

Drugs of Abuse in Urine by GC/MS Following SAMHSA (NIDA) Procedures

Introduction

The Substance Abuse and Mental Health Services Administration (SAMHSA) regulates urine drug-testing programs for the Federal Workplace Drug Testing. This agency was previously known as the National Institute on Drugs and Abuse (NIDA). Procedures and guidelines for the analysis of drugs of abuse are specified by the Department of Health and Human Services (DHHS), Department of Transportation (DOT) and the Department of Defense (DOD). These procedures encompass detailed sample preparation and analysis with reporting by gas chromatography/mass spectrometry (GC/MS).

Techniques for detecting drugs of abuse in biological samples have greatly improved with advances in analytical instrumentation. Benchtop GC/MS systems are the instruments of choice for forensic scientists. These instruments are legally defensible in court as they are able to positively identify the presence of drugs in urine and blood samples. A PerkinElmer® Clarus® GC/MS system was used for all drug analysis demonstrated in this paper.

This paper will demonstrate how to perform the NIDA requirements for six classes of drugs (Table 1). Each drug has unique requirements for extraction, derivatization and analysis. However, there is a basic procedure that each follows. Carefully laid out will be the universal details as well as the individual details for each drug class. Lastly, this paper will show the typical chromatographic results that would be obtained.

Table 1. Drugs of Abuse in Urine Testing Divided into Six Classes of Drugs.

- | | |
|---|---|
| 1 | Amphetamines (Amphetamine and Methamphetamine) |
| 2 | Cannabinoids (11-Nor-Delta 9-Tetrahydrocannabinol-9-Carboxylic Acid, also known as THC-Acid, the primary metabolite of marijuana) |
| 3 | Cocaine Metabolite (Benzoyllecgonine) |
| 4 | 6-Acetyl Morphine (also known as 6-Mono-Acetyl Morphine, the primary metabolite of heroin) |
| 5 | Opiates (Morphine and Codeine) |
| 6 | Phencyclidine (PCP) |

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Overview

The general procedure for the sample preparation and analysis of drugs following SAMHSA procedures is presented in the following sections; specific procedures for each class of drugs are presented in the results section.

The general sample-preparation procedure will begin with the addition of a deuterated internal standard to the urine sample. The pH of the sample is adjusted in preparation for SPE and derivatization. The analytes are extracted from urine using solid phase extraction (SPE). The extract is then derivatized and brought to final volume for GC/MS analysis.

The sample is then ready to be injected into the GC/MS for identification and quantitation. Depending on the concentration and instrument sensitivity, a 1-3 μL injection is performed. The method specifics for the Clarus GC/MS are provided in detail in the following section of this paper.

The final step in the drug analysis is reporting a 'detect' or 'non-detect' condition. TurboMass™ GC/MS software provides the laboratory a reporting package including the 3-ion-ratio report (Figure 1). The 3-ion-ratio report presents all of the data necessary to demonstrate the analytical result.

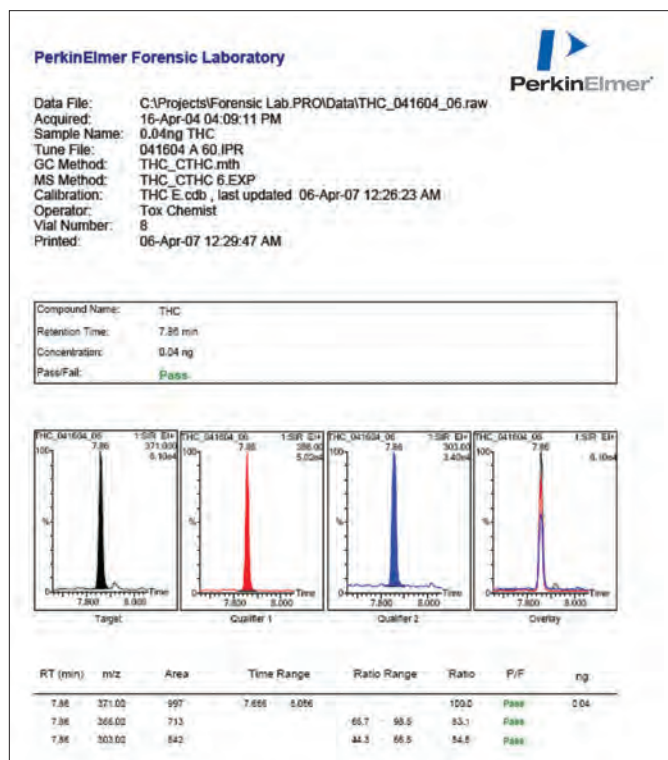


Figure 1. Example of 3-ion-ratio GC/MS report for THC.

