Non-selective Sample Extraction Approaches for Broad-spectrum Drug of Abuse Whole Blood Analysis

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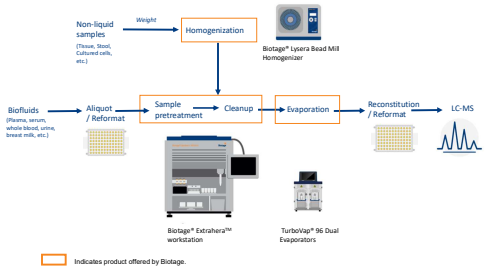
Introduction

Forensic, toxicology, and clinical laboratories conduct broad-spectrum whole blood (WB) screening to identify potential drug exposures. Extraction of drugs of abuse (DoA) panels from WB can be challenging, especially when targeting a large panel of analytes of different classes. The protein precipitation is straightforward but cannot provide sufficient cleanup for WB. Selective techniques, such as solid phase extraction (SPE), offer better sample cleanness; however, the specificity of the interaction makes it difficult to balance the extraction coverage and sample cleanliness. Using non-selective techniques can effectively remove the matrix without compromising the extraction recovery for a wide range of analytes.

Here, we demonstrate an automated sample preparation workflow for the broad-spectrum DoA WB analysis using two non-selective sorbent chemistries (Figure 1): protein and phospholipid depletion (PLD) and supported liquid extraction (SLE).



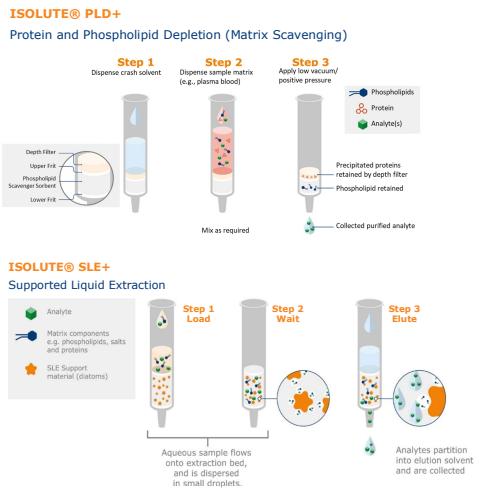


Figure 1. *Automated sample preparation workflow and the working mechanism of PLD and SLE*

Methodology

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 (Figure 2). Sample extraction using the PLD and SLE methods was automated using Biotage® Extrahera™ Sample Prep Workstation (*Figure 1*). Samples were also processed by the protein precipitation (PPT) and the SPE methods for comparison (Table 1). Analytes were spiked at low (5ng/ml) and high concentrations (50ng/ml). Extraction performance was evaluated by recovery, matrix effect, and reproducibility[1].

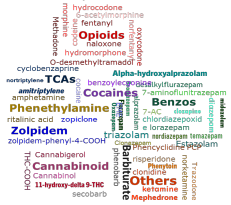


Figure 2. *Analytes spiked into WB for method development.*

Table 1 Sample preparation procedures for different extraction techniques

Steps	PPT	PLD	SLE	SPE-ABN	SPE-CX	SPE-WCX
Pretreatment	ACN	ACN	0.05M ammonium acetate (pH 8.5)	Water	0.05M ammonium acetate (pH 6)	Water
Wash 1			--	Water	0.05M ammonium acetate (pH 6)	Water
Wash 2			--	Water-Methanol (95:5)	Water-Methanol (80:20)	Water-Methanol (80:20)
Wash 3			--	--	Water-Methanol (60:40)	Water-Methanol (60:40)
Elute			EtOAc or DCM-IPA (95:5)	DCM-IPA (95:5)	EtOAc-IPA-NH3OH (78:20:2)	Methanol with 1% formic acid

Results and Discussion

Phospholipid Removal Capability

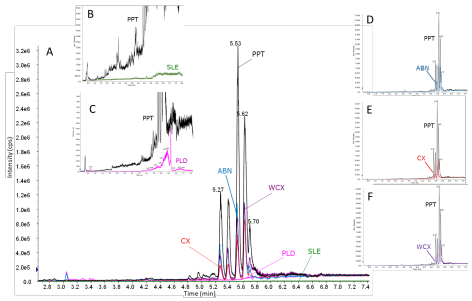


Figure 3. *Phospholipid removal by ISOLUTE® PPT+, ISOLUTE®PLD+, ISOLUTE®SLE+, EVOLUTE® EXPRESS ABN, EVOLUTE® EXPRESS CX, and EVOLUTE® EXPRESS WCX.*
The phospholipid profile was monitored by the MRM transition Q1 184 Da/ Q3 184 Da. (A) Overlay of WB phospholipid profiles after being processed by different sorbent chemistries. (B-F) Comparison of phospholipid removal between PPT and SLE (B), PLD (C), ABN (D), CX (E), and WCX (F).

