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Utilizing HRAM Orbitrap mass spectrometry to quantitate 106 drugs of abuse in urine

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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™ Orbitrap Exploris™ 120 mass spectrometer for forensic and clinical toxicology.

Application benefits

- A complete quantitative workflow for 106 drugs of abuse in urine using the Orbitrap Exploris 120 mass spectrometer
- Assay accuracy and specificity by utilizing the high resolution and mass accuracy of Thermo Scientific™ Orbitrap™ technology to screen and confirm compounds in biological matrices
- Thermo Scientific™ SOLAμ™ SCX SPE plates produce highly reproducible data using small amounts of sample and solvent volumes

INTRODUCTION

According to the United Nations Office on Drugs and Crime report, half a million deaths globally in 2019 were attributed to drug overdoses, highlighting the critical need for accurate and reliable drug testing methods.[1] With the ever-growing number of abused drugs and the increase in overdoses, it is necessary to not only detect but also quantitate and confirm a wide range of analytes with a high level of confidence. It is also of great importance to develop a fast and high-throughput liquid chromatography mass spectrometry (LC-MS/MS) method that can accommodate many drugs of different hydrophilicities, polarities, and chemical structures and can produce baseline separation of isomers. The Orbitrap Exploris 120 mass spectrometer is a high-resolution mass spectrometry platform that offers sensitivity, selectivity, and mass accuracy in the detection and quantitation of drugs of abuse in biological matrices.

This technical note explores the Orbitrap technology in forensic and clinical toxicology for the detection, identification, and quantitation of drugs of abuse in urine. Here, we present a method for quantitative analysis of 106 drugs of abuse with a complete sample preparation workflow and a fast, 7-minute LC-MS/MS method using the Orbitrap Exploris 120 mass spectrometer. The 106 drugs chosen were based on the highest frequency drugs tested from numerous forensic and clinical labs surveyed from around the world.

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, were 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 Thermo Scientific™ 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), 100 µL of MeOH and 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 with elution A. Plates were mixed on a plate vortexer to ensure adequate homogeneity of the samples and then placed directly into the UHPLC system.

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 (P/N 6036.2200) was installed before the column to mitigate the effect of injecting high-organic extracts and to sharpen early-eluting peaks.[2]

Mobile phases consisted of 2 mM ammonium formate with 0.01% formic acid in water and ACN:MeOH (50:50) for mobile phase A and B, respectively. 5 µL of each standard were injected in triplicate and chromatographic separation were 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 Orbitrap Exploris 120 mass spectrometer was used for targeted screening and quantitation with full scan and targeted data-dependent MS/MS scanning used with a mass list for the 106 drugs. The mass list contained the exact mass of the drug, polarity, optimized collision energy, and retention time. A resolution setting of 60,000 (FWHM at *m/z* 200) was used for full scan and 15,000 resolution for the MS^2 scans. An isolation window of *m/z* 1.5 and compound specific collision energies were applied to generate the MS^2 spectra. Instrument setting parameters are listed in Table 2.

Table 2. Orbitrap Exploris 120 MS parameters

Parameter	Value
Source settings	
Positive ion	3,500 V
Negative ion	2,000 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
Full MS scan	
Resolution	60,000
Max injection time	Auto
Scan range	100-500 *m/z*
dd-MS²	
Top N	Positive (4), Negative (2)
Intensity threshold	5.0e4
Dynamic exclusion	1 time, 2 s, ≤5 ppm
Targeted mass list tolerance	≤ 5 ppm, ± 0.5 *m/z*
Isolation window	1.5 min
Collision energy	Compound specific
First mass	40
Resolution	15,000

Figure 1 shows the instrument set-up with the Vanquish Horizon UHPLC system and Orbitrap Exploris 120 mass spectrometer.

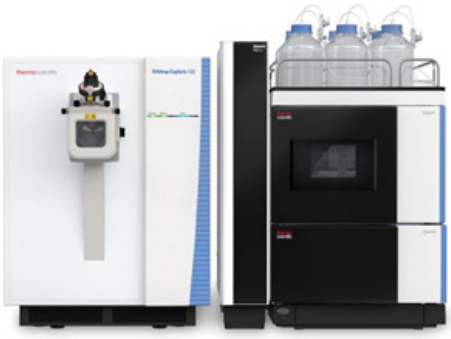

Figure 1. Orbitrap Exploris 120 mass spectrometer and Vanquish Horizon UHPLC system.