Experimental

The experimental section of this paper is broken into a number of different sections and will provide an overview of the extraction and sample-preparation details as well as GC/MS methods. This section includes:

- Reagents List
- Glassware
- Hydrolysis
- Solid Phase Extraction
- Derivatization
- Gas Chromatography
- Mass Spectrometry

The specifics unique to each class of drugs are presented in the results section of the paper – this includes a specific procedure for each of the following:

1. Amphetamine and Methamphetamine
2. Cannabinoids: THC Carboxylic Acid (metabolite of THC)
3. Benzoyllecgonine (Cocaine metabolite)
4. 6-Mono-Acetyl Morphine (metabolite of heroin)
5. Opiates (Morphine and Codeine)
6. PCP (Phencyclidine)

Reagent List

- Drug standards and deuterated internal standards
- BSTFA – Bis(trimethylsilyl)trifluoroacetamide with 1% TMCS, Pierce (Rockford, IL)
- PFPA /PFAA – Pentafluoropropionic acid anhydride, Pierce (Rockford, IL)
- PFPOH – 2,2,3,3,3-Pentafluoropropanol, Pierce (Rockford, IL)
- IOD – Iodomethane, Sigma (St. Louis, MO)
- TMAH – Tetramethyl ammonium hydroxide, 0.2 M in Methanol, Campbell Science Corp (Rockton, IL)
- HFBA – Heptafluorobutyric anhydride, Campbell Science Corp (Rockton, IL)
- DMSO – Dimethyl sulfoxide, Pierce (Rockford, IL)
- MBTFA – n-Methyl-bis-trifluoroacetamide, Pierce (Rockford, IL)
- Acetic Acid, 100 mM – 2.86 mL glacial acetic acid diluted to 500 mL DI water
- Acetate buffer, 100 mM ph 4.5 – 2.93 g sodium acetate trihydrate in 400 mL DI water + 1.62 mL glacial acetic acid, dilute to 500 mL. Adjust to pH 4.5 with 100 mM sodium acetate or 100 mM acetic acid.

- Phosphate buffer, 100 mM pH 6 – 1.7 g Na₂HPO₄ + 12.14 g NaH₂PO₄ dilute to 1000 mL with DI water. Adjust to pH 6 with 100 mM Na₂HPO₄ (raises pH) or 100 mM Na₂HPO₄ (lowers pH).
- Beta-Glucuronidase, Sigma (St. Louis, MO)
- 5000 Fishman units/mL – Dissolve 100,000 Fishman units lyophilized powder into 20 mL acetate buffer 100 mM pH 5.0, make fresh daily.
- Methylene Chloride/Isopropanol/Ammonium Hydroxide (78:20:2) extraction solvent – 40 mL IP-OH + 4 mL of concentrated NH₄OH + 156 mL MeCl₂.

Glassware

All glassware, including autosampler vials and low-volume vial inserts must be silanized to prevent adsorption of sample.

Glassware Silanization Procedure:

- Soak all glassware in 10% DMDCS (dimethyldichlorosilane)/Toluene for 10 min
- Rinse in methanol
- Rinse in hexane
- Air dry

Hydrolysis

Hydrolysis is necessary in some drugs to remove the glucuronide bonding which prevents the solubility and extraction of the drug. This preparation before extraction is usually accomplished either by enzyme or acid hydrolysis of the sample. Both procedures are outlined below.

Enzyme Hydrolysis Procedure:

1. Combine a 1-5 mL urine sample with ISTD, and 2 mL beta-glucuronidase
2. Vortex
3. Heat 3 hours at 65 °C
4. Cool
5. Centrifuge and decant
6. Adjust pH to 6.0 with 700 µL of 1.0 N NaOH.

Acid Hydrolysis Procedure:

1. Combine a 1-5 mL urine sample with ISTD and 500 µL concentrated HCl
2. Vortex
3. Heat 30 min at 120 °C
4. Cool
5. Centrifuge and decant
6. Add 1 mL 7.4 N NH₄OH
7. Vortex
8. Adjust pH to 6.0 with 1-3 mL of 500 mM phosphoric acid.

Solid Phase Extraction

In each of the procedures, the drugs of interest are extracted from the sample matrix by solid phase extraction (SPE) with a polymeric resin cartridge. The drugs are retained as the urine is passed through the resin bed – washing the bed with water will elute salts and other matrix components. Eluting the drugs off the resin bed with a stronger solvent completes the cleanup process. 200-mg extraction cartridges similar to XAD2 resin or CleanScreen ZSDAU020 cartridges were used with success in this paper.

