Clinical, Forensic & Toxicology Article

"The Big Pain": Development of Pain-Free Methods for Analyzing 231 Multiclass Drugs and Metabolites by LC-MS/MS

By Sharon Lupo

As the use of prescription and nonprescription drugs grows, the need for fast, accurate, and comprehensive methods is also rapidly increasing. Historically, drug testing has focused on forensic applications such as cause of death determinations or the detection of drug use in specific populations (military, workplace, probation/parole, sports doping). However, modern drug testing has expanded well into the clinical arena with a growing list of target analytes and testing purposes. Clinicians often request the analysis of large panels of drugs and metabolites that can be used to ensure compliance with prescribed pain medication regimens and to detect abuse or diversion of medications. With prescription drug abuse reaching epidemic levels [1], demand is growing for analytical methods that can ensure accurate results for comprehensive drug lists with reasonable analysis times. LC-MS/MS is an excellent technique for this work because it offers greater sensitivity and specificity than immunoassay and—with a highly selective and retentive Raptor™ Biphenyl column—can provide definitive results for a wide range of compounds.

Typically, forensic and pain management drug testing consists of an initial screening analysis, which is qualitative, quick, and requires only minimal sample preparation. Samples that test positive during screening are then subjected to a quantitative confirmatory analysis. Whereas screening assays may cover a broad list of compounds and are generally less sensitive and specific, confirmation testing provides fast, targeted analysis using chromatographic conditions that are optimized for specific panels. C18 columns are commonly used and generally work well for hydrophobic compounds that separate using dispersive retention. However, not all compounds exhibit this type of interaction and many of today's complex mixtures and difficult matrices require more advanced retention mechanisms. Raptor™ Biphenyl columns exploit the pi-pi (π -π) interactions of fused ring compounds with substituted electron withdrawing groups that are typical of many medications, which results in improved retention for a wider range of structurally diverse drugs and metabolites.

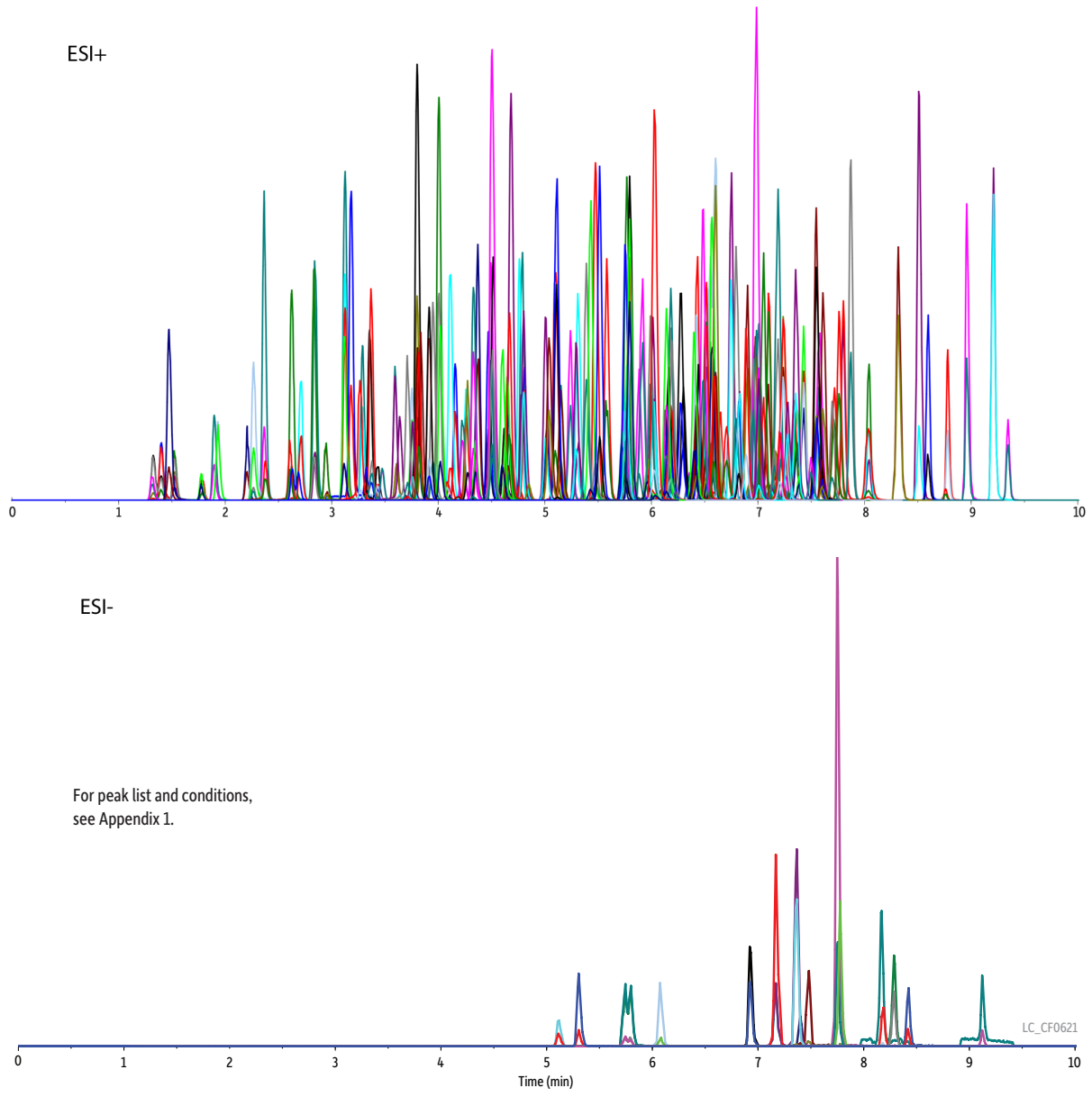
The Raptor™ Biphenyl column was used to develop LC-MS/MS methodology for 231 drugs and drug metabolites because it has the retention required for the wide range of compound classes tested here. It also provides improved selectivity for over 40 structurally similar drugs and metabolites, even those not normally resolved by a C18 column. The comprehensive analysis of 231 compounds shown in this article demonstrates the power and utility of the Raptor™ Biphenyl column for developing multiclass screening assays. Further, the panel-specific confirmation methods discussed later in the article can be paired either with an LC-MS/MS screening method or with traditional immunoassay screening tests. Note that stability experiments and validation in matrix should be performed to demonstrate effectiveness and reproducibility prior to implementing any method for actual sample analysis.

Comprehensive Analysis of 231 Drugs and Metabolites in 10 Minutes

Screening tests often are done by immunoassay, but this approach has several known issues. Immunoassays are less specific, which makes false positives more likely; in addition, they also are less sensitive, which increases the risk of false negatives. The emergence of definitive identification through LC-MS/MS as an alternative to paired screening and confirmational analyses speaks to the strength of LC-MS/MS as a technique [2]. However, even when using LC-MS/MS, separate screening and confirmational analyses are currently still more common than single definitive identification tests. Whether developing a screening assay or advancing to a single definitive analytical method, the power of LC-MS/MS can be maximized by using a Raptor™ Biphenyl column due to its unique retention and selectivity.

Accurately determining long lists of target analytes is a daunting task; in this case, the challenge included compounds from 10 drug classes and over 40 isomers and structurally similar compounds. Development of methodology required careful consideration of many variables, including chemical properties of the target analytes, mobile phase composition and gradient, and detector polarity. The Raptor™ Biphenyl column provided simultaneous analysis of all 231 drugs and metabolites in a fast 10-minute analysis time with an additional 2 minutes for re-equilibration (Figure 1). Good chromatographic separations were achieved and target

Figure 1: Fast, 10-minute analysis of 231 drugs and metabolites on a Raptor™ Biphenyl column by LC-MS/MS.



analytes were positively identified by retention time and optimized precursor and product ions. Polarity switching was used to improve detection with most compounds (209) analyzed using positive electrospray ionization (ESI+) and the remainder (22) using negative electrospray ionization (ESI-). As noted previously, over 40 compounds that shared their precursor ion with at least one other compound were included in this assay and most were chromatographically separated allowing positive identification. Exceptions included noroxycodone and dihydrocodeine, which are distinguished in the confirmatory opioids panel; citalopram and escitalopram, which are R/S enantiomers; and levorphanol (an opioid) and dextrorphan (a hallucinogen), which are enantiomers and can be determined separately in their respective panels.

Optimized Analysis of Separate Panels for Confirmation and Quantification

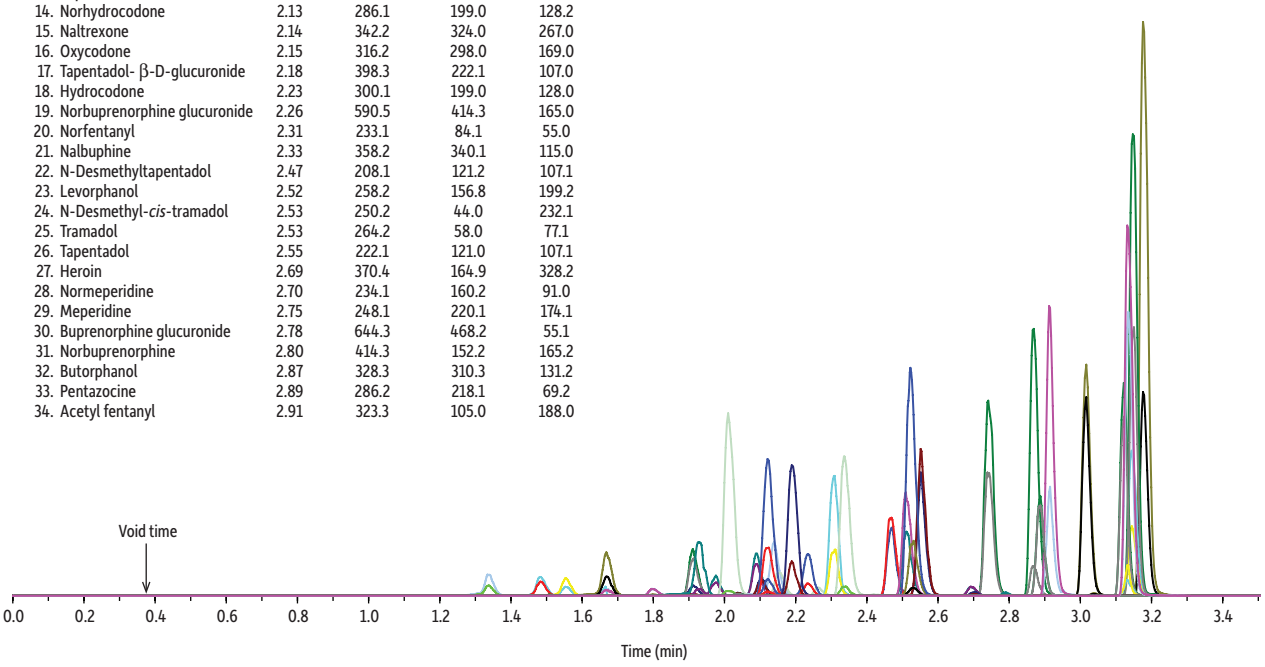
Confirmation methods were developed systematically by first grouping compounds by drug class. Compounds were separated and prepared in water as mixtures of 15-20 analytes; special care was taken to separate isobaric compounds in solutions. Two transitions were identified for each analyte and scouting gradients were run for each mixture using electrospray ionization in both positive and negative ion mode. The scouting gradients were linear (10-100% organic mobile phase), and three separate modifiers were evaluated: 1) 5 mM ammonium acetate, 2) 0.1% formic acid with 5 mM ammonium formate, and 3) 0.1% formic acid. Based on these results, optimal mobile phases, gradient conditions, and polarity were determined for each panel, and analytes were scheduled by retention time using multiple reaction monitoring (MRM).

