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Journal of Chromatography B, 733 (1999) 137–143

JOURNAL OF
CHROMATOGRAPHY B

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Review

Determination of "Ecstasy" components in alternative biological specimens

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Abstract

This paper reviews procedures for the determination of methylenedioxyamphetamine derivatives, MDA, MDMA, MDEA and MBDB in saliva, sweat and hair. For this topic, the international literature appears very poor, particularly for saliva and sweat. MDMA was first reported in hair in 1993. All but one of the reviewed papers reported detection with GC–MS. No references seem to be available for both meconium and vitreous humor. As it has been already reported in these biological specimens, the parent drug is detected in higher concentrations than its metabolites. The main data on sample preparation, work-up, GC column, derivatization and analytical determination are listed. Several references, taken from the forensic practice are used to document the cases. Some new findings, based on the experience of the author, are also added. Some references, dealing with amphetamine and methamphetamine in alternative specimens are listed in the manuscript to give an overview on the stimulants detection. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Reviews; Ecstasy; Methylenedioxyamphetamine

Contents

1. Introduction 137
2. Saliva 138
3. Sweat 139
4. Hair 140
5. Conclusions 143
References 143

1. Introduction

Because of the well known limits in reliability of self-reports on drug use, analytical testing for drugs of abuse is important in most clinical and forensic toxicological situations, both for assessing the reality and cause of an intoxication and for evaluating the level of impairment caused by the drug.

The presence in the body of drugs of abuse can be identified by a variety of laboratory procedures. The standard approach is based on drug screening by immunoassay, followed by confirmation using gas

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PII: S0378-4347(98)00521-0

chromatography–mass spectrometry (GC–MS). The most common biological sample is urine.

The mandatory Guidelines for Federal Workplace Drug Testing in the United States include the identification of both amphetamine and methamphetamine, but not ring-substituted amphetamines, like methylenedioxyamphetamine (MDA), methylene dioxymethamphetamine (MDMA), methylenedioxyethylamphetamine (MDEA) and *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB). Consequently, urine immunoassays have not been specifically developed for these compounds and the performances of the existing ones are very poor [1]. Thus, most of the detection procedures involve GC–MS [2].

Ecstasy and its derivatives are central nervous stimulants popularly abused for their psychotropic effects, including euphoria and alertness. They produce elevated self-awareness and an increased sense of trust. ''Designer amphetamines'' are generally abused orally.

In recent years, alternative specimens to blood and urine have been proposed to document use or exposure to drugs. Hair analysis, has become a useful tool in clinical and forensic toxicology to document long term exposure to drugs [3]. Also, the surveys of drug addicts or prisoners with sweat patches or monitoring driving under influence by saliva analyses have recently been discussed in three reviews [4–6].

The main advantages and disadvantages of ''alternative'' matrices in comparison with urine testing for designer drugs are outlined in Table 1.

General unknown analyses are difficult with sweat, saliva and hair due to low amount of matrix that can be collected and the low concentrations that are measured.

Therefore, most of the papers devoted to alternative specimens are devoted to a single pharmacological class of drugs. Surprisingly, the determination of methylenedioxyamphetamine derivatives has been seldom reported. To date, less than 15 papers are available in the whole literature and no reports concern the analysis of meconium or vitreous humor. Among the alternative specimens, most reports are focussed on hair, with the first detection of MDMA in 1993 [7]. The detection of *O*-dealkylated and *O*-methylated metabolites has never been proposed in alternative specimens.

The present article addresses the determination of methylenedioxyamphetamine derivatives in saliva, sweat and hair using GC–MS and capillary electrophoresis.