Gas Chromatography

A Clarus GC with a capillary injector port was used for this analysis. The GC was fitted with an Elite-5 MS (5% Phenyl/ 95% Methyl Silicone) column of the following dimensions – 12 m x 0.25 mm x 0.25 µm (PerkinElmer Part No. N9316110). The injection-port liner used was a PerkinElmer Siltek™, without glass wool to improve inertness (PerkinElmer Part No. N6502012). The injector port was maintained at a temperature of 250 °C. A pressure-pulsed splitless injection was used to further improve detection limits. This procedure raises the injector pressure during the injection process, creating a narrow band of analytes – the pressure is then reduced to reach the optimal linear velocity for the helium carrier gas. The specific instrument parameters follow:

- Helium carrier – 4 psi
- Instrument timed events:
 - CAR2 set to 15 psi at -0.71 min (raise pressure)
 - SPL2 set to 0 at -0.70 min (splitless injection)
 - CAR2 set to 4 at 1.50 min (operating pressure after injection)
 - SPL2 set to 50 at 1.55 min (open split vent after injection)

The GC oven program will be unique to each class of drugs and is specified in the results section of this paper.

Mass Spectrometry

The mass spectrometer is operated in selected ion monitoring (SIM) mode; in this mode, only specific ions are detected. SIM mode of operation will achieve the lowest detection limits. Specific SIM parameters will be set for each analyte and presented in the results section of this paper. The general MS settings follow:

- Ionization mode – selected ion monitoring mode (SIM)
- Dwell time – 50-150 milliseconds/ion

- Photomultiplier voltage – 600 V
- Ion source temperature – 230 °C

The MS analysis presented here uses a primary ion for quantitation, while the ratio of all three ions in combination with the analyte retention time will confirm identification. The ion ratios must fall within $\pm 20\%$ of the standard ratios. The identification of deuterated internal standards may use only 2 ions – a primary ion and only 1 confirmatory ion.

Results

Amphetamine and Methamphetamine

The full GC/MS methods are presented in the experimental section (Pages 3-4). The specific GC oven program for the analysis of amphetamine and methamphetamine is: starting temperature 60 °C – hold for 1 minute, 40 °C/min to 300 °C – hold for 1 minute.

The GC/MS analysis of amphetamine and methamphetamine following the procedure presented below has demonstrated method limits of quantitation at 500 ng/mL and limits of detection in urine at 50 ng/mL.

Internal standard: d5 or d8-Amphetamine, d5 or d8-Methamphetamine, either d5 or d8 is acceptable.

Available internal standards include d5 (label on side chain), d5 (label on ring), d6, d8, d10, d11.

Extraction Procedure:

1. Combine a 1-5 mL urine sample with, deuterated internal standard, and 2 mL of 100 mM phosphate buffer (pH 6.0).
2. Condition the SPE column by rinsing with 3 mL methanol, followed by 3 mL DI water and 1 mL of phosphate buffer (100 mM, pH 6).
3. Complete the solid phase extraction of the sample by passing the solution from step 1 through the SPE column. Wash column with 3 mL DI water, then 1 mL of 100 mM acetic acid, and 1 mL methanol to remove excess sample matrix.
4. Elute analytes from the SPE column by rinsing with 3 mL methylene chloride: isopropanol: ammonium hydroxide (78:20:2).
5. Concentrate the extract to a low volume at a temperature of < 40 °C. Quantitatively transfer the concentrated extract to a low-volume autosampler vial.
6. Evaporate to dryness at a temperature of < 40 °C.

The sample is prepared for derivatization.

Derivatization Procedure:

Derivatize the sample with the PFPA procedure; addition of PFPOH is not necessary for the amines.

- Reconstitute dried extract in 50 μ L PFPA.
- Cover with N₂, cap, mix, heat 70 °C (20 min), evaporate to dryness < 40 °C.
- Reconstitute in 50 μ L ethyl acetate, inject 1 μ L.

Following derivatization, an optional extraction of the derivative into toluene will further clean up the extract.

When using the PFPA derivatization procedure, the MS SIM ions for the derivatized analytes are:

- Amp: 190, 118, 91
- d5-Amp (ISTD): 194, 122, 123
- d8-Amp (ISTD): 193, 126, 96
- Mamp: 204, 160, 118
- d5-Mamp (ISTD): 208, 120, 163
- d8-Mamp (ISTD): 211, 163, 122

The 119 ion is common in both the analyte and ISTD, therefore should not be used for quantification.

The expected retention times for amphetamine and methamphetamine under the GC conditions prescribed will be approximately 5.07 and 5.48 minutes respectively.

Alternate Derivatization Procedure:

Derivatize the sample with the HFBA procedure.

- Reconstitute dried extract in 50 μ L HFBA.
- Cover with N₂, cap, mix, heat 70 °C (20 min), evaporate to dryness < 40 °C.
- Reconstitute in 50 μ L ethyl acetate, inject 1 μ L.

When using the HFBA derivatization procedure, the MS SIM ions for the derivatized analytes are:

- Amp: 118, 240, 91
- d5-Amp (ISTD): 244, 123, 122
- d8-Amp (ISTD): 126, 243, 96
- Mamp: 118, 210, 254
- d5-Mamp (ISTD): 213, 258
- d8-Mamp (ISTD): 261, 213, 122

The expected retention times for amphetamine and methamphetamine under the GC conditions prescribed will be approximately 5.19 and 5.56 minutes respectively.

