Application Note

Forensic Testing

Agilent

Trusted Answers

Quantitative Determination of Drugs of Abuse in Human Plasma and Serum by LC/MS/MS Using Agilent Captiva EMR—Lipid Cleanup

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Abstract

Agilent Captiva Enhanced Matrix Removal—Lipid (EMR—Lipid) is the second generation of EMR products, and is implemented in convenient SPE cartridge or 96-well plate. This study uses the 96-well plate format for quantitative determination of 24 representative drugs of abuse in human plasma and serum by LC/MS/MS. Samples were prepared by using in-well protein precipitation (PPT) to remove proteins followed by Captiva EMR—Lipid cleanup to remove lipids. The entire sample treatment was done in the 96-well plate as batch process and sample elution was verified by either centrifugation and positive pressure manifold. The entire batch process is easy to use with less than two hours' preparation for 96 samples in the plate. The highly efficient matrix cleanup removes >99 % phospholipids, which reduces matrix ion suppression effect and system contamination. The quantitative method was verified by accuracy and precision runs, delivering exceptional accuracy (100 ±20 %) and precision (RSD <20 %) for all three levels of QCs. The limit of quantitation (LOQ) was 0.1 to 0.5 ng/mL in plasma or serum, and a linear calibration curve gave R^2 >0.99. The method was cross-verified in common human blood matrices including serum and plasma with various anticoagulants.

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[1,2]. This is primarily due to the increasing number of drugs of abuse as well as samples submitted for analysis. There are several advantages of using blood matrices: first, drugs can be detected just after intake, prior to metabolism or filtration. Second, blood is relatively homogeneous since physiological parameters vary within only narrow limits. Third, blood samples are mandatory in cases of driving under the influence of drugs (DUID) testing in a number of European countries and in some states in the United States[3]. Therefore, reliable quantitative determination of DoA in blood matrices is important in routine forensic toxicology analysis.

Sample preparation methods for systematic forensic toxicology analysis include liquid-liquid extraction (LLE), solid-phase extraction (SPE), and supported liquid extraction (SLE). However, these methods can be labor-intensive, time-consuming, and use large amounts of toxic solvents. Agilent Enhanced Matrix Removal—Lipid (EMR—Lipid) sorbent is a novel material that selectively removes major lipid classes from sample matrix without unwanted analyte loss. The lipid removal mechanism is based on the combination of size exclusion and hydrophobic interaction between lipids and the EMR sorbent. The second generation of Captiva EMR—Lipid sorbent is packed in SPE cartridge/plate format, and cleanup is achieved by pass-through elution, leaving analytes in solution for analysis. The phospholipid removal efficacy in biological fluids and the quantitative determination of representative medicinal drugs in human serum were demonstrated by using a Captiva EMR—Lipid 96-well plate for in-well protein precipitation (PPT) and subsequent pass-through cleanup[4,5].

In a previous study, in-well PPT followed by Captiva EMR—Lipid plate cleanup was established and verified for popular DoA analysis in human whole blood[6]. In this study, a similar method was applied to different blood matrices including serum and plasma. The target analytes' chemical properties and structures were listed in the previous Application Note[6]. The method was cross-verified in each blood matrix using one-day accuracy and precision tests as well as analyte recovery and matrix effect evaluation.

Experimental

Reagent and chemicals

All reagents and solvents were HPLC or analytical grade. Acetonitrile (ACN) was from Honeywell (Muskegon, MI, USA). Reagent grade formic acid (FA) was from Agilent (p/n G2453-85060). Ammonium acetate and ammonium hydroxide were from Sigma-Aldrich (St. Louis, MO, USA). Mixed DoA standard stock solution, 1 µg/mL in MeOH, was from Agilent (p/n 5190-0470-1). Human serum, human plasma Li heparin, human plasma Na citrate, and human plasma K_2EDTA were from Biological Specialty Corp. (Colmar, PA, USA). Internal standard (IS) stock solutions, 1 mg/mL in MeOH or ACN, were from Cerilliant (Round Rock, TX, USA).

Standards and solutions

A combined DoA standard stock solution and individual IS stock solutions was used to prepare standard and IS spiking solution. Standard spiking solution was prepared in 20:80 MeOH/water at 200 ng/mL, and it was used to spike calibration standards and QC samples. The IS spiking solution was prepared by diluting individual IS stock solutions with 20:80 MeOH/water at 2 µg/mL, and it was used to spike into samples directly.

