Enhanced Lipids Removal From Biological Matrices to Prepare Samples for LC/MS/MS Analysis

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Introduction

Lipids, especially phospholipids (PPLs), in biological matrices can significantly impact bioanalysis quality by LC/MS/MS. The unremoved phospholipids and matrix interferences can cause significant ion suppression, resulting in lower detection limits and poor method reliability, resulting in lower productivity and eventual financial losses.

Agilent Enhanced Matrix Removal–Lipid (EMR–Lipid) is a series of new products utilizing 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.

Captiva EMR–Lipid is a new pass-through cleanup product implemented in a convenient SPE cartridge or 96-well plate format. The use of Captiva EMR–Lipid products provides > 99 % phospholipids removal and clogging-free, easy elution for in-situ protein precipitation. The 96-well Captiva EMR – Lipid plate was evaluated for the quantitative determination of representative drug compounds in human serum by LC/MS/MS. 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.

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Experimental

Instrument condition

The study was run on an Agilent 1290 Infinity UHPLC system coupled to an Agilent G6490 Triple Quadrupole MS system.

LC/MS/MS conditions

- Agilent InfinityLab Poroshell 120 LC column, EC-C18, 150 x 2.1 mm, 2.7 µm (p/n 699775-902),
- Mobile phase: A) 5 mM ammonium acetate buffer with 0.1 % FA in water, B) 0.1 % FA in Acetonitrile
- 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
8.0	Stop	0.3

- Data acquisition:
  Precursor scan of 184 product ion mode for PPLs removal evaluation; and dMRM mode for quantitation evaluation (refer ref 1&2 for more method detail)

Sample preparation

Phospholipids removal efficiency evaluation was conducted on various biological matrices, while the quantitative bioanalysis of representative drugs was conducted on human serum.

Steps	Operation parameters
Aliquot each sample into 1 mL 96-well collection 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 plate cover and vortex at 2500 rpm	1 min
Add ACN with 1 % FA to Captiva EMR–Lipid plate sitting (on a 1 mL collection plate)	300 µL
Using 96 liquid handler to transfer the entire sample mixture to Captiva EMR-Lipid plate	110 µL
Mixing the sample mixture in EMR-Lipid plate by pipetting	3–5 times
Insert CapiVac collar between EMR-Lipid plate and 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 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 2500 rpm, sonicate, and cap with plate matt	2 min + 5 min

Results and Discussion – Pass Through Cleanup

Simplified Addition of Captiva EMR-Lipids Cleanup after Protein Precipitation

- ✓ Unique frit design assures clogging-free elution
- ✓ Non-dripping feature allows in-situ protein precipitation
- ✓ Easy elution with low vacuum/pressure assures clean eluent
- ✓ 96-well plate elution can be conducted by low-speed centrifugation

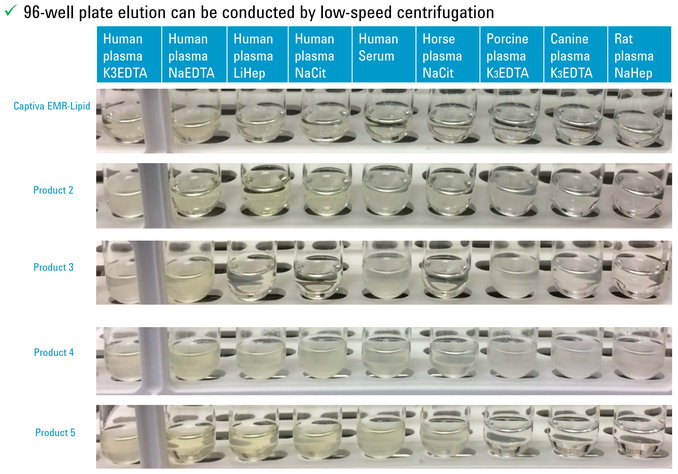

Figure 1. Comparison of sample eluent clarity collected by in-situ PPT.

