Application Note

Forensic Toxicology

Screening, Identification, and Quantitation of 102 Drugs in Human Whole Blood by LC/Q-TOF and LC-QQQ

Authors

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Abstract

An end-to-end workflow for the screening, identification, and quantitation of 102 drugs in whole blood by the combination of liquid chromatography/quadrupole time-of-flight mass spectrometry (LC/Q-TOF) and triple quadrupole mass spectrometry (LC-QQQ) was developed and validated. The suspect drugs included 66 drugs of abuse and some of their metabolites from 15 different classes, and 36 medicinal drugs from 12 different classes. Samples were prepared using in-cartridge protein precipitation (PPT) extraction, followed by Agilent Captiva EMR—Lipid cleanup. Depending on the purpose of analysis, the extracted samples were run on either LC/Q-TOF for screening and identification, or LC-QQQ for quantitation. The Agilent All Ions MS/MS (data-independent acquisition) combined with a compound database was used for suspect screening purposes for the whole blood sample fortified with drugs at 10 and 50 ng/mL. The verified screening method is capable of screening 100% of 102 drugs at 10 and 50 ng/mL. The workflow provides excellent quantitation results, including 95% of analytes within the 70 to 120% recovery window, 98% of analytes with <20% relative standard deviation (RSD), and 93% of analytes within the 60 to 130% matrix effect window. The quantitative method was verified by accuracy and precision runs and delivered exceptional accuracy (100 ±20%) and precision (RSD <20%) for all spiking levels, limits of quantitation (LOQ) of 0.5 to 5 ng/mL in whole blood, and linear calibration curves with R^2 >0.99 for the majority of analytes.

Introduction

In forensic toxicology, the demand for fast and reliable screening and quantitative determination of drugs of abuse (DoA) and misused prescription-type drugs in biological specimens is steadily increasing, due to the increasing number of drugs of abuse, as well as samples submitted for analysis. Traditionally, urine was the sample of choice for screening and identification. However, the metabolites of these drugs had to be identified additionally or even exclusively, adding more complexity and uncertainty to screening and quantitative testing. Therefore, screening and quantitative determination of DoA in whole blood is important and necessary in toxicology analysis.

LC/MS technology, including LC/Q-TOF and LC-QQQ, have been applied as promising techniques, having been increasingly used in forensic toxicology for a wide range of biological samples.[1] Easier sample preparation without derivatization, and shorter analysis time are the major advantages that make them widely accepted. LC-QQQ with multiple reaction monitoring (MRM) is currently adopted mostly for the quantitative analysis of abused drugs.[2-4] MRM methodology is for suspect quantitative analysis and has limitations for drug screening. High resolution mass spectrometry, such as TOF-MS has become an emerging technique for high-throughput forensic toxicological screening.[5,6] The high mass accuracy allows for the use of exact monoisotopic masses and isotopic patterns for drug compound identification. Only TOF-MS analysis of toxicological samples with accurate mass and isotopic pattern matching can be used for suspect screening. Adding fragment ion information can further prevent a false positive and improve screening accuracy. The integration of screening and identification by QTOF-MS was recently investigated.[7-9]

Agilent Captiva EMR—Lipid sorbent was demonstrated to provide efficient and selective cleanup for the complex bio-matrices in forensic testing applications.[9-14] The EMR—Lipid sorbent packed into an SPE cartridge/plate format enables cleanup to be achieved by simply passing the sample through the sorbent. This study investigated the complete workflow for over 100 drugs of abuse and misused prescription drugs subject to screening, identification, and quantitation in human whole blood. Samples were prepared using in-cartridge protein precipitation followed by Captiva EMR—Lipid cartridge cleanup. The prepared matrix samples were then analyzed on LC/Q-TOF for screening and identification, or on LC-QQQ for identification and accurate quantitation. Table 1 shows the tested drugs and their chemical classification, retention times, and MRM parameters on LC-QQQ.

Experimental

Reagent and chemicals

All analytes and isotope-labeled internal standards (IS) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Agilent Technologies (Santa Clara, CA, USA) as either mixed standard stock solutions, individual stock solutions, or standard powder. All other chemical reagents and solvents were LC/MS, HPLC or analytical grade. Acetonitrile (ACN) and methanol (MeOH) were from Honeywell (Muskegon, MI, USA). Reagent-grade formic acid (FA) was from Agilent Technologies (part number G2453-85060). Ammonium acetate and ammonium hydroxide were from Sigma Aldrich (St. Louis, MO, USA). Human whole blood was bought from BIOIVT (Westbury, NY, USA).

Standards and solutions

The commercial individual standard stock solutions were either 1 mg/mL or 100 µg/mL in MeOH or ACN. The commercial mixed standard solutions were 100 µg/mL or 250 µg/mL in MeOH or ACN. For the remaining standards in powder, the 1 mg/mL of stock solutions were prepared in MeOH. All stock solutions were stored in a freezer at –20 °C. Due to the large number of compounds, six intermediate spiking solutions were prepared at 10 µg/mL in 1:1 MeOH/H_2O, containing a group of analytes. A combined standard spiking solution was then prepared in 1:1 MeOH/water at 250 ng/mL, containing all analytes. This spiking solution was used to prepare calibration standards and quality control (QC) samples. The internal standard (IS) spiking solution was prepared by diluting individual IS stock solutions with 20/80 MeOH/water at 1 µg/mL and was used to spike samples directly. All standard solutions were stored in amber glass vials in a freezer at –20 °C for one month.

Mobile phase A was 10 mM ammonium acetate and 0.125% FA in water. Mobile phase B was 10 mM ammonium acetate and 0.125% FA in 95/5 ACN/water. Needle wash was 1:1:1:1 ACN/MeOH/IPA/H_2O with 0.2% FA. The extraction solvent, 95/5 ACN/MeOH, was freshly prepared and kept cold at –20 °C until use. The 80/20 ACN/water was used as the additional elution solution. The reconstitution solution was 90/10 5 mM ammonium acetate buffer/ACN (v/v).

Equipment and materials

Equipment and materials used for sample preparation included:

- Agilent Positive Pressure Manifold 48 Processor (PPM-48) (part number 5191-4101)
- 6 mL cartridge rack for PPM-48 (part number 5191-4103)
- Collection rack, 13 × 100 mm tubes, for PPM-48 (part number 5191-4107)
- Agilent Captiva EMR—Lipid 3 mL cartridge (part number 5190-1003)
- Agilent Captiva filter vial, Regenerated Cellulose (RC), 0.2 µm (part number 5191-5940)
- Multitube vortexer (VWR, PA, USA)
- Glass tubes, 13 × 100 mm and 13 × 85 mm (VWR, PA, USA)
- Eppendorf pipettes and repeater
- SPE TurboVap evaporator

Instrument conditions

The samples were run on an Agilent 1290 Infinity II system consisting of an Agilent 1290 Infinity II binary pump (G4220A), an Agilent 1290 Infinity II high-performance autosampler (G4226A), and an Agilent 1290 Infinity II thermostatted column compartment (G1316C). The LC system was coupled to an Agilent triple quadrupole LC/MS system (G6490) equipped with an Agilent Jet Stream iFunnel electrospray ion source. MassHunter workstation software was used for data acquisition and analysis. Another LC system was coupled to an Agilent 6546A LC/Q-TOF system equipped with an ESI source with Jet Stream technology. Data processing based on the customized Agilent Forensic Toxicology Personal Compounds Database and Library B.07.01 (PCDL) was performed with Agilent SureMassdata format in MassHunter Quantitative Analysis software (for Q-TOF) version 10.1.

Data acquisition was by dynamic multiple reaction monitoring (dMRM) mode for all quantitation work. The precursor and product ions (quantitative and qualitative ions), collision energy (CE), and polarity optimized for each compound are listed in Table 1. The following settings were consistent for all analytes: MS1 and MS2 Res: unit, delta RT window: 2 minutes, cell accelerator voltage: 4V.

LC conditions	
Columns	Agilent ZORBAX Eclipse Plus C18, 100 × 2.1 mm, 1.8 µm (p/n 959758-902) Agilent ZORBAX Eclipse Plus C18 guard, 2.1 × 5 mm, 1.8 µm (p/n 821725-901)
Flow Rate	0.4 mL/min
Column Temperature	40 °C
Injection Volume	5 µL
Mobile Phase	A) 10 mM ammonium acetate buffer with 0.125% FA in water B) 10 mM ammonium acetate and 0.125% FA in 95/5 ACN/water
Needle Wash	1:1:1:1 ACN/MeOH/IPA/H2O with 0.2% FA
Needle Height	3 mm
Gradient	Time (min) %B Flow Rate (mL/min) 0 10 0.4 0.5 10 0.4 8.0 80 0.4 8.01 100 0.5
Stop Time	11 min
Post Time	2 min
QQQ Conditions	
Gas Temperature	220 °C
Gas Flow	18 L/min
Nebulizer	22 psi
Sheath Gas Heater	400 °C
Sheath Gas Flow	12 L/min
Capillary	3,500 V (POS), 3,500 (NEG)
Nozzle Voltage	0 (POS), 0 (NEG)
iFunnel Parameters	High-pressure RF: 120 V (POS), 110 V (NEG) Low-Pressure RF: 60 V (POS), 60 V (NEG)
Data Acquisition	Dynamic multiple reaction monitoring (dMRM)
Acquisition Polarity	Positive and negative

Q-TOF conditions	
Drying Gas (N_2) Temperature	250 °C
Drying Gas Flow	13 L/min
Nebulizer Pressure	30 psig
Sheath Gas Temperature	375 °C
Sheath Gas Flow	12 L/min
Nozzle Voltage	0 V for ESI+ and 2,000 V for ESI–
Capillary Voltage	3,500 V for ESI+ and 5,000 V for ESI–
Skimmer	65 V
Octupole Radio Frequency (RF)	750 V
Fragmentor	125 V

Table 1. Drug analyte classification and data acquisition method parameters on LC-QQQ.

