LC-MS Analysis of Tricyclic Antidepressants in Human Plasma using Supported Liquid Extraction Sample Preparation

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Introduction

Simple, efficient sample preparation techniques are essential to the success of high throughput bioanalytical assays. Techniques such as protein precipitation and solid phase extraction are widely used. Liquid-liquid extraction is also popular, but is difficult to automate successfully, and can present problems such as low analyte recovery and emulsion formation. Supported liquid extraction (SLE) is an alternative to traditional liquid-liquid extraction which is easily automated, and hence suited to high throughput assays. SLE provides high analyte recoveries, while eliminating emulsion formation and other liquid handling issues.

This poster describes the development of a 96-well plate based sample preparation assay using ISOLUTE® SLE+ Supported Liquid Extraction Plates combined with LC-MS for the analysis of three tricyclic antidepressants (TCA's) from human plasma.

Experimental Procedure

Reagents

Imipramine, trimipramine and nortriptyline (see Figure 1a) were purchased from Sigma Chemical Co. (Poole, UK) while the labelled d3 imipramine, trimipramine and nortriptyline (see Figure 1 b) were obtained from Cambridge Isotope Laboratories (Andover, MA, USA). Blank human plasma was obtained through the Welsh Blood Service (Pontyclun, UK) . All solvents were HPLC grade from Fisher Scientific (Loughborough, UK).

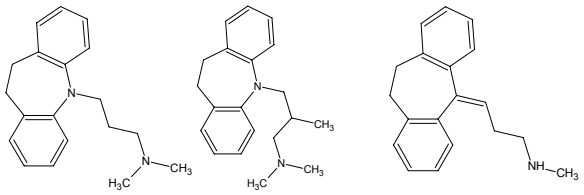

Figure 1a. Imiprimine, trimiprimine and nortryptyline respectively

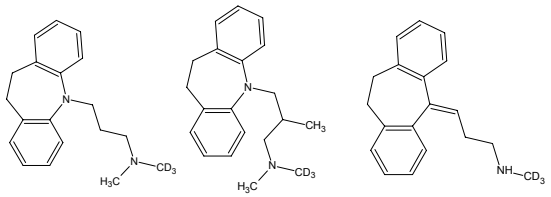

Figure 1b. d_3-Imiprimine, d_3-trimiprimine and d_3-nortryptyline respectively

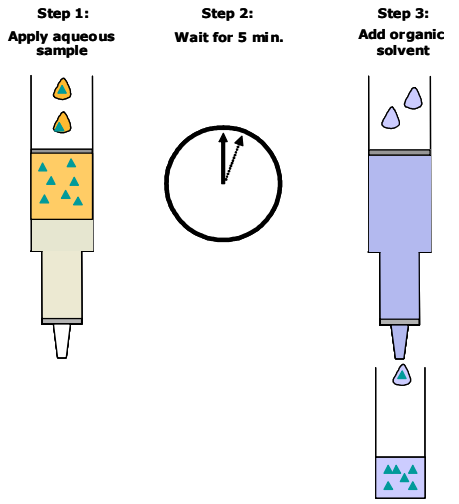

Figure 2. Schematic of the ISOLUTE SLE+ supported liquid extraction procedure. A single well of the 96-well plate is illustrated

Supported Liquid Extraction Procedure

Blank human plasma (100 µL) was spiked with various concentrations of unlabelled TCA's and each respective isotopically labelled internal standard (2 pg/µL). The plasma was then diluted 1:1 (v/v) with 0.5 M NH_4OH prior to loading onto the ISOLUTE SLE+ Supported Liquid Extraction Plate. A pulse of vacuum was applied to initiate flow onto the plate and the samples left to absorb for 5 minutes. Elution was brought about by the addition of 1 mL of 98:2 (v/v) hexane/2-methyl-1-butanol. The eluate was evaporated to dryness and the analytes reconstituted in 200 µL of 80:20 (v/v) methanol/H_2O prior to analysis.

