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Clinical toxicology

Utilizing triple quadrupole mass spectrometry to quantitate 106 drugs of abuse in urine

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Keywords

TSQ Quantis Plus mass spectrometer, Vanquish Horizon UHPLC, TraceFinder software, LC-MS/MS, drugs of abuse, isomers, reversed-phase UHPLC, toxicology, forensic, ANSI, quantitation, fast gradient, high-throughput, urine

Goal

Accurately confirm and quantitate 106 commonly analyzed drugs of abuse extracted from urine using the Thermo Scientific™ Vanquish™ Horizon ultra-high performance liquid chromatography (UHPLC) system coupled with the Thermo Scientific™ TSQ Quantis™ Plus mass spectrometer for forensic and clinical toxicology.

Application benefits

- A complete quantitative workflow for 106 drugs of abuse in urine using the TSQ Quantis Plus mass spectrometer
- A robust and reliable workflow for clinical and forensic toxicology coupled with the sensitivity and reproducibility of triple quadrupole technology
- Highly reproducible extraction with the Thermo Scientific™ SOLAμ™ SCX SPE plates using minimal sample and solvent volumes

Introduction

As the number of drug users has risen 23% worldwide between 2011 and 2021, toxicology labs are analyzing a growing number of samples each year.[1] Robustness and sensitivity are keys to producing reliable and accurate data day after day in high-throughput drug quantitation settings. Moreover, due to growing numbers of drug abusers and overdoses from a wide variety of drug classes, it is necessary to be able to detect and identify drugs and metabolites of varying hydrophilicities and chemical structures, while producing baseline separation of isomers.

In this technical note, we highlight the benefits of using this drug of abuse method coupled with the TSQ Quantis Plus mass spectrometer to achieve reliable and robust data in the quantitation and confirming of 106 drugs of abuse. With the TSQ Quantis Plus mass spectrometer, users can benefit from the 600 SRM/s rate and improved sensitivity, allowing even lower detection levels. Here we present a high-throughput

method for the quantitation of the drugs and their metabolites in urine with a complete sample preparation workflow. This method provides the tools necessary to analyze 106 drugs of abuse from many drug classes in just one method, thereby increasing throughput in the lab.

Experimental

Calibration standards and control samples

A set of 106 non-labeled standard drugs, chosen based on high frequency testing in forensic and clinical labs, was separated into six mixes. A 15-point calibration curve ranging from 0.1 ng/mL to 5,000 ng/mL was prepared by serial dilution in negative human urine. Corresponding internal standards were prepared into six separate mixes of working internal standard solution. 20 µL of 2% formic acid in water and 20 µL of the corresponding internal standard working solution were added to 200 µL of each calibration level, creating internal standard concentrations of 125 ng/mL.

Solid phase extraction

Samples were extracted using SOLAµ SCX SPE plates (P/N 60209-002). Plates were equilibrated with 100 µL of elution solvent (47.5% ACN, 47.5% MeOH, 5% NH_4OH) followed by 100 µL of MeOH and finally conditioned with 100 µL of 2% formic acid. The 240 µL of prepared sample and internal standards were loaded onto the plate. The plate was washed with 100 µL of 2% formic acid. Elution A was performed with 2 x 20 µL of H_2O/MeOH (50:50) into collection wells containing 80 µL of 2% formic acid. The plates were washed once more with 100 µL MeOH. Elution B was performed with 2 x 20 µL of elution solvent into the same collection wells as elution A. Samples were thoroughly mixed on a plate vortexer to ensure adequate homogeneity of the samples and then put directly into the LC.

Liquid chromatography

Analytes were separated with the Vanquish Horizon UHPLC system by a 7 min gradient using a Thermo Scientific™ Accucore™ Vanquish™ C18+ column (1.5 µm, 50 x 2.1 mm, P/N 27101-052130). A strong solvent loop was installed before the column to mitigate the effect of injecting high-organic extracts and to sharpen early-eluting peaks.[2]

Mobile phases A and B consisted of 2 mM ammonium formate with 0.01% formic acid in water and ACN:MeOH (50:50), respectively. 5 µL of each standard were injected in triplicate and chromatographic separation was accomplished using the gradient conditions in Table 1.

Table 1. LC gradient

Time (min)	Flow rate (mL/min)	% A	% B	Curve
0.000	0.5	97	3	5
0.200	0.5	97	3	5
4.250	0.5	1	99	7
5.250	0.5	1	99	5
5.251	0.5	97	3	5
7.000	0.5	97	3	5

Mass spectrometry

The TSQ Quantis Plus mass spectrometer was used for analyte detection and operated in Selected Reaction Monitoring (SRM) mode. Figure 1 shows the instrument set-up with the Vanquish Horizon UHPLC system and TSQ Quantis Plus mass spectrometer. The source and mass spectrometer settings are listed in Table 2. The SRM transitions and compound specific collision energies and RF lens voltages are listed in Appendix 1. A total of 315 transitions were used. The dwell time per transition and the number of transitions per cycle are shown in Figure 2.

Data analysis

Quantitative data for the 106 analytes were processed in Thermo Scientific™ TraceFinder™ 5.2 software.

