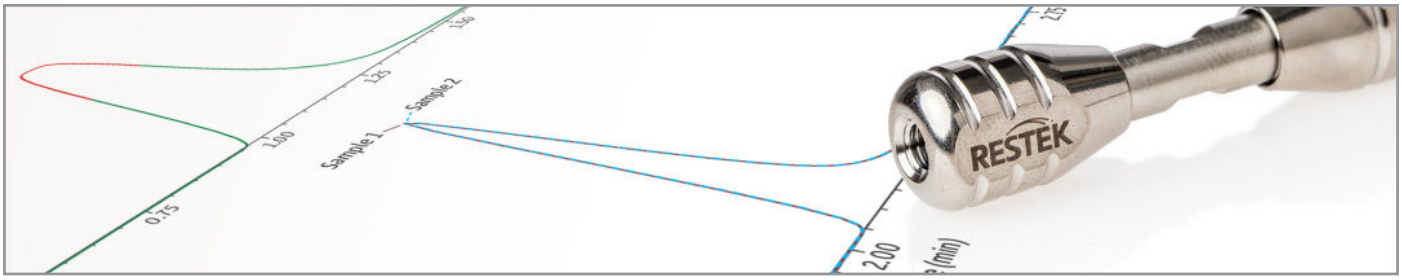
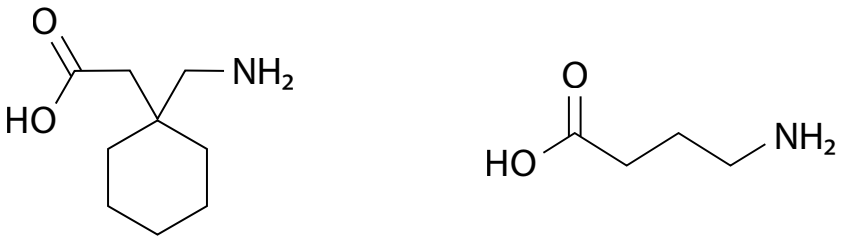


Strategies For Managing High Concentrations of Gabapentin in Urine by LC-MS/MS

Introduction

Gabapentin is an anticonvulsant drug. This compound is a structural analog of the neurotransmitter gamma-aminobutyric acid (GABA).[1] While the primary use of gabapentin is in the treatment of neuropathic pain and seizures, it is commonly prescribed for many off-label uses as well. These include the treatment of anxiety disorders, chronic pain disorders, and restless leg syndrome.[2,3]

Figure 1: Structures of Gabapentin (Left) and GABA (Right)



Related Products

- *Raptor Biphenyl column (cat.# 9309A52, 9309A12, 9309A55)*
- *Raptor Biphenyl EXP Guard Column Cartridge (cat.# 25937)*
- *EXP direct connect holder for EXP guard cartridges (cat.# 25808)*
- *EXP Hand-Tight Fitting (Nut w/Ferrule) (cat.# 25937)*
- *Short-Cap Vial with Grad Marking Spot (cat.# 21142)*
- *Vial Inserts, Glass, Big Mouth (cat.# 21776)*
- *Short Screw Cap, Polypropylene, Screw-Thread (cat.# 24497)*

Gabapentin is prescribed in high doses relative to other therapeutic drugs and is eliminated in urine predominantly in its original form.[1] Due to the high dosages, and the fact that the chemical structure is not modified by any metabolic processes, detection of extremely high concentrations of this compound in urine patient samples is common when analyzed by drug testing assays. Heltsley et al. (2011) reported the mean concentration of gabapentin to be 430.9 µg/mL in patient urine specimens.[1] When analyzed by LC-MS/MS, high concentrations of gabapentin can present significant analytical complications. In this work, we will discuss the analytical challenges associated with high concentrations of gabapentin in urine samples and explore several strategies to address them.

Analytical Challenges of High Gabapentin Levels

Detector Saturation

In targeted mass spectrometry (MS), the detector measures the abundance of ions at selected mass-to-charge ratios (m/z). Saturation of the detector occurs when the intensity of those ions exceeds the upper limit of the detection system. When an analyte saturates the detector, a distorted, flat-topped peak shape is typically displayed in the software. Saturation prevents accurate quantitation of the analyte as the detector response is cut off when it exceeds the upper limit, and the reading is not accurate to what is present in the sample. For samples containing high levels of gabapentin, detector saturation is a concern.

Column Overload

HPLC columns also have limits to analyte concentrations that can be injected while still achieving normal peak shapes due to mass overload. The stationary phase of an HPLC column has a limited number of interaction sites for solute molecules. When there is a greater number of solute molecules seeking interaction sites than are available on the stationary phase, the molecules will continue through the column in the mobile phases until available interaction sites are reached. As analytes move down the column, the sample will be spread across a wider area, which will result in distorted, asymmetric peaks and shifting retention times. High concentrations of gabapentin may cause column overloading and result in unfavorable peak shapes.

Interference

Detector and column overloading of an analyte can negatively affect performance of other nearby eluting compounds. Analytes eluting in the same area as the highly concentrated analyte may suffer from poor peak shape, signal suppression, and retention time shifts. An interference between amphetamine and gabapentin when analyzed by LC-MS/MS has been well documented. [4,5] Gabapentin and amphetamine will elute at very similar retention times under certain chromatographic conditions. This may result in signal suppression and shifting retention times for amphetamine when gabapentin is present in high concentrations.

Experimental

In this section, urine samples spiked with various concentrations of gabapentin and amphetamine were analyzed using an LC-MS/MS method (Method 1) developed for the analysis of 60 drugs of abuse in urine. Samples were fortified with varying amounts of gabapentin and amphetamine in urine as described in Table I. The data was examined to determine how analyte performance was impacted by high levels of gabapentin when analyzed by Method 1. Based on these results, a new method (Method 2) was developed to mitigate the analytical challenges presented by samples with high gabapentin concentrations.