Mobile phase A, 5 mM ammonium acetate buffer with 0.1 % formic acid, was prepared by dissolving 385.3 mg of ammonium acetate into 1 L of Milli-Q water, and then adding 1 mL of FA. Mobile phase B, 0.1 % FA in ACN, was made by adding 1 mL of FA into 1 L of ACN.

A 1 % ammonium hydroxide solution in 95:5 ACN/MeOH was prepared freshly by adding 400 µL of NH_4OH into 40 mL of premixed 95:5 ACN/MeOH. The solvent was kept at –20 °C until use. An 80:20 ACN/water solution was made by mixing 80 mL of ACN with 20 mL of water. A 5 mM ammonium acetate solution was made by dissolving 77.06 mg of ammonium acetate into 200 mL of Milli-Q water. The reconstitution solution was prepared by mixing the above buffer and ACN at a ratio of 8:2.

Equipment and material

Equipment used for sample preparation included:

- CentraCL3R centrifuge (Thermo IEC, MA, USA)
- Multitube vortexer (VWR, PA, USA)
- Eppendorf pipettes and repeater
- SPE Dry 96 evaporator
- Agilent PPM-96 (p/n 5191-4116)
- Agilent Captiva-EMR 96-well plate (p/n 5190-1001)
- Agilent Captiva 96-well 1 mL collection plate (p/n A696001000)
- Agilent Captiva 96-well plate cover, 10/pk (p/n A8961007)

Instrument conditions

The samples were run on an Agilent 1290 Infinity LC system consisting of an Agilent 1290 Infinity binary pump (G4220A), and Agilent 1290 Infinity high-performance autosampler (G4226A), and an Agilent 1290 Infinity thermostatted column compartment (G1316C). The LC system was coupled to an Agilent 6490 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	
Column	InfinityLab Poroshell 120, EC-C8, 100 × 2.1 mm, 2.7 µm (p/n 695775-906(T)) InfinityLab Poroshell 120 guard, EC-C18, 2.1 × 5 mm, 2.7 µm (p/n 821725-911)
Flow rate	0.5 mL/min
Column temperature	60 °C
Injection volume	5 µL
Mobile phase	A) 5 mM ammonium acetate buffer with 0.1 % FA in water B) 0.1 % FA in acetonitrile
Needle wash	1:1:1:1 ACN/MeOH/IPA/H_2O with 0.2 % FA
Gradient	Time (min) %B Flow rate (mL/min) 0 10 0.5 0.5 10 0.5 3.0 50 0.5 4.0 95 0.5 6.0 100 0.5
Stop time	6 minutes
Post time	2 minutes
MS Conditions	
Gas temperature	120 °C
Gas flow	14 L/min
Nebulizer	40 psi
Sheath gas heater	400 °C
Sheath gas flow	12 L/min
Capillary	3,000 V
iFunnel parameters	High-pressure RF: 90 V (POS), 90 V (NEG) Low-pressure RF: 70 V (POS), 60 V (NEG)
Data acquisition	dMRM, positive mode See reference [6] for analyte MRM parameters, and Figure 1 for LC/MS/MS chromatogram at LOQ level of DoA in human plasma, Na citrate.

Calibration standards and QC samples preparation

Calibration curve standards were prepared in corresponding blood matrices using the standard spiking solution of 200 ng/mL in 20:80 MeOH/water. The dynamic range for the calibration curve was from 0.1 to 20 ng/mL in whole blood, including 0.1, 0.5, 1, 5, 10, 15, and 20 ng/mL. These standards were prepared by spiking an appropriate volume of standard spiking solution into matrix blank, and then mixed well by vortexing. Three levels of quality control (QC) samples were run for accuracy and precision method verification tests, including lowest limit of quantitation (LLOQ) of 0.1 or 0.5 ng/mL, mid QC of 1 or 5 ng/mL, and highest limit of quantitation (HLOQ) of 20 ng/mL. All calibration standards and QCs were prepared in 2-mL snap-cap tubes. They were then used to be aliquoted into Captiva EMR—Lipid 96-well plate for extraction.

Sample extraction

The sample preparation procedure is described step-by-step in the previous Application Note[6]. Prior to the sample preparation, a 96-well collection plate was placed under the Captiva EMR—Lipid plate. The plate stack went through the steps until the eluent was collected. Blood samples underwent *in situ* PPT by adding crashing solvent into aliquoted blood samples in Captiva EMR—Lipid plate wells. This addition order significantly improved mixing homogeneity, and ensured complete protein precipitation. The depth filtration design of the Captiva EMR—Lipid plate facilitated sample elution without clogging. For testing and verification in early whole blood study, two sample elution methods were implemented, positive pressure manifold processor (PPM) and centrifugation. In this study, only elution by PPM was used.

