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Drugs of Abuse Monitoring in Blood for Control of Driving Under the Influence of Drugs

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Summary: Driving under the influence of drugs is an issue of growing concern in the industrialized countries as a risk and a cause for road accidents. In forensic toxicology, the increasing number of samples for determination of drugs in blood is mainly due to zero-tolerance laws in several countries and well-trained police officers who can better recognize drivers under the influence of drugs of abuse. This review describes procedures for detection of the following drugs of abuse in whole blood, plasma, and serum: amphetamine, methamphetamine, 3,4-methylenedioxy methamphetamine (MDMA), N-ethyl-3, 4-methylenedioxyamphetamine (MDEA), 3,4-methylenedioxyamphetamine (MDA), cannabinoids (delta-9-tetrahydrocannabinol [THC], 11-hydroxy-delta-9-THC, 11-nor-9-carboxy-delta-9-THC), cocaine, benzoylecgonine, ecgonine methyl ester, cocaethylene, the opiates (heroin, 6-monoacetylmorphine, morphine, or codeine), and methadone as well as gamma-hydroxybutyric acid (GHB), lysergic acid diethylamide (LSD), phencyclidine (PCP), and psilocybin/psilocin. For many of the analytes, sensitive immunologic methods for screening are available. Gas chromatography-mass spectrometry (GC-MS) is still the state-of-the-art method for confirmatory analysis or for screening and confirmation in one step. Liquid chromatography-mass spectrometry (LC-MS) procedures for such purposes are also included in this review. Basic data about the biosample assayed, internal standard, workup, GC or LC column and mobile phase, detection mode, reference data, and validation data of each procedure are summarized in two tables. **Key Words:** Drugs of abuse analysis—Blood—Serum—Driving under the influence.

Driving under the influence of drugs (DUID) is an issue of growing concern in the industrialized countries as a risk and a cause for road accidents. In contrast to alcohol, reliable epidemiologic data are not available. Several reasons can be given for this fact. The frequency of detecting drugs of abuse in blood samples depends largely on the selection of the samples—for instance, from road surveys, from DUID-suspected drivers, from drivers who tested positive for alcohol, from hospitalized drivers after road accidents, or from people who died in road fatalities (1). In addition, driving experiments under the influence of drugs are rare (2). Even the list of drugs of abuse can vary, depending on the context in which the samples are analyzed. Clinical toxicology, forensic toxicology, workplace drug testing, doping analysis, and rehabilitation programs all focus on different drugs of abuse. In this review, methods for the analysis of illicit drugs in blood are covered. In particular, the following drugs and their metabolites are included in this review: amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA), N-ethyl-3, 4-methylene dioxy-amphetamine (MDEA), 3,4-methylenedioxyamphetamine (MDA), delta-9-tetrahydrocannabinol (THC), 11-hydroxy-THC (HO-THC), 11-nor-9-carboxy-THC (THC-COOH), cocaine, benzoylecgonine, ecgonine methyl ester (EME), cocaethylene, heroin, 6-monoace-

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tylmorphine (6-MAM), morphine, methadone, 2-ethylene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), gamma-hydroxy-butyric-acid (GHB), lysergic acid diethylamide (LSD), phencyclidine (PCP), and psilocybin/psilocin.

The European Union project ROSITA (roadside testing assessment, www.rosita.org) has shown that in countries where zero-tolerance laws for DUID are in force (Germany since 1998, Belgium since 1999), the corresponding number of detected cases increases markedly (3). The same situation was reported from Sweden, where a zero-tolerance law for drugs of abuse and special prescribed drugs was also adopted in 1999 (4).

Well-trained police officers are able to recognize DUID drivers. Roadside test devices for oral fluids, sweat, and urine can be used at checkpoints or after accidents to strengthen a first suspicion and to give the police officer the right to proceed with further measures (e.g., to order a blood sample for confirmation in the toxicology laboratory (3,5,6)).

