Quantitation of 106 drugs in urine using a fast 7-minute method by high resolution accurate-mass and Triple Quadrupole Mass Spectrometry

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Abstract

Purpose: The objective of this work was to accurately confirm and quantitate 106 drugs from a wide range of drug classes that are frequently tested for in forensic and clinical research laboratories in urine by liquid chromatography and high-resolution, accurate mass (HRAM) and triple quadrupole mass spectrometry technology.

Methods: Urine samples were spiked with drugs of abuse, extracted on Thermo Scientific™ SOLAμ™ SPE Plates, separated chromatographically, and detected on an HRAM and triple quadrupole mass spectrometers.

Results: All analytes were successfully detected and quantitated at very low concentrations, meeting the sensitivity needs of analytical methodologies for routine laboratories, including those monitoring forensic and sport anti-doping samples.

Introduction

According to the United Nations Office on Drugs and Crime report, half a million death 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 and chemical structures and produce baseline separation of isomers. This work explores the application of Thermo Scientific™ Orbitrap™ Exploris™ MS and Thermo Scientific™ TSQ Quantis™ Plus MS 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. The 106 drugs chosen were based on the highest frequency drugs tested from numerous forensic and clinical research labs around the world that encompass a wide range of drug classes.

Materials and methods

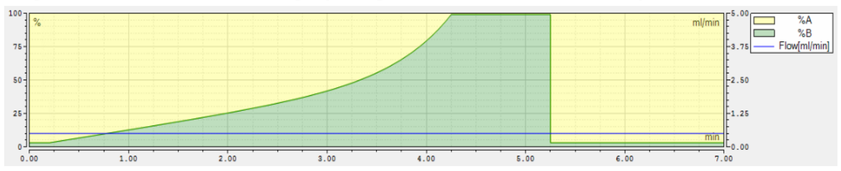
Sample preparation

Non-labeled standards, prepared in six mixes, and corresponding internal standards were spiked into 200 µL of whole blood matrix spanning a concentration range from 0.05 ng/mL to 5,000 ng/mL. Samples were extracted using SOLAμ SCX SPE Plates. Plates were equilibrated with 100 µL of elution solvent (5% ammonium hydroxide, 47.5% acetonitrile, 47.5% methanol (v/v/v)) and 100 µL of MeOH and then conditioned with 100 µL of 2% formic acid in water. 200 µL of sample + 20 µL of 2% formic acid in water + 20 µL ISTD were added to each well. The plates were washed with 100 µL of 2% formic acid in water. Elution A was performed with 2 x 20 µL MeOH/H2O into collection wells containing 80 µL of 2% formic acid in water. The plate was washed again with 100 µL of MeOH. Elution B was performed with 2 x 20 µL elution solvent. Extracted samples were injected in triplicate to determine screening and confirmation cutoffs.

Liquid chromatography

Analytes were separated with the Thermo Scientific™ Vanquish™ Horizon ultra-high performance liquid chromatography (UHPLC) system by a 7 min gradient (Figure 1) using a Thermo Scientific™ Accucore™ Vanquish™ C18+ column (1.5 µm, 50 x 2.1 mm). A strong solvent loop was installed before the column to accommodate high organic samples and give sharper early eluting peaks. Mobile phases consisted of 2 mM ammonium formate with 0.01% formic acid in water and acetonitrile:methanol (50:50) for mobile phase A and B, respectively. A 5-µL aliquot of each standard was injected in triplicate.

Figure 1. UHPLC Chromatographic gradient used for data acquisition.



Mass spectrometry

Targeted screening and quantitation were performed on both a Orbitrap Exploris 120 HRAM mass spectrometer and the TSQ Quantis Plus triple quadrupole mass spectrometer. For HRAM detection, full-scan, targeted, data-dependent MS^2 scanning was used with an inclusion list for the targeted compounds. Resolutions of 60,000 (FWHM at m/z 200) for full scan and 15,000 for MS^2 were employed. An isolation window of m/z 1.5 and compound specific collision energies were applied to generate rich HRAM MS^2 spectra. For TSQ data, an SRM method was used with resolutions (FWHM) of 0.7 for Q1 and 1.2 for Q3 were applied.

The ion source conditions for both instruments are listed in Table 1.

