

Extraction of Commonly Abused Substituted Phenethylamines from Urine and Subsequent Analysis by GC-MS

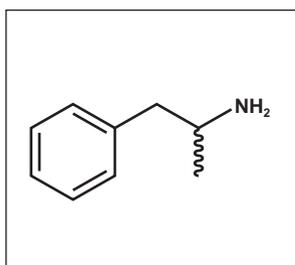
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Abstract

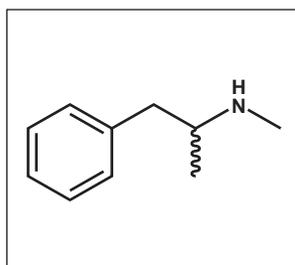
This application note demonstrates a GC-MS method for identifying substituted phenethylamines. An extraction procedure for these compounds was optimized on a Thermo Scientific Servo+ AMP SPE cartridge. A human urine sample was spiked with four commonly abused substituted phenethylamines and analyzed by GC-MS using a Thermo Scientific TraceGOLD TG-5MS column.

Introduction

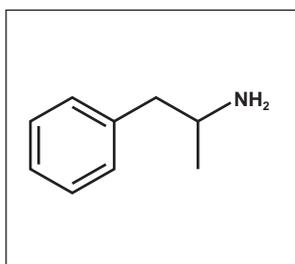
Substituted phenethylamines are chemical compounds with various hydrogen substitutions on the 2-phenylethylamine chemical structure. The most commonly abused substituted phenethylamines are the central nervous system stimulants, such as amphetamine and methamphetamine[1]. Two other substituted phenethylamines, commonly used as recreational drugs, are 3,4-Methylenedioxyamphetamine (3,4-MDA) and 3,4-Methylenedioxymethamphetamine (3,4-MDMA). 3,4-MDA is both a stimulant and a psychedelic. 3,4-MDMA, also known as ecstasy, does not exhibit strong stimulant effects, but induces euphoria and reduces anxiety. Amphetamine and methamphetamine are currently classified as DEA schedule 2 drugs, where as 3,4-MDA and 3,4-MDMA are classified as DEA schedule 1 drugs.



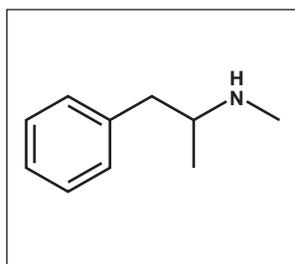
Amphetamine



Methamphetamine



3,4-MDA



3,4-MDMA



Experimental details

| Sample Handling Equipment | Part Number |
|-------------------------------------------------------------------------|-------------|
| Liquid handling hardware: eVol with 50 μ L and 500 μ L syringes | 66002-024 |
| SPE hardware: Universal Vacuum Manifold | 60104-230 |
| SPE cartridges / consumables: | |
| Servo+ AMP 30 mg/1 mL | 60110-701C |
| Servo+ AMP 60 mg/3 mL | 60110-703C |
| Vials and closures: 2 mL clear screw top vials | 60180-599 |

Sample Preparation

| | |
|-----------------|-------------------------------------------------------------------------------------------------------|
| Compounds: | Amphetamine, Methamphetamine, 3,4-MDA, 3,4-MDMA |
| Part Number: | 60110-701C |
| Phase: | HyperSep Servo+ AMP |
| Volume: | 1 mL |
| Bed weight: | 30 mg |
| Conditioning: | 1 mL of MeOH followed by 1 mL of DI H ₂ O |
| Application: | Load at 1 to 2 mL/min |
| Washing: Wash1: | 1 mL of MeOH:Formic Acid (98:2); Wash2: 1 mL of MeOH:H ₂ O:NH ₄ OH (90:9.5:0.5) |
| Elution: | 1 mL of MeOH:NH ₄ OH (95:5) |

Separation Conditions

| | |
|------------------|------------------------------------------------------------------------------|
| Instrumentation: | Thermo Scientific Trace GC Ultra |
| GC Column: | TG-5MS, 30m x 0.25mm x 0.25 μ m |
| Oven temp: | 50 °C (3 min hold) to 250 °C (2 min hold) at 10 °C/min |
| Carrier: | Helium (1.4 mL/min) |
| Injector: | 250 °C split (28 mL/min, 20:1 split ratio) |
| Detector: | FID 300 °C (for SPE elution profiles); MS (for calibration and urine sample) |
| Sample injected: | 1.0 μ L |