Accurate quantitation of drugs and their metabolites in blood samples has acquired a considerably greater value compared with urine investigation during the past few years, particularly in testing drugged drivers (7–13). The number of substances that cannot be reliably determined in blood has decreased with progress in sample preparation, in chromatographic techniques, and in the sensitivity of detectors. Even if pharmacologically based cutoff values cannot (yet) be given for assessment of DUID, the impairing effects of drugs on driving performance can be discussed on the basis of a quantitation in blood. The number of studies concerning the determination of drugs in blood (whole blood, plasma, and serum) has increased greatly during the past few years. In 1998, Moeller et al (14) reviewed the detection and quantification of drugs of abuse in blood. One year later, Staub (15) reviewed chromatographic procedures for determination of cannabinoids in biologic samples, with special attention to blood and alternative matrices, such as hair, saliva, sweat, and meconium. Therefore, the present review is restricted to papers from late 1997 to the beginning of 2001. Different immunoassay procedures that provide preliminary results and chromatographic procedures for the determination of drugs of abuse are reviewed. The principal information on the chromatographic procedures is summarized in Tables 1 and 2 to enable rapid selection of a method suitable for an actual analytical problem. The information about whether a paper deals with a quantitative assay can be taken from the "validation" column. Retention time and mass spectral data are not listed in the tables to save space. The drugs are listed in the tables according to the international nonproprietary names or the common names. The type of biosample used is given in the "sample" column. Because the selection of the internal standard (IS) is important for the precision of a method, this information is given in the "Internal standard" column. For mass spectrometry (MS) procedures, stable isotopes are the most suitable IS because they have the same chemical properties as the corresponding analyte. Sample preparation is summarized in the "Workup" column. The principal information on the gas chromatography (GS) or liquid chromatography (LC) column and mobile phase, as well as on the detection mode, is listed. Validation data, such as recovery, limit of detection, or linearity, are summarized to assess whether a procedure could be useful in solving an actual toxicology case. The limit of quantification is given only if not identical to the lowest linearity value. Because the accuracy and precision of all the reviewed procedures were in the required limits, these data were omitted to save space.

MATRIX TO BE INVESTIGATED

Traditionally, urine was the sample of choice for screening and identification of unknown drugs or poisons because the concentrations of drugs are relatively high compared with other matrices such as blood, saliva, or sweat. However, the metabolites of these drugs had to be identified in addition, or even exclusively. Plasma was used mainly for quantification. Meanwhile, improvements in sample preparation, chromatography, and detection techniques have made whole blood accessible as a screening matrix, especially if particular (e.g., scheduled) drugs must be screened for. Identification and quantification can be performed in one matrix, and even using one procedure. Another advantage of blood is that in most cases the unchanged drug is detectable and the blood matrix is relatively homogeneous, because physiologic parameters vary within only narrow limits. In addition, blood samples are mandatory in cases of DUID in a relevant number of European countries and also in some states in the United States.

The relevant matrices to be analyzed are serum, plasma, or whole blood. Difficulties arise when only aged or hemolyzed blood is available. This situation often occurs when the suspicion of drug use does not arise immediately—for instance, if a blood sample was taken for alcohol determination and if, during investigation, additional suspicion about drug use arises. Procedures describing analyses of postmortem samples are also included in the present review (16–23). Because corresponding sample preparation is the most difficult one, it

TABLE 1. *Gas chromatography procedures for detection or quantification of drugs and/or their metabolites in the given biosample*

Compound	Sample	Internal standard	Workup	Gas chromatography column
THC THC-COOH	Blood	THC-d3 THC-COOH-d3	LLE combined with SPE (Bond Elut THC) PFPOH/PFPA	HP-5MS
Morphine	Plasma	Morphine-d3	LLE-HFB	DB-5MS
Methadone EDDP	Plasma	Proadifen; SKF	SPME (PDMS, 30 and 100 μm)	HP-5
Methadone EDDP	Serum (Saliva, Urine)	Lidocaine	LLE	SP-2250
(Fenfluramine) AM, MA	Blood	MA-d5	SPME (PDMS 100 μm)	PTE-5
COC BZE EME CE	Postmortem blood	COC-d3 BZE-d3 EME-d3 CE-d3	SPE-MTBSTFA	DB-5MS
COC BZE EME Norcocaine	Plasma	COC-d3 BZE-d3 EME-d3 Norcocaine-d5	SPE—PFPA/HFIP	DB-5
LSD	Serum	LSD-d3	LLE-TMS	HP-5MS
GHB GBL	Blood	AMGBL GHB-d6 GBL-d6	LLE/HS	DB-624
GHB GBL	Blood	GHB-d6 GBL-d6	LLE/HS	DB-624
GHB	Blood, serum, urine	Diethylene glycol	LLE-TMS	HP-5
Psilocin	Serum	Morphine-d3	ENHY-SPE-MSTFA	HP-Ultra-1

Compound	Mass spectrometry conditions	Reference data	Validation data	Ref.
THC THC-COOH	EI, SIM	RT, FI, MS	LOD: <1 ng/mL LOQ: 1 ng/mL REC: 80%; 70%	48
Morphine	NICI	RT, FI, MS	LIN: 0.78–50 ng/mL LOD: 0.29 ng/mL REC: 90–95%	33
Methadone EDDP	EI, SIM	RT, FI, MS	LIN: 50–2.000 ng/mL LOD: Methadone: 9 ng/mL EDDP: 5 ng/mL	80
Methadone EDDP	TID	RT	LIN: 0.05–2.0 μg/mL REC: 89–92%	35
(Fenfluramine) AM, MA	EI-SIM	RT, FI, MS	LIN: 0.01–1.0 μg/g LOD: 5–10 ng/g	51
COC BZE EME CE	EI, FSM	RT, FI, MS	LOD: 25–50 ng/mL LOQ: 50–100 ng/mL REC: 58–68%	16
COC BZE EME Norcocaine	PICI	RT, FI	LIN: 1–1.000 ng/mL REC: 69–96%	46
LSD	EI, SIM	RT, FI, MS	LIN: 0.1–10 ng/mL REC: 76%	37
GHB GBL	GC-FID	RT	REC: 82.6–87.6 LOD: 0.5 μg/mL LIN: 5–1.000 μg/mL	34
GHB GBL	EI, FSM	RT, FI, MS	REC: 82.6–87.6 LOD: 0.5 μg/mL LIN: 5–1.000 μg/mL	34
GHB	EI, SIM	RT, FI	LIN: 1–100 mg/L LOD: 0.5 mg/L	32
Psilocin	EI, SIM	RT, FI, MS	LIN: 10–100 ng/mL LOD: 5 ng/mL REC: 98%	39