Table 2. TSQ Quantis Plus MS source and MS settings

Parameter	Value
Source settings	
Positive ion	3,500 V
Negative ion	2,500 V
Sheath gas	55 AU
Aux gas	10 AU
Sweep gas	1 AU
Ion transfer tube temperature	325 °C
Vaporizer temperature	350 °C
Source position	1.2, L/M
MS settings	
Cycle time	0.6
Q1 resolution (FWHM)	0.7
Q3 resolution (FWHM)	1.2
Intensity threshold	5.0e4
Dynamic exclusion	1 time, 2 s, ≤5 ppm
CID gas (mTorr)	1.5
Chromatographic peak width	3

Figure 1. TSQ Quantis Plus mass spectrometer and Vanquish Horizon UHPLC system

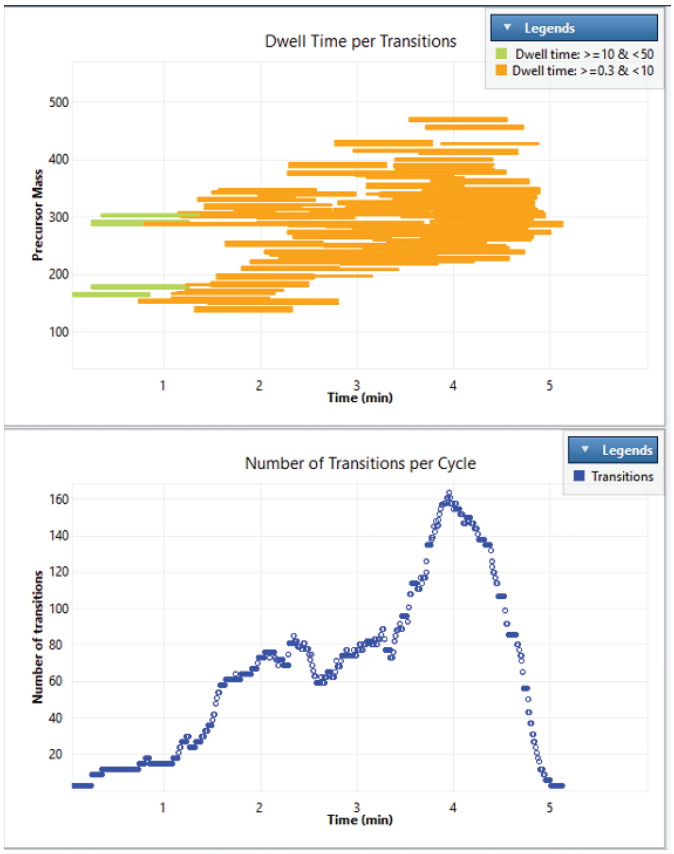

Figure 2. Dwell times of each transition and number of transitions per cycle. The minimum dwell time for any transition was 1.5 ms.

Results and discussion

An overlay of the extracted ion chromatograms is shown in Figure 3, and the baseline separation of selected isomers is depicted in Figure 4. A total of 106 drugs of abuse from various drug classes were successfully detected and quantitated in a single method from the extracted urine samples. The details of calibration and confirmation parameters are listed in Table 3 for each compound. The limit of quantitation (LOQ) for each analyte was determined as the lowest calibration value with % RSD and % Diff as < ±20% for three replicate injections of calibrators. The upper limit of linearity (ULOL) is defined as the highest calibrator level that achieved linearity for the calibration curve. Limit of confirmation (LOC) is defined as the lowest calibration level that produces an ion ratio within 20% of target value.

Calibrations curves for all drugs achieved an R^2 above 0.99. LOQs as low as 0.1 ng/mL were obtained for doxylamine, N-desmethylzopiclone, norketamine, and zopiclone, which far surpasses the industry cutoffs of 10 ng/mL for these drugs. Table 4 includes the quantitative results for the entire panel. Out of the 106 drugs, 39 drugs achieved LOQs lower than 1 ng/mL. Chromatograms of representative compounds at their respective LOQs along with calibrations curves can be seen in Figure 5.

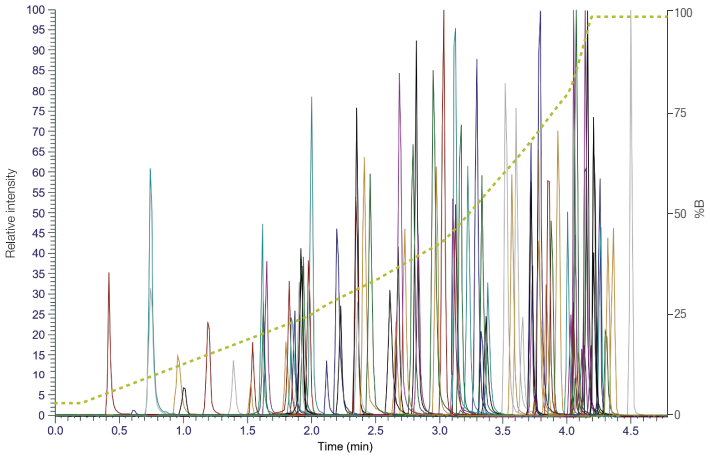

Figure 3. SRM chromatograms of 106 drugs of abuse with the mobile phase gradient overlaid

Table 3. Limits and criteria assigned in TraceFinder software

Parameters	Criteria
Limit of quantitation (LOQ)	Back-calculated concentration on calibration curve within 20%
Limit of confirmation (LOC)	Ion ratio confirmation within 20% (relative) of target value
Upper limit of linearity (ULOL)	Highest calibrator that achieves linearity

