An Automated Sample Preparation Workflow and Toolbox for Bioanalysis

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Introduction

Bioanalysis involves sample preparation techniques designed to meet rigorous standards for sensitivity, specificity, accuracy, and precision. The standards are crucial for evaluating the safety and efficacy of requirements for drug and their metabolites and critical in biomarker analysis and regulatory decision-making processes. One of the biggest challenges is developing a method to extract analyte(s) from biological samples with high recovery, low matrix interferences, and good reproducibility. This is especially difficult when facing diverse analyte structures, complex biometrics, and short project turnaround time. This presentation demonstrates an automated sample preparation workflow with an extraction toolbox containing different sorbent chemistry (Figure 1) to handle sample extraction and cleanup for a wide variety of analytes from complex

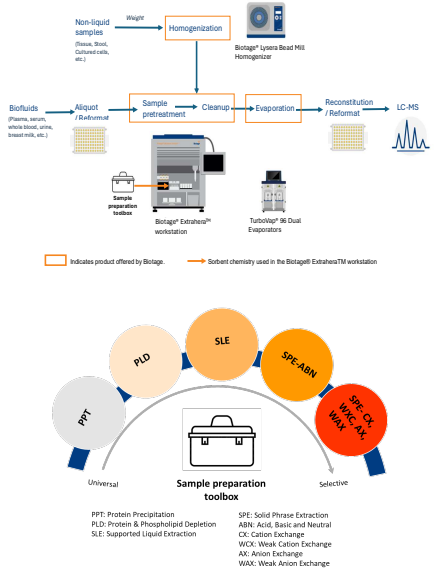

Figure 1. *Automated sample preparation workflow and the extraction toolbox*

Method

A panel of 58 analytes with diverse backbones and a wide range of LogP (-0.6- 7.05) and pKa (0.13-10.47) were spiked into whole blood samples for feasibility testing. Sample extractions were conducted by the Biotage® Extrahera™ using sorbents shown in the toolbox: the ISOLUTE® PPT+, PLD+, SLE+, and the EVOLUTE® EXPRESS ABN, WCX, CX, AX, and WAX. Extraction performance was evaluated by recovery, matrix effect, and reproducibility. The workflow and mechanism of each sorbent chemistry are shown in Figure 2.

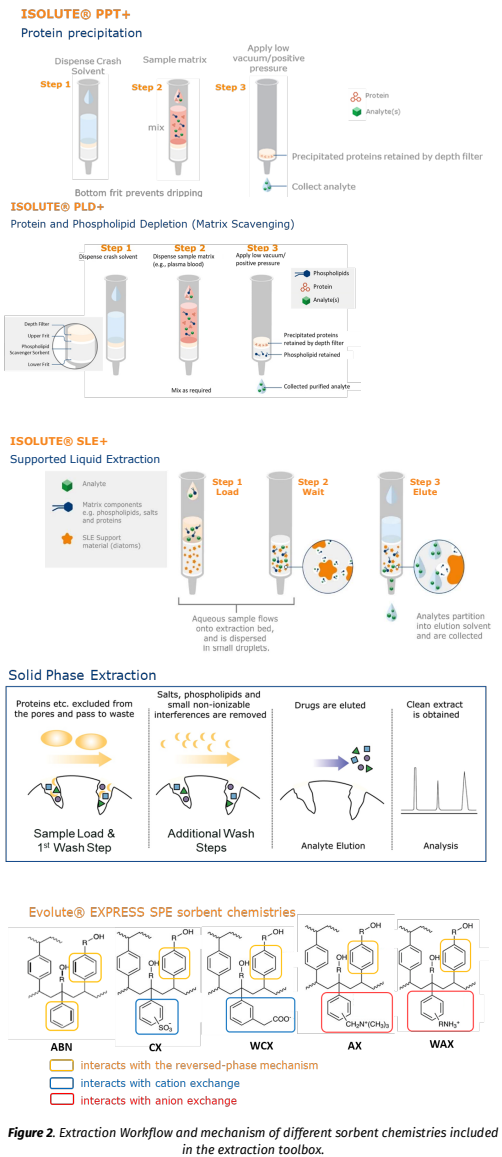

Figure 2. *Extraction Workflow and mechanism of different sorbent chemistries included in the extraction toolbox.*

Results and Discussion

Protein precipitation (PPT) and protein phospholipid depletion (PLD)

As shown in Figure 3, PPT and PLD methods provide standardized and unbiased extraction for a wide range of analytes with good solubility in the crash solvent (e.g., ACN). The matrix-scavenging sorbent in the PLD effectively removed phospholipids and proteins, offering a cleaner sample extract with minimal matrix effects.

