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# The use of supercritical fluid extraction for the determination of amphetamines in hair

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**Abstract**

A laboratory study interested in the analysis of human hair for drugs-of-abuse was conducted to determine if drugs could be detected and quantified from hair. Supercritical fluid extraction (SFE) techniques followed by GC–MS analysis were applied to extract amphetamines from hair. The group of amphetamines included methylenedioxyamphetamine (MDA), methylenedioxymetamphetamine (MDMA), methylenedioxyethylamphetamine (MDEA) and internal standard mephentermine (MP). To validate information on amphetamine use in hair, powdered hair samples free from drugs were collected and soaked in a known amphetamine standard solution. Authentic fortified case hair samples taken from known drug users known to have consumed amphetamines were also analyzed for amphetamine. Results from this study show that amphetamine use can be detected in spiked and authentic fortified human hair using SFE techniques for qualitative and quantitative reproducible results. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

*Keywords:* Hair analysis; Supercritical fluid extraction; Methylenedioxyamphetamine; Methylenedioxyethylamphetamine; Methylenedioxymethamphetamine

## 1. Introduction

Most of the literature dealing with the detection of amphetamine in hair has come from Japanese researchers. This is mainly because of the vast amphetamine consumption in the East which has prompted increased analytical research. In the past, target drugs

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have been amphetamine (AP) and methamphetamine (MA), however, more recently MDA derivatives like MDMA are of particular interest.

Nakahara published a detailed review in 1995 on the detection of amphetamines in hair [1]. Techniques published before 1990 used acid or alkaline hydrolysis or a combination of hydrochloric acid and methanol followed by solid-phase extraction (SPE) or liquid liquid extraction (LLE) and derivatisation. Recently Rohrich and Kauert developed a screening procedure for the simultaneous detection of AP, MA, MDA, MDMA and MDEA based on methanol sonication with methaqualone used as the internal standard [2]. Kintz and Cirimele compared four different procedures for AP, MDA and MDMA (methanol sonication and acid, alkaline and enzymatic hydrolysis) where alkaline hydrolysis was determined to give the greatest recoveries [3].

The detection of amphetamine and the drug ecstasy (MDMA) in hair is becoming of growing interest in the forensic laboratory. Since Moeller et al. first identified MDMA in human hair, it has become one of the most frequently identified, especially in Europe [4]. Therefore, it is included in all screening procedures.

There are various techniques used for the extraction of drugs from hair. Conventional techniques such as solid-phase extraction have been a popular choice, along with alkaline or acid hydrolysis and enzymatic digestion techniques. However, more recent techniques such as supercritical fluid extraction have been applied for the extraction of drugs of abuse from human hair.

Supercritical fluid (SF) is a substance that is above its critical temperature and pressure. Supercritical extraction depends on the ability of the SF to selectively dissolve various amounts of nonvolatile substances [5]. The favorable mass transport properties and the ability to vary the temperature and pressure of the SF, changes the solvent properties enabling the extraction of substances from various matrices. For these reasons, researchers have investigated the use of SFE as an alternative to conventional methods for drug analysis in hair [6,7].

Although, amphetamine and its analogues (e.g. MDMA, MDEA and MDA) have been detected in hair using several extraction techniques, there is no present literature on the extraction of hair for amphetamines by SFE. It was Sachs and Uhl [8] and Sachs and Raff [9] who demonstrated, for the first time, the use of supercritical fluids in the extraction of drugs in hair. Since then there has been studies for the extraction of opiates and cocaine in hair [10,11].

The purpose of this study was first to investigate the use of SFE for the detection of MDA, MDMA and MDEA in human hair. Second, to optimize a method that would extract and identify the selected amphetamines. Also, to determine if this method can be used for the extraction of authentic case samples.

