thermo scientific

TECHNICAL NOTE 65182

Tomorrow's quantitation with LC-MS/MS: fast screening and quantitation of drugs of abuse in urine for forensic toxicology

Authors

Kevin McHale, Kerry Hassell,
Thermo Fisher Scientific,
Somerset, NJ

Claudia Martins,
Thermo Fisher Scientific,
San Jose, CA

Debadeep Bhattacharyya,
Thermo Fisher Scientific,
Boston, MA

Keywords

Drugs of abuse, sensitive quantitation, triple quadrupole MS, UHPLC, separation of isomers, separation of analytes

Goal

Development and implementation of a robust, reliable, reproducible, and sensitive workflow for analysis and quantitation of several drugs of abuse in urine using liquid chromatography-tandem mass spectrometry

Application benefits

- Linearity, chromatographic resolution, and sensitivity for 53 drugs of abuse in urine
- Easy-to-implement workflow that addresses all high productivity/throughput goals with LC-MS/MS technology

Introduction

One of the major challenges for analytical laboratories monitoring drugs of abuse in biological matrices is analyzing hundreds of samples every day while addressing the significant challenge of catching all the attempts that are made to bypass controlled substance laws, as well as identifying and quantifying novel compounds that are appearing in the market. In addition, the typical demands of reducing cost/sample, developing robust, reliable, sensitive methods for all molecule types, and achieving desired sensitivity and robustness continue to pose additional challenges for every analytical laboratory involved in developing quantitation methods. The analytical methods focused on screening and untargeted analysis of drugs of abuse are usually addressed with high-resolution, accurate-mass spectrometry (HRAM).[1,2]

ThermoFisher SCIENTIFIC

In the last few years, multiple quantitation technologies have been developed and used for quantitation of drugs of abuse in biological matrices (such as urine, plasma, oral fluid, etc.).[3] Amongst a host of technologies that are available, liquid chromatography (LC) coupled to mass spectrometry (MS) has gained widespread popularity owing to their increased selectivity, specificity, robustness, and sensitivity.[4,5] In this report, we investigate the feasibility of high-throughput measurements of 53 drugs of abuse and metabolites in forensic toxicology by reducing time-consuming sample preparation steps and employing two-minute UHPLC-MS/MS analyses per sample.

Experimental

Sample preparation

All standards were obtained from Cerilliant (Round Rock, TX) and used as received. Blank urine was obtained from a healthy male volunteer. After centrifugation of urine at 10,000 rpm for 10 min, urine supernatant was spiked with drugs of abuse and metabolites at concentrations equivalent to 0.1, 0.25, 0.5, 1, 2, 5, and 10 times the cutoff concentrations. Prepared urine samples were diluted with equal volume of a stock solution of isotopically labeled standards in 20% methanol prior to LC-MS/MS analyses.

Liquid chromatography

A 2 µL sample was injected onto a 2.1 × 50 mm, 1.9 µm Thermo Scientific™ Hypersil GOLD™ aQ column thermostatted to 40 °C. Compound separation was accomplished with the Thermo Scientific™ Vanquish™ Horizon UHPLC system using a binary reverse-phase gradient as shown in Table 1. Mobile phases were (A) water and (B) acetonitrile, both containing 0.1% formic acid. The LC flow rate was maintained at 1.0 mL/min with no post-column split. The LC effluent was diverted to waste until after the column void to prevent salts from fouling the ion source.

Table 1. LC gradient information for a flow rate of 1 mL/min

Time (min)	%B
0.0	0
0.4	22.5
1.0	80
1.29	80
1.3	0
1.4	0
2.1	0

Mass spectrometry

A Thermo Scientific™ TSQ Quantis™ triple-stage quadrupole mass spectrometer[6] was used for this analysis. All compounds for this study were analyzed in positive ion mode. A total of 210 SRM transitions were monitored using a cycle time of 0.15 s, with most SRM time windows set to a width of 0.1 min (6 s).

Software

Data acquisition, processing, and review was performed using Thermo Scientific™ TraceFinder™ 4.1 software.

Results and discussion

As shown in Table 2, more than 75 drugs of abuse and their metabolites were analyzed and quantified with one LC-MS/MS method having an acquisition time of less than 1.4 min. The comprehensive list of drugs of abuse exclude glucuronides and also include some commonly used antidepressants. While the method ensures high productivity and addresses the critical challenge of achieving throughput goals of most analytical laboratories invested in the analysis and quantitation of drugs of abuse in biological matrices, the data obtained can be complex, especially from the perspective of analysis and review. There are several overlaps between elution times of different analytes, which makes separation between isomers and also separation between analytes challenging. However, additional selectivity and speed offered by the TSQ Quantis instrument enabled the ability to address these challenges in this study.

Table 2 (part 1). List of drugs of abuse identified and quantified in the positive electrospray mode

