



SAMHSA-Compliant LC/MS/MS Analysis of Phencyclidine in Urine with Agilent Bond Elut Plexa PCX and Agilent Poroshell 120

Application Note

Forensic Toxicology

Authors

Irina Dioumaeva, John M. Hughes
Agilent Technologies, Inc.

Abstract

New guidelines from the US Substance Abuse and Mental Health Services Administration (SAMHSA), effective October 2010, allowed LC/MS/MS methods to be used for confirmation of initial drug tests [1]. LC/MS/MS methods are often less complicated than previously employed GC/MS methods because they do not typically require a derivatization step. We present a method for analysis of phencyclidine that meets the most recent SAMHSA guidelines to demonstrate linearity, limit of detection (LOD), accuracy and precision, as well as measurement of matrix effects, extraction recovery, and overall process efficiency. This is one of a suite of six simplified methods covering all classes of SAMHSA-regulated drugs and using premier Agilent products, including Agilent Bond Elut Plexa PCX mixed-mode polymeric SPE sorbent, Agilent Poroshell 120 EC-C18, 2.7 μm superficially porous LC column, Agilent 1200 Infinity LC system, and Agilent 6460 Triple Quadrupole LC/MS system with Agilent Jet Stream Technology (AJST) enhanced electrospray source.



Agilent Technologies

Introduction

Phencyclidine (PCP) is a synthetic drug, a member of the family of dissociative anesthetics. Five to 20 % of administered PCP is excreted unchanged in urine [2]. Therefore, the drug can be detected in its original form and neither hydrolysis nor metabolite measurement are needed. PCP is stable in biological samples. In frozen urine samples, it is preserved for a year, and refrigeration at 4 °C is sufficient for short-term storage.

Phencyclidine has a three-ring structure, with one aryl, one cyclohexane, and one piperidine ring (Figure 1). It is a weak organic base, essentially nonpolar, with a high log P of 4.69. The new SAMHSA confirmation cutoff concentration for phencyclidine is 25 ng/mL, and a LOD at 10% of the cutoff is 2.5 ng/mL [1].

The simple extraction method described in this application note provides reproducible high recoveries of PCP due to the unique properties of the Agilent Bond Elut Plexa polymer. Unlike other polymeric sorbents, Plexa possesses an amide-free hydroxylated particle surface which excludes protein binding. This results in minimized ion suppression and maximum sensitivity. Fast flow and reproducible performance are due to the narrow particle size distribution with no fines to cause blockages.

A Poroshell 120 EC-C18, 3 × 50 mm, 2.7 µm column was chosen due to its high capacity and excellent separation properties. With superficially porous 2.7-µm particles, Poroshell 120 provides similar efficiency to sub-2 µm UHPLC columns but with about 40% less back pressure, thereby allowing users of even 400 bar LC systems to increase resolution and to shorten both analysis and re-equilibration times by applying a higher flow rate.

With a low sample injection volume of 2 µL and no sample preconcentration, the method demonstrates excellent signal-to-noise (S/N) ratios (>200:1 at 2.5 ng/mL, 10% of the SAMHSA confirmation cutoff) due to the enhanced sensitivity of the Agilent 6460 Triple Quadrupole LC/MS system with the AJST electrospray source.

Previous methods from Agilent used the Agilent 6410 Triple Quadrupole LC/MS system and other SPE/LC products and procedures [3,4].

Experimental

Analytes

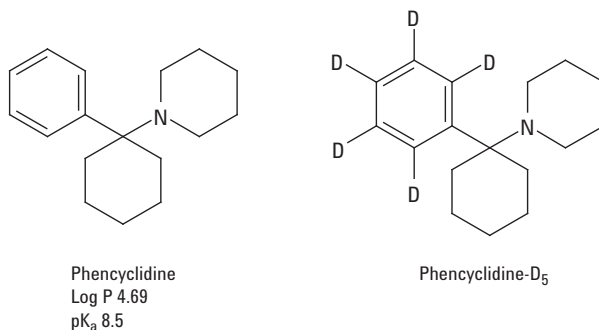


Figure 1. Phencyclidine analytes and their structures.

