0 mg/L benzoylecgonine (mean = 0.31 mg/L, range = 0.065-0.60). 15 cases (65% of those positive) contained benzoylecgonine at concentrations exceeding 2.0 mg/L with 5 cases (22% of those positive) exceeding 10 mg/L (mean = 7.6 mg/L, range = 2.1-23 mg/L). Parent cocaine was detected in the blood of only four cases (cutoff = 0.025 mg/L, mean = 0.13, range = 0.043-0.26 mg/L) and in only one case where the benzoylecgonine concentration exceeded 10 mg/L. Only one case contained ethyl alcohol (0.03%) and ethyl cocaine was not detected in all 23 cases (cutoff = 0.025 mg/L).

Homicide cases were studied for the same period as controls. In contrast to the population with renal disease, cocaine was frequently detected and benzoylecgonine concentrations were much lower. Out of 980 homicide cases, 184 (19%) were positive for cocaine and/or metabolites above the cutoff in the blood. The average blood benzoylecgonine concentration in these cases was 1.2 mg/L (N = 184, range = 0.051-7.2). Only 30 cases (16% of those positive) contained benzoylecgonine above 2.0 mg/L and only 6 cases (3.3% of those positive) above 5.0 mg/L. Parent cocaine was detected in the blood 143 (78%) of the cases positive for benzoylecgonine (mean = 0.25, range = 0.025-7.2) and in ALL 30 cases containing benzoylecgonine above 2.0 mg/L. Ethyl alcohol and ethyl cocaine (mean = 0.094, range = 0.025-0.41) were detected in 87 and 67 cases, respectively.

These findings suggest that in patients with chronic renal failure or end stage kidneys, the polar cocaine metabolite, benzoylecgonine, may accumulate to unusually high concentrations. Careful consideration should be given to the role cocaine abuse may play in patients with renal failure. While cocaine has not been shown to be inherently nephrotoxic, this data supports findings by other investigators who have demonstrated that cocaine users are subject to renal disease. However, in the cases discussed here cocaine toxicity was thought to play a primary role in the cause of death in only a single case and to be contributing in only 4 cases. Based on the association of cocaine abuse with renal disease, the role of cocaine abuse may have been considerably undereported in these cases. Finally, consideration should also be given to the toxicity of benzoylecgonine. Although not centrally active, benzoylecgonine has been reported to have potentially deleterious effects on the vasculature.

KEY WORDS: cocaine, benzoylecgonine, renal disease

72: Localization of Drugs and Metabolites in Human Hair

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Localization of drugs and metabolites in human hair is important in determining the pharmacokinetics of drug incorporation in hair. This information is critical to validate drug testing from hair. Infrared microscopy investigation of laterally microtomed hair has shown to be a valuable tool for imaging this drug distribution. Three dimensional contour maps of drug location are available of the hairs that have been probed by infrared microscopy. Studies have shown that hydrophobic drugs tend to bind to the central core or medulla of the hair while hydrophilic drugs tend to distribute throughout the hair and appear, generally, in lower concentrations per dose. The high spatial resolution available with current infrared microscopy instrumentation allows microprobing of the hair with finite location of the drug distribution within the hair.

Separation of the medullated hair segments from the non-medullated with subsequent GC/MS quantitation have shown that the medulla does play an interactive part in the drug binding in hair. Our studies with aged individuals with

both pigmented and non-pigmented hair have shown that the drug is associated with the medullated portion of the non-pigmented hairs and both the medullated and non-medullated portions of the pigmented hair. This is evidence for a medulla binding as well as binding associated with the pigment of the hair. Microtoming a drug free hair and exposing the center portion to a drug/saline solution shows, by infrared microscopy, a preferential migration of the drug to the medulla of the hair. The infrared microscopic data is not quantitatable but can yield relative concentrations which are confirmed by GC/MS results.

Key Words: Hair analysis, drug distribution, imaging

73: Comparison of *In Vivo* and *In Vitro* Deposition of Rhodamine and Fluoroscein in Hair.

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To investigate the mechanisms involved in the accumulation of drugs or other compounds into hair, we examined the deposition of two fluorescent dyes, rhodamine and fluoroscein in the hair of Balb/C (albino) mice and C57 (pigmented) mice. The deposition patterns of the two dyes were examined by fluorescence microscopy after systemic administration or external loading of each dye into untreated hair under varying conditions. This allowed for the direct comparison of the morphology of internal and external deposition of the same compound as well as a comparison of the deposition of a zwitterionic compound, rhodamine, and an anionic compound fluoroscein.

Twenty-three day old mice were administered rhodamine or fluoroscein i.p. on Wednesdays, Thursdays and Fridays for three weeks. One week after the final dose mice were sacrificed and hair harvested. An additional group was given one 10 mg/kg dose rhodamine or one 100 mg/kg dose of fluoroscein, sacrificed at 1, 2, 24 and 48 hours and skin and hair samples taken. One group of animals was co-administered rhodamine and fluoroscein. Untreated hair was soaked for 1, 5, or 12 hours in solutions of rhodamine or fluoroscein at 100 ug/ml or 1 ug/ml made up in pH 6, pH 9 and pH 3 buffers or methanol. Hairs were then washed briefly with water, dried and fixed on glass slides. After loading, aliquots of hair were washed by soaking in pH 6 phosphate buffer or methanol for 24 hours.

Bands corresponding to the dosage periods along the length of the hair were clearly evident for rhodamine deposited from the systemic circulation. Additionally the pattern of deposition appears to be within the internal protein matrix with little evident deposition in the surface of the hair. The intensity of the fluorescence was not affected by either phosphate buffer or methanol washes. Rhodamine appeared in the hair bulb within 1 hour of the dosing with significantly brighter fluorescence than the surrounding skin.

External loading of rhodamine into the hair resulted in staining of the junctions of cortical scales with nominal internal staining. This pattern persisted even after 12 hours of exposure to the loading solution. This fluorescence was not removed by pH 6 buffer or methanol wash. Fluoroscein followed a similar pattern with maximum fluorescence when loaded at pH 6 and nominal staining when loaded in pH 9 buffer or methanol. Marked diminution of fluorescence occurred after soaking in pH 6 buffer but not after soaking in methanol. This work supported by NIH grant DA09545.

Key Words: Hair Analysis, In Vivo Drug Storage, In Vitro Drug Storage

74: Deposition and Retention of Radiolabeled Serum Constituents in Hair Following Systemic Administration

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To investigate the chemical mechanisms involved in the accumulation of drugs or other compounds into hair, we examined the deposition of radiolabeled serum constituents in the hair of Balb/C (albino) and C57 (pigmented) mice. The extent of *in vivo* incorporation of a normal serum cation ([$^{45}Ca^{++}$]), an anion ([$^{36}Cl^{-}$]), a neutral constituent ([^{14}C]-urea) and a structural component of hair ([^{35}S]-cysteine) were studied to provide a reference framework for the examination of foreign substances deposited in hair from serum. The use of two mouse strains afforded an evaluation of the effect of hair pigmentation on levels of accumulation Additionally, the endogenous content of Mg^{++}, Na^{+} and K^{+} (measured by ICP-AES) was determined, as was their susceptibility to removal. Hair concentration of isotopes were calculated from mean specific activities determined over the treatment period and corrected for quenching and decay.

[$^{45}Ca^{++}$] accumulation (500 ng/mg hair in C57 and 25 ng/mg hair in Balb/C) was unaffected by a 24 hr phosphate buffer extraction. Of the [^{14}C]-urea accumulated (3,500 ng/mg in C57 and Balb/C), 50% was removed by 24 hour extraction in phosphate buffer. Of the accumulated [$^{36}Cl^{-}$] (65 ng/mg in C57; 30 ng/mg in Balb/C), half was removed by 24 hr extraction in phosphate buffer. The accumulated [^{35}S]-cys (210 ng/mg in C57; 110 ng/mg in Balb/C) could not be removed. Endogenous Mg^{++} (350 ng/mg in C57; 75 ng/mg in Balb/C) was stable to 24 hr extraction in phosphate buffer. K^{+} (2,500ng/mg) and Na^{+} (400 ng/mg) concentrations were approximately equal in both strains and were largely extractable.

Based on the accumulation of a neutral serum constituent such as urea, the data suggest that factors other than ionic binding are important in deposition of circulating molecules into hair. The extent and reversibility of ionic binding is dependent upon the chemical nature of the substance. The presence of hair pigmentation greatly increased the accumulation of [$^{45}Ca^{++}$], [$^{36}Cl^{-}$] and [^{35}S]-cys. These data support a multicompartmental nature of drug storage in hair.

This work supported by NIH Grant DA09545.

Key Words: Hair Analysis, Serum Constituents, Drug Storage

75: The Detection of 7-Aminoflunitrazepam in Hair Using Micro-Plate Enzyme Immunoassay

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Introduction: Sexual abuse of both men and women while under the influence of so-called "date-rape" drugs has been the focus of many investigations. One of these drugs, flunitrazepam (Rohypnol, or "Roofies") has recently been banned in the U.S.A. because of its use in various "date-rape" situations. Unfortunately, the detection of flunitrazepam or two of its metabolites, desmethyl-flunitrazepam and 7-aminoflunitrazepam, (7-AF) in a single specimen such as urine or blood is difficult in criminal situations because of the likelihood of single dose ingestion and the length of time since the alleged incident. Hair provides a solution to the second of these problems, in that drugs tend to incorporate into hair and remain there for longer periods of time than either urine or blood. The objective of this paper was to determine whether a commercially available micro-plate enzyme immunoassay system was sufficiently sensitive for the routine screening of 7-AF in hair.

Sample Preparation: Drug free hair was powdered and weighed into 50 mg aliquots. 7-AF was added at various concentrations from 0.05 to 1.0 ng/mg of hair. Following standard sample preparation, the samples were extracted using a mixed-mode solid-phase procedure. The specimens were screened using the methodology described in the benzodiazepine urine micro-plate enzyme imunoassay kit (STC Technologies, Inc.). Incubation time and aliquot size were the major variables in optimization of the assay. The final procedure showed a difference between absorbance value produced by the negative hair and that produced by hair containing 0.05 ng/mg, however, a cut-off of 0.1 ng/mg was selected.

Case Studies: The procedure was run on hair taken from four subjects. Sample A was obtained from an alleged rape victim, sample B was obtained from an emergency room patient whose blood screened positively for benzodiazepines, samples C and D were obtained from corpses whose blood contained flunitrazepam and 7-AF along with other drugs. All the specimens screened positively for benzodiazepines using this assay at 7-AF concentrations approximately equivalent to A:0.2 ng/mg; B: 0.5 ng/mg; C: >1 ng/mg and D: 0.1 ng/mg.

Conclusion: The micro-plate enzyme immunoassay system was sufficiently sensitive for the screening of 7-AF in hair. Confirmatory procedures are under development.

Keywords: flunitrazepam, date-rape-drugs, hair

76: Highly Sensitive Quantitation of Flunitrazepam and 7-Aminoflunitrazepam in Hair.

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Flunitrazepam (FN) known as Rohypnol, Narcozep, and "roofies" is a benzodiazepine mainly used as a hypnotic and anesthetic agent in many European countries and in Mexico. FN is smuggled into the U.S. and has been identified as the compound of choice for "drugging" unsuspecting females and raping them while they are under the influence of this substance. For this reason FN has become known as a "date-rape" drug. The aim of this study was to develop and validate a sensitive NCI-GC-MS method for the quantitation of FN and its major metabolite 7-aminoflunitrazepam (7AFN) in human hair and apply it to real life cases.

Seven point standard curves for FN and 7AFN were prepared by spiking 50 mg aliquots of pulverized drug free hair. The range of the standard curves was 500 fg/mg - 100 pg/mg for 7AFN and 2.5 pg/mg - 100 pg/mg for FN. In addition, two levels of control hairs were prepared for FN (15 and 80 pg/mg) and 7AFN (3 and 40 pg/mg). Our lab received 3 hair samples from suicide cases involving FN and one alleged rape case. All samples were washed in deionized water, dried and pulverized. Internal standards, FN D_7 (100 pg/mg) and 7AFN D_7 (20 pg/mg) were added to 50 mg aliquots of hair, standards and controls. MeOH (3 ml) was added to each vial and they were sonicated for 1 h. MeOH was decanted and 0.1N HCl was added (3 ml). The specimens were incubated for 18 hours at 50°C. The fractions were combined and mixed mode solid phase extraction was performed. Final elution of the drugs was achieved using methylene chloride:isopropanol:NH_4OH (78:20:2, v/v/v). Extracts were evaporated to dryness, and derivatized with HFBA (50 ml). HFBA was evaporated and EtAc (25 ml) was added. A Hewlett Packard GC-MS system comprising a 6890 GC and a 5973 MSD detector (CI with methane) was operated in SIM mode with splitless injection. For FN *m/z* 313, for FN D_7 *m/z* 320, for 7AFN *m/z* 459, for 7AFN D_7 *m/z* 466 ions were monitored.

Standard curves for FN and 7AFN had correlation coefficients of 0.998 and 0.997, respectively. All precision and accuracy values were within acceptable limits. Two samples were negative for FN and 7AFN (including alleged rape), and two were positive (case 1: FN 23 pg/mg, 7AFN 26.07 pg/mg; case 2: 7AFN 48.61 pg/mg). In the second case only 9 mg of gray hair was available for analysis.

Key words: NCI-GC-MS, hair analysis, flunitrazepam, 7-aminoflunitrazepam.

77: Identification of Cocaine, Opiates and Their Metabolites in Nails from Postmortem Cases

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Postmortem fingernail, toenail, and nasal swab samples were utilized for the detection of cocaine, opiates, and their metabolites. This study was designed to evaluate the correlation of these drugs in fingernails (right hand vs. left hand), toenails (right foot vs. left foot), blood, and nasal swabs. Nail clippings were washed with methanol and then solubilized in 0.1 M potassium phosphate (pH 5.0). Drugs were extracted from the nasal swabs by soaking in methanol for 24 hrs. Cocaine and its metabolites were isolated from the solubilized nails and methanol swab extracts by solid phase extraction, while opiates were isolated utilizing a liquid-liquid extraction procedure. After derivatization with MSTFA, quantitation was performed by GC/MS in the SIM mode. The linear range for all analytes was 0.3 to 60.0 ng on column. The limit of quantitation for all analytes was 0.3 ng on column.

The concentration of drugs found in the nails of each hand, foot and nasal swab were compared with those of their corresponding postmortem fluids. Eight cases were screened for cocaine and metabolites and in all of these cases, the fingernails and/or toenails were positive for cocaine, benzoylecgonine, and ecgonine methyl ester. Norcocaine was found in 7 cases and cocaethylene was detected in the toenails of two cases with values of 0.95 and 0.08 ng/mg. Concentration ranges (ng/mg) for nails were as follows: cocaine 4.16 - 397.4; benzoylecgonine 1.8 - 170.3; ecgonine methyl ester 0.19 - 26.98; and norcocaine 0.11 - 32.74. Cocaine was detected in the blood for 5 of the 8 cases. Nasal swabs (2 swabs per nostril) were collected for 7 of the cases and all were positive for cocaine with values ranging from 7.34 to 10,229 ng/swab (mean=2262).

Eight cases were also screened for opiates and in 7 of these, the fingernails and/or toenails were positive for morphine with concentrations ranging from 0.03 - 407.9 ng/mg. In 5 of these cases, 6-monoacetylmorphine and codeine concentrations ranged from 0.18 - 103.6 and 0.06 - 10.4 ng/mg, respectively. Hydromorphone was detected in 2 fingernail specimens with concentrations of 0.02 and 0.44 ng/mg with no drug detected in the corresponding blood. In 6 of the cases, morphine was found in the blood. Nasal swabs were collected for 4 cases and all were positive for morphine with values ranging from 2.29 to 114.9 ng/swab (mean=30.9).

These results demonstrate that nails may provide an alternate matrix for postmortem drug detection in cases where conventional matrices are unavailable. Whenever possible, fingernails are the specimen of choice since in this study, the drug concentrations measured were consistently one order of magnitude greater than drugs detected in toenails.

Keywords: Cocaine, opiates, nails

78: Nail Analysis for Drugs of Abuse

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Fingernail clippings were evaluated as analytical specimens for the detection and quantification of drugs of abuse. Fingernail clippings (2.5-25 mg) were obtained from consenting adults attending a drug clinic, together with information concerning the drugs they had used over the previous six months.

Methods for the surface decontamination and extraction of the specimens were evaluated. The nail clippings were decontaminated by sonication in 0.1% sodium, dodecyl sulfate (SDS) followed by sonication in distilled water and methanol. Whereas the SDS and water washes were discarded, the methanolic washes were analysed for the presence of analytes. The nail clippings were then hydrolysed in 1 M NaOH and the hydrolysates were extracted with organic solvents prior to instrumental analysis. Extracts were analysed by RIA, GC-MS or GC equipped with ECD.

Positive RIA results were obtained with specimens from 6 known drug users. The average cannabinoid concentration in fingernail clippings determined by RIA was 1.03 ng/mg. Using GC-MS, the mean Δ^9-tetrahydrocannabinol concentration in fingernail clippings from a further 14 known cannabis users was 1.44 ng/mg. Finally nail clippings from another 6 known drug users were analysed for diazepam and its mean concentration was found to be 25.71 ng/mg. the limits of detection for the RIA method for cannabinoids and the GC-MS method for Δ^9-tetrahydrocannabinol were determined to be better than 0.1 ng/mg. The limit of detection of the GC-ECD method for diazepam was lower than 0.001 ng/mg. The extraction recoveries for our methods were better than 81%.

Based on these results, fingernails appear to be potentially useful biological specimens for the detection of past drug use in cases of medico-legal interest.

Key Words: Nail, Analysis, Drugs

79: On-site Testing of Saliva for Drugs of Abuse in Suspected Drug Users with DRUGWIPE and Determination of Drug Concentrations in Saliva and Urine by GC/MS

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In the context of roadside testing for driving under the influence of drugs, there is a need for drug assays that can be performed immediately at the site of specimen collection. We evaluated the non-instrumental immunoassay Drugwipe (**DW**) for the detection of cocaine, opiates, amphetamines and cannabinoids in saliva and sweat.

During police actions focused on the control of the possession of illicit drugs, subjects were selected by police staff based on external signs of drug intake and questionnaires. The subject was then examined by a member of the medical staff. A urine sample was screened with Frontline for one or more of the drug classes. DW was applied on the tongue and on the neck or the back. If one of the three assays (urine, saliva, sweat) showed a positive result, a blood sample and a saliva sample were collected. The principal drugs of abuse and their metabolites were quantified in the body fluids with GC/MS after SPE and derivatization. The results are presented in the following table:

	cocaine (6 subjects)	**heroin** (5 subjects)	**amph (speed, XTC)** (15 subjects)	**cannabinoids** (15 subjects)
Saliva + (DW)	5	3	15	4
Saliva + (GC/MS)				
conc. range	6 *22 - 3,500* *ng/ml cocaine*	5 *156 - 3,080* *ng/ml MAM*	15 *5 - 3,000* *ng/ml amph, MDMA, MDEA, MBDB*	10 *1.4 - 42* *ng/ml THC*
Urine +/ number of urine samples	4/4	3/3	12/12	9/11

Saliva is a useful tool to detect illicit drugs after recent abuse. Drugwipe provides satisfactory results for cocaine and amphetamines but lacks sensitivity for heroin and cannabinoids.

Key Words : saliva, drugs of abuse, Drugwipe

80: Anabolic Steroids in Hair of 2 Bodybuilders

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On March 1998, two tourists, coming from Spain by autocar were arrested by the French customs in Strasbourg. In their luggage, the custom-house officers discovered 2050 tablets of Winstrol (stanozolol, 2 mg) and Proviron (mesterolone, 25 mg) and 251 ampules of Winstrol Depot (stanozolol, 50 mg), Testoviron Depot (testosterone, 250 mg), Primobolan Depot (metenolone, 100 mg) and Deca-Durabolin (nandrolone decanoate, 50 mg). Both subjects claimed that the steroids were for personnal use, but this was not accepted by the officers, who considered the drugs as a part of trafficing. To document the pattern of use, the judge in charge of the case requested toxicological analyses, including urine and hair tests. Nandrolone, stanozolol, testosterone and their corresponding metabolites were identified in the urine of both subjects.

50 mg of hair were incubated in 1 ml of 1N NaOH at 95 °C for 10 min, in presence of 5 ng of nandrolone-d_3 and stanozolol-d_3, used as internal standards. Then, the homogenate was extracted with 5 ml of ethyl acetate. After horizontal agitation and centrifugation, the organic phase was removed and evaporated to dryness. The residue was derivatized using 50 μl MSTFA/NH_4I/2-mercaptoethanol (1000/2/5), for 20 min at 60 °C. The drugs were identified by GC/MS in electron impact mode.

The hair tested positive for nandrolone (196 and 260 pg/mg), testosterone (46 and 71 pg/mg) and stanozolol (135 and 156 pg/mg), clearly indicating steroids abuse.

Although not yet recognized by the International Olympic Committee, in sports, hair analysis may be a useful adjunct to conventional drug testing in urine.

Key Words: Anabolic steroids, doping, hair

81: Proof of Steroid Abuse by Detection of Testosterone Esters in Hair

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Two outstanding possibilities of hair analysis are access to retrospective information and identification of substances not excreted in urine. Both aspects are very promising for doping analysis, where the frequency and distribution of out-of-competition control can never be sufficient and exogenous abuse has to be distinguished from endogenous levels of identical substances (testosterone).

Logical scientific approaches for this differentiation (i.e. quantitation of T/E ratios or carbon isotope ratio discrimination) are always controversial due to common biological deviations. Therefore, the incorporation of anabolic agents - especially of exogenous precursors of anabolic steroids (steroid esters) was investigated. Extensive chromatographic procedures (HPLC clean-up, HRMS and MS/MS) were analytical prerequisites for successful sample preparation and identification due to low target concentration and interfering endogenous substances.

Five testosterone esters, nandrolone decanoate, metenolone enantate metandienone, stanozolol and clenbuterol could be identified in hair samples of 5 relevant forensic cases. The identification of 5 different testosterone esters enabled an unambiguous identification of testosterone abuse in three cases.

A comparison with analytical results obtained from urine and other biological tissues leads to conclusions about potential and limitations of steroid analysis in hair.

Keywords: Hair, Steroids, Doping

82: The Effects of Storage Conditions on the Levels of Testosterone and Epitestosterone in Urine Samples

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The effects of various storage conditions on the concentration and the ratio between testosterone and epitestosterone in their free and conjugated forms in a pooled, male urine sample were examined.

Stage 1 was designed to determine the effects of temperature, time, container type and sterility of the urine sample. The experimental design was based on a Taguchi L16 matrix mirrored to reflect the time variable requiring eight levels.

The second series of experiments examined the effects of light, radiation, agitation, storage under nitrogen and the inclusion of a preservative based on a full factorial design with five factors, each at two levels. For these variables, sterile urine samples were stored in a single container type, at 25°C for seven days prior to analysis.

The concentration of the free and conjugated testosterone and epitestosterone were determined by GC-MS using deuterium labelled internal standards after hydrolysis, C8 solid phase extraction followed by liquid-liquid clean up and enol-derivatisation.

The change in these concentrations and ratios are important as unsuitable storage conditions may result in a testosterone/epitestosterone ratio that may exceed the International Olympic Committee level of 6:1 and result in a ban or legal action.

Keywords: testosterone, epitestosterone, urine

83: Urinary Excretion of dl-Fenfluramine in Rat Urine

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Fenfluramine, which is an anorectic agent, is widely abused as a diet pill in Korea. Because it is marketed freely in China, ethnic Koreans and tourists to China took it without knowing that it is controlled under the Act of Psychotropic agent in Korea. Because it's abuse is prevalent, there was a case in which a young mother who had taken it at a dose 6-10 times higher than recommended over an year, killed her one- year son in a state of mental disorder.

