

## Note d'application

# A Simple Dilute and Shoot Method for the UPLC-MS/MS analysis of Pain Management Drugs and Drugs of Abuse From Urine for Forensic Toxicology

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Ce document est une note d'application et ne contient pas de section détaillée concernant l'expérimentation.

*For forensic toxicology use only.*

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## Abstract

This application brief describes a simple dilute and shoot method for the UPLC-MS/MS analysis of pain management drugs and drugs of abuse for forensic toxicology and offers an alternative approach to that described in Waters Application Note. [720006187](#). Sample preparation is simplified from SPE to a single dilution step, which still provides minimal carryover, consistent matrix effects, and precise quantitative data, reaching the required analytical sensitivity for the majority of analytes within the panel. For the compounds requiring more analytical sensitivity, further sample clean up, as described in the original application note, is recommended.

The use of an ACQUITY™ UPLC™ BEH™ C<sub>18</sub> Column allows for a fast analysis of a large panel of compounds, while maintaining all required separations to ensure no interference from isobaric compounds.

The Waters™ Xevo™ TQ-S micro IVD provides accurate quantification of the large panel of analytes over wide

dynamic ranges, simultaneously quantifying some analytes at 2 ng/mL with others at 2500 ng/mL.



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*Figure 1. ACQUITY™ UPLC™ I-Class FL IVD and Xevo TQ-S micro IVD Mass Spectrometer.*

The combination of the sample extraction, chromatography separation and MS/MS detection gives a simple workflow, giving a fast and precise method that can also be automated on a Hamilton STAR or STARlet system.

## Benefits

- Fast and simple method for the analysis of a comprehensive panel of definitive drugs
- Simple dilute and shoot sample extraction method
- Consistent matrix effects and recoveries with minimal carryover
- Precise quantification of a large panel of definitive drugs

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## Introduction

Analyte panels for use in forensic toxicology analysis typically include illicit drugs and common drugs of abuse. Often, multiple methods are used to obtain a comprehensive view of the multiple drug classes. These methods may include immunoassay, GC-MS, LC-MS/MS, or a combination of methods. Waters has developed a method for the quantification of a comprehensive drug panel to achieve the appropriate analytical sensitivity, selectivity, and accuracy for unambiguous identification for forensic toxicology.

This method employs a simple sample extraction procedure using a dilute and shoot approach coupled with a rapid and reproducible chromatographic method using an ACQUITY UPLC BEH C<sub>18</sub> Column that achieves baseline separation for all critical pairs of potentially interfering analytes. A Waters Xevo TQ-S micro IVD Mass Spectrometer with Xtended Dynamic Range (XDR) capabilities provided the analytical sensitivity and dynamic range capabilities required for this diverse group of compounds. Although this method has been shown to provide suitable results for pain management drugs and drugs of abuse, there are some limitations as a result of the simplified dilute and shoot workflow around analytical sensitivity. If low analytical sensitivity is required for these small number of analytes, it is recommended that further sample clean-up is required such as that described in Waters Application Note. [720006187](#).

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## Experimental

### Sample Extraction

All standards were obtained from Cerilliant (Merck Life Sciences, Gillingham, UK), Toronto Research Chemicals (North York, ON) and Cambridge Biosciences UK (Cambridge, UK). A mixed stock solution was prepared in methanol at concentrations of 2, 10, and 25 µg/mL, depending upon the analyte. An internal standard working solution was prepared in 50/50 (v/v) methanol/water at a concentration of 100 ng/mL. Stable isotope labeled internal standards were used for all compounds except in the cases of clonazepam, dehydronorketamine, methedrone, noroxymorphone, and  $\alpha$ -pyrrolidinovalerophenone ( $\alpha$ -PVP) metabolite 1 where stable labeled IS was not readily available. Standards were prepared by diluting the stock solution and spiking dilutions into pooled, blank urine. Quality control (QC) samples were also created by diluting the stock solution and spiking dilutions into pooled, blank urine. All analytes, along with their retention times and calibration ranges are listed in Appendix 1.

Sample extraction can be semi-automated, by utilizing a Hamilton liquid handling robot, enabling sample tracking from the sample tube barcode to the processed sample.

25 µL of urine sample is transferred into a Waters 700 µL Round 96-Well Collection Plate and internal standard working solution is added and thoroughly mixed. Samples are diluted with a distilled water/formic acid solution and mixed prior to injection on the UPLC-MS/MS system.

## LC Conditions

LC system:	ACQUITY UPLC I-Class FL IVD
Column(s):	ACQUITY UPLC BEH C <sub>18</sub> , 1.7 µm, 2.1 x 100 mm
Column temperature:	40 °C ± 2 °C alarm
Injection volume:	20 µL
Mobile phase A:	Water with 0.1% Formic Acid
Mobile phase B:	Acetonitrile with 0.1% Formic Acid

## Gradient Table

Time (min)	Flow rate (mL/min)	%A	%B	Curve
0.00	0.60	98	2	Initial
0.50	0.60	98	2	6
4.50	0.60	45	55	6
4.60	0.60	10	90	11
5.10	0.60	98	2	11

## MS Conditions

MS system:	Xevo TQ-S micro IVD
Ionization mode:	ESI+
Capillary voltage:	0.8 kV

MS method parameters including cone voltage, collision energy and multiple reaction monitoring (MRM) transitions are given in Appendix 1.

## Data Management

MS Software:	MassLynx
Informatics:	TargetLynx™

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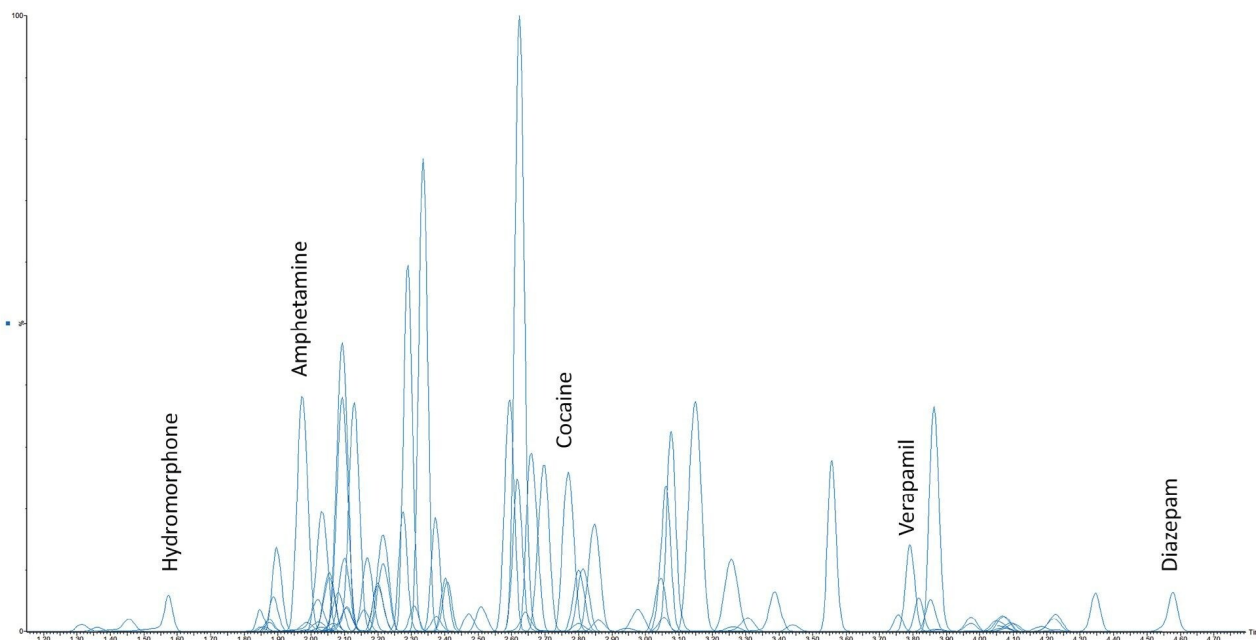
## Results and Discussion

### Chromatography

All test compounds are listed in Appendix 1, along with their retention time windows and calibration ranges. Figure 2 shows the overlaid chromatographic separation of all compounds and Figure 3 shows chromatograms from several groups of analytes with the potential to interfere with each other and these are listed below.

