Quantitative LC/MS/MS Analysis of Drugs in Human Serum With Agilent Captiva EMR–Lipid Cleanup

Application Note

Clinical Research

Authors

Limian Zhao and Derick Lucas
Agilent Technologies, Inc.

Abstract

The Agilent Captiva Enhanced Matrix Removal—Lipid (Captiva EMR—Lipid) is a pass-through lipid cleanup product implemented in a convenient SPE cartridge or 96-well plate format. This study demonstrates the method performance advantages of Captiva EMR—Lipid over protein precipitation (PPT) only for the quantitative determination of nine representative drug compounds in human serum by LC/MS/MS. Samples were prepared using *in situ* PPT for protein removal, followed by Captiva EMR—Lipid cleanup to remove lipids. The entire study was conducted in the 96-well plate as a batch process. The quantitative method was established for the calibration dynamic range of 0.5–200 ng/mL in serum for all nine drug compounds using either isotopic or analog internal standards. Method verification was demonstrated using three-day accuracy and precision runs. The results showed excellent calibration curve linearity with R^2 >0.99, exceptional accuracy for all five levels of QCs (<20 % for Lowest Limit of Quantitation (LLOQ) and <15 % for the other levels), and precision (RSD <15 %). The method selectivity and carryover were evaluated as well. The results demonstrated that the established protocol using *in situ* PPT followed by Captiva EMR—Lipid cleanup provides significant improvements for the reliable quantitative determination of drug compounds in biological matrices.

Agilent Technologies

Introduction

Bioanalysis for quantitative determination of target drug and metabolite compounds in biological matrices normally involves using 96-well plate based, high-throughput sample preparation. Samples are aliquoted directly into 96-well plates, and all sample preparation treatments are conducted in the 96-well format for analyte extraction and matrix cleanup. This process has been widely applied in the bioanalysis industry due to the high productivity and efficiency it provides. Method verification for bioanalytical quantitation usually involves stringent and thorough requirements including calibration curve linearity for duplicated curves, calibration standards accuracy, accuracy and precision for fortified quality control (QC) samples across the dynamic range, analytical method selectivity, carryover, and analyte stability tests during sample treatment. Strict acceptance criteria include 100 ±20 % accuracy for lowest limit of quantitation (LLOQ), 100 ±15 % accuracy for other levels, and ≤20 % RSD for LLOQ, and ≤15 % RSD for other levels[1].

The 96-well plate based sample preparation techniques usually include solid-phase extraction (SPE), liquid-liquid extraction (LLE), solid-supported liquid extraction (SLE), and protein precipitation (PPT)[2]. SPE has a broad range of applications for LC/MS/MS quantification of small molecules in biofluids, and provides efficient matrix cleanup. However, SPE involves more analytical method development studies and multiple steps to trap, then elute target analytes[3]. LLE or SLE can sometimes provide more efficient phospholipid removal than SPE, but the recovery for highly polar analytes is usually a concern[4]. PPT is the simplest and cheapest sample preparation approach for biofluids, and therefore, is widely adopted. Organic solvents such as acetonitrile or methanol are used to crash biofluid samples, removing proteins. This approach does not remove phospholipids, which can cause many problems for the quantification method and instrument maintenance as well[5].

Agilent Captiva Enhanced Matrix Removal—Lipid (Captiva EMR—Lipid) is a series of products using a novel sorbent material that selectively removes major lipid classes from sample matrix without unwanted analyte loss. The lipid removal mechanism is a combination of size exclusion and hydrophobic interaction between the long aliphatic chain of the lipid substances and the EMR—Lipid sorbent. The selective interaction mechanism allows efficient removal of phospholipids and other classes of lipids from biological fluids after PPT. The Captiva EMR—Lipid SPE cartridge/plate format allows *in situ* PPT followed by pass-through cleanup. The phospholipid removal efficiency in biological fluids was thoroughly evaluated and compared in a separate Application Note[6], and Captiva EMR—Lipid demonstrated exceptional phospholipid removal after PPT. In this study, representative small molecule drug compounds were selected to demonstrate the excellent results achieved using the Captiva EMR—Lipid 96-well plate protocol for the quantification of small molecules in biological fluids. Data were also generated using PPT only for a performance comparison. The selected drug compounds vary widely in polarity (hydrophilic to hydrophobic) and functionality (acidic, neutral, and basic). Figure 1 lists the target analyte chemical properties and structures. The method was verified according to standard bioanalytical method verification guidance. In addition, the Captiva EMR—Lipid sorbent batch-to-batch reproducibility was evaluated based on phospholipids removal and analyte recoveries for three separate material lots.