Fenfluramine is administered orally as the racemic mixture, but its optical isomers have different actions. d-Fenfluramine is used as an anorectic agent, while l-fenfluramine is a neuroleptic agent.

To investigate the metabolism of racemic fenfluramine, the urinary excretion of fenfluramine was studied in rats. The enanthiomeric separation of fenfluramine was achieved on achiral columns by gas chromatography using (S)-N-(trifluoroacetyl)-l-prolyl chloride as a derivatizing agent. The assay attempted to distinguish the chirality of fenfluramine after the administration of dl- fenfluramine to rats.

After administration of 15mg/kg of racemic fenfluramine, d-fenfluramine, l-fenfluramine and its metabolites d-norfenfluramine and l-norfenfluramine in urine were well resolved by chromatographic separation of TFP derivatives on DB-5 with retention times of 11.2, 11.8, 8.4, 8.6 min respectively. The amounts of d-fenfluramine were greater than l-fenfluramine, while d-norfenfluramine were smaller than l-norfenfluramine in urine. The ratio of d-norfenfluramine versus d-fenfluramine ranged from 0.6 to 2.8, while the ratio of l-norfenfluramine over l-fenfluramine was 6.9-17.2. This indicated that d-fenfluramine was metabolized at a slower rate than the l-isomer.

Key words: Fenfluramine, Urinary excretion, Enanthiomeric separation

84: A Survey of Methamphetamine and Amphetamine in Postmortem Fluids and Tissues

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Methamphetamine continues to enjoy widespread use in Oklahoma. Tissue drug concentrations, most notably brain, have received increasing interest regarding the interpretation of drug effects. This paper will present data from 52 postmortem cases. The data will be from cases involving various causes and manners of death. The concentrations of methamphetamine and amphetamine were determined in blood, vitreous, liver and brain.

Blood was screened for methamphetamine and amphetamine using radioimmunoassay. Blood and tissue homogenates from positives were confirmed and quantitated with gas chromatography/mass spectrometry (GC/MS) following a liquid-liquid extraction from alkalinized samples to which n-propyamphetamine (NPA) had been added as an internal standard. The extracts were derivatized with pentafluoropropionic anhydride, evaporated to dryness and reconstituted with 50 mcL n-propanol. Analysis of the specimens was achieved with a HP 5890/5971 GC/MS operated in the electron impact mode with temperature programming and selected ion monitoring. Ions monitored were: methamphetamine, 118, 160, 204; amphetamine, 91, 118, 190; NPA, 118, 190, 232. Retention times were sufficiently different for compounds with common ions.

The mean concentrations of the two compounds are listed below (mcg/mL, mcg/G):

	heart blood	femoral blood	subclavian blood	vitreous	liver	brain
methamphetamine	2.39	1.21	2.75	1.38	8.90	5.89
amphetamine	0.27	0.26	0.44	0.27	1.57	0.91

Keywords: Methamphetamine, GC/MS, postmortem

85: Ibogaine-Associated Death in a Female Heroin Addict: Forensic And Toxicological Aspects

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A case involving the death of a young female heroin addict after ibogaine administration is reported. The drug was alleged to have been administered orally at a dose of 23 mg/kg and later on an additional 6 mg/kg was administered in the course of experimental and officially unapproved anti-addictive therapy. The patient collapsed and died about 19 h after ibogaine administration.

Extremely high levels of drug and its active metabolite (12-OH-ibogaine) were found in blood. The following concentrations (mg/L) of ibogaine and its active metabolite 12-OH-ibogain were determined by GC-MS(1): ibogaine in femoral blood 0.710, in heart blood 0.730 , 12-OH-ibogaine in femoral blood 3.900, in heart blood 10.700. In experimental studies the concentration of drugs in blood samples taken 19 h after administration of ibogaine 20 mg/kg were: 0.1-0.2 mg/l for ibogaine and 0.5-1.1 for 12-OH-ibogaine. The circumstances of the case showed that the "therapy" was performed in an unprofessional way and the minimal medical standards were not met.

1. W.L. Hearn, J. Pablo, G. Hime and D.C. Mash, *J.Anal.Toxicol.* **19**: 427-34, 1995.

Key Words: Ibogaine, 12-OH-Ibogamine, Heroin Addiction

86: Unexpected Pitfalls in Toxicological Bioanalysis

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The principle of bioanalysis is based on the comparison between the concentration of xenobiotics in a specimen of a patient or victim and a spiked standard with the same matrix and using about the same concentration. Unfortunately, in practice this apparently easy principle may be undermined by quite a few expected and unexpected problems.

During the last TIAFT meeting in Padua (1997) we previously mentioned the importance of the shaking, evaporation and redissolving steps during the analysis. In this lecture the problems of unexpected adsorption on tubing of the pipettor and on the wall of plastic or glass vessels during the preparation of the stock solutions will be discussed on the basis of some practical examples.

The quality of the reagents might also have a dramatic influence on the results. For instance, the recovery of tricyclic antidepressants depends on the purity of the dichloromethane used during extraction. We noticed the same phenomenon in the reconstitution of the stock solution of a doxyrubicine-analogue using different qualities of methanol.

An other point of concern could be the difference between calves' serum and human serum as an useful matrix for the calibration of several drugs.

Key Words: Adsorption, Reagents, Interferences

87: Toxicologist Dies at Annual Meeting Under Mysterious Circumstances

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This presentation is the second annual murder mystery poster. Should you decide to participate, you will get a packet of information that includes the case background and a few clues. The rest of the clues are found at various vendor booths located at the conference. Your task, should you decide to accept it, will be to collect the remaining clues that will assist you in solving the problem and return them with your answer to the poster site. All participants will receive a prize for their effort and become eligible for the grand prize.

*Editor's note: The answer appears under "Drugs in the News" in the December, 1998, issue of **ToxTalk**.*

Key Words: murder, mystery, clues

88: Headspace GC and Headspace SPME/GC-MS Investigation of Hydrolyzed Hair Samples of Alcoholics, Social Drinkers and Teetotalers

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In order to investigate, whether a steady high alcohol consumption leads to an increasing incorporation of endogenous or nutritive products containing ethyl groups into hair or to a chemical binding of ethyl groups onto hair constituents, hair samples of 50 alcoholics, 10 social drinkers and 10 consequent teetotalers were washed with water and CH_2Cl_2, carefully dried, hydrolyzed with 30% NaOH at 80 °C in closed vessels and analyzed by headspace GC and headspace-solid phase micro extraction (SPME)/GC-MS with t-butanol as internal standard. The following volatile compounds were identified: ethanol (17 – 60 ng/mg), acetone (85 - 230 ng/mg), butan-2-one (4 - 18 ng/mg), a series of further alkane-2-ones and in some cases nicotine.

By preceding 24 h extraction with methanol or aqueous buffer at pH 7.6 in an ultrasonic bath up to 60 % of the ethanol producing compounds were removed from the hair matrix. From the methanol extracts 0 - 12 ng/mg ethanol were formed by hydrolysis after evaporation of methanol and drying. There was no significant difference in the amount of ethanol detected in hydrolyzed hair samples or hair extracts between alcoholics and social drinkers, whereas slightly smaller concentrations were detected in abstinent children's hair. No significant influence of age, sex or hair color was observed.

For identification of extractable ethanol precursors hair extracts were analyzed by GC/MS-SIM for ethyl esters of 15 endogenous or nutritive carboxylic acids after adequate sample preparation. Out of these only p-hydroxybenzoic acid ethyl ester (0.1 – 5.9 ng/mg) as a usual preserving agent in hair care products has been identified, the concentration of which is by far too small to explain the total amount of ethanol formed.

As a conclusion, ethanol liberated from hair by alkaline hydrolysis does not prove chronically elevated alcohol consumption.

Key words: Hair analysis, Ethanol formed in hair hydrolysis, Headspace GC and SPME-GC/MS

89: Headspace-GC/MS for Detection of Gasoline Components in Two Cases of Suicidal Burning

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Suicidal burning has been reported by several authors, but compared to other forms of suicide this form is relatively rare. In two such cases reported by us gasoline served as a fire accelerator.

In the first case the person was found in a barn which had burnt down completely. Some circumstances suggested that gasoline was involved. In the other case a female poured gasoline over her whole body. The use of a highly sensitive headspace-GC/MS method made it possible to detect the special components of the gasoline used. For the detection samples of the lungs, liver, kidneys and blood were used. By means of the matrix-independent multiple headspace extraction (MHE) technique quantitative analyses of the distribution of hydrocarbons in the organs were possible.

The chemical evidence for the gasoline and the exact determination of the type were an important contribution to the criminal investigations and the clearing up of the case, and made it possible to exclude a crime.

Key words: headspace-GC/MS, gasoline, suicidal burning

90: Tissue Distribution of Petrol in a Forensic Case

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A woman killed herself by ingestion of a large amount of petrol (gasoline) in combin-ation with ethanol, trazodone and barbiturates. At autopsy 180 ml of petrol was present in the stomach. Petrol was identified with headspace GC/MS. Benzene, toluene, ethylbenzene, *p*-xylene, *o*-ethyltoluene, *m*-ethyltoluene and 1,2,4-trimethylbenzene, constituents of petrol, were identified in the postmortem samples by headspace GC/MS and quantitatively determined using gas chromatography and flame ionization detection (GC/FID). Blood, liver, kidney, brain tissue, eye fluid, bile and stomach content were collected at autopsy.

Methods Blood (5 mL), kidney, liver, brain (5 g), eye fluid (0.1 mL), bile (1 mL) were added to a headspace vial containing 5 mL of water (10 mL for eye fluid and 9 mL for bile), 2 g of sodium chloride and 100 µg diphenylmethane used as internal standard. After homogenization of the tissue samples, the vials were sealed and sonicated for 15 min. The vials were submitted to headspace GC/FID analysis : after 35 min incubation at 80°C, 2 mL of headspace was auto-matically injected into a Varian gas chromatograph equipped with a DB1301 colomn. The GC/FID procedure was checked for interference by analysis of blank postmortem samples.

Results In the eye fluid no constituents of petrol were found. In the other postmortem samples *p*-xylene, *o*-ethyltoluene, *m*-ethyltoluene and 1,2,4-trimethylbenzene were identified. Benzene and toluene were only detected in the blood and brain tissue.

Keywords : petrol, headspace GC-FID/MS, toxicology

91: Comparison of Post-mortem Blood and Liver Carbon Monoxide Concentrations

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Since suitable blood samples are not always available from badly burned fire victims, a well protected, highly perfused tissue would be useful as an alternative for the determination of percent hemoglobin saturation with carbon monoxide (%COHB). This study compared the %COHB of heart blood to that of femoral blood, liver tissue and liver fluid. The value of the different matrices to predict a potential carbon monoxide fatality was assessed.

Carbon monoxide was quantitated by gas chromatography (GC) using a wide bore open tubular molesieve column and thermal conductivity detector.

Femoral blood was a good predictor of heart blood %COHB ($n = 13$, $r^2 = 0.883$,) at saturations below 55%. Above that, there was greater but equal dispersion both above and below the regression line. Using 35% as a threshold to predict a potentially fatal COHB following exposure to fire, the femoral blood results did not result in a false prediction of a potentially fatal COHB saturation or a nonfatal saturation. Liver fluid was a good predictor of heart blood %COHB ($n = 13$, $r^2 = 0.804$) at saturations below 55%, above which there was greater dispersion about the regression line. In the 13 cases, one resulted in a false positive and one a false negative, thus resulting in an accurate prediction 85% of the time. Comparing homogenized liver to heart blood ($n = 18$, $r^2 = 0.582$), resulted in extreme variation about the regression line at

saturations in excess of 55%. There were no false positive predictions, but 4 false negatives resulting in an accurate prediction 78% of the time.

This study indicates that for the purpose of predicting a potentially fatal carbon monoxide exposure, femoral blood is as useful as heart blood, liver fluid is a good but less reliable predictor, and homogenized liver may be useful in assessing carbon monoxide toxicity, but some fatal exposures may be missed.

Key words: Carbon monoxide, liver, blood.

92: Quantitative Analysis of Hydrogen Cyanide and Carbon Monoxide in Blood of Dead Convicts Caused by Polyurethane Mattress Fire

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The objective of the work was to evaluate quantitatively hydrogen cyanide (HCN) and carbon monoxide (CO) determinations in the blood of 35 fatal cases who were convicts in a state prison. Deaths were caused by inhalation of combustion gases produced during the burning of polyurethane mattresses.

Methods: To determine the amount of hydrogen cyanide, two techniques were carried out simultane-ously: a) Gettler and Goldbaum technique which is based on Prussian blue reaction LOD: 0.1 g) and b) microdiffusion as described by Feldstein and Klendshoj (LOD: 0.2 g).

Carbon monoxide results were expressed as the percentage of carboxihemoglobin over total hemoglobin, showing values between 4 and 18% with an average of 9% for n = 35. Hydrogen cyanide showed values between 2.0 and 7.2 mg per liter of blood with and average of 3.5 mg/l for
n = 35. Other components with toxicogenic relevance such as ethanol, methanol, aldehydes and other volatile compounds gave negative results for the 35 cases. Consumer drugs and psychotropics were negative, as well.

Discussion: The high level of hydrogen cyanide and the low level of carbon monoxide in the 35 cases was remarkable. The high level of HCN in blood can be attributed to the marked polyurethane decom-position produced between 150° and 300°C and the massive amount of hydrogen cyanide evolved above 500C. On the other hand, the low level of CO in blood could be due to the rapid temperature increase (500° to 1000C in 2 to 5 min) of the gases evolved, compared that produced with a fire of an equivalent amount of wood. Other cases of intentional fire of polyurethane mattresses attributed the deaths to carbon monoxide intoxication, with no mention of hydrogen cyanide determination. Authors reporting death cases due to plastic combustion, attributed the main death factor to CO and detected only few cases with sublethal doses of HCN.

Conclusions: In our opinion polymer type and the presence of delaying combustion agents are the main cause of the differences obtained when comparing cases. In this case, the quantitative determination showed that the main toxic action in the fire deaths was hydrogen cyanide and secondary carbon monoxide.

Key words: Hydrogen cyanide, carbon monoxide in blood.

93: Mirtazapine (Remeron): Detection of a New Antidepressant in Postmortem Cases

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Mirtazapine, an analog of mianserin, is a piperazino-azepine compound approved by the U.S. Food and Drug Administration in 1996 for use in the treatment of depression. It has a tetracyclic structure unrelated to selective serotonin reuptake inhibitors, tricyclic antidepressants or monoamine oxidase inhibitors. Mirtazapine administration results in increased release of norepinephrine and serotonin due to blockade of central presynaptic $alpha_2$-adrenergic receptors. After a 15 mg/d dose, peak steady state plasma concentrations of 27-51 ng/mL are reached in approximately 5 days. After a 75 mg/d dose, peak steady state plasma concentrations in the range of 137-225 ng/mL are achieved.

We report 2 postmortem cases in which mirtazapine was detected in heart blood specimens: Case #1- An 82 year old white female was found by nursing staff on the floor of her bedroom at a nursing care facility. Her medications included diet supplements, albuterol, donepezil and mirtazapine. An autopsy was not performed but heart blood was submitted for

toxicological analysis. Case #2- A 70 year old white male was found by nursing staff on the bathroom floor at a nursing facility. Medications included alprazolam, diltiazem and mirtazapine. An autopsy was not performed but heart blood was submitted for toxicological analysis.

Mirtazapine was identified by dual column (RTx-50 and RTx-200) gas chromatography with nitrogen phosphorus detection (HP 6890 GC) after a basic liquid-liquid extraction. Promazine was utilized as the internal standard. Quantitation was achieved by assaying calibrators (200-2000 ng/ml) concurrently with case specimens (r 0.999). Confirmation was achieved by full scan electron impact gas chromatography/mass spectrometry using an HP 5970 MSD with a DB-5 column.

Mirtazapine chromatographed well on the RTx-50 column, with a relative retention time of 0.978 min, eluting near benztropine. By GC/MS, the base peak of mirtazapine was *m/z* 195, with a molecular ion of *m/z* 265, and other prominent ions at *m/z* 194, 208 and 43. The heart blood specimens from each case were subjected to comprehensive toxicological analysis. Mirtazapine was the only drug detected. The mirtazapine blood concentration in case #1 was 280 ng/ml and for case #2, 100 ng/ml. Although the mirtazapine concentration in case #1, was slightly higher than previously reported therapeutic levels, the cause of death was determined to be atherosclerotic heart disease, and the manner, natural.

Key Words: Mirtazapine, Depression, Postmortem

94: Postmortem Tissue Distribution of Mirtazapine (Remeron ®)

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Mirtazapine is a new anti-depressant agent which entered the United States market in April 1996. To date, the literature provides limited information about therapeutic blood concentrations and no information about postmortem levels. During 1998, the Los Angeles County Coroner's Toxicology Laboratory encountered five cases where postmortem tissue distributions of mirtazapine were determined.

The analysis of mirtazapine from postmortem specimens (2 ml sample size) consisted of an n-butylchloride basic extraction procedure with screening and quantitation on a GC/NPD. Linearity was achieved from 0.05 mg/L to 3.0 mg/L with limit of quantitation being 0.05 mg/L. Confirmation of mirtazapine was performed on a GC/MS by comparison with an analytical standard.

The tissue distribution of mirtazapine are in the following concentration ranges: Heart blood 0.10 – 0.32 mg/L, Femoral blood 0.13 – 0.24 mg/L, Vitreous 0.06 – 0.10 mg/L, Liver 0.53 – 2.1 mg/kg, Bile 1.2 – 6.6 mg/L, Urine 0.43 – 2.5 mg/L, and Gastric 0.012 – 2.7 mg total. In all five cases, the concentration of mirtazapine was not directly linked to the cause of death. However, these cases represent the first reported fatalities involving the anti-depressant, mirtazapine, and can be used for postmortem comparisons.

Key Words: Mirtazapine, Anti-depressant, Postmortem Distribution

95: Thirty Case Studies Involving Postmortem Tissue Distributions of Olanzapine (Zyprexa)

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Olanzapine, introduced onto the market in late 1996, is a thienobenzodiazepine derivative which displays efficacy in patients with schizophrenia and related psychoses. To date, the literature provides limited information about therapeutic blood concentrations and no information about postmortem blood levels or tissue distributions. During 1997 & 1998, the Los Angeles County Department of Coroner Toxicology Laboratory encountered thirty cases where olanzapine was detected and postmortem tissue distributions were determined.

The analysis of olanzapine from postmortem specimens (2 ml sample size) consisted of an n-butylchloride basic extraction procedure with screening and quantitation on a GC/NPD. Linearity was achieved from 0.025 mg/L to 3.0 mg/L with the limit of quantitation being 0.01 mg/L. Confirmation of olanzapine was performed on a GC/MS by comparison with an pure analytical standard.

The tissue distribution of olanzapine are in the following concentration ranges with the number of cases associated: Heart blood 0.01 – 4.8 mg/L (n = 29), Femoral blood 0.01 – 1.6 mg/L (n = 24), Vitreous 0.01 – 2.1 mg/L (n = 6), Liver 0.01 – 13 mg/kg (n = 23), Bile 0.02 – 8.4 mg/L (n = 17), Urine 0.03 – 16 mg/L (n = 25), and Gastric 0.001 – 62 mg total (n = 21). A very important point was observed; with any repeat blood analysis, the concentration of olanzapine decreased

substantially, virtually becoming non-detectable over time. Because of this degradation process, the olanzapine concentration must be measured as soon as possible. The case studies reviewed include the decedent's history, postmortem tissue distribution, medical evidence, and cause/mode of death. The information presented about olanzapine, a new anti-psychotic drug, can be useful for postmortem comparisons.

Key Words: Olanzapine, Anti-psychotic, Postmortem Distribution

96: Olanzapine Concentrations in Forensic Investigations

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Olanzapine is a benzodiazepine analog sold in the Unites States under the trade name Zyprexa. It is structurally similar to clozapine and is used to treat schizophrenia. It is reported to have fewer extrapyramidal effects than phenothiazines or butyrophenones. After a single 12.2 mg dose, peak plasma concentrations averaged 0.011 mg/L. The therapeutic steady state concentrations ranged from 0.009 to 0.023 mg/L. Olanzapine is extensively metabolized by demethylation, hydroxylation, N-oxide formation and glucuronidation.

Over a 5-month period, olanzapine was identified in 7 cases investigated by the Office of the Chief Medical Examiner. Olanzapine is extracted under alkaline conditions and chromatographs without derivatization. On a phenylmethyl silicone column, olanzapine has a retention time between nordiazepam and flurazepam. Olanzapine was confirmed in all 7 cases by full-scan electron impact gas chromatography/mass spectrometry; the drug was quantitated using gas chromatography-nitrogen-phosphorus detection.

Five of the 7 cases had causes of death other than olanzapine intoxication; the blood olanzapine concentrations in these cases were between 0.04 and 0.27 mg/L. Urine concentrations were between 0.19 and 0.50 mg/L (n = 3). The cause of death in the sixth case was combined methadone and olanzapine intoxication; the blood and urine olanzapine concentrations were 0.16 and 1.3 mg/L, respectively. The cause of death in the seventh case was olanzapine intoxication; the blood and urine olanzapine concentrations were 0.98 and 28 mg/L respectively.

These data indicate that the "postmortem therapeutic range" for olanzapine is higher than the reported antemortem steady state plasma concentrations. In addition, there was some evidence that olanzapine is unstable in stored blood. In the olanzapine intoxication case, the same blood specimen was analyzed about one month later after storage at $4^{o}C$ and was found to have an olanzapine concentration of 0.16 mg/L. Decreases were also observed after re-analysis of the other blood specimens containing olanzapine.

Key words: olanzapine, postmortem, instability

97: Olanzapine Associated Deaths: A Report Of Five Cases

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Olanzapine is a recently introduced agent for the treatment of schizophrenia. It belongs to the thienobenzodiazepine class, and its chemical structure is similar to clozapine. The mechanism of action, while not firmly established, is proposed to be through a combination of dopamine and serotonin type 2 anatagonism. Therapeutic range of 0.009 to 0.023 mg/L was recently proposed to change to 0.005 to 0.075 mg/L(Xue et al. *Clin Chem* 1998;**44**:A103). A fatal overdose case of olanzapine showed heart blood of 4.9 mg/L and gastric content of 41 mg/L (Elian. *For Sci Int* 1998;**91**:231-5). This report summarizes five cases with detectable concentrations of olanzapine. The following summarizes selected postmortem, toxicological results of chromatographic analyses, cause and manner of death:

Case	Age	Sex	Sample	Olanzapine	Other drugs
1	28	M	Urine	Positive	Bupropion, hydromorphone, morphine,tramadol
2	34	M	Bl. Sub.	0.190 mg/L	Thioridazine and metabolites
3	47	M	Liver	0.3 mg/kg	Valproic acid
4	44	M	Bl. Il	1.02 mg/L	Ephedrine/pseudoephrine
5	42	M	Bl. Ht.	1.24 mg/L	Alcohol

The above blood concentrations of 0.19 to 1.24 mg/L were between those of previously reported therapeutic range and a single overdose case. The medical examiners designated the following cause and manner of death for the above cases: 1. pulmonary embolus/natural, 2. arteriosclerotic heart disease/natural, 3. injuries due to fall/suicide, 4. undetermined sudden death/undetermined, and 5. schizophrenia associated sudden death/natural. This case report is intended to add to the olanzapine toxicological literature and would hopefully enhance future cases interpretation involving olanzapine.

