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TECHNICAL NOTE 73168

Quantification of 78 drugs of abuse in human blood by liquid chromatography – HRAM Orbitrap mass spectrometry for clinical research

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Keywords

Drugs of abuse, blood, Orbitrap, Q Exactive Focus

Goal

Implementation of an analytical method for the quantification of several drugs of abuse in human blood in one assay using a Thermo Scientific™ Q Exactive™ Focus hybrid quadrupole-Orbitrap™ mass spectrometer

Application benefits

- Simple offline sample preparation by protein precipitation
- 78 analytes in a single quantitative method

Introduction

The world of analytical testing of illicit drugs experiences several challenges due to rapid development of unregulated designer and synthetic compounds. The difficulty for forensic testing facilities is the fact that most or all of these compounds are not detected under normal testing methods, resulting in the requirement of a more specific and selective LC-MS based approach. In this report, an analytical method for clinical research is described for quantification of 78 drugs of abuse in human blood. The typical LC-MS methodologies involve use of LC-MS/MS technology, also referred to as triple quadrupole mass spectrometers. While triple quadrupole MS systems offer sensitive data for targeted quantitation assays, most samples often have analytes that are not confirmed/identified and hence, require both identification and quantitation capabilities. High-resolution, accurate-mass (HRAM) spectrometers are equipped with high-resolution features that offer every user the unique ability to screen and quantify with high efficiency.

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Whole blood samples were extracted by offline internal standard addition and protein precipitation. Extracted samples were injected onto a Thermo Scientific™ UltiMate™ 3000 RSLC system fitted with a binary high-pressure mixing pump. The LC system was connected to a Q Exactive Focus hybrid quadrupole-Orbitrap mass spectrometer. Heated electrospray ionization (HESI) with positive-negative polarity switching was used. Detection was performed with a resolution of 35,000 (FWHM) @ *m/z* 200 in Full MS mode covering an *m/z* range between 100 and 600. Method performance was evaluated using home-made calibrators and controls in whole blood in terms of limit of quantification (LOQ) and linearity of response within the calibration ranges for each analyte.

Experimental

Target analytes

A list of the analytes and corresponding internal standards included in this quantification method are reported in Table 1.

Table 1 (part 1). Analytes and corresponding internal standards

Analyte	Internal Standard
1-(3-Chlorophenyl) piperazine	1-(3-Chlorophenyl) piperazine-D8
6-MAM	6-MAM-D3
7-Aminoclonazepam	7-Aminoclonazepam-D3
α-Hydroxyalprazolam	α-Hydroxyalprazolam-D5
Alprazolam	Alprazolam-D5
Amitriptyline	Amitriptyline-D3
Amphetamine	Amphetamine-D11
Anhydroecgonine methyl ester	Cocaine-D3
Baclofen	MDMA-D5
Benzoylecgonine	Benzoylecgonine-D3
Buprenorphine	Buprenorphine-D4
Carisoprodol	Fentanyl-D5
Chlordiazepoxide	Chlordiazepoxide-D5
cis-Tramadol	*cis*-Tramadol-13C,D3
Clonazepam	Clonazepam-D4
Clonidine	Methamphetamine-D11
Cocaine	Cocaine-D3
Codeine	Codeine-D3
Cyclobenzaprine	Cyclobenzaprine-D3
Desipramine	Desipramine-D3
Diazepam	Diazepam-D5
Dihydrocodeine	Dihydrocodeine-D6
Doxepin	Doxepin-D3
Duloxetine	Cyclobenzaprine-D3
Ecgonine methyl ester	Ecgonine methyl ester-D3
EDDP	EDDP-D3
Fentanyl	Fentanyl-D5
Gabapentin	Gabapentin-D10
Hydrocodone	Hydrocodone-D3

