

Analysis of clozapine and norclozapine in plasma using automated online sample preparation and LC-MS/MS

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Introduction

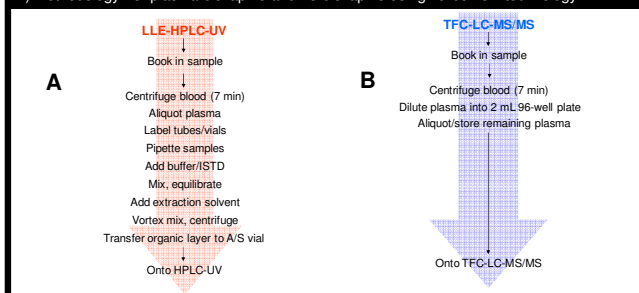
Clozapine is a dibenzodiazepine drug used in the treatment of schizophrenia. It is uniquely effective in patients resistant to therapy with other antipsychotics. In addition to mandatory haematological monitoring to minimize the risk of agranulocytosis, there is a large variation (50-fold) in clozapine dose requirement that complicates dose assessment. Current methodology in our laboratory for plasma clozapine and norclozapine measurement involves off-line liquid-liquid extraction (LLE) with manual transfer of the extract to an HPLC-UV system¹ (Figure 1A). Clozapine *N*-oxide is not extracted.

Aim

Multiplexed, automated online sample preparation (Figure 2) coupled with a triple quadrupole tandem mass spectrometer (MS/MS) was investigated in order to simplify sample preparation for the analysis of plasma clozapine and norclozapine (Figure 1B).

FIGURE 1.

- A) Current methodology for analysis of plasma clozapine and norclozapine
B) Methodology for plasma clozapine and norclozapine using TurboFlow technology

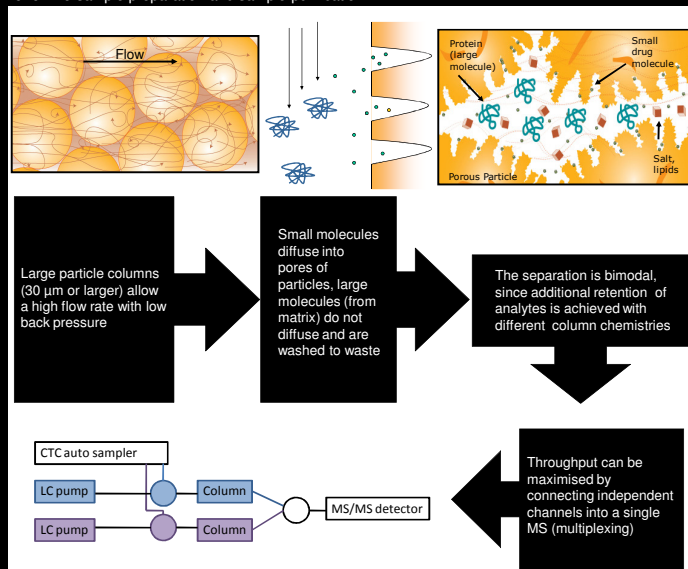


Method

- Calibration (n = 6, range 0.05 - 2 mg/L both analytes in newborn calf serum) and IQC (0.15, 0.40, and 1.20 mg/L both analytes in drug-free pooled human plasma) solutions were prepared. Centrifuged plasma (50 µL) was diluted with 950 µL of diluent (15 µg/L clozapine-D₈, 3 µg/L clozapine *N*-oxide) and mixed thoroughly. A portion (10 µL) of the mixture was injected and directly analyzed by TLX-MS/MS.
- MS analysis: Thermo Scientific TSQ Vantage (positive mode APCI; corona discharge current 3 µA; vaporizer and capillary temperatures 300 °C; auxiliary, sheath and ion sweep gas settings 10, 20 and 0 arbitrary units, respectively).
- Calibration and IQC solutions, and plasma blanks, were analyzed at the beginning and end of each batch.

FIGURE 2.