## 2. Experimental

### *2.1. Materials*

Chloroform and isopropyl alcohol AnalaR grade were supplied by Merck (Poole, UK). Ethyl acetate chromatographic (HPLC) grade by Analytical Sciences Lab-Scan,

Dublin, Ireland. Pentafluoropropionic anhydride (PFPA) was supplied by Fluka Chemika. $CO_2$ air products by Walton-on-Thames, UK. SFE vials 6 ml Hypovial™ were from Pierce, Oud-Beyerland, The Netherlands, butyl rubber septa from Pierce and Warner, Chester, UK and columns for SFE were 3 cm×4.6 mm I.D. stainless steel tubing.

### *2.2. Stock solutions*

Drug and internal standard stock solutions were prepared in methanol (1 μg/ml and 10 μg/ml).

### *2.3. Sample conditions and preparation*

It is necessary to wash the hair to remove any exogenous contaminates, prior cosmetic treatments and grease that can interfere with analysis or create false positives. The samples are first washed with sodium dodecyl sulfate (detergent) followed by a dichloromethane wash, a methanol wash and a final wash with distilled water. Each stage was repeated twice and followed by a 15-min sonication at room temperature and dried at room temperature.

After being washed and dried, blank hair samples were finely cut and weighed out into 50-mg samples. The blank sample was spiked with 10 ng/mg of drug standards (e.g. MDA, MDMA and MDEA) in a glass vial. To ensure the hair was coated sufficiently, 3 ml of MeOH was added to the vial, sealed and sonicated for 15 min then opened and left overnight to dry at room temperature. The internal standard for all drugs was added to the hair during hair spiking preparation or after extraction for recovery purposes.

It is possible that this method of spiking is not the most ideal, as most drugs coat the surface of the hair and possibly do not incorporate into the hair structure. However, it is accurate enough for the estimation of exact concentration levels and is the only way to spike hair samples with known concentrations.

### *2.4. SFE parameters*

A Gilson 308 ‘master’ pump, which controlled a 306 ‘slave’ pump and regulated the pressure, $CO_2$ amount, modifier and flow-rate was used. A Gilson 811B dynamic mixer combined the solvent with $CO_2$ and a Gilson 821-pressure regulator controlled by the 308 pump regulated the pressure.

Placed in-line between the 811B mixer and the extraction cell, an HPLC 7125 Rheodyne valve was housed inside an 831 Gilson temperature regulator (oven) for the application of the gas and modifier to the cell. A second valve located below the 7125 valve, was used to switch the extraction from static to dynamic (continuous) mode.

SFE pressure conditions were set at 3800 psi with the flow-rate set at 2 ml/min and temperature 70°C. The modifier was chloroform and isopropyl alcohol (90:10 v/v) pumped at 10% in 90% $CO_2$ in dynamic extraction mode for 30 min. Derivatisation was carried out by adding 50 μl of PFPA:ethyl acetate (1:1 v/v) and analysis was by GC–MS.

### *2.5. GC–MS parameters*

A Hewlett-Packard gas chromatography model 5890 fitted with a VG Analytical VG70-250S mass spectrometer was used for analysis. The GC was fitted with a HP-5 X-link 5% PH Me silicone capillary column 30 m×0.25 μm, 0.88-μm film thickness.

The selected ions were monitored in the electron ionization mode at 70 eV and the trap current was set at 200 μA. The initial temperature was 55°C held for 2 min and final temperature was 280°C held for 5 min. The oven rise was at a rate of 20 C° per min. The ions monitored were *m*/*z* 218 mephentermine (internal standard), *m*/*z* 325 MDA, *m*/*z* 339 MDMA and *m*/*z* 353 MDEA.

## 3. Results and discussions

Under the SFE parameters developed from amphetamines, hair samples were extracted and analyzed using GC–MS (Fig. 1). At 10 ng/mg, hair samples produced recoveries at an average of 84% for MDA, 71% for MDMA and 77% for MDEA. These results are summarized in Table 1.