Morphine and Hydromorphone	Methedrone and MDMA
Dehydronorketamine, Ethylone, and Butylone	Codeine and Hydrocodone
Naloxone and 6-Acetylmorphine	Dihydrocodeine and Noroxycodone
Methamphetamine and Phentermine	Normeperidine and PVP Metabolite 1

In all cases shown, either baseline chromatographic separation is achieved, or selective MRMs are employed and therefore these compounds will not interfere with one another. The UPLC separation was modified slightly when compared to Application Note [720006187](#) due to the less selective sample clean-up of this dilution method. A temporary hold was employed at the start of the gradient to allow for salts and other matrix components to be diverted to waste before analytes were eluted from the column.



*Figure 2. Chromatographic Separation of all Definitive Drug Compounds*

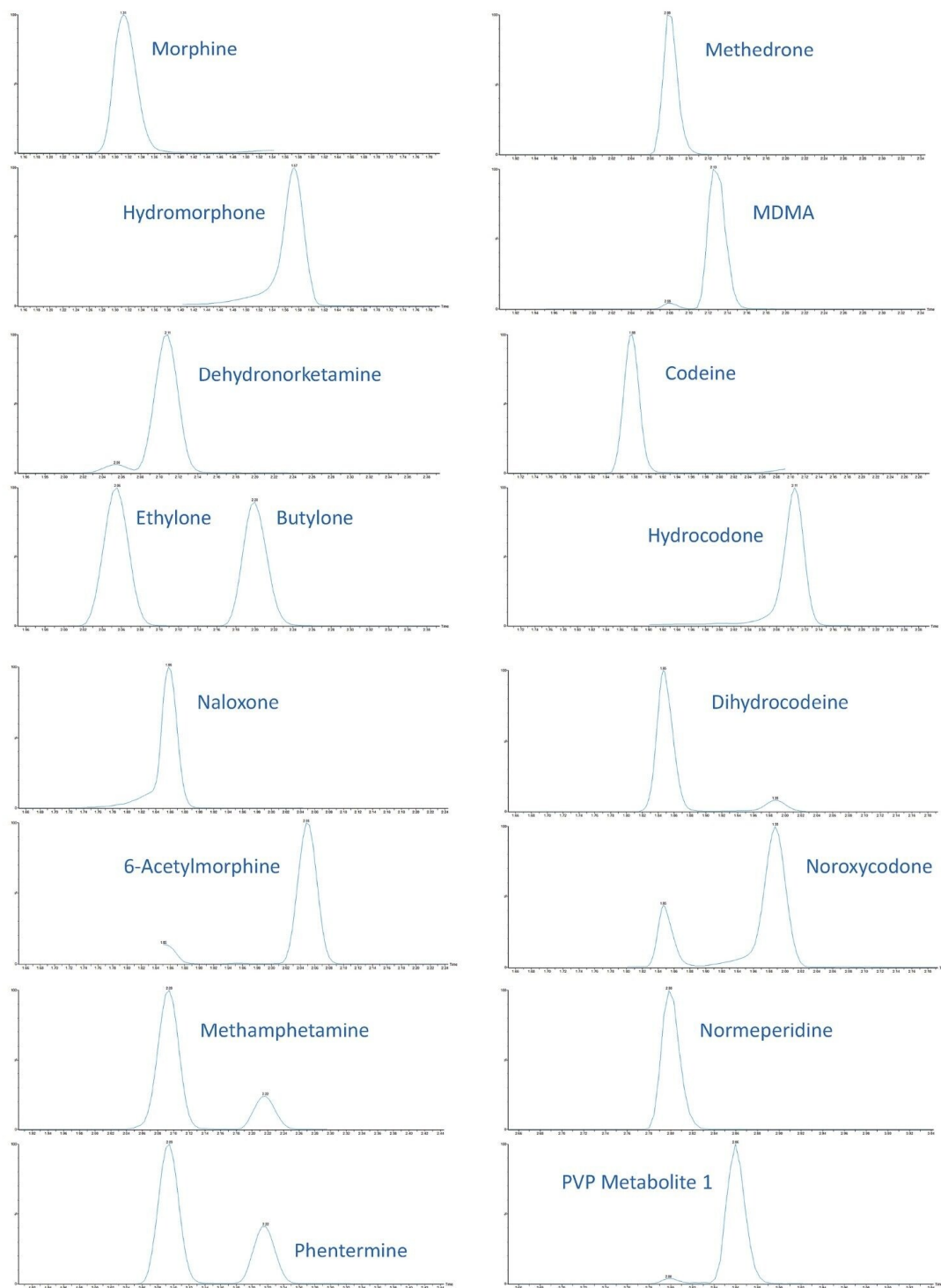


Figure 3. Selected Chromatograms of compounds with the potential to interfere with one another.

Seven-point calibration lines were extracted in duplicate and analyzed for each analysis run. Calibrators at the higher range were between 25–2,500 ng/mL, calibrators at the mid-range were between 10–1,000 ng/mL and calibrators at the lower range were between 2–200 ng/mL and are shown in more detail in Appendix 1. All calibration lines had a correlation coefficient ( $r^2$ ) of >0.99 and at least 75% of calibration points were within  $\pm 15\%$  of their nominal value ( $\pm 20\%$  at the calibrator 1 level) with the exception of N-desmethylzopiclone, norpropoxyphene, and zopiclone. Further investigation is needed for these compounds to identify whether solubility/stability improvements can be made when preparing the calibrators. N-desmethylzopiclone, norpropoxyphene, and zopiclone were only monitored qualitatively.

QC samples at the higher range were at concentrations of 25, 75, 187.5, and 1875 ng/mL, QC samples at the mid-range were at concentrations of 10, 30, 75, and 750 ng/mL and QC samples at the lower range were at concentrations of 2, 6, 15, and 150 ng/mL and are shown in more detail in Appendix 1. At least 66% of QC samples were within  $\pm 15\%$  of their nominal value ( $\pm 20\%$  at the QC1 level) for all runs.

## Precision Performance

Precision of the method was evaluated by extracting and analyzing five replicates of each QC sample (concentrations above) on each of five occasions (n=25 replicates). Figure 4a to 4d shows a summary of results obtained for each QC level.