Final optimized chromatographic conditions and results for each panel are shown in Figure 2 (opioids/metabolites), Figures 4–6 (antianxiety drugs/metabolites and barbiturates), Figure 7 (nonsteroidal anti-inflammatory drugs [NSAIDs]), Figure 8 (stimulants), Figure 9 (antiepileptic), Figure 10 (antipsychotics), Figure 11 (antidepressants), and Figure 12 (hallucinogens). While the determination of some common pain panel components is relatively straightforward, the analyses of several groups (opioids/metabolites, antianxiety drugs/metabolites and barbiturates, and NSAIDs) warrant further discussion.

Opioids are used for pain management and are among the most commonly prescribed—and abused—drugs in the world. As clinicians frequently test patients to monitor compliance, these analytes are key components in drug testing methods. These compounds present several analytical difficulties: the presence of structural isomers; poor sensitivity for buprenorphine and norbuprenorphine; and poor retention for noroxymorphone, morphine, and hydromorphone. With regard to these challenges, the chromatographic performance of the Raptor™ Biphenyl column is exceptional (Figure 2). The use of this column under gradient elution with acetonitrile and water mobile phases modified with 0.1% formic acid allowed the separation of all structural isomers. Use of LC-MS/MS provided additional specificity, which is important because 15 analytes share five precursor ions (M+H). Regarding the sensitivity issue that is common with buprenorphine and norbuprenorphine, the 2.7 µm superficially porous particle (SPP) silica in the Raptor™ Biphenyl column produced narrow peaks that improved response. The choice of acidic mobile phase for this panel also increased sensitivity compared to the use of a buffered mobile phase (Figure 3). As a final note for opioids analysis, the alternate retention mechanisms of the Raptor™ Biphenyl column increased retention of noroxymorphone, morphine, and hydromorphone. The increased separation of these small, polar target analytes from hydrophilic matrix interferences allows more accurate quantification.

Figure 2: Opioids/metabolites panel confirmation analysis on the Raptor™ Biphenyl column.

Peaks	t_R (min)	Precursor Ion	Product Ion	Product Ion
1. Noroxymorphone	1.35	288.1	270.1	212.9
2. Morphine	1.50	286.2	152.1	165.0
3. Oxymorphone	1.57	302.1	227.2	198.2
4. Hydromorphone	1.67	286.2	184.9	156.9
5. Norcodeine	1.80	286.1	151.9	115.0
6. (-)-Dihydrocodeine	1.91	302.2	199.0	128.2
7. (-)-Naloxone	1.92	328.3	310.1	212.3
8. Codeine	1.97	300.2	152.0	165.1
9. O-Desmethyl-*cis*-tramadol	2.01	250.1	58.0	42.0
10. Noroxycodone	2.04	302.1	227.0	197.9
11. Desomorphine	2.09	272.1	152.1	165.0
12. 6-Acetylmorphine	2.10	328.2	211.0	165.0
13. 6-β-Naltrexol	2.12	344.3	326.1	308.1
14. Norhydrocodone	2.13	286.1	199.0	128.2
15. Naltrexone	2.14	342.2	324.0	267.0
16. Oxycodone	2.15	316.2	298.0	169.0
17. Tapentadol- β-D-glucuronide	2.18	398.3	222.1	107.0
18. Hydrocodone	2.23	300.1	199.0	128.0
19. Norbuprenorphine glucuronide	2.26	590.5	414.3	165.0
20. Norfentanyl	2.31	233.1	84.1	55.0
21. Nalbuphine	2.33	358.2	340.1	115.0
22. N-Desmethyltapentadol	2.47	208.1	121.2	107.1
23. Levorphanol	2.52	258.2	156.8	199.2
24. N-Desmethyl-*cis*-tramadol	2.53	250.2	44.0	232.1
25. Tramadol	2.53	264.2	58.0	77.1
26. Tapentadol	2.55	222.1	121.0	107.1
27. Heroin	2.69	370.4	164.9	328.2
28. Normeperidine	2.70	234.1	160.2	91.0
29. Meperidine	2.75	248.1	220.1	174.1
30. Buprenorphine glucuronide	2.78	644.3	468.2	55.1
31. Norbuprenorphine	2.80	414.3	152.2	165.2
32. Butorphanol	2.87	328.3	310.3	131.2
33. Pentazocine	2.89	286.2	218.1	69.2
34. Acetyl fentanyl	2.91	323.3	105.0	188.0
35. Fentanyl	3.01	337.3	188.0	105.1
36. Buprenorphine	3.03	468.3	55.1	414.2
37. Norpropoxyphene	3.10	326.4	252.0	91.1
38. Sufentanil	3.12	387.2	238.1	111.1
39. Propoxyphene	3.13	340.3	265.9	58.1
40. EMDP	3.13	264.2	235.2	220.3
41. Mitragynine	3.14	399.3	174.2	159.0
42. EDDP	3.15	278.2	234.3	249.2
43. Methadone	3.18	310.2	264.9	105.1



LC_CF0610

Column
Dimensions: 100 mm x 2.1 mm ID
Particle Size: 2.7 µm
Guard Column: Raptor™ Biphenyl EXP® guard column cartridge 5 mm, 2.1 mm ID, 2.7 µm (cat.# 9309A0252)
Temp.: 30 °C

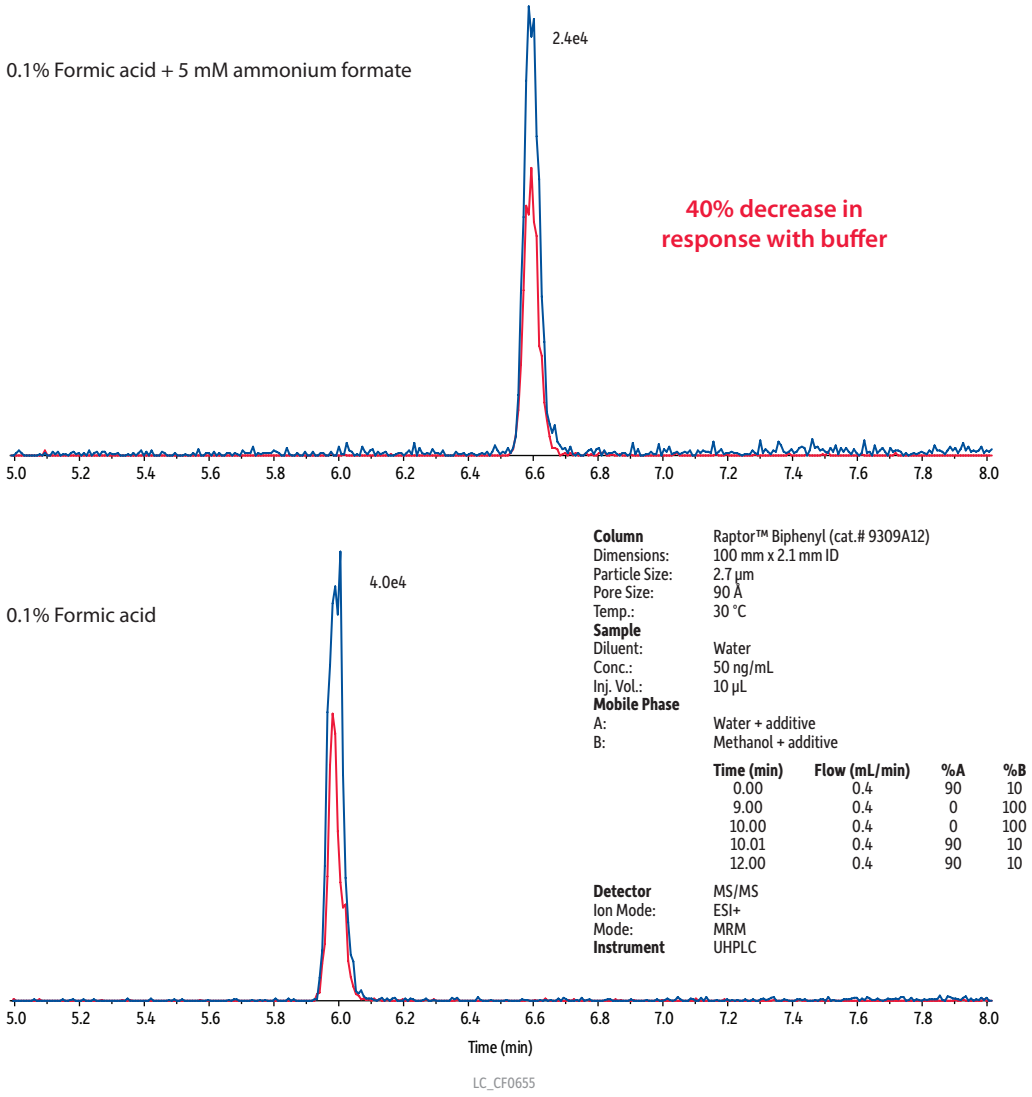
Sample
Diluent: Water
Conc.: 25 ng/mL
Inj. Vol.: 5 µL

Mobile Phase
A: Water + 0.1% formic acid
B: Acetonitrile + 0.1% formic acid

Time (min)	Flow (mL/min)	%A	%B
0.00	0.6	98	2
2.00	0.6	75	25
3.50	0.6	10	90
3.51	0.6	98	2
5.50	0.6	98	2

Detector MS/MS
Ion Mode: ESI+
Mode: Scheduled MRM
Instrument UHPLC

Figure 3: Use of an acidic mobile phase without buffer improves the response of opioids such as buprenorphine.



Column	Raptor™ Biphenyl (cat.# 9309A12)
Dimensions:	100 mm x 2.1 mm ID
Particle Size:	2.7 µm
Pore Size:	90 Å
Temp.:	30 °C
Sample	
Diluent:	Water
Conc.:	50 ng/mL
Inj. Vol.:	10 µL
Mobile Phase	
A:	Water + additive
B:	Methanol + additive

Time (min)	Flow (mL/min)	%A	%B
0.00	0.4	90	10
9.00	0.4	0	100
10.00	0.4	0	100
10.01	0.4	90	10
12.00	0.4	90	10

