Technical Note: 10163

High-Throughput Confirmation and Quantitation of Phencyclidine (PCP) in Urine Using the DSQ II GC/MS

Jason Cole, Matthew Lambing, Trisa Robarge, Thermo Fisher Scientific, Austin, TX, USA

Key Words

- DSQ II GC/MS
- ToxLab 2.0 Software
- PCP
- Toxicology
- Urine Drug Testing

Overview

Phencyclidine (PCP) is a commonly abused hallucinogenic drug. Although used legitimately as a veterinary tranquilizer, the human use of the drug is prohibited in the United States and elsewhere. PCP is commonly tested in urine at workplace drug testing laboratories, while less commonly used matrices include hair, sweat, oral fluids, and blood. Toxicologists in other disciplines may also test for PCP use, for such diverse applications as driving impairment analyses, postmortem investigations, and clinical toxicology. Analytical methods for PCP range from immunoassay techniques to gas chromatography/mass spectrometry (GC/MS). The methodology presented here focuses on the use of the DSQ™ II GC/MS system for the confirmation and quantitation of PCP (Figure 1) extracted from urine.

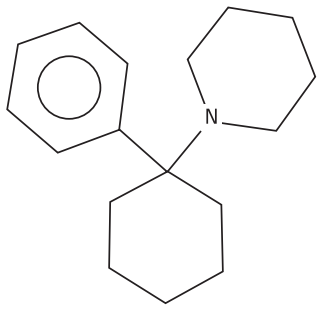

Figure 1: Chemical Structure of PCP

A 2 mL urine sample size was used, with PCP-D5 as the deuterated internal standard. Samples were extracted using solid phase extraction. The extracted eluate was evaporated under a nitrogen stream and the resulting residue was reconstituted in ethyl acetate. This final solution of PCP and its deuterated analog were then injected onto a DSQ II single stage quadrupole GC/MS system. A calibrator at the cutoff threshold concentration of 25 ng/mL, a level established by the United States Substance Abuse and Mental Health Services Administration (SAMHSA), was used as a single point calibrator. The resulting method demonstrated excellent precision, no interference for a number of tested compounds and provided linearity from 5 to 5,000 ng/mL, with a limit of detection and limit of quantitation of 5 ng/mL.

Introduction

After the administration of PCP, the user generally experiences a feeling of lethargy and disorientation and can experience hallucinations. PCP undergoes oxidative metabolism in the body, forming at least three different metabolites. However, enough of the compound remains in its native state to allow for the testing of the parent compound as an indicator of its use.[1] Due to its lack of highly polar functional groups, PCP lends itself readily to GC/MS analysis, with no need for derivatization.[2] Confirmation of PCP use utilizing GC/MS is currently mandated by SAMHSA, and a fully validated method is described here.

The DSQ II, a single stage quadrupole mass spectrometer with a curved prefilter that minimizes background noise derived from excited neutrals, was used for this analysis. Coupled to a TRACE GC Ultra™ gas chromatograph and an AS3000 autosampler, this GC/MS system represents the standard for confirmatory analyses of drug use. ToxLab™ 2.0 software provided automated sample analysis and quantitation, and the method was fully validated, including assessments of precision, interference, and linearity. This method describes the GC/MS confirmation and quantitation of PCP in human urine, and it does not include other matrices or any metabolites of PCP. The method was validated to include precision (both inter- and intra-day), linearity, carryover, and specificity.

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Methods

To provide a comprehensive view of PCP method development and validation, methods for sample preparation, acquisition, and analysis are described in detail below. Sample preparation plays a critical role in method validation in that many certifying bodies recommend or require method validation performed in matrix. In this case, solid phase extraction is used due to its ease of use and the cleanliness of the resultant extracts.[3]

Sample Preparation

Known negative urine was collected and used for sample preparation. A sample size of 2 mL was selected. Calibrators and linearity samples were spiked with appropriate amounts of PCP (Cerilliant, Round Rock, TX). Single point calibration at 25 ng/mL was used for calculation of all quantitative amounts. A commercial control (Medical Analysis Systems, Level G3, Freemont, CA) calibrated to represent 125% of 25 ng/mL (31.25 ng/mL) was used as the positive control for the batch, and as objective proof of calibration accuracy. A second control at 40% of 25 ng/mL (10 ng/mL), obtained by the dilution of a standard from a second source (Alltech Associates, Deerfield, IL), was used to check the accuracy of the calibrator. All batches contained an unextracted standard, calibrator, negative control, 40% control and the 125% control. PCP-D5 (Cerilliant) was used as the deuterated internal standard, and was added to each sample at a final concentration of 25 ng/mL. An unextracted standard was prepared by adding 100 µL of 500 ng/mL PCP standard solution and 100 µL of 500 ng/mL PCP-D5 internal standard solution to a labeled tube, yielding the equivalent of a 25 ng/mL sample. The purpose of the unextracted standard is to demonstrate recovery and to prep the GC/MS system. The unextracted standard is not subjected to the extraction steps but instead proceeds directly to the dry-down step, at which point it rejoins the rest of the samples for reconstitution and analysis.