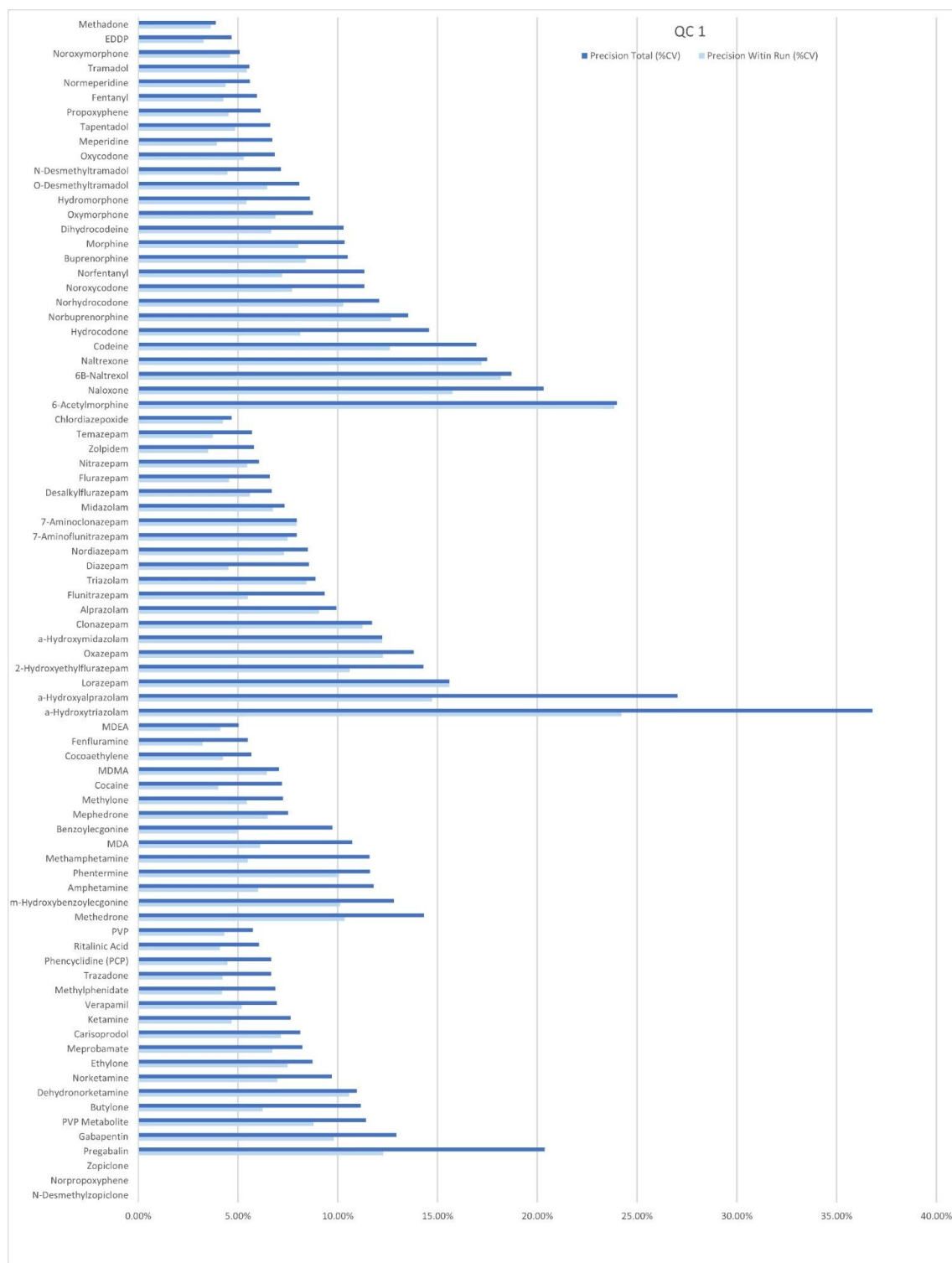


Figure 4a. Precision Performance summary of the method for all compounds of the QC 1 sample

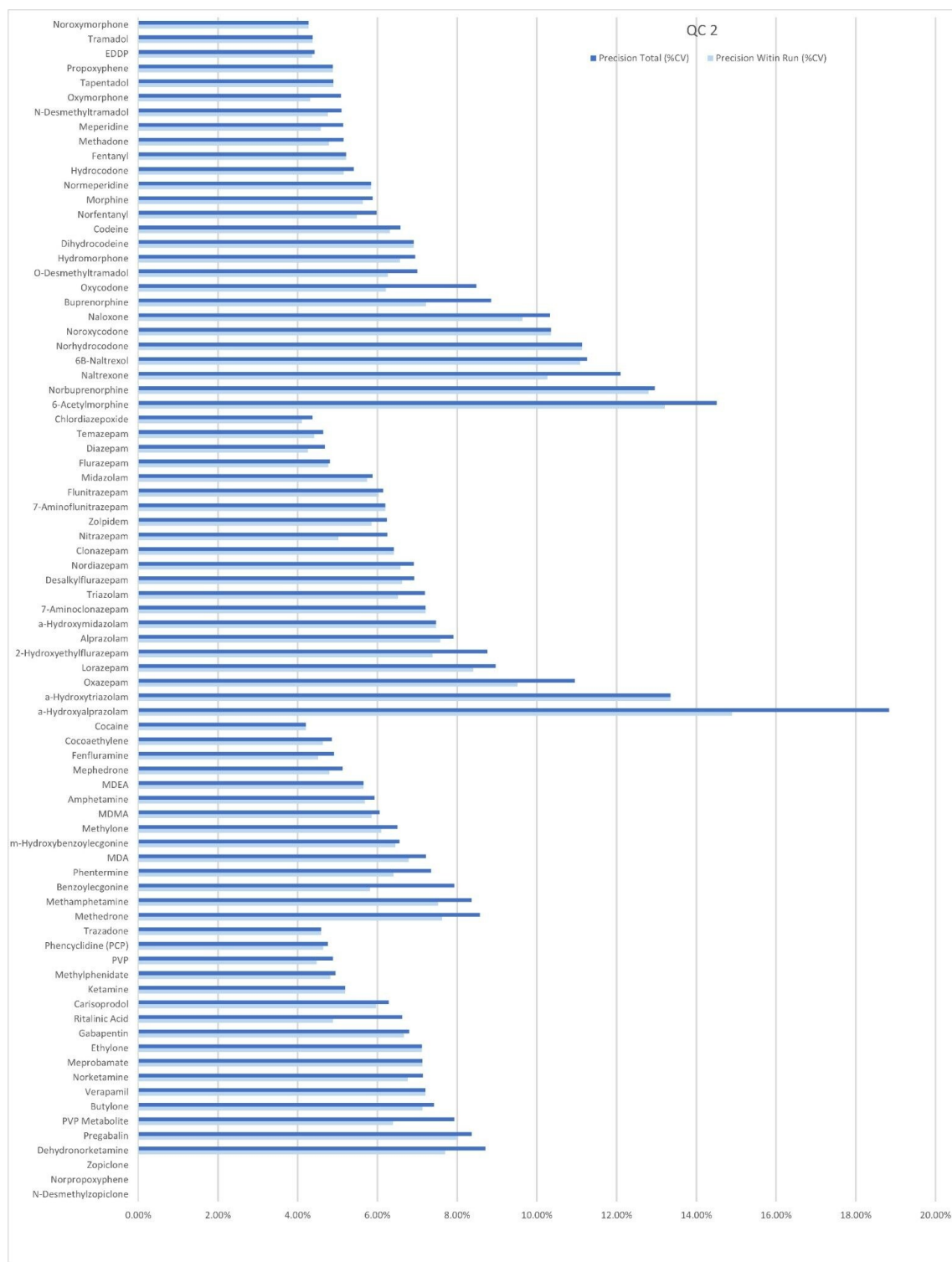


Figure 4b. Precision Performance summary of the method for all compounds of the QC 2 sample.