Compound	Retention Time (min)	RT Window (min)	Precursor (*m/z*)	Product (*m/z*)	Collision Energy (V)	RF Lens (V)
Nicotine_D4	0.275	0.3	167.15	134.09	22	101
Nicotine*	0.275	0.3	163.12	130.07	22	101
Nicotine*	0.275	0.3	163.12	132.08	16	101
Cotinine_D3	0.325	0.3	180.12	80.05	24	128
Cotinine*	0.325	0.3	177.1	80.05	24	128
Cotinine*	0.325	0.3	177.1	98.06	21	128
Morphine_D6	0.473	0.12	292.18	165.07	39	214
Oxymorphone*	0.497	0.12	302.14	198.09	46	177
Oxymorphone*	0.497	0.12	302.14	227.1	29	177
Oxymorphone_D3	0.497	0.12	305.16	230.11	29	177
Hydromorphone_D6	0.526	0.1	292.18	185.06	31	218
Mor_HMor_NorCod_NorHC*	0.575	0.32	286.14	128.06	57	186
Mor_HMor_NorCod_NorHC*	0.575	0.32	286.14	152.06	59	186
Mor_HMor_NorCod_NorHC*	0.575	0.32	286.14	157.07	42	186
Mor_HMor_NorCod_NorHC*	0.575	0.32	286.14	165.07	40	186
Mor_HMor_NorCod_NorHC*	0.575	0.32	286.14	185.06	31	186
Mor_HMor_NorCod_NorHC*	0.575	0.32	286.14	199.08	29	186
Naloxone_D5	0.601	0.1	333.18	212.07	38	179
Pregabalin*	0.603	0.1	160.13	55.05	22	83
Pregabalin*	0.603	0.1	160.13	97.1	16	83
Gabapentin*	0.604	0.1	172.13	95.09	23	96
Gabapentin*	0.604	0.1	172.13	137.1	16	96
Gabapentin_D10	0.604	0.1	182.19	147.16	16	96
Codeine*	0.61	0.1	300.16	152.06	61	219
Codeine*	0.61	0.1	300.16	165.07	41	219
Codeine_D3	0.61	0.1	303.18	165.07	41	219
DiHCod_NorOC*	0.62	0.14	302.14	128.06	61	152
DiHCod_NorOC*	0.62	0.14	302.14	187.08	25	152
DiHCod_NorOC*	0.62	0.14	302.14	199.08	33	152
DiHCod_NorOC*	0.62	0.14	302.14	227.1	29	152
D3-Methylone	0.627	0.1	211.12	163.1	18	93
Methylone*	0.627	0.1	208.1	132.04	27	93
Methylone*	0.627	0.1	208.1	160.08	18	93
Naloxone_6-MAM*	0.627	0.16	328.15	165.07	38	179
Naloxone_6-MAM*	0.627	0.16	328.15	211.08	26	179
Naloxone_6-MAM*	0.627	0.16	328.15	212.07	38	179
Naloxone_6-MAM*	0.627	0.16	328.15	253.11	27	179
Amphetamine*	0.631	0.1	136.11	91.05	18	53
Amphetamine*	0.631	0.1	136.11	119.09	10	53
Amphetamine_D5	0.631	0.1	141.14	93.07	18	53
Oxycodone_D3	0.651	0.1	319.17	244.13	29	167
Oxycodone*	0.651	0.1	316.15	241.11	29	167
Oxycodone*	0.651	0.1	316.15	256.11	26	167

*Isomers with the same *m/z*

Table 2 (part 2). List of drugs of abuse identified and quantified in the positive electrospray mode

Compound	Retention Time (min)	RT Window (min)	Precursor (*m/z*)	Product (*m/z*)	Collision Energy (V)	RF Lens (V)
6-MAM_D3	0.655	0.1	331.17	211.08	26	184
MDA*	0.655	0.1	180.1	133.06	18	62
MDA*	0.655	0.1	180.1	135.04	19	62
MDA_D5	0.655	0.1	185.13	140.07	19	62
O-DesmethylTramadol*	0.662	0.1	250.18	42.05	65	95
O-DesmethylTramadol*	0.662	0.1	250.18	58.05	17	95
O-DesmethylTramadol_13C	0.662	0.1	251.19	58.05	17	95
O-Desmethyltramadol_D6	0.662	0.1	256.22	64.1	17	90
Hydrocodone_D3	0.667	0.1	303.19	202.1	30	227
Hydrocodone*	0.667	0.1	300.16	128.06	59	227
Hydrocodone*	0.667	0.1	300.16	199.08	30	227
Methamphetamine_D5	0.673	0.1	155.16	92.06	20	55
MDMA*	0.682	0.1	194.12	105.07	25	82
MDMA*	0.682	0.1	194.12	163.08	13	82
MDMA_D5	0.682	0.1	199.15	107.08	25	82
7-Hydroxyquetiapine*	0.687	0.1	400.17	208.03	44	217
7-Hydroxyquetiapine*	0.687	0.1	400.17	269.07	22	217
MethAMP_Phentermine*	0.695	0.14	150.13	65.04	39	55
MethAMP_Phentermine*	0.695	0.14	150.13	91.05	20	55
MethAMP_Phentermine*	0.695	0.14	150.13	105.07	19	55
MethAMP_Phentermine*	0.695	0.14	150.13	119.09	12	55
Phentermine_D5	0.701	0.1	155.16	96.08	20	55
7-Aminoclonazepam*	0.713	0.08	286.07	222.1	25	165
7-Aminoclonazepam*	0.713	0.08	286.07	250.1	20	165
7-Aminoclonazepam_D4	0.713	0.08	290.1	226.13	25	165
Benzoylecgonine*	0.72	0.1	290.14	105.03	31	152
Benzoylecgonine*	0.72	0.1	290.14	168.1	20	152
Benzoylecgonine_D3	0.72	0.1	293.16	171.12	20	152
MDEA*	0.731	0.1	208.13	77.05	42	84
MDEA*	0.731	0.1	208.13	135.04	24	84
MDEA_D5	0.731	0.1	213.16	135.04	24	84
Methylphenidate_D9	0.737	0.1	243.2	93.13	20	95
Norfentanyl*	0.75	0.1	233.16	56.05	26	124
Norfentanyl*	0.75	0.1	233.16	84.08	18	124
Norfentanyl_D5	0.75	0.1	238.2	84.08	18	124
D3-Dextrorphan	0.776	0.1	261.21	157.07	36	148
Dextrophan*	0.776	0.1	258.19	157.07	36	148
Dextrophan*	0.776	0.1	258.19	199.11	25	148
Zopiclone*	0.777	0.1	389.11	217.03	33	114
Zopiclone*	0.777	0.1	389.11	245.02	17	114
Zopiclone_D4	0.777	0.1	393.14	245.02	17	114

*Isomers with the same *m/z*

Table 2 (part 3). List of drugs of abuse identified and quantified in the positive electrospray mode