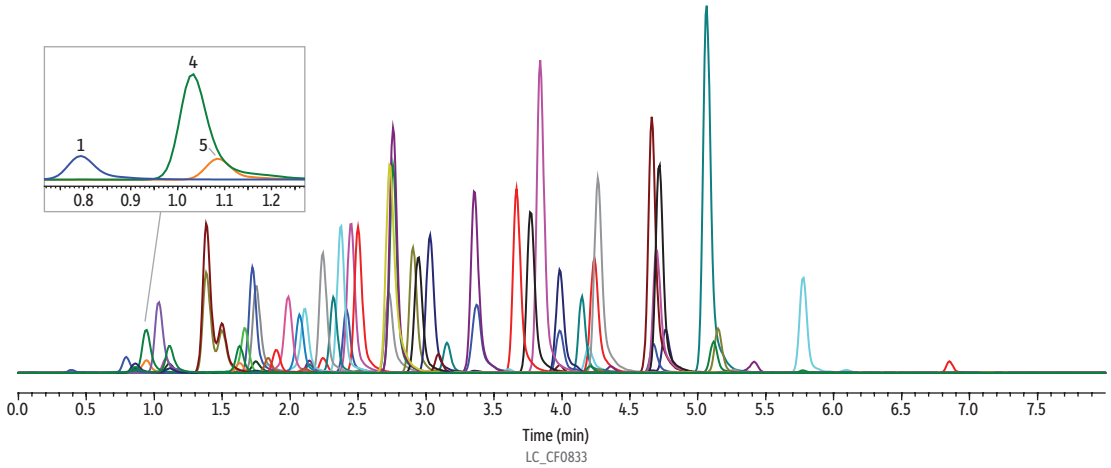
Table I: Analyte Concentrations of Samples 1, 2, and 3 in Urine

Sample	Gabapentin Concentration (µg/mL)	Amphetamine Concentration (µg/mL)
1	0.1	0.1
2	250	0.1
3	500	-

Method 1—Instrument Conditions

The instrument conditions and an example chromatogram containing all target analytes in Method 1 are shown below. Under these conditions, gabapentin and amphetamine elute at similar retention times, with gabapentin at 1.09 minutes and amphetamine at 1.03 minutes.

Figure 2: Method 1 Instrument Conditions



	Peaks	tR (min)	Precursor	Product 1	Product 2
1.	Pregabalin	0.80	160.1	142.1	55.0
2.	Morphine	0.85	285.9	152.0	165.1
3.	Oxymorphone	0.95	302.0	284.1	227.0
4.	Amphetamine	1.03	135.9	91.1	65.1
5.	Gabapentin	1.09	172.1	154.1	136.9
6.	Hydromorphone	1.11	286.2	185.1	157.0
7.	Methamphetamine	1.38	150.1	91.2	119.0
8.	Phentermine	1.50	150.1	91.2	133.2
9.	Noroxycodone	1.63	301.9	227.0	197.1
10.	Naloxone	1.66	327.9	310.1	212.1
11.	O-Desmethyltramadol	1.72	250.1	58.1	-
12.	Norhydrocodone	1.75	285.9	199.1	128.1
13.	MDMA	1.75	194.1	162.9	134.9
14.	Codeine	1.84	300.1	165.1	215.0
15.	6-Acetylmorphine	1.89	328.0	165.1	211.1
16.	Oxycodone	1.97	315.9	298.1	241.1
17.	Naltrexone	2.06	342.1	324.1	267.1
18.	Hydrocodone	2.10	300.1	199.1	171.1
19.	Desmethylvenlafaxine	2.14	264.0	58.1	107.0
20.	6-B-Naltrexol	2.23	344.0	326.1	308.2
21.	Ritalinic acid	2.32	220.1	84.1	55.9
22.	N-Desmethyltapentadol	2.37	208.1	107.0	121.0
23.	Norketamine	2.41	223.9	125.0	89.2
24.	Hydroxybupropion	2.44	256.1	238.0	138.9
25.	Norfentanyl	2.49	233.1	84.0	55.1
26.	4'-Hydroxy nitazene	2.73	369.1	100.2	72.0
27.	7-Hydroxyquetiapine	2.73	399.9	269.0	208.0
28.	Tramadol	2.75	264.1	58.0	-
29.	Normeperidine	2.91	233.9	160.1	56.0
30.	Benzoylecgonine	2.95	290.1	168.1	77.0

	Peaks	tR (min)	Precursor	Product 1	Product 2
31.	Zolpidem phenyl-4-carboxylic acid	3.03	337.9	265.1	219.2
32.	Meprobamate	3.08	219.1	158.1	55.0
33.	7-Aminoclonazepam	3.15	286.0	121.1	249.9
34.	Venlafaxine	3.36	278.0	260.2	121.0
35.	Mirtazapine	3.37	266.1	195.1	106.9
36.	Norbuprenorphine	3.61	414.0	101.1	222.9
37.	LSD	3.67	324.1	223.1	208.1
38.	9-Hydroxyrisperidone	3.77	427.1	207.1	110.0
39.	Acetyl fentanyl	3.84	322.9	188.2	105.0
40.	Citalopram	3.98	324.9	109.1	233.8
41.	Desmethyldoxepin	3.99	266.0	115.0	107.0
42.	Trazodone	4.14	372.1	176.1	148.0
43.	Haloperidol	4.20	376.9	123.1	95.0
44.	Dextromethorphan	4.21	272.0	215.1	171.1
45.	PCP	4.25	244.2	86.1	159.2
46.	Fentanyl	4.26	337.0	188.0	105.1
47.	Norfluoxetine	4.27	296.0	134.0	29.9
48.	Buprenorphine	4.37	468.2	55.0	396.1
49.	EDDP	4.66	278.1	234.1	249.1
50.	Nortriptyline	4.68	264.1	91.2	115.0
51.	Cyclobenzaprine	4.69	276.0	215.0	189.1
52.	Sufentanil	4.72	387.0	238.0	110.9
53.	Amitriptyline	4.77	278.0	202.1	91.2
54.	Methadone	5.05	310.0	265.1	105.0
55.	Lorazepam	5.12	320.8	275.0	302.9
56.	Oxazepam	5.15	287.0	241.0	268.5
57.	Dehydro aripiprazole	5.27	446.0	285.1	98.1
58.	α-hydroxyalprazolam	5.41	325.0	297.0	216.0
59.	Temazepam	5.78	301.0	255.0	282.5
60.	Δ9-THC-COOH	6.85	345.1	327.0	299.2

