ANALYSIS OF TRICYCLIC ANTIDEPRESSANT DRUGS IN PLASMA FOR CLINICAL RESEARCH

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INTRODUCTION

The quantitative analysis of tricyclic antidepressants (TCAs) in plasma is crucial in order to undertake pharmacokinetic studies and monitor therapy efficiently.

Waters has developed a clinical research LC-MS/MS method for the simultaneous analysis of the following TCAs in plasma; amitriptyline, clomipramine, clozapine, desipramine, doxepin, imipramine, maprotiline, norclomipramine, norclozapine, nordoxepin, normaprotiline, nortrimipramine, nortriptyline, protriptyline, trimipramine (10-500 ng/mL); normaprotiline and trimipramine (20-1000 ng/mL) and clozapine and norclozapine 50-2500 ng/mL).

METHODS

Materials and Sample Preparation

- Plasma calibrators and quality control (QC) materials were prepared in-house using pooled human plasma supplied by BioIVT (West Sussex, UK).
- Concentrated stock solutions were prepared from certified powders and solutions supplied by Cambridge Bioscience (Cambridgeshire, UK), Merck Life Science (Dorset, UK) and Toronto Research Chemicals (Ontario, Canada).
- Stable-labeled internal standards were supplied by ALSACHIM (Illkirch-Graffenstaden, France), 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 1500 r.p.m. for 3 minutes, then centrifuged for 2 minutes at 16100g.
- 25µL of supernatant was transferred to a 1mL 96-well collection plate and 475µL water added.

LC-MS/MS Parameters

- Using an ACQUITY™ UPLC™ I-Class FTN System, samples were injected onto an XSelect™ Premier HSS T3 C18 2.5µm, 2.1 x 100mm Column, using a methanol/water/5mM ammonium formate gradient and analyzed with a Waters Xevo™ TQ-S micro Detector in positive ESI, using Multiple Reaction Monitoring.
- The run time is 4.0 minutes (approximately 4.5 minutes injection-to-injection).

Gradient table

Time (min)	Flow Rate (mL/min)	% A	% B	Curve
0	0.50	40	60	Initial
1.5	0.50	40	60	6
3.0	0.50	0	100	6
3.14	0.50	0	100	11
3.15	0.50	40	60	11

RESULTS

Analytical separation

Baseline resolution was achieved for isobaric compounds (nortriptyline and protriptyline, m/z 264.1) and interfering qualifier transitions (281.2>86.1) of imipramine and nortrimipramine, using the XSelect Premier HSS T3 C18 Column. **Figure 1** is a chromatogram of a pooled plasma mid-level calibrator sample, showing the separation across all the 15 TCAs.

Fast quantification of 15 TCAs in 4 minutes

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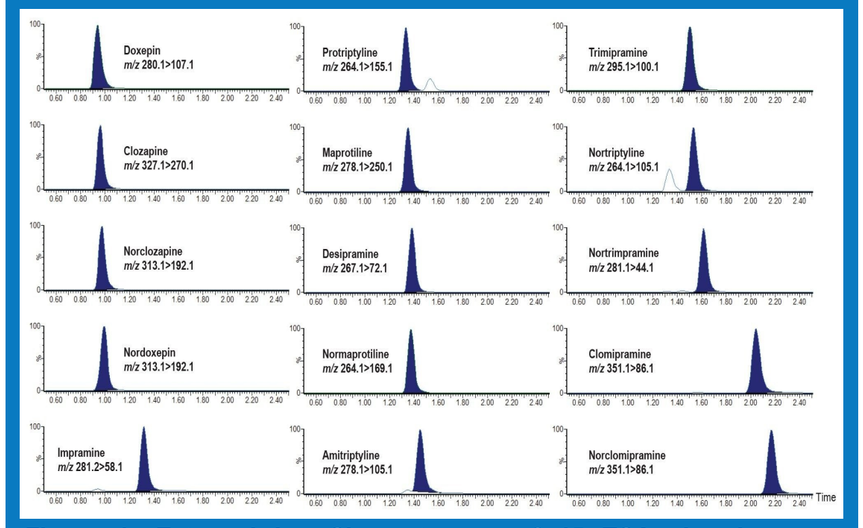

