Application Note

Clinical Research

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Fast LC/MS/MS Analytical Method with Alternating Column Regeneration for the Analysis of 125 Various Drugs and Their Metabolites in Urine in Clinical Research

Authors

Andre Szczesniewski and
Carrie J. Adler
Agilent Technologies, Inc.

Abstract

This research outlines a highly sensitive, specific, and fast LC/MS/MS analytical method that was developed for the quantitation of 125 drugs from a variety of drug classes over a wide dynamic range. The alternate column regeneration (ACR) hardware configuration was used to significantly increase the sample throughput. The ability to combine many analytes into a single run coupled with a fast analytical method and ACR could improve turnaround time in a clinical research laboratory.

Introduction

Liquid chromatography triple quadrupole mass spectrometry (LC/MS/MS) is well suited for the rapid analysis of large numbers of analytes using a single method. A highly sensitive, specific, and fast LC/MS/MS analytical method has been developed for the quantitation of 125 analytes (see Appendix A) of the following drug classes: antidepressants, benzodiazepines, opioids, muscle relaxants, hallucinogens, and stimulants. The described method achieves high analytical sensitivity and is capable of quantitating analytes over a wide dynamic range. In addition, the ACR hardware configuration was used to significantly increase the sample throughput. The analytical methodology was developed on an Agilent 1290 Infinity II UHPLC and 6470 TQ mass spectrometer with a 7.35-minute analysis time (5-minute gradient + 1.5-minute equilibration + 0.85-minute injection). ACR reduced the analysis time by 30% to 5.1 minutes, injection to injection. The ability to combine many analytes into a single run coupled with a fast analytical method and ACR could significantly improve turnaround time in a clinical research laboratory.

Experimental

Chemicals and reagents

Optima-grade methanol, acetonitrile, and isopropanol were from Fisher Scientific (Hampton, NH) and ammonium formate and formic acid were purchased from Agilent Technologies (Santa Clara, CA). Clinical Laboratory Reagent Water (CLRW) was from a MilliQ Advantage A10 system manufactured by MilliporeSigma. Stock standards for drugs, metabolites, and deuterated internal standards were purchased from Cerilliant Corporation (Round Rock, TX).

LC configuration and parameters

Agilent 1290 Infinity II Binary pumps (G7120A)		
Agilent 1290 Infinity II Multisampler (G7167B)		
Agilent 1290 Infinity Multicolumn Thermostated (G7116B) TCC with 2-position 10-port valve		
Needle Wash	50/20/20/10 IPA/MeOH/ACN/H_2O	
Autosampler Temperature	5 °C	
Injection Volume	1 µL	
Analytical Column	2 Agilent Poroshell 120 EC-C18, 2.1 x 100 mm, 2.7 µm, LC columns (p/n 695775-902)	
Column Temperature	55 °C	
Mobile Phase A	H_2O + 5 mM ammonium formate + 0.01% formic acid	
Mobile Phase B	Methanol + 0.01% Formic Acid	
Flow Rate, Eluent	0.35 mL/min	
Flow Rate, Regeneration	0.50 mL/min	
Gradient	**Eluent pump** Time (min) %B 0.00 12 0.30 12 1.20 40 2.90 70 3.30 98 4.00 98 4.01 12	**Regeneration pump** Time (min) %B 0.00 98 2.00 98 2.01 12
Valve Position V1	0.00 min – current position 4.70 min – next position	
Stop Time	5.00 min	

MS configuration and parameters

Agilent 6470 Triple Quadrupole Mass Spectrometer	
Ionization Mode	Positive and Negative
Drying Gas Temperature	300 °C
Drying Gas Flow	9 L/min
Nebulizer Pressure	30 psi
Sheath Gas Temperature	380 °C
Sheath Gas Flow	11 L/min
Nozzle Voltage	500 V
Capillary Voltage, Positive	3,750 V
Capillary Voltage, Negative	3,500 V
Delta EMV, Positive	0 V
Delta EMV, Negative	100 V

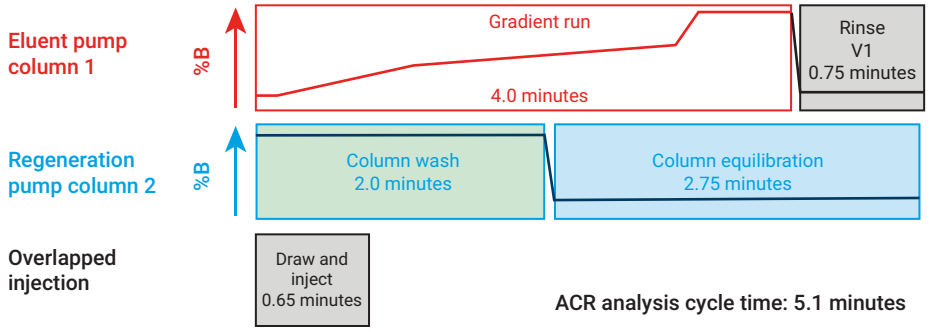

Figure 1. Graphical timeline for ACR analysis.

