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Review

Determination of drugs of abuse in blood

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Abstract

The detection and quantitation of drugs of abuse in blood is of growing interest in forensic and clinical toxicology. With the development of highly sensitive chromatographic methods, such as high-performance liquid chromatography (HPLC) with sensitive detectors and gas chromatography–mass spectrometry (GC–MS), more and more substances can be determined in blood. This review includes methods for the determination of the most commonly occurring illicit drugs and their metabolites, which are important for the assessment of drug abuse: Methamphetamine, amphetamine, 3,4-methylenedioxymethamphetamine (MDMA), *N*-ethyl-3,4-methylenedioxyamphetamine (MDEA), 3,4-methylenedioxy-amphetamine (MDA), cannabinoids (delta-9-tetrahydrocannabinol, 11-hydroxy-delta-9-tetrahydrocannabinol, 11-nor-9-carboxy-delta-9-tetrahydrocannabinol), cocaine, benzoylecgonine, ecgonine methyl ester, cocaethylene and the opiates (heroin, 6-monoacetylmorphine, morphine, codeine and dihydrocodeine). A number of drugs/drug metabolites that are structurally close to these substances are included in the tables. Basic information about the biosample assayed, work-up, GC column or LC column and mobile phase, detection mode, reference data and validation data of each procedure is summarized in the tables. Examples of typical applications are presented.

Keywords: Reviews; Drugs of abuse

Contents

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1. Introduction

The list of ''drugs of abuse'' can vary, depending on who is performing the analysis: clinical toxicology, forensic toxicology, workplace testing, doping analysis in humans and animals, or rehabilitation programs focus on different ''drugs of abuse''. In this review, only methods for the analysis of the most frequently abused illicit drugs are covered, in particular, methamphetamine (MA), amphetamine (A), 3,4-methylenedioxymethamphetamine (MDMA), *N*-ethyl-3,4-methylenedioxyamphetamine (MDEA), 3,4-methylenedioxyamphetamine (MDA), cannabinoids (delta-9-tetrahydrocannabinol, 11-hydroxy-delta-9-tetrahydrocannabinol, 11-nor-9-carboxy-delta-9-tetrahydrocannibinol), cocaine, benzoylecgonine, ecgonine methyl ester, cocaethylene and the opiates (heroin, 6-monoacetylmorphine, morphine, codeine and dihydrocodeine).

The analysis of blood samples has acquired a considerably greater value in comparison to urine investigation over the last few years, particularly in forensic toxicology. Improved sample preparation and chromatographic techniques, along with highly sensitive detectors, have lead to a decrease in the number of substances that cannot be determined in blood. Clinical and forensic questions can be solved more effectively when, in addition to the analytical results of urine samples, quantitative determination in blood can be achieved. The number of studies concerning the determination of drugs in blood (whole blood, plasma and serum) has greatly increased over the last six years, so that a review seems necessary. A large number of reviews describing the determination of drugs in blood deals with only particular substances or substance classes or special analytical systems [1–8]. The determination of lysergic acid diethylamide (LSD) in blood is not discussed in this review, as it has its own chapter in this special volume. To simplify the rapid selection of a method suitable for a given analytical problem, this review is divided according to the different drug classes.

1.1. Choice of references

The Medline database on CD-ROM (Silver Platter, Version 2.0, Boston, London, Amsterdam, 1991–1997) was chosen as the basis for the literature survey. A period of six years, from January 1991 to April 1997, was included. Only papers written in English were considered. In addition, papers from the most relevant journals on toxicological and analytical methods, published between January and April 1997, which have not yet been included in Medline, were searched. Furthermore, the ''bibliography section'' of the Journal of Chromatography between 1991–1997 was searched, because there are journals included that are not registered in Medline.

1.2. Matrix to be investigated

Today, several different biological matrices are used in clinical and forensic toxicology for the detection of drugs and poisons. Besides urine and blood as the classical matrices, hair, sweat, saliva and meconium have become important. The use of hair as a matrix will be discussed in the review of Kintz and Sachs [9], the use of sweat and saliva in the review of Kidwell [10] and that of meconium in the review of Moore et al. [11], all in this volume.

Traditionally, urine was the sample of choice for the screening and identification of unknown drugs or poisons, as the concentrations of drugs are relatively high in urine. However, the metabolites of these drugs had to be identified in addition or even exclusively. Plasma was the sample of choice for quantification. However, improvements in sample preparation, chromatography and in detector techniques have made blood accessible as a screening

matrix. Identification and quantification can be performed in one matrix. Another advantage of blood is that the matrix is relatively homogeneous, since physiological parameters vary within only narrow limits. Another great advantage of blood as a matrix is that drugs can be detected just after intake prior to metabolism and/or filtration.

The most relevant matrices to be analyzed are serum, plasma and whole blood. Difficulties arise when only aged or hemolyzed blood is available. Refs. [1,12–45] deal with these problems. Postmortem samples were analyzed in [18,20,22,26,29,33, 35–37,39,41,46,47].

Little information about the partition of drugs between plasma and red blood cells is available in the reviewed papers. Garrett et al. [48] found no significant differences in the detection of MDMA and MDA between plasma and erythrocytes of dog blood at concentrations of about 100 ng/ml. delta-9-Tetrahydrocannabinol (THC) [49] is almost 100% protein-bound, being distributed between lipoproteins and albumin at a ratio of 6:4. Very little THC enters the red blood cells. Bailey [50] determined the binding of cocaine and cocaethylene in human serum. Information about the distribution of other drugs between whole blood and plasma/serum was not available.

The stability of drugs in stored blood samples was investigated by Giorgi and Meeker [51] over a five-year period. They found that cocaine (COC) and benzoylecgonine (BZE) had poor stability. Methamphetamine was fairly stable, whereas unconjugated morphine showed wide variation throughout the study.

2. Methods for the analysis of drugs of abuse in blood

2.1. Published reviews

The detection of drugs of abuse has been reviewed with a main focus on the determination of single drug classes or analytical methods. Campíns-Falcó et al. [6] reviewed the detection of amphetamine and methamphetamine by high-performance liquid chromatography (HPLC). They focused mainly on sample clean-up and derivatization steps. Clauwaert et al. [7] surveyed the HPLC determination of cocaine and its main metabolites, paying special attention to blood, but also to other matrices. Cone and Darwin [5] reviewed the simultaneous detection of cocaine, opiates and metabolites in small volumes of biological samples (extraction, derivatization, chromatographic conditions, detection mode, data acquisition). An overview of gas chromatography–mass spectrometry (GC–MS) methods for the detection and quantitation of cannabinoids, cocaine, cocaine metabolites, amphetamines and opiates was published by Cody and Foltz [3]. Maurer [8] reviewed the systematic toxicological analysis of drugs and their metabolites by GC–MS, mostly in urine. Of the large variety of drugs that are relevant in clinical and forensic toxicology, methods in blood are given for central stimulants (cocaine, methamphetamine), opiates (6-MAM), and THC-COOH. Binder [4] extensively reviewed the analysis of ''misused'' illicit drugs and pharmaceuticals in biological fluids by LC until 1994, with a few citations from 1995. An overview of the analysis of opiates by GC–MS was given by Wasels and Belleville [2], with emphasis on the hydrolysis, extraction and derivatization of the compounds. Bronner and Xu [1] reviewed GC–MS methods for the detection of THC-COOH in biological samples. They focused mainly on derivatization, detection techniques and internal standards.