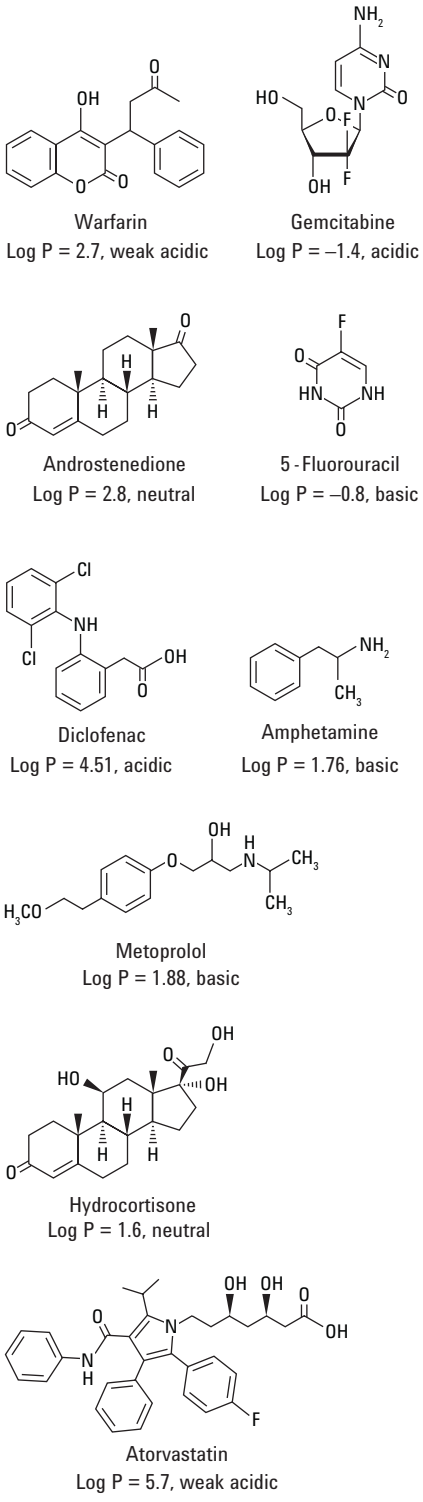

Figure 1. Chemical structures and properties of the representative drug compounds.

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). Chemical standards and other chemicals were purchased either as pure powder or standard stock solution from Sigma (St. Louis, MO, USAI). Human serum was bought from Biological Specialty Corporation (Colmar, PA, USA).

Standards and solutions

Standard and internal standard (IS) stock solutions were made in either methanol or DMSO at 2.0 mg/mL. A combined standard spiking solution was prepared in 1:1 ACN/water at 25 µg/mL, respectively. The IS working solution to add to the aliquoted samples was 2 µg/mL in 2:8 ACN/water.

A 1 % FA in ACN solution was prepared by adding 200 µL of FA into 20 mL of acetonitrile. This solution was used for protein precipitation.

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

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 preceding buffer and ACN at a ratio of 9:1. An 80:20 ACN/water solution was made by mixing 80 mL of ACN with 20 mL of water.

HPLC conditions

Parameter	Value
Column	Agilent InfinityLab Poroshell 120 LC column, EC-C18, 150 × 2.1 mm, 2.7 µm (p/n 699775-902) Agilent InfinityLab Poroshell 120 guard column, EC-C18, 5 × 2.1 mm, 2.7 µm (p/n 821725-911)
Flow rate	0.3 mL/min
Column temperature	30 °C
Autosampler temperature	4 °C
Injection volume	8 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, flushing time: 7.5 seconds
Gradient	Time (min) %B Flow rate(mL/min) 0 6 0.3 2.5 40 0.3 7.0 90 0.3 7.01 100 0.3
Stop time	8 minutes
Post time	3 minutes

MS conditions

Parameter	Value
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: 150 V (POS), 90 V (NEG) Low-pressure RF: 60 V (POS), 60 V (NEG)
Data acquisition	dMRM

Equipment and materials

Equipment used for sample preparation

- CentraCL3R centrifuge (Thermo IEC, MA, USA)
- Eppendorf pipettes and repeater
- ViaFlo 96 Liquid Handler (Integra, Hudson, NH, USA)
- Captiva vacuum collar (p/n A796)
- Vacuum pump (Gast, Benton Harbor, MI, USA)
- CentriVap concentrator, cold trap, and vacuum gauge (Labconco, Kansas City, MO, USA)
- Agilent Captiva EMR—Lipid 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 UHPLC system consisting of:

- Agilent 1290 Infinity binary pump (G4220A)
- Agilent 1290 Infinity high performance autosampler (G4226A)
- Agilent 1290 Infinity thermostatted column compartment (G1316C)

The UHPLC system was coupled to an Agilent G6490 Triple Quadrupole LC/MS system equipped with an Agilent Jet Stream iFunnel electrospray ionization source. Agilent MassHunter workstation software was used for data acquisition and analysis.

See Table 1 for analyte dMRM parameters, and Figure 2 for chromatogram and peak identification.

Table 1. LC-Triple quadrupole dMRM parameters and retention times for target analytes.

