APPLICATION NOTE

Gas Chromatography/
Mass Spectrometry

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Opiates 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 2000 ng/mL each for codeine and morphine. If morphine is detected above 2000 ng/mL, then an additional quantification for 6-acetylmorphine is suggested. 6-AM is a unique metabolite indicating the use of heroin. 6-AM cutoff level is 10 ng/mL.

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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 (SPE), 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 quantitation using three ion ratio reporting software.

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

Beta-glucuronidase

Sodium Hydroxide

Hydrochloric Acid (conc.)

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_2PO_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: d3-codeine, d3-morphine, d3-6-acetylmorphine.

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 SQ8 MS, 270 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.

PFPA SIM ions:	Codeine: 282,445, 446	d3-Codeine: 285, 448	RT: 7.16 min
	Morphine: 414, 430, 577	d3-morphine: 417, 580	RT: 7.35 min
	6-acetylmorphine: 414, 361, 473	d3-6-acetylmorphine: 417, 479	
BSTFA SIM ions:	Codeine: 371, 234, 343	d3-Codeine: 374, 237	
	Morphine: 429, 287, 324	d3-Morphine: 432, 290	

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).

Hydrolysis

Hydrolysis is necessary in some drugs to remove 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

Combine 3-5 mL urine sample with ISTD and 2 mL beta-glucuronidase, vortex, heat 3 hours at 65 °C.

Cool, centrifuge, decant and keep top layer. Adjust pH to 6.0 with 700 uL 1.0 N NaOH.

Acid Hydrolysis

Combine 3-5 mL urine sample with ISTD and 500 uL concentrated HCl, vortex, heat 30 min at 120 °C.

Cool, centrifuge, decant and keep top layer. Add 1 mL 7.4 N NH_4OH, vortex. Adjust pH to 6.0 with 1-3 mL of 500 mM phosphoric acid.

Experimental

Extraction Procedure: 3 mL urine + ISTD. Hydrolyze sample to break glucuronide bonding.

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. Derivitize with 50 uL PFPA, add 25 uL PFPOH. Cover with N2, 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.

Alternative Derivitization Procedure

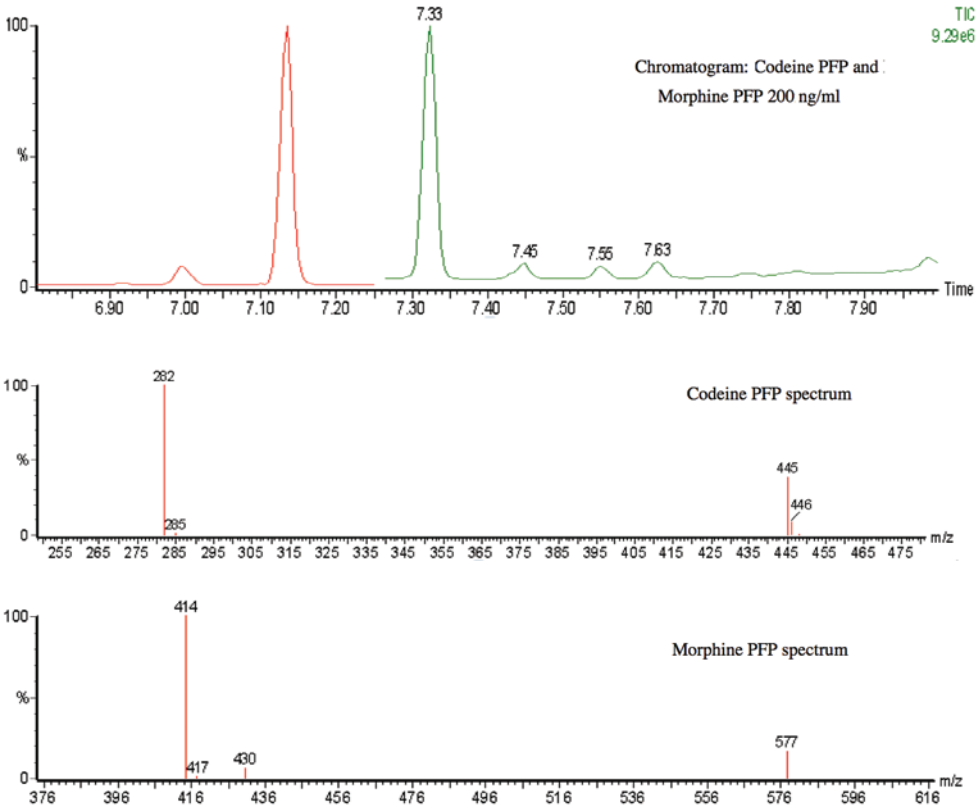
Reconstitute dried extract with 50 uL ethyl acetate, add 50 uL BSTFA with 1% TMCS.

Cover with N2, cap, mix, heat 70 °C (20 min), cool, do not evaporate, inject BSTFA solution directly.