2.2. Screening methods for drugs of abuse in blood

For urine screening, usually immunoassays (IA) are used to differentiate between negative and presumably positive samples. Positive results must be confirmed by a second independent method that is at least as sensitive as the screening test and that provides the highest level of confidence in the result. Without doubt, GC–MS is the most widely used method for confirmation of positive screening tests [52–55] as it provides high levels of specificity and sensitivity. Some authors tried to establish IA prescreening methods for blood samples, often using the IAs developed for urine samples. Lillsunde et al. [12] used an immunological screening method after acetone precipitation of the plasma proteins. They found sufficient sensitivity for opiates, amphetamines and

cocaine/cocaine metabolites. The drugs were quantitated after extraction and derivatization with heptafluorobutyric anhydride (HFBA) by GC–MS. However, the procedure was not sensitive enough for low concentrations of cannabinoids. Diosi and Harvey [32] used the EMIT d.a.u. (Palo Alto, CA, USA) urine assay to screen with an autoanalyzer after methanolic precipitation for the drugs discussed in this review. They compared the results with GC–MS determinations. However, the concentrations listed in the table are mostly above the low ng/ml range, which would be necessary for sensitive detection. Asselin and Leslie [56] also used methanolic supernatants from whole blood to screen, in addition to other drugs, for amphetamines and opiates. With slight modifications of the IA procedure, they reported good results for these two compound groups. Perrigo and Joynt [40] tested the enzyme-linked immunosorbent assay (ELISA) technique on whole blood samples for COC and metabolites, cannabinoids, amphetamines and opiates. They reported an improved sensitivity to EMIT methods. Moriya and Hashimoto [13] reported a screening with TRIAGE™ (Merck, Germany; or Biosite Diagnostics, San Diego, CA, USA) after protein precipitating in whole blood with sulfosalicylic acid. However, the detection limits for the drugs in question are not low enough to exclude their presence, which is often the critical question in forensic cases. Apparently, immunological methods for screening purposes are presently not sensitive enough to cover the detection of the drugs reviewed in this paper in blood samples. Nevertheless, as will be discussed later, certain drugs can be detected with sufficient sensitivity by immunological methods, which means, in most cases, a simplification and reduction of costs. However, special sample preparation (deproteinization, etc.) is necessary for the use of urine IAs for detection in blood. In addition, confirmation of IAs is indispensable. Since there is no longer an advantage in sample preparation when using urine IAs for blood, it seems to be more reasonable to directly perform chromatographic procedures.

Neill et al. [45] described a GC–MS screening method for the identification of 120 drugs of interest to road safety. No extraction method was given. Most of the drugs reviewed here are listed with their retention times, two diagnostic ions, and ion ratios. Separate analyses were performed with underivatized, methylated and trifluoroacetylated (MBTFA) drugs. Wang et al. [57] developed a method for the simultaneous measurement of cocaine, heroin and their metabolites in plasma, saliva, urine and hair. The samples were extracted by solid-phase extraction (SPE), derivatized with N,O-bis(trimethylsilyl)trifluoroacetamide–trimethylchlorosilane (BSTFA–TMCS) and analyzed by GC–MS. Chee and Wan [58] described the separation of seventeen drugs, including codeine, methamphetamine and amphetamine using capillary zone electrophoresis. More information on this technique can be found in the review of Tagliaro [59] in this volume.

2.3. Amphetamines and designer drugs

2.3.1. Non-chromatographic methods

Simonick and Watts [17] published a study in which they used the Abbott TDx amphetamine/methamphetamine II (Irving, TX, USA) fluorescence polarization immunoassay (FPIA) method, originally designed for urine screening, to determine the level of D-methamphetamine in hemolyzed whole blood. Their blood calibration curve showed linearity in a range from 25–100 ng/ml. Comparison of the results obtained with those determined by radioimmunoassay (RIA) and GC–MS showed that the test was reliable for the screening of blood.

Capillary electrophoresis (CE) proved to be a technique with a higher separation efficiency within short analysis times. However, because of insufficient sensitivity, only a few studies on the determination of drugs of abuse have been published. Chee and Wan [58] described the separation of seventeen drugs, including codeine, methamphetamine and amphetamine using capillary zone electrophoresis (CZE). CZE and micellar electrokinetic capillary chromatography (MEKC) were used by Hyoetylaeinen et al. [60] for the determination of amphetamine and opiates in human serum. The analytes could be screened by a short-capillary method in less than 2 min. For details on the CE technique, refer to the corresponding review of Tagliaro [59] in this volume.

2.3.2. Chromatographic methods

Liquid–liquid procedures for extraction are predominant in the determination of amphetamines and designer drugs [16,61–64]. Concerning the chromatographic techniques, GC and HPLC procedures are fairly well balanced. Many different detectors have been used for HPLC. For GC, MS detection was predominant. Tables 1 and 2 highlight reported methods for this substance group.

2.3.2.1. *Gas chromatographic methods*. In the time interval examined, four GC–MS procedures [14,16,63,65] and two GC–negative ion chemical ionization (NICI)–MS procedures [61,62] were published. In addition, a method using flame ionization detection after derivatization, with trifluoroacetic anhydride, was published by Kumazawa et al. [66], with the main emphasis of the study being on the method of extraction. Cheung et al. [64] described a method for the simultaneous determination of amphetamine, methamphetamine and their hydroxylated metabolites in plasma, using a GC–nitrogen–phosphorus detection (NPD) system. Amazingly, no procedures have been reported for the detection of designer drugs in blood or serum by GC–MS.

2.3.2.2. *Liquid chromatographic methods*. Three studies have been published concerning HPLC procedures, in which the detection of the analytes was carried out by means of fluorescence detection (FLD) [67–69]. All of these studies use precolumn solid-phase derivatization to improve the detection of the analyte. Bowyer et al. [70] also carried out a precolumn solid-phase derivatization to detect amphetamine. In order to determine MDMA and its metabolites in plasma, studies have been described which used spectrophotometric detection. Garrett et al. [48] worked at a wavelength of 280 nm and detected concentrations down to 2.7 ng/ml of MDMA and 1.6 ng/ml of MDA in plasma. Helmlin et al. [71] used a diode array detector. Their limits of quantitation (LOQs) were 5 and 7 ng/ml, respectively. Michel et al. [15] used electrochemical detection (ED) for the quantitation of MDMA, MDA and MDEA in microsamples of whole blood. Fig. 1, taken from reference [15], shows LC–ED chromatograms of whole blood samples spiked with different concentrations of MDA, MDMA and MDEA and measured at different detector sensitivities. Bogusz et al. [72] also described an analytical system, using UV spectrometry and diode array detection (DAD), to determine amphetamine and its analogues in serum. In the same study, atmospheric pressure chemical ionization (APCI) MS was also used for detection. The mass spectrometric detection method turned out to be far more specific and sensitive. This topic is further discussed in the review of Maurer [73] in this volume. The separation of optical isomers of amphetamine and designer drugs are mostly carried out in urine or animal blood [74].

