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Forensic

Rapid and Sensitive Analysis of a 93-Compound Forensic Panel in Urine using the QTRAP®/Triple Quad™ 4500 LC-MS/MS System

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Overview

In this technical note, we describe a rapid and sensitive analysis of a comprehensive panel of forensic compounds in human urine using the ExionLC™ AC HPLC system and the QTRAP®/Triple Quad™ 4500 LC-MS/MS system (Figure 1). This forensic panel contains 93 compounds, and a total of 212 MRM transitions (including internal standards) are monitored. Some compounds ionize preferentially in positive mode, while others ionize preferentially in negative mode; therefore this method takes advantage of rapid polarity switching. The total LC runtime is 6.5 minutes, which can be further accelerated for a smaller panel of compounds. Sample preparation is based on enzymatic hydrolysis and a simple "dilute and shoot" methodology.

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.

There are challenges associated with the monitoring of large numbers of compounds in a single MRM method. Ideally, cycle times should remain as small as possible, to ensure that a large number of data points are acquired across each LC peak. However, as the number of analytes in a panel increases, the cycle time increases accordingly. In order to maintain the desired cycle time, one may choose to decrease the dwell times for individual MRM transitions, however this will inevitably decrease 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. The Scheduled MRM™ algorithm has been re-optimized in the Analyst 1.6.3 software to provide superior data quality at high levels of MRM multiplexing (Figure 2).

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.
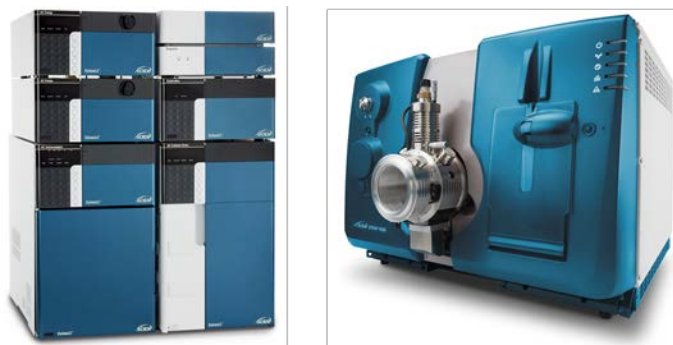

Figure 1: The SCIEX ExionLC™ AC HPLC system (left) and the SCIEX QTRAP® 4500 LC-MS/MS System (right).
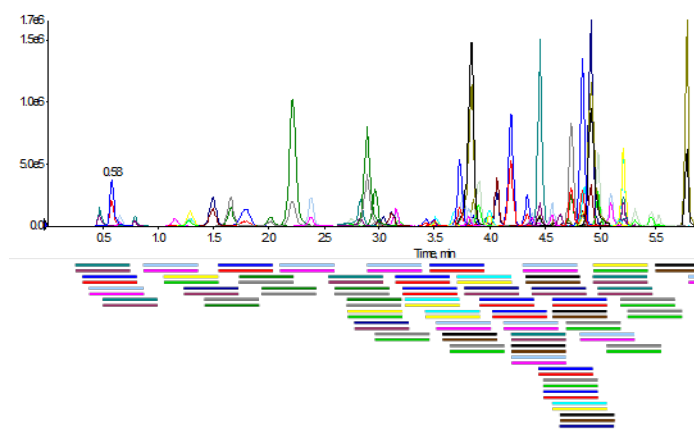

Figure 2: The Scheduled MRM™ algorithm allows the detection of many more MRMs using fast LC while maintaining optimal cycle time and dwell times.

Materials and Methods

Compound list and spiking solutions

Table 1 lists the 93 compounds plus internal standards in the extended panel. All were procured from Cerilliant Corporation (Round Rock, TX). Two spiking solutions in methanol were prepared: one for analytes (**SA**) and the other for internal standards (**SIS**). Concentrations of all the analytes in the spiking solution **SA** are listed in Table 1. Compounds in black font are in the regular panel (72 analytes) and the ones in blue font are the additional 21 analytes in the extended panel (93 analytes). Internal standards are shown in grey background.

Calibrator preparation

Blank human urine was spiked with solution **SA** to prepare calibrators. Four levels of calibrators were prepared. Actual concentrations varied for each compound, however the concentration ratio between these calibrators was always (in descending order): 20:6:2:1. For instance, the four different concentrations (in descending order) for fentanyl in the calibrators were: 20, 6, 2 and 1 ng/mL, while those of gabapentin were: 1000, 300, 100 and 50 ng/mL.

Sample preparation

1. 100 μL urine sample was mixed with 25 μL IMCS Rapid Hydrolysis Buffer, 20 μL IMCSzyme and 10 μL **SIS**. Both IMCS Rapid Hydrolysis Buffer and IMCSzyme were acquired from IMCS (Columbia, SC). Hydrolysis time was typically between 30 and 60 min at 55°C.