Column Raptor Biphenyl (cat.# 9309A52)
Dimensions: 50 mm x 2.1 mm ID
Particle Size: 2.7 µm
Pore Size: 90 Å
Guard Column: Raptor Biphenyl EXP guard column cartridge 5 mm, 2.1 mm ID, 2.7 µm (cat.# 9309A0252)
Temp.: 45 °C
Standard/Sample
Diluent: 90:10 Water:methanol, both with 0.1% formic acid
Conc.: 500 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
6.00	0.6	25	75
7.00	0.6	0	100
8.00	0.6	0	100
8.01	0.6	90	10
9.00	0.6	90	10

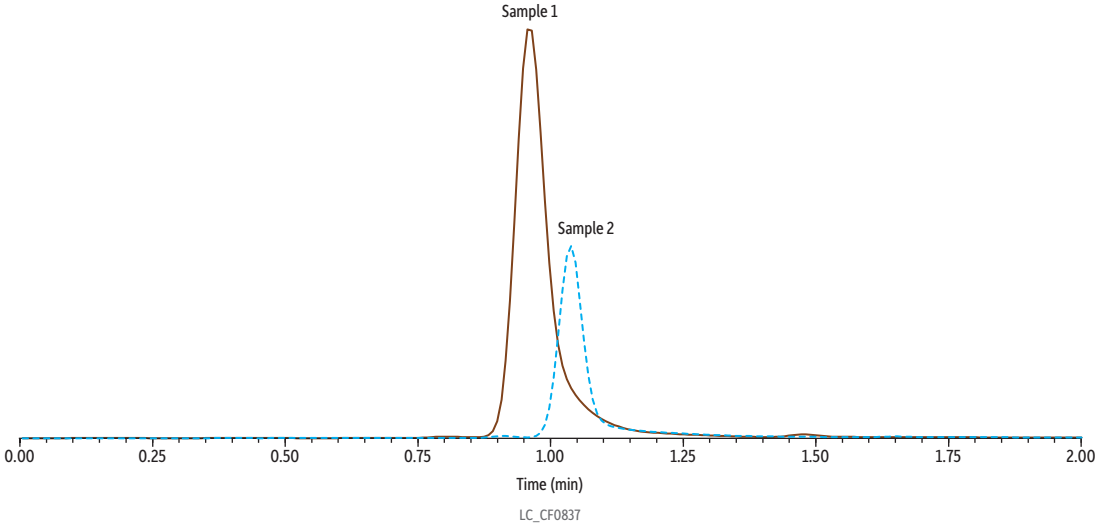
Max Pressure: 300 bar
Detector Shimadzu 8045 LC-MS/MS
Ion Mode: ESI+
Mode: MRM
Instrument Shimadzu Nexera X2
Sample Preparation Control urine (20 µL) was added to a 1.5 mL microcentrifuge tube along with 20 µL of a premade enzyme hydrolysis master mix. The sample was vortexed for 10 seconds and left to incubate at room temperature for 20 minutes. After the incubation, 260 µL of the diluent (water, 0.1 % formic acid:methanol, 0.1 % formic acid 90:10 [v/v]) was added. A 100 µL aliquot was added to a vial insert (cat.# 21776) in a 2.0 mL amber short-cap vial (cat.# 21142) and capped with a 9 mm short screw cap (cat.# 24497) and injected on the LC-MS/MS for analysis.

Method 1—Results

Amphetamine

In Figure 3, Sample 1 (0.1 µg/mL of gabapentin and amphetamine) and Sample 2 (250 µg/mL of gabapentin and 0.1 µg/mL of amphetamine) were analyzed using Method 1. When compared, the peak height and area for amphetamine are significantly lower in Sample 2 than in Sample 1. This indicates that, under these conditions, the high concentration of gabapentin present in Sample 2 is significantly suppressing the signal of amphetamine. Additionally, in Sample 2, amphetamine experienced a shifted retention time.

Figure 3: Amphetamine in Sample 1 and Sample 2, Analyzed Using Method 1



Peaks	Conc. (µg/mL)	t_R (min) (Sample 1)	t_R (min) (Sample 2)	Peak Area (Sample 1)	Peak Area (Sample 2)	Peak Height (Sample 1)	Peak Height (Sample 2)
1. Amphetamine	0.1	0.96	1.03	3351017	1257485	720647	335805

Column Raptor Biphenyl (cat.# 9309A52)
Dimensions: 50 mm x 2.1 mm ID
Particle Size: 2.7 µm
Pore Size: 90 Å
Guard Column: Raptor Biphenyl EXP guard column cartridge 5 mm, 2.1 mm ID, 2.7 µm (cat.# 9309A0252)
Temp.: 45 °C
Standard/Sample
Diluent: 90:10 Water:methanol, both with 0.1% formic acid
Inj. Vol.: 5 µL
Mobile Phase
A: Water, 0.1% formic acid
B: Methanol, 0.1% formic acid

