Application Note

Forensics

Agilent Ultivo ESI for High-Throughput Detection of Drugs in Urine and Serum

Authors

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Abstract

This application note describes a seven-minute method for the detection of 68 drugs of abuse in urine and serum using the Agilent Ultivo triple quadrupole LC/MS (LC/TQ) coupled to an Agilent 1290 Infinity II LC. The Ultivo triple quadrupole LC/MS provides exceptional sensitivity with minimal sample preparation for all 68 drugs studied in each matrix, with quantitation limits of 10 ng/mL or lower for all analytes. Outstanding precision was observed with %RSD less than 20% for 10 replicate injections at the quantitation limit for each analyte. All analytes showed linear calibration curves, with R^2 >0.98, and analytes that had limits of quantitation (LOQs) of 0.1 ng/mL demonstrated linearity for four orders of magnitude (0.1 to 1,000 ppb).

Introduction

Forensic toxicology laboratories require sensitive, robust, and reliable methods for the routine testing of drugs in human specimens. This Application Note presents a sensitive and precise method for analyzing 68 drugs in human serum and urine in a quick seven-minute method using the Ultivo triple quadrupole LC/MS with electrospray ionization (ESI). The analytes in this method include multiple drug classes such as opiates/opioids, benzodiazepines, stimulants, and others. This fast method was enabled using dynamic multiple reaction monitoring (dMRM), which allows the user to collect sufficient datapoints for specific MRM transitions during a set time window based on an analyte's retention time.

The Ultivo LC/TQ's early maintenance feedback (EMF) software features, VacShield technology enabling the quick change of an ion injector, and Ultivo's small, stackable design make it an ideal platform for the high-throughput laboratory. The exceptional robustness of Ultivo in the forensic toxicology space has been highlighted previously.[1] However, in this method, we highlight Ultivo's ability to detect drugs in human serum and urine at exceedingly low levels.

Experimental

Reagents and chemicals

All reagents were HPLC or LC/MS grade. Acetonitrile and methanol were purchased from Honeywell (Morristown, NJ, USA), and ultrapure water was sourced from a Milli-Q Integral System with an LC-Pak Polisher and a 0.22 µm point-of-use membrane filter cartridge (EMD Millipore, Billerica, MA, USA). Analytes standards were purchased from Cerilliant. Human serum and MS Gold Urine were obtained from Golden West Biologicals (Temecula, CA, USA).

Sample preparation

Human serum and urine were spiked with analytes and internal standards prior to sample preparation. Serum was prepared by taking 250 µL of serum and crashing it with 500 µL of cold acetonitrile then vortexing for one minute.[1] The serum was then centrifuged for four minutes at 10,000 rpm, then 500 µL of serum supernatant was diluted with 500 µL of water.[1] Urine samples were diluted 10x with ultrapure water before analysis. Calibration standards from 0.1 to 1,000 ng/mL were prepared in series with prepared serum.

Table 1. Agilent 1290 Infinity II LC parameters.

Parameter	Value
Column	Agilent Poroshell 120 EC-C18, 2.1 × 100 mm, 2.7 µm
Column Temperature	55 °C
Injection Volume	5 µL
Mobile Phase	A) 0.2% formic acid in water B) 0.5 mM ammonium fluoride in methanol
Flow Rate	0.5 mL/min
Gradient	Time (min) %B 0 10 0.5 15 3.0 50 4.0 95 6.0 95
Total Run Time	7.0 minutes

Instrumentation

Agilent 1290 Infinity II LC

- Agilent 1290 Infinity II high-speed pump (G7120A)
- Agilent 1290 Infinity II multisampler with cooler (G7167B)
- Agilent 1290 Infinity II multicolumn thermostat (G7116B)

Agilent Ultivo triple quadrupole LC/MS system

- Electrospray ion source (G1948B)

Method

Table 1 summarizes the 1290 Infinity II LC conditions, and Table 2 summarizes the Ultivo ion source and instrument parameters. Optimized MS parameters for the compounds of interest and internal standards (ISs) are listed in the Appendix. dMRM was used for data collection. Agilent MassHunter Quantitative Analysis software 10.1 with the Quant-My-Way feature was used to accelerate and streamline the data analysis and review process.