2. After hydrolysis was complete, 0.2 mL methanol and 0.625 mL water were added to the mixture.

3. The mixture was then centrifuged at 21,000 *g* for 10 min.

4. The supernatant was transferred to a glass vial with insert for analysis by LC-MS/MS.

Liquid Chromatography

Liquid Chromatography analysis was performed on the SCIEX ExionLC™ AC HPLC system at 30°C. Separation was achieved using a Phenomenex Kinetex Phenyl-Hexyl column (50 × 4.6 mm, 2.6 μm, 00B-4495-E0), with a Phenomenex SecurityGuard ULTRA UHPLC Phenyl (AJ0-8774) and ULTRA holder (AJ0-9000). Mobile phase A (MPA) was ammonium formate in water. Mobile phase B (MPB) was formic acid in methanol. The LC flow rate was 1 mL/min and the LC run-time was 6.5 min. Injection volume was 5 μL.

For the autosampler, the needle rinse solution was methanol:acetonitrile:isopropanol (1:1:3, v/v/v). The Rinse sequence was as follows:

- Rinsing volume: 1 mL
- Needle stroke: 54 mm
- Rinsing speed: 35 μL/sec
- Sampling speed: 15 μL/sec
- Rinse dip time: 5 sec
- Rinse mode: before and after aspiration.

MS and MS/MS condition

- Curtain gas (CUR): 30
- Collision gas (CAD): Medium
- IonSpray Voltage (IS): 2500 V (positive) and -4500 V (negative)
- Temperature (TEM): 650°C
- Ion Source Gas 1 (GS1): 60
- Ion Source Gas 2 (GS2): 50

The Declustering Potential (DP), Collision Energy (CE), and Collision Cell Exit Potential (CXP) voltages were optimized for each individual component, as described in Table 2 below.

General scan timings for Scheduled MRM™ algorithm and MRM table

- Target Scan Time (positive) = 0.2 sec
- Target Scan Time (negative) = 0.05 sec
- MRM detection window = 20 sec

Results and Discussion

Sample preparation

IMCSzyme was selected as the hydrolysis enzyme for this study. Using this enzyme, the hydrolysis of glucuronide-conjugated analytes was completed in a shorter time frame compared to other traditional beta-glucuronidase enzymes. Furthermore, less interferences were observed when using IMCSzyme, which proved beneficial for LC column life and MS maintenance.

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Table 1: List of analytes and internal standards, and their concentrations in spiking solution (for preparation of calibrators)

Compounds	(ng/mL)	Compounds	(ng/mL)	Compounds	(ng/mL)	Compounds	(ng/mL)
6-MAM	1000	Gabapentin	10000	Naltrexone	5000	Secobarbital	10000
7-Aminoclonazepam	5000	Hydrocodone	5000	N-desmethyltapentadol	5000	THC-COOH	2000
7-Hydroxymitragynine	1000	Hydromorphone	5000	Norbuprenorphine	2000		
Acetyl Fentanyl	200	Imipramine	5000	Norcodeine	5000	6-MAM-d3	
Alpha-Hydroxyalprazolam	5000	JWH-018 4-OH pentyl	1000	Nordiazepam	5000	Amphetamine-d5	
Alpha-Hydroxymidazolam	5000	JWH-018 pentanoic acid	1000	Norfentanyl	200	Benzoylecgonine-d3	
Alpha-Hydroxytriazolam	5000	JWH-019 6-OH hexyl	1000	Norhydrocodone	5000	Buprenorphine-d4	
Alpha-PPP	1000	JWH-073 3-OH butyl	1000	Normeperidine	5000	Carisoprodol-d7	
Alpha-PVP	1000	JWH-073 butanoic acid	1000	Noroxycodone	5000	Codeine-d6	
Alprazolam	5000	JWH-081 5-OH pentyl	1000	Norpropoxyphene	10000	Fentanyl-d5	
AM-2201 4-OH pentyl	1000	JWH-122 5-OH pentyl	1000	Nortriptyline	5000	Hydrocodone-d6	
Amitriptyline	5000	JWH-210 5-OH pentyl	1000	O-Desmethyltramadol	5000	Hydromorphone-d6	
Amphetamine	10000	JWH-250 4-OH pentyl	1000	Oxazepam	5000	JWH 018 4-OH pentyl-d5	
Benzoylecgonine	5000	Lorazepam	5000	Oxycodone	5000	JWH 019 6-OH hexyl-d5	
Buphedrone	1000	MDA	10000	Oxymorphone	5000	MDPV-d8	
Buprenorphine	2000	MDEA	10000	PCP	2500	Meperidine-d4	
Carisoprodol	10000	MDMA	10000	Pregabalin	10000	Mephedrone-d3	
Clomipramine	5000	MDPV	1000	Propoxyphene	10000	Meprobamate-d7	
Codeine	5000	Meperidine	5000	Protriptyline	5000	Methadone-d3	
Cotinine	5000	Mephedrone	1000	RCS4-4-OH-pentyl	1000	Methamphetamine-d5	
Cyclobenzaprine	5000	Meprobamate	10000	Ritalinic Acid	5000	Methylone-d3	
Desalkylflurazepam	5000	Methadone	10000	Sufentanil	200	Mitragynine-d3	
Desipramine	5000	Methamphetamine	10000	Tapentadol	5000	Morphine-d6	
Desmethyldoxepin	5000	Methedrone	1000	Temazepam	5000	Nordiazepam-d5	
Dextromethorphan	5000	Methylone	1000	Tramadol	5000	Nortriptyline-d3	
Diazepam	5000	Methylphenidate	5000	Zolpidem	5000	Oxycodone-d6	
Dihydrocodeine	5000	Midazolam	5000	Amobarbital/pentobarbital	10000	Oxymorphone-d3	
Doxepin	5000	Mitragynine	1000	Butabarbital	10000	THC-COOH-d3	
EDDP	10000	Morphine	5000	Butalbital	10000	Butalbital-d5	
Fentanyl	200	Naloxone	5000	Phenobarbital	10000	Secobarbital-d5	