More detailed information about the detection of

Table 1
GC methods for the determination of amphetamine and its analogues in blood

Substance	Matrix	Detection	Extraction	Internal standard	Derivatization	Column	LOD (ng/ml)	LOQ (ng/ml)	Linearity (ng/ml)	Reference
A, MA[a]	W	MS	Extrelut	4-Methoxy-MA-d5	Heptafluorobutyl chloride	XTI-5	2	?	5–1000	[42]
A, MA	B	MS	HS–SPME	MA-d5	HFBA	PTE-5	10	?	10–2000	[14]
A, MA	B	MS	LLE	A-d3	Perfluorooctanoyl chloride	OV-1	A: 11 MA: 13	A: 22 MA: 34	A: 14–2700 MA: 15–3000	[16]
A	P	NICI–MS	LLE	A-d5	Pentafluorobenzoyl chloride	DB-5	0.035	0.05	0.04–4.8	[61]
A, MA	P	NICI–MS	LLE	A-d6, MA-d6	HFBA	DB-1	?	0.1	0.1–5	[62]
A, MA	W	FID	Bond Elute SCX	?	TFA	?	?	?	?	[66]
A, MA	P	NPD	LLE	*p*-methyl-A/*p*-methyl-MA	Propionaldehyde–$NaBH_4$	HP Ultra 1	?	?	5–500	[63]
A, MA[a]	P	NPD	LLE	*N*-Methylphenthylamine	HFBA	HP-5	1	?	1–30	[64]

[a] In addition, the hydroxylated metabolites of amphetamine and methamphetamine can be determined.

Table 2
LC methods for the determination of amphetamine and its analogues in blood

Substance	Matrix	Detection	Extraction	Internal standard	Derivatization	Mobile phase	Stationary phase	Wavelength (nm)	LOD (ng/ml)	LOQ (ng/ml)	Linearity (ng/ml)	Reference
MDMA, MAD[a]	P	DAD	Adsorbex SCX	MA	–	ACN–H_2O–H_3PO_4–hexylamine	Spherisorb ODS-1	Maxima at 200, 236, 284	?	MDA:5 MDMA:7	?	[71]
A, MA, MDMA, MDA, MDEA	S	DAD/UV	LLE	BEA or one of the amphetamines not present in the sample	Phenylisothiocyanate	NH_4 formate Buffer–ACN	Superspher Select B a ECOcart	245–250	A, MDA: 100 MA, MDMA, MDEA:50	?	5–1000	[72]
MDMA, MDA	P	UV	LLE	MDMA for MDA MDA for MDMA	–	MeOH–acetate buffer	Zorbax CN/ ODS Hyposil	280	MDA: 1.6 MDMA: 2.7	?	?	[48]
A, MA, MDMA, MDA, MDEA	S	UV	LLE	BEA or one of the amphetamines not present in the sample	Phenylisothiocyanate	NH_4 formate buffer–ACN	Superspher Select B a ECOcart	250	A, MDA: 30 MA, MDMA, MDEA:10	?	5–1000	[72]
A, MA	P	FLD	On-line precolumn extraction	2-Amino-4-phenyl-butane	Precolumn SP derivatization with a 9-fluorenyl-acetate-activated ester	Gradient elution: ACN–H_2O–SDS	Supelcosil LC-18-DB	254/305–395, ex/em	?	?	?	[68]
A	P	FLD	SDS ion-pairing extraction	–	Precolumn SP derivatization with a FMOC–L-prolyl-tagged reagent	ACN–H_2O	Supelcosil C_{18} DB	254/313, ex/em	?	?	150–1000	[108]
A	P	FLD	LLE	Tryptamine	*o*-Phthaldialdehyde and 3-Mercaptopropionic acid	Gradient elution: phosphate buffer–MeOH	Supelco LC-18	340/440, ex/em	0.2	?	11–460	[70]
A, MA	P	FLD	LLE	–	Precolumn SP derivatization with 9-fluorenyl-methylchloroformate	ACN–phosphate buffer	Nova-Pak phenyl	260/315, ex/em	?	0.5	0.5–80	[67]
A	P	FLD	–	–	Precolumn SP derivatization with a 9-fluorene-acetyl tag	Gradient elution: ACN–H_2O–SDS	LiChrospher C_{18} RP/Supelcosil LC–ABZ	254/313, ex/em	4	?	2–40	[69]
MDMA, MDA, MDEA	W	ED	LLE	MDEA	–	MeOH–sodium acetate buffer	Whatman silica Partisphere	–	1	?	1–1000	[15]
A, MA, MDMA, MDA, MDEA	S	APCI–MS	LLE	A-d10, MA-d10, MDEA-d7, MDMA-d5	Phenylisothiocyanate	NH_4 formate buffer–ACN	Superspher Select B a ECOcart	–	A, MDA: 5 MA, MDMA, MDEA:1	?	5–1000	[72]

[a]In addition, the hydroxylated metabolites of methoxyamphetamine and methoxymethamphetamine can be determined.

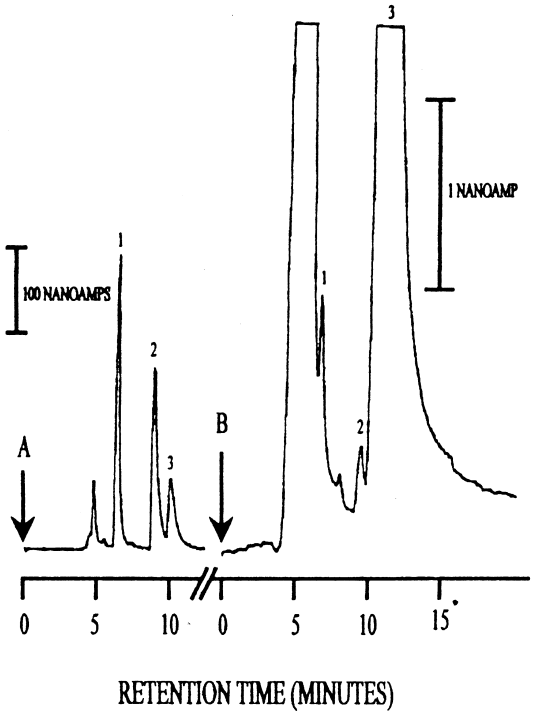

Fig. 1. LC–ED chromatograms of whole blood samples spiked with different concentrations (500 and 1 ng/ml) of MDA (1), MDMA (2) and MDMA (3), measured at different detector sensitivities (1 μA/V and 5 nA/V) (taken from Ref. [15]).

amphetamines, and about problems arising from pharmaceuticals that are metabolized to amphetamine or methamphetamine, is given in the review of Kraemer and Maurer [75] in this volume.

2.4. Cannabinoids

2.4.1. Non-chromatographic methods

No direct immunological methods, without sample pretreatment, have been reported for the cannabinoids. This is probably due to the fact that the IAs only crossreact with the THC-COOH, and that the concentrations of both the psychoactive drug and its metabolite are in the low ng/ml range. Therefore, the IAs are not sensitive enough without a concentration step. Immunological screening after acetone precipitation has been described by Lillsunde et al. [12]. They reported positive cases with 20 ng/ml THC and 5 ng/ml THC-COOH. Goodall and Basteyns [29] used a FPIA urine test prescreening method with a threshold of 25 ng/ml for presumptive positives. From 217 samples tested, they reported a predictive value of 90% for a positive screening and of 99% for a negative screening. The cross-reactivity of the FPIA to THC is very low; consequently, single consumption would probably not be detected, even in a narrow time frame with sample collection. In conclusion, immunological screening as a prescreening method for exclusion of cannabinoid consumption cannot be recommended.