To each spiked 2 mL specimen, 2 mL of 0.1 M phosphate buffer (pH 6.0) were added. Each sample was extracted by solid phase extraction on Thermo Scientific HyperSep™ Verify™ CX columns. The extraction columns were conditioned with sequential rinses of the following: 3 mL methanol, 3 mL DI water, and 1 mL 0.1 M phosphate buffer. Between each conditioning step, the columns were aspirated but were not allowed to dry. The samples were loaded onto the column and extracted under low vacuum (″ 3 in. Hg). After the samples were loaded, the columns were washed sequentially with 3 mL of DI water, 1 mL of 0.1 M acetic acid, and 3 mL of methanol. The columns were then dried under high vacuum for five minutes. The sample eluates were collected in clean tubes under low vacuum (″ 1 in. Hg) with 3 mL of elution solvent (methylene chloride: isopropanol: ammonium hydroxide, 78:20:2 v:v:v). This solution was made fresh daily.[3]

The extracts were evaporated to dryness at 40 °C under nitrogen. Caution was taken to prevent excessive drying of the extracts. For analysis, 100 µL of ethyl acetate were added to the dried extracts, and the resulting samples were vortexed and transferred to autosampler vials with glass inserts and loaded onto the AS3000 autosampler for GC/MS analysis. Table 1 summarizes sample prep, extraction, and derivatization steps.

Instrumental Analysis

The DSQ II mass spectrometer used for this analysis was configured with a 250 L/s turbomolecular pump, and the TRACE GC Ultra was equipped with a standard split/splitless injector. A 5 mm i.d. deactivated glass liner was used in the injector and glass wool was used in the liner. The split/splitless injector temperature was set to 250 °C. A 1 µL injection volume was programmed on the AS 3000 auto sampler, and a 10:1 split injection was used. The analytical column was a TRACE™ TR-5MS 15 m x 0.25 mm i.d. x 0.25 µm film which was installed 64 mm into the injection port (Figure 2).

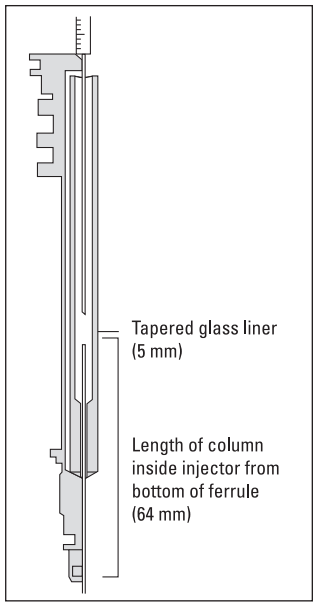

Figure 2: Column installation in GC split/splitless injection port (not to scale)

Sample Preparation and Hydrolysis	Extraction	Concentration
1. Label 13 x 100 mm screw top culture tubes	1. Condition SPE columns with the following: a. 3 mL methanol b. 3 mL DI water c. 1 mL pH 6, 0.1 M phosphate buffer	1. Blow down samples at < 40 °C under N_2 stream until dry
2. Add 2 mL of blank urine, QC sample or donor specimen	2. Apply samples and extract under low vacuum	2. Reconstitute with 100 µL ethyl acetate
3. Spike calibrator and low QC with PCP	3. Rinse with the following: d. 3 mL DI water e. 1 mL 0.1 M acetic acid f. 3 mL of methanol	3. Transfer resulting solutions to autosampler vials with inserts for GC/MS analysis
4. Add 100 µL of working PCP-D5 internal standard to each tube	4. Dry columns at high vacuum for 5 minutes	
5. 2 mL pH 6, 0.1 M phosphate buffer	5. Elute PCP extracts with 3 mL 78:20:2 methylene chloride: isopropanol: ammonium hydroxide and collect in labeled screw top culture tubes	
6. Vortex		
7. Prep vacuum manifold for sample extraction		