Analyte	RT (min)	Delta RT (min)	Polarity	Precursor ion (*m/z*)	Product ion			
					Quant ion	CE (v)	Qual ion	CE (v)
5-Fluorouracil	1.5	2	Negative	129	59.1	29	42.1	17
Gemcitabine	1.7	2	Positive	264.1	112.2	17	95.1	49
Amphetamine	3.7	2	Positive	136.1	119.1	5	91	21
Amphetamine-D5 (IS)	3.7	2	Positive	141.1	124.1	5	93	13
Metoprolol	4.1	2	Positive	268.2	77	69	56.1	41
Hydrocortisone	4.7	2	Positive	363.2	121.1	25	91	73
Androstenedione	6.1	2	Positive	287.2	109.1	29	97.1	25
Warfarin	6.1	2	Positive	309.1	251	15	163	8
Atorvastatin	6.3	2	Positive	559.3	440.2	25	250.1	49
Diclofenac	6.7	2	Negative	294	249.9	9	35.1	45
Progesterone-D9 (IS)	7.4	2	Positive	324.3	113.2	29	100	29

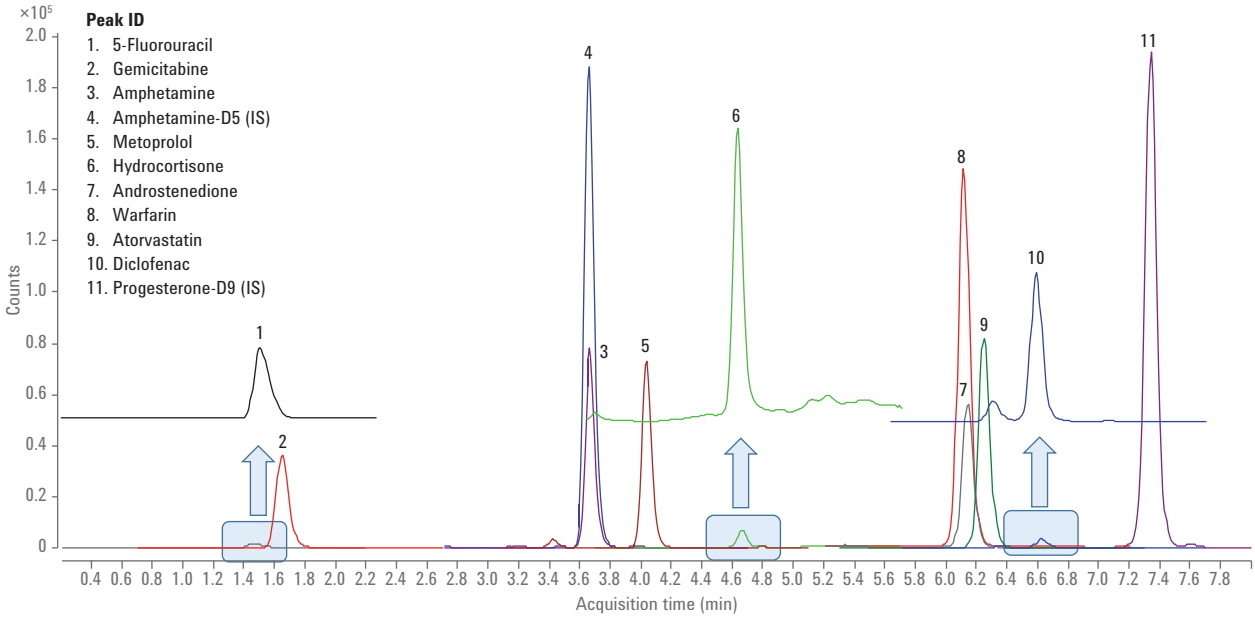

Figure 2. LC/MS/MS chromatogram (DMRM) for a human serum sample fortified with a 50 ng/mL drug standard and 200 ng/mL IS standard. Samples were extracted by protein precipitation followed by Agilent Captiva EMR—Lipid cleanup. Refer to the sample preparation section for details.

Calibration standards and QC sample preparation

Calibration curve standards were prepared in serum using the standard working solution of 25 µg/mL in 1:1 ACN/water. To reduce the solvent spiking impact on the matrix, a fresh intermediate sample, 250 ng/mL in serum, was prepared each time for calibration standards spiking. The dynamic range for the calibration curve was 0.5–200 ng/mL, including 0.5, 1, 5, 10, 50, 100, 150, and 200 ng/mL. These standards were prepared by spiking an appropriate volume of intermediate serum sample into serum blank, then vortexing to mix well. Five levels of QC samples were run for accuracy and precision method verification tests. These included 0.5 ng/mL (LLOQ), 2 ng/mL (low QC), 50 ng/mL (mid QC), 150 ng/mL (high QC), and 200 ng/mL (highest limit of quantitation (HLOQ)). These QC samples were prepared by spiking an appropriate volume of intermediate serum sample as well. All calibration standards and QCs were prepared in the 2-mL snap cap tubes. Then, they were aliquoted into 96-well plate for extraction.

Sample extraction

Table 2 describes, in detail, the sample preparation procedure. Before the addition of serum sample into the crashing solvent in a Captiva EMR—Lipid plate, it is important to aliquot serum samples in a collection plate, then add the IS solution. First, this facilitates the simultaneous transfer of multiple samples into the EMR—Lipid plate, allowing simultaneous PPT within the wells, and improving sample reproducibility. Second, the addition of IS solution into biological matrix followed by mixing is important to allow IS to properly equilibrate, and bind to the protein before extraction to ensure identical behavior of the internal standard and target analyte[4].

Table 2. Human serum sample preparation procedure using protein precipitation followed by Agilent Captiva EMR—Lipid cleanup.