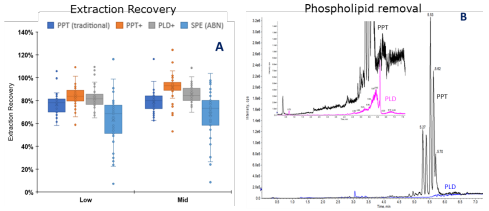

Figure 3. *Extraction recovery of analytes extracted by PPT and PLD methods (A) and phospholipid removal capability of the PLD method (B).*
Dots in the box plot indicate individual analytes. Low and Mid indicate the experiments were conducted in 2 concentrations (5 and 50 ng/ml)

Supported Liquid Extraction (SLE)

Like liquid-liquid extraction, SLE separates the analyte from the biomatrix by supporting its partition from aqueous into water-immiscible organic fractions. Hydrophobic analytes with high solubility in organic solvents show high extraction recoveries in the SLE methods (Figure 4A). Understanding analytes' chemical properties (Log P and pKa) is crucial to choosing appropriate pretreatment buffers and elution solvents for good extraction performance (Figure 5). SLE is effective in removing phospholipids from biological samples.

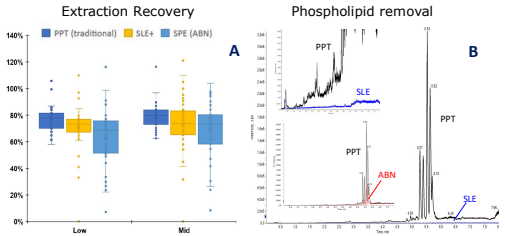

Figure 4. *Extraction recovery of analytes extracted by PPT and PLD methods (A) and phospholipid removal capability of the PLD method (B).*
Dots in the box plot indicate individual analytes. Low and Mid indicate the experiments were conducted in 2 concentrations (5 and 50 ng/ml)

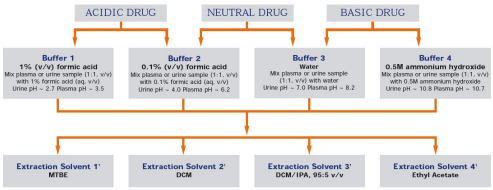

Figure 5. *ISOLUTE® SLE+ supported liquid extraction method selection guidance.*

Solid Phase Extraction (SPE)

Analyte retention on the polymeric SPE depends on the size-fit of the analyte to the sorbent pore and the analyte-sorbent interaction regarding the reverse-phase mechanism and/or ion exchange. The SPE methods are more selective than other methods (e.g., PPT, PLD, and SLE). Therefore, method development, including sorbent chemistry selection, washing, and elution procedures, is highly reliant on knowledge of analytes' structure, chemical properties, and matrices (Figure 6).

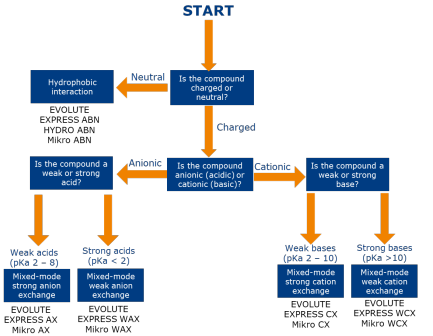

Figure 6. *Solid phase extraction sorbent chemistry selection flowchart.*

Conclusions

Sample preparation method development should start with simple and generic matrix scavenging techniques (PPT, PLD, and SLE) and move toward the more selective SPE method when the results do not meet the requirements. The automated sample preparation workflow using the Biotage® Extrahera™ and the extraction toolbox streamlined the method development workflow in Bioanalysis.

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