Table 1: Sample Prep, Extraction and Concentration Summary

Programmed carrier gas flow started with an initial flow rate of 2.5 mL/min of helium. At 1.9 minutes, the flow was ramped to 12.5 mL/min, to get the heavy matrix compounds through the column as quickly as possible. The initial temperature on the TRACE GC Ultra was set to 175 °C. The high temperature at the beginning of the analytical run allowed the PCP to elute from the column as quickly as possible. The initial oven hold time was 1 minute, after which the GC temperature ramped at 60 °C/min to a final temperature of 310 °C for 0.65 min, for a total run time of 3.9 minutes, a PCP retention time of 1.77 minutes and a total inject-to-inject time of 6.9 minutes. The DSQ II source temperature was set to 300 °C, and the mass spectrometer was tuned using default *AutoTune* parameters except for the emission current, which was changed from 100 µA to 60 µA. These tune settings were used for acquisition, with a detector gain of 3 x 10^5.

For initial mass spectrometer method development, high concentrations of PCP and PCP-D5 were injected and analyzed in electron impact full scan to determine appropriate masses for selected ion monitoring (SIM). The set of SIM masses and dwell times used to detect PCP and its deuterated internal standard are shown in Table 2. Mass 200 was used as the quantitation mass for PCP, and mass 205 was the quantitation mass for internal standard, PCP-D5. A narrow SIM width enhances sensitivity and builds on the mass stability and resolution of the DSQ II, while a short dwell time provides quantitative precision across the narrow GC peak that results from the use of fast GC. Table 2 summarizes instrument parameters for the validated method.

Sample Processing and Result Derivation

For sample acquisition, peak detection and quantitation, ToxLab 2.0 software was utilized. By incorporating all of the vital components of analyses into a unified workflow-oriented application, ToxLab 2.0 provides an integrated solution to PCP GC/MS confirmation. To make use of ToxLab 2.0 for method validation, an instrument method was created for the mass spectrometer, autosampler, and GC. A processing method for component identification and quantitation was also created. In ToxLab 2.0, these methods were integrated into a single master method, which also allows the user to establish criteria specific to the method. Batch creation was performed through the Batch Wizard function of ToxLab 2.0, which greatly simplified and streamlined sample entry, particularly for the longer validation batches (Figure 3). This highlights the applicability of this software to routine analysis of toxicological samples.[4]

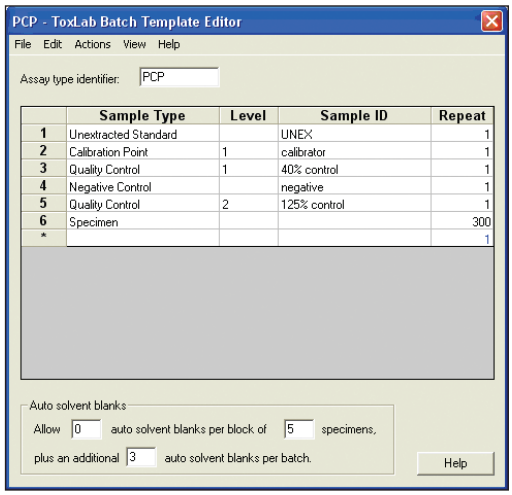

Figure 3: ToxLab 2.0 Batch Template Editor, showing framework for PCP batches

DSQ II	
Source Temp (°C):	300
Acquisition Time (min):	1.95
Detector Gain:	3 x 10^5
Start Time (min):	1.55
PCP Mass (*m/z*):	200.0 186.0 142.0
PCP-D5 Mass (*m/z*):	205.0 248.0
Width (amu):	0.5
Dwell Time (ms):	10

TRACE GC Ultra	
Oven Method	
Initial Temp (°C):	175
Initial Time (min):	1.0
Rate (°C /min):	60
Final Temperature (°C):	310
Final Hold Time (min):	0.65
SSL Method	
Temperature (°C):	250
Mode:	Split
Split Ratio:	10:1
Split Flow:	25
Constant Septum Purge:	on
Carrier Method	
Initial Value (mL/min):	2.5
Initial Time:	1.90
Rate #1 (mL/min2):	999.9
Final Value (mL/min):	12.5
Hold Time #1 (min):	5
Gas Saver:	off