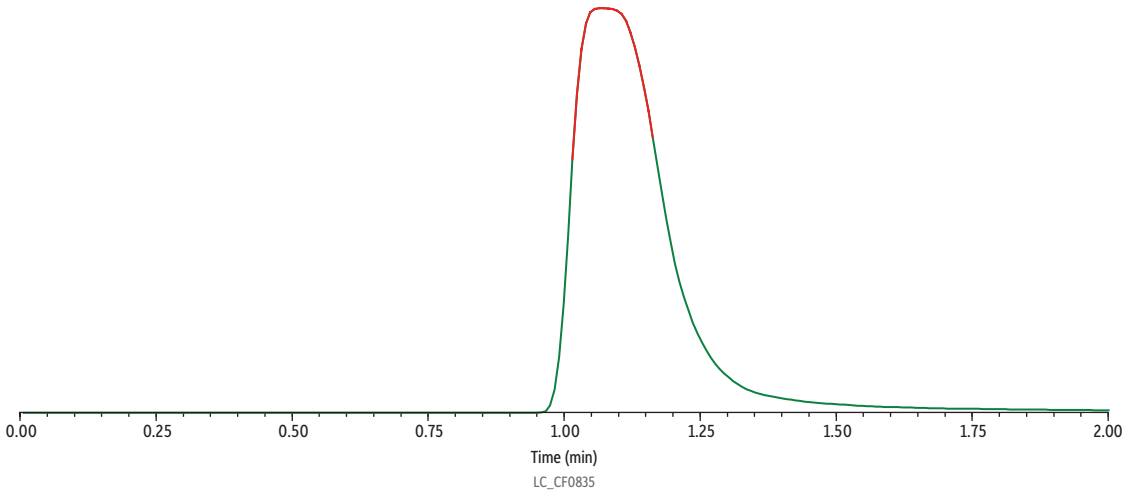
Time (min)	Flow (mL/min)	%A	%B
0.00	0.6	90	10
6.00	0.6	25	75
7.00	0.6	0	100
8.00	0.6	0	100
8.01	0.6	90	10
9.00	0.6	90	10

Max Pressure: 300 bar
Detector Shimadzu 8045 LC-MS/MS
Ion Mode: ESI+
Mode: MRM
Instrument Shimadzu Nexera X2
Sample Preparation Control urine (20 µL) was added to a 1.5 mL microcentrifuge tube along with 20 µL of a premade enzyme hydrolysis master mix. The sample was vortexed for 10 seconds and left to incubate at room temperature for 20 minutes. After the incubation, 260 µL of the diluent (water, 0.1 % formic acid:methanol, 0.1 % formic acid 90:10 [v/v]) was added. A 100 µL aliquot was added to a vial insert (cat.# 21776) in a 2.0 mL amber short-cap vial (cat.# 21142) and capped with a 9 mm short screw cap (cat.# 24497) and injected on the LC-MS/MS for analysis.
Notes Sample 1 contained 0.1 µg/mL of gabapentin (not shown) and 0.1 µg/mL of amphetamine.
Sample 2 contained 250 µg/mL of gabapentin (not shown) and 0.1 µg/mL of amphetamine.

Gabapentin

In Figure 4, Sample 3 (500 µg/mL of gabapentin) was analyzed using Method 1. The high concentration of gabapentin results in a wide peak that is tailing significantly. The apex of the peak is beginning to flatten, indicating that the detector is being overloaded.

Figure 4: Gabapentin in Sample 3 Analyzed Using Method 1

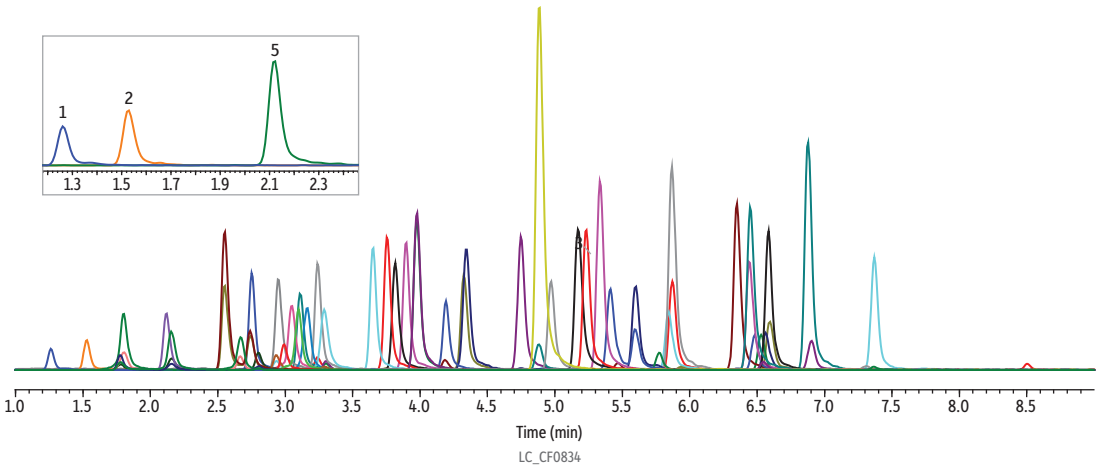


Peaks	tR (min)	Precursor	Product 1	Product 2
1. Gabapentin	1.09	172.1	154.1	136.9

Method 2—Instrument Conditions

The instrument conditions and an example chromatogram containing all target analytes in Method 2 are shown below. Under these conditions, gabapentin and amphetamine are chromatographically resolved, with gabapentin at 1.53 minutes and amphetamine at 2.12 minutes.