AM, amphetamine; AMGBL, alpha-methylene-gamma-butyrolactone; BZE, benzoylecgonine; CE, capillary electrophoresis; COC, cocaine; COOH, carboxy metabolite; EDDP, methadone metabolite 2-ethylene-1,5-dimethyl-3,3-diphenylpyrrolidine; EI, electron impact ionization; EME, ecgonine methyl ester; ENHY, enzymatic hydrolysis/enzymatically hydrolyzed; FI, fragment ions; FID, flame ionization detector; GBL, gamma-butyrolactone; GC, gas chromatography; GHB, gamma-hydroxybutyric acid; HFB, heptafluorobutyric anhydride; HFIP, hexafluoroisopropanol; HP, Hewlett-Packard; HS, head space; LIN, linearity; LLE, liquid-liquid extraction; LOD, limit of detection; LOQ, limit of quantification; LSD, lysergic acid diethylamide; MA, methamphetamine; MS, mass spectrometry; MSTFA, N-methyl-N-trimethylsilyltrifluoroacetamide; MTBSTFA, N-methyl-N-(tert.-butyldimethylsilyl)-trifluoroacetamide; NICI, negative-ion chemical ionization; PDMS, polydimethylsiloxane; PFPA, pentafluoropropionic acid anhydride; PFPOH, pentafluoropropanol; PICI, positive ion chemical ionization; REC, recovery; RT, retention time; SIM, single-ion monitoring; SPE, solid-phase extraction; SPME, solid-phase microextraction; THC, tetrahydrocannabinol; TID, thermionic detector; TMS, trimethysilyated.

TABLE 2. *Liquid chromatography procedures for detection or quantification of drugs and/or their metabolites in the given biosample*

Compound	Sample	Internal standard	Workup	Stationary phase	Mobile phase	Detection mode	Validation data	Ref.
Morphine M3G M6G	Plasma	Deuterated analogs	SPE	Inertsil Silica (50 × 3. 5 μm)	Formic acid/water/ACN (1:10:90)	MS-MS	LIN: 0.5–50 (MO) 1.0–10 (M6G) 10–100 (M3G) LOD: 0.25 (MO. M3G) 0.5 (M6G) REC: 70–93%	45
Morphine M3G M6G	Serum	MO-d3	SPE	LiChrospher (30 × 5 mm; 5 μm)	ACN/ammonium formate (6:94) 1% formic acid	MS-MS	LIN: 1–20 (MO) 2–100 (M6G) 5–500 (M3G)	42
Morphine M3G M6G	Plasma	M-d3 M3G-d3	SPE	YMC-ODS-AQ 3 (150 × 2 mm; 3 μm)	0.1% formic acid/ACN (95:5)	MS-MS	LIN: 0.5–10 (MO) 0.25–10 (M6G, M3G)	43
Morphine M3G M6G COD CODG 5-MAM	Blood	M-d3 M3G-d3 M6G-d3 COD-d3 CODG-d3	SPE	ODS-3 (150 × 3 mm; 5 μm)	1 mmol/L ammonium fomiate (pH3)/ACN	MS	LIN: 1–500 ng/mL LOD: 0.5 (morphine) 5 (M3G), 1 (M6G) 1 (COD), 1 (CODG) 0.5 (6-MAM) REC: 70–84%	44
MO, COD; BZE: AM (and related substances)	Serum Urine	MO-d3, COD-d3, BZE-d3, AM-d5	SPE	Flow injection	ACN/10 mmol/L Ammonium acetate (1:1) Flow injection	MS-MS	LIN: 2-12–1,000 ng/mL LOD: 1–4 ng/mL LOQ: 2–12 ng/mL REC: >86%	41
MDMA, MDEA, MBDB, MDA	Serum	—	LLE	RP-18-AB (125 × 4, 5 μm)	20 mmol/L phosphate buffer (pH 3.8)/ACN (85:15)	FD	LIN: 10–500 ng/mL LOQ: 7–11 ng/mL LOD: 2–3 ng/mL REC: 55%	38
Cocaine Norcocaine BZE EME	Plasma (rat) Urine	Bupivacaine	SPE SPE-FBC (EME)	C8 (4.6 × 250, 5 μL + cyanopropyl (4.6 × 250. 5 μL)	ACN/Water/TFA (28:72:0.1)	DAD	LIN: 25–2,000 ng/mL LOD: 25 ng/mL 50 ng/mL (EME) 50–2,000 ng/mL (EME) REC: 80–86%	40
Cocaine (+12 metabolites)	B (rat) (amniotic fluid/placental and fetal tissues)	COD-d3 EME-d3 BZE-d3	SPE	Zorbax SB-C18 (150 × 2.1 mm; 5 μm) Or: Eclipse XDB-C8 (150 × 2.1 mm; 5 μm)	2.5 mmol/L ammonium acetate (pH 2.7)/ACN (97:3 MeOH/ACN (50:50) 20 mmol/L ammonium acetate (pH 2.7)	MS-MS	LIN: 0.01–2.50 ppm LOD: 0.2–1.25 ppb REC: 48–100%	47