AS 3000	
Sample Volume (µL):	1
Plunger Strokes:	3
Viscous Sample:	Yes
Sampling Depth in Vial:	Bottom
Injection Depth:	Standard
Pre-Inj Dwell Time (sec):	0
Post-Inj Dwell Time (sec):	0
Sample Rinses:	0
Pre-Injection Solvent Rinses:	0
Post-Inj Solvent Rinses	
Solvent A (50:50 EtOAc:$MeCl_2$):	5
Solvent B (50:50 EtOAc:$MeCl_2$):	5

Table 2: Instrument method summary for the SIM analysis of PCP on the DSQ II

Concentration calculations were based on a single point calibrator at 25 ng/mL, using PCP-D5 as the internal standard. Linear calibration including the origin created the calibration curve, and calculated amounts were based on this curve. All validation batches had to conform to quality control (QC) criteria, including quantitative and qualitative bounds checking.

Quantitative criteria for the batch included acceptable quantitation ranges for all samples in each batch. All calculated amounts for QC samples and study samples had to fall within ± 20% of the expected concentration in order to accept the sample. Failure of a QC sample within a batch would mean the entire batch would need to be repeated. In addition to this quantitative window, negative controls were evaluated based on two additional criteria. One means of assessing a negative control is a quantitative value for PCP less than the method limit of detection (LOD), which in this case is 5.0 ng/mL. An alternate criterion for negative controls is that the calculated amount must be less than a pre-determined percentage of the method cutoff. For this method, a level of 5% of the cutoff (1.25 ng/mL) was used as a second criterion, and all negative controls were evaluated for compliance to both criteria.

Qualitative criteria included ion ratio and retention time target ranges based on the calibrator, along with peak shape considerations. These criteria were applied to all sample types. Ion ratio ranges for the batch were developed based on the appropriate ratios from the 25 ng/mL calibrator. Ratios were defined as follows:

$$ion\ ratio = \frac{area\ of\ qual\ ion}{area\ of\ quant\ ion} \times 100$$

Ratios were calculated for PCP-D5 (248:205) and PCP (186:200 and 242:200), and for each ratio, an acceptable range of ± 20% was established. Similarly, the target retention time for PCP and PCP-D5 was set using a ± 2% retention time window based on the calibrator retention time. Peak symmetry requirements required the peaks to be >90% symmetrical at 50% peak height.

Each validation batch was reviewed for compliance with these criteria, and for a study batch to be accepted, it had to comply with all of these QC criteria.

Results

The analysis of PCP in urine using the DSQ II GC/MS system was thoroughly validated through rigorous determination of linear range, carryover, precision, and specificity. All validation batches had to conform to quality control (QC) criteria as described above.

Four separate batches were prepared and analyzed: one for linearity/carryover, one for specificity, and two for precision. Each batch included the appropriate quality controls and calibration standards, along with validation samples prepared according to Table 3. Carryover was assessed during the course of the linearity study. Precision analyses were performed on two separate batches analyzed on two separate days, while specificity assessed potential interference from a number of compounds. Limits of detection and quantitation were determined both analytically and statistically. The DSQ II demonstrated excellent intra- and inter-day precision, linearity from 5 to 5,000 ng/mL with carryover seen only after the 5,000 ng/mL sample, and no interference was seen for this assay for the compounds tested. With 6.9 minute inject to inject times, the method also provides a productive means of performing this confirmation.

Spiked Concentration (ng/mL)	Calculated Concentration (ng/mL)
40% Control	9.50
Negative Control	0
125% Control	30.86
5	5.27
Negative	0
10	9.64
Negative	0
25	24.8
Negative	0
50	49.3
Negative	0
100	98.2
Negative	0
250	248
Negative	0
500	491
Negative	0.1
1000	1000
Negative	0.4
2500	2540
Negative	0.4
5000	5060
Negative	4.3

Table 3: Linearity and carryover study results for PCP

Linear Range Determination

The determination of assay linearity was performed at concentrations across a broad dynamic range. The linearity batch, as with every validation batch, included an unextracted standard, a negative control (blank urine and internal standard), the 25 ng/mL calibrator, a 40% control sample (10 ng/mL) and a 125% commercial control sample (31.25 ng/mL). To evaluate method linearity, samples at 5, 10, 25, 50, 100, 250, 500, 1,000, 2,500, 5,000 ng/mL were prepared and extracted, along with the calibrator and controls. These samples were then injected 7 times each, and the resulting 70 data points were quantified based on the 25 ng/mL calibrator. All 70 quantitative values were within ± 20% of their expected concentrations, and a regression analysis comparing the average quantitative value for each level to its nominal value was found to have a correlation coefficient of 0.99998 (Figure 4). At the lowest level, 5 ng/mL, the coefficient of variation (CV) of the calculated amount was 2.4%, with an average concentration of 5.3 ng/mL. Chromatography for the quantitation ion and all qualifiers was exceptional, as shown in Figure 5.

