Supported Liquid Extraction (SLE): A Novel Sample Preparation Method for Small Molecule Extraction from Biological Matrices Prior to High Pressure Liquid Chromatography Mass Spectrometry Analysis

Victor E. Vandell, Ph.D., Frank Kero, Ph.D. and Elena Gairloch, Biotage USA, 10430 Harris Oaks Blvd. Charlotte, NC. 28269
Lee Williams, Ph.D., Biotage GB Limited, Dyffryn Business Park, Ystrad Mynach, CF82 7RJ, UK

Introduction

Supported Liquid Extraction (ISOLUTE SLE+) is a relatively novel sample preparation methodology that can be used to extract a variety of basic, acidic and neutral small drug molecules from biological matrices (e.g. urine, serum, whole blood, and oral fluid). Typically, endogenous interferences like proteins, phospholipids, lysophospholipids, and ionic salts can cause clogging on HPLC columns and ion suppression in cases where a mass detector is used. Classical methods like solid phase extraction and liquid-liquid extraction are typically employed to extract small analytes from biological matrices. These classical methods can be laborious and inconsistent in their application. Supported Liquid Extraction is a faster and more efficient method that cleans up >95% of proteins, phospholipids and salts from biological matrices prior to injection onto a HPLC column and subsequent detection by UV-Vis or mass spectrometry. This poster will demonstrate the utility of SLE for extracting a variety of basic, acidic and neutral drugs in biological fluids that are typically analyzed in forensic and clinical laboratories.

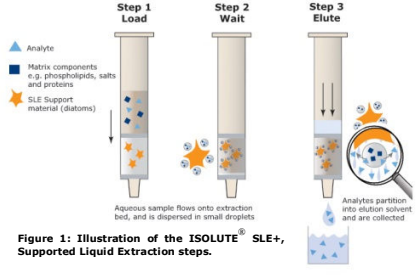


Figure 1: Illustration of the ISOLUTE® SLE+, Supported Liquid Extraction steps.

What is Supported Liquid Extraction?

Supported Liquid Extraction (ISOLUTE® SLE+) is a modified diatomaceous sorbent that has a high adsorption affinity for aqueous solutions and the analytes solubilized in the aqueous solution. The sample preparation methodology of Supported Liquid Extraction works on the same chemical premises as a liquid-liquid extraction experiment, but is carried out on a solid phase.

The desired target analyte(s) will be solubilized in an aqueous biological matrix. Also solubilized in the matrix are endogenous interferences like proteins, lipids, phospholipids, salts and other unwanted components that will cause problems with the chromatographic separation and subsequent detection of the desired analytes.

The development of a sample preparation method using SLE will enable the user to load the aqueous matrix, containing the target analytes and interferences onto a pre-packed SLE column or 96 well plate. Any water-immiscible organic solvent (e.g. dichloromethane, ethyl acetate, diethyl ether, etc.) can then be gravity fed through the column or well to extract and collect the target analytes. The water-soluble endogenous interferences (i.e. proteins, lysophospholipids, phospholipids and salts) are retained on the sorbent, which can subsequently be discarded. The process takes only 3 steps (Figure 1).

Why is Sample Preparation Important?

The endogenous interferences found in all biological matrices are complex molecules that are abundant and can be problematic for qualitative and quantitative analysis for target small molecules of interest. The injection of proteins onto an HPLC system can cause build-up of the proteins on the head of an HPLC column resulting in clogging and poor performance. The first level of sample preparation is often a simple "protein crash" and inject. This method is known as the "dirtiest" sample preparation method because it does not get rid of all of the proteins like albumin which is the most abundant protein in most biological matrices (Figure 2 and Figure 3A). Another limitation with protein crash is that it does not address the lipids and phospholipids which are also present in most biological samples (Figure 2). The phospholipids and lipids are a major source of ion suppression which can be observed in a mass spectrometer. The phospholipids and lysophospholipids compete for charge in the mass spectrometer source and result in lower signal response for the target analytes. Ion suppression becomes extremely problematic when lower limits of detection for target ions are desired. Most clinical assays require maximum sensitivity from the mass spectrometer to detect desired analytes at lower limits of detection and quantitation. Other interferences like salts, urea and creatinine (Figure 2) can also build up on the head of HPLC columns and cause ion suppression in the mass spectrometer ion source. Hence, laboratories that conduct even a modest number of day to day testing from biological fluids will require sample preparation techniques that alleviate these endogenous interferences which can lead to costly equipment replacement and instrument down time.

