APPLICATION NOTE

Gas Chromatography/
Mass Spectrometry

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Benzoylecgonine 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 100 ng/mL for benzoylecgonine, the major metabolite of cocaine.

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Overview

The general procedure for the 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

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 deuterarated internal standards are available from Cerillant (Round Rock, TX). Internal standard: d3-BE.

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 .200 mm x 0.33 µm (Cat. No. N9316110). Helium carrier – 2 mL/min.

GC oven: Start temperature 100 °C. Hold for 0.5 min, then 20 °C/min to 310 °C. Hold 4 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 Clarus SQ 8 MS, 255 L/sec turbomolecular pump, EI mode.

All data is collected in selected ion monitoring mode (SIM) acquiring 20-50 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.

PFPA SIM ions:

BE: 300,421,316	d3-BE: 303,424	RT: 5.17 min

BSTFA SIM Ions:

BE: 240,361,256	d3-BE: 243,364	RT: 4.76 min

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).

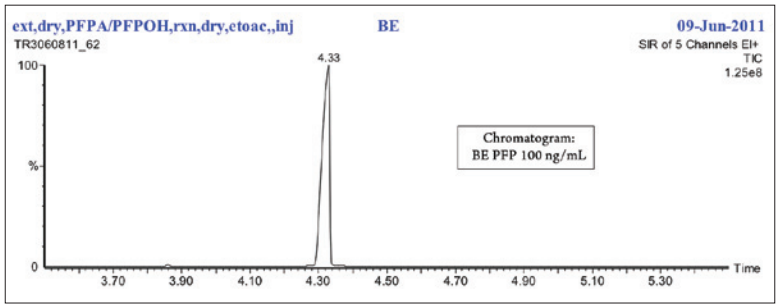
Experimental

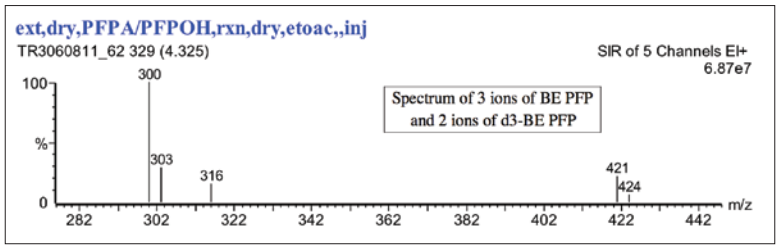
Extraction Procedure: 1-2 mL urine + ISTD + 2 mL 100 mM phosphate buffer (pH 6). SPE column extract: Condition column with 3 mL methanol, then 3 mL DI water, then 1 mL 100mM phosphate buffer (pH6). Extract sample, wash column with 3 mL DI water, then 1 mL 100 mM HCl, then 1 mL methanol. Elute column with 3 mL Methylene chloride: Isopropanol:Ammonium Hydroxide (78:20:2) into conical tube. Evaporate to dryness <50 °C. Reconstitute in 50 uL PFPA. Add 25 uL PFPOH. 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.

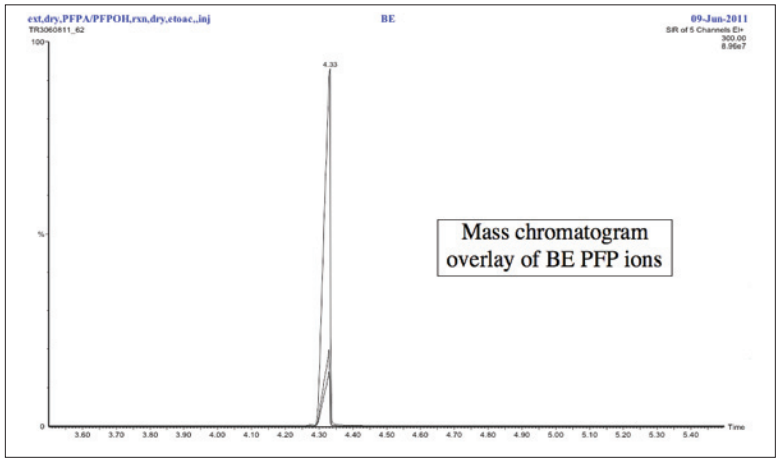
Hint: PFPA will sputter if any hydroxyl is present; therefore dry any water or alcohol before derivitization.

Calibration Range: method confirmation cutoff level = 100 ng/mL. 10% cutoff (10 ng/mL), 40% cutoff (40 ng/mL), 100% cutoff (100 ng/mL), 125% cutoff (125 ng/mL), 500% cutoff (500 ng/mL), 1000% cutoff (1000 ng/mL).