Key words: Olanzapine, schizophrenia agent, postmortem analyses

98: Olanzapine Concentrations in Clinical Serum and Forensic Blood Specimens – When does Therapeutic become Toxic?

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Olanzapine (Zyprexa) is a new drug for the treatment of schizophrenia that has been reported to provide favorable anti-psychotic efficacy with minimal adverse side effects. Very little has been reported in the literature with respect to plasma concentrations following clinical dosing. A therapeutic range of 9 to 23 ng/mL has been proposed. Similarly very little has been reported about olanzapine concentrations contributing to, or causing death. In one reported case of suicide involving olanzapine, the olanzapine concentration in whole blood was 4100 ng/mL.

The objective of this paper is to present the concentrations of olanzapine in 1655 clinical samples together with concentrations of olanzapine detected in post-mortem whole blood specimens received by NMS.

The analysis of olanzapine was performed by the solid phase extraction of 1.0 ml of buffered serum or blood, followed by GC separation with nitrogen-phosphorus detection.

The analysis of 1655 patient samples showed that 70 % of positive serum values were below 40 ng/mL, with a mean and median of 36 and 26 ng/mL, respectively, and only 10% were above 70 ng/mL. The mean olanzapine concentration in 22 forensic whole blood specimens was 318 ng/mL with a range of 6 ng/mL to 1600 ng/mL. Case studies in which olanzapine toxicity is believed to have contributed to death will also be presented.

From these results it appears that the therapeutic range may be higher than those previously reported. The results also suggest that due to the absence of any extensive literature relating olanzapine concentrations and toxicity, potential toxicity at concentrations above 80 ng/mL should be considered.

Key Words: Olanzapine, concentrations, toxicity

99: A Zolpidem Overdose Case

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A 36 year old female was found dead in bed in her secured home. An apparent suicide note was found posted on the refrigerator door. The decedent had a history of psychiatric illnesses including: paranoid disorder, depression with panic episodes, and post-tramatic stress disorder. As a result, she had been home bound. For the above disorders, she was medicated with risperidone, and sertraline. Previously, the decedent was also prescribed zolpidem (Ambien), a hypnotic agent.

The postmortem examination showed an obese individual weighing 262 lbs with an unremarkable external examination. The heart weighed 400 gm and was grossly normal. Examination of the respiratory system revealed white foam within the larynx and upper tracheal. Histological diagnoses included intra-alveolar pulmonary edema and hepatic steatoisis. Toxicological analyses showed the presence of caffeine, risperidone, and zoplidem in urine, and in the blood, 9-hydroxyrisperidone, 5.6 mg/L, and zolpidem, 4.5 mg/L. The latter corresponded to a toxic concentration. The cause of death was determined to zolpidem overdose, and manner of death - suicide.

Key words: Zolpidem, hypnotic, postmortem analyses

100: Caveats Associated with the Evaluation of Nifedipine in Post-Mortem Blood and Stomach Contents

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Nifedipine, a peripheral vasodilator drug available since 1975 in Germany, has been marketed in over 100 countries. Deaths associated with acute poisoning with nifedipine are rare. Only three reports involving four fatalities exist, all of which involve unusual circumstances or significant predisposing medical conditions.

In our medicolegal investigations, nifedipine is detected by a neutral extraction of postmortem blood and stomach contents using HPLC with UV-DAD detection. Nifedipine was not detected using a standard GC/NP and GC/MS screening method of blood for basic drugs, partly because nifedipine is thermally unstable.

In a review of our coroner investigations since 1986, nifedipine was found in five cases, two of which attributed the cause of death to an overdose of nifedipine.

In the first case, a 32 year old woman presented to an emergency department feeling shaky and having chest and severe abdominal pain. She developed a sudden and rapid decline of blood pressure, became hypotensive, oliguric, and died of a cardiac arrest within one-half hour. A post-mortem examination found more than 30 partially digested tablets in the proximal GI tract, and no anatomical cause of death. Nifedipine was detected in the stomach contents, and at a concentration of 0.56 mg/L in presumed cardiac blood.

In the second case, a 73 year old woman was found dead in bed. No anatomical cause of death was found, however nifedipine was detected in the stomach contents, and at a concentration of 3.1 mg/L in cardiac blood.

Recently, an unsuccessful attempt at poisoning for the purpose of a residential looting was revealed when the intended victim discovered three tablets, later identified as nifedipine, in the bottom of a cup of coffee.

Evaluating the role of nifedipine in forensic casework requires suspicion about nifedipine, the application of an analytical method specific for nifedipine, and knowledge about the acute and chronic effects of nifedipine.

Keywords: nifedipine, post mortem blood, HPLC

101: Therapeutic, Toxic, and Lethal Concentrations of Citalopram

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Citalopram belongs to the second generation of antidepressants and was registered in Denmark in 1989. Citalopram is marketed under the following tradenames: Cipramil®, Seropram®, Cipram®. It is a selective serotonin reuptake inhibitor (SSRI) without cardiovascular adverse effects. Only little information on citalopram in forensic cases exists in the literature and as citalopram is manufactured by a Danish company we found it of interest to make a survey on the occurrence of citalopram in medico legal cases in Denmark with a population of 5.2 million.

The survey includes all medico legal cases involving citalopram examined in Denmark during the period 1989-1997. Citalopram was involved in 86 cases. We have tried to make an interpretation of the citalopram concentrations found in this investigation. We suggest the following levels for letal, toxic and therapeutic concentrations. Letal: 2.0- 6.2 mg/kg ; Toxic: 0.4-0.9 mg/kg; Therapeutic: 0.03-0.6 mg/kg. In the suicide cases in which only citalopram was found, the blood concentrations were 10 times higher than therapeutic concentrations. This indicates that citalopram possesses a low toxicity range when used correctly.

Key words: Citalopram, fatal concentrations, toxic concentrations.

102: Moclobemide Toxicity and Serotonin Reuptake Inhibitors.

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Moclobemide is a reversible inhibitor of monoamine oxidase A and is widely used to treat depression. There are few mentions of any significant toxicity with this drug alone, even in overdose, although interactions of moclobemide with clomipramine and serotonin reuptake inhibitors (SSRI) are known when these drugs are taken to excess.

The author has recognised that adverse interactions of moclobemide occur with SSRI even when SSRIs are used in therapeutic amounts. As a consequence poisoning cases may not be identified. This paper reviews the Victorian toxicological experience with moclobemide.

Deaths associated with moclobemide were reviewed for the period 1993 to end 1997. There were 101 cases of which seven were attributed to death from moclobemide toxicity alone or in combination with a SSRI. There were three females, and the age range was 22 to 38, median 29 years. There was no significant natural disease in any of these seven cases.

In six of these cases a SSRI was present: fluoxetine (1 case), sertraline (2 cases) and paroxetine (3 cases). In all of these cases they were present in concentrations consistent with normal therapeutic use. In the other case only a therapeutic concentration of diazepam was present. The femoral blood concentrations of moclobemide ranged from 8 to 105 mg/L, median 39 mg/L. Two cases were listed as suicide, but in all seven cases excessive moclobemide had been ingested. In at least two of the cases circumstances strongly suggested a serotonin syndrome. Details of the cases will be presented.

These cases illustrate that moclobemide is not safe in overdose, particularly when combined with therapeutic doses of a SSRI. Caution is also advised when patients are switched from a SSRI to moclobemide, or vice versa.

Keywords: moclobemide, toxicity, serotonin reuptake inhibitors

103: Study of Amlodipine Influence on Amiodarone Toxicity in Laboratory Animals

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Amiodarone, an iodinated derivative of benzofluran was originally introduced as an antianginal agent, but is now increasingly used for its potent antiarrhythmic actions. Its use is accompanied by substantial toxicity, liver injury being common with this agent. The aim of this study was to examine the effects of amlodipine, a dihydropyridine calcium antagonist, on liver injury and on serum lipids changes induced by amiodarone.

The study was made with white male rats, weighing 190 ± 30 g, kept in the same environment conditions and receiving the same standardised food. They were divided into lots of 6, as follow: one control lot, another lot treated with amiodarone (10 mg/kg b/w), another lot treated with amlodipine alone (10 mg/kg b/w) and a fourth one treated with amiodarone and amlodipine (10 mg/kg b/w each). Both drugs were administered intraperitoneally, once a day, for 5 days each week, for a period of 12 weeks. The following parameters were assessed in all the animals: the serum transaminases (ASAT, ALAT), the alkaline phosphatase, the serum cholesterol, the liver weight. Also, a histo-pathological examination of the liver tissues samples was performed on all the animals.

Amiodarone produced liver damage in rats as manifested by the rise of serum levels of ASAT and ALAT, rise of alkaline phosphatase, as well as by histological changes that resembled that of alcoholic hepatitis. Amiodarone also produced a significant increase in the serum cholesterol levels. Associating amiodarone with amlodipine resulted in marked potentation of amiodarone toxicity.

These findings raise the possibility that this association may similarly enhance susceptibility to amiodarone liver injury in humans; accordingly we suggest that clinicians to be aware of this potential interaction and counsel patients to exercise caution when taking both these medication.

Key Words: amiodarone, amlodipine, liver toxicity

104: Fatal Amitriptiline Poisoning: Studies Using Chemical, TLC, GC/MS and Surface Ionization MS-methods. - WITHDRAWN

105: A Case of Propofol Abuse

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Propofol (2,6-diisopropylphenol) is a drug used as an anesthetic agent. It is structurally unrelated to other common anesthetic agents such as opioids, barbiturates, benzodiazepines and halogenated liquids. An initial inducing dose is given to the patient and is followed by an infusion to maintain anesthesia. Blood propofol concentrations associated with anesthesia range from 2 to 4 mg/L.

In the presented case, a 50 year-old white female surgical nurse was found unconscious in a bathroom near her duty station at a major military medical institution. A syringe was found in her arm. In her purse were found 5 empty and 1 unused vials of Diprivan. Other empty vials were found among her possessions. The patient had a medical history of migraine headaches and insomnia, but no history of depression or other psychiatric disorders. A blood specimen was collected from the patient and sent to the Division of Forensic Toxicology for comprehensive testing for therapeutic and abused drugs.

No common therapeutic or abused drugs were detected in the blood specimen using a combination of radioimmunoassay and gas chromatography-nitrogen phosphorus detection. Since propofol does not contain a nitrogen atom, a gas chromatography/mass spectrometry (GC/MS) method was developed to identify and quantify propofol in the blood. To 2 mL blood were added 2 mL saturated borate buffer, 40 g methylphenidate (internal standard, I.S.) and 0.15 mL chloroform: ethyl acetate (70:30). After mixing and centrifuging, 2 L were injected into the GC/MS. A 30 m x 0.53 mm ID DB-5 column was used to achieve analytical separation. The oven temperature began at 100°C for one minute and increased at 30°C to 270°C. Three ions at m/z = 163, 178 and 117 were monitored for qualitative identification; m/z = 163 and 84 (I.S.) were used for quantification. In this case, the blood propofol concentration was 0.24 mg/L. A previously reported propofol fatality had a propofol concentration of 0.22 mg/L.

Key Words: propofol, abuse, gas chromatography/mass spectrometry

106: Kinetics of Alcohol in Blood and Breath: Results of Experiments on Human Volunteers

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This experiment was set up to follow alcohol kinetics in the mouth cavity, breath and blood by performing many analyses on some individuals of which one had an antrum resection. Three healthy test persons had an intraveneous catheter from which blood could be drawn at short intervals. The Belgian Road Safety Institute put a calibrated Breath Analyzer Seres 679 TB at our disposal. In the first range of experiments (1995) a dilution of pure alcohol was given in a constant volume to all test persons.

In a second experiment (1996 and 1997) some modifications were made: the dose of alcohol was expressed in gram per kilogram of body mass and the test person was administered a fixed volume of beverage consisting of different concentrations of jenever in beer. Doses of 0.3 and 0.6 grams of alcohol per kg body weight were administered before and after a meal. Immediately thereafter a blood sample was drawn and a breath analysis was performed every three minutes. All data were collected and absorption-elimination curves were plotted. Special attention was paid to mouth alcohol.

Main conclusions Mouth alcohol decreases following first order kinetcs and the rate is dependant on the kind of beverage. Alcohol from whisky disappears more slowly than alcohol from beer. Mouth alcohol is completely eliminated after flushing the mouth at least 10 times with 20 mL of water. Alcohol on a fasting stomach can cause pylorus spasms which slows down the absorption process. In the test person with slow stomach emptying in the fasting state, we did not notice a significant change in absorption rate after taking a meal. On the contrary, in this particular case, absorption was even faster.

The patient who underwent a resection of the antrum showed no absorption curve. Transit to the intestine was so rapid that Cmax was reached within 3 min.

In these patients the elimination curve shows very clear Michaelis-Menten kinetics. Under no circumstances may the BAC be calculated from the BrAC by using a fixed ratio as 2100 for the US and 2300 for Belgium. In fact, the ratio varies for every individual and varies in time for the same individual. Also the speed of drinking and the kind of beverage influences the ratio.

Key words_: BAC, BrAC, mouth alcohol.

107: Metaldehyde Determination in Body Fluids by Headspace Solid-Phase Microextraction (SPME) and Gas Chromatography-Mass Spectrometry in a Case of Acute Intoxication

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A 47-year-old woman was admitted to an Intensive Care Unit after attempted suicide by ingestion of a molluscicide containing metaldehyde. On arrival the patient was unconscious, with bilateral reactive mydriasis and tonic-clonic seizures. She was mechanically ventilated after oro-tracheal intubation. Her body temperature rose to 40 °C in the first days of the clinical course, and a status epilepticus also occurred. Among other diagnostic assessments, this laboratory was requested to assay for metaldehyde in blood, urine and dialysis fluid sampled from 12 hours after admission to the 4th day of admission.

A simple, fast and reliable method based on headspace Solid-Phase Microextraction (SPME) and Gas Chromatography-Mass Spectrometry (GC-MS) was developed. SPME was carried out with a 65-µm Carbowax/Divinylbenzene fiber on 0.5-ml-aliquots of each biological fluid, saturated with NaCl and heated at 70 °C for 15 min in sealed vials. GC-MS analysis was performed on a FFAP (acid-modified polyethylene glycol phase) capillary column, in Full Scan acquisition mode (20-250 u.) for identification and Selected Ion Monitoring mode (m/z 89, 117, 131) for quantification. The assay was sufficiently linear over a metaldehyde range of 0.1-200 µg/ml and had a sensitivity of about 0.01 µg/ml for all biological fluids.

Blood and urine metaldehyde levels peaked about 24 hours after admission (10.5 and 18 µg/ml for blood and urine respectively) and were detectable until day 4 (0.6 and 1.3 µg/ml for blood and urine respectively). Dialysis fluid, sampled only on day 4, had very low metaldehyde levels (0.1 µg/ml).

Therapy (barbiturate infusion and haemodialysis) resulted in improvement of the patient's nervous functions. She was extubated on day 7 and discharged on day 9, after her complete recovery.

Key Words : Metaldehyde intoxication, SPME, GC/MS.

108: Detection of Methanol Impurities in Body Fluids by Headspace Solid-Phase Microextraction (SPME) and GC-MS to Identify the Source of Exposure in Two Fatalities Involving Methanol

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Two men were found comatous in their home. The first (Subject A) died immediately after admission to hospital, and the second (Subject B) 24 h later.

Overall evaluation of circumstantial, anatomical and histopathological as well as microbiological and toxicological data indicated that the cause of both deaths was methanol intoxication by oral intake (methanol concentrations were 0.95 g/l, 0.80 g/l, 0.83 g/l, 0.90 g/l, and 0.60 g/l in Subject A's urine, heart blood, femoral blood, bile, and gastric content respectively ; 3.54 g/l, 3.70 g/l, and 3.58 g/l in Subject B's heart blood, femoral blood, and gastric content respectively).

In an attempt to identify the source of methanol exposure, judicial authorities subsequently asked for new comparative toxicological analyses of the above autopsy biological specimens and of samples of a liquid labelled as "methanol", confiscated later in the factory where the two men were employed.

A method based on headspace Solid-Phase Microextraction (SPME) coupled with Gas Chromatography-Mass Spectrometry (GC-MS) was developed to assay for possible low-boiling (volatile) substances in biological specimens and confiscated liquids. Another GC/MS method was developed to analyse ether extracts of the same samples, to search for possible high-boiling substances.

Toxicological findings indicated that the confiscated liquid was in fact methanol, containing the volatile impurities isobutyl alcohol and nitromethane and less than 1% of ricin oil, the latter characterized by the presence of three steroids: campesterol, stigmasterol, and beta-sitosterol. These impurities were due to chemical products used for model-making propellents manufactured by the factory. All autopsy biological specimens from both subjects also contained detectable amounts of isobutyl alcohol, nitromethane, and the above three vegetal steroids.

These findings indicated that the confiscated "methanol" represented the most probable source of fatal exposure for both subjects.

Key Words : Methanol intoxication, SPME, GC/MS.

109: A Fatality Involving GHB

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An eighteen year-old male arrived at the hospital unresponsive and in respiratory failure. He was pronounced dead 30 minutes later. At autopsy, no anatomic cause of death was identified. Toxicology tests for alcohol, comprehensive drug screen and gamma hydroxybutyric was requested on the admission and postmortem specimens. GHB was converted into the lactone form, gamma butylrolactone (GBL) by the addition of 10% trichloroacetic acid and heat. GBL was then extracted with chloroform and injected into a gas chromatography mass spectrometer. Delta-valerolactone was used as the internal standard. The results were as follows:

	Alcohol	GHB	Cocaine	Cocaethylene	Benzoylecgonine
Postmortem Blood	0.16 g/dL	309.4 mg/L	0.04 mg/L	0.02 mg/L	0.03 mg/L
Antemortem Blood		300.3 mg/L			
Vitreous Humor	0.20 g/dL				
Urine	0.22 g/dL				

The lack of a substantial difference in the concentration of GHB between the postmortem and antemortem blood suggests no GHB production from postmortem decomposition in this case.

The cause of death was ruled as an overdose due to combination effect of GHB, cocaine and alcohol. The manner of death was accident.

Key Words: GHB, Antemortem blood, Postmortem blood

110: The Detoxification Metabolism of 4-O-Methylpyridoxine (MPN), a Causative Substance of Ginkgo Seed Poisoning.

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Ginkgo seed poisoning sometimes occurs in Japan and China. The symptoms are mainly convulsions and loss of consciousness. 4-O-Methylpyridoxine, MPN, the causative substance has been identified. MPN toxicity shows species differences between guinea pig and rat. The LD50 of MPN in guinea pigs was 200 times lower than that of rats. We tried to clarify the species differences in metabolism of MPN.

Methods After phenobarbital (PB) or beta-naphtoflavone (B-NF) was administered once a day for five days to each guinea pig and rat, the animals were killed and liver microsomes and brain S9 fractions were prepared. The demethylation of MPN activity was determined using Nash's method. Further, the P450 isozyme involved in the demethylation of MPN was identified using anti-rat P450 antibodies (CYP1A2, 2B1, 2C11, 3A2).

Results 1. Demethylation of MPN in rat and guinea pigs liver microsomes was dependent on NADPH and significantly inhibited by octylamine. This activity was induced with PB and inhibited with B-NF in both rats and guinea pigs and differences between rats and guinea pig liver microsomes were not observed. The P450 isozyme which catalyzed the demethylation of MPN belongs to the CYP2B and 3A subfamily by inhibition experiments using anti-rat P450 antibodies.

2. The demethylation of MPN in brain S9 from animals which were pretreated with PB was higher than that of B-NF or control in both rats and guinea pigs. A significant difference in MPN demethylation activity between rat and guinea pigs brain S9 was observed in which the demethylation activity of rat brain S9 was 10-50 fold higher than that of guinea pigs brain S9.

Therefore, we concluded that one of the factors in species toxicity differences might due to the gap of metabolism in brain. We also present the analytical method of MPN in biological fluids. We will report the elimination rate of MPN in rats and guinea pigs brain, and pharmacokinetics of MPN.

Key Words: Ginko seed poisoning, O-Methylpyridoxine, Cytochrome P450

111: Limitations in the Use of Pharmacokinetic Calculations for Forensic Toxicology

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Pharmacokinetic retrograde calculations and total body-burden calculations are a common request in the field of forensic toxicology. Although theoretically appropriate, biological, pharmacological and toxicological considerations, in addition to postmortem changes make such calculations inaccurate, limiting their application. Two recent cases from our laboratory support that conclusion:

Case 1: A 52 month-old boy, who weighed 17.2 kg, and his 28 month-old sister, who weighed 14.5 kg, were found dead, suffocated. The suspect confessed to having given Dimetapp Elixir TM (brompheniramine (brphen) and phenylpropanolamine (PPA)) to the victims some time before the incident. The postmortem concentration of PPA and brphen were 2.17 and 0.17 mg/L and 1.87 and 0.15 mg/L for the boy and girl respectively. Both the prosecution and the defense became interested in determining the "total amount of Dimetapp given to the victims" and its dose equivalence. The volume of distribution (Vd) of brphen reported in the literature is 11.7 + 3.1 L/kg and the Vd of PPA for children 6 to 12 years old, weighing 20 to 40 kg is 2.5 ± 0.3 and 2.8 ± 0.4 L/kg depending on the dose. The recommended doses of brphen and PPA for children 2 to 6 years old are 0.73 mg and 5.0 mg respectively. Using pharmacokinetic parameters, it was estimated that the amount of PPA given to the boy was 16 to 24 times higher than recommended and the amount of brphen 34 to 59 times higher than recommended. The amount of PPA estimated to have been given to the girl was 12 to 17 times higher than recommended and the amount of brphen 26 to 44 times higher than recommended.

Case 2: A 17-year old female was involved in a stressful domestic situation. She was found disoriented, pale and feverish, and was taken to the emergency room where she died 3 hours later, approximately 12 hours after ingesting an unknown amount of acetaminophen and aspirin. The postmortem blood salicylate and acetaminophen levels were 0.86 and 0.48 mg/L respectively. Her clinical history is consistent with hepatotoxic effects of acetaminophen. Due to insistent family requests, pharmacokinetic calculations were made. The results suggest the dose of acetaminophen taken by the decedent to be between 106 and 337 kg, while the dose of salicylate could vary between 14 g and 6.2 $\times 10^8$ kg.

Key Words: Post-mortem, Pharmacokinetics, Dose, Retrograde calculations.

112: Long Term Stability of Abused Drugs and Anti-Abuse Chemotherapeutical Agents Stored at -20°C.

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Stability is an important consideration in the use of specimens for accurate determination of analyte concentrations. To determine the long-term stability for analytes routinely analyzed by mass spectrometry in this laboratory, quality control (QC) results were plotted versus time. The time required for the initial concentration to reach a specified level of deviation (i.e. 10%) was then determined from the slopes. QCs were prepared at 3 concentrations in drug-free matrix and stored at approximately -20°C; urines were fortified with 2% sodium fluoride; plasmas (except for those containing cocaine) were prepared with heparin; those used for cocaine and metabolites were fortified with sodium flouride and potassium citrate. Data are summarized below for the low concentration QCs.

Analyte	Maximum Days of Storage	Intercept $(ng)(mL)^{-1}$	Slope $(ng)(mL)^{-1}(day)^{-1}$	Days to Reach 10% Deviation
Cocaine (plasma)	765	26.0	0.00428	608
Benzoylecgonine (plasma)	765	26.4	-0.00269	981
Ecgonine Methyl Ester (plasma)	765	26.0	-0.00338	769
Norcocaine (plasma)	765	24.0	0.00229	1048
Ibogaine (plasma)	475	25.7	0.00298	863
Methadone (urine)	384	25.8	-0.00564	457
EDDP (urine)	280	25.6	0.02505	102
EMDP (urine)	280	23.5	-0.00293	802
LAAM (plasma)	650	10.1	0.00053	1906
norLAAM (plasma)	650	10.8	0.00070	1543
dinorLAAM (plasma)	650	10.3	0.00052	1981

Both positive and negative slopes were found. With the exception of EDDP concentrations were within 10% of initial for at least one year, and in many cases, several years.

