Streamlined Method Development using Supported Liquid Extraction (SLE+) prior to LC-MS/MS Analysis

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Introduction

It is well known that traditional liquid-liquid extraction (LLE) provides very clean extracts prior to LC/MS analysis. Supported liquid extraction is analogous to traditional LLE, however, analyte partitioning takes place using an inert support material, rather than two immiscible liquids. This provides excellent extraction efficiencies while alleviating many of the tedious liquid handling issues associated with LLE.

This poster aims to provide more generic methods for the extraction of various acidic, basic or neutral drugs therefore simplifying the whole SLE+ method development process. We have devised extraction screening protocols based on the combination of two loading pHs and four extraction solvents. The loading pH is dictated by the types of analytes being tested: For acidic analytes the suggested plasma loading pHs are approximately 3.2 and 6.1; for neutral analytes the suggested pHs are approximately 6.1 and 8.0; and finally for basic analytes 8.0 and 10.5. Full buffer details are given in the experimental section.

Experimental Procedure

Reagents

Non-steroidal anti-inflammatory drugs (NSAIDs), β-blockers, corticosteroids and formic acid were purchased from Sigma Chemical Co. (Poole, UK). Blank human plasma was obtained through the Welsh Blood Service (Pontyclun, UK). All solvents were HPLC grade from Fisher Scientific (Loughborough, UK).

Sample Preparation

Supported Liquid Extraction Procedure

Plate: ISOLUTE SLE+ Supported Liquid Extraction Plate 200 mg, part number 820-0200-P01

Sample pre-treatment:-

Acidic analytes (NSAIDs):- Plasma pre-treatment 1:1 v/v with either 1% formic acid or 0.1% formic acid aq. This sample dilution results in loading pH's of approximately 3.2 and 6.1, respectively.

Neutral analytes (Corticosteroids):- Plasma pre-treatment 1:1 v/v with either H_2O or 0.1% formic acid aq. This sample dilution results in loading pH's of approximately 6.1 and 8.0, respectively.

Basic analytes (β-blockers):- Plasma pre-treatment 1:1 v/v with either H_2O or 0.5M ammonium hydroxide. This sample dilution results in loading pH's of approximately 8.0 and 10.5, respectively.

Sample Application: The pre-treated plasma was loaded onto the plate, a pulse of vacuum applied to initiate flow and the samples left to absorb for 5 minutes.

Analyte Elution: Addition of 1 mL of various water immiscible extraction solvents. The extraction solvents evaluated were DCM, 95:5 (v/v) DCM/IPA, MTBE and EtOAc.

Post Extraction: The eluate was evaporated to dryness and the analytes reconstituted in 500 µL of appropriate H_2O/MeOH mixtures prior to analysis.

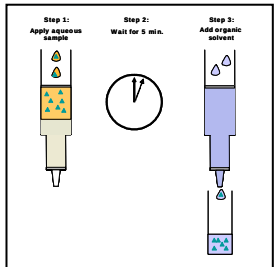

Figure 1*. Schematic of ISOLUTE SLE+ Supported Liquid Extraction procedure. A single well of the 96-well plate is shown*

HPLC Conditons

Instrument:	Waters 2795 Liquid Handling System (Waters Assoc., Milford, MA, USA).
Column:	Zorbax Eclipse XDB C18 3.5 µm analytical column (100 x 2.1 mm id, 3.5 µm) (Agilent Technologies, Berkshire, UK).
Guard Column:	C8 guard column (Agilent Technologies, Berkshire, UK).
Mobile Phase:	0.1% formic acid aq and MeCN (acetonitrile) at a flow rate of 0.25 mL/min using various gradients.

Mass Spectrometry

Instrument: Ultima Pt triple quadrupole mass spectrometer (Waters Assoc., Manchester, UK) equipped with an electrospray interface for mass analysis. Positive and negative ions were acquired in the multiple reaction monitoring mode (MRM).