Standards and curve preparation

Standards were spiked into drug-free human urine solution (10%), and 10 µL were injected into the LC/MS/MS system. The 10-point calibration curve was prepared by serial dilution, with concentrations ranging from 1 to 1,000 ng/mL. Internal standards were added at 125 ng/mL.

Data analysis

Data acquisition was performed using MassHunter Acquisition Software (B.09.00). Data were analyzed using MassHunter Quantitative Analysis Software (B.09.00) and Qualitative Analysis Software (B.09.00). All analytes were normalized to 124 internal standards.

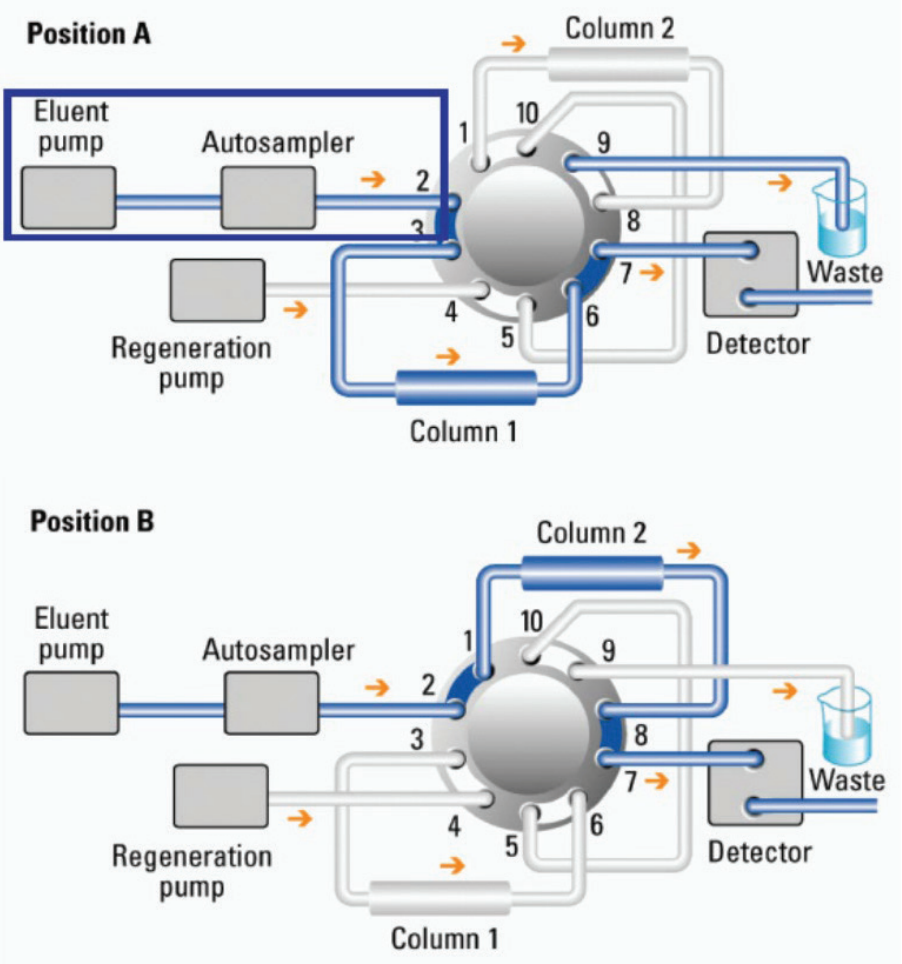

Figure 2. Alternating column regeneration (ACR) valve configuration.

