Analysis of Phencyclidine in Urine to U.S. SAMHSA Guidelines with LC/MS/MS and GC/MS

Application Note

Forensics & Toxicology

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Abstract

The U.S. Substance Abuse and Mental Health Services Administration (SAMHSA) guideline cutoff level for phencyclidine (PCP) in urine is 25 ng/mL. New guidelines from SAMHSA offer the option of LC/MS/MS as an alternative to GC/MS. In this study, we used Agilent Bond Elut Certify mixed-mode solid phase extraction (SPE) for sample preparation in an analysis of PCP by LC/MS/MS and GC/MS. Bond Elut Certify is ideal for PCP extraction from urine because it meets all requirements for linearity, limit of detection (LOD), accuracy, and precision. In addition, LC/MS/MS or GC/MS can be applied with the same sample preparation method, maximizing the convenience of instrument choice in the laboratory. GC/MS was used in splitless and split injection modes.

Agilent Technologies

Introduction

Agilent Bond Elut Certify mixed-mode SPE is very versatile, with well balanced reversed-phase characteristics along with cation-exchange capability. By using its cation-exchange chemistry, basic compounds such as phencyclidine (PCP, pKa = 8.29) can be extracted from human urine, leaving other interferences behind. A newly improved sample preparation method using Bond Elut Certify mixed-mode SPE meets the needs of many laboratories for environmentally friendly solvents, and reduced solvent use and sample amounts, compared to many other methods [1,2]. Also, application data for LC/MS/MS, and GC/MS with split and splitless injection modes, support wider applicability in forensics laboratories.

Materials and Methods

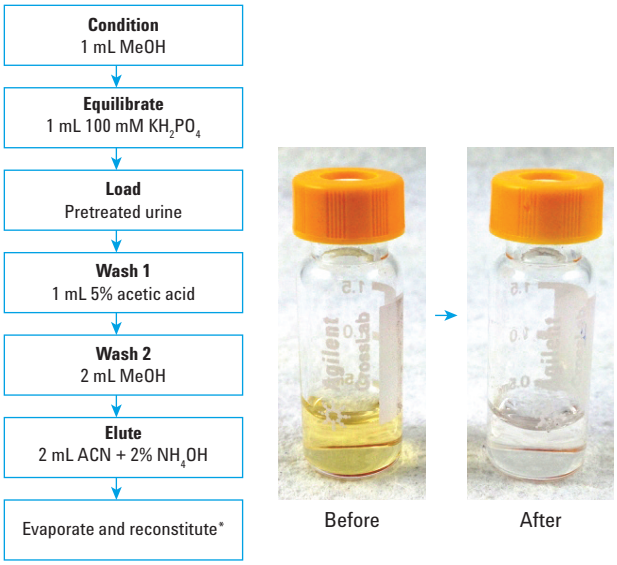
Acetonitrile, methanol, formic acid:	LC/MS grade
Water:	Milli-Q filtered or LC/MS grade
KH_2PO_4, NH_4OH:	Reagent grade
Acetic acid:	Premium quality
Analytes:	PCP and PCP-d5 from Sigma-Aldrich, Corp.
Sample preparation:	Agilent Bond Elut Certify, 130 mg, 3 mL, 50/pk (p/n 12102051)
QC samples:	Liquichek Urine Toxicology Control, Level C2, from Bio-Rad Laboratories, Inc. (PCP concentration 19 ng/mL)

Parameters of the LC/MS/MS and GC/MS instruments are shown in greater detail in Appendix A.

Sample preparation using Agilent Bond Elut Certify mixed-mode SPE

PCP and internal standard were spiked in 1 mL human urine at the desired concentration levels, and 0.5 mL 100 mM KH_2PO_4 was added to adjust the pH to 6.0 ± 0.5. A double blank urine sample was prepared without spiking any compounds into the human urine. QC samples and blank urine sample were prepared by spiking internal standard only.

The solid phase extraction workflow is outlined in Figure 1. A positive-pressure manifold was used throughout the process and high pressure was applied for 2 minutes between the Wash 2 and elute steps. The sample cleanup effect is evident.



* Reconstitute in 0.5 mL 30:70 ACN:H_2O + 0.1% formic acid for LC/MS/MS; reconstitute in 0.5 mL hexane for GC/MS.

Figure 1. Sample preparation steps (left) and urine sample before and after Agilent Bond Elut Certify mixed-mode solid phase extraction (right).

Results and Discussion

Excellent calibration curve linearity was achieved by LC/MS/MS and GC/MS, with $R^2 \geq 0.9996$ over the concentration range of 1 to 500 ng/mL (Figure 2). The limits of quantitation (LOQ) were 1 ng/mL for LC/MS/MS and 5 ng/mL for GC/MS. Data from LC/MS/MS and GC/MS are summarized in Table 1. Excellent accuracy and precision were obtained, demonstrating the performance of Bond Elut Certify mixed-mode SPE. All QC samples run in the beginning, middle, and end of the batch were within a ± 20% accuracy range, further confirming the robustness of this method. The chromatograms obtained from LC/MS/MS and GC/MS with split and splitless injection modes are shown in Figure 3.