Results





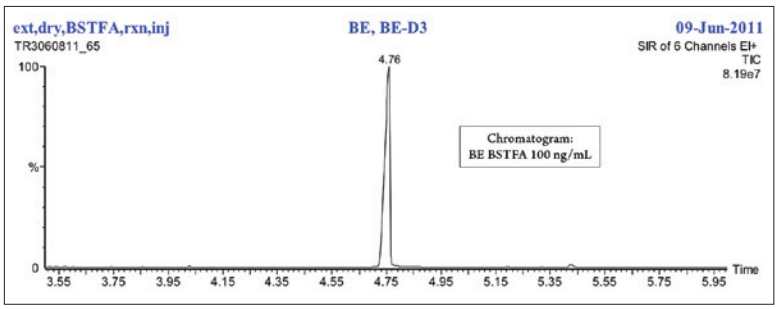


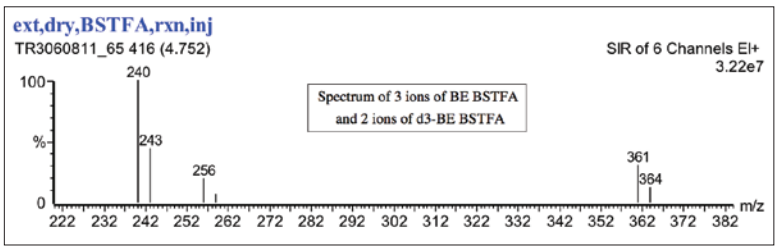
Alternative derivitization using BSTFA.

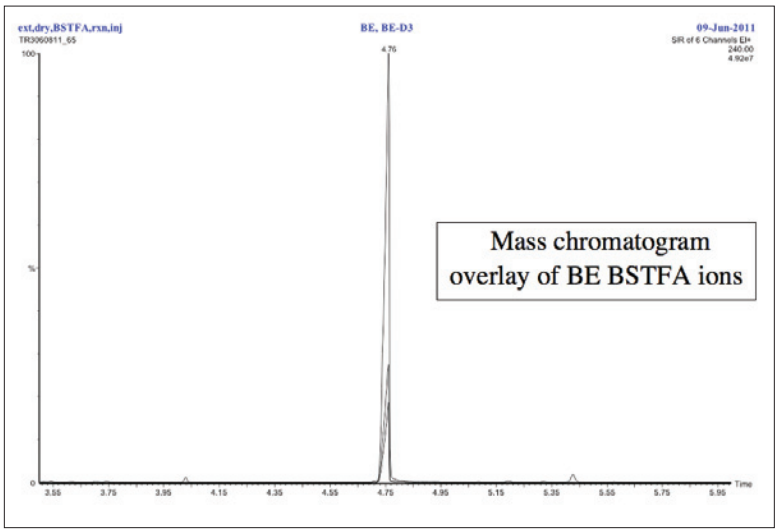
Experimental

Extraction Procedure: 1-2 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 HCl, 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 of BSTFA with 1% TMCS. Cover with N_2, cap, mix, heat 70 °C (20 min). Inject 1 uL of BSTFA solution (Do not evaporate BSTFA solution).

Results







Limit of Quantitation: 10 ng/mL from 1 mL urine

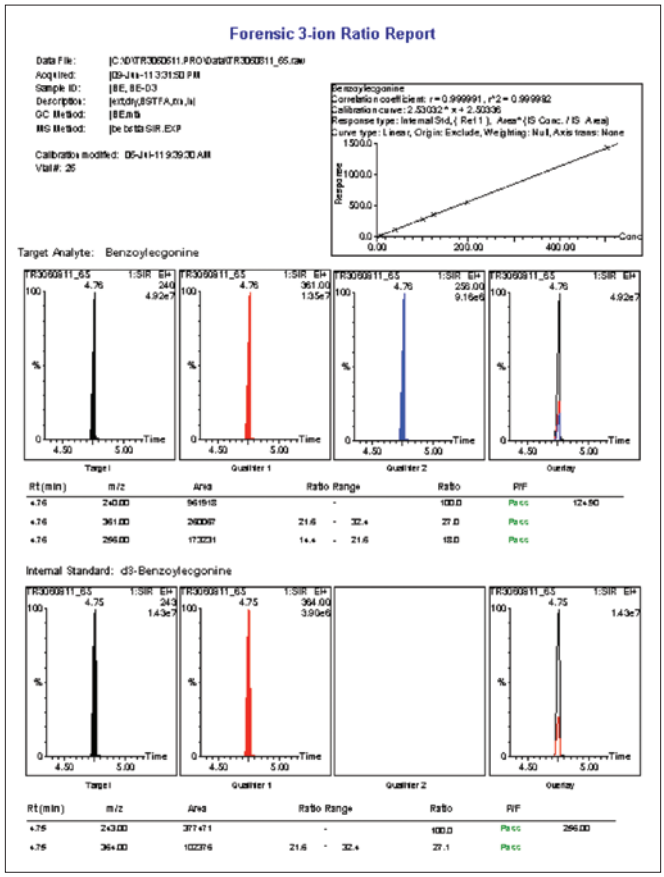
Limit of Detection: < 2 ng/mL from 1 mL urine

Linear Correlation coefficient (R2) >0.999 10 ng/mL – 1000 ng/mL

Conclusions

This application has presented the analysis of benzoylecgonine 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 Clarus SQ 8 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.



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2. Mandatory Guidelines for Federal Workplace Drug Testing Programs, Fed Reg, 73: 71857 (Nov. 25, 2008).

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4. Pierce Catalog (Rockford, IL).

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