Drug Compound	Drug Class	RT (min)	ESI Polarity	Precursor Ion	Product Ion			
					Quant ion	CE (V)	Qual ion	CE (V)
Ecgonine methyl ester	Alkaloid	0.78	POS	200.1	182.1	19	81.9	23
Strychnine		3.56	POS	335.2	184.1	40	156.1	40
Cocaethylene		5.00	POS	318.2	196.1	19	82.0	39
Lidocaine	Aminoethylamide	3.67	POS	235.2	86.1	23	58.1	39
Phenylpropanolamine	Amphetamine	1.62	POS	152.1	117.0	19	134.1	7
Ephedrine		2.18	POS	166.1	148.1	11	115.2	35
Amphetamine		2.83	POS	136.1	91.1	20	65.0	40
MDA		3.07	POS	180.1	163.1	4	105.1	24
Phendimetrazine		3.11	POS	192.1	115.0	35	91.0	43
Methamphetamine		3.15	POS	150.1	91.1	20	119.1	8
MDMA		3.30	POS	194.1	163.1	8	105.1	24
Diethylpropion		3.34	POS	206.2	105.0	19	77.0	55
Phentermine		3.40	POS	150.1	91.0	40	65.1	48
MDEA		3.65	POS	208.1	163.1	8	105.1	24
Primidone	Anticonvulsant	3.90	POS	219.1	162.1	11	91.0	39
Carbamazepine		5.89	POS	237.1	194.0	23	192.1	23
Citalopram		5.59	POS	325.2	109.1	35	262.1	23
Chlorpheniramine	Antihistamine	4.89	POS	275.1	230.0	15	167.1	55
Diphenhydramine		5.51	POS	256.2	167.1	19	165.1	51
Risperidone	Antipsychotics	4.85	POS	411.2	191.1	43	69.0	71
Quetiapine		5.42	POS	384.2	253.1	23	221.1	55
Phenobarbital	Barbiturate	4.98	NEG	231.1	41.9	19	132.9	15
Butabarbital		5.14	NEG	211.1	42.0	27	N/A	
Butalbital		5.44	POS	223.1	41.9	27	180.0	11
Amobarbital		5.97	NEG	225.1	42.0	27	N/A	
Secobarbital		6.30	NEG	237.1	42.0	19	N/A	
7-Aminoclonazepam	Benzodiazepine	4.09	POS	286.1	121.0	31	222.1	27
Chlordiazepoxide		5.03	POS	300.1	282.1	31	227.1	35
Midazolam		5.44	POS	326.1	291.1	31	223.1	47
Flurazepam		5.47	POS	388.2	315.1	31	134.0	55
Demoxepam		5.68	POS	287.1	105.0	23	179.9	23
Oxazepam		6.14	POS	287.1	241.1	20	104.1	40
Nitrazepam		6.22	POS	282.1	236.1	24	180.1	40
Lorazepam		6.30	POS	321.0	275.0	20	229.1	32
Alprazolam		6.35	POS	309.1	205.1	55	281.1	23
2-Hydroxyethylflurazpam		6.37	POS	333.1	109.0	39	119.0	80
Clonazepam		6.41	POS	316.1	214.0	51	270.0	27
Triazolam		6.44	POS	343.1	239.1	51	308.0	31
Desalkylflurazepam		6.67	POS	289.1	225.9	35	140.0	39
Nordiazepam		6.76	POS	271.1	140.0	31	165.1	35
Temazepam		6.80	POS	301.2	255.1	16	177.0	44
Clobazam		6.97	POS	301.1	259.1	23	224.0	39
Diazepam		7.49	POS	285.1	193.1	32	154.1	24

Drug Compound	Drug Class	RT (min)	ESI Polarity	Precursor Ion	Product Ion			
					Quant ion	CE (V)	Qual ion	CE (V)
Atenolol	Beta blocker	1.34	POS	267.2	190.1	19	145.1	23
Metoprolol		4.13	POS	268.2	56.1	31	77.0	75
Propranolol		5.16	POS	260.2	56.0	31	116.1	15
Norbuprenorphine	Buprenorphine	4.78	POS	414.3	55.1	79	101.0	47
Buprenorphine		5.71	POS	468.3	55.1	67	100.9	47
Meprobamate	Carisoprodol	4.72	POS	219.1	158.1	7	54.9	23
Carisoprodol		6.36	POS	261.2	55.0	31	176.1	7
Benzoylecgonine	Cocaine	3.60	POS	290.1	168.1	19	77.0	71
Cocaine		4.51	POS	304.2	182.1	16	82.0	48
m-Hydroxybenzoylecgonine	Cocaine metabolite	3.20	POS	306.1	168.1	19	65.0	79
Zopiclone	Cyclopyrrolone	5.47	POS	389.1	174.9	80	254.7	35
Norfentanyl	Fentanyl	3.77	POS	233.2	84.1	23	55.4	43
Fentanyl		5.40	POS	337.2	188.1	23	105.1	43
Doxylamine	Histamine H1 antagonist	3.78	POS	271.2	182.0	19	167.0	39
Hydroxyzine		6.10	POS	375.2	201.0	19	165.3	80
Prednisone	Hormone	5.41	POS	359.1	147.2	33	341.2	9
Clonidine	Imidazole	2.47	POS	230	74.0	79	72.9	80
Zolpidem	Imidazopyridine	4.66	POS	308.2	235.5	39	65.0	80
Normeperidine	Meperidine	4.50	POS	234.2	160.1	15	56.1	31
Meperidine		4.55	POS	248.2	174.1	16	220.1	20
Methadone		6.38	POS	310.2	105.0	28	265.2	12
Ketamine	NMDA antagonist	3.74	POS	238.1	125.0	31	89.0	71
Morphine	Opiate	1.02	POS	286.2	152.1	79	153.0	47
Hydromorphone		1.39	POS	286.2	184.9	31	157.1	51
Dihydrocodeine		2.27	POS	302.2	199.1	35	128.1	79
Naloxone		2.44	POS	328.2	310.2	19	212.2	51
Codeine		2.48	POS	300.2	128.1	60	165.1	40
6-Acetylmorphine		3.12	POS	328.2	211.0	31	165.0	59
Hydrocodone		3.24	POS	300.2	128.1	60	171.1	40
Dextromethorphan		5.31	POS	272.2	171.1	47	128.1	80
EDDP		5.94	POS	278.2	234.1	35	115.0	80
Oxymorphone	Oxycodone	1.15	POS	302.1	284.0	19	227.1	31
Oxycodone		3.01	POS	316.2	241.1	28	256.1	24
Proadifen	P450 inhibitor	7.20	POS	354.2	91.1	40	167.1	40
PCP	Phencyclidine	5.17	POS	244.2	91.1	36	86.2	8
Acepromazine	Phenothiazine	5.78	POS	327.2	86.0	21	58.0	45
Promethazine		5.85	POS	285.1	71.0	47	86.0	19
Chlorpromazine		6.57	POS	319.1	58.1	45	86.0	21
Ritalinic acid	Phenylacetic acid	3.44	POS	220.1	84.0	31	56.1	59
Verapamil	Phenylalkylamine	6.26	POS	455.3	165.1	28	150.1	48
Norpropoxyphene	Propoxyphene	6.15	POS	326.2	252.2	3	91.0	51
Propoxyphene		6.29	POS	340.2	58.1	15	266.2	7
Paroxetine	Selective serotonin reuptake inhibitor	5.91	POS	330.2	70.1	31	192.1	19
Fluvoxamine		6.06	POS	319.2	200.0	23	71.1	19
Fluoxetine		6.38	POS	310.1	117.0	59	91.0	80
Sertraline		6.51	POS	306.1	159.0	31	275.0.	11

Drug Compound	Drug Class	RT (min)	ESI Polarity	Precursor Ion	Product Ion			
					Quant ion	CE (V)	Qual ion	CE (V)
Methylphenidate	Stimulant	4.21	POS	234.2	84.1	27	56.1	67
cis-Tramadol	Tramadol	4.17	POS	264.2	56.1	75	58.1	35
N-desmethyl-cis-tramadol		4.21	POS	250.2	232.1	7	121.1	31
Trazodone	Triazolopyridine	4.98	POS	372.2	176.1	23	148.1	36
Clozapine	Tricyclic dibenzodiazepine	5.28	POS	327.1	270.1	23	192.0	55
Doxepin		5.66	POS	280.2	107.0	27	77.0	59
Dothiepin		6.01	POS	296.2	202.0	63	222.6	31
Desipramine		6.01	POS	267.2	72.0	19	44.1	55
Cyclobenzaprine		6.12	POS	276.2	215	51	58.1	19
Imipramine		6.13	POS	281.2	86.1	19	58.1	43
Nortriptyline		6.17	POS	264.2	91.0	27	105.0	23
Amitriptyline		6.28	POS	278.2	91.0	27	105.1	35
Clomipramine		6.73	POS	315.2	86.1	15	58.1	51
Amphetamine-D5		2.81	POS	141.1	124.1	5	93.0	13
Oxycodone-D6		2.98	POS	322.2	304.2	19	262.0	27
Hydrocodone-D6		3.18	POS	306.2	202.1	35	128.1	80
Cocaine-D3		4.51	POS	307.2	185.1	30	82.0	48
Butalbital-D5		5.42	NEG	228.1	42.0	23	185.0	7
Alprazolam-D5		6.32	POS	314.1	286.2	31	210.0	55
Diazepam-D3		7.45	POS	290.1	198.1	32	154.1	24

Figure 1 shows the LC/QQQ chromatogram at the LOQ of DoA in human whole blood extract.

The Q-TOF was tuned and calibrated at a simultaneous high resolution and extended dynamic range of low mass range (*m/z* 1,700) with SureMass optimization enabled. For suspect screening of drugs in samples, data-independent acquisition (DIA) was performed through All Ions MS/MS data acquisition of full spectra in MS mode (*m/z* 50 to 1,000 under ESI+ and *m/z* 40 to 1,000 under ESI-) with four scan segments (at collision energies 0, 10, 20, and 40 V) at a scan rate of 4 spectra/second. Reference ions with *m/z* of 121.05087 (protonated purine) and 922.00980 (protonated HP-0921) in the positive mode and *m/z* of 119.03632 (deprotonated purine) and 966.00072 (formate ion adducted HP-0921) in the negative mode were selected as means

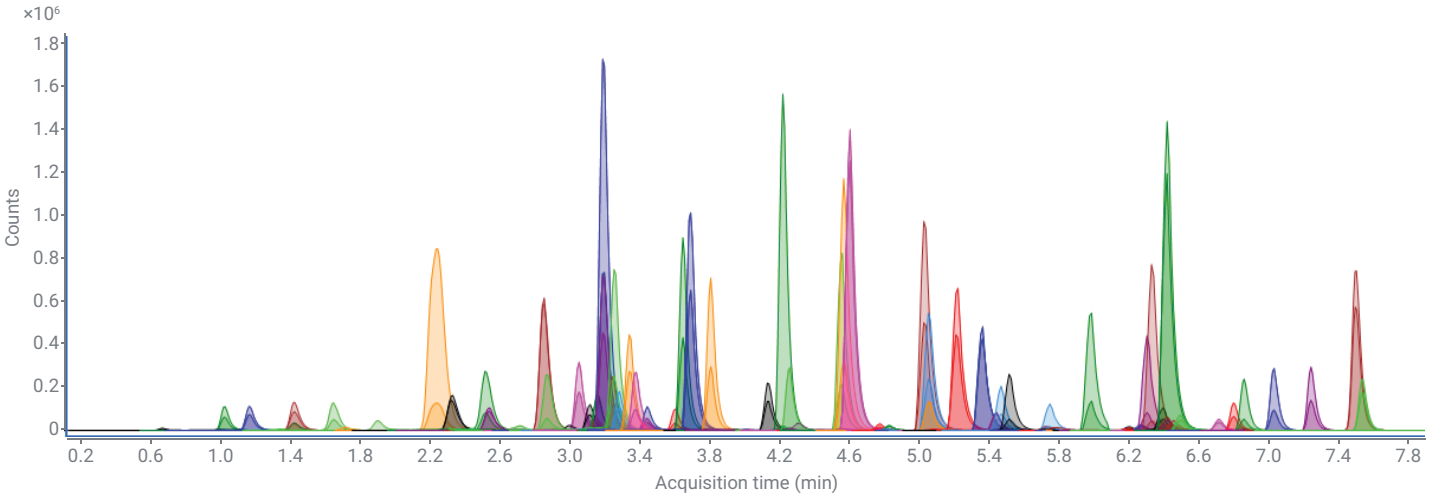

Figure 1. LC-QQQ chromatogram (dMRM) for human whole blood samples fortified at 1 ng/mL of DoA in human whole blood. Samples were extracted by in-cartridge protein precipitation followed by Agilent Captiva EMR—Lipid cleanup.

of ion correction for accurate mass measurements. The reference solution was introduced into a dual-ESI source using an Agilent 1200 isocratic pump and a 100:1 splitter set at a flow rate of 10 µL/min.

