Application Note

Forensic Testing

A Comprehensive Workflow for a Large Panel of Drugs of Abuse in Human Whole Blood by LC/MS/MS

By Captiva EMR–Lipid Cleanup

Author

Limian Zhao
Agilent Technologies, Inc.

Abstract

This study demonstrates the application of Captiva EMR–Lipid 6 mL cartridges for the quantitative determination of 67 common drugs of abuse (DoA) and metabolites in human whole blood by LC/MS/MS. Samples were prepared using in-cartridge protein precipitation (PPT) to remove proteins, followed by Captiva EMR–Lipid cleanup to remove lipids. Large sample size (1 mL) was used to correspond the common sample size used in forensic testing labs. The highly efficient matrix cleanup results in >99% phospholipid removal, which reduces the matrix ion suppression effect and system contamination. The method provided satisfactory 60 to 120% recoveries with acceptable reproducibility of <20% RSD for over 95% of tested drug compounds. The quantitative method's robustness 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 1 ng/mL in whole blood, and linear calibration curves. The method selectivity and carryover were evaluated as well. The results demonstrate that the established protocol using in-cartridge PPT followed by Captiva EMR–Lipid cleanup provides a reliable workflow for quantitative determination of DoA compounds in human whole blood.

Introduction

In forensic toxicology, the demand for fast and reliable screening and quantitative determination of drugs of abuse (DoA) in biological specimens is steadily increasing.[1-3] This is primarily due to the increasing number of drugs of abuse, as well as samples submitted for analysis. Reliable quantitative determination of DoA in blood matrices is therefore important in regular toxicology analysis.

The Agilent Captiva EMR–Lipid 96-well plate was used for high throughput forensic testing in whole blood, plasma, and serum.[4,5] However, a large sample size, 0.5 to 1 mL, was requested more in forensic labs. To accommodate this requirement, the method was modified for 1 mL extraction using Captiva EMR–Lipid 6 mL cartridges. The study also extended the method applicability to include more drugs of abuse and metabolites for quantitative analysis in whole blood.

Experimental

Reagents and chemicals

All reagents and solvents were HPLC or analytical grade. Acetonitrile (ACN) and methanol (MeOH) were from Honeywell (Muskegon, MI, USA). Reagent grade formic acid (FA) was from Agilent (part number G2453-85060). Ammonium acetate was from Sigma-Aldrich (St. Louis, MO, USA). DoA mixed or individual standard stock solutions and isotopic internal standard (IS) stock solutions were from Sigma-Aldrich and Agilent. Human whole blood K3EDTA was from BIOIVT, (Westbury, NY, USA).

Standards and solutions

A combined DoA standard stock solution and individual IS stock solutions were used to prepare the standard and IS spiking solutions. The standard spiking solution was prepared in 50/50 MeOH/water at 1 µg/mL, and it was used to spike calibration standards and QC samples. All drug compounds were prepared at 1 µg/mL in the solution, except the five barbiturate drugs, phenobarbital, butabarbital, butalbital, amobarbital, and secobarbital. These compounds were prepared at higher concentration, 5 µg/mL, due to their relative low instrument sensitivity. The IS spiking solution was prepared by diluting individual IS stock solutions with 20/80 MeOH/water at 1 µg/mL, and it was spiked into samples directly.

A 5 M ammonium acetate stock solution was made by dissolving 192.7 g of ammonium acetate in 500 mL of Milli-Q water and then mixing well. The stock solution was stored in a refrigerator at 4 °C.

Mobile phase A, which is 10 mM ammonium acetate and 0.125% FA in water, was prepared by adding 4 mL of 5 M ammonium acetate stock solution and 2.5 mL of FA into 2 L of Milli-Q water. Mobile phase B, 10 mM ammonium acetate and 0.125% FA in 95/5 ACN/water, was made by adding 4 mL of 5 M ammonium acetate stock solution into 100 mL of water, then adding 1,900 mL of ACN, and finally 2.5 mL of FA. The prepared mobile phases were mixed thoroughly.

Needle wash, 1:1:1:1 ACN:MeOH:IPA:H_2O with 0.2% FA, was prepared by combining 250 mL of ACN, MeOH, IPA, and Milli-Q water, adding 2 mL of FA, and then mixing well.

The reconstitution solution, 9:1 5 mM ammonium acetate buffer:ACN was made by adding 180 µL of 5 M ammonium acetate stock solution into 180 mL of water, and then mixing with 20 mL of ACN. A 80/20 ACN/water solution was made by mixing 80 mL of ACN with 20 mL of water.

Equipment and materials

Equipment and materials used for sample preparation included:

- Agilent positive pressure manifold 48 processor (PPM-48) (part number 5191-4101)
- Cartridge rack for PPM-48, 6 mL (part number 5191-4103)
- Collection rack, 13 × 100 mm tubes, for PPM-48 (part number 5191-4107)
- Agilent Captiva EMR–Lipid 6 mL cartridge (part number 5190-1004)
- 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 condition

The samples were run on an Agilent 1290 Infinity LC consisting of an Agilent 1290 Infinity binary pump (G4220A), an Agilent 1290 Infinity high-performance autosampler (G4226A), and an Agilent 1290 Infinity thermostatted column compartment (G1316C). The LC system was coupled to an Agilent G6490 triple quadrupole LC/MS (G6490A) system, equipped with an Agilent Jet Stream iFunnel electrospray ionization source. Agilent MassHunter workstation software was used for data acquisition and analysis.

