Quantitative Analysis of 58 Antipsychotics and Antidepressants in Human Urine by LC-MS/MS

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Abstract & Introduction

Human mental health disorders have become modern-day living diseases and contribute significantly to worldwide morbidity and mortality. This is reflected in fast growing numbers of antipsychotics (APs) and antidepressants (ADs) entering the market and the rising rate of prescriptions. These drugs are prescribed for a wide range of psychiatric disorders such as schizophrenia, bipolar disorder, dysthymia, social anxiety disorder, obsessive-compulsive disorder, and chronic pain. In the forensic setting, due to their abusive potential, the detection of these drugs is critical in determining their involvement in intoxications and suicides. In the clinical setting, analysis of APs and ADs in blood or urine is necessary to ensure suitable therapeutic concentration and to monitor patient compliance. It has been reported that the patient's adherence to prescribed APs is quite low, and thus new clinical recommendations have been released regarding when and how to use urine testing procedures to help monitor adherence. By combining a simple sample preparation procedure and a fast chromatographic elution with the Raptor Biphenyl column, a highly specific and accurate method was established for simultaneous measurement of 28 AP and 30 AD drugs in human urine.

Methods

Table 1: Analytical Conditions for Shimadzu Nexera X2 UHPLC + SCIEX Triple Quad 4500 MS/MS

Analytical Column	Raptor Biphenyl 2.7µm, 50 mm x 3.0 mm (Restek Cat.# 9309A5E)	
Guard Column	Raptor Biphenyl EXP Guard Colum Cartridge 2.7um, 5 mm x 3.0 mm (Cat.# 9309A0253)	
Mobile Phase A	Water, 0.1% formic acid + 5mM ammonium formate	
Mobile Phase B	Methanol, 0.1% formic acid + 5mM ammonium formate	
Gradient	**Time (min)**	**%B**
	0.00	60
	0.20	60
	3.50	100
	3.51	60
	5.50	60
Flow Rate	0.6 mL/min	
Injection Volume	2 µL	
Column Temp.	30°C	
Ion Mode	Scheduled MRM in positive ESI	

Sample Preparation

Drug-free human urine (BioIVT) was fortified with 58 analytes to prepare the calibration standards and QC samples. Bupropion-D9 was used as the internal standard for quantification of all 58 compounds. The urine sample (50 µL) was mixed with 15 µL of IMCSzyme®, 20 µL of reaction buffer, and 10 µL of internal standard solution (1 µg/mL in methanol). Hydrolysis was performed at 45°C (water bath) for 30 minutes and then 400 µL of acetonitrile was added, vortexed to mix, and centrifuged at 4000 rpm for 10 minutes. The supernatant was diluted 2-fold with water and injected for analysis.

Results & Discussion

(1) Chromatographic Performance: Chromatographic carryover was initially problematic for accurate measurement of AP and AD drugs. In addition, the existence of isobaric compounds (maprotiline vs. amitriptyline; protriptyline vs. nortriptyline) is an added difficulty as chromatographic separation is required for these analytes. By specifically evaluating these issues, it was found that by rinsing the injector and needle externally and internally with a 50/50 methanol/DMSO solution coupled with a gradient elution starting with high content of organic mobile phase could greatly reduce the carryover while maintaining chromatographic separation of the isobaric compounds as shown in Figure 1. A fast, efficient separation was achieved for the simultaneous analysis of 58 analytes with a 3.5 minute gradient and a 5.5 minute total run time.

(2) Linearity: The linearity test showed that the majority of compounds (43 out of 58) could be quantified in the range of 10-2500 ng/mL with either quadratic or linear regression (1/x weighted). Additional compounds could be quantified in the range of 20-2500 ng/mL, 10-500 ng/mL, or 10-1000 ng/mL (Table 2). Due to their relatively high carryover, olanzapine and desmethylolanzapine were quantified in the range of 25-2500 and 35-2500 ng/mL, respectively. All compounds showed good linearity with r values of 0.998 or greater, and the % deviations <15%.

(3) Accuracy & Precision: Precision and accuracy was assessed with the simultaneous quantification of 58 drugs and metabolites, evaluated at 3 QC levels prepared at suitable concentrations. The method accuracy for all analytes was demonstrated with %recovery <15% of the nominal concentration for all QC levels. The %RSD was <10% demonstrating acceptable method precision.

Figure 1: Analysis of Fortified Human Urine (2500 ng/mL)