For the linear calibration of each drug, the method proved linear over a concentration range of 0 to 20 ng/mg of hair investigated. The limit of detection for MDA was 0.02

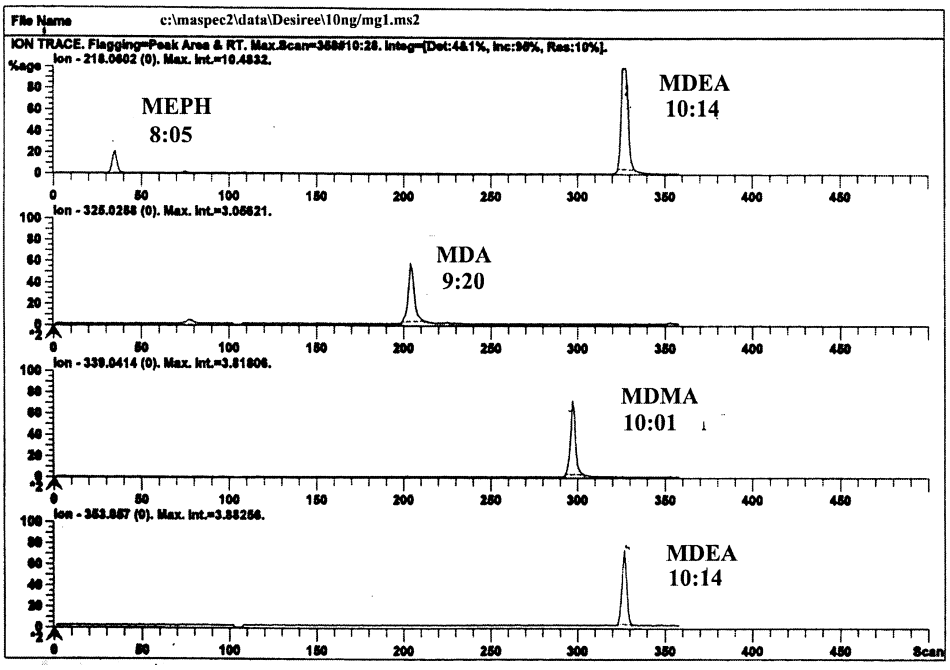


Fig. 1. Chromatography of a 10-ng/mg hair sample of MDA, MDMA and MDEA extracted using supercritical fluid extraction.

Table 1
The average recovery of amphetamines from hair

| Drug | % AVG recovery | Unextracted STD PAR | PAR range |
|---|---|---|---|
| MDA | 84.2 | 1.6722 | 1.0976 – 1.5090 |
| MDMA | 71.2 | 0.7352 | 0.4071 – 0.7345 |
| MDEA | 77 | 0.3785 | 0.2294 – 0.3690 |

ng/mg and 0.1 ng/mg for MDMA and MDEA. Table 2 shows the method validation for MDA, MDMA and MDEA.

The reproducibility of this method was investigated and was found to be relatively reproducible from sample to sample extraction. The results are summarized in Table 3.

Intra and inter-day reproducibility produced comparable results from sample to sample and day to day. Tables 4 and 5 shows the peak area ratios produced for day one and day two for MDA, MDMA and MDEA.

For 10 ng/mg MDA, MDMA and MDEA for day one, the standard deviation was 0.595, 0.200 and 0.148, the mean 2.102, 1.804 and 0.692 and the RSD 28%, 11% and 21%. For MDA, MDMA and MDEA for day two, the standard deviation was 0.507, 0.565 and 0.214, the mean 2.5022, 2.1318, 1.2960 and RSD 20%, 26% and 16%.

There was some variation between the two days but comparable MDEA for day one is much lower than for day two. A possible explanation for this could be because of the

Table 2
Method validation for amphetamines in hair

| Drug | Correlation coefficient ($r^2$) | Conc. range ng/mg | LOD ng/mg | Intercept *B* | Slope *A* |
|---|---|---|---|---|---|
| MDA | 0.9703 | 0.02–20 | 0.02 | 0.4736 | 0.085 |
| MDEA | 0.9775 | 0.02–20 | 0.1 | 0.3535 | 0.0106 |
| MDMA | 0.9938 | 0.02–20 | 0.1 | 0.2870 | 0.0581 |