Blue font: extended panel.   Grey background: IS.

Table 2-1: MRM Table (part 1)

Analyte	Q1	Q3	RT (min)	DP	EP	CE	CXP
6-MAM-d3 IS	331.1	165	1.79	100	10	49	8
Amphetamine-d5 IS	141.1	93	1.7	30	10	21	8
Benzoylecgonine-d3 IS	293.1	171.2	2.17	80	10	25	12
Buprenorphine-d4 IS	472.3	400.2	2.77	120	10	52	18
Carisoprodol-d7 IS	267.1	180	2.99	35	10	11	12
Codeine-d6 IS	306.2	152.2	1.6	100	10	83	10
Fentanyl-d5 IS	342.3	105.1	2.59	90	10	50	8
Hydrocodone-d6 IS	306.2	202.1	1.85	100	10	39	14
Hydromorphone-d6 IS	292.1	185.1	1.2	100	10	39	12
JWH 018 4-OH pentyl-d5 IS	363.1	155.1	4.44	100	10	35	14
JWH 019 6-OH hexyl-d5 IS	377.2	155.1	4.42	90	10	27	12
MDPV-d8 IS	284.1	134.1	2.28	70	10	27	15
Meperidine-d4 IS	252.2	224.1	2.26	86	10	29	16
Mephedrone-d3 IS	181.1	148.1	1.98	46	10	26	6
Meprobamate-d7 IS	226.1	165.1	2.33	26	10	11	8
Methadone-d3 IS	313.2	105.1	3.29	60	10	35	14
Methamphetamine-d5 IS	155.2	92	1.84	45	10	25	6
Methylone-d3 IS	211.1	163.1	1.7	60	10	25	12
Mitragynine-d3 IS	402.2	174.1	2.81	71	10	40	12
Morphine-d6 IS	292.1	152	1.01	90	10	75	12
Nordiazepam-d5 IS	276.1	140	4	95	10	40	10
Nortriptyline-d3 IS	267.1	233.1	3.45	56	10	20	14
Oxycodone-d6 IS	322.1	247.1	1.79	75	10	40	8
Oxymorphone-d3 IS	305.1	230.1	1.1	90	10	40	8
PCP-d5 IS	249.3	96.1	2.5	56	10	41	8
6-MAM 1	328.1	165	1.8	100	10	48	6
6-MAM 2	328.1	211.1	1.8	100	10	34	8
7-Aminoclonazepam 1	286.1	121.1	2.27	80	10	37	13
7-Aminoclonazepam 2	286.1	250.1	2.27	80	10	29	13
7-Hydroxymitragynine 1	415.2	190.1	2.4	85	10	50	8
7-Hydroxymitragynine 2	415.2	226.1	2.4	85	10	30	10
Acetyl fentanyl 1	323.1	188.1	2.38	90	10	31	10
Acetyl fentanyl 2	323.1	105.1	2.38	90	10	50	8
Alpha-Hydroxyalprazolam 1	325.1	297.1	3.5	60	10	40	15
Alpha-Hydroxyalprazolam 2	327.1	299.1	3.5	60	10	35	15
Alpha-Hydroxymidazolam 1	342.1	203.1	3.66	60	10	36	10

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Table 2-2: MRM Table (part 2)

