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.
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 Na₂HPO₄ + 12.14 g Na₂HPO₄ dilute to 1000 mL with DI water.

Adjust to pH6 with 100 mM Na₂HPO₄ (raises pH) or 100 mM NaH₂PO₄ (lowers pH).

Methylene Chloride/Isopropanol/Ammonium Hydroxide (78:20:2) extraction solvent = 40 mL IP-OH + 4 mL con NH₄OH + 156 mL MeCl₂. Make fresh daily.

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

Internal standard: d5-PCP

**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**

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
4. Pierce® Catalog (Rockford, IL).