In addition to evaluating quantitative performance, the ratios of the qualifier ions to the quantitation ion for both PCP and PCP-D5 were also evaluated across the concentration range. For PCP, *m/z* 200 served as the quantitation mass, while *m/z* 186 and 242 were used for confirmation. *m/z* 205 was used as the quantitation mass for the internal standard, with *m/z* 248 used as the PCP-D5 confirmatory ion. The acceptable ion ratio ranges were calculated based on the appropriate ratios from the 25 ng/mL calibrator, and a relative range of ± 20% was used as evaluation criteria for the 70 linearity injections. For each of these injections, the ion ratios were calculated and all were found to be within the acceptable range, indicating excellent linearity of ion ratios across the concentration range.

An additional component of the linearity study included a determination of the carryover limit for the method. To do so, a negative control was injected following each set of linearity samples. These negatives were evaluated for acceptability according to the batch criteria described above. Under these constraints, significant carryover was seen only after the 5,000 ng/mL level. The use of a split injection coupled with a combination of syringe rinse steps ensures minimal carryover.

Finally, for the batch to be considered acceptable, the quality control for the batch had to meet QC standards described above. For the 40% control, the calculated value was 9.50 ng/mL, a -5% deviation from the target and well within the ± 20% quantitation range, and the ion ratios were also within the ± 20% target range. The 125% control was calculated to be 30.9 ng/mL, -1% deviation from nominal and well within ± 20%, and the ion ratios met their criteria. The negative control showed no signs of the presence of PCP. Table 3 includes a summary of the linearity/carryover study for PCP on the DSQ II.

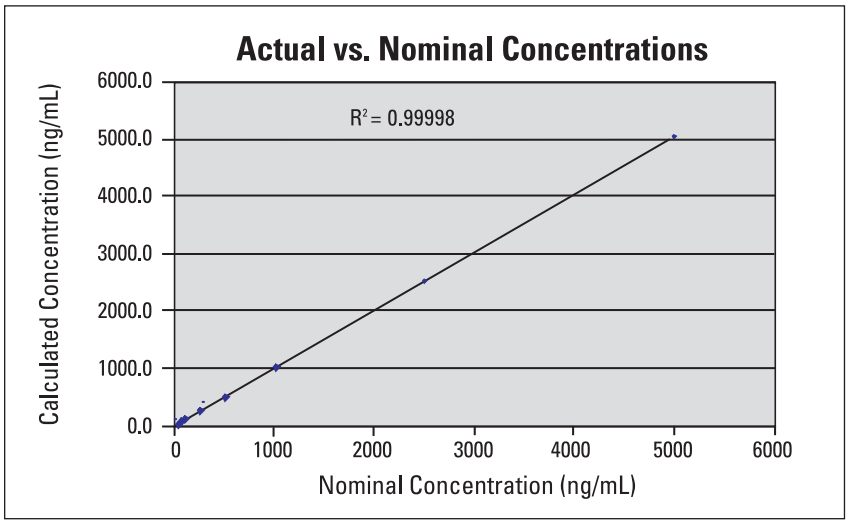

Figure 4: Linearity study results, comparing average concentrations for replicates at 9 different levels to the nominal amounts at each level. The regression analysis for this study gave a correlation coefficient of 0.99998 across all 9 levels.

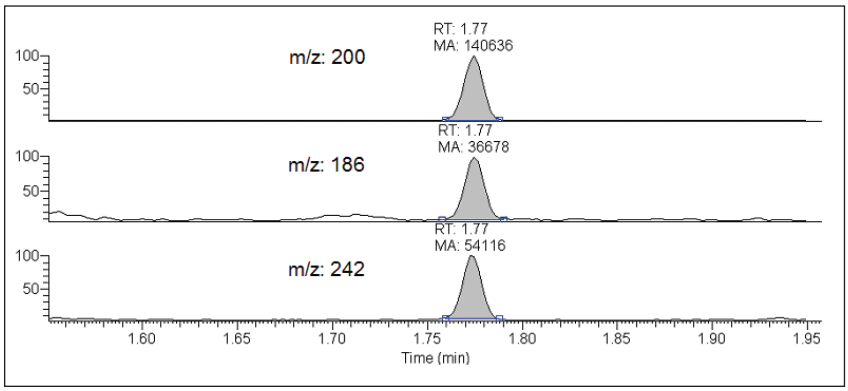

Figure 5: *m/z* 200, 186, and 242 from the 25 ng/mL level, showing good chromatography and signal intensity at the limit of detection for this method