Sample preparation

Human whole blood with K_3EDTA was used as a sample matrix control for method development and verification tests. To emulate a common sample size used in forensic lab, 0.5 mL of whole blood was used for sample preparation. Prior to extraction, EMR—Lipid 3 mL cartridges were set up on the PPM-48 processor with labeled collection tubes beneath. Human whole blood was taken from the refrigerator and warmed to room temperature for 10 minutes. Control blood samples were then spiked appropriately with standard and/or internal standard solutions. Samples were vortexed for two minutes before extraction. The following procedure was then followed to prepare the sample:

1. An aliquot of 0.5 mL of whole blood sample was transferred into an EMR—Lipid cartridge, and then 2 mL of cold crashing solvent 95/5 ACN/MeOH was added. It was important to only take the crashing solvent from the freezer right before addition. The use of cold crashing solvent improved the protein precipitation efficiency in whole blood. As whole blood is highly viscous and contains more proteins, samples usually stay in the cartridges without gravity flow.
2. Sample mixtures were settled for 5 to 10 minutes. Low level pressure (2 to 5 psi) was then applied gradually for sample elution. It was important to control the flow rate at 3 to 5 seconds per drop. When working with multiple cartridges at once, the pressure should always be adjusted to the ones with the fastest flow, being careful not to apply any sudden high-pressure spikes.
3. After all cartridges appeared dry, an aliquot of 625 µL of 80/20 ACN/water was added for additional elution. Low-level pressure was used to control the flow rate until no visible liquid was left in cartridges. High-level pressure (6 to 12 psi) was applied to dry the sorbent bed completely.
4. The collection tubes were removed, and the eluent was mixed well by gently vortexing for 10 seconds. An aliquot of 2 mL eluent was transferred to another tube for N_2 drying at 35 °C.
5. The dried sample was reconstituted into 640 µL of reconstitution solution. Samples were vortexed for two minutes, sonicated for 10 minutes, and then transferred to RC filter vials for filtration. The filtrate in the filter vial was then injected for analysis.

Calibration standards and QCs

To minimize the impact of standard spiking to the whole blood matrix, an intermediate spiking solution of 250 ng/mL was prepared in whole blood. This intermediate solution was then used for all calibration curve standards and QC sample spiking. The dynamic range for the calibration curve ranged from 0.5 to 50 ng/mL, including 0.5, 1, 5, 10, 20, 40, and 50 ng/mL. The dynamic range for five barbiturate drugs were adjusted to 5 to 250 ng/mL, with a corresponding five times higher concentration for each level. This adjustment was due to low instrument sensitivity caused by poor ionization and fragmentation of this class of drugs. The dynamic range of fentanyl ranged from 0.05 to 5 ng/mL, with a corresponding ten times lower concentration for each level. This was due to the ten times lower concentration of fentanyl in the mixed standard stock solution. These standards were prepared by spiking an appropriate volume of intermediate standard spiking solution into the whole blood blank, and vortexing well.

Prespiked QC samples were run for method verification tests, including low QC, mid QC, and high QC levels for each analyte, depending on the calibration range of different analyte. These QC samples were prepared by spiking an appropriate volume of intermediate spiking solution into the sample whole blood blank. An appropriate volume of IS spiking solution (1 µg/mL in 20/80 MeOH/water) was then spiked into calibration standards and QC samples to generate the final IS concentration of 50 ng/mL in whole blood. All samples were vortexed gently for thorough mixing, and samples were then ready for sample preparation.

Suspect screening and identification (LC/Q-TOF)

Whole blood samples spiked with drugs of abuse standards were prepared for LC/Q-TOF analysis using the developed sample preparation method. A whole blood blank was spiked in the same way as QC samples prepared for LC-QQQ quantitation. The same intermediate standard spiking solution was used to spike the whole blood matrix control for 10 and 50 ng/mL levels, except 50 and 250 ng/mL for five barbiturate drugs and 1 and 5 ng/mL for fentanyl. An additional 1 ng/mL level sample was spiked to collect data for buprenorphine, due to the requirement of low-level screening. This standard spiking resulted in positive findings for all 102 drugs in whole blood samples. All spiked samples were then prepared using the aforementioned method.

To verify the developed screening method practically, whole blood blank samples were spiked by another scientist. Sample spiking information, including number of drugs, drug type and drug concentration, was completely blind to the scientist who ran the samples on LC/Q-TOF for screening

and identification. These samples were treated as unknown samples, and eight of these unknown samples were prepared together with control blank. After LC/Q-TOF screening and identification, results were verified with the scientist who prepared the samples.

Quantitation method verification (LC-QQQ)

Quantitation method verification was performed through an assessment of method analytical sensitivity and selectivity, recovery and matrix effect and accuracy and precision. Both calibration standards and QCs were prespiked appropriately. Samples and blank were aliquoted into EMR—Lipid 3 mL cartridges, including double matrix blank, matrix blank (spiked with IS), a set of calibration standards, three matrix blanks and six replicates of QC samples at each spiking level, respectively.

Analyte recovery and matrix effect studies included six replicates of prespiked samples at 10 and 50 ng/mL levels in whole blood, six replicates of matrix matched samples at equivalent levels post spiked during reconstitution step, and six replicates of neat standard at equivalent levels. The analyte peak areas were used for recovery and matrix effect assessment. The ratios of analyte peak areas in prespiked samples versus matrix-matched samples were used for analyte recovery calculation, while the ratios of analyte peak areas in matrix-matched samples versus neat standards were used for matrix-effect calculation.

Results and discussion

Sample preparation optimization

Sample preparation plays a critical role in the success of the entire workflow. It is expected that the sample preparation method provides satisfactory recovery and reproducibility for analytes, as well as efficient matrix cleanup. A simplified and robust sample preparation method is important for the method to be consistently transferred person-to-person and lab-to-lab. EMR—Lipid products have been demonstrated to provide efficient matrix removal by passing through cleanup after traditional PPT. In this study, the sample workflow was investigated further based on analyte recovery, reproducibility, and matrix effect.

In-cartridge versus offline PPT

The in-cartridge PPT and offline PPT were compared for workflow feasibility and convenience, and analyte recovery.

In comparison to offline PPT, the in-cartridge PPT showed to be beneficial for simplified operation with less sample transferring steps. Given the high viscosity of whole blood, the EMR—Lipid cartridge held the whole blood intact after addition in the cartridge. No whole blood breakthrough was observed for the many cartridges tested. Even with the large amount of precipitates generated in the cartridge, no cartridge was observed with significant clogging. Variations of flow rate could be observed cartridge by cartridge, which was due to the variations of instant PPT and precipitate particulate size. These variations were usually insignificant, and the flow rate could be well-controlled by adjusting the vacuum or positive pressure control based on the cartridges with the fastest flow. The recovery results comparison also indicated that the used in-cartridge PPT improved the majority of analyte recoveries by 20 to 25%. The average recovery of 102 analytes increased from 62% for offline PPT to 86% for in-cartridge PPT, and the average RSD reduced from >20% for offline PPT to <10% for in-cartridge PPT. As a result, the in-cartridge PPT was confirmed for both feasibility and performance improvement on sample preparation workflow and was chosen for the optimized method.

EMR—Lipid elution

EMR—Lipid cartridges provided an efficient pass-through cleanup for unwanted matrix interferences, especially for lipids. The additional elution step was recommended in order to improve the complete analyte elution for high recoveries.[10-13] The impact of this additional elution was investigated for analyte recovery and reproducibility comparison. The results indicated that the additional elution improved recoveries by 10 to 20% for many drug compounds. This was especially true for 6-acetylmorphine, ritalinic acid, benzoylecgonine, doxylamine, risperidone, chlorpheniramine, midazolam, acepromazine, and triazolam, where recoveries increased by more than 50%. The additional elution on the EMR—Lipid cartridge was used in the final optimal method.

LC/Q-TOF screening and identification

Data acquisition

Agilent All Ions MS/MS data-independent acquisition (DIA), was used to acquire data. The collision cell fragmented all ions with different collision energy (CE) settings: 0, 10, 20, and 40 V respectively, to form a number of fragment ions for each pseudo-molecular ion at a defined mass range of *m/z* 50 to 1,000 for ESI+ and *m/z* 40 to 1,000 for ESI−. Therefore, the individual compound was identified using the Agilent PCDL with the accurate mass of all ions (pseudo-molecular ion and fragments), isotope fidelity, retention time, and coelution of the pseudo-molecular ion and fragment ions (Table 2). For isobaric analytes pairs, methamphetamine/phentermine, methylphenidate/normeperidine, hydromorphone/morphine, and codeine/hydrocodone, chromatographic separation was a key factor on compound differentiation, where retention time (RT) was a critical parameter for identification, as demonstrated in Table 2, column D.

Table 2. Analyte identification parameters on LC/Q-TOF.