Table 1 (part 2). Analytes and corresponding internal standards

Analyte	Internal Standard
Hydromorphone	Hydromorphone-D3
Imipramine	Imipramine-D3
JWH-018 5-Pentanoic acid	JWH-018 4-hydroxypentyl-D5
JWH-073 3-Hydroxybutyl	JWH-018 4-hydroxypentyl-D5
JWH-073 4-Butanoic acid	JWH-018 4-hydroxypentyl-D5
Ketamine	Ketamine-D4
Levamisole	Cocaine-D3
Lorazepam	1-(3-Chlorophenyl) piperazine-D8
MBDB	MBDB-D5
MDA	MDA-D5
MDEA	MDEA-D5
MDMA	MDMA-D5
Meperidine	Meperidine-D4
Mephedrone	Mephedrone-D3
Meprobamate	Desipramine-D3
Metaxalone	Desipramine-D3
Methadone	Methadone-D3
Methamphetamine	Methamphetamine-D11
Methylphenidate	(±)-threo-Ritalinic acid-D10
Midazolam	Midazolam-D4
Morphine	Morphine-D3
Naloxone	Hydrocodone-D3
Naltrexone	Oxymorphone-D3
Naphyrone	Mephedrone-D3
N-Desmethyl-*Cis*-Tramadol	Cyclobenzaprine-D3
N-Desmethyltapentadol	Normeperidine-D4

Table 1 (part 3). Analytes and corresponding internal standards

Analyte	Internal Standard
Norbuprenorphine	Norbuprenorphine-D3
Nordiazepam	Nordiazepam-D5
Norfentanyl	Norfentanyl-D5
Norhydrocodone	Hydrocodone-D3
Norketamine	Ketamine-D4
Normeperidine	Normerperidine-D4
Noroxycodone	Oxycodone-D6
Norpseudoephedrine	Amphetamine-D11
Nortriptyline	Nortriptyline-D3
Oxazepam	Oxazepam-D5
Oxycodone	Oxycodone-D6
Oxymorphone	Oxymorphone-D3
Pregabalin	Gabapentin-D10
Propoxyphene	Propoxyphene-D5
Protriptyline	Protriptyline-D3
Quetiapine	Quetiapine-D8
Ritalinic acid	(±)-threo-Ritalinic acid-D10
Tapentadol	Meperidine-D4
Temazepam	Temazepam-D5
Trazodone	Trazadone-D6
Triazolam	1-(3-Chlorophenyl) piperazine-D8
Zaleplon	Zolpidem-D6
Zolpidem	Zolpidem-D6

Sample preparation

Calibrators at nine different concentration levels were prepared by spiking 1:50 (v/v) negative whole blood with methanolic solutions containing all the analytes of interest to a final concentration range from 0.02 to 200 ng/mL. Controls at six different concentration levels in blood from 0.05 to 150 ng/mL were prepared using the same approach but using a separate dilution branch. Samples of 50 µL of blood were extracted by protein precipitation using 100 µL of acetonitrile containing the internal standards. Precipitated samples were vortex-mixed, and centrifuged. Then, 100 µL of the supernatant were dried down under gentle nitrogen flow and reconstituted using 100 µL of water/methanol 90/10 (v/v). The reconstituted supernatant was transferred onto a clean vial and preserved in the autosampler set at 4 °C prior to injection.

Liquid chromatography

The LC separation was achieved on an UltiMate 3000 RSLC system fitted with a Thermo Scientific™ Accucore™ Phenyl Hexyl 100 × 2.1 mm (2.6 µm) analytical column at 40 °C. Chromatographic separation was obtained by gradient elution using water and acetonitrile both with 0.1% formic acid. Details of the analytical method are reported in Table 2. Total runtime was 17.0 minutes.

Table 2. LC method description

Gradient Profile			
Time (min)	**Flow Rate (mL/min)**	**A (%)**	**B (%)**
0.0	0.5	99	1
1.0	0.5	99	1
10.0	0.5	1	99
11.5	0.5	1	99
11.6	0.5	99	1
17.0	0.5	99	1
Other Parameters			
Injection volume (µL)		40	
Column temperature (°C)		40	

Mass spectrometry

Detection was performed on a Q Exactive Focus hybrid quadrupole-Orbitrap mass spectrometer fitted with a heated-electrospray source operated in positive ionization mode. Data were acquired in full scan mode using a resolution of 35,000 (FWHM) at *m/z* 200 and covering a mass range between 100 and 600 *m/z*. A summary of the MS conditions is reported in Table 3. Chromatograms were extracted using a mass accuracy window of 5 ppm.