2. Saliva

The passing of drugs from blood to saliva was suggested as early as the mid-1960s. Saliva has increasingly been used as an alternative specimen in pharmacokinetic studies, therapeutic drug monitoring and for investigating use of illicit drugs. In recent years, particular interest has been expressed for this biological sample by law enforcement agencies for roadside testing of potentially intoxicated drivers. Saliva is probably the only other body fluid that might parallel blood in some regards and that may be related to behavioral performance [8,9]. Abuse of designer amphetamines represents a growing problem with described clinical implications [10] but

Table 1
Comparison of urine, sweat, saliva and hair for ring-substituted amphetamines analysis

Parameter	Urine	Sweat	Saliva	Hair
Sample collection	Privacy concerns	Non invasive	Non invasive	Non invasive
Detected compound	Parent drug and metabolites	Parent drug>> metabolites	Parent drug>> metabolites	Parent drug>> metabolites
Window of detection	2–3 Days	1 Week	Some hours	Some months
Drug concentration	High	Low	Low	Low
Associated problems	Adulteration	Limited sample	Limited sample	Environmental contamination
Units	ng/ml	ng/patch	ng/ml	ng/mg

only a few studies on their detection in saliva have been published [6,11,12].

Samyn and co-workers [6,11] have recently presented some reports on the detection of these drugs. In a controlled study [6] on the excretion of MDMA and MDEA, only traces of the non-metabolized drugs were recovered in the saliva of the subjects. Their levels remained detectable for several hours after ingestion of the compounds. However the analytical methodology was not described. In a large roadside study examining injured drivers [6], traces of MDA, MDMA and MDEA could be detected in saliva. More recently [11], MDMA MDEA and MBDB were found by GC–MS after screening the saliva with the Drugwipe (Securetec, Germany), a non-instrumental immunoassay. Ten subjects tested positive after the saliva was brought to pH 6.0, addition of deuterated internal standards, solid-phase extraction, elution with ethyl acetate–NH_4OH and derivatization with heptafluorobutyric anhydride (HFBA). The target drugs were separated on a HP-5MS capillary column and detected in electron impact mode. Concentration ranges were 14–264, 219–6280, 4–268 and 510 ng/ml for MDA (n=8), MDMA (n=9), MDEA (n=6) and MBDB (n=1), respectively.

After a single oral administration of 100 mg of MBDB to one subject, the excretion of the parent drug and its major metabolite, 3,4-(methylenedioxyphenyl)-2-butanamine (BDB) was evaluated by Kintz [12]. Fifteen saliva specimens were collected for 24 h in plastic tubes, without any stimulation. Alkaline extraction (1 ml 1 M NaOH) of 1 ml of the specimen in ethyl acetate (5 ml) was achieved in presence of MDEA-d_5, used as internal standard, but today, a pentadeuterated analog of MBDB is available on the market. Derivatization was done with HFBA, generating by GC–MS the following ions: MBDB (m/z 176, 268 and 403) and BDB (m/z 135, 176 and 389). The column used was a HP-5MS (30 m×0.25 mm I.D.) and the carrier gas was helium (1 ml/min). The injector temperature was 240°C and splitless injection was used. The oven was held at 100°C for 2 min, then increased to 280°C at 20°C/min and held for 3 min. Both MBDB and BDB were detectable during the first 17 h, and the peak saliva concentrations were observed at 2 h, at 1083 and 146 ng/ml for MBDB and BDB, respectively. At all times, higher concentrations of the parent compound were observed as compared to its metabolite, as is the case for other drugs in saliva [13].

3. Sweat

Researchers have known since 1911 [14] that drugs are excreted by the body in sweat, but until recently, no one has been able to develop a practical solution to the problem of collecting sweat in a suitable way for analysis. Occlusive bandages, consisting of one to three layers of filter paper or pieces of cotton, gauze or towel were proposed to collect sweat. Significant advances have been made during the past years with the development a ''sweat patch'' technology, which was proposed by PharmChem Labs. (Menlo Park, USA). However, little attention has been paid to sweat as a sample for detecting ring-substituted amphetamines. In their recent review, Kidwell et al. [4] did not mention the detection of ecstasy or related compounds in sweat.