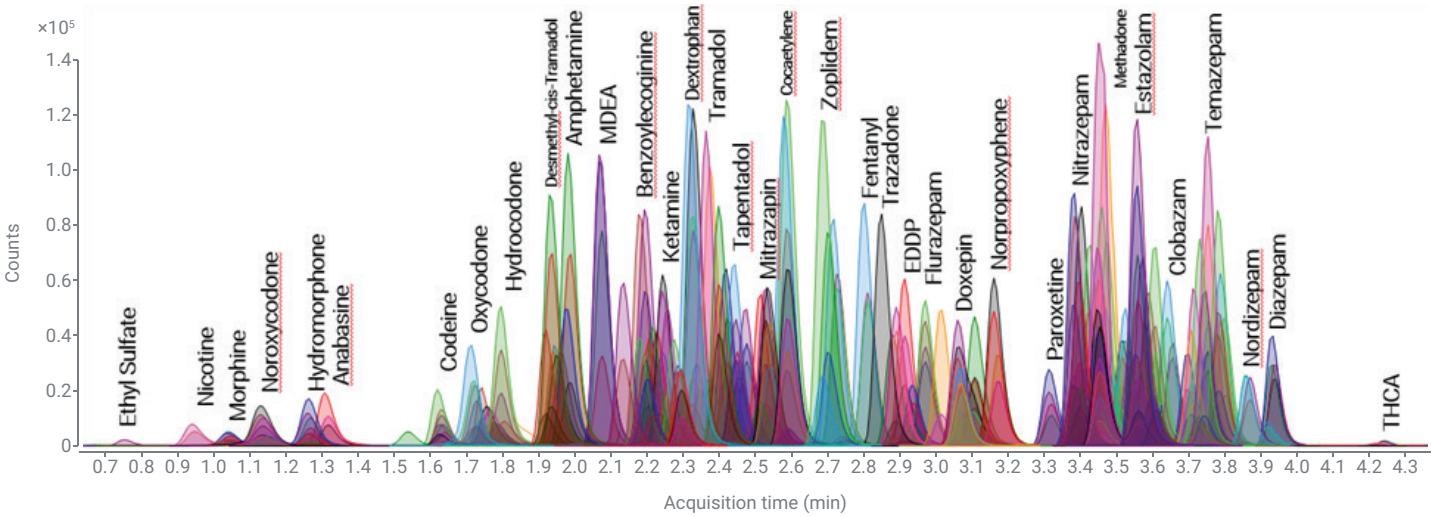

Figure 3. Example chromatogram with 117 analytes in 4.5 minutes.

Results and discussion

Columns comparison test

To ensure for peak retention time reproducibility, both analytical columns used in this method were tested by repeating five injections over each column. As shown in Figure 4, both columns had virtually identical retention times and response.

Quantitation results

Examples of quantitation results are shown in Table 1. These results were based on a 10-point calibration curve ranging from 1 to 1,000 ng/mL for all compounds. Sensitivity was analyzed down to 1 ng/mL, 1 pg on-column injection, and 104 of the analytes produced a signal to noise better than 10 at that level.

Linearity results are shown in the example calibration curves in Figure 5. Of all the analytes, 112 compounds were linear from the 1 to 1,000 ng/mL analysis range. Quadratic fit was used for the few other compounds. Excellent reproducibility was observed for majority of analytes (CV <15%) for all techniques and configurations.

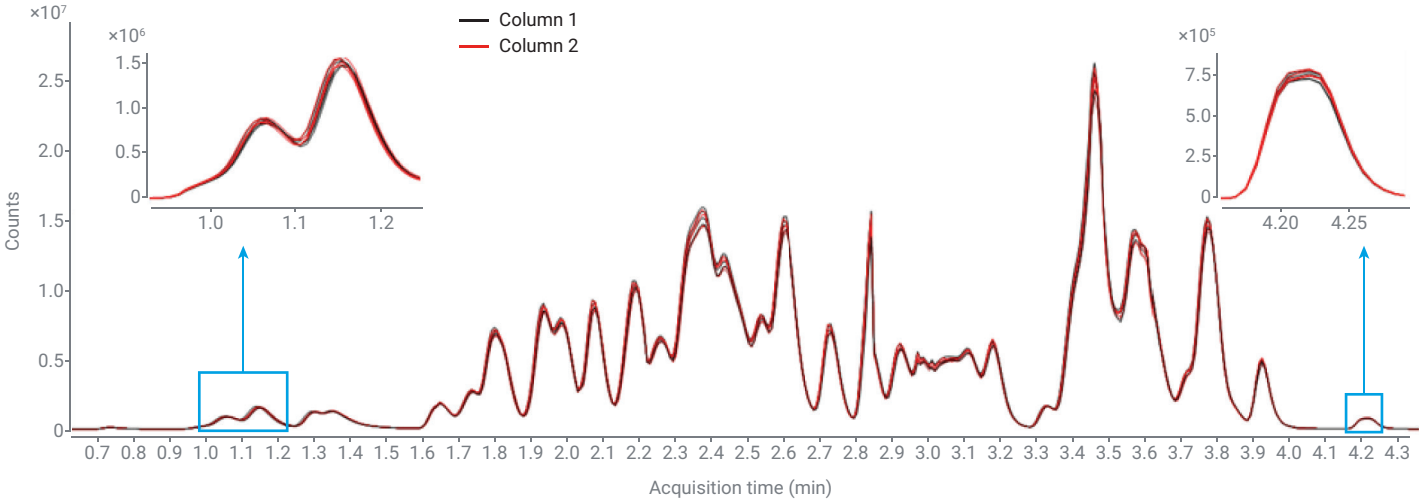

Figure 4. Overlay of 10 total ion chromatogram (TIC) traces, comparing column 1 and column 2.

Table 1. Examples of quantitative results.

