QUANTITATIVE ANALYSIS OF MULTIPLE PSYCHOTHERAPEUTIC DRUGS IN HUMAN SERUM USING UPLC /MS/MS

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OBJECTIVE

To develop a simple UPLC®/MS/MS method for the simultaneous quantitation of multiple psychotherapeutic drugs in human serum and assess its utility with authentic samples.

INTRODUCTION

- Psychotherapeutic drugs are commonly prescribed for the treatment of depressive, anxiety, bipolar and eating disorders
- The use of antidepressants has increased greatly in recent years
- Analysis of psychotherapeutics can be useful in clinical, post-mortem and forensic toxicology

METHOD

SAMPLE PREPARATION

Protein precipitation using acetonitrile containing internal standards Clomipramine-D3, Doxepin-D3 and Imipramine-D3.

CHROMATOGRAPHY

ACQUITY UPLC® system

Column: Waters ACQUITY UPLC® BEH C18 (2.1 x 100mm, 1.7µm), 40°C

Mobile phase: A=5mM Ammonium acetate + 0.05% formic acid
B=Acetonitrile

Gradient: 10% B up to 100% B over 5 minutes (8 minute cycle time)

MASS SPECTROMETRY

Waters Quattro Premier™ XE mass spectrometer used in electrospray positive ionisation mode with a collision cell pressure of 3.5 x 10^{-3} mbar (argon). A quantifier and qualifier ion was monitored for each compound.

Compound	Precursor ion (*m/z*)	Product ion (*m/z*)	Cone voltage (V)	Collision energy (eV)
Amitriptyline	278	91	35	25
Clomipramine	315	86	35	20
Norclomipramine	301	72	35	15
Clomipramine-d3	318	89	35	20
Desipramine	267	72	35	15
Doxepin	280	107	35	25
Doxepin-d3	283	107	40	25
Imipramine	281	86	30	15
Imipramine-d3	284	89	30	15
Nortriptyline	264	91	35	20
Protriptyline	264	155	40	20
Trimipramine	295	100	35	15
Citalopram	325	109	40	25
Fluoxetine	310	148	25	8
Norfluoxetine	296	134	20	5
Fluvoxamine	319	71	30	15
Paroxetine	330	192	45	20
Sertraline	306	159	25	25
Venlafaxine	278	58	30	20
Trazodone	372	148	35	35

Table 1. MRM conditions used for the psychotherapeutic drug analysis (only quantifier ion conditions shown).

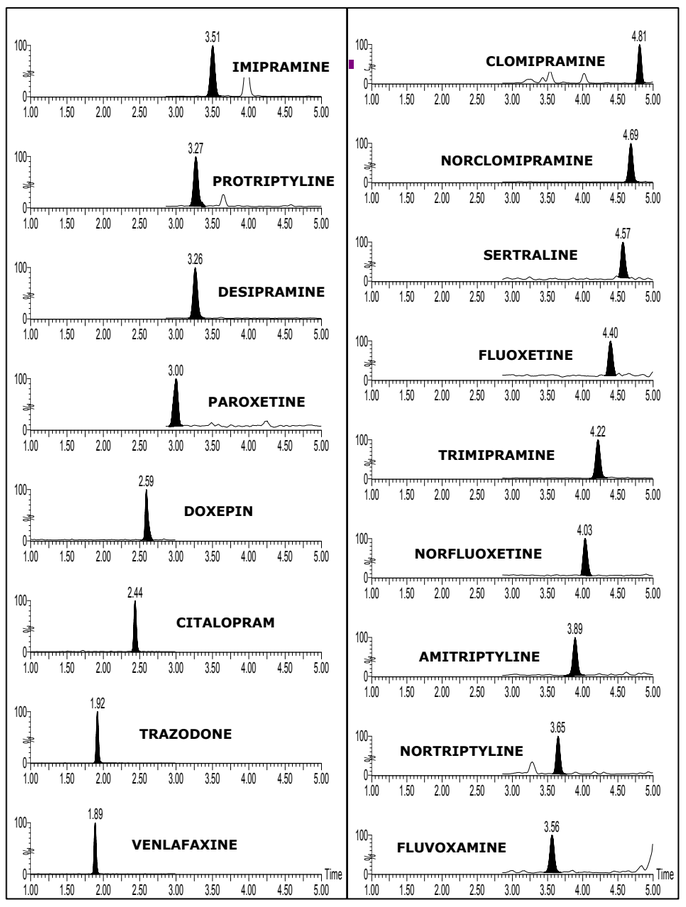

Figure 1. MRM chromatograms for all compounds which were obtained with a 10µL injection of a 2µg/L serum calibrator (only quantifier ion trace shown)

RESULTS

A preliminary validation was performed. Intra-assay precision and accuracy results were good, with CV's < 15% and > 88% respectively. The use of a simple protein precipitation was demonstrated to be very efficient and gave reproducible extraction recoveries > 93% for all analytes.

Figure 2. shows a typical standard curve for venlafaxine in serum. Responses were linear for all compounds over the investigated range (coefficient of determination, r^2 for all compounds was ≥ 0.996).