Method verification

Usually, for the method to be completely verified in one matrix with three-day accuracy and precision runs, only cross-verification is needed when applying the method to different matrices. Therefore, method cross-verification was performed through one-day accuracy and precision (A&P) runs for each additional blood matrix in this study, given the complete verification in whole blood.

Both calibration standards and QCs were prespiked appropriately. Samples were aliquoted into a Captiva EMR—Lipid plate in the following sequence: double matrix blank, matrix blank (spiked with IS), first set of calibration standards, 2 to 3 matrix blanks, LLOQs (n = 6), mid QCs (n = 6), HLOQ (n = 6), 2 to 3 carryover matrix blanks, double matrix blank, matrix blank, second set of calibration standards, 2 to 3 matrix blanks.

Analyte recovery and matrix effect

Analyte absolute recoveries were studied by comparing the analytes' responses (peak areas) between pre- and post spiked QC samples at low (1 ng/mL in serum or plasma) and high (10 ng/mL in serum or plasma) levels. Prespiked QCs were spiked appropriately into matrix blank directly, then prepared with the developed method. Post spiked QCs were spiked into the matrix blanks after extraction. In detail, post spiking happened during the sample reconstitution step by using appropriate neat standard solution to reconstitute dried matrix blanks. Matrix effect was studied by comparing the analytes' responses (peak areas) between post spiked QC samples and corresponding neat standards made in reagent blank.

Results and discussion

This study is focused on the demonstration of using Captiva EMR—Lipid cleanup for quantitative determination of forensic toxicological drugs of abuse in biological matrices for toxicological applications.

Method protocol—sample-first protein precipitation

It is demonstrated that the addition order, by adding crashing solvent into high viscous whole blood sample, provides excellent mixing homogeneity for efficient protein precipitation[6,7]. For less viscous bio-fluids such as serum and some plasma, the bio-fluid mixing resistance is not as significant as a highly viscous matrix such as whole blood. However, the sample-first PPT for bio-fluid provides improved mixing homogeneity over solvent-first PPT. Adding the sample first also enables *in situ* spiking of the IS using the Captiva EMR—Lipid plate directly followed by the addition of a crashing solvent. Sample-first addition order requires no additional sample transfers, thus simplifying the workflow. The excellent mixing homogeneity requires no additional pipette mixing for complete protein precipitation, which was recommended previously[4,5]. Additionally, the above modifications reduce sample cross-contamination risk on the 96-well plate, which is important for high-throughput testing, especially for automated sample preparing operations. Removing the transfer step also reduces the number of consumable used such as collection plates and pipette tips. Based on the above considerations, the new sample-first PPT was used for serum and plasma sample analysis.