Exp. Conc. (ng/mL)	Nicotine		Morphine		Fentanyl		Buprenorphine		Hydrocodone	
	Resp.	Final Conc.	Resp.	Final Conc.	Resp.	Final Conc.	Resp.	Final Conc.	Resp.	Final Conc.
1	200	1.55	570	1.14	870	1.44	27	0.97	217	1.10
2.5	368	2.31	1,042	2.98	2,465	2.31	64	2.62	568	2.24
5	830	4.56	1,171	3.71	6,029	4.30	117	4.94	1402	4.98
10	1,686	8.56	2,514	8.77	14,477	8.87	251	10.41	2803	9.52
25	4,578	22.43	6,312	24.58	38,876	22.64	631	26.78	7697	25.54
50	8,992	44.09	12,293	50.69	83,930	48.17	1,229	51.33	14,557	49.65
100	19,647	94.60	24,286	98.99	170,035	94.79	2,428	99.98	28,752	100.11
250	53,201	257.86	59,423	260.40	451,903	261.34	5,942	251.24	70,769	264.18
500	104,254	497.40	115,458	514.02	865,763	515.45	11,545	501.76	125,968	498.01
1,000	205,825	1,010.14	192,631	998.16	1,556,435	984.16	19,263	996.85	209,324	999.18

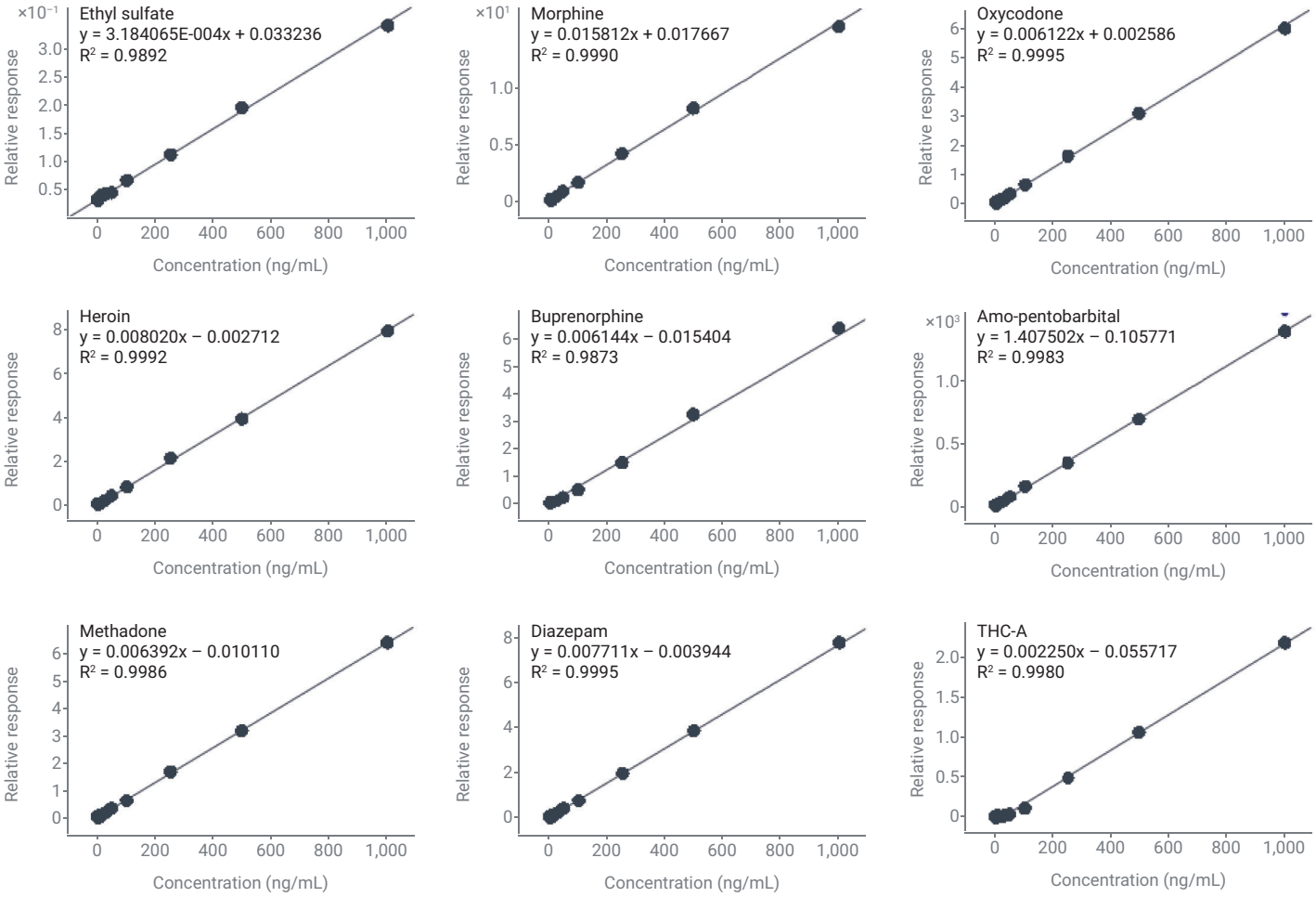

Figure 5. Examples of quantitative calibration curves.

Conclusion

This fast, sensitive, simple, specific, and accurate analytical LC/MS/MS method was developed and verified for the simultaneous measurement of 125 various drugs and their metabolites in urine. The use of ACR reduced the analysis turnaround time by 30%. Future work will include testing multiple sources of human urine for interferences that may impact the quantitation of any of the compounds in the analytical method.