Step	Operation parameter
Aliquot each sample into 1 mL 96-well plate.	100 µL
Add IS working solution to each sample except control blank, or 50:50 ACN/water to control blank.	10 µL
Cover with a plate cover, and vortex at 2,500 rpm.	1 minute
Add ACN with 1 % FA to an Agilent Captiva EMR—Lipid plate sitting on another 1-mL collection plate.	300 µL
Using a 96 liquid handler, transfer the entire sample mixture to an EMR—Lipid plate.	110 µL
Mix the sample mixture in the EMR—Lipid plate by pipetting.	3–5 times
Insert the CapiVac collar between the EMR—Lipid plate and the collection plate.	
Add make-up solution (80:20 ACN/water) to each sample.	300 µL
Apply appropriate vacuum for gradual and steady elution.	2–4 inch Hg
At the end, apply a higher vacuum to drain the cartridge bed.	8–10 inch Hg
Remove the collection plate, and evaporate to dryness with CentriVap.	40 °C
Reconstitute with 10:90 ACN/5 mM ammonium acetate buffer.	100 µL
Vortex at 2,500 rpm, sonicate, and cap with plate matt.	2 minutes + 5 minutes

Analytical method verification

Method verification was conducted through three-day accuracy and precision (A and P) runs. The sequence of the verification runs included:

- Double matrix blank
- Matrix blank (spiked with IS)
- First set of calibration standards
- 2–3 Matrix blanks
- LLOQs (n = 6)
- Low QCs (n = 6)
- Mid QCs (n = 6),
- High QCs (n = 6)
- HLOQ (n = 6)
- 2–3 Carryover matrix blanks
- Double matrix blank
- Matrix blank
- Second set of calibration standards
- 2–3 Matrix blanks

In all, there were 56–58 samples included in each verification run.

Matrix ion suppression study

The impact of matrix ion suppression on target analytes was evaluated using the standard postcolumn infusion method[7]. A neat standard solution prepared in 10:90 ACN/5 mM ammonium acetate buffer at 20 ng/mL was infused post column by a syringe pump through a T-connector, combining with the flow from the LC column to the MS detector. Figure 3 shows a diagram for the standard post column infusion setup. Matrix blanks, prepared under various cleanup protocols, were then injected onto the LC system using the previously mentioned analytical method. The target analyte MRM channels were scanned for the entire 8-minute chromatography window to monitor the effect of matrix on analyte response.

Results and Discussion

This study demonstrates the use of Captiva EMR—Lipid for the quantitative determination of small molecules in biological matrices.

Analytical method verification

Quantitation results for three-day accuracy and precision runs were analyzed based on the acceptance criteria for accuracy and precision at LLOQ and other levels, such as ≥80 % for accuracy and ≤20 % RSD for LLOQ level, and ≥85 % for accuracy and ≤15 % RSD for LLOQ level. There were a total of 16 calibration points. Any point that exceeded the accuracy acceptance level could not be used for calibration calculation, but the total unused calibration points could not be more than 20 % of total calibration points, otherwise, it would fail the run. The used calibration points have to be ≥13 for 16 total calibrators.

Table 3 shows the intra-day calibration curve standards results. All compounds gave acceptable calibration curve linearity and accuracy results. The cleaned matrix ensured analyte response consistency, resulting in tight and linear duplicated calibration curves. Conversely, samples with PPT only contain high abundant phospholipids[6], causing inconsistent analyte responses over the run resulting in divergent and nonlinear duplicate curves (Figure 4). These divergent curves, and over 30 % unusable calibration points fail the run. Table 4 lists the intra-day QC accuracy and precision results, and Figure 5 shows the inter-day results. Both intra-day and inter-day accuracy and precision results met the acceptance criteria.

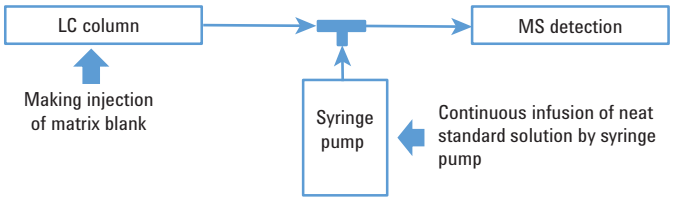

Figure 3. Standard post column infusion setup diagram for the matrix ion suppression evaluation and comparison study.

Table 3. Three-day accuracy and precision runs calibration curve standards results summary.

Analyte		5-Fluorouracil	Gemcitabine	Amphetamine	Metoprolol	Hydrocortisone	Warfarin	Androstenedione	Atorvastatin	Diclofenac
Calibration curve	Regression	Linear	Linear	Linear	Linear	Linear	Linear	Linear	Linear	Linear
	Weight	$1/x^2$	$1/x^2$	$1/x^2$	$1/x^2$	$1/x^2$	$1/x^2$	$1/x$	$1/x^2$	$1/x$
IS used for quantification		Am-D5	Am-D5	Am-D5	Am-D5	Am-D5	Am-D5	Pro-D9	Pro-D9	Pro-D9
Day 1	Points used (total points)	14 (16)	15 (16)	16 (16)	16 (16)	15 (16)	15 (16)	16 (16)	15 (16)	16 (16)
	R^2	0.9929	0.9912	0.9958	0.9907	0.9966	0.9914	0.9989	0.9965	0.9988
	Average accuracy	97.6	98.7	100.0	100.0	101.5	98.5	100.0	100.2	100.0
	RSD (n = 16)	8.5	7.3	3.8	5.2	5.8	8.2	2.5	3.9	3.9
Day 2	Points used (total points)	14 (16)	15 (16)	15 (16)	14 (16)	15 (16)	15 (16)	16 (16)	15 (16)	15 (16)
	R^2	0.9918	0.9964	0.9975	0.9961	0.9948	0.9912	0.9985	0.9958	0.9981
	Average accuracy	101.6	99.7	99.9	99.0	99.1	99.6	100.0	98.8	101.7
	RSD (n = 16)	5.8	2.7	2.3	6.3	6.1	6.8	3.7	6.0	8.1
Day 3	Points used (total points)	16 (16)	16 (16)	16 (16)	16 (16)	16 (16)	16 (16)	16 (16)	16 (16)	15 (16)
	R^2	0.9926	0.9936	0.9953	0.9928	0.9910	0.9929	0.9993	0.9933	0.9971
	Average accuracy	100.5	99.2	99.7	99.5	99.4	98.9	100.0	100.0	100.6
	RSD (n = 16)	5.0	5.1	4.9	6.7	4.8	2.6	2.2	6.1	2.8