Key Words: stability, quality control

113: Determining Diabetic Status from HbA_{1c} in Postmortem Blood

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Diabetes is one of the most common diseases and death from diabetic ketoacidosis (DKA), an acute complication, is common in death investigation practice. A diabetic history and high vitreous glucose and blood acetone concentrations are helpful for establishing DKA as the cause of death. HbA_{1c} which is roughly proportional to the integrated average blood glucose level, is very widely used to monitor and manage treatment of diabetic patients. We decided to test whether HbA_{1c} in postmortem blood would be a reliable indicator of the deceased's diabetic status.

Blood samples from diabetic and non-diabetic cases were assayed for HbA_{1c} with the Biorad Variant™ ion exchange HPLC system using the procedure recommended by the manufacturer. The reference range for non-diabetics is up to 6%. Diabetics with values less than 8% are considered to be in good control. The results from 13 diabetics were mean 8.9%, range 6.2-12.1 and for 16 non-diabetics mean 5.4%, range 4.5-6.4.

One of the diabetics was a gestational diabetic who died unexpectedly within a few days of delivery. Her HbA_{1c} was 7.1%. In addition to these cases there were three more that apparently died in DKA and had not been previously diagnosed as diabetic. The HbA_{1c} values were12.2, 12.6 and 13.8%. Other results were consistent with DKA at the time of death: vitreous glucose was greater than 600 mg/dL in all three and two of the three cases had high blood acetone levels.

For another four cases HbA_{1c} could not be measured due either to the presence of variant hemoglobins or to the presence of unusual or unresolved peaks. Sample degradation may be responsible for some of these problems.

Keywords: Diabetes, Diabetic Ketoacidosis, Glycosylated Hemoglobin

114: Comparison of the Results from Benzodiazepine Urine Immunoassay with Quantitative GC Blood Analysis

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The presence of benzodiazepines is usually tested by screening urine samples by immunochemical techniques followed by confirmation by chromatographic methods such as GC, HPLC or GC/MS. On many occasions it is necessary to perform also a quantitative benzodiazepine determination in the blood. This study investigates if post mortem blood samples alone could be used for quantitative screening for benzodiazepines by advanced GC methods.

In the present study, 514 such successive medical examiner's cases were selected where urine and blood were available. Urine specimens were analysed by Emit d.a.u. Benzodiazepine Assay (ETSPLUS) using a 200 ng cutoff-value. Blood samples (1 ml) were screened for 21 benzodiazepine drugs or metabolites by a dual-column gas chromatographic method using DB-5 and DB-17 capillary columns, EC detectors and advanced software for the interpretation of the dual-column results. The limit of detection of the GC method was from 3 to 50 ng/ml depending on the compound.

The results are summarised in the following table:

URINE (ETS):	negative	positive	negative	positive	invalid*	invalid*	Total
BLOOD (GC):	negative	positive	positive	negative	negative	positive	
No. of cases:	284	149	48	4	26	3	514

* no result obtained by ETS

The following drugs were detected in cases where urine was negative and blood positive: diazepam (18 times: range 20-700 ng/ml), desmethyldiazepam (18 times: range 20-7000 ng/ml), oxazepam (13 times: range 10-400 ng/ml), temazepam (19 times: range 10-300 ng/ml), alprazolam (2 times: 20 ng/ml), and lorazepam (1 time: 10 ng/ml). In three of

the cases where urine was positive and blood negative, oxazepam and temazepam were found and in one case only oxazepam was found after further analysis by GC/MS. The results suggest that in post mortem forensic toxicology benzodiazepines are more reliably detected in the blood by a modern GC than in urine by immunoassay. Furthermore, the GC method used allows simultaneous quantitation of the drugs.

Keywords: Benzodiazepine screening, Immunoassay, Gas chromatography

115: Detection of Benzodiazepines in Blood Using Capillary GC-ECD: Recovery, Day-to-Day and Within-Day Variations

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The benzodiazepine drugs are extensively prescribed and are the focus of a number of deaths associated with poisoning world-wide. It is therefore indispensable to have reliable methods for their detection, identification and quantification at the low levels encountered in body fluids. We recently have described the identification and quantification of 29 benzo-diazepines using capillary GC-ECD following extraction of these drugs from post-mortem blood with Na_2CO_3, diethylether and H_3PO_4 [Bruneel N., Tytgat J. & Daenens P. in: *Proceedings of the 34th Triennial Meeting of TIAFT*, Eds. Sachs H., Bernhard W. & Jeger A., Molinapress, Leipzig, Germany, 1996, pp. 122-126]. Based on this method, we report here the parameters affecting recovery, day-to-day and within-day variations for 7 selected benzodiazepines: alprazolam, bromazepam, brotizolam, clotiazepam, flunitrazepam, lorazepam and tetrazepam. In particular, we investigated whether an additional clean-up of the blood extract with 0.1 % H_3PO_4 was beneficial for extraction recovery of these drugs. Recovery was determined by comparing the representative peak heights of extracted blood with peak heights of standards prepared in methanol of the same concentration.

The following recoveries were obtained with and without 0.1 % H_3PO_4 clean-up respectively (n=5): 77 and 51 % (alprazolam), 57 and 22 % (bromazepam), 100 and 60 % (brotizolam), 91 and 60 % (clotiazepam), 75 and 58 % (flunitrazepam) and 31 and 18 % (lorazepam). Both day-to-day and within-day variations generally demonstrated a coefficient of variation less than 10 %.

Key Words: Benzodiazepines, Gas Chromatography, Blood

116: Case Report: Death of an Infant Involving Benzocaine

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A case is presented of a four month old Hispanic male found unresponsive in bed. Efforts to resuscitate were unsuccessful. The toxicological evaluation revealed benzocaine at a concentration of 3.48 mg/L and methemaglobin of 36% (normal 0.4 – 1.5). The coroner investigated the source of the benzocaine and discovered that the child was being treated with "Zenith Goldline Allergen Ear Drops" 1.4% w/v benzocaine and 5.4% w/v antipyrine. There was an admission that on the day prior to the child's death, he was treated with 3X the prescribed dose by a caregiver.

Benzocaine is available in many over the counter as well as prescription drugs. It's potential for inducing methemaglobinemia has long been recognized. Methema-globinemia occurs when more than 1% of the heme iron is oxidized to the ferric form. The oxidized hemoglobin (methemoglobin) is incapable of reversibly binding oxygen. Infants less than 6 months are at increased risk for methemaglobinemia due to lower levels of enzyme systems needed to reduce ferrihemoglobin to ferrohemoglobin.

There are no reported blood benzocaine blood concentrations in the literature for the cases where death or illness from benzocaine has been reported. We also determined the blood benzocaine concentrations in 4 other cases and found levels from 0.46 – 1.49 mg/L. Three of the four were polydrug users from the same Washington State county where benzocaine was purportedly used to "cut" cocaine. Methemglobin in these cases ranged from 36 – 69%. However, elevated methemoglobin can occur as an artifact in post mortem blood.

Key Words: Benzocaine, post mortem blood, infant

117: Cocaine and Cocaine Metabolite Concentrations in Postmortem Fluids

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Elevated concentrations of cocaine, benzoylecgonine, and other cocaine metabolites were identified in the postmortem fluids of an individual found dead in the rural West Texas town of Alpine. No drug paraphernalia was found at the scene and no intravenous injection sites were noted at autopsy. An ongoing police investigation, subsequent to the autopsy and initial toxi-cological evaluation, suggested that the decedent had a history of "body-packing". No direct evidence for this conclusion was determined by investigation or at autopsy. Body packing is the procedure by which illegal drugs are concealed in the body by swallowing drug-filled balloons, condoms, etc. for the purpose of transporting the drugs while avoiding detection.

Femoral blood (preserved and unpreserved), bile, urine, vitreous humor, and stomach contents were submitted for routine toxicological testing including analysis for alcohols, basic drugs, cocaine, and opiates. The urine was screened for cocaine and opiates using the Abbott TDx FPIA assays. The positive cocaine metabolite result was confirmed using a combined cocaine, benzoylecgonine, cocaethylene, ecgonine methyl ester, morphine, codeine, and 6-monoacetylmorphine gas chromatography/mass spectrometry assay using solid phase extraction. All samples were also subjected to an alkaline drug screen using gas chromatography with a flame ionization detector (FID) followed by mass spectrometry for the confirmation of positive results. Cocaine and benzoylecgonine concentrations in the femoral blood were measured as high as 38.1 mg/L and 26.1 mg/L, respectively, with a benzoylecgonine concentration in the stomach contents of 673.5 mg/L. A significant loss of cocaine was noted in those blood samples that were not preserved with sodium fluoride.

The cause of death was ruled as cocaine intoxication and the manner was accidental. The cocaine concentrations reported here are the highest determined in any recent case submitted to this office. An additional interesting finding in this case was the identification of benzoyl-ecgonine in the alkaline blood extract by GC/FID with subsequent full scan mass spectral confirmation.

Keywords: Cocaine, Body Packing, Postmortem Concentrations

118: Cocaine Transport and Metabolism by the Colonic T84 Epithelial Cell Line

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Cocaine abuse during pregnancy has a profound effect on the fetus and neonate. Cocaine permeability and metabolism in gastrointestinal tract was studied using epithelial monolayers of human colonic T84 cells. T84 monolayers were maintained in 75 cm^2 tissue culture flasks, in DMEM/Ham's F-12 medium containing 6 % newborn calf serum. The cells were passaged upon confluence (every 7-8 days). For the experiment, cells were plated on 1.0 mm collagen type I coated tissue culture inserts in 24 well plates. Cells (passages 47-55) were grown to confluence as determined by steady-state trans epithelial resistance of 180 Qcm^2.

On the day of the experiment, the culture medium from the upper (U) and lower (L) chamber was removed and replaced with serum free medium. Cocaine HCl (500-4000 ng/ml) was added to U. After incubation with cocaine, samples were obtained from both U and L at 30 and 60 min. The samples were analyzed for cocaine, benzoylecgonine (BE), norcocaine (NC) and ecgonine methyl ester (EME) using solid phase extraction and GC/MS with corresponding deuterated internal standards. All studies were run in duplicates. Each experiment was done in a new passage.

Results Cocaine incubated with media alone for 30 and 60 min showed no metabolism. Data for cocaine and its metabolites in U and L at 60 min respectively are shown below. Results: 1) Linear increase of cocaine permeability across T84 cells was observed with increasing concentration in U. 2) Significant amounts of BE and EME were detected in U whereas only small amounts were seen in L. 3) NC was not detected.

ng added to U	Cocaine (U)	BE (U)	EME (U)	Cocaine (L)	BE (L)	Total conc. (U+L)
100	35.65±4.4	2.35±2.1	1.36±09	16.0±2.3	9.53	61.91±3.3
200	100.1	18.2	1.66	31.8	8.2	158.3
400	173.6±36.9	22.8±13.1	1.8	46.4±10.6	4.5±4.9	251.7±27.5
800	285.3	58.5	8.73	139.8	14.8	504.0

Conclusions 1) Concentration dependent cocaine transport occurs across colonic monolayers. 2) Appearance of BE and EME in U in the presence, but not absence of T84 cells, suggests cellular metabolism of cocaine. 3) Significant amounts of cocaine and metabolites (37-40%) may be retained by the monolayer.

Key words: cocaine permeability, T84 cell line, GC-MS

119: Fenproporex Abuse by Truck Drivers in Brazil

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The use of Fenproporex (N-2-cyanoethylamphetamine) by truck drivers to improve long distance driving is subject of much speculation in Brazil. The drug has been used as anorectic and about 60% of the world production are consumed in Brazil. In a preliminary study we found that amphetamines are the psychoactive drugs most used while driving, except alcohol. The aim of this study is to obtain more information about fenproporex abuse by truck drivers. A total of 3,538 urine samples were submitted to "Laboratório de Análises Toxicológicas da Universidade de S. Paulo", for analysis of fenproporex and its metabolite amphetamine. The samples were collected in two different ways: 2,810 in private transport companies with urine testing programs and 728 from truck drivers in six different roads. Sample donors of the two groups were not suspected to have been driving under the influence of drugs.

Capillary gas chromatography with NPD and gas chromatography/ mass spectrometry (GC/MS) were utilized for urine drug analysis. The obtained results were: 1.58% of all analyzed samples were found to be positive. Of all positives cases 62.5 % were for fenproporex and amphetamine and 37.5% for the presence of amphetamine. The frequency at which drugs were encountered according to the samples origin was: 4.94% of the samples collected of the truck drivers in the road were found to be positive (61% for fenproporex and amphetamine and 39% for the presence of amphetamine) and 0.71% of positive cases were in the samples collected in the companies (65% for fenproporex and amphetamine and 35% for the presence of amphetamine). The percentage of positive cases was markedly different but the frequency at which drugs were encountered was quite similar regardless of the origin.

Our results show that urine testing programs could be an important instrument to reduce the use of psychoactive substances by workers and confirm fenproporex abuse by truck drivers in Brazil.

Key Words: Fenproporex; Amphetamine; Truck drivers

120: *In vitro* Studies on the Influence of Cytochrome P450 Isoenzymes on the Human or Rat Metabolism of the Designer Drugs MDMA and MDE.

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In vitro studies have been described indicating that the demethylenation of MDMA was catalyzed by polymorphic CYP2D6 (Tucker G.T. et al. *Biochem. Pharmacol.* 47, 1993, 1151). The resulting catechol and/or its oxidation products are claimed to be responsible for the neuro- and/or hepatotoxic effects. However, Colado et al. (*Br. J. Pharmacol.* 11, 1995, 1281) showed that in CYP2D1/6 deficient rats the catechols of MDMA and MDA could also be formed. The aim of our studies was to elucidate, which metabolic pathway is catalyzed by the main CYP isoforms CYP1A2, CYP2C, CYP2D1/6, and/or CYP3A. In addition, we studied, whether structure analogies could be found and whether differences in humans or rats could be observed. The influence of cytosolic enzymes was also studied. Racemic MDMA and MDE were incubated with human or rat liver microsome and/or cytosol preparations with and without addition of the corresponding cosubstrates and/or specific inhibitors. The metabolites were analyzed by GC-MS (for details cf. *Ther. Drug Monit.* 18, 1996, 465).

In humans and rats the N-dealkylation of MDMA was predominantly catalyzed by CYP1A2 and CYP2C and of MDE by CYP3A. First, we were not able to demonstrate in microsomes the influence of CYP's on the demethylenation of MDMA and MDE to the corresponding catechols. To check, whether this effect was due to the instability of the catechols, we incubated the drugs with both, microsomes and cytosol. Thus, the catechols could be stabilized *in statu nascendi* by COMT methylation. In this way, we were able to show, that CYP2D1/6 and CYP3A were predominantly responsible for the demethylenation of MDE in humans and rats, and of MDMA in rats. Interestingly, we have found that the drugs could also be demethylenated in cytosol in presence of NAD.

In conclusion, our data show, that the toxicologically relevant demethylation of designer drugs is catalyzed not only by the polymorphic CYP2D1/6, but also by the non-polymorphic main isoenzyme CYP3A. Therefore, a polymorphism (poor vs. extensive metabolizer) in this presumed toxification step could not be concluded. Enantioselective studies are in progress.

Keywords: Designer Drugs, CYP Isoenzymes, Metabolism

121: Methylephedrine Findings after Intake of Metamfepramone - Studies on the Metabolism and the Toxicological Detection of Metamfepramone using GC-MS.

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Methylephedrine (ME), a sympathomimetic amine, is ingredient of many over-the-counter cold medications. ME abuse has been reported in some asian countries. Kunsman et al. reported ME findings in drug testing samples in the U.S.A. (1). In our lab, ME was found in urine of patients denying its intake. The only known medication was metamfepramone (*R,S*-2-dimethylaminopropiophenone, MP), a sympathomimetic used as antihypotonic or as cold medication. To study, whether and how long ME, methylpseudoephedrine, ephedrine (EP) and/or pseudoephedrine (PE) can be detected in urine after intake of MP, we reinvestigated the metabolism of MP, its detection within our STA procedure and the duration of detectability.

The metabolites were identified in urine after cleavage of conjugates, extraction and derivatization by acetylation using GC-MS. Besides the parent compound, the following metabolites could be identified in urine: ME, EP, PE, nor-EP, nor-MP, hydroxy-nor-MP and hydroxy-nor-EP. Differentiation of the ME and EP diastereomers was achieved after trifluoroacetylation. Three partly overlapping metabolic pathways could be postulated: 1) reduction of the ketogroup, 2) one- and two-fold N-demethylation and 3) ring hydroxylation.

After intake of 20 mg of MP, its main metabolite ME could be detected for about 140 h, EP and PE for about 132 h (n = 3). Nor-MP, the MP specific metabolite could only be detected for about 52 h. Therefore, in the time window from 52 to 140 h differentiation of MP intake from ME, EP and/or PE use was not possible. The analytical recoveries were 55 % for MP, 98 % for ME and 75 % for PE and the LOD's were 50 ng/mL for MP and 10 ng/mL for ME, EP and PE.

1. G.W. Kunsman, R. Jones, B. Levine and M.L. Smith; Methylephedrine Concentrations in Blood and Urine Specimens, Abstracts to the SOFT annual meeting, October 5-9, 1997, Utah

Key Words: (Methyl)ephedrine, Metamfepramone, GC-MS

122: Studies on the Metabolism of 2C-B

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The psychodelic drug 2C-B (4-bromo-2,5-dimethoxyphenethylamine) is a hallucinogenic phenalkylamine of which the abuse in Europe is increasing significantly during the recent years. Although 2C-B is active at much lower dosages, its physicochemical and pharmacodynamic profile can be compared to that of mescaline (3,4,5-trimethoxyphenethylamine). In order to be able to detect the abuse of 2C-B by analysing biological specimens, the metabolic pathways of 2C-B were evaluated in this study.

To obtain preliminary information *in vitro* studies were performed by incubating 2C-B in rat liver homogenates. GC/MS analysis indicated that under these conditions several metabolites were formed. The identity of some these metabolites could be revealed. These results were compared with those obtained from a so-called *in vivo* study, i.e. drug-of-abuse testing of a urine specimen of a subject abusing 2C-B.

This way the presence of the parent compound and of 4-bromo-2,5-dimethoxyphenylacetic acid in the urine specimen of the abuser was shown and confirmed using reference standards. Furthermore the formation of 4-bromo-2,5-dimethoxybenzoic acid and of 4-bromo-5-hydroxy-2-methoxyphenethylamine was indicated by GC/MS.

Considering the known biotransformations of mescaline in humans, it can be concluded that the metabolism of 2C-B in humans follows similar pathways compared to that of mescaline, i.e. oxidative deamination leading to phenylacetic and benzoic acid-like metabolites and O-demethylation of the aromatic methoxy groups.

KEY WORDS: metabolism , 2C-B, 4-bromo-2,5-dimethoxyphenethylamine

123: A Procedure for the Identification and Quantitation of Clobenzorex

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Drugs that are metabolized to amphetamine or methamphetamine are potentially significant concerns in the interpretation of amphetamine positive urine drug testing results. One of these compounds, clobenzorex, is an anorectic drug that is available in many countries. Clobenzorex (2-Chlorobenzyl-amphetamine) is metabolized to amphetamine by the body and excreted in the urine. Following administration, the parent compound was detectable for several hours, up to approximately one day, while amphetamine was detected for days. Because of complications with interpretation of amphetamine positive drug tests, the viability of a current amphetamine procedure was evaluated for identification and quantitation of this compound.

Clobenzorex was monitored by acquiring four prominent ions in the mass spectrum at m/z 91, 118, 125 and 364. All 4 ions were evaluated to assess the best 3 ions to select for routine monitoring. Interference was occasionally seen at m/z 91 and/or m/z 118 but in most cases, particularly at low concentrations, either ion gave acceptable results. HP-1 and DB-17 capillary columns were used to ensure elimination of interference from related amines.

Several attempts were made to quantitate clobenzorex using either methamphetamine-d11, fenfluramine, benzphetamine or diphenylamine as internal standard. Results using these internal standards proved to be unacceptable due to poor accuracy and reproducibility of the quantitative results (>20% of target levels). The compound, 3-Chlorobenzyl-amphetamine (3-Cl-clobenzorex) was synthesized in our lab and evaluated in this study for the quantitation of clobenzorex. This 3-chloro analog of clobenzorex was selected for its anticipated similar behavior in the extraction and derivatization to the compound of interest. The compound proved to behave as predicted and was chromatographically separated from clobenzorex.

Using a calibrator at 25 ng/mL, the limit of detection (LOD) was determined to be 1 ng/mL, the limit of quantitation (LOQ) was 5 ng/mL and the linear range from 5 to 100 ng/mL. When using a calibrator at 10 ng/mL, the LOD and LOQ were 1 ng/mL and the linear range was shown to be 1 to 25 ng/mL. All characteristics (variability, accuracy, etc.) were determined using 3-Cl-clobenzorex as the internal standard. Precision studies showed 3-Cl-clobenzorex to produce accurate and reliable quantitative results (within-run RSD < 6.1%, between-run RSD < 6.0%).

Key Words: Clobenzorex, Precursor Drugs, GC/MS, Amphetamine

124: Separation of Amphetamine and Methamphetamine Stereoisomers in Urine by Chiral High Pressure Liquid Chromatography.

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An enantiomeric separation of amphetamine and methamphetamine in urine by chiral high pressure liquid chromatography (HPLC) column is described. The method involved liquid/liquid extraction of 2 mL of urine and derivatization of the amphetamines with benzoyl chloride. The analytes were separated on a (S)-indole carboxylic acid and (R)-1-napthyl-ethylamine (Phenomenex, CA) chiral HPLC column and detected at a wavelength of 220 nm with a diode array detector. Hexane:2-propanol (90:10) was used as the mobile phase with a flow rate of 1.0 mL/min. Under these conditions, the retention times of d-amphetamine, l-amphetamine, d-methamphetamine, l-methamphetamine, and N-methylphenethyl amine (internal standard) were 13.3, 14.0, 8.6, 8.9, and 9.8 min., respectively. Quantitation was performed with by the method of peak height ratios.

Calibration curves were linear from 50 to 5,000 ng/mL with a LOD of 20 ng/mL and a LOQ of 50 ng/mL. The overall within run precision of the method yielded CVs of 6% (d-amphetamine), 8% (l-amphetamine), 4% (d-methamphetamine), and 6% (l-methamphetamine) at 500 ng/mL (n=5) and 2% (d-amphetamine), 5% (l-amphetamine,

3% (d-methamphetamine), and 3% (l-methamphetamine) at 2500 ng/mL (n = 5). The overall recovery of the method was 97% at analyte concentrations of 500 ng/mL and 102% at analyte concentrations of 2500 ng/mL. Popular phenylisopropylamine drugs were found not to interfere with the method. The results of enantiomeric analysis of 30 urines from methamphetamine abusers by the presented method and chiral GC/MS were compared by linear regression analysis which yielded good correlation for each enantiomer: r^2 s ranged from 0.952 to 0.974. An advantage of the chiral HPLC method over the chiral GC/MS method was the greater accuracy in determining the ratio of the amphetamine optical isomers as there was no contribution to apparent isomer content due to contamination of d-TPC in l-TPC GC/MS derivatizing reagent.

