Biotage

Extraction of Drugs from Plasma Using ISOLUTE SLE+ Supported Liquid Extraction Plates

L. Williams, H. Lodder, S. Merriman, A. Howells, S. Jordan, J. Labadie, M. Cleeve, C. Desbrow, and R. Calverley, Argonaut Technologies Ltd.
M. Burke, MFB Consulting LLC

Liquid-liquid extraction (LLE) is widely used for preparation of biological fluid samples (plasma, urine) prior to LC–MS analysis. The technique uses simple methodology, and provides clean extracts for introduction to the mass spectrometer.

Traditional liquid-liquid extraction is labour 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 automatable 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 of the procedure, is compared with the traditional technique.

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.

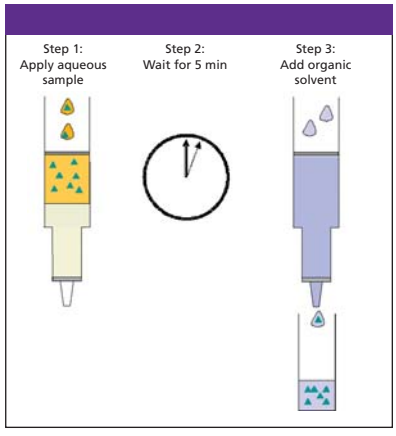

Figure 1: The supported liquid extraction process using the ISOLUTE SLE+ Supported Liquid Extraction Plate (single well shown).

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 Nortriptyline is reported.

Experimental Details

Sample (ISOLUTE SLE+ and LLE): 100 mL human plasma diluted 1:1 with 0.5M NH_4OH

Analytes (ISOLUTE SLE+ and LLE): Imipramine, Trimipramine, Nortriptyline, 10 ng/mL spiked plasma concentration

Extraction solvent (ISOLUTE SLE+ and LLE): hexane:2-methyl-1-butanol (98 :2, v/v), 1 mL

ISOLUTE SLE+ Procedure

1. Dispense prebuffered sample (200 µL)
2. Apply vacuum (−15"Hg / −0.5 bar) for 2–10 s to initiate loading.
3. Wait 5 min for sample to completely absorb.
4. Apply extraction solvent (1 × 1 mL).
5. Allow solvent to flow for 5 min under gravity.
6. Apply vacuum (−15"Hg / −0.5 bar) for 2 min 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 × 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 × 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 elec-

trospray 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.

Table I.

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

Table II: Results (shown graphically in Figure 2)

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

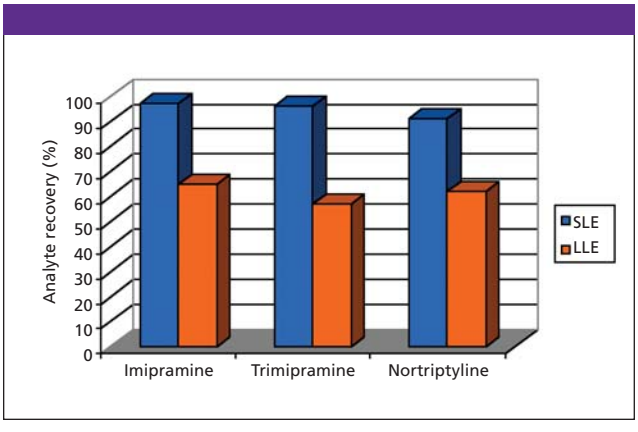

Figure 2: Comparison of analyte recovery by SLE and LLE.

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: prebuffered 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 s to initiate loading.
3. Wait 5 min for sample to completely absorb.
4. Apply water immiscible extraction solvent (3 × 330 µL) to each well.
5. Allow solvent to flow for 5 min under gravity.
6. Apply vacuum (−15"Hg / −0.5 bar) for 2 min 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 × 330 µL) to each well.
3. Remove plate from Quadra 96

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4. Cap plate
5. Mix (2 min)
6. Centrifuge to separate layers (10 min total)
7. Uncap plate

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8. Replace plate on Quadra 96
9. Transfer 900 µL extraction solvent to collection plate

Off-line steps. Total time estimated at 15 min (includes capping, transfer steps, centrifuge spin up/down, decapping)

Results

Table III.

Technique	SLE	LLE
# Off line steps	None	4
Total extraction time	12.5 min	22.5 min
Potential productivity	4 plates per h	2 plates per h

Conclusions

1. Supported liquid extraction (SLE) using the ISOLUTE SLE+ plate is an easily automated technique, providing 2 × 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).

Biotage
1725 Discovery Drive, Charlottesville, Virginia 22911
tel . (434) 979-2319, (434) 220-2687
ordermailbox@biotage.com, www.biotage.com