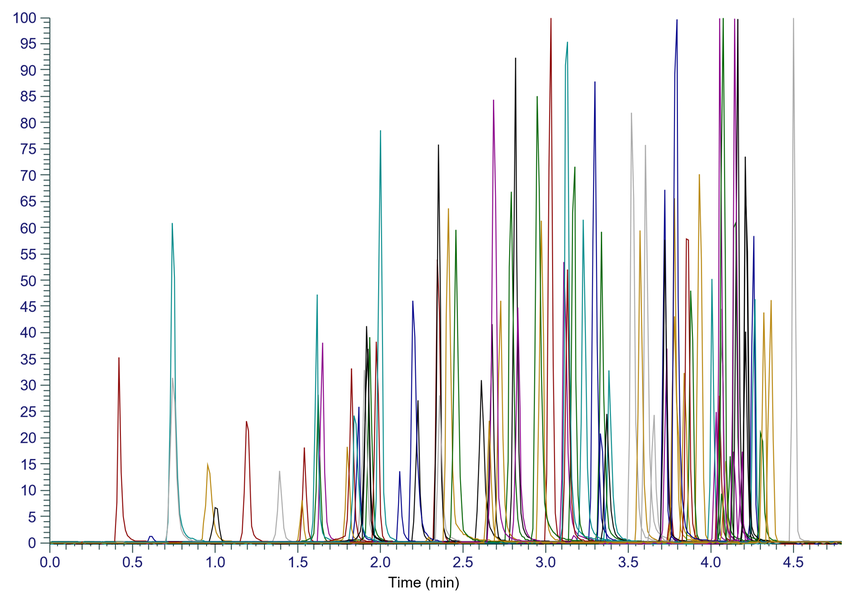
Table 1. Ion source conditions for Orbitrap Exploris 120 and TSQ Quantis Plus Mass Spectrometers.

Source Parameters	Value
Positive Ion	3500 V
Negative Ion	2000 V
Sheath Gas	55 AU
Aux Gas	10 AU
Sweep Gas	1 AU
Ion Transfer Tube Temp	325 ° C
Vaporizer Temp	350 ° C
Source Position	1.2, L/M

Data analysis

Data was acquired and processed with Thermo Scientific TraceFinder ™ software, version 5.2. TraceFinder software utilizes its Compound Database which stores information including molecular formula, exact mass, retention time and fragment ions for all compounds of interest. A mass window of 5ppm was used as the precursor ion criteria of HRAM data.

Figure 2. Combined extracted chromatogram of the 106 drugs



Results

All 106 analytes are depicted in the extracted ion chromatogram in Figure 2. The limit of quantitation (LOQ), upper limit of linearity (ULOL), limit of identification (LOI), and limit of confirmation (LOC) were evaluated for each of the 106 analytes. Table 2 shows the criteria for each of these limits. Table 3 defines the specific parameters for the Orbitrap data.

Table 2. Criteria assigned in TraceFinder software for limits.

Parameter	Criteria
Limit of Quantitation (LOQ)	Back-calculated concentration on calibration curve within 20%.
Upper Limit of Linearity (ULOL)	Highest calibrator that achieves linearity
Limit of Identification (LOI)	**Orbitrap**: IP = passing isotopic pattern score (70) FI = presence of diagnostic fragment ions LS = passing library score (70)
Limit of Confirmation (LOC)	**Triple Quadrupole**: Ion ratio confirmation within 20% (relative) of target value

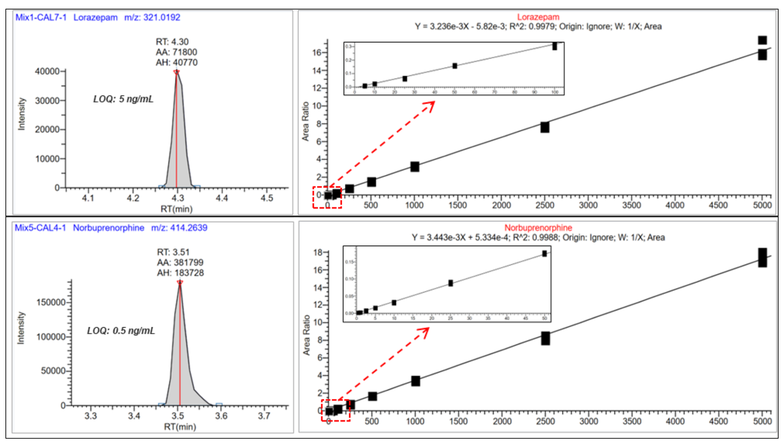
Table 3. Criteria assigned in TraceFinder software for Orbitrap confirmation.

