Extraction of Tricyclic Anti-depressants from Plasma Using ISOLUTE® SLE+ Supported Liquid Extraction Plates

Application Note

Introduction

Traditional Liquid-liquid extraction (LLE) is widely used for preparation of biological fluid samples (plasma, urine) prior to LC-MS/MS analysis.

LLE is labor intensive, very difficult to automate, and is therefore not well suited to high throughput bioanalytical sample preparation. Supported-liquid extraction (SLE) provides an easier to automate alternative to LLE. Problems such as emulsion formation and automated pipetting of liquid layers are eliminated, as the two phases are never in direct contact with each other.

This Application Note describes the development of an automated procedure for high throughput supported-liquid extraction of three tricyclic antidepressant drugs from human plasma, using the ISOLUTE® SLE+ Supported-liquid Extraction Plate. Analyte recovery, along with the speed and efficiency are compared to traditional LLE.

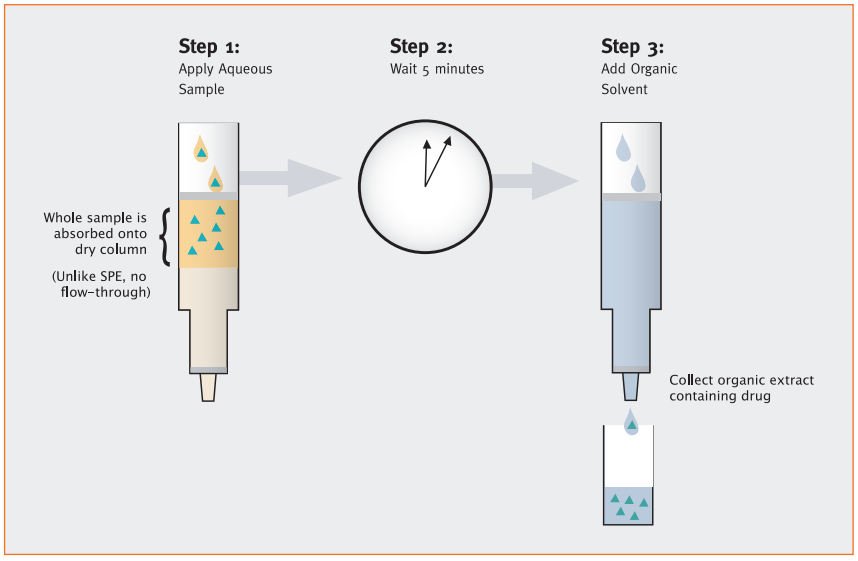

Figure 1. The supported-liquid extraction process using the ISOLUTE SLE+ supported-liquid extraction plate (single well shown).

The ISOLUTE SLE+ plate consists of 96 extraction wells each containing a modified form of diatomaceous earth. When the aqueous biological fluid sample is applied, it spreads over the surface of the packing material, and is absorbed. Analytes of interest remain on the surface of the support, forming the interface for extraction (equivalent to the phase interface in LLE). When the water immiscible extraction solvent is applied, analytes are efficiently desorbed, and the solvent is collected. This process is shown schematically in **Figure 1**.

1. Analyte Recovery

Extraction efficiency using the ISOLUTE SLE+ plate was investigated, and compared to the equivalent LLE procedure (carried out in glass vials). Analyte recovery for the tricyclic antidepressants Imipramine, Trimipramine and Nortryptiline are reported.

Experimental Details

Sample (ISOLUTE SLE+ and LLE):	100 µL human plasma diluted 1:1 with 0.5 M NH_4OH
Analytes (ISOLUTE SLE+ and LLE):	Imipramine, Trimipramine, Nortryptiline, 10 ng/mL spiked plasma concentration
Extraction solvent (ISOLUTE SLE+ and LLE):	Hexane:3-methyl-1-butanol (98:2, v/v), 1 mL

ISOLUTE SLE+ procedure

1. Dispense pre-buffered sample (200 µL).
2. Apply vacuum (-15 ”Hg / -0.5 bar) for 2-10 seconds to initiate loading.
3. Wait 5 minutes for sample to completely absorb.
4. Apply extraction solvent (1 x 1 mL).
5. Allow solvent to flow for 5 minutes under gravity.
6. Apply vacuum (-15 ”Hg / -0.5 bar) for 2 minutes to complete elution.
7. Evaporate to dryness. Reconstitute in mobile phase prior to analysis.

