Sample Matrix Cleanup using Protein and Phospholipid Depletion Technique (PLD) for Drugs of Abuse LC-MS/MS Analysis in Whole Blood

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Introduction

Clinical analysis encompasses a wide range of analytes, whether they are endogenous compounds such as amino acids, vitamins, and hormones or exogenous compounds ("xenobiotics"), such as prescribed and/or illicit drugs and their metabolites. The diversity of these analytes' properties coupled with the complexity of sample matrices presents challenges in LC-MS/MS analysis, as it is difficult to develop a method that can remove biomatrix interferences effectively while maintaining good recovery across wide ranges of analytes. Sample extractions methods using solid phase extraction (SPE) are tailored to specific compound classes, and the extraction procedures are rather tedious and time-consuming. This underlines the need for a simple, fast, cost-effective, and automatable sample preparation approach for clinical analysis.

In this investigation, we aim to demonstrate a streamlined, effective, and automated protein and phospholipid depletion (PLD) sample preparation workflow that offers high analyte recovery, low matrix effect, and excellent reproducibility for a wide range of analytes in LC-MS/MS analysis.

PLD Automated Workflow

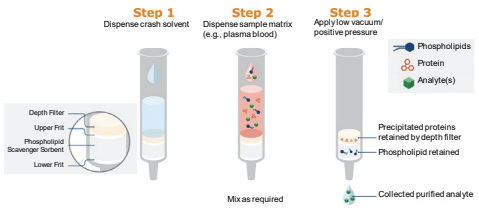

Figure 1. Working mechanism of PLD for sample cleanup.

Methodology

To assess the performance of this workflow, we chose whole blood as the sample biomatrix due to its viscous and complex nature. Samples were extraction using ISOLUTE® PLD+ on the Biotage® Extrahera™ automated sample preparation workstation (*Figures 1 and 2*). A panel of 58 analytes with diverse structures and wide ranges of LogP (-0.6-7.05) and pKa (0.13-10.47) were spiked into whole blood at low (~5ng/ml) and medium (mid) concentrations (~50ng/ml) to assess the feasibility.

Extraction performance was evaluated by recovery, matrix effect, and reproducibility[1]. The recovery is calculated as the ratio (%) of peak areas between the pre-and post-spiked samples. The matrix effect is evaluated by the ratio of peak areas between the post-spiked sample and the same analyte concentration spiked in the reconstitution solution. Matrix effect value of <1 indicates ion suppression, whereas matrix effect value of >1 represents signal enhancement by matrix. Reproducibility is the RSD (%) of studied sample replicates(n=5). The same spiked samples were processed by traditional protein precipitation and different SPE chemistries for comparison.

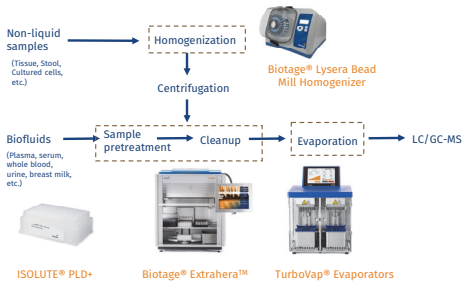

Figure 2. Automated sample preparation workflow.

LC/MS Conditions

Instrument: Shimadzu Nexera UHPLC using coupled to an AB Sciex 5500 triple quadrupole MS system.

Column: Restek Biphenyl 2.7 µm (100 x 2.1 mm)
Mobile Phase: A: 0.1% formic acid (aq)
Mobile Phase B: 0.1% formic acid (MeOH)
Injection volume: 5 µL
Flow rate: 0.45 mL min
Gradient: 10% B to 100% B (0.1 min to 8.5 min), 100% B to 10% B (8.5 min to 10.5 min)

Acquisition parameters: Optimized MRM transitions were optimized for each analyte. Available upon request.

Results

Phospholipid Removal Capability

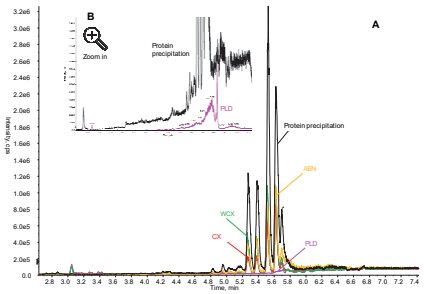

Figure 3. *Phospholipid removal by ISOLUTE® PLD+, traditional protein precipitation, and SPE chemistries: EVOLUTE® EXPRESS CX, WCX and ABN.*