Table 2. Agilent Ultivo ion source and mass analyzer parameters.

Parameter	Value
Gas Temperature	350 °C
Gas Flow	12 L/min
Nebulizer Pressure	50 psi
Capillary Voltage	2,000 V(+)
Cycle Time	500 ms

Results and discussion

Method sensitivity and precision

Good chromatographic separation and peak shape was observed for all analytes studied, owing to the reliable performance of the 1290 Infinity II LC system and the Poroshell 120 EC-C18 column used in this method (Figure 1).

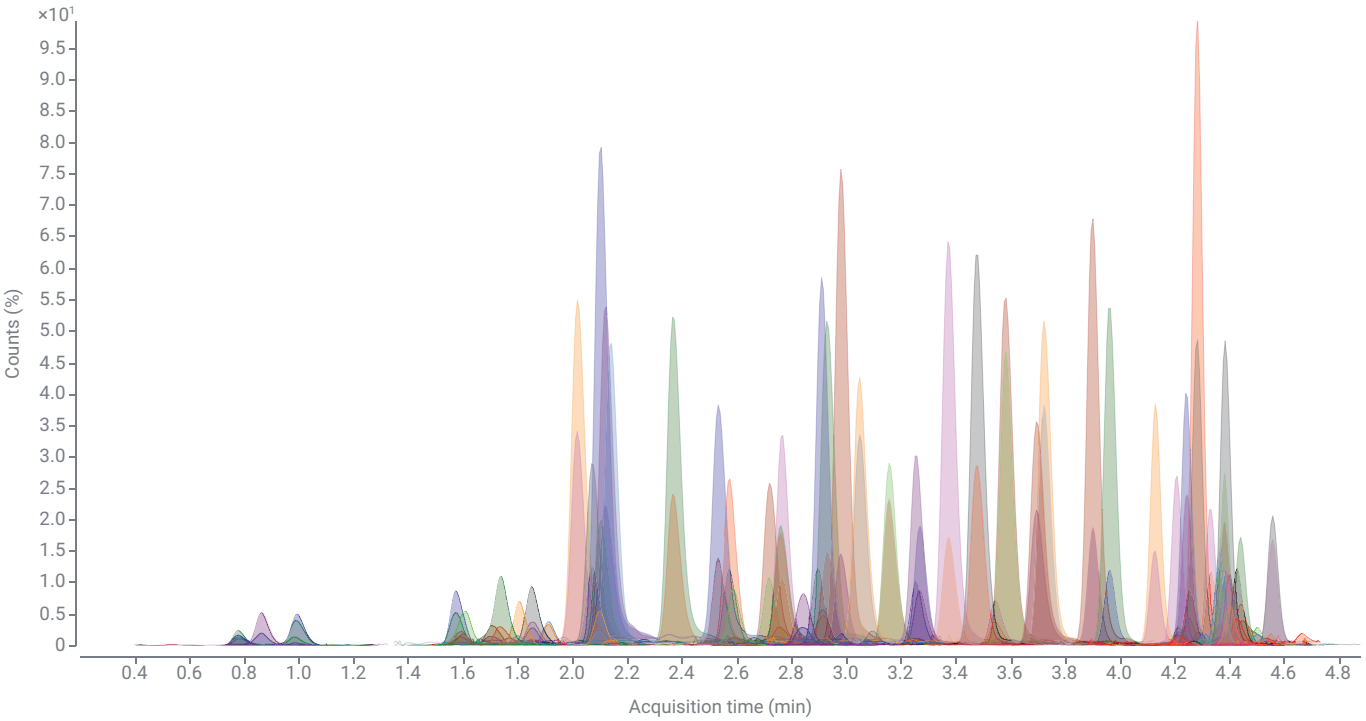

Figure 1. Chromatogram of analytes at 10 ppb in urine.

Excellent sensitivity was observed with all compounds having an LOQ of 10 ng/mL or lower. The LOQ for each compound was defined as having seven out of 10 replicate injections with accuracy of 80 to 120%, and a signal-to-noise ratio (S/N) greater than 10. Table 3 shows the quantitation limits for individual compounds.