Compound Name	Formula	Adduct	RT (min)	RT Diff., 10 ng/mL	Target Pseudo molecular Ion	Accurate Fragment 1	Accurate Fragment 2	Accurate Fragment 3	Accurate Fragment 4	Precursor Mass Accuracy (ppm), 10 ng/mL	Number of Verified Ions, 10 ng/mL
A[1]	B	C	D	E	F	G	H	I	J	K	L
2-Hydroxyethylflurazpam	$C_{17}H_{14}ClFN_2O_2$	$[M+H]^+$	5.61	0.004	333.08006	211.07918	109.04481	140.02567	140.02567	1.15	5
6-Acetylmorphine	$C_{19}H_{21}NO_4$	$[M+H]^+$	2.42	0.008	328.15433	165.06988	211.07536	181.06479	58.06513	0.70	3
7-Aminoclonazepam	$C_{15}H_{12}ClN_3O$	$[M+H]^+$	3.51	0.008	286.07417	121.07603	250.09749	222.10257	94.06513	0.36	3
Acepromazine	$C_{19}H_{22}N_2OS$	$[M+H]^+$	4.99	0.012	327.15256	58.06513	86.09643	222.09134	254.06341	1.30	4
Alprazolam	$C_{17}H_{13}ClN_4$	$[M+H]^+$	5.62	0.001	309.09015	205.07603	281.07143	240.04488	219.09168	0.07	4
Amitriptyline	$C_{20}H_{23}N$	$[M+H]^+$	5.45	0.004	278.19033	91.05423	233.13248	105.06988	117.06988	0.70	1
Amobarbital[2]	$C_{11}H_{18}N_2O_3$	$[M-H]^-$	5.13	0.006	225.12447	41.99854	182.11865	68.99820	85.00435	-1.90	1
Amphetamine	$C_9H_{13}N$	$[M+H]^+$	2.32	0.007	136.11208	91.05423	65.03858	119.08553	63.02293	0.98	3
Atenolol	$C_{14}H_{22}N_2O_3$	$[M+H]^+$	1.18	0.003	267.17032	145.06479	56.04948	190.08626	74.06004	0.32	2
Benzoylecgonine	$C_{16}H_{19}NO_4$	$[M+H]^+$	2.97	0.005	290.13868	105.03349	168.10191	77.03858	82.06513	0.53	3
Buprenorphine[3]	$C_{29}H_{41}NO_4$	$[M+H]^+$	5.02	0.007	468.31084	55.05423	396.21803	414.26389	84.08078	-2.32	2
Butabarbital[2]	$C_{10}H_{16}N_2O_3$	$[M-H]^-$	4.35	0.009	211.10882	41.99854	168.10300	85.00435	124.11317	-0.26	1
Butalbital[2]	$C_{11}H_{16}N_2O_3$	$[M-H]^-$	4.61	0.014	223.10882	41.99854	180.10300	85.00435	136.11317	1.96	1
Carbamazepine	$C_{15}H_{12}N_2O$	$[M+H]^+$	5.10	0.002	237.10224	194.09643	193.08860	179.07295	192.08078	1.39	5
Carisoprodol	$C_{12}H_{24}N_2O_4$	$[M+H]^+$	5.58	0.004	261.18088	55.05423	62.02366	176.12812	97.10118	0.51	5
Chlordiazepoxide	$C_{16}H_{14}ClN_3O$	$[M+H]^+$	4.68	0.004	300.08982	282.07925	247.11040	227.04963	283.08708	-0.48	5
Chlorpheniramine	$C_{16}H_{19}ClN_2$	$[M+H]^+$	4.29	0.010	275.13095	230.07310	167.07295	201.03398	118.06513	0.36	4
Chlorpromazine	$C_{17}H_{19}ClN_2S$	$[M+H]^+$	5.70	0.002	319.10302	58.06513	86.09643	214.04180	246.01387	1.64	5
cis-Tramadol	$C_{16}H_{25}NO_2$	$[M+H]^+$	3.54	0.002	264.19581	58.06513	246.18524			-0.01	3
Citalopram	$C_{20}H_{21}FN_2O$	$[M+H]^+$	4.78	0.002	325.17107	109.04481	262.10265	234.07135	116.04948	0.43	5
Clobazam	$C_{16}H_{13}ClN_2O_2$	$[M+H]^+$	6.05	0.004	301.07383	224.09441	259.06327	105.03349	153.02092	0.28	4
Clomipramine	$C_{19}H_{23}ClN_2$	$[M+H]^+$	5.86	0.016	315.16225	58.06513	86.09643	227.04963	242.07310	0.48	5
Clonazepam	$C_{15}H_{10}ClN_3O_3$	$[M+H]^+$	5.51	0.003	316.04835	214.04180	241.05270	270.05544	207.09166	-0.19	5
Clonidine	$C_9H_9Cl_2N_3$	$[M+H]^+$	1.99	0.010	230.02463	159.97153	144.96063	123.99485	132.96063	-0.37	5
Clozapine	$C_{18}H_{19}ClN_4$	$[M+H]^+$	4.65	0.003	327.13710	270.07925	192.06820	227.03705	84.08078	0.79	4
Cocaethylene	$C_{18}H_{23}NO_4$	$[M+H]^+$	4.26	0.006	318.16998	82.06513	196.13321	105.03349	91.05423	1.33	4
Cocaine	$C_{17}H_{21}NO_4$	$[M+H]^+$	3.79	0.003	304.15433	82.06513	182.11756	105.03349	77.03858	0.48	3
Codeine	$C_{18}H_{21}NO_3$	$[M+H]^+$	1.89	0.011	300.15942	165.06988	153.06988	199.07536	181.06479	0.56	1
Cyclobenzaprine	$C_{20}H_{21}N$	$[M+H]^+$	5.30	0.008	276.17468	215.08553	216.09335	231.11683	58.06513	0.58	3
Demoxepam	$C_{15}H_{11}ClN_2O_2$	$[M+H]^+$	4.90	0.001	287.05818	241.02999	207.06835	77.03858	123.99485	-0.51	3
Desalkylflurazepam	$C_{15}H_{10}ClFN_2O$	$[M+H]^+$	5.82	0.005	289.05385	140.02567	226.09008	165.02092	214.04180	0.64	5
Desipramine	$C_{18}H_{22}N_2$	$[M+H]^+$	5.21	0.003	267.18558	72.08078	44.04948	193.08860	208.11208	0.67	3
Dextromethorphan	$C_{18}H_{25}NO$	$[M+H]^+$	4.57	0.006	272.20089	171.08044	147.08044	213.12739	173.09609	1.20	5
Diazepam	$C_{16}H_{13}ClN_2O$	$[M+H]^+$	6.59	0.007	285.07892	193.08860	154.04180	91.05423	222.11515	1.54	3
Diethylpropion	$C_{13}H_{19}NO$	$[M+H]^+$	2.79	0.007	206.15394	105.06988	77.03858	100.11208	79.05423	0.22	3
Dihydrocodeine	$C_{18}H_{23}NO_3$	$[M+H]^+$	1.81	0.054	302.17507	199.07536	171.08044	201.09101	183.08044	-1.32	3
Diphenhydramine	$C_{17}H_{21}NO$	$[M+H]^+$	4.72	0.001	256.16959	167.08553	152.06205	165.06988	166.07770	0.07	5
Dothiepin	$C_{19}H_{21}NS$	$[M+H]^+$	5.18	0.002	296.14675	203.08553	221.04195	223.05760	217.10118	0.08	5
Doxepin	$C_{19}H_{21}NO$	$[M+H]^+$	4.86	0.005	280.16959	107.04914	115.05423	91.05423	77.03858	0.45	3
Doxylamine	$C_{17}H_{22}N_2O$	$[M+H]^+$	3.51	0.009	271.18049	167.07295	182.09643	90.09134	72.08078	0.52	5
Ecgonine methyl ester	$C_{10}H_{17}NO_3$	$[M+H]^+$	0.66	0.013	200.12812	82.06513	182.11756	68.04948	91.05423	-2.34	1
EDDP	$C_{20}H_{23}N$	$[M+H]^+$	5.10	0.003	278.19033	234.12773	186.12773	219.10425	249.15120	1.34	5

Compound Name	Formula	Adduct	RT (min)	RT Diff., 10 ng/mL	Target Pseudo molecular Ion	Accurate Fragment 1	Accurate Fragment 2	Accurate Fragment 3	Accurate Fragment 4	Precursor Mass Accuracy (ppm), 10 ng/mL	Number of Verified Ions, 10 ng/mL
A[1]	B	C	D	E	F	G	H	I	J	K	L
Ephedrine	$C_{10}H_{15}NO$	$[M+H]^+$	1.79	0.019	166.12264	148.11208	91.05423	115.05423	133.08860	0.08	5
Fentanyl[3]	$C_{22}H_{28}N_2O$	$[M+H]^+$	4.63	0.001	337.22744	105.06988	188.14338	216.13829	132.08078	0.98	3
Fluoxetine	$C_{17}H_{18}F_3NO$	$[M+H]^+$	5.59	0.004	310.14133	44.04948	148.11208			1.27	2
Flurazepam	$C_{21}H_{23}ClFN_3O$	$[M+H]^+$	4.78	0.004	388.15864	315.06950	317.08515	287.06038	271.04279	1.06	4
Fluvoxamine	$C_{15}H_{21}F_3N_2O_2$	$[M+H]^+$	5.33	0.007	319.16279	71.05028	258.11003	200.06816	55.05537	0.25	4
Hydrocodone	$C_{18}H_{21}NO_3$	$[M+H]^+$	2.57	0.001	300.15942	199.07536	171.08044	141.06988	181.06479	0.20	3
Hydromorphone	$C_{17}H_{19}NO_3$	$[M+H]^+$	1.15	0.000	286.14377	185.05971	157.06479	153.06988	181.06479	0.70	2
Hydroxyzine	$C_{21}H_{27}ClN_2O_2$	$[M+H]^+$	5.36	0.001	375.18338	166.07770	201.04655	165.06988	173.12845	0.00	5
Imipramine	$C_{19}H_{24}N_2$	$[M+H]^+$	5.31	0.005	281.20123	58.06513	86.09643	193.08860	208.11208	1.52	3
Ketamine	$C_{13}H_{16}ClNO$	$[M+H]^+$	3.14	0.007	238.09932	125.01525	179.06221	207.05712	220.08875	1.34	3
Lidocaine	$C_{14}H_{22}N_2O$	$[M+H]^+$	3.11	0.005	235.18049	86.09643	58.06513			0.64	3
Lorazepam	$C_{15}H_{10}Cl_2N_2O_2$	$[M+H]^+$	5.49	0.003	321.01921	275.01373	229.05270	303.00865	163.00527	0.87	3
MDA	$C_{10}H_{13}NO_2$	$[M+H]^+$	2.44	0.006	180.10191	105.06988	163.07536	77.03858	135.04406	0.52	4
MDEA	$C_{12}H_{17}NO_2$	$[M+H]^+$	2.98	0.003	208.13321	163.07536	77.03858	135.04406	105.06988	0.70	5
MDMA	$C_{11}H_{15}NO_2$	$[M+H]^+$	2.65	0.005	194.11756	105.06988	163.07536	77.03858	135.04406	0.44	5
Meperidine	$C_{15}H_{21}NO_2$	$[M+H]^+$	3.85	0.012	248.16451	70.06513	220.13321	174.12773	91.05423	0.95	4
Meprobamate	$C_9H_{18}N_2O_4$	$[M+H]^+$	3.99	0.006	219.13393	55.05423	158.11756	97.10118	69.06988	2.43	3
Methadone	$C_{21}H_{27}NO$	$[M+H]^+$	5.56	0.005	310.21654	105.03349	265.15869	77.03858	91.05423	0.38	5
Methamphetamine	$C_{10}H_{15}N$	$[M+H]^+$	2.59	0.004	150.12773	91.05423	119.08553	65.03858	63.02293	0.85	5
Methylphenidate	$C_{14}H_{19}NO_2$	$[M+H]^+$	3.55	0.022	234.14886	84.08078	56.04948	91.05423	55.05423	0.91	2
Metoprolol	$C_{15}H_{25}NO_3$	$[M+H]^+$	3.53	0.008	268.19072	56.04948	103.05423	74.06004	116.10699	0.23	5
m-Hydroxybenzoylecgonine	$C_{16}H_{19}NO_5$	$[M+H]^+$	2.51	0.006	306.13360	121.02841	168.10191	93.03349	82.06513	-0.22	4
Midazolam	$C_{18}H_{13}ClFN_3$	$[M+H]^+$	4.84	0.004	326.08548	291.11663	249.08225	223.07918	209.06353	1.12	5
Morphine	$C_{17}H_{19}NO_3$	$[M+H]^+$	0.94	0.018	286.14377	165.06988	153.06988	157.06479	181.06479	2.67	3
Naloxone	$C_{19}H_{21}NO_4$	$[M+H]^+$	1.78	0.022	328.15433	310.14377	212.07061	253.10973	268.13321	0.36	5
N-desmethyl-*cis*-tramadol	$C_{15}H_{23}NO_2$	$[M+H]^+$	3.57	0.002	250.18016	58.06513	232.16959			0.63	1
Nitrazepam	$C_{15}H_{11}N_3O_3$	$[M+H]^+$	5.36	0.002	282.08732	180.08078	207.09168	236.0944	190.06513	-1.02	5
Norbuprenorphine	$C_{25}H_{35}NO_4$	$[M+H]^+$	4.11	0.005	414.26389	101.09609	83.08553	57.06988	187.07536	-0.66	3
Nordiazepam	$C_{15}H_{11}ClN_2O$	$[M+H]^+$	5.94	0.004	271.06327	140.02567	165.02092	208.09950	91.05423	-0.50	4
Norfentanyl	$C_{14}H_{20}N_2O$	$[M+H]^+$	3.16	0.006	233.16484	84.08078	55.05423	56.04948	94.06513	0.78	3
Normeperidine	$C_{14}H_{19}NO_2$	$[M+H]^+$	3.81	0.000	234.14886	42.03383	160.11208	56.04948	91.05423	0.58	2
Norpropoxyphene	$C_{21}H_{27}NO_2$	$[M+H]^+$	5.38	0.010	326.21146	44.04948	91.05423			0.61	2
Nortriptyline	$C_{19}H_{21}N$	$[M+H]^+$	5.36	0.003	264.17468	91.05423	105.06988	233.1325	117.06988	-0.37	4
Oxazepam	$C_{15}H_{11}ClN_2O_2$	$[M+H]^+$	5.35	0.003	287.05818	104.04948	241.05270	269.04762	163.00527	-2.18	4
Oxycodone	$C_{18}H_{21}NO_4$	$[M+H]^+$	2.36	0.009	316.15433	241.10973	298.14377	212.10699	226.08626	0.75	5
Oxymorphone	$C_{17}H_{19}NO_4$	$[M+H]^+$	1.02	0.005	302.13868	227.09408	284.12812	198.09134	199.09649	1.11	4
Paroxetine	$C_{19}H_{20}FNO_3$	$[M+H]^+$	5.08	0.003	330.15000	70.06513	192.11830	135.06046	109.04481	0.86	3
PCP	$C_{17}H_{25}N$	$[M+H]^+$	4.45	0.006	244.20598	86.09643	91.05423	159.11683	81.06988	-0.10	4
Phendimetrazine	$C_{12}H_{17}NO$	$[M+H]^+$	2.54	0.017	192.13829	91.05423	115.05423	144.08078	146.09643	0.14	4
Phenobarbital[2]	$C_{12}H_{12}N_2O_3$	$[M-H]^-$	4.14	0.007	231.07752	188.07170	85.00435			0.61	1
Phentermine	$C_{10}H_{15}N$	$[M+H]^+$	2.84	0.007	150.12773	91.05423	65.03858	133.10118	105.06988	1.00	4
Phenylpropanolamine	$C_9H_{13}NO$	$[M+H]^+$	1.42	0.031	152.10699	91.05423	117.06988	134.09643	115.05423	-0.38	2
Prednisone	$C_{21}H_{26}O_5$	$[M+H]^+$	4.68	0.004	359.18530	147.08044	237.12739	171.08044	341.17474	0.32	2
Primidone	$C_{12}H_{14}N_2O_2$	$[M+H]^+$	3.26	0.003	219.11280	91.05423	162.09134	119.08553	117.06988	-0.42	2