Reference

1. June Feng *et al.* Simultaneous Determination of Multiple Drugs of Abuse and Relevant Metabolites in Urine by LC-MS-MS, *J. of AT* September **2007**, *31*.

Appendix A

Analyte and internal standard mass spectrometer specifications. All analyzed using a cell accelerator voltage (CAV) of 4 and using a 0.4-minute retention time window in dynamic MRM scan mode.

Analyte	Precursor Ion	Product Ion (No. 1/ No. 2)	Retention Time (min)	Frag (V)	CE (V) (No. 1/ No. 2)	Polarity
2-OH-Ethylflurazepam	333.1	211/109	3.61	135	40/28	positive
6-Monoacetyl Morphine	328.2	211/165	1.79	135	28/48	positive
7-Aminoclonazepam	286.1	222/121	2.34	125	28/36	positive
7-Aminoflunitrazepam	284.1	227/135	2.55	125	28/32	positive
7-OH-Mitragynine	415.2	234.1	2.75	120	40	positive
a-OH-Alprazolam	325.1	297/216	3.51	130	28/48	positive
a-OH-Triazolam	359.1	330.9/175.9	3.42	140	32/28	positive
a-PVP	232.2	91/77	2.42	105	28/56	positive
Alprazolam	309.1	281/205	3.65	135	28/48	positive
Amitriptyline	278.2	105/91	3.51	100	24/28	positive
Amo/Pentobarbital	225.1	182/42	3.41	100	12/24	negative
Amphetamine	136.1	119.1/91	1.95	65	8/20	positive
Anabasine	163.1	120/80	1.31	95	16/28	positive
Benzolyecgonine	290.1	168/77	2.19	100	20/72	positive
Bromazepam	316	86/58.1	3,75	115	16/56	positive
Buprenorphine	468.3	83.7/55.1	3.58	155	48/72	positive
Buprenorphine Glucuronide	644.4	55.1	2.78	160	44/100	positive
Butabarbital	211.1	168/42.1	3.01	90	12/28	negative
Butalbital	223.1	180/42.1	3.14	90	8/24	negative
Carisoprodol	261.2	224/51.1	3.55	165	28/96	positive
Chlordiazepoxide	300.1	282/227	3.80	95	28/24	positive
Citalopram	325.2	262/109	2.90	120	20/28	positive
Clobazam	301.1	259/224	3.55	95	24/36	positive
Clomipramine	315.2	86/58.1	3.75	95	16/60	positive
Clonazepam	316.1	86.1/58.1	3.75	120	16/60	positive
Cocaethylene	318.2	196/82	2.58	110	20/36	positive
Cocaine	304.2	182/77	2.33	115	20/72	positive
Codeine	300.2	152/115	1.65	130	76/92	positive
Cotinine	177.1	98/80	1.73	110	24/28	positive
Cyclobenzaprine	276.2	216/215	3.39	110	28/48	positive
Desalkylflurazepam	289.1	226/139.9	3.70	135	32/36	positive
Desipramine	267.2	72.1/44.1	3.47	80	16/64	positive
Desmethyldoxepin	266.2	107/77	3.10	90	24/64	positive
Dextromethophran	272.2	171/128	2.97	130	44/76	positive
Dextrophan	258.2	199/157	2.24	125	28/44	positive
Diazepam	285.1	193/154	3.94	130	36/28	positive
Dihydrocodeine	302.2	199/128	1.63	135	36/80	positive
Doxepin	280.2	107/77	3.05	105	24/68	positive
EDDP	279.2	250.1/235	2.93	135	28/36	positive
Estrazolam	295.1	267/205	3.53	120	28/44	positive