Intra- and Inter-day Precision

Instrument precision and method precision were measured by extracting two separate precision batches and running these batches on two different days. The precision study was designed to indicate precision at the 40% level, at the cutoff and at the 125% level. Coefficients of variation (CV) were calculated for the average concentrations at each level, and these CVs had to be less than 10% for each concentration. As with the linearity batch, the precision batches had to comply with the QC criteria, and all controls were acceptable. To gauge inter-day precision, the percent difference in the average quantitation amounts at each level had to be less than 10%.

The method described above provides excellent quantitative precision, with CVs all less than 3%, and percent differences all less than 4%. Table 4 includes a summary of the precision results for PCP on the DSQ II.

Specificity

To determine assay specificity, an interference study was also performed. A number of compounds with potential to interfere with the immunoassay screening test for PCP were included in this test, as were a range of other drugs. Table 5 describes the drugs and their respective concentrations. Dextromethorphan, diphenhydramine and venlafaxine were assessed individually. The other drugs were analyzed together. For each interference test, the potential interferent was spiked into a blank urine sample, a 10 ng/mL sample and a 31.25 ng/mL sample at the concentration specified. All negatives met the negative control criteria for PCP, and each 40% and 125% control quantitated within 20% of the target concentration, showing that none of the potential interferents tested affected quantitation. Also, all ion ratios were checked against the ion ratios of the calibrator and each were within 20% of the calibrator ion ratios, showing no interference with the confirming ions as well. The interference batch also complied with all applicable QC criteria, and the results of the specificity batch were accepted as demonstrating the assay to be free of interference from the tested compounds.

Drug	Concentration (ng/mL)
Dextromethorphan	5000
Diphenhydramine	5000
Venlafaxine	10000
Ethosuximide	2000
alpha-Methyl-alpha-propylsuccinimide	2000
Metharbital	2000
Barbital	2000
Methsuximide	2000
Phensuximide	2000
Normethsuximide	2000
Mephenytoin	2000
Ethotoin	2000
Mephobarbital	2000
PEMA	2000
Phenobarbital	4000
Methyl PEMA	2000
10,11-Dihydrocarbamazepine	2000
Primidone	2000
Phenytoin	2000
Carbamazepine	4000
4-Methylprimidone	2000
Caffeine	800
Methadone	600
Cocaine	4600
Codeine	1000
6-Monoacetylmorphine	1500
Diacetylmorphine	1500
dl-Glutethimide	2000
Lidocaine	2000
dl-Methadone (primary metabolite)	2000
dl-Methadone	2000
Methaqualone	2000
Desipramine	2000

Table 5: List of compounds tested for potential interference, along with concentrations tested

Concentration	CV for Batch 1	CV for Batch 2	Inter-batch Percent Difference
10 ng/mL	2%	2%	0%
25 ng/mL	1%	1%	1%
31.25 ng/mL	1%	1%	3%

Table 4: Results of precision study showing intra-day coefficients of variations of less than 3% and percent differences for inter-batch calculated amounts of less than 4%

Conclusion

The analysis of PCP on the DSQ II was completed with a PCP retention time of less than two minutes. The validated method is sensitive, with a wide dynamic range, ranging from 5 to 5,000 ng/mL. All samples tested in this range gave calculated amounts that were within 20% of the nominal values, based on a one-point calibration curve at 25 ng/mL. Across this range, all samples also gave ion ratios which were within 20% of the ion ratios of the calibrator. A series of replicate injections at the reported LOD of 5 ng/mL gave a coefficient of variation of 2.6% and an average calculated value of 5.3 ng/mL, demonstrating remarkable sensitivity even when using a split injection technique. Method precision and specificity were also excellent, with coefficients of variation all less than 3% at three different concentrations. Because all method development and validation was performed in extracted urine matrix, the results demonstrate that the DSQ II is able to adequately handle matrix contamination if a sufficient amount of sample preparation is done. These results also accurately reflect method development and validation as they would be performed within a working laboratory.

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