Table 3. Quantitation limits for each analyte in urine and serum.

Analyte	LOQ (ng/mL)	
	Urine	Serum
Benzodiazepines		
2-Hydroxyethylflurazepam	2	2
7-Aminoclonazepam	2	1
7-Aminoflunitrazepam	0.5	0.5
Alprazolam	0.2	0.5
Chlordiazepoxide	0.5	0.2
Clonazepam	5	2
Desalkylflurazepam	5	5
Diazepam	1	0.5
Flunitrazepam	1	1
Flurazepam	0.2	0.2
Lorazepam	5	5
Midazolam	0.5	1
Nitrazepam	2	1
Nordiazepam	5	1
Oxazepam	5	5
Temazepam	1	0.5
Triazolam	1	0.5
α-Hydroxyalprazolam	10	5
α-Hydroxymidazolam	1	2
α-Hydroxytriazolam	10	5
Stimulants		
Amphetamine	0.5	0.2
Benzoylecgonine	0.2	0.5
Cocaethylene	0.5	0.1
Cocaine	0.5	0.2
MDA	1	0.5
MDEA	0.5	0.2
MDMA	1	0.2
Meprobamate	1	0.5
Methamphetamine	1	0.2
Methylphenidate	0.5	0.5
m-Hydroxybenzoylecgonine	2	0.5
Phentermine	0.5	1
Ritalinic acid	0.5	0.5
Zopiclone	1	0.2

Analyte	LOQ (ng/mL)	
	Urine	Serum
Opiates/Opioids		
6-Acetyl Morphine	1	1
Buprenorphine	5	1
Codeine	2	2
Dihydrocodeine	1	0.5
EDDP	0.5	0.2
Fentanyl	0.2	0.1
Heroin	5	2
Hydrocodone	1	0.5
Hydromorphone	0.5	1
Meperidine	0.5	0.2
Methadone	0.5	0.5
Morphine	1	2
Naloxone	1	0.2
Naltrexone	2	0.5
N-desmethyltramadol	5	5
Norbuprenorphine	5	5
Norfentanyl	0.5	0.2
Normeperidine	1	0.2
Norpropoxyphene	5	2
o-Desmethyltramadol	0.2	0.2
Oxycodone	0.5	0.5
Oxymorphone	1	1
Propoxyphene	1	0.5
Tapentadol	0.5	0.5
Tramadol	0.5	0.5
Others		
Carisoprodol	0.5	0.5
Gabapentin	2	10
Ketamine	0.5	0.2
Norketamine	1	1
PCP	0.2	0.1
Pregabalin	5	10
Trazodone	0.5	0.1
Verapamil	1	0.1
Zolpidem	0.2	0.1

Figure 2 shows the distribution of LOQs for serum and urine. More compounds were detected below 500 ppt in serum than in urine, which is likely due to the reduced dilution factor in the serum sample preparation compared to the sample preparation for urine.

Figure 3 shows the distribution of precision for 10 replicate injections for each analyte at its LOQ. Most of the compound RSDs for serum and urine fall within 5 to 15% at the LOQ.

Many of the compounds in this method had been analyzed on an earlier Agilent LC/TQ platform. From the 64 compounds analyzed in urine that overlap with compounds analyzed on an Agilent 6420 triple quadrupole LC/MS in a 2013 Application Note,[2] 97% of the compounds were at least 2x more sensitive on the Ultivo ESI, with 50% at least 10x more sensitive, and 20% at least 20x more sensitive.

Method linearity

Exceptional linearity was observed with R^2 values greater than 0.98 for all compounds in serum and urine matrices, with 10 replicate injections for each calibration level. Calibration curves were created using the ratio between the analyte response and its correlating IS, and either had no weighting or were weighted 1/x. Calibration levels ranged from the detection limit to 1,000 ppb. For some analytes, linearity was observed for four orders of magnitude (0.1 to 1,000 ppb). Figure 4 shows some calibration curves.