Compound	Retention Time (min)	RT Window (min)	Precursor (*m/z*)	Product (*m/z*)	Collision Energy (V)	RF Lens (V)
Tramadol*	0.797	0.1	264.2	42.05	65	80
Tramadol*	0.797	0.1	264.2	58.05	16	80
Tramadol_13C	0.797	0.1	265.21	58.05	16	80
Tramadol-13C-D3	0.797	0.1	268.23	58.05	16	80
Methylphenidate_Normeperidine*	0.8	0.2	234.15	56.05	46	95
Methylphenidate_Normeperidine*	0.8	0.2	234.15	84.08	20	95
Methylphenidate_Normeperidine*	0.8	0.2	234.15	91.05	45	95
Methylphenidate_Normeperidine*	0.8	0.2	234.15	160.11	14	95
Tapentadol*	0.808	0.1	222.19	107.05	24	117
Tapentadol*	0.808	0.1	222.19	121.07	20	117
Tapentadol_D3	0.808	0.1	225.2	107.05	24	117
Meprobamate*	0.831	0.08	219.13	97.1	19	56
Meprobamate*	0.831	0.08	219.13	158.12	10	56
Meprobamate_D7	0.831	0.08	226.18	165.16	10	56
Alpha-PVP*	0.834	0.1	232.17	91.05	22	145
Alpha-PVP*	0.834	0.1	232.17	126.13	24	145
D8-alpha-PVP	0.834	0.1	240.2	91.05	22	145
Normeperidine_D4	0.839	0.1	238.17	164.14	14	95
Cocaine*	0.842	0.1	304.15	82.07	32	151
Cocaine*	0.842	0.1	304.15	182.12	20	151
D8-MDPV	0.85	0.12	284.2	134.17	27	130
MDPV*	0.85	0.12	276.16	126.13	27	130
MDPV*	0.85	0.12	276.16	135.05	25	130
9-Hydroxyrisperidone*	0.851	0.12	427.214	207.113	27	195
9-Hydroxyrisperidone*	0.851	0.12	427.214	110.06	42	195
Meperidine*	0.855	0.1	248.16	174.13	20	129
Meperidine*	0.855	0.1	248.16	220.13	23	129
Meperidine_D4	0.855	0.1	252.19	224.16	23	129
Zolpidem*	0.855	0.1	308.18	235.12	34	219
Zolpidem*	0.855	0.1	308.18	263.12	26	219
Zolpidem_D7	0.855	0.1	315.22	242.16	34	219
Norbuprenorphine*	0.862	0.1	414.26	165.07	65	225
Norbuprenorphine*	0.862	0.1	414.26	187.08	38	225
Norbuprenorphine_D3	0.862	0.1	417.28	187.08	38	225
Cocaethylene*	0.91	0.1	318.17	82.07	32	157
Cocaethylene*	0.91	0.1	318.17	196.13	21	157
Cocaethylene_D3	0.91	0.1	321.19	199.15	21	157
PCP*	0.941	0.12	244.21	86.1	10	65
PCP*	0.941	0.12	244.21	91.05	27	65
PCP_D5	0.941	0.12	249.24	86.1	10	65
Zaleplon*	0.945	0.1	306.135	236.093	25	160
Zaleplon*	0.945	0.1	306.135	264.124	20	160

*Isomers with the same *m/z*

Table 2 (part 4). List of drugs of abuse identified and quantified in the positive electrospray mode

Compound	Retention Time (min)	RT Window (min)	Precursor (*m/z*)	Product (*m/z*)	Collision Energy (V)	RF Lens (V)
alpha_OH-Alprazolam*	0.955	0.1	325.09	216.08	39	244
alpha_OH-Alprazolam*	0.955	0.1	325.09	297.07	26	244
alpha-OH Alprazolam_D5	0.955	0.1	330.12	302.1	26	244
Fentanyl*	0.958	0.12	337.23	105.07	38	182
Fentanyl*	0.958	0.12	337.23	188.14	23	182
Fentanyl_D5	0.958	0.12	342.26	188.14	23	182
Oxazepam*	0.975	0.08	287.06	104.05	36	187
Oxazepam*	0.975	0.08	287.06	241.05	24	187
Oxazepam_D5	0.975	0.08	292.09	246.08	24	187
Desalkylflurazepam*	0.977	0.08	289.05	140.03	31	218
Desalkylflurazepam*	0.977	0.08	289.05	226.09	29	218
Citalopram*	0.977	0.12	325.17	109.05	26	162
Citalopram*	0.977	0.12	325.17	262.1	19	162
Citalopram-D6	0.977	0.12	331.22	109.05	26	162
D3-Doxepin	0.978	0.12	283.19	107.05	22	136
Doxepin*	0.978	0.12	280.17	107.05	22	136
Doxepin*	0.978	0.12	280.17	235.11	16	136
Buprenorphine*	0.982	0.1	468.31	396.21	40	234
Buprenorphine*	0.982	0.1	468.31	414.26	34	234
Buprenorphine_D4	0.982	0.1	472.34	400.22	40	234
Carisoprodol*	0.988	0.08	261.18	97.1	18	73
Carisoprodol*	0.988	0.08	261.18	176.13	9	73
Carisoprodol_D7	0.988	0.08	268.22	183.17	9	73
Lorazepam*	0.989	0.08	321.02	229.05	32	185
Lorazepam*	0.989	0.08	321.02	275.01	22	185
Lorazepam_37Cl	0.989	0.08	323.02	277	22	185
Mitragynine*	1.002	0.12	399.23	174.1	29	185
Mitragynine*	1.002	0.12	399.23	226.14	22	185
Alprazolam*	1.01	0.08	309.09	205.08	42	225
Alprazolam*	1.01	0.08	309.09	281.07	27	225
Alprazolam_D5	1.01	0.08	314.12	286.1	27	225
Nordiazepam*	1.011	0.08	271.06	140.03	28	148
Nordiazepam*	1.011	0.08	271.06	208.1	28	148
Nordiazepam_D5	1.011	0.08	276.09	140.03	28	148
D3-Desipramine	1.028	0.12	270.2	75.1	15	121
Desipramine*	1.028	0.12	267.19	72.08	15	121
Desipramine*	1.028	0.12	267.19	193.09	37	121
EDDP*	1.029	0.15	278.19	234.13	30	160
EDDP*	1.029	0.15	278.19	249.15	21	160
EDDP_D3	1.029	0.15	281.21	234.13	30	160
D3-Imipramine	1.032	0.12	284.22	89.11	17	136
Imipramine*	1.032	0.12	281.2	58.07	34	136
Imipramine*	1.032	0.12	281.2	86.1	17	136