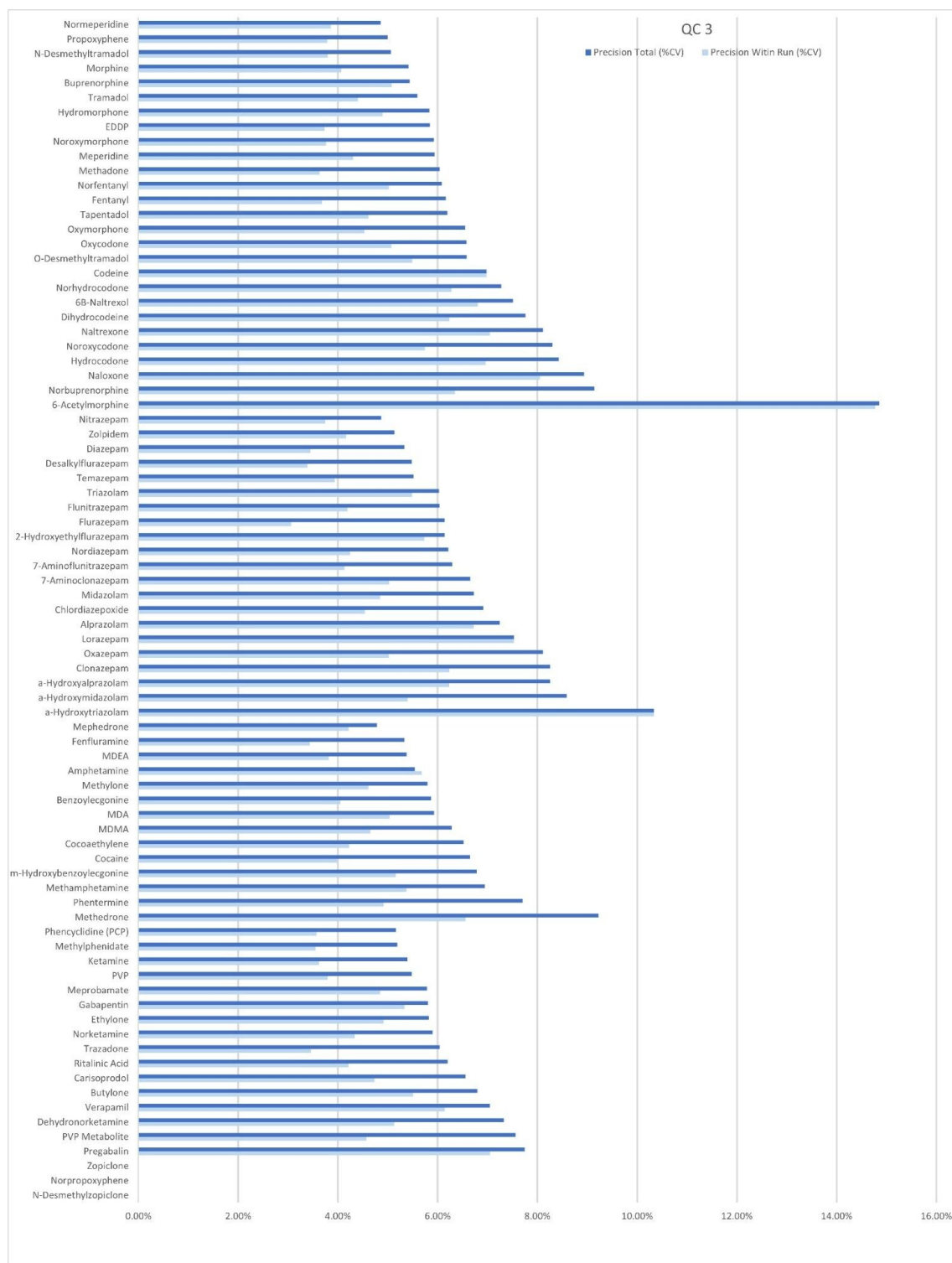


Figure 4c. Precision Performance summary of the method for all compounds of the QC 3 sample.

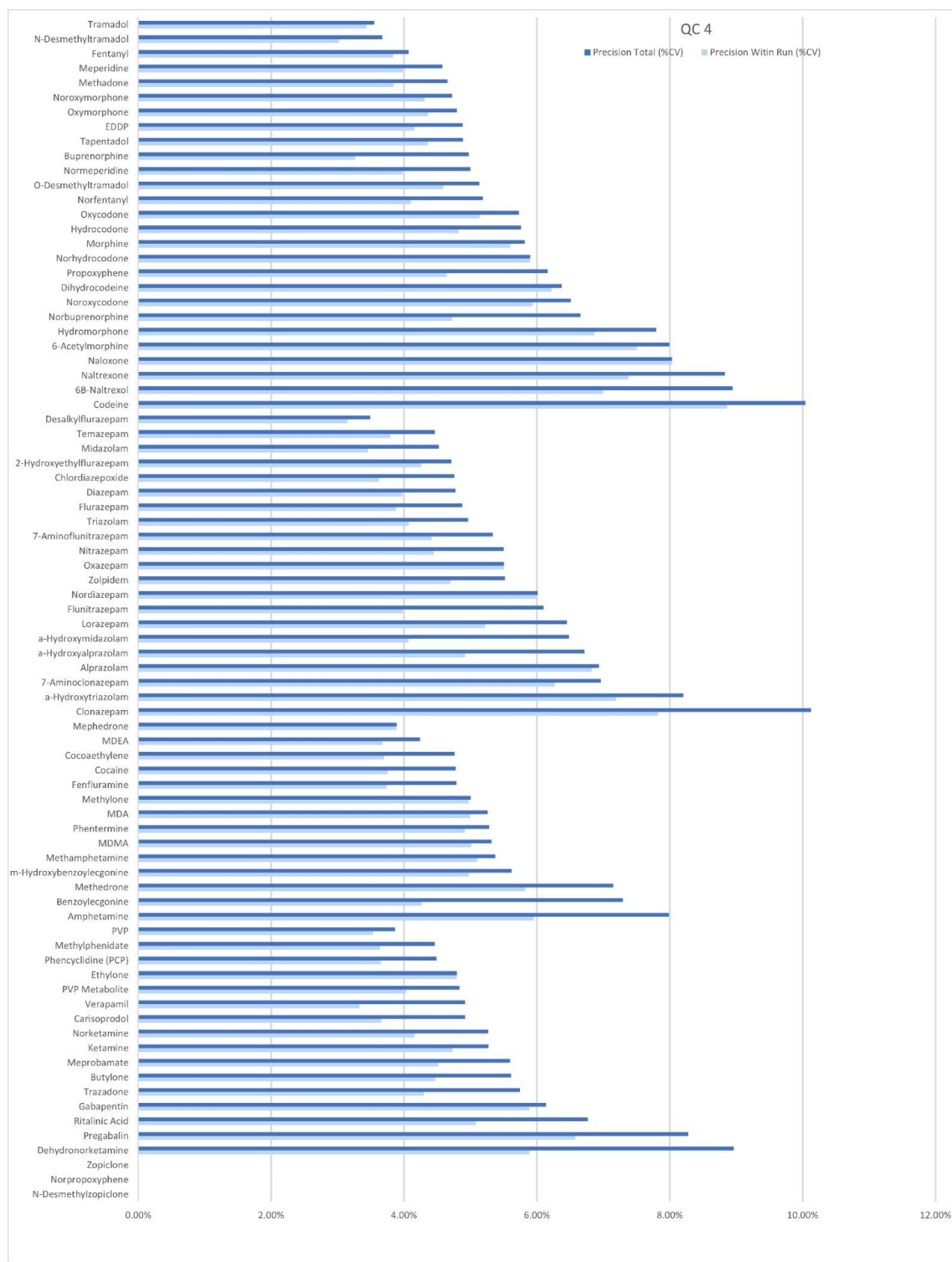


Figure 4d. Precision Performance summary of the method for all compounds of the QC 4 sample.

## Analytical Sensitivity

Ten replicates of low level samples at or below the calibrator 1 concentration were extracted and analyzed on each of four occasions and their %CVs calculated. An acceptance criteria of <20% CV was obtained for the majority of analytes. In the case of lorazepam, 6-acetylmorphine,  $\alpha$ -hydroxyalprazolam,  $\alpha$ -hydroxytriazolam, naloxone, naltrexone, and pregabalin where this acceptance criteria was not met, it is recommended to employ the sample extraction method described in Waters Application Note: [720006187](#), if lower concentrations are required.

## Carryover

No significant carryover was observed when comparing the mean of six blank matrix samples to those following samples at double the Calibrator 7 concentration for all analytes with the exception of 7-aminoclonazepam and 7-aminoflunitrazepam, which were both found to be at 38.8% of the calibrator 1 sample peak area. Further testing would be required to evaluate the concentration at which carryover becomes insignificant for these analytes.

## Matrix Factor

Matrix Factor was between 0.85 and 1.15 (Matrix effects within 15%) when comparing extracted urine samples from six different individuals to control samples in water and post spiked at low and high concentration samples for all analytes with the exception of phentermine and noroxymorphone. A summary of Matrix Factor data for all analytes can be seen in Appendix 2.