Data analysis

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

RESULTS AND DISCUSSION

The 106 drugs elute between 0.45 and 5 minutes (Figure 2) with baseline separation of isomeric pairs (Figure 3). The details of detection, calibration, and identification are listed in Table 3.

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 identification (LOI) is defined as the lowest calibration level with passing isotopic pattern, fragment ion, and library match scores.

The drug calibration and identification data are listed in Table 4. The data shows LOQs as low as 0.1 ng/mL for metabolites 1-(3-chlorophenyl)piperazine and norketamine. 89 out of the 106 drugs had upper limits of linearity (ULOL) of 5,000 ng/mL. All drugs had an R^2 above 0.99.

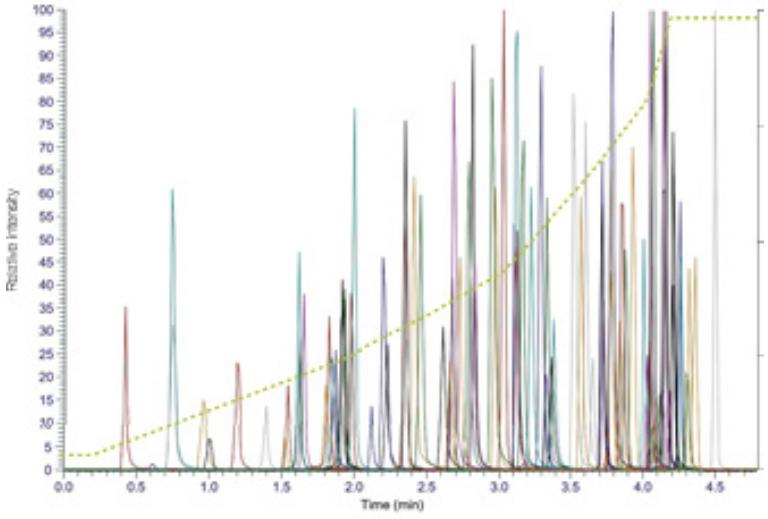

Figure 2. Extracted ion chromatogram of 106 drugs of abuse with the mobile phase gradient overlaid.