Desolvation Temperature:	350 °C
Ion Source Temperature:	100 °C
Collision Gas Pressure:	2.3 x 10^{-3} mbar

Results

Analytes were selected to give a broad range of polarities (LogP) and pKa values within each suite. The appropriate screening procedures listed above were then carried out. Figures 2-7 show the respective recoveries obtained for the three analyte suites using various combinations of pH loading and extraction solvent conditions.

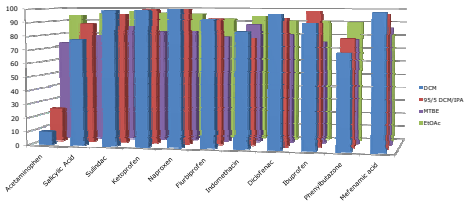

Figure 2. NSAID recovery chart comparing various extraction solvents with a 1% formic acid:plasma load.

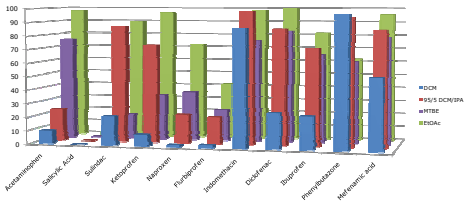

Figure 3. NSAID recovery chart comparing various extraction solvents with a 0.1% formic acid:plasma load.

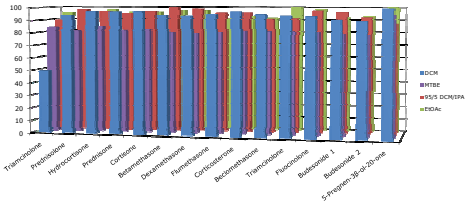

Figure 4. Corticosteroid recovery chart comparing various extraction solvents with a 0.1% formic acid:plasma load.

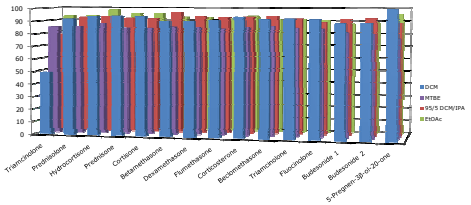

Figure 5. Corticosteroid recovery chart comparing various extraction solvents with an H_2O:plasma load.

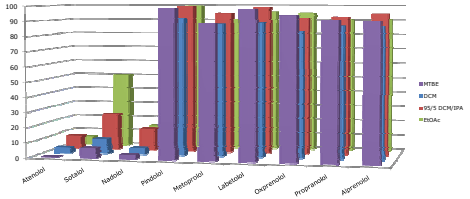

Figure 6. β-blocker recovery chart comparing various extraction solvents with an H_2O:plasma load.

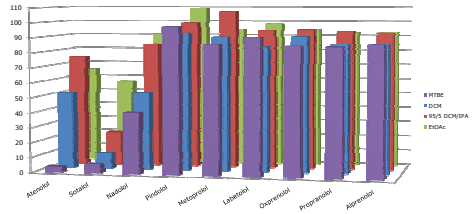

Figure 7. β-blocker recovery chart comparing various extraction solvents with a 0.5M NH_4OH:plasma load.

Conclusions

- NSAID recoveries for equivalent extraction solvents were generally higher for the lower pH loading conditions.
- Corticosteroid recoveries were consistently high using all extraction solvent and pH loading combinations. Triamcinolone, however, only gave 50% recoveries when using DCM as the extraction solvent.
- 6/9 β-blockers showed consistently high recoveries for all extraction solvents at both loading pH's. For the 3 most polar analytes (atenolol, sotalol and nadalol) better recoveries were obtained when using combinations of more polar extraction solvents at a higher loading pH.
- By using the suggested protocol (screening two pH loading conditions combined with four extraction solvents) good recoveries and RSD's were obtained for the majority of analytes used in this study.
- For very polar analytes, assuming they have adequate solubility in the extraction solvents, more precise pH control is required (analytes must be in their unionized forms).

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