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Determination of amphetamine and methylenedioxy-amphetamine-derivatives in hair

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Abstract

Two GC/MS-procedures for the detection of amphetamine and its methylenedioxy-derivatives (MDA, MDMA and MDE) in hair are presented. In these methods a methanol sonication extraction technique was applied. The extracted drugs were derivatized either with propionic acid anhydride (PSA) or trifluoroacetic acid anhydride (TFA). PSA-derivatives are more stable than TFA-derivatives, but the latter provide more specific mass-spectrometric information, and, therefore, seem to be preferable for amphetamine determination. The detection limit for all compounds was in a range of about 0.01 ng/mg, if at least 50–100 mg of hair were analyzed, independent of the derivatization used.

Keywords: Amphetamine/Ecstasy abuse; Hair analysis; Methanol sonication extraction technique; PSA/TFA derivatization; GC/MS analysis

1. Introduction

The abuse of amphetamine and its methylenedioxy-derivatives such as methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA) and methylenedioxyethylamphetamine (MDE) has increased enormously during the last few years. Recent trends among young people, such as the techno and rave scene, are closely connected to the abuse of stimulant and hallucinogenic drugs of the methylenedioxyamphetamine type ('Ecstasy') [1–4]. The number of seizures in

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Germany has increased from 4000 Ecstasy-pills in 1991 to 239051 in 1994 [5]. Hair-analysis, a useful tool for the detection of a multiple drug abuse, has to focus on these compounds. Various extraction procedures for the detection of amphetamine-derivatives in hair have been described in the literature, particularly in Japanese publications [6–10]. Most of these methods dealt with strongly basic (NaOH) or acidic (HCl/methanol) conditions. The approach of the present work was to apply a methanol sonication extraction technique, which has been successfully used for the isolation of THC, cocaine and opiates from hair [11], to amphetamine analysis. The extracted drugs could be derivatized either with propionic acid anhydride (PSA) or with trifluoroacetic acid anhydride (TFA).

2. Experimental

2.1. Sample material

Drug-free hair samples, which were spiked with amphetamine, MDA, MDMA and MDE were used to evaluate the experimental conditions. Moreover, authentic hair samples from individuals with suspected drug abuse were analyzed.

Hair samples were collected from the back of the head (vertex posterior). A strand (about 5 mm in diameter) was cut as close as possible to the scalp, fixed with a string and enveloped in alumina foil. Root and tip of the hair strand were marked. The samples were stored under dry conditions at room temperature until analysis.

2.2. Methanol sonication extraction procedure

The total length of the hair samples was measured and special features such as colouring, bleaching etc. were noted. If the hair was long enough, usually the first proximal 6 cm of the hair were used for analysis. The hair segment was placed in a polypropylene vial and washed for 5 min each with water (5 ml), acetone (5 ml) and petroleum ether (5 ml). After drying, the hair was cut into small pieces of about 1 mm. 50 to 200 mg were used for analysis. The hair was transferred to a polypropylene vial and 4 ml methanol and 200 ng methaqualone as internal standard (20 μl of a solution containing 10 ng/μl) were added. The closed vial was sonicated (120 W) for 5 h at 50°C. The methanolic extract was then transferred to a silanized glass vial and the solvent was evaporated after acidifying the mixture with one drop of acetic acid.

2.3. Derivatization procedures

Propionic acid anhydride derivatization: The extract was dissolved in 0.05 ml of propionic acid anhydride (PSA) and the mixture was incubated at 100°C for 1 h. The exceeding propionic acid anhydride was evaporated under reduced pressure at 90°C. For GC-MS analysis the residue was dissolved in 0.05 ml of water-free ethyl acetate containing 1% PSA.

Table 1

Methaqualone (internal standard)	m/z = 235 (target), 250	Rt: 12.0 min
Amphetamine-PSA	m/z = 44 (target), 91, 100, 118, 191	Rt: 6.7 min
MDA-PSA	m/z = 44 (target), 135, 162, 235	Rt: 9.6 min
MDMA-PSA	m/z = 58 (target), 114, 135, 162, 249	Rt: 10.3 min
MDE-PSA	m/z = 72 (target), 128, 135, 162, 263	Rt: 10.6 min
Amphetamine-TFA	m/z = 91, 118, 140 (target), 231	Rt: 5.2 min
MDA-TFA	m/z = 135 (target), 162, 275	Rt: 7.1 min
MDMA-TFA	m/z = 135, 154 (target), 162, 289	Rt: 8.0 min
MDE-TFA	m/z = 135, 162, 168 (target), 303	Rt: 8.4 min

Dwell time per ion = 40 ms.