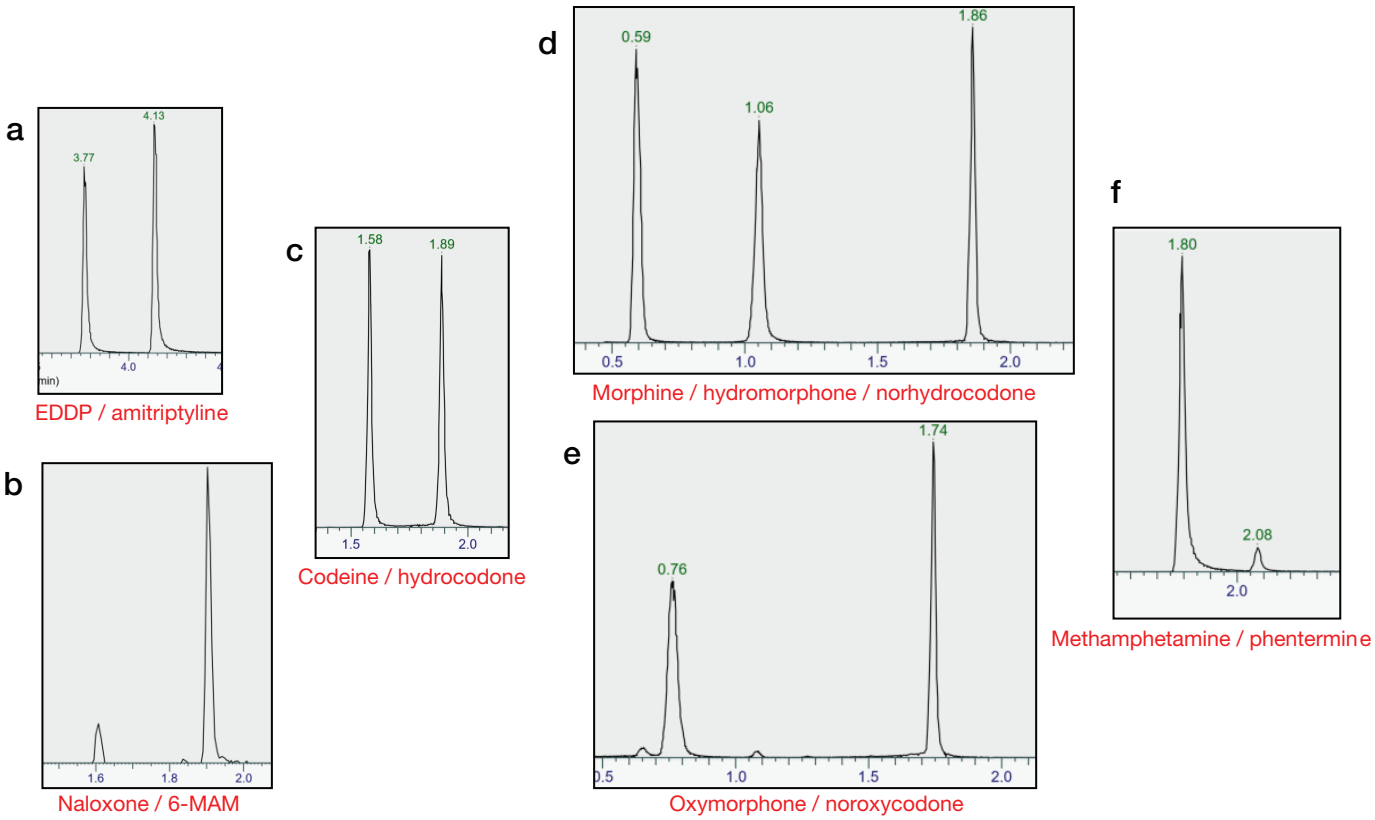

Figure 4. Separation of isomers (a) EDDP and amitriptyline, (b) naloxone and 6-MAM, (c) codeine and hydrocodone, (d) morphine, hydromorphone, and norhydrocodone, (e) oxymorphone and noroxycodone, (f) methamphetamine and phentermine.

NOTE: Methylphenidate and normeperidine also separated but are not shown.

Table 4. Calibration and confirmation results of the 106 analytes in urine. LOQ, ULOL, and LOC are in units of ng/mL. L=Linear curve, Q=Quadratic curve

Compound	*m/z*	t_R (min)	Polarity	LOQ	ULOL	R^2	CAL type	LOC
1-(3-Chlorophenyl)piperazine*	197.084	2.65	+	2.5	5000	0.9987	L	0.25
25I-NBOMe*	428.072	4.37	+	2.5	5000	0.9985	Q	1
2-hydroxyethylflurazepam	333.080	4.35	+	0.25	2500	0.9977	L	2.5
6-acetylmorphine	328.154	2.05	+	0.25	5000	0.9993	L	0.25
6-beta-Naltrexol	344.186	2.07	+	0.25	5000	0.9993	L	0.25
7-aminoclonazepam	286.074	2.79	+	1	2500	0.9995	Q	1
7-aminoflunitrazepam	284.119	3.18	+	0.5	2500	0.9994	Q	1
9-hydroxyrisperidone	427.214	3.27	+	5	2500	0.9995	Q	0.5
Acetaminophen	152.071	1.24	+	10	5000	0.9969	L	1
a-hydroxyalprazolam	325.085	4.27	+	2.5	5000	0.9988	Q	2.5
a-hydroxymidazolam	342.080	4.18	+	1	5000	0.9983	Q	2.5
a-hydroxytriazolam	359.046	4.27	+	5	1000	0.9959	L	10
Alprazolam	309.090	4.36	+	0.5	5000	0.9982	Q	1
Amitriptyline	278.190	4.26	+	5	5000	0.9995	L	2.5
Amobarbital	225.125	4.04	-	25	5000	0.9940	L	50
Amphetamine	136.112	1.82	+	5	2500	0.9919	L	25
Benzoylecgonine	290.139	2.46	+	0.25	2500	0.9991	Q	0.25
Buprenorphine	468.311	4.04	+	1	500	0.9970	L	2.5
Bupropion	240.115	3.27	+	0.25	5000	0.9989	L	0.1
Butalbital	223.109	3.70	-	25	5000	0.9980	Q	25