Key Words: Amphetamines, Urine drug testing, Chiral HPLC

125: Mass Spectrometric Identification of Some Sulphur-Containing Phenalkylamine Designer Drugs

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The continuous search for new phenalkylamine designer drugs has provided the drugs-of-abuse market with drugs such as substituted 3,4-methylenedioxyphenalkyl and 2,5-dimethoxy-phenalkylamine analogues. The most recent development in Europe is the marketing of sulphur containing phenalkylamine designer drugs. In this study the mass spectrometric characteristics of two alkylthio-phenalkylamine compounds are described which have been found on the European drugs-of-abuse market. The identity of these alkylthiophenalkylamines have been confirmed by comparing them either with reference compounds or if not available as reference compounds by ^1H and ^{13}C nuclear magnetic resonance spectroscopy.

The first compound is *para* -methylthioisopropylamine, also known as *para* -methylthio-amphetamine (MTA). If not derivatized, its electron ionisation (EI) mass spectrum proved to be almost identical to that of 4-hydroxy-3-methoxyamphetamine (HMA). However, because both compounds have different functional groups, most derivatisation methods result in end-products, which can be distinguished by GC/MS analysis. This is demonstrated by the EI mass spectra of non-derivatized MTA and HMA, as well as their *N* -TFA and *N* -TFA-*O* -TMS derivatives.

The second compound is 4-ethylthio-2,5-dimethoxyphenethylamine (2C-T-2). In general 2,5-dimethoxyphenethylamine analogues substituted at the 4-position have EI mass spectra with closely related fragmentation patterns. This is demonstrated by the EI mass spectra of the *N* -TFA derivatives of 2,5-dimethoxyphenethylamine, 4-bromo-2,5-dimethoxyphenethyalkylamine (2C-B) and 2C-T-2.

Because the EI mass spectra of the *N* -TFA derivatives of *para*-methylthioamphetamine and of 2C-T-2 are characteristic, it can be concluded that these sulphur containing phenalkyl-amine designer drugs can easily be identified using mass spectrometry.

Key Words: para -methylthioamphetamine, 4-ethylthio-2,5-dimethoxyphenethylamine, 2C-T-2

126: Detection and Quantification of Drugs in Urine and Serum by Time of Flight Secondary Ion Mass Spectrometry

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Detection and quantification of drugs in biological specimens is often time consuming due to extensive sample preparation. A fast method for simultaneous detection and quantification with minimal work-up of the specimens would be an important advance in emergency situations.

In the last few years Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS), one of the most sensitive analytical techniques in surface science, has also become a powerful tool for analysing biomolecules adsorbed on a noble metal. In the present work, the performance of ToF-SIMS for the detection of several drugs (cocaine, morphine, scopolamine, LSD and buprenorphine) in serum and urine after liquid-liquid extraction was evaluated. The extraction

procedures and the sample preparation were investigated in order to establish an analytical protocol. Comparative investigations of the nature of the metal substrate, used to absorb the extracts, show for the first time an optimum emission of secondary ions obtained from gold substrates.

In a first step, buprenorphine, which is rather difficult to detect in routine clinical and forensic analytical toxicology was selected for in-depth investigation. In a first approach extraction yield in urine was found to be 40 – 50%. The limit of detection (LOD) has been determined to be 1 ng/ml and the standard deviation (SD) was 0.05. This assay was used for comparison of ToF-SIMS and LC/MS results in the case of several fatal buprenorphine intoxications.

Key Words: ToF-SIMS, Buprenorphine.

127: Application of Surface Ionization Organic Mass Spectrometry (SIOMS) to Forensic Toxicology: Identification and Highly Sensitive Quantitation of Phencyclidine (PCP) in Human Body Fluids

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Surface ionization (SI), an ionization of organic compounds on an incandescent metal surface, has been used for detection of drugs and toxic compounds by gas chromatography (GC). SI specifically ionizes tertiary amines; thus this method is very useful for analysis of abused drugs containing tertiary amines. Recently, Fujii and Arimoto have developed new surface ionization organic mass spectrometry techniques (SIOMS), based on the SI principle. In this study, we have applied the SIOMS to analysis of phencyclidine (PCP) in human body fluids.

A quadrupole GC/MS system of QP 5050A with a GC-17A gas chromatograph (Shimadzu) was used. A rhenium ribbon filament was placed on the tip of a direct inlet probe. The filament was heated resistively, and continuous oxygen introduction was employed to keep the condition of the surface constant. We spiked 25 ng each of PCP and pethidine (internal standard, IS) into 1 mL of human body fluids and extracted by solid-phase extraction using Bond Elut glass cartridges before SIOMS analyses.

We compared the peaks of PCP and IS obtained by SIOMS with those obtained by the conventional electron impact (EI) MS. When 500 pg each of PCP and IS was injected, SIOMS gave sharp peaks in total ion chromatograms, while such peaks were hardly detectable in the EI mode. A calibration curve for PCP obtained from mass chromatograms by SIOMS was linear in the range of 5 to 200 pg on-column. Because of its high sensitivity and high specificity, this method seems quite promising for forensic toxicology.

Key Words: Phencyclidine, Surface Ionization, Organic mass spectrometry

128: QUANTITATIVE DETERMINATION OF (DESPROPIONYL)-BEZITRAMIDE IN POSTMORTEM SAMPLES BY LIQUID CHROMATOGRAPHY COUPLED TO ELECTROSPRAY IONIZATION TANDEM MASS SPECTROMETRY

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A sensitive and highly specific method for the quantitative determination of (despropionyl)-bezitramide in postmortem samples using liquid chromatography combined with electrospray ionization tandem mass spectrometry (LC-ESI-MS-MS) is presented. The method results from a simple methodological transfer of a liquid chromatographic method with fluorescence detection (LC-FL) previously developed in our laboratory (DeBaere, Lambert, DeLZeenheer, *Anal. Chem.* 1997, 69: 5186-5192).

A liquid-liquid back extraction procedure using *n*-hexane-isoamyl alcohol (93:7, v/v) as the extraction solvent was performed for a basic sample clean-up. N-methyldespropionyl-bezitramide was used as the internal standard. Chromatographic separation was achieved on a Hypersil ODS (C18) 5-µm column, using a 80:20 (v,v) mixture of 1.0 mM ammonium acetate and methanol/ acetonitrile (50:50, v/v)-1.0 mM ammonium acetate as the mobile phase. To obtain as

high a sensitivity and selectivity as possible, a selected reaction monitoring mass spectrometric technique in the daughter ion mode was applied. In addition low energy collision-activated dissociation (CAD) product ion spectra were recorded for a few samples.

Calibration graphs were prepared for blood and urine and good linearity is achieved over a concentration range of 1 to 150 ng/mL ($r > 0.9994$). The intra- and interassay coefficients of variation (CV%) for the analysis of quality control samples were: within-day CV% 6.4 and 1.6%, between-day CV% 10.2 and 7.4% for the 10 and 50 ng/ml levels in blood, respectively. At the same concentration levels percent of targets are within 12.1% and 9.2%.

Postmortem samples (blood, urine, stomach contents, bile, liver and kidney) from three fatalities, all suspected victims from a drug overdose, were analyzed. The following results were found for fatality 1,2 and 3 respectively: blood 106 ng/mL, 6 ng/mL, NA (matrix not available); urine 2.5 ng/mL, < LOD of 0.7 ng/mL, NA; stomach contents 5.1 μg/g, 3.5 μg/g, NA; bile 621 ng/g, NA, NA; liver 2 μg/g, 0.3 μg/g, 2.5 μg/g; kidney 1.7 μg/g, 0.2 μg/g, 0.3 μg/g. The results obtained with LC-ESI-MS/MS are in close agreement with those obtained using the LC-FL method. Moreover the isolates' identity and structure is fully confirmed by the CAD product ion spectra, thus allowing unequivocal conclusions about the prior intake of bezitramide by the three subjects.

Key Words: despropionyl-bezitramide, postmortem samples, LC-ESI-MS-

129: Direct Analysis of Drugs in Urine using REMEDi/MS/MS: Qualitative and Quantitative Applications

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Identification of therapeutic and illicit drugs in urine is essential for a variety of settings including pre-employment, probable cause and in hospital emergency departments. The REMEDi HS from Bio-Rad Laboratories is an automated HPLC system, which uses a scanning ultraviolet (UV) detector to identify a broad spectrum of drugs. The purpose of this study was to modify the REMEDi to make it suitable for atmospheric pressure ionization mass spectrometry and to demonstrate the utility of this system in a qualitative and quantitative mode of analysis.

The standard REMEDi mobile phase was changed to an ammonium acetate/acetonitrile mix to make it compatible with positive ion electrospray ionization. Sample preparation consisted of adding 200 uL of the REMEDi internal standard mixture (an ammonium acetate buffer containing retention time markers) to 1 mL of urine. After centrifugation to pellet out any solids present, samples were automatically injected into the REMEDi which had been interfaced with an ion trap mass spectrometer (Finnigan LCQ).

The REMEDi/MS system detected 18 drugs from a variety of classes including amphetamines, benzoylecgonine, antidepressants, benzodiazepines, opiates and antihistamines directly from urine specimens using both a full scan mode and a data dependant MS/MS mode of analysis. The ion trap was under automatic gain control with full scan and MS/MS targets of 5×10^7 and 2×10^7 respectively. In the data dependant mode, the LCQ collected full scan spectra from 50 to 500 amu and when any ion exceeded 1×10^6 counts, it automatically switched to collect daughter ion spectra. With electrospray ionization most drugs only exhibit a molecular ion in the full scan mode, consequently MS/MS is necessary for unequivocal identification.

The quantitative ability of this system was demonstrated for the cocaine metabolite benzoylecgonine (BE) using benzoylecgonine-d_3 as the internal standard. Quantification was performed using selected reaction monitoring. The parent/daughter ions for benzoylecgonine and benzoylecgonine-d_3 were 290/168 and 293/171 respectively. Using 200 ng/mL benzoylecgonine-d_3 as the internal standard the method was linear from 30 to 10,000 ng/mL (r squared = 0.999).

Key Words: Automated analysis of drugs, HPLC/tandem mass spectrometry, REMEDi

130: Direct Analysis of Urine Specimens for Succinylcholine by Electrospray LC-MS-MS

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Succinylcholine (SC) is a depolarizing neuromuscular blocking agent typically used as an adjunct to surgical anesthesia. SC poisoning presents the forensic toxicologist with the difficult task of trying to identify its presence in biofluids and tissues. Previous analytical techniques for analyzing SC have relied on the formation of

an ion-pair complex with bromothymol blue prior to thin-layer chromatographic analysis and/or gas chromatographic procedures.

A rapid and sensitive liquid chromatography-tandem mass spectrometry (LC-MS-MS) procedure has been developed for the direct detection and identification of SC in urine specimens. This qualitative procedure alleviates the time-consuming preparative techniques that older procedures have employed, as well as provides for a mass spectral identification of the poisoning agent.

The procedure's mobile phase consisted of methanol:water:methanesulfonic acid (20:80:0.01) through a Hamilton PRP-1 analytical column. The mass spectrometer was optimized using a solution of SC and was operated in the daughters of 385 m/z mode with argon as the collision gas. Ionization was through an atmospheric pressure ionization source with an electrospray interface. Resulting daughter spectra allowed for the unequivocal identification of succinylcholine. The detection limit of SC was under 1 mg/L. The method was validated using SC-spiked urine specimens, as well as specimens collected from surgery patients who had received SC during anesthesia.

Key Words: Succinylcholine, LC-MS-MS, Urine

131: Analysis of LSD and Metabolites in Biological Samples by Immunoaffinity Purification followed by GC/MS/MS Combined with NICI

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A sensitive and highly specific method for the analysis of LSD in urine has been developed. In order to obtain clean extracts satisfactory for the chromatographic analyses, monoclonal LSD-antibody (CEDIA LSD Assay^{R})-resin was mixed with urine samples directly into the extraction columns. After incubation at room temperature for 30 minutes, the urine matrix was washed out with phosphate buffer and water. LSD was released from the antibodies by methanol elution. After evaporation, LSD and the internal standard (IS) LSD-d_3 were derivatized with heptafluorobutyrylimidazole (HFBI), followed by GC/MS/MS detection combined with NICI. Methane and argon were use as ionization and collision gases, respectively. Based on multiple reaction monitoring of several ions, e.g. m/e 499 (LSD), 502 (IS) as parent ions, producing 456 as daughter ions (both from LSD and IS), LSD down to 0.1 pmol/ml could be detected from 2 ml urine sample.

The metabolite nor-LSD could also be isolated using the same immunoaffinity reagent followed by derivatization and GC/MS/MS-detection and NICI, while iso-LSD and iso-nor-LSD had both very low antibody affinity.

The same procedure was tested for the analysis of LSD in whole blood at similar concentration levels. After precipitation of red cells with cold ethanol followed by centrifugation, the supernatant was incubated with LSD-antibody-resin. The cleaner sample extracts obtained both for urine and blood samples after immunoaffinity purification, both improved sensitivity and simplified the instrument maintenance procedures.

Key words: LSD, immunoaffinity purification, GC/MS/MS confirmation

132: Detection of Underivatized Lysergic Acid Diethylamide (LSD) in Urine Using Gas Chromatography/Ion Trap Tandem Mass Spectrometry

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In the Department of Defense (DoD) urinalysis drug-testing program, 190,000 urine specimens are tested monthly for lysergic acid diethylamide (LSD). Although LSD usage is known to be popular in some branches of the armed forces, less than 0.01% of the urine samples are determined to be positive for LSD as compared to 7% for the other drugs tested. The ability to routinely detect the low levels of LSD in a cost-effective manner would greatly assist the drug deterrence program for this illicit substance.

To that end, a gas chromatography/tandem mass spectrometry (GC/MS/MS) method was developed using an internal ionization ion trap MS for the detection of underivatized LSD. Following solid phase extraction of 5mL of urine, extracts are injected into a temperature-programmable injector with a 0.5mm I.D. direct-injection insert. The injection port

is optimized to reduce analyte loss by injecting at low temperatures followed by rapid heating. Collision-induced dissociation (CID) of the isolated 323 m/z parent ions of LSD and lysergic acid methylpropylamide (LAMPA, internal standard) is achieved by resonant dissociation for 30msec at 0.61v. The product ions 280, 222, and 196 m/z are monitored for both LSD and LAMPA with 222 m/z as the quantifying ion.

The method was linear over the concentration range 20-2000 pg/mL with a correlation coefficient of 0.999. The observed limit of detection was 20 pg/mL with an 80 pg/mL limit of quantitation. Intra-day stability assessed over 12 injections gave a %CV=5.0. The method was successfully applied to both DoD Quality Control Laboratory samples and previously confirmed LSD positive samples.

Ion trap MS/MS has been demonstrated as a favorable tool for low level drug confirmation in biological samples. It provides the necessary sensitivity with lower complexity of operation over alternate instrumental methods.

Key Words: LSD, Ion Trap, Tandem Mass Spectrometry

133: Determination of 2-oxo-3-hydroxy-LSD in Urine by GC/MS

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Only 1 to 3 % of an LSD dose (typically 50 µg) is excreted unchanged in urine. Therefore urinary LSD concentrations are very low (rarely above 10 ng/mL) and detectable for only 12 - 24 hours after intake (at a cutoff of 0.5 ng/mL). Recently, certain unpublished LC/MS/MS observations have indicated that 2-oxo-3-hydroxy-LSD is present in urine at much higher concentrations. The cross reactivity of 2-oxo-3-hydroxy-LSD in the EMIT, CEDIA and KIMS immunoassays is 1.7, 1.8 and 11 % respectively.

LSD and 2-oxo-3-hydroxy-LSD have been quantified in urine by GC/MS (Hewlett Packard 5970) after solid phase extraction on SPEC.PLUS MP1 (3 mL, 30 mg, Ansys, Irvine, CA, USA) disks using the method recommended for amphetamines and derivatization by BSTFA. Lysergic acid methyl propyl amide (LAMPA) was used as the internal standard for quantitation. For 2-oxo-3-hydroxy-LSD-diTMS, m/z of 309 and 499 were used as quantitation and confirmation ions respectively.

In four samples containing between 0.26 and 7.0 ng/mL of LSD, 2-oxo-3-hydroxy-LSD concentrations between 8.0 and 28.5 ng/mL were found, i.e. between 4.3 and 41 times higher than the LSD concentration. High peaks of 2-oxo-3-hydroxy-LSD was also observed in full scan chromatograms of two more samples that we analyzed some years ago. The concentrations were not higher after enzymatic hydrolysis, which suggests that 2-oxo-3-hydroxy-LSD is not conjugated. These results indicate that 2-oxo-3-hydroxy-LSD in present in urine at higher concentrations than LSD, which will facilitate the confirmation of positive screenings and could increase the detection time.

In conclusion, we developed a simple GC/MS method for quantifying 2-oxo-3-hydroxy-LSD in urine and found it at concentrations 4 to 40 times higher than LSD. Further studies will be needed to confirm these results on a larger number of samples and to document the excretion kinetics of this metabolite.

Key Words: LSD, 2-oxo-3-hydroxy-LSD, GC/MS

134: Determination of Gamma-hydroxybutyric Acid (GHB) in Plasma and Urine by Headspace Solid-Phase Microextraction (SPME) and Gas Chromatography-Positive Ion Chemical Ionization-Mass Spectrometry

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Gamma-hydroxybutyric Acid (GHB) is an endogenous constituent of mammalian brain and has been used, in therapeutics, as an intravenous anaesthetic and in the treatment of various diseases. However, as evidenced by many reported cases of intoxication, GHB is also a widespread drug of abuse, probably causing physical dependence.

A new method of detecting toxic, therapeutic and sub-therapeutic levels of GHB in plasma and urine samples by headspace Solid-Phase Microextraction (SPME) and Gas Chromatography-Positive Ion Chemical Ionization-Mass Spectrometry (GC-PICI-MS) is reported. The method was carefully evaluated and optimized in terms of choice of SPME

fiber, working pH, absorption and desorption times, absorption temperature, effect of salts, location of the fiber in the GC injector, and choice of CI reagent gas.

The main steps in SPME of 0.5-ml-aliquots of plasma and urine samples included GHB conversion to its lactonic form Gamma-butyrolactone (GBL) at 80°C in acid conditions in the presence of D_6-GBL as internal standard, addition of solid phosphate buffer to adjust pH to 6-7, and headspace adsorption with a 50-µm Carbowax/TPR-100 fiber for 15 min at 70°C. GC-PICI-MS analysis with splitless injection was carried out using a 25-m FFAP (acid-modified polyethylene glycol phase) capillary column, methane as CI reagent gas, and Selected Ion Monitoring (SIM) of ionic species at m/z 86, 87, 88 (GBL) and 92, 93, 94 (D_6-GBL).

The assay was linear over a plasma GHB range of 1-100 µg/ml ($n = 5$, $r = 0.999$) and a urine GHB range of 5-150 µg/ml ($n = 5$, $r = 0.998$). Intra- and inter-assay relative standard deviations (n=5) at 5 and 50 µg/ml were below 5%. Limits of detection were 0.05 and 0.1 µg/ml for plasma and urine respectively, and practically dictated by their endogenous GHB levels.

Compared with previous GC and GC/MS methods for GHB analysis, the method presented here is simpler, faster and more specific, and may be conveniently applied for emergency toxicology and therapeutic drug monitoring purposes.

Key Words : Gamma-hydroxybutyric Acid (GHB), SPME, GC/MS.

135: Sensitive Determinations of Volatile Organic Compounds in Body Fluids by Capillary Gas Chromatography with Cryogenic Oven Trapping

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The determination of volatile organic compounds (VOCs) in body fluids is frequently required in forensic science practice. VOCs are usually measured by gas chromatography (GC) with a headspace method. In most of the previous reports, conventional packed columns, which give relatively low sensitivity and poor separation, were used. With wide-bore capillary columns, only a 0.10.5 ml volume of headspace vapor can be injected; with medium-bore capillary columns, split injection giving 15% efficiency has to be used.

Recently, a microcomputer-controlled device for lowering oven temperature below 0°C has become available for new types of gas chromatographs. This device was originally designed for rapid cooling of an oven to reduce the time for analysis. In the present studies, we have used it for trapping VOCs inside a capillary column at cryogenic oven temperatures; as much as 5 ml of headspace vapor could be injected into a medium-bore capillary column without any loss, giving sensitivity 1050 times higher compared with that by the previous headspace GC methods. In addition to the above high sensitivity, much better resolution (separation) of compounds could be also achieved, probably because VOCs were trapped, at a cryogenic oven temperature, in a quite narrow zone at the front end of a capillary column.

The method is appropriate for wide use in forensic and environmental toxicology applications requiring high sensitivity and high resolution, because it is simple and requires no special GC operations. Some successful data have obtained by the present capillary GC with cryogenic oven trapping for analyses of chloroform, solvent thinner compounds, hydrogen cyanide, ethanol and xylenes in human body fluids. A discussion of the method's utility and limitations will be presented.

Key Words: Cryogenic oven trapping, Volatile organic compounds, Gas chromatography

136: SPEC Discs for Rapid Broad Spectrum Drug Screening in Urine

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Broad spectrum drug screening in analytical toxicology requires that all relevant substances be isolated, detected and identified, regardless of their structure and/or polarity. To this end, we developed a systematic SPE approach for drug

isolation from biological fluids [Chen et al., *J. Forensic Sci.* 37 (1992) 61-71]. Since speed and cost-effectiveness are key issues, we have evaluated disc-format extraction for the above purpose.

Discs were SPEC.PLUS.C18AR/MP3 columns, providing hydrophobic and cation exchange interaction. Blank human urine was spiked at 2 mg/L with pentobarbital, secobarbital, methaqualone, diazepam, phencyclidine, lidocaine, methadone, imipramine and codeine, thus representing a variety of drug classes. Urine specimens (2 mL), were diluted with 2 mL phosphate buffer pH 5.0, and the mixture was applied to the preconditioned disc. Washing was done with 1 mL water. Acidic and neutral drugs were eluted with 1 mL ethyl acetate-acetone 1:1, followed by eluting basic drugs with 1 mL ammoniated ethyl acetate. Extracts were examined by GC-FID to check cleanliness, recoveries and reproducibilities.

The discs showed good extraction efficiencies for all drugs, with little or no matrix interferences. Recoveries were 75-100% with CV's around 5%. As compared to our standard SPE method mentioned above, the disc procedure allowed a reduction of both elution volumes and total processing times by some 65%.

Key Words: SPE, Disc Extraction, Systematic Toxicological Analysis

137: Fatalities due to Alcohol and Drugs: The Jordanian Experience

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A study of fatal poisoning due to alcohol and drugs was carried out, to examine the mortality resulting from alcohol and drugs in the Greater Amman County, Jordan.

Methods A retrospective review of all autopsy records and deaths certificates issued by the department of forensic medicine at Jordan University Hospital was carried out. Cases were identified and analyzed according to age, race, sex, manner of death of the victims along with blood alcohol concentration, and drugs detected at autopsies.

Results A total of 60 cases involving drugs and alcohol were identified, during the 18 years (1978-1996) in the 6109 postmortem cases examined in our department. 40 were males (66.6%), 7 were females (11.6%) and 13 were children ≤5 years (21.6%). The majority of the victims were Jordanians 51 (85%) and 9(15%) were of other nationalities. Alcohol seems to be equally prevalent as other drugs in causing fatal poisoning in Jordan. A significant gender difference in alcohol cases (100% males) in comparison to drugs (58.8% males) was observed. The adult male to female ratio was 5.7:1 in all cases. It was found that 76.6% and 23.3% of the fatalities were due to accidental and suicidal ingestion, respectively, of alcohol and drugs.