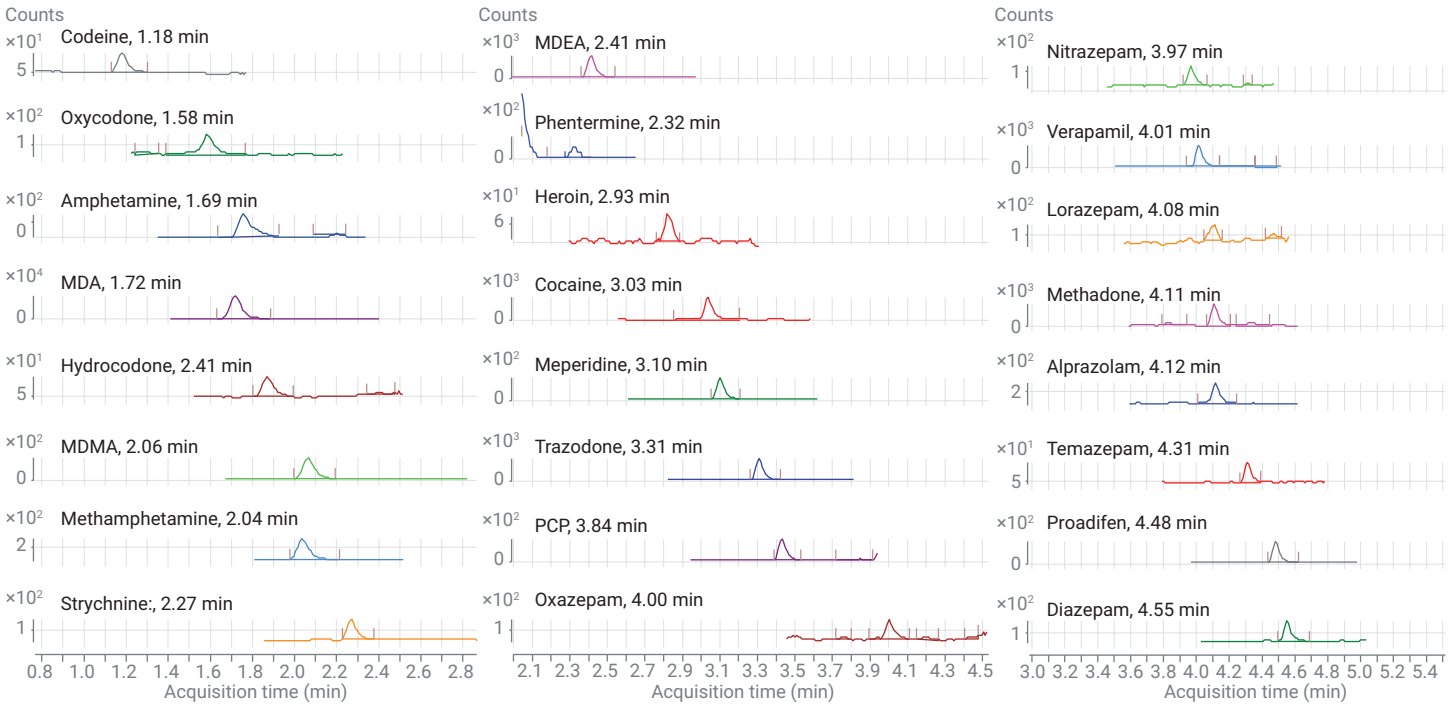

Figure 1. LC/MS/MS chromatogram (DMRM) for human plasma Na citrate fortified at LOQ level with DoA in human plasma Na citrate (0.1 ng/mL except amphetamine and heroin, with 0.5 ng/mL).

Elution by centrifugation and positive pressure were both tested and verified for the DoA analysis in whole blood[7]. Results showed that equivalent elution can be achieved by either elution technique. In this study, only positive pressure elution was used for method cross-verification. Figure 2 shows the pictures for sample batch processing on a Captiva EMR—Lipid 96-well plate. Figures 2A and 2B show the in-well PPT sample homogeneity and consistency in the Captiva EMR—Lipid plate. Figures 2C and 2D show the dried precipitates residue in the Captiva EMR—Lipid plate after elution, and consistent sample eluent collected in the collection plate.