Table 4. Calibration and confirmation results of the 106 analytes in urine. LOQ, ULOL, and LOC are in units of ng/mL. L=Linear curve, Q=Quadratic curve *(Continued)*

Compound	*m/z*	t_R (min)	Polarity	LOQ	ULOL	R^2	CAL type	LOC
Carbamazepine	237.102	4.06	+	0.5	2500	0.9988	Q	2.5
Carisoprodol	261.181	4.29	+	10	2500	0.9989	Q	5
Chlordiazepoxide	300.090	3.96	+	2.5	2500	0.9971	Q	10
Chlorpheniramine	275.131	3.52	+	1	5000	0.9996	Q	0.25
Citalopram	325.171	3.87	+	0.5	2500	0.9996	Q	1
Clonazepam	316.048	4.22	+	5	2500	0.9980	L	2.5
Clozapine	327.137	3.88	+	2.5	2500	0.9988	Q	2.5
Cocaethylene	318.170	3.38	+	0.25	5000	0.9994	Q	0.1
Cocaine	304.154	3.01	+	5	5000	0.9994	Q	2.5
Codeine	300.159	1.68	+	2.5	5000	0.9995	L	0.5
Cotinine	177.102	0.75	+	5	5000	0.9994	Q	5
Cyclobenzaprine	276.175	4.20	+	1	5000	0.9996	Q	0.5
Desipramine	267.187	4.22	+	5	5000	0.9996	L	1
Dextromethorphan	272.201	3.75	+	2.5	5000	0.9996	Q	1
Diazepam	285.079	4.62	+	2.5	5000	0.9995	Q	1
Dihydrocodeine	302.175	1.65	+	0.25	5000	0.9982	Q	1
Diphenhydramine	256.170	3.82	+	5	2500	0.9993	Q	5
Doxepin	280.170	3.92	+	2.5	2500	0.9992	Q	1
Doxylamine	271.181	2.78	+	0.1	5000	0.9978	L	0.25
EDDP	278.190	3.93	+	2.5	5000	0.9987	L	0.25
Fentanyl	337.227	3.74	+	0.25	5000	0.9995	L	0.25
Fluoxetine	310.141	4.34	+	10	5000	0.9994	L	1
Flurazepam	388.159	3.88	+	0.5	5000	0.9971	L	0.5
Gabapentin	172.133	1.73	+	50	5000	0.9972	Q	25
Haloperidol	376.147	4.03	+	2.5	5000	0.9965	L	2.5
Hydrocodone	300.159	2.02	+	0.25	2500	0.9994	Q	0.5
Hydromorphone	286.144	1.30	+	5	5000	0.9969	L	5
Imipramine	281.201	4.21	+	2.5	5000	0.9996	Q	1
Isotonitazene	411.239	4.15	+	2.5	500	0.9916	L	25
Ketamine	238.099	2.54	+	0.5	5000	0.9981	L	2.5
Lorazepam	321.019	4.33	+	5	5000	0.9974	L	0.5
LSD	324.207	3.30	+	2.5	5000	0.9994	L	5
MDA	180.102	1.99	+	2.5	5000	0.9994	L	1
MDEA	208.133	2.30	+	1	1000	0.9995	Q	2.5
MDMA	194.118	2.04	+	0.5	5000	0.9983	L	5
Meperidine	248.165	3.16	+	1	5000	0.9995	L	5
Meprobamate	219.134	3.33	+	10	5000	0.9983	Q	0.25
Methadone	310.217	4.29	+	1	5000	0.9995	L	5
Methamphetamine	150.128	1.95	+	1	2500	0.9986	L	1
Methylphenidate	234.149	2.82	+	0.25	2500	0.9997	Q	0.5
Mitragynine	399.228	3.89	+	1	500	0.9902	L	1
Morphine	286.144	0.75	+	1	1000	0.9982	Q	2.5
Naloxone	328.154	1.85	+	0.5	2500	0.9979	Q	0.5
Naltrexone	342.170	2.00	+	2.5	5000	0.9994	Q	0.1
N-desmethyl-Tapentadol	208.170	2.92	+	0.25	5000	0.9988	L	0.25
N-desmethylzopiclone	375.097	2.78	+	0.1	1000	0.9991	L	1
Nicotine	163.123	0.44	+	2.5	5000	0.9996	L	1
Norbuprenorphine	414.264	3.46	+	0.5	1000	0.9989	Q	1

Table 4. Calibration and confirmation results of the 106 analytes in urine. LOQ, ULOL, and LOC are in units of ng/mL. L=Linear curve, Q=Quadratic curve *(Continued)*