Table 3. MS settings

Source type:	Heated electrospray ionization (HESI)
Vaporizer temperature:	350 °C
Spray voltage (positive mode):	4000 V
Sheath gas:	50 AU
Sweep gas:	0 AU
Auxiliary gas:	10 AU
Data acquisition mode:	Full scan *m/z* 100–600
Resolution:	35,000 (FWHM) @ *m/z* 200

Method evaluation

The method performance was evaluated in terms of lower (LLOQ) and upper (ULOQ) limits of quantification, and linearity range for each analyte. Three validation batches, including calibrators in duplicate at the beginning and at the end of the run and controls in between extracted and injected in replicates of five, were run on three different days for the analytical validation of the method. LLOQ evaluation was based on the acceptance criterion of the percentage bias between nominal and back-calculated concentration being within ±20% for the lowest calibrator and ±15% for the others. Acceptance criterion for all the controls was the same bias to be within ±20%.

Data analysis

Data were acquired and processed using Thermo Scientific™ TraceFinder™ 4.1 software.

Results and discussion

The method proved to be linear in the calibration ranges specified in Table 4. Representative chromatograms for the lowest calibrator for baclofen (1 ng/mL), EDDP (0.2 ng/mL), and norbuprenorphine (1 ng/mL) are reported in Figure 1. Representative calibration curves for the same analytes are reported in Figure 2.

Table 4 (part 1). Calibration ranges

Analyte	Calibration Range (ng/mL)
1-(3-Chlorophenyl)piperazine	2–200
6-MAM	1–200
7-Aminoclonazepam	10–200
α-Hydroxyalprazolam	10–200
Alprazolam	1–200
Amitriptyline	0.2–200
Amphetamine	10–200
Anhydroecgonine methyl ester	20–200
Baclofen	1-100
Benzoylecgonine	1–200
Buprenorphine	1–200
Carisoprodol	10–200
Chlordiazepoxide	0.2–200
cis-Tramadol	0.02–200
Clonazepam	1–200
Clonidine	0.2–200
Cocaine	0.2–200
Codeine	0.1–200
Cyclobenzaprine	0.2–200
Desipramine	2–200
Diazepam	0.2–200
Dihydrocodeine	0.2–200
Doxepin	1–200
Duloxetine	2–200
Ecgonine methyl ester	10–200
EDDP	0.2–200
Fentanyl	1–200
Gabapentin	0.2–200
Hydrocodone	1–200

Table 4 (part 2). Calibration ranges

Analyte	Calibration Range (ng/mL)
Hydromorphone	1–200
Imipramine	1–200
JWH-018 5-Pentanoic acid	2–200
JWH-073 3-Hydroxybutyl	20–200
JWH-073 4-Butanoic acid	10–200
Ketamine	1–200
Levamisole	2–200
Lorazepam	2–200
MBDB	0.1–200
MDA	0.1–200
MDEA	1–200
MDMA	1–200
Meperidine	0.2–200
Mephedrone	*0.2–200*
Meprobamate	20–200
Metaxalone	1–200
Methadone	1–200
Methamphetamine	1–200
Methylphenidate	0.1–200
Midazolam	0.2–200
Morphine	1–200
Naloxone	1–200
Naltrexone	20–200
Naphyrone	2–200
N-Desmethyl-*Cis*-Tramadol	2–200
N-Desmethyltapentadol	0.02–200
Norbuprenorphine	1–200
Nordiazepam	1–200
Norfentanyl	0.2–200

Table 4 (part 3). Calibration ranges

Analyte	Calibration Range (ng/mL)
Norhydrocodone	10–200
Norketamine	0.2–200
Normeperidine	10–200
Noroxycodone	2–100
Norpseudoephedrine	10–200
Nortriptyline	0.1–200
Oxazepam	2–200
Oxycodone	1–200
Oxymorphone	2–200
Pregabalin	10–200
Propoxyphene	2–200
Protriptyline	2–200
Quetiapine	0.2–200
Ritalinic acid	1–200
Tapentadol	0.2–200
Temazepam	20–200
Trazodone	0.2–200
Triazolam	1–200
Zaleplon	2–200
Zolpidem	20–200