Compound Name	Formula	Adduct	RT (min)	RT Diff., 10 ng/mL	Target Pseudo molecular Ion	Accurate Fragment 1	Accurate Fragment 2	Accurate Fragment 3	Accurate Fragment 4	Precursor Mass Accuracy (ppm), 10 ng/mL	Number of Verified Ions, 10 ng/mL
A[1]	B	C	D	E	F	G	H	I	J	K	L
Proadifen	$C_{23}H_{31}NO_2$	$[M+H]^+$	6.33	0.004	354.24276	91.05423	209.13248	105.06988	167.08553	-0.02	3
Promethazine	$C_{17}H_{20}N_2S$	$[M+H]^+$	5.03	0.004	285.14200	86.09643	198.03720	71.07295	56.04948	0.26	4
Propoxyphene	$C_{22}H_{29}NO_2$	$[M+H]^+$	5.49	0.007	340.22711	58.06513	266.19033	91.05423	143.08553	0.64	3
Propranolol	$C_{16}H_{21}NO_2$	$[M+H]^+$	4.42	0.004	260.16451	56.04948	116.10699	183.08044	74.06004	0.72	4
Quetiapine	$C_{21}H_{25}N_3O_2S$	$[M+H]^+$	4.76	0.001	384.17402	221.10733	253.07940	210.03720	247.12298	0.22	5
Risperidone	$C_{23}H_{27}FN_4O_2$	$[M+H]^+$	4.25	0.006	411.21908	191.11789	110.05984	69.03349	82.06513	-0.49	2
Ritalinic acid	$C_{13}H_{17}NO_2$	$[M+H]^+$	2.82	0.000	220.13321	84.08078	56.04948	85.08860	91.05423	0.27	2
Secobarbital[2]	$C_{12}H_{18}N_2O_3$	$[M-H]^-$	5.39	0.007	237.12447	41.99854	194.11865	85.00435	150.12882	-2.02	1
Sertraline	$C_{17}H_{17}Cl_2N$	$[M+H]^+$	5.67	0.006	306.08108	158.97628	275.03888	129.06988	122.99960	-0.70	5
Strychnine	$C_{21}H_{22}N_2O_2$	$[M+H]^+$	2.87	0.000	335.17540	184.07569	156.08078	264.10191	222.09134	0.13	3
Temazepam	$C_{16}H_{13}ClN_2O_2$	$[M+H]^+$	5.95	0.002	301.07383	255.06835	283.06327	177.02092	193.08860	0.41	5
Trazodone	$C_{19}H_{22}ClN_5O$	$[M+H]^+$	4.24	0.007	372.15856	148.05237	176.08184	78.03383	133.07603	0.40	5
Triazolam	$C_{17}H_{12}Cl_2N_4$	$[M+H]^+$	5.70	0.005	343.05118	239.03888	315.03245	308.08233	253.06345	-0.30	5
Verapamil	$C_{27}H_{38}N_2O_4$	$[M+H]^+$	5.47	0.002	455.29043	165.09101	150.06753	303.20671	105.06988	0.85	4
Zolpidem	$C_{19}H_{21}N_3O$	$[M+H]^+$	4.03	0.006	308.17574	235.12298	263.11789	236.13080	92.04948	1.32	5
Zopiclone	$C_{17}H_{17}ClN_6O_3$	$[M+H]^+$	3.38	0.006	389.11234	217.02706	245.02198	111.99485	139.00527	0.19	5

[1] Column symbol.

[2] The data listed for amobarbital, butabarbital, butalbital, phenobarbital and secobarbital was 50 ng/mL spiked in whole blood.

[3] The data listed for buprenorphine and fentanyl was 1 ng/mL spiked in whole blood.

Suspect screening parameters and identification criteria

Agilent MassHunter Quantitative Analysis 10.1 with integrated Agilent SureMass was used for the screening analysis.[15] The screening parameters were mass accuracy of a pseudo-molecular ion and its fragment ions, the retention time, the minimum number of verified ions, chromatographic coelution of the pseudo-molecular ion and fragment ions, and the minimum response threshold. A positive screening finding can be achieved by mass accuracy of a pseudo-molecular ion ≤5 ppm, retention time of ±0.4 minutes and the minimum response threshold of S/N >3. The identification criteria were ≤5 ppm for mass accuracy of a pseudo-molecular ion and its fragment ions, ±0.4 minutes for retention time, the minimum of two verified ions including a pseudo-molecular ion and at least one fragment ion, suspect analyte peaks from a pseudo-molecular ion, or fragment ion(s) in the extracted SureMass chromatograms being fully overlapped, and the minimum response threshold of S/N >3. When the pseudo-molecular ion was set as a quantifier and at least one fragment ion was set as the qualifier for each compound, the ion ratio (≤30%) was also evaluated as an additional screening parameter.

Analyte database

The suspect screening analyte database, the Agilent Personal Compounds Database and Library (PCDL), was established based on information about 102 drug compounds, including the precursor accurate mass, the four most abundant fragments, fragment ions in the form of $[M+H]^+$ or $[M-H]^-$, MS/MS spectra collected at different CEs, and retention times. All information is shown in Table 2.

Data analysis workflow and results

In the initial evaluation by the automated workflow, for samples fortified at 50 ng/mL, 93 out of 97 drugs in ESI+ mode were identified as meeting the acceptance criteria, and four drugs were indicated for further review. In ESI– mode, four out of five drugs were identified meeting the criteria, and one drug was indicated for further review. For samples fortified at 10 ng/mL, 89 out of 97 drugs in ESI+ mode were identified meeting the criteria, and eight drugs were indicated for further review. In ESI– mode, all five drugs were indicated for further review. Buprenorphine and fentanyl fortified at 1 ng/mL were identified meeting the criteria.

The drugs that met the identification criteria were confirmed to have pseudo-molecular ions and at least one fragment ion meeting the mass accuracy, retention time, coelution, and S/N criteria. Drugs that required

further review involved either a lack of qualified fragment ions or were misintegrated for either the pseudo-molecular ion or fragment ion peaks. After correction of the integration, these drugs were identified meeting the criteria. The screening and identification criteria were confirmed to demonstrate the optimal detection scenario with minimal false negatives. After manual review, the method was able to screen 102 drugs and identify 100 drugs (98%) at 50 ng/mL and identify 93 drugs (91%) at 10 ng/mL. For buprenorphine and fentanyl, the method was able to identify at 1 ng/mL. No false negatives resulted in the automated workflow at either 10 or 50 ng/mL in whole blood extract.

Blind spiking

The suspect screening method was further evaluated by a blind-spiking experiment. Unknown samples prepared by another scientist were analyzed by the developed Q-TOF screening method to identify the drugs incurring positive results. This was to mimic a practical situation for drug screening in an actual situation. All 16 drugs were positively identified in the provided samples with no false negatives or false positives. Fifteen drugs were successfully identified in the spiked samples at both 10 and 50 ng/mL, and butalbital was identified at 250 ng/mL. See Table 3 for drug screening results in blind-spiked samples.

Table 3. Drug screening results in the blind spiked human whole blood samples by LC-QTOF.

