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Review

Determination of amphetamine, methamphetamine and amphetamine-derived designer drugs or medicaments in blood and urine

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Abstract

This paper reviews procedures for the determination of amphetamine, methamphetamine and amphetamine-derived designer drugs or medicaments in blood and urine. Papers published from 1991 to early 1997 were taken into consideration. Gas chromatographic and liquid chromatographic procedures with different detectors (e.g., mass spectrometer or diode array) were considered as well as the seldom used thin-layer chromatography and capillary electrophoresis. Enantioselective procedures are also discussed. A chapter deals with amphetamine-derived medicaments, e.g. anoretics, antiparkinsonians or vasodilators, which are metabolized to amphetamine or methamphetamine. Differentiation of an intake of such medicaments from amphetamine or methamphetamine intake is discussed. Basic information about the biosample assayed, internal standard, work-up, GC column or LC column and mobile phase, detection mode, reference data and validation data of each procedure is summarized in Tables. Examples of typical applications are presented.

Keywords: Reviews; Amphetamine; Methamphetamine

Contents

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1. Introduction

Amphetamine (AM, *R,S*-1-phenyl-2-propanamine) and methamphetamine (MA, *R,S*-*N*-methyl-1-phenyl-2-propanamine) are powerful stimulants of the central nervous system. They are drugs of abuse as well as doping agents in sports. The *S*-(+)-enantiomers of AM and MA have five times more psychostimulant activity than the *R*-(−)-enantiomers. Methylenedioxy derivatives of amphetamine or methamphetamine (so called designer drugs) are abused to enhance understanding, communicativeness and empathy, while hallucinogenic effects are rare. Nichols [1] described these substances as entactogens, a new drug class different from hallucinogenic phenylethylamines and phenylpropanamines. N-Substituted derivatives are therapeutically used as anorectics, antiparkinsonians or vasodilators. The structures of N-substituted amphetamine derivatives are shown in Fig. 1a and the structures of N-substituted methamphetamine derivatives are shown in Fig. 1b. The structures and the main metabolic pathways of methylenedioxyphenylalkylamine designer drugs are shown in Fig. 2.

Immunoassays are frequently used for urine screening for amphetamines in order to differentiate between negative and presumptively 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, gas chromatography–mass spectrometry (GC–MS) is the most widely used method for confirmation of positive screening tests [2–6], since it provides high levels of specificity and sensitivity. The mandatory Guidelines for Federal Workplace Drug Testing in the US also demand GC–MS as confirmation method [7]. Besides GC–MS, further methods like GC with other detectors, high-performance liquid chromatography (HPLC), high-performance thin-layer chromatography (HPTLC) or capillary electrophoresis (CE) have been used.

The great number of publications on amphetamine analysis published in the last five years indicates that it was necessary to improve the methods of extraction and derivatization, and the instrumental techniques. Reports on false positive MA results by GC–MS stimulated these efforts.

In this paper procedures are critically reviewed for the determination of amphetamine, methamphetamine and amphetamine-derived designer drugs or medicaments in blood and urine. Detection in alternative matrices like hair [8], sweat and saliva [9] or meconium [10] is discussed in other reviews in this Special Volume.

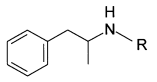
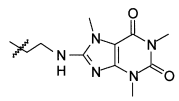
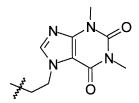
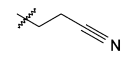
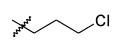
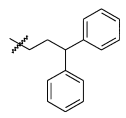
1.1. Choice of the references

The reviewed references were selected by on-line searching the Medline database, the Chemical Abstract Services and the Current Contents. The period from January 1991 to early 1997 was taken into consideration. Only papers written in English were considered.

2. GC and LC procedures for the determination of amphetamine, methamphetamine and amphetamine-derived designer drugs

GC and LC procedures for the determination of amphetamine, methamphetamine and amphetamine-derived designer drugs published in the last five years are critically reviewed in this chapter. The principal information on each procedure is summarized in Tables 1 and 2 to simplify the rapid selection of a method suitable for an actual analytical problem. The information, whether a paper deals with a quantitative assay, can be taken from the ‘‘Valida-

(a)

	R
Amphetamine	**H**
Amfetaminil	
Clobenzorex	
Ethylamphetamine	C_2H_5
Fencamine	
Fenethylline	
Fenproporex	
Mefenorex	
Prenylamine	

(b)

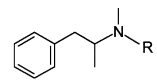
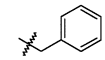
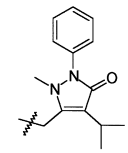
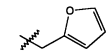
	R
Methamphetamine	**H**
Benzphetamine	
Dimethylamphetamine	CH_3
Famprofazone	
Furfenorex	
Selegiline	

Fig. 1. (a) Structures of amphetamine and amphetamine-derived medicaments. (b) Structures of methamphetamine and methamphetamine-derived medicaments.

tion'' 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 non-proprietary names (INNs) or the common names. If metabolites were determined additionally ''+ M'' is given in the ''Compound'' column. If further drugs were determined ''a.o.'' (''and others'') was added. The kind of matrix used is given in the ''Sample'' column (B: blood, P: plasma, S: serum, U: urine etc.). Since the selection of the internal standard (I.S.) is of importance for the precision of a method, this information is given in the ''Internal standard'' column. Presence of drug, used as I.S., in the sample must be excluded. For MS procedures, stable isotopes are the most suitable I.S.s, since they have the same analytical properties as the corresponding analyte.

The sample preparation is concisely summarized in the ''Work-up'' column. The principal information on the GC column or LC column and mobile phase as well as on the detection mode is listed. Validation data like recovery (REC), limit of detection (LOD) or linearity (LIN) are summarized for easy estimation, whether a procedure is suitable to solve an actual toxicological case. The limit of quantification (LOQ) is only given, if not identical to the lowest linearity value. Since the precision of all the reviewed procedures was better than 20%, as recommended for analyses in biosamples, these data were omitted to save space.

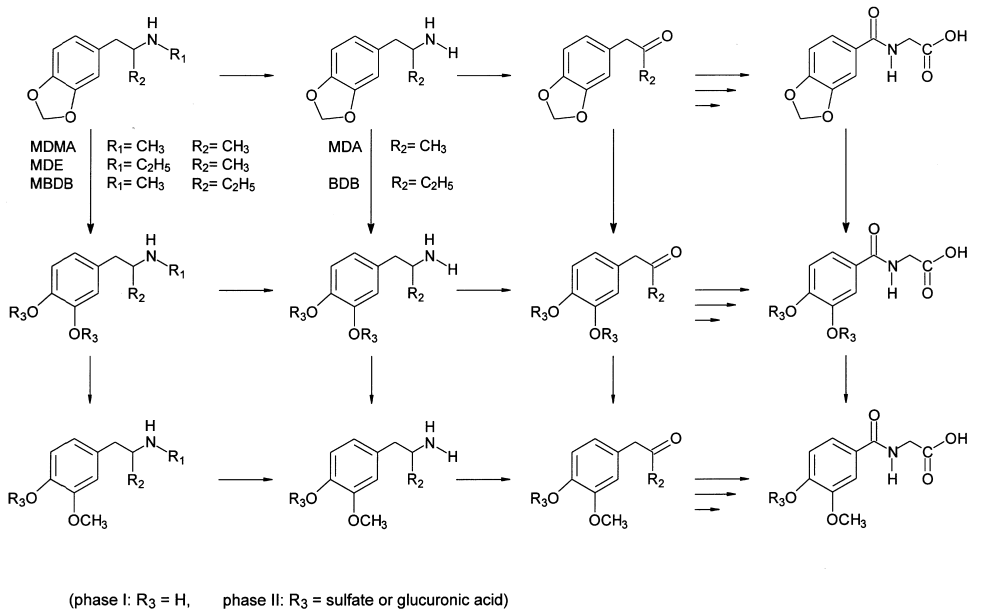


Fig. 2. Metabolic pathways of the methylenedioxyphenylalkylamine designer drugs MDMA, MDE(A), MDA, MBDB and BDB. The glycine conjugates were only formed by the propylamines. The hydroxy metabolites were excreted as glucuronide or sulfate conjugates. (Taken from Ref. [50]).

2.1. Achiral procedures

2.1.1. GC procedures

Papers on GC procedures with different detectors for the determination of amphetamines are reviewed in this chapter. Papers on this topic published before 1991 were reviewed in 1992 by Cody [4], who discussed the determination of methamphetamine enantiomer ratios in urine by GC–MS and by Maurer [5], who reviewed the systematic toxicological analysis of drugs and their metabolites by GC–MS. In 1994 Goldberger and Cone [3] reviewed confirmatory tests for drugs in the workplace by GC–MS. Two papers published after 1991 concerning amphetamines are discussed therein. In their 1995 review on the screening for drugs of abuse Braithwaite et al. [2] surprisingly considered only one reference more recent than 1991 concerning chromatographic procedures for amphetamines.

2.1.1.1. *Sample preparation*. Suitable sample preparation is an important prerequisite for chromatography in biosamples. It involves isolation and, if necessary, cleavage of conjugates and/or derivatization of the amphetamines and their metabolites. Isolation was performed by liquid–liquid extraction (LLE) usually at an alkaline pH, at which the amphetamines are unionized [11–29] or by solid-phase extraction (SPE) [30–40]. Further details on LLE and SPE are discussed in the review of Franke and de Zeeuw [41] in this Special Volume.