ACN, acetonitrile; BZE, benzoylecgonine; COD, codeine; CODG, codeine glucuronide; DAD, diode-array detector; EME, ecgonine methyl ester; FBC, fluorobenzoylchloride; FD, fluorescence detector; LIN, linearity; LLE, liquid-liquid extraction; LOD, limit of detection; LOQ, limit of quantification; M3G, morphine-3-glucuronide; M6G, morphine-6-glucuronide; MAM, monoacetylmorphine; MBDB, R,S-N-methyl-benzo-dioxolyl-butanamine; MDA, 3,4-methylenedioxyamphetamine; MDEA, 4-methylenedioxyamphetamine; MDMA, 3,4-methylenedioxy methamphetamine; MO, morphine; MS, mass spectrometry; ODS, octadecyl silyl; REC, recovery; RP, reversed phase; SPE, solid-phase extraction; TFA, trifluoroacetanhydride.

can easily be adapted to less problematic matrices (e.g., to slightly hemolyzed blood).

Little information about the partition of drugs between plasma and red blood cells is available in the reviewed literature. Garrett et al (24) found no significant difference for MDMA and MDA between plasma and erythrocytes of dog blood at concentrations of about 100 ng/mL. Delta-9-THC is almost completely bound to plasma proteins, being distributed between lipoproteins and albumin in a 6:4 ratio. Very little THC enters the red blood cells (25). Bailey (26) determined the binding of cocaine and cocaethylene in human serum by equilibrium dialysis. Scatchard analysis suggested a high-affinity binder and a low-affinity binder for cocaine. Supplementation of serum with specific proteins suggested that the high-affinity binding was due to alpha-1-acid glycoprotein, whereas the low-affinity binding was due to albumin, inasmuch as such supplementation increased the ratio of bound to free drug for both cocaine and cocaethylene (26). The same author also investigated the binding of therapeutic drugs to serum of different mammalian species and found that the variations between drugs are much greater than variations between species (27). Information about the distribution of the other drugs between blood cells and plasma or serum is not available.

The stability of some drugs in stored blood samples was investigated by Giorgi and Meeker (28) over a 5-year period. They found that the esters cocaine and benzoylecgonine had poor stability. Methamphetamine was fairly stable, whereas unconjugated morphine showed wide variation throughout the study. Skopp et al (29) investigated the stability of morphine and its glucuronides in subcompartments of blood. The blood-to-plasma ratio of free morphine was unaffected by variations in hematocrit and water content, whereas the corresponding ratios for the morphine glucuronides were strongly influenced. They stated that conclusions drawn from pharmacokinetic studies and transferred to parent drug to metabolite ratios resulting from forensic blood samples may be biased by the particular biologic matrix under investigation.

SAMPLE PREPARATION

Sample preparation is probably the most critical step in each procedure, at least when quantification must be made in addition to qualitative analysis. Sample preparation methods for systematic toxicologic analysis (STA) for drugs and/or metabolites were reviewed by Maurer (30). Drummer (31) reviewed chromatographic screening techniques in STA for (mostly) basic drugs. He divided the possible procedures into liquid–liquid extractions, solid-phase extractions (SPE), and other techniques. Polettini (31a) described sample preparation methods for the analysis of drugs and poisons in biosamples by hyphenated chromatographic and spectroscopic techniques. For extraction of drugs of abuse from blood, plasma, or serum, liquid-liquid extraction procedures have been used (32–38) as well as SPE procedures (16,39–47). Combinations of liquid–liquid extraction and SPE were seldom used (48). Allen and Oliver proposed supercritical fluid extraction for isolation of cocaine, benzoylecgonine, and EME from blood and urine (49). This method showed good results also in comparison with usual SPE procedures. However, it is not very widespread in forensic laboratories and plays no role in daily analysis. Sample pretreatment for SPE depends on the sample type: whole blood and tissue (homogenates) need deproteinization and filtration/centrifugation steps before application to the SPE columns. Whatever SPE column is used, the analyst should keep in mind that there are large differences from batch to batch, and that the same absorbents from different manufacturers may also lead to different results (50). Therefore, use of a suitable IS is recommended. If mass selective detectors were used, deuterated IS is recommended (16,33,34,37,39,41–48,51). In Tables 1 and 2, the type of extraction procedure, as well as that used for derivatization, is listed in the Workup column.