LC conditions

Parameter	Value
Column	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:H_2O 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 minutes
Post Time	2 minutes

MS conditions

Parameter	Value
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	dMRM
Acquisition Polarity	Positive and negative

See Table 1 for target analytes and method settings and Figure 1 for LC/MS/MS chromatogram of human whole blood sample spiked at the 10 ng/mL of DoA and prepared by the developed method.

Table 1. List of DoA analytes and instrument method conditions.

Analyte	Drug Class	Retention Time (min)	ESI Polarity	Precursor Ion (*m/z*)	Product Ion (*m/z*)			
					Quant Ion	CE (V)	Qual Ion	CE (V)
Ecgonine methyl ester	Alkaloid	0.782	POS	200.1	182.1	19	81.9	23
Morphine	Opiate	1.016	POS	286.2	152.1	79	153	47
Oxymorphone	Oxycodone	1.146	POS	302.1	284	19	227.1	31
Hydromorphone	Opiate	1.393	POS	286.2	184.9	31	157.1	51
Phenylpropanolamine	Amphetamine	1.623	POS	152.1	117	19	134.1	7
Ephedrine	Amphetamine	2.183	POS	166.1	148.1	11	115.2	35
Dihydrocodeine	Opiate	2.27	POS	302.2	199.1	35	128.1	79
Naloxone	Opiate	2.436	POS	328.2	310.2	19	212.2	51
Codeine	Opiate	2.477	POS	300.2	128.1	60	165.1	40
Amphetamine-D5	IS	2.806	POS	141.1	124.1	5	93	13
Amphetamine	Amphetamine	2.826	POS	136.1	91.1	20	65	40
Oxycodone	Oxycodone	3.009	POS	316.2	241.1	28	256.1	24
MDA	Amphetamine	3.073	POS	180.1	163.1	4	105.1	24
Phendimetrazine	Amphetamine	3.113	POS	192.1	115	35	91	43
6-Acetylmorphine	Opiate	3.117	POS	328.2	211	31	165	59
Methamphetamine	Amphetamine	3.154	POS	150.1	91.1	20	119.1	8
Hydrocodone-D6	IS	3.182	POS	306.2	202.1	35	128.1	80
m-Hydroxybenzoylecgonine	Cocaine metabolite	3.2	POS	306.1	168.1	19	65	79
Hydrocodone	Opiate	3.242	POS	300.2	128.1	60	171.1	40
MDMA	Amphetamine	3.298	POS	194.1	163.1	8	105.1	24
Diethylpropion	Amphetamine	3.339	POS	206.2	105	19	77	55
Phentermine	Amphetamine	3.403	POS	150.1	91	40	65.1	48
Strychnine	Alkaloid	3.559	POS	335.2	184.1	40	156.1	40
Benzoylecgonine	Cocaine	3.603	POS	290.1	168.1	19	77	71
MDEA	Amphetamine	3.648	POS	208.1	163.1	8	105.1	24
Norfentanyl	Fentanyl	3.765	POS	233.2	84.1	23	55.4	43
7-Aminoclonazepam	Benzodiazepine	4.093	POS	286.1	121	31	222.1	27
cis-Tramadol	Tramadol	4.173	POS	264.2	56.1	75	58.1	35
N-desmethyl-*cis*-tramadol	Tramadol	4.214	POS	250.2	232.1	7	121.1	31
Normeperidine	Meperidine	4.504	POS	234.2	160.1	15	56.1	31
Cocaine	Cocaine	4.512	POS	304.2	182.1	16	82	48
Cocaine-D3	IS	4.512	POS	307.2	185.1	30	82	48
Meperidine	Meperidine	4.55	POS	248.2	174.1	16	220.1	20
Meprobamate	Carisoprodol	4.721	POS	219.1	158.1	7	54.9	23
Norbuprenorphine	Buprenorphine	4.783	POS	414.3	55.1	79	101	47
Trazodone	Triazolopyridine	4.975	POS	372.2	176.1	23	148.1	36
Phenobarbital	Barbiturate	4.983	NEG	231.1	41.9	19	132.9	15
Cocaethylene	Alkaloid	5.001	POS	318.2	196.1	19	82	39
Chlordiazepoxide	Benzodiazepine	5.027	POS	300.1	282.1	31	227.1	35
Butabarbital	Barbiturate	5.14	NEG	211.1	42	27		
PCP	Phencyclidine	5.174	POS	244.2	91.1	36	86.2	8
Clozapine	Tricyclic dibenzodiazepine	5.279	POS	327.1	270.1	23	192	55
Dextromethorphan	Opiate	5.307	POS	272.2	171.1	47	128.1	80
Fentanyl	Fentanyl	5.4	POS	337.2	188.1	23	105.1	43
Butalbital-D5	IS	5.419	NEG	228.1	42	23	185	7