Figure 5: Method 2 Instrument Conditions



Peaks	tR (min)	Precursor	Product 1	Product 2	Peaks	tR (min)	Precursor	Product 1	Product 2
1. Pregabalin	1.26	160.1	142.1	55.0	31. Venlafaxine	4.75	278.0	260.2	121.0
2. Gabapentin	1.53	172.1	154.1	136.9	32. 7-aminoclonazepam	4.88	286.0	121.1	249.9
3. Morphine	1.78	285.9	152.0	165.1	33. 4'-Hydroxy nitazene	4.89	369.1	100.2	72.0
4. Oxymorphone	1.80	302.0	284.1	227.0	34. Norbuprenorphine	4.92	414.0	101.1	222.9
5. Amphetamine	2.12	135.9	91.1	65.1	35. 7-Hydroxyquetiapine	4.96	399.9	269.0	208.0
6. Hydromorphone	2.15	286.2	185.1	157.0	36. 9-Hydroxyrisperidone	5.17	427.1	207.1	110.0
7. Methamphetamine	2.55	150.1	91.2	119.0	37. LSD	5.23	324.1	223.1	208.1
8. Noroxycodone	2.66	301.9	227.0	197.1	38. Acetyl fentanyl	5.34	322.9	188.2	105.0
9. Phentermine	2.74	150.1	91.2	133.2	39. Mirtazapine	5.41	266.1	195.1	106.9
10. O-Desmethyltramadol	2.75	250.1	58.1	-	40. Citalopram	5.46	324.9	109.1	233.8
11. Norhydrocodone	2.80	285.9	199.1	128.1	41. Desmethyldoxepin	5.60	266.0	115.0	107.0
12. Codeine	2.92	300.1	165.1	215.0	42. Haloperidol	5.77	376.9	123.1	95.0
13. MDMA	2.95	194.1	162.9	134.9	43. Dextromethorphan	5.84	272.0	215.1	171.1
14. 6-Acetylmorphine	2.96	328.0	165.1	211.1	44. PCP	5.86	244.2	86.1	159.2
15. Oxycodone	3.05	315.9	298.1	241.1	45. Fentanyl	5.87	337.0	188.0	105.1
16. Ritalinic acid	3.12	220.1	84.1	55.9	46. Norfluoxetine	5.93	296.0	134.0	29.9
17. Naloxone	3.13	327.9	310.1	212.1	47. EDDP	6.35	278.1	234.1	249.1
18. Naltrexone	3.15	342.1	324.1	267.1	48. Trazodone	6.45	372.1	176.1	148.0
19. 6-B-Naltrexol	3.24	344.0	326.1	308.2	49. Cyclobenzaprine	6.46	276.0	215.0	189.1
20. Hydrocodone	3.29	300.1	199.1	171.1	50. Nortriptyline	6.48	264.1	91.2	115.0
21. Desmethylvenlafaxine	3.30	264.0	58.1	107.0	51. Lorazepam	6.52	320.8	275.0	302.9
22. N-Desmethyltapentadol	3.65	208.1	107.0	121.0	52. Buprenorphine	6.55	468.2	55.0	396.1
23. Norfentanyl	3.75	233.1	84.0	55.1	53. Amitriptyline	6.56	278.0	202.1	91.2
24. Benzoylecgonine	3.82	290.1	168.1	77.0	54. Sufentanil	6.58	387.0	238.0	110.9
25. Hydroxybupropion	3.89	256.1	238.0	138.9	55. Oxazepam	6.60	287.0	241.0	268.5
26. Tramadol	3.97	264.1	58.0	-	56. Methadone	6.88	310.0	265.1	105.0
27. Meprobamate	4.19	219.1	158.1	55.0	57. α-Hydroxyalprazolam	6.90	325.0	297.0	216.0
28. Norketamine	4.20	223.9	125.0	89.2	58. Dehydro aripiprazole	7.31	446.0	285.1	98.1
29. Normeperidine	4.32	233.9	160.1	56.0	59. Temazepam	7.36	301.0	255.0	282.5
30. Zolpidem Phenyl-4-carboxylic acid	4.34	337.9	265.1	219.2	60. Δ9-THC-COOH	8.50	345.1	327.0	299.2

Column Raptor Biphenyl (cat.# 9309A12)
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 5 mm, 2.1 mm ID, 2.7 µm (cat.# 9309A0252)
Temp.: 45 °C
Standard/Sample
Diluent: 90:10 Water:mobile phase B
Conc.: 500 ng/mL
Inj. Vol.: 2 µL
Mobile Phase
A: Water, 10 mM ammonium formate
B: 90:10 Methanol:2-propanol (v/v), 0.1% formic acid

Time (min)	Flow (mL/min)	%A	%B
0.00	0.5	90	10
7.00	0.5	25	75
9.00	0.5	0	100
10.00	0.5	0	100
10.01	0.5	90	10
11.00	0.5	90	10

