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)

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, r² for all compounds was ≥ 0.996).

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.

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 injection 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.

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.

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.