2.4.2. Chromatographic methods

2.4.2.1. *Gas chromatographic methods*. In contrast to the amphetamines and their designer drug analogues, the cannabinoids are mainly determined by GC–MS. Table 3 shows detailed data of the reviewed papers on this topic. Moeller et al. [76] quantitated THC and THC-COOH simultaneously in serum. The extraction was carried out by SPE. The primary metabolite, 11-hydroxy-THC (OH-THC), can be determined within the same procedure, also using deuterated THC as the internal standard, because the hydroxy analogue is not commercially available in its deuterated form at present. Fig. 2, taken from this reference, shows typical single ion monitoring (SIM) chromatograms of extracts of serum samples spiked with THC, OH-THC and THC-COOH. Kemp et al. [77] used liquid–liquid extraction with hexane–ethyl acetate for the determination of THC, OH-THC, THC-COOH, cannabinol, cannabidiol and four other metabolites, with excellent sensitivity and reproducibility. Goodall and Basteyns [29] also used GC–MS for quantitation after liquid–liquid extraction. The procedure was successfully used for aged whole blood. They used an additional prescreening step with FPIA and discussed the stability of the cannabinoids in aged blood samples. The combination of GC and tandem mass spectrometry (MS–MS) further improves the sensitivity 10–100-fold, compared to SIM methods. Nelson et al. [78] described examples of the application of GC–MS and GC–MS–MS methods for the detection of THC and its active metabolite, OH-THC, in plasma, down to limits of detection (LODs) of 0.01 and 0.02 ng/ml, respectively. Shaw et al. [79] measured THC and THC-COOH using a high energy dynode detector system, retrofitted to a GC–

Table 3
GC methods for the determination of cannabinoids in blood

Substance	Matrix	Detection	Extraction	Internal standard	Derivatization	Column	LOD (ng/ml)	LOQ (ng/ml)	Linearity (ng/ml)	Reference
THC, THC-COOH	W	MS	Extrelut/ACN, Bond Elut Certify clean-up	Deuterated analogues	BSTFA	CP-Sil5-CB	pg level	?	0–100	[41]
THC, THC-COOH, OH-THC	S	MS	Bakerbond C_{18}	Deuterated analogoues	Iodomethane	HP1	THC: 0.3 THC-COOH: 3	?	THC: 1–15 THC-COOH: 3–60	[76]
THC, THC-COOH, OH-THC	W,P	MS	LLE	Deuterated analogues	BSTFA–TMCS	HP 1	THC, OH-THC: 0.2 THC-COOH: 2	THC, OH-THC: 0.2 THC-COOH: 2	THC, OH-THC: 0–25 THC-COOH: 0–100	[29]
THC, THC-COOH, OH-THC[a]	P	MS	LLE	Deuterated analogues	BSTFA–TMCS	HP 5	THC: 1.6 OH-THC: 0.9 THC-COOH: 0.6	THC: 3.5 OH-THC: 2.2 THC-COOH: 1.0	0–100	[77[]
THC, OH-THC	P	MS–MS	Bond Elut Certify	Deuterated analogues	Tri-Sil TBT	HP 5	THC: 0.01 OH-THC: 0.02	THC: 0.05 OH-THC: 0.1		[78]
THC, THC-COOH	P	NICI–MS–HED	EDTA Vacutainer tubes	Deuterated analogues	TFA	Supelco SPB 5		THC, THC-COOH: 0.8	THC: 0.08–10 THC-COOH: 0.1–25	[79]

[a] More cannabinoids are detectable with this method.

Table 4
LC methods for the determination of cannabinoids in blood

Substance	Matrix	Detection	Extraction	Internal standard	Derivatization	Mobile phase	Stationary phase	Wavelength (nm)	LOD (ng/ml)	LOQ (ng/ml)	Linearity (ng/ml)	Reference
THC, OH-THC	B	ED	LLE	?	–	ACN–MeOH–sulfuric acid	Spherisorb C_8	–	1	?	THC: 2–15 OH-THC: 3–21	[30]
THC, THC-COOH	B	ED	ACN, Bond Elut Certify clean-up	*n*-Octylhydroxy-benzoate Phenylbutazon	–	ACN–H_2O–H_2SO_4	LiChrosorb RP8	–	THC: 2.5 THC-COOH: 1.0	?	0–100	[41]
THC, THC-COOH	B	UV	ACN, Bond Elut Certify clean-up	*n*-Octylhydroxy-benzoate Phenylbutazon	–	ACN–H_2O–H_2SO_4	LiChrosorb RP8	212, 220	THC: 20 THC-COOH: 10	?	0–100	[41]
THC[a]	P	HPTLC and fluorimetric scanning densitometry	C_{18} Sep-Pak	?	Dansyl chloride	Isooctane–ethyl acetate–acetic acid	Silica gel, HPTLC	340	<0.5	?	0–500	[84]

[a] More cannabinoids are detectable with this method.

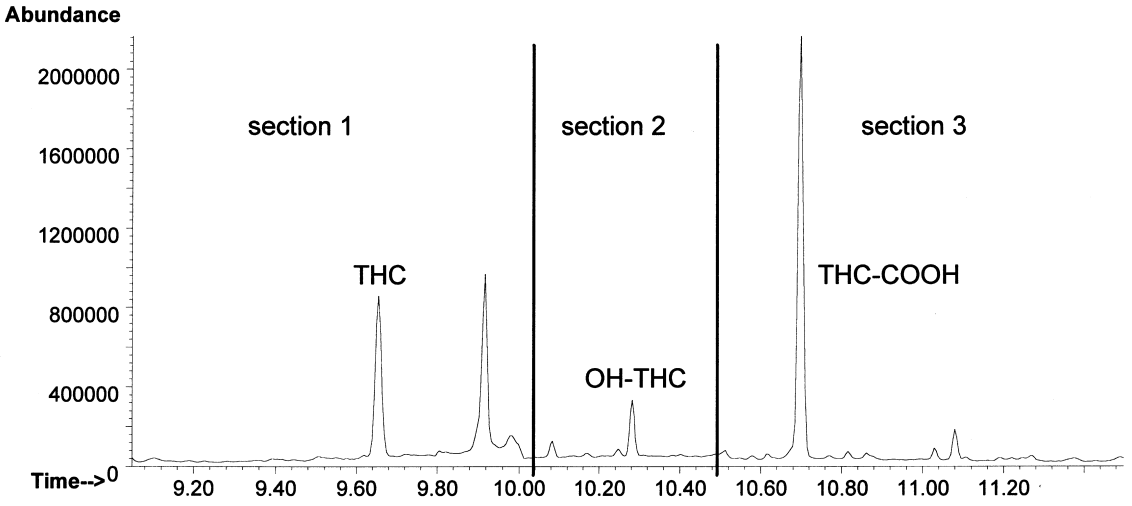

Fig. 2. Typical SIM chromatograms of extracts of serum samples spiked with THC (2 ng/ml), OH-THC (5 ng/ml) and THC-COOH (20 ng/ml). SIM section 1: *m/z* 313, 328, 316, 331; SIM section 2: *m/z* 313, 314, 358; SIM section 3: *m/z* 313, 357, 372, 375 (taken from ref. [76]).

MS system operating in the NICI mode. They improved their LOD for THC in plasma by about sixfold over that obtained with the same GC–MS system without the new detector (0.08 vs. 0.5 ng/ml).