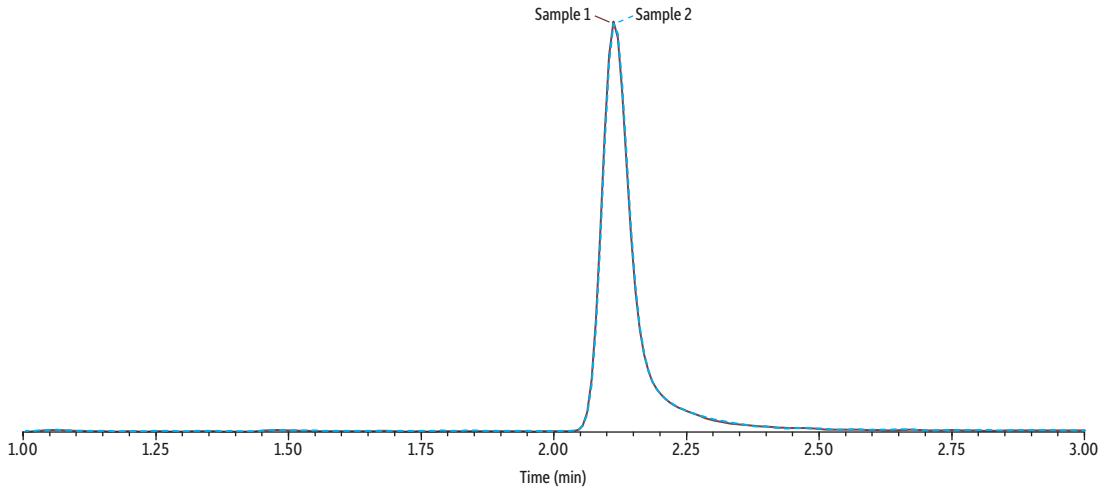
Max Pressure: 390 bar
Detector Shimadzu 8045 LC-MS/MS
Ion Mode: ESI+
Mode: MRM
Instrument Shimadzu Nexera X2
Sample Preparation Control urine (20 µL) was added to a 1.5 mL microcentrifuge tube along with 20 µL of a premade enzyme hydrolysis master mix. The sample was vortexed for 10 seconds and left to incubate at room temperature for 20 minutes. After the incubation, 260 µL of the diluent (water:mobile phase B [v/v]) was added. A 100 µL aliquot was added to a vial insert (cat.# 21776) in a 2.0 mL amber short-cap vial (cat.# 21142) and capped with a 9 mm short screw cap (cat.# 24497) and injected on the LC-MS/MS for analysis.

Method 2—Results

Amphetamine

In Figure 6, Sample 1 (0.1 µg/mL of gabapentin and amphetamine) and Sample 2 (250 µg/mL of gabapentin and 0.1 µg/mL of amphetamine) were analyzed using Method 2. When compared, the peak height and area for amphetamine are consistent in Sample 1 and Sample 2 despite the high concentration of gabapentin in Sample 2. This indicates that, under these conditions, gabapentin is sufficiently separated from amphetamine so that its signal is not being suppressed.

Figure 6: Amphetamine in Sample 1 and Sample 2 Analyzed Using Method 2

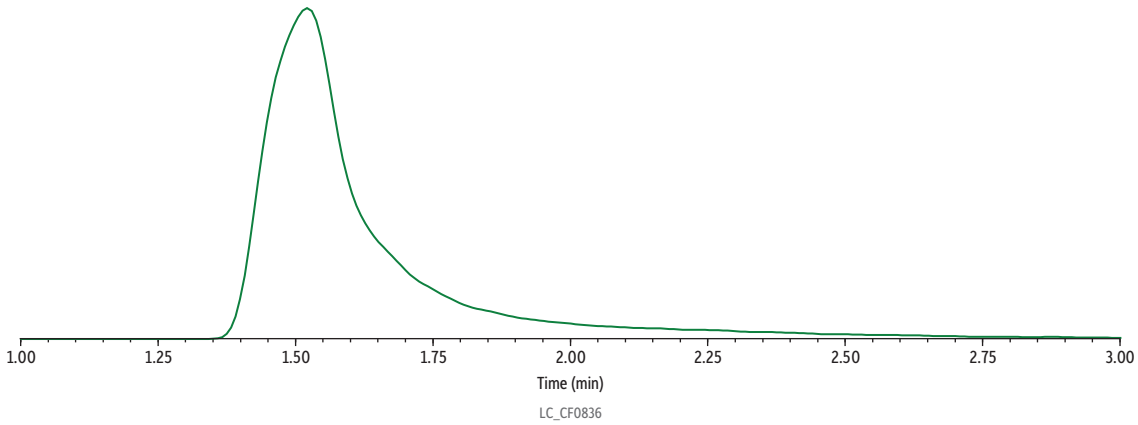


Peaks	Conc. (µg/mL)	t_R (min) (Sample 1)	t_R (min) (Sample 2)	Peak Area (Sample 1)	Peak Area (Sample 2)	Peak Height (Sample 1)	Peak Height (Sample 2)
1. Amphetamine	0.1	2.12	2.12	1361087	1372573	318247	318376

Gabapentin

In Figure 7, Sample 3 (500 µg/mL of gabapentin) was analyzed using Method 2. Under these method conditions, the peak shape of gabapentin has improved compared to those used in Method 1 (Figure 4). The peak is not as wide, and the tailing has significantly improved. The collision energy was deoptimized for gabapentin to mitigate detector saturation.

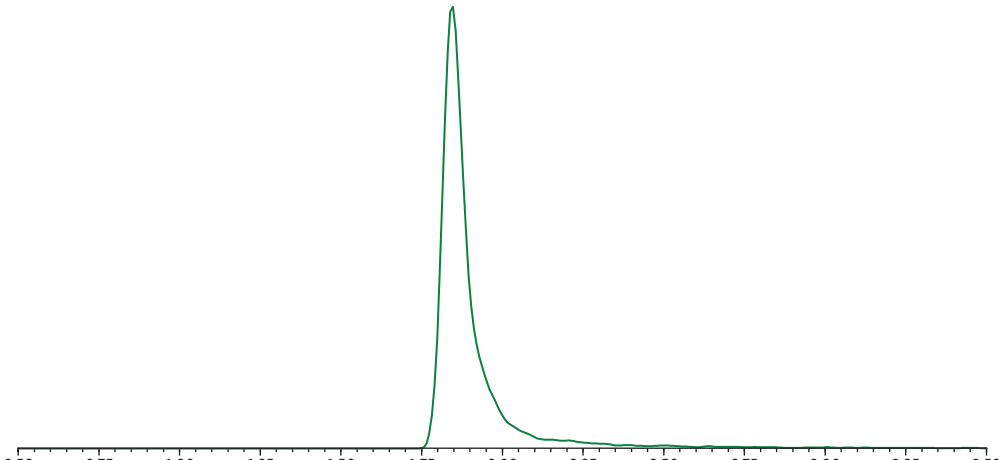
Figure 7: Gabapentin in Sample 3 Analyzed Using Method 2