Calibration Range

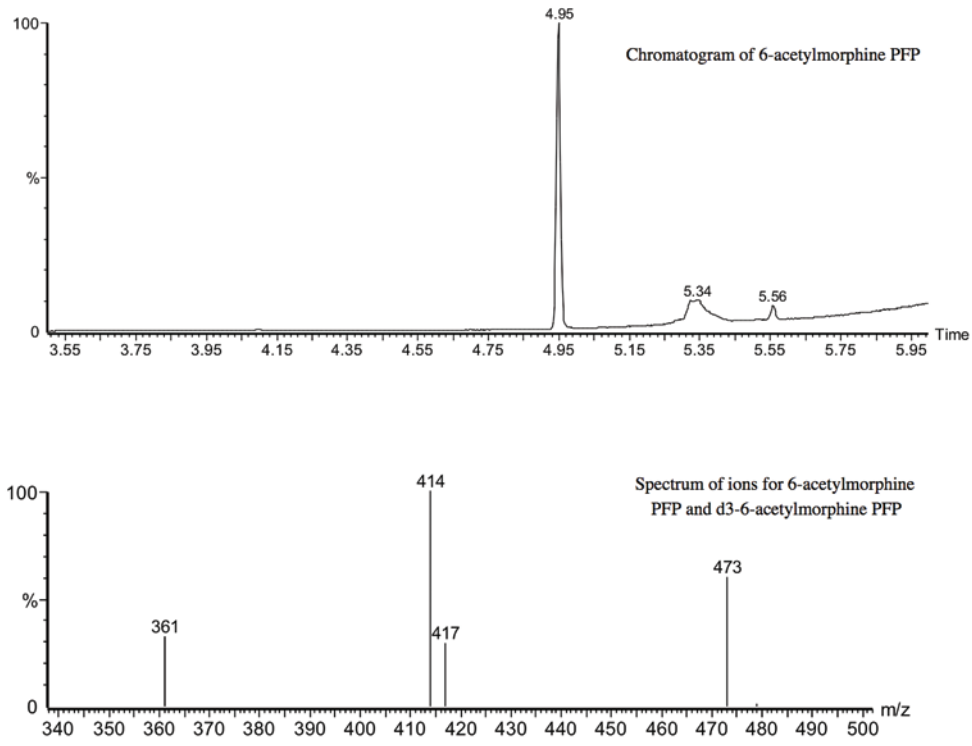
10% cutoff (200 ng/mL), 40% cutoff (800 ng/mL), 100% cutoff (2000 g/mL), 125% cutoff (2500 ng/mL), 500% cutoff (10000 ng/mL), 1000% cutoff (20000 ng/mL)

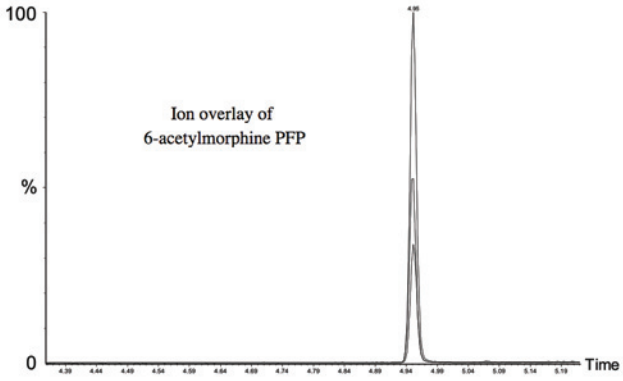
Results



Limit of Quantitation: 20 ng/mL from 1mL urine.
Limit of Detection: < 2 ng/mL from 1 mL urine.
Linear Correlation coefficient (R^2) >0.999 20 ng/mL – 20000 ng/mL.

Results for 6-acetylmorphine PFP

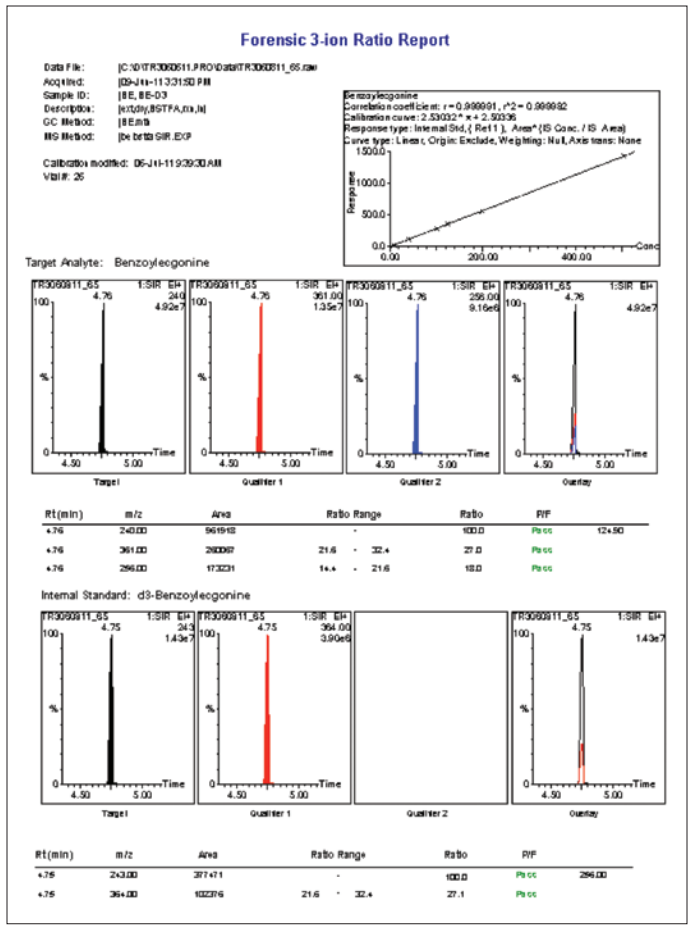




Conclusion

The GC/MS analysis of codeine and morphine in this application has demonstrated the limit of quantitation at or below 20 ng/mL in urine a 10 fold factor lower than the limit of quantitation 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 the reporting capability required to present three-ion-ratio data in a format that is simple and easy to understand.



An example of a customizable three-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).

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