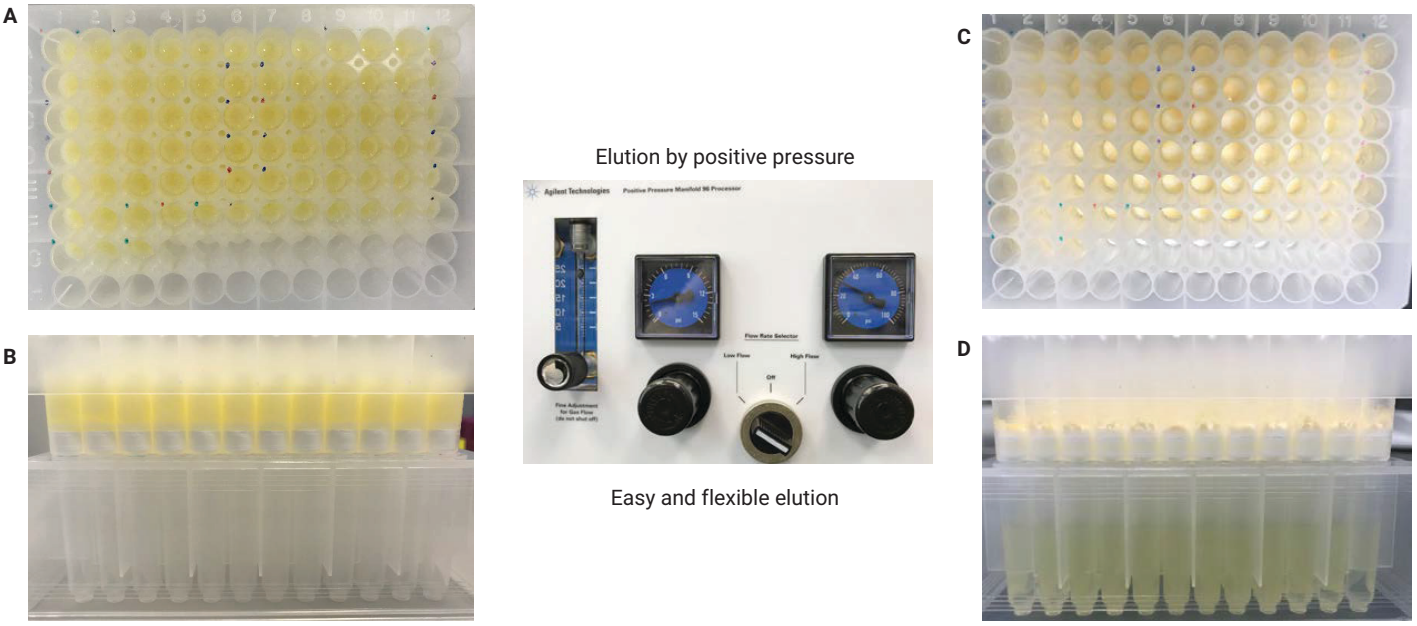

Figure 2. Batch processing on a 96-well Captiva EMR—Lipid plate with human plasma samples using in-well PPT followed by pass-through EMR—Lipid cleanup. A) In-well PPT view from top of Captiva EMR—Lipid plate; B) in-well PPT view from side of Captiva EMR—Lipid plate. C) Dried precipitates in wells (after elution) view from top of EMR—Lipid plate; D) consistent sample eluent collected in collection plate.

Analyte recovery and matrix effect

The results of analyte recovery and matrix effect for DoA in multiple blood matrices, which were the mean value of six replicates from spiking levels of 1 and 10 ng/mL, respectively, in matrix are shown in Figure 3. Given the variations of different types of blood matrix, the analyte recovery and matrix effect are consistent; however, different sample types can give matrix effects for several analytes. Lower recoveries were found for some analytes; however, method precision and analytical sensitivity make them acceptable.

Method verification

Four common blood matrices were included in this study: human serum, human plasma Li Heparin, human plasma Na citrate, and human plasma K_2EDTA. Both serum and plasma are important parts of blood separated from blood cells. The main difference between plasma and serum lies in their clotting factors. Plasma still contains the clogging substance, fibrinogen, but serum does not. Therefore, anticoagulant is added in plasma to prevent clotting, while serum does not have anticoagulant. Both serum and plasma can be sample matrices for DoA screening and confirmative analysis.

