A CLINICAL RESEARCH LC-MS/MS METHOD FOR THE ANALYSIS OF ANTIDEPRESSANTS IN PLASMA

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B-339

INTRODUCTION

Depression is common globally, with an estimated 3.8% of the population affected. The condition can impact individuals' ability to function in work, social and family settings. Many antidepressant drugs are currently prescribed, encompassing selective serotonin reuptake inhibitors (SSRIs), serotonin-nonresponsive reuptake inhibitors (SNRIs) and tetracyclic antidepressants (TeCAs).

However pharmacokinetic and drug interactions are known, therefore a reliable quantitative clinical research method may play a role in researching the effects of their administration.

Waters has developed a clinical research LC-MS/MS method for the simultaneous analysis of the following antidepressants in plasma; citalopram, desmethylfluoxetine, duloxetine, fluoxetine, fluvoxamine, O-desmethylvenlafaxine, sertraline and venlafaxine (10-1000 ng/mL); mirtazapine (5-500 ng/mL) and trazodone (30-3000 ng/mL).

METHODS

Materials and Sample Preparation

- Plasma calibrators and quality control materials were prepared in house using pooled human plasma supplied by BioIVT (West Sussex, UK).
- Concentrated stock solutions were prepared from certified powders supplied by Cambridge Bioscience (Cambridgeshire, UK), Merck Life Science (Dorset, UK) and Toronto Research Chemicals (Ontario, Canada).
- Stable labelled internal standards were supplied by Cambridge Bioscience (Cambridgeshire, UK), Merck Life Science (Dorset, UK) and Toronto Research Chemicals (Ontario, Canada).
- 50µL of sample was added to a microcentrifuge tube followed by 150µL of working internal standard in acetonitrile.
- Tubes were placed on a multitube vortex mixer at 2500 r.p.m. for 30 seconds, then centrifuged for 2 minutes at 16100g.
- 50 µL of supernatant was transferred to a 1mL 96-well plate Recovery vial (p/n: 186002481), and 450 µL water added.

LC-MS/MS Parameters

- Using an ACQUITY™ UPLC™ I-Class FTN System, samples were injected onto an XSelect™ Premier HSS T3 2.5µm, 2.1 x 100mm Column (p/n: 186009831), using a water/acetonitrile/ammonium acetate gradient and analyzed with a Xevo™ TQD Mass Spectrometer in ESI+, using MRM mode.
- The run time is 5.0 minutes (approximately 5.7 minutes injection-to-injection).

RESULTS

Linearity, Analytical Sensitivity and Carryover

- Linear fits were established over 7.7-1300 ng/mL for O-Desmethylvenlafaxine and duloxetine. Citalopram, desmethylfluoxetine, fluvoxamine, sertraline and venlafaxine were all determined to have quadratic fits over 7.7-1300 ng/mL, similarly mirtazapine was deemed quadratic over 3.8-650 ng/mL and trazodone over 23-3900 ng/mL.
- The analytical sensitivity of the method was assessed by extracting and quantifying 10 replicates of low concentration samples over 5 days. ≤20%CV precision and ≤15% bias was achieved at concentrations equivalent to or lower than the lowest concentration calibrator, with the exception of fluvoxamine (17.6% bias).
- No system carryover was observed following analysis of plasma samples containing the highest concentration calibrators.

Matrix Effects and Ion Suppression

- Matrix effect investigations were evaluated at low and high concentrations for all 10 analytes, using six individual plasma samples.
- Normalized matrix factor calculations, based on the analyte:internal standard response ratio demonstrated that the internal standards compensated for any ion suppression observed, with mean matrix factors in the range 0.90-1.07.
- Post-column infusion experiments revealed that analytes eluted in regions free of significant ion suppression.

Precision

- Low, mid and high concentration plasma pools were analysed in replicates of 5, on 5 occasions (n=25), to assess repeatability and total precision.
- Reproducibility and total precision was determined to be ≤10.0% CV for the entire panel and concentrations tested (Figure 1 shows total precision).