Procedure Without Derivatization:

Without derivatization, the masses are very low and un-unique. Additionally, the amphetamine and methamphetamine are volatile and can be lost to evaporation.

Without derivatization, the MS SIM ions for the analytes are:

- Amp: 44, 91, 134
- d5-Amp (ISTD): 49
- Mamp: 58, 91, 148
- d5-Mamp (ISTD): 63

The expected retention times for amphetamine and methamphetamine under the GC conditions prescribed will be approximately 4.49 and 4.71 minutes respectively.

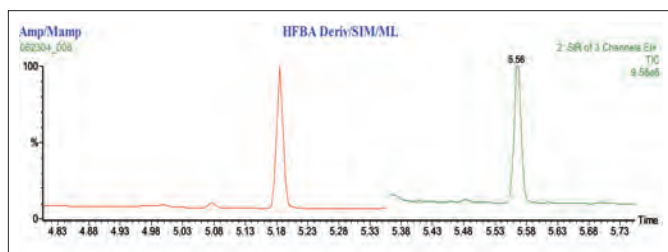


Figure 2. Amphetamine and Methamphetamine HFBA derivative (50 ng/mL in urine) with separate SIM chromatogram windows.

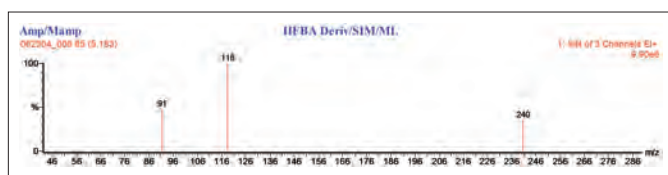


Figure 3. Amphetamine HFBA derivative.

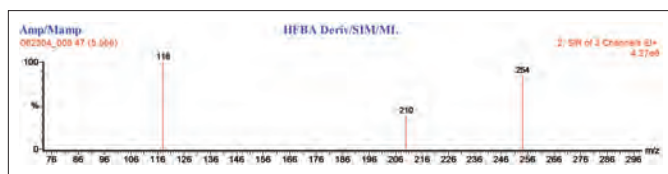


Figure 4. Methamphetamine HFBA derivative.

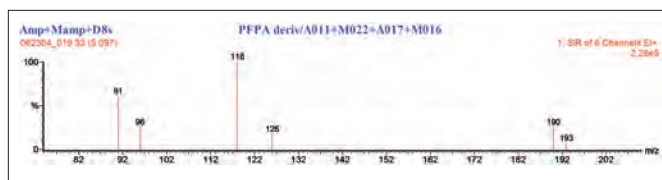


Figure 5. Amphetamine and d8 ISTD PFPA derivative.

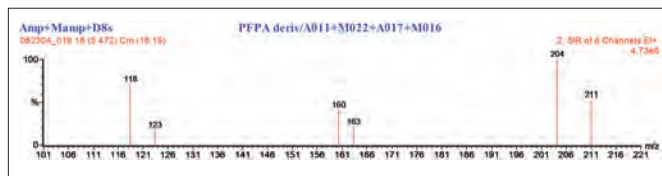


Figure 6. Methamphetamine and d8 ISTD PFPA derivative.

Cannabinoids: THC Carboxylic Acid (metabolite of THC)

The full GC/MS methods are presented in the experimental section (Pages 3-4). The specific GC oven program for the analysis of THC carboxylic acid is: starting temperature 180 °C, 20 °C/min to 285 °C – hold 2 minutes.

The GC/MS analysis of THC carboxylic acid following the procedure presented below has demonstrated method limits of quantitation at 15 ng/mL and limits of detection in urine at 1 ng/mL.

Extraction Procedure:

1. Combine a 1-5 mL urine sample with deuterated TCH-COOH (ISTD) and 200 µL of 10 N NaOH – vortex to ensure complete homogenization.
2. Hydrolyze for 30 min at 60 °C, procedural details presented on Page 3.
3. Once cool, adjust the pH to 3.5 with 2 mL glacial acetic acid
4. Condition the SPE column by rinsing with 3 mL methanol, followed by 3 mL DI water, and 1 mL of 100 mM HCl.
5. Complete the solid phase extraction of the sample by passing the solution from step 1 through the SPE column. Wash column with 3 mL DI water, then 2 mL of 100 mM HCl : acetonitrile (70:30), dry the column, and finally rinse with 200 µL hexane, to remove excess sample matrix.

6. Elute column with 3 mL hexane : ethyl acetate (50:50)
7. Concentrate the extract to a low volume at a temperature of < 40 °C. Quantitatively transfer the concentrated extract to low-volume autosampler vial.
8. Evaporate to dryness at a temperature of < 40 °C.

The sample is prepared for derivatization.

Derivatization Procedure:

Derivatize the sample with the TMAH/IOD procedure. Please note: in order to ensure a complete reaction, make a fresh batch of the derivatization reagent daily by combining 1 mL DMSO with 50 µL TMAH.