Conclusion Fatal poisoning due to alcohol and drugs had a minor contribution to the total deaths among Jordanians in comparison to other societies. The risk of alcohol related deaths was noticed only among adult males, children ≤ 5 years exhibited a high risk of fatal poisoning with drugs. Suicide by drug was not a common problem as was expected.

Key words: Fatalities, Alcohol, Drugs, Jordan.

138: Categorization of Medicinal Drugs Affecting Driving-Related Psychomotor Performance.

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In order to provide physicians and pharmacists with a scientific base for guiding their patients on the effects of drugs on driving performance, an attempt was made to categorize 179 medicinal drugs from 9 therapeutic classes listed in the Belgian "Commented Repertory of Drugs-1997", based on literature data from about 500 references. These 179 molecules are available in 241 (single drug) and 100 (associations) registered medicines. The package insert mentions the possible influence or absence of effect on driving for 78% (single) and 48% (associations) of these medicines.

The classification was based on the system of Wolschrijn et al (1991) : 7 categories ranging from no effect (I) over minor and moderate (II1,II2) to severe effects (III), completed with the respective * categories for assumed classes with insufficient scientific data. A total of 28/179 molecules (16%) were considered having no influence (I/I*) : 1/33 hypnotics-sedatives (0/4 barbiturates and 1/25 benzodiazepines), 0/10 anticonvulsants, 0/25 antidepressants, 0/28

neuroleptics, 0/19 narcotic analgesics and antitussives, 6/24 antihistamines (4/20 H1 and 2/4 H2), 11/20 beta blockers, 3/10 antidiabetics and 7/10 central stimulants.

The categorization of the molecules proved to be problematic. The lack of study data is reflected in the number of * categories (50%), this fraction being variable in the different drug groups e.g. antihistamines 0%, benzodiazepines 32%, neuroleptics 71%. The diversity in the study protocols makes an unequivocal classification impossible and emphasizes the need for standardization. It is obvious that the effect on driving ability is dose-dependent and time-related, which makes the use of a single category inadequate; co-ingestion of other medicines or alcohol, and development of tolerance further complicate the problem. Moreover the study data seldom relate the observed psychomotor performances with measured plasma concentrations, while this relation may be important in future traffic legislation. Physicians and pharmacists should take these considerations into account when estimating the driving abilities of their patients.

Key words : medicinal drugs, driving ability, psychomotor performance

139: Drugs and Driving: Blood Drug Concentration vs. Clinical Signs of Intoxication

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After an experimental phase of checks on drivers based on clinical and toxicological analyses carried out during the summer weekends of 1994 and 1995, a permanent checking service, set up in collaboration with the Italian Road Police, was activated during the period July 1997-May 1998.

Toxicological and forensic assessments, including clinical observations and sampling of biological fluids, were carried out on 1073 drivers. Of these 952 supplied blood samples and 858 urine samples. There were 317 drivers reported for driving under the influence of alcohol (BAC > 80 mg %). In 147 drivers, psychoactive drugs were found in biological fluids, 74 of them in blood.

The present work aims at assessing the correlation between drug presence and concentration in the blood, and the type of physical and behavioural alterations recorded during the clinical examination. Analysis of clinical data on the driver population in question involved assessment of the following parameters : blood pressure, heart rate, ocular clinical signs (e.g., nystagmus, photomotor reflex, myosis, mydriasis), slurred speech, motor coordination, and the presence of other clinical signs indicating states of intoxication or withdrawal symptoms.

Toxicological analyses revealed the presence of the following psychoactive substances or classes of substances in drivers' blood samples : cannabinoids (n = 39, THC-COOH concen-tration range 0.3-125 ng/ml), cocaine (n = 30, benzoylecgonine concentration range 18-890 ng/ml), amphetamine and analogues (n = 9, MDMA concentration range 21-266 ng/ml) and opiates (n = 6, concentration range of total morphine 20-621 ng/ml).

Key Words : Drugs and driving, psychomotor performance, blood drug concentration.

140: Horror Trips Due to Atropine-Tainted Cocaine and Immediately Drug Users Became Colleagues of the Police

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On the night of October 28 to 29, 1996 about 30 cocaine users were admitted to hospitals in the city of Zurich or treated by physicians. All showed the same symptoms of a severe drug intoxication. This kind of intoxication was never seen before, and the symptoms lasted up to 24 or even 48 hours.

The drug scene immediately turned crazy, the drug users were totally confused and the intoxicated persons suffered from mortal terror. During the following days more intoxications were registered, but after a few days no further cases were noted.

It was highly interesting - especially from the point of view of drug politics - to note that the behavior of drug addicts changed immediately. Drug users became "colleagues" of the police, were willing to bring the drugs to the police station, suddenly knew the names of the dealers, the prices, and the locations where the deals took place. Also quite new was the spontaneous readiness to file a declaration with the police. The information of the drug users enabled the police to arrest the responsable drug dealers within a few days.

Six different whitish powders were submitted to our laboratory. The analyses revealed that five powders contained atropine (1 - 11 %, mean 7 %), cocaine (41 - 61 %, mean 49 %), procaine (6 - 14 %, mean 9 %), and dimethylterephthalate (not quantified). One powder contained cocaine only (57 %).

At first, GC/MS analysis was not able to find atropine. Only cocaine, procaine and dimethylterephthalate were identified. But in the two dimensional HPTLC four spots were detected. With this method the fourth compound could be identified as atropine. Only when looking closer at the cocaine peak in the GC/MS run atropine was found, because atropine was hidden under the cocaine peak when using our standard DB-5ms column. The quantitations were made on a GC-NPD with DB-17 column. This method was able to separate all three basic substances.

Key words: Atropine, Adulterated Cocaine, Intoxication

141: Driving Under the Influence of Atropine-Adulterated Cocaine

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For a short time in October 1996, cocaine adulterated with atropine (mean content 7 %) appeared on the illicit drug market in Zurich. Subsequently, four cases of suspected driving under the influence of drugs proved to be related to atropine, as was shown by our investigations. Two of these cases will be presented in detail.

Case 1: A 30-year-old man called for the police claiming that his little daughter had been kidnapped. The police found him nervously walking around his car and talking to not existing persons whereas his daughter sat crying on the back-seat.

Case 2: The police observed a 30-year-old man driving unsafely and sometimes even stopping on the highway. His behaviour was striking: apathy, apparent confusion, difficulties in walking. A subsequent interrogation by the police was impossible.

In both cases the medical examination revealed mydriasis, slurred speech, bloodshot conjunctivas, in case 1 furthermore disorientation, in case 2 agitation.

Atropine was detected in toxic concentrations in whole blood (0.003 and 0.009 mg/L, respectively) and urine (6.6 and 27.0 mg/L, respectively) taken from the drivers a few hours after the event. Identification was carried out by GC/MS, quantitation by GC using nitrogen-phosphorus-detection. The intake of cocaine was proven in both cases. Additionally, in case 2, 0.020 mg/L of free morphine were detected in blood by GC/MS.

Conclusions: Atropine-adulterated cocaine produced severe intoxications with long lasting symptoms, e.g. hallucinations, disorientation, confusion, impaired vision, resulting in a severe driving impairment even at a time when direct impairment by cocaine could be ruled out. In case 2 a combined effect of atropine and heroin/morphine had to be taken into account.

Key Words: Atropine, Adulterated Cocaine, Driving Impairment

142: Forensic Quality Assurance: Limit of Identification and Analytical Sensitivity Definition for the Determination of THC in Serum Using GCMS (R. E. Aderjan) - WITHDRAWN

143: Driving Under the Influence of Drugs in the Nordic Countries.

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The purpose of this study was to determine if the high occurrence of drugs other than alcohol (35 –40%) among Norwegian drivers suspected to be under drug influence, is different compared to other Nordic countries. Blood samples received by Nordic forensic institutes during one week, from drivers suspected by the police to be driving under the influence of alcohol or drugs (Denmark: n = 255, Finland: n = 270, Iceland: n = 40, Sweden: n = 86, Norway: n = 149), were analyzed for alcohol and drugs, e.g. benzodiazepines (BZD), cannabinoides, amphetamines, cocaine, opiates and a number of antidepressant drugs. The same analytical cut-off levels were used at the different institutes.

For more than 40% of the Norwegian cases, the primary suspicion by the police was directed towards drugs, which were detected in more than 70% of these cases. In only 0-3% of the cases from Denmark, Finland and Iceland, drugs were suspected, while the corresponding frequency from Sweden was 17%. However, evidential breath analyses are used for about 3/4 of the Swedish drivers suspected to be under the influence of alcohol, consequently, the Swedish material might be different from the rest.

BAC's below the legal limits were found in 32, 18 and 2% of the Norwegian, Icelandic and Finnish cases, respectively (BAC <0.05%), in 10% of the Danish cases (BAC <0.08%) and in 20% of the Swedish cases (BAC <0.02%). The highest frequencies of drugs were found in the Norwegian and Swedish cases with no alcohol detected (80 - 83%). For cases with BAC's above the legal limits, similar frequencies of drug detections (19 – 22%) were obtained for the five countries. BZD, THC and amphetamine were among the most frequently detected drugs. Our results show that differences between Norway and the other Nordic countries with regard to drugs and driving, are connected to the selection criteria made by the police and probably due to greater focus on drugs combined with driving in Norway.

Key Words: DUI, Drugs, driving in Nordic countries.

144: Testing for Illicit Drugs in Sweat and Saliva of Drivers

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Urine has been the favored testing medium for detecting and deterring drug use. However, the short window of detection for many drugs in urine coupled with the personal inconvenience in collection has led to the search for alternative matrices for analysis. We have shown that sweat, collected by a simple forehead wipe, can detect cocaine use/exposure at approximately twice the rate of hair analysis and much better than urinalysis for individuals in a drug treatment program. Likewise, in a randomly selected university population, sweat testing showed twice the rate for cocaine use/exposure than did hair analysis (Kidwell, et al. *For. Sci. Int.* 1997, 84:75-86). Using data from the first study, cocaine levels >15 ng/wipe appear to be indicative of cocaine use rather than exposure. A ratio of benzoylecgonine to cocaine of greater than 0.1 also appears to support use rather than exposure.

Although sweat testing is thought to measure both use and exposure, it still could provide useful information in driving under the influence cases. A positive sweat result, coupled with subjective observations of driving behavior and personnel demeanor, will provide powerful evidence to arrest a driver, impound the car, and conduct more extensive testing back at the station. To evaluate sweat wipes for detecting drug use/exposure in roadside testing, sweat wipes and saliva samples were collected from volunteer drivers who were not detained by officers for any alcohol, drug, or traffic violation at a special enforcement roadblock. In a separate collection, samples were also obtained from commercial truck drivers at an interstate weigh station. The saliva samples were analyzed by an enzymatic assay for alcohol and all had alcohol levels lower than 0.01%. Both the saliva samples and sweat wipes were analyzed by CI-GC/MS for cocaine and its metabolites, amphetamine class drugs, and opiates.

7-29% of the non-commercial drivers tested positive for cocaine (n = 182) whereas 1.5-6% of the commercial drivers were positive using 15 ng/wipe cocaine and 2.5 ng/wipe BE as the cut-off levels (n = 136). Approximately 1% of the saliva samples were positive for cocaine, demonstrating recent usage. The individuals with positive saliva samples also had the highest sweat positives. The saliva results indicate that individuals often do not show obvious signs of stimulant use.

Keywords: sweat testing, saliva, driving under the influence, cocaine

145: Rohypnol In Dade County: Patterns of Use, Psychomotor Impairment And Results of Florida Legislation.

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Benzodiazepines are central nervous system (CNS) depressant drugs often detected in samples from driving under the influence (DUI) offenders. They are associated with marked psychomotor impairment and represent annually up to 20% of all Dade County (DUI) samples analyzed in our laboratory. Flunitrazepam emerged in the mid-90's as an illegal drug

recreationally abused and associated with date-rapes. Following an alarming increase in incidents associated with the abuse of Rohypnol, the House of Representatives Committee on Crime and Punishment passed House Bill 91 in February 1997, reclassifying flunitrazepam from Schedule IV to Schedule I of the controlled substance list. We report the dramatic drop in DUI cases involving flunitrazepam compared to 1996 and 1995. The patterns of use were studied and in cases where Drug Recognition Examinations (DRE) were available, the DRE reports revealed the classical CNS depressant psychomotor impairment.

Flunitrazepam was confirmed in 4 out of 301 samples in 1997, the was present in nearly 10% of all DUI cases and represented close to 44% of all benzodiazepine cases in 1995 and 1996. It was the only benzodiazepine confirmed in these urine samples 90% of the time. It was most often found in combination with cocaine and marijuana (37%) or marijuana alone (33%). Very few cases involved flunitrazepam and cocaine alone (<8%).

In all cases, coordination was poor, evidenced by failure to perform standardized roadside tests (Rhomberg, finger to nose, walk and turn, and one leg stand). Speech was slurred, eyes were bloodshot and watery, mydriasis and horizontal gaze nystagmus were most often noted. A possible decrease in mean blood pressure was compensated by an increase in mean pulse pressure.

Interestingly, the reclassification of flunitrazepam to Schedule I coincides with a dramatic drop in the incidence of flunitrazepam in DUI samples from Dade County, FL.

Keywords: Flunitrazepam, psychomotor impairment, driving under the influence

146: Status of Anti-Drugged Driving Efforts in Florida: Perspectives from Research, Law Enforcement Training, and Legal

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Due to the illegal nature of drugs of abuse, accurate estimates of their use in most population groups are extremely difficult to obtain. There are no national statistics on the use by drivers and very few community estimates. Research conducted by the USF College of Public Health estimated the prevalence of drug impaired adult drivers arrested for DUI, the number of drivers and passengers injured or killed in a motor vehicle crash, and the number of juvenile drug impaired drivers; all in Hillsborough County, Florida during the same time period.

Current collaborative law enforcement training focuses on the development, evaluation, and dissemination of cost efficient training for law enforcement agencies utilizing new urine drug testing technology to facilitate the detection of drugged drivers. On-site urine drug testing, in combination with drug recognition training, provides a new opportunity for law enforcement.

Florida law provides that should a person be proven guilty of driving while his normal faculties were impaired by drugs he would suffer the same penalties as the "drunk driver". But the proof necessary to obtain such convictions remains elusive. Unfortunately, due to the fact that urine tests do not readily provide quantifiable results, it is unlikely that a defendant can be convicted of driving under the influence on the basis of evidence of impairment and the presence of an unquantified amount of illicit drugs in his urine. Efforts are underway to adopt a "per se" law in Florida allowing a person to be found guilty of driving under the influence if he was operating a motor vehicle while any illicit drugs were present in his system.

Due to increasing aswareness of drugged drivers as a public health problem requiring attention, a new approach being investigated is the feasibility of drug testing high risk drivers prior to applying for renewal of a drivers license.

Key Words: Drug impaired drivers, anti-drugged driving efforts, drugs and driving.

147: The Use of the Addictive Substances by the Students of High Schools in Krakow.

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Sixty-one health young (18 -20 years) male students from two high schools: one located in downtown and the other in the suburb of Kraków, were asked to fill out a questionnaire concerning their drinking and smoking habits. Additionally,

urine samples were taken from each student. The students answered the following questions: How often do you drink? What kind of alcohol do you drink?, Did you ever lose consciousness after alcohol consumption?, Do you smoke, how many cigarettes per day, since when? In the urine samples, amphetamine, opiates, tetrahydrocannabinols and cocaine were measured by fluorescence polarization immunoassay and cotinine by HPLC.

The studies showed that more than 50% students drink only occasionally, once a week or less and the most popular beverage was beer. An alarming fact was the consumption of strong alcohol beverages by the students. The smoking habit was less popular than expected, 23 % (questionnaire and level of cotinine in urine). The use of narcotics was the most dangerous habit observed among Polish youth, being more frequent than ever before. Amphetamine was the most common drug and was detected in 17% of the studied groups. In 7% of the students tetrahydrocanabinol (marihuana) was detected in urine samples. Fortunately none of the studied group used opiates or cocaine. We did not observed any differences in drinking alcohol, smoking cigarettes and using drugs between the students from the two schools.

We conclude that, great attention must be paid to young people who are using „soft" drugs that can be a gateway to the use of opiates or cocaine.

Key Words: Substance abuse, alcohol, high school students.

148: Prevalence of Chemical Poisoning And Drug Abuse In Japan

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The prevalence of chemical poisoning and drug abuse in Japan was studied. On the basis of the annual reports of the Japanese National Research Institute of Police Science, there were 3,195 case reports in 1996. Carbon monoxide poisoning had the highest incidence about 66% of total cases, followed by pesticides (22%) and drugs (7. 0%). The data indicated that pesticide poisoning tended to decrease with concomitant decrease in paraquat poisoning in Japan. The number of paraquat poisoning cases (281) in 1996 was about one half of that (479 cases) in 1993. In contrast, the number of drug poisoning including sedative hypnotics gradually increased (about 40%) over the past 5 years. Other cases of poisoning involved alkaloids and volatile substances including propane, thinner and cyanides. Poisoning which are unique to Japan are tetrodotoxin poisonings from eating globefish.

In 1996, persons arrested for illegal use of stimulants (mainly methamphetamine), marijuana and narcotics were 17,101, 1,481 and 444, respectively. The problem of stimulant abuse has occurred in our country since World War II as three peaks in 1945, 1980 and 1996. In the past decade, gradual increases in marijuana abuse were observed, and smuggling of marijuana resin significantly increased. From 1996, there was a dramatic rise in illegal use of LSD. In all types of drug abuse, There were marked increases in illegal use of drugs by foreigners. The problem has come to be as serious as in other countries.

Key Words: drug abuse, chemical poisoning, Japan

149: Cocaine Hydrochloride Content and Adulterants in Street Samples Seized in the City of San Paulo, Brazil

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The presence of pharmacologically active adulterants and inert diluents in illicit drugs trafficked in any part of the world is very common. For cocaine in its hydrochloride form (white powder) this is specially true due to the several possibilities of adulteration and dilution this drug presents. Besides being a fraud, the practice of adulterating cocaine may contribute to the toxic effects or even their amplification observed in the abuse of the drug specially for users whose daily intake is several grams. The determination of cocaine content and adulterants in street samples is not only of clinical value but also important for forensic purposes related to the geographical distribution and allocation of the drug. So the objective of this study was to determine the purity and the presence of active adulterants in samples of cocaine hydrochloride trafficked in the city of San Paulo, Brazil in the year of 1996.

In the 2,105 samples of "white powder" seized in this period by the police authorities, 233 were collected on a random basis to be submitted to full analysis in the Toxicology Laboratory of University of San Paulo. Gas-liquid chromatography (FID), thin-layer chromatography and GC-MS for confirmatory analysis were used for the determination of cocaine contents in the samples as well as their adulterants.

The results were as follows: of all samples seized, 2.28% were negative for cocaine and any other pharmacologically active compound; of all samples analyzed in our laboratories 187 (80.26% of the total) consisted of white powder with no more of 30 per cent cocaine; in 24 samples (10.30%) cocaine purity ranged from 30 to 50 per cent and in 13 street samples (5.58 %) cocaine purity was between 60 to 80 per cent. In only 9 analyses (3.80 %) was the purity of the cocaine greater than 80 per cent.

Only local anesthetics were found as active adulterants: Lidocaine was found in 8 samples and procaine in only 2. Chemical analysis of street samples of cocaine (white powder) seized in the city of San Paulo, Brazil, showed purity that ranged from 1 to 96.42 per cent with an average of 20.02 per cent.

Key words: Cocaine Hydrochloride, Adulterants, Purity.

150: Fatal Poisonings In The City of Tashkent (Usman Kh. Khasanov) - WITHDRAWN

152: Quantitation of Dimethylphosphate (DMP) as a Metabolite in Cases of Acute Organophosphate Insecticide Intoxications

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In this paper we describe a sensitive gas chromatography-mass spectrometry (GC/MS) method for the detection of dimethylphosphate (DMP) in human blood, urine, and gastric fluid. DMP is a stable metabolite of some organophosphate compounds, like mevinphos, dicrotophos, monocrotophos, dichlorvos, phosphamidon and trichlorfon. This procedure can also be used in post-mortem material as heart blood, liver, kidney, muscles, fat tissues and brain tissues.

DMP was extracted with acetonitrile from a 0.1 mL aliquot of the sample. Deuterated dimethylphosphate (DMP-d6) was used as an internal standard. The extract was washed once with 0.02 mL heptane and 0.02 mL water. Then 0.05 mL ethanol was added to the aqueous phase to allow cold evaporation under a stream of nitrogen. 0.2 mL acetonitrile and dry potassium carbonate were added to the residue, followed by 0.01 mL pentaflurobenzyl-bromide (PFB-Br) for derivatization. After incubation for 30 min at 90°C, 0.05 mL of the extract were evaporated and disolved in 0.05 mL acetone (water free). Finally 1 µL of extract was analysed by GC/MS. The limit of detection was 0.06 mg/L serum. Calibration curves were linear over a range of 0.5-8.0 mg/L. The average recovery of DMP from human serum was 60 %. No changes in DMP concentration were observed in spiked human serum up to 5 days at 4°C.

In a case of acute phosphamidon intoxication, 3.5 mg DMP /L blood and 33.5 mg DMP /L urine were detected with the described method.

This method provides excellent tool in the determination of the water soluble metabolites of organophsphate insecticides in the field of forensic as well as clinical toxicology.

Key Words: Dimethylphosphate, organophosphate intoxication, GC/MS

153: Methods for the Quantification of Parathion in Tissues A Comparison Between Two Clean-Up Procedures

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Acute intoxications by organophosphate pesticides are frequent in Portugal. Parathion ($C_{10}H_{14}NO_5PS$) is the product responsible for the greatest number of lethal cases studied (> 50%) in the Laboratory of Toxicology of the Institute

of Legal Medicine of Coimbra. Blood is the sample most used in toxicological analysis, because it shows a better correlation with the symptomatology and also it is one of the simplest matrices, with the exception of urine. However, in some fatal cases it is not possible to collect blood samples and urine is seldom present. Thus, the toxicological tests in such cases must be undertaken on organs of the cadaver.

The authors describe two methods of parathion determination in liver, kidney and heart from analyte-free tissues and compared their accuracy and precision. All tissue samples were fortified to parathion concentrations of 0.01, 0.1, 1.0 and 10.0 mg/g. The extraction was carried out by hexane. In one of the methods (Method A), the clean-up was performed by solvent partitioning and by column chromatography with florisil. In the other (Method B), the clean-up was carried out by thin-layer chromatography. Gas chromatography was used in the identi-fication and quantification of parathion in both methods.

In method A a higher recovery rate (70.2 % to 79.0%) than in method B was generally obtained. However, in the latter one the lowest concentration presented better results in all the tissues, specially in the liver (80.4%). In the remaining concentration levels the recoveries vary from 68.7% to 78.1%. The coefficients of variation were 8.2% and 8.7% with ranges of 4.3% to 11.1% and 4.5% to 10.0% for methods A and B, respectively.

Although, in general, method A presents better results, method B, being simpler, is the one used in undertaking routine analyses for parathion determination, except in cases where the samples have a great deal of fat, since the insecticide may not be well differentiated from the fat in these cases.

Key Words: Parathion, clean-up procedures, tissue quantification.

154: Experimental Studies with Parathion Insecticide in Rabbits

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Parathion ($C_{10}H_{14}NO_5PS$) is an insecticide which is highly toxic for mammals (the lethal dosis for man is from 20 to 100 mg). It has a high toxicity because it is a powerful colinesterase inhibitor. In Portugal's Central Region, this insecticide is responsible for the majority of fatal intoxications. Practically all fatalities are the result of suicides through ingestion of commercial formulations with parathion (> 90%).