Compound	*m/z*	t_R (min)	Polarity	LOQ	ULOL	R^2	CAL type	LOC
Norcyclobenzaprine	262.160	4.17	+	2.5	5000	0.9997	Q	2.5
Nordiazepam	271.063	4.50	+	1	2500	0.9982	Q	0.1
Norfentanyl	233.165	2.60	+	0.25	2500	0.9990	Q	0.1
Norfluoxetine	296.126	4.31	+	5	5000	0.9996	Q	5
Norhydrocodone	286.144	2.02	+	0.25	5000	0.9992	L	2.5
Norketamine	224.084	2.69	+	0.1	5000	0.9995	L	0.25
Normeperidine	234.149	3.12	+	0.25	5000	0.9993	L	1
Noroxycodone	302.139	1.88	+	1	5000	0.9995	L	0.25
Nortriptyline	264.175	4.31	+	2.5	5000	0.9990	Q	5
O-desmethyltramadol	250.180	2.13	+	0.5	2500	0.9997	Q	0.5
Olanzapine	313.148	2.23	+	0.25	5000	0.9977	Q	0.1
Oxazepam	287.058	4.26	+	5	2500	0.9985	Q	0.25
Oxycodone	316.154	1.92	+	0.25	2500	0.9950	L	1
Oxymorphone	302.139	0.85	+	0.5	5000	0.9993	Q	1
Paroxetine	330.150	4.15	+	1	5000	0.9981	L	1
Pentobarbital	225.125	4.06	-	25	5000	0.9960	L	0.5
Phencyclidine	244.206	3.56	+	0.5	5000	0.9988	Q	5
Phenobarbital	231.078	3.32	-	10	5000	0.9928	L	1
Phentermine	150.128	2.29	+	5	5000	0.9989	Q	0.05
Phenytoin	251.083	4.12	-	50	5000	0.9926	Q	100
Pregabalin	160.133	1.58	+	1	5000	0.9986	L	1
Propranolol	260.165	3.67	+	0.5	1000	0.9996	Q	0.5
Pseudoephedrine	166.123	1.64	+	0.25	5000	0.9997	L	0.25
Quetiapine	384.174	3.90	+	5	1000	0.9968	L	1
Ritalinic Acid	220.133	2.40	+	10	5000	0.9990	Q	1
Secobarbital	237.125	4.22	-	25	5000	0.9923	L	25
Sertraline	306.081	4.42	+	10	5000	0.9996	L	1
Tapentadol	222.185	2.94	+	0.5	5000	0.9996	L	0.25
Temazepam	301.074	4.44	+	0.25	5000	0.9984	L	0.25
Topiramate	340.106	3.60	+	10	5000	0.9947	L	5
Tramadol	264.196	2.84	+	1	5000	0.9980	L	5
Trazodone	372.159	3.49	+	2.5	5000	0.9990	L	0.1
Triazolam	343.051	4.38	+	2.5	5000	0.9993	Q	1
Venlafaxine	278.212	3.45	+	0.5	1000	0.9995	L	1
Verapamil	455.290	4.21	+	10	2500	0.9989	Q	1
Zolpidem	308.176	3.26	+	0.5	5000	0.9989	Q	0.1
Zolpidem-COOH	338.150	2.47	+	0.25	2500	0.9995	Q	0.25
Zopiclone	389.112	2.79	+	0.1	5000	0.9987	L	0.5

*Each target analyte used its own labeled internal standard except for 1-(3-Chlorophenyl)piperazine, 25I-NBOMe, and N-desmethyl-Tapentadol, which used the internal standards Norketamine-d4, Chlordiazepoxide-d5, and Zolpidem-COOH-d4, respectively.

