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

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AIMS: Traditional liquid-liquid extraction and the diatomaceous earth column based alternative, supported liquid extraction (SLE), have long been used in forensic and toxicological drug determination. However, neither of these traditionally used techniques is amenable to automation or high throughput applications.
This poster demonstrates the applicability of SLE in the high throughput 96 well plate format for the extraction of a range of acidic and basic drugs (e.g. NSAIDs, and tricyclic antidepressants) from biological fluids (e.g. plasma, urine, saliva). High analyte recoveries with low rsds are achieved. The effect of various extraction solvents on ion suppression in LC-MS is investigated, and compared to data obtained from the equivalent liquid-liquid extraction procedure. This poster will also demonstrate 96 samples can be extracted simultaneously in less than 13 minutes.

METHODS:

Experiment 1: Extraction of drugs from biological fluids using SLE

- *Supported Liquid Extraction*

Biological fluid samples (e.g. plasma, urine, saliva) spiked with either NSAIDS (500 ng/mL) or tricyclic antidepressants (10 ng/mL) were extracted using the ISOLUTE SLE+ Supported Liquid Extraction Plate, utilizing the methodology described below.

- *NSAIDs*

Analytes: Sulindac, flurbiprofen, ibuprofen
Sample: biological fluid (100 µL) diluted with 1:1 (v/v) with water adjusted to pH 2.5 with formic acid
Extraction solvent: DCM:IPA (90:10, v/v, 1 mL)
Extract was evaporated to dryness and reconsitituted in mobile phase for LC-UV analysis (see poster for analytical conditions).

- *Tricyclic antidepressants*

Analytes: imipramine, trimipramine, nortryptiline
Sample: biological fluid (100 µL) diluted with 1:1 (v/v) 0.5M NH_4OH
Extraction solvent: Hexane: 2-methyl-1 butanol (98:2, v/v, 1 mL)
Extract was evaporated to dryness and reconsitituted in mobile phase for LC-MS-MS analysis (see poster for analytical conditions)

Experiment 2. Quantification of LC-MS-MS ion suppression

Ion suppression effects were quantified by FIA LC-MS-MS. This approach allows quantification of ion suppression due to extractable endogenous components of human plasma. The effect of a range of extraction solvents was investigated, and the resulting suppression compared to traditional liquid-liquid extraction.

- *Supported Liquid Extraction*

Sample: human plasma (100 µL) diluted 1:1 with HPLC grade water
Extraction solvent: various, 1 mL
Procedure: sample was loaded onto an ISOLUTE SLE+ plate, and extracted using standard conditions (see poster for details).

- *Liquid-liquid Extraction*

Sample: human plasma (100 µL) diluted 1:1 with HPLC grade water
Extraction solvent: various, 1 mL
Procedure: sample was dispensed into a 2 mL extraction vial, and extraction solvent was added. Vial was agitated for 2 minutes, and left to stand for approximately 10 minutes for layers to separate. Organic layer removed.

- *FIA LC-MS-MS Conditions*

Solvent extraction from SLE and LLE were evaporated to dryness, and reconstituted in 1 mL of 1 ng/mL caffeine solution in mobile phase.
Mobile phase: Water/ACN/MeOH/Formic acid (50/45/5/0.1, v/v)
Injection volume 5 µL
Caffeine MRM transition: 195>138

RESULTS: Typical data for analyte recovery and ion suppression using ISOLUTE SLE+ procedures are shown in table 1 and 2 respectively.

Analyte	Sulindac	Flurbiprofen	Ibuprofen	Imipramine	Trimipramine	Nortriptyline
Average recovery (n=8)	96%	91%	86%	97%	96%	91%
RSD	3%	3%	2%	4%	2%	4%

Table 1: *Analyte recovery form human plasma using supported liquid extraction*

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

Table 2: *Effect of different extraction solvents on ion suppression due to extracted endogenous plasma components*

CONCLUSIONS: High throughput supported liquid extraction is suitable for extraction of acidic and basic drugs from biological fluid samples, giving high, reproducible analyte recoveries.LC-MS-MS ion suppression using this technique is lower or equivalent to that for liquid-liquid extraction, for a range of extraction solvents.

KEYWORDS: *Sample preparation, Supported liquid extraction, High throughput*

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