Cleavage of conjugates is not necessary, if detection of only unchanged AM or MA is required. However, for the detection of designer drugs in urine, cleavage of conjugates is indispensable. The main metabolites excreted in urine are the hydroxy-methoxy metabolites, which are completely conjugated (see also Fig. 2) [25,42,43]. Studies on the metabolism of MDE [42] have shown that the hydroxy methoxy metabolite could be detected for 7–8 days after ingestion whereas the parent compound was only detectable for about 2–3 days. Similar problems arise from amphetamine-derived medicaments as discussed in Section 4. Conjugates can be cleaved by gentle but time-consuming enzymatic hydrolysis [18,20,35] in drug abuse or doping control studies. In toxicological analysis, especially in emergency cases, it is preferable to

Table 1
GC procedures for the identification and/or quantification of amphetamine, methamphetamine and amphetamine-derived designer drugs or medicaments

Compound	Sample	Internal standard	Work-up	Column	Detection mode	Reference data	Validation data	Ref.
Achiral procedures								
AM MA	U	MA-d5	HS-SPE	FSC DB-1 40-250/25°C	CI, SIM	RI, FI	REC: ? LIN: 200–100 000 ng/ml LOD: 100 ng/ml	[53]
AM MA	U	MA-d8	SPE	FSC DB-5 60-260/25°C	CI, SIM		REC: ? LOD: 2.4 ng/ml (MA) 8.6 ng/ml (MA)	[38]
AM MA a.o.	U	MA-d8	SPE, HFB	FSC DB-5	EI, Scan	MS	REC: 99–114% LIN: 50–5000 ng/ml LOD: 0.7 ng/ml (AM) 2.4 ng/ml (MA)	[39]
AM MA Hydroxy-AM Hydroxy-MA	U, B, PSM PSM	4-Methoxy-MA-d5	SPE, HFB (extractive)	XTI-5 Restek 60–290/20°C	EI, SIM	RT, FI	REC: 75–97% (U) 62–105% (B) LIN: 5–1000 ng/ml LOD: ?	[31]
AM MA	B	MA-d5	HS-SPME, HFB	FSC PTE-5 45-270/25°C	EI, SIM	FI	REC: 100% LIN: 10–2000 ng/ml LOD: 10 ng/ml	[40]
AM MA (MDMA, MDA)	U	*N*-propyl-AM	LLE, PRCF (extractive)	FSC DB-1 85-210/10°C	EI, scan, SIM	RT, MS, FI	REC: 84–120% LIN: ? LOD: 5 ng/ml LOQ: 50 ng/ml	[16]
AM MA (MDMA, MDA)	U	AM-d6 MA-d9	LLE, PRCF (extractive)	FSC DB-1 85-210/10°C	EI, scan, SIM	RT, MS, FI	REC: 88–100% LIN: ? LOD: 25 ng/ml LOQ: 100 ng/ml	[16]
AM	P	AM-d5	LLE, PFB	FSC DB-5 90-300/30°C	CI, SIM (Scan)	FI (MS)	REC: ? LIN: 0.035–4.775 ng/ml LOD: ? LOQ: 0.05 ng/ml	[17]
AM MA Dimethamphetamine Ethylamphetamine Phentermine Pseudoephedrine a.o.	U	MDMA-d5	EHY, SPE, TFA	FSC HP-5 Ultra 2 100-290/20°C	EI, scan	RRT, FI	REC: 91% (AM) LIN: ? LOD: th. conc.	[35]
AM MA Nor-selegiline	P	AM-d6	LLE, HFB	FSC DB-1 70-120-170/60; 5	CI, SIM (scan)	FI (MS)	REC: 104–110% LIN: ?–5 ng/ml LOD: ? LOQ: 0.1–0.25 ng/ml	[29]
AM MA MDMA MDA MDE	U		SPE	Supelco SPB-5 50-250/20°C	CI, SIM	FI	REC: ? LOD: 2100 ng/ml (AM) 900 ng/ml (MA)	[30]

(Cont.)

Table 1. Continued

Compound	Sample	Internal standard	Work-up	Column	Detection mode	Reference data	Validation data	Ref.
AM MA MDMA MDA MDE	U		SPE, HFB	Supelco SPB-5 50-250/20°C	EI, SIM	FI (MS)	REC: ? LOD: 10 ng/ml (AM) 9 ng/ml (MA)	[30]
AM MA MDMA MDA MDE	U		SPE, HFB	Supelco SPB-5 50-250/20°C	CI, SIM	FI (MS)	REC: ? LOD: 95 ng/ml (AM) 90 ng/ml (MA)	[30]
AM MA MDMA MDA Ephedrine Norephedrine Phentermine a.o.	B	4-Chloro-amphetamine; *N*-ethyl-3,4-methylene-dioxy-AM	LLE, HFB	FSC HP-5 120-320/15°C	EI, SIM	RT, FI	REC: ? LOD: ?	[11]
MBDB MDMA MDA MDE	U	MDMA-d5 MDA-d5 MDE-d5	LLE, TFA	FSC HP-5 85-280/30	EI, SIM	RT, FI (MS)	REC: ? LIN: 50–5000 ng/ml (MBDB) 200–1000 ng/ml LOD: ?	[12]
AM MA MDMA	U	AM-d5 MA-d9	SPE, TCA	FSC DB-5 180-250/15°C	EI, SIM	FI (MS)	REC: >65% LIN: 250–4000 ng/ml LOD: 50 ng/ml	[32]
AM MA Ethylamphetamine MDMA, MDE, MMDA, 4-methoxy-AM, trimethoxy-AM, DOET, DOB dimethoxy-AM	U B PS	4-Chloro-amphetamine	SPE, HFB	PC 2% SP-2110/ 1% SP-2510 120-210/10°C	EI, Scan	RT, MS	REC: ? LIN: ? LOD: ?	[34]
MDMA MDA, MDE Hydroxy-methoxy-MA	U F		EHY, LLE, TFA	FSC DB-5 100-300/20°C	CI, SIM	FI	REC: 66–96% LIN: 2–1000 ng/ml LOD: 2 ng/ml	[20]
MDE+M	U		AHY, LLE, AC	FSC HP-1 100-310/30°C	EI, Scan	RI, MS	REC: 65–85% LOD: 5–10 ng/ml	[25]
MDA MDMA Ephedrine Pseudoephedrine Phentermine	U	AM-d3 MA-d5	SPE, HFB, CB	FSC HP-5 60-225-260/25; 70°C 60-225-260/20, 70°C	EI, SIM (scan)	RT, FI (MS)	REC: ? LOD: ?	[36]
AM MA	U	Benzylamine	LLE, PFBS	FSC DB-5 105-270/15°C	ECD		REC: 75–95% LIN: 1–50 ng/ml LOD: 10 ng/ml	[23]
AM MA Phentermine	U	Ethyl-AM	LLE, CIF	FSC DB-5 85-165-290/20; 25°C	NPD		REC: >83% LIN: 200–? ng/ml LOD: 4–20 ng/ml	[22]

Table 1. Continued

Compound	Sample	Internal standard	Work-up	Column	Detection mode	Reference data	Validation data	Ref.
Phenmetrazine (Ephedrine, norephedrine)								
AM MA Ethylamphetamine Phentermine Pseudoephedrine a.o.		Diphenyl-amine	LLE	FSC HP-5 90-180-300/10; 30°C	FID, NPD	RT	REC: 50–100% LOD: 200–400 ng/ml	[26]
AM a.o.	B	Procaine	SPE	FSC Ultra-1 100-280/5°C	NPD	RT	REC: 48–62% LOD: 50 ng/ml	[37]
AM MA Nor-selegiline	P	1-Phenyl-2-pentylamine	LLE, PFB	FSC HP-2 90-100-190-280/10; 5; 70	NPD	–	REC: ? LIN: 1.5–70 ng/ml LOD: ? LOQ: 1.5 ng/ml	[28]
AM MA (a.o.)	U	AM-d5; 4-Phenyl-butanamine	LLE, HFB	FSC HP-5 100-180/20°C	FTIR/EI	RT, FI, IR	REC: 85–88% LIN: 100–5000 ng/ml LOD: 25 ng/ml	[24]
AM MA	U	4-Phenyl-butanamine	LLE, CDFA	FSC DB-5 100-200/20°C	FTIR	IR	REC: 80% LIN: 10–2500 ng/ml (AM) 40–2500 ng/ml (MA) LOD: 10 ng/ml	[19]
AM MA MDA MDMA Phentermine Pseudoephedrine a.o.	B		LLE	FSC BP-5 100-310/7.5°C	NPD	RRT	REC: 67–100% LOD: 50 ng/ml (MA)	[13]
AM MA Ethylamphetamine, MDMA, MDE, MMDA, 4-methoxy-AM, trimethoxy-AM, DOET, DOB dimethoxy-AM	U B PS	4-Chloroamphetamine	SPE, HRB	FSC SE-54 120-280/10°C	ECD, NPD	RT	REC: ? LOD: ?	[34]
AM MA MDMA MDA Ephedrine Norephedrine Phentermine a.o.	B	4-Chloro-amphetamine *N*-ethyl-3,4-methylene-dioxy-AM	LLE, HFB	FSC HP-5 120-320/15°C	NPD, ECD	RT	REC: 45–126% LOD: 10–100 ng/ml	[11]
Chiral procedures								
AM	U		LLE, TPC	FSC HP-5 60-175-275-320/30; 11; 20°C	EI, SIM	FI	REC: ? LOD: ?	[27]

(Cont.)

Table 1. Continued

Compound	Sample	Internal standard	Work-up	Column	Detection mode	Reference data	Validation data	Ref.
AM MA	U	AM-d6 MA-d6	SPE, TPC	FSC 5 220-245-290/5;	EI, SIM	RT, FI	REC: 88–91% LOD: ?	[33]
AM MA	U	AM-d3 AM-d6 MA-d5 MA-d6 MA-d9	LLE, MCF	FSC DB-5 FSC DB-17 90-200/5°C 160-270/3°C	EI, SIM	FI	REC: 85–92% LIN: 50–6000 ng.ml LOD: 9.5 ng/ml 6.7 ng/ml	[21]
MDMA MDA Hydroxy-methoxy-AM Hydroxy-methoxy-MA	U Brain	3,4-Methylene dioxypropyl-AM	EHY, LLE, HFBP	FSC DB-5 100-300/30°C	CI, SIM	FI	REC: 67–94% LIN: 5-1000 ng/ml LOD: ? LOQ: 5 ng/ml	[18]

cleave the conjugates by rapid acid hydrolysis [5,44]. However, the formation of artifacts during this procedure must be considered [45].