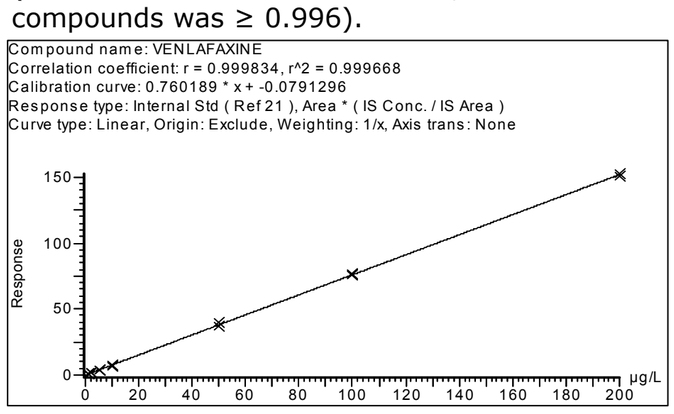

Figure 2. Typical response for venlafaxine extracted from serum. All compounds were quantified by reference to one of the three internal standards

The limits of detection were assessed for all compounds and found to range between 0.1 - 1.0 µg/L, which is below the limits required for this analysis. Figure 3. shows a patient sample containing citalopram at the lower therapeutic range.

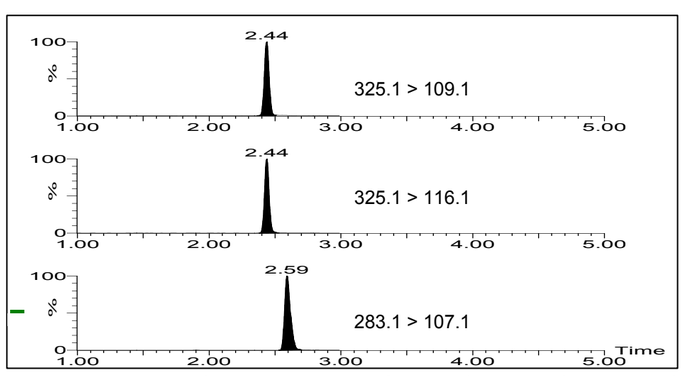

Figure 3. MRM chromatograms for citalopram (quan and qual ion shown in top and middle trace, respectively) in a patient sample at the lower therapeutic range (~20 µg/L). Doxepin-D3 (bottom trace) was used as the internal standard.

Matrix effects were assessed by the comparison of six different patient serum samples spiked with the psychotherapeutic compounds after extraction against the equivalent concentration solvent standards. Matrix effects were found to be acceptable with the norclomipramine response being most affected (+10%). The post-column infusion of all compounds was performed during the injection of solvent blank and extracted serum. This showed the extraction was efficient in removing any significant matrix effects. An example shown in Figure 4.

As part of the validation, patient samples (n=23) were analysed by the newly developed UPLC/MS/MS method and the established HPLC/UV method, with the results showing good agreement.

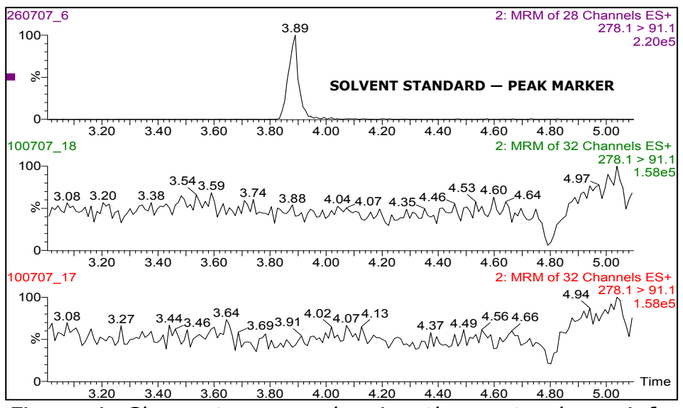

Figure 4. Chromatograms showing the post-column infusion of amitriptyline during the injection of extracted serum blank and a solvent blank (middle and bottom chromatograms respectively). Top chromatogram shows amitriptyline retention time.

DISCUSSION

The developed method offers a significant decrease in total analysis time over the established HPLC/UV method. The time required for the preparation of a batch of samples was greatly reduced *i.e.*, ~ 30 minutes when using protein precipitation compared with 2 hours for the liq/liq extraction. A 2.5-fold reduction in chromatographic run time was also achieved.

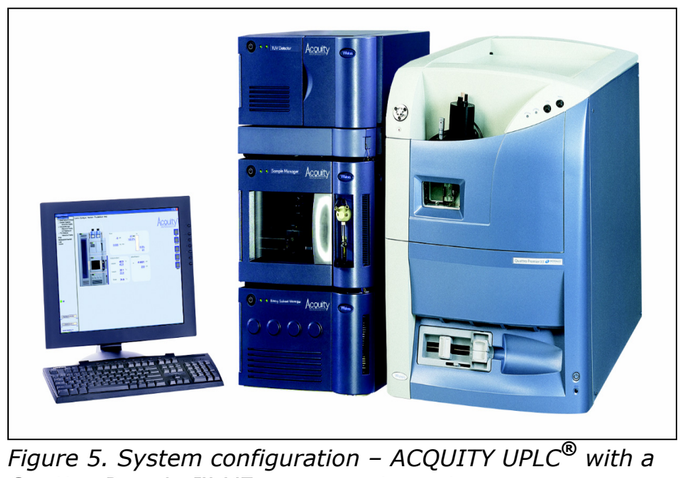

Figure 5. System configuration – ACQUITY UPLC® with a Quattro Premier™ XE mass spectrometer

CONCLUSION

The developed methodology has been shown to be accurate and precise in the screening and quantitation of psychotherapeutic drugs in a single 8 minute chromatographic run.

This method has been applied successfully to the analysis of clinical samples and the results compared against an established HPLC/UV method.

The new UPLC/MS/MS method resulted in greater laboratory efficiency and therefore sample capacity.

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