High throughput extraction of drugs from biological fluids using an improved supported liquid extraction plate

Lee Williams, Scott Merriman, Helen Lodder, Anne Howells, Steve Jordan, Claire Desbrow, Matthew Cleeve, Richard Calverley

Argonaut Technologies, now a Biotage company, Dyffryn Industrial Estate, Ystrad Mynach, Mid Glamorgan, CF82 7RJ, UK

Introduction

Liquid-liquid extraction (LLE), based on the transfer of drug species from the aqueous sample into a water immiscible organic solvent, is often used for preparation of biological fluid samples (plasma, urine). Traditionally carried out in glass vials or microtitre plates, the technique is popular as it can provide extracts with low levels of co-extracted matrix material, leading to minimal ion suppression in LC-MS analysis. However, this traditional technique is labour intensive, and although procedures have been successfully automated, relatively expensive instrumentation is required. Manual intervention at various stages of the procedure (for mixing or centrifuging) is necessary, and the problems of foaming and emulsion formation are not entirely eliminated.

Supported liquid extraction (SLE) is an alternative sample preparation technique which is analogous to traditional LLE, but can be easily automated without the problems presented by LLE.

ISOLUTE® SLE+ plates utilize a proprietary inert support material packed into individual wells of a 96-well extraction plate. When the biological fluid sample is applied, the sample is absorbed by the support, spreading over a high surface area as it migrates down the bed. The water immiscible extraction solvent is applied to the wells and the analytes of interest efficiently partition into the organic solvent at the interface between the two phases as the solvent migrates down the bed. Efficient extraction is achieved without the need for agitation of the plate, with the extract collected in a 96-well collection plate. This procedure is shown schematically in **Figure 1**.

This presentation describes the application of this technique for extraction of a range of drugs from biological fluids. The effect of a range of extraction solvents and modifiers on LC-MS matrix effects will be investigated.

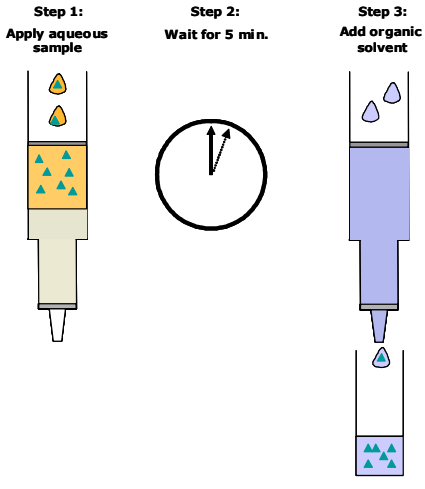

Figure 1. Schematic of the ISOLUTE SLE+ supported liquid extraction procedure. A single well of the 96-well plate is illustrated

1. Extraction of drugs from biological fluids using ISOLUTE SLE+ plates

Generic procedures have been developed for the extraction of both acidic and basic drugs from biological fluids. To enhance recovery of ionizable compounds, pH adjustment is used to neutralize ionizable groups (as in LLE). Water immiscible extraction solvent is chosen based on solubility of the analytes. The same solvent systems as existing LLE methods can be used.

Using the generic procedures described below, previous work1 has shown that 96 samples can be extracted in 12.5 minutes using a Tomtec Quadra 320 system equipped with a vacuum manifold.

Experimental Procedure

1a. Extraction of acidic drugs

Analytes: sulindac, flurbiprofen, ibuprofen, 500 ng/mL
Sample: human plasma (100 µL) diluted 1:1 with water adjusted to pH 2.5 with formic acid
Extraction solvent: DCM:IPA (90:10 v/v, 1 mL)
Extracts were evaporated to dryness and reconstituted in mobile phase for LC-UV analysis.

Analyte	Sulindac	Flurbiprofen	Ibuprofen
Average recovery (n=8)	96	91	86
RSD	3	3	2

1b. Extraction of basic drugs

Analytes: imipramine, trimipramine, nortriptyline, 10 ng/mL
Sample: human plasma (100 µL) diluted 1:1 with 0.5 M NH4OH
Extraction solvent: hexane:2-methyl-1 butanol (98:2, v/v,1 mL)
Extracts were evaporated to dryness and reconstituted in mobile phase for LC-MS/MS analysis.