Derivatization of the amphetamines is necessary to improve their GC properties, to form more characteristic mass spectral fragment ions, to introduce halogen atoms for sensitive negative chemical ionization (NCI) or electron capture detection (ECD), to differentiate functional groups, or to form diastereomers for chiral analysis. In the reviewed papers, numerous different procedures were used (for details see Tables 1 and 2). As shown in the ''Validation'' column all derivatization procedures applied lead to similar analytical results.

Nevertheless, some authors stated that their procedures had specific advantages. Leis et al. [46] stated that the pentafluorobenzoate derivatives were superior to the fluoroacetyl derivatives with respect to sample handling due to their lower volatility. Meatherall [16] stated that the propylchloroformate derivatives were very stable and gave no interferences with sympathomimetic amines. N-Propylation by reductive alkylation lead to derivatives with excellent chromatographic properties as stated by Jacob III et al. [15]. Jonsson et al. [22] claimed as an advantage of derivatization with methylchloroformate, that it can take place under aqueous conditions during extraction thus avoiding an evaporation step. However, a washing step was necessary since the reagent caused rapid degradation of the nitrogen–phosphorous selective detector (NPD). Brooks and Smith [47] suggested a method employing preextraction aqueous acetylation, where only the amino group was acetylated whereas the hydroxy groups of e.g., ephedrine or pseudoephedrine were not acetylated. These monoacetates caused no formation of MA-like artifacts even at elevated injector temperatures.

For detection of amphetamines as part of general screening procedures in urine combined trifluoroacetylation and trimethylsilylation [35] or simple acetylation [5,25,44,48–50] were used. The molecular mass of acetyl derivatives does not increase very much, in contrast to HFB etc., so that compounds with relatively high molecular mass and several derivatizable groups can be measured with low-priced mass selective detectors with a mass range only up to 650 u. Further details on derivatization for GC are discussed in the review of Segura et al. [51] in this Special Volume.

Evaporation steps after extraction and/or derivatization may lead to loss of the amphetamines because of their high volatility. Therefore, addition of hydrochloric acid before evaporation is recommended to form less volatile hydrochlorides [34,52]. However, this can also cause problems. In our experience, traces of the acid in the GC system may impair detection of basic drugs. Evaporation of aqueous HCl requires high temperatures or longer evaporation times, so that evaporation losses of the amphetamines may increase. Use of alcoholic solutions of HCl (e.g., methanolic, isopropanolic) may help. However, it should be possible to renounce the use of HCl. Dallakian et al. [30] reported that evaporation at 48°C without addition of HCl did not lead to any loss of amphetamine. This is in accord-

Table 2
LC procedures for the identification and/or quantification of amphetamine, methamphetamine and amphetamine-derived designer drugs or medicaments

Compound	Sample	Internal standard	Work-up	Stationary phase	Mobile phase	Detection mode	Validation data	Ref.
Achiral procedures								
AM MA Ephedrine Methylephedrine a.o.	U		SPE	ODS (150×4.6 mm I.D.)	Gradient elution: ammonium acetate buffer (pH 3)– ACN	TS-MS	REC: 88–99% LOD: 50–400 ng/ml (Scan) 2–40 ng/ml (SIM)	[90]
AM MA MDA MDMA Ephedrine a.o.	S, U	Deuterated I.S.	LLE	Superspher Select B EcoCart (125×3 mm I.D.)	ACN–ammonium formate buffer (pH 3) (55:45)	APCI–MS	REC: ? LOD: 1–5 ng/ml (SIM)	[70]
AM MDE β-Phenylethylamine a.o.	B,U Gastric contents Tissues		AHY, LLE	Aluspher RP- select B (125×4 mm I.D., 5 μm)	Gradient elution: 0.0125 *M* NaOH in MeOH– aqueous 12.5 m*M* NaOH	DAD 225–350	REC: ? LOD: ?	[67]
AM Hydroxy-AM MA Hydroxy-MA	U		EHY, SPE	Microsorb MV- phenyl (250×4.6 mm I.D., 5 μm)	ACN–MeOH–phosphate buffer (pH 3) (5:15:80)	UV 215	REC: ? LOD: ?	[82]
AM MA a.o.	U		SPE, NQS	Hibar LiChrospher RP8 100 (250×4 mm I.D., 5 μm)	Phosphate buffer (pH 3, cont. methanesulfonic acid–ACN) (45:55)	UV 211	REC: ? LOD: 60 ng/ml	[83]
AM	P		SPE, 9-FA (on-line)	Supelcosil LC- -ABZ (150×4 mm I.D., 5 μm)	Gradient elution: ACN–water–SDS	FL (254/313)	REC: 100% LOD: 200 ng/ml LIN: 2000–40 000 ng/ml	[92]
AM MA	U	β-Phenyl-ethylamine	SPE, NQS	Hypersil ODS (250×4 mm I.D., 5 μm)	Gradient elution: ACN–water (propylamine)	UV 250–500 nm	REC: 100% 60% LOD: 100 ng/ml 400 ng/ml LIN: 900–9500 ng/ml (AM) 3000–30 000 ng/ml (MA)	[71]
AM	U	1-Methyl- 3-phenyl-propyl-amine	SPE, DNBT	Microsorb ODS (100×4.6 mm I.D., 5 μm)	ACN–10 m*M* phosphate buffer (pH 2.5) (55:45)	UV 220	REC: 99% LOD: 14 ng/ml LIN: 10–4000 ng/ml	[75]
AM MA	U	β-Phenylethylamine	SPE, NQS	Hypersil ODS (250×4 mm I.D., 5 μm)	Gradient elution: ACN–water (propylamine)	DAD 280, (450)	REC: 88% (AM) 87% (MA) LOD: 4 ng/ml (AM) 2 ng/ml (MA) (UV 280) LIN: 300–4000 ng/ml	[76]
AM Ephedrine (a.o.)	U	–	SPE	LiChrospher 60 RP-select B (240×4 mm I.D., 5 μm)	Gradient elution: ACN–potassium phosphate buffer (pH 3.2)	DAD (190–370)	REC: 82–100% 83–100% LOD: ?	[77]

(Cont.)

Table 2. Continued

Compound	Sample	Internal standard	Work-up	Stationary phase	Mobile phase	Detection mode	Validation data	Ref.
AM, MA, norephedrine, hydroxy-AM, hydroxy-MA	U	β-Phenylethylamine	EHY, LLE, DNS; NDA	Inertsil ODS-2 (250×4.6 mm I.D.)	Gradient elution: imidazole buffer (pH 7)–THF–ACN	TCPOCL, postcolumn	REC: >90, >90, >90, 63–70, 63–70% LOD: 30 fmol (DNS) 0.3–1.5 fmol	[63]
AM MA	U S	2-Amino-4-phenyl-butane	SPE: 9-FA (on-line)	Supelcosil LC-18-DB (250×4.6 mm I.D., 5 μm)	Gradient elution: ACN–water–SDS	FL (254, 305–395)	REC: ? LOD: 0.5 ng/ml (AM)	[78]
AM hydroxy-AM hydroxy-MA	U	MA	EHY, SPE	Microsorb PhenylRP	ACN–MeOH, potassium phosphate buffer (pH 3) (5:10:85)	UV 215	REC: 94–103% LOD: ? LIN: 1600–16 000 ng/ml LOQ: 920 ng/ml 810 ng/ml	[79]
AM hydroxy-AM	MD, P, B,S	Tryptamine	LLE, OPA (or microdialysates, MD)	Supelco LC18 (250×4.6 mm I.D., 5 μm)	Gradient elution: methanol–potassium phosphate buffer (pH 5.5)	FL (340/440)	REC: 99% LOD: ? LIN: 11–460 ng/ml MD: 1.35–27 ng/ml LOQ: 1.5 pmol MD: 370 fmol	[65]
AM MA	U	β-Phenyl-ethylamine	SPE, NQS	LiChrospher Si-60 (125×4 mm I.D., 5 μm)	Ethanol–chloroform–ethyl acetate–*n*-hexane (1:22:32:45)	DAD 280, (450)	REC: (C_{18}, UV 280) 100% 80% LOD: ? LIN: 630–12 580 ng/ml	[80]
AM	U		–	OD-MP Spheri-5 RP-18 (100×4.6 mm I.D., 5 μm) 60°C	0.1 *M* SDS–3% pentane-1-ol	UV 260	REC: ? LOD: 4130 ng/ml	[91]
AM MA	U		SPE, OPA; FMOC; *o*-acetyl-salicylic-acid (on-line)	LiChrospher C-18 (250×4.6 mm I.D., 5 μm)	Gradient elution: ACN–water	UV 254	REC: ca. 100% LOD: ?	[85]
AM MA Nor-selegiline	P		LLE, 9-fluorenyl-methyl chloroformate	Nova Pack Phenyl (150×4 mm I.D., 4 μm)	ACN–phosphate buffer (pH 6)	FL (260/315)	REC: 74–90% 72–90% 57–72% LOD: ? LIN: 0.5–80 ng/ml LOQ: 0.5 ng/ml	[69]
AM	U		DanCl	Vercopak Inertsil 5-ODS-80A (250×3.2 mm I.D., 5 μm)	ACN–water (70:30)	FL (343/500)	REC: ? LOD: 0.048 μ*M* LIN: 0.05–10 μ*M*	[95]
AM MA Ephedrine Pseudoephedrine Phenylpropanolamine	U		SPE, 9-fluorenyl-methyl chloro-formate (on-line)	LiChrospher 100 RP 18 (125×4 mm I.D., 5 μm)	Gradient elution: ACN–water	FL (264/313)	REC: 50–65% LOD: 5–25 ng/ml LIN: 500–10 000 ng/ml	[87]
Ethylamphetamine	U	MA	SPE, BC	Chiralcel OB-H 55°C	Hexane–2-propanol (90:10)	UV 220	REC: ? LOD: ?	[88]