*Isomers with the same *m/z*

Table 2 (part 5). List of drugs of abuse identified and quantified in the positive electrospray mode

Compound	Retention Time (min)	RT Window (min)	Precursor (*m/z*)	Product (*m/z*)	Collision Energy (V)	RF Lens (V)
Temazepam*	1.037	0.08	301.07	177.02	39	174
Temazepam*	1.037	0.08	301.07	255.07	23	174
Temazepam_D5	1.037	0.08	306.11	260.1	23	174
Aripiprazole*	1.045	0.12	448.155	285.092	26	243
Aripiprazole*	1.045	0.12	448.155	176.071	31	243
Duloxetine*	1.045	0.12	298.13	44.05	12	80
Duloxetine*	1.045	0.12	298.13	154.07	7	80
Duloxetine-D3	1.045	0.12	301.15	47.07	12	80
Cyclobenzaprine*	1.05	0.15	276.17	58.07	21	133
Cyclobenzaprine*	1.05	0.15	276.17	215.09	40	133
D3-Cyclobenzaprine	1.05	0.15	279.19	215.09	40	133
Norpropoxyphene*	1.055	0.12	326.21	44.05	12	84
Norpropoxyphene*	1.055	0.12	326.21	252.17	8	84
Norpropoxyphene_D5	1.055	0.12	331.24	252.17	8	84
D3-Nortriptyline	1.069	0.12	267.2	233.13	15	128
Nortriptyline*	1.069	0.12	264.17	91.05	23	128
Nortriptyline*	1.069	0.12	264.17	233.13	15	128
Amitriptyline*	1.071	0.12	278.19	91.05	26	161
Amitriptyline*	1.071	0.12	278.19	233.13	18	161
D3-Amitriptyline	1.071	0.12	281.21	233.13	18	161
Propoxyphene*	1.077	0.12	340.23	58.07	15	96
Propoxyphene*	1.077	0.12	340.23	266.19	9	96
Propoxyphene_D5	1.077	0.12	345.26	266.19	9	96
Methadone*	1.085	0.14	310.22	105.03	32	105
Methadone*	1.085	0.14	310.22	265.16	15	105
Methadone_D3	1.085	0.14	313.24	268.18	15	105
Diazepam*	1.093	0.08	285.08	154.04	28	218
Diazepam*	1.093	0.08	285.08	193.09	32	218
Diazepam_D5	1.093	0.08	290.11	198.12	32	218
JWH-073 N-(4-hydroxybutyl)*	1.144	0.08	344.165	155.049	23	168
JWH-073 N-(4-hydroxybutyl)*	1.144	0.08	344.165	127.054	43	168
JWH-018 N-(5-hydroxypentyl)*	1.151	0.08	358.18	155.049	22	180
JWH-018 N-(5-hydroxypentyl)*	1.151	0.08	358.18	127.054	46	180
JWH-018 N-(5-hydroxypentyl)_D5	1.151	0.08	363.21	155.049	22	180
JWH-122 N-(5-hydroxypentyl)*	1.192	0.08	372.2	115.054	58	185
JWH-122 N-(5-hydroxypentyl)*	1.192	0.08	372.2	169.065	22	185
UR-144 N-(5-hydroxypentyl)*	1.209	0.08	328.25	97.1	26	150
UR-144 N-(5-hydroxypentyl)*	1.209	0.08	328.25	125.096	17	150
THC-COOH_D3_Pos	1.265	0.08	348.22	302.2	20	154
THC-COOH_pos*	1.265	0.08	345.21	193.12	26	154
THC-COOH_pos*	1.265	0.08	345.21	299.2	20	154

*Isomers with the same *m/z*

Separation of isomers

Developing LC-MS/MS methods with short run times to ensure high productivity requires an efficient UHPLC pump, a robust LC column, and a triple quadrupole MS that can operate at very high scan speeds with remarkable reproducibility. At 1 mL/min with a 1.9 µm particle column, the observed LC peak widths were typically about ~1.3 s at the base (Figure 1). The LC flow rate is with no split to the TSQ Quantis HESI source.

Opiate isomers have the same precursor *m/z* and many generate the same product ions. Hence, it is necessary to chromatographically separate these compounds. Four opiate isomers that have a molecular weight of 286 are easily separated by UHPLC at 1 mL/min, and the TSQ Quantis instrument has sufficient acquisition speed to accurately quantify these compounds (Figure 2). Multiple SRMs were needed for some of the analytes, especially those with isomers, to ensure ideal separation within the required run time.

