APPLICATION NOTE

Gas Chromatography/
Mass Spectrometry

Author

Timothy D. Ruppel

PerkinElmer, Inc.
Shelton, CT 06484 USA

Sympathomimetic Amines in Urine by SAMHSA GC/MS

Introduction

The United States Department of Health and Human Services (DHHS), Substance Abuse and Mental Health Services Administration (SAMHSA) regulates urine drug testing programs in the Mandatory Guidelines for the Federal Workplace Drug Testing Program. These Mandatory Guidelines require a laboratory to conduct two analytical tests before a urine specimen can be reported positive for a drug, the initial drug test and the confirmatory drug test. The initial drug test is performed by immunoassay screening for the five drug classes (i.e., amphetamines, cocaine, opiates, phencyclidine, and marijuana). Examples of immunoassay screening would include radioimmunoassay (RIA), enzyme immunoassay (EIA, EMIT) or others.

Samples found positive to the immunoassay screening are subjected to a second confirmatory test by chromatographic separation and identification by mass spectrometry. SAMHSA defines the Method Quantification Cutoff Level as 250 ng/mL for each of 5 amines (AMP, MAMP, MDA, MDMA, MDEA).

PerkinElmer®
For the Better

Overview

The general procedure for drug confirmatory test in urine follows the 7 steps listed below:

1. Add a deuterated internal standard to the urine.
2. Adjust urine pH.
3. Hydrolyze urine (opiates and cannabinoids only).
4. Extract drugs from urine using solid phase extraction (SFE), evaporate to dryness.
5. Derivitize the extract (except for PCP), evaporate to dryness.
6. Reconstitute extract into organic solvent.
7. Inject 1-3 µL into gas chromatograph/mass spectrometer for identification and quantification using 3 ion ratio report.

Glassware

All glassware, including autosampler vials and low volume vial inserts must be silanized to prevent adsorption of sample. Soak all glassware in 10% DMDCS/Toluene for 10 min. Rinse in methanol, rinse in hexane, air dry.

Reagents List

PFPA = PFAA = Pentafluoropropionic acid anhydride, Campbell Scientific (Rockton, IL).

HFBA = Heptafluorobutyric anhydride, Campbell Scientific (Rockton, IL).

TFAA = trifluoroacetic anhydride, Campbell Scientific (Rockton, IL).

Acetic Acid, 100 mM = 2.86 mL glacial acetic acid diluted to 500 mL DI water.

Phosphate buffer, 100 mM pH6 = 1.7 g Na_2HPO_4 + 12.14 g Na_2HPO_4 dilute to 1000 mL with DI water, adjust to pH6 with 100 mM Na_2HPO_4 (raises pH) or 100 mM Na_2HPO_4 (lowers pH).

Methylene Chloride/Isopropanol/Ammonium Hydroxide (78:20:2) extraction solvent = 40 mL IP-OH + 4 mL con NH_4OH + 156 mL $MeCl_2$. *Make fresh daily.*

Drug standards and deuterated internal standards are available from Cerillant (Round Rock, TX).

Internal standard: d5 or d8-Amphetamine d5 or d8-Methamphetamine.

Instrumentation

Gas Chromatograph: PerkinElmer Clarus 680 GC.

Injector: Capillary injector using pressure pulsed splitless injection, 250 °C.

Injection port liner: Siltek™ with wool (Cat. No. N6502010).

GC Column: Elite-5 (5% Phenyl/95% Methyl Silicone) – 12 m x 0.200 mm x 0.33 µm (Cat. No. N9316110).

Helium carrier: 2 mL/min.

GC oven: Start temperature 60 °C hold for 1 min, then 40 °C/min to 300 °C hold 1 min at 300 °C = 8 min.

Pressure pulsed, splitless injection: This procedure raises the injector pressure during the injection process to put more sample onto the column in a narrow band and then reduces the carrier gas to normal operational linear gas velocity for chromatography. This is accomplished with timed events such as the following:

CAR2 set to 5 mL/min at -0.71 min (raise pressure before injection).

SPL2 set to 0 at -0.70 min (splitless injection).

CAR2 set to 2 mL/min at 0.7 min (operating flow after injection).

SPL2 set to 50 at 0.8 min (open split vent after injection).

Mass Spectrometer: PerkinElmer SQ8 MS, 255 L/sec turbomolecular pump, EI mode.

All data is collected in selected ion monitoring mode (SIM) acquiring 20-30 msec per ion.