Peaks	t_R (min)	Precursor	Product 1	Product 2
1. Gabapentin	1.53	172.1	154.1	136.9

The peak shape of gabapentin can be further improved by moving to a larger-bore column if desired. In Figure 8, 500 µg/mL of gabapentin is shown on a Raptor Biphenyl 50 x 4.6 mm, 2.7 µm column. The flow rate and injection volume have been adjusted to reflect the use of a larger-bore column. While a larger-bore column was not selected for use in the redeveloped method, this is another strategy method developers may employ to further improve the peak performance of gabapentin.

Figure 8: Gabapentin in Sample 3 Analyzed Using a Larger-Bore Column



Peaks	t_R (min)	Precursor	Product 1	Product 2
1. Gabapentin	1.84	172.1	154.1	136.9

Discussion

Method Development

Method 1 was redeveloped to more effectively handle samples with high levels of gabapentin. Several strategies were employed to accomplish this, such as choosing a longer column length, switching mobile phase additives, and decreasing the injection volume. Carryover was also addressed. These strategies are discussed below.

Column Length

Shorter column lengths, such as 30 or 50 mm, are often preferred as they are capable of shorter runtimes and reduced back pressure. While longer column lengths have more resolving power than shorter columns, they may require longer analysis times and result in higher back pressures. While Method 1 utilized a 50 mm column length, Method 2 used a 100 mm column length. The additional column length allowed for more chromatographic space between gabapentin and amphetamine, which was necessary to reduce suppression in samples with high gabapentin levels.

Mobile Phase Additives

The original method used formic acid as a mobile phase additive. When formic acid was swapped for ammonium formate, the elution order for early eluting compounds was affected. This allowed gabapentin to elute before amphetamine, which successfully mitigated much of the suppression caused by the high concentration of gabapentin.

Injection Volume

As discussed, chromatographic overload is a concern when high concentrations of gabapentin are encountered. One way to mitigate chromatographic overload is by decreasing the sample injection volume. This can also help with detector saturation. For this reason, the injection volume was decreased from 5 µL to 2 µL. This allowed for an improved peak shape for gabapentin even at high concentrations. It is important to consider that lowering the injection volume can raise the limit of detection (LOD) or limit of quantification (LOQ) for other analytes in the method. The performance of the other analytes in the method should be verified to ensure that acceptable LODs/LOQs are still achievable.

Carryover

High concentrations of analyte may result in carryover between samples. 2-Propanol may be added to a mobile phase to help mitigate carryover by washing contaminants off the analytical column more efficiently. For this reason, the composition of Mobile Phase B was altered from 0.1% formic acid in methanol to 0.1% formic acid, 90:10 methanol:2-propanol (v/v).

Deoptimization of MRM Transition

When performing analysis by LC-MS/MS, it is recommended that method developers perform compound tuning to determine the most optimal mass spectrometry parameters for individual analytes. These parameters include precursor/product ions; collision energies; and other voltages. Compound tuning, or optimization, is generally considered good practice as it helps to improve analyte sensitivity. There are unique scenarios, however, in which using non-optimized settings for an analyte may be acceptable. In this work, deoptimization was utilized for gabapentin to mitigate saturation of the mass detector by adjusting settings to reduce the number of product ions hitting the detector. Method developers using deoptimization to help reduce detector saturation should ensure that the necessary detection limits can still be achieved, and that proper identification of the analyte is not affected.

Column Diameter

Although a narrow-bore column (2.1 mm) was chosen for the redeveloped method, a larger-bore (4.6 mm) column was also explored. Larger column IDs offer increased space in the flow path and additional interaction sites for solute molecules to bind to. This can help to mitigate the distorted peak shapes and retention times that result from column overload, which was observed on smaller ID columns. Though the 4.6 mm column ID was successful in improving the peak performance when a large concentration of gabapentin was present, method developers should be aware of the drawbacks that may accompany using a large column ID in this scenario. Larger-bore columns may require an increased injection volume to achieve the same sensitivity as a narrow-bore column. The downside to increasing injection volumes is the increased introduction of matrix on the column that can affect chromatography, decrease column lifetimes, and enhance matrix interferences. The flow rate may also need to be increased to achieve similar analysis times to methods using a narrow-bore column. Increased flow rates may negatively impact ionization efficiency which can reduce sensitivity and require increased solvent usage over narrow-bore columns.

Chromatographic Performance

While the redeveloped method successfully mitigated interference between gabapentin and amphetamine, the performance of the other analytes in the method should also be tested. To ensure that the rest of the analytes still met analysis goals, critical isobars were examined. The reproducibility of the method was also verified by assessing lot-to-lot column variation.

Separation of Isobars

The analyte list contained nine groups of isobaric compounds that share a molecular weight. For quantitative methods to be both precise and rugged, a resolution of 1.5 or greater must be achieved. To ensure that the redeveloped method still achieved adequate resolution of critical isobars, the resolution for each isobar group was calculated (Table II). All isobar groups had a resolution of 1.5 or better when analyzed using the redeveloped method.