Drug standards were purchased from Cerilliant Corporation as 1 mg/mL (phencyclidine) and 100 µg/mL (phencyclidine-D₅) solutions in methanol.

Materials and instrumentation

SPE

- Agilent Bond Elut Plexa PCX cartridges, 30 mg, 3 mL (p/n 12108303)
- Agilent vacuum manifold VacElut 20 (p/n 12234100)
- Agilent stopcock valves (p/n 12234520)
- Agilent 2 mL autosampler vials (p/n 5182-0716) or silanized vials (p/n 5183-2072)
- Agilent screw caps for autosampler vials (p/n 5182-0717)

LC

- Agilent Poroshell 120 EC-C18 3 × 50 mm, 2.7 µm (p/n 699975-302)
- Agilent 1260 Infinity LC (G1379B microdegasser, 1312B binary pump in low delay volume configuration, G1367E autosampler, and G1330B thermostat)

MS

- Agilent 6460A Triple Quadrupole LC/MS system with AJST electrospray ionization source

Sample preparation

Pretreatment

Spike 1 mL of urine with ISTD at 50 ng/mL; use of 12 × 75 mm glass tubes is recommended. Add 1 mL of 2% formic acid, vortex; centrifuge if cloudy.

Extraction

1. Condition Bond Elut Plexa PCX column with 0.5 mL methanol – soak, then let drip.
2. Load sample/supernatants.
3. Wash 1: 1 mL 2% formic acid.
4. Wash 2: 1 mL of methanol.
5. Dry 5–10 minutes under vacuum (10–15 in Hg).
6. Elute with 1 mL ethyl acetate: methanol: ammonium hydroxide (80:20:5), freshly prepared. Let eluate drip into collection vials, then apply low vacuum (2–3 in Hg).
7. Evaporate under stream of nitrogen to dryness.
8. Reconstitute in 1 mL initial mobile phase (10% methanol, 90% water, 0.1% formic acid).

LC/MS/MS

LC conditions

Mobile phase A	0.1% formic acid in water	
Mobile phase B	0.1% formic acid in methanol	
Flow rate	0.8 mL/min	
Gradient	Time (min)	% B
	0.0	10
	0.5	10
	2.5	70
	2.51	90
	5.5	90
	5.51	10
Stop time	5.6 min	
Post time	2 min	
Max pump pressure	400 bar	
Injection volume	2 µL	
Injection with needle wash		
Needle wash	Flush port 75:25 methanol:water for 10 s	
Disable overlapped injection		
No automatic delay volume reduction		

MS conditions

ES source parameters

Ionization mode	Positive
Capillary voltage	3,000 V
Drying gas flow	10 L/min
Drying gas temperature	350 °C
Nebulizer gas	35 psi
Sheath gas flow	12 L/min
Sheath gas temperature	400 °C
Nozzle voltage	0 V

MS parameters

Scan type	MRM
Pre-run script	SCP_MS DiverterValveToWaste() {MH_Acq_Scripts.exe}
Time segments	#1: 1.2 min - diverter valve to MS
Delta EMV (+)	200 V

Results and Discussion

At acidic pH, the tertiary amine of phencyclidine was protonated, and the analyte was efficiently retained on Plexa PCX polymeric sorbent by a combination of hydrophobic interaction and a strong cation exchange.

A 100% methanol wash eliminated most matrix interferences without PCP loss from the SPE column. A strong base was added to the organic eluent to break the ionic interaction between the analyte and the strong cation exchange sorbent. PCP recovery is optimized with a two-component organic eluent consisting of 80% ethyl acetate and 20% methanol, with 5% NH₄OH added shortly before sample elution.

The Poroshell 120 EC-C18 3 × 50 mm, 2.7 µm column provided fast separation of phencyclidine in urine extract and good peak shape (Figure 2). The LC separation started with a low fraction of organic solvent (10%) to allow salts and other polar components of urine to elute at the beginning of the sample run. Each sample run started with diverting the first portion of flow to waste to minimize source contamination. Data collection started at 1.2 minutes, immediately after the diverter valve switch. A flow rate of 0.8 mL/min allowed short retention and re-equilibration times.