Am = Amphetamine; Pro = Progesterone

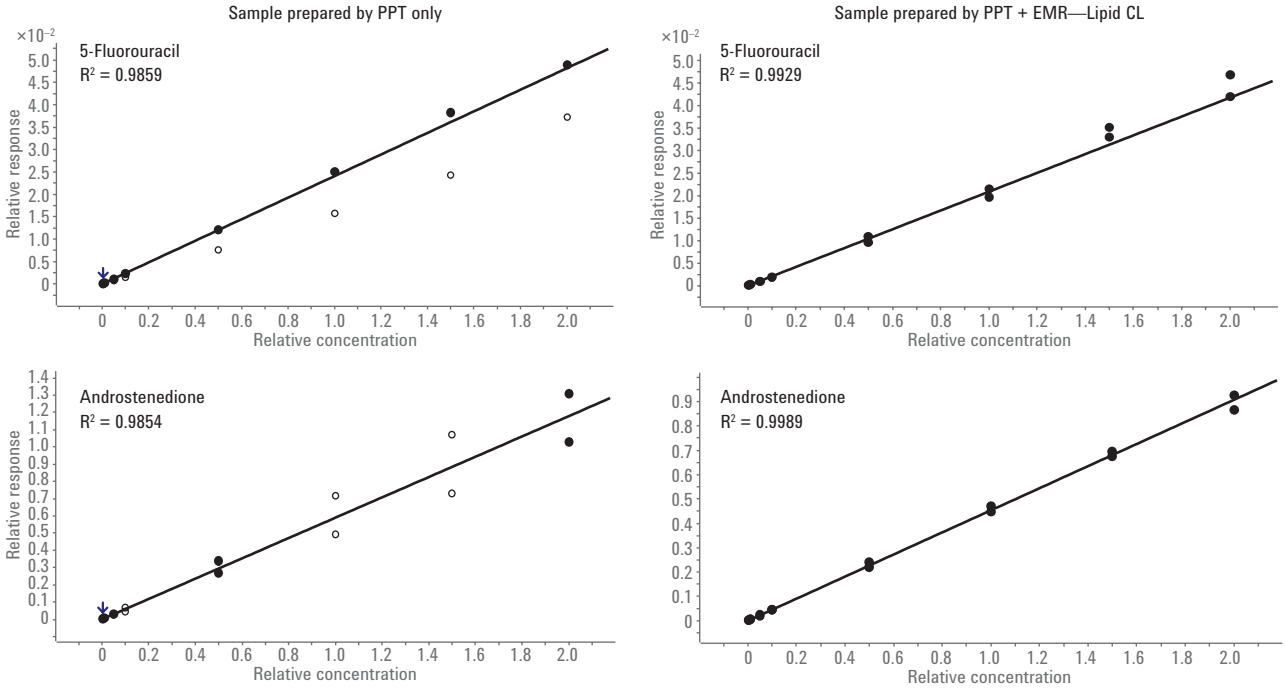

Figure 4. Duplicated calibration curves linearity comparison for sample using PPT only and PPT followed by Agilent Captiva EMR—Lipid cleanup.

Table 4. Three-day accuracy (%Ac) and precision (%RSD) runs QC samples intra-day results summary, n = 6 at each level.