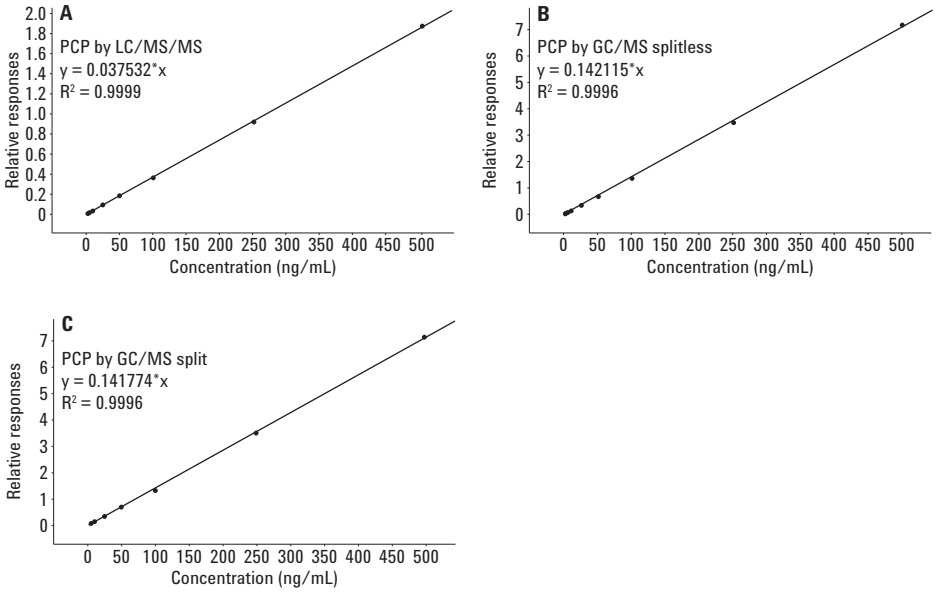


Figure 2. Calibration curves from 1 to 500 ng/mL in urine. A) LC/MS/MS, B) GC/MS with pulsed splitless injection mode, and C) GC/MS with pulsed split injection mode.

Table 1. Summary of LC/MS/MS and GC/MS accuracy and precision data for analysis of PCP in urine.

LC/MS/MS data

R^2	LOQ	Accuracy (% recovery)*(ng/mL)				Precision (% RSD)*(ng/mL)			
0.9999	1 ng/mL	5	10	25	500	5	10	25	500
		101%	92.9%	100%	100%	2.6%	0.7%	0.4%	0.2%

GC/MS data (pulsed splitless injection mode)

R^2	LOQ	Accuracy (% recovery)*(ng/mL)			Precision (% RSD)*(ng/mL)		
0.9996	5 ng/mL	10	25	500	10	25	500
		95.2%	96.0%	97.4%	3.6%	1.6%	2.6%

GC/MS data (pulsed split injection mode)

R^2	LOQ	Accuracy (% recovery)*(ng/mL)			Precision (% RSD)*(ng/mL)		
0.9996	5 ng/mL	10	25	500	10	25	500
		101%	99.0%	101%	2.5%	4.4%	3.9%

* Accuracy and precision data are based on six data points.