Compound Name	Adduct	BS1[1] 10 ng/mL	RT Diff., 10 ng/mL	Precursor Mass Accuracy (ppm), 10 ng/mL	Number of Verified Ions, 10 ng/mL	BS2 50 ng/mL	RT Diff., 50 ng/mL	Precursor Mass Accuracy (ppm), 50 ng/mL	Number of Verified Ions, 50 ng/mL
Chlordiazepoxide	$[M+H]^+$	Positive	0.001	1.60	5	Positive	0.001	2.19	5
Cyclobenzaprine	$[M+H]^+$	Positive	0.011	1.63	4	Positive	0.000	0.40	4
Morphine	$[M+H]^+$	Positive	0.021	0.87	2	Positive	0.001	0.23	5
Quetiapine	$[M+H]^+$	Positive	0.002	1.93	5	Positive	0.001	1.71	5
		BS2 10 ng/mL				BS2 50 ng/mL			
3-Hydroxyethylflurazepam	$[M+H]^+$	Positive	0.002	-0.65	3	Positive	0.003	1.10	5
7-Aminoclonazepam	$[M+H]^+$	Positive	0.001	-0.80	3	Positive	0.001	0.81	4
Ephedrine	$[M+H]^+$	Positive	0.031	0.74	3	Positive	0.011	1.71	5
Ketamine	$[M+H]^+$	Positive	0.002	0.90	3	Positive	0.001	0.14	3
		BS3 10 ng/mL				BS3 50 ng/mL			
Demoxepam	$[M+H]^+$	Positive	0.001	0.93	4	Positive	0.002	0.25	4
Doxylamine	$[M+H]^+$	Positive	0.020	1.02	4	Positive	0.005	1.61	4
Propranolol	$[M+H]^+$	Positive	0.004	1.30	4	Positive	0.003	1.33	4
Butalbital	$[M-H]^-$	Positive	0.005	0.53	1	Positive	0.000	2.27	2
		BS4 10 ng/mL				BS4 50 ng/mL			
cis-Tramadol	$[M+H]^+$	Positive	0.007	1.37	3	Positive	0.004	1.24	3
Diethylpropion	$[M+H]^+$	Positive	0.010	1.30	3	Positive	0.005	0.98	5
Midazolam	$[M+H]^+$	Positive	0.000	0.44	5	Positive	0.002	0.62	5
Naloxone	$[M+H]^+$	Positive	0.045	0.24	5	Positive	0.012	0.80	5

[1] BS, blind spiking.

LC-QQQ quantitation

The quantitation method on LC-QQQ was evaluated for analyte recovery and matrix effect, analytical sensitivity and selectivity, calibration range, and accuracy and precision. Figure 2 shows the average recoveries of 102 compounds for samples prespiked at 10 ng/mL and 50 ng/mL in whole blood. Given that the common acceptance window for recovery is 70 to 120%, only two of 102 analytes, ritalinic acid and strychnine, were shown with <70% recovery. One analyte (amobarbital) was shown with >120% recovery. This indicates that 97% of drugs generated acceptable recoveries using the optimized workflow. For the three compounds with recoveries outside of the acceptance window, their recoveries were close to the border line with <20% RSD. Therefore, relatively low or high recoveries did not impact the quantitation of these three compounds.

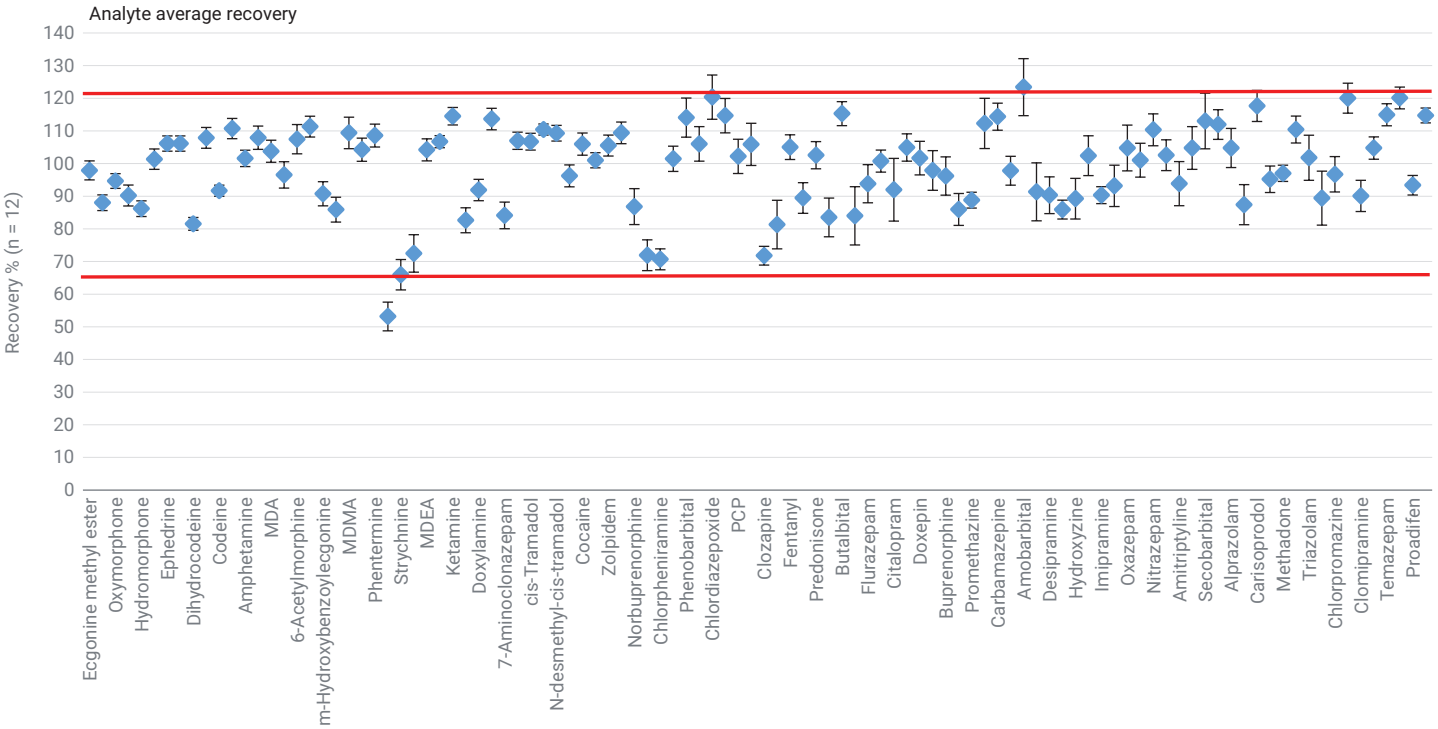

Figure 2. Average recovery for 102 analytes in human whole blood samples fortified at 10 ng/mL and 50 ng/mL. Average recoveries were calculated by the ratio of peak areas in prespiked samples to peak areas in the corresponding level matrix-matched samples, based on six replicates of fortified samples at each level.

Figure 3 shows the average matrix effects of 102 compounds for samples post spiked at 10 ng/mL and 50 ng/mL in whole blood blank extract. For the 102 analytes, seven compounds showed <60% matrix suppression, and the remaining 95 compounds showed reasonable matrix effects within a 60 to 140% window. Five of the seven compounds with <60% matrix effect were very polar drugs in the early eluted retention time (RT) window of less than two minutes. This was exactly the RT window where matrix salts were eluted.

As the PPT extraction and EMR—Lipid cleanup did not remove matrix salts efficiently, there was an expectation to see the early-eluted compounds show significant matrix ion suppression. To mitigate the significant matrix effect on these polar compounds, dried sample extract residue was reconstituted into a greater volume of reconstitution solution, resulting in a final two times dilution of the original sample. Given the acceptable method sensitivity, the dilution of matrix compromised the matrix ion suppression for the polar analytes.

The other two compounds, nitrazepam, with <60% matrix effect was eluted at 6.2 minutes, and doxylamine, with >140% matrix effect had an RT at 3.8 minutes. Their suppressed or enhanced matrix effects were probably linked to a specific matrix interference coeluted in the same RT window. Overall, >93% of analytes generated reasonable matrix effects using the optimized workflow.

The optimized method was then verified for method quantitation of suspect drugs in whole blood. The results shown in

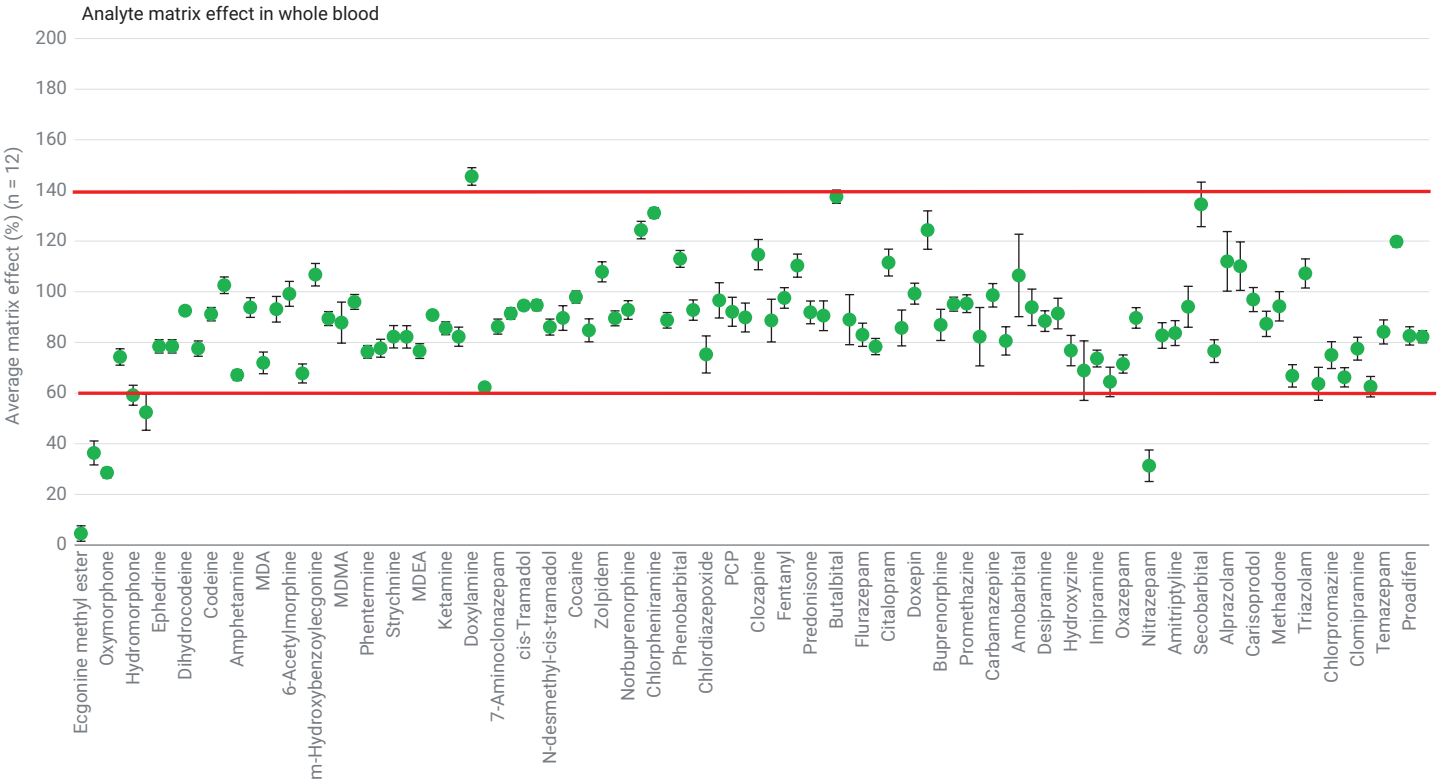

Figure 3. Average matrix effect for 102 analytes in human whole blood samples fortified at 10 ng/mL and 50 ng/mL. Average matrix effects were calculated by the ratio of peak areas in matrix-matched samples to peak areas in the corresponding level neat standards, based on six replicates of fortified samples at each level.

