Simultaneous Extraction of Acidic, Basic and Neutral Drugs using 96-well Supported Liquid Extraction (SLE) and LC-MS/MS

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Introduction

Simple, efficient sample preparation techniques are essential to the success of high throughput bioanalytical assays. Liquid-liquid extraction is widely used, but is difficult to automate successfully. Typical challenges encountered with LLE are low analyte recovery and emulsion formation. Supported liquid extraction (SLE) using ISOLUTE® SLE+ plates is analogous to traditional liquid-liquid extraction (LLE), however, as a flow-through technique has differences and advantages. SLE alleviates the liquid handling problems associated with LLE and is therefore far more amenable to high throughput assays. Higher analyte recoveries have also been reported when directly comparing LLE and SLE[1].

The use of pH adjustment and appropriate extraction solvents are crucial when performing LLE and SLE. This poster investigates the effect of loading pH and extraction solvent on the recovery of nine analytes extracted from human plasma, using ISOLUTE SLE+ plates. The analyte test mix was selected to incorporate acidic, basic and neutral functionalities along with wide ranging pK and LogP values. Analyte properties and structures are shown on **Table 2**, **page 3**.

Experimental Procedure

Reagents

All analytes (see **Table 2**, **page 3**), formic acid, 3-methyl-1-butanol and ammonium hydroxide 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).

Supported Liquid Extraction Procedure

Sample: Blank human plasma (100 µL) was spiked with the analyte suite at 50 pg/µL. The plasma was then diluted 1:1 with various pH buffers prior to loading onto the ISOLUTE SLE+ Supported Liquid Extraction Plate (part number 820-0200-P01). The buffers included in this study were; 1% (v/v) aqueous formic acid, 0.1% (v/v) aqueous formic acid, H_2O and 0.5M aqueous NH_4OH.
Sample Application: The pre-treated plasma was loaded on to the plate. Vacuum was applied to initiate flow (-15" Hg / 0.5 bar) for 2-10 seconds to initiate loading. The samples were left to absorb for 5 minutes.

Analyte Extraction: Addition of 1 mL of various water immiscible extraction solvents. The extraction solvents tested were: 98:2 (v/v) hexane/3-methyl-1-butanol, 90:10 (v/v) DCM/IPA, EtOAc, DCM and MTBE.
Post Extraction: The eluate was evaporated to dryness and the analytes reconstituted in 1 mL of 80:20 (v/v) H_2O/MeOH prior to analysis.

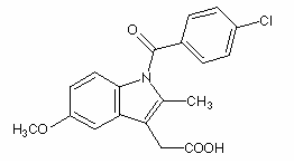
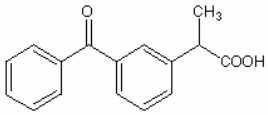
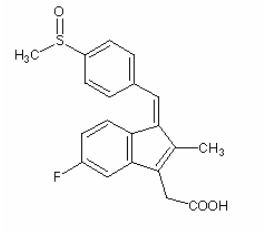
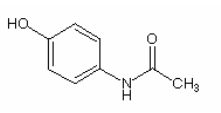
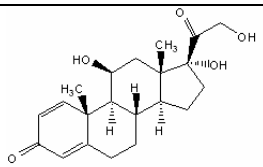
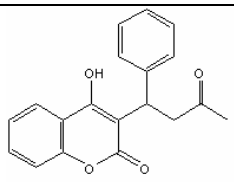
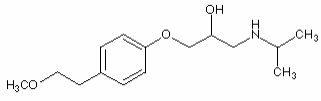
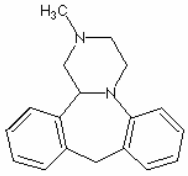
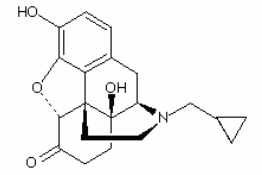
HPLC Conditons
Instrument: Waters 2795 Liquid Handling System (Waters Assoc., Milford, MA, USA).
Column: Zorbax Eclipse XDB C18 3.5 µm analytical column (100 x 2.1 mm ID) (Agilent Technologies, Berkshire, UK).
Guard Column: C8 guard column (Agilent Technologies, Berkshire, UK).
Mobile Phase: 0.1% aqueous formic acid and MeCN at a flow rate of 0.25 mL/min.
Gradient: 80%, 0.1% aqueous formic acid and 20% MeCN increasing to 90% MeCN over 6 minutes. The high organic mobile phase was held for 1.5 minutes then returned to the initial starting conditions.
Injection Volume: 25 µL
Temperature: Room temperature