Analyte	Drug Class	Retention Time (min)	ESI Polarity	Precursor Ion (*m/z*)	Product Ion (*m/z*)			
					Quant Ion	CE (V)	Qual Ion	CE (V)
Midazolam	Benzodiazepine	5.438	POS	326.1	291.1	31	223.1	47
Butalbital	Barbiturate	5.444	POS	223.1	41.9	27	180	11
Flurazepam	Benzodiazepine	5.472	POS	388.2	315.1	31	134	55
Demoxepam	Benzodiazepine	5.675	POS	287.1	105	23	179.9	23
Buprenorphine	Buprenorphine	5.708	POS	468.3	55.1	67	100.9	47
EDDP	Opiate	5.939	POS	278.2	234.1	35	115	80
Amobarbital	Barbiturate	5.969	NEG	225.1	42	27		
Oxazepam	Benzodiazepine	6.143	POS	287.1	241.1	20	104.1	40
Norpropoxyphene	Propoxyphene	6.152	POS	326.2	252.2	3	91	51
Nitrazepam	Benzodiazepine	6.22	POS	282.1	236.1	24	180.1	40
Verapamil	Phenylalkylamine	6.264	POS	455.3	165.1	28	150.1	48
Propoxyphene	Propoxyphene	6.29	POS	340.2	58.1	15	266.2	7
Secobarbital	Barbiturate	6.3	NEG	237.1	42	19		
Lorazepam	Benzodiazepine	6.304	POS	321	275	20	229.1	32
Alprazolam	Benzodiazepine	6.345	POS	309.1	205.1	55	281.1	23
Carisoprodol	Carisoprodol	6.361	POS	261.2	55	31	176.1	7
2-Hydroxyethylflurazpam	Benzodiazepine	6.367	POS	333.1	109	39	119	80
Methadone	Methadone	6.382	POS	310.2	105	28	265.2	12
Clonazepam	Benzodiazepine	6.406	POS	316.1	214	51	270	27
Triazolam	Benzodiazepine	6.441	POS	343.1	239.1	51	308	31
Desalkylflurazepam	Benzodiazepine	6.666	POS	289.1	225.9	35	140	39
Nordiazepam	Benzodiazepine	6.755	POS	271.1	140	31	165.1	35
Temazepam	Benzodiazepine	6.801	POS	301.2	255.1	16	177	44
Clobazam	Benzodiazepine	6.974	POS	301.1	259.1	23	224	39
Proadifen	P450 inhibitor	7.203	POS	354.2	91.1	40	167.1	40
Diazepam-D3	IS	7.449	POS	290.1	198.1	32	154.1	24
Diazepam	Benzodiazepine	7.486	POS	285.1	193.1	32	154.1	24

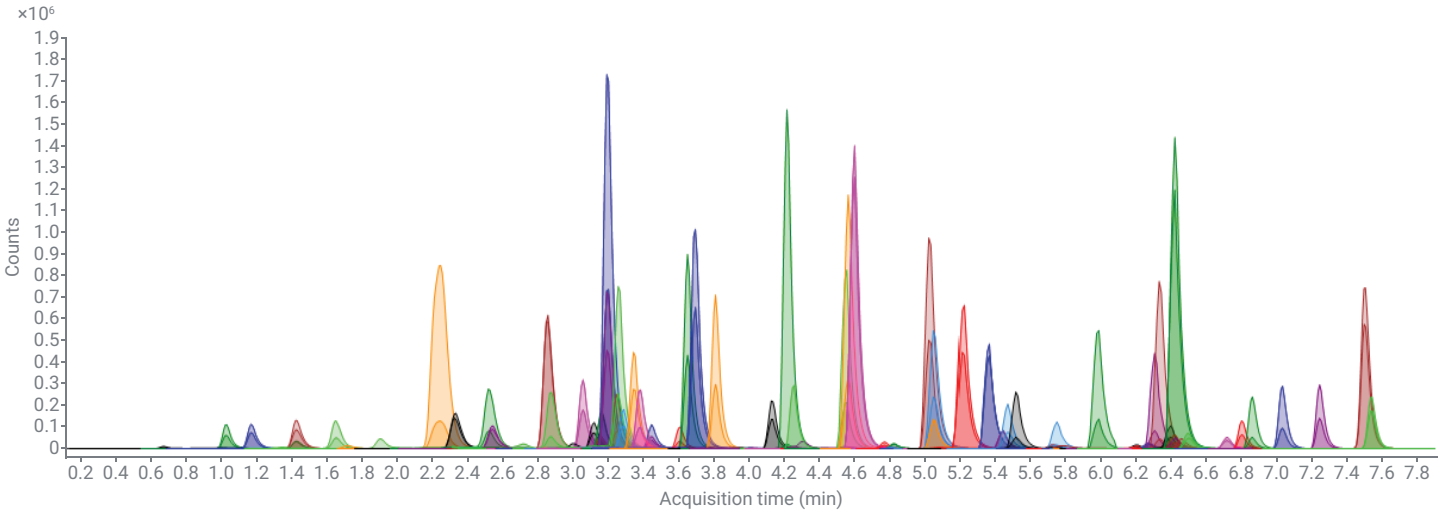

Figure 1. LC/MS/MS chromatogram (DMRM) for human whole blood samples fortified at 1 ng/mL of DoA in human whole blood. Samples were extracted by in-cartridge protein precipitation followed by Agilent Captiva EMR–Lipid cleanup. Refer to the sample preparation section for details.