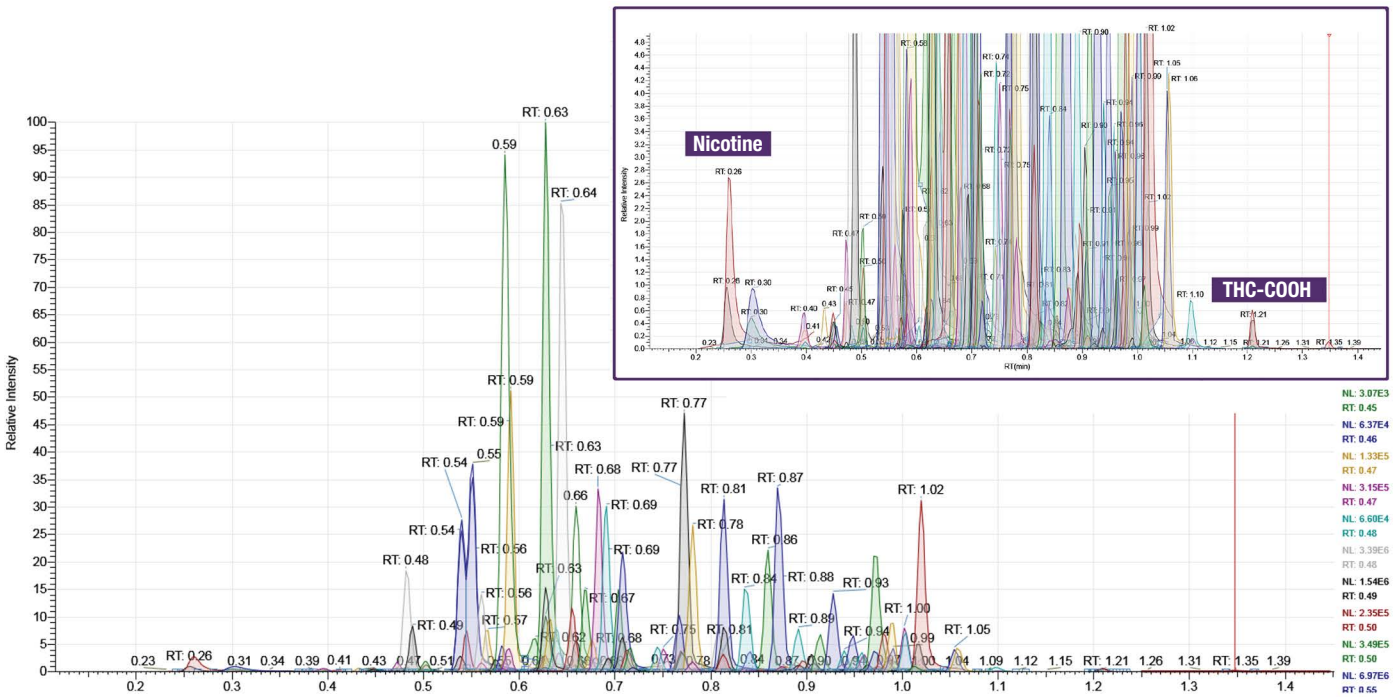

Figure 1. SRM chromatograms of 53 drugs of abuse in under 1.4 minutes (THC-COOH elutes at 1.21 min, inset)

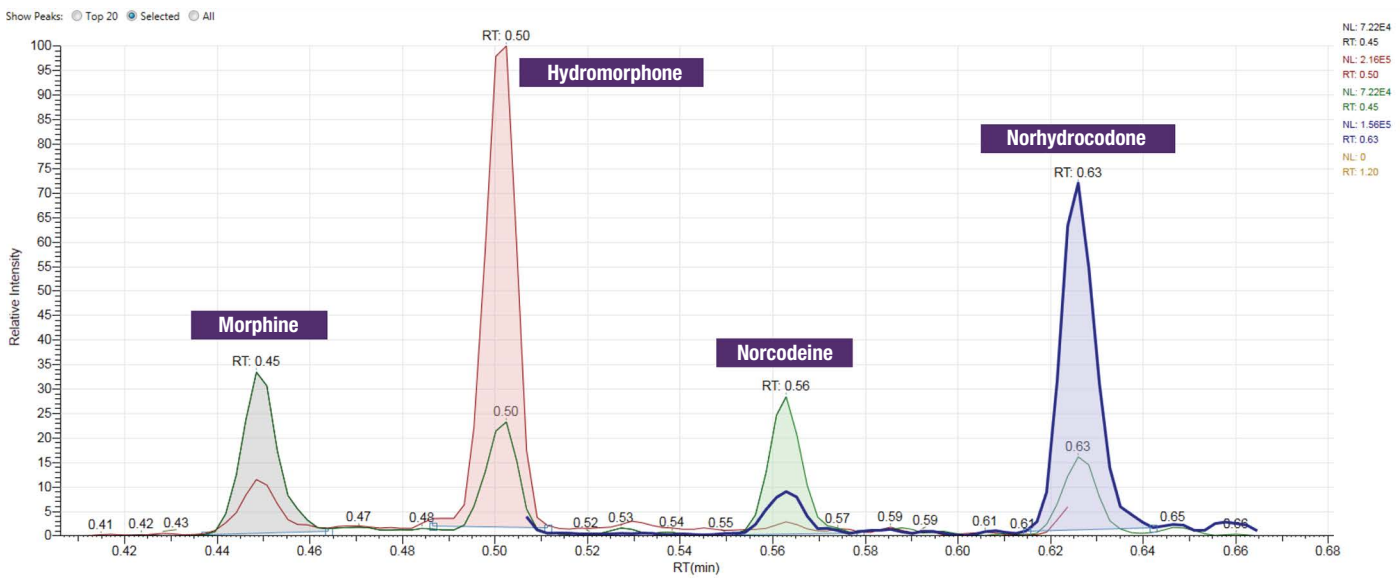

Figure 2. Opiate isomers at *m/z* 286 are well separated in under 12 s (typical LC peak = 1.3 s wide)

Separation of analytes

Optimization of MS parameters was critical to ensure high quality quantitation data for every analyte in the sample within the short run time. Setting the SRM cycle time to 0.15 s allowed 8–10 acquisition points under each LC peak, as can be seen for the elution of 6-monoacetylmorphine (6-MAM) (Figure 3). Based on earlier published reports,[7] nine points measured under a Gaussian peak integrated with 0.1% relative abundance will result in measurement errors of less than 3%. The acquisition speed and detection efficiency of the TSQ Quantis instrument are critical in such situations, especially for narrow LC peaks (as indicated in the case of 6-MAM (Figure 3)).