2.4.2.2. *Liquid chromatographic methods*. Detection of cannabinoids using LC procedures are rare. Only three papers could be found in the review time frame. Table 4 summarizes important data of these papers. Gerostamoulos and Drummer [30] developed a HPLC assay to detect THC and OH-THC using ED. The detection limit for both was 1.0 ng/ml. Abdul Rahman et al. [41] used several extraction methods with different solvents, compared their recoveries and concluded that acetonitrile (ACN) deproteinization, followed by Bond Elut Certify II clean-up, gave the cleanest extracts with the best recoveries (>85%). Comparison of HPLC with UV and ED detection with GC–MS showed advantages for the latter method for reasons of sensitivity and specificity. For practical forensic purposes, a LOQ of below 1 ng/ml does not seem to be useful. Inter-laboratory studies in about 50 laboratories have confirmed the statistical analytical error at this concentration to be in the range of 45%, as predicted by the Horwitz function [80,81]. In addition, due to the long terminal half-time of the cannabinoids [82,83], a close time relation to consumption or even psychoactivity is questionable.

2.4.2.3. *Thin-layer chromatographic methods*. Alemany et al. [84] extracted cannabinoids with C_{18} Sep-Pak cartridges and derivatized them with dansylchloride. The derivatives were developed on high-performance thin layer chromatography (HPTLC) silica plates and quantified by fluorescence densitometry at 340 nm. They determined the detection limit to be less than 0.5 ng/ml. However, this technique could not assert itself against the other chromatographic techniques.

2.5. Cocaine

2.5.1. Non-chromatographic methods

Poklis et al. [85] described the application of an EMIT d.a.u. IA for urine testing on the Syva ETS plus analyzer (Palo Alto, CA, USA) for the detection of the cocaine metabolite, BZE, in human serum. The assay cut-off concentration for BZE was 50 ng/ml. Poklis et al. [85] found that the within-run and between-run precisions of the assay were suitable for qualitative clinical determinations of BZE. Also, Diosi and Harvey [32] developed a modified procedure for drugs of abuse, using extraction–precipitation of whole blood and screening with EMIT

d.a.u. reagents. The presence of drugs was subsequently confirmed by GC–MS.

The screening of blood for cocaine metabolites by enzyme IA is limited (necessarily) to the detection of BZE, because the cross-reactivity of the antibodies is tailored to the analysis of urine samples. The cross-reactivity for COC is rather poor. However, in the majority of practical forensic cases, BZE is also a main compound in blood, due to its much longer half-life. By modifying a commercial RIA for urine samples that had a high cross-reactivity for COC, Henderson et al. [86] used blood as a matrix to quantitate BZE. They found that BZE was stable for long periods of time in blood spots. Yee et al. [87] developed a procedure to estimate BZE in whole blood, using FPIA. It allowed the handling of large numbers of samples without the need to evaporate the extraction solvent. Again, it should be clearly stated that IA results must be confirmed, preferably by GC–MS.

2.5.2. Chromatographic methods

2.5.2.1. *Gas chromatographic methods*. In most cases, GC separation is followed by MS detection. The advantage of this analytical method is the simultaneous quantification of the parent drug, the metabolites and additionally of the compound cocaethylene (COCE) [27,33,35,57], which is only produced in the human body. Most authors used SPE for extraction [25,27,28,37,38,57,88,89] and the deuterated analogues as internal standards [25,27,35,37,90]. Derivatization is required to detect the metabolites. A simple, one-step procedure was developed by Marinetti-Sheff [38] that was suitable for the determination of COC, BZE, COCE and other metabolites from antemortem and postmortem blood. Postmortem samples from forensic cases were also analyzed with good sensitivity (LOD=25 ng/ml) by Corburt and Koves [37]. Virag et al. [91] and Hime et al. [33] described the detection of COC, COCE and BZE by GC–NPD. The latter authors described five cases of lethal COC intoxication. Table 5 highlights reported procedures for the gas chromatographic determination of cocaine and its metabolites.

2.5.2.2. *Liquid chromatographic methods*. The number of GC and LC procedures for the detection of COC and metabolites in blood is almost equal. The topic was reviewed by Clauwaert et al. [7]. Fernandez et al. [47] described a HPLC procedure with UV detection after SPE extraction and reported six postmortem cases, where they only found BZE. However, the only specific detector for LC is, as for GC, the mass spectrometer. The coupling of LC with MS is unfortunately still expensive and, therefore, not widely used. Therefore, only a few papers were published using this sophisticated method. Sosnoff et al. [92] found BZE in dried blood spots by LC–APCI–MS–MS. They used the residual material from blood spots of newborns (typically 10–15 μl of whole blood) that had been tested for inborn metabolic disorders and endocrinopathies. A positive RIA screening was confirmed by the described LC–MS method. For details on this method, see the review of Maurer [73] in this volume. Table 6 highlights reported procedures for the liquid chromatgraphic determination of cocaine and its metabolites.

2.6. Opiates

2.6.1. Non-chromatographic methods

For the detection of opiates in whole blood, plasma or serum, non-chromatographic methods are rare. In some papers, the use of IAs as a first step has been reported [12,13,32,40,56,93–95]. However, positive results must be confirmed by a second independent method that is at least as sensitive as the screening test and that provides the highest level of confidence in the result.

Hyoetylaeinen et al. [60] analyzed morphine analogues using CE. The rapidity of the fast MEKC method and the good separation and repeatability make the technique useful for both the screening and simultaneous determination of drugs. Petrovska et al. [96] used isotachophoresis, a special kind of microanalytical technique with capillary separation, to determine morphine in serum. However, an extraction was necessary to concentrate morphine and, furthermore, the minimum tested concentration was approximately 70 ng/ml. For details on the CE technique, refer to the corresponding review of Tagliaro [59] in this volume.

2.6.2. Chromatographic methods

Narcotic analgesics of the opiate type were preferably screened by chromatographic methods. Tables 7 and 8 highlight detailed data on the results obtained.

Table 5
GC methods for the determination of cocaine and its metabolites in blood

Substance	Matrix	Detection	Extraction	Internal standard	Derivatization	Column	LOD (ng/ml)	LOQ (ng/ml)	Linearity (ng/ml)	Reference
COC, BZE[a,d]	W	MS	Clean Screen	?	PFPA–HFIP	?	1 ng on column	?	?	[38][c]
COC, BZE, EME	W,P	MS	Clean Screen	Deuterated analogues	MTBSTFA	DB 5	?	?	2.5–2000	[25]
COC, BZE, EME, COCE	W/P	MS	Bond Elut Certify	Deuterated analogues	PFPA–PFP	HP Ultra 2	COC: 27/27[b] BZE: 13/14[b] EME: 35/59[b] COCE: 22/25[b]	COC: 35/35[b] BZE: 17/21[b] EME: 45/109[b] COCE: 37/40[b]	0–1000	[27]
COC, BZE, EME, Ecgonine	P	MS	Cation-exchange	Isopropyl-ecgonine	PFPA–PFP	DB 1	5–10	?	250–2000	[89]
COC, BZE, EME[a]	P	MS	Clean Screen	Deuterated analogues	BSTFA–TMCS	HP 1	1	?	3.1–1000	[88]
COC, BZE, EME, COCE[a,d]	P	MS	Clean Screen	Deuterated analogues	BSTFA–TMCS	HP 1	COC: 0.1 BZE: 0.5	?	1–100	[57]
COC, BZE	B	MS	Amberlite XAD 2	Deuterated analogues	Diazopropane	DB 5	COC: 2.5 BZE: 20	COC: 50 BZE: 50	COC: 50–50 000 BZE: 50–50 000	[37]
COC, BZE	B	MS	Bond Elut C_{18}	COC-d3	Iodomethane-d3 or PFPA/HFIP	CP-Sil-5	HFIP-deriv.: BZE: 20; COC: ? Iodomethane-deriv.: BZE: 40; COC:?	?	HFIP-deriv.: 25–1000 iodomethane-deriv.: 50–1000	[28]
COC, BZE, EME, Ecgonine, COCE[a]	W	MS	LLE	Deuterated analogues	Propyliodide–nitrobenzoylchloride	Econocap BP 5	?	?	10–1000	[35]
BZE, EME, Ecgonine[a]	W	MS	LLE	Deuterated analogues	Propyliodide–nitrobenzoylchloride	?	?	?	10–10 000	[39][c]
COC, BZE[d]	B	MS, MS–MS	SPEC MP3 microcolumn	Deuterated analogues	BSTFA–TMCS	?	1	5	1–100	[90][c]
COC, BZE	P	NPD	Bond Elut Certify	BZE propylester	Ox alylchloride	HP Ultra 2		4	4–2000	[91]
COC, COCE	W	NPD	LLE	Propylbenzoyl-ecgonine	–	DB 17	20	50	50–10 000	[33]