Figure 1: Extraced ion Chromatogram of 15 TCAs in plasma calibrator sample

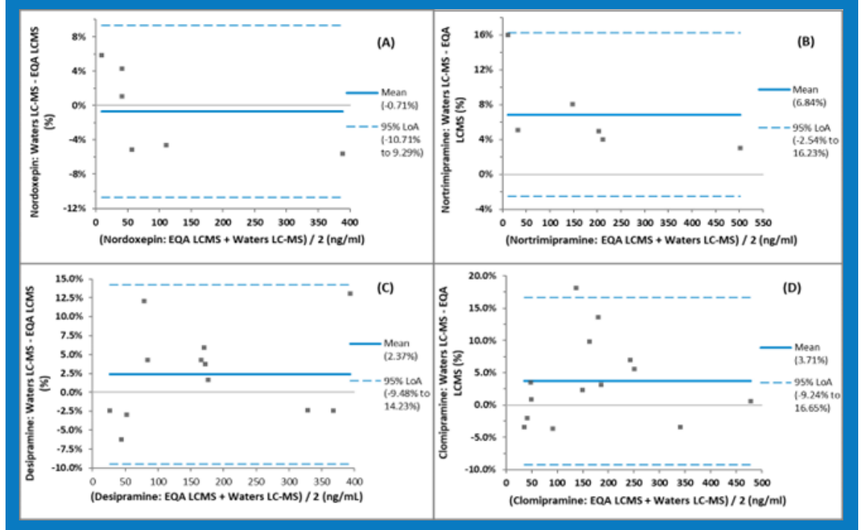

Figure 3: Comparison of Waters LC-MS/MS method to EQA scheme MS method mean using Bland-Altman fit for selected analytes

Linearity, Analytical Sensitivity and Carryover

- The calibration lines of all analytes exhibited coefficient of determination (r^2) of >0.995.
- Analytical sensitivity studies were performed by extracting and quantifying 10 replicates of four levels of low concentration samples prepared in plasma, over five days (n=40). The Lower Limit of the Measuring Interval (LLMI, ≤20%CV precision and ≤15% bias) was achieved at concentrations equivalent to the lowest calibrator for all analytes.
- No significant carryover was observed from highest calibrator sample ng/mL into subsequent blank samples.

Matrix Effects

- Matrix effect investigations were conducted at both low and high concentrations for all 15 analytes, using six different plasma samples.
- The normalized matrix factor calculations, based on the ratio of analyte to internal standard response, showed that the internal standards effectively compensated for any observed ion suppression, with mean matrix factors ranging from 0.88 to 1.07.
- Post-column infusion experiments indicated that the analytes eluted in regions free from significant ion enhancement or suppression.

Precision

- Low, mid and high concentration QC plasma pools were analysed in replicates of 5, on 5 occasions (n=25), to assess repeatability and total precision.
- Total precision and reproducibility was determined to be ≤8.0% CV for the all analytes and concentrations tested.

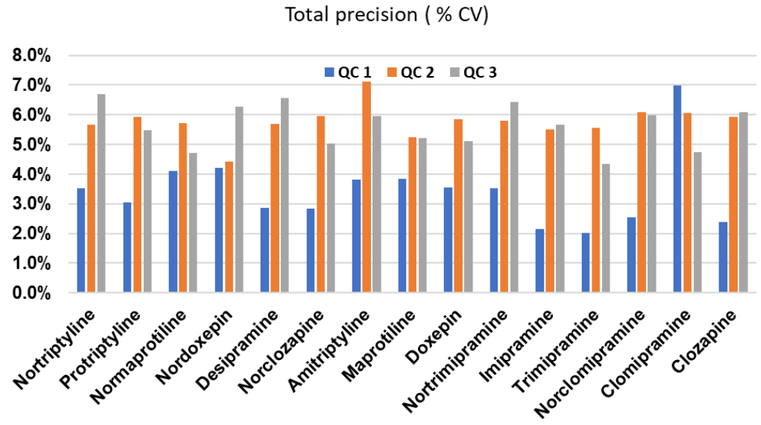

Figure 2: Total precision for TCAs

Interference Testing

- Potential interference from endogenous compounds (albumin, bilirubin, creatinine, cholesterol, triglycerides and uric acid) was assessed.
- The recoveries for the low and high pools were within the acceptance criteria (15% of nominal concentration), except for amitriptyline, clomipramine, clozapine, imipramine, norclomipramine, nortriptyline and protriptyline, which exhibited some interference (17%) from triglycerides at low concentration samples.

Accuracy and EQA agreement

- External quality assurance (EQA) serum samples (LGC, UK) assessed for accuracy showed a mean method bias of <14.75% using Bland-Altman agreement, demonstrating very good agreement with the EQA LC-MS mean values (**Figure 3).**

CONCLUSION

- **A clinical research method utilizing UHPLC-MS/MS has been developed to analyze 15 tricyclic antidepressant drugs from just 50µL of plasma.**
- **The separation of isobaric compounds was achieved using Waters XSelect Premier HSS Technology.**
- **The method features a simple, fast, and cost-effective protein precipitation sample extraction, with a 4-minute run time, precision of ≤8.0% RSD, and no carryover.**
- **Any observed matrix effects were effectively compensated for by stable-labeled internal standards.**

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