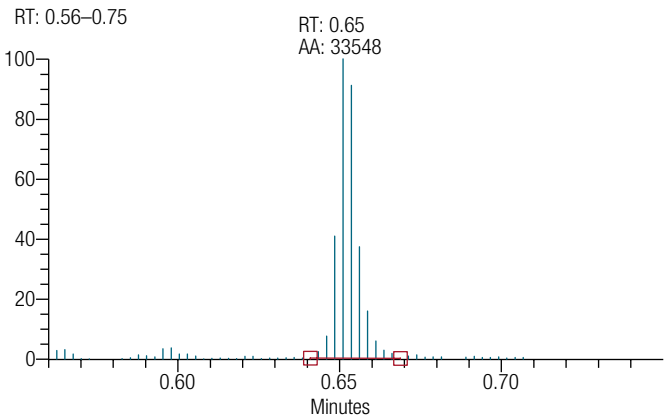

Figure 3. SRM acquisition points under LC peak – 6-MAM at 10 ng/mL

As Figure 4 highlights, the elution of 6-MAM and buprenorphine occur at the times of higher numbers of SRM transitions, hence, with the lowest duty cycles and dwell times. With a fast UHPLC method, analytes like 6-MAM and buprenorphine elute with many other compounds. The increased number of SRM transitions with low SRM dwell times enables increased productivity.

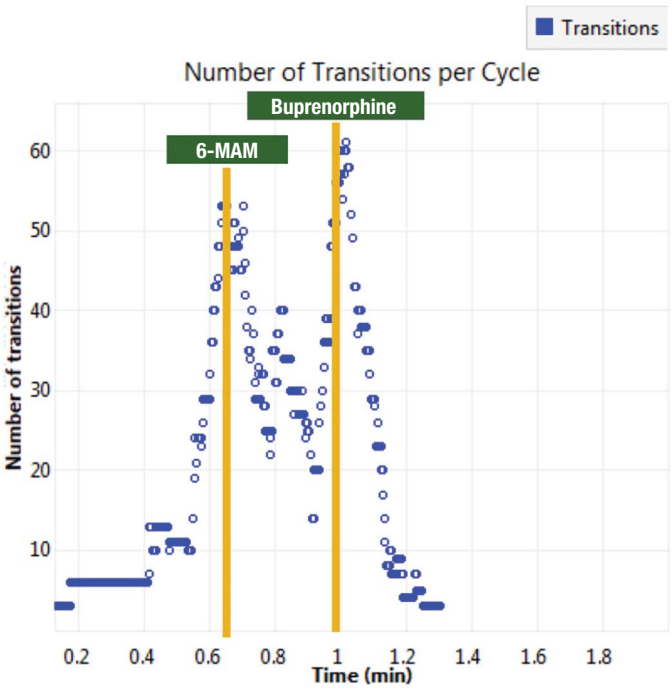

Figure 4. Retention times of 6-MAM and buprenorphine enabled by increased number of SRM transitions offered by the TSQ Quantis instrument with short dwell times

The acquisition speed and detection efficiency of the TSQ Quantis MS are critical in such situations, especially for narrow LC peaks (as indicated in the case of 6-MAM (Figure 3)). With a fast UHPLC method, 6-MAM and buprenorphine elute with many other compounds, which requires the flexibility of a short dwell time. Figure 5 indicates that at the elution times for 6-MAM and buprenorphine, the minimum dwell times were at 1.62 and 0.82 ms, respectively. Furthermore, LC retention times were remarkably consistent with very little variation (less than 0.005 min = 0.3 s) over the range of injections performed highlighting the performance of the Vanquish Horizon UHPLC system. This retention time consistency allowed narrow Timed SRM windows of 0.1 min (6 s) for most compounds to maximize detection efficiency without compromising LC peak measurements.