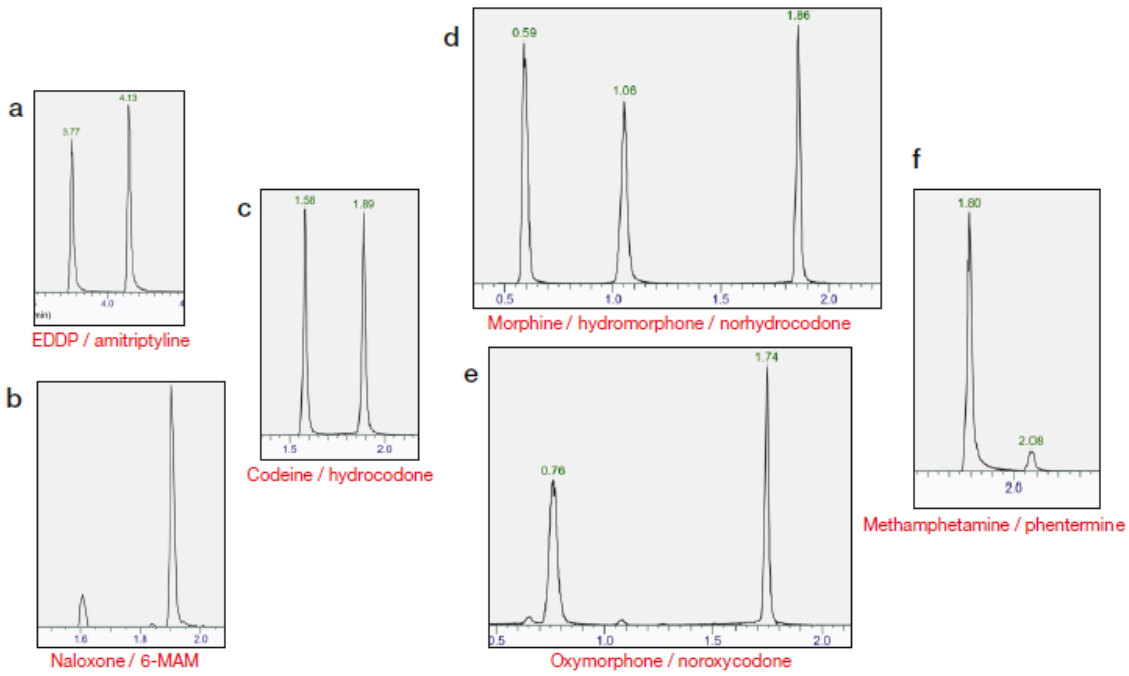

Figure 3. Separation of isomers (A) EDDP and amitriptyline, (B) naloxone and 6-MAM, (C) codeine and hydrocodone, (D) morphine, hydromorphone, and norhydrocodone, (E) oxymorphone and noroxycodone, (F) methamphetamine and phentermine. NOTE: Methylphenidate and normeperidine were also separated but are not shown.

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 identification (LOI)	IP = passing isotopic pattern score (70) FI = presence of diagnostic fragment ions LS = passing library score (70)
Upper limit of linearity (ULOL)	Highest calibrator that achieves linearity
Precursor ion	*m/z* <5 ppm mass deviation
Retention time	30 s window
Isotopic pattern (IP)	<10 ppm mass deviation, <20% intensity deviation, fit >70%
Fragment ion (FI)	At least 2 fragments with <10 ppm mass deviation in MS^2 spectra
mzVault™ HRAM library (LS)	Reverse search with >70% match of $ddMS^2$ spectra

Table 4. Calibration and confirmation results of the 106 analytes in urine. LOQ, ULOL, and LOI 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	LOI
1-(3-Chlorophenyl)piperazine	197.0840	2.65	+	0.1	5000	0.9988	L	0.5
25I-NBOMe	428.0717	4.37	+	10	1000	0.9980	Q	2.5
2-hydroxyethylflurazepam	333.0801	4.35	+	0.25	5000	0.9970	L	2.5
6-acetylmorphine	328.1543	2.05	+	0.5	5000	0.9994	L	0.5
6-beta-Naltrexol	344.1856	2.07	+	1	5000	0.9994	L	0.5
7-aminoclonazepam	286.0742	2.79	+	5	2500	0.9988	Q	0.05