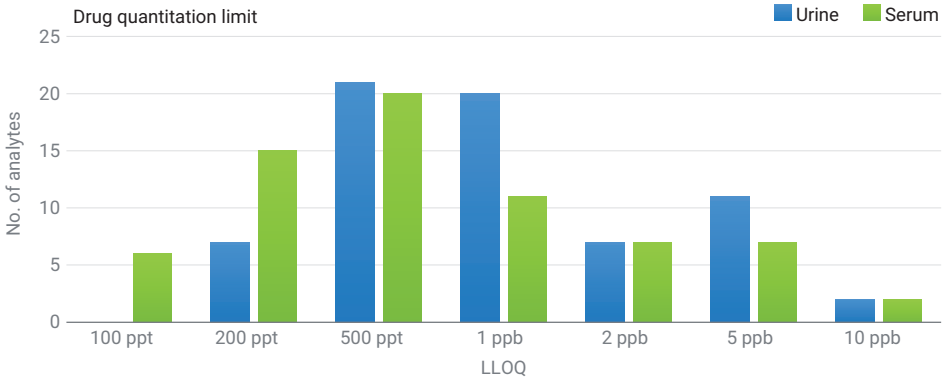

Figure 2. Distribution of LOQs of the 68 drugs included in this analytical method.

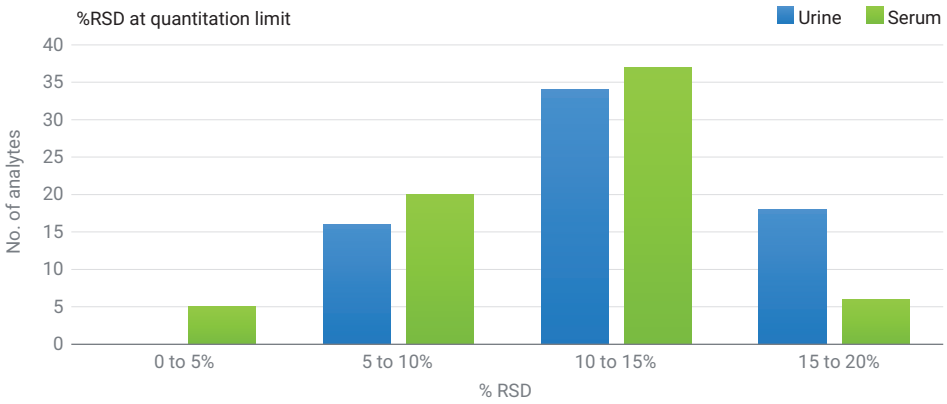

Figure 3. Distribution of the precision of 10 replicate injections at the LOQ for each compound.