Table 3
Reproducibility of amphetamines in hair at 2 ng/mg

| Drug | Mean | Range | SD | %RSD |
|---|---|---|---|---|
| MDA | 1.2761 | 1.2309–1.3241 | 0.038 | ±3 |
| MDMA | 1.1079 | 0.9470–1.2779 | 0.165 | ±14 |
| MDEA | 0.6522 | 0.5770–0.7230 | 0.059 | ±9 |

Table 4
Day one reproducibility of each drug

| Drug | Unextracted STD | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|
| MDA | 2.119 | 1.913 | 1.1897 | 2.7397 | 2.2025 | 2.4690 |
| MDMA | 2.0746 | 2.068 | 1.788 | 1.939 | 1.6371 | 1.5929 |
| MDEA | 2.0549 | 0.456 | 0.677 | 0.855 | 0.7037 | 0.7699 |

Table 5
Day two reproducibility of each drug

| Drug | Unextracted STD | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|
| MDA | 3.3182 | 2.1934 | 2.1173 | 2.6670 | 3.3232 | 2.2103 |
| MDMA | 2.5970 | 1.7711 | 1.8597 | 1.9111 | 3.133 | 1.9840 |
| MDEA | 2.0833 | 1.1376 | 1.2789 | 1.1837 | 1.6681 | 1.2103 |

spiking of the blank hair. Spiking blank hair with a drug does not incorporate into the hair as fortified hair samples, therefore not all the time will there be precise consistency from batch to batch. However, it is accurate enough for estimation of exact concentration levels and is the only way to spike hair samples with known concentrations. Also the sensitivity of the GC–MS from day to day can be a possible contributory factor.

Hair samples taken from subjects participating in the self-report study for amphetamine use were analyzed under SFE conditions. The samples were too small to be separated into segments of root or tip so they were analyzed in bulk or full. Before analysis, the hair samples were washed and dried under the aforementioned wash conditions and the colour, weight and length were applicable of each hair sample recorded. Table 6 lists twenty cases analyzed for MDA, MDMA and MDEA.

Table 6
Case samples analyzed for MDA, MDMA and MDEA

| Case # | MDA (ng/mg) | MDMA (ng/mg) | MDEA (ng/mg) | Length (cm) | Weight (mg) |
|---|---|---|---|---|---|
| A99-A | 0.255 | 0.638 | 0.396 | 11 | 60 |
| A99-B | 0.125 | 0.288 | 0.067 | 4 | 30 |
| A99-C | 0.252 | 0.153 | 0.171 | 11 | 40 |
| A99-D | ND[a] | 0.117 | ND | 11 | 70 |
| A99-E | ND | 0.659 | 0.286 | 11 | 19 |
| A99-F | ND | ND | ND | 12 | 78 |
| A99-G | ND | ND | ND | 11 | 60 |
| A99-H | 0.274 | 0.53 | 0.11 | 5 | 35 |
| A99-I | 0.061 | 0.336 | 0.082 | 4 | 20 |
| A99-J | ND | ND | ND | 11 | 53 |
| A99-K | ND | 3.88 | ND | 11 | 60 |
| A99-L | ND | ND | ND | 11 | 21 |
| A99-M | 0.059 | 1.15 | ND | 12 | 10 |
| A99-N | ND | ND | ND | 11 | 40 |
| A99-O | ND | ND | ND | 2 | 66.5 |
| A99-P | ND | ND | ND | 2 | 7 |
| A99-Q | 2.14 | 6.44 | ND | 8.5 | 10 |
| A99-R | ND | 0.71 | ND | 11 | 9 |
| A99-S | ND | 1.62 | ND | 11 | 10 |
| A99-T | ND | ND | ND | 11 | 52.5 |

[a] ND=Not detected.

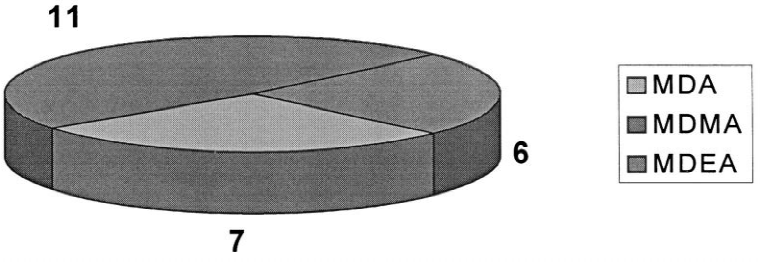


Fig. 2. Chart of overall positive cases.

