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

Xiang He[1], Casey Burrows[1], Matthew Noestheden[2], Michael Jarvis[2], Adrian Taylor[2], and Alexandre Wang[1]
[1]SCIEX, 1201 Radio Rd, Redwood City, CA 94065, USA; [2]SCIEX, 71 Four Valley Drive, Concord, Ontario, L4K 4V8 Canada.

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)
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 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 (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
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

Analyte	Q1	Q3	RT (min)	DP	EP	CE	CXP
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
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

Analyte	Q1	Q3	RT (min)	DP	EP	CE	CXP
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
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
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

Analyte	Q1	Q3	RT (min)	DP	EP	CE	CXP
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
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

Table 2. List of MRMs (212 MRMs in total).

RESULTS and DISCUSSION

1. We achieved fast separation of various isobaric compounds in the panel despite short LC runtime (Figure 1).
2. We achieved a minimum of 10 data points across the LC peak when the majority of the MRM transitions had over 15 or more data points.
3. We observed strong MRM signal at lowest calibrator level, suggesting the possibility of reaching even lower LOQ (Figure 2). Excellent linearity and reproducibility was observed throughout the dynamic range assessed in this effort.
4. It was essential to utilize polarity switching to accommodate more than 200 MRMs within the one short data acquisition method. In the current panel with 93 compounds, the LC runtime is 6.5 minutes (Figure 3). With a smaller panel (e.g. 72 compounds), we can easily reduce the LC runtime to 5.5 minutes.

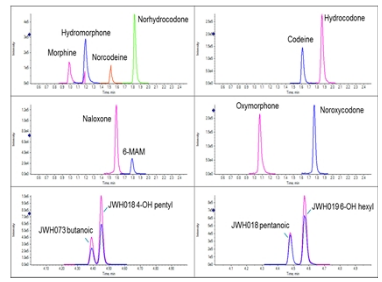

Figure 1. LC separation of isobaric compounds.

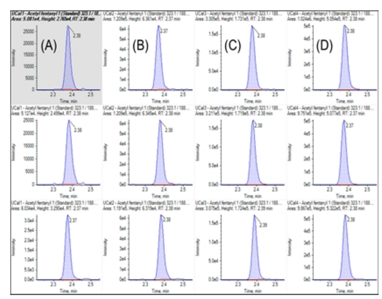

Figure 2. XICs of the quantifier MRM of acetyl fentanyl (n=3, 323.1 à 188.0 m/z). (A) 1 ng/mL; (B) 2 ng/mL; (C) 6 ng/mL; (D) 20 ng/mL.

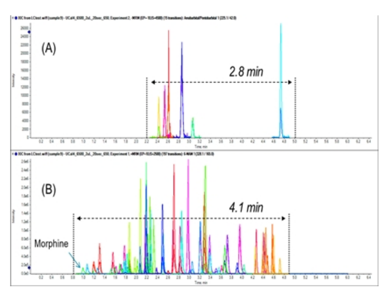

Figure 3. Elution profile of all the 93 compounds in the panel. (A) Compounds in negative mode. (B) Compounds in positive mode.

CONCLUSIONS

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.