Analyte	Q1	Q3	RT (min)	DP	EP	CE	CXP
Alpha-Hydroxymidazolam 2	342.1	168.1	3.66	60	10	53	11
Alpha-Hydroxytriazolam 1	359.1	331.1	3.24	90	10	37	12
Alpha-Hydroxytriazolam 2	359.1	239.1	3.24	90	10	60	12
alpha-PPP 1	204.1	105	1.86	80	10	30	9
alpha-PPP 2	204.1	133	1.86	80	10	26	14
alpha-PVP 1	232.1	91	2.21	80	10	30	10
alpha-PVP 2	232.1	126.1	2.21	80	10	32	12
Alprazolam 1	309.1	281.1	3.72	80	10	35	10
Alprazolam 2	309.1	205.1	3.72	80	10	50	10
AM 2201 4-OH pentyl 1	376.1	155.1	4.42	65	10	31	10
AM 2201 4-OH pentyl 2	376.1	127.1	4.42	65	10	71	8
Amitriptyline 1	278.1	233.1	3.38	70	10	23	12
Amitriptyline 2	278.1	91	3.38	70	10	27	7
Amphetamine 1	136	119	1.71	40	10	12	10
Amphetamine 2	136	91	1.71	40	10	40	8
Benzoylecgonine 1	290.1	168	2.17	80	10	37	12
Benzoylecgonine 2	290.1	105	2.17	80	10	40	8
Buphedrone 1	178.1	131.1	1.87	50	10	28	11
Buphedrone 2	178.1	132.1	1.87	50	10	22	12
Buprenorphine 1	468.3	55	2.8	120	10	85	8
Buprenorphine 2	468.3	414.2	2.8	120	10	46	18
Carisoprodol 1	261.1	176	2.97	35	10	11	12
Carisoprodol 2	261.1	97	2.97	35	10	24	7
Clomipramine 1	315.1	86.1	3.95	26	10	23	12
Clomipramine 2	315.1	58.1	3.95	26	10	67	16
Codeine 1	300.1	215.1	1.62	100	10	35	12
Codeine 2	300.1	165	1.62	100	10	50	12
Cotinine 1	177	80	1.78	70	10	28	8
Cotinine 2	177	98	1.78	70	10	25	9
Cyclobenzaprine 1	276.1	215.1	3.2	80	10	58	12
Cyclobenzaprine 2	276.1	216.1	3.2	80	10	34	12
Desalkylflurazepam 1	289.1	140	3.61	90	10	40	10
Desalkylflurazepam 2	289.1	226	3.61	90	10	38	16
Desipramine 1	267.1	72	3.28	70	10	50	8
Desipramine 2	267.1	193	3.28	70	10	50	12
Desmethyldoxepin 1	266.1	107	2.82	70	10	29	8

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Table 2-3: MRM Table (part 3)

Analyte	Q1	Q3	RT (min)	DP	EP	CE	CXP
Desmethyldoxepin 2	266.1	235.1	2.82	70	10	21	10
Dextromethorphan 1	272.1	171.1	2.69	70	10	50	12
Dextromethorphan 2	272.1	215.1	2.69	70	10	30	14
Diazepam 1	285.1	193	4.27	80	10	40	10
Diazepam 2	285.1	154	4.27	80	10	35	8
Dihydrocodeine 1	302.1	199.1	1.57	95	10	44	14
Dihydrocodeine 2	302.1	201.1	1.57	95	10	40	14
Doxepin 1	280.1	107	2.81	70	10	45	10
Doxepin 2	280.1	115	2.81	70	10	60	8
EDDP 1	278.1	234.1	2.71	80	10	50	12
EDDP 2	278.1	186	2.71	80	10	46	10
Fentanyl 1	337.1	188.1	2.6	90	10	31	10
Fentanyl 2	337.1	105.1	2.6	90	10	50	8
Gabapentin 1	172.1	137	1.32	50	10	20	10
Gabapentin 2	172.1	95	1.32	50	10	30	8
Hydrocodone 1	300.1	199	1.87	100	10	50	12
Hydrocodone 2	300.1	128	1.87	100	10	70	10
Hydromorphone 1	286.1	185	1.21	100	10	39	10
Hydromorphone 2	286.1	157	1.21	100	10	51	8
Imipramine 1	281.1	86	3.28	80	10	25	6
Imipramine 2	281.1	58	3.28	80	10	51	6
JWH 018 4-OH pentyl 1	358.1	155.1	4.45	100	10	37	14
JWH 018 4-OH pentyl 2	358.1	127.1	4.45	100	10	63	10
JWH 018 Pentanoic acid 1	372.1	155.1	4.48	90	10	33	14
JWH 018 Pentanoic acid 2	372.1	127.1	4.48	90	10	71	10
JWH 019 6-OH hexyl 1	372.2	155.1	4.57	90	10	27	12
JWH 019 6-OH hexyl 2	372.2	127.1	4.57	90	10	69	10
JWH 073 3-OH butyl 1	344.1	155.1	4.39	100	10	33	14
JWH 073 3-OH butyl 2	344.1	127.1	4.39	100	10	70	8
JWH 073 Butanoic acid 1	358.2	155.1	4.38	90	10	33	10
JWH 073 Butanoic acid 2	358.2	127.1	4.38	90	10	65	10
JWH 081 5-OH-pentyl 1	388.1	185.1	4.4	100	10	31	18
JWH 081 5-OH-pentyl 2	388.1	114.1	4.4	100	10	100	12
JWH 122 5-OH pentyl 1	372.1	169.1	4.59	90	10	32	14
JWH 122 5-OH pentyl 2	372.1	141.1	4.59	90	10	40	12
JWH 210 5-OH-pentyl 1	386.1	183.1	4.71	100	10	45	18