In this paper we describe metabolism and toxicokinetic studies following oral administration of a standard and a commercial formulation of parathion to the rabbit. Parathion and its metabolites aminoparathion ($C_{10}H_{16}NO_3PS$) and paraoxon ($C_{10}H_{14}NO_6P$) were analysed in biological products (blood, heart, vitreous humour, kidney, liver, lung, small intestine) after 30 min., 1, 2, 4, 8, and 24 hours. Gas chromatography with a nitrogen phosphorus detector and a mass spectrometry detector were used in the determination of the analytes.

The absorption process of parathion was very fast and this compound had a high elimination rate. The three compounds were detected in the different specimens. A low bioavailability and an interindividual variability in parathion metabolism were found for all analytes. Paraoxon presented the lowest concentrations in all samples. Aminoparathion was found in higher concentrations in the liver and kidneys. In the vitreous humour, the concentration levels were lower than in the other substracts. Generally speaking, the concentration levels of parathion and its metabolites increased up to 2 hours and decreased after 4 hours.

Although the determinations of the different specimens/blood partition coefficients (PCs) are not a reliable biomarker to evaluate the elapsed time between ingestion and death, the PCs can help the diagnostic interpretation of the poisoning cases by this insecticide.

Key Words: Parathion, metabolism, toxicokinetic.

155: What Human Hair Can Tell about Environmental Pollution at the Aral Sea

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The environment of the Aral Sea region has changed dramatically during the last 40 years. Shortsighted irrigation practices let the Aral Sea surface shrink to one half of the original surface, causing a 90 % decrement of air humidity and

an increment of salty dust in the air by windblown sediments. Moreover, the remains of water reaching the Aral Sea were contaminated with fertilizers and pesticides used in the intensively cultivated fields. At the same time, the state of health of people living in the affected regions deteriorated considerably. Many children showed an impairment of respiratory, renal, digestive, and blood systems. At the National laboratory of health in Luxembourg, trace elements in hair specimens originating from the Aral Sea region of Kazakhstan (n = 6) were measured by X-ray fluorescence spectrometry.

Concentrations of the element Cl were up to 4 times higher compared to values found in European hair. Analyses of Kazakh drinking water samples (n = 12) revealed that concentrations of chloride and sodium, as well as the number of bacteria in specimens from the Aral sea region were far above maximal permitted levels in Europe. This indicated that surface water was highly contaminated with salt originating very likely from the former Aral Sea ground. The high sodium concentration and high number of bacteria in drinking water could be at least partly responsible for the impaired state of health of the local Kazakh population. In addition, chloride could theoretically be sequestered into hair, when consumed daily due to high concentrations in drinking water. These findings were supported by the fact that children living in Almaty, were asymtomatic. The former Kazakh Capital is situated more than 700 km east of Aral Sea region and is supplied with clean fresh water from the near-by mountain range.

Another hypothesis to explain the high levels of the element Cl in Kazakh hair samples could be the high organochlorine (PCBs, DDT, lindane) body burdens. A Swedish team had measured levels of organochlorines in blood of Kazakh children (n = 12) being 1 to 2 orders above Western Europe reference values. To check this hypothesis, a method for analyzing organochlorine compounds in hair had to be developed. Preliminary results indicate that there are no significant differences between organochlorine concentrations found in Kazakh (n = 2) and in European (n = 3) hair analyzed so far. Measured concentrations of lindane, DDT, PCB Nos. 138, 153, and 180 were between 0.5 to 5 pg/mg hair. Further studies are scheduled in order to elucidate if and to which extent relations between blood and hair organochlorine concentrations can be found.

Key Words: hair analysis, polychlorinated compounds, environmental pollutants

156: Infrared Spectroscopy in Diagnostic Environmental Toxicology: Case Reports

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Objectives This work was undertaken to demonstrate the utility of infrared spectroscopy in the identification of materials used in patient care. In the cases described herein, it was suspected that these materials had been tampered with or utilized inappropriately.

Methods Infrared spectra of submitted materials were collected using a Nicolet model 860 Fourier transform infrared (FT-IR) spectrometer. Liquids and solutions were analyzed as thin films between barium fluoride windows, and solids and tissue specimens were placed on aluminum-coated glass slides for use with a microscope accessory.

Results The infrared absorption spectrum of a material can often be used to uniquely identify that material. In the first example, the presence of an antibiotic inadvertently added to a patient's intravenous fluid supply was confirmed using infrared spectroscopy. There were no adverse reactions associated with the extra medication. In another case, the efficacy of a prescription steroid hormone was questioned. Infrared spectra confirmed the identity and purity of the drug, and its unsatisfactory performance was linked to improper weighing during the preparation of the capsule. The final example is a case in which a foreign material remaining after a surgical procedure caused discomfort to the patient and slow recovery. Infrared spectra were consistent with a polyamide coating from one of the instruments. These cases are examples of the utility of FT-IR in resolving questions presented to the toxicologist.

Key Words: Infrared Spectroscopy, Therapeutic Drugs, Polymers

157: Quantitative Determination of LSD and a Major Metabolite, 2-Oxo-3-Hydroxy-LSD in Human Urine by Solid Phase Extraction and Gas Chromatography/Tandem Mass Spectrometry

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LSD continues to be a widely used drug of abuse. One reason for the continued use may be the difficulty in detecting LSD use by urine analysis. We have developed a method for the confirmation of LSD use which not only quantifies the parent LSD, but also quantifies a major human metabolite, 2-oxo-3-hydroxy-LSD, which is generally present in human urine at much higher concentrations and for longer periods of time than the parent LSD. Sample preparation is achieved by solid-phase extraction using Certify Bond Elut® extraction cartridges. Confirmatory identification is accomplished by the trimethylsilyl derivatization of LSD and 2-oxo-3-hydroxy-LSD followed by GC/MS/MS analysis with positive ion chemical ionization detection. LAMPA and 2-oxo-3-hydroxy-LAMPA are used as internal standards. With selected reaction monitoring, both compounds give linear calibration curves from 10 pg/mL to 5000 pg/mL.

Thirty-nine human urine samples from a forensic workplace drug testing program which had previously been confirmed to contain LSD were reanalyzed by this method. Upon reanalysis, the thirty-nine samples showed an average LSD concentration of 319 pg/mL with an average 2-oxo-3-hydroxy-LSD concentration of 4083 pg/mL, (approximately 12 times higher than the average LSD concentration). Additional experiments utilizing clinical samples in which subjects were dosed with LSD indicated that while LSD concentrations typically fall below current confirmation limits (and Department of Defense approved cutoff levels) after 12-24 hours post-administration, 2-oxo-3-hydroxy-LSD concentrations were detectable up to 96 hours post-administration.

This method provides a more effective method for the detection of LSD use by the quantitation of the LSD human metabolite, 2-oxo-3-hydroxy-LSD, which is commonly present at higher concentrations and provides a longer window of detection than LSD.

Key Words: LSD, Metabolite, Quantitative Analysis

158: LSD Casework at the RCMP Laboratory: Screening of Pre and Post-mortem Biological Specimens by RIA, EMIT and ELISA and Confirmation by GC/MS or GC/MS/MS

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The Royal Canadian Mounted Police forensic laboratory system has been involved in the analysis of LSD in pre and post-mortem fluids (blood, serum, plasma, urine, bile) and tissues (stomach contents, liver homogenates) as well as food extracts for approximately six years.

A number of screening techniques have been evaluated and compared: Abuscreen RIA (Roche Diagnostics Systems), Coat-A-Count RIA (Diagnostics Products Corp.), EMIT II (Syva and Behring Diagnostics) and ELISA (STC Technologies, Inc.). Methanolic extracts of blood, serum and plasma samples were prepared before assay. Fifteen samples previously screened LSD positive by Abuscreen RIA and confirmed by GC/MS were also found to be positive by Coat-A-Count RIA, EMIT and ELISA. However, a number of samples that screened positive by EMIT were not necessarily positive by either RIA assay or ELISA. The EMIT procedure was modified to exclude a large percentage of the false positive samples.

A quantitative confirmation of LSD was initially performed by manual solid-phase extraction followed by GC/MS (Varian 3400 GC coupled to Finnegan TSQ 7000 MS) on the trimethylsilyl derivative of LSD and LSD-d_3. The limit of quantitation ranged from 0.25 ng/mL to 0.50 ng/mL. A cleaner, faster and more sentitive confirmation method was then developed using the Zymark Rapid Trace solid phase extractor followed by GC/MS/MS on the trimethylsilyl derivative of LSD and LAMPA. The limit of quantitation was found to be 0.1 ng/mL. The latter method was also modified to accomodate LSD extraction from liver homogenates.

Results from selected criminal cases will be presented.

Key words: LSD, screening, GC/MS

159: Evaluation of an LC/MS System for the Determination of LSD, iso-LSD, and 2-Oxo-3-hydroxy-LSD in Urine from LSD Users

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Lysergic acid diethylamide (LSD) is one of the most difficult drugs of abuse to detect by urinalysis because of the very low concentrations of the parent drug excreted in the urine. Several methods for confirmation of LSD in urine have employed GC/MS or GC/MS/MS. Instrumentation which utilizes tandem mass spectrometry (MS/MS) often produces an improvement in specificity and signal-to-noise ratio which can be important in measuring trace concentrations of drugs in biological tissues. However, the cost of MS/MS instrumentation is prohibitive for many toxicology laboratories. We report a relatively rapid and sensitive analytical method for the quantitation of LSD, iso-LSD and 2-oxo-3-hydroxy-LSD in urine using liquid chromatography and single-stage mass spectrometry (LC/MS). 2-Oxo-3-hydroxy-LSD is a recently identified metabolite of LSD that is generally present in urine from LSD users at higher concentrations than LSD. Iso-LSD is not a metabolite of LSD, but is often detected in the urine from an LSD user because of its presence as a contaminant in LSD sold on the street.

Internal standards (LSD-d_3 and 2-oxo-3-hydroxy-LAMPA) were added to urine samples which were then subjected to solid-phase extraction and analyzed by electrospray LC/MS using a Hewlett-Packard series 1100 LC-MSD. Calibration curves for each analyte were linear from 50 pg/mL to 5000 pg/mL with correlation coefficients (r^2) greater than 0.99. Quality control samples fortified with 250 pg/mL LSD quantitated within 20% of the target concentration with a coefficient of variation of 2.3%. Urine samples (n = 8) previously confirmed positive for LSD by GC/MS/MS have been analyzed using the above method. LSD concentrations for positive samples ranged from 102 - 844 pg/mL. Iso-LSD concentrations for positive samples ranged from 52 - 574 pg/mL. 2-Oxo-3-hydroxy-LSD concentrations for positive samples ranged from 203 - 4288 pg/mL.

Key Words: LSD, LC/MS

160: Application of ChromatoProbe and Ion Trap Chemical Ionization/Mass Spectrometry (CI/MS) for Detection of Gamma-Hydroxybutryric Acid (GHB)

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Procedures for rapid, sensitive and specific identification of gamma-Hydroxybutryric acid (GHB) are presented showing confirming results in samples collected from a clandestine laboratory. The ChromatoProbe is a sample introduction device fitted to a temperature programmable injector of an Ion Trap GC/MS. Both solid and liquid samples suspected of containing GHB are quickly analyzed without extraction or derivatization giving positive confirmation by CI/MS. The method uses less then 1 uL of liquid or a few grains of solid placed into a microvial. Sample delivery is controlled by the injector temperature and the split flow ratio. Starting from a temperature of 70 degrees C, the injector is programmed at a rate of 200 degrees C/min to adequately vaporize the sample for transfer to the MS. The GHB is separated from a matrix by either using an analytical column and programming the GC oven, or using a short (1 – 2 meter) column and applying MS/MS. Flow rates through the injector split vent are maintained at 25mL/min. This minimizes the introduction of air into the injector when changing samples. Positive-ion acetonitrile (CH3CN) chemical ionization with full scan MS gives confirming protonated molecules (MH+) and an adduct ion thus providing a high degree of specificity for identification. The ChromatoProbe with Ion Trap GC/MS can also be utilized for the analysis of GHB in urine or blood of persons suspected of being under the influence, adulterated beverages, or clandestine samples.

Key Words: GHB, Ion Trap CI/MS, ChromatoProbe

161: Quantitation of Clenbuterol in Plasma and Urine Specimens Using GC-MS

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Clenbuterol, a $_2$ agonist, is used as a bronchodilator for the treatment of asthma and other respiratory conditions. Due to its growth stimulatory effects - which lead to enhanced muscle and decreased fat deposition - clenbuterol has been abused by athletes to increase performance. As a result, the International Olympic Committee and the Association of Official Racing Chemists included clenbuterol on their lists of banned substances. Commercially, clenbuterol has been used to enhance lean body mass in animal production.

A simple and sensitive procedure utilizing GC-MS for the identification and quantitation of clenbuterol in plasma and urine has been developed. This improved method utilizes trimethylboroxine for the derivatization of clenbuterol thereby yielding abundant diagnostic ions with high *m/z* values.

Linear quantitative response curves have been generated for derivatized clenbuterol over a concentration range of 5-200 ng/mL. The extraction efficiency at four representative points of the standard curve exceeded 90 % in both specimen types (plasma and urine). Linear regression analyses of the standard curve in both specimen types exhibited correlation coefficients ranging from 0.996 to 1.000. The LOD and LOQ values for plasma specimens were determined to be 0.5 and 1.5 ng respectively. For urine specimens, LOD and LOQ values were 0.17 and 0.6 ng, respectively. Precision and accuracy (within-run and between-run) studies reflected a high level of reliability and reproducibility of the method. In addition to its reliability, sensitivity, and simplicity, the procedure requires less time, only one mL of sample, and minimal amounts of extraction solvents.

The applicability of the method for the detection and quantitation of clenbuterol in biological tissues such as kidney, lung, and liver samples, was demonstrated successfully.

Key Words: Clenbuterol Quantitation, Plasma, Urine, GC-MS.

167: Screening for Barbiturates by Capillary Electrophoretic Techniques

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In Systematic Toxicological Analysis (STA), powerful analytical methods are required to screen samples for the presence of toxicologically relevant substances. Capillary Electrophoresis (CE) is a modern separation technique which provides outstanding efficiency and fast analysis. Our objective was to evaluate and compare two CE techniques, namely Capillary Zone Electro-phoresis (CZE) and Micellar Electrokinetic Chromatography (MEKC) for STA. Initially, the methods were developed for a group of 25 barbiturates.

To enhance the reproducibility of the data and to create a standardisable identification parameter, migration times were converted to effective mobilities, which were then corrected with 5 external reference barbiturates. The data were used to evaluate the Identification Power (IP) of the methods by calculating the Mean List Length, Discriminating Power and Discriminating Number.

The CE methods were very promising for the application in STA, providing fast analyses (within 5 min for CZE and 10 min for MEKC). The two methods showed low correlation and hence complement each other. Also, especially the CZE method showed low correlation with existing RP-HPLC and GC for STA. Reproducibilities were significantly improved using corrected effective mobilities instead of migration times. Both methods had good IP's but combinations of methods such as CZE plus MEKC, HPLC or GC and MEKC plus GC provided considerably better IP's than the individual methods.

Finally, CZE and MEKC appeared to be very favourable in STA when analysing a group of structurally similar compounds. In further studies, the two methods (CZE and MEKC) will be evaluated for compounds from different chemical classes.

Key words: Capillary electrophoresis, Systematic Toxicological Analysis, Barbiturates.

168: Detection of Medicinal Materials by Direct Reaction Gas Liquid Chromatography

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The search for new methodological approaches for determination of the presence of potent medicines in biological materials is a urgent problem in forensic - medical toxicology. We demonstrate a method for direct reaction gas - liquid chromatography for determination of the nature of some potent drugs through the formation of acetyl- and silyl-derivates, formed directly in the injection port of the gas chromatograph as the result of interaction of the initial preparations directly with acetylating or silylating reagents.

For this purpose, it is necessary to collect in a micro syringe 1 µL acetyl anhydride or trimethylchlorsilane (TMS), then 2 µL 0.2 % alcohol solutions of the drug or of an extract from the biological material. This mixture is injected to the chromatograph evaporation device ("Hewlett-Packard" - HP5890 with FID, glass column 2 m x 3 mm with 5 % SE - 30 on Inerton AW, injection port temperature 270°C). Thus, paracetamol at 200°C column temperature in the presence of acetyl anhydride completely derivatizes; at 235°C tazepam will form acetyltazepam with 100% efficiency, and clopheline is only 28% converted to acetylclopheline. With trimethylchlorsilane phencarolum is completely converted to the trimethilsilyl derivative. Use of these micro chemical reactions in forensic - toxicological analysis has allowed skipping a stage of extraction in preparations from biological materials, and detection of the presence of tazepam and clopheline in stomach contents. A small quantity phencarolum (up to 1 mg) was detected in the liver of a corpse.

The method of direct reaction chromatography may be applied to determination of the nature of medicinal materials in a variety of biological materials, especially if the small quantity of substance present does not allow determination by other methods.

Key Words: Direct Reaction Gas Chromatography; Acetylating; Silylating.

169: Selective Determination Of Trace Amounts Of Psychotropic Preparations By Surface Ionization Mass-Spectrometry: Derivatives Of Phenothiazine.

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At present the phenomenon of Surface Ionization (SI) of organic compounds belonging to classes of amines, hydrazines and their derivatives is well studied and the general rules of ion formation are formulated which permit forecasting the ion composition in the mass-spectra and the intensities of their fragment ions [Rasulev U.Kh., Zandberg E.Ya. "*Progress in Surface Science*", Vol. 28, No. 3/4, P.181-412, 1988].

This work demonstrates the possibilities of the SI mass-spectrometry in highly sensitive and selective analysis of psychotropic preparations from phenothiazine derivatives. Chlorpro-mazine manufactured in Russia was tested as a representative of the aliphatic group of phenothiazines, tioproperazine of pyperazine group, and periciazine (manufactured in France) was chosen as a representative of the pyperidine group.

The experiments have shown that the regularities of formation of the SI mass-spectra obey the general rules. However, the presence of two or more heteroatoms in a molecule leads to additional channels of molecule dissociation. In spite of this the mass-spectra are of few lines. Their composition and the temperature dependencies of ion currents demonstrate a high efficiency of formation of quasimolecular ions [M-H]+ and of fragments ions [M-Ri]+ (where M is a molecule, H is a hydrogen atom, Ri is a radical) which do not contain uncoupled electrons.

Under optimum experimental conditions the [M-H]+ ion intensities may reach up to 60% for chlorpromazine, 50% for tioproperazine and 10-15% for periciazine. The peculiarities of mass-spectra formation connected with the possible pathways of decay of molecules on the surface are discussed. The estimations show the amount of substance necessary for reliable identification was only 1 ng.

Key Words: phenothiazine derivatives, surface ionization mass-spectrometry.

170: Determination of Common Drugs of Abuse in Body Fluids Using One Isolation Procedure and Liquid-Chromatography-Atmospheric Pressure Chemical Ionization Mass Spectrometry.

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The method of determination of opiate agonists (morphine, morphine-3-glucuronide, morphine-6-glucuronide, 6-monoacetylmorphine, codeine, codeine-6-glucuronide, dihydrocodeine, dihydromorphine, buprenorphine, methadone, tramadol), cocaine and its metabolites (benzoylecgonine and ecgonine methyl ester), ibogaine and lysergic acid diethylamide in serum, blood, urine and other biological matrices is presented.

0.5-1.5 ml aliquots of biological fluids were spiked with appropriate deuterated internal standards and extracted using a common solid phase extraction method (C-18 cartridges). The extracts were subjected to liquid chromatographic-atmospheric pressure chemical ionization mass spectrometric examination (positive ions), using selected ion monitoring procedures. These procedures were developed after analysis of full-scan mass spectra of examined compounds. The extraction method appeared very universal; the recoveries ranged from 73% to 98% for all drugs but EME (41%), the LOD's ranged from 0.1 to 2.5 µg/ for all drugs but C6G (100 µg/l). Overall precision was 2-11% RSD. The extracts analyzed by HPLC-DAD and full-scan GC-MS appeared very clean. The procedure was applied for routine forensic casework.

Key Words: LC-APCI-MS, Solid Phase Extraction, Drugs of Abuse

171: Determination of Flunitrazepam and Its Metabolites in Blood by Means of High Performance Liquid Chromatography-Atmospheric Pressure Chemical Ionization Mass Spectrometry.

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A selective assay of flunitrazepam (F) and its metabolites: 7-aminoflunitrazepam (7-AF), N-desmethylflunitrazepam (N-DF) and 3-hydroxyflunitrazepam (3-OHF) with liquid chromatography atmospheric pressure chemical ionization (LC-APCI-MS, positive ions) was developed. The drugs were isolated from serum, blood or urine by solid phase extraction. F-d3 and 7AF-d3 were used as internal standards. The drugs were separated on an ODS column in acetonitrile-50 mM ammonium formate buffer, pH 3.0 (45:55). After analysis of mass spectra taken in full scan mode, a selected-ion monitoring detection was applied with following ions: m/z 284 (7-AF and F), 287 (7AF-d3 and F-d3), 314 (F), 300 (N-DF and 3-OHF), 317 (F-d3), 330 (3-OHF).

The limits of detection (µg/l) were: 0.2 for F and 7-AF, 1 for N-DF and 3-OHF. The method was linear in range 1-500 µg/l; the recoveries ranged from 92 to 99%. The method was applied for determination of F and metabolites in clinical and forensic samples. In 8 positive forensic samples, blood concentrations (µg/l) of F ranged from 4 to 48, concentrations of 7-AF 4 to 48. In 4 cases N-DF was detected (2-31 µg/l) and in 2 cases 3-OHF (10-12 µg/l). LC-APCI-MS seems to be a method of choice for these compounds.

Key Words: LC-APCI-MS, Flunitrazepam, Flunitrazepam Metabolite

172: HPLC/MS/MS Analysis of Flunitrazepam, Metabolites, and other Benzodiazepines in Urine and Whole Blood.

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Flunitrazepam (FNP), des-methyl-flunitrazepam, 7-amino-flunitrazepam, and 7-acetamido-flunitrazepam are analyzed by HPLC/MS/MS using a Waters C18 Symmetry column (3.9mm x 150mm), ammonium acetate buffer (pH 4.8, 40mM acetate, 40% v/v acetonitrile), 1.0mL/min. The internal standard is d5-nordiazepam. MS instrumentation includes

Shimadzu LC10A/d pumps, P-E SciEx API-300 triple quadrupole mass spectrometer run in the MS/Ms mode, with SciEx MassChrom quantitation software. Ion transitions used are as follows: flunitrazepam (FNP): 314.2/205.1; 7-amino-FNP: 284.1/227.1; des-methyl-FNP: 300.1/254.1; 3-hydroxy-FNP: 330.1/284.1; 7-acetamido-FNP: 326.2/227.2; d5-nordiazepam: 276.1/213.0.

Blood, standards, and hydrolyzed urine samples are mixed with internal standard, then extracted with n-butyl chloride. The organic layer is separated and dried under nitrogen. The organic layer is then reconstituted with mobile phase and analyzed in duplicate.

LOD is less than 1 ng/mL for 14 benzodiazepines and metabolites tested. LOQ is 2 ng/mL for the same group of benzodiazepines and metabolites.

Keywords: HPLC/MS/MS, flunitrazepam, benzodiazepines

173: Solid Phase Extraction and HPLC Analysis of Scilliroside in Rodenticide.

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Scilliroside is a cardioactive glycoside from red squill (*Urginea maritima*) used as rodenticide by ancient Egyptians and Roman. Only few reports were found concerning methods for analysis of the sclilliroside. We describe here a detection method for scilliroside using a solid phase extraction technique and reverse phase HPLC with diode array detector.