Analyte	Imipramine	Trimipramine	Nortriptyline
Average recovery (n=8)	97	96	91
RSD	4	2	4

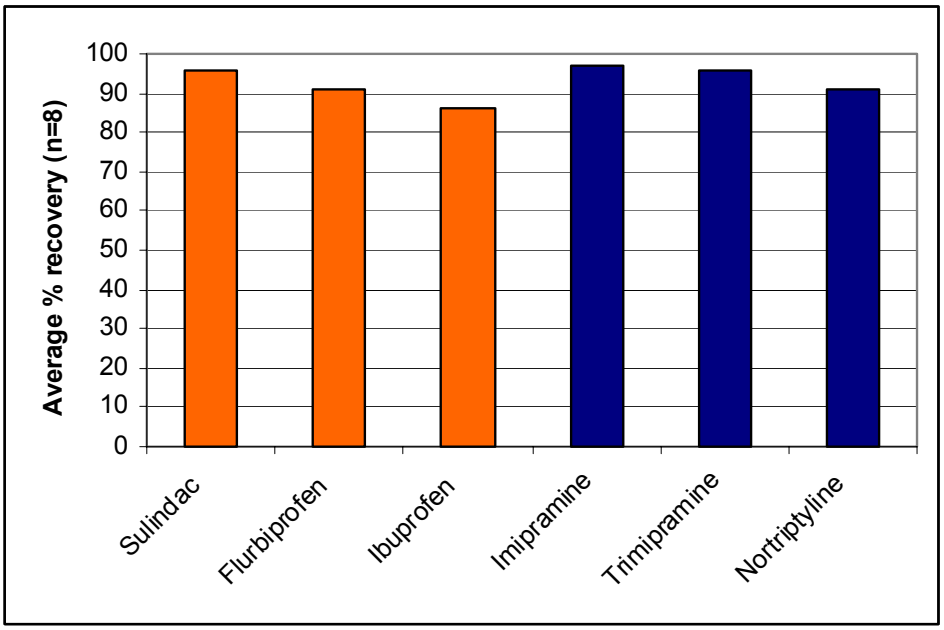

Figure 2. Recovery of acidic and basic drugs using ISOLUTE SLE+ plates

2. Effect of choice of extraction solvent on ion suppression in LC-MS-MS

Traditional liquid-liquid extraction is popular as it can provide extracts with low levels of co-extracted matrix material, leading to minimal ion suppression in LC-MS analyses. This is because the components of human plasma which can cause ion suppression (e.g salts, proteins, phospholipids) tend to have low solubility in the water immiscible solvent systems used in LLE and ISOLUTE SLE+ procedures.

One of the main limitations of these extraction techniques is that the range of analytes that can be extracted is limited to those with relatively high solubility in water immiscible solvents. In order to increase solubility (and enhance analyte recovery) it is often necessary to add a small proportion of more polar solvent modifier to the extraction solvent. This can have the adverse effect of increasing the amount of endogenous material co-extracted with the analyte, leading to dirtier extracts.

In order to quantify extract cleanliness, the degree of ion suppression of extracts due to endogenous plasma components was measured using flow injection analysis (FIA) LC-MS-MS. Blank human plasma samples were extracted using both ISOLUTE SLE+ and the equivalent LLE procedure.

A number of common water immiscible extraction solvents were investigated (Figure 3). The effect of increasing modifier concentration was also examined (Figure 4).