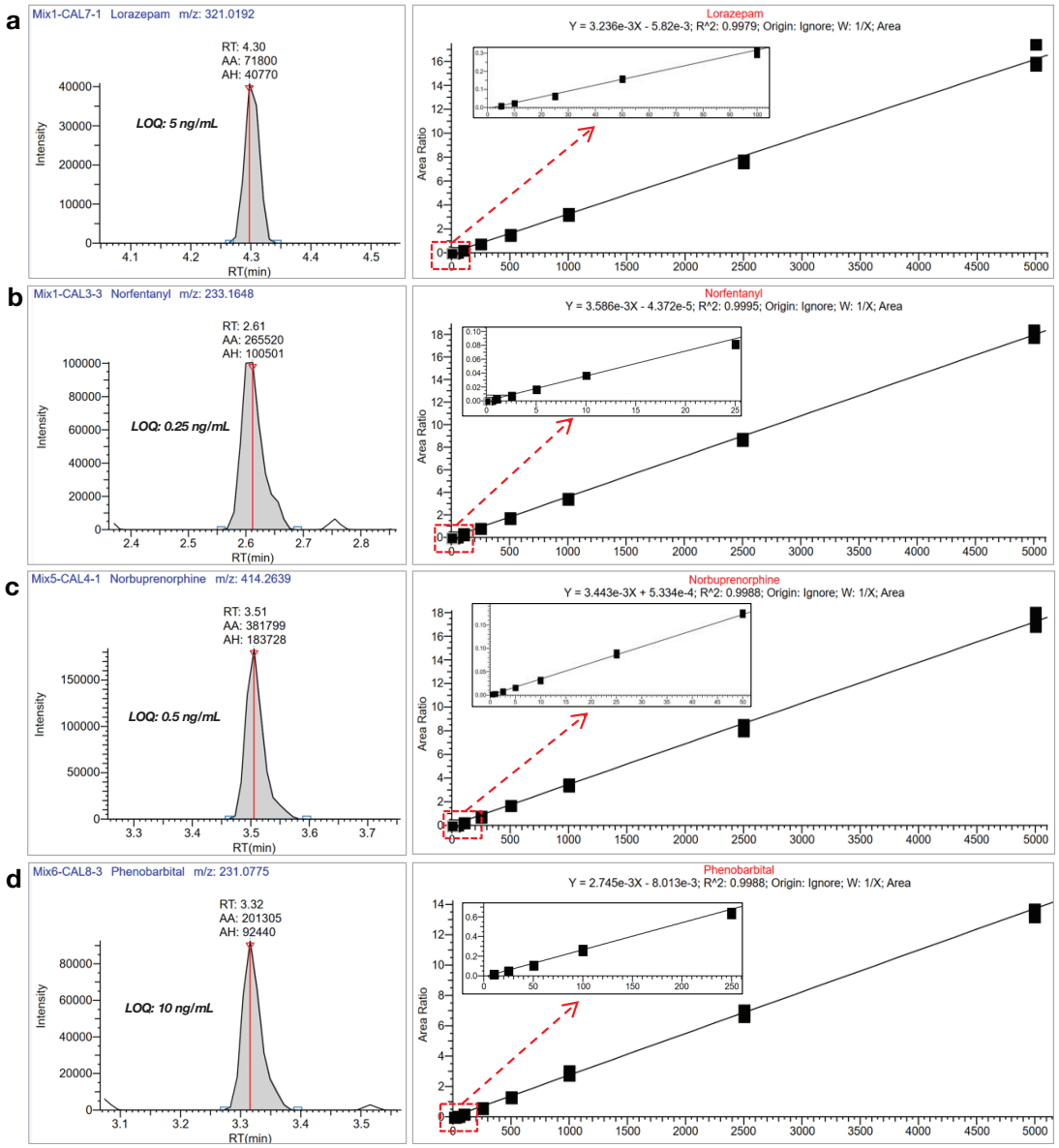

Figure 5. SRM chromatograms of (a) lorazepam, (b) norfentanyl, (c) norbuprenorphine, and (d) phenobarbital at their respective LOQ levels along with calibration curves

Conclusions

This LC-MS/MS method provides a complete solution for drugs of abuse testing in a single run. Developed around 106 of the most frequently tested drugs of abuse, this method works for a wide range of drug classes, allows for polarity switching, and produces baseline separation of 15 common isomers. A complete workflow was presented that involved sample preparation using SOLAμ SCX SPE plates followed by injection on the Vanquish Horizon UHPLC system and TSQ Quantis Plus mass spectrometer. Using the TSQ Quantis Plus mass spectrometer, sensitivity and reproducibility were observed in low LOQs with many of the analytes producing LOQs of less than 1 ng/mL. This method also demonstrates the wide dynamic range achievable with this mass spectrometer with linearity between LOQs as low as 0.1 ng/mL and ULOLs up to 5,000 ng/mL.

This 7-minute method is highly flexible for toxicology and clinical research labs. Because it was built to separate and identify many different drug classes and isomers, compounds can easily be added. Furthermore, TraceFinder software allows the compound parameters in the method as well as the curated compound database to be changed with only a few clicks, making this method very customizable.

References

1. United Nations Office on Drugs and Crime: Special Points of Interest; 2023.
2. Thermo Fisher Scientific Technical Note 74138: Evaluation of custom injection programs and larger internal diameter capillary for strong solvent sample effects mitigation in liquid chromatography. https://assets.thermofisher.com/TFS-Assets/CMD/Technical-Notes/tn-74138-hplc-custom-injection-programs-tn74138-en.pdf

Appendix 1. MS parameters for all target analytes and internal standards

Compound	Precursor *(m/z)*	Product (*m/z*)	Collision energy (V)	RF lens (V)
1-(3-Chlorophenyl)piperazine	197.08	154.04	20	100
		118.07	34	
25I-NBOMe	428.07	91.05	41	173
		121.06	23	
2-hydroxyethylflurazepam	333.08	109.04	30	187
		211.08	37	
2-hydroxyethylflurazepam-d4	337.11	113.07	30	187
6-acetylmorphine	328.15	165.07	38	184
		211.08	25	
6-acetylmorphine-d3	331.17	165.07	38	184
6-beta-Naltrexol	344.19	308.16	26	150
		254.12	32	
6-beta-Naltrexol-d3	347.30	329.10	22	140
7-aminoclonazepam	286.07	222.10	25	175
		121.08	32	
7-aminoclonazepam-d4	290.10	226.13	25	175
7-aminoflunitrazepam	284.12	135.09	28	176
		227.10	25	
7-aminoflunitrazepam-d7	291.16	138.11	28	176
9-hydroxyrisperidone	427.21	207.11	27	195
		110.06	42	
9-hydroxyrisperidone-d4	431.30	211.10	28	185
a-hydroxyalprazolam	325.09	279.07	24	192
		297.07	26	
a-hydroxyalprazolam-d5	330.12	302.10	26	192
a-hydroxymidazolam	342.08	203.04	27	195
		324.07	22	
a-hydroxymidazolam-d4	346.20	328.10	21	163
a-hydroxytriazolam	359.05	176.03	27	185
		331.03	28	
a-hydroxytriazolam-37Cl-d4	365.15	336.97	28	185
Acetaminophen	152.07	110.06	17	88
		65.04	31	
Acetaminophen-d4	156.10	114.09	17	88
Alprazolam	309.09	281.07	27	165
		205.08	41	
Alprazolam-d5	314.12	286.10	27	165
Amitriptyline	278.19	91.05	26	161
		233.13	18	
Amitriptyline-d3	281.21	233.13	18	161
Amobarbital	225.12	42.00	18	105
		182.12	13	
Amobarbital-d5	230.16	187.15	13	105
Amphetamine	136.11	91.05	18	65
		119.09	9.5	
Amphetamine-d5	141.14	96.09	18	65
Benzoylecgonine	290.14	168.10	20	149
		105.03	31	

Appendix 1. MS parameters for all target analytes and internal standards *(Continued)*

