

Rapid and Sensitive Analysis of a 93-Compound Forensic Panel in Urine



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INTRODUCTION

Liquid Chromatography coupled to Tandem Mass Spectrometry (LC-MS/MS) is a widely used analytical tool for simultaneous quantitation of multiple compounds in forensic samples. Multiple Reaction Monitoring (MRM) detection is the gold standard for quantitation purposes because of its speed, specificity and sensitivity. All these attributes are critical for quantitative analysis of a comprehensive forensic compound panel. However, as the number of analytes in a panel increases and the same total cycle time is maintained, the scanning time of each individual MRM will inevitably decrease, affecting data quality. Therefore, we have employed the Scheduled MRM™ algorithm to intelligently monitor MRMs only during the appropriate retention time windows, thus decreasing the number of concurrent MRMs monitored at any point in time, allowing both the cycle time and dwell time to remain optimal.

In this study we present a rapid, robust and sensitive analysis of a comprehensive forensic panel consisting of 93 compounds in human urine using the QTRAP®/Triple Quad™ 4500 LC-MS/MS system. Owing to the inclusion of several barbiturates in the panel which ionize preferentially in negative mode, a polarity switching method has been implemented. Due to a high number of MRM transitions (212 MRMs in total, including the internal standards) and a short LC runtime (6.5 min), a newly optimized Scheduled MRM™ algorithm is used.

MATERIALS and METHODS

Compound list and spiking solutions:

Table 1 lists all the compounds and internal standards in the panel. The total number of monitored analytes is 93 (regular panel: 72; extended panel: 21 in blue font). Internal standards are shown in grey background.

Compounds (ng/mL)	Compounds (ng/mL)	Compounds (ng/mL)	Compounds (ng/mL)	Compounds (ng/mL)			
6-AMM	1000	Galoperin	1000	Naloxone	5000	Saccharin	10000
Hydrocodone	5000	Hydrocodone	5000	N-desmethylpropofol	5000	THC-COOH	2000
Hydroxyzine	1000	Hydroxyzine	5000	Nitazepan	2000		
Asyl Fentanyl	200	Impasine	5000	Norcodeine	5000	6-AMM-d5	
Alpha-Hydroxypropizolam	5000	JWH-018 4-OH pentyl	1000	Nordazepam	5000	Alprazolam-d5	
Alpha-Hydroxypropizolam	5000	JWH-018 pentanoic acid	1000	Nortofentyl	200	Benzylegonine-d5	
Alpha-PPP	1000	JWH-073 3-OH butyl	1000	Norpropidine	5000	Carisoprodol-d7	
Alpha-PVP	1000	JWH-073 butanoic acid	1000	Norazocodone	5000	Codeine-d5	
Alprazolam	5000	JWH-081 5-OH pentyl	1000	Fentanyl-d5	10000	Fentanyl-d5	
AMA-2001 4-OH pentyl	1000	JWH-012 5-OH pentyl	1000	Nortofentyl	5000	Hydrocodone-d5	
Amphetamine	5000	JWH-012 5-OH pentyl	1000	O-desmethyltramadol	5000	Hydroxyzine-d5	
Amphetamine	1000	JWH-250 4-OH pentyl	1000	Oxazepam	5000	JWH-018 4-OH pentyl	
Benzylegonine	5000	Lorazepam	5000	Oxycodone	5000	JWH-018 5-OH butyl	
Buprenorphine	1000	MDA	10000	Oxycodone	5000	MDPV-d5	
Buprenorphine	2000	MDA	10000	PCP	2500	Mipradofedine-d4	
Carisoprodol	10000	MDMA	10000	Pregabalin	10000	Mipradofedine-d5	
Companazine	5000	MDPV	1000	Propoxyphene	10000	Mipradofedine-d7	
Codeine	5000	Meprobamate	5000	Propoxyphene	5000	Methadone-d5	
Cocaine	5000	Mephedrone	1000	RS-4 4-OH pentyl	1000	Methamphetamine-d5	
Cyclobenzaprine	5000	Meprobamate	10000	Ritalin acid	5000	Methylone-d5	
Desallylfentanyl	5000	Methadone	5000	Sufentanil	200	Methylone-d5	
Despropylfentanyl	5000	Methamphetamine	10000	Taperindol	5000	Morphine-d5	
Desmethyldiazepam	5000	Methone	10000	Temazepam	5000	Morphine-d5	
Dextromethorphan	5000	Methyone	1000	Tramadol	5000	Nortofentyl-d5	
Diazepam	5000	Methylphenidate	5000	Zolpidem	5000	Oxycodone-d5	
Dihydrocodone	5000	Midazolam	5000	Amobarbital/pentobarbital	10000	Dipropionyl-d5	
Doxin	5000	Mirtazapine	1000	Butabital	10000	THC-COOH-d5	
EDOP	10000	Morphine	5000	Butabital	5000	Subabital-d5	
Fentanyl	200	Naloxone	5000	Phenobarbital	10000	Saccharin-d5	

Blue font: extended panel. Grey background: IS.