SCREENING PROCEDURES

Screening Procedures Using Immunoassays

Within the past few years, progress has been made in immunoassay screening procedures for the most relevant drugs of abuse in blood. Kaferstein and Sticht (52) compared the enzyme multiplied immunoassay technique (EMIT) after acetone precipitation of the plasma proteins with a direct microtiter procedure (MTP plates) and found that the latter system was much more sensitive for cannabinoids and amphetamines. They stated that sensitivity of the EMIT assay for amphetamines was unacceptable due to losses during sample preparation. However, the greater sensitivity of the MTP immunoassay for cannabinoids was combined with a loss of specificity. Sensitivity for cocaine and opiates was comparable, but the specificity of the EMIT assay was substantially lower. Iwersen and Schmoldt (53) tested direct applicability of CEDIA DAU urine immunoassays to serum or

whole blood for amphetamines, benzoylecgonine, benzodiazepines, methadone, opiates, and THC-COOH acid on the BM/Hitachi 911 analyzer with unpretreated samples. Sensitivity, specificity, and comparison of CEDIA semiquantitation with GC-MS quantitative results were performed on 500 original serum and whole blood samples. The data provided sufficient documentation to use the CEDIA urine-screening technique without any adaptation as a sensitive serum/whole blood screening for benzoylecgonine, benzodiazepines, methadone, opiates, and THC-COOH. Serum screening for amphetamines is not sensitive enough in the unchanged urine mode. It requires some adaptation to a serum mode (probably a higher sample volume combined with protein precipitation of the sample). Moore et al (20) used a commercially available enzyme-linked immunosorbant assay (ELISA) to evaluate a screening procedure for the detection of nine classes of abused drugs in postmortem blood and tissue specimens. They compared it with radioimmunoassay (RIA) methods and found that ELISA was an adequate alternative to RIA for screening of postmortem specimens, including blood and tissue.

Screening Procedures Using Chromatographic Techniques

Every positive immunoassay result must be confirmed by a second analytic method, usually by chromatographic techniques with specific and sensitive detectors. However, if the prevalence of positive samples is high (e.g., in clinical toxicology), specific chromatographic procedures should be used also for screening purposes (54,55). Several papers on screening procedures for drugs of abuse in blood have been published, most of them dealing with LC-MS techniques. Bogusz et al (56) determined opiates, opioids, cocaine and metabolites, and LSD by liquid chromatography–chemical ionization–mass spectrometry (LC-CI-MS) after SPE. Weinmann and Svoboda (41) presented a fast analytic approach for the simultaneous quantitative screening for illicit drugs in serum and urine without tedious chromatographic separation steps by combining SPE followed by flow-injection analysis (FIA) with ionspray-ionization and tandem mass spectrometry (MS-MS) detection. However, practical application of this method is rare. The application of LC-MS for screening purposes was presented in several reviews published in the past few years (31a,57–60). Marquet (58) denounced the current practice of publishing poorly defined LC-MS procedures without any validation. He concluded that virtually all drugs of abuse can be sensitively and specifically determined using LC-MS, although the gold standard in this field is still GC-MS due to its wide acceptance, identity of the analytic conditions required for the different families (e.g., no change of chromatographic column or mobile phase required), reliability, and affordability of the instruments.

CANNABINOIDS

Delta-9-THC and its metabolites HO-THC and THC-COOH are the most prevalent drugs of abuse in blood samples analyzed in DUID cases in the European Union (ROSITA-WP4). Sanctions concerning the zero-tolerance legislation are linked to the presence of THC in blood in Belgium, Germany, and Sweden.

Immunoassays

Immunologic screening procedures including detection of cannabinoids have been presented by different authors (20,21,52,53). More details on the cited references were given above.

Chromatographic Procedures

Staub (15) reviewed chromatographic procedures for determination of cannabinoids in biologic samples with special attention to blood and alternative matrices such as hair, saliva, sweat, and meconium. Basic information about the biosample assayed, sample preparation, workup, GC or LC column and mobile phase, detection mode, reference, and validation data are summarized in the tables. As already concluded in a more extensive review (61), most of the procedures used SPE with GC-MS detection. In the time frame of the present review, only two papers were published on THC determination in blood using chromatographic procedures. Felgate and Dinan (48) used conventional liquid–liquid extraction followed by a cleanup using polar solid-phase cartridges for isolation of THC and THC-COOH from whole blood. The analytes were determined by GC-MS in the selected ion monitoring (SIM) mode. The limit of detection was less than 1 ng/mL, and extraction efficiencies were greater than 80% for THC and 70% for THC-COOH. The high-performance liquid chromatography (HPLC) method of Kramer and Kovar (62) with electrochemical detection after automated on-line SPE seems to be rather exotic. The limits of quantification for THC and THC-COOH were 5 ng/mL, which is not sufficient for routine use in a forensic context.