Table 2-4: MRM Table (part 4)

Analyte	Q1	Q3	RT (min)	DP	EP	CE	CXP
JWH 210 5-OH-pentyl 2	386.1	155.1	4.71	100	10	41	6
JWH 250-4-OH pentyl 1	352.1	121.1	4.25	90	10	35	12
JWH 250-4-OH pentyl 2	352.1	91	4.25	90	10	65	8
Lorazepam 1	321.1	275.1	3.4	80	10	40	10
Lorazepam 2	321.1	229.1	3.4	80	10	40	10
MDA 1	180.1	105	1.87	40	10	30	8
MDA 2	180.1	133.1	1.87	40	10	25	10
MDEA 1	208.1	163.1	2.08	100	10	20	12
MDEA 2	208.1	105.1	2.08	100	10	35	8
MDMA 1	194	163	1.97	100	10	20	10
MDMA 2	194	135	1.97	100	10	26	10
MDPV 1	276.1	175.1	2.29	70	10	30	8
MDPV 2	276.1	205.1	2.29	70	10	25	10
Meperidine 1	248.1	220.1	2.26	86	10	28	10
Meperidine 2	248.1	174	2.26	86	10	26	10
Mephedrone 1	178.1	145.1	2.02	46	10	26	6
Mephedrone 2	178.1	144.1	2.02	46	10	38	10
Meprobamate 1	219	158	2.33	26	10	11	8
Meprobamate 2	219	97.2	2.33	26	10	19	6
Methadone 1	310.1	265	3.3	80	10	25	18
Methadone 2	310.1	105.1	3.3	80	10	33	14
Methamphetamine 1	150	119	1.85	60	10	15	8
Methamphetamine 2	150	91	1.85	60	10	45	6
Methedrone 1	194.1	161.1	1.88	50	10	26	6
Methedrone 2	194.1	146.1	1.88	50	10	36	6
Methylone 1	208.1	160.1	1.7	60	10	25	12
Methylone 2	208.1	132.1	1.7	60	10	32	14
Methylphenidate 1	234.1	84.1	2.21	31	10	60	8
Methylphenidate 2	234.1	56	2.21	31	10	65	8
Midazolam 1	326.1	291.1	3.66	101	10	45	22
Midazolam 2	326.1	249.1	3.66	101	10	49	18
Mitragynine 1	399.2	174.1	2.79	71	10	59	12
Mitragynine 2	399.2	159.1	2.79	71	10	37	12
Morphine 1	286	152	1.02	90	10	75	12
Morphine 2	286	165	1.02	90	10	50	12
Naloxone 1	328.1	212.1	1.6	90	10	49	14

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Table 2-5: MRM Table (part 5)

Analyte	Q1	Q3	RT (min)	DP	EP	CE	CXP
Naloxone 2	328.1	253.1	1.6	90	10	35	18
Naltrexone 1	342.1	267.2	1.75	86	10	39	18
Naltrexone 2	342.1	282.1	1.75	86	10	37	20
N-desmethyl-Tapentadol 1	208.1	107	2.18	120	10	30	10
N-desmethyl-Tapentadol 2	208.1	121	2.18	120	10	25	10
Norbuprenorphine 1	414.3	55	2.43	141	10	90	12
Norbuprenorphine 2	414.3	83	2.43	141	10	70	12
Norcodeine 1	286.1	152	1.53	80	10	75	10
Norcodeine 2	286.1	165	1.53	80	10	58	10
Nordiazepam 1	271.1	140.1	4.03	71	10	37	10
Nordiazepam 2	271.1	165.1	4.03	71	10	37	14
Norfentanyl 1	233.1	84.1	2.12	50	10	23	8
Norfentanyl 2	233.1	150.1	2.12	50	10	23	12
Norhydrocodone 1	286.1	199	1.83	70	10	39	11
Norhydrocodone 2	286.1	241.1	1.83	70	10	32	11
Normeperidine 1	234.1	160	2.27	45	10	30	14
Normeperidine 2	234.1	42	2.27	45	10	45	6
Noroxycodone 1	302.1	187.1	1.77	40	10	33	10
Noroxycodone 2	302.1	227.1	1.77	40	10	40	11
Norpropoxyphene 1	308.1	100	2.85	50	10	18	10
Norpropoxyphene 2	308.1	44	2.85	50	10	50	6
Nortriptyline 1	264.1	233.1	3.39	56	10	17	4
Nortriptyline 2	264.1	117.1	3.39	56	10	25	4
O-Desmethyltramadol 1	250.1	58.1	1.8	55	10	97	19
O-Desmethyltramadol 2	250.1	42	1.8	55	10	97	4
Oxazepam 1	287.1	241.1	3.55	76	10	31	18
Oxazepam 2	287.1	269.2	3.55	76	10	24	22
Oxycodone 1	316.1	241.1	1.79	75	10	38	8
Oxycodone 2	316.1	256.1	1.79	75	10	33	8
Oxymorphone 1	302	227.1	1.1	86	10	39	18
Oxymorphone 2	302	198.1	1.1	86	10	57	14
PCP 1	244.1	159.1	2.5	56	10	19	10
PCP 2	244.1	91.1	2.5	56	10	41	8
Pregabalin 1	160.1	55.1	1.27	58	10	31	8
Pregabalin 2	160.1	97.1	1.27	58	10	19	8
Propoxyphene 1	340.1	266.1	3.05	46	10	20	10