Key Words

- Servo+ AMP
- TraceGOLD TG-5MS
- Substituted phenethylamines
- Amphetamine
- Methamphetamine
- 3,4-MDA
- 3,4-MDMA
- Drugs of abuse
- Clinical

MS Conditions (if applicable)

| | |
|----------------------------|--------------------------------------------|
| Instrumentation: | Thermo Scientific Trace GC Ultra and DSQII |
| Transfer line temperature: | 290 °C |
| Source temperature: | 230 °C |
| Ionization conditions: | El |
| Emission current: | 100.00 |
| Electron energy: | -70.00 |
| Filament delay: | 7 minutes |
| Ion Range: | Scan 48 - 600 |
| Scan time: | Scan 3sec |

Solutions

Amphetamine (1 mg/mL in Methanol), Methamphetamine (1 mg/mL in Methanol), 3,4-MDA (1 mg/mL in Methanol) and 3,4-MDMA (1 mg/mL in Methanol) was purchased from Alltech Associates Inc.

Derivatization reagent used was MSTFA with 1% TMCS (p/n TS-48915).

Standards were prepared in house by making a stock solution at the highest calibration level and then diluting to achieve the various lower level standards.

Data Processing

| | |
|-----------|----------|
| Software: | Xcalibur |
|-----------|----------|

Results

Both a sample extraction method and GC-MS method were developed for the identification of commonly abused substituted phenethylamines. The extraction procedure developed in this application note was the result of SPE optimization. This optimization was done in order to maximize the recovery of the analytes.

A HyperSep Servo+ AMP SPE cartridge (30 mg/1mL) was conditioned with 1mL of MeOH followed by 1 ml of H₂O. A 1 mL aliquot of H₂O was spiked with 20 µg of amphetamine and 20 µg of methamphetamine and loaded on to the HyperSep Servo+ AMP SPE cartridge. The eluant from the loading was collected. Washes with increasing strengths of MeOH in H₂O/NH₄OH 95:5 (v/v) were applied, starting from 10% MeOH/90% H₂O/NH₄OH [95:5] (v/v) and increasing in MeOH content by 10% each. Finally four 1mL aliquots of 95% MeOH / 5% NH₄OH (v/v) were used to wash the SPE cartridge. Each eluant from the washings was collected and analyzed by GC-FID. By plotting the area/detector response for each of these eluants generates an elution profile. This data is shown in Figure 1, where L indicates the loading of the SPE cartridge, the numeric indicators are the ratios of MeOH to H₂O used in the washes, and the four elutions are marked with E1 – E4. These results show an optimum wash profile of 90% MeOH/10% H₂O/NH₄OH [95:5] (v/v) before amphetamine and methamphetamine start to elute.

To demonstrate the robustness of the method and the HyperSep Servo+ AMP SPE cartridges, percent recoveries of amphetamine and methamphetamine were calculated. Using 60 mg/3 mL cartridges, 2 ml of dilute urine (1:1 dilution) spiked with 20 µg each of amphetamine and methamphetamine, and an internal standard (naphthalene) was loaded onto the SPE devices. Nine SPE cartridges were loaded in this manner. All of the cartridges were then washed with 2 mL of 90% MeOH/10% H₂O/NH₄OH [95:5] (v/v) and then eluted with 1mL of 95% MeOH/5% NH₄OH (v/v). These samples, along with a standard

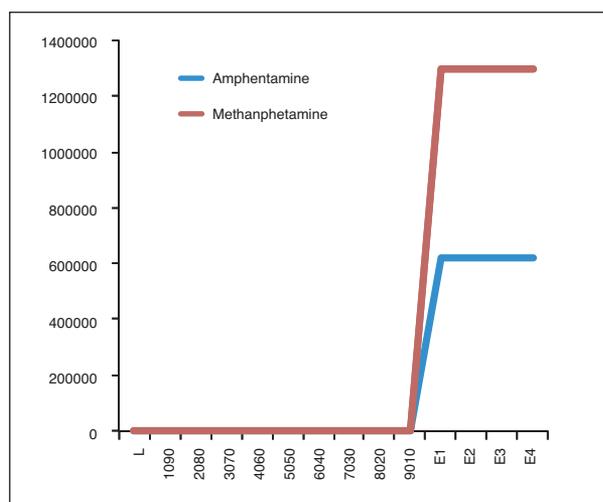


Figure 1: Elution profile of amphetamine and methamphetamine

prepared with 20 µg each of amphetamine, methamphetamine, and naphthalene were analyzed by GC-FID. Using the results from these analysis percent recoveries were calculated resulting, on average with a percent recovery of 85% for amphetamine and 97% for methamphetamine.