Calibration standards and quality control (QC) samples preparation

An intermediate solution of 250 ng/mL in whole blood was prepared by diluting a standard spiking solution of 1 µg/mL in 1:1 MeOH:water with whole blood. The concentration of all of five barbiturate drugs were five times of the rest of drugs in the spiking solution. Calibration curve standards and QC samples were then prepared in whole blood using the intermediate spiking solution of 250 ng/mL in whole blood. The dynamic range for the calibration curve was from 0.5 to 50 ng/mL, including 0.5, 1, 5, 10, 20, 40, 50 ng/mL. These standards were prepared by spiking an appropriate volume of intermediate spiking solution into the whole blood blank, and then vortexing. Four levels of quality control (QC) samples were run for method verification tests, including low QC of 1 ng/mL, mid QC 1 of 5 ng/mL, mid QC 2 of 10 ng/mL, and high QC of 50 ng/mL. 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 the samples were vortexed gently for thorough mixing, and were then ready for sample preparation.

Sample preparation

The sample preparation procedure is described step-by-step in Figure 2. Before sample preparation, the appropriate quantity of Captiva EMR–Lipid 6 mL cartridges were placed onto the PPM-48 6 mL cartridge rack, with labeled collection tubes underneath, in the collection rack. All of cartridges went through the steps until the eluent was collected. Whole blood was aliquoted into the cartridges, followed by crashing solvent. Due to the high viscosity of whole blood, samples were held intact in the cartridge without moving into the sorbent bed until the addition of the crashing solvent. Cold 95/5 ACN/MeOH was used as crashing solvent for highly efficient protein precipitation. Low-level pressure (1 to 5 psi) was applied to initiate and maintain the elution flow at 3 to 5 seconds per drop. Flow variations between cartridges are normal for in-cartridge PPT due to the large amount of precipitates generated, but the variations are usually insignificant. Adjusting pressure for flow rate control by the fastest eluting cartridges is always recommended. Once the primary sample elution indicated no visible liquid left in the cartridge, an additional elution was conducted by adding 2 mL of 80/20 ACN/water to each cartridge. Low-level pressure was applied to maintain the appropriate flow, as recommended. When no visible liquid was left in the cartridge, high-level pressure (6 to 9 psi) was applied for at least 30 seconds to ensure the complete elution. If desired, an even higher-level pressure (10 to 15 psi) can be applied for certain cartridges. The cartridge rack was then removed and the eluent in collection tubes was ready for post-treatment.

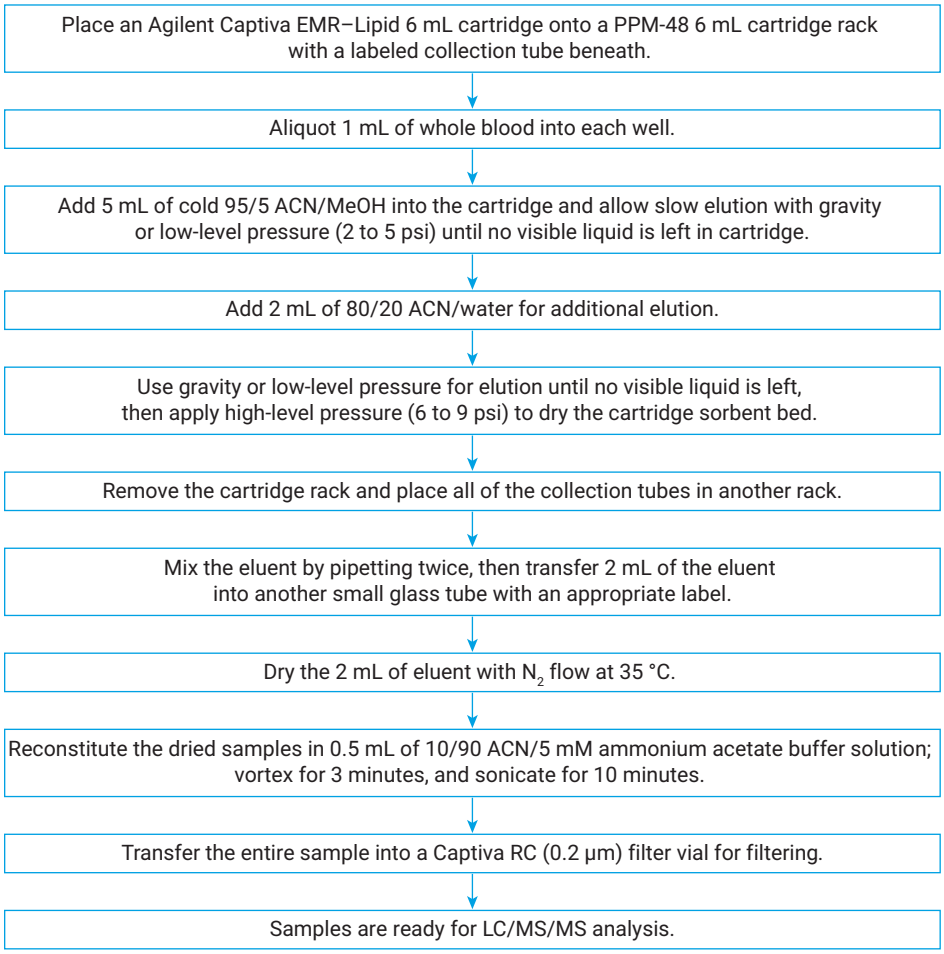

Figure 2. Human whole blood samples preparation procedure using in-cartridge protein precipitation followed by Agilent Captiva EMR–Lipid cleanup.

