

A TurboFlow On-Line Extraction LC-MS Method for Screening and Quantification of Multiple Therapeutic Drugs in Human Urine

Barbora Brazdova, Marta Kozak; Thermo Fisher Scientific, San Jose, CA, USA



Overview

Purpose: Develop a quantitative, efficient method for the analysis of 30 therapeutic drugs in urine supporting research and pharmacology applications.

Methods: A 9 minute LC method utilizing Thermo Scientific TurboFlow technology coupled with a Thermo Scientific TSQ Quantum Access triple quadrupole mass spectrometer.

Results: Quantitation of 30 therapeutic 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.

Introduction and Methodology

LC-MS is an efficient, sensitive, accurate and precise technique for the analysis of a large number of compounds from various classes such as antidepressants, hypnotics, stimulants, cardiacs, antihistamines and others. Implementation of TurboFlow™ technology (Figures 1-4) allows a user to minimize sample preparation and increases LC-MS sensitivity.

TurboFlow – High turbulence liquid chromatography (HTLC) is a system for online sample preparation and sample purification

High column velocities and large particles create turbulence within the column

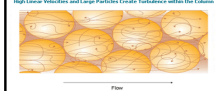


Figure 1. Large particle columns (30 µm or larger) allow high flow rate with low back pressure.

Turbulence compensates the difference in diffusion rates of small and large molecules

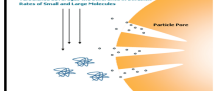


Figure 2. Small molecules diffuse into pores of particles, large molecules (from matrix) do not diffuse and are washed to waste.

Particle Chemistry: Small, Small molecules with large molecules flow to waste

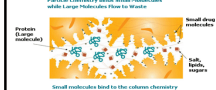


Figure 3. The separation is bimodal, since additional retention time is achieved with different column chemistries.

Figure 4. Transcend TLX™ system and TSQ Series triple quadrupole MS.



Methods

Internal Standards: Eight internal standards were used in the study: Nicotine-d₄, Cotinine-d₄, Midazolam-d₄, Diphenhydramine-d₃, Promethazine-d₃, Norfluoexetine-d₃, Chlorpromazine-d₃, Fluoxetine-d₆ for the respective compounds. To the rest of the compounds the internal standard with the closest retention time was assigned.

Sample Preparation: 100 µL of urine was diluted with 100 µL of MeOH containing internal standards in concentration of 100ng/mL. Sample was vortexed, centrifuged and 10 µL of supernatant was injected onto LC-MS system.

LC Method (Table 2):

Loading Solvent A: 10 mM Ammonium Formate in 0.1% Formic Acid

Loading Solvent B: 50 mM Ammonium Acetate

Loading Solvent C: 10 mM Ammonium Acetate in MeOH

Loading Solvent D: 0.3% formic acid in ACN:IPA:Acetone = 1:1:1 v/v/v

Eluting Solvent A: 10 mM Ammonium Acetate in 0.1% Formic Acid

Eluting Solvent B: 0.1% Formic Acid in Acetonitrile

TurboFlow Column: Thermo Scientific Cyclone MAX, 0.5 x 50 mm

Analytical Column: Thermo Scientific Hypersil GOLD PFP, 100 x 2.1 mm, 5 µm

MS Conditions:

Samples were analyzed using a TSQ Quantum Access™ triple quadrupole mass spectrometer equipped with a Thermo Scientific Ion Max ESI+ source in an SRM data acquisition mode. Two SRM transitions with scan time of 10 msec were collected for each analyte (Table 1.).

Table 1. List of SRM transitions collected for each analyte.

