

Antidepressants and Neuroleptics Quantitation Using Tandem Mass Spectrometry and Automated Online Sample Preparation

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Introduction

Liquid chromatography-mass spectrometry (LC/MS) is a powerful technique applied in clinical research for the analysis of a broad number of analytes. Offline sample preparation techniques (solid phase extraction and liquid-liquid extraction) are widely used but are often time consuming and labor intensive. The Thermo Scientific Transcend system powered by TurboFlow™ technology provides an alternative approach simplifying sample preparation.

Goal

To develop a fast and efficient LC-MS/MS method using Thermo Scientific TurboFlow technology for the analysis of 18 antidepressants and neuroleptics.

Experimental

Sample Preparation

A 100 µL aliquot of serum or plasma sample was mixed with 300 µL of methanol containing internal standards (Venlafaxine-d6 and Sertraline-d3) at 100 ng/mL. The resulting mixture was thoroughly vortexed, allowed to stand for 10 minutes at room temperature and then centrifuged at 4 °C for 10 minutes.

Chromatography and Mass Spectrometry

High pressure LC (HPLC) was performed using the Transcend™ TLX system. Serum and plasma samples were extracted using a TurboFlow Cyclone P (0.5 x 50 mm) extraction column. Chromatographic separation was performed using a Thermo Scientific Hypersil GOLD column (50 x 3 mm, 3 µm particle size). Gradient elution was used. Total analysis time was 8 minutes.

The TurboFlow method conditions were as follows:

Eluent A:	0.1% Formic acid in water
Eluent B:	0.1% Formic acid in methanol
Eluent C:	Acetonitrile, isopropanol and acetone (45/45/10, v/v/v)
Eluent D:	Acetonitrile, water (90/10, v/v)

The analytical LC conditions were as follows:

Eluent A:	0.1% Formic acid in water
Eluent B:	0.1% Formic acid in methanol

The entire LC effluent from the sample injections was directed to the Thermo Scientific Ion Max source, utilizing heated electrospray ionization (HESI), on a Thermo Scientific TSQ Quantum Access MAX triple stage quadrupole mass spectrometer in positive ion selected reaction monitoring (SRM) mode.

Key Words

- TurboFlow Technology
- TSQ Quantum Access MAX
- Triple Quadrupole
- Clinical Research

Results and Discussion

For each analyte, linearity and quantitative results were obtained using SRM transitions. Quantitation of the 18 drugs was performed with a calibration range of 5 to 500 ng/mL for 5 compounds, 10 to 1000 ng/mL for 9 compounds, 2 to 200 ng/mL for 3 compounds, and 1 to 100 ng/mL for 1 compound. The R^2 value for

each of the calibration curves was above 0.998, which indicates an excellent linear fit over the dynamic range. Figure 1 shows the chromatogram of the lowest calibration standard. Calibration curves for risperidone and clozapine are reported in Figure 2. Table 1 displays the calibration ranges and method precision for all analyzed drugs.