Recovery, Matrix Effect, and Reproducibility

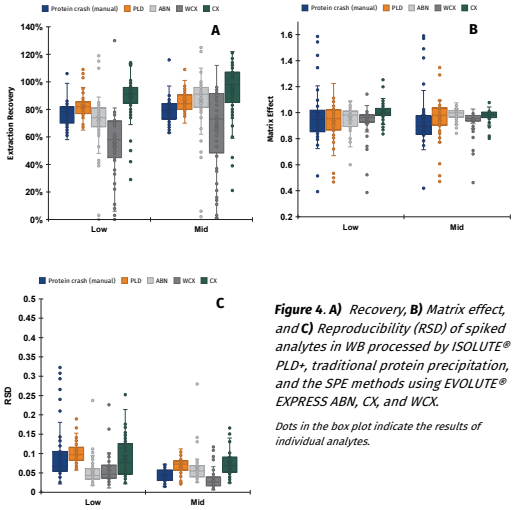

Figure 4. A) *Recovery,* ***B)*** *Matrix effect, and* ***C)*** *Reproducibility (RSD) of spiked analytes in WB processed by ISOLUTE® PLD+, traditional protein precipitation, and the SPE methods using EVOLUTE® EXPRESS ABN, CX, and WCX.*

Dots in the box plot indicate the results of individual analytes.

Solvent to Sample Ratio

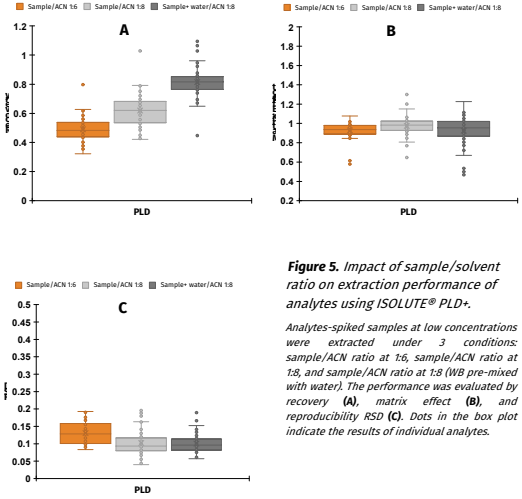

Figure 5. *Impact of sample/solvent ratio on extraction performance of analytes using ISOLUTE® PLD+.*

Analytes-spiked samples at low concentrations were extracted under 3 conditions: sample/ACN ratio at 1:6, sample/ACN ratio at 1:8, and sample/ACN ratio at 1:8 (WB pre-mixed with water). The performance was evaluated by recovery ***(A)****, matrix effect* ***(B)****, and reproducibility RSD* ***(C)****. Dots in the box plot indicate the results of individual analytes.*

Discussion

The traditional protein precipitation method co-extracted high levels of phospholipids (Figure 3), whereas the PLD method removed more than 99% phospholipids (Figure 3 A and B). SPE methods (ABN, CX, and WCX) removed part of the phospholipids but were not comparable with PLD. Although sample cleanliness can be improved by increasing SPE wash strength, it may impact the recovery of a large panel of analytes of different properties.

Extraction by PLD delivered excellent recovery, matrix effect, and reproducibility across diverse analytes (Figure 4A, B, and C). In contrast, analytes extracted by the SPE methods presented substantial variances in the extraction recovery across the investigated panel, indicating that certain SPE conditions may work for a small group of compounds but not others.

The sample-to-solvent ratio significantly influenced the extraction recovery using the PLD technique. The sample to extraction solvent ratio should not be lower than 1:6 for most biofluids, ideally 1:8, or even higher for biofluids with high viscosity, like whole blood (Figure 5A). Dilution of whole blood samples with water (1:1, v/v) greatly improved extraction recovery while maintaining good matrix effects (Figure 5B). The small amount of water improved sample fluidity and facilitated better extraction efficiency and reproducibility (Figure 5C).

The PLD extraction technique is simpler and quicker than SPE. The extraction of a full 96-well plate by PLD only required 2 steps and 45 minutes, while the SPE extraction techniques required 5 steps and 75 minutes. Automating the workflow improves the method's reproducibility and robustness. It also reduces the labor and analyst time spent on the bench.

Conclusion

The automated PLD workflow offers a simple, fast, and effective solution to extract a diverse range of analytes for LC-MS/MS analysis with less interference from biomatrix components such as protein and phospholipids. The optimized procedure proved effective for a complex biomatrix, whole blood, and can be readily applied to other biomatrices such as serum, plasma, and cell/tissue homogenates.

Reference

1. Matuszewski BK, et. al. Anal Chem. 2003 Jul 1;75(13):3019-30.
2. Little JL, et al. Journal of Chromatography B. 2006 Apr 3;833(2):219-30.

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