Trifluoroacetic acid anhydride derivatization: 0.05 ml of trifluoroacetic acid anhydride (TFA) were added to the extract and the mixture was incubated at 40°C for 0.5 h. The excess trifluoroacetic acid anhydride was evaporated at room temperature in a slight nitrogen stream. For GC/MS analysis the dry residue was dissolved in 0.05 ml of water-free ethyl acetate containing 1% TFA.

2.4. GC / MS analysis

For GC/MS analysis a capillary column HP-5 MS (methyl silicone, 30 m, 0.25 mm i.d., 0.25 μm film thickness) was used. The carrier gas was He (constant flow: 1 ml/min). Injection volume: 1 μl (splitless injection). Injector temperature: 250°C. Oven temperature program: 2 min at 60°C, 40°C/min to 170°C, 8°C/min to 270°C, 13 min isothermally at 270°C. Transfer line temperature: 280°C. Ionisation: EI (70 eV). The ions were measured in the selected ion monitoring (SIM)-mode (Table 1).

2.5. Quantification

The quantification of amphetamine, MDA and MDMA was based on peak area ratios. For quantification the peak areas of the ions specified as 'target' were used. In routine analysis a two-point calibration curve was used for each drug by measuring blank hair samples of 100 mg, which were spiked with 100 ng and 500 ng of each drug. In each series of hair analyses a drug free hair sample was assayed. MDE was quantified by using the MDMA calibration.

2.6. Instrumentation and reagents

The GC/MS measurements were performed on a Hewlett Packard gas chromatograph HP 5890 II coupled with a mass-spectrometer HP 5972 (Mass Selective Detector) and an auto-sampler HP 7673 GC/SFC. For sonication the Transsonic Digital S ultrasonic bath (Elma, Germany) was used. Polypropylene vials (30-ml) with screw caps were purchased from Sarstaedt (Nümbrecht, Germany). The following reagents were used: Methanol (99.8%), acetone p.a., petroleum ether

40C p.a., ethyl acetate p.a., propionic acid anhydride and trifluoroacetic acid anhydride (99.5%) were purchased from Merck (Darmstadt, Germany), D-amphetamine sulphate, methylenedioxyamphetamine hydrochloride and methylenedioxymethamphetamine hydrochloride from Sigma (St. Louis, MO, USA) and methylenedioxyethylamphetamine (1 mg/ml in methanol) from Radian (Austin, TX, USA).

3. Results and discussion

3.1. Analytical procedures

The methanol sonication extraction of hair makes possible the simultaneous analysis of amphetamine, MDA, MDMA and MDE. Additionally, other commonly used drugs such as opiates (heroin, 6-monoacetylmorphine, morphine, dihydrocodeine and codeine), methadone, cocaine and tetrahydrocannabinol (THC) can also be detected within one analysis. The presented methanol sonication extraction technique was previously applied to the determination of heroin, 6-monoacetylmorphine, morphine, cocaine and THC in hair using PSA derivatization [11]. It has been demonstrated that this method did not produce artefacts of the parent drugs. Furthermore, the experimental procedure is relatively simple. Methanol seems to provide a good extractability for drugs from hair in solvent extraction techniques, as was shown by Rothe and Pragst [12] for the extraction of heroin, 6-monoacetylmorphine and morphine. The extraction procedure [11] could be applied to amphetamine analysis without any modifications. The loss of amphetamine during the evaporation of the methanolic extract is prevented by acidifying the mixture with acetic acid. In most of the previously published extraction methods for amphetamines strongly basic (NaOH) or acidic (HCl/methanol) conditions were used [6–10], which caused hydrolysis of compounds such as heroin or 6-monoacetylmorphine. In contrast, the method presented prevents hydrolysis and, therefore, enables the simultaneous extraction of amphetamines and other drugs from a hair sample in one step.