Mass Spectrometry
Instrument: Ultima Pt triple quadrupole mass spectrometer (Waters Assoc., Manchester, UK) equipped with an electrospray interface for mass analysis. Positive ions were acquired in the multiple reaction monitoring mode (MRM)
Desolvation Temperature: 350 °C
Ion Source Temperature: 100 °C
Collision Gas Pressure: 2.9 x 10^{-3} mbar.
The base peak in each compound spectrum was attributed to the protonated molecular ion $[M+H]^+$ and were subsequently used as the precursor ions in the resulting MRM transitions. The MRM transitions were split into 5 scan functions depending on analyte retention. Full MRM transitions and ionization conditions are shown below in **Table 1**.

***Table 1**. Quattro Ultima Pt mass spectrometer parameters*

Scan Function	Analyte	MRM Transition	Cone Voltage (V)	Collision Energy (eV)
1	Acetaminophen	152.1 > 110.1	40	12
	Naltrexone	342.1 > 324.1	40	19
2	Metoprolol	268.1 > 116.1	35	17
3	Mianserin	265.0 > 208.0	35	19
	Prednisolone	361.3 > 343.2	35	10
4	Sulindac	357.0 > 233.0	50	25
5	Ketoprofen	255.1 > 209.1	35	11
	Warfarin	309.1 > 163.0	65	13
	Indomethacin	358.1 > 139.0	35	15

***Table 2**. Analyte properties and structures.*

Analyte	LogP	pK_a	Structure	Analyte	LogP	pK_a	Structure
Acidic Analytes							
Indomethacin	4.27	4.50		Ketoprofen	3.12	4.45	
Sulindac	3.42	4.70					
Neutral Analytes							
Acetaminophen	0.46	9.38**		Prednisolone	1.62	N/A	
Warfarin	2.60	5.08					
Basic Analytes							
Metoprolol	1.88	9.56		Mianserin	3.67	8.30	
Naltrexone	1.92	8.13					

Results

The analyte suite was selected to incorporate acidic, basic and neutral functionalities with varying pK_a and LogP values (see **Table 2**, **page 3**) in order to assess the role of loading pH and extraction solvent polarity. All extractions were spiked with the analyte suite at 50 pg/µL (n=7).

Table 3 shows the pH conditions obtained when diluting plasma (1:1) with the various buffers investigated. **Figure 1** shows analyte recoveries comparing the various pH loading conditions and extraction solvents.

***Table 3**. pH conditions when mixing plasma/buffer 1:1 v/v*

Plasma/buffer 1:1 v/v	**pH**
1% (v/v) aqueous formic acid	3.2
0.1% (v/v) aqueous formic acid	6.3
H_2O	8.0
0.5 M NH_4OH	10.4