Method verification

Method verification was performed through accuracy and precision (A&P) runs. One set of calibration standards, six replicates of QC samples at each level, and matrix blank samples were prepared appropriately. Three replicates of QC samples at each level were run first, and the calibration curve standards followed. The rest of QC samples were then run after the calibration standards. Matrix blank samples were run for method selectivity evaluation.

Analyte absolute recovery

Analyte absolute recoveries were studied by comparing the instrument responses (peak areas) between prespiked and postspiked QC samples at low (1 ng/mL in whole blood) and high (10 ng/mL in whole blood) levels. Prespiked QCs were spiked in whole blood directly and samples were prepared with the developed method. Postspiked QCs were spiked during the sample reconstitution step using appropriate neat standard solution to reconstitute dried matrix blank samples.

Results and Discussion

Sample preparation method

Human whole blood is considered a highly viscous bodily fluid, therefore whole blood sample handling and preparation are challenging. Unlike plasma and serum, whole blood contains blood cells and extra proteins, which generate more precipitates during PPT. Adding whole blood to the Captiva EMR–Lipid cartridge first followed by the crashing solvent, is recommended. Adding the whole blood first provides better PPT efficiency, simplifies the workflow, and reduces the risk of sample loss and cross-contamination.[6] Cold crashing solvent 5/95 MeOH/ACN was used to rupture red blood cells and release their contents (cytoplasm) into the surrounding blood plasma. The elution flow in the cartridge is important. If more precipitates are generated in the cartridge, gravity elution might not be feasible for some samples. Positive pressure or vacuum may be applied when necessary. Using samples with the fastest flow for flow control is recommended for vacuum or pressure adjustment. This is to avoid any high or sudden external forces resulting in fast flow and compromising the efficiency of lipid removal on the EMR–Lipid sorbent. The use of additional elution with 2 mL of 80/20 ACN/water is also important in achieving good recoveries for all compounds. Results indicate that the additional elution step improved recoveries by 10 to 30% for some analytes. Finally, the use of Captiva RC filter vial after reconstitution not only assures particle-free samples for injection on the sub-2 µm LC column, but also, greatly simplifies the potential extra steps resulting from filtration. Samples can be injected in the filter vial directly after filtration, alleviating the need for another transfer. The Captiva filter vial is designed for convenient sample filtration and injection. The only recommendation is that the autosampler needle height needs to be increased to 2 to 3 mm. Figure 3 shows the cartridge and collection tubes setting for the sample treatment in Captiva EMR–Lipid 6 mL cartridges on PPM-48.

Analytes recovery and reproducibility

Analyte recoveries were evaluated, and the results are shown in Figure 4. All drugs, except strychnine, showed absolute recoveries within the acceptance range of 60 to 120%, with <20% RSD. For strychnine, the only compound with <60% recovery, the method provides good reproducibility of 8.1% RSD and reasonable sensitivity. With the appropriate correction using internal standards, the quantitation results for this analyte were acceptable.

Method verification

The optimized method was then verified by A&P run to collect the complete quantitative results. The results shown in Table 2 include calibration curve data, limit of quantitation (LOQ), accuracy and precision data for three spiking levels. Quantitation results from A&P run demonstrated excellent method accuracy and precision results, meeting the acceptance criteria, defined as accuracy of 100 ± 20%, and RSD ≤20%. A calibration range of 0.5 to 50.0 ng/mL in whole blood was established for most analytes. Modified calibration range exceptions include 1.0 to 50.0 ng/mL for amphetamine and 5.0 to 50.0 ng/mL for methamphetamine, dextromethorphan, and lorazepam, due to either low sensitivity or positive matrix contributions. The exceptions for phenobarbital, butabarbital, butalbital, amobarbital, and secobarbital were 5.0 to 250.0 ng/mL, due to low sensitivity and higher detection limit requirements for the barbiturate drugs in whole blood. For fentanyl, the exception was 0.05 to 5.0 ng/mL, due to compound high sensitivity and low detection limit requirements. Linear regression and weight of $1/x^2$ were used for all of analyte calibration curves with correlation coefficient R^2 >0.99 for most analytes. The method provided acceptable precision for all analytes at different spiking concentrations across the calibration range, with sufficient sensitivity to meet the desired LOQ.

Figure 3. Human whole blood in-cartridge protein precipitation in Agilent Captiva EMR–Lipid 6 mL cartridges followed with pass through cleanup.