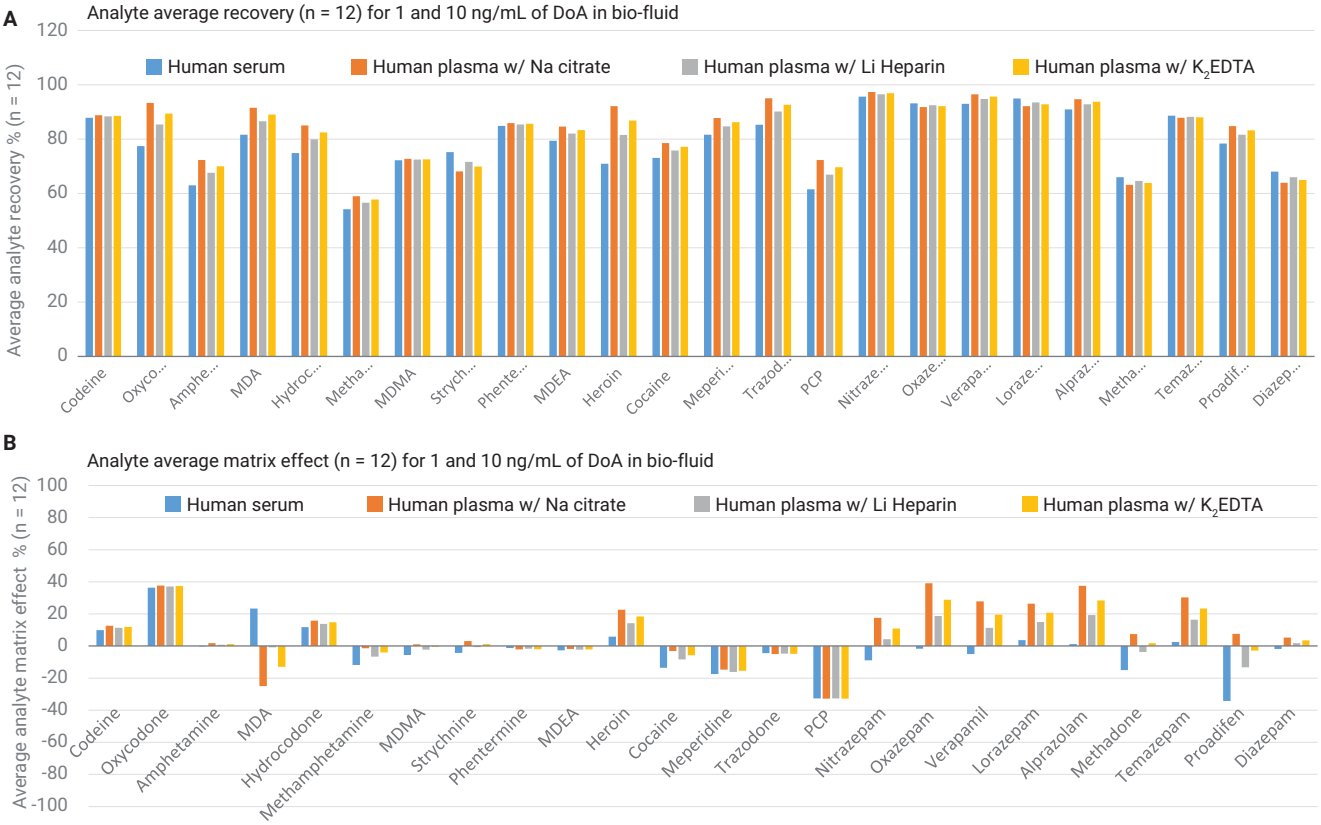

Figure 3. Average recovery (A) and matrix effect (B) for 1 ng/mL (n = 6) and 10 ng/mL (n = 6) of DoA in bio-fluid using in-well PPT followed by Captiva EMR—Lipid cleanup.

The results shown in Table 1 include calibration curve data, LOQ, and accuracy and precision data for method cross-verification in each matrix. The calibration range was 0.1 (0.5) to 20 ng/mL in blood matrices.

For calibration curve regression, the linear regression fit was used with $1/x^2$ weighting. An LOQ of 0.1 ng/mL in blood was achieved for a majority of analytes, except heroin, in all of matrices due to low sensitivity, and amphetamine and lorazepam in certain matrices due to matrix interferences. All of the quantitation results demonstrated method transfer to various blood matrices.

Table 1. Cross-verification data.