Compound	Precursor *(m/z)*	Product *(m/z)*	Collision energy (V)	RF lens (V)
Benzoylecgonine-d8	298.19	171.12	20	149
Buprenorphine	468.31	55.04	47	234
		396.21	39	
Buprenorphine-d4	472.34	400.24	39	234
Bupropion	240.12	184.05	13	89
		131.07	27	
Bupropion-d9	249.20	184.97	13	83
Butalbital	223.11	180.10	11	100
		42.00	17	
Butalbital-d5	228.14	185.13	11	100
Carbamazepine	237.10	194.10	19	117
		192.08	23	
Carbamazepine-d10	247.20	204.10	21	115
Carisoprodol	261.18	176.13	9	80
		97.10	16	
Carisoprodol-d7	268.23	183.17	9	80
Chlordiazepoxide	300.09	227.05	25	218
		282.10	25	
Chlordiazepoxide-d5	305.20	232.00	25	112
Chlorpheniramine	275.13	167.05	39	91
		230.07	17	
Chlorpheniramine-d6	281.20	230.10	17	92
Citalopram	325.17	109.05	27	162
		262.10	20	
Citalopram-d6	331.21	109.05	27	162
Clonazepam	316.05	270.06	26	185
		214.04	38	
Clonazepam-d4	320.10	274.00	25	170
Clozapine	327.13	192.07	44	162
		270.08	24	
Clozapine-d4	331.20	272.10	23	155
Cocaethylene	318.17	196.13	20	157
		82.07	30	
Cocaethylene-d8	326.30	204.10	20	133
Cocaine	304.15	182.12	20	152
		82.07	29	
Cocaine-d3	307.17	85.09	29	152
Codeine	300.16	152.06	61	219
		165.07	40	
Codeine-d6	306.30	152.00	67	167
Cotinine	177.10	80.05	24	128
		98.06	21	
Cotinine-d3	180.12	80.05	24	128
Cyclobenzaprine	276.18	215.09	42	138
		216.09	25	
Cyclobenzaprine-d3	279.19	215.09	42	138
Desipramine	267.19	72.08	16	122
		193.09	38	

Appendix 1. MS parameters for all target analytes and internal standards *(Continued)*

Compound	Precursor *(m/z)*	Product (*m/z*)	Collision energy (V)	RF lens (V)
Desipramine-d3	270.20	75.10	16	122
Dextromethorphan	272.20	171.08	38	123
		215.14	24	
Dextromethorphan-d3	275.22	171.08	38	123
Diazepam	285.08	154.04	27	218
		193.09	32	
Diazepam-d5	290.11	198.12	32	218
Dihydrocodeine	302.18	199.08	33	172
		128.06	61	
Dihydrocodeine-d6	308.30	202.10	33	168
Diphenhydramine	256.20	167.00	13	69
		165.00	40	
Diphenhydramine-d3	259.20	167.00	13	70
Doxepin	280.17	107.05	23	136
		235.11	17	
Doxepin-d3	283.19	107.05	23	136
Doxylamine	271.18	167.00	34	86
		182.00	16	
Doxylamine-d5	276.20	187.10	16	84
EDDP	278.19	234.13	31	175
		249.15	24	
EDDP-d3	281.21	234.13	31	175
Fentanyl	337.23	105.07	37	182
		188.14	24	
Fentanyl-d5	342.26	188.14	24	182
Fluoxetine	310.14	44.05	12	105
		148.11	9.5	
Fluoxetine-d6	316.10	154.10	9	87
Flurazepam	388.16	315.07	23	190
		317.09	19	
Flurazepam-d4	392.20	319.10	23	150
Gabapentin	172.13	95.09	23	89
		137.10	16.5	
Gabapentin-d10	182.196	147.16	16.5	89
Haloperidol	376.15	123.02	38	178
		165.07	23	
Haloperidol-d4	380.20	127.00	39	148
Hydrocodone	300.16	199.08	29	227
		128.06	55	
Hydrocodone-d6	306.20	202.10	29	227
Hydromorphone	286.14	185.06	31	217
		157.07	42	
Hydromorphone-d3	289.10	185.00	30	180
Imipramine	281.20	58.07	33	126
		86.10	17	
Imipramine-d3	284.22	89.12	17	126
Isotonitazene	411.30	100.10	22	160
		72.00	36	

Appendix 1. MS parameters for all target analytes and internal standards *(Continued)*

Compound	Precursor *(m/z)*	Product *(m/z)*	Collision energy (V)	RF lens (V)
Isotonitazene 13C6	417.30	100.10	22	162
Ketamine	238.10	125.02	29	102
		179.06	16	
Ketamine-d4	242.12	129.04	29	102
Lorazepam	321.02	275.01	22	185
		229.05	31	
Lorazepam-37Cl-d4	327.07	280.97	22	127
LSD	324.21	223.12	25	147
		207.09	44	
LSD-d3	327.30	226.10	24	138
MDA	180.10	105.07	23	72
		135.04	19	
MDA-d5	185.13	110.10	23	72
MDEA	208.13	105.07	26	94
		163.08	13	
MDEA-d5	213.17	163.08	13	94
MDMA	194.12	105.07	25	86
		163.08	13	
MDMA-d5	199.15	107.08	25	86
Meperidine	248.17	174.13	21	144
		220.13	22	
Meperidine-d4	252.19	224.16	22	144
Meprobamate	219.13	97.10	14	66
		158.12	9	
Meprobamate-d3	222.15	161.14	9	66
Methadone	310.22	105.03	28	124
		265.16	15.5	
Methadone-d3	313.24	268.18	15.5	124
Methamphetamine	150.13	91.05	19	79
		119.09	11.5	
Methamphetamine-d5	155.16	92.06	19	79
Methylphenidate	234.15	84.08	20	115
		56.05	43	
Methylphenidate-d4	238.20	88.00	20	106
Mitragynine	399.23	174.09	29	185
		226.14	22	
Mitragynine-d3	402.30	177.10	31	184
Morphine	286.14	152.06	58	214
		165.07	38	
Morphine-d6	292.18	165.07	38	214
Naloxone	328.15	212.07	38	179
		268.13	27	
Naloxone-d5	333.19	212.07	38	179
Naltrexone	342.17	270.11	27	160
		267.13	29	
Naltrexone-d3	345.19	270.11	27	160
N-desmethyl-Tapentadol	208.20	107.00	24	96
		121.00	19	

Appendix 1. MS parameters for all target analytes and internal standards *(Continued)*