OPIATES

Immunoassays

Got et al (63) compared a morphine-specific antibody RIA and a nonspecific opiate RIA for detection or quantitation of opiates in biologic fluids in cases of opiate

overdose. They could show a persistent opiate concentration with rebound after oral ingestion, suggesting a slow release of opiates from the gastrointestinal tract (e.g., in bodypackers). Moreover, intravenous and oral kinetic data were similar for the two RIAs, except for the ratio between total and unchanged morphine concentrations. The nonspecific morphine assay gave a 3- to 16-fold higher concentration ratio than the specific morphine assay, but with parallel kinetics. They concluded that the current, routine nonspecific morphine immunoassays could be a valuable analytic tool for investigating opiate toxicokinetics. According to our experience with the morphine-specific RIA (DPC), there is a close relation between the free morphine concentrations determined by RIA and GC-MS. The results of the CEDIA-DAU studies of Iwersen and Schmoldt (53) and of the MTP immunoassay versus EMIT studies of Kaferstein and Sticht (52), which also included opiates, were described above.

Chromatographic Procedures

LC is the preferred method for the analysis of opiates, because parent compound and the conjugates can be determined in one run without cleavage of conjugates (42–45). Determination of morphine conjugates may be useful, because morphine-6-glucuronide is even more pharmacologically potent than morphine, and some further conclusions could be drawn from the conjugate ratios (e.g., that low ratios of M3G-morphine and M6G-morphine in blood of heroin overdose victims indicated a short survival time after drug intake (64)). Moreover, advantages in the MS detection after LC separation allow sensitive and specific determination of the analytes. For details on this technique, Marquet's review (58) is recommended. Pichini et al (65) reviewed papers on the determination of opiates and their metabolites in different biologic matrices using LC-MS. Details on several LC-MS procedures can be found in Table 2.

If only free morphine is to be determined, GC-MS methods can also be used. Leis et al (33) extracted morphine from plasma using liquid–liquid extraction with ethyl acetate and derivatized it by heptafluorobutyric anhydride. The derivatives were measured by GC-negative ion chemical ionization (NICI) MS with no further purification. The use of the NICI technique allowed quantification down to 0.78 ng/mL. More details on the use of the NICI technique in clinical and forensic toxicology, doping control, and biomonitoring can be found in Maurer's review (66).

AMPHETAMINE AND DERIVATIVES

Amphetamine and derivatives were the second most frequent drug group testing positive in all DUID cases in the EU ROSITA project in 1999 and 2000. The combination of amphetamines with cannabinoids represented the most frequent drug combination. Information on the toxicokinetics of amphetamines, including the role of CYP450 isoenzymes in their metabolism, can be found in the recent review by Kraemer and Maurer (67).

Immunoassays

Collison et al (17) investigated the optimal immunoassay cutoff values for screening of postmortem blood for drugs of abuse with a coated tube RIA. The aim was to ensure that the results with this RIA would be equal to or better than those with the previously used double-antibody RIA. Immunoassay results (positive or negative) in blood were compared with confirmed results by GC-MS alone or in combination with GC using either a nitrogen phosphorus selective detector (NPD) or flame ionization detector (FID). Four to seven potential cutoff concentrations were evaluated for the drug classes opiates, amphetamines, cocaine and metabolites, and barbiturates. Three hundred fifty postmortem blood specimens and liver homogenates were tested. The cutoffs chosen for the coated tube RIA using this approach were 5 ng/mL morphine, 25 ng/mL methamphetamine, 500 ng/mL benzoylecgonine, and 500 ng/mL secobarbital. These cutoffs corresponded to a sensitivity and specificity of 94% and 96% for opiates, 93% and 86% for amphetamines, 91% and 96% for cocaine and metabolites, and 91% and 87% for barbiturates. The double-antibody RIAs were run on the same specimens with cutoffs of 20 ng/mL morphine, 50 ng/mL methamphetamine, 50 ng/mL benzoylecgonine, and 1,000 ng/mL phenobarbital. The sensitivity and specificity values for the double-antibody immunoassay were more than 99% and 96% for opiates, 83% and 89% for amphetamines, 98% and 97% for cocaine, and 79% and 95% for barbiturates (17). Other procedures that also include amphetamines were discussed above.