Procedures Powder of scilimurine containing 1% scilliroside was dissolved in 1% acetic acid solution. The mixture was applied to the Extrelut column, and was eluted with dichrolo-methane/iso-propanol (85:15 v/v). The eluent was evaporated completely and was dissolved again with mixture of methanol and an equal volume of phenacetin solution (0.2 mg/mL, I.S.) for HPLC analysis. The HPLC systems consisted of a LC-6A pump, Inertsil ODS-3V (4.6 mm x 150 mm) column and a diode array detector. Acetonitrile and water (23:77) were used for the mobile phase.

Results The retention time of scilliroside was observed at 12.6 min (wavelength: 250 nm), and the internal standard was at 7.0 min (wavelength: 301 nm). This method can useful for determining scilliroside from biological materials such as blood and urine.

Key Words: Scilliroside, SPE, HPLC

174: Sensitivity Limits of Liquid Chromatography/Mass Spectrometry (LC-MS/MS) in Toxicology Applications Performed with an Ion Trap

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A major application of LC-MS/MS in toxicology is the determination of small drug molecules and their metabolites in body fluid matrices. Using the MS/MS and MSn capabilities of ion trap technology significantly improves the selectivity and specificity for those samples in crude mixtures. As well, LC-MS/MS reduces often also the requirements for sample preparation and purification. The requirements for the LC separation are greatly reduced, i.e. a time consuming chromatography can be avoided in many cases. Finally, the sensitivity limits are usually lower than those of the UV detection in HPLC or classical mass spectrometers used in toxicology.

In this report, results on the determination of plasma neuroleptics and some investigations into the limits of detection of compounds difficult to identify like buprenorphine, LSD and pancuronium will be presented.

Neuroleptics represent a very heterogenous pharmacological group of drugs (phenothiazines, thioxanthenes, butyrophenones, etc) with a wide range of therapeutic and toxic concentrations (from ng/ml up to µg/ml). They are frequently used in suicide attempts but all existing systematic toxicological analysis procedures are just sensitive enough to find only the most concentrated ones (i.e. chlorpromazine, prothipendyl). More sensitive methods are generally focused on one specific compound like haloperidol or benperidol. Using liquid chromatography coupled with an ion trap mass spectrometry detector, we have developed a method to screen and identify the most relevant compounds in plasma. After a classical screening extraction procedure with Bond Elut Certify SPE column, 5 µl of the extract were injected into a C18 HPLC column (Phenomenex Jupiter 50 x 2.0 mm i.d.). Separation of neuroleptics was achieved with a mobile phase 2 mM

formic acid pH 3.5/acetonitrile (50/50 ; v/v) at 0.5 ml/min. With multiple reactions monitoring (MRM), specific mass transitions allowed the identification of twelve of the most relevant neuroleptics. Limits of detection varied from 0.1 to 1 ng/ml depending on the drug.

Key Words : Sensitivity – Liquid Chromatography and Mass Spectrometry – Ion Trap

175: Validation of an Automated Solid-Phase Extraction (SPE) Method for the Routine Quantitative Determination of Urinary Opiates

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Sample throughput, automation and quality of analysis are key factors in clinical and forensic toxicology laboratories. Using the ASPEC robot (Gilson, France), we have developed and validated an off-line automated solid-phase extraction method for the determination of urinary opiates.

With morphine and codeine as model compounds, all solvents volumes and flows were optimized on a C18 SPE column leading to recoveries of nearly 100%. Final analysis involved GC-MS after derivatization with trifluoroacetic anhydride (TFAA).

Using selected ion monitoring (SIM) and deuterated morphine as internal standard, limit of detection (LOD) of 7 ng/ml and limit of quantitation (LOQ) of 8.5 ng/ml were achieved for both compounds. Linearity was good from the LOQ up to 20,000 ng/ml. At 200 ng/ml, intra- and inter-day variabilities were respectively 0.5 and 2.2 % for morphine and 6.1% and 8.8 % for codeine. These results highlight the need of deuterated codeine for a better reproducibility of these analysis. The same method was found to be suitable for plasma samples.

Key Words : Automation – Sample Preparation – Urinary Opiates

176: Confirmation of Keto-opioids in Urine by GC-MS. 1

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A GC-MS method is described for the urinary confirmation of the keto-opioids: hydrocodone, hydromorphone, oxycodone and oxymorphone along with codeine, morphine and 6-monoacetyl-morphine(6-MAM).

The urine was hydrolysed with E. coli 2β-glucuronidase, alkaninized to pH 9 and extracted with methylene dichloride/trifluoroethanol (92:8). The dried extract was subjected to sequential derivatization. First, the keto-opioids were converted to their respective oximes with the addition of 2% methoxyamine HCl in pyridine. The reaction occurs at room temperature and is complete within 15 minutes. Next, propionic anhydride was added to the pyridine. The available hydroxyl groups at O_3 and O_6 are converted to propionyl esters. The reaction occurs at 56°C and is complete within 15 minutes. The excess derivitizing reagents were evaporated. The residue was purified by liquid extraction from 100 μL of 15% ammonium hydroxide into 1 mL of hexane/chloroform (3:1). The organic phase is dried, reconstituted in 50 μL of ethyl acetate and 1 μL analysed by GC-MS in full scan, m/z 50 to 550 at 1 scan/s.

Good separation was obtained between all opioids on a 15 m x 0.25 mm DB-1 column (J&W Scientific); a unique mass spectrum is obtained for each. Using naltrexone as the internal standard for the keto-opioids and nalorphine for the others, the day-to-day CVs for hydrocodone, hydromorphone, oxycodone, oxymorphone, codeine and morphine are 7.6, 8.7, 10.9, 8.3, 8.6 and 6.7% at 300 ng/mL and 9.1, 6.0, 10.2, 9.0, 8.3, and 7.9% at 1500 ng/mL. CVs for 6-MAM are 12.0% at both 30 and 150 ng/mL. The method is linear from 25 to 2,000 ng/mL.

Key Words: opiates, confirmation, urine, GC-MS

177: A Gas Chromatographic Positive Ion Chemical Ionization Mass Spectrometric Method that Uses Solid Phase Extraction for Determination of Cocaine, Benzoylecgonine, Ecgonine Methyl Ester and Norcocaine

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The gas chromatographic-mass spectrometric method to determine cocaine, benzoyl-ecgonine (BZE), ecgonine methyl ester (EME) previously described by Crouch et al. (*J. Anal. Toxicol.* **19**: 352, 1995) has been modified to also allow determination of norcocaine. Analytes were isolated from human plasma utilizing a modification of the previously described solid-phase extraction. Hexafluoroisopropanol and pentafluoroproprionic anhydride replaced *N*-methyl-*N*-(*tert*-butyldimethylsilyl)-trifluoroacetamide as derivatizing reagents. Quantitation of cocaine and its metabolites using deuterium labeled internal standards was determined after separation of the compounds by capillary chromatography. Analysis was performed by positive ion chemical ionization mass spectrometry using methane and ammonia as the reagent gases. The ions monitored for the analytes and respective internal standards were: cocaine (d_0/d_3), m/z 304/307; BZE (d_0/d_3), m/z 440/443; EME (d_0/d_3), m/z 346/349; and norcocaine (d_0/d_5), m/z 453/458.

Linearity of the method was established from 1.0 to 1000 ng/mL with r^2 for 6 runs of 0.992-0.997. Recovery of cocaine and each of its metabolites was approximately 80%. Intra-run precision and accuracy were determined for the limit of quantitation (LOQ) calibrators at 1 ng/mL and quality control samples (QCs) at 25, 100 and 250 ng/mL. At the LOQ accuracy was within 10% of target for all analytes and % CVs ranged from 9 to 18%. QCs had accuracy within 15% of target and CVs were also within 15%. Inter-run precision and accuracy were also determined with the accuracy at the LOQ ranged from 100 to 120%, and the CVs for the LOQ ranged from 0 to 10%. The inter-run accuracy for the QCs were within 12% and the CVs ranged from 4 to 15%. Stability data suggest the analytes are stable for 24 hours at room temperature and following 2 freeze-thaw cycles.

Key Words: GC/MS CIPI, SPE, Cocaine

178: Accelerated Solvent Extraction: A New Tool for Toxicological Screening of Postmortem Samples?

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Accelerated Solvent Extration (ASE^{TM}) is a new extraction method that significantly streamlines sample preparation. The solvent is pumped into the extraction cell containing the sample, which is than brought to an elevated temperature and pressure. Minutes later, the extract is transferred from the cell to a standard collection vial for cleanup or analysis. The entire extraction process is fully automated and performed in minutes for fast and easy extraction with low solvent consumption.

Previously, the extraction of biological materials such as liver, kidney an other tissues required large amounts of solvents. Funnel extraction can use from 200 mL to 400 mL of solvent for most tissue samples. Recent and anticipated changes in environmental regulations will cause severe restrictions on the amount of solvent usage in laboratories worldwide. ASE was developed to meet the new requirements for reducing solvent usage in the preparation of tissue samples. The objective of this study was to evaluate wether this technique can be used for toxicological screening of postmortem samples such as liver and urine after disperging them with a solid phase.

Results The use of ASE for the extraction of postmortem samples provides a more convenient, faster, and less solvent intensive method than previously available. Recoveries by ASE are equivalent to recoveries from other more solvent intense methods such as classic funnel extraction. ASE can extract a 4g sample of tissue in about 8 minutes with a total solvent consumption of approximately 10mL.

Conclusions Accelerated solvent extraction seems to be a promising technique for a rapid and automated extraction of solid and liquid postmortem samples. Further experiments should focus on the study of the influence of the number of purging steps, the pressure and the amount of solvent used during the extraction procedure on the extraction recovery and the purity of the extract.

Keywords : accelerated solvent extraction, toxicological screening, postmortem samples.

179: Improved Solid Phase Extraction Method for Systematic Analysis in Biological Fluids (T Soriano) WITHDRAWN

179: Analysis of Clozapine in Plasma, Post-Mortem Blood and Liver Tissue by LC with Electrospray MS Detection

M P Heenan and S G G Russell, ESR, P. O. Box 30547, Lower Hutt, New Zealand ADDED

180: Comparison of Three Different Digestion Procedures for Systematic Toxicological Analysis In Tissues (T Soriano) WITHDRAWN

180: Ion-Pairing Technique in Liquid-Liquid Extraction for quantification of Drugs in Serum

(A Hassoun) ADDED

181: Automated Analysis of Controlled Drugs in Biological Samples

Bridin Brady†, John Cornish†, Siobhán Moane‡* and Liam Regan†, †State Laboratory, Dublin 15, Ireland.‡Chemistry Dept., Trinity College, Dublin 2, Ireland.

Existing state-of-the-art confirmatory GC-MS techniques for drugs of abuse such as heroin, ecstacy and cocaine in blood and urine consist of sample preparation which is complicated and time-consuming, especially when the number of samples is large. It involves sample purification to eliminate endogenous compounds, followed by analyte pre-concentration by solid-phase extraction (SPE) and derivatisation prior to injection onto the GC-MS system. Automation of the SPE procedure greatly speeds up analysis, thereby increasing sample throughput and improving reproducibility of results.

The work reported here has successfully automated the existing manual opiate SPE method using a Gilson Automated Sample Preparation with Extraction Cartridges (ASPEC XL) system. The method extracts morphine, codeine, dihydrocodeine and the main metabolite of heroin, 6-monoacetylmorphine (6-MAM), from blood and urine using mixed-mode IST Confirm HCX cartridges. Following off-line derivatisation, the samples are separated by capillary GC and detected by an ion-trap MS in electron ionisation mode. Quantitation is based on peak area ratios of analyte to deuterated internal standards, from calibration curves in the range 0.25-5.0 μg/ml morphine, codeine and dihydrocodeine and 0.05-1.0 μg/ml 6-MAM for urine analysis and 0.05-1.0 μg/ml morphine, codeine and dihydrocodeine and 0.01-0.2 μg/ml 6-MAM for blood analysis.

The method has been optimised and evaluated, with limits of detection of 0.05 μg/ml for the four drugs in both blood and urine, based on a signal-to-noise ratio of 3:1. Recoveries of morphine, codeine, dihydrocodeine and 6 -MAM from blood were 59%, 58%, 57% and 56%, respectively. Recoveries from urine were 87%, 87%, 91% and 58%, respectively. Good correlation was obtained between real samples determined by the manual and automated methods.

Key Words: Opiates, Biological Matrices, Automated SPE.

182: Solid Phase Extraction on the Zymark Rapid Trace Workstation for the Screening of Basic Drugs

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A solid phase extraction procedure was designed to replace a current liquid-liquid extraction used for screening basic drugs in forensic samples. The goal was to perform solid phase extraction on the Zymark Rapid Trace Workstation while obtaining results comparable to a liquid-liquid extraction. The SPE procedure was first performed manually and then incorporated on the Rapid Trace Workstation by using copolymeric bonded extraction columns.

Urine samples were prepared by centrifuging 5 ml of urine followed by the addition of 2 ml of 100 mM phosphate buffer (pH 6.0). Blood samples were prepared by centrifuging 2 ml of blood and 3 ml DI H_2O followed by the addition of 2 ml of 100 mM phosphate buffer (pH 6.0). Liver samples were prepared by homogenizing 3 g tissue in 6 ml DI H_2O, adding 300 l of 1.0 M acetic acid and centrifuging for 15 min at 3000 rpm. Internal standard was added to all supernatants after being transferred to clean test tubes. Three standards containing 30 drugs were prepared in 5 ml of negative urine. Concentrations of individual drugs ranged from 0.2 mg/L to 0.8 mg/L. All samples were then extracted by the "Therapeutic and Abused Drugs" procedure found in the United Chemical Technologies, Inc. application manual. An extra wash step with 20% acetonitrile in water was required to obtain clean extracts. Samples were identified by GC-NPD and GC/MS.

The percent recovery of the thirty-drug profile varied from 73% to 100%, with the exception of diazepam. The limit of detection was found to be 0.04 mg/L to 0.16 mg/L. All 30 drugs were recovered on the GC-NPD; however, alprazolam, nordiazepam and mefloquine were not detected on the GC/MS.

All drugs that were reported positive by the liquid-liquid extraction procedure were found by both the manual and automated SPE procedures. Two drugs, caffeine and nicotine, were not always recoverable by SPE. However, the GC-NPD and GC/MS relative responses for the SPE procedure were 3×10^5 and 2×10^7, respectively, in comparison to 8×10^4 and 1×10^7 for the liquid-liquid procedure. Due to the above responses with SPE, some drugs at lower concentrations were found that were not recovered in the liquid-liquid extraction; e.g., benzoylecgonine, cotinine, desethyl-lidocaine, desmethylverapamil, desmethyltramadol, diphenhydramine, norpropoxyphene, phenyl-propanolamine, thioridazine, tramadol, trimethoprim.

Based on our results of increased response and the fact that the Rapid Trace offers a less labor-intensive extraction, the SPE procedure is preferred over the liquid-liquid procedure for basic drugs in forensic samples.

Key Words: Solid Phase Extraction, Basic Drugs, Postmortem analysis

183: Evaluation of the CV-1000 for Automated Aliquotting of Urine Specimens for Forensic Urine Drug-Testing.

R. H. Barry Sample*, Michael S. Feldman, Peter Sagona, SmithKline Beecham Clinical Laboratories.

In Forensic Urine Drug-Testing (FUDT) laboratories, many of the processes may be automated to increase throughput, reduce manual handling, and increase accuracy. For example, automated chemistry analyzers are capable of testing 100-300 specimens per hour while automated extraction platforms may process up to 300 specimens per hour. Specimen processing (receiving, accessioning, aliquotting) has been the most challenging to automate and remains the most labor-intensive process in FUDT labs. This report describes an evaluation of a new automated aliquotter (CV-1000) for preparing specimens for immunoassay analysis using an Olympus AU-800 or AU-5200 analyzer. The CV-1000 can also be configured to prepare aliquot samples for the Hitachi 747 analyzer.

The CV-1000 was evaluated for the following: carryover/cross-contamination using a fluoroscein dye solution, which can detect 250 PPM of contamination, and a high concentration of the "drugs of abuse"; maximum fill level of specimen bottles for safe liquid handling; bar-code reading accuracy of specimen bottles and destination tubes; accuracy of the LIS interface and batch reports; QC dispensing; guarding and access; through-put; and accuracy as compared to hand-poured specimens analyzed in parallel. This study demonstrated sustained throughput of 800-850 specimens per hour with no measurable carryover or cross-contamination with properly filled specimen bottles. The throughput is based on running 10% QC samples -- throughput can exceed 1000 samples per hour with no QC samples. The parallel analysis of more than 3000 specimens showed no significant difference between hand-poured and aliquotted (CV-1000) specimens.

Key Words: Urine Drug-Testing, Automation

184: Thin Layer Chromatography Screening of Amphetamines, Opiates and Cannabinoids Using Fluorescence and Colorimetric Detection

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Biological and non-biological materials containing multidrug mixtures are very often the subject of forensic toxicological analysis. A simple and sensitive screening system for the detection of cannabinoids, amphetamines and opiates simultaneously present in non-biological materials and in appropriate extracts of biological material by thin-layer

chromatography (TLC) is presented. A combination analysis of 7 cannabinoids, amphetamine and 13 related compounds, and 8 opiates by colour reactions and as dansyl derivatives was performed. Dansylation was carried out on plates.

For underivatized drugs four independent mobile phases and a sequence of different spray reagents were used. Visualization of chromatograms can lead to coloured spots (with Fast Blue B salt, Fast Blue RR salt, ninhydrin, Marquis reagent) or to fluorescent derivatives (with 2-amino-methyl-1,3-propandiol buffer and potassium hexacyanoferrate (III), and fluorescamine).

The screening of dansylated cannabinoids, phenylethylamines, morphine and heroin was based on four other mobile phases used in succession. In the first phase cannabinoids (R_F 5-8) were developed, in the second cannabinoids (R_f19-30) and amphetamines (R_f 6-21), in the third amphetamines (R_f 20-62), and in the fourth morphine and heroin (R_f 28 and 53 respectively). This method generally enabled the differentiation of cannabinoids, amphetamines and opiates from other drugs, biological and non-biological matrices, and in most cases from each other. Fluorescent dansyl derivatives of compounds studied were often sensitive enough to be detected in hundred nanogram quantities or less by the TLC screening method described.

Screening by examination of dansylated drugs, followed by confirmation using appropriate chromatographic systems for underivatized drugs, identifies the drugs.

Key Words: Drugs, TLC screening, Colorimetric and Fluorescence Detection

185: Supercritical Fluid Extraction (SFE) of Diazepam from Whole Blood Using Fillers

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In forensic toxicology, rapid extraction and determination of drugs are often very important. Liquid-liquid extraction (LLE) is complex and time-consuming and is often replaced by solid phase extraction (SPE). However, adjustment of pH and deproteinization of the sample are still required and problems of chemical pollution and cost effectiveness arise. SFE gives shorter extraction times and causes little chemical pollution but is not readily applied to samples with a high moisture content [Evaluation of drying agents for off-line SFE., M. D. Burford et al., *J. Chromatogr.* A 657 (1993) 413-427]. Previously, we have used SFE combined with freeze-drying for the extraction of biological samples with high water contents [Application of SFE with freeze-drying to analysis of benzodiazepines and their metabolites in blood samples., K. Takaichi et al., TIAFT 1997, Padova, Italy, p 96].

This study concerned the direct extraction of drugs from blood by SFE combined with fillers. The aim was to develop a simple, fast and inexpensive SFE extraction method which does not require deproteinization and which is not affected by liquid-liquid distribution ratios. Cellulose powder (0.2 g), the filler (appropriate amount), and absorbent cotton (0.2 g) were sequentially packed into the extraction vessel (10 ml). A blood sample (1 ml) containing 16 ng of diazepam as the target drug was added to the vessel, which was then set in the device. As the supercritical fluid passed through successive layers of the filler materials in the extraction vessel, protein in the blood was filtered out. At the same time, a colorless and transparent filtrate was easily obtained without the need for deproteinization. Fifteen kinds of drying agents and adsorbents, such as molecular sieves, were used as fillers. Quantitative analysis of the extracts was carried out by GC/MS.

The results showed that recovery of diazepam from whole blood was high as long as the extraction conditions were considered. Filling materials which gave good recoveries were molecular sieves, diatomaceous earth, silica gel, magnesium sulfate (anhydrous), and sodium sulfate (anhydrous). In particular, when molecular sieve 5A was used, recovery was 100%. This SFE method was simpler, faster, and less expensive than conventional methods.

Key Words. SFE, drugs, whole blood

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Society of Forensic Toxicologists
San Juan, Puerto Rico
October 10-14, 1999

PRELIMINARY MEETING SCHEDULE

SATURDAY, October 9, 1999	
2:00 p.m. - 6:30 p.m.	CAP Inspectors Workshop
5:00 p.m. - 8:00 p.m.	Registration
7:00 p.m. – 11:00 p.m.	NLCP Inspectors Workshop
Pre conference Tours available	

SUNDAY October 10. 1999	
8:00 a.m. - 5:00 p.m.	Registration
8:30 a.m.- 12:00 p.m.	Workshop #1 Drugs and Driving Update
2:00 p.m.- 6:00 p.m.	Workshop #2 Pathology for Toxicologists
12:00 p.m. - 6:00 p.m.	ABFT Board Meeting
2:00 p.m.- 6:00 p.m.	Workshop #3 Workplace Drug Testing: Issues in Puerto Rico
6:30 p.m. – 10:00 p.m.	Welcoming Reception

MONDAY October 11, 1999	
7:00 a.m. - 8:00 am	SOFT Fun Run/Walk
8:00 a.m. - 5:00 p.m.	Registration
8:00 a.m.- 12:00 p.m..	ABFT Examination
8:00 a.m.- 12:00 p.m..	Exhibit set up
8:30 a.m.- 12:00 p.m.	Workshop #4 New Pharmaceuticals – Pharmacology and Toxicology
12:00 noon - 6:00 p.m.	S.O.F.T. Board of Directors Meeting
2:00 p.m. - 6:00 p.m.	Exhibits Open
2:00 p.m.- 6:00 p.m.	Workshop #5 Investigation of Drug-facilitated Sexual Battery
7:00 p.m. - 10:00 p.m.	President's Reception/ Elmer Gordon Forum

TUESDAY, October 12, 1999	
8:00 a.m. - 5:00 p.m.	Registration
8:00 a.m.- 10:00 p.m..	Poster set up
8:30 a.m.- 12:00 p.m.	Scientific Session
12:00 noon - 2:00 p.m.	Luncheon
12:00 p.m. - 6:00 p.m.	Exhibits and Posters Open
2:00 p.m.- 5:00 p.m.	Scientific Session
7:00 p.m. - 10:00 p.m.	*Puertorrican Night*

WEDNESDAY October 13, 1999	
7:00 a.m.- 8:30 a.m.	ABFT Breakfast Buffet
8:00 p.m. - 12:00 p.m.	Exhibits and Posters Open
8:30 a.m.- 12:00 p.m.	Scientific Session
12:00 noon - 2:00 p.m.	Luncheon
2:00 p.m.- 5:00 p.m.	Scientific Session
3:00 p.m. - 5:00p.m.	Vendor's Meeting
6:30 p.m. 11:00 p.m.	Farewell Banquet/ Vendors Recognition *Night*

THURSDAY, October 14, 1999	
8:30 a.m.- 12:00 p.m.	Scientific Session
12:00 noon - 1:00 p.m.	Luncheon
1:00 p.m.- 3:00 p.m.	Scientific Session/Closing
3:00 p.m. - 6:00p.m.	San Juan Photo-Marathon
Post conference tours available	