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## Conclusion

This application brief shows an overview for the UPLC-MS/MS analysis of pain management drugs and drugs of abuse for forensic toxicology. Sample preparation has been simplified to a dilute and shoot approach to allow for a fast extraction technique. Coupled with UPLC separation and detection on the Xevo TQ-S micro IVD, a method for the analysis of a large panel of pain management drugs and drugs of abuse has been shown. Results were shown to be precise, with consistent matrix effects and minimal carryover for the majority of analytes within this large panel. A more substantial sample extraction technique is recommended for certain compounds to improve analytical sensitivity such as that shown in Waters Application Note. [720006187](#).

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## References

1. Danaceau JP, Freeto S, Calton L. A Comprehensive Method for the Analysis of Pain Management Drugs and Drugs of Abuse Incorporating Simplified, Rapid Mixed-Mode SPE with UPLC-MS/MS for Forensic Toxicology. Waters Application Note [720006187](#). 2019 March.
2. Rosano TG, Rumberger JM, Wood M. Matrix Normalization Techniques for Definitive Urine Drug Testing. *Journal of Analytical Toxicology*, 2021;45:901–912.

## Appendix 1

Compound	MRM (m/z)	Cone (V)	Collision (kV)	Time window (min)	Conc. range (ng/mL)
Amphetamine	136.1>119.1 (91.1)	10	6 (14)	1.75–2.15	25–2500 (25, 75, 187.5, 1875)
[ <sup>2</sup> H <sub>11</sub> ]-Amphetamine	147.1>130.1 (97.1)	10	6 (14)		
MDA	180.2>163.1 (105.1)	10	6 (20)	1.85–2.25	25–2500 (25, 75, 187.5, 1875)
[ <sup>2</sup> H <sub>5</sub> ]-MDA	185.2>168.1 (110.1)	10	6 (20)		
MDEA	208.2>163.1 (105.1)	10	10 (24)	2.10–2.50	25–2500 (25, 75, 187.5, 1875)
[ <sup>2</sup> H <sub>5</sub> ]-MDEA	213.2>163.1 (105.1)	10	10 (24)		
MDMA	194.2>163.1 (105.1)	10	10 (22)	1.95–2.45	25–2500 (25, 75, 187.5, 1875)
[ <sup>2</sup> H <sub>5</sub> ]-MDMA	199.2>165.1 (105.1)	10	10 (22)		
Methamphetamine	150.1>91.1 (119.1)	10	6 (14)	1.90–2.30	25–2500 (25, 75, 187.5, 1875)
[ <sup>2</sup> H <sub>14</sub> ]-Methamphetamine	164.1>98.1 (130.1)	10	6 (14)		
Phentermine	150.1>133.1 (91.1)	10	8 (14)	2.05–2.45	25–2500 (25, 75, 187.5, 1875)
[ <sup>2</sup> H <sub>5</sub> ]-Phentermine	155.1>138.1 (96.1)	10	8 (14)		
Alprazolam	309.2>205.1 (281.1)	25	40 (24)	3.90–4.30	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>5</sub> ]-Alprazolam	314.2>210.1 (286.1)	25	40 (24)		
Clonazepam (uses [ <sup>2</sup> H <sub>5</sub> ]-Alprazolam as ISTD)	316.2>270.1 (241.1)	25	32 (22)	3.80–4.20	10–1000 (10, 30, 75, 750)
Diazepam	285.2>154.1 (193.1)	25	25 (28)	4.40–4.80	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>5</sub> ]-Diazepam	290.2>154.1 (198.1)	25	25 (28)		
Flunitrazepam	314.2>268.1 (239.1)	25	25 (28)	4.05–4.45	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>7</sub> ]-Flunitrazepam	321.2>275.1 (246.1)	25	25 (28)		
Lorazepam	323.2>277.1 (229.1)	25	16 (25)	3.85–4.25	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>4</sub> ]-Lorazepam	327.2>281.1 (233.1)	25	16 (25)		
Nitrazepam	282.2>236.1 (180.1)	25	20 (35)	3.65–4.05	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>5</sub> ]-Nitrazepam	287.2>241.1 (185.1)	25	20 (35)		
Oxazepam	289.2>243.1 (104.1)	25	18 (35)	3.80–4.20	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>5</sub> ]-Oxazepam	294.2>248.1 (109.1)	25	18 (35)		
Temazepam	301.2>255.1 (177.1)	25	18 (35)	4.15–4.55	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>5</sub> ]-Temazepam	306.2>260.1 (177.1)	25	18 (35)		
Benzoyllecgonine	290.2>168.1 (105.1)	35	12 (25)	2.20–2.60	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>6</sub> ]-Benzoyllecgonine	298.2>171.1 (110.1)	35	12 (25)		
Cocaethylene	318.2>196.1 (105.1)	25	16 (30)	2.85–3.25	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>6</sub> ]-Cocaethylene	326.2>204.1 (105.1)	25	16 (30)		
Cocaine	304.2>182.1 (82.1)	25	16 (26)	2.60–3.00	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>3</sub> ]-Cocaine	307.2>185.1 (85.1)	25	16 (26)		
6-Acetyl morphine	328.2>165.1 (211.1)	25	35 (22)	1.85–2.25	2–200 (2, 6, 15, 150)
[ <sup>2</sup> H <sub>6</sub> ]-6-Acetyl morphine	334.2>165.1 (211.1)	25	35 (22)		



Compound	MRM (m/z)	Cone (V)	Collision (kV)	Time window (min)	Conc. range (ng/mL)
7-Aminoclonazepam	286.2>121.1 (222.1)	25	26 (20)	2.20–2.60	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>6</sub> ]-7-Aminoclonazepam	290.2>121.1 (226.1)	25	26 (20)		
7-Aminoflunitrazepam	284.2>135.1 (227.1)	25	25 (20)	2.45–2.85	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>7</sub> ]-7-Aminoflunitrazepam	291.2>138.1 (230.1)	25	25 (20)		
Buprenorphine	468.4>55.1 (101.1)	25	50 (42)	3.25–3.65	2–200 (2, 6, 15, 150)
[ <sup>2</sup> H <sub>4</sub> ]-Buprenorphine	472.4>59.1 (101.1)	25	50 (42)		
Butylone	222.2>174.1 (146.1)	10	15 (22)	2.00–2.40	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>3</sub> ]-Butylone	225.2>177.1 (149.1)	10	15 (22)		
Carisoprodol	261.2>176.1 (158.1)	10	6 (8)	3.90–4.30	10–1000 (10, 30, 75, 750)
[ <sup>13</sup> C <sub>3</sub> ]-Carisoprodol	264.2>179.1 (161.1)	10	6 (8)		
Chlordiazepoxide	300.2>227.1 (283.1)	25	20 (10)	2.80–3.20	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>5</sub> ]-Chlordiazepoxide	305.2>232.1 (288.1)	25	20 (10)		
Codeine	300.2>215.1 (165.1)	25	22 (40)	1.70–2.10	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>6</sub> ]-Codeine	306.2>218.1 (165.1)	25	22 (40)		
Dehydronorketamine (Uses [ <sup>2</sup> H <sub>4</sub> ]-Norketamine for ISTD)	222.2>142.1 (177.1)	10	22 (15)	1.95–2.35	10–1000 (10, 30, 75, 750)
Desalkylflurazepam	289.2>140.1 (226.1)	40	26 (25)	4.05–4.45	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>4</sub> ]-Desalkylflurazepam	293.2>140.1 (230.1)	40	26 (25)		
N-Desmethyl tramadol	250.2>44.1 (232.1)	10	10 (6)	2.45–2.85	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>3</sub> ]-N-Desmethyl tramadol	253.2>47.1 (235.1)	10	10 (6)		
O-Desmethyl tramadol	250.2>58.1 (No Qual)	25	10	1.90–2.30	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>6</sub> ]-O-Desmethyl tramadol	256.2>64.1 (No Qual)	25	10		
N-Desmethyl zopiclone	375.2>245.1 (331.1)	10	15 (6)	2.30–2.70	Monitored only
[ <sup>2</sup> H <sub>4</sub> ]-N-Desmethyl zopiclone	379.2>245.1 (335.1)	10	15 (6)		
Dihydrocodeine	302.2>199.1 (128.1)	25	28 (60)	1.65–2.05	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>6</sub> ]-Dihydrocodeine	308.2>202.1 (128.1)	25	28 (60)		
EDDP	278.2>234.1 (249.1)	25	26 (20)	3.40–3.80	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>3</sub> ]-EDDP	281.2>234.1 (249.1)	25	26 (20)		
Ethylone	222.2>174.1 (146.1)	25	15 (25)	1.85–2.25	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>5</sub> ]-Ethylone	227.2>179.1 (151.1)	25	15 (25)		
Fenfluramine	232.2>159.1 (109.1)	25	18 (35)	2.90–3.30	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>5</sub> ]-Fenfluramine	237.2>159.1 (109.1)	25	18 (35)		
Fentanyl	337.2>188.1 (105.1)	25	20 (35)	3.10–3.50	2–200 (2, 6, 15, 150)
[ <sup>2</sup> H <sub>5</sub> ]-Fentanyl	342.2>188.1 (105.1)	25	20 (35)		
Flurazepam	388.2>315.1 (100.1)	25	18 (25)	3.20–3.60	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>4</sub> ]-Flurazepam	392.2>319.1 (100.1)	25	18 (25)		