Chromatographic Procedures

A detailed overview of chromatographic techniques for the determination of amphetamines can be found in the review by Kraemer and Maurer (68). The detection of Ecstasy (MDMA) and related compounds in alternative matrices was reviewed in 1999 by Kintz and Samyn (69). Only a few new procedures for determination of amphetamines in blood have been published in the meantime. In

a study by Bogusz et al (70), amphetamine, methamphetamine, MDA, MDEA, and MDMA, as well as eight other sympathomimetic amines, were extracted from serum or urine with ether, derivatized with phenylisothiocyanate, and subjected to HPLC separation in isocratic mode. Two detection techniques were applied: atmospheric pressure chemical ionization (APCI) MS and UV spectrometry as diode array detection (DAD) or single wavelength detection at 250 nm. The derivatives were well separated and showed good chromatographic behavior. Full-scan mass spectra of drugs examined by means of APCI with collision-induced dissociation (APCID) contained protonated molecular ions (M+H)+ and fragments typical for particular drugs. APCI-LC-MS appeared very selective for differentiation of all drugs involved. The quantitation with APCI was performed using SIM of (M+H)+ ions and selected fragments of drugs involved and their deuterated analogues. The limits of detection ranged from 0.001 mg/L (methamphetamine, MDMA, and MDEA) to 0.005 mg/L (amphetamine and MDA). Using HPLC-DAD, the spectra of MDMA and MDE were practically identical, with maxima of 236 to 240 nm. This lack of selectivity justifies the use of other detectors, such as MS. Other amphetamines showed slightly different spectra, with maxima of 245 to 250 nm. The limits of detection in UV detection amounted to 0.01 to 0.03 mg/L (single wavelength detector at 250 nm) or 0.05 to 0.1 mg/L (DAD) (70). Amphetamines are relatively volatile. Thus, headspace solid-phase microextraction was also used for GC-MS determination of fenfluramine, amphetamine, and methamphetamine (51). Details of this procedure can be found in Table 1.

COCAINE

Cocaine was the second most frequent drug seized in Germany in 1999, but the number of cocaine users who were detected while driving was small (3). The reasons for this discrepancy have not been elucidated. Detection of cocaine and its selected metabolites benzoylecgonine, EME, and cocaethylene has already been discussed as part of screening procedures. If evidence of recent smoking of crack is required, special procedures including the detection of anhydroecgonine methyl ester (methylecgonidine, AEME) have been proposed. Toennes et al (71) used a procedure consisting of mixed phase SPE and GC-MS after tert-butyldimethylsilylation for detection of AEME in serum. Special care had to be taken to prevent volatile AEME from evaporating. Recently, norcocaine, as a primary pharmacologically active metabolite of oxidative metabolism of cocaine, has been found worth monitoring, not only in postmortem blood samples (46,72). Other interesting aspects of the elimination of cocaine and metabolites in plasma, saliva, and urine after repeated oral administration have been published by Jufer et al (72). They observed a terminal elimination phase for cocaine metabolites, with half-life estimates ranging from 14 to 52 hours. These terminal elimination half-lives greatly exceeded previous data from studies of acute cocaine administration. These data suggest that cocaine accumulates in the body with chronic use, resulting in a prolonged terminal elimination phase for cocaine and metabolites.

Immunoassays

As discussed above, cocaine abuse can be screened for in blood by immunoassay methods. Because benzoylecgonine is also a main compound in blood after intake of cocaine, antibodies designed for its analysis of urine may also work for plasma analysis. Moore et al (20) included ELISA and coated tube RIA for detection of benzoylecgonine in their screening of postmortem blood samples.

Chromatographic Procedures

Most of the procedures published in the past 3 years for determination of cocaine and its metabolites in blood were LC procedures employing DAD (73), fluorescence detection (40), or mass selective detection (47,56). GC-MS methods were more seldom published (16,46). Chasin and Midio (16) investigated the coingestion of cocaine and ethanol, which is known to increase the risk of morbidity and mortality by the formation of the transesterification product cocaethylene. The aim was to study the role of ethanol as an agent of interaction in lethal cocaine intoxication and to establish its influence in postmortem cocaine concentrations. They developed and validated a GC-MS method for quantification of cocaine and its biotransformation products benzoylecgonine, EME, and the biomarker of the interaction, cocaethylene, in whole blood. The GC positive-ion chemical ionization MS method of Spanbauer et al (46) also included the detection of norcocaine. More details on these procedures can be found in Table 1.

MISCELLANEOUS

In this section some information was gathered on the determination of LSD, GHB, PCP, and psilocin in blood using immunoassays or chromatographic techniques. These analytes are seldom included in DUID case investigations, probably because there are no satisfactory analytic methods. Nevertheless, they can markedly impair

driving ability and are of forensic interest. Because few papers have been published on this topic in the time frame of this review, the papers on a particular drug are concisely discussed, without further subdivision.