- Reconstitute the dried extract with 100 µL of the derivatizing reagent, let stand for 2 min at room temperature.
- Add 10 µL iodomethane, let stand 5 min at room temperature. Once the appropriate amount of reagent is added, the sample will become cloudy. If the sample is not cloudy, add an additional 5 µL of iodomethane.
- Add 1 mL 0.1 M HCl.
- Vortex to clear cloudiness.
- Add 1 mL iso-octane and vortex, this will create two phases in the extract.
- Pipette upper layer into 2-mL autosampler vial.
- Evaporate to dryness.
- Reconstitute in 100 µL of iso-octane, transfer into low volume insert.

When using the TMAH/IOD derivatization procedure, the MS SIM ions for the derivatized analytes are:

- THC-COOH: 313, 357, 372
- d3-THC-COOH (ISTD): 316, 360, 375

The expected retention time for TCH-COOH under the GC conditions prescribed will be approximately 5.75 minutes.

Alternate Derivatization Procedure:

Derivatize the sample with BSTFA with 1% TMCS.

- Reconstitute dried extract by adding 50 µL ethyl acetate and 50 µL BSTFA with 1% TMCS.
- Cover with N₂, cap, mix, heat at 70 °C for 20 min, cool.
- Do not concentrate the BSTFA solution.

When using the BSTFA and 1% TMCS derivatization procedure, the MS SIM ions for the derivatized analytes are:

- THC-COOH: 371, 473, 488
- d3-THC-COOH(ISTD): 374, 476, 491

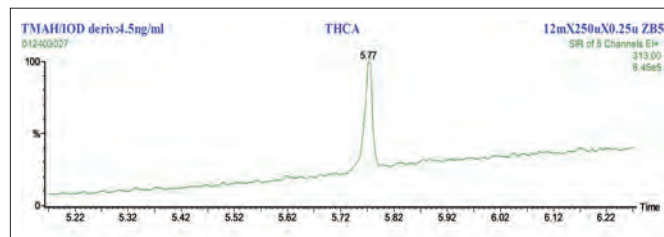


Figure 7. THC acid TMAH/IOD derivative, full SIM scan (4.5 ng/mL in urine).

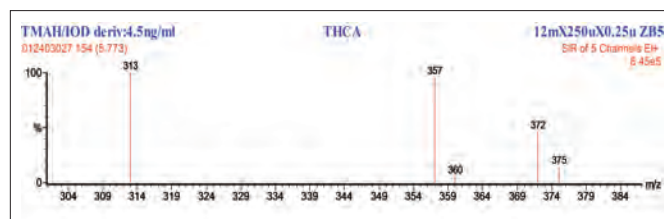


Figure 8. Spectrum of THC acid TMAH/IOD derivative, SIM.

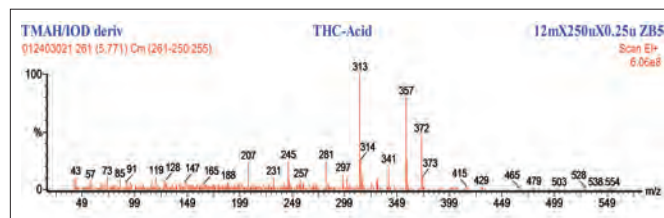


Figure 9. Spectrum of THC acid TMAH/IOD derivative, full scan.

Benzoylcgonine (Cocaine metabolite)

The full GC/MS methods are presented in the experimental section (Pages 3-4). The specific GC oven program for the analysis of benzoylecgonine is: starting temperature 130 °C – hold for 1.5 min, 20 °C/min to 280 °C – hold 1 min.

The GC/MS analysis of benzoylecgonine following the procedure presented below has demonstrated method limits of quantitation at 100 ng/mL and limits of detection in urine at 10 ng/mL.

Extraction Procedure:

1. Combine a 1-5 mL urine sample with deuterated benzoylecgonine (internal standard) and 2 mL of 100 mM phosphate buffer (pH 6.0).
2. Condition the SPE column by rinsing with 3 mL methanol, followed by 3 mL DI water, and 1 mL of phosphate buffer (100 mM, pH 6).
3. Complete the solid phase extraction of the sample by passing the solution from step 1 through the SPE column. Wash column with 3 mL DI water, then 1 mL of 100 mM HCl, and 1 mL methanol to remove excess sample matrix.
4. Elute analytes from the SPE column by rinsing with 3 mL of methylene chloride : isopropanol : ammonium hydroxide (78:20:2).
5. Concentrate the extract to a low volume at a temperature of < 40 °C. Quantitatively transfer the concentrated extract to a low-volume autosampler vial.
6. Evaporate to dryness at a temperature of < 40 °C.

Derivatization Procedure:

Derivatize sample with the PFPA/PFPOH procedure:

- Reconstitute dried extract in 50 µL PFPA.
- Add 25 µL PFPOH.
- Cover with N₂, cap, mix, heat 70 °C (20 min).