Class/Compound	MH+	SRM	Class/Compound	SRM	MH+
Antidepressants			Others		
Citalopram	325.1	109.2, 262.1	Dextromethorphan	272.2	215.1, 171.1
Fluoxetine*	351.1	310.2, 44.5	Topiramate	357.1	264.0, 184.1
Norfluoexetine*	337.1	296.1, 134.1	Orphenadrine	270.1	181.0, 165.0
Mirtazapine	266.1	195.1, 194.1	Lidocaine	235.1	86.3, 58.4
Paroxetine	330.1	192.1, 70.4	Phenteramine	150.1	91.3, 65.4
Sertraline	306.1	159.0, 275.0	Mesoridazine	387.2	126.2, 98.2
Trazodone	372.1	176.1, 148.1	Midazolam	326.0	291.1, 223.1
Venlafaxine	278.2	58.5, 121.2	Chlorpromazine	319.1	86.3, 58.5
Antihistamines			Sedatives		
Diphenhydramine	256.1	167.1, 165.1	Trifluoperazine	408.1	141.1, 113.1
Chlorpheniramine	275.1	230.1, 167.1	Cardiac		
Pheniramine	241.2	196.1, 167.1	Diltiazem	415.2	178.1, 150.1
Cetirizine	389.1	200.9, 165.1	Metoprolol	268.1	116.2, 191.1
Promethazine	285.1	198.1, 240.1	Verapamil	455.3	165.1, 303.2
Stimulants			Sedatives		
Nicotine	163.2	130.2, 117.2	Doxylamine	271.11	182.1, 167.1
Cotinine	177.1	80.2, 98.1	Hydroxyzine	375.2	201.0, 166.1
Internal Standards					
Nicotine-d ₄	167.2	121.1, 134.1	Chlorpromazine-d ₃	322.1	61.3, 89.2
Cotinine-d ₄	180.2	80.2, 101.1	Promethazine-d ₃	288.1	89.2, 197.9
Fluoxetine-d ₆	315.9**	44.3	Midazolam-d ₄	330.1	227.0, 295.1
Norfluoexetine-d ₃ *	343.1**	140.2	Diphenhydramine-d ₃	259.0	167.0, 165.0

*ACN adduct was used as a precursor ion. **Only one SRM transition was collected

Table 2: LC Method

Step	Start	Sec	Flow	Grad	% A	% B	% C	% D	Temp	Loop	Flow	Grad	% A	% B
1	00:00	20	1.5	Step	-	100	-	-	-----	out	0.6	Step	98	2.0
2	00:20	3	0.1	Step	-	100	-	-	-----	out	0.6	Step	98	2.0
3	00:23	60	0.2	Step	-	-	100	-	T	in	0.6	Step	98	2.0
4	01:23	30	1.5	Step	-	-	-	100	-----	in	0.6	Step	83	17
5	02:03	30	1.5	Step	-	-	-	100	-----	in	0.6	Step	65	35
6	02:33	30	1.5	Step	-	-	-	100	-----	in	0.6	Ramp	55	45
7	03:03	30	1.5	Step	-	-	50	50	-----	in	0.6	Ramp	45	55
8	03:33	30	1.5	Step	-	-	100	-	-----	in	0.6	Ramp	35	65
9	04:03	30	1.5	Step	-	-	100	-	-----	in	0.6	Ramp	25	75
10	04:33	40	1.5	Step	-	-	100	-	-----	in	0.65	Step	-	100
11	05:13	60	0.9	Step	-	-	75	25	T	in	0.05	Step	-	100
12	06:13	30	3.0	Step	40	35	25	-	-----	in	0.65	Step	-	100
13	06:43	80	3.0	Step	-	-	-	-	-----	out	0.65	Step	0	100
14	08:03	47	3.0	Step	-	-	-	-	-----	out	0.65	Step	98	2.0

Calibration Standards were prepared in house by spiking urine with analytes to concentrations of 1, 5, 10, 50, 100, 250, 500 and 1000 ng/mL.

QC samples were prepared in house by spiking urine with analytes to concentrations of 2, 15, 20, 200 and 800 ng/mL. Calibration Standards and QC samples were processed with the sample preparation procedure and analyzed with LC-MS method.

Intra assay variability was obtained by processing and analyzing 5 replicates of each QC sample.

Inter assay variability was obtained by processing and analyzing 5 replicates of each QC sample in 3 different batches.