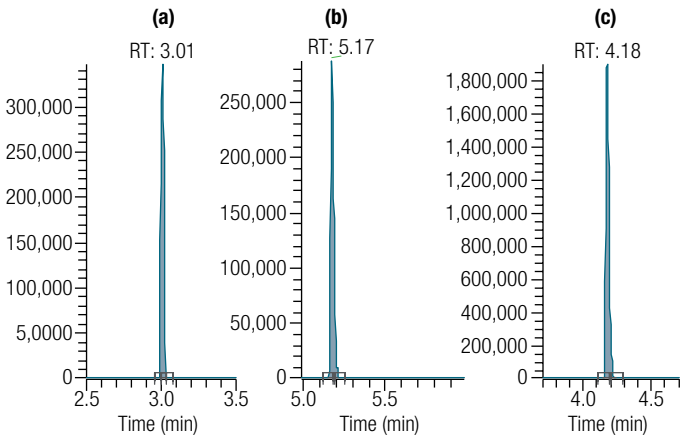

Figure 1. Representative chromatograms of the lowest calibrator for (a) baclofen (1 ng/mL), (b) EDDP (0.2 ng/mL), and (c) norbuprenorphine (1 ng/mL)

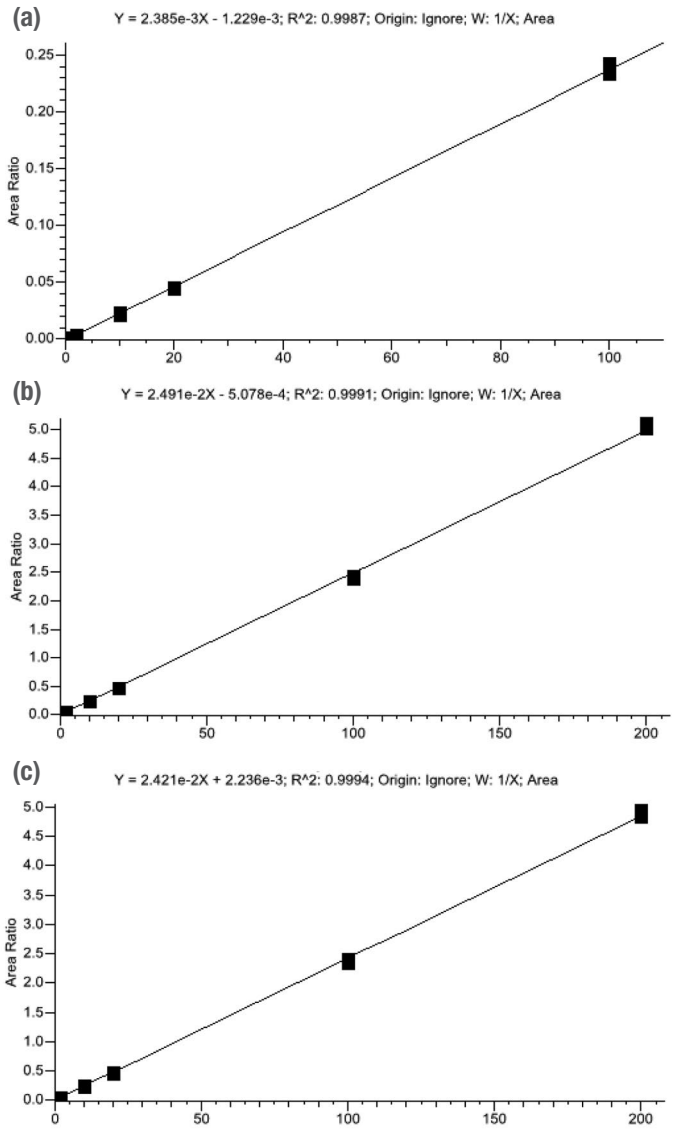

Figure 2. Representative calibration curves for (a) baclofen, (b) EDDP, and (c) nobuprenorphine

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Conclusions

A liquid chromatography – Orbitrap HRAM mass spectrometry method for clinical research for the quantification of 78 different drugs of abuse in human blood was implemented. The method was evaluated on an UltiMate 3000 RSLC system connected to a Q Exactive Focus hybrid quadrupole-Orbitrap mass spectrometer. The method offers a quick and simple offline protein precipitation with concomitant internal standard addition. The described method meets research laboratory requirements in terms of sensitivity and linearity of response.

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