[a] More cocaine metabolites are detectable with this method.
[b] Values are given for whole blood and for plasma.
[c] Only abstract available.
[d] Opiates are also detectable.

Table 6
LC methods for the determination of cocaine and its metabolites in blood

Substance	Matrix	Detection	Extraction	Internal standard	Derivative	Mobile phase	Stationary phase	Wavelength (nm)	LOD (ng/ml)	LOQ (ng/ml)	Linearity (ng/ml)	Reference
COC, BZE[c]	P/S	DAD	Altech Toxclean	Nalorphine	–	MeOH–ACN–CH_3COONH_4	Adsorbospher HS C_{18}	225, 232, 239, 254, 289	?	?	100–1500	[100]
COC, BZE	S	DAD	Baker Narc 2	Benzoctamine	–	MeOH–phosphate buffer–TEA	SGX CN	210–400	?	50	20–6000	[65]
COC, BZE[a]	S	DAD	Clean Screen	Bupivacaine	–	THF–phosphate buffer–TFA	SPS C_8	?	1	?	50–1000	[109]
COC, COCE	S	PDA	LLE	Protriptyline	–	ACN–MeOH–phosphate buffer	Supelcosil LC-PCN	214, 230	?	10	10–2000	[110]
COC, BZE	P	UV	Bond Elut	Methaqualone	–	MeOH–phosphate buffer	LiChrospher RP 18	235	12.5	?	50–20 000	[47]
COC, BZE[a]	P	UV	Bond Elut C_8	Atropine	–	ACN–phosphate buffer–TEA	Nucleosil C_{18}	230	COC: 24 BZE: 32	COC: 70 BZE: 93	0–2000	[111]
COC, BZE	P	FLD	LLE	–	–	K-phosphate–MeOH–THF	Bio-Gel PRP 70-5	230/315, ex/em	1	?	1.5–500	[112]
BZE	W	APCI–MS–MS	LLE	*N*-Methyl-d3-BZE	–	Ammonium acetate–MeOH–H_2O	Perkin-Elmer C_{18} column		2	4–5	0–100	[92]

[a] More cocaine metabolites are detectable with this method.
[b] Only abstract available.
[c] Opiates can also be determined.

Table 7
GC methods for the determination of opiates in blood

Substance	Matrix	Detection	Extraction	Internal standard	Derivatization	Column	LOD (ng/ml)	LOQ (ng/ml)	Linearity (ng/ml)	Reference
MOR[d]	W	MS	Clean Screen	?	PFPA–HFIP	?	1 ng on column	?	?	[38][c]
MOR, COD, 6-MAM[a]	P/W	MS	ASTED	Nalorphine	PFPA	HP Ultra 1	15	?	60–1500	[21]
MOR, COD, 6-MAM, DHC	P/W	MS	Chromabond C_{18}	Methaqualone	PAA	DB-1	<5	?	?	[34]
HER, COD, MOR, 6-MAM[a,d]	P	MS	Clean Screen	Deuterated analogues	BSTFA–MCS	HP 1	1	?	1–100	[57]
HER, MOR, 6-MAM	B/P	MS	ZS DAU 020	Deuterated analogues	MBTFA	RTX 5	1	?	HER: 1–250 MOR, 6-MAM: 1–500	[23]
MOR, COD, 6-MAM, DHC	S/B	MS	Amchro C_{18} ec	Deuterated MOR, COD	PFPA–PFP	OV-1	<1	?	1–1500	[22]
MOR	B	MS	Extrelut, Bond Elut	Nalorphine	MTBSTFA or DETMDS or EDMSI	OV-1	?	?	?	[43]
COD, MOR[a]	B	MS	Bond Elut C 18	Deuterated analogues	PFPA	HP-1	8	?	COD: 30–1200 MOR: 15–600	[44]
MOR, COD, 6-MAM[d]	B	MS, MS–MS	SPEC MP3 microcolumn	Deuterated analogues	BSTFA–MCS	?	1	5	1–100	[90][c]
Opiates	B	MS, MS–MS	?	?	?	RTX-5	?	<1	?	[113][c]
DHC[a]	S	MS–MS	LLE	COD, MOR	PFPA	DB 5	?	DHC 2	DHC: 0.5–500	[98]
MOR, COD[a,b]	P	NICI–MS	?	Deuterated analogous	HFBA	?	<0.02	?	?	[114][c]
MOR, COD	B	ED	LLE	Nalorphine	HFBA	1.5% OV 17+1.95% OV 202 on Chromosorb W-HP	MOR: 40 COD: 100	?	0–10 000	[36]
MOR, COD, 6-MAM[a]	P/W	NPD	ASTED	Nalorphine	BSTFA	HP Ultra 1	15	?	60–1500	[21]
MOR, COD	B	NPD	LLE	Nalorphine	BSTFA	HP 1	MOR: 40 COD: 100	?	0–10 000	[36]
COD, DHC	P	SID	Sep-Pak C_{18}	Dimemorfan	–	DB 17	2.5	?	COD: 4.5–7.2 DHC: 3–76	[97]

[a] More opiates are detectable with this method.
[b] The glucuronides of morphine are also detectable.
[c] Only an abstract is available.
[d] Cocaine and its metabolites are also detectable.