A primary ion is used for identification and quantitation while 2 additional ions are used for confirmation of identification.

Three ion ratio chromatograms must all apex within ±2 scans of standard retention time. Ion ratios must fall within ±20% of standard ratios. Deuterated internal standards may use only 2 ions, a primary ion and only 1 confirmation ion.

Solid Phase Extraction

Drugs are extracted from the urine 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 can remove salts and other contaminants. Eluting the drugs off the resin bed with a stronger solvent completes the cleanup process from the urine. Extraction cartridges used were Supra-Clean SPE Columns C18-S 200 mg/3 mL 50 u (Cat. No. N9306462).

Experimental

Extraction Procedure: 2 mL urine + ISTD + 2 mL 100 mM phosphate buffer (pH 6).

SPE column extract: Condition column with 3 mL methanol, then 3 mL DI water, then 1 mL 100 mM phosphate buffer (pH6).

Extract sample, wash column with 3 mL DI water, then 1 mL 100 mM Acetic Acid, then 1 mL methanol.

Elute column with 3 mL Methylene chloride:Isopropanol: Ammonium Hydroxide (78:20:2) into conical tube.

Evaporate to dryness <50 °C. Reconstitute in 50 uL derivitization reagent. Cover with N_2, cap, mix, heat 70 °C (20 min), Evaporate to dryness <50 °C. Reconstitute in 100 µL ethyl acetate, transfer to low volume autosampler vial insert, inject 1 µL.

Several derivitization reagents are available, each creating different ions. Reagents: PFPA, HFBA, TFAA and others

PFPA SIM ions:

Amp: 190, 118, 91	d5-Amp: 194, 123
d8-Amp: 193, 126	RT: 3.11 min
Mamp: 204, 160, 118	d5-Mamp: 208, 163
d8-Mamp: 211, 163	RT: 3.49 min

HFBA SIM ions:

Amp: 118, 240, 91	d5-Amp: 244, 123
d8-Amp: 243, 126	RT: 3.29 min
Mamp: 118, 210, 254	d5-Mamp: 258, 213
d8-Mamp: 261, 213	RT: 3.59 min

TFAA SIM ions:

Amp: 118, 140, 91	d5-Amp: 144, 123
d8-Amp: 143, 126	RT: 3.10 min
Mamp: 154, 118, 110	d5-Mamp: 158, 113
d8-Mamp: 161, 113	RT: 3.48 min

Hint: PFPA evaporates to dryness easier than HFBA, evaporates at 50 °C easily in ~5min.

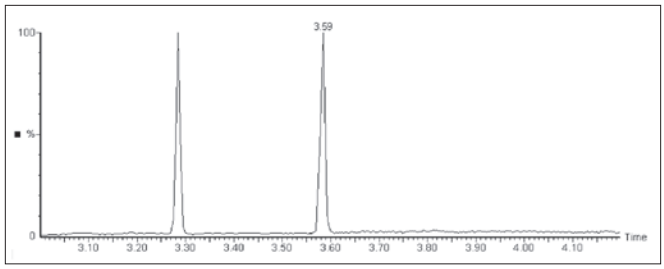
Hint: HFBA can be diluted (1:9) into hexane to evaporate faster. TFAA can be diluted (1:9) into hexane to evaporate faster.

Hint: PFPA will sputter if any hydroxyl is present; therefore dry completely any water or alcohol before derivitization.

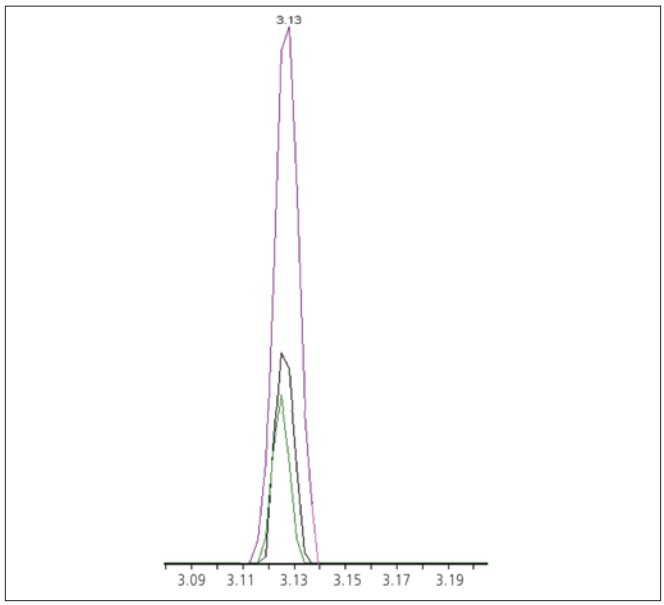
Calibration Range: method confirmation cutoff level = 250 ng/mL.