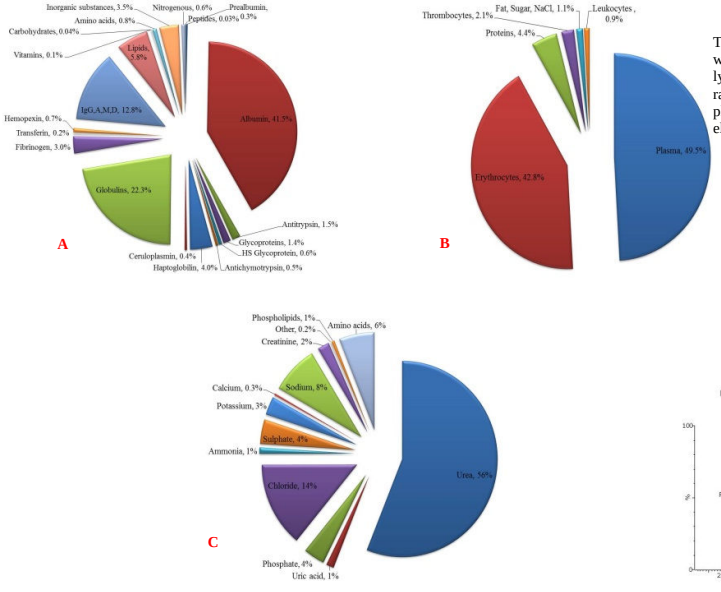


Figure 2. Endogenous components of Plasma (A), Whole Blood (B) and Urine (C).

The Cleanliness of Supported Liquid Extraction

Supported Liquid Extraction cleanliness has been tested and demonstrated through a number of applied assays in the clinical, forensic and pharmaceutical areas. The cleanliness of SLE was demonstrated using gel electrophoresis to show the elimination of proteins from raw serum. A comparison of protein precipitation at various crash solvent ratios (Figure 3A) shows that even at an optimal ratio of 1:6 (serum:acetonitrile) there are proteins still present, mainly albumin. The gel slab with the same raw serum loaded onto an SLE cartridge (Figure 3b) shows >99% elimination of proteins.

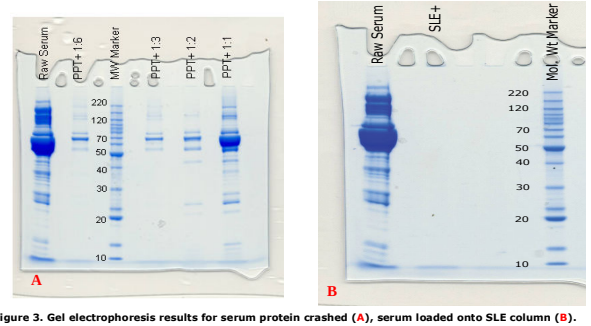
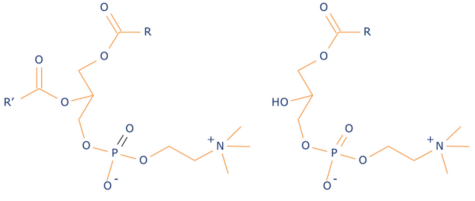


Figure 3. Gel electrophoresis results for serum protein crashed (A), serum loaded onto SLE column (B).

Testing of the ISOLUTE SLE+ sorbent for cleanliness of phospholipids and lysophospholipids (Figure 4) was conducted using targeted mass spectrometry. The mass transitions for monitoring phospholipids and lysophospholipids (e.g. 496>194Da, 704>184Da) were run on a triple quadrupole mass spectrometer for raw plasma under different sample pretreatment and elution conditions to demonstrate cleanliness of phospholipids as compared to raw plasma that was just protein crashed. Again, protein crash does not eliminate the phospholipids and hence can result in huge ion suppression effects for samples (Figure 5).



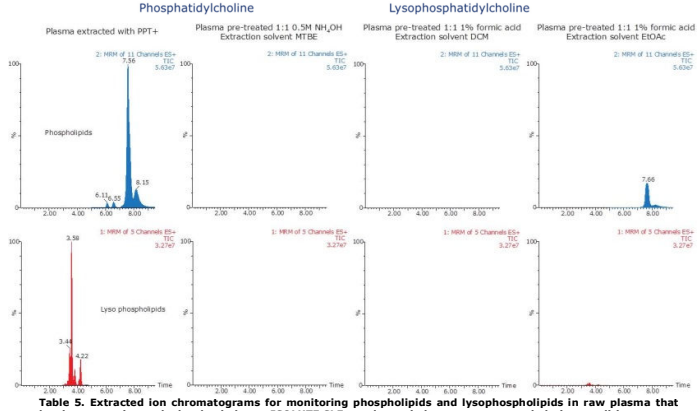


Table 5. Extracted ion chromatograms for monitoring phospholipids and lysophospholipids in raw plasma that has been protein crashed or loaded onto ISOLUTE SLE+ under varied pre-treatment and elution conditions.

Supported Liquid Extraction Formats

The ISOLUTE SLE+ comes in a variety of formats relative to the volume of sample needing to be prepared. The 96 well plate formats can be used to prepare samples ranging from 50uL to 400uL in volume. The cartridge format can be used to prepare samples ranging from 100uL up to 10mL. For more information on supported liquid extraction visit our website or call one of our world-wide offices to reach a technical representative near you.

http://www.biotage.com

Part Number: P80