Analyte	Precursor Ion	Product Ion (No. 1/ No. 2)	Retention Time (min)	Frag (V)	CE (V) (No. 1/ No. 2)	Polarity
Ethyl Glucuronide	223.1	176/175	3.73	220	40/52	positive
Ethyl Sulfate	125	96.9/79.9	0.76	80	16/40	negative
Fentanyl	337.2	188/105	2.81	110	24/44	positive
Flunitrazepam	314.1	268/239	3.42	115	28/40	positive
Fluoxetine	310.1	148/44.1	3.58	105	4/12	positive
Fluphenazine	438.2	171.1/143	3.92	135	28/36	positive
Flurazepam	388.2	317/315	3.01	100	20/24	positive
Gabapentin	172.1	154.1/137	1.81	105	12/16	positive
Heroin	370.2	165/58.1	2.25	150	60/32	positive
Hydrocodone	300.2	199/128	1.77	130	32/72	positive
Hydromorphone	286.2	185/157	1.26	130	32/44	positive
Imipramine	281.2	86.1/58.1	3.42	80	16/48	positive
Ketamine	238.1	179/124.9	2.23	70	16/36	positive
Lorazepam	321	274.9/229	3.61	105	20/36	positive
Maprotiline	278.2	250.1/191	3.50	110	20/40	positive
MDA	180.1	163/105	1.96	90	8/24	positive
MDEA	208.1	163/77	2.08	80	12/56	positive
MDMA	194.1	165/139	3.09	215	32/60	positive
MDPV	276.2	135/126.1	2.45	105	28/28	positive
Meperidine	248.2	220/174	2.47	115	24/20	positive
Mephedrone	178.1	176/152	3.09	220	44/36	positive
Meprobamate	219.1	204/203	3.46	150	24/36	positive
Methadone	310.2	265.1/105	3.45	75	12/32	positive
Methamphetamine	150.1	91/65	1.99	70	20/48	positive
Methylone	208.1	160/132	1.80	65	20/32	positive
Methylphenidate	234.1	84/56.1	2.37	70	24/56	positive
m-OH-Benzoylecgonine	306.1	168/120.9	1.91	100	20/32	positive
Mianserine	265.2	250/235	3.06	220	20/32	positive
Midazolam	326.1	291/249	3.73	135	28/44	positive
Mitragynine	399.2	226/174	2.88	135	24/36	positive
Mitrazapine	266.2	195/72.1	2.52	105	28/20	positive
Morphine	286.2	201/151.9	1.04	135	28/76	positive
Naloxone	328.2	291/249	3.73	135	32/44	positive
Naltrexone	342.2	324.1/55.1	1.75	125	20/40	positive
n-Desmethyl-*cis*-Tramadol	250.2	44.1/42.1	2.44	90	20/92	positive
n-Desmethylclobazam	287.1	244.9/210	3.41	100	20/36	positive
n-Desmethylclomipramine	301.2	72.1/44.1	3.78	80	16/64	positive
n-Desmethylcyclobenzaprine	262.2	216/215	3.45	75	24/48	positive
n-Desmethylmirtazapine	252.1	209/195	2.50	110	24/24	positive
n-Desmethyltapentadol	208.2	107/77	2.47	140	24/60	positive
n-Desmethyltrimipramine	281.2	86.1/44.1	3.60	70	16/56	positive
Nicotine	163.1	130/117	0.93	70	20/28	positive
Nitrazepam	282.1	236/180	3.39	115	28/44	positive
Norbuprenorphine	414.3	83/55.1	2.75	150	56/76	positive
Norbuprenorphine Glucuronide	590.3	414.2/83.1	2.18	165	40/76	positive
Norcodeine	286.2	268/152	1.04	115	20/76	positive
Nordiazepam	271.1	164.9/139.9	3.87	115	32/32	positive

Analyte	Precursor Ion	Product Ion (No. 1/ No. 2)	Retention Time (min)	Frag (V)	CE (V) (No. 1/ No. 2)	Polarity
Norfentanyl	233.2	84.1/55.1	2.25	100	20/40	positive
Norhydrocodone	286.2	199/128	1.27	115	28/64	positive
Norketamine	224.1	207/124.9	2.28	105	8/28	positive
Normeperidine	234.1	160/42.1	2.53	105	16/36	positive
Normorphine	272.1	164.9/152	1.04	115	56/72	positive
Noroxycodone	302.1	284/227	1.15	105	16/32	positive
Noroxymorphone	288.1	270/213	1.16	100	16/32	positive
Norpropoxyphene	308.1	100/44.1	3.17	70	12/24	positive
Norsertraline	275	158.1/122.9	3.70	85	20/56	positive
Nortriptyline	264.2	105/91	3.56	75	24/20	positive
o-Desmethyl-*cis*-Tramadol	250.2	58.1/42.1	1.94	65	16/100	positive
Oxazepam	287.1	219.1/41.1	2.58	100	20/56	positive
Oxycodone	316.2	298.1/241	1.73	105	20/32	positive
Oxymorphone	302.1	284.1/227	1.13	105	20/28	positive
Paroxetine	330.2	192/70	3.32	115	20/36	positive
PCP (Phencyclidine)	244.2	91/86	2.72	40	48/8	positive
Pentazocine	286.2	218.1/69.1	2.58	105	20/28	positive
Phenobarbital	231.1	188/42.1	2.73	85	8/16	negative
Phentermine	150.1	133/91	2.19	85	8/24	positive
Pregabalin	160.1	92/91.6	2.72	80	20/16	positive
Propoxyphene	340.2	266.1/58.1	3.38	35	4/16	positive
Protriptyline	264.2	191/155	3.45	90	36/20	positive
Ritalinic Acid	220.1	84/56.1	2.21	75	20/56	positive
Secobarbital	237.1	194/42.1	3.56	80	12/24	negative
Sertraline	306.1	159	3.7	95	24	positive
Tapentadol	222.2	107/77	2.40	75	28/56	positive
Temazepam	301.1	255/177	3.74	85	20/48	positive
THC	315.2	238.9/177	3.61	170	36/64	positive
THC-A	343.2	299.2/245	4.24	125	24/36	negative
THC-OH	331.2	313.1/193	4.22	85	12/24	positive
Tramadol	264.2	58.1/42.1	2.32	65	20/100	positive
Triazolam	343.1	308/239	3.61	130	28/48	positive
Trazadone	372.2	176/148	2.89	125	24/40	positive
Trimipramine	295.2	100/58.1	3.55	75	16/44	positive
Zaleplon	306.1	264/236	3.07	120	24/32	positive
Zolpidem	308.2	236/235	2.69	135	28/40	positive
Zolpidem Phenyl-4-Carboxylic Acid	338.2	266/265	2.13	140	32/40	positive
Zopiclone	389.1	245/217	2.29	82	12/32	positive

