Supported Liquid Extraction: Automate those Tiresome Bioanalytical LLE Protocols

L. Williams, H. Lodder, S. Merriman, A. Howells, S. Jordan, J. Labadie, M. Cleeve, C. Desbrow, R. Calverley and M. Burke[1]

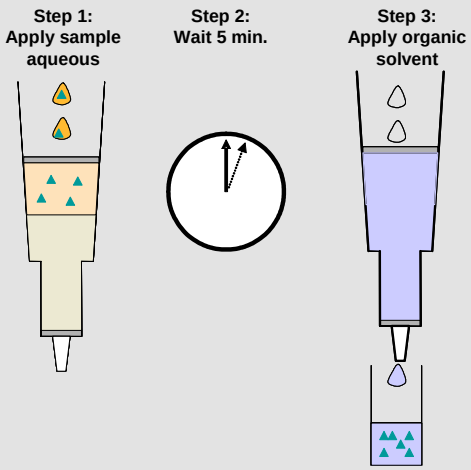
Argonaut Technologies Ltd., now a Biotage company, New Road, Hengoed, Mid Glamorgan, CF82 8AU, UK.
[1]MFB Consulting LLC, 5560 N. Via Velásquez, Tucson, AZ 85715

Introduction

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 LLE is labor intensive, very difficult to automate, and 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 poster describes the development of an automatable procedure for high throughput SLE, using ISOLUTE® SLE (a 96-well plate). The speed and efficiency of the procedure, along with analyte recovery and extract cleanliness will be 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 packing material, 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 is shown schematically in **Figure 1**.

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

1. Automation Efficiency

The speed and ease of automation of a typical supported liquid extraction (SLE) procedure (schematic above) was investigated. This was compared to the equivalent LLE procedure, using the same sample and extraction solvent volumes.

Experimental Procedure

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

IST Sample Preparation

Biotage

Outline 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 333 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

Outline LLE Procedure

1. Dispense aqueous sample (200 µL) to each well.
2. Dispense water immiscible extraction solvent (3 x 333 µL) to each well.
3. Remove plate from Quadra 96

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4. Cap plate
5. Mix (2 mins)
6. Centrifuge to separate layers (10 minutes 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 minutes (inc. 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/hour	2 plates/hour

2. 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 Procedure

Sample (SLE and LLE): 100 µL human plasma diluted 1:1 with 0.5M NH_4OH
Analytes (SLE and LLE): Imipramine, trimipramine, nortriptyline, 10 ng/mL spiked plasma concentration
Extraction Solvent (SLE and LLE): hexane/2-methyl-1-butanol (98:2, v/v), 1 mL

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.

LLE 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

Guard Column: Eclipse XDB-C18 (2.1 x 12.5 mm, 5 µm)
Analytical Column: Eclipse XDB-C18 (2.1 x 50 mm, 3.5 µm)
Mobile Phase: H2O/ACN/NH4OH (10/90/0.1, v/v)
Isocratic
Flow Rate: 0.25 mL/minute
Injection Volume: 5-20 µL **Temperature:** Ambient

MS Conditions

Instrument: Quattro Ultima Pt triple quadrupole
Ionization: Electrospray, +ve, source temperature 100 °C
Desolvation Temperature: 350 °C

Analyte	MRM Transitions	Collision Energy (eV)
Imipramine	281.1>86.1	15
Trimipramine	295.1>100.1	15
Nortriptyline	264.1>233.1	13

Results (shown graphically in **Figure 2**)