The extracted amphetamines can be derivatized either with PSA or TFA. It is an advantage of derivatization with TFA that more specific mass-spectrometric information is obtained. The fragmentation of amphetamine TFA derivatives under EI conditions leads to characteristic mass spectra (Fig. 1). In comparison, the EI mass spectra of the propionylated amphetamines (Fig. 2) exhibit fragmentation patterns, which are rather similar to the mass spectra of other phenylethylamines. PSA as well as TFA derivatives are susceptible to hydrolysis. Therefore, the extracts must be dissolved in water free ethyl acetate containing small amounts (1–5%) of PSA or TFA, respectively, to ensure the stability of the derivatized amphetamines. In fact, the derivatives are most stable in pure PSA or TFA, but the injection of pure PSA or TFA damages the GC column. Hence, the addition of 1% PSA or TFA is a compromise between the prevention of hydrolysis of the derivatized drugs and preserving the GC column. The PSA-derivatives are obviously more stable than the derivatives with TFA. TFA-derivatives of amphetamine, MDA, MDMA or MDE

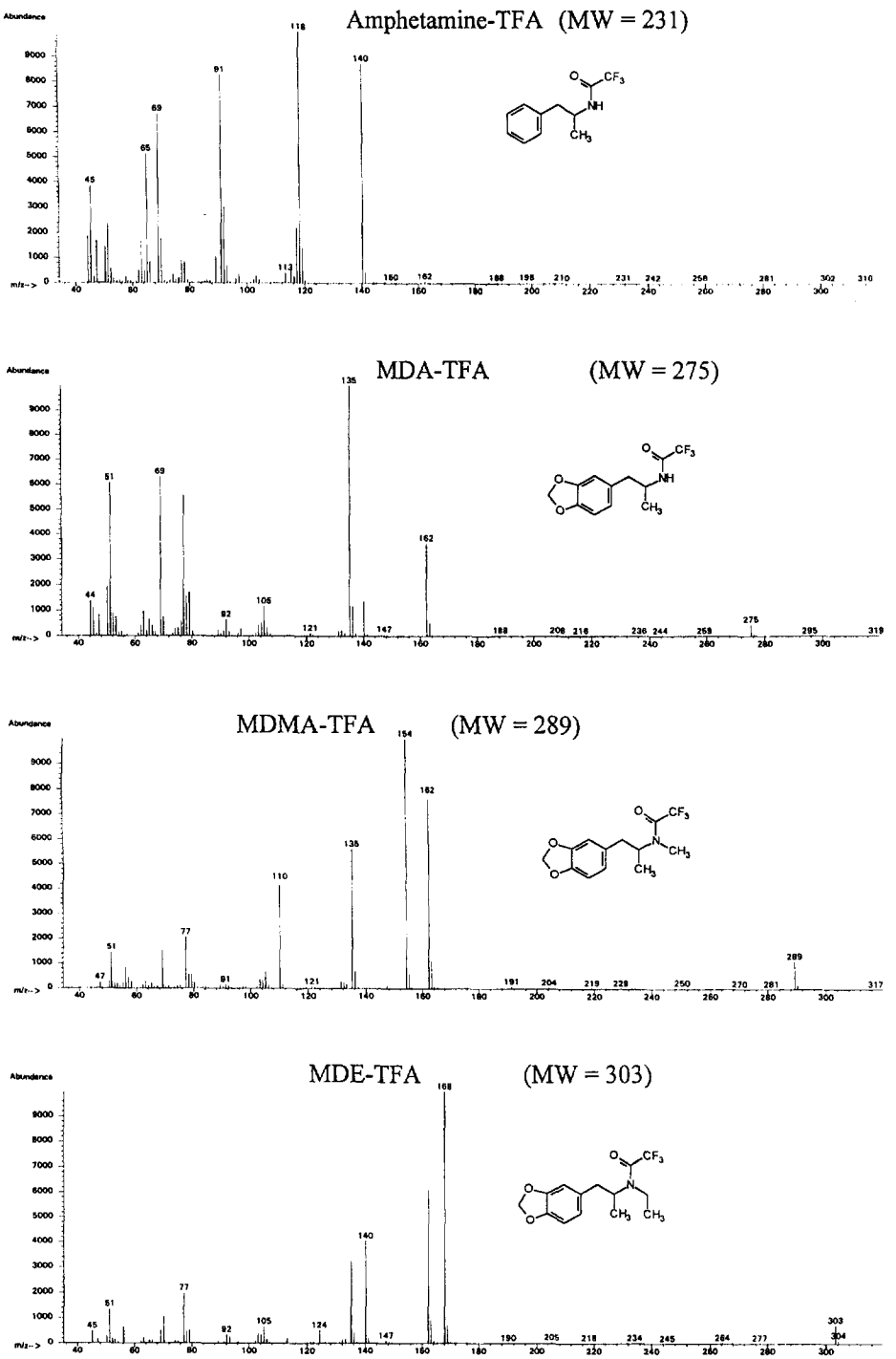

Fig. 1. EI mass spectra of trifluoroacetylated amphetamine, MDA, MDMA and MDE.