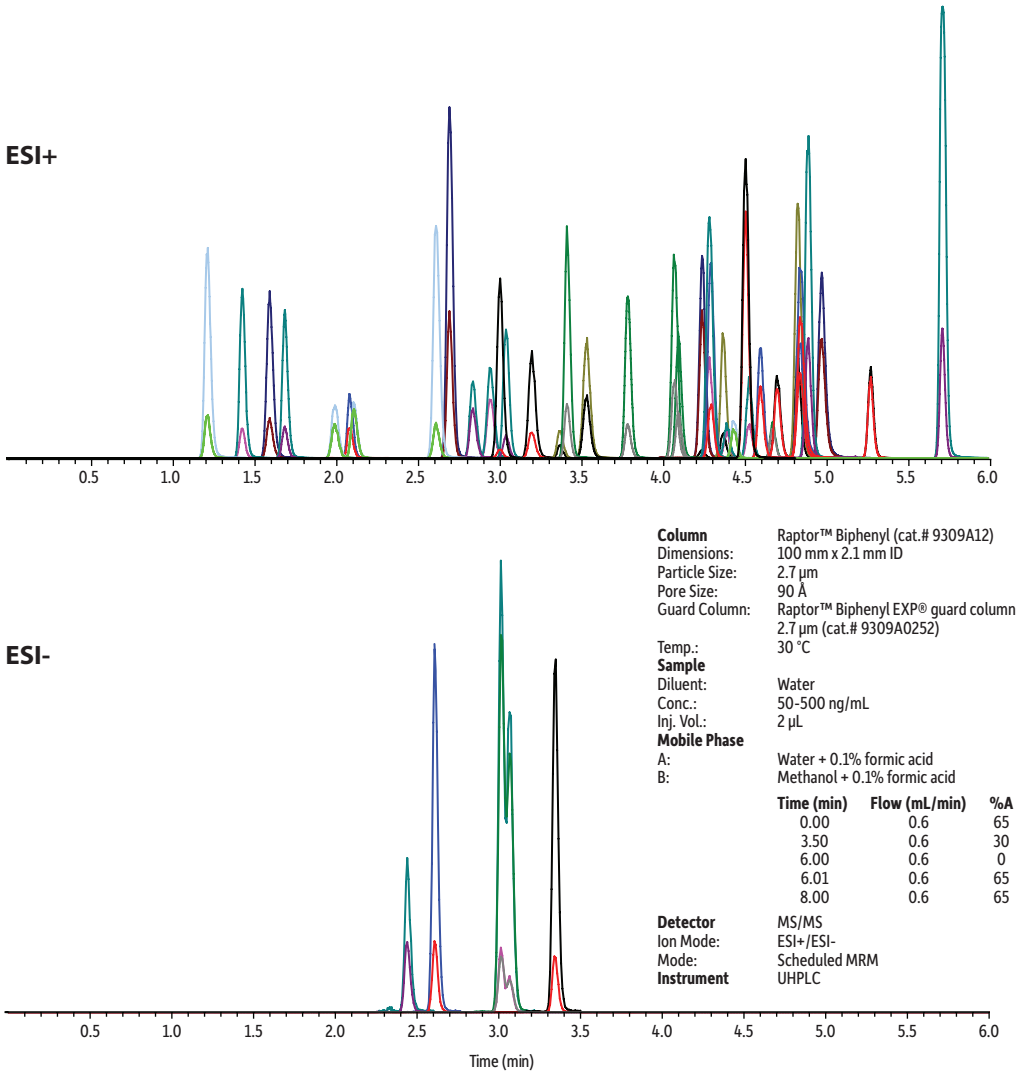
Detector	MS/MS
Ion Mode:	ESI+
Mode:	MRM
Instrument	UHPLC

Antianxiety drugs, such as benzodiazepines, muscle relaxers, hypnotics, sedatives, and z-drugs, along with barbiturates are often abused in conjunction with other drugs, most commonly opioids [3]. Like opioids, this group also presents chromatographic challenges, namely that barbiturates are detected in negative ion mode, whereas most other drugs are detected in positive ion mode. This significantly complicates the analysis and requires polarity switching and the use of an instrument with sufficient data acquisition speed. In addition, the barbiturates amobarbital and pentobarbital are positional isomers and can be extremely difficult to resolve. If resolution between the isomers is not critical, the Raptor™ Biphenyl column allows the combined analysis of antianxiety drugs and barbiturates in just 8 minutes. The column's unique selectivity provides ~40% resolution between amobarbital and pentobarbital and efficient peak capacity, which allows optimal use of instrument data speed. Analytical conditions and results for this combined panel are shown in Figure 4. If resolution of barbiturate isomers is critical, antianxiety drugs and barbiturates can also be analyzed separately. By using the Raptor™ C18 column for the barbiturates analysis, amobarbital and pentobarbital are almost completely resolved in 6 minutes (Figure 5), while the analysis of the antianxiety drugs can be completed on the Raptor™ Biphenyl column in 5.5 minutes (Figure 6). This simpler approach improves the resolution of barbiturate isomers and is suitable for slower mass spectrometers that lack the speed required for combined analysis.

Figure 4: Combined antianxiety drugs/metabolites and barbiturates panel confirmation analysis on the Raptor™ Biphenyl column.

	Peaks	t_R (min)	Conc. (ng/mL)	Precursor Ion	Product 1	Product 2
1.	7-Aminonitrazepam	1.23	50	252.2	121.1	94.2
2.	Norketamine	1.44	50	224.1	125.0	89.1
3.	Zolpidem phenyl-4-carboxylic acid	1.61	50	338.2	265.1	219.0
4.	Ketamine	1.70	50	238.1	125.1	89.1
5.	Zopiclone	2.01	50	389.2	245.0	217.2
6.	Meprobamate	2.10	50	219.1	158.2	97.0
7.	7-Aminoclonazepam	2.12	50	286.1	121.2	250.1
8.	Phenobarbital*	2.44	500	230.9	187.8	85.0
9.	Butalbital*	2.61	500	223.0	180.0	84.9
10.	Zolpidem	2.63	50	308.2	235.2	218.9
11.	Diphenhydramine	2.71	50	256.1	167.0	152.1
12.	7-Aminoflunitrazepam	2.85	50	284.1	135.0	227.1
13.	Chlordiazepoxide	2.96	50	300.1	227.0	282.0
14.	Flurazepam	3.02	50	388.2	315.2	183.0
15.	Amobarbital*	3.02	500	225.0	182.0	84.8
16.	Buspirone	3.05	50	386.3	122.1	95.0
17.	Pentobarbital*	3.07	500	225.0	182.0	84.9
18.	Midazolam	3.21	50	326.2	291.1	248.9
19.	Secobarbital*	3.35	500	237.0	193.9	84.8
20.	Carisoprodol	3.38	50	261.1	176.0	62.0
21.	Cyclobenzaprine	3.43	50	276.2	215.0	189.0
22.	α-Hydroxymidazolam	3.54	50	342.2	324.1	202.9
23.	Metaxalone	3.80	50	222.1	161.1	77.1
24.	N-Desmethylflunitrazepam	4.08	50	300.1	254.1	198.1
25.	Lorazepam	4.11	50	321.1	275.0	229.0
26.	Oxazepam	4.25	50	287.1	268.8	241.2
27.	Nitrazepam	4.30	50	282.1	180.1	235.9
28.	Clonazepam	4.31	50	316.1	270.0	214.1
29.	Desalkylflurazepam	4.38	50	289.0	140.1	104.0
30.	α-Hydroxytriazolam	4.40	50	359.1	330.9	175.9
31.	2-Hydroxyethylflurazepam	4.45	50	333.1	211.1	109.0
32.	Methaqualone	4.52	50	251.1	132.2	91.1
33.	α-Hydroxyalprazolam	4.54	50	325.1	297.0	216.2
34.	Nordiazepam	4.61	50	271.0	139.9	208.0
35.	Phenazepam	4.68	50	349.1	206.2	179.0
36.	Zaleplon	4.71	50	306.1	264.0	236.0
37.	Flunitrazepam	4.84	50	314.2	267.9	239.1
38.	Estazolam	4.85	50	295.1	266.9	205.1
39.	Triazolam	4.86	50	343.1	307.9	315.0
40.	Temazepam	4.90	50	301.1	255.1	282.9
41.	Alprazolam	4.98	50	309.1	280.9	204.9
42.	Diazepam	5.28	50	285.1	153.9	192.9
43.	Prazepam	5.72	50	325.2	139.9	271.0

*Analysis performed in negative ion mode.



Column
Dimensions: 100 mm x 2.1 mm ID
Particle Size: 2.7 µm
Pore Size: 90 Å
Guard Column: Raptor™ Biphenyl EXP® guard column cartridge 2.7 µm (cat.# 9309A0252)
Temp.: 30 °C

Sample
Diluent: Water
Conc.: 50-500 ng/mL
Inj. Vol.: 2 µL

Mobile Phase
A: Water + 0.1% formic acid
B: Methanol + 0.1% formic acid

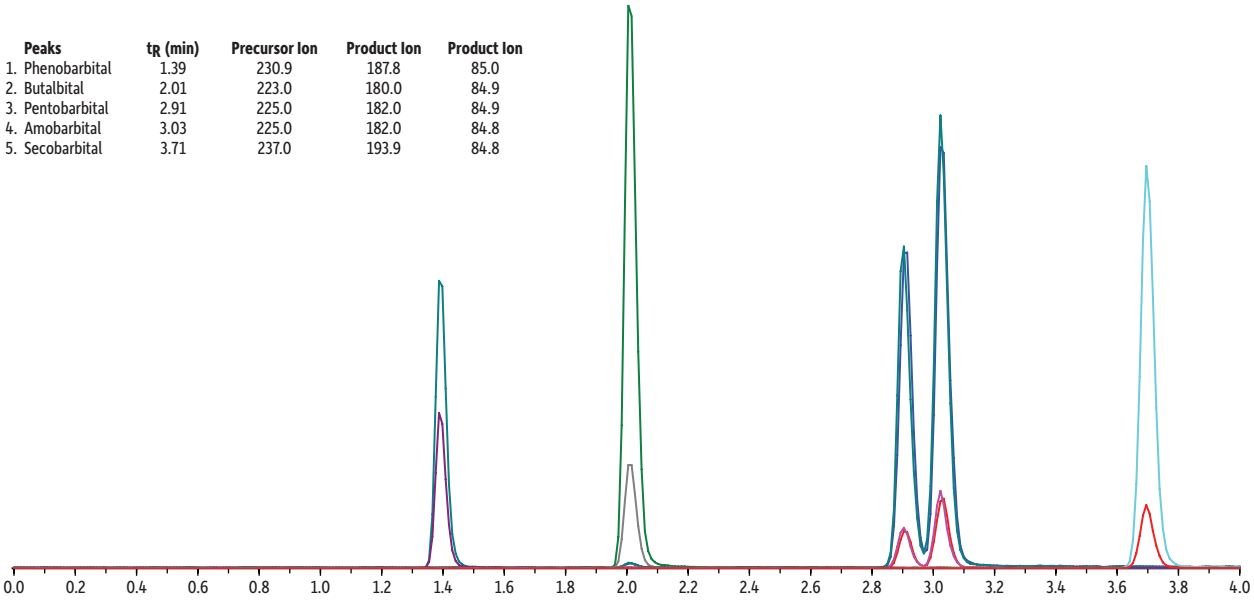
Time (min)	Flow (mL/min)	%A	%B
0.00	0.6	65	35
3.50	0.6	30	70
6.00	0.6	0	100
6.01	0.6	65	35
8.00	0.6	65	35

Detector MS/MS
Ion Mode: ESI+/ESI-
Mode: Scheduled MRM
Instrument UHPLC

LC_CF0657

Figure 5: Barbiturates analyzed separately with improved resolution in 6 minutes on the Raptor™ C18 column.