Results and Discussion – PPL Removal

Highly Selective and Efficient Lipids Removal

- ✓ >99% PPLs removal demonstrated in various biological matrices
  - Various matrices and resources
  - Various anti-coagulants
- ✓ Superior to or equivalent to other cleanup products PPLs removal performance

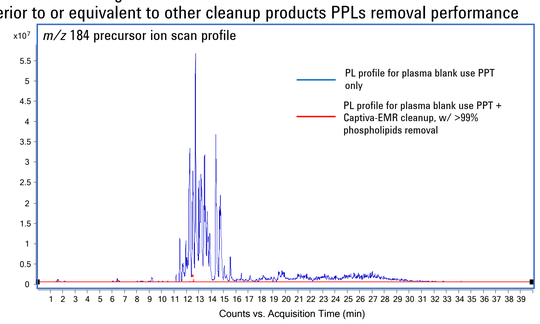

Figure 2. Overlapped chromatograms for phospholipids profile by monitoring precursor ion scan for 184.

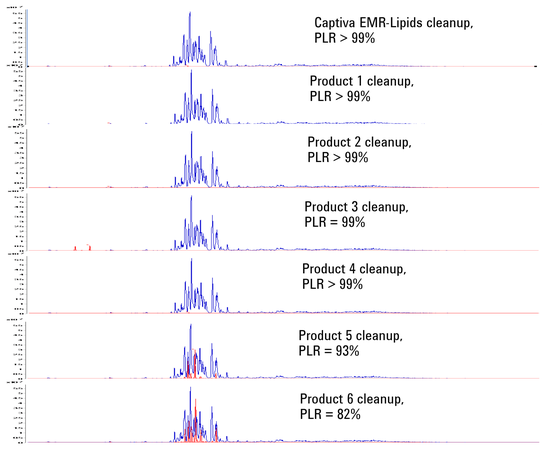

Figure 3. Phospholipids removal efficiency comparison among various cleanup methods after PPT of human plasma Na Heparin.

Results and Discussion – Quantitation of Representative Drugs

Representative Drug Compounds for Quantitative Bioanalysis in Human Serum

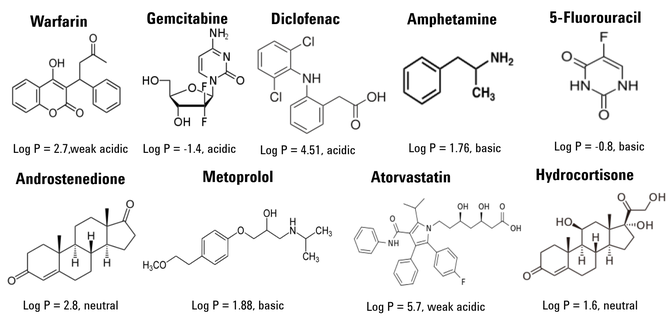

Figure 4. Chemical structures and properties of the representative drug compounds.

Captiva EMR-Lipid cleanup improves calibration curve linearity

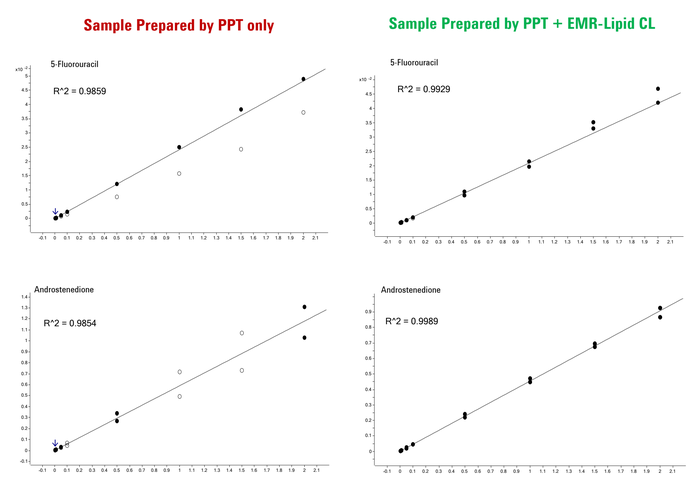

Figure 5. Duplicated calibration curves linearity comparison for sample using PPT only and PPT followed by EMR-Lipid cleanup. 0.5 – 200 ng/mL in human serum