Table 1. List of analytes and internal standards, and their concentrations in spiking solution.

Sample preparation

Blank human urine was used to prepare calibrators. Urine sample was hydrolyzed at 55°C. After hydrolysis, methanol and water were added to the mixture. The mixture was then centrifuged and the supernatant was transferred to glass vial for LC-MS/MS analysis.

LC-MS/MS

Phenomenex Kinetex Phenyl-hexyl column were used. Mobile phase A (MPA) was ammonium formate in water and mobile phase B (MPB) was formic acid in methanol. The LC flowrate was 1 mL/min and the LC runtime was 6.5 min. Injection volume was 5 µL.

Data acquisition was done with Analyst 1.6.3 using Scheduled MRM™ and polarity switch. Table 2 shows the MRMs in the method (212 in total).

Analyte	Q1	Q3	RT	SP	CP	Q1	Q3	RT	SP	CP
Alprazolam-d5	146.1	101	1.7	20	11	146.1	101	1.7	20	11
Alprazolam-d5	231.1	152.2	2.27	10	20	231.1	152.2	2.27	10	20
Alprazolam-d5	267.1	186	2.39	10	12	267.1	186	2.39	10	12
Alprazolam-d5	267.1	186	2.39	10	12	267.1	186	2.39	10	12
Alprazolam-d5	342.1	201	2.59	10	10	342.1	201	2.59	10	10
Alprazolam-d5	342.1	201	2.59	10	10	342.1	201	2.59	10	10
Alprazolam-d5	381.1	239	2.71	10	10	381.1	239	2.71	10	10
Alprazolam-d5	381.1	239	2.71	10	10	381.1	239	2.71	10	10
Alprazolam-d5	462.1	276	2.91	10	10	462.1	276	2.91	10	10
Alprazolam-d5	462.1	276	2.91	10	10	462.1	276	2.91	10	10
Alprazolam-d5	543.1	321	3.11	10	10	543.1	321	3.11	10	10
Alprazolam-d5	543.1	321	3.11	10	10	543.1	321	3.11	10	10
Alprazolam-d5	624.1	366	3.31	10	10	624.1	366	3.31	10	10
Alprazolam-d5	624.1	366	3.31	10	10	624.1	366	3.31	10	10
Alprazolam-d5	705.1	411	3.51	10	10	705.1	411	3.51	10	10
Alprazolam-d5	705.1	411	3.51	10	10	705.1	411	3.51	10	10
Alprazolam-d5	786.1	456	3.71	10	10	786.1	456	3.71	10	10
Alprazolam-d5	786.1	456	3.71	10	10	786.1	456	3.71	10	10
Alprazolam-d5	867.1	501	3.91	10	10	867.1	501	3.91	10	10
Alprazolam-d5	867.1	501	3.91	10	10	867.1	501	3.91	10	10
Alprazolam-d5	948.1	546	4.11	10	10	948.1	546	4.11	10	10
Alprazolam-d5	948.1	546	4.11	10	10	948.1	546	4.11	10	10
Alprazolam-d5	1029.1	591	4.31	10	10	1029.1	591	4.31	10	10
Alprazolam-d5	1029.1	591	4.31	10	10	1029.1	591	4.31	10	10
Alprazolam-d5	1110.1	636	4.51	10	10	1110.1	636	4.51	10	10
Alprazolam-d5	1110.1	636	4.51	10	10	1110.1	636	4.51	10	10
Alprazolam-d5	1191.1	681	4.71	10	10	1191.1	681	4.71	10	10
Alprazolam-d5	1191.1	681	4.71	10	10	1191.1	681	4.71	10	10
Alprazolam-d5	1272.1	726	4.91	10	10	1272.1	726	4.91	10	10
Alprazolam-d5	1272.1	726	4.91	10	10	1272.1	726	4.91	10	10
Alprazolam-d5	1353.1	771	5.11	10	10	1353.1	771	5.11	10	10