Peaks	t_R (min)	Precursor Ion	Product Ion	Product Ion
1. Phenobarbital	1.39	230.9	187.8	85.0
2. Butalbital	2.01	223.0	180.0	84.9
3. Pentobarbital	2.91	225.0	182.0	84.9
4. Amobarbital	3.03	225.0	182.0	84.8
5. Secobarbital	3.71	237.0	193.9	84.8



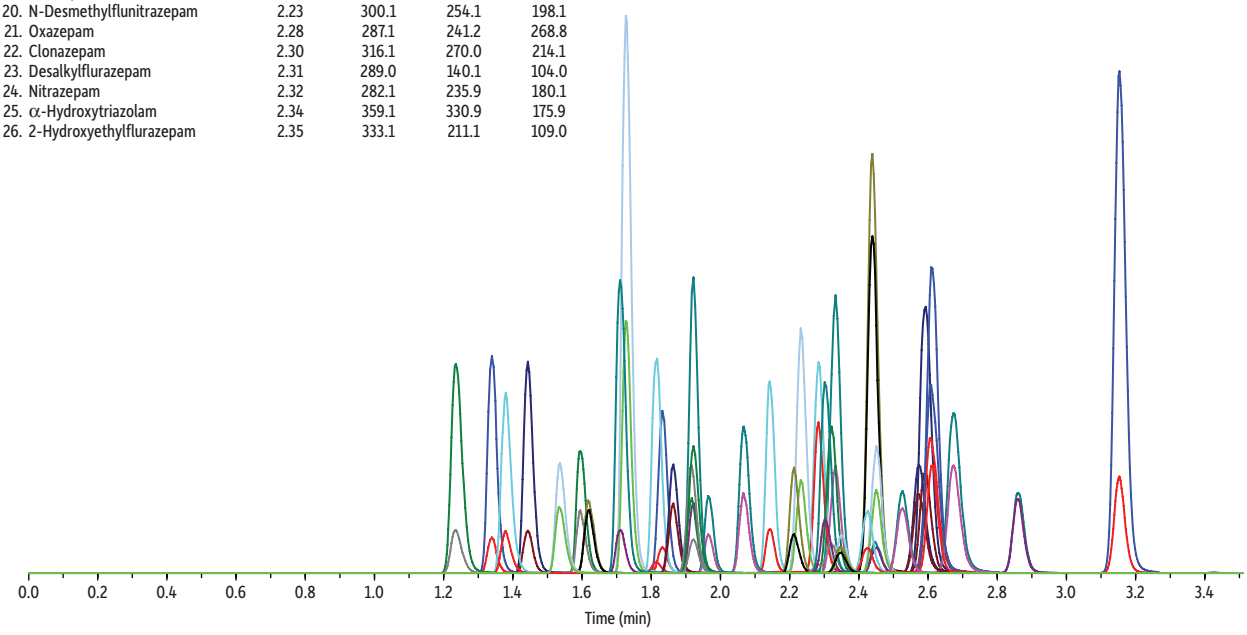
Column	Raptor™ C18 (cat.# 9304A12)
Dimensions:	100 mm x 2.1 mm ID
Particle Size:	2.7 µm
Temp.:	50 °C
Sample	
Diluent:	Water
Conc.:	500 ng/mL
Inj. Vol.:	5 µL
Mobile Phase	
A:	Water + 0.1% formic acid
B:	Acetonitrile + 0.1% formic acid

Time (min)	Flow (mL/min)	%A	%B
0.00	0.6	80	20
4.00	0.6	72	28
4.01	0.6	80	20
6.00	0.6	80	20

Detector	MS/MS
Ion Mode:	ESI-
Mode:	MRM
Instrument	UHPLC

Figure 6: Antianxiety drugs/metabolites analyzed separately (without barbiturates) in 5.5 minutes on the Raptor™ Biphenyl column.

Peaks	t_R (min)	Precursor Ion	Product Ion	Product Ion
1. 7-Aminonitrazepam	1.22	252.2	121.1	94.2
2. Norketamine	1.34	224.1	125.0	89.1
3. Zolpidem Phenyl-4-carboxylic acid	1.38	338.2	265.1	219.0
4. Ketamine	1.45	238.1	125.1	89.1
5. Zopiclone	1.54	389.2	245.0	217.2
6. Meprobamate	1.59	219.1	158.2	97.0
7. 7-Aminoclonazepam	1.60	286.1	121.2	250.1
8. Zolpidem	1.72	308.2	235.2	218.9
9. Diphenhydramine	1.74	256.1	167.0	152.1
10. Flurazepam	1.83	388.2	315.2	183.0
11. Buspirone	1.86	386.3	122.1	95.0
12. 7-Aminoflunitrazepam	1.87	284.1	135.0	227.1
13. Chlordiazepoxide	1.88	300.1	227.0	282.0
14. Midazolam	1.92	326.2	291.1	248.9
15. Cyclobenzaprine	1.94	276.2	215.0	189.0
16. Carisoprodol	1.96	261.1	176.0	62.0
17. α-Hydroxymidazolam	2.03	342.2	324.1	202.9
18. Metaxalone	2.14	222.1	161.1	77.1
19. Lorazepam	2.20	321.1	275.0	229.0
20. N-Desmethylflunitrazepam	2.23	300.1	254.1	198.1
21. Oxazepam	2.28	287.1	241.2	268.8
22. Clonazepam	2.30	316.1	270.0	214.1
23. Desalkylflurazepam	2.31	289.0	140.1	104.0
24. Nitrazepam	2.32	282.1	235.9	180.1
25. α-Hydroxytriazolam	2.34	359.1	330.9	175.9
26. 2-Hydroxyethylflurazepam	2.35	333.1	211.1	109.0
27. α-Hydroxyalprazolam	2.42	325.1	297.0	216.2
28. Methaqualone	2.44	251.1	132.2	91.1
29. Nordiazepam	2.44	271.0	139.9	208.0
30. Phenazepam	2.44	349.1	206.2	179.0
31. Zaleplon	2.53	306.1	264.0	236.0
32. Triazolam	2.58	343.1	307.9	315.0
33. Flunitrazepam	2.59	314.2	267.9	239.1
34. Estazolam	2.61	295.1	266.9	205.1
35. Temazepam	2.61	301.1	255.1	282.9
36. Alprazolam	2.68	309.1	280.9	204.9
37. Diazepam	2.85	285.1	153.9	192.9
38. Prazepam	3.14	325.2	271.0	139.9



LC_CF0615

Column
Dimensions: 100 mm x 2.1 mm ID
Particle Size: 2.7 µm
Guard Column: Raptor™ Biphenyl EXP® guard column cartridge 5 mm, 2.1 mm ID, 2.7 µm (cat.# 9309A0252)
Temp.: 30 °C

Sample
Diluent: Water
Conc.: 10 ng/mL
Inj. Vol.: 5 µL

Mobile Phase
A: Water + 0.1% formic acid
B: Methanol + 0.1% formic acid

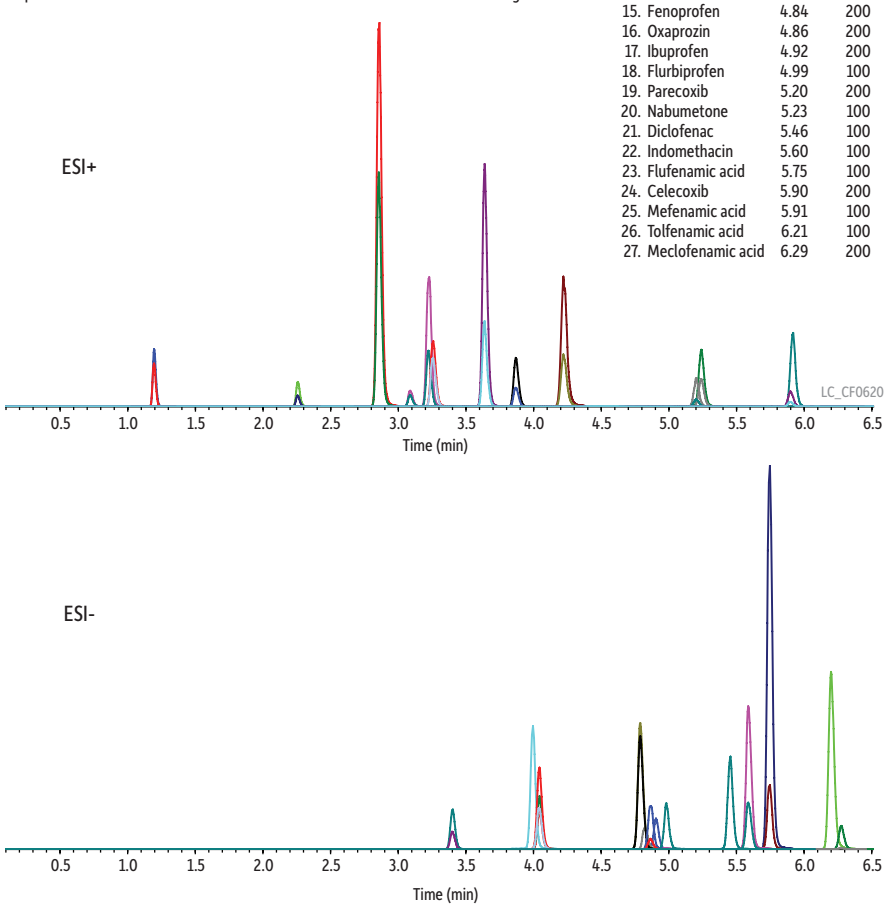
Time (min)	Flow (mL/min)	%A	%B
0.00	0.6	70	30
1.50	0.6	20	80
3.00	0.6	5	95
3.50	0.6	5	95
3.51	0.6	70	30
5.50	0.6	70	30

Detector MS/MS
Ion Mode: ESI+
Mode: Scheduled MRM
Instrument UHPLC

The optimized NSAIDs panel in Figure 7 is the final panel that warrants additional discussion regarding performance of the confirmational method. Although NSAIDs are generally considered safe, toxicity can occur due to their availability, widespread use, and their inclusion in combination drug formulations [4]. The primary challenge with NSAID analysis is that it is a diverse group of drugs and most detection methods are optimized for a single drug or one or more of its metabolites. For this panel, the combination of the Raptor™ Biphenyl column, use of scheduled polarity switching, and selection of mobile phases that maximized sensitivity in both positive and negative ion modes allowed the simultaneous detection of 27 NSAIDs (including acetaminophen) in one fast ~8-minute analysis.

Figure 7: NSAIDs panel confirmation analysis on the Raptor™ Biphenyl column.

Peaks	t_R (min)	Conc. (ng/mL)	Precursor Ion	Product Ion	Product Ion	ESI Polarity
1. Acetaminophen	1.19	100	152.2	93.0	65.0	Positive
2. Tenoxicam	2.26	100	338.1	121.0	95.0	Positive
3. Etoricoxib	2.84	100	359.2	280.0	279.2	Positive
4. Lornoxicam	3.08	200	373.1	121.1	95.0	Positive
5. Ketorolac	3.22	100	256.1	104.9	77.0	Positive
6. Piroxicam	3.26	100	332.1	95.2	78.1	Positive
7. Loxoprofen	3.41	100	245.0	83.2	80.9	Negative
8. Tolmetin	3.64	100	258.1	119.1	91.0	Positive
9. Sulindac	3.83	100	357.2	233.1	248.0	Positive
10. Ketoprofen	4.00	100	253.0	209.0	--	Negative
11. Naproxen	4.05	200	229.0	168.9	184.9	Negative
12. Salsalate	4.05	100	256.8	136.8	92.9	Negative
13. Meloxicam	4.22	100	352.1	115.0	141.0	Positive
14. Etodolac	4.80	100	286.0	241.9	212.0	Negative
15. Fenoprofen	4.84	200	240.9	92.8	--	Negative
16. Oxaprozin	4.86	200	292.0	219.8	102.0	Negative
17. Ibuprofen	4.92	200	205.0	160.9	--	Negative
18. Flurbiprofen	4.99	100	243.0	199.0	196.8	Negative
19. Parecoxib	5.20	200	371.2	234.0	165.1	Positive
20. Nabumetone	5.23	100	229.1	171.1	128.1	Positive
21. Diclofenac	5.46	100	293.9	249.8	--	Negative
22. Indomethacin	5.60	100	356.0	311.8	296.9	Negative
23. Flufenamic acid	5.75	100	279.9	236.1	176.2	Negative
24. Celecoxib	5.90	200	382.2	362.0	300.1	Positive
25. Mefenamic acid	5.91	100	242.1	224.1	--	Positive
26. Tolfenamic acid	6.21	100	259.9	215.8	--	Negative
27. Meclofenamic acid	6.29	200	293.9	257.8	177.7	Negative