A S/N ratio >200:1 for the 2.5 ng/mL peak (Figure 2, upper panel) illustrates state-of-the-art performance of the 6460 Triple Quadrupole LC/MS system, capable of reliably detecting PCP at a small fraction (10%) of the SAMHSA cutoff concentration. Being very hydrophobic, phencyclidine has the potential to adhere to any active surfaces. To avoid carryover, we recommend using the external needle wash flush port option of the high performance autosampler, and running a mobile phase blank after samples, which appear from screening results to have a high concentration. If needed, the needle wash can be increased from 10 to 20 seconds.

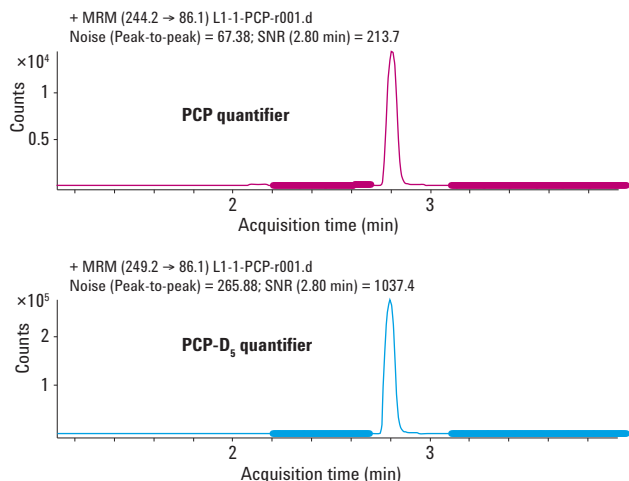


Figure 2. MRM extracted ion chromatograms for PCP (2.5 ng/mL) and PCP-D₅ (50 ng/mL) in urine extract. Agilent Poroshell 120 EC-C18 3 × 50 mm, 2.7 μm, column. Noise regions are shown in bold.

SAMHSA guidelines require one quantifier and at least one qualifier ion for both target compound and ISTD. A third transition for target analyte (Table 1) is provided for additional confidence. Agilent MassHunter Quantitative software automatically calculates qualifier ion ratios, highlighting those out of acceptable range.

Figure 3 shows an example calibration curve for extracted urine standards at five concentration levels of phencyclidine. Calibration standards were prepared by spiking negative urine at 2.5, 25, 100, 250, and 1,000 ng/mL. Deuterated internal standard PCP-D₅ was added at 50 ng/mL. Excellent linear fit with $R^2 > 0.999$ demonstrates the linearity of the method across a broad dynamic range of concentrations, as required by SAMHSA guidelines.

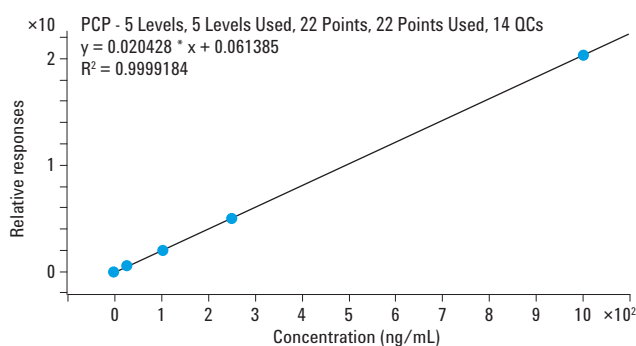


Figure 3. Example calibration curve for phencyclidine in urine extract. Calibration range 2.5 to 1000 ng/mL. Linear fit, $R^2 > 0.999$.

Table 1. MRM Transitions.

Compound	Precursor	Product	Fragmentor	Collision energy
PCP	244.2	86.1	80	7
PCP	244.2	159.1	80	7
PCP	244.2	91.1	80	34
PCP-D ₅	249.2	164.1	80	7
PCP-D ₅	249.2	86.1	80	7

Normal, rather than dynamic, MRM scan type can be used with this method, since dynamic MRM has no advantages for detection of a single compound.