Alprazolam-d5	1353.1	771	5.11	10	10	1353.1	771	5.11	10	10
Alprazolam-d5	1434.1	816	5.31	10	10	1434.1	816	5.31	10	10
Alprazolam-d5	1434.1	816	5.31	10	10	1434.1	816	5.31	10	10
Alprazolam-d5	1515.1	861	5.51	10	10	1515.1	861	5.51	10	10
Alprazolam-d5	1515.1	861	5.51	10	10	1515.1	861	5.51	10	10
Alprazolam-d5	1596.1	906	5.71	10	10	1596.1	906	5.71	10	10
Alprazolam-d5	1596.1	906	5.71	10	10	1596.1	906	5.71	10	10
Alprazolam-d5	1677.1	951	5.91	10	10	1677.1	951	5.91	10	10
Alprazolam-d5	1677.1	951	5.91	10	10	1677.1	951	5.91	10	10
Alprazolam-d5	1758.1	996	6.11	10	10	1758.1	996	6.11	10	10
Alprazolam-d5	1758.1	996	6.11	10	10	1758.1	996	6.11	10	10
Alprazolam-d5	1839.1	1041	6.31	10	10	1839.1	1041	6.31	10	10
Alprazolam-d5	1839.1	1041	6.31	10	10	1839.1	1041	6.31	10	10
Alprazolam-d5	1920.1	1086	6.51	10	10	1920.1	1086	6.51	10	10
Alprazolam-d5	1920.1	1086	6.51	10	10	1920.1	1086	6.51	10	10
Alprazolam-d5	2001.1	1131	6.71	10	10	2001.1	1131	6.71	10	10
Alprazolam-d5	2001.1	1131	6.71	10	10	2001.1	1131	6.71	10	10
Alprazolam-d5	2082.1	1176	6.91	10	10	2082.1	1176	6.91	10	10
Alprazolam-d5	2082.1	1176	6.91	10	10	2082.1	1176	6.91	10	10
Alprazolam-d5	2163.1	1221	7.11	10	10	2163.1	1221	7.11	10	10
Alprazolam-d5	2163.1	1221	7.11	10	10	2163.1	1221	7.11	10	10
Alprazolam-d5	2244.1	1266	7.31	10	10	2244.1	1266	7.31	10	10
Alprazolam-d5	2244.1	1266	7.31	10	10	2244.1	1266	7.31	10	10
Alprazolam-d5	2325.1	1311	7.51	10	10	2325.1	1311	7.51	10	10
Alprazolam-d5	2325.1	1311	7.51	10	10	2325.1	1311	7.51	10	10
Alprazolam-d5	2406.1	1356	7.71	10	10	2406.1	1356	7.71	10	10
Alprazolam-d5	2406.1	1356	7.71	10	10	2406.1	1356	7.71	10	10
Alprazolam-d5	2487.1	1401	7.91	10	10	2487.1	1401	7.91	10	10
Alprazolam-d5	2487.1	1401	7.91	10	10	2487.1	1401	7.91	10	10
Alprazolam-d5	2568.1	1446	8.11	10	10	2568.1	1446	8.11	10	10
Alprazolam-d5	2568.1	1446	8.11	10	10	2568.1	1446	8.11	10	10
Alprazolam-d5	2649.1	1491	8.31	10	10	2649.1	1491	8.31	10	10
Alprazolam-d5	2649.1	1491	8.31	10	10	2649.1	1491	8.31	10	10
Alprazolam-d5	2730.1	1536	8.51	10	10	2730.1	1536	8.51	10	10
Alprazolam-d5	2730.1	1536	8.51	10	10	2730.1	1536	8.51	10	10
Alprazolam-d5	2811.1	1581	8.71	10	10	2811.1	1581	8.71	10	10
Alprazolam-d5	2811.1	1581	8.71	10	10	2811.1	1581	8.71	10	10
Alprazolam-d5	2892.1	1626	8.91	10	10	2892.1	1626	8.91	10	10
Alprazolam-d5	2892.1	1626	8.91	10	10	2892.1	1626	8.91	10	10
Alprazolam-d5	2973.1	1671	9.11	10	10	2973.1	1671	9.11	10	10
Alprazolam-d5	2973.1	1671	9.11	10	10	2973.1	1671	9.11	10	10
Alprazolam-d5	3054.1	1716	9.31	10	10	3054.1	1716	9.31	10	10
Alprazolam-d5	3054.1	1716	9.31							