A SIMPLE LC-MS/MS METHOD FOR SIMULTANEOUS ANALYSIS OF 35 ANTI-PSYCHOTICS IN HUMAN PLASMA FOR CLINICAL RESEARCH

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INTRODUCTION

Anti-psychotic drugs (APDs) are frequently used in combination with other drugs, leading to potential pharmacokinetic and pharmacodynamic drug interactions. Many of these interactions have not been adequately studied in clinical research.

Waters has developed a simple, cost-effective and robust LC-MS/MS method for clinical research which is based on protein precipitation for the simultaneous analysis of 35 APDs in human plasma

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 of analyte standards and stable-labeleld internal standards were prepared from certified powders and solutions supplied by LGC Standards (Teddington, UK), Merck Life Science (Dorset, UK) and Toronto Research Chemicals (Ontario, Canada), British Pharmacopoeia Chemical Reference Substances (Norwich, UK).
- 50µL of sample was added to a microcentrifuge tube followed by 100µL of working internal standard in a mixture of 70:30 (v:v) MeOH:0.1M $ZnSO_4$(aq).
- Samples were vortex mixed briefly before centrifugation at 18,000g for five minutes at room temperature.
- 100µL of the supernatant was transferred into a 96-well plate and then diluted with 100µL distilled pure water.
- The samples in the plate were mixed on a shaker for three minutes at 850rpm and room temperature prior to analysis by LC-MS/MS.

LC-MS/MS Parameters

- Using an ACQUITY™ UPLC™ I-Class System with FL Sample Manager, samples were injected onto an Waters XSelect™ HSS C18 SB XP, 100 Å, 2.5 µm, 2.1 mm x 30 mm Column, with mobile phases of water and methanol containing 2 mM ammonium acetate/0.1% formic acid.
- All analytes were analysed with a Waters Xevo™ TQ-S micro Mass Spectrometer in positive ESI, using Multiple Reaction Monitoring.
- The run time is 4.3 minutes (approximately 4.9 minutes injection-to-injection).

Gradient table

Time (min)	Flow Rate (mL/min)	% A	% B	Curve
0.0	0.60	90	10	Initial
0.5	0.60	90	10	6
3.0	0.60	25	75	6
3.1	0.60	0	100	11
3.8	0.60	90	10	11

Fast quantification of 35 APDs in 4.3 minutes

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Index	Analyte	RT	Index	Analyte	RT	Index	Analyte	RT
1	Sulpiride	0.99	4	Paliperidone	1.95	7	Levomepromazine	2.58
2	Pipamperone	1.42	5	Quetiapine	2.25	8	Zotepine	2.74
3	Amisulpride	1.66	6	Promethazine	2.41	9	Thioridazine	2.88

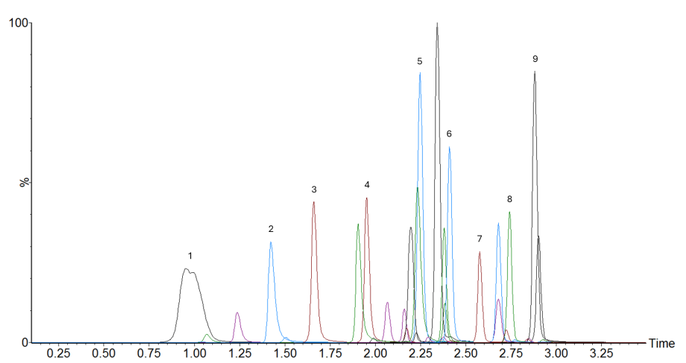

Figure 1: Chromatography of all 35 compounds on the ACQUITY UPLC I-Class System XSelect HSS C18 SB XP Column. The earliest eluting compound is sulpiride at 0.97 minutes and the latest eluting compound is flupentixol at 2.93 minutes.

RESULTS

Linearity, Analytical Sensitivity and Carryover

- Linear regression (1/x weighting) provided the best fit, with coefficients of determinations (r^2) higher than 0.99 for all analytes.
- Analytical sensitivity was assessed by extracting and quantifying 10 replicates at four low concentration levels prepared in plasma, over three consecutive days (n = 30). The Lower Limit of the Measuring Interval (LLMI, ≤20% CV and ≤15% bias), was achieved at concentrations corresponding to the lowest calibrator for all analytes.
- No significant system carryover (<25% Calibrator 1 mean peak area) was observed across all analytes following analysis of samples at twice the concentration of the high QC.

Matrix Effects and Recoveries

- Matrix effect investigations were conducted at both low and high concentrations for all 35 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 or enhancement, with mean matrix factors ranging from 0.88 to 1.14.
- Recoveries were evaluated at low and high QC concentrations in plasma for six individuals (n=6) taken. The overall mean recovery for each concentration for all analytes were in the range of 85%–115%. The mean extraction efficiencies across the 35 analytes ranged from 52.2%–96.9%.

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 (Figure 2) and reproducibility was determined to be ≤8.7% CV for the all analytes and concentrations tested.

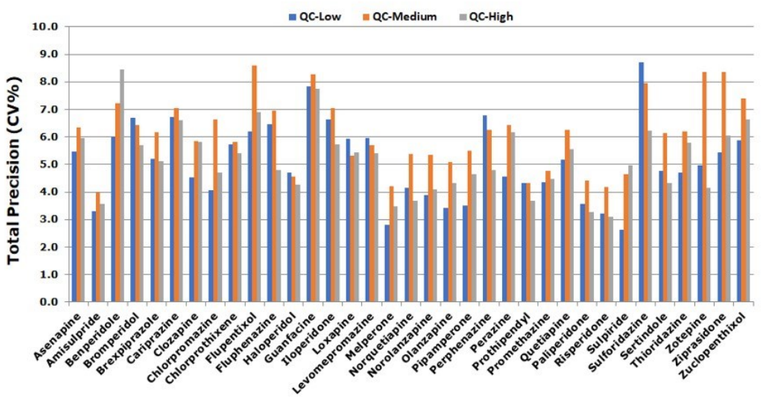

Figure 2: Total precision for 35 APDs

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), No significant interferences were observed for all 35 APD analytes.

CONCLUSION

- **A clinical research method utilizing LC-MS/MS has been developed to analyze 35 anti-psychotic drugs from just 50µL of plasma.**
- **The separation of all compounds was achieved using Waters XSelect HSS C18 SB XP Column, 100 Å, 2.5 µm 2.1 mm x 30 mm.**
- **The method features a simple, fast, and cost-effective protein precipitation sample extraction, with a 4.3-minute run time, precision of ≤8.7% RSD, and no carryover.**
- **Any observed matrix effects were effectively compensated for by stable-labeled internal standards.**

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