Table 4 include calibration curve data, limit of quantitation (LOQ), accuracy, and precision data. Quantitation results demonstrated excellent method accuracy and precision, with all spiking levels meeting the acceptance criteria (defined as an accuracy of 100 ±20%, and RSD ≤20%). An LOQ of 0.5 ng/mL in whole blood was established for the majority of analytes, except raised LOQ (≥1 ng/mL) for lorazepam, phenobarbital, butabarbital, nortriptyline and zopiclone due to low sensitivity of the compound, and for amphetamine, methamphetamine, and dextromethorphan due to matrix contribution or interference. Given the various linearities for different analytes, different calibration ranges were established with appropriate spiking levels for quantitation verification. Groups with a different calibration range and corresponding spiking level for QC samples are listed in Table 4 as the annotation. Linear regression and a weight of $1/x^2$ were used for most analyte calibration curves with correlation coefficient R^2 >0.99. The calibration curves for five barbiturate analytes (butalbital, amobarbital, secobarbital, phenobarbital, and butabarbital) fit well to quadratic regression using $1/x^2$ weighting with R^2 >0.99. Method selectivity was evaluated for the whole blood matrix blank with matrix contribution less than 20% of LOQ, except for amphetamine, methamphetamine, and dextromethorphan, which showed as positive in the matrix blank. The LOQ for these three drugs were thus raised significantly to accommodate the positive contribution from matrix blank. Given to a completely drug-free whole blood control blank, these analytes should be able to establish a broader calibration range with lower LOQ.

Table 4. Quantitation results summary for 102 analytes in human whole blood by LC-QQQ.

Drug Compound	Quantitation Group	Internal Standard	LOQ (ng/mL)	Calibration Range (ng/mL)	Calibration Curve R^2	Low QC		Mid QC		High QC	
						Accuracy	RSD	Accuracy	RSD	Accuracy	RSD
Fentanyl	5	Cocaine-D3	0.05	0.05 to 20	0.9922	104	7.1	93	4.5	92	9.0
Ecgonine methyl ester	1	Amphetamine-D5	0.5	0.5 to 200	0.9947	100	2.4	100	3.6	86	5.3
Morphine	1	Amphetamine-D5	0.5	0.5 to 200	0.9939	111	3.2	112	3.6	104	5.6
Oxymorphone	1	Amphetamine-D5	0.5	0.5 to 200	0.9928	104	5.1	98	5.3	95	7.2
Atenolol	1	Amphetamine-D5	0.5	0.5 to 200	0.9912	113	3.8	101	4.4	105	4.9
Hydromorphone	1	Amphetamine-D5	0.5	0.5 to 200	0.9936	112	4.8	98	3.9	103	4.4
Phenylpropanolamine	1	Amphetamine-D5	0.5	0.5 to 100	0.9946	101	5.9	109	5.0	95	5.0
Ephedrine	1	Amphetamine-D5	0.5	0.5 to 100	0.999	109	4.2	103	3.1	99	4.7
Dihydrocodeine	1	Amphetamine-D5	0.5	0.5 to 200	0.9935	110	4.7	98	5.6	112	3.0
Naloxone	1	Amphetamine-D5	0.5	0.5 to 200	0.9972	109	3.7	102	3.2	93	7.0
Clonidine	1	Amphetamine-D5	0.5	0.5 to 200	0.9958	113	6.5	96	4.6	104	6.5
Codeine	1	Amphetamine-D5	0.5	0.5 to 100	0.9947	114	3.0	110	4.0	101	5.8
Oxycodone	2	Oxycodone-D6	0.5	0.5 to 50	0.9954	113	7.1	107	7.0	97	10.4
MDA	2	Amphetamine-D5	0.5	0.5 to 50	0.9922	99	6.3	114	8.1	103	7.7
Phendimetrazine	1	Amphetamine-D5	0.5	0.5 to 200	0.9951	109	5.9	101	8.9	115	7.9
6-Acetylmorphine	2	Amphetamine-D5	0.5	0.5 to 50	0.9926	110	2.9	115	9.4	100	5.3
m-Hydroxybenzoylecgonine	1	Amphetamine-D5	0.5	0.5 to 100	0.9956	137	10.1	102	9.7	100	6.7
Hydrocodone	1	Hydrocodone-D6	0.5	0.5 to 200	0.991	102	12.3	97	8.9	100	6.5
MDMA	1	Hydrocodone-D6	0.5	0.5 to 200	0.9932	118	10.4	101	11.7	96	7.1
Diethylpropion	2	Hydrocodone-D6	0.5	0.5 to 50	0.9981	114	8.6	109	6.9	101	7.3
Phentermine	1	Hydrocodone-D6	0.5	0.5 to 200	0.9967	101	10.0	100	10.4	91	8.1
Ritalinic acid	1	Hydrocodone-D6	0.5	0.5 to 200	0.9947	113	10.4	95	7.0	101	10.6
Strychnine	1	Hydrocodone-D6	0.5	0.5 to 100	0.9976	111	10.5	101	7.4	126	7.5
Benzoylecgonine	1	Hydrocodone-D6	0.5	0.5 to 100	0.9931	111	8.1	109	4.8	105	8.3
MDEA	2	Hydrocodone-D6	0.5	0.5 to 50	0.9923	110	9.1	108	5.5	100	9.2
Lidocaine (Lignocaine)	2	Cocaine-D3	0.5	0.5 to 50	0.9954	102	4.6	102	2.5	107	5.9
Ketamine	2	Cocaine-D3	0.5	0.5 to 50	0.9991	104	4.6	95	6.7	91	6.7

Drug Compound	Quantitation Group	Internal Standard	LOQ (ng/mL)	Calibration Range (ng/mL)	Calibration Curve R^2	Low QC		Mid QC		High QC	
						Accuracy	RSD	Accuracy	RSD	Accuracy	RSD
Norfentanyl	2	Cocaine-D3	0.5	0.5 to 50	0.9998	94	3.5	108	3.7	111	6.9
Doxylamine	2	Cocaine-D3	0.5	0.5 to 50	0.9933	100	5.9	107	3.4	106	5.1
Primidone	1	Cocaine-D3	0.5	0.5 to 200	0.9971	98	9.0	89	4.8	91	4.8
7-Aminoclonazepam	2	Cocaine-D3	0.5	0.5 to 50	0.9929	100	6.4	103	8.3	88	9.6
Metoprolol	2	Cocaine-D3	0.5	0.5 to 50	0.9956	95	9.5	106	2.3	104	9.3
cis-Tramadol	1	Cocaine-D3	0.5	0.5 to 100	0.9949	97	10.8	96	6.0	95	9.0
Methylphenidate	2	Cocaine-D3	0.5	0.5 to 20	0.9919	98	6.6	105	8.5	100	8.5
N-desmethyl-*cis*-tramadol	2	Cocaine-D3	0.5	0.5 to 50	0.9952	100	11.4	110	6.8	98	3.8
Normeperidine	2	Cocaine-D3	0.5	0.5 to 20	0.9937	92	7.9	92	5.4	94	9.4
Cocaine	2	Cocaine-D3	0.5	0.5 to 50	0.9906	109	4.2	111	5.7	113	9.7
Meperidine	2	Cocaine-D3	0.5	0.5 to 50	0.9974	95	11.5	103	6.4	107	8.5
Zolpidem	2	Cocaine-D3	0.5	0.5 to 50	0.9857	101	6.1	105	9.3	97	10.7
Meprobamate	1	Cocaine-D3	0.5	0.5 to 200	0.9965	103	9.8	102	5.7	100	7.1
Norbuprenorphine	1	Cocaine-D3	0.5	0.5 to 200	0.9959	56	18.7	113	6.2	103	9.6
Risperidone	1	Hydrocodone-D6	0.5	0.5 to 100	0.9993	95	10.5	97	11.6	112	4.3
Chlorpheniramine	1	Hydrocodone-D6	0.5	0.5 to 100	0.9931	105	7.9	101	5.0	114	4.5
Trazodone	2	Cocaine-D3	0.5	0.5 to 50	0.9916	91	13.3	97	7.1	106	5.7
Cocaethylene	2	Cocaine-D3	0.5	0.5 to 50	0.9924	130	16.3	131	17.0	115	16.0
Chlordiazepoxide	2	Cocaine-D3	0.5	0.5 to 50	0.9958	103	11.9	99	14.0	96	12.0
PCP	1	Cocaine-D3	0.5	0.5 to 100	0.9909	85	4.0	116	10.5	98	7.3
Propranolol	2	Cocaine-D3	0.5	0.5 to 50	0.9955	85	13.8	100	19.7	100	8.8
Clozapine	1	Cocaine-D3	0.5	0.5 to 100	0.9926	101	9.0	102	10.0	110	4.3
Prednisone	2	Cocaine-D3	0.5	0.5 to 50	0.9926	91	12.2	105	12.9	96	8.5
Quetiapine	2	Cocaine-D3	0.5	0.5 to 50	0.9979	98	7.1	105	12.8	98	8.3
Midazolam	2	Cocaine-D3	0.5	0.5 to 50	0.9909	101	8.7	103	13.0	94	15.5
Butalbital	1	Cocaine-D3	0.5	0.5 to 200	0.9913	93	7.7	96	4.2	96	14.0
Flurazepam	2	Cocaine-D3	0.5	0.5 to 50	0.9913	107	9.9	106	9.1	104	16.9
Diphenhydramine	2	Cocaine-D3	0.5	0.5 to 50	0.9939	103	7.4	104	9.1	103	10.9
Citalopram	2	Cocaine-D3	0.5	0.5 to 50	0.9923	106	13.2	99	10.4	85	8.2
Doxepin	2	Cocaine-D3	0.5	0.5 to 50	0.987	94	4.3	94	7.3	92	14.4
Demoxepam	2	Cocaine-D3	0.5	0.5 to 50	0.9973	113	4.1	94	9.9	93	10.6
Buprenorphine	2	Cocaine-D3	0.5	0.5 to 50	0.9963	104	11.2	103	11.8	92	18.6
Acepromazine	2	Cocaine-D3	0.5	0.5 to 50	0.9856	87	14.2	102	9.1	110	7.7
Promethazine	1	Cocaine-D3	0.5	0.5 to 100	0.995	100	13.8	97	10.2	105	15.9
Carbamazepine	2	Cocaine-D3	0.5	0.5 to 50	0.9906	88	10.2	92	5.8	107	11.5
Paroxetine	1	Cocaine-D3	0.5	0.5 to 200	0.9922	101	11.6	98	13.6	105	14.0
EDDP	2	Cocaine-D3	0.5	0.5 to 50	0.9901	114	5.6	114	15.6	95	12.4
Amobarbital	1	Butalbital-D5	0.5	0.5 to 200	0.9909	79	16.1	100	12.4	111	4.0
Dothiepin (Dosulepin)	1	Cocaine-D3	0.5	0.5 to 100	0.9941	103	9.0	77	11.5	92	10.8
Desipramine	2	Cocaine-D3	0.5	0.5 to 50	0.9928	86	7.7	89	13.6	101	8.8
Fluvoxamine	2	Cocaine-D3	0.5	0.5 to 50	0.992	99	9.0	96	7.8	93	6.4
Hydroxyzine	2	Cocaine-D3	0.5	0.5 to 20	0.9882	109	11.2	98	15.7	97	14.2
Cyclobenzaprine	2	Diazepam-D3	0.5	0.5 to 50	0.9992	92	10.6	102	15.0	118	6.5
Imipramine	2	Cocaine-D3	0.5	0.5 to 20	0.9918	75	12.8	101	6.3	103	9.2
Oxazepam	2	Cocaine-D3	0.5	0.5 to 50	0.9971	114	10.7	112	8.2	107	11.4
Norpropoxyphene	2	Cocaine-D3	0.5	0.5 to 20	0.9892	55	22.2	104	14.5	97	16.1