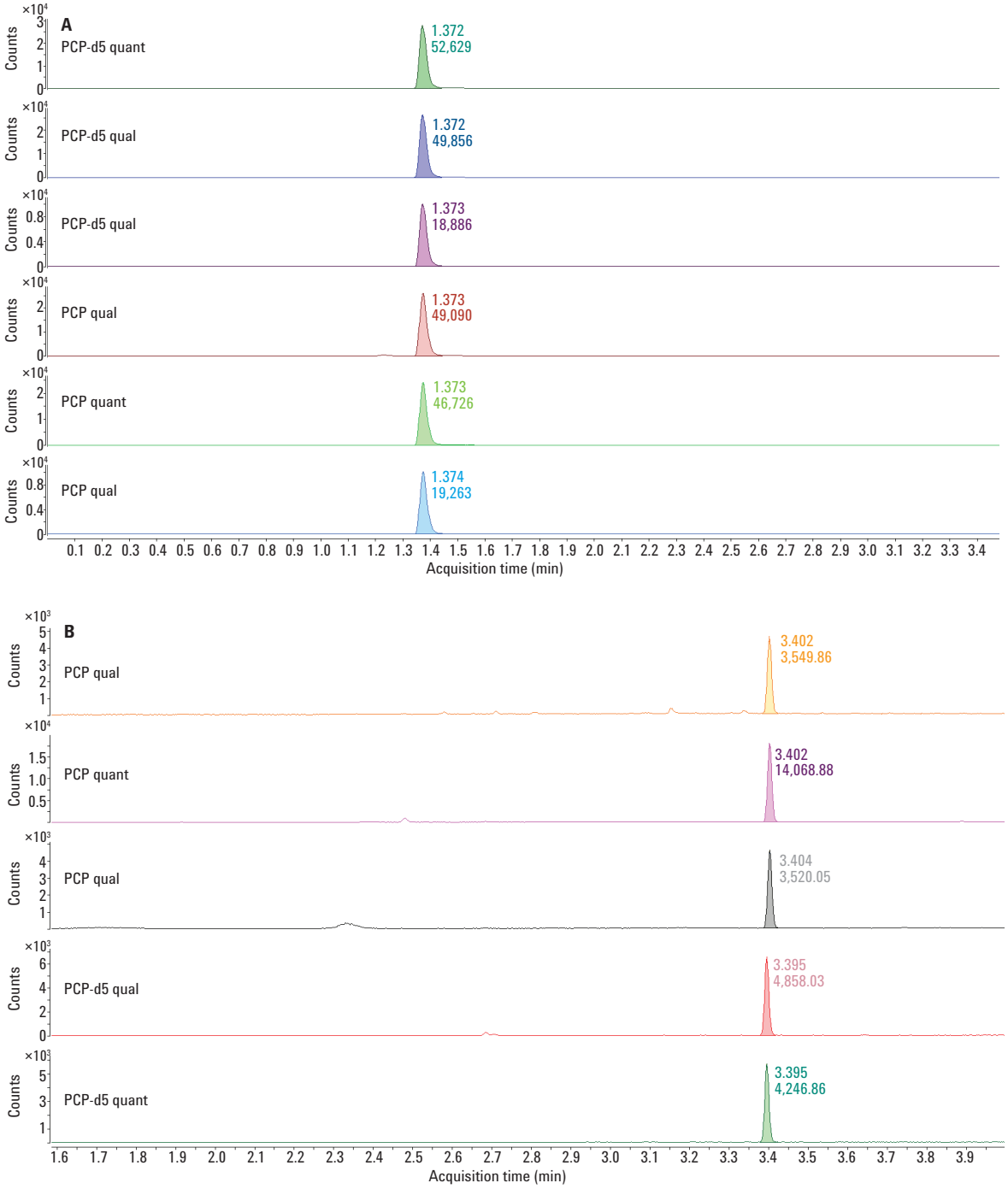


Figure 3. Chromatograms of PCP in urine at the cutoff level (25 ng/mL) by A) LC/MS/MS, B) GC/MS with pulsed splitless injection, and C) GC/MS with pulsed split injection (*continued*).