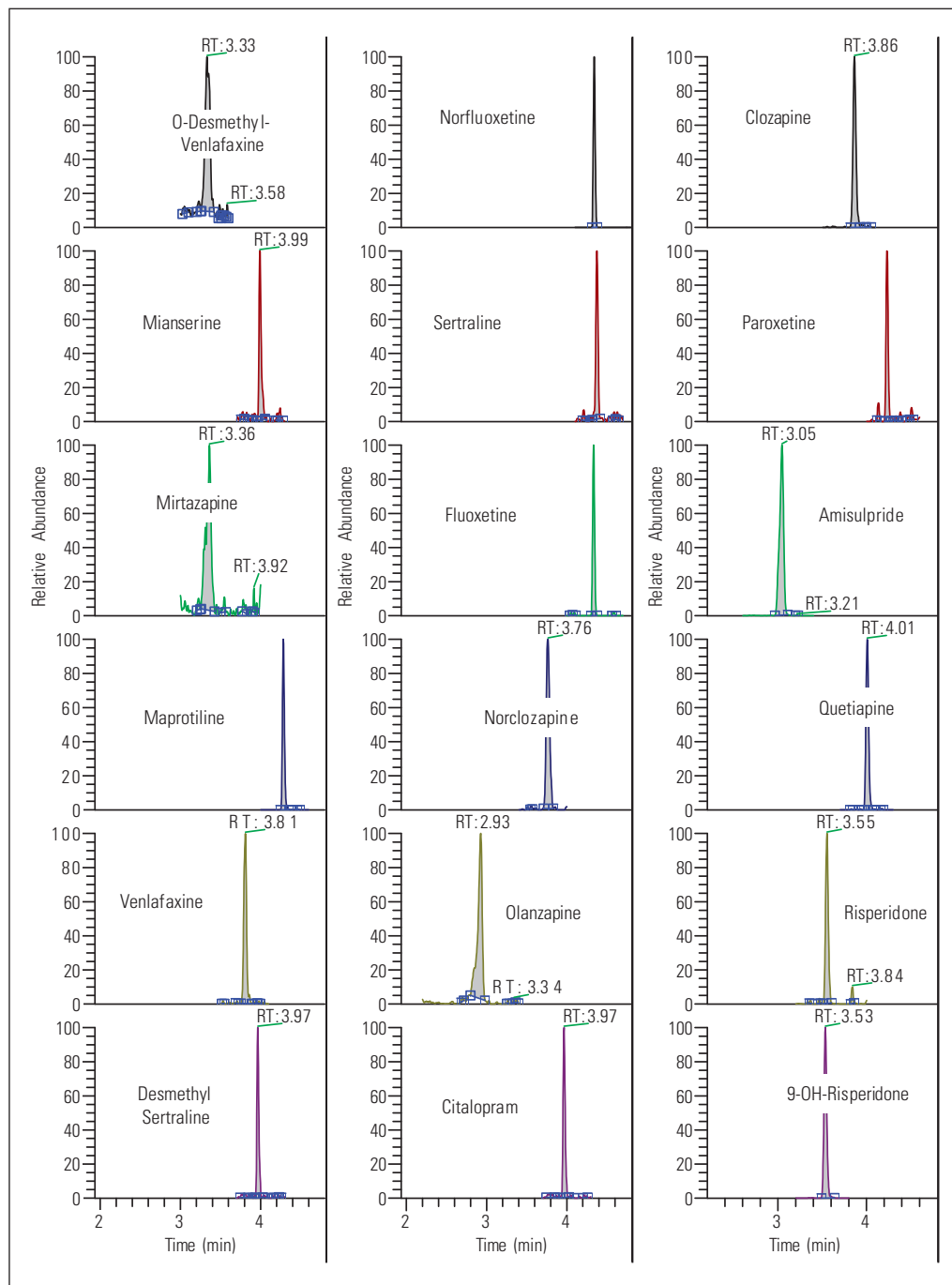


Figure 1. Representative chromatograms for the methods at the low end of the calibration curve