When using the PFPA/PFPOH derivatization procedure, the MS SIM ions for the derivatized analytes are:

- BE: 300, 421, 316
- d3-BE (ISTD): 303, 424

The expected retention time for benzoylecgonine under the GC conditions prescribed will be approximately 6.53 minutes.

Alternate Derivatization Procedure:

1. Reconstitute the dried extract in 50 µL ethyl acetate.
2. Add 50 µL of BSTFA with 1% TMCS.
3. Cover with N₂, cap, mix, heat 70 °C (20 min).
4. Inject 1 µL of BSTFA solution (Do not evaporate BSTFA solution).

When using the BSTFA with 1% TMCS derivatization procedure, the MS SIM ions for the derivatized analytes are:

- BE: 240, 361, 256
- d3-BE (ISTD): 243,364, 259

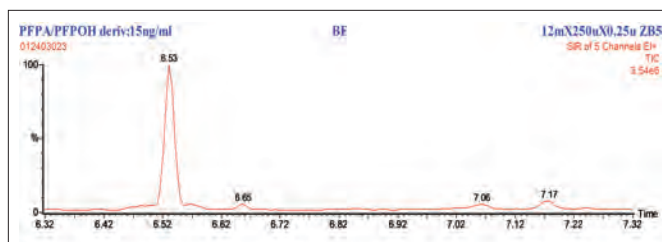


Figure 10. SIM scan of PFPA/PFPOH derivative showing all ions.

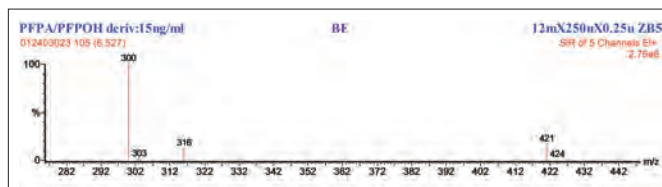


Figure 11. SIM spectrum of BE (15 ng/mL in urine).

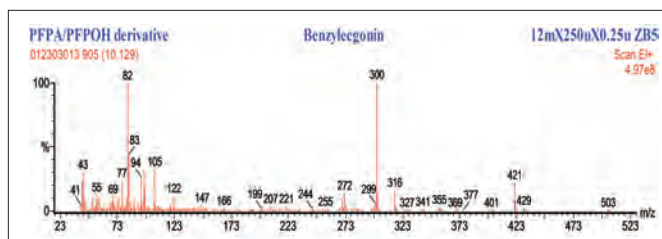


Figure 12. Full scan spectrum of Benzylecgonine showing prominent ions used in SIM.

6-Mono-Acetyl Morphine (metabolite of heroin)

The full GC/MS methods are presented in the experimental section (Pages 3-4). The specific GC oven program for the analysis of 6-mono-acetyl morphine (MAM) is: starting temperature 140 °C – hold for 1.5 minutes, 25 °C/min to 290 °C – hold 4 minutes.

The GC/MS analysis of 6-mono-acetyl morphine following the procedure presented below has demonstrated method limits of quantitation at 10 ng/mL and limits of detection in urine at 1 ng/mL.

The analysis of MAM requires the use of silanized glassware to prevent loss of analytes. Furthermore, the analytes, once extracted, are subject to additional losses. Analyze extracted samples immediately. These samples are not stable for storage or transport.

Extraction Procedure:

1. Combine a 1-5 mL urine sample with, deuterated internal standard, and 2 mL of 100 mM phosphate buffer (pH 6.0).
2. Condition the SPE column by rinsing with 3 mL methanol, followed by 3 mL DI water, and 1 mL of phosphate buffer (100 mM, pH 6).
3. Complete the solid phase extraction of the sample by passing the solution from step 1 through the SPE column.
4. Wash column with 3 mL DI water, then 1 mL of 100 mM acetic acid pH 4.5, and 1 mL methanol to remove excess sample matrix.
5. Elute analytes from the SPE column by rinsing with 3 mL methylene chloride: isopropanol: ammonium hydroxide (78:20:2).
6. Concentrate the extract to a low volume at a temperature of < 40 °C. Quantitatively transfer the concentrated extract to a low-volume autosampler vial.
7. Evaporate to dryness at a temperature of < 40 °C.

The sample is prepared for derivatization.

Derivatization Procedure:

Derivatize the sample with the PFPA/PFPOH procedure.

- Reconstitute dried extract in 50 µL PFPA. Add 25 µL of PFPOH.
- Cover with N₂, cap, mix, heat 70 °C (20 min), evaporate to dryness at a temperature < 40 °C.
- Reconstitute in 50 µL ethyl acetate.

When using the PFPA/PFPOH derivatization procedure, the MS SIM ions for the derivatized analytes are:

- 6-MAM: 414, 361, 473
- d3-6-MAM (ISTD): 417, 479

The expected retention time for 6-mono-acetyl morphine under the GC conditions prescribed will be approximately 6.52 minutes.