For each analyte calibration range, limit of detection, intra- and inter-assay variability with QC samples with concentration within determined compound specific calibration range were obtained (Figures 5-6, Tables 3-5).

Results

Figure 5. Chromatographs of the lowest calibration standard

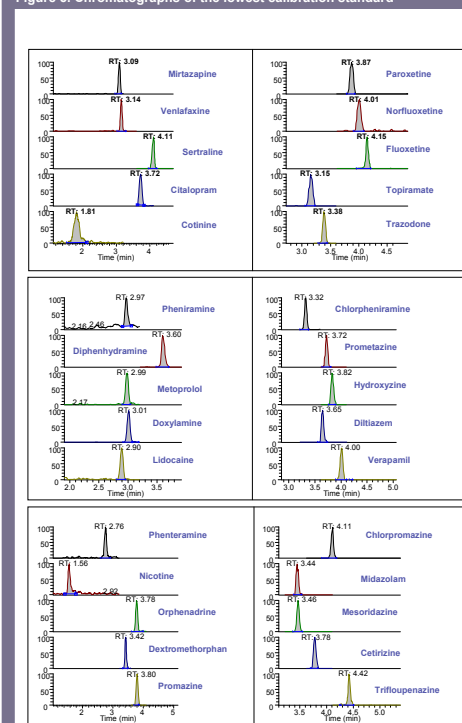


Figure 6. Examples of calibration curves

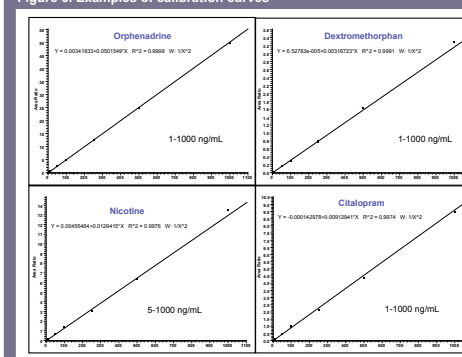


Table 3. Intra-assay variability (%RSD) for 30 analyzed compounds

Class/Compound	QC1 (2ng/mL)	QC2 (15 ng/mL)	QC3 (20 ng/mL)	QC4 (200 ng/mL)	QC5 (800 ng/mL)
Antidepressants					
Citalopram	10.7	7.6	5.2	5.1	3.4
Fluoxetine	-	-	10.4	7.0	5.7
Norfluoexetine	-	-	16.4	15.5	13.2
Mirtazapine	13.6	6.4	7.3	6.7	6.6
Paroxetine	-	-	10.2	14.0	4.3
Sertraline	-	-	8.0	4.6	3.6
Trazodone	11.7	4.3	4.2	5.1	3.8
Venlafaxine	13.6	6.6	3.1	4.3	4.3
Sedatives					
Doxylamine	14.4	4.8	8.5	10.1	8.8
Hydroxyzine	17.9	10.6	6.8	12.5	5.7
Stimulants					
Nicotine	-	10.7	8.8	6.3	3.7
Cotinine	-	12.0	12.2	7.1	4.5
Cardiacs					
Diltiazem	10.1	7.6	4.9	9.6	1.9
Metoprolol	-	10.0	6.5	3.3	2.0
Verapamil	-	8.7	5.3	7.1	6.3
Antihistamines					
Diphenhydramine	7.1	7.4	2.9	1.8	2.3
Chlorpheniramine	8.2	6.8	6.3	8.0	6.6
Pheniramine	7.6	3.3	5.3	3.0	3.2
Cetirizine	-	15.4	12.7	9.2	8.8
Promethazine	-	-	-	4.6	5.4
Others					
Dextromethorphan	8.6	5.3	3.9	3.4	3.0
Topiramate	-	-	-	13.2	9.9
Orphenadrine	7.2	4.3	2.4	3.9	2.3
Lidocaine	11.5	7.6	6.1	6.2	4.6
Phenteramine	-	11.1	5.7	3.6	3.0
Mesoridazine	-	3.4	3.4	4.9	3.4
Midazolam	14.3	5.4	5.1	5.0	3.5
Promazine	-	17.1	6.6	9.7	9.9
Chlorpromazine	-	8.0	5.6	2.1	3.6
Trifluoperazine	-	7.9	7.3	7.2	5.9