Compound	MRM (m/z)	Cone (V)	Collision (kV)	Time window (min)	Conc. range (ng/mL)
Gabapentin	172.2>137.1 (95.1)	10	14 (20)	1.70–2.10	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>3</sub> ]-Gabapentin	182.2>147.1 (105.1)	10	14 (20)		
Hydrocodone	300.2>199.1 (171.1)	25	24 (34)	1.85–2.25	25–2500 (25, 75, 187.5, 1875)
[ <sup>2</sup> H <sub>6</sub> ]-Hydrocodone	306.2>202.1 (174.1)	25	24 (34)		
Hydromorphone	286.2>185.1 (157.1)	25	30 (40)	1.40–1.80	25–2500 (25, 75, 187.5, 1875)
[ <sup>2</sup> H <sub>6</sub> ]-Hydromorphone	292.2>185.1 (157.1)	25	30 (40)		
α-Hydroxyalprazolam	325.2>297.1 (205.1)	25	22 (45)	3.70–4.10	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>5</sub> ]-α-Hydroxyalprazolam	330.2>302.1 (210.1)	25	22 (45)		
2-Hydroxyethylflurazepam	333.2>109.1 (194.1)	25	25 (20)	3.90–4.30	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>4</sub> ]-2-Hydroxyethylflurazepam	337.2>113.1 (194.1)	25	25 (20)		
α-Hydroxymidazolam	342.2>168.1 (203.1)	25	25 (35)	3.10–3.50	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>4</sub> ]-α-Hydroxymidazolam	346.2>168.1 (203.1)	25	25 (35)		
α-Hydroxytriazolam	359.2>176.1 (141.1)	25	25 (35)	3.70–4.10	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>4</sub> ]-α-Hydroxytriazolam	363.2>176.1 (141.1)	25	25 (35)		
Ketamine	238.2>125.1 (179.1)	25	25 (15)	2.20–2.60	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>4</sub> ]-Ketamine	242.2>129.1 (183.1)	25	25 (15)		
Meperidine	248.2>174.1 (220.1)	25	15 (16)	2.65–3.05	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>4</sub> ]-Meperidine	252.2>178.1 (224.1)	25	15 (16)		
Mephedrone (4-MMC)	178.2>145.1 (91.1)	25	16 (30)	2.10–2.50	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>3</sub> ]-Mephedrone (4-MMC)	181.2>148.1 (91.1)	25	16 (30)		
Meprobamate	219.2>158.1 (97.1)	10	6 (12)	2.90–3.30	10–1000 (10, 30, 75, 750)
[ <sup>13</sup> C <sub>3</sub> ]-Meprobamate	222.2>161.1 (100.1)	10	6 (12)		
Methadone	310.2>265.1 (105.1)	25	10 (25)	3.70–4.10	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>9</sub> ]-Methadone	319.2>268.1 (105.1)	25	10 (25)		
Methedrone (Uses [ <sup>2</sup> H <sub>3</sub> ]-Mephedrone for ISTD)	194.1>161.1 (146.1)	10	18 (25)	1.90–2.30	10–1000 (10, 30, 75, 750)
Methylone	208.2>160.1 (132.1)	10	15 (25)	1.70–2.10	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>3</sub> ]-Methylone	211.2>163.1 (135.1)	10	15 (25)		
Methylphenidate	234.2>84.1 (91.1)	25	15 (40)	2.45–2.85	25–2500 (25, 75, 187.5, 1875)
[ <sup>2</sup> H <sub>9</sub> ]-Methylphenidate	243.2>93.1 (91.1)	25	15 (40)		
m-Hydroxybenzoyllecgonine	306.2>168.1 (121.1)	25	15 (25)	2.00–2.40	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>3</sub> ]-m-Hydroxybenzoyllecgonine	309.2>171.1 (121.1)	25	15 (25)		
Midazolam	326.2>291.1 (223.1)	25	24 (35)	3.10–3.50	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>4</sub> ]-Midazolam	330.2>295.1 (227.1)	25	24 (35)		
Morphine	286.2>201.1 (165.1)	25	22 (35)	1.15–1.55	25–2500 (25, 75, 187.5, 1875)
[ <sup>2</sup> H <sub>6</sub> ]-Morphine	292.2>201.1 (165.1)	25	22 (35)		