Table 4. Calibration and confirmation results of the 106 analytes in urine. LOQ, ULOL, and LOI 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	LOI
7-aminoflunitrazepam	284.1194	3.18	+	1	5000	0.9989	Q	0.05
9-hydroxyrisperidone	427.2140	3.27	+	10	2500	0.9985	Q	1
Acetaminophen	152.0706	1.24	+	25	5000	0.9988	Q	0.05
a-hydroxyalprazolam	325.0851	4.27	+	1	5000	0.9975	L	2.5
a-hydroxymidazolam	342.0804	4.18	+	2.5	5000	0.9980	Q	2.5
a-hydroxytriazolam	359.0461	4.27	+	2.5	5000	0.9969	Q	10*
Alprazolam	309.0902	4.36	+	1	5000	0.9987	Q	2.5
Amitriptyline	278.1903	4.26	+	5	5000	0.9998	L	2.5
Amobarbital	225.1245	4.04	-	5	5000	0.9990	L	5
Amphetamine	136.1121	1.82	+	1	5000	0.9976	L	2.5
Benzoylecgonine	290.1387	2.46	+	0.25	2500	0.9987	Q	0.05
Buprenorphine	468.3108	4.04	+	2.5	250	0.9962	Q	0.5*
Bupropion	240.1150	3.27	+	0.25	5000	0.9994	L	0.25
Butalbital	223.1088	3.70	-	10	5000	0.9996	L	10
Carbamazepine	237.1022	4.06	+	0.5	5000	0.9992	Q	1
Carisoprodol	261.1809	4.29	+	100	2500	0.9983	Q	50
Chlordiazepoxide	300.0898	3.96	+	0.5	2500	0.9970	Q	2.5
Chlorpheniramine	275.1310	3.52	+	2.5	5000	0.9994	Q	0.5
Citalopram	325.1711	3.87	+	0.5	5000	0.9981	Q	0.5
Clonazepam	316.0484	4.22	+	5	2500	0.9960	Q	2.5*
Clozapine	327.1371	3.88	+	2.5	2500	0.9984	Q	2.5*
Cocaethylene	318.1700	3.38	+	0.5	5000	0.9990	L	0.25
Cocaine	304.1543	3.01	+	2.5	5000	0.9996	L	0.5
Codeine	300.1594	1.68	+	2.5	5000	0.9988	L	2.5
Cotinine	177.1022	0.75	+	10	5000	0.9997	Q	0.05
Cyclobenzaprine	276.1747	4.20	+	0.5	5000	0.9999	Q	0.5
Desipramine	267.1856	4.22	+	10	5000	0.9994	L	1
Dextromethorphan	272.2009	3.75	+	5	2500	0.9995	Q	0.5
Diazepam	285.0789	4.62	+	2.5	5000	0.9970	Q	2.5
Dihydrocodeine	302.1751	1.65	+	0.5	5000	0.9974	Q	1
Diphenhydramine	256.1696	3.82	+	2.5	5000	0.9992	L	5
Doxepin	280.1696	3.92	+	0.25	5000	0.9992	L	2.5
Doxylamine	271.1805	2.78	+	5	5000	0.9991	Q	5*
EDDP	278.1903	3.93	+	2.5	5000	0.9995	L	2.5
Fentanyl	337.2274	3.74	+	0.25	5000	0.9980	L	0.25
Fluoxetine	310.1413	4.34	+	2.5	5000	0.9992	L	2.5
Flurazepam	388.1586	3.88	+	10	2500	0.9988	Q	5
Gabapentin	172.1332	1.73	+	5	5000	0.9980	Q	1
Haloperidol	376.1474	4.03	+	25	5000	0.9985	Q	25
Hydrocodone	300.1594	2.02	+	0.25	5000	0.9980	L	0.5
Hydromorphone	286.1438	1.30	+	1	5000	0.9997	L	1
Imipramine	281.2012	4.21	+	0.5	5000	0.9993	L	1
Isotonitazene	411.2391	4.15	+	5	500	0.9938	Q	2.5
Ketamine	238.0993	2.54	+	1	5000	0.9987	L	1