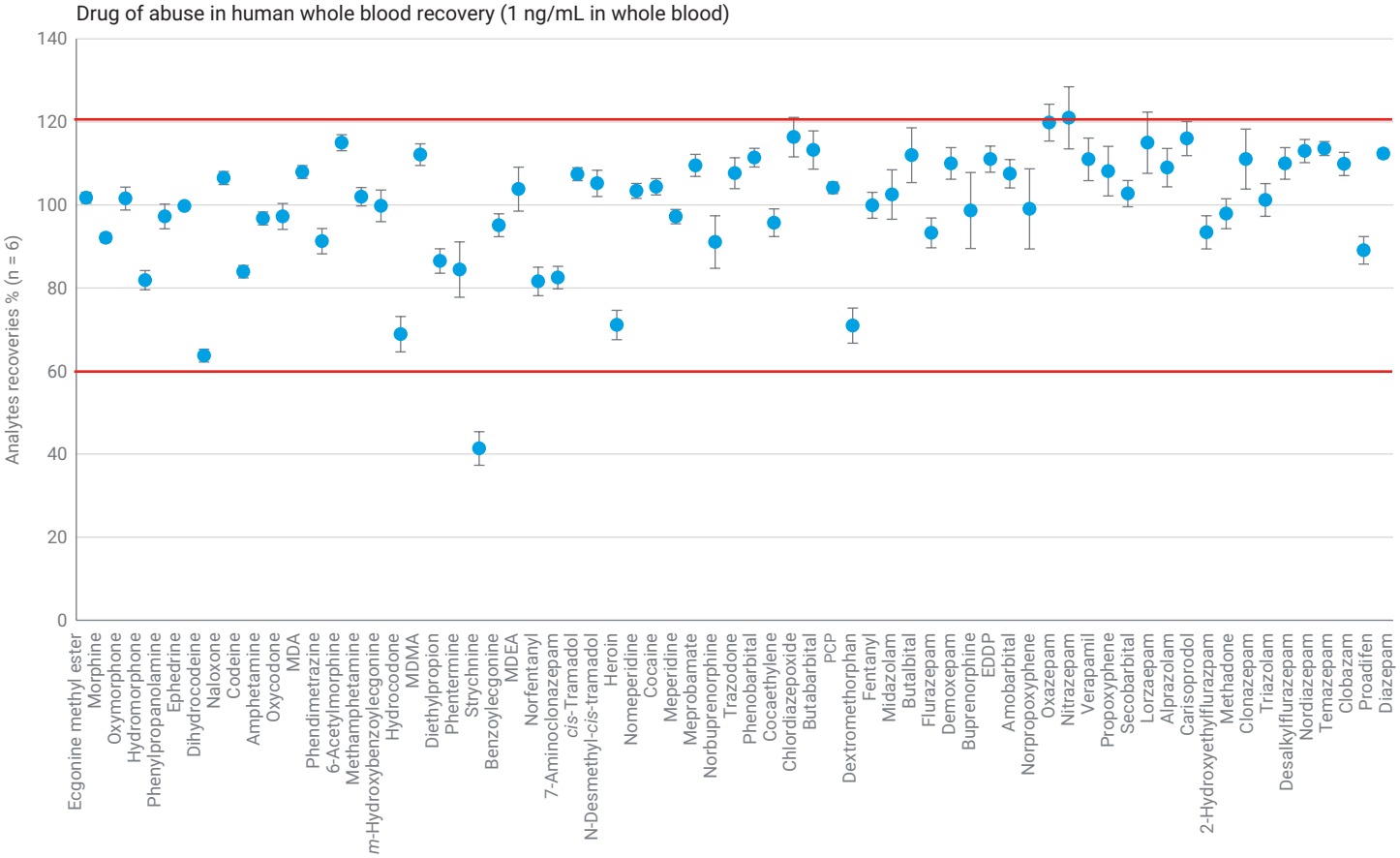

Figure 4. Analyte recovery for 67 drugs of abuse spiked with 1 ng/mL in human whole blood using the in-cartridge PPT followed with Captiva EMR–Lipid cleanup.

Table 2. Method quantitation results for 67 DoA analytes in whole blood.