Table 8
LC methods for the determination of opiates in blood

Substance	Matrix	Detection	Extraction	Internal standard	Derivatization	Mobile phase	Stationary phase	Wavelength (nm)	LOD (ng/ml)	LOQ (ng/ml)	Linearity (ng/ml)	Reference
MOR, COD[a,b]	P	DAD	Clean Screen	Codeine	–	Phosphate buffer–ACN	LiChrospher Diol	integration at 230	?	1	1–100	[115]
MOR, COD, 6-MAM[d]	P/S	DAD	Altech Toxclean	Flufenamic acid	–	MeOH–ACN–CH_3COONH_4	Adsorbospher HS C_{18}	225/239/254/289	?	?	100–1500	[100]
MOR[b]	P	ED	Sep-Pak C_{18}	Hydromorphone	–	MeOH–ACN–phosphate buffer–cetrimide	C_8/C_{18} μ-Bondapak	–	5.7	?	5.7–625	[116]
MOR[b]	P	ED	Sep-Pak C_{18}	?	–	NaH_2PO_4–SDS–ACN	C_{18} Nucleosil RP	–	0.6	?	0.6–28.5	[117]
MOR, COD[a]	P	ED	C_{18}	–	–	HSA–KH_2PO_4–H_3PO_4–MeOH–ACN	CP-tm-Spher C_8	–	5	?	?	[118]
MOR	P	ED	Sep-Pak/LLE	?	–	ACN–NaCl–NaAc–EDTA	C_{18} microbore	–	0.05	?	1–10	[99]
MOR[a]	P	ED	Baxter C_{18}	Naltrexone	–	MeOH–Na_2HPO_4–HSA	C_{18}/Spherisorb RP C_8	–	1.2	?	1.6–130	[119]
MOR, 6-MAM	P/W	ED	Sep-Pak C_{18}	Nalorphine	–	NH_4-Ac–ACN	μ Bondapak Phenyl	–	MOR: 1 6-MAM: 4	?	2–100	[19]
MOR[a,b]	B	ED/UV	C_{18} Sep-Pak	Nalorphine	–	ACN–SDS–NaH_2PO_4	Nova-Pak C_{18}	210	10	?	10–5000	[18]
MOR, COD[a,b]	P	ED/UV	Sep-Pak light C_{18}	?	–	phosphate buffer–SDS–ACN	Spherisorb S3 ODS 2	214	MOR: 0.14 COD: 6		MOR: 0.3–45.6 COD: 7.5–1200	[120]
MOR[b]	S	ESI–MS	Baker Ethyl SPE	Naltrexone or COD	–	Gradient elution H_2O–MeOH	Supelcosil ABZ	–	?	10	10–1000	[107]
MOR[b]	S	ESI–MS	Sep-Pak light C_{18}	External standard	–	Linear gradient ACN–formic acid	ODS C_{18}	–	0.2	0.84	0.8–2000	[104]
MOR[b]	P	FLD	Online: OSP-2	?	–	Linear gradient K-phosphate–ACN	LiChrospher 60 RP select B	210/350, ex/em	1	?	0.5–100	[121]
MOR[a,b]	P	FLD	Bond Elut C_8	Nalorphine	–	Phosphate buffer–SDS–ACN	μBondapak C_{18}	210/340, ex/em	10	?	0–2000	[122]
COD	P	FLD	Bond Elut Certify	Nalophine	–	ACN–NH_4-phosphate	YMC C_8	214/345, ex/em	5	?	10–300	[123]
MOR, 6-MAM	P	FLD	Bond Elut	Nalorphine	Dansyl chloride	*n*-hexane–2-propanol–NH_3	Spherisorb S3W, Spherisorb 3 CN	340/500, ex/em	?	Mor: 10 6-MAM: 25	MOR: 10–250 6-MAM: 25–250	[102]
MOR[b]	S	FLD	Bond Elut C_8	?	–	Isocratic steps ACN–TEAP buffer	Nucleosil 5 C_{18}	245/345, ex/em	<5	?	10–1000	[101]
MOR[b]	P	FLD	Bond Elut C_8	Dihydro-carbamazepine	–	SDS–H_3PO_4–ACN	Nova-Pak C_{18}	245/335, ex/em	5–10	?	10–500	[103]
MOR[b]	P	FLD/ED	Nonpolar/polar C_2	Noroxymorphone	–	MeOH–phosphate buffer	Nova-Pak Phenyl	210/335, ex/em	?	1	1–30	[124]
MOR, COD	B	FLD/UV	LLE	Nalorphine	–	ACN–NaH_2PO_4	Nova-Pak Phenyl	UV: 210; FLD:220/370, ex/em	MOR: 100 COD: 60	?	100–3000	[20]
MOR, COD	P	UV	Altech C_{18}	Quinine	–	MeOH–ACN–NH_4-acetate	Adsorbosphere HS C_{18}, ODS	241	MOR: 200 COD: 100	?	177–21 300	[105]
MOR, COD[b]	P	UV	Bond Elut SCX	?	–	ACN–phosphate buffer	LiChrospher 100 CM_8/III	230, 255, 280	0.5	?	10–50	[125]
MOR[b]	P	UV	Sep-Pak C_{18}	Hydromorphone	–	ACN–SDS–NaH_2PO_4	Bondapak C_{18}/ Corasil, Nova-Pak C_{18}	210	?	3.8	3.8–151	[126]
MOR, COD, 6-MAM	B	UV/ED	LLE	Nalorphine	–	ACN–NaH_2PO_4	Nova-Pak Phenyl	210	MOR: 100 COD: 60 6-MAM: 10 (ED)	7	500–3000	[127]

[a] More opiates are detectable with this method.
[b] The glucuronides of morphine are also detectable.
[c] Only an abstract is available.
[d] Cocaine and its metabolites are also detectable.

Due to the varying chemical nature of the compounds to be determined, with different lipid solubilities (e.g. basic, phenolic and/or amphoteric character), extraction methods are the most critical point. For ''general unknown analysis'', which is usually performed in urine in clinical and forensic toxicology, liquid–liquid extraction (LLE) is preferred. For the target analysis of blood for specific drugs, SPE is used more frequently.

2.6.2.1. *Gas chromatographic methods*. In GC–MS analysis, derivatization is required to overcome the poor chromatographic behavior of morphine. Silylation or fluoroacetylation are the preferred methods. An interesting method for the determination of opiates, in plasma and whole blood, using automated sample preparation, was described by Krogh et al. [21]. They used on-line dialysis as a purification step. The authors quantitated the substances using GC–NPD and GC–MS in parallel.

Geier et al. [34] compared different SPE phases and the usefulness of precipitation as a pretreatment method for the simultaneous determination of morphine, 6-MAM, codeine and dihydrocodeine (DHC). Seno et al. [97] compared a GC–MS procedure with GC–NPD after the same sample preparation for the detection of codeine and DHC. They found that their GC–MS procedure was ten-times more sensitive. Wang et al. [57] described a method that was used for the analysis of plasma, saliva, hair and urine. The detection of heroin in plasma was described by Goldberger et al. [23]. The heroin disappeared with a half-life of approximately 6.3 min. Therefore, the detection of heroin can be useful in clinical studies, but not in forensic samples. DHC, which is an important drug of abuse in a number of countries, can be determined with sufficient sensitivity together with other opiates by GC–MS [22,34,97] or GC–MS–MS methods [98]. In Fig. 3, taken from this reference, daughter-ion MS–MS spectra of pentafluoro-1-propanol (PFP)-derivatized DHC, codeine, dihydromorphine and morphine are shown. Lee and Lee [36] used GC–ED after derivatization with HFBA, as well as NPD detection after derivatization with BSTFA, for the determination of morphine and codeine in blood and bile. They stated that both methods were equally sensitive. However,