Table 2. Continued

Compound	Sample	Internal standard	Work-up	Stationary phase	Mobile phase	Detection mode	Validation data	Ref.
AM MA	U	β-Phenyl-ethylamine	SPE, NQS; OPA; 9-fluorenyl-methyl chloroformate (on-line)	Hypersil ODS (250×4 mm I.D., 5 μm) (NQS) or LiChrospher 100 RP 18 (125×4 mm I.D., 5 μm) (OPA, 9-fluorenyl-methylchloro-formate)	Gradient elution: ACN–water (propylamine)	UV 280 FL (345/445) FL (264/313)	REC: 100/31 66/–% 98/86% LOD: 25/50 ng/ml 10 ng/ml 1 ng/ml/1 ng/ml LIN: 400–4000 ng/ml 500–10 000 ng/ml	[96]
MA MDMA	P	MDA MDMA	LLE	Zorbax CN (150×4 mm I.D., 5 μm)	MeOH–acetate buffer (50:50) pH 4.54	UV 280	REC: ? LOD: 2.7 ng/ml 1.6 ng/ml	[61]
AM, MA, MDMA, MDA, 4-methoxy-AM, phentermine, MMDA, trimethoxy-AM, MDE, DOBP, DOM, DOB, DOET, Mescalin	PS			Spherisorb ODS-1 (125×4 mm I.D., 3 μm)	Gradient elution: ACN–water–H_3PO_4–hexylamine	DAD 198/205	REC: ? LOD: ?	[73]
MDMA MDA	U		SPE	Spherisorb ODS-1 (125×4 mm I.D., 3 μm) 40°C	ACN–water–H_3PO_4–hexylamine	DAD 198/205	REC: 98% 99% LOD: 56 ng/ml 1.3 ng/ml LIN: 500–17 000 ng/ml 80–1600 ng/ml	[73]
MDMA, MDA,	B	MDE	LLE	Whatman silica Partisphere (250×4.6 mm I.D., 5 μm)	MeOH–sodium acetate buffer pH 4.25	ECH	REC: 82–86%, 91–100% LOD: 1.6 ng/ml, 2.7 ng/ml LIN: 10–1000 ng/ml	[62]
MDMA, Hydroxy-methoxy-MA Di-hydroxy-MA MDA Hydroxy-methoxy-AM Di-hydroxy-AM	P, U	MA	AHY, EHY, SPE	Spherisorb ODS-1 (150×4.6 mm I.D., 3 μm)	ACN–water (cont. H_3PO_4, hexylamine) (96:904)	DAD 200	REC: 99% MDMA 100% MDA 90% hydroxy-methoxy-MA 68% hydroxy-methoxy-Am LOQ: 7 ng/ml (MDMA), 5 ng/ml (MDA), 15 ng/ml (hydroxy-methoxy-MA, hydroxy-methoxy-AM)	[43]
AM MA MDMA MDA	U		SPE, NQS	Hibar LiChrospher 100 RP 8 (250×4 mm, 5 μm)	Phsophate buffer (pH 3, cont. methanesulfonic acid)–ACN (48:52)	UV 480	REC: 80–85% LOD: 40–60 ng/ml	[86]
AM MA MDA MDMA Ephedrine a.o.	S, U	Deuterated I.S.	LLE, PIT	Superspher Select B EcoCart (125×3 mm I.D.)	ACN–ammonium formate buffer (pH 3) (60:40)	DAD (−) UV 250	REC: ? LOD: 50–100 ng/ml 10–30 ng/ml	[70]
Chiral procedures								
AM MA HO-MA	U	*N*-Ethyl-aniline	SPE	Ultron ES-PhCD β-CD phenylcarbamate bonded silica (150×6 mm I.D., 5 μm)	ACN–MeOH–ammonium (pH 6) (10:30:60)	TS-MS	REC: ? LOD: 10–20 ng/ml (Scan) 0.5–0.8 ng/ml (SIM)	[89]

(Cont.)

Table 2. Continued

Compound	Sample	Internal standard	Work-up	Stationary phase	Mobile phase	Detection mode	Validation data	Ref.
AM	P		SPE, FLEC (on-line)	Supelcosil C18-DB (250×4.6 mm I.D.)	ACN–water (50:50)	FL (254/313)	REC: ? LOD: ? LIN: 150–10 000 ng/ml	[84]
AM	P		SPE, FLEC (on-line)	Supelcosil C18-DB (250×4.6 mm I.D.)	ACN–water (50:50)	FL (254/313)	REC: ? LOD: ? LIN: 150–10 000 ng/ml	[84]
AM MA	S	Aniline sulfate	LLE, FLEC	Adsorbosphere HS C18 (150×4.6 mm I.D., 3 μm)	Acetate buffer (pH 3.6)–ACN–THF (46:39:15)	FL (265/330)	REC: ? LOD: ? LIN: 5–250 ng/ml	[68]
MA AM Hydroxy-MA Hydroxy-AM	PS U	*n*-Propyl-aniline	EHY, BC, SPE	Chiralcel OB/OJ combined 48°C	*n*-Hexane–ethanol (89:11) or *n*-hexane–2-propanol (90:10)	UV 220	REC: ? LOD: ? LIN: 2500–25 000 ng/ml	[72]
AM MA HO-MA		*N*-Ethyl-aniline	SPE	Ultron ES-PhCD β-CD phenyl-carbamate bonded silica (150×6 mm I.D., 5 μm)	ACN–MeOH–phosphate buffer pH 6 (10:30:60)	UV 200	REC: ? LOD: 50–100 ng/ml LIN: 200–20 000	[89]
AM	PS U		SPE DNBC	Supelcosil LC-(*S*)-naphthylurea column (250×4.6 mm I.D.)	Hexane–2-propanol–ACN (97:1:2)	UV 240	REC: ? LOD: 100 ng/ml	[81]
AM MA Norephedrine	PS, U		LLE	Daicel Crownpack CR (+) (150×4 mm I.D.)	Aqueous $HClO_4$ (pH 1.8)	DAD 200, 254	REC: ? LOD: ? LOQ: 30 ng/ml	[66]
AM, MA, hydroxy-AM, hydroxy-MA	U	*o*-Amino-phenol	EHY, LLE, FLEC	Absorbosphere HS C18 (150×4.6 mm I.D., 3 μm)	ACN–acetate buffer (pH 3.6)–THF (25:54:21, for HO metabolites: 26:59:21)	FL (265/330)	REC: ? LIN: 5–100 ng/ml	[64]

ance with our results concerning LLE of AM from urine. Careful evaporation allowed recoveries of over 80% with coefficients of variation (C.V.s) of less than 5% [49].

2.1.1.2. *GC–MS*

Amphetamine and methamphetamine. Most of the GC–MS procedures for the determination of amphetamine and methamphetamine in blood and urine followed the same principles (Table 1): after LLE or SPE followed by derivatization, the analytes were separated on fused-silica capillary (FSC) columns and detected in the single-ion monitoring (SIM) mode, most often using deuterated I.S.s [14,17,21,27,29,31–33,36,38,40,53]. Scan mode is seldom used [16,35,39] (cf. ''Pitfalls of GC–MS procedures'' later in this section). In the following the pros and cons of the different procedures are discussed.

One reason for developing new procedures seems to be the reports on false positive MA results in presence of extremely high concentrations of other sympathomimetic amines [7,36,54]. Hornbeck et al. [54] described, that at GC injection port temperatures higher than 300°C, ephedrine or pseudoephedrine were partly converted into MA after derivatization with heptafluorobutyric anhydride (HFB), 4-carbethoxyhexafluorobutyryl chloride (CB) or *N*-trifluoroacetyl-*S*-prolyl chloride (TPC). Some authors stated however, that their procedures were not susceptible to such artifact formation [14,16,35,36,38]. This topic will further be discussed later in this section.

Other authors simplified the sample preparation

procedures. Hara et al. [31] presented an extractive derivatization procedure using SPE columns. Yashiki et al. [53] and Nagasawa et al. [40] used head space SPE as a simple and rapid extraction method. The relatively low sensitivity (LOD: 100 ng/ml) [53] is sufficient for confirmation of immunoassays.

Some authors included in their procedures the detection of nor-selegiline [29] or of the hydroxy metabolites of AM and MA [31], however without cleavage of conjugates. In some papers detection of AM and MA is part of screening procedures for several drugs [35,39,44,48,49].

Designer drugs. Amphetamine-derived designer drugs are also detectable by GC–MS after suitable sample preparation. Lillsunde and Korte [34] analysed 12 ring- and N-substituted amphetamines in blood, urine and seized material after SPE and HFB derivatization. They used NPD and ECD for quantification and MS for identification. Unfortunately, the authors published no recovery data. Gan et al. [32] used SPE for isolation of AM, MA and MDMA from urine samples. The extracts were back extracted into chlorobutane and then trichloroacetylated. The retention time of AM was identical to that of phentermine. However, the mass spectra of the two compounds were sufficiently different so that the presence of phentermine could not be misinterpreted as that of AM. Therefore, the authors stressed that the ion m/z 91 should not be used for the identification of AM. Ephedrine, pseudoephedrine, norephedrine and norpseudoephedrine did not interfere (cf. ''Pitfalls of GC–MS procedures'' later in this section). Lim et al. [20] studied the disposition of MDMA and its main metabolites in rats and mice using GC–MS based on perfluorotributylamine-enhanced ammonia positive-ion chemical ionization. This is the only recent paper using this unusual technique for the detection of amphetamines. The method of Meatherall [16] is claimed to cover also designer drugs. However, validation data were only given for AM and MA.