DoA Compound	IS	LOQ (ng/mL)	Cal. Range (ng/mL)	Cal. Curve R^2	Low QC		Mid QC		High QC	
					Accu. %	RSD %	Accu. %	RSD %	Accu. %	RSD %
Ecgonine methyl ester	Amphetamine-D5	0.5	0.5 to 50.0	0.9929	106	8.2	101	3.9	100	3.6
Morphine	Amphetamine-D5	0.5	0.5 to 50.0	0.9898	107	4.4	102	2.6	99	3.9
Oxymorphone	Amphetamine-D5	0.5	0.5 to 50.0	0.9958	104	8.3	99	2.9	103	3.4
Hydromorphone	Amphetamine-D5	0.5	0.5 to 50.0	0.9913	101	5.7	108	2.5	112	4.1
Phenylpropanolamine	Amphetamine-D5	0.5	0.5 to 50.0	0.9953	107	6.1	104	3.9	101	3.9
Ephedrine	Amphetamine-D5	0.5	0.5 to 50.0	0.9972	103	5.0	102	1.4	102	3.1
Dihydrocodeine	Amphetamine-D5	0.5	0.5 to 50.0	0.9935	101	5.7	104	3.1	108	3.1
Naloxone	Amphetamine-D5	0.5	0.5 to 50.0	0.9932	111	6.2	109	3.5	95	7.4
Codeine	Amphetamine-D5	0.5	0.5 to 50.0	0.9990	103	6.6	102	2.2	95	4.3
Amphetamine[1]	Amphetamine-D5	1.0	1.0 to 50.0	0.9937	104	14.3	107	2.0	102	1.6
Oxycodone	Amphetamine-D5	0.5	0.5 to 50.0	0.9954	103	4.9	105	3.0	98	4.8
MDA	Amphetamine-D5	0.5	0.5 to 50.0	0.9934	111	3.5	110	5.8	89	3.9
Phendimetrazine	Amphetamine-D5	0.5	0.5 to 50.0	0.9911	97	6.7	102	5.6	106	9.0
6-Acetylmorphine	Amphetamine-D5	0.5	0.5 to 50.0	0.9937	109	2.5	111	3.2	99	10.0
Methamphetamine[1]	Amphetamine-D5	5.0	5.0 to 50.0	0.9884	110	9.1	113	2.7	101	4.1
m-Hydroxybenzoylecgonine	Amphetamine-D5	0.5	0.5 to 50.0	0.9967	114	8.9	107	7.0	114	5.2
Hydrocodone	Amphetamine-D5	0.5	0.5 to 50.0	0.9952	103	7.4	116	3.0	106	6.8
MDMA	Amphetamine-D5	0.5	0.5 to 50.0	0.9973	112	5.6	104	6.1	101	6.6
Diethylpropion	Hydrocodone-D6	0.5	0.5 to 50.0	0.9941	104	12.0	109	15.9	111	10.1
Phentermine	Cocaine-D3	0.5	0.5 to 50.0	0.9965	100	7.3	98	6.0	87	9.1
Strychnine	Hydrocodone-D6	0.5	0.5 to 50.0	0.9963	98	6.9	106	10.1	116	7.0
Benzoylecgonine	Cocaine-D3	0.5	0.5 to 50.0	0.9975	97	9.4	98	8.4	94	3.2
MDEA	Cocaine-D3	0.5	0.5 to 50.0	0.9937	104	6.4	113	5.7	101	8.2
Norfentanyl	Cocaine-D3	0.5	0.5 to 50.0	0.9962	100	4.7	108	11.3	94	9.1
7-Aminoclonazepam	Cocaine-D3	0.5	0.5 to 50.0	0.9908	104	9.9	99	6.4	105	12.2
cis-Tramadol	Cocaine-D3	0.5	0.5 to 50.0	0.9948	101	7.0	105	8.6	83	7.6
N-desmethyl-*cis*-tramadol	Cocaine-D3	0.5	1.0 to 50.0	0.9973	94	10.2	101	9.0	94	8.6
Normeperidine	Cocaine-D3	0.5	0.5 to 50.0	0.9972	101	4.6	104	7.8	82	9.9
Cocaine	Cocaine-D3	0.5	0.5 to 50.0	0.9952	98	4.8	100	12.1	80	9.7
Meperidine	Cocaine-D3	0.5	0.5 to 50.0	0.9928	102	5.2	102	6.9	80	9.0
Meprobamate	Cocaine-D3	0.5	0.5 to 50.0	0.9938	94	11.4	87	12.6	94	4.0
Norbuprenorphine	Cocaine-D3	0.5	0.5 to 50.0	0.9915	103	6.1	101	8.8	93	12.7
Phenobarbital	Butalbital-D5	0.5	5.0 to 250.0	0.9974	106	11.7	107	8.0	95	8.3
Trazodone	Cocaine-D3	0.5	0.5 to 50.0	0.9927	104	4.6	104	7.4	84	6.9
Cocaethylene	Diazepam-D3	0.5	0.5 to 50.0	0.9924	97	9.6	85	8.4	94	6.0
Chlordiazepoxide	Cocaine-D3	0.5	0.5 to 50.0	0.9920	100	14.8	103	8.6	97	10.6
Butabarbital	Butalbital-D5	5.0	5.0 to 250.0	0.9913	96	12.0	113	6.0	100	7.7
PCP	Cocaine-D3	0.5	0.5 to 50.0	0.9985	110	5.4	102	11.8	91	10.2
Clozapine	Cocaine-D3	0.5	0.5 to 50.0	0.9918	102	10.2	89	10.5	91	13.5
Dextromethorphan	Cocaine-D3	5.0	5.0 to 50.0	0.9984	90	14.8	85	15.4	84	10.4
Fentanyl	Cocaine-D3	0.05	0.05 to 5.0	0.9917	105	8.4	99	10.9	97	11.1
Midazolam	Cocaine-D3	0.5	0.5 to 50.0	0.9986	102	6.3	108	7.4	96	7.4
Butalbital	Butalbital-D5	5.0	5.0 to 250.0	0.9937	98	9.8	101	10.9	104	12.2
Flurazepam	Cocaine-D3	0.5	0.5 to 50.0	0.9842	104	11.2	107	8.2	107	8.2