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Table 2-6: MRM Table (part 6)

Analyte	Q1	Q3	RT (min)	DP	EP	CE	CXP
Propoxyphene 2	340.1	91	3.05	46	10	60	8
Protriptyline 1	264.1	155	3.2	76	10	29	10
Protriptyline 2	264.1	191	3.2	76	10	43	16
RCS4-4-OH-pentyl 1	338.2	135	4.16	90	10	40	8
RCS4-4-OH-pentyl 2	338.2	77	4.16	90	10	75	8
Ritalinic Acid 1	220.1	84	2.07	50	10	60	8
Ritalinic Acid 2	220.1	56	2.07	50	10	62	8
Sufentanil 1	387.1	238	3.01	46	10	27	16
Sufentanil 2	387.1	111.1	3.01	46	10	49	10
Tapentadol 1	222.1	107	2.19	100	10	40	8
Tapentadol 2	222.1	121	2.19	100	10	26	8
Temazepam 1	301.1	255.1	3.9	70	10	50	8
Temazepam 2	301.1	177.1	3.9	70	10	50	10
Tramadol 1	264.1	58.1	2.17	66	10	103	8
Tramadol 2	264.1	42.2	2.17	66	10	103	6
Zolpidem 1	308.1	235.1	2.49	85	10	60	12
Zolpidem 2	308.1	219	2.49	40	10	77	6
THC-COOH-d3 IS	346.1	302.1	4.73	-110	-10	-28	-11
Butalbital-d5 IS	228.1	42	2.74	-65	-10	-40	-7
Secobarbital-d5 IS	242.1	42	3.06	-90	-10	-40	-7
Amobarbital/Pentobarbital 1	225.1	42	2.86	-60	-10	-40	-7
Amobarbital/Pentobarbital 2	225.1	182	2.86	-60	-10	-18	-10
Butabarbital 1	211.1	42	2.53	-60	-10	-40	-7
Butabarbital 2	211.1	168	2.53	-60	-10	-18	-9
Butalbital 1	223.1	42	2.6	-65	-10	-40	-7
Butalbital 2	223.1	180	2.6	-65	-10	-16	-10
Phenobarbital 1	231.1	42.1	2.42	-70	-10	-40	-7
Phenobarbital 2	231.1	188	2.42	-70	-10	-14	-10
Secobarbital 1	237.1	42.1	3.06	-90	-10	-40	-7
Secobarbital 2	237.1	194.1	3.06	-77	-10	-17	-10
THC-COOH 1	343.1	299.1	4.73	-110	-10	-28	-11
THC-COOH 2	343.1	245.1	4.73	-110	-10	-36	-9

Blue font: negative mode.