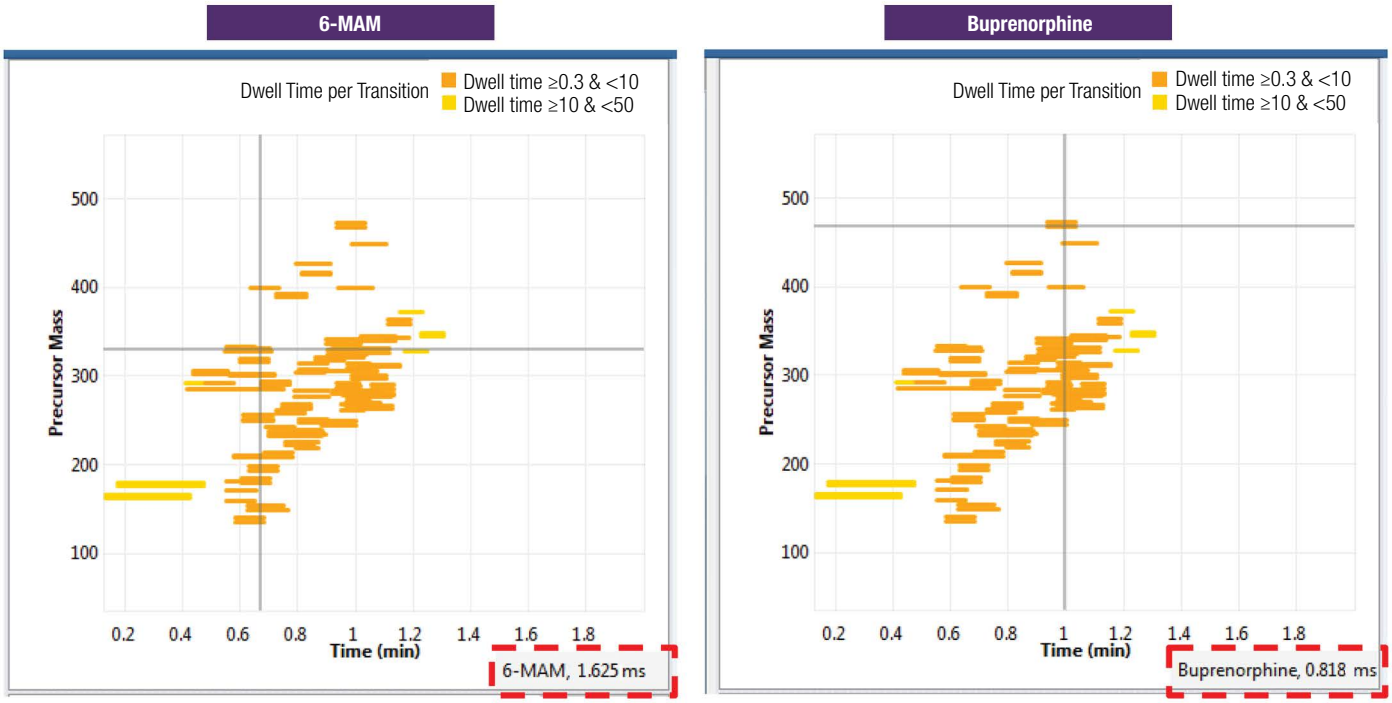

Figure 5. Map of precursor mass over time (min) showing elution of 6-MAM (left) and buprenorphine (right) of 1.62 and 0.82 ms, respectively

Sensitivity

One of the major goals for performing quantitation analysis with triple quadrupoles is to achieve high sensitivity. While the TSQ Quantis instrument is a mid-range triple quadrupole MS, and despite the complexity of the matrix (urine in this study) and complications of overlapping analytes in the short run time of the method, sensitivity demands of all the analytes were addressed with remarkable ease. As an example, multiple injection profiles of buprenorphine (Figure 6) and 6-MAM (Figure 7) are shown below. The robustness data over five injections were achieved with 1:2 dilution of the urine sample with 2 µL injections.

For LC-MS/MS based quantitation, buprenorphine can pose some challenges as a synthetic opioid, owing to its poor fragmentation efficiency. Even with this fast UHPLC method, and the fact buprenorphine elutes with many other compounds, the TSQ Quantis instrument offers sufficient speed, sensitivity, and selectivity to quantify buprenorphine at 5 ng/mL in 1:2 diluted urine with %CV of ~17% (Figure 6). These data were achieved with 60 simultaneous SRM transitions with 0.15 s SRM cycle time (Figure 4).

6-MAM is the primary metabolite of heroin, thereby making it a marker for heroin. Similar to buprenorphine, 6-MAM elutes with many other analytes and thus requires a short dwell time (Figures 4 and 5). High speed, sensitivity, and selectivity offered by the TSQ Quantis instrument allow robust, reproducible, and sensitive quantitation of 6-MAM at 10 ng/mL in 1:2 diluted urine (Figure 7). The %CV value was at 8.5% with 50 simultaneous SRM transitions with 0.15 s SRM cycle time.

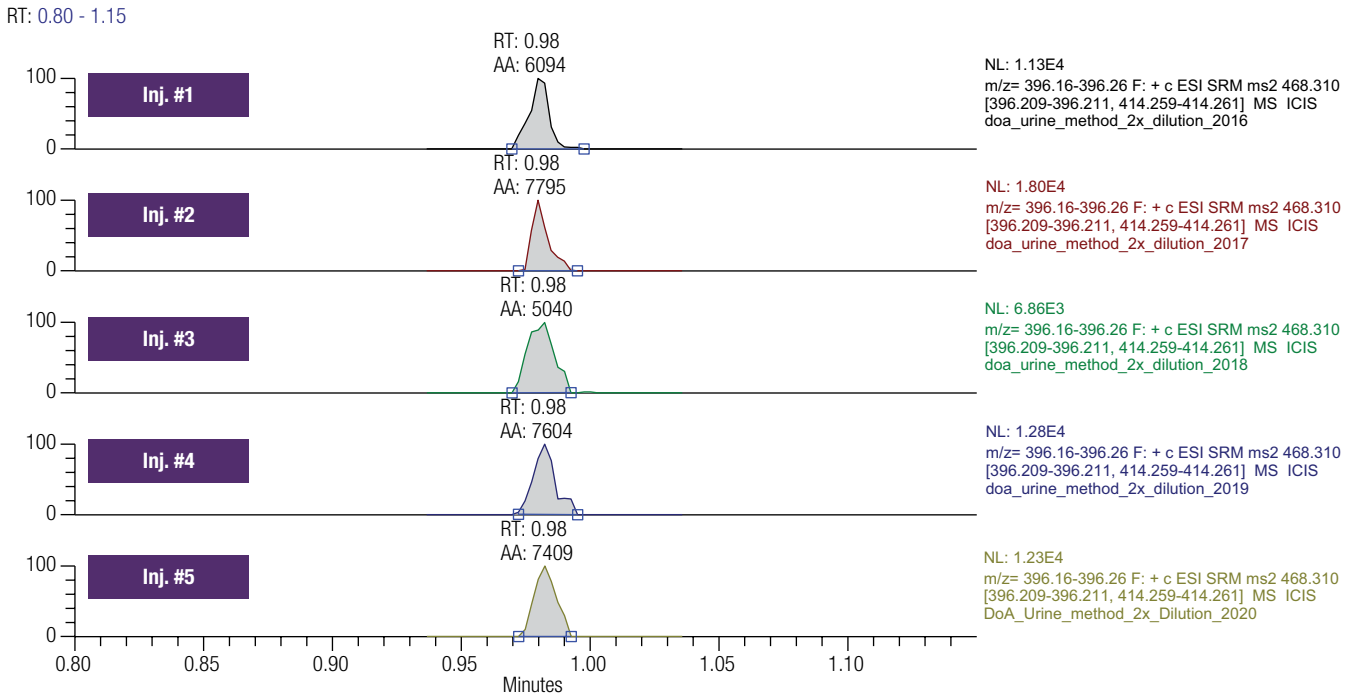

Figure 6. Reproducibility of buprenorphine at 5 ng/mL with a dwell time of 0.82 ms