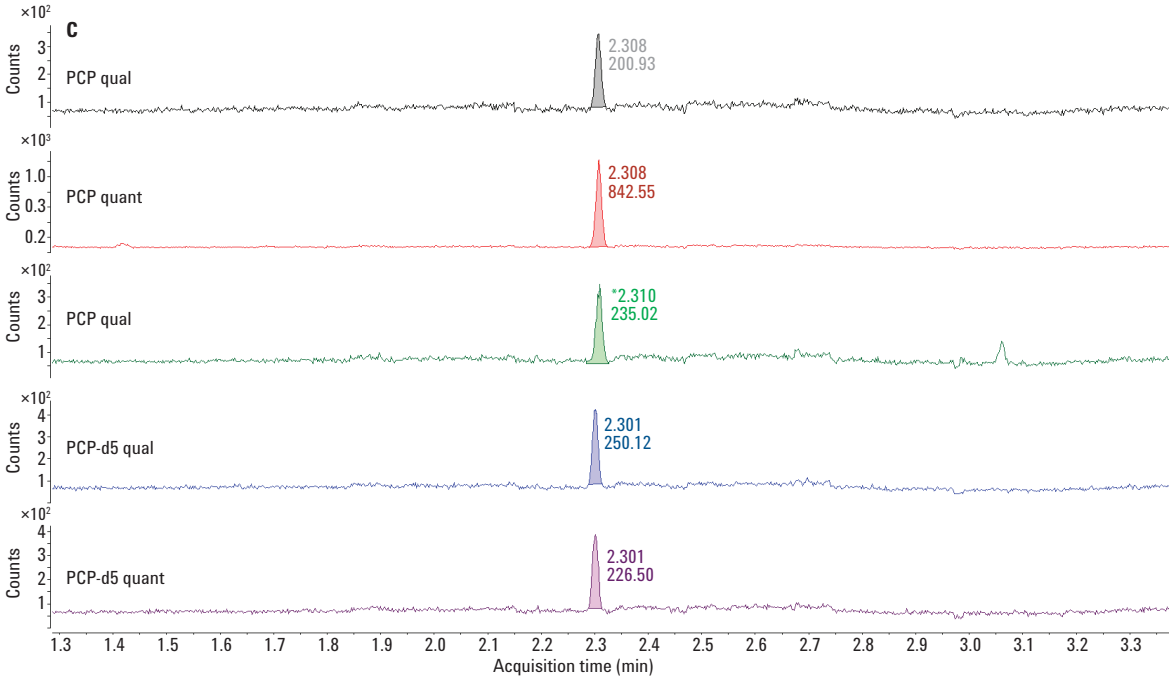


Figure 3. Chromatograms of PCP in urine at the cutoff level (25 ng/mL) by A) LC/MS/MS, B) GC/MS with pulsed splitless injection, and C) GC/MS with pulsed split injection.

Conclusions

Agilent Bond Elut Certify mixed-mode SPE was successfully used for extraction and cleanup of PCP in urine for forensic applications, showing instrument-independent performance with LC/MS/MS and GC/MS with split or splitless injection. Excellent linearity was obtained ($R^2 \geq 0.9996$) from 1 to 500 ng/mL for all instrument configurations. This simple and robust sample preparation method provided high accuracy (100 ± 4 %) and precision (% RSD ≤ 4.4 %) at the cutoff level. All third-party QC samples were within ± 20% accuracy, demonstrating the validity of the method. This ability to use a single sample preparation procedure for multiple instrument platforms or configurations fits laboratory needs, regardless of instrument preference.

References

1. A. Ishii, *et al*. *Int. J. Legal Med.* **108**, 244 (1996).
2. C. Moore, C. Coulter, K. Crompton. Detection of Phencyclidine in Human Oral Fluid Using Solid Phase Extraction and Liquid Chromatography with Tandem Mass Spectrometric Detection. Application note, Agilent Technologies Publication 5989-8084EN (2008) http://www.chem.agilent.com/library/applications/5989-8084en.pdf

Appendix A

Instrument conditions

LC/MS/MS conditions

System:	Agilent Infinity 1260 Infinity LC with Agilent 6460 Triple Quadrupole LC/ MS with Agilent JetStream ESI
Column:	Agilent Pursuit XRs Ultra Diphenyl, 2.0 × 50 mm, 2.8 µm (p/n A7521050X020)
A:	0.1% formic acid
B:	ACN + 0.1% formic acid
Flow rate:	0.6 mL/min
Injection volume:	5 µL
Sample solvent:	30:70 ACN:H_2O + 0.1% formic acid
Gas temperature:	300 °C
Gas flow:	7 L/min
Sheath gas temperature:	250 °C
Sheath gas flow:	8 L/min
Capillary:	3,500 V (+)
Nozzle voltage:	0 V

Gradient:

Time (min)	% B
0	5
1	95
1.5	95
1.6	5
3.5	5

MRM

Compound	Precursor	Product
PCP	244.2	86.1
		91.1*
		159.1
PCP-d5	249.2	86.1*
		96.1
		164.2

*Quantitative MRM transition

GC/MS conditions with pulsed splitless injection

GC/MS:	Agilent 7890A GC with an Agilent 5975C Series GC/MSD
Column:	Agilent J&W DB-5ms Ultra Inert, 15 m × 0.25 mm, 0.25 µm (p/n 122-5512UI)
Septum:	Bleed/temp optimized nonstick, 11-mm septa, 2 packs of 50/pk (p/n 5183-4757)
Liner:	Agilent Ultra Inert splitless single taper with glass wool, 25/pk (p/n 5190-3167)
Carrier gas:	He
Flow rate:	1.0 mL/min, constant flow
Inlet temperature:	250 °C
Septum purge:	3 mL/min, switched mode
Injection mode:	Pulsed splitless
Injection volume:	1 µL
Pulse pressure:	35 psi until 0.5 min
Purge flow to split vent:	50 mL/min at 0.75 min
Sample wash:	Max, 2 times
Sample pumps:	5
Oven temperature:	Initial hold at 100 °C for 0.5 min, ramp to 300 °C at 80 °C /min, hold at 300 °C for 1 min Run time: 4 min

SIM

	m/z	Dwell time
PCP	186	20
	200*	20
	242	20
PCP-d5	246	20
	248*	20

*Quantitative ions

GC/MS conditions with pulsed split injection

Parameters are the same as GC/MS conditions with pulsed splitless injection, with some variations as below.

Injection mode:	Pulsed split
Split ratio:	10:1
Split flow:	10 mL/min
Oven temperature:	Initial hold at 150 °C for 0.5 min, ramp to 300 °C at 80 °C/min, hold at 300 °C for 1 min Run time: 3.375 min

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