		5-Fluorouracil		Gemcitabine		Amphetamine		Metoprolol		Hydrocortisone		Warfarin		Androstenedione		Atorvastatin		Diclofenac	
Analyte		%Ac	%RSD	%Ac	%RSD	%Ac	%RSD	%Ac	%RSD	%Ac	%RSD	%Ac	%RSD	%Ac	%RSD	%Ac	%RSD	%Ac	%RSD
LLOQ (0.5 ng/mL)	Day 1	103.0	8.0	97.2	5.4	94.4	5.0	105.1	12.2	100.4	2.8	105.9	8.6	99.7	6.9	98.8	8.7	99.9	6.0
	Day 2	97.4	5.9	87.2	5.6	93.2	9.5	93.8	9.7	87.5	10.4	87.5	5.1	86.0	10.5	103.5	6.5	90.3	11.3
	Day 3	98.4	5.6	88.6	4.2	98.2	2.6	96.7	7.1	87.7	7.3	90.3	4.5	90.3	7.5	92.3	14.1	94.2	14.1
Low QC (2 ng/mL)	Day 1	103.7	10.0	90.5	5.5	96.1	4.2	99.7	6.4	103.3	5.2	88.7	6.0	95.8	4.7	94.5	7.3	89.9	14.2
	Day 2	97.4	6.5	87.2	7.5	93.2	3.6	93.8	3.6	87.5	4.1	87.5	8.6	86.0	7.0	103.5	6.1	90.3	15.0
	Day 3	85.8	6.1	95.2	4.6	100.3	3.8	101.9	10.8	97.9	3.7	99.3	6.2	99.3	6.2	93.8	6.8	91.2	8.1
Mid QC (50 ng/mL)	Day 1	107.0	6.3	94.7	3.3	97.9	5.0	107.5	6.9	103.1	4.7	92.2	3.8	104.3	3.8	86.6	12.2	97.3	6.7
	Day 2	101.5	7.1	94.3	7.0	100.5	5.0	102.5	9.5	92.5	14.6	97.7	10.4	97.7	3.4	95.9	13.0	93.3	6.5
	Day 3	85.8	7.6	95.2	4.2	100.3	6.1	101.9	5.6	97.9	6.5	99.3	5.2	99.3	5.2	93.8	7.1	91.2	8.6
High QC (150 ng/mL)	Day 1	109.2	11.9	102.8	3.8	98.9	4.1	95.6	4.9	108.7	7.6	101.5	8.0	94.2	3.7	92.5	7.8	96.4	10.5
	Day 2	104.4	5.6	99.4	9.3	100.0	5.1	92.3	5.1	101.7	7.8	105.1	6.6	93.4	6.5	93.4	5.1	94.8	6.1
	Day 3	110.1	3.1	99.5	6.4	99.7	4.1	92.7	4.4	103.3	6.9	103.6	8.9	105.8	8.0	98.2	7.4	102.7	9.0
HLOQ (200 ng/mL)	Day 1	108.7	4.3	106.9	4.3	101.1	5.6	101.3	8.4	112.5	2.6	107.0	8.4	97.6	3.6	85.4	14.2	93.1	4.5
	Day 2	104.5	6.6	101.7	7.4	102.7	4.6	94.1	8.7	109.0	4.9	108.5	7.2	101.5	5.0	95.5	8.9	100.9	4.5
	Day 3	100.5	6.4	100.7	3.9	99.5	4.6	90.0	1.8	107.3	6.0	110.6	6.7	110.6	6.7	105.8	5.9	110.0	3.4

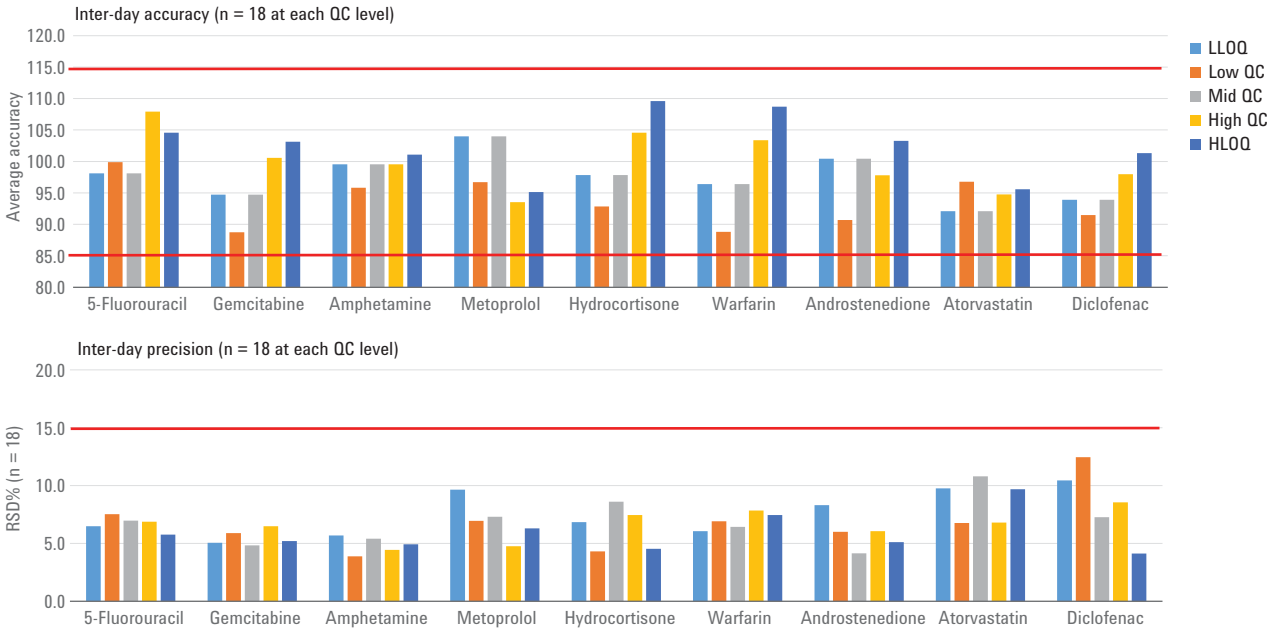

Figure 5. Method verification interday accuracy and precision results summary.