Table 4. Calibration and confirmation results of the 106 analytes in urine. LOQ, ULOL, and LOI 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	LOI
Lorazepam	321.0192	4.33	+	5	5000	0.9979	L	10*
LSD	324.2070	3.30	+	2.5	5000	0.9998	Q	0.5
MDA	180.1019	1.99	+	2.5	5000	0.9978	L	2.5
MDEA	208.1332	2.30	+	1	5000	0.9991	L	0.5
MDMA	194.1176	2.04	+	1	5000	0.9991	L	2.5
Meperidine	248.1645	3.16	+	1	5000	0.9994	L	0.5
Meprobamate	219.1339	3.33	+	25	5000	0.997	L	25
Methadone	310.2165	4.29	+	2.5	5000	0.9994	L	0.5
Methamphetamine	150.1277	1.95	+	0.5	5000	0.9990	L	0.1
Methylphenidate	234.1489	2.82	+	0.25	5000	0.9989	L	0.5
Mitragynine	399.2278	3.89	+	1	500	0.9946	Q	1*
Morphine	286.1438	0.75	+	0.25	5000	0.9994	L	1
Naloxone	328.1543	1.85	+	0.5	2500	0.9986	Q	0.5
Naltrexone	342.1700	2.00	+	1	5000	0.9994	Q	2.5
N-desmethyl-Tapentadol	208.1696	2.92	+	0.25	5000	0.9986	L	0.05
N-desmethylzopiclone	375.0967	2.78	+	1	500	0.9968	Q	5
Nicotine	163.1230	0.44	+	1	5000	0.9997	L	0.05
Norbuprenorphine	414.2639	3.46	+	0.5	5000	0.9988	L	5
Norcyclobenzaprine	262.1595	4.17	+	2.5	5000	0.9998	L	2.5
Nordiazepam	271.0633	4.50	+	0.5	5000	0.9915	L	1
Norfentanyl	233.1648	2.60	+	0.25	5000	0.9995	L	0.1
Norfluoxetine	296.1257	4.31	+	5	5000	0.9984	Q	10
Norhydrocodone	286.1438	2.02	+	0.5	5000	0.9980	L	0.25
Norketamine	224.0837	2.69	+	0.1	5000	0.9996	L	0.5
Normeperidine	234.1489	3.12	+	0.25	5000	0.9991	L	0.25
Noroxycodone	302.1387	1.88	+	1	5000	0.9968	L	2.5
Nortriptyline	264.1747	4.31	+	5	5000	0.9996	Q	2.5
O-desmethyltramadol	250.1802	2.13	+	0.5	5000	0.9998	L	0.05
Olanzapine	313.1481	2.23	+	0.5	5000	0.9988	Q	1
Oxazepam	287.0582	4.26	+	1	5000	0.9974	L	2.5
Oxycodone	316.1543	1.92	+	0.5	5000	0.9988	L	1
Oxymorphone	302.1387	0.85	+	2.5	5000	0.9994	Q	0.05
Paroxetine	330.1500	4.15	-	0.25	5000	0.9982	L	0.5
Pentobarbital	225.1245	4.06	+	2.5	5000	0.9987	L	100
Phencyclidine	244.2060	3.56	-	1	5000	0.9981	L	1
Phenobarbital	231.0775	3.32	+	10	5000	0.9988	L	10
Phentermine	150.1277	2.29	+	5	5000	0.9979	Q	5
Phenytoin	251.0826	4.12	-	100	5000	0.9949	Q	500
Pregabalin	160.1332	1.58	+	2.5	5000	0.9973	Q	0.05
Propranolol	260.1645	3.67	+	2.5	2500	0.9992	Q	0.05
Pseudoephedrine	166.1226	1.64	+	5	5000	0.9984	L	5
Quetiapine	384.1740	3.90	+	5	5000	0.9967	Q	5
Ritalinic Acid	220.1332	2.40	+	5	5000	0.9994	Q	0.05
Secobarbital	237.1245	4.22	-	5	5000	0.9979	L	50
Sertraline	306.0811	4.42	+	5	5000	0.9996	L	5
Tapentadol	222.1852	2.94	+	0.5	5000	0.9998	L	0.5

Table 4. Calibration and confirmation results of the 106 analytes in urine. LOQ, ULOL, and LOI 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	LOI
Temazepam	301.0738	4.44	+	0.5	5000	0.9971	L	0.5
Topiramate	340.1061	3.60	+	5	5000	0.9956	L	25
Tramadol	264.1958	2.84	+	1	5000	0.9985	L	0.05
Trazodone	372.1586	3.49	+	2.5	1000	0.999	Q	2.5
Triazolam	343.0512	4.38	+	2.5	5000	0.999	Q	2.5*
Venlafaxine	278.2115	3.45	+	0.5	5000	0.9996	L	0.5
Verapamil	455.2904	4.21	+	10	2500	0.9989	Q	10*
Zolpidem	308.1757	3.26	+	0.5	5000	0.9985	L	0.5*
Zolpidem-COOH	338.1499	2.47	+	0.5	5000	0.9990	L	0.25
Zopiclone	389.1123	2.79	+	2.5	5000	0.9994	L	1

* IP not included in LOC due to matrix contaminant

Extracted ion chromatograms of representative compounds at their respective LOQs along with calibrations curves can be seen in Figure 4. To demonstrate the mass accuracy across the peak, several of the compounds are displayed in "stick mode" and have the accurate m/z and delta ppm above each scan (Figure 5).

Mass accuracy across the peak is at around 1 ppm or less, even at very low concentrations. Internal standard showed reproducibility across triplicate injections of calibrators with 7-aminoflunitrazepam-d7 having an % RSD of 4.54 as depicted in Figure 6.