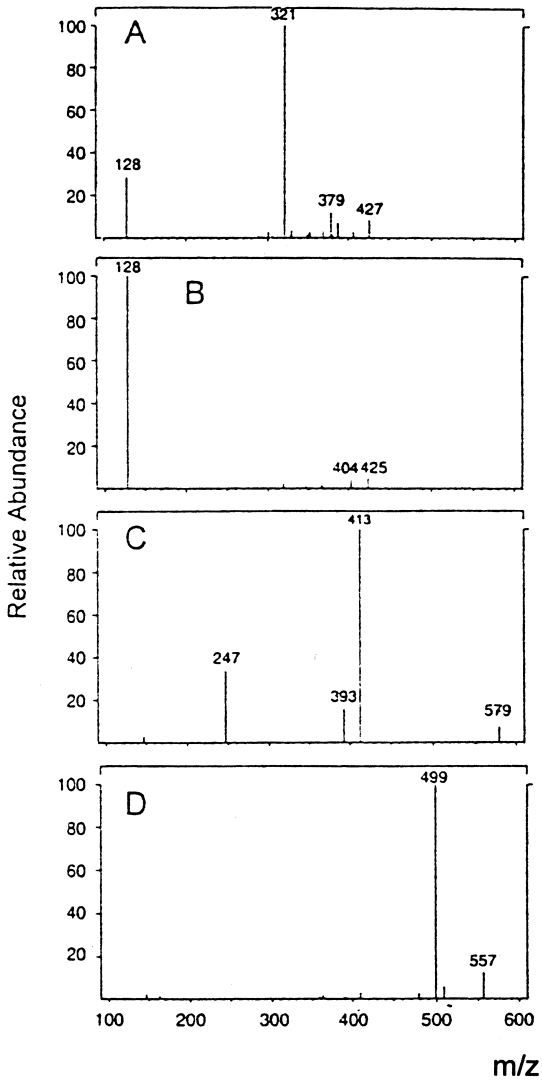

Fig. 3. Daughter-ion MS–MS spectra of (A) 6-PFP-DHC (parent ion 427), (B) PFP-codeine (parent ion 425), (C) di-PFP-dihydromorphine (parent ion 579) and (D) di-PFP-morphine (parent ion 557), measured in extracts of spiked serum samples (taken from ref. [98]).

as can be seen clearly in Table 7, MS methods are preferable, due to their higher specificity [21,97].

2.6.2.2. *Liquid chromatographic methods*. LC procedures are more often used for the determination of opiates than GC–MS methods. LC methods in combination with ED or FLD are comparable in sensitivity with GC–MS methods, and have the

advantage that they additionally cover the glucuronides of morphine. However, the specificity of GC–MS methods cannot be reached.

An extremely sensitive method for the detection of endogenous morphine in plasma (80 pg/ml) using HPLC and ED was described by Liu et al. [99]. The stability of 6-MAM in frozen samples and in samples at room temperature was studied, with good recoveries and sensitivity (1 ng/ml) [19]. A comparative study of different SPE methods for the opiates, COC and BZE was performed by Theodoridis et al. [100]. They tested nine different SPE cartridges, and found that Alltech Toxiclean gave the best results. In a variety of methods, low sample volume [101–103] or a short analysis time [104,105] and good sensitivity are emphasized.

It should be stated that mass spectrometric detection is still more specific than most of the LC detectors used, such as UV, DAD or FLD. As described above, the coupling of LC with MS is still expensive and not widely used. Nevertheless, three publications [104,106,107] appeared in the last two years, proving that LC–MS is the method of choice, if the glucuronides of morphine are to be covered. In all other cases, GC–MS is preferable.

3. Conclusions and perspectives

The detection of drugs of abuse in urine, the primary goal in the eighties, has grown to industrial dimensions, mostly due to the availability of immunoassays. With the development of more specific, especially chromatographic methods with high sensitivity, the interest of forensic and clinical toxicologists has focused on other biological fluids, particularly blood, hair, sweat, saliva and meconium. The complex metabolism of these drugs in the human body has serious consequences for their exact detection and quantitation.

Depending on the chemical nature of the drugs to be detected, tendencies to special sample preparations, separation methods and detection methods seem to crystallize. The availability of deuterated analogues of the drugs of abuse has really pushed the GC–MS methods, because steps that were critical previously, such as sample pretreatment, became less important. Therefore, GC–MS is the method of choice for the identification and quantification of most drugs of abuse. The exceptions are the glucuronides of morphine, which cannot be detected by GC–MS. In this case, liquid chromatographic techniques are preferable. The method of choice for this drug metabolite is the coupling of LC with MS.

Furthermore, the limited amount of samples has to be taken into account. This is an additional reason against splitting samples for screening and subsequent confirmation using different methods. A universal procedure for the screening of drugs of abuse in blood still does not exist. However, with the further improvement of extraction, separation and detection techniques, such a procedure will possibly be available in the future.

4. List of abbreviations

A	amphetamine
ACN	acetonitrile
APCI–MS	atmospheric pressure chemical ionization mass spectrometry
ASTED	automated sequential trace enrichment of dialysate
B	blood
BEA	*N*-benzyl-1-phenylethylamine
BSTFA	*N*,*O*-bis(trimethylsilyl)trifluoroacetamide
BZE	benzoylecgonine
COC	cocaine
COCE	cocaethylene
COD	codeine
CZE	capillary zone electrophoresis
DAD	diode array detection
DETMDS	diethyl tetramethyldisilazane
DHC	dihydrocodeine
ED	electrochemical detection
EDMCS	ethyl dimethylchlorosilane
EDMSI	ethyl dimethylsilylimidazole
EME	ecgonine methyl ester
ESI–MS	electrospray ionization mass spectrometry
FLD	fluorescence detection
FMOC	9-fluorenyl methyl chloroformate
FPIA	fluorescence polarization immunoassay
HED	high energy dynode detector

HER	heroin
HFBA	heptafluorobutyric anhydride
HFIP	hexafluoroisopropanol
HPLC	high-performance liquid chromatography
HPTLC	high-performance thin-layer chromatography
HSA	heptane sulphonic acid
HS-SPE	head space solid-phase microextraction
IA	immunoassay
LLE	liquid–liquid extraction
LOD	limit of detection
LOQ	limit of quantitation
MA	methamphetamine
6-MAM	6-monoacetylmorphine
MBTFA	*N*-methyl-bis-trifluoroacetamide
MDA	3,4-methylenedioxyamphetamine
MDEA	*N*-ethyl,3-4-methylenedioxyamphetamine
MDMA	3,4-methylenedioxymethamphetamine
MeOH	methanol
MEKC	micellar electrokinetic capillary chromatography
MOR	morphine
MS	mass spectrometry
MTBSTFA	*N*-methyl-*N*-(*tert*.-butyldimethylsilyl)-trifluoroacetamide
NICI–MS	negative ion chemical ionization mass spectrometry
NPD	nitrogen–phosphorous detection
OH-THC	11-hydroxy-delta-9-tetrahydrocannabinol
P	plasma
PAA	propionic acid anhydride
PDA	photodiode array
PFP	pentafluoro-1-propanol
PFPA	pentafluoropropionic anhydride
S	serum
SDS	sodium dodecyl sulphate
SID	surface ionisation detection
SIM	single ion monitoring
SP	solid-phase
SPE	solid-phase extraction
TEA	triethylamine
TEAP	tetraethylammonium phosphate
TFA	trifluoroacetic anhydride
THC	delta-9-tetrahydrocannabinol
THC-COOH	11-nor-9-carboxy-delta-9-tetrahydrocannabinol
THF	tetrahydrofuran
TMAH	tetramethylammonium hydroxide
TMCS	trimethylchlorosilane
UV	ultraviolet
W	whole blood

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