Method evaluation

Method performance metrics in Table 2 were calculated according to the principles proposed by Matuszewski *et al.* and widely accepted as an industry standard approach for LC/MS/MS methods [5]. Extraction procedure and LC/MS/MS measurement were performed for five replicates of negative urine spiked pre-extraction at the cutoff level, and five replicates of negative urine extract reconstituted in initial mobile phase and then fortified at 25 ng/mL with PCP (spiked post-SPE). The third measurement was of initial mobile phase (the reconstitution solvent) fortified to correspond to the cutoff concentration of 25 ng/mL in urine (spiked mobile phase).

Process efficiency (absolute recovery) is a ratio of a peak area of target analyte in urine sample spiked pre-SPE to its peak area in matrix-free spiked mobile phase. Extraction recovery is a ratio of a peak area of target analyte in urine extract spiked pre-SPE to its peak area in an extracted negative urine sample spiked post-SPE. Matrix effect is a ratio of a peak area of target analyte in urine extract spiked post-SPE to its peak area in spiked mobile phase. Accuracy is a ratio of a measured concentration calculated using the calibration curve to the expected concentration in a sample spiked with a known amount of target analyte. Precision or coefficient of variation (CV) is a measure of reproducibility and is calculated as a percent standard deviation over the mean of the five measurements.

Table 2 shows high extraction recovery for phencyclidine (85%) together with very good accuracy (93%) and precision (0.5 %). Matrix effect of 98% indicates only minor ion suppression of the signal due to matrix interferences (2%), thus confirming an exceptional cleanliness of Plexa PCX-processed extracts.

Table 2. Method performance for phencyclidine, n = 5.

	%
Process efficiency	83
Extraction recovery	85
Matrix effect	98
Accuracy	93
Precision (CV)	0.5

Conclusions

The solid phase extraction procedure coupled with LC/MS/MS detection method described in this application note is SAMHSA-compliant and provides accurate, precise and reproducible results for forensic toxicology or other analytical environments with similar requirements for legally defensible data. The hardware setup is the same as in the other 2011 SAMHSA methods from Agilent. These methods are intended for all users of Agilent 1100 and Agilent 1200 LC series since the back pressure in the LC system does not exceed 400 bar. Source parameters can be easily modified to use this method with other models of Agilent Triple Quadrupole LC/MS systems. Electronic copies of the LC/MS/MS acquisition and quantitation methods are available from Agilent Technologies.

References

1. SAMHSA (2010) Manual for Urine Laboratories, National Laboratory Certification Program, 1 October 2010. U. S. Department of Health and Human Services.
2. R. Baselt (2008) Disposition of Toxic Drugs and Chemicals in Man. 8th edition. Atlas Books, Ashland, OH, USA.
3. P. Moorman and J. Hughes (2010) "Phencyclidine in Urine by LC/Triple Quadrupole Mass Spectrometry (LC/MS/MS)". SOP, Agilent Technologies, Inc. Publication number 5990-5864EN.
4. J. Hughes and P. Moorman (2011) "Confirmation by Triple Quadrupole LC/MS/MS for HHS-compliant Workplace Urine Drug Testing". Agilent Technologies, Inc. Seminar available from www.agilent.com/chem.
5. B. K. Matuszewski, M. L. Constanzer, and C.M. Chavez-Eng (2003) Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. *Analytical Chemistry*, 75: 3019-3030.

For More Information

These data represent typical results. For more information on our products and services, visit our Web site at www.agilent.com/chem.

www.agilent.com/chem

Agilent shall not be liable for errors contained herein or for incidental or consequential damages in connection with the furnishing, performance, or use of this material.

Information, descriptions, and specifications in this publication are subject to change without notice.

© Agilent Technologies, Inc., 2013
Printed in the USA
February 15, 2013
5990-9626EN



Agilent Technologies