Introduction

Lipids, especially phospholipids (PLs), 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% phospholipid removal and clogging-free, easy elution for in-situ protein precipitation. The 98-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.

For Research Use Only. Not for use in diagnostic procedures.

Experimental

Instrument condition

The study was run on an Agilent 1290 Infinity UHPLC system coupled to an Agilent 6490 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,
- Captiva EMR–Lipid is a new pass-through cleanup product implemented in a convenient SPE cartridge from biological fluids after PPT.
- The interaction between the long aliphatic chain of the lipid substances and the EMR–Lipid sorbent. The sorbent material that selectively removes major lipid classes from sample matrix without unwanted productivity and eventual financial losses.
- Lipids, especially phospholipids (PPLs), in biological matrices can significantly impact bioanalysis.
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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 phospholipid profile by monitoring precursor ion scan for 184.

Results and Discussion – Quantitation of Representative Drugs

Captiva EMR-Lipid cleanup improves method accuracy and precision

Figure 5. 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 6. Stacked post-columns infusion profiles comparison and demonstration of matrix 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