The matrix blank run before the lowest calibration standard was used for method selectivity. Matrix coeluted interference response should be less than 20 % of target analyte response at the LLOQ level. An interference is present with the amphetamine peak, which could occasionally contribute >20 % of amphetamine peak response at 0.5 ng/mL. Hydrocortisone was present in trace levels in the serum blank, but the response was less than 20 % of compound's response at 0.5 ng/mL level. Carryover was evaluated using the matrix blanks after the highest calibration standard. Androstenedione, Atorvastatin, and Diclofenac usually showed some trace carryover. The trace carryover was addressed by increasing the needle port washing time, and using a longer post LC gradient column flushing time.

Based on the 3-day accuracy and precision runs, the method was verified for the determination of multiple drug compounds with a single method. IS (internal standard) was used in this verified method. Usually, stable isotope-labeled IS is highly recommended in bioanalysis. When stable isotope-labeled IS is not available, structurally similar analog IS should be implemented[4]. In this method, two IS compounds were used for nine drug compounds, and only amphetamine had stable isotope-labeled IS, amphetamine-D5. Amphetamine-D5 and progesterone-D9 were used as IS for the rest of the compounds. However, the established method was easily verified using the structurally irrelevant IS for many compounds, which is attributed to the cleaner sample matrix and reduced ion suppression effect. This benefit makes the method development and verification easier and more cost-effective.

Matrix ion suppression

The matrix effect was evaluated by a standard post column infusion study, where possible matrix effects were assessed by continuous post column infusion of the analyte after injection of a processed serum blank onto the LC column. Any variation of signal intensity at or near the retention times of the analyte indicate the presence of substances from the matrix interfering with the analysis.

The post column infusion study was conducted with injections of serum matrix blank prepared by PPT only, PPT followed by Captiva EMR—Lipid cleanup, and PPT followed by other lipid-removing product cleanup. The profiles were overlapped and compared to the target analyte chromatogram in Figure 6 to show the different ion suppressions on the target analytes. There are three major zones for possible ion suppression, RT 1–2 minutes (zone 1), RT 3.5–6 minutes (zone 2), and RT 6.2–8 minutes (zone 3). Zone 1 shows suppression in all profiles, possibly caused by the salts from matrix. Zone 2 is primarily caused by the lysophospholipids from matrix, while zone 3 is primarily caused by the glycerophospholipids from matrix. The profile for PPT plus Captiva EMR—Lipid cleanup sample shows a smooth analyte trace without significant dips in zones 2 and 3, indicating the efficient phospholipids removal. However, profiles for PPT only and PPT plus other lipid-removing product cleanup samples, show significant dips in zones 2 and 3, indicating phospholipids ion suppression. The ion suppression on target analytes in these zones, especially for analytes sitting on the edges of dips, will impact the analytical method reliability of the quantification of these analytes.

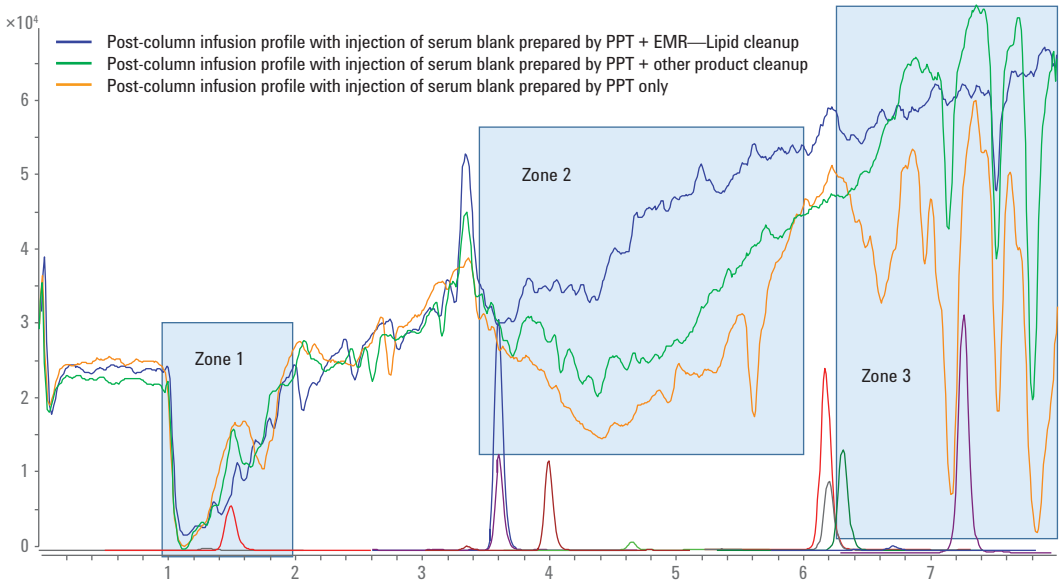

Figure 6. Standard post column infusion profiles comparison and demonstration of matrix ion suppression effect on target analytes.