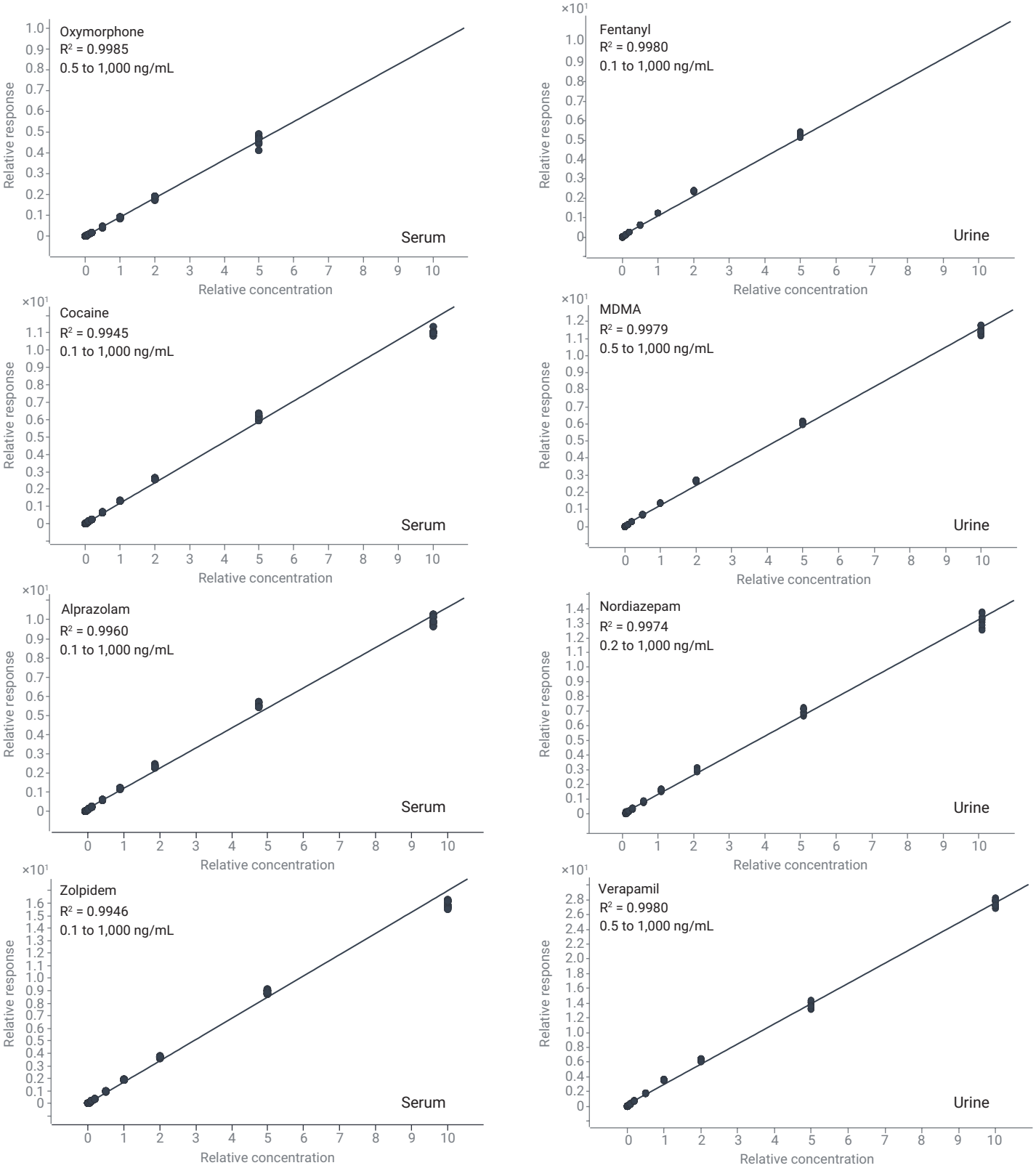

Figure 4. Select calibration curves of analytes at concentrations ranging from 0.1 to 1,000 ng/mL.

Conclusion

The Agilent Ultivo LC/TQ with a standard ESI source can be used to detect drugs of several drug classes in human urine and serum with excellent sensitivity and precision, and minimal sample preparation. The Ultivo LC/TQ with ESI is robust, cost-effective, and space-conscious, and is well suited to the high-throughput forensic toxicology laboratory environment.

References

1. Sosienski, T. Robustness of the Agilent Ultivo Triple Quadrupole LC/MS with Standard ESI Ion Source for High-Throughput Testing of Drugs in Serum. *Agilent Technologies Application Note*, publication number 5994-0737EN, **2019**. https://www.agilent.com/cs/library/technicaloverviews/public/application-human-serum-forensic-drugs-ultivo-5994-0737en-agilent.pdf
2. Stone, P.; McCann, K. Comprehensive LC/MS Analysis of Illicit and Pain Management Drugs Including Their Metabolites, in Urine. *Agilent Technologies Application Note*, publication number 5991-1667EN, **2013**. https://www.chem-agilent.com/pdf/5991-1667EN.pdf