Turboflow technology and multiplexing - High turbulence liquid chromatography (HTLC): a system for online sample preparation and sample purification

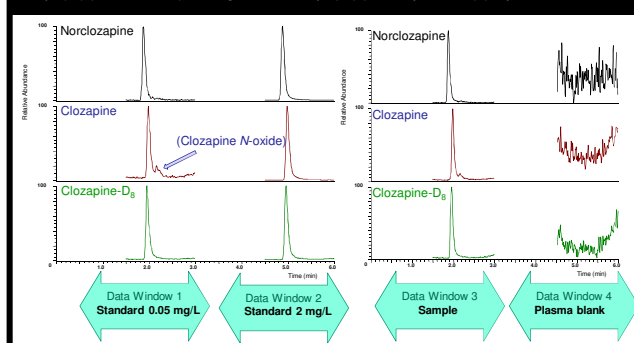


Results

- In-source conversion of clozapine *N*-oxide to clozapine was a potential source of interference in patient samples.² Clozapine *N*-oxide was therefore chromatographically resolved from clozapine and norclozapine (Figure 3).
- No matrix effects were observed (evaluated as described by Bonfiglio *et al.*³)
- Carry-over was shown to be < 0.5 % for both analytes.
- Inter- and intra-assay precision was less than 10 % (RSD) for both clozapine and norclozapine at each concentration tested. For both analytes, calculated IQC solution concentrations were 93 - 104 % of nominal values.
- TFC recovery was 69 and 60 % for clozapine and norclozapine.

FIGURE 3.

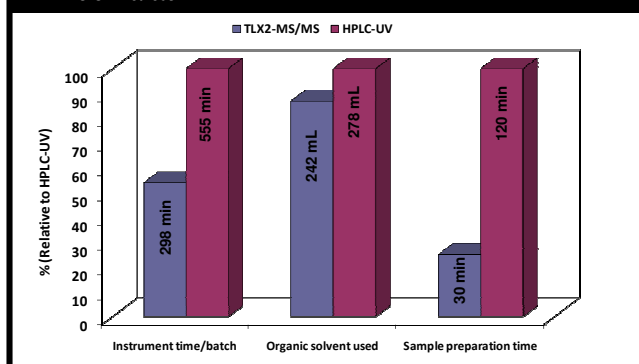
Representative TLX2-MS/MS chromatograms showing (1) standard 1 (0.05 mg/L each analyte), (2) standard 6 (2.00 mg/L each analyte), (3) a sample, and (4) a plasma blank.



Method comparison

- Retrospective analysis of external quality assurance serum samples (United Kingdom National External Quality Assurance Scheme Psychoactive Drug Scheme) for clozapine and norclozapine showed good correlation with consensus means (Clozapine: $y = 1.07x - 0.007$, $R^2 = 0.989$; norclozapine: $y = 0.91x + 0.008$, $R^2 = 0.996$).
- Comparison of results (n = 50) from the existing LLE-HPLC-UV method and the multiplexed TLX2-MS/MS method also showed good correlation. (Clozapine: $y = 0.94x - 0.036$, $R^2 = 0.942$; norclozapine: $y = 0.827x - 0.006$, $R^2 = 0.888$).
- The savings made using the TLX2-MS/MS method are shown in Figure 4.

FIGURE 4. Batch analysis comparison between multiplexed TLX2-MS/MS and existing LLE-HPLC-UV methods.



Conclusions

- The use of turbulent flow chromatography for automated online sample preparation and tandem MS detection allowed the selective analysis of clozapine and norclozapine in plasma.
- Use of this method eliminated 2 hrs of sample preparation time for a typical batch analysis. By multiplexing the instrument, time required was reduced from >9 h (HPLC-UV) to <5 h.
- In addition to the staff time saved, there were significant cost savings associated with reduced consumable requirements such as tubes, pipettes, vials and solvents, and in the volumes of blank serum/plasma required for standard and IQC preparation.

References

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- D. M. Peiris *et al.*, *J. Mass. Spectrom.* **39**, 600-606 (2004).
- R. Bonfiglio *et al.*, *Comm. Mass Spec.* **13**, 1175-1185 (1999).