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

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

2a. Results: Effect of different extraction solvents

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

2b. Results: Effect of increasing modifier concentration

Extraction solvent	% ion suppression	
	ISOLUTE SLE+	LLE
DCM	24	17
DCM + 2% IPA	22	21
DCM + 5% IPA	22	19
DCM + 10% IPA	35	47
DCM + 20% IPA	59	49

Biotage

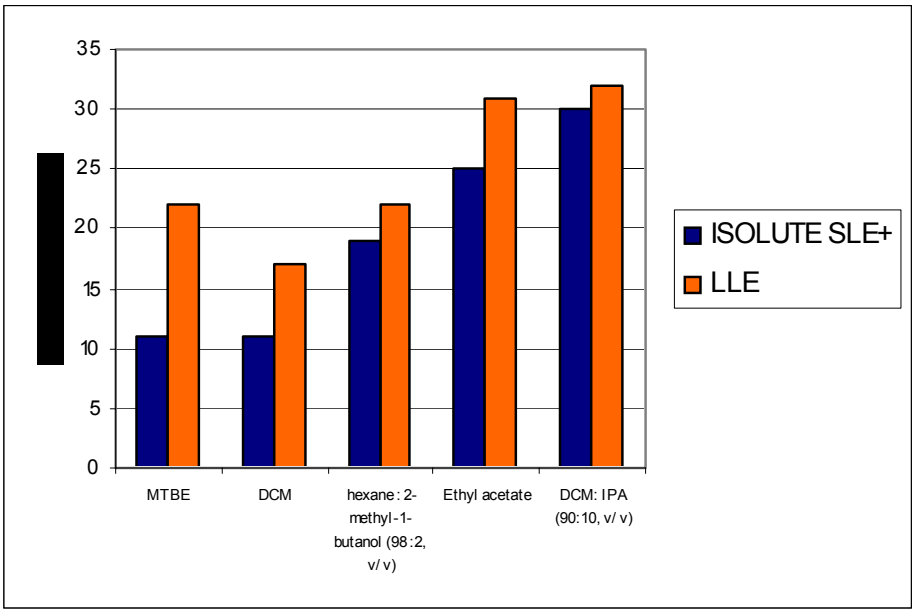

Figure 3: Effect of different extraction solvents on ion suppression

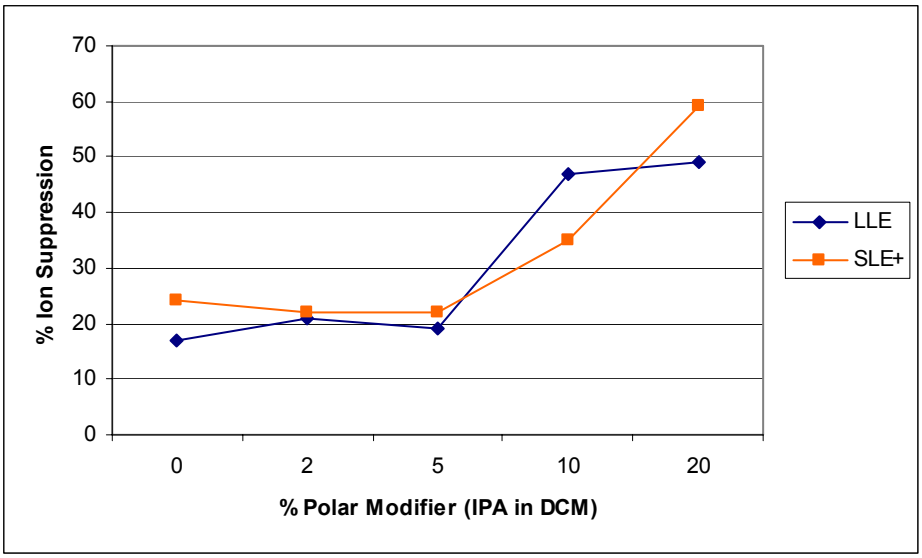

Figure 4. Effect of increasing polar solvent modifier concentration on ion suppression

Overall Conclusions

1. ISOLUTE SLE+ Supported Liquid Extraction plates can be used as alternative to liquid-liquid extraction for high throughput sample preparation of drugs in biological fluids
2. Generic SLE procedures have been developed for both basic and acidic drugs which deliver high analyte recoveries and low RSDs, using 'LC-MS friendly' pH adjustment protocols.
3. The degree of ion suppression is dependant on extraction solvent used. However, in general, ISOLUTE SLE+ extracts exhibit lower suppression than the equivalent LLE extract using the same solvent system.
4. Ion suppression increases with increasing concentration of polar modifier used in the extraction solvent. This is true for both ISOLUTE SLE+ and LLE procedures.

www.biotage.com

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