LSD

LSD can be screened for by ELISA and by coated tube RIA (20). Confirmation can be achieved by different chromatographic techniques. Reuschel et al (74) recently reviewed advances in chromatographic and MS methods for the determination of LSD and its metabolites in physiologic specimens. They concluded that detecting LSD continues to be a challenge for toxicology laboratories due to the very low concentrations. However, they showed that immunoaffinity extraction, CI-GC-MS and MS-MS detection, and LC-MS techniques have proven particularly effective for this purpose. In addition, a major metabolite of LSD, 2-oxo-3-hydroxy-LSD, has been identified and found to be present in far higher concentrations than LSD in most LSD-positive urine samples. Recently, Rohrich et al (75) determined LSD in blood (and hair samples) by HPLC with fluorometric detection after extraction with immunoaffinity extraction units that are commercially available for urine analysis (LSD ImmunElute).

GHB and Gamma-Butyrolactone

In the past few years there has been an increase in the abuse of GHB and gamma-butyrolactone (GBL). The abuse of this "liquid Ecstasy" stems primarily from its euphoric and sedative properties, but these substances are also misused by bodybuilders as steroid alternatives. Recently, there has also been an increase in the use of GHB and GBL in crimes of drug-facilitated sexual assault. GHB is a federally controlled Schedule I substance in the United States. Also, in DUID cases, GHB could be detected (32).

Immunoassays for GHB or GBL detection are not available. Therefore, HS-GC-FID (34) or GC-MS (32,34) assays have been developed for determination. LeBeau et al (34) determined GHB directly after liquid–liquid extraction or after cyclization of GHB to GBL. Both extracts were screened using automated headspace GC-FID. GHB-d6 or GBL-d6 was used as IS. Full-scan GC-MS quantitation was performed. Its sensitivity has proven useful for the toxicologic investigation of cases of drug-facilitated sexual assault. Couper and Logan (32) described a simple liquid–liquid extraction procedure for the analysis of GHB in biologic fluids without conversion to its lactone GBL. After derivatization to its di-TMS derivative, GHB was detected using GC-MS and diethylene glycol as the IS. GHB was detected also in the blood of two DUID cases. It has been documented that GHB is artifactually elevated in postmortem blood specimens (76). In a letter to the editor, LeBeau et al (77) warned about elevated GHB levels in citrate-buffered antemortem blood samples. Even if the particular circumstances are not quite clear, these authors stated that they will not report positive GHB results from such blood samples unless a urine specimen from the individual was also collected, supporting the GHB result. This seems to be necessary to avoid false-positive GHB results.

Phencyclidine

As discussed above, Moore et al (20) tested an automated microplate immunoassay for screening of nine classes of drugs of abuse in postmortem blood and tissues. An ELISA assay for PCP was performed as well as the conventional RIA. They confirmed the immunoassay results using GC-MS. Ishii et al (78) described a method for determination of PCP using GC/surface ionization organic MS (SIOMS) producing much higher sensitivity than the conventional GC/electron impact MS. Thus, they established a detailed procedure for measurements of PCP in body fluids by both full-scan and SIM of SIOMS using pethidine as an IS. Good linearity was found in the range of 0.25 to 10 ng/mL of whole blood or urine, when measured in the full-scan mode, and in the range of 0.025 to 1.0 ng/mL of whole blood by SIM. They could detect PCP from rat whole blood 2 hours after subcutaneous injection of PCP (1 mg/kg) by mass chromatography. The mean PCP concentration in rat blood was 48 ng/mL. This method is sensitive but not in widespread use in forensic laboratories. Therefore, its use in daily laboratory work is not very likely. In addition, applicability of this method to authentic cases has not been shown.

Psilocybin and Psilocin

Psilocybin and psilocin, active compounds of some so-called magic mushrooms (e.g., *Psilocybe cubensis*, *Panaeolus subalteatus*, or *Stropharia coronilla*), have hallucinogenic effects. Sticht and Kaferstein (39) reported the detection of psilocin in serum in a subject after magic mushroom intake. The concentration of psilocin in serum was too low for detection with REMEDi HS. Therefore, determination by a GC-MS procedure in the

SIM mode was used. After enzymatic cleavage of conjugates, the free psilocin was extracted using SPE and silylated before GC-MS determination. The concentration of free psilocin was 18 ng/mL; that of total psilocin was 52 ng/mL of serum. Detection of psilocybin by GC-MS is not possible because of its phosphoric acid ester structure. It is to be expected that LC-MS will be a suitable technique for determination of psilocybin and psilocin in biomatrices (31,79).

CONCLUSIONS

DUID is a growing concern in industrialized countries, and the number of blood samples that have to be analyzed in forensic laboratories is growing steadily due to more restrictive traffic laws. This has led to developments in the analytic toxicology of various drugs. In the past few years, progress has been made in the use of immunologic methods for screening for drugs of abuse in blood. GC-MS remains the gold standard for confirmation of positive immunoassay results and is the most widely used technique in forensic laboratories worldwide. Exceptions include confirmation of the glucuronides of morphine, which are more suitable for LC-MS analysis. More LC-MS procedures have been developed for screening and confirmation of the drugs of abuse in blood. However, there is still a need for validated procedures to increase the role of LC-MS in this field.

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