Liquid-liquid Extraction procedure

1. Dispense pre-buffered sample (200 µL).
2. Add extraction solvent (1 x 1 mL).
3. Mix thoroughly.
4. Allow layers to separate.
5. Remove organic layer.
6. Evaporate to dryness. Reconstitute in mobile phase prior to analysis.

Analytical Conditions

HPLC CONDITIONS:
HPLC was performed using a Waters® Alliance 2795 liquid handling system. Chromatography was achieved using a Zorbax® Eclipse XDB-C18 3.5 µm analytical column (2.1 x 50 mm) equipped with a narrow bore guard column (both Agilent Technologies) at a flow rate of 0.25 mL/min. An isocratic mobile phase was employed, consisting of H_20/ACN/NH_4OH (10/90/0.1, v/v). Separations were carried out under ambient temperatures and injection volumes ranged between 5-20 µL.

MS CONDITIONS:
The entire column effluent was directed into a Quattro Ultima® Pt triple quadrupole mass spectrometer equipped with an electrospray 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.

Analyte	MRM transitions	Dwell time (s)	Cone Voltage (V)	Collision Energy (eV)
Imipramine	281.1>86.1	0.1	40	15
Trimipramine	295.1>100.1	0.1	40	15
Nortriptyline	264.1>233.1	0.1	40	13

Analyte	Analyte Recovery (% RSD)	
	SLE	LLE
Imipramine	97% (4)	65% (4)
Trimipramine	96% (2)	57% (4)
Nortriptyline	91% (4)	62% (5)

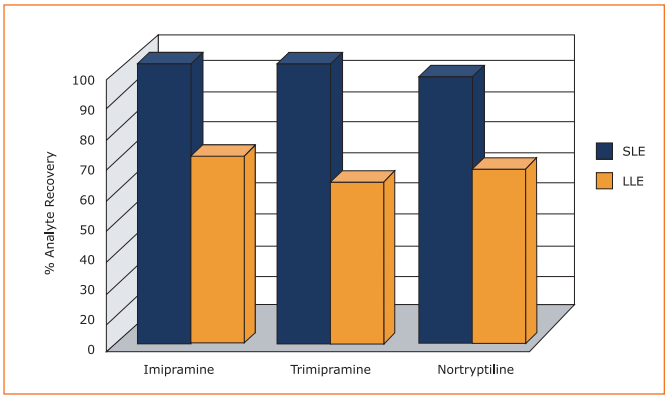

Figure 2. Comparison of analyte recovery using ISOLUTE SLE+ and LLE

2. Automation Efficiency

The speed and ease of automation of a typical supported-liquid extraction procedure using ISOLUTE SLE+ plates was investigated. This was compared to the equivalent LLE procedure, using the same sample and extraction solvent volumes.

Experimental Details

Sample:	Pre-buffered human plasma sample, 200 µL
Extraction solvent:	Water immiscible solvent, 1 mL
Liquid handling:	Quadra 96® Model 320 equipped with vacuum manifold

ISOLUTE SLE+ procedure

1. Dispense aqueous sample (max 200 µL) to each well.
2. Apply vacuum (-15 ”Hg / -0.5 bar) for 2-10 seconds to initiate loading.
3. Wait 5 minutes for sample to completely absorb.
4. Apply water immiscible extraction solvent (3 x 330 µL) to each well.
5. Allow solvent to flow for 5 minutes under gravity.
6. Apply vacuum (-15 ”Hg / -0.5 bar) for 2 minutes to complete elution.
7. Collect 1 mL extraction solvent in collection plate

Liquid-liquid extraction procedure

1. Dispense aqueous sample (200 µL) to each well.
2. Dispense water immiscible extraction solvent (3 x 330 µL) to each well.
3. Remove plate from Quadra 96
4. Cap plate
5. Mix (2 minutes)
6. Centrifuge to separate layers (10 minutes total)
7. Uncap plate
8. Replace plate on Quadra 96
9. Transfer 900 µL extraction solvent to collection plate

Steps 4–7 are off-line. Total time estimated at 15 minutes (includes capping, transfer steps, centrifuge spin up/down, decapping)

Results

Technique	SLE	LLE
Off line steps	None	4
Total extraction time	12.5 minutes	22.5 minutes
Potential productivity	4 plates per hour	2 plates per hour

Conclusions

1. Supported-liquid extraction (SLE) using the ISOLUTE SLE+ plate is an easily automated technique, providing 2 x increased sample throughput compared to traditional LLE.

2. ISOLUTE SLE+ supported-liquid extraction Plates can give significantly higher analyte recoveries than traditional LLE using the same extraction conditions (sample and solvent).

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