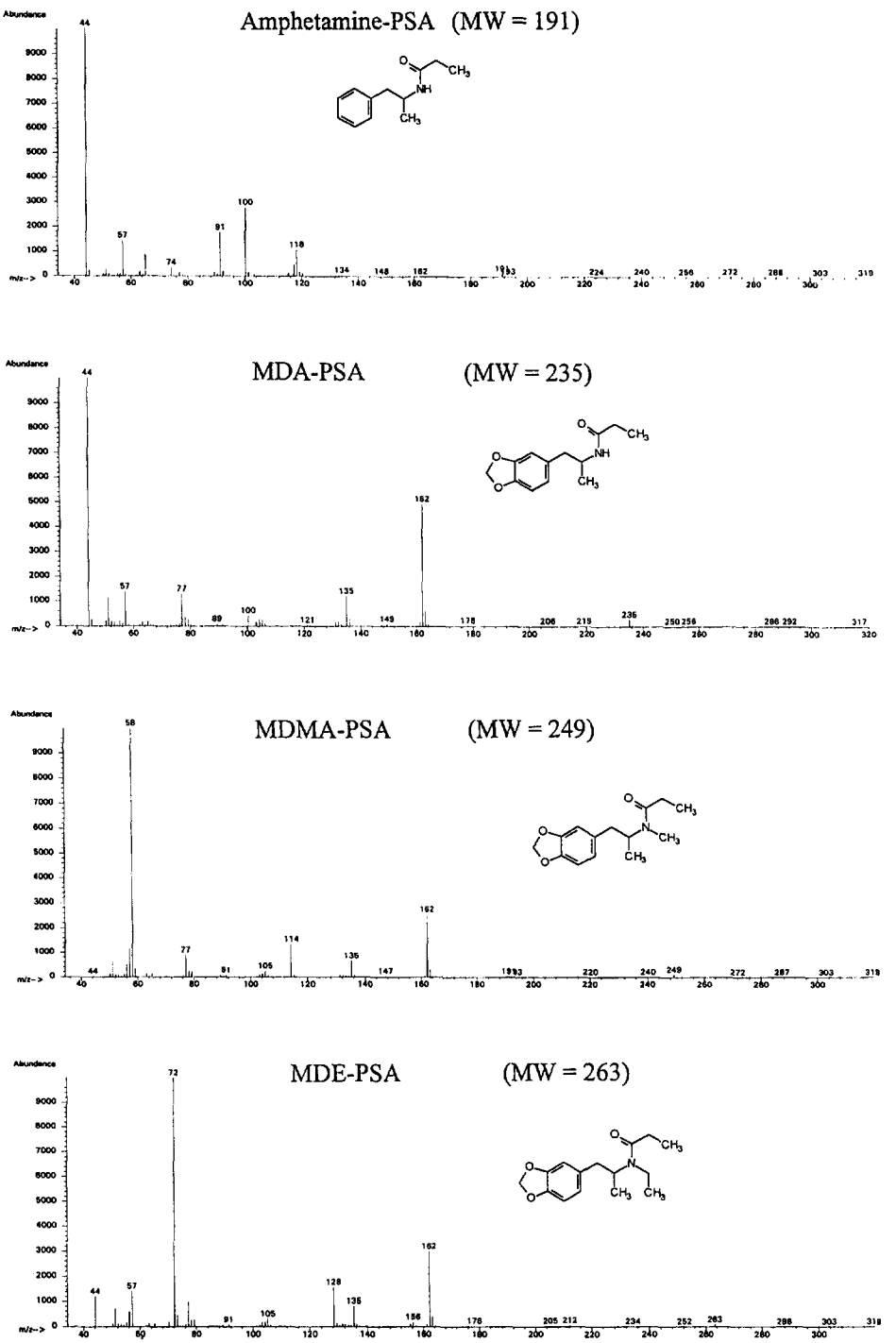

Fig. 2. EI mass spectra of propionylated amphetamine, MDA, MDMA and MDE.