Parameter	Criteria
Isotopic Pattern	< 10 ppm mass deviation, < 20% intensity deviation, fit > 70%
Fragment Ion	At least 2 fragments with < 10 ppm mass deviation in MS^2 spectra
mzVault HRAM Library	Reverse search with > 70% match of ddMS2 spectra

As shown in Table 4, the LOQs were as low as 0.1 ng/mL for several compounds such as 1-(3-chlorophenyl)piperazine and norketamine for orbitrap data and N-desmethylzopiclone, doxylamine, norketamine for triple quadrupole data. 89 out of the 106 drugs had upper limits of linearity (ULOL) of 5,000 ng/mL on the Orbitrap Exploris 120 MS. All compound LOQs, except for buprenorphine, were at or well below industry standards.

Figure 3 depicts the extracted ion chromatograms of two of the analytes, lorazepam and norbuprenorphine, at their respective LOQ concentrations with their corresponding calibration curves obtained from Orbitrap Exploris 120 MS.

Figure 3. Chromatograms of lorazepam and norbuprenorphine at their respective LOQ levels and calibration curves from the Orbitrap Exploris 120 MS.



Conclusions

This fast 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 positively and negatively ionized species. A complete workflow was presented that involved sample preparation using SOLAμ SCX SPE plates. Linearity was achieved from LOQs as low as 0.1 ng/mL to ULOLs of 5,000 ng/mL which exemplifies the sensitivity and wide dynamic range of these instruments. This method is fast and 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.

References

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

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Table 4. Calibration and confirmation results of the 106 analytes in urine. LOQ, ULOL, and LOC are in units of ng/mL.