Compound	MRM (m/z)	Cone (V)	Collision (kV)	Time window (min)	Conc. range (ng/mL)
Naloxone	328.2>253.1 (212.1)	25	22 (35)	1.65–2.05	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>5</sub> ]-Naloxone	333.2>258.1 (212.1)	25	22 (35)		
6β-Naltrexol	344.2>308.1 (254.1)	10	25 (30)	1.85–2.25	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>3</sub> ]-6β-Naltrexol	347.2>311.1 (254.1)	10	25 (30)		
Naltrexone	342.2>270.1 (324.1)	25	22 (16)	1.85–2.25	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>3</sub> ]-Naltrexone	345.2>270.1 (327.1)	25	22 (16)		
Norbuprenorphine	414.3>101.1 (83.1)	25	35 (45)	2.75–3.15	2–200 (2, 6, 15, 150)
[ <sup>2</sup> H <sub>3</sub> ]-Norbuprenorphine	417.3>101.1 (83.1)	25	35 (45)		
Nordiazepam	271.2>140.1 (165.1)	60	25 (25)	3.85–4.25	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>5</sub> ]-Nordiazepam	276.2>140.1 (165.1)	60	25 (25)		
Norfentanyl	233.2>84.1 (177.1)	25	14 (10)	2.20–2.60	2–200 (2, 6, 15, 150)
[ <sup>2</sup> H <sub>5</sub> ]-Norfentanyl	238.2>84.1 (182.1)	25	14 (10)		
Norhydrocodone	286.2>199.1 (171.1)	25	24 (34)	1.85–2.25	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>3</sub> ]-Norhydrocodone	289.2>202.1 (174.1)	25	24 (34)		
Norketamine	224.2>125.1 (179.1)	10	20 (14)	2.15–2.55	2–200 (2, 6, 15, 150)
[ <sup>2</sup> H <sub>4</sub> ]-Norketamine	228.2>129.1 (183.1)	10	20 (14)		
Normeperidine	234.2>160.1 (131.1)	25	12 (20)	2.65–3.05	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>4</sub> ]-Normeperidine	238.2>164.1 (135.1)	25	12 (20)		
Noroxycodone	302.2>187.1 (227.1)	25	20 (25)	1.80–2.20	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>3</sub> ]-Noroxycodone	305.2>190.1 (230.1)	25	20 (25)		
Noroxymorphone (Uses [ <sup>2</sup> H <sub>3</sub> ]-Oxymorphone for ISTD)	288.2>213.1 (173.1)	10	25 (20)	1.20–1.60	10–1000 (10, 30, 75, 750)
Norpropoxyphene	326.2>252.1 (118.1)	10	5 (5)	3.55–3.95	Monitored only
[ <sup>2</sup> H <sub>5</sub> ]-Norpropoxyphene	331.2>257.1 (118.1)	10	5 (5)		
Oxycodone	316.2>298.1 (241.1)	25	15 (25)	1.85–2.25	25–2500 (25, 75, 187.5, 1875)
[ <sup>2</sup> H <sub>3</sub> ]-Oxycodone	319.2>301.1 (244.1)	25	15 (25)		
Oxymorphone	302.2>227.1 (242.1)	25	25 (22)	1.30–1.70	25–2500 (25, 75, 187.5, 1875)
[ <sup>2</sup> H <sub>3</sub> ]-Oxymorphone	305.2>230.1 (245.1)	25	25 (22)		
Phencyclidine (PCP)	244.2>86.1 (159.1)	10	10 (12)	2.95–3.35	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>5</sub> ]-Phencyclidine (PCP)	249.2>86.1 (164.1)	10	10 (12)		
Pregabalin	160.2>125.1 (107.1)	10	10 (14)	1.70–2.10	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>6</sub> ]-Pregabalin	166.2>131.1 (112.1)	10	10 (14)		
Propoxyphene	340.2>266.1 (143.1)	10	20 (6)	3.60–4.00	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>5</sub> ]-Propoxyphene	345.2>271.1 (147.1)	10	20 (6)		
Ritalinic acid	220.2>84.1 (56.1)	25	15 (38)	2.15–2.55	25–2500 (25, 75, 187.5, 1875)
[ <sup>2</sup> H <sub>10</sub> ]-Ritalinic acid	230.2>93.1 (61.1)	25	15 (38)		

Compound	MRM (m/z)	Cone (V)	Collision (kV)	Time window (min)	Conc. range (ng/mL)
Tapentadol	222.2>107.1 (121.1)	25	15 (20)	2.50–2.90	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>3</sub> ]-Tapentadol	225.2>107.1 (121.1)	25	15 (20)		
Tramadol	264.2>58.1 (No Qual)	10	15	2.40–2.80	10–1000 (10, 30, 75, 750)
[ <sup>13</sup> C <sub>1</sub> <sup>2</sup> H <sub>3</sub> ]-Tramadol	268.2>58.1 (No Qual)	10	15		
Trazodone	372.2>148.1 (176.1)	50	28 (16)	2.85–3.25	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>6</sub> ]-Trazodone	378.2>150.1 (182.1)	50	28 (16)		
Triazolam	343.2>308.1 (239.1)	25	24 (40)	4.00–4.40	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>4</sub> ]-Triazolam	347.2>312.1 (243.1)	25	24 (40)		
Verapamil	455.3>165.1 (303.1)	25	25 (22)	3.60–4.00	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>6</sub> ]-Verapamil	461.3>165.1 (309.1)	25	25 (22)		
Zolpidem	308.2>235.1 (92.1)	25	30 (50)	2.65–3.05	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>6</sub> ]-Zolpidem	314.2>235.1 (92.1)	25	30 (50)		
Zopiclone	389.2>245.1 (112.1)	10	14 (55)	2.30–2.70	Monitored only
[ <sup>2</sup> H <sub>4</sub> ]-Zopiclone	393.2>245.1 (112.1)	10	14 (55)		
α-Pyrrolidinovalerophenone (PVP)	232.2>91.1 (126.1)	25	20 (22)	2.50–2.90	10–1000 (10, 30, 75, 750)
[ <sup>2</sup> H <sub>4</sub> ]-α-Pyrrolidinovalerophenone (PVP)	240.2>91.1 (134.1)	25	20 (22)		
α-Pyrrolidinovalerophenone (PVP) Metabolite 1 (Uses [ <sup>2</sup> H <sub>4</sub> ]-PVP for ISTD)	234.2>173.1 (117.1)	25	22 (25)	2.65–3.05	10–1000 (10, 30, 75, 750)

## Appendix 2

Compound	QC1 (%CV)		QC2 (%CV)		QC3 (%CV)		QC4 (%CV)		Matrix factor (range)
	Within-run	Total	Within-run	Total	Within-run	Total	Within-run	Total	
Amphetamine	6.01	11.80	5.68	5.93	5.68	5.54	5.95	7.99	0.92 (0.83–0.99)
MDA	6.11	10.73	6.78	7.22	5.04	5.93	4.99	5.26	0.96 (0.90–1.00)
MDEA	4.12	5.04	5.65	5.65	3.81	5.38	3.68	4.24	0.96 (0.92–1.00)
MDMA	6.44	7.05	5.86	6.06	4.65	6.28	5.01	5.32	0.94 (0.87–1.02)
Methamphetamine	5.49	11.59	7.52	8.36	5.38	6.95	5.10	5.37	0.97 (0.93–1.02)
Phentermine	10.05	11.62	6.41	7.34	4.91	7.71	4.91	5.28	1.23 (1.08–1.44)
Alprazolam	9.05	9.92	7.58	7.91	6.72	7.24	6.82	6.94	0.91 (0.78–0.99)
Clonazepam	11.22	11.72	6.41	6.41	6.23	8.26	7.83	10.13	0.94 (0.84–1.06)
Diazepam	4.53	8.55	4.25	4.68	3.45	5.33	3.98	4.77	0.96 (0.91–0.99)
Flunitrazepam	5.49	9.33	6.03	6.14	4.19	6.03	3.98	6.10	0.94 (0.87–1.00)
Lorazepam	15.59	15.59	8.40	8.97	7.53	7.53	5.22	6.45	1.03 (0.90–1.20)
Nitrazepam	5.46	6.05	5.02	6.24	3.75	4.87	4.45	5.50	0.96 (0.84–1.01)
Oxazepam	12.26	13.80	9.51	10.95	5.02	8.11	5.50	5.50	0.97 (0.91–1.05)
Temazepam	3.74	5.70	4.42	4.64	3.93	5.52	3.79	4.46	0.98 (0.93–1.01)
Benzoylcegonine	4.98	9.72	5.81	7.93	4.04	5.87	4.27	7.30	0.99 (0.89–1.39)
Cocaoethylene	4.25	5.66	4.63	4.85	4.22	6.52	3.70	4.76	0.95 (0.76–1.01)
Cocaine	4.00	7.20	4.21	4.21	3.98	6.65	3.75	4.78	1.00 (0.96–1.03)
6-Acetylmorphine	23.86	24.00	13.21	14.51	14.78	14.85	7.50	8.00	1.05 (0.92–1.22)
7-Aminoclonazepam	7.94	7.94	7.20	7.20	5.02	6.65	6.27	6.96	0.85 (0.70–0.96)
7-Aminoflunitrazepam	7.48	7.94	6.20	6.20	4.13	6.29	4.41	5.34	1.04 (0.80–1.44)
Buprenorphine	8.39	10.50	7.22	8.85	5.08	5.44	3.27	4.98	0.96 (0.93–1.00)
Butylone	6.23	11.16	7.13	7.42	5.51	6.80	4.47	5.61	0.97 (0.89–1.04)
Carisoprodol	7.14	8.12	5.96	6.28	4.73	6.56	3.66	4.92	0.96 (0.93–0.99)
Chlordiazepoxide	4.23	4.68	4.10	4.37	4.54	6.92	3.62	4.76	0.94 (0.86–0.99)
Codeine	12.62	16.96	6.32	6.58	6.98	6.98	8.86	10.04	0.98 (0.84–1.09)
Dehydronorketamine	10.57	10.96	7.70	8.71	5.13	7.33	5.89	8.96	1.00 (0.87–1.18)
Desalkylflurazepam	5.58	6.70	6.62	6.92	3.39	5.48	3.15	3.49	0.96 (0.83–1.02)
N-Desmethyltramadol	4.47	7.16	4.76	5.09	3.80	5.06	3.02	3.67	0.98 (0.92–1.01)
O-Desmethyltramadol	6.45	8.06	6.26	7.00	5.49	6.58	4.59	5.13	0.96 (0.94–0.99)
Dihydrocodeine	6.67	10.30	6.91	6.91	6.24	7.76	6.22	6.37	0.98 (0.91–1.03)
EDDP	3.28	4.69	4.36	4.42	3.73	5.84	4.15	4.88	0.96 (0.92–1.00)
Ethylone	7.49	8.74	7.12	7.12	4.91	5.82	4.80	4.80	1.00 (0.95–1.05)
Fenfluramine	3.23	5.50	4.51	4.91	3.44	5.33	3.74	4.79	0.97 (0.93–0.99)
Fentanyl	4.26	5.94	5.21	5.21	3.68	6.16	3.84	4.07	0.98 (0.94–1.02)
Flurazepam	4.55	6.58	4.77	4.81	3.06	6.14	3.88	4.88	0.98 (0.95–1.05)
Gabapentin	9.80	12.93	6.66	6.80	5.33	5.81	5.89	6.14	0.94 (0.83–1.19)