Using GC-MS, an instrument calibration was performed for amphetamine, methamphetamine, 3,4-MDA and 3,4-MDMA. This was an external calibration curve and no internal standards were used. Standards were first derivatized using MSTFA with 1% TMCS to form the TMS derivatives for easier analysis. Using 5 different calibration levels and triple injections, percent relative standard deviation calculated from the detector response are shown in Table 1. Figures 2-5 show the linear correlation for the calibration curve.

| Compound | 5 µg/mL | 10 µg/mL | 20 µg/mL | 30 µg/mL | 40 µg/mL |
|---------------------|---------|----------|----------|----------|----------|
| Amphetamine-TMS | 3.9% | 0.8% | 2.0% | 1.0% | 2.2% |
| Methamphetamine-TMS | 1.6% | 7.3% | 1.3% | 1.5% | 1.6% |
| 3,4-MDA-TMS | 4.9% | 1.7% | 2.4% | 1.6% | 2.7% |
| 3,4-MDMA-TMS | 2.3% | 9.1% | 0.9% | 3.6% | 2.4% |

Table 1: Calibration Table showing %RSD

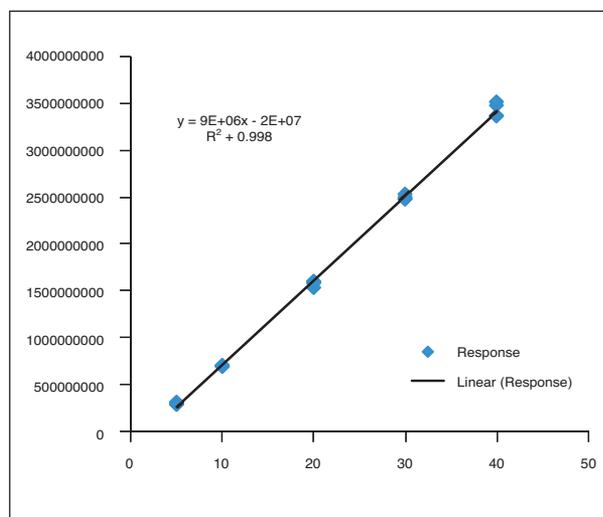


Figure 2: Calibration Curve for Amphetamine-TMS

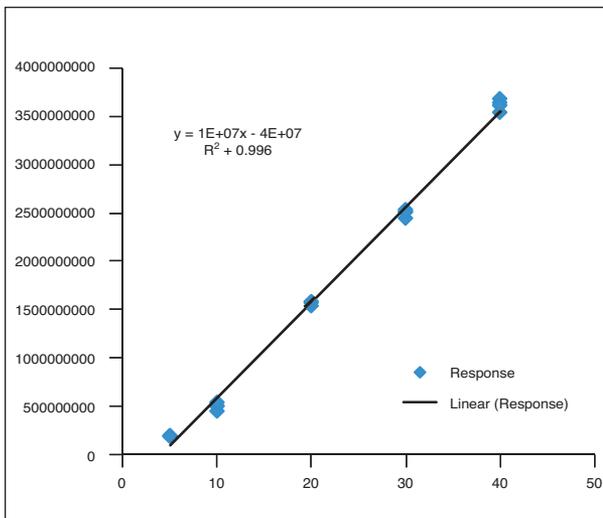


Figure 3: Calibration Curve for Methamphetamine-TMS

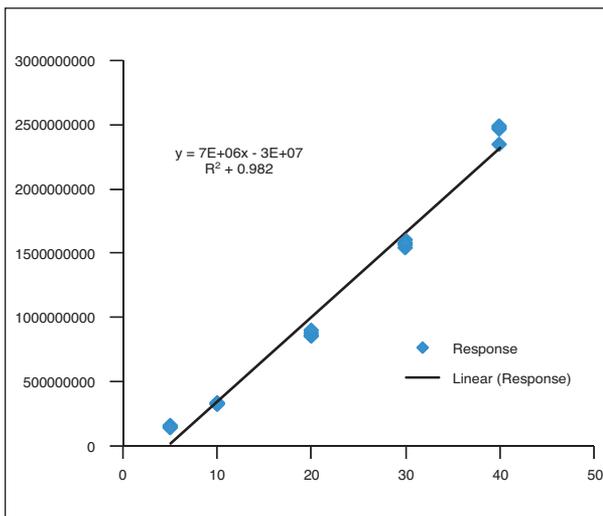


Figure 4: Calibration Curve for 3,4-MDA-TMS

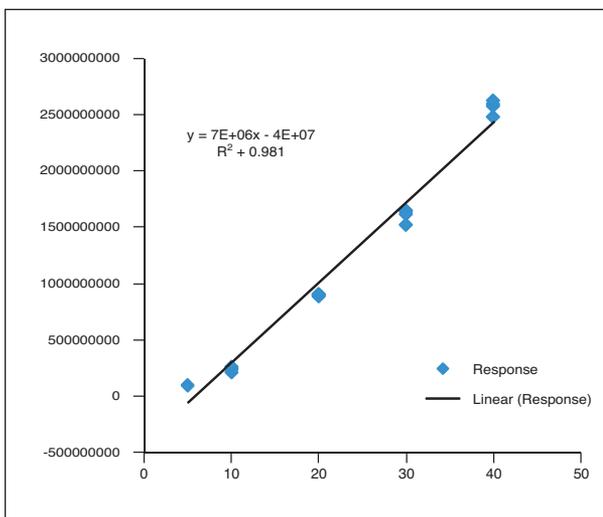


Figure 5: Calibration Curve for 3,4-MDMA-TMS