In a study [15] conducted in a detoxification center, a patch was applied for five days to 20 addicts. After elution of the pad with methanol in presence of deuterated internal standards, a portion of the solvent was evaporated to dryness, derivatized by HFBA and analyzed by GC–MS. MDEA (121 ng/patch) and its metabolite MDA (22 ng/patch) were detected in sweat collected from one subject. Other subjects were positive for opiates, cannabis or/and benzodiazepines.

A cumulative excretion study of MBDB and BDB was done after oral administration of 100 mg MBDB [12]. Eight patches were applied to the outer portion of the upper arm and periodically removed over a period of 72 h. Drugs were eluted with methanol in presence of MDEA-d_5, derivatized with HFBA and analyzed by GC–MS. The increase in drug concentration was constant during the first 36 h and was followed by a decrease for the next period of time (Fig. 1). Again, the parent drug was detected in higher concentrations than its metabolite.

Four ''techno ravers'' agreed to wear a patch for 28 h during a weekend of dance music. The skin site selected for patch placement was gently cleansed with a 70% isopropanol swab before application to the outer portion of the left arm. No urine was

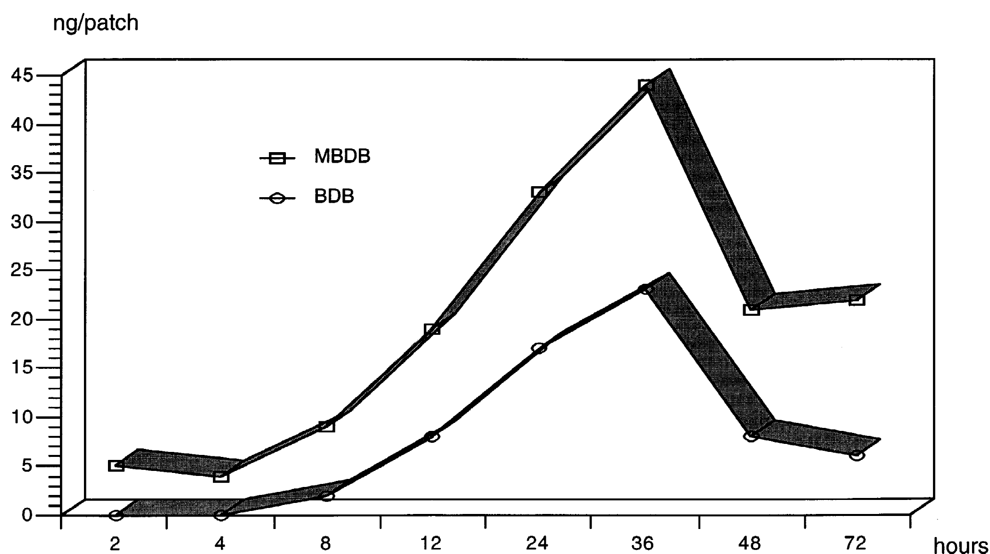

Fig. 1. Cumulative excretion of MBDB and BDB in sweat after oral administration of 100 mg MBDB.

collected at the same time. Subjects were orally informed of the procedure. The medico–legal consequences were avoided by direct cooperation with the subjects. After removal of the patch, the pad was stored at −20°C until analysis. The next week, the pad was placed in an extraction tube, along with 5 ml of methanol and 100 ng of the following internal standards: MDA-d_5, MDMA-d_5, MDEA-d_5 and MBDB-d_5. After 30 min agitation, the methanol was evaporated to dryness and the residue derivatized with HFBA at 70°C for 20 min. After evaporation, the residue was dissolved in ethyl acetate, and a portion was injected into the HP-5MS capillary column coupled to a mass selective detector. The analytical procedure was the same that was used in Ref. [12]. Results are presented Table 2 and indicate that during rave participants generally take mixtures of both MDMA and MDEA (sold as ecstasy).