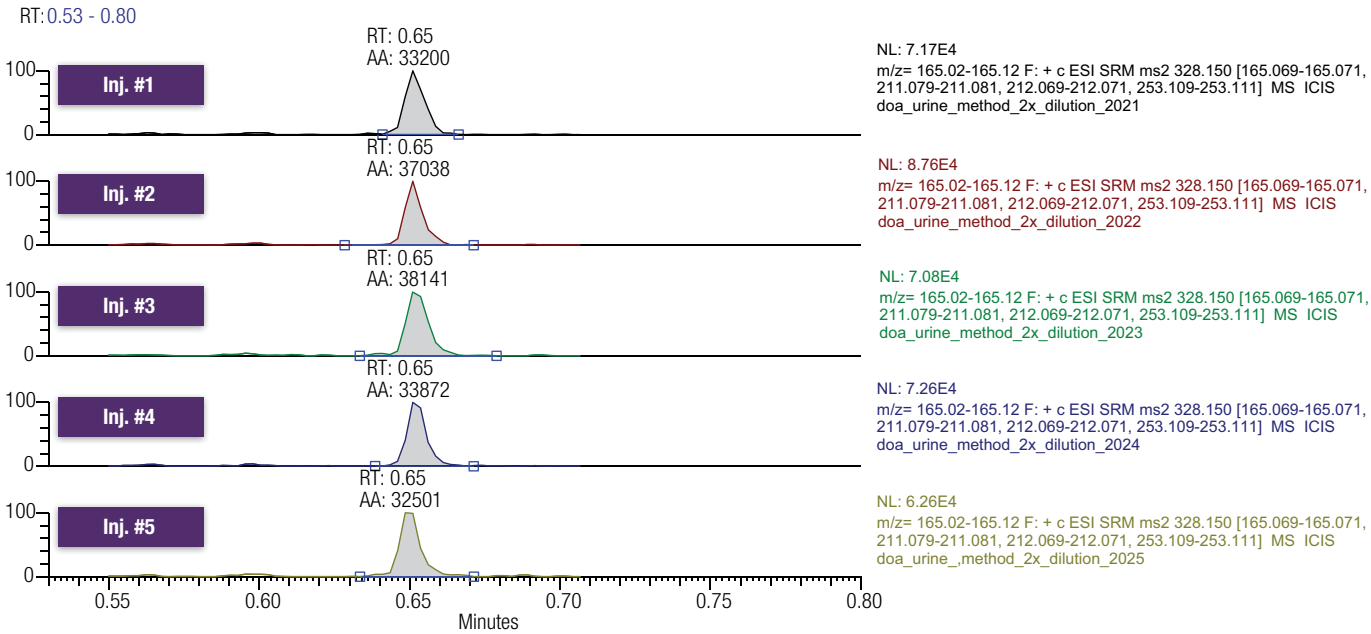

Figure 7. Reproducibility of 6-MAM at 10 ng/mL with a dwell time of 1.63 ms

thermo scientific

Conclusion

Analysis and quantitation of drugs of abuse in biological matrices can pose several challenges, especially with the growing demands of increasing throughput and high sensitivity. LC-MS/MS with liquid chromatography and triple quadrupole mass spectrometry offers several advantages in performing robust, reproducible, fast, and sensitive quantitation of drugs of abuse across several biological matrices, especially urine. In this study, we demonstrate the highly reproducible chromatographic performance of the Vanquish Horizon UPHLC system along with the outstanding speed and sensitivity of the TSQ Quantis mass spectrometer to perform confident quantitation of several drugs of abuse and metabolites in diluted urine for forensic toxicology samples in ~2 minutes per sample.

Diligent LC method development allowed for the baseline separation of most isomeric and isobaric compounds measured by UHPLC-MS/MS in under 1.4 minutes. Most target compounds had LLOQs at or below the designated cutoff levels in diluted urine. Overlapping signals of analytes and isomers are amongst the complexities typically observed for methods with such short run times. In this method, outstanding chromatographic resolution offered by the Vanquish Horizon UHPLC system and the increased speed and sensitivity offered by the TSQ Quantis triple quadrupole mass spectrometer enables ideal separation and quantitative efficiency for each of the target isomers (as shown for the opiate isomers) and also for each of the target analytes (as discussed for 6-MAM and buprenorphine).

References

1. Li, X., et al. Rapid screening of drugs of abuse in human urine by high-performance liquid chromatography coupled with high resolution and high mass accuracy hybrid linear ion trap-Orbitrap mass spectrometry. *Journal of Chromatography A* **2013,** *1302,* 8.
2. Heuett, N. V., et al. Analysis of drugs of abuse by online SPE-LC high resolution mass spectrometry: Communal assessment of consumption. *Science of The Total Environment* **2015,** *511,* 12.
3. DeNardi, C. D., et al. Quantification of drugs of abuse in oral fluid using online TurboFlow™ sample extraction. *MSACL*. Salzberg, Austria, MSACL, **2017.**
4. McHale, K. J. and K. Hassell. Increasing Sample Throughput for Detecting Drugs of Abuse and Metabolites in Urine by UHPLC-MS/MS for Forensic Toxicology. *ASMS.* San Antonio, Thermo Fisher Scientific, **2016.**
5. Van Natta, K. L. and M. Kozak. Evaluation of a method for Forensic Quantitative Screening of over 120 drugs of abuse on a triple quadrupole mass spectrometer. Thermo Scientific Poster Note 64378 https://assets.thermofisher.com/TFS-Assets/CMD/posters/PN-64378-LC-MS-TSQ-Endura-Forensic-120-Drugs-MSACL2015-PN64378-EN.pdf **2017.**
6. http://www.thermofisher.com/Altis-Quantis "Confident Quantitation." 2017.
7. Chesler, S. N. and S. P. Cram. Effect of Peak Sensing and Random Noise on the Precision and Accuracy of Statistical Moment Analyses from Digital Chromatographic Data. *Anal. Chem.* **1971,** *43,* 12.

For Forensic Use Only.

Find out more at **thermofisher.com**

ThermoFisher SCIENTIFIC