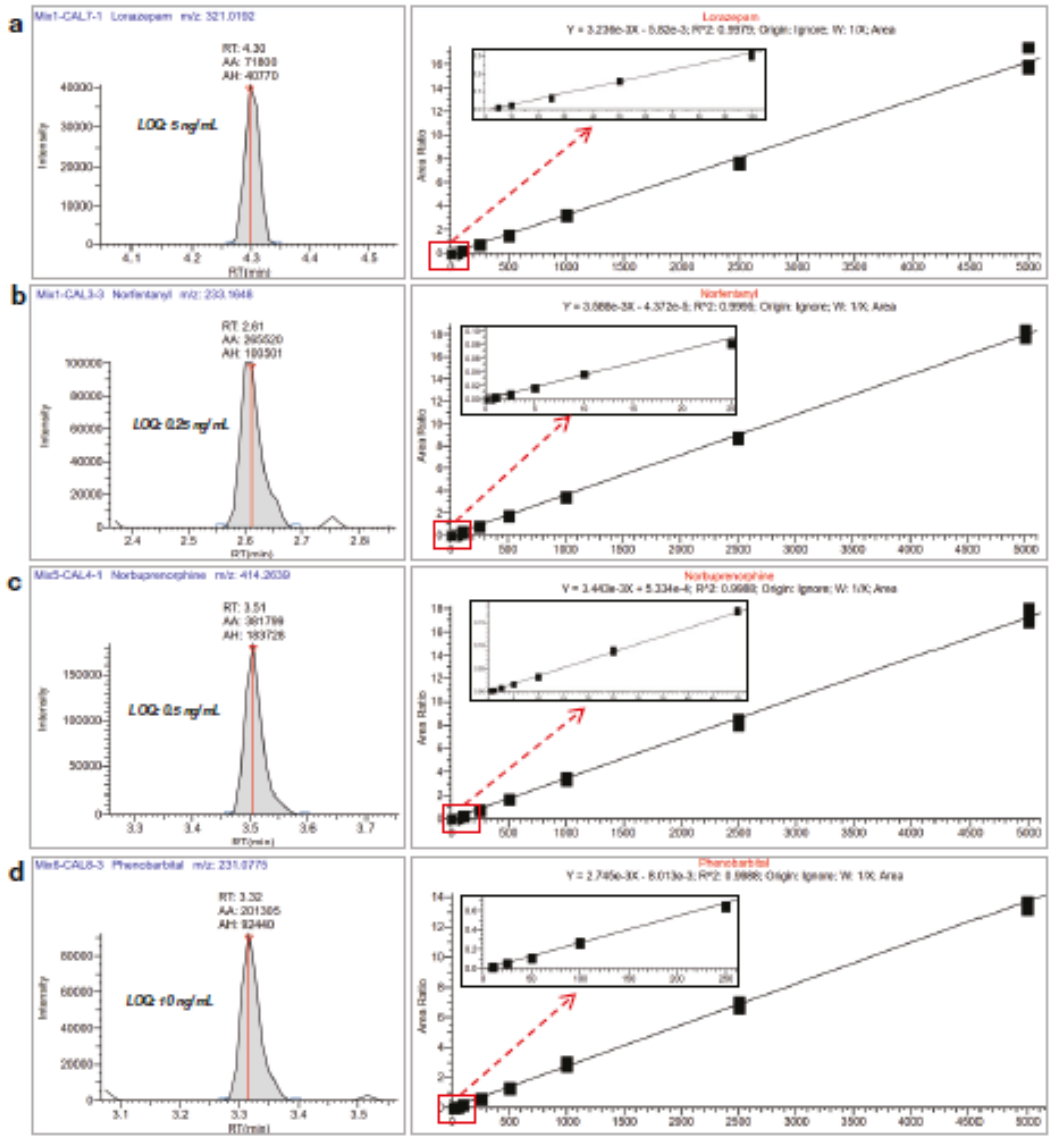

Figure 4. Extracted ion chromatograms of (a) lorazepam, (b) norfentanyl, (c) norbuprenorphine, and (d) phenobarbital at their respective LOQ levels and calibration curves.