Liquid Chromatography

Liquid Chromatography was performed using a Waters 2795 Liquid Handling System (Waters Assoc., Milford, MA, USA). Chromatographic separation was achieved on a Zorbax Eclipse XDB C18 3.5 µm analytical column (50 x 2.1 mm) equipped with a C8 guard column (both Agilent Technologies, Berkshire, UK). An isocratic mobile phase of 90:10 (v/v) acetonitrile/water modified with 0.25 % ammonium hydroxide was employed at a flow rate of 0.3 mL/min. Injection volumes were set to 25 µL and all separations were performed at ambient temperature.

Mass Spectrometry

The entire column effluent was directed into a Quattro 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) using a desolvation temperature of 350 °C, an ion source temperature of 100 °C and collision gas pressure of 2.5 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. See **Table 1** for full MRM transitions and ionization conditions.

Table 1. Quattro Ultima Pt mass spectrometer parameters.

Analyte	MRM Transition	Dwell (s)	Cone Voltage (V)	Collision Energy (eV)
Imipramine	281.1→86.1	0.1	40	13
d_3-imipramine	284.2→89.0	0.1	40	13
Trimipramine	295.1→100.1	0.1	40	15
d_3-trimipramine	298.2→103.0	0.1	40	15
Nortriptyline	264.1→233.1	0.1	40	15
d_3-nortriptyline	267.1→233.1	0.1	40	15

Results
Method Validation

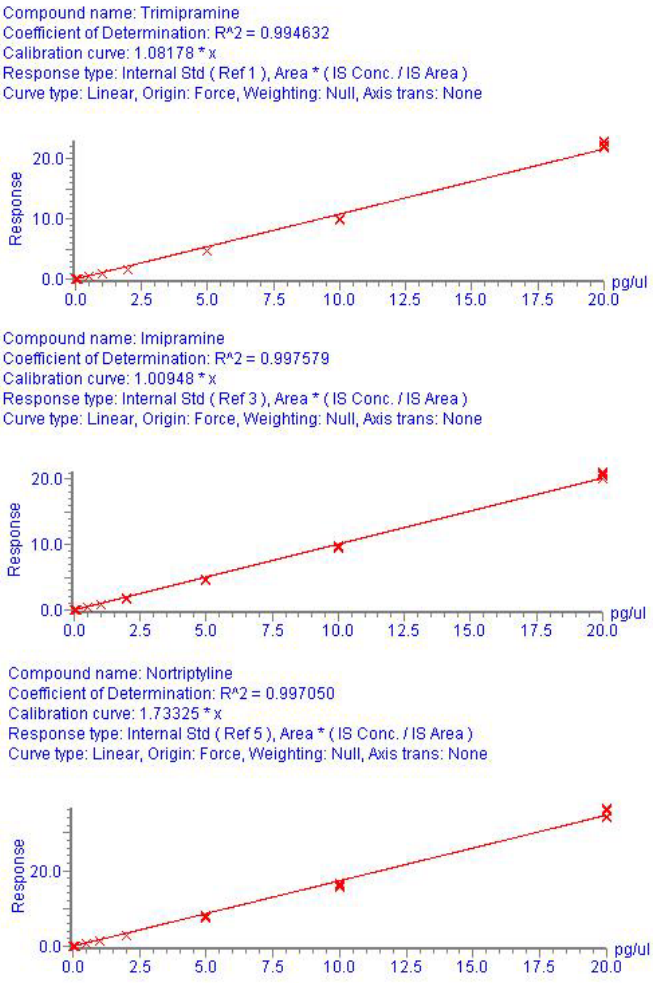

Figure 3. Calibration curves for tricyclic antidepressants

Internal standard characterization

Deuterated standards were characterized using UV, LC-UV and full scan LC-ES/MS and compared to the corresponding unlabeled standards (structures shown in **Figure 1a and 1b**). Calibration curves were constructed from mixtures of each labelled TCA at a fixed concentration (2 pg/µL) along with respective unlabelled TCA's in the ranges 0.005-20 pg/µL. In all cases the plots of response ratio versus concentration ratio were highly linear (coefficients of determination >0.99).
See **Figure 3** for calibration curves for imipramine, trimipramine and nortriptyline.