	Human plasma Li Heparin					Human plasma Na citrate					Human plasma K_2EDTA					Human serum				
DoA analyte	LOQ*	R^2	Conc.*	Accur. %**	RSD% **	LOQ*	R^2	Conc.*	Accur. %**	RSD% **	LOQ*	R^2	Conc.*	Accur. %**	RSD% **	LOQ*	R^2	Conc.*	Accur. %**	RSD% **
Codeine	0.1	0.9967	0.1	92	6.5	0.1	0.9968	0.1	103	6.0	0.1	0.9985	0.1	98	2.5	0.1	0.9917	0.1	88	4.4
			1	98	5.7			1	96	4.1			1	103	4.2			1	91	7.4
			20	100	3.3			20	102	3.5			20	102	3.4			20	99	9.1
Oxycodone	0.1	0.9949	0.1	99	6.3	0.1	0.9939	0.1	100	9.0	0.1	0.9936	0.1	93	5.6	0.1	0.9797	0.1	84	3.2
			1	104	4.7			1	95	4.1			1	96	1.9			1	101	11.4
			20	110	3.7			20	105	3.1			20	107	2.8			20	115	3.5
Amphetamine	0.1	0.9971	0.1	98	6.5	0.5	0.9972	0.1	100	4.4	0.1	0.9980	0.1	92	5.3	0.1	0.9963	0.1	97	4.7
			1	96	1.4			1	100	4.4			1	99	2.6			1	100	5.3
			20	100	4.9			20	100	3.8			20	102	2.5			20	100	3.8
MDA	0.1	0.9978	0.1	88	6.2	0.1	0.9991	0.1	105	6.3	0.1	0.9968	0.1	104	3.6	0.1	0.9863	0.1	88	8.1
			1	101	3.3			1	97	6.9			1	101	2.8			1	105	6.4
			20	107	3.2			20	100	2.1			20	105	2.0			20	111	5.7
Hydrocodone	0.1	0.9951	0.1	98	9.2	0.1	0.9947	0.1	103	13.0	0.1	0.9913	0.1	87	9.3	0.1	0.9855	0.1	102	12.9
			1	99	4.4			1	91	6.0			1	102	7.4			1	106	7.5
			20	108	5.1			20	103	3.3			20	106	3.2			20	116	6.1
Methamphet-amine	0.1	0.9988	0.1	93	5.4	0.1	0.9982	0.1	105	5.1	0.1	0.9977	0.1	91	7.6	0.1	0.9928	0.1	104	5.6
			1	93	2.2			1	94	6.8			1	97	5.4			1	100	5.7
			20	97	4.5			20	100	5.7			20	101	2.7			20	94	6.9
MDMA	0.1	0.9989	0.1	94	5.0	0.1	0.9955	0.1	100	4.7	0.1	0.9979	0.1	102	3.3	0.1	0.9957	0.1	85	3.6
			1	96	3.1			1	94	3.5			1	100	5.1			1	91	7.9
			20	101	3.7			20	101	5.3			20	103	4.1			20	99	7.1
Strychine	0.1	0.9984	0.1	100	8.3	0.1	0.9930	0.1	109	7.8	0.1	0.9967	0.1	101	6.6	0.1	0.9929	0.1	96	9.7
			1	101	5.0			1	93	6.9			1	100	5.7			1	96	3.6
			20	108	3.6			20	102	4.3			20	108	3.1			20	101	6.7
Phentermine	0.1	0.9948	0.1	97	7.9	0.1	0.9952	0.1	97	6.0	0.1	0.9941	0.1	102	7.5	0.1	0.9968	0.1	98	5.7
			1	109	4.0			1	104	4.9			1	97	3.8			1	106	5.3
			20	100	4.6			20	91	2.9			20	97	3.9			20	102	11.1
MDEA	0.1	0.9993	0.1	99	4.5	0.1	0.9985	0.1	99	5.6	0.1	0.9984	0.1	99	6.6	0.1	0.9983	0.1	98	3.8
			1	98	3.5			1	94	6.7			1	101	3.6			1	106	4.2
			20	100	3.7			20	94	3.0			20	99	3.7			20	100	4.1
Heroin	0.5	0.9848	0.5	99	10.3	0.5	0.9932	0.5	101	10.3	0.5	0.9908	0.5	107	7.7	0.5	0.9802	0.5	93	11.2
			5	103	6.6			5	113	6.7			5	108	9.8			5	112	11.2
			20	101	10.4			20	102	10.1			20	106	3.8			20	111	4.3
Cocaine	0.1	0.9981	0.1	93	11.7	0.1	0.9948	0.1	96	11.8	0.1	0.9979	0.1	100	6.9	0.1	0.9959	0.1	88	16.7
			1	98	2.0			1	100	4.6			1	98	3.9			1	95	5.7
			20	100	2.1			20	95	2.4			20	100	2.5			20	102	5.9
Meperidiene	0.1	0.9988	0.1	100	6.3	0.1	0.9983	0.1	101	4.0	0.1	0.9991	0.1	100	2.3	0.1	0.9924	0.1	111	5.7
			1	99	4.2			1	94	5.2			1	103	4.1			1	113	5.3
			20	102	5.3			20	96	2.4			20	104	2.9			20	98	8.4

* Concentration unit: ng/mL in human plasma or serum.

** n = 6 replicates

Table 1. Cross-verification data (continued).

