

Screening and Quantification of Multiple Drugs in Urine Using Automated Online Sample Preparation and Tandem Mass Spectrometry

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Key Words

- Transcend System
- TSQ Quantum Access MAX
- ToxID Software
- Clinical Research

Introduction

Liquid chromatography-mass spectrometry (LC-MS) is a sensitive, accurate, and precise technique applied in clinical research for the analysis of a large number of compounds and metabolites from various drug classes, such as antidepressants, hypnotics, stimulants, cardiacs, and antihistamines. Thermo Scientific Transcend system powered by TurboFlow™ technology provides an alternative separation technique for complex biomatrices, simplifying sample preparation, increasing LC-MS/MS sensitivity, and reducing ion suppression.

Goal

To develop a fast and efficient LC-MS/MS method using Thermo Scientific TurboFlow technology for the analysis of 30 drugs and metabolites in urine.

Experimental

Sample Preparation

Eight internal standards were used in the study for the corresponding compounds: nicotine-d4, cotinine-d4, midazolam-d4, diphenhydramine-d3, promethazine-d3, norfluoxetine-d6, chlorpromazine-d3, and fluoxetine-d6. For the other compounds, the internal standard with the closest retention time was assigned.

Human urine samples (100 µL) were diluted with 100 µL of methanol containing the internal standards in concentrations of 100 ng/mL. The samples were vortexed and centrifuged. Then, 10 µL of the supernatant was injected onto the TurboFlow column.

HPLC

HPLC analysis was performed using the Transcend™ system with a TurboFlow Cyclone MAX column (0.5 x 50 mm) and a Thermo Scientific Hypersil GOLD PFP analytical column (100 x 2.1 mm; 5 µm). Total analysis time was 9 minutes.

Mass Spectrometry

MS analysis was carried out on a Thermo Scientific TSQ Quantum Access MAX triple stage quadrupole mass spectrometer equipped with a Thermo Scientific Ion Max source and an electrospray ionization (ESI) probe. Two selected reaction monitoring (SRM) transitions with scan times of 10 msec were collected for each analyte.

Results and Discussion

Quantitation of 30 drugs in urine was performed in 9 minutes with a calibration range of 1-1000 ng/mL for 14 compounds, 5-1000 ng/mL for 9 compounds, 10-1000 ng/mL for 5 compounds and 50-1000 ng/mL for 2 compounds. Figure 1 shows the chromatograms of the lowest calibration standard. Table 1 displays the calibration ranges and method precision for all analyzed drugs.

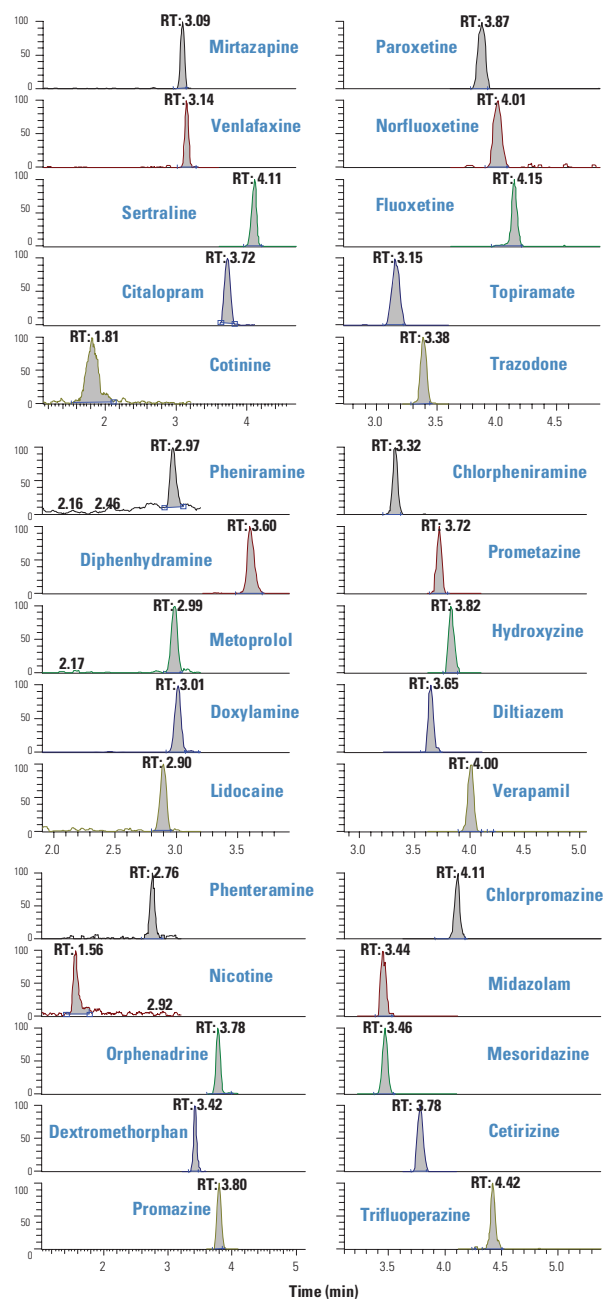


Figure 1: Chromatograms of the lowest calibration standard.

Within-day and between-days precisions were determined with QC samples prepared by spiking blank urine to three concentrations: twice the lowest standard concentration (QC1), the middle of the calibration range concentration (QC2), and 80% of the highest standard concentration (QC3).

Table1: Calibration ranges, within-day precision, and between-days precision for the lowest QC sample.

Analyte	Calibration range (ng/mL)	Within-day (%RSD)	Between-days (%RSD)
Citalopram	1-1000	10.7	9.3
Fluoxetine	10-1000	10.4	9.1
Norfluoxetine	10-1000	16.4	11.0
Mirtazapine	1-1000	13.6	12.5
Paroxetine	10-1000	10.2	14.6
Sertraline	10-1000	8.0	15.7
Trazodone	1-1000	11.7	10.6
Venlafaxine	1-1000	13.6	12.1
Diphenhydramine	1-1000	7.1	7.1
Chlorpheniramine	1-1000	8.2	7.2
Pheniramine	1-1000	7.6	5.6
Cetirizine	5-1000	15.4	15.1
Promethazine	50-1000	4.6	4.2
Nicotine	5-1000	10.7	7.1
Cotinine	5-1000	12.0	8.1
Dextromethorphan	1-1000	8.6	10.9
Topiramate	50-1000	13.2	10.4
Orphenadrine	1-1000	7.2	9.1
Lidocaine	1-1000	11.5	9.4
Phenteramine	10-1000	11.1	13.8
Mesoridazine	5-1000	3.4	4.4
Midazolam	1-1000	14.3	12.2
Chlorpromazine	5-1000	8.0	15.0
Promazine	5-1000	17.1	10.8
Trifluoperazine	5-1000	7.9	17.5
Diltiazem	1-1000	10.1	10.2
Metoprolol	5-1000	10.0	8.5
Verapamil	5-1000	8.7	9.2
Doxylamine	1-1000	14.4	11.7
Hydroxyzine	1-1000	17.9	14.0

Conclusion

An efficient (9 minute), sensitive (LOQ of 1-50 ng/mL), and precise LC-MS/MS method using TurboFlow technology was developed for the quantitation of 30 drugs in human urine. In clinical research, TurboFlow technology simplifies sample preparation and improves method robustness and sensitivity.

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