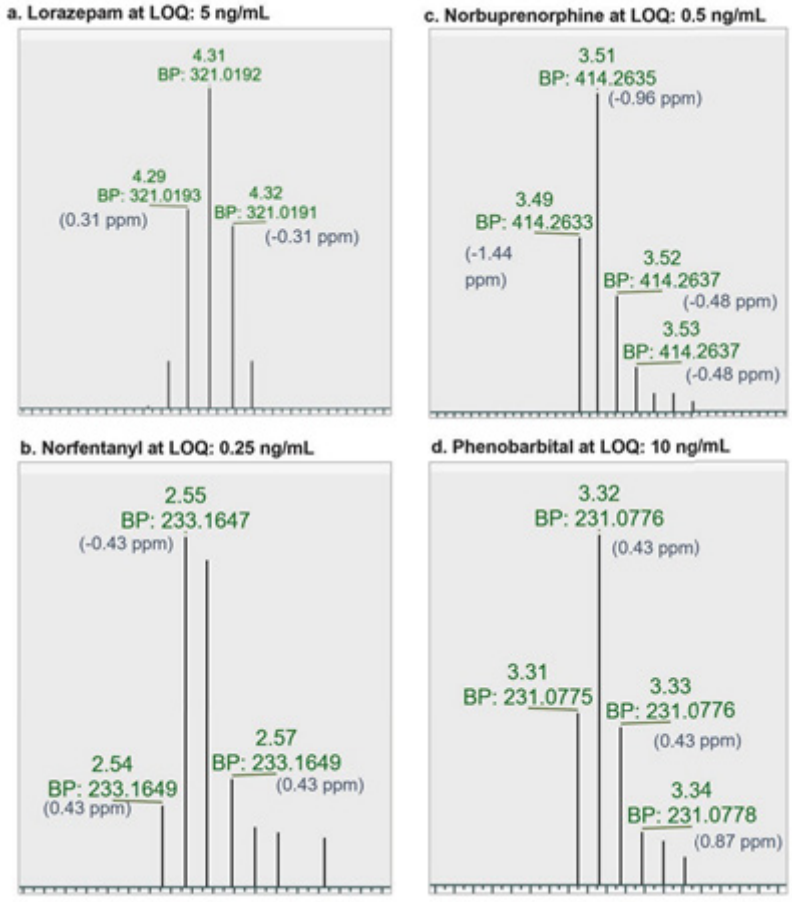

Figure 5. Mass accuracy of (a) lorazepam, (b) norfentanyl, (c) norbuprenorphine, and (d) phenobarbital across the peak at their respective LOQ concentrations.

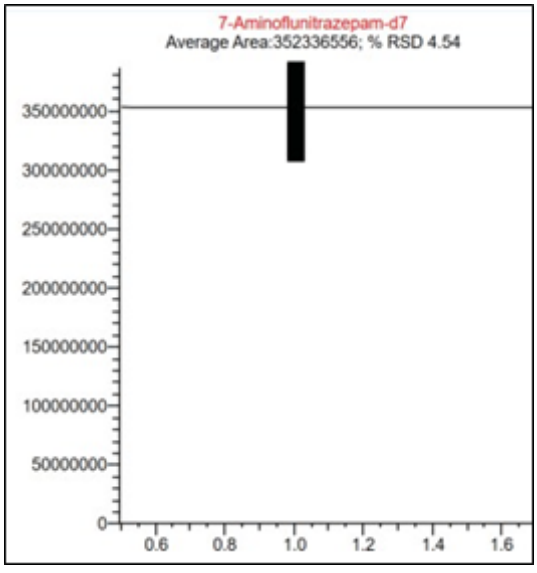

Figure 6. Reproducibility of internal standard 7-aminoflunitrazepam-d7 across triplicates of 15 calibrators.

CONCLUSIONS

This fast, robust, and quantitative method was developed around 106 of the most tested drugs of abuse worldwide that come from a wide range of drug classes and include positive and negative polarities. A complete workflow was presented that involved sample preparation using SOLAμ SCX SPE plates followed by injection on the Vanquish Horizon UHPLC system and Orbitrap Exploris 120 mass spectrometer. Linearity was achieved from LOQs as low as 0.1 ng/mL to ULOLs 5,000 ng/mL, which exemplifies the wide dynamic range of this instrument.

Compounds are confidently confirmed by accurate *m/z*, retention time, isotopic pattern calculated from the empirical formula, and library spectral matching. This method is fast and highly flexible for toxicology and clinical labs. Because it was built to separate and identify many different drug classes and isomers, compounds can easily be added. Furthermore, the TraceFinder software allows the compound parameters in the method as well as the curated library to be changed with only a few clicks, making this method very customizable.

REFERENCES

(1) United Nations Office on Drugs and Crime. World Drug Report 2021.
(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.

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