Simultaneous detection of MDMA, MDE, MDA, BDB, MBDB and their metabolites as part of a general screening procedure in urine by full scan GC–MS after LLE at pH 8–9 and acetylation was reported by Maurer et al. [25,50]. The metabolic pathways of these methylenedioxyphenylalkylamine designer drugs are shown in Fig. 2. Fig. 3 shows the reconstructed mass chromatograms indicating the compounds which were identified in urine of a patient who had ingested an unknown mixture of designer drugs (taken from Ref. [50]). As shown, all the drugs and their metabolites were sufficiently separated. Since their mass spectra are quite different the given compounds could be confirmed.

This GC–MS procedure has the further advantage that most of the toxicologically relevant drugs like AM and MA, amphetamine-derived medicaments as well as barbiturates, benzodiazepines, opioids, analgesics, antidepressants, neuroleptics, antiparkinsonians, anticonvulsants, antihistamines, betablockers, antiarrhythmics and laxatives can simultaneously be detected by starting the macro for generation of the corresponding selective mass chromatograms followed by library search of the peak underlying full mass spectrum [44,48,49,55,56].

Pitfalls of GC–MS procedures. Today, GC–MS is the golden standard in toxicological analysis. Nevertheless, some pitfalls of GC–MS procedures must be considered. The high temperatures of the GC injection port and column may lead to formation of artifacts from the analyte [45]. Special problems arising during the analysis of amphetamines were reported. The CB, HFB and TPC derivatives of ephedrine or pseudoephedrine can partially be converted to MA when heated to 300°C in the injection port of the GC [54]. The HFB derivative of ephedrine was also shown to give MA peak interferences because of contaminants in the derivatizing reagent [7]. However, this appears to have been an isolated incident. In 1992 Thurman et al. [36] studied the possible interferences of sympathomimetic amines with AM and MA analysis. After LLE, the extracts were HFB or CB derivatized and analyzed by GC–MS in the SIM mode. CB derivatives lead only to false positive MA results, when high concentrations (>5 μg/ml) of ephedrine or pseudoephedrine were present in the specimen. As a consequence to the discussion on false positive MA results, the National Institute on Drug Abuse (NIDA) instructed its certified laboratories, that in specimens, which are positive for MA (>500 ng/ml), also AM of at least 200 ng/ml must be present. However, Valentine et al. [57] showed that in urine samples of volunteers, treated with *S*-(+)-MA, usually the AM concentrations did not reach the 200 ng/ml cutoff, even

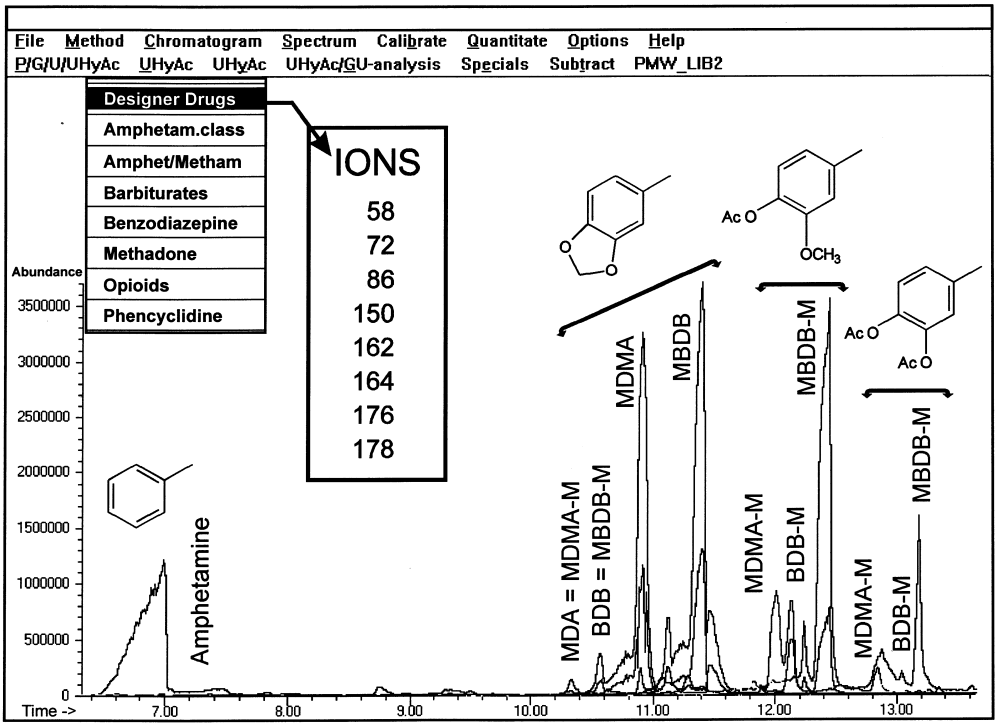


Fig. 3. Typical mass chromatograms with the ions *m*/*z* 58, 72, 86, 150, 162, 164, 176 and 178 indicating the presence of amphetamine, MDMA, MBDB and their main metabolites in urine. The merged mass chromatograms can be differentiated by their colours on a colour screen (taken from Ref. [50]).

when MA was higher than 500 ng/ml. Therefore, it can be concluded that there are a large number of false negatives. ElSohly et al. [58] oxidized interfering amines to small molecules leaving the amphetamines intact. This procedure may be useful for confirmation of immunoassays but not for screening of unknown drugs. Brooks and Smith [47] suggested a method employing preextraction aqueous acetylation, where only the amino group was acetylated whereas the hydroxy groups of e.g., ephedrine or pseudoephedrine were not acetylated. These monoacetates caused no formation of MA-like artifacts even at elevated injector temperatures.

Other authors stated that they had no problems with interfering sympathomimetic amines [11,13,26,35]. Nevertheless, it seems that this discussion has led to a loss of confidence in GC–MS as the most specific method. However, as the NIDA stated, ''not the method per se, but rather inadequately challenging the ruggedness of an application of a specific method'' was the problem.

Other pitfalls may arise from using the SIM mode with only a few diagnostic ions. At this point, it should clearly be stated, that the method is the more specific the more ions are chosen for SIM. Therefore, the question arises, whether the full scan mode using modern highly sensitive benchtops should be prefered, at least for qualitative analysis. Reconstructed mass chromatography can indicate the presence of e.g., amphetamines. Library search of the full mass spectra underlying the positive peaks in such mass chromatograms can be performed for confirmation. Automation by so-called macros is possible [45,55].

2.1.1.3. *GC with other detectors*

Amphetamine and methamphetamine. A mass spectrometer is the most specific GC detector for drug testing. Nevertheless, some papers were pub-

lished concerning drug testing using GC with less specific detectors. It should clearly be stated, that especially in forensic cases or doping control GC–MS confirmation is required.

Zweipfenning et al. [37] stated, that the use of Bond-Elut Certify SPE columns produced very clean extracts from whole blood which were suitable for simple GC–NPD. As the authors stated in their conclusions, the method needed further validation and fine-tuning. Szebeni et al. [28] quantified AM, MA and the nor metabolite of selegiline in pig plasma after LLE and pentafluorobenzoylation using GC–NPD. Recently, Jonsson et al. [22] reported GC detection using NPD of amphetamines (AM, MA, phentermine, phenmetrazine, ephedrine, norephedrine; ethylamphetamine as I.S.) after derivatization with methyl chloroformate as derivatizing agent. An advantage of this procedure is, that derivatization can take place under aqueous conditions during extraction, thus avoiding an evaporation step. However, a washing step was necessary since the reagent caused rapid deterioration of the NPD.

GC with ECD was used after ion-pairing LLE and pentafluorobenzenesulfonylation for the detection of AM and MA in urine and liver tissue [23]. Good recoveries (79–95%) and a linearity range from 1–50 ng/ml were achieved.

Fourier transformation infra red spectroscopy (FTIR) is a further detection mode for GC which can be coupled in-line with MS. Platoff Jr. et al. [24] described such a technique for qualitative/quantitative GC–FTIR and quantitative GC–MS determination of AM, MA and related analogues in human urine. The simultaneous use of both techniques should provide more specificity than each single technique. Only Kalasinsky et al. [19,59] used GC with FTIR spectroscopy for detection of amphetamines without MS detection. The authors stated that the main drawback of the GC–FTIR technique was that the detector responded to everything that was eluting from the column and that the extracts needed to be very clean. Since there are no advantages of this technique over GC coupled with MS, it could not prevail on the market.

Designer drugs. On the determination of amphetamine-derived designer drugs using other than MS detectors only a few papers were published. Drummer et al. [13] determined AM, MA, MDMA, MDA, phentermine, pseudoephedrine and other drugs of forensic interest in blood after LLE using GC–NPD. The LOD for MA was 50 ng/ml. Unfortunately, no LOD data were given for the designer drugs. Lillsunde and Korte [34] analysed 12 ring- and N-substituted amphetamines in blood, urine and seized material by GC–NPD or ECD. Unfortunately, no recovery data were reported. Again, GC–NPD or ECD results should be confirmed by GC–MS.

2.1.2. LC procedures

Papers on achiral LC procedures with different detectors for the determination of amphetamines are reviewed in this section. In 1994 Campins-Falco et al. [60] reviewed amphetamine and methamphetamine determinations in biological samples by HPLC. Surprisingly, only six references were more recent than 1991.

