Ultra-rapid, fully-automated plasma clozapine and norclozapine analysis using AC Extraction Plate™ technology and flow-injection MS/MS

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The analysis of plasma clozapine and *N*-desmethylclozapine (norclozapine) for therapeutic drug monitoring (TDM) purposes is well-established (Couchman *et al*., 2010). To minimize analysis times and thus facilitate rapid reporting of results, we have (i) fully automated sample preparation using novel AC Extraction Plates™ (Tecan Schweiz AG) and a Tecan Freedom EVO® 100 liquid-handling platform (Tecan Schweiz AG), and (ii) minimised extract analysis times by using flow-injection MS/MS rather than HPLC-UV or LC-MS/MS.

Clozapine, norclozapine, and deuterated internal standards (clozapine-D_8 and norclozapine-D_8) were extracted from plasma samples (100 µL) and extracts (100 % methanol) were analysed directly using MS/MS (20 µL injection volume, 0.5 mL/min methanol carrier flow, selected reaction monitoring mode). Data acquisition time as 10 seconds per injection. Results were compared with those obtained using manual liquid-liquid extraction and LC-MS/MS.

Typical batch preparation time was reduced from approximately 2.5 hours (manual liquid-liquid extraction, 100 tubes/batch) to approximately 30 minutes for a 96-well plate. Extract analysis time was reduced from 5 minutes per sample by HPLC-UV or LC-MS/MS, to 1 minute per sample (the minimum cycle time of the autosampler available).

Sample extracts (N = 50) prepared using the AC Extraction plate, but analysed by both FIA-MS/MS and LC-MS/MS showed excellent agreement ($y = 1.001x - 0.0016$, $R^2 = 0.999$ and $y = 0.968x + 0.006$, $R^2 = 0.991$ for clozapine and norclozapine, respectively). Validation data

for the FIA-MS/MS method showed excellent intra- and inter-plate accuracy (95-104 % nominal concentrations, N = 8 at three concentrations each analyte) and precision (CV < 6 %). Matrix effects were observed for both clozapine and norclozapine, but were compensated for by the internal standards. Overall process efficiency (PE) for 8 independent analyte-free matrices was 56–70 % and 66–77 % for clozapine and norclozapine, respectively. However, mean relative PE was 98 and 99 % for clozapine and norclozapine, respectively (Matuzewski *et al*., 2003). Results for external quality assurance (EQA) samples (N = 14) using the FIA-MS/MS method compared well with target ('spiked') concentrations and our previously reported results ($y = 1.006x - 0.013$, $R^2 = 0.991$ and $y = 0.976x + 0.006$, $R^2 = 0.993$ for clozapine and norclozapine, respectively; reported results produced using manual liquid-liquid extraction with HPLC-UV).

This work illustrates the application of novel AC Extraction Plate technology to an ultra-high-throughput and fully-automated TDM application. In the future, this workflow will be combined with an internal calibration approach (Couchman *et al*., 2013) to give very rapid sample turnaround. AC Extraction Plate sample preparation technology has many potential applications in the analysis of lipohilic analytes such as many drugs and endogenous analytes such as vitamin D metabolites and steroids.

References

Couchman L *et al*. *Ther Drug Monit* 2010; **32**: 438-47.

Matuzewski *et al*. *Anal Chem* 2003; **75**: 3019-30.

Couchman L *et al*. *Anal Bioanal Chem* 2013; **405**:9455-66.