Table 2
Sweat concentrations (ng/patch) observed from four subjects after a rave party

Subject	MDA	MDMA	MDEA	MBDB
1	41	159	281	Not detected[a]
2	13	222	Not detected	Not detected
3	26	138	171	Not detected
4	56	431	244	Not detected

[a] Not detected: under the limit of quantification (5 ng/patch).

4. Hair

Almost all the literature dealing with amphetamines in hair comes from Japanese researchers, and again, until recently, the detection of ring-substituted amphetamines is not well documented. No mention was reported in a review by Moeller [16] in 1992. Three years later, in a review by Nakahara [17], only one reference was available for MDMA [7]. After a first screening procedure reported in 1995 [18], four major methods have been published in the last period [19–22], all based on GC–MS. These four procedures are summarized in Table 3. In all cases, a decontamination step was included. The range of hair concentrations that are reported in the literature is summarized in Table 4. Urinary metabolites of MDMA, such as 4-hydroxy-3-methoxyamphetamine and 4-hydroxy-3-methoxymethamphetamine were never reported in human hair.

Fig. 2 shows a typical chromatogram obtained from a stimulant drug abuser using the procedure adopted in our laboratory [22].

When comparing the four different procedures for hair preparation (methanol sonication, acid hydrolysis, alkaline hydrolysis and enzymatic hydrolysis) Kintz and Cirimele [23] demonstrated that best recoveries were observed after alkaline hydrolysis. However, it was not possible to determine which

Table 3
GC–MS procedures for the detection of ring-substituted amphetamines in hair (PAA: propionic acid anhydride, PFPA: pentafluoropropionic anhydride)

Ref.	Drug	Hydrolysis	Work-up	Derivatization	Column	Ions (*m*/*z*)	Limit of detection (pg/mg)
[19]	MDA	Methanol sonication	–	PAA	HP-5MS	44, 135, 162, 235	10
	MDMA					58, 114, 135, 249	10
	MDEA					72, 128, 135, 263	10
[20]	MDA	Methanol–5 *M*	SPE Bond Elut	PFPA	TC-1	135, 162, 325	100
	MDMA	HCl (20:1)	Certify			204, 162, 135, 339	100
	MDEA					218, 162, 135, 353	100
[21]	MDA	1 *M* NaOH	SPE Bond Elut	PFPA	HP-1	135, 162	40
	MDMA		Certify			204, 162	40
	MDEA					218, 190	100
	MBDB					218, 176	40
[22]	MDA	1 *M* NaOH	L/L ethyl	HFBA	HP-5MS	135, 240, 275	50
	MDMA		acetate			210, 254, 389	20
	MDEA					240, 268, 403	20
	MBDB					176, 268, 403	20

method performed best, based on recoveries, precision and practicality. Lower concentrations were observed after methanol sonication together with heavily laden chromatograms, but this non-selective procedure can be used of screening drugs of abuse from different classes.

Hair analysis for methylenedioxy derivatives as a diagnostic tool was recently reported in two real cases of intoxication [24,25]. After extraction with cyclohexane under alkaline conditions, acetylation and separation on a CP-Sil 5 capillary column, MDMA and MDA findings in hair were used for the diagnosis of toxic hepatitis after ecstasy abuse [24]. In the second case, lethal monointoxication by MDEA of a chronic abuser was confirmed by hair. MDEA (17 ng/mg) and its metabolite MDA (0.3 ng/mg) were extracted from hair after acid hydrolysis (2 ml 0.6 *M* HCl) in presence of deuterated internal standards. Purification was achieved with Chromabond Drug 200 mg solid-phase extraction. Before GC–MS, the residue was trifluoroacetylated. A DB-5MS capillary column (30 m×0.25 mm I.D., 0.25 μm film thickness) was used with a temperature program (2 min at 60°C, 30°C/min to 310°C, 7 min at 310°C). Ions monitored were: MDA (*m*/*z* 162, 275), MDMA (*m*/*z* 110, 154, 289) and MDEA (*m*/*z* 168, 303).