2.1.2.1. *Sample preparation*. As described for GC procedures (Section 2.1.1.1), suitable sample preparation is also an important prerequisite for liquid chromatography (LC) in biosamples. Procedures for extraction or cleavage of conjugates are in principle the same as described for the GC methods. Isolation of the amphetamines was performed by LLE [61–70] or SPE [43,71–90]. Amphetamines do not show high UV absorbance or natural fluorescence. Additionally, primary and secondary amines often show poor chromatographic performance, which can be improved by derivatization. Nevertheless, some authors got useful results without derivatization [43,61,62,66,67,70,73,79,82,89,91]. To improve both chromatographic behaviour and detectability of the amphetamines, a great number of procedures involving precolumn or postcolumn derivatization using different reagents have been developed. Table 2 gives an overview of the LC methods published in the last five years.

Solid-phase derivatization is a selective reaction between the analyte in solution and the reagent immobilized on a solid support. This can be an elegant alternative to derivatization in solution. The reactor can several times be used, since large excess of reagent is present, and since only the reagent, that reacts with the analyte, is consumed. No excess reagent, which could interfere with the detection, is eluted with the mobile phase. No additional hard-

ware is necessary other than a small reactor column. No additional dead volume is introduced in the system other than that usually introduced with a guard column. Different tags were used. In 1992 Zhou et al. [92] described a resin-based derivatization reagent, containing a 9-fluorenacetyl tag on a controlled pore substrate for the direct injection of amphetamine in plasma. Bourque and Krull [93] used a polymeric ester for the immobilization of the 3,5-dinitrobenzoyl group. Confirmation and quantitation of AM in urine was accomplished using a polymer containing two labelling moieties, a 3,5-dinitrobenzoyl and a *p*-nitrobenzoyl group. Such mixed-bed multiderivatization approaches using polymeric reagents for derivatization of amines in HPLC detection have earlier been described by Gao et al. [94]. In 1992 Szulc and Krull [85] studied the quantitation of AM and MA in urine using mixed-bed polymeric *o*-nitobenzophenone reagents for the on-line derivatization. They immobilized *o*-nitrobenzophenone, 9-fluorenylmethyl chloroformate and *o*-acetylsalicylic acid in one reactor. Unfortunately, no validation data were given. It has to be noted that variations in the amount ratios of polymeric reagents in the reactor will lead to varying ratios of the final derivatives. Therefore, the approximate reactivity of each polymeric reagent, the percent derivatizations and the overall rates for each reagent towards the substrate must be known. On-line SPE and derivatization can also be used for automatization as described by Bourque et al. [78]. They immobilized 9-fluoreneacetic acid (9-FA) on a controlled pore, polystyrene divinylbenzene support. Fig. 4 taken from Ref. [78], shows typical HPLC–FL chromatograms detecting AM and MA at different concentration levels (different outlines of lines) in an on-line derivatized urine sample. Other authors stated that on-line derivatization was not suitable for routine analysis because of the limited lifetime of the reactor column [95]. These authors derivatized AM with dansyl chloride for fluorescence detection. They renounced extraction procedures. Urine was directly dansylated and injected. As shown in Table 2, the sensitivity of the procedure was not as good as that of procedures employing SPE or LLE. Fisher and Bourque [75] used off-line derivatization with a polymeric 1-hydroxybenzotriazole reagent containing a 3,5-dinitrobenzylic ester for the detection of AM.

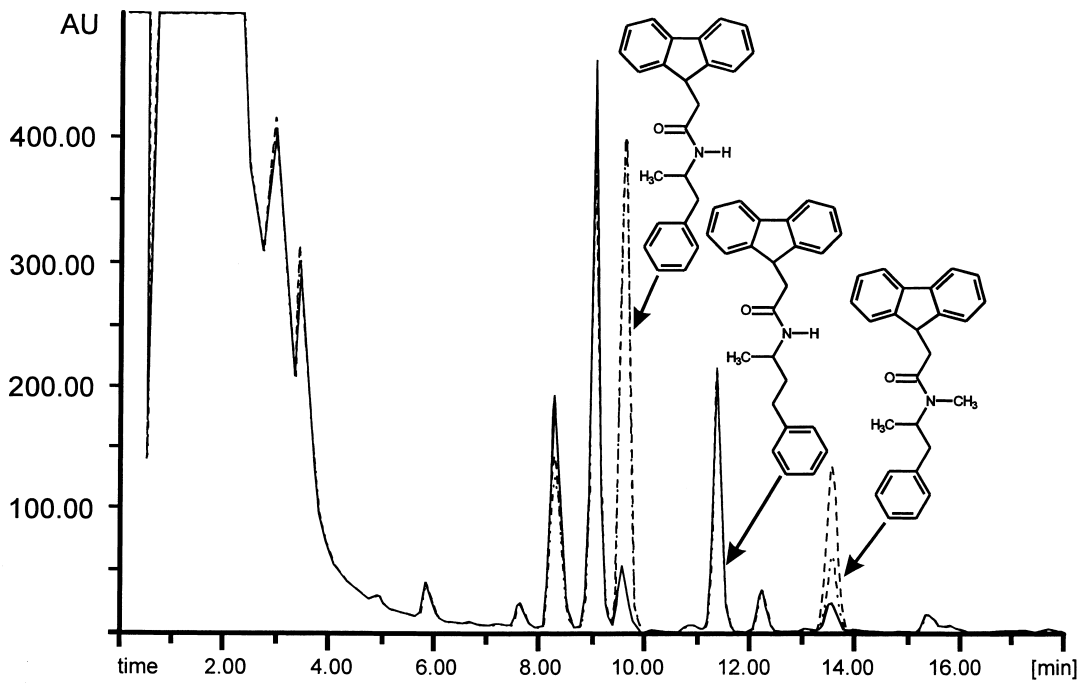


Fig. 4. HPLC–FL chromatogram detecting AM and MA in an on-line derivatized urine sample (taken from Ref. [78]).

In Refs. [71,87,96] the use of ODS commercial packing materials instead of immobilized reagents in solid supports was described, thus avoiding the required synthesis of the solid-phase reagent.

2.1.2.2. *LC–MS*. A mass spectrometer is the most specific detector also for LC. It seems questionable, whether LC–MS is necessary for specific detection of amphetamines taking into consideration that they are very suitable for GC–MS analysis. Since some methodological problems associated with GC–MS were reported (cf. ''Pitfalls in GC–MS procedures'' in Section 2.1.1.2), the development of inert LC–MS methods is maybe of interest. Actually, a few papers on LC–MS detection of amphetamines were published. For more details see the corresponding review on LC–MS of Maurer [97] in this Special Volume.

Amphetamine and methamphetamine. Tatsuno et al. [90] reported the simultaneous determination of several illicit drugs in urine by thermospray LC–MS. Extraction was performed using SPE, but derivatization was not used. In 1997, Bogusz et al. [70] used atmospheric-pressure chemical ionization (APCI) LC–MS and LC with diode array detection (DAD) for the determination of phenylisothiocyante derivatives of AM and its analogues, and other sympathomimetic amines in serum, blood and urine. The APCI mass spectra were very specific for all the drugs tested.

Designer drugs. The APCI LC–MS and LC–DAD procedures of Bogusz et al. [70] allowed the simultaneous determination of phenylisothiocyante derivatives also of designer drugs. The APCI mass spectra were very specific for all the drugs tested. However, the UV spectra for example of MDMA and MDE were nearly identical. Therefore, the authors concluded that specific MS detection was preferable over simple UV absorbance detection.

2.1.2.3. *LC with other detectors*

Amphetamine and methamphetamine. Since the most specific MS detection for LC has not been widely available, some authors used other less specific detectors for their procedures. The amphetamines were usually separated on reversed-phase (RP) stationary phases. Only in one procedure normal-phase separation was used [80]. In this work Campins-Falco et al. [60] tested six different packing materials for SPE and compared the results with LLE. They found SPE more suitable for the extraction of amphetamines. AM and MA were derivatized by sodium 1,2-naphtoquinone 4-sulphonate (NQS) separated on normal-phase column and detected using DAD. Since normal-phase columns are not widely used, Molins Legua et al. [76] from the same working group proposed in a succeeding paper RP separation of AM and MA after fast NQS derivatization. At the chosen high reaction pH, the derivatization was complete within 10 min at room temperature [71,76,96]. Usually, the NQS derivatization was performed at 70°C and lasted 20 min [80,83] or even 1 h [86]. The fast NQS derivatization was used by Herraez-Hernandez et al. [96], again from the same working group, for on-line derivatization of amphetamines in urine. In the same paper 9-fluorenylmethyl chloroformate and *o*-phthaldialdehyde derivatization in the on-line mode was tested. The lowest LOD (1 ng/ml) was achieved by the 9-fluorenylmethyl chloroformate derivatization, which was proposed by the authors as the derivatization of choice for routine analysis. La Croix et al. [69] determined, besides AM and MA, the nor metabolite of selegiline after off-line derivatization with 9-fluorenylmethyl chloroformate.

Ion pairing of the analytes was also used to separate the amphetamines at acidic pH. Ferrara et al. [83] studied HPLC–UV absorbance detection of drugs of abuse in urine after SPE and off-line NQS derivatization for confirmation of positive enzyme immunoassay (EMIT) results. Efficient hydrophobic interaction between the analytes and the stationary phase (RP-8) was achieved by ion-pairing the positively charged amphetamine and methamphetamine with methanosulfonic acid. The procedure of Tedeschi et al. [86] from the same working group included the detection of MDMA and MDA using the same extraction and derivatization procedure. However, separation was now achieved on an RP C_{18} column.

Postcolumn addition of several different chemoluminescence reagents for detection of amphetamines was proposed [63,74]. Micellar LC was used by Carretero et al. [91] to study the detection of banned substances in sport. This technique allows direct sample injection of biological material into the column, because the micellar aggregates allow the solubilization of sample (plasma) proteins and other

compounds. In addition, it allows the elution of both hydrophilic and hydrophobic substances. However, slow mass transfer between the mobile and stationary phases results in poor chromatographic efficiency of micellar solvents. Addition of a medium chain alcohol (e.g., pentane-1-ol) as an organic modifier could improve the chromatographic efficiency. Peak tailing could be avoided by increase of the separation temperature (60°C).

