APPLICATION NOTE

Gas Chromatography/
Mass Spectrometry

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Phencyclidine (PCP) 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 25 ng/mL for PCP.

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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 (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 quantitation using 3 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

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 NaH_2PO_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-PCP

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 GC/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.

SIM ions: PCP: 200,242,186
d5-PCP: 205, 248
RT: 5.17 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 (pH6).

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.

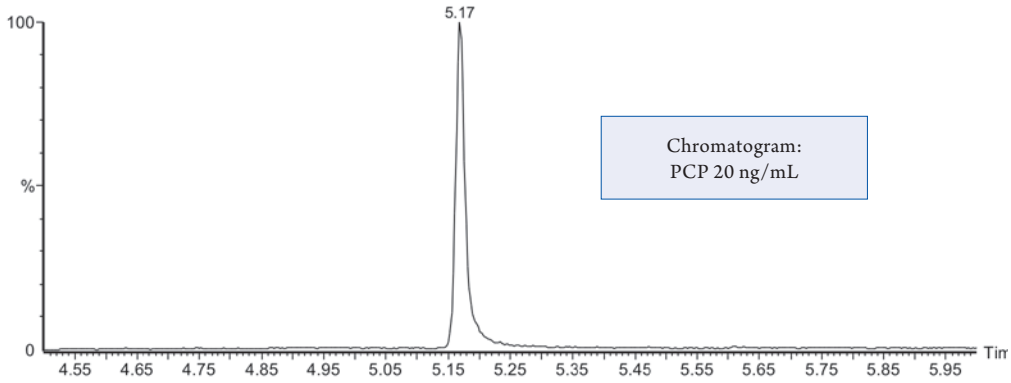
Elute column with 3 mL Methylene Chloride/Isopropanol/ Ammonium Hydroxide (78:20:2) into conical tube.

Evaporate to dryness <50 °C. Derivitization is not necessary for PCP. Reconstitute in 100 µL ethyl acetate, transfer to low volume autosampler vial insert, inject 1 µL.

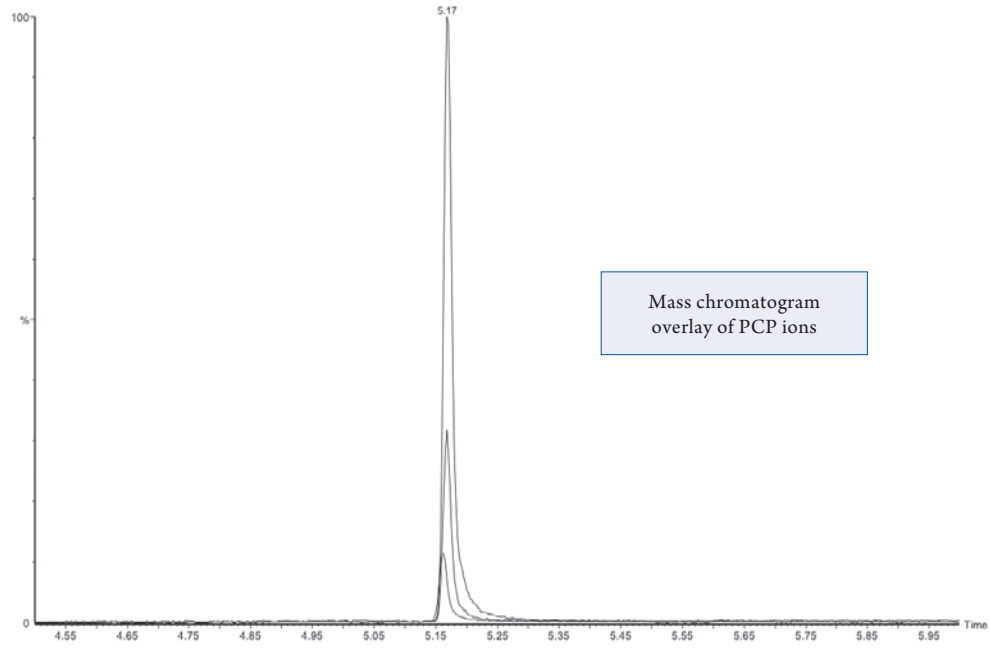
Calibration Range

10% cutoff (2.5 ng/mL), 40% cutoff (10 ng/mL), 100% cutoff (25 ng/mL), 125% cutoff (31.25 ng/mL), 500% cutoff (125 ng/mL), 1000% cutoff (250 ng/mL)

Results



Chromatogram:
PCP 20 ng/mL



Mass chromatogram
overlay of PCP ions

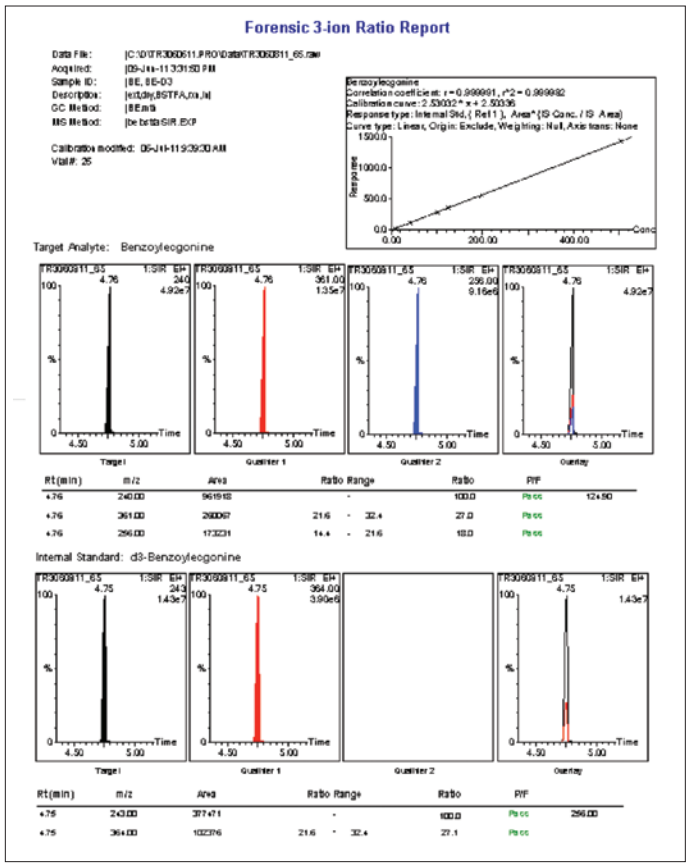
Limit of Quantitation: 2.5 ng/mL from 1 mL urine
Limit of Detection: <1.0 ng/mL from 1 mL urine
Linear Correlation coefficient (R2) >0.999 2.5 ng/mL - 250 ng/mL

Conclusion

The GC/MS analysis of PCP in this application has demonstrated the limit of quantitation and limit of detection at or below 2.5 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 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 the reporting capability required to present 3-ion-ratio data in a format that is simple and easy to understand.

An example of a customizable 3-ion-ratio report:



References

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2. Mandatory Guidelines for Federal Workplace Drug Testing Programs, 73Fed Reg, 71857 (Nov 25, 2008).
3. Mandatory Guidelines for Federal Workplace Drug Testing Programs, 75Fed Reg, 22809 (April 30, 2010).
4. Pierce® Catalog (Rockford, IL).

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