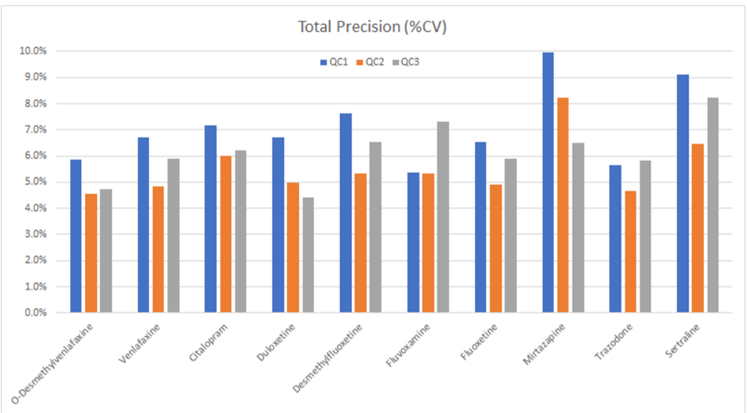


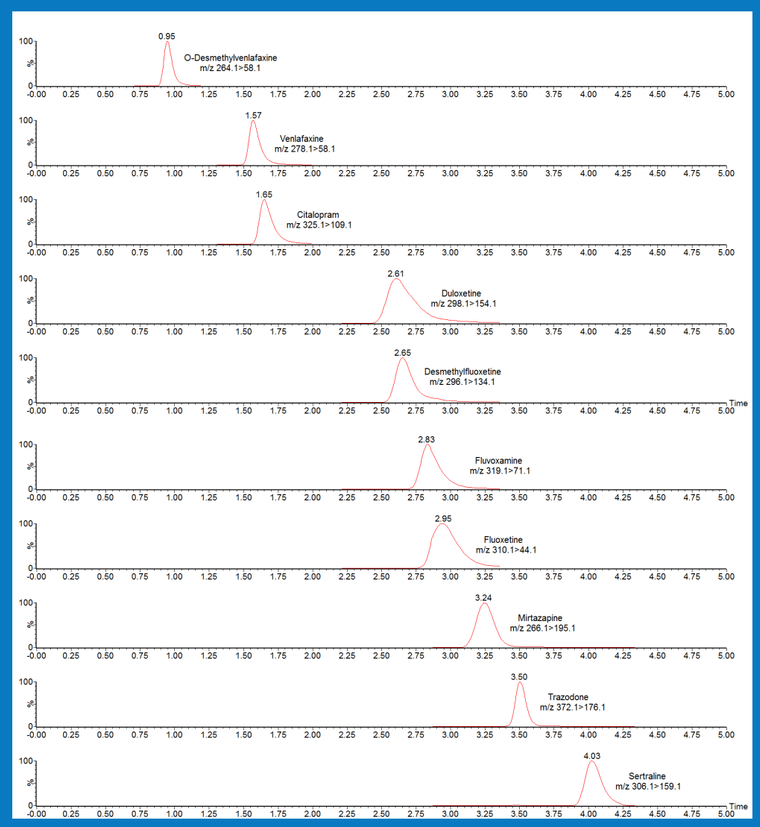
Figure 1. *Total Precision*

Interference Testing

- Potential interference from endogenous compounds (albumin, bilirubin, creatinine, cholesterol, triglycerides and uric acid) was assessed at low and high concentrations.
- A substance was deemed to interfere if a recovery range of 85-115% was exceeded; recoveries ranged from 86.9-112.9%.
- Additionally, full chromatographic resolution of O-Desmethylvenlafaxine from isobaric tramadol was established.

Note: ACQUITY™, UPLC™, Xevo™ and XSelect™ are trademarks owned by Waters Technologies Corporation.

Multiplexing of 10 antidepressants in 5 mins



Chromatogram showing the analysis of 10 antidepressant drugs

CONCLUSION

- **This quantitative method for clinical research demonstrates very good precision with minimal matrix effects.**
- **Using a small sample volume, of 50 µL, the method allows simultaneous quantification of a panel of antidepressants in a short run time.**
- **Sample preparation is simple, fast and inexpensive.**

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