Table 2
Intra-assay precision of analyses ($n = 5$) of spiked hair containing 500 ng amphetamine, MDA and MDMA

	n	Range (ng)	Mean (ng)	SD (ng)	CV (%)
Amphetamine-PSA	5	483–530	504	15	3.1
MDA-PSA	5	465–538	505	26	5.1
MDMA-PSA	5	454–551	498	36	7.3
Amphetamine-TFA	5	475–526	499	17	3.4
MDA-TFA	5	472–538	501	24	4.8
MDMA-TFA	5	441–512	486	25	5.7

could not be detected 2–3 days after the extracts were dissolved in water-free ethyl acetate (TFA 1%). In contrast, the PSA-derivatives are stable in solution for at least 6 days. However, since the GC/MS analyses are usually performed within 24 h after the preparation of the hair extracts, the lower stability of the TFA-derivatives is tolerable. Therefore, the TFA-derivatization seems to be preferable for amphetamine determinations because of its higher mass-spectrometric specificity compared to PSA-derivatives.

For evaluating precision and accuracy of the method used a five-point calibration curve was constructed for each drug by measuring blank hair samples of 100 mg, which were spiked with 10 ng, 50 ng, 100 ng, 250 ng and 500 ng of amphetamine, MDA and MDMA. The spiked samples were extracted as described above and derivatized either with PSA or TFA. The calibration was linear in the range tested. The correlation coefficients were > 0.9. The calibration curve for trifluoroacetylated amphetamine is presented in Fig. 3. The intra-assay precision was determined by analyzing five spiked hair samples containing 500 ng amphetamine, MDA and MDMA in one series (Table 2). The coefficient of variation (CV) ranged from 3.1% (amphetamine-PSA) to 7.3% (MDMA-PSA). Inter-assay precision data were obtained from analyses of spiked hair samples (500 ng amphetamine, MDA and MDMA), performed on seven different days (Table 3). As expected, the within-day

Table 3
Inter-assay precision determined by analyzing spiked hair samples (500 ng amphetamine, MDA and MDMA) at seven different days

	n	Range (ng)	Mean (ng)	SD (ng)	CV (%)
Amphetamine-PSA	7	418–661	524	71	13.5
MDA-PSA	7	389–548	480	62	13.0
MDMA-PSA	7	357–615	481	76	15.9
Amphetamine-TFA	7	463–512	493	21	4.2
MDA-TFA	7	406–564	513	65	12.6
MDMA-TFA	7	409–665	518	102	19.7