Compound	Precursor *(m/z)*	Product (*m/z*)	Collision energy (V)	RF lens (V)
N-desmethylzopiclone	375.10	245.02	18	94
		331.11	10	
N-desmethylzopiclone-d4	379.20	245.00	18	91
Nicotine	163.12	130.07	21	101
		117.06	27	
Nicotine-d4	167.15	134.09	21	101
Norbuprenorphine	414.26	115.04	86	225
		83.04	46	
Norbuprenorphine-d3	417.28	83.04	46	225
Norcyclobenzaprine	262.20	215.00	38	120
		231.10	16	
Norcyclobenzaprine-d3	265.10	215.00	39	122
Nordiazepam	271.06	140.03	28	185
		208.10	28	
Nordiazepam-d5	276.10	140.03	28	185
Norfentanyl	233.17	84.08	18	125
		56.05	27	
Norfentanyl-d5	238.20	84.08	18	125
Norfluoxetine	296.13	134.10	7.5	88
		30.03	12	
Norfluoxetine-d6	302.13	140.05	7.58	73
Norhydrocodone	286.14	199.08	29	186
		128.06	56	
Norhydrocodone-d3	289.16	202.09	29	186
Norketamine	224.08	125.02	27	97
		179.06	15	
Norketamine-d4	228.16	129.01	26	80
Normeperidine	234.15	56.05	22	123
		160.11	14.5	
Normeperidine-d4	238.17	164.14	14.5	123
Noroxycodone	302.14	284.13	17	151
		187.08	25	
Noroxycodone-d3	305.20	287.10	16	109
Nortriptyline	264.18	91.05	23	128
		233.13	15.5	
Nortriptyline-d3	267.19	233.13	15.5	128
O-desmethyltramadol	250.18	58.05	17	100
		42.05	70	
O-desmethyltramadol-d6	256.22	64.09	17	100
Olanzapine	313.15	198.02	41	148
		256.09	23	
Olanzapine-d8	321.20	261.10	24	152
Oxazepam	287.06	241.05	23	187
		104.05	35	
Oxazepam-d5	292.09	246.08	23	187
Oxycodone	316.15	241.11	29	167
		256.13	26	
Oxycodone-d6	322.19	262.17	26	167

Appendix 1. MS parameters for all target analytes and internal standards *(Continued)*

Compound	Precursor *(m/z)*	Product (*m/z*)	Collision energy (V)	RF lens (V)
Oxymorphone	302.14	227.09	28	141
		198.09	45	
Oxymorphone-d3	305.16	230.11	28	141
Paroxetine	330.15	192.12	21	157
		70.07	28	
Paroxetine-d6	336.20	198.10	21	149
Pentobarbital	225.12	42.00	18	105
		182.12	13	
Pentobarbital-d5	230.10	187.10	13	95
Phencyclidine	244.21	86.10	12	81
		91.05	31	
Phencyclidine-d5	249.43	86.10	12	81
Phenobarbital	231.08	42.00	16	105
		188.07	10	
Phenobarbital-d5	236.11	193.10	10	105
Phentermine	150.13	91.05	20	61
		65.04	40	
Phentermine-d5	155.16	96.08	20	61
Phenytoin	251.10	208.00	17	152
		102.00	22	
Phenytoin-d10	261.10	106.00	22	160
Pregabalin	160.13	55.05	22	83
		83.09	17	
Pregabalin-d6	166.17	103.14	16	83
Propranolol	260.20	116.10	18	115
		183.10	18	
Propranolol-d7	267.30	116.10	19	130
Pseudoephedrine	166.12	115.05	27	61
		117.07	20	
Pseudoephedrine-d3	169.10	115.00	28	63
Quetiapine	384.17	221.11	38	157
		253.08	23	
Quetiapine-d8	392.30	258.10	23	171
Ritalinic Acid	220.13	84.08	20	95
		56.05	39	
Ritalinic Acid-d10	224.20	88.10	20	95
Secobarbital	237.12	42.00	21	107
		194.12	12	
Secobarbital-d5	242.16	199.15	12	107
Sertraline	306.08	158.98	28	89
		275.04	13	
Sertraline-d3	309.20	158.90	28	80
Tapentadol	222.19	107.05	26	136
		77.04	46	
Tapentadol-d3	225.20	107.05	26	136
Temazepam	301.07	255.07	23	174
		177.02	39	
Temazepam-d5	306.11	260.10	23	174

Appendix 1. MS parameters for all target analytes and internal standards *(Continued)*

Compound	Precursor *(m/z)*	Product *(m/z)*	Collision energy (V)	RF lens (V)
Topiramate	357.20	282.00	12	88
		264.00	15	
Topiramate-d12	369.30	270.10	15	91
Tramadol	264.20	58.00	17	103
		42.05	15	
Tramadol-13C-d3	268.20	42.05	15	103
Trazodone	372.16	148.05	33	183
		176.08	24	
Trazodone-d6	378.20	182.10	24	171
Triazolam	343.05	308.08	26	187
		239.04	43	
Triazolam-37Cl-d4	349.15	314.05	27	186
Venlafaxine	278.21	58.07	20	102
		121.06	28	
Venlafaxine-d6	284.30	64.10	21	96
Verapamil	455.29	165.09	27	210
		303.21	26	
Verapamil-13C3	458.40	165.10	27	209
Zolpidem	308.18	235.12	35	220
		263.12	26	
Zolpidem-d6	314.30	235.10	36	184
Zolpidem-COOH	338.15	265.10	37	205
		293.09	27	
Zolpidem-COOH-d4	342.20	269.10	36	197
Zopiclone	389.11	245.02	17	114
		217.03	33	
Zopiclone-d4	393.14	245.02	17	114