Using the generalized rank annihilation method (GRAM), identification and quantification of even partly unresolved peaks was possible [77]. AM, ephedrine and 12 other drugs could be separated in only 8.5 min on an RP column. Unfortunately, no further data on detection limits or linearity ranges were given.

Screening procedures for several classes of drugs using a single LC system were seldom published. Lambert et al. [67] tested alumina-based HPLC packing material for separation of more than 130 toxicologically relevant substances under alkaline conditions and DAD. The used stationary phase was stable in the pH range from pH 2–12, thus allowing the alkaline chromatographic conditions. Analytes with phenolic hydroxy groups or carboxy groups could not be covered in this screening because of the poor retention of such compounds under these conditions. The authors stated that a universal LC separation is not possible, and that they were working on a second LC system for the acidic drugs. The authors claimed to have successfully applied their procedure in 500 extracts of fresh or postmortem specimens. However, no validation data at all were given in the paper, so that the actual usability in forensic and clinical toxicology cannot be estimated. In another paper of the same working group [98], the authors admitted that their procedure is working correctly only when using an ''in home'' library. Therefore, reproducibility and reliability, seem unguaranteed.

Designer drugs. Only a few papers were published dealing with the determination of designer drugs by LC. Garrett et al. [61] separated MDA and MDMA on a Zorbax CN column using methanol–acetate buffer as mobile phase. They used MDMA as I.S. for MDA determination and vice versa. However, the twofold back extraction LLE procedure seems to be quite laborious. The authors tested their procedure for stability studies and protein binding studies. For toxicological applications it should be noted that MDA is a metabolite of MDMA and that a different I.S. should be selected. Helmlin and Brenneisen [73] determined psychotropic phenylalkylamine derivatives in biological matrices. After SPE on cation-exchange columns the analytes were separated on an RP C_{18} column with acetonitrile–water–phosphoric acid–hexylamine as mobile phase. It is well known that basic compounds can show tailing effects on RP columns due to interactions with residual silanol groups. The addition of an amine modifier to the mobile phase as a masking agent for silanol groups improved the peak shape of such analytes. Helmlin et al. [43] described the analysis of MDMA and its metabolites in plasma and urine by HPLC after SPE and separation on RP column with DAD and GC–MS confirmation. The authors used MA as I.S. The procedure was used for measuring the analytes in blood and urine of volunteers participating in a controlled study. However, the method seems to be unsuitable for routine analysis in clinical or forensic toxicology, since amphetamine was too polar and interfered with the biological matrix. In our experience, AM is often additionally present in urine samples positive for MDMA and other designer drugs.

Electrochemical detection was proposed by Michel et al. [62] for the determination of the designer drugs MDA, MDMA and MDE in whole blood and other biological tissues. The procedure had a good LOD of 1 ng/ml.

It should be kept in mind, that LC–UV results should be confirmed by a second independent method like GC–MS or LC–MS.

2.2. Chiral procedures

Amphetamine and its derivatives are chiral compounds. The *S*-(+)-enantiomers of AM and MA have five times more psychostimulant activity than the *R*-(−)-enantiomers. Most of the immunoassays as well as the confirmation tests do not allow such differentiation. There are medicaments on the market which contain only the *R*-(−)-enantiomer of methamphetamine (e.g., Vicks Nasal Inhaler, [4]) or which are metabolized to the *R*-(−)-enantiomers of methamphetamine and amphetamine (e.g., selegiline, [56]). For differentiation of the intake of these medicaments from an abuse of (meth)amphetamine,

enantioselective GC and LC procedures have been published [50,56,64,66,68,72,81,84,89]. The separation of AM or MA enantiomers can also be useful in the differentiation of AM or MA metabolically formed from amphetamine like medicaments from intake of illicit AM or MA (cf. Section 4).

Separation of enantiomers can be accomplished by using a chiral stationary phase or by forming diastereomers by derivatizing the enantiomers with a chiral reagent prior to their chromatographic separation. The diastereomers can be separated using standard achiral stationary phases.

2.2.1. GC procedures

2.2.1.1. *Sample preparation*. For the GC separation of the amphetamine enantiomers different chiral derivatization reagents were used: trifluoroacetyl-*S*-prolyl chloride (TPC) [27,33,56], heptafluorobutyryl-*S*-prolyl chloride (HFBP) [18] and 1*R*,2*S*,5*R*-(−)-menthyl-chloroformate (MCF) [21]. As demonstrated by Maurer et al. [99], chiral GC columns are also suitable for separation of enantiomers of AM and MA extracted from urine samples of patients treated with selegiline. The disadvantages of the chiral columns are the relative thermal lability, the insufficient separation power [18] and the laborious handling. The gas chromatograph must first be equipped with the chiral column, which can be used only for a specific analytical problem. Using GC–MS with direct interfaces, change of column needs a lot of time, since the MS must also be brought down and the vacuum must be completely restored. A drawback of the TPC reagent is that it is contaminated by the *R*-enantiomer by 0.7% [33] or even more. Furthermore, it is known, that the enantiomers react at different rates with the TPC reagent, but using stable isotopes as I.S. this should be compensated.

2.2.1.2. *GC–MS*

Amphetamine and methamphetamine. Ellerbe et al. [33] checked urine reference material of the National Institute of Standards and Technology (NIST) using isotope dilution GC–MS with a deuterium labeled standard after SPE and TPC or HFB derivatization. Unfortunately, the authors gave no validation data for the TPC method. Tetlow and Merrill [27] also used TPC as derivatization reagent for the separation of AM enantiomers. Again, validation data were not given. Maurer and Kraemer [56] used TPC derivatization for the differentiation of the intake of selegiline from abuse of methamphetamine or amphetamine after intake of therapeutic doses (10 mg) of selegiline. Hughes et al. [100] used MCF derivatization for sensitive and enantioselective determination of AM and MA.

Designer drugs. Since commercially available chiral GC columns did not provide sufficient separation power for the separation of the enantiomers of MDMA and its three main metabolites, since *R*-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid did not give quantitative conversion, and since TPC was found to be unsuitable for aqueous derivatization of amines, Lim et al. [18] developed a procedure using HFBP as chiral reagent. After enzymatic cleavage of conjugates, LLE and this derivatization, the enantiomers of the analytes could be separated and quantified in the range of 5–1000 ng/ml. GC–MS was operated in the NCI SIM mode using 3,4-methylenedioxy-*N*-propyl-amphetamine as I.S. However, the HFBP reagent was not commercially available and had to be synthesized by the authors.

2.2.1.3. *GC with other detectors*

Amphetamine and methamphetamine. Van Bocxlaer et al. [101] used TPC derivatization for the differentiation of α-phenylethylamine as an endogenous putrefactant from the racemic α-phenylethylamine as an addition to amphetamine street drugs in urine of persons who were known to be drug users and were found dead. They used FID and FTIR for detection.

2.2.2. LC procedures

2.2.2.1. *Sample preparation*. For the separation of the enantiomers of amphetamines using LC, chiral derivatization or use of chiral columns are suitable. Since thermolability of stationary phases is no problem in LC and since change of column is easier to perform, chiral stationary phases are used [44,66,72,81,88,89] as frequently as chiral reagents [64,68,84,102].

2.2.2.2. *LC–MS*

Amphetamine and methamphetamine. Recently, LC–MS was employed for enantioselective analysis

of amphetamines. Katagi et al. [89] determined the AM and MA enantiomers after separation on a β-cyclodextrin column by thermospray LC–MS (TS-LC–MS).

2.2.2.3. *LC with other detectors*

Amphetamine and methamphetamine. Hutchaleelaha and coworkers [68,102] and Sukbuntherng et al. [64] of the same working group used (−)-1-(9-fluorenyl)ethyl chloroformate to form fluorescent diastereomers, which could be separated on achiral columns and sensitively be determined by fluorescence detection. Determination of the enantiomers of MA and AM as well as the determination of the enantiomers of their hydroxy metabolites in urine after enzymatic cleavage of conjugates was reported [64]. Zhou and Krull [84] used 9-fluorenylmethyl chloroformate-*S*-prolyl immobilized on a solid-phase polystyrene bed for simultaneous extraction from plasma and derivatization. In all the procedures, the formed diastereomers were separated on RP 18 columns and the fluorescence was detected.

A chiral column (Chiralcel OB-H) for the enantioselective separation of benzoyl derivatives of ethylamphetamine was used by Nagai et al. [88]. They studied the time-lapse changes of the enantiomers of *rac*-ethylamphetamine and the stereoselective metabolism in rat urine. Recently, they applied this HPLC–UV method for the same analytes in human urine. In a former study, Nagai and Kamiyama [72] had successfully applied Chiralcel OB and OJ columns for the enantiomer separation of benzoyl derivatives of MA and its metabolites.

Other types of chiral columns were seldom used. Makino et al. [66] separated the enantiomers of amphetamines on a chiral crown ether column and detected them using UV–DAD. Palfrey and Labib [81] reported the use of a *S*-(−)-naphthylurea column and UV detection.

3. Alternative techniques for the determination of amphetamine, methamphetamine and amphetamine-derived designer drugs

3.1. Thin-layer chromatography

Thin-layer chromatography (TLC) is a relatively inexpensive procedure and is easily available. A great disadvantage of TLC is the lack of quantitative results without significant effort and coupling to other techniques. Kovar and coworkers [103–105] used coupling of high-performance TLC with FTIR detection after automated multiple development for the determination of designer drugs in urine. However, this expensive system is not very widely used and could not prevail on the market. Gerhards and Szigan [106] used the Toxi-Lab system for screening of amphetamines, but GC–MS was necessary for confirmation. Lillsunde and Korte [107] also described TLC procedures with GC–MS confirmation. They even tried to conduct a comprehensive drug screening in urine using SPE and combined TLC and GC–MS. However, the use of nine different TLC systems with different detection reagents seems to be quite laborious. If GC–MS was anyway necessary, it would be more comprehensive to screen and confirm in one step using GC–MS.