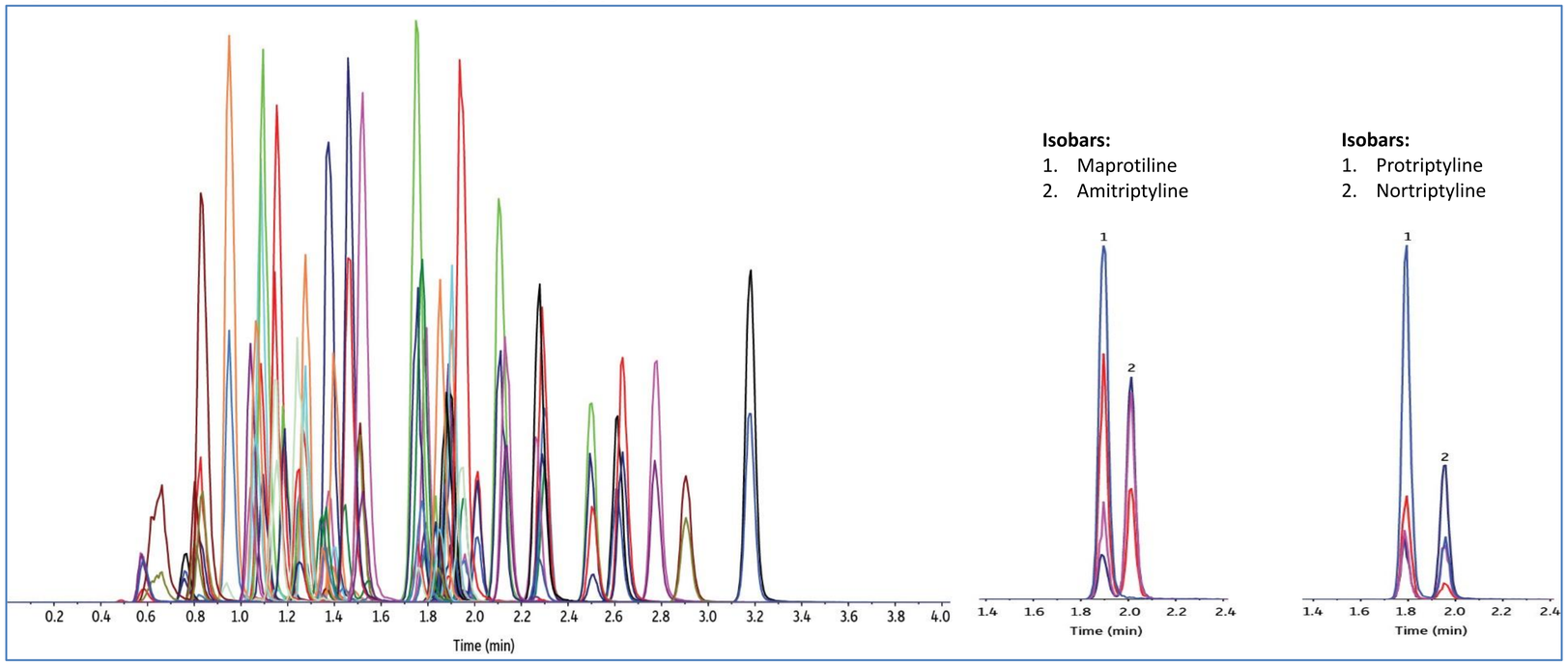

Table 2: Analyte Retention Times & Detection Range

Analyte	RT	Range (ng/mL)
Desmethylolanzapine	0.58	35 - 2500
Phenelzine sulfate	0.59	10 - 2500
Olanzapine	0.65	25 - 2500
Lamotrigine	0.76	10 - 2500
Molindone	0.81	10 - 2500
Hydroxybupropion	0.83	10 - 2500
7-Hydroxyquetiapine	0.84	10 - 2500
Bupropion-D9 (IS)	0.94	10 - 2500
Bupropion	0.95	10 - 2500
Venlafaxine	1.04	10 - 2500
Reduced haloperidol	1.07	10 - 2500
Milnacipran	1.09	10 - 2500
Desmethylmirtazapine	1.10	10 - 2500
9OH-Risperidone	1.15	10 - 2500
Mirtazapine	1.16	10 - 2500
N-desmethylclozapine	1.19	10 - 2500
Droperidol	1.24	10 - 2500
Clozapine	1.25	10 - 2500
Didesmethyl citalopram	1.26	20 - 2500
Desmethylcitalopram	1.27	10 - 2500
Escitalopram	1.28	10 - 2500
Fluvoxamine	1.35	10 - 2500
Haloperidol	1.36	10 - 2500
Norfluoxetine	1.37	20 - 2500
Isocarboxazid	1.38	10 - 2500
Fluoxetine	1.39	20 - 2500
Desmethyldoxepin	1.40	10 - 2500
Doxepin	1.45	10 - 2500
Trazodone	1.46	10 - 2500
Oxcarbazepine	1.51	10 - 2500
Risperidone	1.52	10 - 2500
Quetiapine	1.75	10 - 2500
Asenapine	1.76	20 - 2500
Ziprasidone	1.78	10 - 2500
Protriptyline	1.79	10 - 2500
Desipramine	1.83	10 - 2500
Paroxetine	1.85	10 - 500
Iloperidone	1.85	10 - 2500
Duloxetine	1.86	20 - 2500
Amoxapine	1.88	10 - 2500
Carbamazepine	1.89	10 - 2500
Maprotiline	1.90	10 - 2500
Imipramine	1.91	10 - 2500
Nortriptyline	1.95	10 - 2500
Loxapine	1.95	10 - 2500
Amitriptyline	2.01	20 - 2500
Trimipramine	2.11	10 - 2500
Pimozide	2.13	10 - 500
Chlorpromazine	2.26	10 - 2500
Dehydroaripiprazole	2.28	10 - 500
Clomipramine	2.29	10 - 2500
Sertraline	2.30	10 - 500
Fluphenazine	2.50	10 - 1000
Aripiprazole	2.51	10 - 2500
Perphenazine	2.61	10 - 2500
Trifluoperazine	2.63	10 - 500
Prochlorperazine	2.78	10 - 2500
Thiothixene	2.91	10 - 2500
Thioridazine	3.18	10 - 1000

Conclusions

It was demonstrated that simultaneous measurement of 58 AP and AD drugs and their metabolites in urine can be achieved with a simple sample preparation procedure and a fast 5.5-minute LC-MS/MS analysis using the Raptor Biphenyl column. The major carryover issue was addressed and resolved in this study with proper injection needle rinsing and LC elution conditions. The established method provides high-throughput and accurate determination for a variety of available mental health drugs, and is suitable for both clinical and forensic monitoring of AP and AD drugs in human urine.