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

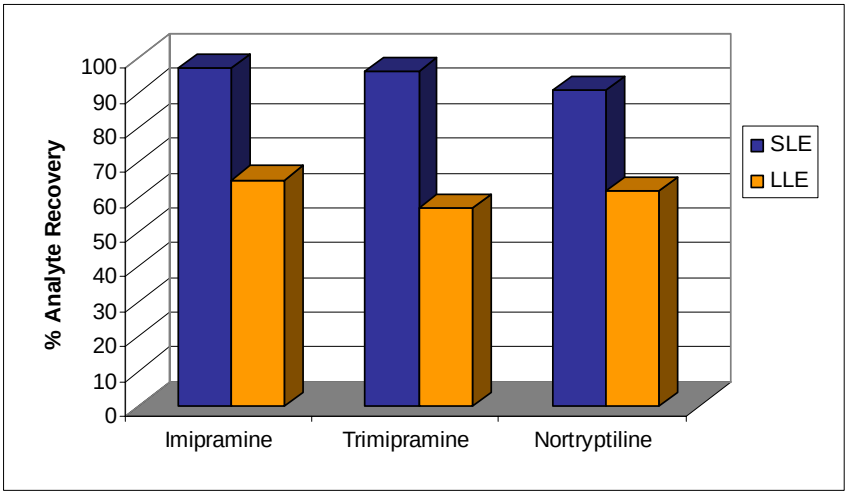

Figure 2. Comparison of analyte recovery by SLE and LLE

Sample Preparation

Biotage

3. Extract Cleanliness

In order to quantify extract cleanliness, the degree of ion suppression of extracts due to matrix components was measured using flow injection analysis (FIA) LC-MS/MS. Blank human plasma samples were extracted using both SLE and the equivalent LLE procedure. A number of common water immiscible extraction solvents were investigated.

Experimental Procedure

Sample (SLE and LLE): 100 µL human plasma diluted 1:1 with water (HPLC grade)
Extraction Solvent (SLE and LLE): Various, 1 mL
Procedure: As described in **Section 2**.

FIA LC-MS-MS Conditions
Samples: Reconstituted in 1mL of 1 ng/µL caffeine solution in mobile phase.
Mobile Phase: H2O/ACN/MeOH/Formic acid (50/45/5/0.1, v/v)
Injection Volume: 5 µL
Caffeine MRM Transition: 195>138

Results (shown graphically in **Figure 3**)

Extraction solvent	% ion suppression	
	SLE	LLE
MTBE	11	22
DCM	11	17
Ethyl acetate	25	31
DCM: IPA (90:10, v/v)30	30	32
Hexane/2-methyl-1-butanol (98 :2, v/v)	19	22

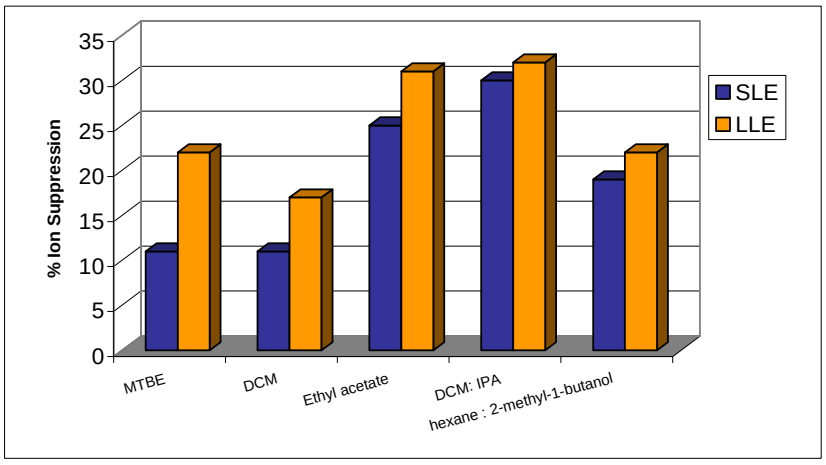

Figure 3. Comparison of ion suppression caused by SLE and LLE extracts

Overall Conclusions

1. Supported liquid extraction (SLE) using the ISOLUTE SLE plate is an easily automated technique, providing 2 times increased sample throughput compared to traditional LLE.
2. SLE can give significantly higher analyte recoveries than traditional LLE using the same extraction conditions (sample and solvent).
3. Extracts obtained by SLE exhibit similar or lower ion suppression than LLE extracts, for a range of extraction solvents.

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United States and Canada
Tel: +1 434 979 2319
Toll-Free: +1 800 446 4752
ordermailbox@biotage.com

United Kingdom, EIRE
Biotage
Tel: +44 1992 501535
order@eu.biotage.com

Sweden
Biotage
Tel: +46 18 56 59 00
order@eu.biotage.com

Japan
Biotage
Tel: +81 422 281233
order@biotage.co.jp