3.2. Capillary electrophoresis

CE is a relatively new separation technique based on the mobility differences exhibited by different molecules in an electric field. It is a simple, fast and highly efficient technique suitable for separation of a wide variety of analytes. Some papers have been published in the last five years, dealing with the CE analysis of amphetamines [108–114]. Separation of enantiomers was also described [115,116]. Details on this technique are discussed in the review of Tagliaro et al. [117] in this Special Volume.

4. Interpretation of amphetamine and methamphetamine findings: discrimination between abuse of amphetamines or legitimate intake of a medication

It is well known that AM and MA can also be metabolically formed from their derivatives like amphetaminil, ethylamphetamine, dimethylamphetamine, famprofazone [118–121], fencamine, furfenorex, benzphetamine, prenylamine, fenethylline, mefenorex [48,122], clobenzorex [44], fenproporex [49] or selegiline [56,123]. Studies were performed on the metabolism of these medicaments in order to find specific metabolites suitable for differentiation [4,44,48,49,56,124]. Fig. 5 shows metabolic path-

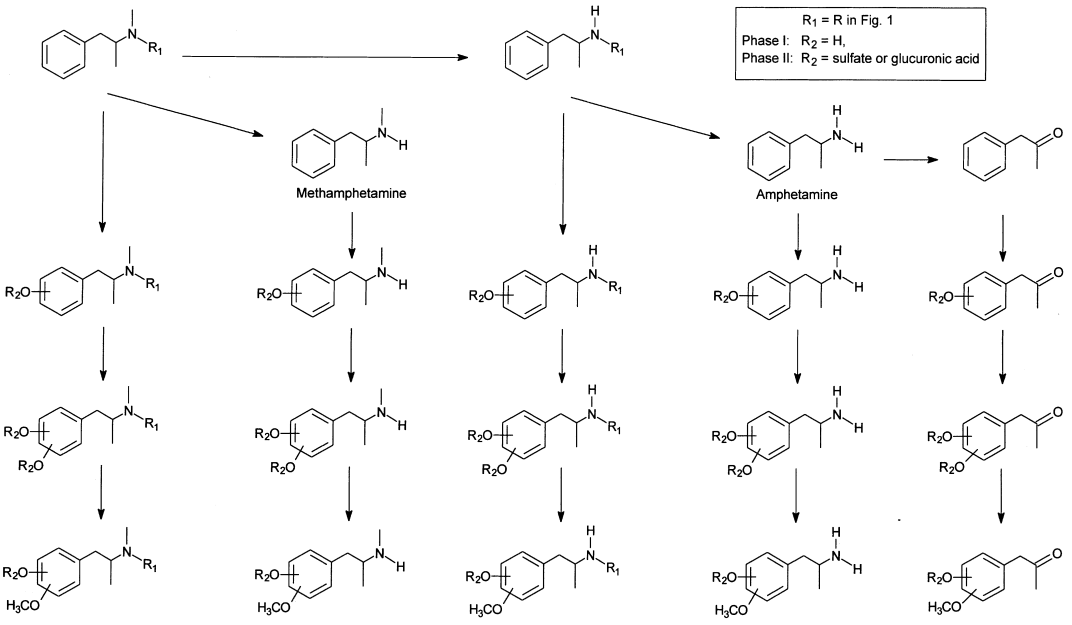


Fig. 5. Predominant metabolic pathways of amphetamine and methamphetamine-derived medicaments (overlapping pathways not indicated by arrows).

ways of N-substituted amphetamine and methamphetamine-derived medicaments. The main metabolic pathways are: (1) one- or twofold ring hydroxylation, followed by methylation of one of the hydroxy groups, (2) N-demethylation and/or N-dealkylation to AM or MA, (3) oxidative deamination (overlapping pathways not indicated by arrows). However, not all of the shown metabolites can be detected in every case. The parent compounds are usually detectable only for a few hours after ingestion and are not useful as a target compound for differentiation. The corresponding hydroxy metabolites, which are not N-dealkylated and therefore specific for the taken drug, can be detected for a much longer time. It should be noted that common procedures for confirmation of positive amphetamine immunoassay results are not suitable for the detection of such metabolites, since they do not include cleavage of conjugates and since the analyte is extracted at a strong alkaline pH. The (metabolic) introduction of a phenolic hydroxy group into a phenylalkylamine derivative increases the acidity of the compound and thereby changes the extractive properties of it. The resulting phenolbases are best extracted at pH 8–9.

However, in the late phase of excretion, AM or MA are often the only metabolites which can be detected in urine. In such urine samples differentiation of illicit AM or MA intake cannot be differentiated from the intake of such medicaments.

To say it clearly and unambiguously, every positive AM or MA result in urine can be caused by intake of legal medicaments. In a late phase of excretion of such medicaments, differentiation from abuse of illicit AM and/or MA is not always possible, regardless which method is employed.

Studies on enantiomeric profiles for AM and/or MA metabolically formed from amphetamine-derived medicaments, as described for fenproporex by Cody and Valtier [124], will show whether enantioselective differences allow differentiation [125].

5. Conclusions and perspectives

In the last five years numerous papers appeared concerning the determination of AM, MA and amphetamine-derived designer drugs or medicaments in urine and blood. Using different detectors, determinations down to low ng/ml range are possible. Most of the papers deal with the confirmation of positive prescreenings using immunoassay. False positive

MA results using GC–MS were an important stimulus for the development of both GC and LC procedures, which are not susceptible to such false positives. However, as far as loss of confidence on GC–MS procedures is concerned, it should be kept in mind, what was stated by the NIDA: ''not the method per se, but rather inadequately challenging the ruggedness of an application of a specific method'' was the problem.

Some papers were published on the determination and differentiation of amphetamine-derived designer drugs. The methylenedioxy derivatives undergo extensive metabolism to dihydroxy and hydroxy methoxy metabolites. Therefore, these metabolites must also be detected especially in the later phase of excretion.

Other papers reported the determination and differentiation of amphetamine-derived medicaments. Differentiation of intake of illicit AM or MA from intake of such medicaments should be performed by full scan EI mass spectrometry allowing the detection of specific metabolites. However, excretion studies showed, that in a late phase of excretion of such medicaments, differentiation from abuse of illicit AM and/or MA is not possible, regardless which method is employed. Studies should be performed to prove whether enantiomeric profiles for AM and/or MA metabolically formed from amphetamine-derived medicaments will allow the differentiation.

6. List of abbreviations

AC	Acetylated
ACN	Acetonitrile
AHY	Acid hydrolysis of conjugates
AM	Amphetamine
a.o.	and others
APCI	Atmospheric pressure chemical ionization
B	Blood
BC	Benzoylchloride
CDFA	Chloro-difluoro-acetylated
CI	Chemical ionization
DAD	Diode array detector/detection
DanCl	Dansyl chloride (derivatized)
DNBC	Dinitrobenzoyl chloride (derivatized)
DNBT	Dinitrobenzoylbenzotriazole
DOB	4-Bromo-2,5-dimethoxyamphetamine
DOBP	4-Bromo-2,5-dimethoxyphenylethylamine
DOET	2,5-Dimethoxy-4-ethylamphetamine
DOM	2,5-Dimethoxy-4-methylamphetamine
ECH	Electrochemical detection
EHY	Enzymatic cleavage of conjugates
EI	Electron impact ionization
9-FA	9-Fluoreneacetyl tagged
FI	Fragment ion
FLEC	(−)-1-(9-Fluorenyl)ethyl chloroformate
FSC	Fused-silica capillary
GC	Gas chromatography
GC–MS	Gas chromatography–mass spectrometry
HFB	Heptafluorobutyrated
HFBP	Heptafluorobutyryl-S-prolyl chloride (derivatized)
HPLC	High-performance liquid chromatography
HS-SPME	Head space-solid-phase microextraction
INN	International non-proprietary name (WHO)
I.S.	Internal standard
LC	Liquid chromatography
LC–MS	Liquid chromatography–mass spectrometry
LLE	Liquid–liquid extraction
M	Metabolite
MA	Methamphetamine
MCF	1*R*,2*S*,5*R*-(−)-Methyl-chloroformate (derivatized)
MDA	3,4-Methylenedioxyamphetamine
MDE	3,4-Methylenedioxyethylamphetamine
MDMA	3,4-Methylenedioxymethamphetamine
MS	Mass spectrometry, mass spectrum
NCI	Negative chemical ionization
NPD	Nitrogen–phosphorous selective detector/detection
NQS	1,2-Naphtoquinone 4-sulphonate
OPA	*o*-Phthaldialdehyde
P	Plasma
PFB	Pentafluorobenzoylated
PFBS	Pentafluorobenzenesulfonyl (derivatized)
PIT	Phenylisothiocyanate
POCL	Peroxyoxalate chemoluminescence
PR	N-Propylated

PRCF	Propychloroformate (derivatized)
PS	Pure substance
PSM	Porcine skeletal muscle
RI	Retention index
RP	Reversed-phase
RT	Retention time
SDS	Sodium dodecyl sulphate
SIM	Single-ion monitoring
SPE	Solid-phase extraction
TCA	Trichloroacetylated
TCPOCL	2,4,6-Trichlorophenyl)-oxalate chemoluminescence
TFA	Trifluoroacetylated
th. conc.	Therapeutic concentrations detectable
TPC	*S*-(−)-Trifluoroacetylprolyl chloride (derivatized)
TS	Thermospray ionization
U	Urine

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