Rapid Analysis of Clozapine and Norclozapine in Plasma for Clinical Research

Brown, H., Roberts, S., Balloch, S., Danaceau, J., Vukovic, J., and Hammond, G.

Clinical Business Unit, Waters Corporation, Wilmslow, United Kingdom.

Background: Protein precipitation is combined with liquid chromatography (LC) and tandem mass spectrometry for high throughput analysis of clozapine (CLZ) and norclozapine (NCLZ), for use in clinical research.

Methods: Matrix-matched calibrators and quality control samples were prepared in human plasma, using stocks prepared from certified materials. Samples (50µL) were treated with internal standard in acetonitrile, and 20µL of supernatant was diluted with 30% methanol (aq). With an injection cycle of 1.2 minutes, the sample was separated by LC in a gradient of buffered water, methanol and isopropanol using a Waters XBridge™ Premier BEH™ UPLC™ Column, on an ACQUITY™ UPLC I-Class FTN System, followed by detection using a Xevo™ TQ-S micro Mass Spectrometer.

Results: Having verified the absence of significant carryover, the linear measurement interval (LMI) was determined to be 12-4946 ng/mL for CLZ and 4.5-3765 ng/mL for NCLZ, defined as the range with no significant 2^{nd}/3rd order terms ($p<0.05$), $r^2 \geq 0.997$ by linear regression, and measurable with ≤20% imprecision (relative standard deviation, RSD %) and ≤15% deviation from nominal concentrations. Total imprecision of measurement for four levels of controls within the LMI was ≤3.2% (n=25), with ≤4.8% deviation from nominal. The limit of detection and lower limit of quantification was 4.4 and 13.3 ng/mL for CLZ, and 14.7 and 44.5 ng/mL for NCLZ, determined by regression of five calibration datasets. Functional sensitivity experiments corroborated this by showing ≥85% recovery of compounds from pooled plasma enriched with 12.0 ng/mL CLZ and 4.5 ng/mL NCLZ, measurable with ≤20% imprecision (n=50). Internal standardization of CLZ and NCLZ with $^2H_4$-CLZ was applied, which compensated for the negligible residual matrix effects in six plasma samples within the LMI, with mean recovery normalized to solvent standards of 100.3% for CLZ (range 99.0-100.8) and 104.1% for NCLZ (103.6-104.7). Agreement with comparator LC-MS/MS analytical methods was established, and EQA z-scores ≤2.00 for 11/11 distributions were achieved.

Conclusion: The analytical method meets performance requirements and is suitable for use in clinical research where high-throughput of samples is imperative.