Although sometimes proposed for urine, chiral analysis of amphetamine related substances has not been extensively studied in hair. Only recently, Tagliaro et al. [26] proposed a procedure applicable to hair using capillary electrophoresis with native β-cyclodextrin (15 m*M*) as the chiral selector. The optimized conditions were: phosphate, pH 2.5, un-

Table 4
Range of concentrations (ng/mg) observed in hair

Ref.	MDA	MDMA	MDEA	MBDB
[19]	0.04–1.23 (n=6)	0.05–2.91 (n=9)	0.80–3.07 (n=5)	NR[a]
[20]	0.13–0.79 (n=5)	0.15–12.51 (n=7)	NR	NR
[21]	0.05–0.89 (n=16)	0.1–8.3 (n=16)	0.12–15.0 (n=13)	0.21–1.3 (n=2)
[22]	0.4–8.0 (n=13)	0.3–42.7 (n=14)	0.6–69.3 (n=6)	1.41–3.09 (n=2)

[a] NR: Not reported.

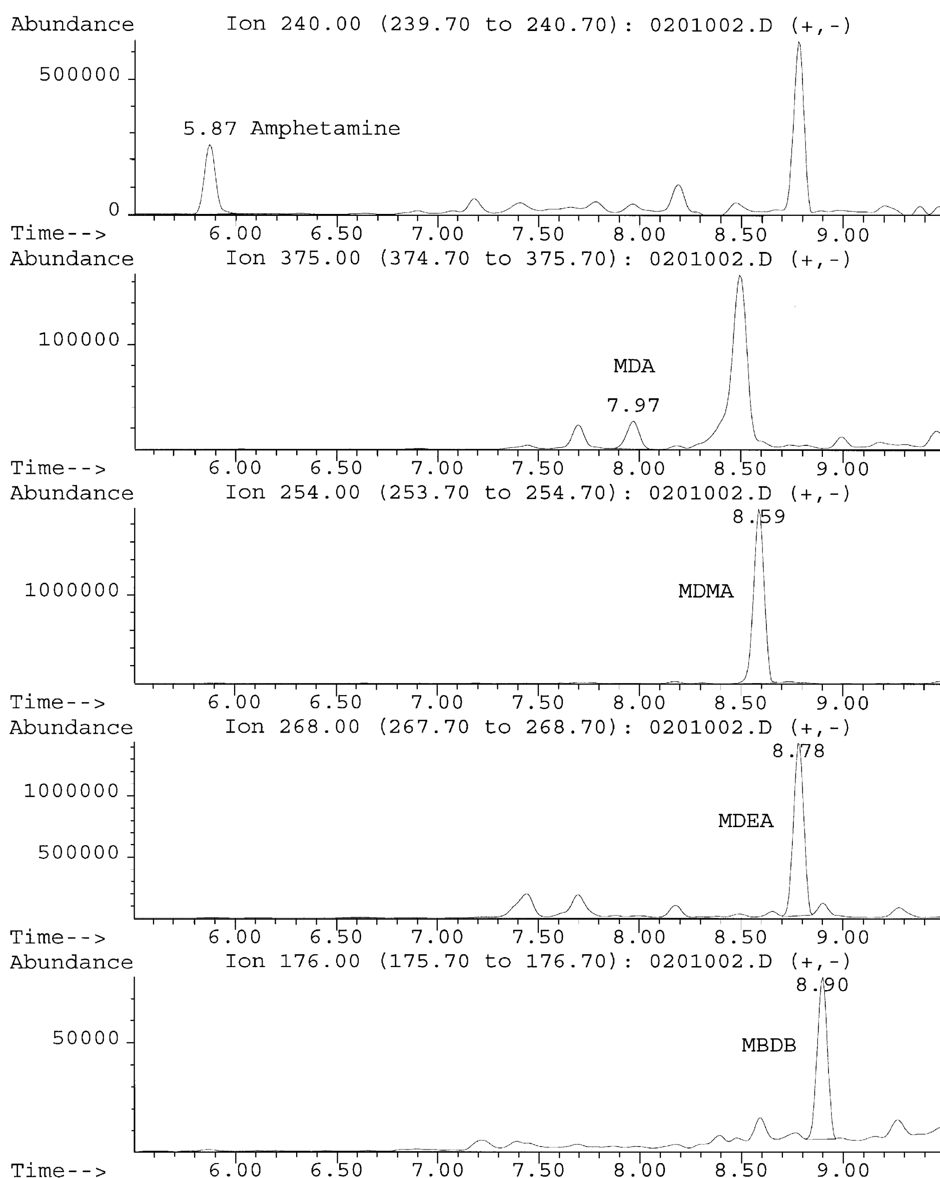

Fig. 2. Chromatogram obtained after alkaline extraction (1 *M* NaOH) and derivatization with HFBA of a hair specimen obtained from a designer drugs abuser. The chromatogram was recorded in total ion current mode and selected ions were chosen for identification and quantitation. Concentrations simultaneously measured were 3.56 ng/mg (amphetamine, t_R 5.87 min, m/z 240), 4.88 ng/mg (MDA, t_R 7.97 min, m/z 375), 33.81 ng/mg (MDMA, t_R 8.59 min, m/z 254), 39.32 ng/mg (MDEA, t_R 8.78 min, m/z 268) and 3.09 ng/mg (MBDB, 8.90 min, m/z 176).

coated capillary (45 cm×50 μm I.D.), potential 10 kV. To improve the concentration sensitivity, the authors adopted a field-amplified sample stacking procedure. Good resolution with excellent chiral selectivity and efficiency was obtained for all the analytes. To the best of my knowledge, this paper [26] is the only alternative to GC that has been published in the whole literature, as demonstrated by a review of Tagliaro et al. [27].

However, these authors have recently submitted a