Recovery, Matrix Effect, and Reproducibility

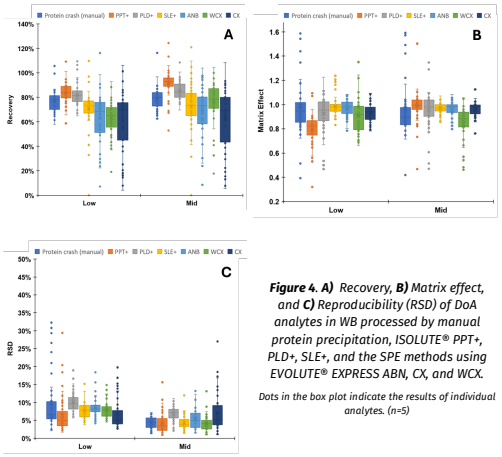


Figure 4. A) *Recovery,* ***B)*** *Matrix effect, and* ***C)*** *Reproducibility (RSD) of DoA analytes in WB processed by manual protein precipitation, ISOLUTE® PPT+, PLD+, SLE+, and the SPE methods using EVOLUTE® EXPRESS ABN, CX, and WCX.*
Dots in the box plot indicate the results of individual analytes. (n=5)

The PLD method demonstrated excellent extraction performance for diverse analytes, with high recoveries, minimal matrix effects, and good reproducibility (Figure 4). The phospholipid scavenging sorbent in PLD removed 99% of the phospholipids (Figure 3), effectively improving ion suppression. The SLE method achieved high extraction recovery for analytes more soluble in organic solvents, with superior matrix effect and reproducibility.

On the other hand, when considering extraction recovery across a wide range of analytes, the SPE methods are effective for only a limited set of analytes and have limited phospholipid removal capability.

Automated versus Manual Procedures

Table 2. Comparison of the run time regarding different extraction techniques

	Manual			Automated		
Methods	Total Time (min)	Bench Time* (min)	Walk Away** Time (min)	Total Time (min)	Bench Time (min)	Walk Away Time (min)
SLE	55	50	5	55	5	50
PLD	55	55	0	55	5	50
PPT	55	55	0	55	5	50
CX	75	75	0	75	5	70
WCX	75	75	0	75	5	70
ABN	75	75	0	75	5	70

** "Hands-on" analyst time; ** Free time for the analyst*
The run time is estimated based on 96-well plate.

Method Development Tips and Tricks

PLD

- Ensure the solubility of analytes in the crash solvent.
- Dilute WB with water 1:1 to improve extraction efficiency.
- Optimize the ratio between the sample and extraction solvent. (Recommend 1:8 or higher for WB)
- Ensure good mixing between solvent and sample.
- Recommend using acetonitrile as extraction solvent and avoid using high concentrations of alcohols.

SLE

- Understand the chemical properties of analyte panels (Log P and pKa).
- Pretreat WB with the appropriate buffer to neutralize compounds and facilitate better solubility in organic solvent.
- Avoid strong acidic and basic pretreatment conditions.
- Optimize the elution conditions by comparing different organic solvents.
- Only load 75% or lower capacity to avoid breakthrough.
- Add acids to the eluted fraction to prevent the loss of volatile compounds during evaporation.

Conclusions

The non-selective sample preparation techniques, **PLD** and **SLE**, are more suitable for broad-spectrum DoA whole blood analysis than the **SPE** methods. While SPE methods are highly selective for specific analytes, PLD and SLE focus on removing biomatrix components without being analyte-specific. The PLD method is advantageous in standardized and universal procedures and better recovery regardless of structure diversity, while SLE offers cleaner sample matrix and better reproducibility.

Automated workflows provide better reproducibility than manual methods and can significantly reduce the time scientists spend on bench operations.

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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