Appendix

Compound Name	ISTD?	Precursor (*m/z*)	Product (*m/z*)	RT (min)	Fragmentor (V)	CE (V)	Polarity
2-Hydroxyethylflurazepam	No	333.1	211.1	4.35	120	44	+
2-Hydroxyethylflurazepam	No	333.1	109	4.35	120	32	+
2-Hydroxyethylflurazepam-d_4	Yes	337.1	215.2	4.35	130	44	+
6-Acetylmorphine	No	328.2	211	1.90	110	28	+
6-Acetylmorphine	No	328.2	165	1.90	110	44	+
6-Acetylmorphine-d_3	Yes	331.2	165.1	1.90	130	48	+
7-Aminoclonazepam	No	286.1	222.1	2.90	110	28	+
7-Aminoclonazepam	No	286.1	121	2.90	110	36	+
7-Aminoclonazepam-d_4	Yes	290.1	121	2.90	120	32	+
7-Aminoflunitrazepam	No	284.1	227.1	3.26	110	28	+
7-Aminoflunitrazepam	No	284.1	135	3.26	110	32	+
Alprazolam	No	309.1	281	4.37	130	28	+
Alprazolam	No	309.1	205	4.37	130	48	+
Alprazolam-d_5	Yes	314.1	286.1	4.37	130	28	+
Amphetamine	No	136.1	119	2.00	50	4	+
Amphetamine	No	136.1	91	2.00	50	16	+
Benzoylecgonine	No	290.1	168.1	2.56	90	20	+
Benzoylecgonine	No	290.1	77	2.56	90	65	+
Benzoylecgonine-d_3	Yes	293.2	171.1	2.56	90	20	+
Buprenorphine	No	468.3	414.1	4.27	180	40	+
Buprenorphine	No	468.3	55	4.27	180	65	+
Buprenorphine-d_4	Yes	472.3	58.9	4.27	190	64	+
Carisoprodol	No	261.2	176	4.33	60	4	+
Carisoprodol	No	261.2	55	4.33	60	32	+
Carisoprodol-d_7	Yes	268.2	183.1	4.33	80	4	+
Chlordiazepoxide	No	300.1	282.1	4.43	100	24	+

Compound Name	ISTD?	Precursor (*m/z*)	Product (*m/z*)	RT (min)	Fragmentor (V)	CE (V)	Polarity
Chlordiazepoxide	No	300.1	227	4.43	100	28	+
Chlordiazepoxide-d_5	Yes	305.1	286.1	4.43	90	28	+
cis-Tramadol-^{13}C, d_3	Yes	268.2	58	2.89	70	28	+
Clonazepam	No	316.1	270	4.22	110	28	+
Clonazepam	No	316.1	213.9	4.22	110	48	+
Clonazepam-d_4	Yes	320.1	274.1	4.22	120	28	+
Cocaethylene	No	318.2	196.1	3.36	110	20	+
Cocaethylene	No	318.2	82	3.36	110	36	+
Cocaethylene-d_8	Yes	326.2	204.2	3.36	110	20	+
Cocaine	No	304.2	182.1	2.92	110	20	+
Cocaine	No	304.2	77	2.92	110	65	+
Cocaine-d_3	Yes	307.2	185.1	2.92	100	16	+
Codeine	No	300.2	165.1	1.58	110	56	+
Codeine	No	300.2	127.9	1.58	110	64	+
Codeine-d_6	Yes	306.2	152	1.58	110	65	+
Desalkylflurazepam	No	289.1	226.1	4.40	120	32	+
Desalkylflurazepam	No	289.1	140	4.40	120	36	+
Diazepam	No	285.1	193	4.55	120	36	+
Diazepam	No	285.1	154	4.55	120	28	+
Diazepam-d_5	Yes	290.1	198.1	4.55	110	40	+
Dihydrocodeine	No	302.2	199	1.56	120	36	+
Dihydrocodeine	No	302.2	127.9	1.56	120	65	+
EDDP	No	278.2	234.1	3.90	110	36	+
EDDP	No	278.2	219.1	3.90	110	48	+
EDDP-d_3	Yes	282.2	235.1	3.90	140	36	+
Fentanyl	No	337.2	188.2	3.72	140	24	+
Fentanyl	No	337.2	104.9	3.72	140	48	+
Fentanyl-d_5	Yes	342.3	105	3.72	100	44	+
Flunitrazepam	No	314.1	268.2	4.24	120	28	+
Flunitrazepam	No	314.1	239	4.24	120	40	+
Flurazepam	No	388.2	317.1	3.96	110	16	+
Flurazepam	No	388.2	315.1	3.96	110	24	+
Gabapentin	No	172.1	154.1	1.70	70	12	+
Gabapentin	No	172.1	137	1.70	70	16	+
Heroin	No	370.2	268.2	2.86	140	32	+
Heroin	No	370.2	165.1	2.86	140	64	+
Heroin-d_9	Yes	379.2	272.2	2.86	160	32	+
Hydrocodone	No	300.2	199	1.58	140	32	+
Hydrocodone	No	300.2	128	1.58	140	65	+
Hydrocodone-d_6	Yes	306.2	202.1	1.58	140	32	+