Compound	Orbitrap Exploris 120			TSQ Quantis Plus		
	LOQ	ULOL	LOI	LOQ	ULOL	LOC
1-(3-Chlorophenyl)piperazine	0.1	5000	0.5	2.5	5000	0.25
25I-NBOMe	10	1000	2.5	2.5	5000	1
2-hydroxyethylflurazepam	0.25	5000	2.5	0.25	2500	2.5
6-acetylmorphine	0.5	5000	0.5	0.25	5000	0.25
6-beta-Naltrexol	1	5000	0.5	0.25	5000	0.25
7-aminoclonazepam	5	2500	0.05	1	2500	1
7-aminoflunitrazepam	1	5000	0.05	0.5	2500	1
9-hydroxyrisperidone	10	2500	1	5	2500	0.5
Acetaminophen	25	5000	0.05	10	5000	1
a-hydroxyalprazolam	1	5000	2.5	2.5	5000	2.5
a-hydroxymidazolam	2.5	5000	2.5	1	5000	2.5
a-hydroxytriazolam	2.5	5000	10*	5	1000	10
Alprazolam	1	5000	2.5	0.5	5000	1
Amitriptyline	5	5000	2.5	5	5000	2.5
Amobarbital	5	5000	5	25	5000	25
Amphetamine	1	5000	2.5	5	2500	25
Benzoylecgonine	0.25	2500	0.05	0.25	2500	0.25
Buprenorphine	2.5	250	0.5*	1	500	2.5
Bupropion	0.25	5000	0.25	0.25	5000	0.1
Butalbital	10	5000	10	25	5000	25
Carbamazepine	0.5	5000	1	0.5	2500	2.5
Carisoprodol	100	2500	50	10	2500	5
Chlordiazepoxide	0.5	2500	2.5	2.5	2500	10
Chlorpheniramine	2.5	5000	0.5	1	5000	0.25
Citalopram	0.5	5000	0.5	0.5	2500	1
Clonazepam	5	2500	2.5*	5	2500	2.5
Clozapine	2.5	2500	2.5*	2.5	2500	2.5
Cocaethylene	0.5	5000	0.25	0.25	5000	0.1
Cocaine	2.5	5000	0.5	5	5000	2.5
Codeine	2.5	5000	2.5	2.5	5000	0.5
Cotinine	10	5000	0.05	5	5000	5
Cyclobenzaprine	0.5	5000	0.5	1	5000	0.5
Desipramine	10	5000	1	5	5000	1
Dextromethorphan	5	2500	0.5	2.5	5000	1
Diazepam	2.5	5000	2.5	2.5	5000	1
Dihydrocodeine	0.5	5000	1	0.25	5000	1
Diphenhydramine	2.5	5000	5	5	2500	5
Doxepin	0.25	5000	2.5	2.5	2500	1
Doxylamine	5	5000	5	0.1	5000	0.25
EDDP	2.5	5000	2.5	2.5	5000	0.25
Fentanyl	0.25	5000	0.25	0.25	5000	0.25
Fluoxetine	2.5	5000	2.5	10	5000	1
Flurazepam	10	2500	5	0.5	5000	0.5
Gabapentin	5	5000	1	50	5000	25
Haloperidol	25	5000	25	2.5	5000	2.5
Hydrocodone	0.25	5000	0.5	0.25	2500	0.5
Hydromorphone	1	5000	1	5	5000	5
Imipramine	0.5	5000	1	2.5	5000	1
Isotonitazene	5	500	2.5	2.5	500	2.5
Ketamine	1	5000	1	0.5	5000	5
Lorazepam	5	5000	10*	5	5000	5
LSD	2.5	5000	0.5	2.5	5000	0.25
MDA	2.5	5000	2.5	2.5	5000	5
MDEA	1	5000	0.5	1	1000	1
MDMA	1	5000	2.5	0.5	5000	0.5
Meperidine	1	5000	0.5	1	5000	1
Meprobamate	25	5000	25	10	5000	2.5
Methadone	2.5	5000	0.5	1	5000	0.5
Methamphetamine	0.5	5000	0.1	1	2500	0.1
Methylphenidate	0.25	5000	0.5*	0.25	2500	0.25
Mitragynine	1	500	1	1	500	1
Morphine	0.25	5000	1	1	1000	1
Naloxone	0.5	2500	0.5	0.5	2500	1
Naltrexone	1	5000	2.5	2.5	5000	2.5
N-desmethyl-Tapentadol	0.25	5000	0.05	0.25	5000	0.1
N-desmethylzopiclone	1	500	5	0.1	1000	0.1
Nicotine	1	5000	0.05	2.5	5000	5
Norbuprenorphine	0.5	5000	5	0.5	1000	2.5
Norcyclobenzaprine	2.5	5000	2.5	2.5	5000	0.25
Nordiazepam	0.5	5000	1	1	2500	1
Norfentanyl	0.25	5000	0.1	0.25	2500	0.25
Norfluoxetine	5	5000	10	5	5000	5
Norhydrocodone	0.5	5000	0.25	0.25	5000	0.5
Norketamine	0.1	5000	0.5	0.1	5000	0.1
Normeperidine	0.25	5000	0.25	0.25	5000	0.25
Noroxycodone	1	5000	2.5	1	5000	1
Nortriptyline	5	5000	2.5	2.5	5000	1
O-desmethyltramadol	0.5	5000	0.05	0.5	2500	1
Olanzapine	0.5	5000	1	0.25	5000	0.5
Oxazepam	1	5000	2.5	5	2500	5
Oxycodone	0.5	5000	1	0.25	2500	1
Oxymorphone	2.5	5000	0.05	0.5	5000	0.05
Paroxetine	0.25	5000	0.5	1	5000	2.5
Pentobarbital	2.5	5000	100	25	5000	25
Phencyclidine	1	5000	1	0.5	5000	5
Phenobarbital	10	5000	10	10	5000	10
Phentermine	5	5000	5	5	5000	5
Phenytoin	100	5000	500	50	5000	100
Pregabalin	2.5	5000	0.05	1	5000	1
Propranolol	2.5	2500	0.05	0.5	1000	0.5
Pseudoephedrine	5	5000	5	0.25	5000	0.25
Quetiapine	5	5000	5	5	1000	1
Ritalinic Acid	5	5000	0.05	10	5000	1
Secobarbital	5	5000	50	25	5000	25
Sertraline	5	5000	5	10	5000	1
Tapentadol	0.5	5000	0.5	0.5	5000	0.25
Temazepam	0.5	5000	0.5	0.25	5000	0.25
Topiramate	5	5000	25	10	5000	5
Tramadol	1	5000	0.05	1	5000	5
Trazodone	2.5	1000	2.5	2.5	5000	0.1
Triazolam	2.5	5000	2.5*	2.5	5000	1
Venlafaxine	0.5	5000	0.5	0.5	1000	1
Verapamil	10	2500	10*	10	2500	1
Zolpidem	0.5	5000	0.5*	0.5	5000	0.1
Zolpidem-COOH	0.5	5000	0.25	0.25	2500	0.25
Zopiclone	2.5	5000	1	0.1	5000	0.5

* IP not included in LOI due to matrix contaminant