LC performance

A Phenomenex Kinetex Phenyl-Hexyl column (50 × 4.6 mm, 2.6 μm) was used for LC separation. A guard column was used for LC column protection. Using a rapid 6.5-minute LC gradient, we achieved separation of various isobaric compounds in the panel. In addition, the size and dimension of this column yielded less than 3000 psi column pressure at 1 mL/min flow rate throughout the gradient while still maintaining the narrow LC peak width. Under these conditions, at 20% organic content in the injected solution, we did not notice any peak front-tailing for those early eluters including gabapentin, morphine and oxymorphone. Figure 3 shows the elution profile of all the compounds, in which the top trace shows the extracted ion chromatograms (XICs) for the barbiturates and THC-COOH (negative mode) and the bottom trace the XICs for the remaining compounds in positive mode. Figure 4 shows the LC separation of several isobaric compounds in the panel.
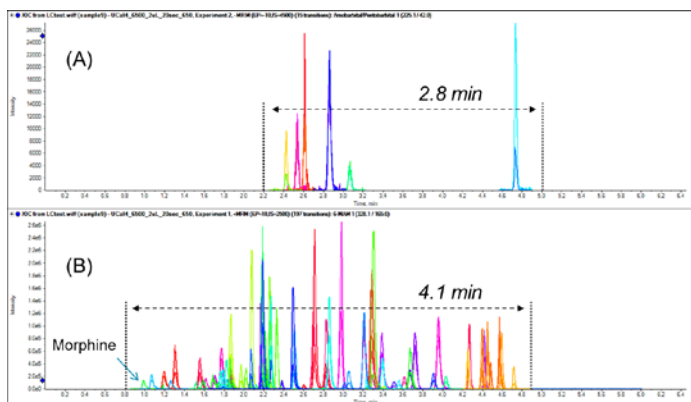
Figure 3: Elution profile of all the 93 compounds in the panel. (A) Compounds in negative mode. (B) Compounds in positive mode.
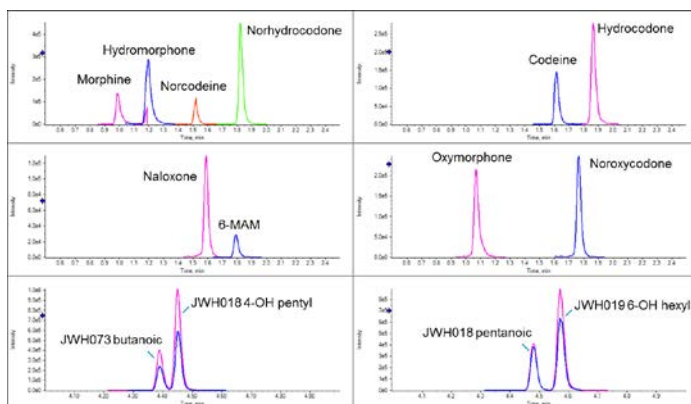
Figure 4: LC separation of isobaric compounds.

Scheduled MRM™

We designed the LC gradient to ensure that the retention times of the various compounds were evenly distributed throughout the gradient to reduce the MRM concurrency. This enabled maximum sampling of every LC peak, while maintaining optimal dwell times for each MRM transition. The re-optimized *Scheduled* MRM™ algorithm in Analyst® 1.6.3 ensured optimal data quality even during regions of the chromatogram when the MRM concurrency was very high. Most MRM transitions had 15 or more data points across the LC peaks. A minimum of 10 data points was achieved across every peak (Figure 5).
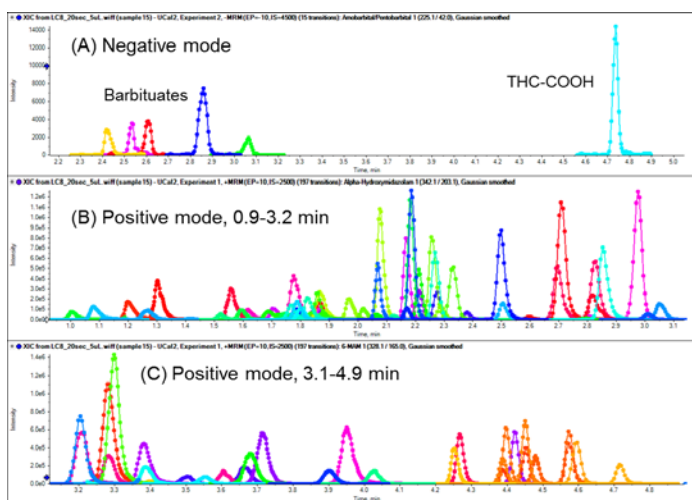

Figure 5: Representative chromatogram, highlighting the number of data points across each LC peaks. (A) Negative mode. (B) and (C) Positive mode.