Compound Name	ISTD?	Precursor (*m/z*)	Product (*m/z*)	RT (min)	Fragmentor (V)	CE (V)	Polarity
Hydromorphone	No	286.2	185	1.00	120	32	+
Hydromorphone	No	286.2	157.1	1.00	120	48	+
Hydromorphone-d_6	Yes	292.2	185.1	1.00	130	36	+
Ketamine	No	238.1	220.1	2.71	70	12	+
Ketamine	No	238.1	125	2.71	70	36	+
Lorazepam	No	321	303	4.20	100	12	+
Lorazepam	No	321	274.9	4.20	100	20	+
MDA	No	180.1	163.1	2.05	60	8	+
MDA	No	180.1	105	2.05	60	24	+
MDA-d_5	Yes	185.1	168.1	2.05	60	8	+
MDEA	No	208.1	163	2.35	70	12	+
MDEA	No	208.1	104.9	2.35	70	28	+
MDMA	No	194.1	163	2.11	60	8	+
MDMA	No	194.1	105.1	2.11	60	24	+
MDMA-d_5	Yes	199.1	165	2.11	70	8	+
Meperidine	No	248.2	220.1	3.15	110	24	+
Meperidine	No	248.2	174.1	3.15	110	20	+
Meperidine-d_4	Yes	252.2	224.2	3.15	100	20	+
Meprobamate	No	219.1	158.1	3.53	60	4	+
Meprobamate	No	219.1	55	3.53	60	24	+
Methadone	No	310.2	265.2	4.28	90	16	+
Methadone	No	310.2	104.9	4.28	90	28	+
Methadone-d_9	Yes	319.3	268.1	4.28	80	12	+
Methamphetamine	No	150.1	91	2.09	60	16	+
Methamphetamine	No	150.1	65	2.09	60	48	+
Methylphenidate	No	234.1	84	2.98	80	24	+
Methylphenidate	No	234.1	56	2.98	80	60	+
Methylphenidate-d_9	Yes	243.2	93.1	2.98	80	24	+
m-Hydroxybenzoylecgonine	No	306.1	168	2.08	100	20	+
m-Hydroxybenzoylecgonine	No	306.1	121	2.08	100	32	+
Midazolam	No	326.1	291.1	4.37	150	28	+
Midazolam	No	326.1	249.1	4.37	150	44	+
Midazolam-d_4	Yes	330.1	295.2	4.37	150	32	+
Morphine	No	286.2	165.1	0.78	100	48	+
Morphine	No	286.2	152	0.78	100	65	+
Morphine-d_3	Yes	289.2	152	0.78	120	64	+
Naloxone	No	328.2	310.2	1.58	110	20	+
Naloxone	No	328.2	212.1	1.58	110	44	+
Naltrexone	No	342.2	324.2	1.78	120	20	+
Naltrexone	No	342.2	55.1	1.78	120	48	+