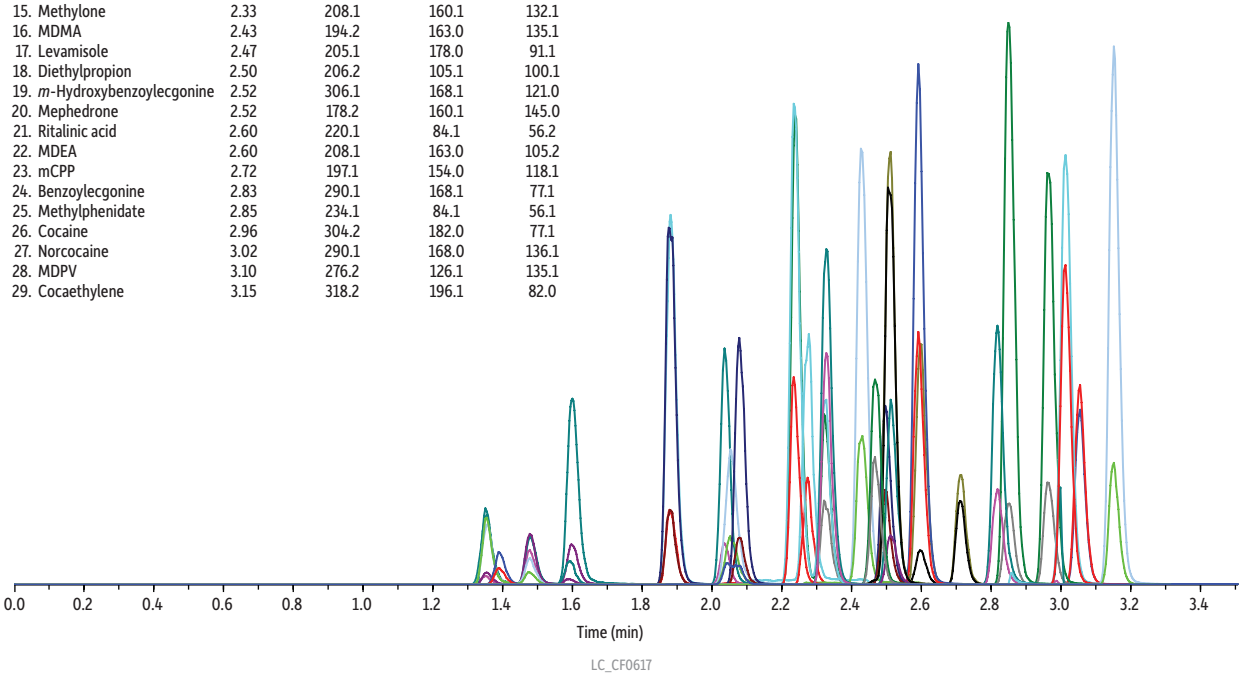
Column
Dimensions: 100 mm x 2.1 mm ID
Particle Size: 2.7 µm
Guard Column: Raptor™ Biphenyl EXP® guard column cartridge 5 mm, 2.1 mm ID, 2.7 µm (cat.# 9309A0252)
Temp.: 30 °C
Sample
Diluent: Water
Inj. Vol.: 5 µL
Mobile Phase
A: Water + 0.1% formic acid + 5 mM ammonium formate
B: (95:5) Acetonitrile:water + 0.1% formic acid + 5 mM ammonium formate

Time (min)	Flow (mL/min)	%A	%B
0.00	0.6	95	5
1.00	0.6	70	30
6.50	0.6	40	60
6.51	0.6	95	5
8.50	0.6	95	5

Detector MS/MS
Ion Mode: ESI+/ESI-
Mode: Scheduled MRM
Instrument UHPLC
Notes
1. Positive and negative polarity was collected simultaneously from a single injection.
2. Oxaprozin displays enhanced sensitivity using positive polarity.

Figure 8: Stimulants panel confirmation analysis on the Raptor™ Biphenyl column.

	Peaks	tR (min)	Precursor Ion	Product Ion	Product Ion
1.	Nicotine	1.35	163.2	117.1	130.1
2.	Norcotinine	1.36	163.1	80.1	118.1
3.	*trans*-3-Hydroxycotinine	1.41	193.1	80.0	134.0
4.	Anabasine	1.50	163.2	120.1	80.1
5.	Lisdexamfetamine	1.58	264.2	84.1	91.0
6.	BZP	1.64	177.2	91.1	65.1
7.	1S,2R-(+)-Ephedrine	1.88	166.2	148.1	115.0
8.	Amphetamine	2.03	136.2	91.0	65.1
9.	DMAA	2.06	116.1	57.1	99.2
10.	Methylephedrine	2.08	180.2	162.0	115.1
11.	Cotinine	2.15	177.2	80.0	98.1
12.	Methamphetamine	2.24	150.3	91.1	119.0
13.	MDA	2.28	180.1	163.1	105.2
14.	Phentermine	2.33	150.2	91.1	133.1
15.	Methylone	2.33	208.1	160.1	132.1
16.	MDMA	2.43	194.2	163.0	135.1
17.	Levamisole	2.47	205.1	178.0	91.1
18.	Diethylpropion	2.50	206.2	105.1	100.1
19.	*m*-Hydroxybenzoylecgonine	2.52	306.1	168.1	121.0
20.	Mephedrone	2.52	178.2	160.1	145.0
21.	Ritalinic acid	2.60	220.1	84.1	56.2
22.	MDEA	2.60	208.1	163.0	105.2
23.	mCPP	2.72	197.1	154.0	118.1
24.	Benzoylecgonine	2.83	290.1	168.1	77.1
25.	Methylphenidate	2.85	234.1	84.1	56.1
26.	Cocaine	2.96	304.2	182.0	77.1
27.	Norcocaine	3.02	290.1	168.0	136.1
28.	MDPV	3.10	276.2	126.1	135.1
29.	Cocaethylene	3.15	318.2	196.1	82.0



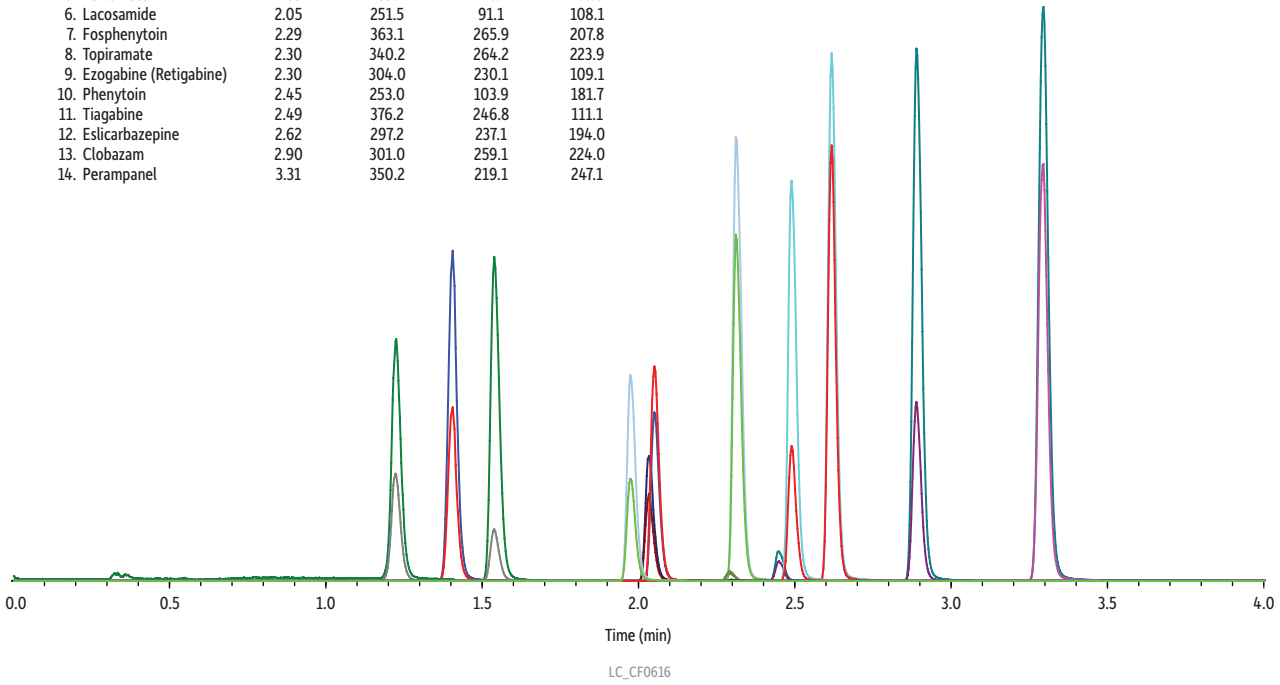
Column Raptor™ Biphenyl (cat.# 9309A12)
Dimensions: 100 mm x 2.1 mm ID
Particle Size: 2.7 µm
Guard Column: Raptor™ Biphenyl EXP® guard column cartridge 5 mm, 2.1 mm ID, 2.7 µm (cat.# 9309A0252)
Temp.: 30 °C
Sample
Diluent: Water
Conc.: 25 ng/mL
Inj. Vol.: 5 µL
Mobile Phase
A: Water + 0.1% formic acid + 5 mM ammonium formate
B: Methanol + 0.1% formic acid + 5 mM ammonium formate

Time (min)	Flow (mL/min)	%A	%B
0.00	0.6	95	5
3.50	0.6	10	90
3.51	0.6	95	5
5.50	0.6	95	5

Detector MS/MS
Ion Mode: ESI+
Mode: Scheduled MRM
Instrument UHPLC

Figure 9: Antiepileptic panel confirmation analysis on the Raptor™ Biphenyl column.