Analytical sensitivity

The processed urine sample had a final dilution factor of 10. With only 5 μL injection volume (equivalent of 0.5 μL unprocessed urine), all compounds were detected with ease. For a few analytes such as fentanyl/norfentanyl/acetyl fentanyl/sufentanil, buprenorphine/norbuprenorphine, 6-MAM and THC-COOH, the lowest calibrator concentration was between 1 and 10 ng/mL. For instance, for acetyl fentanyl, the concentration at the lowest calibrator level was 1 ng/mL, which is equivalent to a mere 0.5 pg on-column. Figure 6 shows XICs of the quantifier MRM of acetyl fentanyl (323.1 → 188.0 m/z). Three replicate set of injections were shown (row 1 to 3). Figure 7 shows XICs for THC-COOH in negative mode. As can be observed from the data shown, in both cases, the analyte MRM signal was strong at the lowest calibrator level, suggesting the possibility of reaching even lower LOQ. Figure 8 shows typical calibration curves for the following analytes: codeine, nortriptyline, temazepam and THC-COOH. Excellent linearity and reproducibility was observed across the entire concentration ranges assessed in this effort.
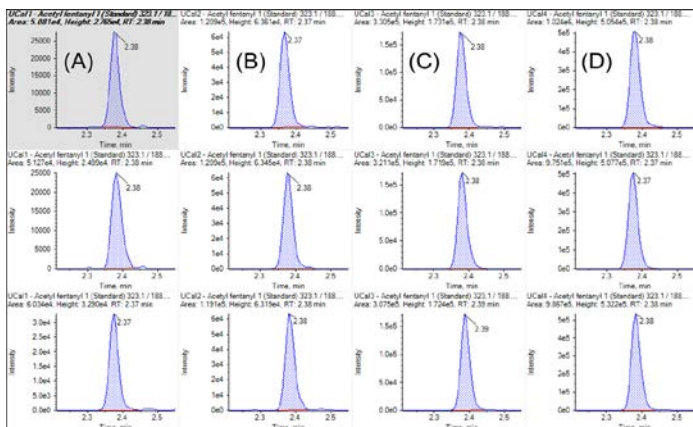
Figure 6: Extracted ion chromatograms (XICs) of the quantifier MRM of acetyl fentanyl (n=3, 323.1 → 188.0 m/z). (A) 1 ng/mL; (B) 2 ng/mL; (C) 6 ng/mL; (D) 20 ng/mL.
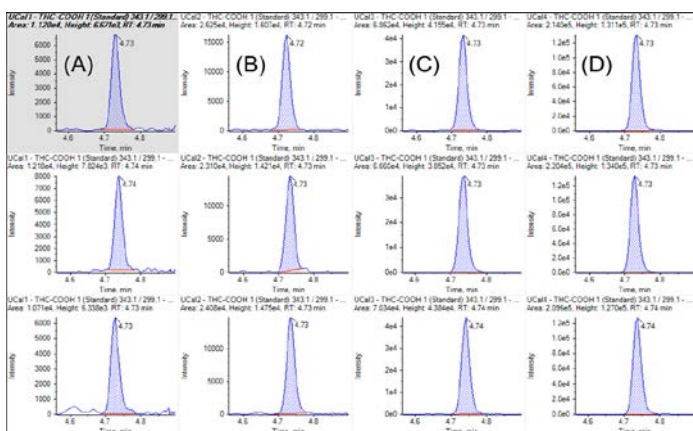

Figure 8: Typical calibration curves for selected compounds.

Figure 7: Extracted ion chromatograms (XICs) of the quantifier MRM for THC-COOH (n=3, 343.1 → 299.1 m/z). (A) 10 ng/mL; (B) 20 ng/mL; (C) 60 ng/mL; (D) 200 ng/mL.

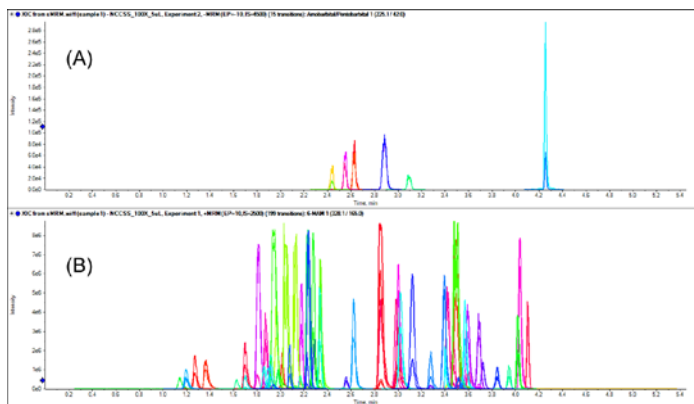**Figure 9: Elution profile of a smaller panel (72 compounds). (A) Compounds in negative mode. (B) Compounds in positive mode.**

Polarity Switching and Sample Throughput

It was essential to employ rapid polarity switching in order to accommodate 93 compounds (more than 200 MRMs) within a single, rapid data acquisition method. Compared to two separate injections (one for each polarity), this rapid polarity switching method offers a significant improvement in sample throughput. In the current extended panel consisting of 93 compounds, the LC runtime is 6.5 minutes. With a smaller panel (e.g. 72 compounds), we can easily reduce the LC runtime to 5.5 minutes (Figure 9).

Conclusion

A rapid and sensitive method for the LC-MS/MS analysis of a 93-compound forensic panel in human urine was developed using the SCIEX ExionLC™ AC HPLC system and the SCIEX QTRAP®/Triple Quad™ 4500 LC-MS/MS system. The method takes advantage of the re-optimized *Scheduled* MRM™ algorithm, and the fast polarity switching capability of the 4500 series, to deliver high throughput and high-quality data. This method utilized a dilute-and-shoot sample preparation procedure. Excellent linearity and precision were observed for all the compounds across the relevant calibration range.