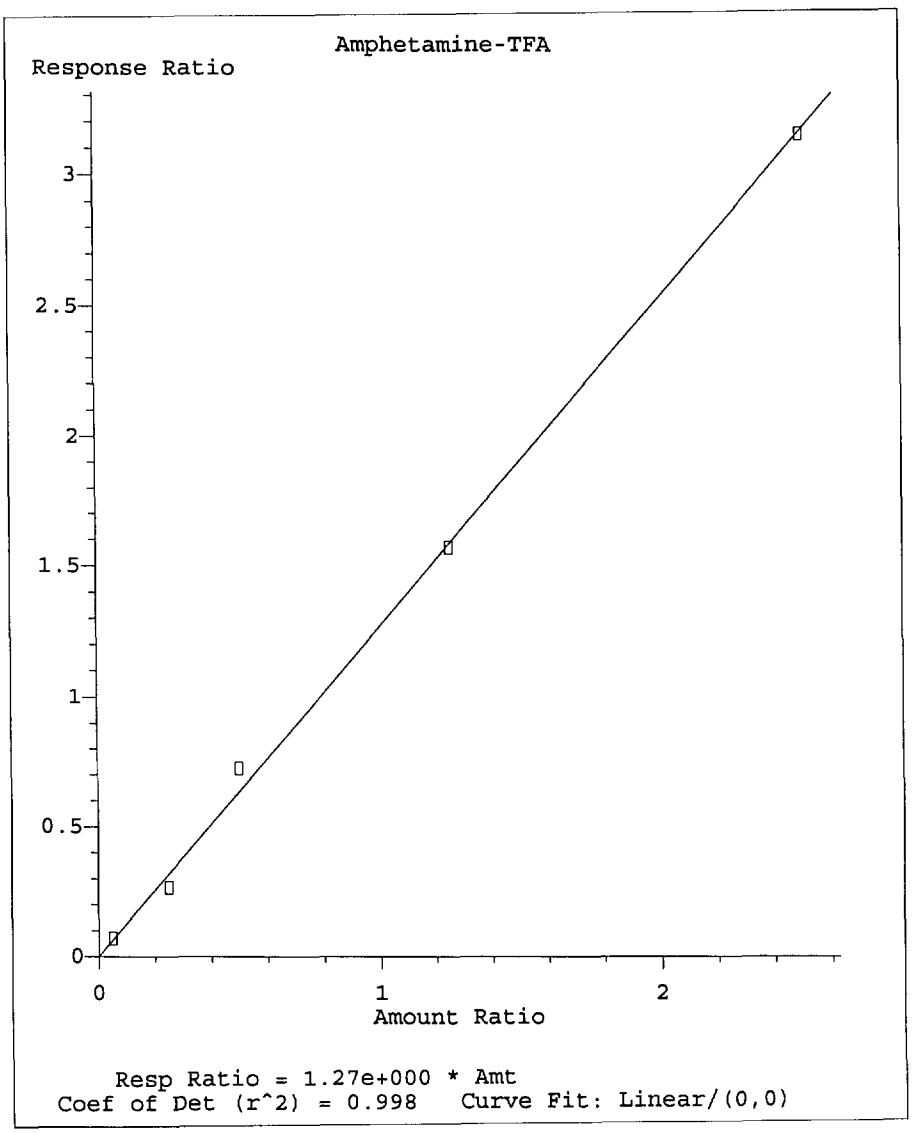

Fig. 3. Calibration curve for the quantification of trifluoroacetylated amphetamine in hair.

reproducibility of the method was better than the day-to-day reproducibility. The inter-assay coefficients of variation were about 2–3 times higher than the intra-assay coefficients. The detection limit for all compounds was in a range of about 0.01 ng/mg if at least 50–100 mg of hair were used for analysis, independent of the derivatization procedure applied (PSA or TFA). It has to be mentioned that for routine analyses a two-point calibration curve (100 ng and 500 ng of each drug) is

Table 4
Amphetamine and its methylenedioxy-derivatives in authentic hair samples of drug abusers

	Amphetamine	MDMA	MDA	MDE
Number of positive findings	23	9	6	5
Minimum concentration (ng/mg)	0.02	0.05	0.04	0.80
Maximum concentration (ng/mg)	6.52	2.91	1.23	3.07
Average (ng/mg)	0.84	1.41	0.40	1.47
Median (ng/mg)	0.31	1.27	0.16	1.20

mostly used for quantification, which seems reasonably precise because of the proven linearity of the calibration.

3.2. Findings in authentic hair samples of drug abusers

A total of 303 hair samples were analyzed for illicit drugs between January 1995 and February 1996. 28 of these samples (9.2%) contained amphetamine and/or methylenedioxy-amphetamine-derivatives (see Table 4). 17 of these samples were obtained from persons who had been asked by the authorities to provide services such as hair donation for reissuing confiscated driving licences. Eleven samples were taken at autopsies. Considering the increasing use of amphetamine and particularly of 'Ecstasy', this number of 28 amphetamine-positive samples seems remarkably low. In contrast, THC was found in 70 samples (23%) and cocaine in 116 samples (38%). These findings may suggest that amphetamines were used only occasionally, probably at special events such as rave parties. Hair samples of occasional users may not necessarily contain measurable amphetamine concentrations.

Amphetamine itself could be detected in 23 (82%) of the 28 amphetamine-positive hair samples. MDMA was found in 9 (32%), MDA in 6 (21%) and MDE in 5 (18%) cases. The concentrations of amphetamine were in a range of 0.02–6.52 ng/mg (mean 0.84 ng/mg), for MDMA in a range of 0.05–2.91 ng/mg (mean 1.41 ng/mg), for MDA of 0.04–1.23 ng/mg (mean 0.40 ng/mg) and for MDE of 0.8–3.07 ng/mg (mean 1.47 ng/mg). The clearly lower MDA concentrations compared to those of MDMA and MDE may be explained by the fact that MDA is also a metabolite of MDMA and MDE. If MDA occurs as a metabolite of MDMA or MDE, the MDA concentrations in blood are low [13], which may also lead to lower MDA concentration in hair.

4. Conclusion

The presented methanol sonication extraction technique enables the simultaneous extraction of amphetamines besides other drugs from a hair sample in one analysis. Especially after TFA derivatization amphetamines can be detected with high specificity and sensitivity. Because of it's reliability and low experimental

effort, the method is particularly recommended for laboratories dealing with a larger number of hair samples.

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