Compound Name	ISTD?	Precursor (*m/z*)	Product (*m/z*)	RT (min)	Fragmentor (V)	CE (V)	Polarity
N-Desmethyltramadol	No	250.2	232.2	3.08	70	4	+
N-Desmethyltramadol	No	250.2	77	3.08	70	65	+
Nitrazepam	No	282.1	236.1	4.21	120	28	+
Nitrazepam	No	282.1	180	4.21	120	44	+
Nitrazepam-d_5	Yes	287.1	185.2	4.21	110	44	+
Norbuprenorphine	No	414.3	101.2	3.64	160	40	+
Norbuprenorphine	No	414.3	83	3.64	160	60	+
Nordiazepam	No	271.1	208.1	4.50	110	32	+
Nordiazepam	No	271.1	139.9	4.50	110	32	+
Norfentanyl	No	233.2	84.1	2.76	80	16	+
Norfentanyl	No	233.2	55.1	2.76	80	40	+
Norfentanyl-d_5	Yes	238.2	84.2	2.74	80	16	+
Norketamine	No	224.1	207	2.76	70	8	+
Norketamine	No	224.1	124.9	2.76	70	24	+
Normeperidine	No	234.1	160.1	3.25	80	16	+
Normeperidine	No	234.1	56.1	3.25	80	24	+
Norpropoxyphene	No	326.2	252.1	4.26	60	0	+
Norpropoxyphene	No	326.2	90.9	4.26	60	52	+
o-Desmethyltramadol	No	250.2	232.2	2.12	70	8	+
o-Desmethyltramadol	No	250.2	58	2.12	70	16	+
Oxazepam	No	287.1	269.1	4.20	100	16	+
Oxazepam	No	287.1	241	4.20	100	24	+
Oxycodone	No	316.2	298.2	1.72	100	16	+
Oxycodone	No	316.2	241.1	1.72	100	32	+
Oxymorphone	No	302.1	284.2	0.86	100	20	+
Oxymorphone	No	302.1	227	0.86	100	28	+
PCP	No	244.2	91	3.58	60	48	+
PCP	No	244.2	86	3.58	60	8	+
PCP-d_5	Yes	249.2	86	3.58	70	8	+
Phentermine	No	150.1	133.1	2.52	50	8	+
Phentermine	No	150.1	91	2.52	50	20	+
Phentermine-d_5	Yes	155.2	96	2.52	60	24	+
Pregabalin	No	160.1	142.1	1.74	60	8	+
Pregabalin	No	160.1	55	1.74	60	24	+
Propoxyphene	No	340.2	266.2	4.23	80	4	+
Propoxyphene	No	340.2	58.1	4.23	80	24	+
Propoxyphene-d_5	Yes	345.3	57.9	4.23	70	16	+
Ritalinic acid	No	220.1	84	2.57	80	20	+
Ritalinic acid	No	220.1	56	2.57	80	52	+
Tapentadol	No	222.2	106.9	3.05	90	32	+

Compound Name	ISTD?	Precursor (*m/z*)	Product (*m/z*)	RT (min)	Fragmentor (V)	CE (V)	Polarity
Tapentadol	No	222.2	77	3.05	90	56	+
Tapentadol-d_3	Yes	225.2	107	3.05	70	28	+
Temazepam	No	301.1	283.1	4.42	100	12	+
Temazepam	No	301.1	255.1	4.42	100	24	+
Tramadol	No	264.2	58	2.90	70	16	+
Tramadol	No	264.2	56	2.90	70	64	+
Trazodone	No	372.2	176.1	3.68	140	24	+
Trazodone	No	372.2	147.9	3.68	140	40	+
Triazolam	No	343.1	308.1	4.35	120	28	+
Triazolam	No	343.1	239.1	4.35	120	48	+
Verapamil	No	455.3	303.2	4.12	140	28	+
Verapamil	No	455.3	165	4.12	140	28	+
Zolpidem	No	308.2	236.1	3.47	130	28	+
Zolpidem	No	308.2	235.1	3.47	130	40	+
Zolpidem-d_7	Yes	315.2	242.2	3.47	130	40	+
Zopiclone	No	389.1	245.1	2.83	60	16	+
Zopiclone	No	389.1	111.9	2.83	60	64	+
Zopiclone-d_4	Yes	393.1	245.1	2.83	60	16	+
α-Hydroxyalprazolam	No	325.1	296.9	4.30	110	28	+
α-Hydroxyalprazolam	No	325.1	205.1	4.30	110	52	+
α-Hydroxymidazolam	No	342.1	324.1	4.40	120	24	+
α-Hydroxymidazolam	No	342.1	203	4.40	120	28	+
α-Hydroxytriazolam	No	359.1	239	4.25	130	52	+
α-Hydroxytriazolam	No	359.1	176.2	4.25	130	28	+

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