Results and Discussion – Quantitation of Representative Drugs

Captiva EMR-Lipid cleanup improves method accuracy and precision

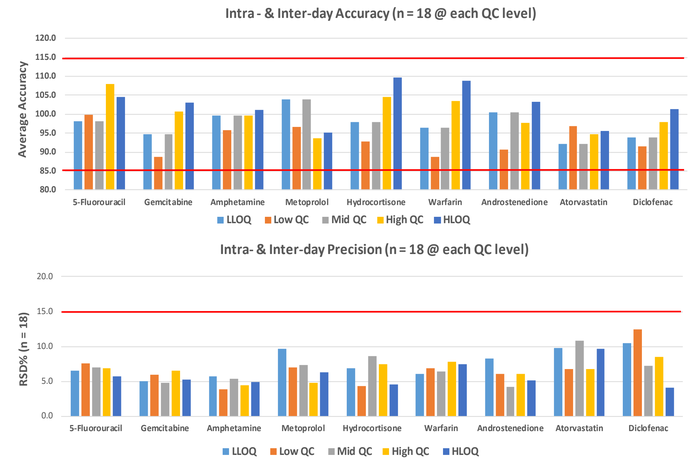

Figure 6. Method verification inter-day accuracy and precision results summary.

Results and Discussion – Matrix Ion Suppression

Captiva EMR-Lipid cleanup significantly reduces matrix ion suppression

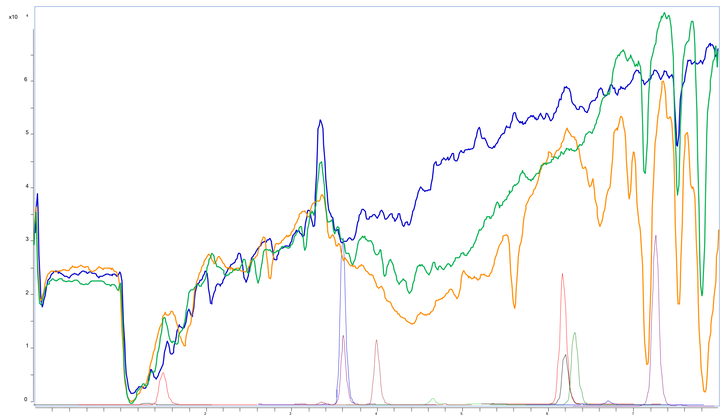

Figure 7. Standard post-column infusion profiles comparison and demonstration of matrix ion suppression effect on target analytes.

Conclusions

The use of Captiva EMR-Lipid products for bioanalysis provides

- ✓ Easy accommodation to traditional in-situ or offline protein precipitation work flow;
- ✓ Frits optimized to resist clogging and provide easy elution.
- ✓ Highly selective and efficient lipids removal (>99%), and thus significantly reduced matrix ion suppression;
- ✓ Exceptional quantitative results for easy method validation under standard criteria;
- ✓ Overall improved productivity by reducing instrument downtime and prolonging column lifetime.

References

1. L. Zhao, D. Lucas. Efficiency of Biological Fluid Matrix Removal using Captiva EMR-Lipid Cleanup. *Agilent Technologies Application Note*, publication number 5991-8006EN, 2017.
2. L. Zhao, D. Lucas. Quantitative LC/MS/MS Analysis of Drugs in Human Serum With Agilent Captiva EMR-Lipid Cleanup. *Agilent Technologies Application Note*, publication number 5991-8007EN, 2017.
3. US Food and Drug Administration, *Guidance for Industry Bioanalytical Method Validation,* 2001.

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