Internal Standard	Precursor Ion	Final conc in sample	Retention Time (min)	Frag (V)	CE (V) (No. 1/ No. 2)	Polarity
2-OH-Ethylflurazepam-D4	337.1	113	3.61	160	32	positive
6-Monoacetyl Morphine-D3	331.2	165	1.79	160	48	positive
6-Monoacetyl Morphine-D6	334.2	165	1.78	155	44	positive
7-Aminoclonazepam-D4	290.1	121	2.33	140	36	positive
7-Aminoflunitrazepam-D7	291.2	138	2.52	135	32	positive
a-OH-Alprazolam-D5	330.1	302	3.50	135	32	positive
a-OH-Triazolam-D4	363.1	335	3.41	135	32	positive
a-PVP-D8	240.2	91	2.41	110	28	positive
Alprazolam-D5	314.1	286	3.64	135	32	positive
Amitriptyline-D3	281.2	91	3.51	105	28	positive
Amo/Pentobarbital-D5	230.2	42	3.40	100	24	negative
Amphetamine-D5	141.1	96.1	1.95	65	16	positive
Amphetamine-D8	144.2	97.1	1.94	65	16	positive
Anabasine-D4	167.1	84	1.30	95	28	positive
Benzoylecgonine-D3	293.2	171	2.19	110	20	positive
Benzoylecgonine-D8	298.2	171	2.18	105	20	positive
Buprenorphine-D4	472.3	59.1	3.50	155	56	positive
Butalbital-D5	228.1	42.1	3.13	90	24	negative
Carisoprodol-D7	268.2	51	3.45	165	96	positive
Chlordiazepoxide-D5	305.1	286	3.78	90	28	positive
Citalopram-D6	331.2	109	2.90	125	28	positive
Clobazam-$^{13}C_6$	307.1	265	3.58	100	24	positive
Clomipramine-D3	318.2	89.1	3.75	100	20	positive
Clonazepam-D4	320.1	89.1	3.75	105	20	positive
Cocaethylene-D3	321.2	199.1	2.58	105	20	positive
Cocaethylene-D8	326.1	204.1	2.57	115	20	positive
Cocaine-D3	307.2	185	2.32	105	20	positive
Codeine-D3	303.2	115	1.64	130	88	positive
Codeine-D6	306.2	152	1.64	130	84	positive
Cotinine-D3	180.1	101	1.72	110	24	positive
Cyclobenzaprine-D3	279.2	215	3.39	110	48	positive
Desalkylflurazepam-D4	293.1	139.9	3.69	135	36	positive
Desipramine-D3	270.2	75.1	3.47	80	16	positive
Desmethyldoxepin-D3	269.2	107	3.10	90	24	positive
Dextromethophran-D3	275.2	171	3.97	130	48	positive
Dextrophan-D3	261.2	157	2.23	130	44	positive
Diazepam-D5	290.1	198	3.93	135	40	positive
Dihydrocodeine-D6	308.2	202	1.62	130	40	positive
Doxepin-D3	283.2	107	3.05	105	24	positive
EDDP-D3	282.2	235	2.93	135	36	positive
Estrazolam-D5	300.1	210	3.52	120	52	positive
Ethyl Sulfate-D5	130	97.9	0.75	80	20	negative
Fentanyl-D5	342.3	188.1	2.79	125	24	positive
Flunitrazepam-D7	321.1	275	3.42	135	28	positive
Fluoxetine-D6	316.2	44.1	3.57	120	16	positive
Gabapentin-D10	182.2	164.1	1.78	115	12	positive
Heroin-D9	379.2	61.1	2.23	150	36	positive