Peaks	t_R (min)	Precursor Ion	Product Ion	Product Ion
1. Pregabalin	1.24	160.2	142.1	55.0
2. Gabapentin	1.42	172.2	154.0	137.1
3. Levetiracetam	1.54	171.1	125.9	69.2
4. Zonisamide	1.98	212.9	132.1	77.1
5. Felbamate	2.03	239.1	117.1	177.8
6. Lacosamide	2.05	251.5	91.1	108.1
7. Fosphenytoin	2.29	363.1	265.9	207.8
8. Topiramate	2.30	340.2	264.2	223.9
9. Ezogabine (Retigabine)	2.30	304.0	230.1	109.1
10. Phenytoin	2.45	253.0	103.9	181.7
11. Tiagabine	2.49	376.2	246.8	111.1
12. Eslicarbazepine	2.62	297.2	237.1	194.0
13. Clobazam	2.90	301.0	259.1	224.0
14. Perampanel	3.31	350.2	219.1	247.1



Column
Dimensions: 100 mm x 2.1 mm ID
Particle Size: 2.7 µm
Guard Column: Raptor™ Biphenyl EXP® guard column cartridge 5 mm, 2.1 mm ID, 2.7 µm (cat.# 9309A0252)
Temp.: 40 °C

Sample
Diluent: Water
Conc.: 25 ng/mL
Inj. Vol.: 5 µL

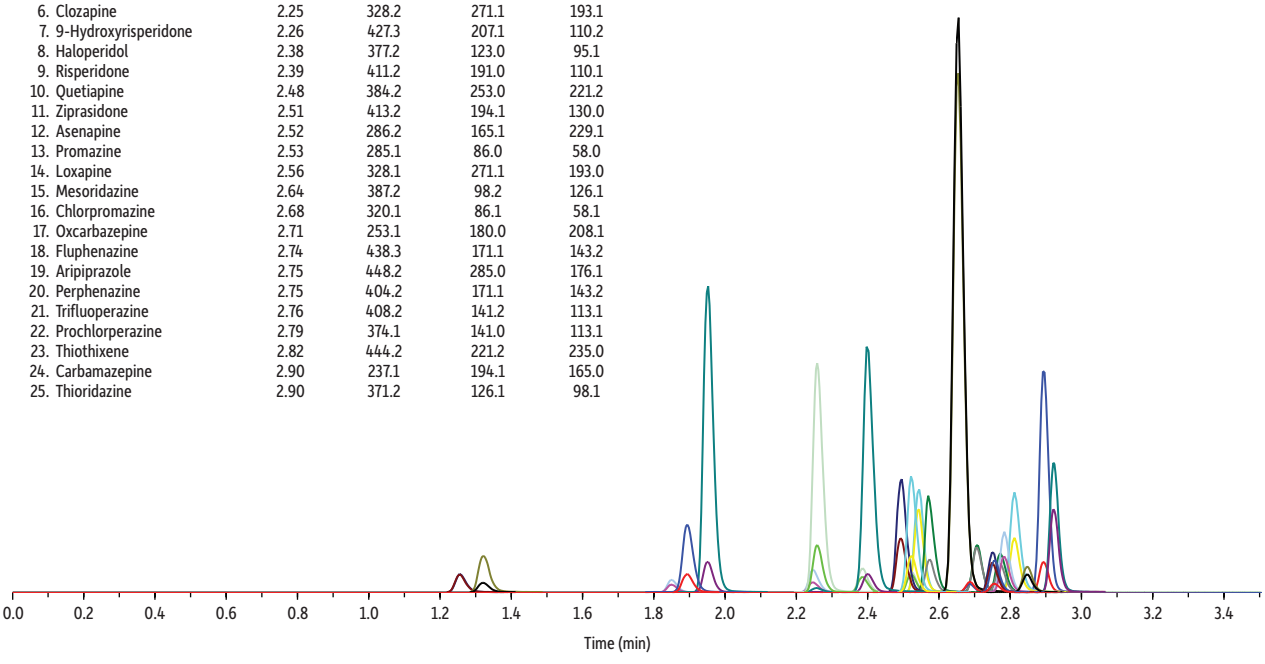
Mobile Phase
A: Water + 0.1% formic acid
B: Methanol + 0.1% formic acid

Time (min)	Flow (mL/min)	%A	%B
0.00	0.6	90	10
2.00	0.6	20	80
3.50	0.6	0	100
3.51	0.6	90	10
5.50	0.6	90	10

Detector MS/MS
Ion Mode: ESI+
Mode: Scheduled MRM
Instrument UHPLC

Figure 10: Antipsychotics panel confirmation analysis on the Raptor™ Biphenyl column.

Peaks	t_R (min)	Precursor Ion	Product Ion	Product Ion
1. Desmethylolanzapine	1.27	299.1	256.1	198.0
2. Olanzapine	1.32	313.2	256.1	198.1
3. Lamotrigine	1.86	256.0	211.1	145.0
4. 7-Hydroxyquetiapine	1.90	400.3	269.0	208.0
5. Molindone	1.95	277.2	100.1	70.2
6. Clozapine	2.25	328.2	271.1	193.1
7. 9-Hydroxyrisperidone	2.26	427.3	207.1	110.2
8. Haloperidol	2.38	377.2	123.0	95.1
9. Risperidone	2.39	411.2	191.0	110.1
10. Quetiapine	2.48	384.2	253.0	221.2
11. Ziprasidone	2.51	413.2	194.1	130.0
12. Asenapine	2.52	286.2	165.1	229.1
13. Promazine	2.53	285.1	86.0	58.0
14. Loxapine	2.56	328.1	271.1	193.0
15. Mesoridazine	2.64	387.2	98.2	126.1
16. Chlorpromazine	2.68	320.1	86.1	58.1
17. Oxcarbazepine	2.71	253.1	180.0	208.1
18. Fluphenazine	2.74	438.3	171.1	143.2
19. Aripiprazole	2.75	448.2	285.0	176.1
20. Perphenazine	2.75	404.2	171.1	143.2
21. Trifluoperazine	2.76	408.2	141.2	113.1
22. Prochlorperazine	2.79	374.1	141.0	113.1
23. Thiothixene	2.82	444.2	221.2	235.0
24. Carbamazepine	2.90	237.1	194.1	165.0
25. Thioridazine	2.90	371.2	126.1	98.1



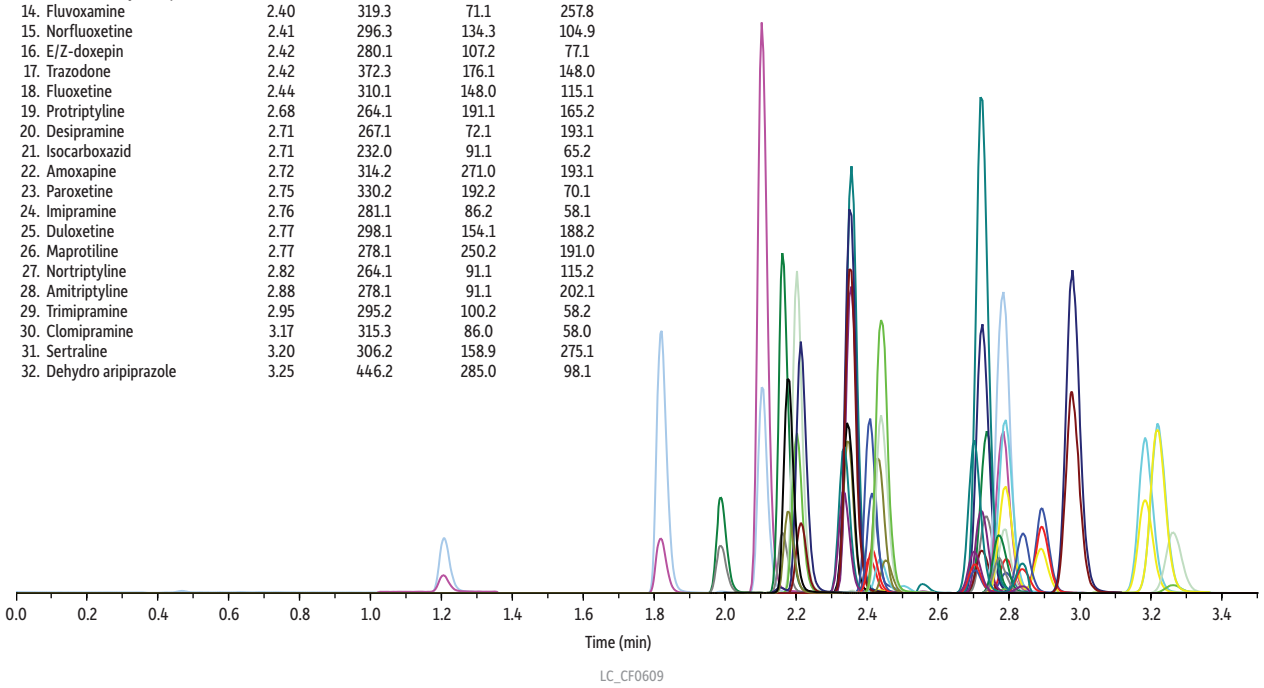
Column Raptor™ Biphenyl (cat.# 9309A12)
Dimensions: 100 mm x 2.1 mm ID
Particle Size: 2.7 µm
Guard Column: Raptor™ Biphenyl EXP® guard column cartridge 5 mm, 2.1 mm ID, 2.7 µm (cat.# 9309A0252)
Temp.: 30 °C
Sample
Diluent: Water
Conc.: 2.5 ng/mL
Inj. Vol.: 5 µL
Mobile Phase
A: Water + 0.1% formic acid
B: Methanol + 0.1% formic acid

Time (min)	Flow (mL/min)	%A	%B
0.00	0.6	85	15
3.00	0.6	0	100
3.01	0.6	85	15
5.00	0.6	85	15

Detector MS/MS
Ion Mode: ESI+
Mode: Scheduled MRM
Instrument UHPLC

Figure 11: Antidepressants panel confirmation analysis on the Raptor™ Biphenyl column.

Peaks	t_R (min)	Precursor Ion	Product Ion	Product Ion
1. Phenelzine sulfate	1.19	137.2	105.1	77.1
2. O-desmethylvenlafaxine	1.81	264.1	58.1	107.0
3. (+/-)-Hydroxybupropion	1.98	256.0	130.2	166.0
4. Bupropion	2.09	240.0	184.1	130.2
5. N-desmethylmirtazapine	2.15	252.1	195.1	209.2
6. Venlafaxine	2.16	278.4	260.4	121.2
7. Milnacipran	2.19	247.2	100.1	129.1
8. Mirtazapine	2.20	266.1	195.1	72.1
9. Didesmethylcitalopram	2.32	297.1	261.9	109.1
10. N-desmethylcitalopram	2.33	311.1	262.1	109.1
11. Citalopram	2.34	325.2	109.1	262.0
12. Escitalopram	2.34	325.3	109.1	261.9
13. E/Z-desmethyldoxepin	2.39	266.1	107.1	115.0
14. Fluvoxamine	2.40	319.3	71.1	257.8
15. Norfluoxetine	2.41	296.3	134.3	104.9
16. E/Z-doxepin	2.42	280.1	107.2	77.1
17. Trazodone	2.42	372.3	176.1	148.0
18. Fluoxetine	2.44	310.1	148.0	115.1
19. Protriptyline	2.68	264.1	191.1	165.2
20. Desipramine	2.71	267.1	72.1	193.1
21. Isocarboxazid	2.71	232.0	91.1	65.2
22. Amoxapine	2.72	314.2	271.0	193.1
23. Paroxetine	2.75	330.2	192.2	70.1
24. Imipramine	2.76	281.1	86.2	58.1
25. Duloxetine	2.77	298.1	154.1	188.2
26. Maprotiline	2.77	278.1	250.2	191.0
27. Nortriptyline	2.82	264.1	91.1	115.2
28. Amitriptyline	2.88	278.1	91.1	202.1
29. Trimipramine	2.95	295.2	100.2	58.2
30. Clomipramine	3.17	315.3	86.0	58.0
31. Sertraline	3.20	306.2	158.9	275.1
32. Dehydro aripiprazole	3.25	446.2	285.0	98.1



Column	Raptor™ Biphenyl (cat.# 9309A12)
Dimensions:	100 mm x 2.1 mm ID
Particle Size:	2.7 µm
Guard Column:	Raptor™ Biphenyl EXP® guard column cartridge 5 mm, 2.1 mm ID, 2.7 µm (cat.# 9309A0252)
Temp.:	30 °C
Sample	
Diluent:	Water
Conc.:	10 ng/mL
Inj. Vol.:	5 µL
Mobile Phase	
A:	Water + 0.1% formic acid
B:	Methanol + 0.1% formic acid

Time (min)	Flow (mL/min)	%A	%B
0.00	0.6	90	10
1.50	0.6	40	60
3.50	0.6	30	70
3.51	0.6	90	10
5.50	0.6	90	10