Supported Liquid Extraction Validation

Previous work[1] investigated TCA recovery using ISOLUTE SLE+ plates compared with the equivalent LLE procedure. Recoveries in excess of 90 % were obtained with ISOLUTE SLE+, compared with approximate recoveries of around 60 % for the LLE procedure.

In this study, precise and accurate quantitation was provided through the use of isotopically labelled internal standards.
The method was validated for all compounds by replicate analysis (n = 7) through the SLE and LC/ES-MS/MS procedures on
different days using blank human plasma spiked at various concentrations. Acceptable inter- and intra-day precision and accuracy were obtained for all compounds as shown in **Table 2**. Respective LOQ and LOD for all analytes are shown in **Table 3**. These were derived by extrapolating a signal to noise ratio of 10:1 and 3:1 for the LOQ and LOD, respectively.

Table 2. Method validation summary

Analyte	Concentration pg/µl	Day 1 precision (RSD)	Day 1 accuracy (% added)	Day 2 precision (RSD)	Day 2 accuracy (% added)
Imipramine	0.1	0.1003 ± 0.004 (3.9%)	100	0.0986 ± 0.004 (4.2%)	99
	0.5	0.487 ± 0.019 (3.8%)	97	0.533 ± 0.027 (5%)	107
	2	1.85 ± 0.03 (1.5%)	93	1.80 ± 0.03 (1.7%)	90
	20	19.88 ± 0.44 (2.2%)	99	21.46 ± 0.83 (3.9%)	107
Trimipramine	0.1	0.100 ± 0.002 (2.2%)	100	0.0868 ± 0.004 (4.4%)	87
	0.5	0.479 ± 0.006 (1.4%)	96	0.488 ± 0.021 (4.2%)	98
	2	1.74 ± 0.03 (1.7%)	87	1.79 ± 0.02 (1.3%)	90
	20	19.58 ± 0.30 (1.6%)	98	20.68 ± 0.38 (1.8%)	103
Nortriptyline	0.1	0.091 ± 0.009 (9.5%)	91	0.0898 ± 0.005 (5.8%)	90
	0.5	0.457 ± 0.023 (5.0%)	91	0.541 ± 0.03 (5.6%)	108
	2	1.68 ± 0.06 (3.5%)	84	1.94 ± 0.04 (2%)	97
	20	18.28 ± 0.84 (4.6%)	91	18.56 ± 0.72 (3.9%)	93

Blank human plasma spiked with various concentrations of TCA's on two separate days. The values shown are means ± SD (n = 7) with the relative standard deviation (RSD) shown in parenthesis

Table 3. Limits of quantitation and limits of detection

Analyte	Limit of quantitation pg/µl (LOQ)	Limit of detection pg/µl (LOD)
Imipramine	0.01	0.004
Trimipramine	0.01	0.004
Nortriptyline	0.1	0.03

Overall Conclusions

1. Sample processing using the ISOLUTE SLE+ plate was simple, with 96 samples processed in approximately 15 minutes using an 8-port manual pipettor for all liquid dispensing steps.
2. Minimal ion suppression was observed, and excellent analyte recovery and linearity were obtained for TCA's at all concentration levels, indicating no loss of compound at low concentrations through interaction with the supported liquid extraction media.
3. LOQ and LOD of 0.01 and 0.004 pg/µL respectively were achieved for imipramine and trimipramine. For nortriptyline, LOQ of 0.1 and LOD of 0.03 pg/µL were achieved.
4. Good inter- and intra-day precision and accuracy were obtained, showing that supported liquid extraction using the ISOLUTE SLE+ can be used for high sensitivity bioanalytical assays.

Reference:

[1] 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
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