10% cutoff (25 ng/mL), 40% cutoff (100 ng/mL), 100% cutoff (250 ng/mL), 125% cutoff (312.5 ng/mL), 500% cutoff (1250 ng/mL), 1000% cutoff (2500 ng/mL).

Results



Chromatogram: HFBA derivatives of amphetamine and methamphetamine (100 ng/mL in urine).



Mass chromatogram overlay of amhetamine PFPA ions.

Limit of Quantification: 25 ng/mL from 2 mL urine.

Limit of Detection: <2.5 ng/mL from 2 mL urine.

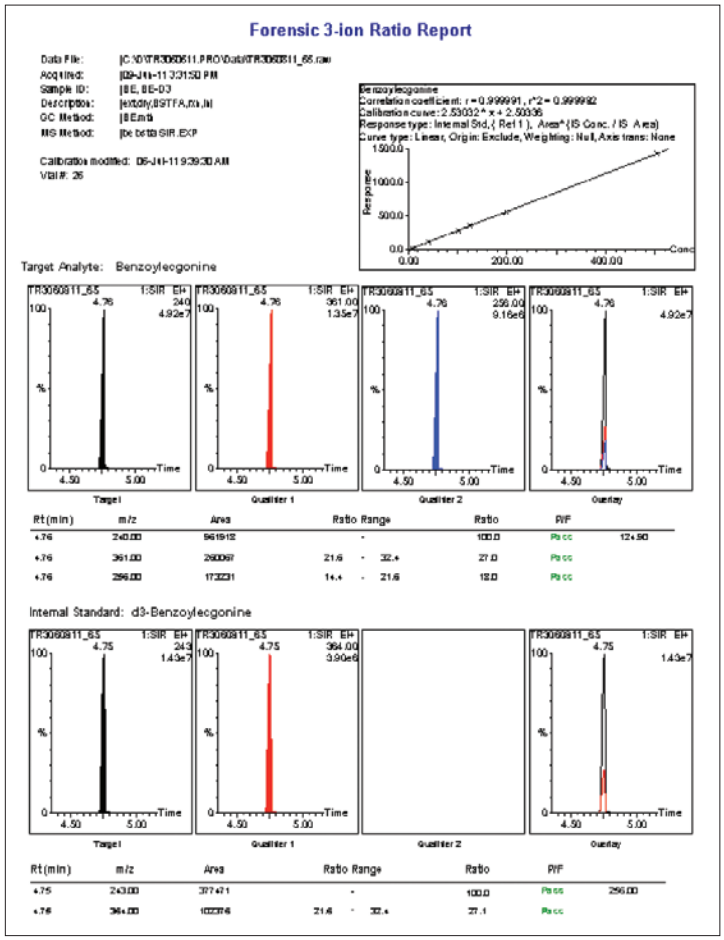
Linear Correlation Coefficient (R2) >0.999 25 ng/mL – 2500 ng/mL.

Conclusions

This application has presented the analysis of amphetamine related drugs in urine for the testing requirements of the Federal Workplace Drug Testing Program. Forensic and clinical laboratories can use the same method for toxicology samples in non-regulated drug testing. Fast sample throughput was increased through the use of a short GC column, fast flow rate into the mass spectrometer, very fast cooling GC oven and autosampler pre-rinsing options.

The PerkinElmer SQ8 GC/MS system operating in SIM mode provided the sensitivity and spectral data necessary to generate legally defensible results. The TurboMass GC/MS software includes 3-ion ratio confirmation calculations and reporting to present data in a format that is simple and easy to understand.

Example of a customizable 3-ion ratio report



Example of a customizable 3-ion ratio report.

References

1. Disposition of Toxic Drugs and Chemicals in Man, 8th Ed, Randall C. Baselt, Biomedical Publications, 2008.
2. Mandatory Guidelines for Federal Workplace Drug Testing Programs, Fed Reg, 73: 71857 (Nov 25, 2008).
3. Mandatory Guidelines for Federal Workplace Drug Testing Programs, Fed Reg, 75: 22809 (April 30, 2010).
4. Pierce Catalog (Rockford, IL).

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

009784A_01