A 1 mL urine sample was spiked with 10 µg of amphetamine, methamphetamine, 3,4-MDA and 3,4-MDMA. This sample was then diluted with 1 mL of deionized H₂O. Following the optimized SPE method for the HyperSep Servo+ AMP SPE cartridge, the urine sample was extracted. The extraction was evaporated and derivatized with 1 mL of MSTFA with 1% TMCS. After mixing and 15 minutes at 40°C, the eluant was analyzed via the GC-MS method described above. The resulting chromatogram is shown in Figure 6.

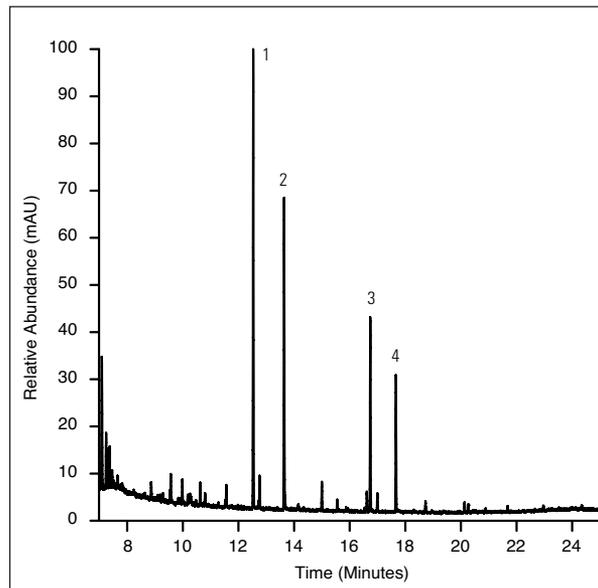


Figure 6: Analysis of spiked urine sample by GC-MS following clean-up with a 60 mg / 3 mL HyperSep Servo+ AMP cartridge and derivatization. Peak identification: 1. Amphetamine (TMS), 2. Methamphetamine (TMS), 3. 3,4-MDA (TMS), 4. 3,4-MDMA (TMS)

Conclusion

In this application note an SPE method for the extraction of commonly abused substituted phenethylamines from urine was developed. The optimized extraction for these compounds was achieved on a HyperSep Servo+ AMP SPE column. A GC-MS method was also developed for the identification of amphetamine, methamphetamine, 3,4-MDA and 3,4-MDMA. These compounds were extracted from a spiked urine sample, derivatized, and identified by GC-MS and quantified by a standard calibration. The TraceGOLD TG-5MS capillary columns provided the inertness and low bleed characteristics necessary for quantifying these drugs of abuse.

References

1. Mack, Avram H.; Frances, Richard J.; Miller, Sheldon I. (2005). *Clinical Textbook of Addictive Disorders*, Third Edition. New York: The Guilford Press. p. 207

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