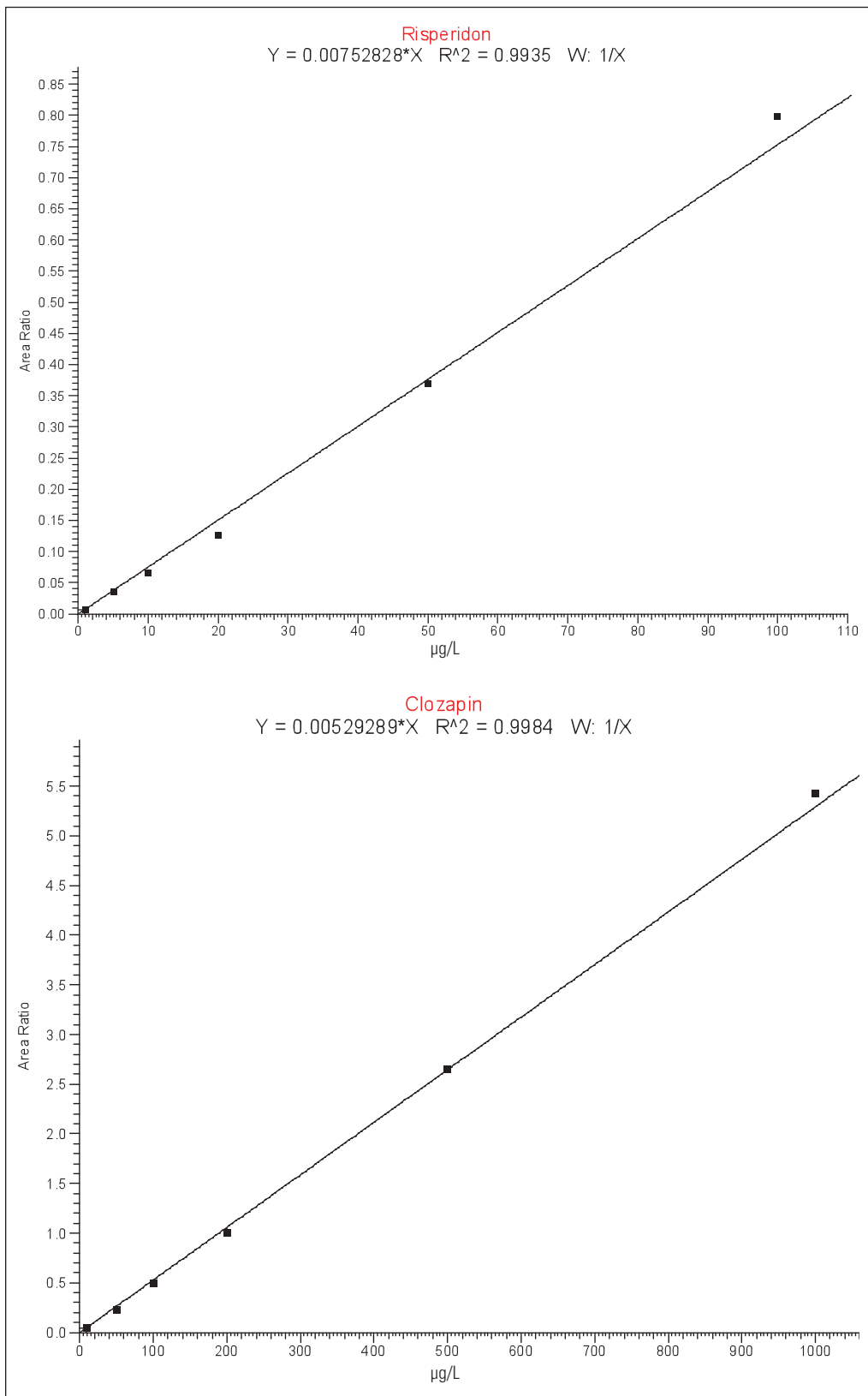


Figure 2. Calibration curves for risperidone and clozapine

Table 1. Calibration ranges and method precision for all the analytes

Analyte	Calibration range (ng/mL)	Within-day (%RSD)*	Between-days (%RSD)**
9-OH-Risperidone	5-500	7.1	5.5
Amisulpride	10-1000	3.9	3.4
Citalopram	5-500	4.9	5.1
Clozapine	10-1000	5.8	4.3
Desmethyl Sertraline	2-200	6.3	6.0
Fluoxetine	10-1000	3.4	3.4
Maprotiline	10-1000	4.2	4.1
Mianserine	5-500	6.2	5.1
Mirtazapine	2-200	5.9	4.4
Norclozapine	10-1000	5.9	3.6
Norfluoxetine	10-1000	6.5	5.0
O-Desmethyl-Venlafaxine	10-1000	4.3	4.8
Olanzapine	5-500	6.2	3.3
Paroxetine	5-500	6.2	5.3
Quetiapine	10-1000	5.2	3.5
Risperidone	1-100	5.8	5.4
Sertraline	2-200	4.5	3.4
Venlafaxine	10-1000	4.5	3.4

* Replicates analyzed each day = 10

** Days averaged = 10

Conclusion

A fast and analytically sensitive method for the detection of 18 antidepressants and neuroleptics is described. The Transcend TLX automated online sample preparation system allows minimal sample preparation and time saving in the absence of SPE sample preparation for clinical research laboratories.

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