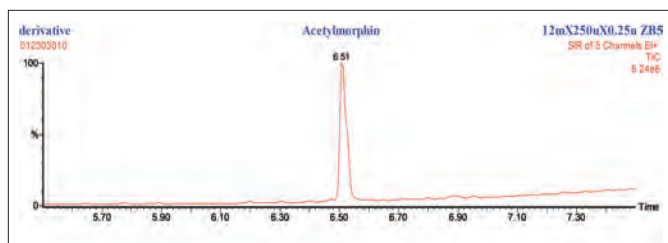


Figure 13. 6-MAM (10 ng/mL in urine).

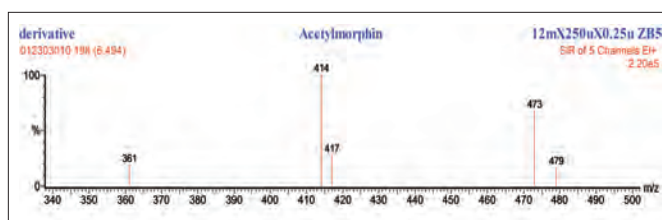


Figure 14. 6-MAM (10 ng/mL in urine).

Opiates (Morphine and Codeine)

The full GC/MS methods are presented in the experimental section (Pages 3-4). The specific GC oven program for the analysis of morphine and codeine is: starting temperature 115 °C – hold for 0.5 minutes, 20 °C/min to 305 °C – hold 0.5 minutes.

The GC/MS analysis of morphine and codeine following the procedure presented below has demonstrated method limits of quantitation at 300 ng/mL and limits of detection in urine at 20 ng/mL.

Extraction Procedure:

1. Combine a 5 mL urine sample with d3-morphine and d3-Codeine (ISTD).
2. Hydrolyze sample to break glucuronide bonding, procedural details presented on Page 3.
3. Condition the solid phase extraction column by washing with 3 mL methanol, followed by 3 mL DI water, and 1 mL of phosphate buffer (100 mM, pH 6).
4. Complete the solid phase extraction of the sample by passing the mixture from step 1 through the SPE column. Wash the column with 3 mL DI water, then 2 mL of 100 mM acetate buffer (pH 4.5) and 3 mL of methanol to remove excess sample matrix.
5. Elute the analytes from the column by rinsing with 3 mL methylene chloride : isopropanol : ammonium hydroxide (78:20:2).
6. Concentrate the extract to low volume at a temperature of < 40 °C. Quantitatively transfer the concentrated extract to low-volume autosampler vial.
7. Evaporate to dryness at a temperature of < 40 °C.

The sample is prepared for derivatization.

Derivatization Procedure:

Derivatize the sample with the PFPA/PFPOH procedure.

- Reconstitute dried extract in 50 μ L PFPA.
- Add 25 μ L PFPOH.
- Cover with N_2 , cap, mix, heat 70 $^{\circ}C$ (20 min).
- Evaporate to dryness.
- Reconstitute in 50 μ L ethyl acetate.

When using the PFPA/PFPOH derivatization procedure, the MS SIM ions for the derivatized analytes are:

- Morphine: 414, 430, 577
- d3-Morphine(ISTD): 417, 580
- Codeine: 282, 445, 446
- d3-Codeine (ISTD): 285, 448

The expected retention time for morphine and codeine under the GC conditions prescribed will be approximately 7.16 and 7.35 minutes respectively.

Alternate Derivatization Procedure:

Derivatize the sample with the BSTFA and 1% TMCS procedure.

- Reconstitute dried extract with 50 μ L ethyl acetate and 50 μ L BSTFA with 1% TMCS.
- Cover with N_2 , cap, mix, heat 70 $^{\circ}C$ (20 min), cool – do not concentrate the BSTFA solution.

When using the BSTFA and 1% TMCS derivatization procedure, the MS SIM ions for the derivatized analytes are:

- Morphine: 429, 287, 324
- d3-Morphine: 432, 290, 327
- Codeine: 371, 234, 343
- d3-Codeine: 374, 237, 346

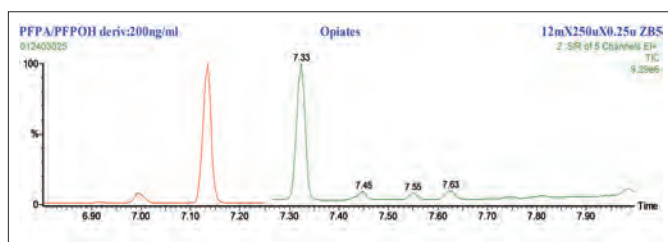


Figure 15. Codeine and Morphine SIM (200 ng/mL in urine).