Table 4. Calibration Range and Limit of Detection (LOD) for 30 analyzed compounds

Class/Compound	Cal range (ng/mL)	LOD (ng/mL)	Class/Compound	Cal range (ng/mL)	LOD (ng/mL)
Antidepressants			Others		
Citalopram	1-1000	1	Dextromethorphan	1-1000	1
Fluoxetine	10-1000	1	Topiramate	50-1000	5
Norfluoexetine	10-1000	5	Orphenadrine	1-1000	1
Mirtazapine	1-1000	1	Lidocaine	1-1000	1
Paroxetine	10-1000	5	Phenteramine	10-1000	10
Sertraline	10-1000	1	Mesoridazine	5-1000	1
Trazodone	1-1000	1	Midazolam	1-1000	1
Venlafaxine	1-1000	1	Chlorpromazine	5-1000	5
Antihistamines			Sedatives		
Diphenhydramine	1-1000	1	Promazine	5-1000	5
Chlorpheniramine	1-1000	1	Trifluoperazine	5-1000	5
Pheniramine	1-1000	1	Cardiac		
Cetirizine	5-1000	1	Diltiazem	1-1000	1
Promethazine	50-1000	50	Metoprolol	5-1000	1
Stimulants			Sedatives		
Nicotine	5-1000	5	Doxylamine	1-1000	1
Cotinine	5-1000	1	Hydroxyzine	1-1000	1

Table 5. Inter-assay variability (%RSD) for 30 analyzed compounds

Class/Compound	QC1 (2ng/mL)	QC2 (15 ng/mL)	QC3 (20 ng/mL)	QC4 (200 ng/mL)	QC5 (800 ng/mL)
Antidepressants					
Citalopram	9.3	5.6	4.7	4.1	3.1
Fluoxetine	-	-	9.1	8.1	7.2
Norfluoexetine	-	-	11.0	13.2	9.5
Mirtazapine	12.5	5.6	5.6	6.2	7.1
Paroxetine	-	-	14.6	7.0	4.8
Sertraline	-	-	15.7	12.4	8.5
Trazodone	10.6	4.5	4.6	4.8	4.4
Venlafaxine	12.1	5.4	4.1	5.5	5.3
Sedatives					
Doxylamine	11.7	13.1	10.7	9.9	10.0
Hydroxyzine	14.0	8.5	9.0	9.3	10.3
Stimulants					
Nicotine	-	7.1	6.2	4.3	3.4
Cotinine	-	8.1	8.3	4.2	4.9
Cardiacs					
Diltiazem	10.2	8.7	5.5	6.8	6.9
Metoprolol	-	8.5	6.1	4.1	4.3
Verapamil	-	9.2	6.2	7.8	7.0
Antihistamines					
Diphenhydramine	7.1	5.8	3.5	2.3	3.1
Chlorpheniramine	7.2	12.8	10.8	11.2	11.8
Pheniramine	5.6	5.3	4.6	3.8	4.9
Cetirizine	-	15.1	12.3	9.5	9.7
Promethazine	-	-	-	4.2	5.3
Others					
Dextromethorphan	10.9	3.7	4.8	3.7	4.9
Topiramate	-	-	-	10.4	6.1
Orphenadrine	9.1	4.7	3.3	3.3	4.4
Lidocaine	9.4	5.1	5.8	5.5	5.2
Phenteramine	-	13.8	9.7	6.4	7.7
Mesoridazine	-	4.4	3.9	3.9	2.2
Midazolam	12.2	5.3	5.4	3.9	6.2
Promazine	-	10.8	10.7	11.6	12.5
Chlorpromazine	-	15.0	13.3		