Compound	QC1 (%CV)		QC2 (%CV)		QC3 (%CV)		QC4 (%CV)		Matrix factor (range)
	Within-run	Total	Within-run	Total	Within-run	Total	Within-run	Total	
Hydrocodone	8.12	14.58	5.15	5.41	6.96	8.43	4.82	5.76	0.97 (0.88–1.01)
Hydromorphone	5.40	8.61	6.57	6.94	4.90	5.83	6.86	7.79	0.97 (0.84–1.12)
a-Hydroxyalprazolam	14.72	27.01	14.89	18.84	6.23	8.26	4.92	6.72	0.98 (0.85–1.14)
2-Hydroxyethylflurazepam	10.58	14.30	7.38	8.76	5.73	6.14	4.26	4.71	0.99 (0.92–1.08)
a-Hydroxymidazolam	12.22	12.23	7.47	7.47	5.40	8.59	4.07	6.48	0.95 (0.86–1.05)
a-Hydroxytriazolam	24.22	36.80	13.36	13.36	10.34	10.34	7.19	8.21	1.06 (0.89–1.29)
Ketamine	4.69	7.63	5.19	5.19	3.62	5.39	4.73	5.27	0.96 (0.91–0.99)
Meperidine	3.94	6.72	4.58	5.14	4.30	5.94	3.98	4.58	0.98 (0.94–1.04)
Mephedrone	6.50	7.51	4.80	5.12	4.22	4.78	3.88	3.89	0.98 (0.92–1.00)
Meprobamate	6.73	8.22	7.13	7.13	4.85	5.79	4.52	5.60	0.97 (0.91–1.01)
Methadone	3.63	3.88	4.78	5.15	3.63	6.04	3.84	4.65	0.98 (0.93–1.02)
Methedrone	10.34	14.32	7.62	8.57	6.56	9.22	5.83	7.15	1.05 (0.98–1.24)
Methylone	5.45	7.26	6.09	6.50	4.61	5.80	4.97	5.00	0.96 (0.91–1.03)
Methylphenidate	4.20	6.87	4.82	4.95	3.55	5.19	3.64	4.47	1.01 (0.95–1.06)
m-Hydroxybenzoylecgonine	10.12	12.82	6.45	6.55	5.16	6.78	4.98	5.62	0.98 (0.90–1.03)
Midazolam	6.74	7.32	5.74	5.88	4.85	6.72	3.46	4.52	0.95 (0.83–1.00)
Morphine	8.02	10.33	5.63	5.88	4.07	5.42	5.60	5.82	0.97 (0.90–1.01)
Naloxone	15.75	20.31	9.64	10.32	8.05	8.93	8.03	8.03	1.04 (0.91–1.24)
6B-Naltrexol	18.16	18.70	11.08	11.25	6.81	7.51	7.00	8.95	0.94 (0.83–1.07)
Naltrexone	17.21	17.47	10.26	12.10	7.05	8.11	7.38	8.83	1.01 (0.82–1.18)
Norbuprenorphine	12.67	13.54	12.80	12.96	6.35	9.14	4.72	6.66	1.03 (0.92–1.26)
Nordiazepam	7.30	8.50	6.57	6.91	4.24	6.21	6.00	6.01	0.92 (0.84–0.97)
Norfentanyl	7.19	11.32	5.49	5.97	5.02	6.08	4.10	5.19	0.96 (0.88–1.01)
Norhydrocodone	10.27	12.08	11.14	11.14	6.28	7.27	5.90	5.90	1.01 (0.93–1.13)
Norketamine	6.97	9.70	6.76	7.14	4.34	5.90	4.16	5.27	0.98 (0.93–1.07)
Normeperidine	4.38	5.59	5.84	5.84	3.86	4.86	3.97	5.00	0.97 (0.93–1.03)
Noroxycodone	7.71	11.33	10.35	10.35	5.75	8.30	5.93	6.51	1.00 (0.80–1.12)
Noroxymorphone	4.59	5.08	4.27	4.27	3.76	5.92	4.31	4.72	0.72 (0.54–0.89)
Oxycodone	5.29	6.83	6.21	8.49	5.07	6.58	5.14	5.73	0.97 (0.91–1.05)
Oxymorphone	6.87	8.75	4.31	5.08	4.53	6.55	4.36	4.80	1.02 (0.99–1.10)
Phencyclidine (PCP)	4.47	6.66	4.64	4.75	3.57	5.16	3.65	4.49	0.98 (0.92–1.01)
Pregabalin	12.28	20.37	8.02	8.37	7.05	7.74	6.58	8.28	0.98 (0.90–1.08)
Propoxyphene	4.52	6.13	4.88	4.88	3.79	5.00	4.64	6.16	0.98 (0.93–1.01)
Ritalinic Acid	4.08	6.04	4.89	6.62	4.21	6.20	5.08	6.77	0.98 (0.93–1.02)
Tapentadol	4.86	6.61	4.90	4.90	4.61	6.19	4.36	4.89	0.97 (0.93–1.00)
Tramadol	5.43	5.56	4.38	4.38	4.40	5.59	3.44	3.55	0.93 (0.87–1.02)
Trazadone	4.21	6.66	4.59	4.59	3.46	6.04	4.30	5.74	0.94 (0.88–1.01)

Compound	QC1 (%CV)		QC2 (%CV)		QC3 (%CV)		QC4 (%CV)		Matrix factor (range)
	Within-run	Total	Within-run	Total	Within-run	Total	Within-run	Total	
Triazolam	8.43	8.88	6.52	7.20	5.49	6.02	4.07	4.96	0.96 (0.87–1.04)
Verapamil	5.19	6.94	7.20	7.20	6.14	7.05	3.33	4.92	0.96 (0.89–1.00)
Zolpidem	3.50	5.80	5.86	6.24	4.16	5.14	4.70	5.52	0.98 (0.94–1.02)
PVP	4.32	5.74	4.48	4.88	3.79	5.48	3.53	3.87	0.96 (0.93–1.00)
PVP Metabolite 1	8.77	11.42	6.39	7.93	4.57	7.56	4.03	4.83	0.98 (0.92–1.12)

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