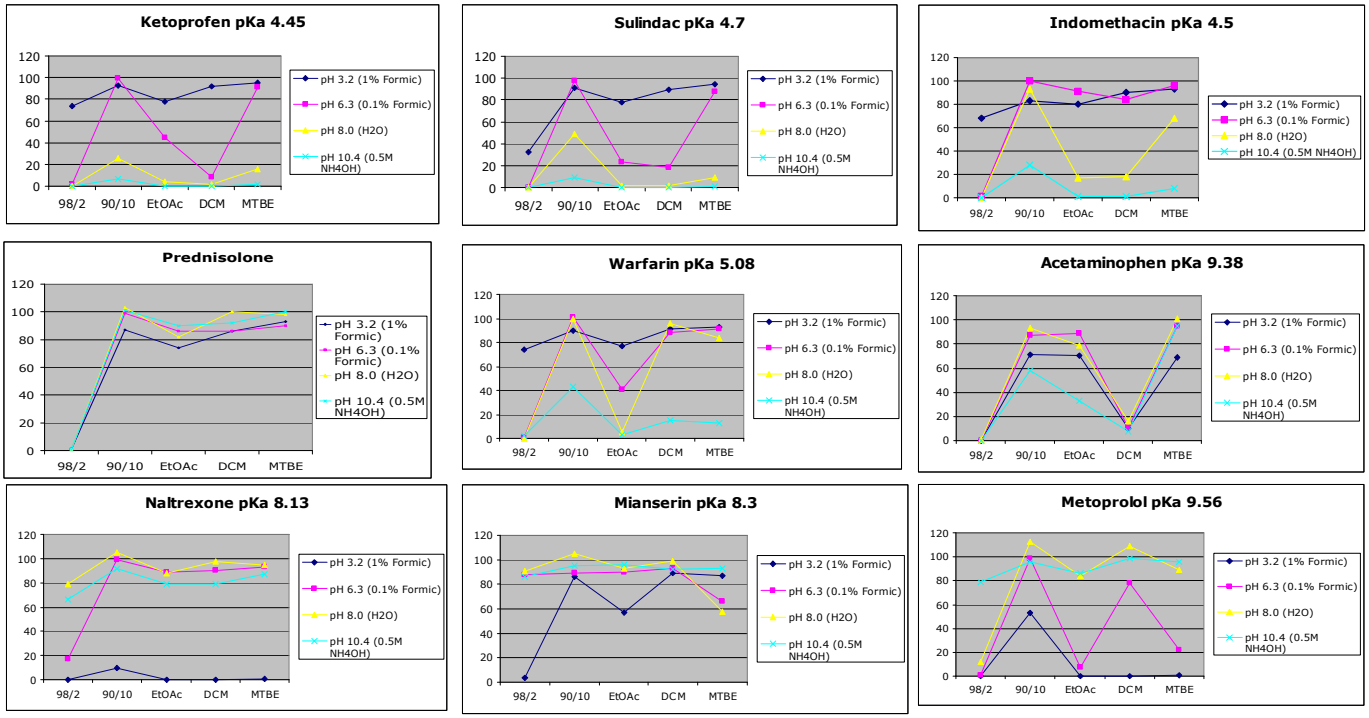


***Figure 1**. Comparison of loading pH and elution solvent on analyte recoveries.*
Extraction solvents: 98:2 (v/v) hexane/3-methyl-1-butanol, 90:10 (v/v) DCM/IPA, Ethyl acetate, DCM and MTBE

In most cases the 98:2 hexane/3-methyl-butanol gives the lowest recoveries even when using the optimum pH conditions for the various analytes.

For the acidic and basic analytes the best recoveries were seen when the analytes were in their neutral form, or partially ionized. Good recoveries were still possible when the analytes were fully ionized but only with the more polar extraction solvents. At pH conditions remote from the pK_a values the polarity of the extraction solvent was not sufficient to elute the analytes. The neutral analyte, prednisolone, showed similar recoveries for each of the extraction solvents at the various pH conditions.

Table 4 shows the recoveries and relative RSD's obtained when loading using 0.1% (v/v), aqueous formic acid and extracting with 90:10 (v/v) DCM/IPA These conditions represent a compromise loading pH and extraction with the most polar solvent in this study. Recoveries were >85% with respective RSD's <10% for all analytes. At this loading pH both the acids and the bases are almost fully ionized.

***Table 4**. Analyte recoveries (n=7) using loading conditions of 0.1% (v/v) aqueous formic acid/plasma (~ pH 6.4) and 90:10 (v/v) DCM/IPA as the extraction solvent*

Analyte	**% Recovery (RSD %)**
Ketoprofen, pK_a 4.45	99 (3)
Sulindac, pK_a 4.70	98 (2)
Indomethacin, pK_a 4.50	100 (3)
Acetaminophen, pK_a 9.38	87 (3)
Prednisolone	99 (2)
Warfarin, pK_a 5.08	101 (5)
Mianserin, pK_a 8.30	89 (7)
Naltrexone, pK_a 8.13	99 (2)
Metoprolol, pK_a 9.56	99 (2)

Conclusions

- Solubility of the analytes in the extraction solvent is very important when using both supported liquid extraction and traditional LLE. The extraction solvent 98:2 (v/v) hexane/3-methyl-butanol gives lower recoveries for the majority of the analytes. The solubility of the analytes in the other solvents varies.
- Generally, the best recoveries were obtained when the analytes were not fully ionized.
- When the analytes were fully ionized, good recoveries are still attainable with careful extraction solvent selection.
- At pH values remote from the pKa and with analytes fully ionized, elution becomes very difficult even when polar solvents are used.
- Prednisolone is the only 'true' neutral analyte in the suite. This shows similar recoveries between the fully range of loading conditions for the same extraction solvent.
- Acetaminophen and warfarin behave as weak acids
- Finally it was possible to select a loading pH and extraction solvent which gave recoveries >85% for all the acidic, basic and neutral analytes in the suite analyzed.

References

[1] Supported Liquid Extraction: Automate those Tiresome Bioanalytical LLE Protocols. L Williams et al, Biotage GB Ltd. Presented at Eastern Analytical Symposium, 2005

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