Drug Compound	Quantitation Group	Internal Standard	LOQ (ng/mL)	Calibration Range (ng/mL)	Calibration Curve R^2	Low QC		Mid QC		High QC	
						Accuracy	RSD	Accuracy	RSD	Accuracy	RSD
Nitrazepam	1	Cocaine-D3	0.5	0.5 to 100	0.992	95	10.7	102	16.6	88	17.2
Verapamil	1	Cocaine-D3	0.5	0.5 to 100	0.9915	81	12.5	94	8.7	98	7.0
Amitriptyline	2	Cocaine-D3	0.5	0.5 to 50	0.9891	108	11.2	99	7.1	96	8.4
Propoxyphene	2	Cocaine-D3	0.5	0.5 to 20	0.9987	106	11.5	92	9.4	97	11.8
Secobarbital	4	Butalbital-D5	0.5	1 to 200	0.9905	102	9.6	108	10.1	111	8.7
Alprazolam	2	Aprazolam-D5	0.5	0.5 to 50	0.9919	100	11.3	94	18.8	85	13.0
Carisoprodol	2	Diazepam-D3	0.5	0.5 to 20	0.9927	116	19.6	114	9.7	97	18.4
2-Hydroxyethylflurazpam	2	Aprazolam-D5	0.5	0.5 to 50	0.9974	99	10.6	101	18.9	108	10.5
Methadone	2	Diazepam-D3	0.5	0.5 to 50	0.9978	129	13.5	113	11.1	116	9.7
Fluoxetine	2	Diazepam-D3	0.5	0.5 to 50	0.9946	78	14.6	83	11.8	98	8.7
Clonazepam	2	Aprazolam-D5	0.5	0.5 to 50	0.9935	114	5.6	98	6.3	97	9.7
Triazolam	2	Diazepam-D3	0.5	0.5 to 50	0.9957	93	15.9	104	13.0	107	14.7
Sertraline	1	Diazepam-D3	0.5	0.5 to 100	0.9943	96	13.6	96	9.2	108	14.5
Chlorpromazine	1	Diazepam-D3	0.5	0.5 to 200	0.9955	98	9.3	88	6.8	109	15.0
Desalkylflurazepam	2	Diazepam-D3	0.5	0.5 to 50	0.9941	119	5.2	110	4.8	107	6.5
Clomipramine	2	Diazepam-D3	0.5	0.5 to 50	0.9866	84	3.6	96	8.8	113	10.9
Nordiazepam	2	Diazepam-D3	0.5	0.5 to 50	0.9922	105	11.7	109	10.7	104	4.0
Temazepam	1	Diazepam-D3	0.5	0.5 to 100	0.991	110	5.7	106	6.4	103	3.9
Clobazam	1	Diazepam-D3	0.5	0.5 to 200	0.9962	108	3.4	96	1.9	107	6.9
Proadifen	1	Diazepam-D3	0.5	0.5 to 100	0.9983	102	2.2	95	1.7	104	5.6
Diazepam	2	Diazepam-D3	0.5	0.5 to 50	0.9984	108	1.6	105	2.4	104	2.9
Lorazepam[2]	4	Diazepam-D3	1	1 to 100	0.9876	122	5.3	91	6.6	102	17.8
Amphetamine[1]	3	Amphetamine-D5	5	5 to 200	0.9942	104	7.0	N6	4.2	101	4.0
Methamphetamine[1]	3	Amphetamine-D5	5	5 to 100	0.9884	96	12.3	93	17.2	94	8.0
Phenobarbital[2]	3	Cocaine-D3	5	5 to 200	0.9904	104	11.9	96	5.4	99	10.5
Butabarbital[2]	3	Butalbital-D5	5	5 to 200	0.9913	108	4.4	96	11.0	97	6.9
Dextromethorphan[1]	3	Cocaine-D3	5	5 to 100	0.9977	139	24.7	111	15.0	91	12.9
Nortriptyline[2]	3	Cocaine-D3	5	5 to 100	0.9957	91	17.0	86	13.1	77	3.8
Zopiclone[2]	6	Cocaine-D3	20	20 to 200	0.9972	99	10.9	103	8.2	95	6.3

Quantitation group 1: Calibration range = 0.5-200 or 0.5-100 ng/mL; Low QC = 0.5 ng/mL, Mid QC = 5 ng/mL, High QC = 50 ng/mL
Quantitation group 2: Calibration range = 0.5-50 or 0.5-20 ng/mL; Low QC = 0.5 ng/mL, Mid QC = 1 ng/mL, High QC = 10 ng/mL
Quantitation group 3: Calibration range = 5-200 or 5-100 ng/mL; Low QC = 5 ng/mL, Mid QC = 10 ng/mL, High QC = 50 ng/mL
Quantitation group 4: Calibration range = 1-200 or 1-100 ng/mL; Low QC = 1 ng/mL, Mid QC = 5 ng/mL, High QC = 50 ng/mL
Quantitation group 5: Calibration range = 0.05-20 ng/mL; Low QC = 0.05 ng/mL, Mid QC = 0.5 ng/mL, High QC = 5 ng/mL
Quantitation group 6: Calibration range = 20-200 ng/mL; Low QC = 20 ng/mL, Mid QC = 50 ng/mL, High QC = 200 ng/mL

[1] Target compound was found positive in matrix blank, which either resulted in raised LOQ, or completely messed up the calibration curve.

[2] Target compound has either poor sensitivity or selectivity in matrix, which resulted in raised LOQ.

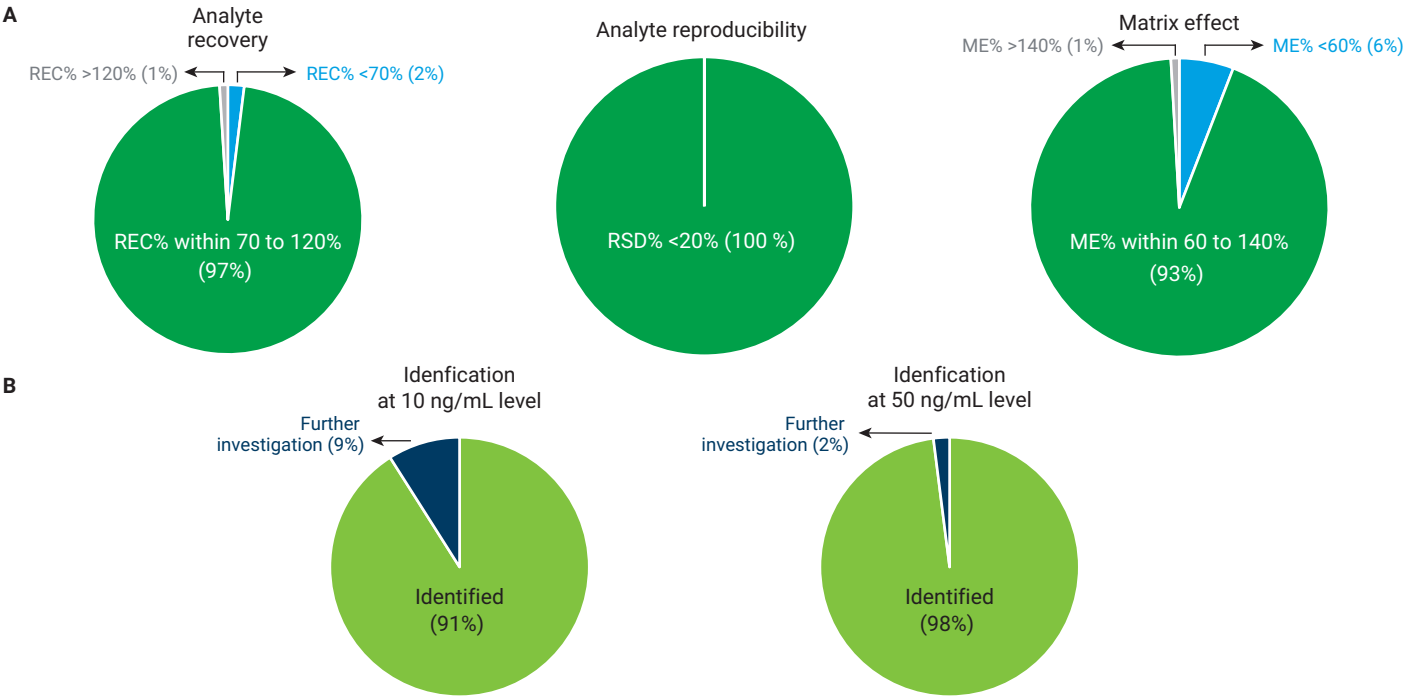

Figure 4. Statistical results for quantitative analysis on LC-QQQ (A) and screening analysis on LC/Q-TOF (B).

Conclusion

An end-to-end workflow, including sample preparation, instrument analysis, and data processing, was developed and verified for over 100 drugs of abuse and medicinal drugs in human whole blood screening, with identification on LC/Q-TOF and quantitation on LC-QQQ. Sample preparation method based on in-cartridge PPT followed by EMR—Lipid cleanup provided simple but efficient analyte extraction and matrix cleanup. With the increased resolution and dynamic range, the LC/Q-TOF workflow proved to reduce the workload for suspect screening in routine practice. Based on the identification criteria used in this study, the method screened 102 drugs in human whole blood and identified 91% and 98% of targets respectively at the 10 and 50 ng/mL levels. In addition, buprenorphine and fentanyl were identified at 1 ng/mL. Quantitation on LC-QQQ was based on dynamic MRM detection. The results showed verified quantitative accuracy and precision, acceptable analyte recovery and matrix effects, and calibration range and linearity.

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