The Advantages of Automating Sample Preparation through Supported Liquid Extraction Plates

Sample preparation can be accomplished by several methodologies including, protein precipitation (PPT), solid phase extraction (SPE), and Liquid-liquid extraction (LLE). In many cases the use of automated liquid handlers and 96-well plates is desired for high throughput. When faced with the challenges of higher performance and limited resources, developing a robust, cost effective sample preparation protocol can be a difficult process.

Now available in the 96-well plate format, supported liquid extraction (SLE) is emerging as a preferred technique for aqueous samples because it is easily automated, provides clean extracts, method development is fast and simple, and they are generally lower cost than most SPE plates. The 2 step extraction method is simple to optimise and can be applied to a wide variety of acidic, basic and neutral compounds.

This article outlines the SLE technique compared with traditional LLE for the application of drugs in human plasma.

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SAMPLE PREPARATION TECHNIQUES

Traditionally used for the preparation of biological fluids such as plasma and urine prior to LC-MS or GC-MS analysis, LLE is used for a wide range of applications, including forensic and toxicological drug determination, nuclear reprocessing, or processing and fine organic compounds production. This technique employs simple methodology to produce clean extracts that can be directly introduced into an LC-MS or GC-MS analytical platform for processing. However, LLE can be labour-intensive and difficult to automate since it often requires off-line processing such as mixing or centrifuging. Complete separation of the organic and aqueous layers is also difficult utilising traditional plates. Due to advances in manufacturing, supported liquid extraction can now be done in high throughput 96-well plates compatible with most automated systems, requiring no off-line steps. The flow-through design allows for complete separation of the organic and aqueous layers and thus, no loss of analyte or contamination from the water layer.

SLE can offer additional benefits to the laboratory by preventing the sample and the immiscible extraction solvent from coming into direct contact. This step eliminates the problems of contamination, automated pipetting of liquid layers and emulsion formation, which are common during the sample preparation process. Analyte recoveries are maximised due to the particularly effective mechanisms used by SLE and cleaner extracts are produced compared to those delivered by traditional methods. The new SLE+ plate, developed by Biotage, is a 96-well plate featuring an optimised grade of diatomaceous earth. Through advances in manufacturing, it provides reproducible flow characteristics from well to well. Through this technique, aqueous biological fluid samples are introduced into the extraction well and spread over the surface of the well support forming an extremely thin layer that is subsequently absorbed into the dry column. Following this step, the analytes remain on the surface of the well support, forming the interface for the extraction. After several minutes, water immiscible extraction solvent is added, the analytes are efficiently desorbed and the organic extract is collected *(Figure 1)*. This process prevents well blockage, which can lead to loss of valuable samples in automated sample preparation, and means that analyte recoveries are significantly higher due to high extraction efficiency and the elimination of emulsion formation.

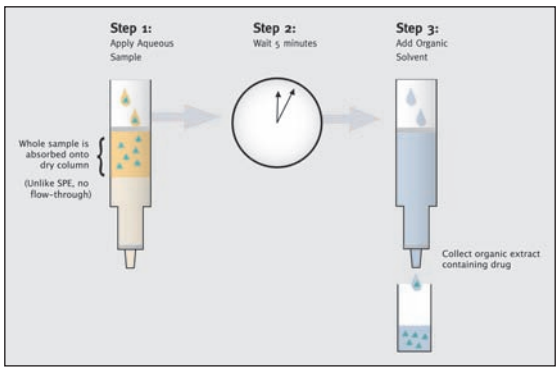

Figure 1. The Biotage SLE procedure.

EXTRACTION OF DRUGS FROM PLASMA USING SLE

In order to test the extraction efficiency of this technique, an experiment was developed using SLE plates. Results were compared to those achieved using conventional LLE carried out in glass vials. The plates used for this application were the ISOLUTE® SLE+ Plates from Biotage.

A 100µL human plasma was diluted with 0.5M ammonium hydroxide, with analytes of imipramine, tripramine, nortryptiline and a 10ng/mL spiked plasma concentration. The extraction solvent used was hexane: 2-methyl-1-butanol, 1mL. SLE requires the use of the same water immiscible extraction solvents and sample pre-treatment conditions as those required for LLE. This means that existing LLE methods are easily transferable, thus reducing method development for SLE.

The pre-buffered sample was dispensed into the 96-well plate of the SLE plates, which was optimised for the simultaneous extraction of samples. The plate was subsequently processed using a vacuum manifold or an automated liquid handling system with vacuum capability for 2-10 seconds to initiate loading. As soon as the sample was completely absorbed, the hexane was applied and allowed to flow for five minutes under gravity. The vacuum was re-applied for two minutes to complete elution and the sample was evaporated to dryness and reconstituted in mobile phase prior to analysis.

A liquid handling system was employed to perform HPLC analysis while an analytical column equipped with a narrow bore guard column was used to execute chromatography analysis. Separations were carried out under ambient temperatures and injection volumes ranged between 5-20µL. The entire column effluent was directed into a triple quadruple mass spectrometer featuring an electro-spray interface. Positive ions were acquired in the Multiple Reaction Monitoring (MRM) mode using a desolvation temperature of 350°C and a source temperature of 100°C.

The sample used to test the speed and ease of automation of SLE was 200µL pre-buffered human plasma, with 1mL water immiscible extraction solvent and a liquid handling model equipped with a vacuum manifold. The aqueous sample with a maximum of 200µL was dispensed to each well and a vacuum was applied to commence loading. Once the sample was absorbed, the water immiscible extraction solvent was applied to each well and allowed to flow under gravity. The vacuum was then re-applied to complete elution and the extraction solvent was collected in the collection plate.

By employing this advanced SLE technique, analyte recovery was significantly increased in comparison to recovery achieved with conventional LLE techniques *(Figure 2)*.

Productivity was significantly increased as a result of the fully automated SLE technique. Actions such as capping the plate, mixing and centrifuging the layers of the sample and the extraction solvent, allowing the layers to separate prior to evaporation and uncapping the plate are completely eliminated.

Analyte	Analyte Recovery (% RSD)	
	SLE	LLE
Imipramine	97% (4)	65% (4)
Trimipramine	96% 92)	57% (4)
Nortriptyline	91% (4)	62% (5)

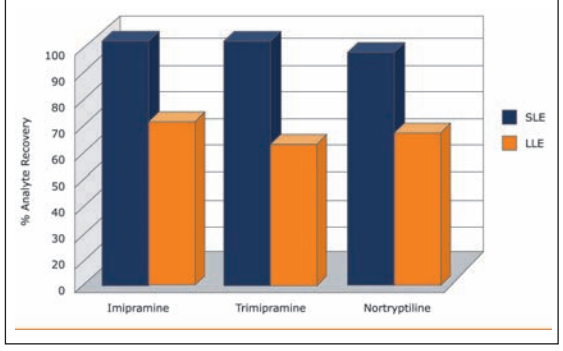

Figure 2. Comparison of analyte recovery using ISOLUTE® SLE+ and traditional LLE.

CONCLUSION

While LLE has proven an effective sample preparation technique for a wide range of applications, its requirement for offline steps poses challenges where high throughput analysis is needed. Advanced SLE techniques pioneered by Biotage are emerging as a viable alternative technique that can increase sample throughput by up to 100%. The removal of manual steps decreases extraction time, while analyte recovery is increased, overall increasing laboratory productivity.