Internal Standard	Precursor Ion	Final conc in sample	Retention Time (min)	Frag (V)	CE (V) (No. 1/ No. 2)	Polarity
Hydrocodone-D3	303.2	199	1.76	135	32	positive
Hydrocodone-D6	306.2	202	1.75	140	36	positive
Hydromorphone-D3	289.2	185	1.25	140	36	positive
Hydromorphone-D6	292.2	185	1.25	140	36	positive
Imipramine-D3	284.2	58	3.41	80	48	positive
Ketamine-D4	242.1	128.9	2.22	65	36	positive
Lorazepam-D4	325.1	279	3.62	100	24	positive
MDA-D5	185.1	168	1.95	95	8	positive
MDEA-D5	213.2	163	2.07	85	12	positive
MDEA-D6	214.2	166	2.07	80	12	positive
MDMA-D5	199.1	170	2.97	215	32	positive
MDPV-D8	284.2	134.5	2.45	105	32	positive
Meperidine-D4	252.2	224.1	2.47	110	24	positive
Mephedrone-D3	181.1	179	3.09	220	44	positive
Meprobamate-D3	222.2	203	3.45	175	40	positive
Meprobamate-D7	226.2	105	3.45	145	16	positive
Methadone-D3	313.2	268	3.45	75	16	positive
Methadone-D9	319.3	268.1	3.44	75	16	positive
Methamphetamine-D5	155.2	92	1.98	70	20	positive
Methamphetamine-D8	158.2	93	1.97	70	20	positive
Methylone-D3	211.1	163	1.79	60	20	positive
Methylphenidate-D9	243.2	93.1	2.36	90	24	positive
Mianserine-D3	268.2	61.1	3.06	115	32	positive
Midazolam-D4	330.1	295.1	3.71	145	32	positive
Mitragynine-D3	402.2	177	2.87	135	36	positive
Morphine-D3	289.2	152	1.06	130	76	positive
Morphine-D6	292.2	152	1.03	135	80	positive
Naloxone-D5	333.2	315.1	3.62	115	20	positive
Naltrexone-D3	345.2	327.1	1.72	110	24	positive
n-Desmethyl-*cis*-Tramadol-D3	253.2	44.1	2.43	90	20	positive
n-Desmethylclobazam-$^{13}C_6$	293.1	251	3.40	105	20	positive
n-Desmethylclomipramine-D3	304.2	75.1	3.78	70	16	positive
n-Desmethylcyclobenzaprine-D3	265.2	215	3.44	80	44	positive
Nicotine-D4	167.1	121	0.92	70	32	positive
Nitrazepam-D5	287.1	241	3.37	110	28	positive
Norbuprenorphine Glucuronide-D3	593.3	417.2	2.17	150	40	positive
Norbuprenorphine-D3	417.3	55.1	2.74	150	88	positive
Norbuprenorphine-D4	418.3	55.1	2.74	160	92	positive
Norcodeine-D3	289.2	152	1.03	115	80	positive
Nordiazepam-D5	276.1	213	3.85	120	32	positive
Norfentanyl-D5	238.2	84.1	2.24	100	20	positive
Norhydrocodone-D3	289.2	202.1	1.26	115	28	positive
Norketamine-D4	228.1	129	2.27	105	32	positive
Normeperidine-D4	238.2	164.1	2.53	80	16	positive
Noroxycodone-D3	305.2	287.1	1.14	95	16	positive
Norpropoxyphene-D5	313.1	44.1	3.15	60	20	positive
Norsertraline-$^{13}C_6$	281.1	158.9	3.70	135	16	positive

Internal Standard	Precursor Ion	Final conc in sample	Retention Time (min)	Frag (V)	CE (V) (No. 1/ No. 2)	Polarity
Nortriptyline-D3	267.2	91	3.56	80	28	positive
o-Desmethyl-*cis*-Tramadol-D6	256.2	64.1	1.93	65	16	positive
Oxycodone-D3	319.2	301.1	1.71	115	20	positive
Oxymorphone-D3	305.2	287.1	1.12	105	20	positive
Paroxetine-D6	336.2	76.1	3.31	130	36	positive
PCP-D5	249.2	86.1	2.71	45	12	positive
Pentazocine-$^{13}C_3$	289.2	218.1	2.58	120	20	positive
Phenobarbital-D5	236.1	42.1	2.72	85	16	negative
Phentermine-D5	155.2	96	2.17	85	24	positive
Pregabalin-D6	166.2	139	2.61	220	28	positive
Propoxyphene-D11	351.3	64.1	3.37	35	16	positive
Propoxyphene-D5	345.3	58.1	3.75	35	8	positive
Protriptyline-D3	267.2	191	3.45	90	36	positive
Ritalinic Acid-D10	230.2	93.1	2.19	80	20	positive
Secobarbital-D5	242.1	42.1	3.56	80	24	negative
Sertraline-D3	309.1	159	3.70	100	28	positive
Tapentadol-D3	225.2	107	2.39	85	28	positive
Temazepam-D5	306.1	260	3.72	95	20	positive
THC-D3	318.2	242	3.75	170	36	positive
THC-A-D3	346.2	302.2	4.24	120	24	negative
THC-A-D9	352.2	308.2	4.233	120	24	negative
THC-OH-D3	334.2	46.2	4.21	115	44	positive
Triazolam-D4	347.1	243	3.59	140	48	positive
Tramadol-$^{13}C_1$-D3	268.2	58.1	2.31	65	20	positive
Trazadone-D6	378.2	182	2.84	135	28	positive
Trimipramine-D3	298.2	103.1	3.55	75	20	positive
Zaleplon-D4	310.2	240	3.06	115	32	positive
Zopiclone-D4	393.1	245	2.29	82	12	positive
Zolpidem-D6	314.2	235	2.68	135	40	positive
Zolpidem-D7	315.2	236	2.68	140	40	positive

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RA44370.592662037

Printed in the USA, June 16, 2021
5994-3595EN

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