Detector	MS/MS
Ion Mode:	ESI+
Mode:	Scheduled MRM
Instrument	UHPLC

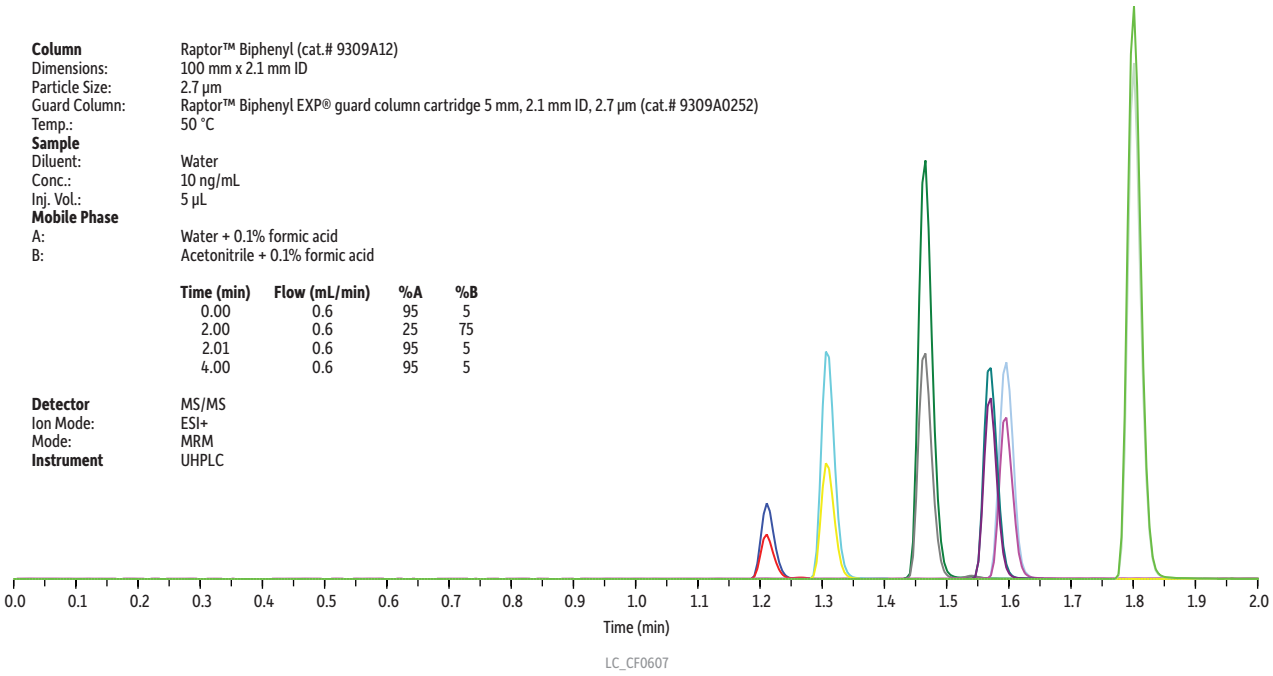
Figure 12: Hallucinogens panel confirmation analysis on the Raptor™ Biphenyl column.

Peaks	t_R (min)	Precursor Ion	Product Ion	Product Ion
1. 2-Oxo-3-hydroxy-LSD	1.21	356.3	237.0	222.0
2. Dextrorphan	1.31	258.2	157.0	201.2
3. LSD	1.46	324.3	223.1	208.0
4. Dextromethorphan	1.57	272.2	215.1	170.9
5. PCP	1.60	244.2	86.1	159.1
6. 25I-NBOMe	1.80	428.1	121.2	91.1

Column Raptor™ Biphenyl (cat.# 9309A12)
Dimensions: 100 mm x 2.1 mm ID
Particle Size: 2.7 µm
Guard Column: Raptor™ Biphenyl EXP® guard column cartridge 5 mm, 2.1 mm ID, 2.7 µm (cat.# 9309A0252)
Temp.: 50 °C
Sample
Diluent: Water
Conc.: 10 ng/mL
Inj. Vol.: 5 µL
Mobile Phase
A: Water + 0.1% formic acid
B: Acetonitrile + 0.1% formic acid

Time (min)	Flow (mL/min)	%A	%B
0.00	0.6	95	5
2.00	0.6	25	75
2.01	0.6	95	5
4.00	0.6	95	5

Detector MS/MS
Ion Mode: ESI+
Mode: MRM
Instrument UHPLC



Conclusion

As the demand for clinical and forensic drug testing increases, many drug testing facilities are turning to LC-MS/MS for its increased speed, sensitivity, and specificity. The methods shown here provide fast, accurate analysis of 231 drugs and drug metabolites. In this case, optimized method conditions and the use of a Raptor™ Biphenyl column allowed many problematic analytes—including over 40 isomers—to be identified and reported with confidence. The strong retention and unique selectivity of this column provide a powerful alternative to frequently used C18 columns.

References

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Appendix 1: Peak list and instrument conditions for Figure 1

	Peaks	t_R (min)	Conc. (ng/mL)	Precursor Ion	Product Ion	Product Ion
1.	*trans*-3-Hydroxycotinine	1.32	25	193.1	80.0	134.0
2.	Norcotinine	1.33	25	163.1	80.1	118.1
3.	Nicotine	1.40	25	163.2	130.1	117.1
4.	BZP	1.47	25	177.2	91.1	65.1
5.	Anabasine	1.53	25	163.2	120.1	80.1
6.	Noroxymorphone	1.78	25	288.1	270.1	212.9
7.	Lisdexamfetamine	1.79	25	264.2	84.1	91.0
8.	Phenelzine sulfate	1.90	25	137.2	105.1	77.1
9.	Acetaminophen	1.93	100	152.2	93.0	65.0
10.	Pregabalin	2.21	25	160.2	142.1	55.0
11.	Cotinine	2.27	25	177.2	80.0	98.1
12.	Morphine	2.27	25	286.2	152.1	165.0
13.	(+)-Ephedrine	2.37	25	166.2	148.1	115.0
14.	Oxymorphone	2.38	25	302.1	227.2	198.2
15.	Hydromorphone	2.61	25	286.2	184.9	156.9
16.	Amphetamine	2.63	25	136.2	91.0	65.1
17.	DMAA	2.65	25	116.1	57.1	99.2
18.	Gabapentin	2.71	25	172.2	154.0	137.1
19.	Methylephedrine	2.83	25	180.2	162.0	115.1
20.	Levetiracetam	2.84	25	171.1	125.9	69.2
21.	Desmethylolanzapine	2.94	25	299.1	256.1	198.0
22.	Norcodeine	2.96	25	286.1	151.9	115.0
23.	Olanzapine	3.12	25	313.2	256.1	198.1
24.	Methamphetamine	3.13	25	150.3	91.1	119.0
25.	MDA	3.18	25	180.1	163.1	105.2
26.	(-)-Naloxone	3.27	25	328.3	310.1	212.3
27.	Phentermine	3.29	25	150.2	91.1	133.1
28.	Noroxycodone	3.32	25	302.1	227.0	197.9
29.	Methylone	3.36	25	208.1	160.1	132.1
30.	O-Desmethyl-*cis*-tramadol	3.37	25	250.1	58.0	42.0
31.	(-)-Dihydrocodeine	3.38	25	302.2	199.0	128.2
32.	Norhydrocodone	3.43	25	286.1	199.0	128.2
33.	Codeine	3.43	25	300.2	152.0	165.1
34.	6-Acetylmorphine	3.47	25	328.2	165.0	211.0
35.	MDMA	3.60	25	194.2	163.0	135.1
36.	Desomorphine	3.61	25	272.1	152.1	165.0
37.	Oxycodone	3.64	25	316.2	298.0	169.0
38.	Naltrexone	3.71	25	342.2	324.0	267.0
39.	Levamisole	3.75	25	205.1	178.0	91.1
40.	Hydrocodone	3.76	25	300.1	199.0	128.0
41.	Diethylpropion	3.80	25	206.2	105.1	100.1
42.	Mephedrone	3.80	25	178.2	145.0	160.1
43.	2-Oxo-3-hydroxy-LSD	3.82	25	356.3	237.0	222.0
44.	*o*-Desmethylvenlafaxine	3.84	25	264.1	58.1	107.0
45.	6-β-Naltrexol	3.91	25	344.3	326.1	308.1
46.	Tapentadol-β-D-glucuronide	3.91	25	398.3	222.1	107.0
47.	7-Aminonitrazepam	3.95	25	252.2	121.1	94.2
48.	MDEA	4.00	25	208.1	163.0	105.2
49.	*m*-Hydroxybenzoylecgonine	4.02	25	306.1	168.1	121.0
50.	Lamotrigine	4.09	25	256.0	211.1	145.0
51.	Ritalinic acid	4.11	25	220.1	84.1	56.2
52.	N-Desmethyltapentadol	4.16	25	208.1	121.2	107.1
53.	Norbuprenorphine glucuronide	4.18	25	590.5	414.3	165.0
54.	Zonisamide	4.23	25	212.9	132.1	77.1
55.	mCPP	4.26	25	197.1	154.0	118.1
56.	Norketamine	4.28	25	224.1	125.0	89.1
57.	Nalbuphine	4.33	25	358.2	340.1	115.0
58.	Norfentanyl	4.33	25	233.1	84.1	55.0
59.	(+/-)-Hydroxybupropion	4.35	25	256.0	130.2	166.0
60.	Tapentadol	4.37	25	222.1	107.1	121.0
61.	Lacosamide	4.47	25	251.5	91.1	108.1
62.	Dextrorphan	4.50	25	258.2	157.0	201.2
63.	Levorphanol	4.50	25	258.2	156.8	199.2
64.	7-Hydroxyquetiapine	4.51	25	400.3	269.0	208.0
65.	Molindone	4.51	25	277.2	100.1	70.2
66.	Tramadol	4.52	25	264.2	58.0	77.1
67.	Zolpidem phenyl-4-carboxylic acid	4.60	25	338.2	265.1	219.0
68.	Felbamate	4.62	25	239.1	117.1	177.8
69.	N-Desmethyl-*cis*-tramadol	4.65	25	250.2	44.0	232.1
70.	Ketamine	4.66	25	238.1	125.1	89.1
71.	Benzoylecgonine	4.67	25	290.1	168.1	77.1
72.	Methylphenidate	4.68	25	234.1	84.1	56.1
73.	Normeperidine	4.76	25	234.1	160.2	91.0
74.	Meprobamate	4.79	25	219.1	158.2	97.0
75.	Meperidine	4.80	25	248.1	220.1	174.1
76.	Bupropion	4.81	25	240.0	184.1	130.2
77.	Heroin	4.85	25	370.4	328.2	164.9
78.	Cocaine	5.01	25	304.2	182.0	77.1
79.	7-Aminoclonazepam	5.02	25	286.1	121.2	250.1
80.	Zopiclone	5.04	25	389.2	245.0	217.2
81.	N-Desmethylmirtazapine	5.10	25	252.1	195.1	209.2
82.	Norcocaine	5.11	25	290.1	168.0	136.1
83.	Phenobarbital*	5.11	500	230.9	187.8	85.0
84.	Buprenorphine glucuronide	5.12	25	644.3	468.2	55.1
85.	MDPV	5.15	25	276.2	135.1	126.1
86.	Venlafaxine	5.15	25	278.4	260.4	121.2
87.	Milnacipran	5.24	25	247.2	100.1	129.1
88.	Pentazocine	5.29	25	286.2	218.1	69.2
89.	Mirtazapine	5.31	25	266.1	195.1	72.1
90.	Butalbital*	5.31	500	223.0	180.0	84.9
91.	Norbuprenorphine	5.35	50	414.3	152.2	165.2
92.	LSD	5.39	25	324.3	223.1	208.0
93.	Butorphanol	5.43	25	328.3	310.3	131.2
94.	Fosphenytoin	5.43	25	363.1	265.9	207.8
95.	Topiramate	5.44	25	340.2	264.2	223.9
96.	Cocaethylene	5.48	25	318.2	196.1	82.0
97.	Clozapine	5.48	25	328.2	271.1	193.1
98.	9-Hydroxyrisperidone	5.51	25	427.3	207.1	110.2
99.	Acetyl fentanyl	5.58	25	323.3	188.0	105.0
100.	Didesmethyl citalopram HCl	5.73	25	297.1	261.9	109.1
101.	7-Aminoflunitrazepam	5.75	25	284.1	135.0	227.1
102.	Amobarbital*	5.75	500	225.0	182.0	84.8
103.	Zolpidem	5.76	25	308.2	235.2	218.9
104.	N-Desmethylcitalopram	5.77	25	311.1	262.1	109.1
105.	Isocarboxazid	5.77	25	232.0	91.1	65.2
106.	Pentobarbital*	5.79	500	225.0	182.0	84.9
107.	Escitalopram	5.80	25	325.3	109.1	261.9
108.	Citalopram	5.80	25	325.2	109.1	262.0
109.	Diphenhydramine	5.80	25	256.1	167.0	152.1
110.	Desmethyldoxepin	5.88	25	266.1	107.1	115.0
111.	Ezogabine	5.91	25	304.0	230.1	109.1
112.	Doxepin	5.96	25	280.1	107.2	77.1
113.	Haloperidol	5.96	25	377.2	123.0	95.1
114.	Fluvoxamine	5.97	25	319.3	71.1	257.8
115.	Trazodone	5.99	25	372.3	176.1	148.0
116.	Norfluoxetine	6.01	25	296.3	134.3	104.9
117.	Fentanyl	6.02	25	337.3	188.0	105.1
118.	Oxcarbazepine	6.02	25	253.1	180.0	208.1
119.	Risperidone	6.03	25	411.2	191.0	110.1
120.	Buprenorphine	6.04	50	468.3	55.1	414.2
121.	Fluoxetine	6.05	25	310.1	148.0	115.1
122.	Secobarbital*	6.08	500	237.0	193.9	84.8
123.	Phenytoin	6.12	25	253.0	103.9	181.7
124.	Carisoprodol	6.13	25	261.1	176.0	62.0
125.	Flurazepam	6.14	25	388.2	315.2	183.0
126.	Chlordiazepoxide	6.14	25	300.1	282.0	227.0
127.	Dextromethorphan	6.16	25	272.2	215.1	170.9
128.	PCP	6.18	25	244.2	86.1	159.1
129.	Tenoxicam	6.18	25	338.1	121.0	95.0
130.	Buspirone	6.20	25	386.3	122.1	95.0
131.	Quetiapine	6.28	25	384.2	253.0	221.2
132.	Norpropoxyphene	6.29	25	326.4	252.0	91.1
133.	Propoxyphene	6.30	25	340.3	265.9	58.1
134.	Asenapine	6.35	25	286.2	165.1	229.1
135.	Ziprasidone	6.40	25	413.2	194.1	130.0
136.	Protriptyline	6.42	25	264.1	191.1	165.2
137.	Promazine	6.42	25	285.1	58.0	86.0
138.	Midazolam	6.43	25	326.2	291.1	248.9
139.	Desipramine	6.43	25	267.1	72.1	193.1
140.	Amoxapine	6.44	25	314.2	271.0	193.1
141.	Sufentanil	6.48	25	387.2	238.1	111.1
142.	Imipramine	6.51	25	281.1	86.2	58.1
143.	Paroxetine	6.51	25	330.2	192.2	70.1
144.	Loxapine	6.52	25	328.1	271.1	193.0
145.	Maprotiline	6.52	25	278.1	250.2	191.0
146.	Duloxetine	6.52	25	298.1	154.1	188.2
147.	Metaxalone	6.52	25	222.1	161.1	77.1
148.	Carbamazepine	6.55	10	237.1	194.1	165.0
149.	Cyclobenzaprine	6.56	25	276.2	215.0	189.0
150.	EDDP	6.57	25	278.2	234.3	249.2
151.	Nortriptyline	6.58	25	264.1	91.1	115.2
152.	Mitragynine	6.59	25	399.3	174.2	159.0
153.	Eslicarbazepine	6.60	25	297.2	194.0	237.1
154.	Amitriptyline	6.65	25	278.1	91.1	202.1
155.	α-Hydroxymidazolam	6.70	25	342.2	324.1	202.9
156.	Trimipramine	6.75	25	295.2	100.2	58.2
157.	N-Desmethylflunitrazepam	6.80	25	300.1	254.1	198.1
158.	Lorazepam	6.82	25	321.1	275.0	229.0
159.	Mesoridazine	6.83	25	387.2	126.1	98.2
160.	Tiagabine	6.89	25	376.2	246.8	111.1
161.	Ketorolac	6.90	25	256.1	104.9	77.0
162.	Chlorpromazine	6.91	25	320.1	58.1	86.1