paper [28] on the simultaneous determination of MDMA, MDA and MDEA in hair by high-performance liquid chromatography with direct fluorescence detection. After incubation in 0.25 *M* HCl, 100 mg of hair were extracted with a commercial liquid–liquid method. Isocratic reversed-phase liquid chromatography was carried out on a column (250×4.6 mm I.D.) packed with spherical 5 μm poly(styrene–divinylbenzene) particles with a mobile phase composed of 0.1 *M* potassium phosphate, pH 3–acetonitrile (82:18). The excitation and the emission wavelengths were set to 285 and 320 nm, respectively. The limit of detection was less than 1 ng/mg.

Due to the very few specific publications on the analysis of ecstasy components in alternative specimens, the reader may be interested in obtaining some additional information on stimulants analysis. As amphetamine and methamphetamine are of interest in the mandatory Guidelines for Federal Workplace Drug Testing in the United States, some publications report their detection in alternative specimens. In the last few months, several complete reviews were published, dealing with saliva [4,6], sweat [4] and hair [22,27].

5. Conclusions

Saliva, sweat and hair analyses are today to be considered as useful adjuncts to conventional drug testing. Methods for evading urine analysis are unable to affect these specimens, and they can be more easily obtained with less embarrassment and close supervision, either by the medical staff or the Police.

In the last few years, the abuse of designer drugs has become a widespread habit of young people at techno and rave parties. The popularity of ecstasy has greatly increased and it is assumed that the number of people having used such compounds has tripled in the last five years.

Surprisingly, the detection of ring-substituted amphetamines in alternative specimens does not appear to be very documented.

A review of the international literature and meeting presentations from 1980 to early 1998 was done, and only three, two and ten references are available for saliva, sweat and hair, respectively. When no references are available for meconium and vitreous humor, the detection of MDMA and MDA in fingernails appears rather as a curiosity [29].

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