Agilent Captiva EMR—Lipid sorbent batch-to-batch reproducibility

Captiva EMR—Lipid sorbent batch-to-batch reproducibility was evaluated for phospholipids removal and analyte recovery at the 1 ng/mL level using 1-mL cartridges packed with three different manufacturing lots. All three sorbent lots gave >99 % removal for phospholipids. Captiva EMR—Lipid sorbent consistency and batch-to-batch reproducibility was also demonstrated by the consistent analyte recovery results shown in Figure 7.

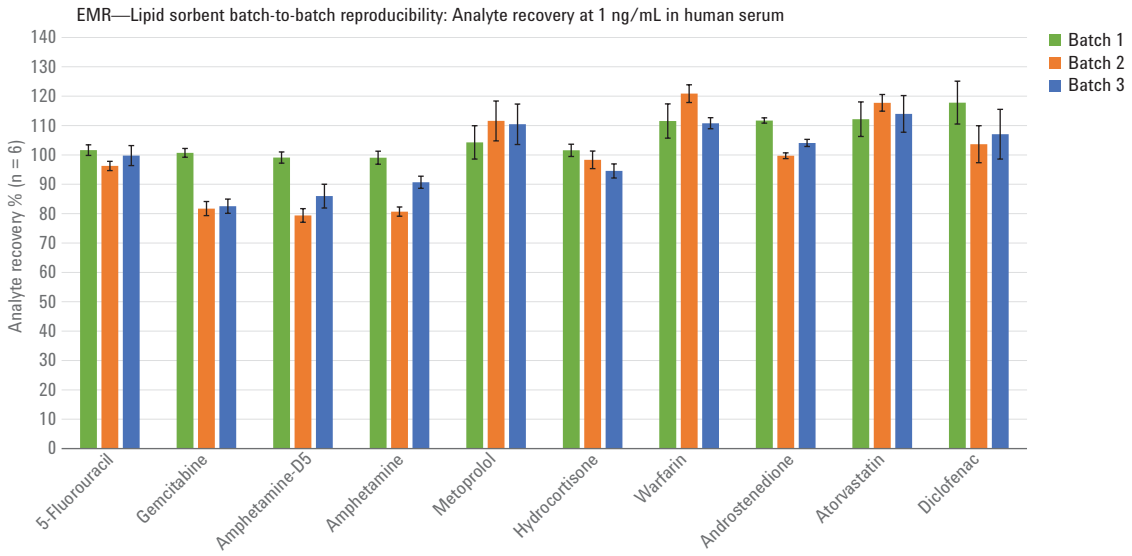

Figure 7. Agilent Captiva EMR—Lipid sorbent batch-to-batch reproducibility demonstrated as analyte recoveries at 1 ng/mL in human serum.

Conclusions

A sample preparation method using PPT followed by Agilent Captiva Enhanced Matrix Removal—Lipid cleanup was verified for the quantitative determination of nine representative drug compounds in human serum. Three-day accuracy and precision runs verified that the Captiva EMR—Lipid method provides superior dynamic range and calibration linearity over PPT only, and delivers exceptional intra- and inter-day accuracy and precision. PPT-only samples contained lipids that decreased analytical method sensitivity and failed the method verification due to poor calibration curve accuracy. The standard post column infusion study showed that the matrix ion suppression was significantly reduced in comparison with PPT only and PPT followed by alternative lipid removal product cleanups. The lipid selective sorbent delivers cleaner matrix without unwanted analyte retention, and improves established method reliability, as demonstrated by the superior quantitative results. The cleaner matrix can potentially allow the use of analog IS or even structure-irrelevant IS instead of expensive stable isotope-labeled IS, making the method verification easier, and sample analysis more cost-effective. The unique sorbent chemistry also allows lipid removal for other complex sample types and future applications will explore multiclass, multiresidue analysis in meats and other foods.

References

1. US Food and Drug Administration, *Guidance for Industry Bioanalytical Method Validation* **2001**.
2. Bylda, C.; Thiele, R. Recent advances in sample preparation techniques to overcome difficulties encountered during quantitative analysis of small molecules from biofluids using LC-MS/MS. *The Analyst* **2014**, *139*, 2265–2276.
3. León, Z.; *et al.* Solid-phase extraction liquid chromatography-tandem mass spectrometry analytical method for the determination of 2-hydroxy-4-methoxybenzophenone and its metabolites in both human urine and semen. *Analytical and Bioanalytical Chemistry* **2010**, *398*, 831–843.
4. Mulvana, D.E. Critical topics in ensuring data quality in bioanalytical LC-MS method development. *Bioanalysis* **2010**, *2*, 1051–1072.
5. Michopoulos, F.; *et al.* Extraction methods for the removal of phospholipids and other endogenous material from a biological fluid. *Bioanalysis* **2011**, *3*, 2747–2755.
6. Zhao, L.; Lucas, D. Efficiency of Biological Fluid Matrix Removal using Captiva EMR—Lipid Cleanup. *Agilent Technologies Application Note*, publication number 5991-8006EN, **2017**.
7. Bonfiglio, R.; *et al.* The effects of sample preparation methods on the variability of the electrospray ionization response for model drug compounds. *Rapid Communications in Mass Spectrometry* **1999**, *13*, 1175–1185.

www.agilent.com/chem

For Research Use Only.
Not for use in diagnostic procedures

This information is subject to change without notice.

Published in the USA, July 26, 2017
5991-8007EN

Agilent Technologies