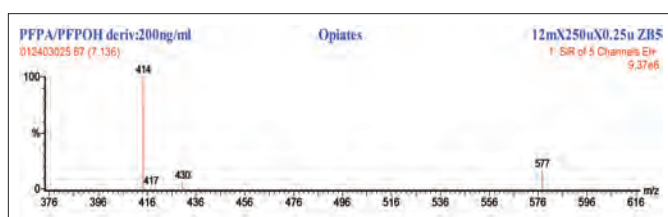


Figure 16. Spectrum of Morphine SIM (200 ng/mL in urine).

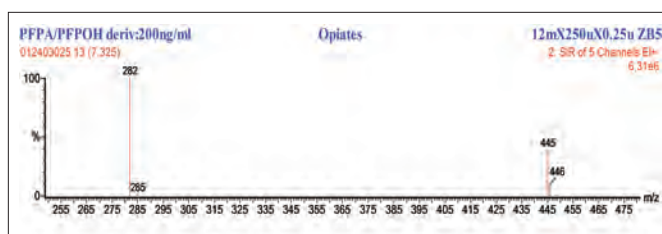


Figure 17. Spectrum of Codeine SIM (200 ng/mL in urine).

PCP (Phencyclidine)

The full GC/MS methods are presented in the experimental section (Pages 3-4). The specific GC oven program for the analysis of PCP is: starting temperature 120 $^{\circ}C$, 10 $^{\circ}C$ /min to 210 $^{\circ}C$, 40 $^{\circ}C$ /min to 280 $^{\circ}C$.

The GC/MS analysis of PCP following the procedure presented below has demonstrated method limits of quantitation at 25 ng/mL and limits of detection in urine at 2.5 ng/mL.

Extraction Procedure:

Please note derivatization is unnecessary in this analysis.

1. Combine a 1-5 mL urine sample with deuterated PCP (ISTD) and 2 mL of 100 mM phosphate buffer (pH 6.0).
2. Condition the SPE column by rinsing with 3 mL methanol, followed by 3 mL DI water and 1 mL phosphate buffer (100 mM, pH 6).
3. Complete the solid phase extraction of the sample by passing the solution from step 1 through the SPE column. Wash the column with 3 mL DI water, then 1 mL of 100 mM acetic acid and 1 mL methanol to remove excess sample matrix.
4. Elute analytes from the SPE by rinsing with 3 mL methylene chloride : isopropanol : ammonium hydroxide (78:20:2).
5. Concentrate the extract to a low volume at a temperature of < 40 $^{\circ}C$. Quantitatively transfer the concentrated extract to a low-volume autosampler vial.
6. Evaporate to dryness at a temperature of < 40 $^{\circ}C$.
7. Reconstitute in 100 μ L ethyl acetate.

When analyzing PCP, the MS SIM ions are:

- PCP: 186, 200, 242
- d5-PCP(ISTD): 205, 248

The expected retention time for PCP under the GC conditions prescribed here will be approximately 7.10 minutes.

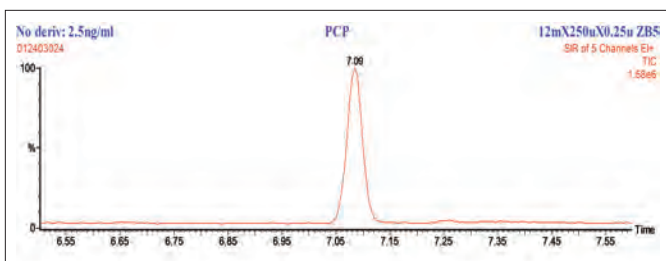


Figure 18. SIM scan of PCP (2.5 ng/μL in urine) showing all ions.

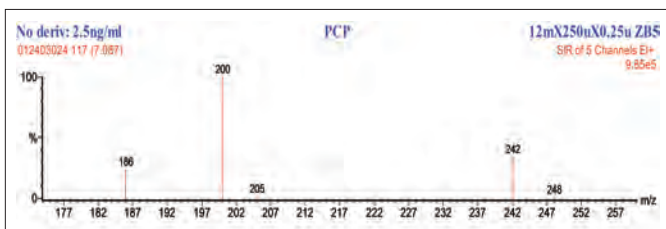


Figure 19. SIM spectrum of PCP (2.5 ng/mL in urine).

Conclusion

This application note has presented the analysis of drugs of abuse in urine for the testing requirements of the Federal Workplace Drug Testing program. Analyzing biological samples requires careful handling extraction, cleanup and derivatization. The testing program divides drugs of abuse into 6 distinct classes. There is commonality in the analysis of all 6 drug classes; however, each drug also has unique analytical requirements. The detailed sample-preparation and analytical methods for each class were presented.

The PerkinElmer Clarus GC/MS system operating in SIM mode provided the sensitivity and spectral data necessary to generate legally-defensible results. Furthermore, TurboMass GC/MS software includes the reporting capability required to present 3-ion-ratio data in a format that is simple and easy to understand.