DoA analyte	Human plasma Li Heparin					Human plasma Na citrate					Human plasma K_2EDTA					Human serum				
	LOQ*	R^2	Conc.*	Accur. %**	RSD% **	LOQ*	R^2	Conc.*	Accur. %**	RSD% **	LOQ*	R^2	Conc.*	Accur. %**	RSD% **	LOQ*	R^2	Conc.*	Accur. %**	RSD% **
Trazodone	0.1	0.9955	0.1	101	3.1	0.1	0.9976	0.1	95	4.0	0.1	0.9962	0.1	84	6.2	0.1	0.9920	0.1	101	5.6
			1	94	4.4			1	95	3.3			1	97	3.5			1	101	2.8
			20	101	3.5			20	97	4.4			20	104	2.7			20	102	3.0
PCP	0.1	0.9987	0.1	100	3.4	0.1	0.9983	0.1	102	1.6	0.1	0.9985	0.1	99	2.5	0.1	0.9857	0.1	107	8.0
			1	96	5.5			1	97	4.8			1	100	2.9			1	112	10.2
			20	98	4.8			20	95	1.4			20	99	3.2			20	92	11.9
Nitrazepam	0.1	0.9858	0.1	94	9.3	0.1	0.9870	0.1	115	2.7	0.1	0.9938	0.1	100	13.6	0.1	0.9913	0.1	96	14.5
			1	107	3.9			1	109	4.6			1	105	8.2			1	112	5.6
			20	97	3.4			20	86	2.8			20	100	7.2			20	100	8.4
Oxazepam	0.1	0.9870	0.1	100	3.6	0.1	0.9783	0.1	99	10.0	0.1	0.9842	0.1	99	10.6	0.1	0.9936	0.1	98	11.2
			1	108	7.1			1	96	7.3			1	89	6.1			1	103	4.0
			20	111	9.0			20	107	10.0			20	88	5.5			20	105	7.3
Verapamil	0.1	0.9944	0.1	103	6.3	0.1	0.9934	0.1	103	2.7	0.1	0.9950	0.1	98	4.7	0.1	0.9973	0.1	111	5.1
			1	91	8.0			1	93	8.2			1	91	7.3			1	103	10.3
			20	105	3.3			20	104	4.0			20	105	3.6			20	99	6.5
Lorazepam	0.5	0.9935	0.5	95	12.7	0.1	0.9882	0.5	101	12.3	0.5	0.9778	0.5	103	9.9	0.5	0.9936	0.5	98	12.7
			5	112	4.9			5	107	6.3			5	111	4.8			5	105	3.6
			20	105	4.1			20	111	8.0			20	102	7.0			20	107	6.4
Alprazolam	0.1	0.9943	0.1	103	10.0	0.1	0.9936	0.1	99	4.2	0.1	0.9921	0.1	85	10.9	0.1	0.9951	0.1	92	12.5
			1	106	5.7			1	100	6.4			1	98	5.2			1	103	7.2
			20	105	6.3			20	98	4.3			20	99	5.0			20	100	4.9
Methadone	0.1	0.9963	0.1	94	5.3	0.1	0.9966	0.1	102	5.3	0.1	0.9963	0.1	104	4.2	0.1	0.9919	0.1	109	5.5
			1	89	6.3			1	87	9.3			1	99	5.3			1	102	9.8
			20	5.3	7.5			20	95	6.7			20	100	2.7			20	93	5.4
Temazepam	0.1	0.9862	0.1	105	11.0	0.1	0.9930	0.1	117	6.7	0.1	0.9821	0.1	91	14.4	0.1	0.9941	0.1	101	9.8
			1	98	6.8			1	102	6.3			1	90	8.1			1	98	4.2
			20	105	6.1			20	98	2.1			20	97	3.4			20	97	10.8
Proadifen	0.1	0.9888	0.1	111	5.1	0.1	0.9887	0.1	111	3.9	0.1	0.9933	0.1	101	4.8	0.1	0.9835	0.1	119	10.1
			1	84	4.3			1	85	6.8			1	92	3.4			1	104	11.3
			20	111	6.3			20	100	8.1			20	102	3.6			20	92	8.4
Diazepam	0.1	0.9959	0.1	94	8.5	0.1	0.9967	0.1	109	8.3	0.1	0.9974	0.1	97	8.5	0.1	0.9977	0.1	113	6.2
			1	95	3.3			1	99	3.9			1	102	3.2			1	102	6.9
			20	100	4.7			20	100	5.7			20	102	2.1			20	102	5.9

* Concentration unit: ng/mL in human plasma or serum.
** n = 6 replicates

Matrix cleanup and impact on instrument detection system

Matrix cleanup in various blood matrices was demonstrated in a previous study[4] where >99 % of phospholipids was removed by Captiva EMR—Lipid cleanup. The removal of phospholipids improved the method reliability and quantitation consistency; it also significantly reduced system contamination and carryover. The injection of cleaner samples into the LC/MS/MS detection system reduces system contamination, which could result in shorter cycle time and increased sample testing throughput[7]. These effects were seen for serum and plasma samples, which provided potential for improved analytical throughput.

Conclusions

A sample preparation method using in-well PPT followed by Captiva EMR—Lipid cleanup was cross-verified to quantitatively determine 24 representative DoA compounds in human blood matrices. One-day accuracy and precision evaluation in each blood matrix verified that the method was transferred successfully to other matrices. The developed protocol on 96-well plate format fits well for fast and automatable sample preparation needs in high-throughput labs, while the convenient in-well PPT followed with Captiva EMR—Lipid cleanup simplifies the workflow while providing efficient sample extraction and lipid cleanup. Cleaner samples for analysis also reduce the time needed to clean the detection system, which can lead to higher sample testing throughput. Protein precipitation, by adding the plasma or serum sample first followed by the crashing solvent, improved sample mixing homogeneity and thus protein removal, and reduced transfer steps.

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