Appendix 1: Peak list and instrument conditions for Figure 1 (Continued)

Peaks	t_R (min)	Conc. (ng/mL)	Precursor Ion	Product Ion	Product Ion
163. Loxoprofen*	6.93	200	245.0	83.2	80.9
164. Clomipramine	6.95	25	315.3	86.0	58.0
165. Oxazepam	6.96	25	287.1	241.2	268.8
166. Methadone	6.98	25	310.2	264.9	105.1
167. Sertraline	6.98	25	306.2	158.9	275.1
168. Dehydro aripiprazole	7.01	25	446.2	285.0	98.1
169. Clonazepam	7.01	25	316.1	270.0	214.1
170. Nitrazepam	7.01	25	282.1	235.9	180.1
171. Lornoxicam	7.02	100	373.1	121.1	95.0
172. 25I-NBOMe	7.05	25	428.1	121.2	91.1
173. Salsalate*	7.05	100	256.8	136.8	92.9
174. Desalkylflurazepam	7.09	25	289.0	140.1	104.0
175. Tolmetin	7.10	25	258.1	119.1	91.0
176. α-Hydroxytriazolam	7.12	25	359.1	330.9	175.9
177. Fluphenazine	7.16	25	438.3	171.1	143.2
178. 2-Hydroxyethylflurazepam	7.16	25	333.1	211.1	109.0
179. Ketoprofen*	7.17	100	253.0	209.0	-
180. Naproxen*	7.17	200	229.0	168.9	184.9
181. EMDP	7.19	25	264.2	235.2	220.3
182. Aripiprazole	7.20	25	448.2	285.0	176.1
183. Perphenazine	7.22	25	404.2	171.1	143.2
184. Etoricoxib	7.24	25	359.2	280.0	279.2
185. Methaqualone	7.24	25	251.1	91.1	132.2
186. α-Hydroxyalprazolam	7.25	25	325.1	297.0	216.2
187. Trifluoperazine	7.28	25	408.2	141.2	113.1
188. Piroxicam	7.34	25	332.1	95.2	78.1
189. Ibuprofen*	7.34	200	205.0	160.9	-
190. Prochlorperazine	7.36	25	374.1	141.0	113.1
191. Etodolac*	7.36	50	286.0	241.9	212.0
192. Nordiazepam	7.37	25	271.0	139.9	208.0
193. Phenazepam	7.39	25	349.1	206.2	179.0
194. Fenoprofen*	7.40	200	240.9	92.8	-
195. Zaleplon	7.43	25	306.1	264.0	236.0
196. Clobazam	7.43	25	301.0	259.1	224.0
197. Flurbiprofen*	7.48	200	243.0	199.0	196.8
198. Thiothixene	7.50	25	444.2	221.2	235.0
199. Flunitrazepam	7.55	25	314.2	267.9	239.1
200. Meloxicam	7.55	25	352.1	115.0	141.0
201. Triazolam	7.58	25	343.1	307.9	315.0
202. Estazolam	7.58	25	295.1	266.9	205.1
203. Celecoxib	7.60	25	382.2	362.0	300.1
204. Parecoxib	7.60	25	371.2	234.0	165.1
205. Temazepam	7.61	25	301.1	255.1	282.9
206. Sulindac	7.70	25	357.2	233.1	248.0
207. Alprazolam	7.72	25	309.1	280.9	204.9
208. Flufenamic acid*	7.75	50	279.9	236.1	176.2
209. Thioridazine	7.76	25	371.2	126.1	98.1
210. Diclofenac*	7.78	100	293.9	249.8	-
211. Nabumetone	7.80	25	229.1	171.1	128.1
212. Oxaprozin	7.87	25	294.1	103.0	234.2
213. HU-211	8.03	25	387.3	155.9	128.1
214. JWH-200	8.04	25	385.3	155.0	127.1
215. Diazepam	8.04	25	285.1	192.9	153.9
216. Mefenamic acid	8.04	25	242.1	224.1	-
217. Tolfenamic acid*	8.17	50	259.9	215.8	-
218. Meclofenamic acid*	8.18	200	293.9	257.8	177.7
219. (+/-)-CP 47,497*	8.27	200	317.1	245.1	159.0
220. Indomethacin*	8.29	50	356.0	311.8	296.9
221. Perampanel	8.32	25	350.2	219.1	247.1
222. (+/-)-CP 47,497 C8 homologue*	8.42	200	331.1	259.0	159.1
223. 11-Nor-9-carboxy-delta-9-THC*	8.44	25	343.0	298.9	244.8
224. Prazepam	8.51	25	325.2	271.0	139.9
225. XLR-11	8.60	25	330.3	125.2	144.0
226. Δ9-THC	8.76	25	315.2	193.0	123.2
227. UR-144	8.78	25	312.3	125.1	214.1
228. JWH-250	8.96	25	336.3	120.9	91.1
229. THCA-A*	9.12	25	357.1	312.9	245.0
230. JWH-073	9.21	25	328.2	155.0	127.1
231. JWH-018	9.35	25	342.3	127.1	155.1

* Analyzed using negative polarity.

Column
Dimensions: 100 mm x 2.1 mm ID
Particle Size: 2.7 µm
Guard Column: Raptor™ Biphenyl EXP® guard column cartridge 5 mm, 2.1 mm ID, 2.7 µm (cat.# 9309A0252)
Temp.: 30 °C

Sample
Diluent: Water
Inj. Vol.: 5 µL

Mobile Phase
A: Water + 0.1% formic acid + 2 mM ammonium formate
B: Methanol + 0.1% formic acid + 2 mM ammonium formate

Time (min)	Flow (mL/min)	%A	%B
0.00	0.6	95	5
9.00	0.6	0	100
10.00	0.6	0	100
10.01	0.6	95	5
12.00	0.6	95	5

Detector MS/MS
Ion Mode: ESI+/ESI-
Mode: Scheduled MRM
Instrument UHPLC
Notes

1. Positive and negative polarity data were collected simultaneously from a single injection.
2. Ibuprofen, fosphenytoin, and topiramate degrade quickly in solution.
3. Levorphanol and dextrorphan are enantiomers and are not resolved.
4. Escitalopram is a pure enantiomer and citalopram is a racemic mixture of both enantiomers. These compounds are not resolved.
5. DMAA consists of 4 stereoisomers, 2 of which are partially resolved.
6. Doxepin and desmethylydoxepin consist of a mixture of *cis/trans* isomers. The isomers are fully resolved and the retention time identifies the main isomer component.
7. Lornoxicam, meloxicam, etodolac, oxaprozin, indomethacin, and mefenamic acid can be detected by positive or negative polarity. The polarity chosen in this chromatogram provides optimal response.

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