DoA Compound	IS	LOQ (ng/mL)	Cal. Range (ng/mL)	Cal. Curve R^2	Low QC		Mid QC		High QC	
					Accu. %	RSD %	Accu. %	RSD %	Accu. %	RSD %
Demoxepam	Cocaine-D3	0.5	0.5 to 50.0	0.9946	94	13.4	111	17.7	92	14.1
Buprenorphine	Cocaine-D3	0.5	0.5 to 50.0	0.9963	97	10.8	116	2.0	100	22.3
EDDP	Cocaine-D3	0.5	0.5 to 50.0	0.9921	101	10.1	103	4.5	90	6.3
Amobarbital	Butalbital-D5	5.0	5.0 to 250.0	0.9909	101	13.0	106	7.6	100	8.2
Oxazepam	Cocaine-D3	0.5	0.5 to 50.0	0.9978	114	17.1	101	8.7	95	7.1
Norpropoxyphene	Cocaine-D3	0.5	0.5 to 50.0	0.9922	99	2.7	105	6.0	91	13.9
Nitrazepam	Diazepam-D3	0.5	0.5 to 50.0	0.9955	97	20.3	87	17.6	98	24.9
Verapamil	Diazepam-D3	0.5	0.5 to 50.0	0.9958	110	9.2	90	10.0	90	11.1
Propoxyphene	Diazepam-D3	0.5	0.5 to 50.0	0.9934	108	12.2	113	10.9	96	8.4
Secobarbital	Butalbital-D5	5.0	5.0 to 250.0	0.9917	103	12.4	108	9.6	100	10.2
Lorazepam	Diazepam-D3	5.0	5.0 to 50.0	0.9876	109	6.3	99	3.8	103	7.6
Alprazolam	Diazepam-D3	0.5	0.5 to 50.0	0.9919	91	4.6	101	7.7	94	13.4
Methadone	Diazepam-D3	0.5	0.5 to 50.0	0.9929	112	4.2	107	7.7	94	8.7
Carisoprodol	Diazepam-D3	0.5	0.5 to 50.0	0.9927	104	9.9	97	11.0	104	10.7
2-Hydroxyethylflurazpam	Diazepam-D3	0.5	0.5 to 50.0	0.9903	104	10.7	103	6.1	99	8.5
Clonazepam	Diazepam-D3	0.5	0.5 to 50.0	0.9935	104	9.8	111	4.6	103	8.4
Triazolam	Diazepam-D3	0.5	0.5 to 50.0	0.9876	105	6.8	107	12.8	109	11.1
Desalkylflurazepam	Diazepam-D3	0.5	0.5 to 50.0	0.9936	99	3.8	90	12.4	100	16.8
Nordiazepam	Diazepam-D3	0.5	0.5 to 50.0	0.9948	105	13.6	93	5.0	94	9.6
Temazepam	Diazepam-D3	0.5	0.5 to 50.0	0.9942	103	6.6	94	6.7	97	4.3
Clobazam	Diazepam-D3	0.5	0.5 to 50.0	0.9941	97	6.6	94	5.3	99	6.2
Proadifen	Diazepam-D3	0.5	0.5 to 50.0	0.9919	113	3.5	87	2.5	87	9.1
Diazepam	Diazepam-D3	0.5	0.5 to 50.0	0.9950	104	1.1	100	2.0	98	3.1

Matrix cleanup and impact on instrument detection system

It was reported that Captiva EMR–Lipid cleanup provides >99% of phospholipid removal in whole blood matrix.[4,6] The removal of phospholipids not only improved method reliability and quantitation result consistency, but also significantly reduced the system contamination and carryover. The results showed that running biological samples with Captiva EMR–Lipid cleanup can reduce detection system contamination and therefore instrument downtime for cleaning, as well as shorten the sample testing cycle time using a shorter LC gradient and system washing time.[6] These benefits significantly improve sample testing throughput and overall lab productivity.

Conclusions

A robust sample preparation method using protein precipitation followed by Agilent Captiva EMR–Lipid cleanup was verified for quantitative determination of 67 common DoA compounds in human whole blood. The developed method provides excellent quantitative results, including calibration curve linearity, accuracy and precision, and analyte recovery. This method, based on the use of Captiva EMR–Lipid 6 mL cartridges, allows for large sample sizes (1 mL) for forensic sample analysis, and provides a streamlined workflow and efficient sample matrix cleanup.

References

1. Mali, N.; Karpe, M.; Kadam, V. A Review on Biological Matrices and Analytical Methods Used for Determination of Drug of Abuse, *J. Applied Pharmaceutical Science* **2011**, *06*, 58–65.
2. Saito, K. *et al.* Analysis of Drugs of Abuse in Biological Specimens, *J. Health Science* **2011**, *57(6)*, 472–487.
3. Moeller, M.R.; Steinmeyer, S.; Kraemer, T. Determination of Drugs of Abuse in Blood, *J. Chromatography B* **1998**, *713*, 91–109.
4. Limian Z. Quantitative Determination of Drugs of Abuse in Human Whole Blood by LC/MS/MS Using Agilent Captiva EMR–Lipid cleanup, *Agilent Technologies Application Note*, publication number 5991-9251EN, **2018**.
5. Limian Z. Quantitative Determination of Drugs of Abuse in Human Plasma and Serum by LC/MS/MS Using Agilent Captiva EMR–Lipid cleanup, *Agilent Technologies Application Note*, publication number 5991-9312EN, **2018**.
6. Limian, Z.; Megan, J. Protein Precipitation for Biological Fluid Samples Using Agilent Captiva EMR–Lipid 96-well Plates, *Agilent Technologies Application Note*, publication number 5991-9222EN, **2018**.

www.agilent.com/chem

For Forensic Use.

RA.6516087963

This information is subject to change without notice.

Printed in the USA, August 14, 2020
5994-2215EN

Agilent

Trusted Answers