Out of the 20 samples analyzed, five were positive for MDA, MDMA and MDEA, four were positive for MDMA only, two were positive for MDA and MDMA only, one was positive for MDMA and MDEA only and eight were not found to contain drugs. Samples that were not detected may have contained drugs below the limit of detection. Below in Fig. 2 are the results summarized for the case samples positive overall for all three drugs. Samples that were listed as not detected are not included. There were seven overall MDA positives, 11 MDMA positives and six MDEA positives.

The self-report data was compared to the SFE findings from the hair samples to find if a correlation existed. It was found that some of the subjects that reported no use or little use (e.g. half an ecstasy tablet monthly) over the past 12 months were found to be negative and others were found to be positive with low levels detected. Fig. 3 summarizes the results of the self-report data to the hair findings. Out of the 20 hair samples analyzed, 11 (55%) were positive for all or either MDA, MDMA or MDEA and said yes to consuming ecstasy. Three (15%) were also positive but disagreed with having taken any ecstasy and six (30%) were negative for MDA, MDMA or MDEA, but agreed with having consumed ecstasy in the past 12 months. There was no one that claimed to be negative and was found to be negative.

| | | SELF-REPORT | |
|---|---|---|---|
| | | YES | NO |
| LAB | YES | 11 (55%) | 3 (15%) |
| | NO | 6 (30%) | 0 (0%) |

Fig. 3. Self-report data compared to hair analysis for MDA, MDMA and MDEA.

## 4. Conclusion

Supercritical fluid extraction is a good technique that can be used as an alternative extraction method to conventional techniques. Among its many advantages, SFE is fast, inexpensive and produces less solvent waste. The use of SFE for the extraction of hair has been reported in various studies. Despite the controversial aspects of hair testing, the use of hair analysis is rapidly expanding.

The aim of this study was to determine if amphetamines, MDA, MDMA and MDEA, could be extracted from hair samples using SFE, to optimize a method for extraction and its application to authentic hair samples. The results demonstrate that MDA, MDMA and MDEA can be extracted from hair under the developed SFE conditions. Recoveries better than 84%, 71% and 77% were produced for MDA, MDMA and MDEA. Based on spiked hair samples, the method was found to be linear for a concentration range of 0.02 to 20 ng/mg of hair. The correlation coefficient ($r^2$) for MDA, MDMA and MDEA was 0.9703, 0.9775 and 0.9938 showing the linearity of the method. The method was also found to be reproducible from sample to sample and on a day to day basis.

It has also been demonstrated that by the application of a polar modifier directly through the system, better extraction conditions are provided for the drugs of interest. By varying the temperature and pressure, optimal conditions increased the extraction potential. Temperature and pressure are the two main parameters that effect SFs. An increase in pressure at a constant temperature, increasing the density and mass transfer of the SF allows more polar and higher-molecular-weight analytes of interest to be eluted.

Authentic hair samples taken from subjects participating in a self-report study were analyzed using the developed SFE techniques. Out of the 20 samples analyzed, seven were positive overall for MDA, 11 for MDMA and six for MDEA. Samples were positive for either MDA or MDMA or a combination of MDA, MDMA and MDEA. Samples that were not detected may not have been negative, but had levels too low to detect using this method. The results from the hair analysis were compared to the self-report data collected to find if a correlation existed between the two. It was found that with some of the subjects, a correlation did exist between what they had reported as having consumed from month to month and hair findings and with others it did not. The accuracy of the self-report data was questionable because of the possibility of under or over reporting consumption and whether or not what was taken contained MDA, MDMA or MDEA.

It can be concluded that, SFE is an appropriate method for the extraction of amphetamines in hair. It is fast (30 min), reproducible and can be applied to authentic hair samples with results comparable to those generally observed in hair analysis.

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