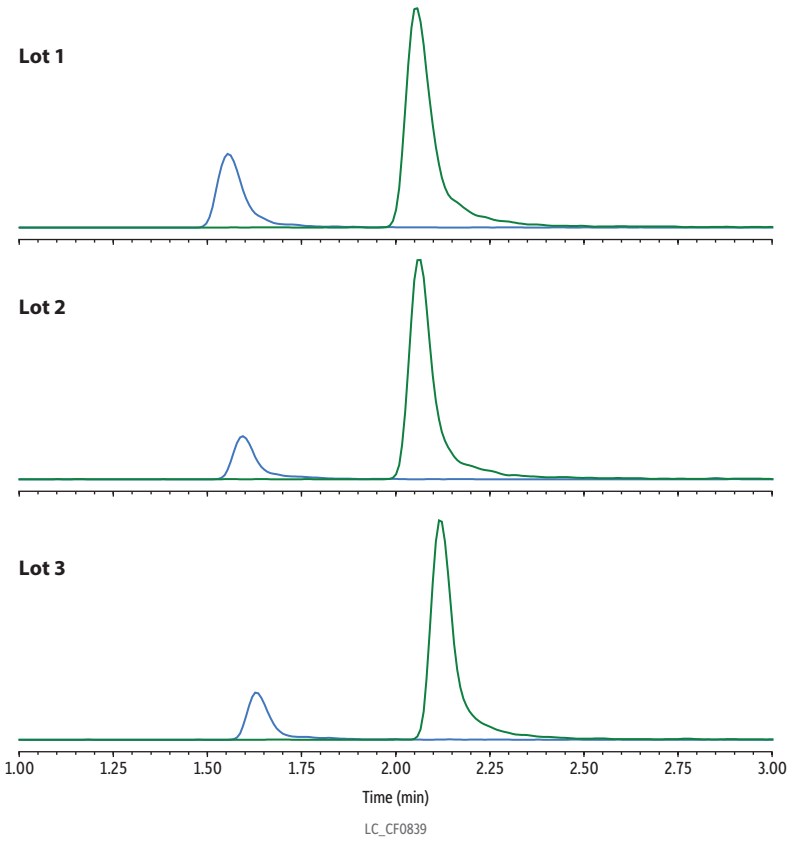
Table II: Compound Name, Shared Molecular Weight, Analyte Retention Time, Peak Width, and Calculated Resolution between Isobar Groups

Isobar Group	Analyte	Molecular Weight (g/mol)	tR (min)	Peak Width	Resolution
1	Methamphetamine	149.2	2.55	0.106	1.7
	Phentermine		2.74	0.119	
2	Venlafaxine	277.4	4.75	0.113	14.2 1.9
	EDDP		6.35	0.112	
	Amitriptyline		6.56	0.109	
3	Naloxone	327.3	3.13	0.111	1.5
	6-Acetylmorphine		2.96	0.107	
4	Morphine	285.3	1.78	0.101	3.3 5.5 18.6
	Hydromorphone		2.15	0.122	
	Norhydrocodone		2.80	0.112	
	7-aminoclonazepam		4.88	0.111	
5	Codeine	299.3	2.93	0.115	2.9
	Hydrocodone		3.29	0.126	
6	o-Desmethylvenlafaxine	263.4	3.30	0.108	6.1 22.6
	Tramadol		3.97	0.110	
	Nortriptyline		6.48	0.112	
7	Mirtazapine	265.3	5.41	0.125	1.6
	Desmethyldoxepin		5.60	0.114	
8	Oxymorphone	301.3	1.80	0.121	7.5
	Noroxycodone		2.66	0.107	
9	Citalopram	324.4	5.46	0.105	12.7
	α-Hydroxyalprazolam		6.90	0.121	

Lot-to-Lot Reproducibility

The chromatographic separation of gabapentin and amphetamine that is featured in the redeveloped method is critical for preventing interference. Given the unique mobile phase composition of Mobile Phase B, the method was tested across three different column lots to ensure that the separation was reproducible. The results of this study are shown in Figure 9. Though minor differences in retention times for both analytes were observed, the chromatographic separation between gabapentin and amphetamine was maintained on all columns. The performance method was consistent across all three lots.

Figure 9: Lot-to-Lot Reproducibility for Gabapentin and Amphetamine Separation



Peaks	Lot 1 t_R (min)	Lot 2 t_R (min)	Lot 3 t_R (min)
1. Gabapentin	1.55	1.59	1.63
2. Amphetamine	2.05	2.06	2.12

Pregabalin

Pregabalin is another anticonvulsant drug that is structurally related to gabapentin. Like gabapentin, pregabalin is prescribed in very high doses and is eliminated in urine in its original form , which may be detected in urine at elevated concentrations. No specific interference for pregabalin has been widely reported, however, the same analytical complications associated with high levels of gabapentin may also occur for pregabalin due to their structural and metabolic similarities. In the instrument conditions described for Method 2, pregabalin is the first eluting analyte and is well resolved from other early eluting analytes. For samples containing high concentrations of pregabalin, this will help to prevent analyte suppression or retention time shifting of other nearby eluting analytes.

Conclusion

In this work, the effect of high concentrations of gabapentin in urine samples was investigated. An LC-MS/MS method developed for the analysis of 60 drugs of abuse in urine was tested to determine if amphetamine was negatively affected by high levels of gabapentin. The results of these experiments initiated the development of a second method with the purpose of mitigating the impact of high levels of gabapentin. The redeveloped method employed several strategies, including a longer column length, alternative mobile phase composition, and reduced injection volume. The redeveloped method successfully resolved gabapentin from amphetamine, which improved the performance of both analytes. The method is also suitable to handle samples with large concentrations of pregabalin.

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This method has been developed for research use only; it is not suitable for use in diagnostic procedures without further evaluation.

Raptor Biphenyl HPLC Column

- Ideal for bioanalytical testing applications like drug and metabolite analyses.
- Heightened selectivity and retention for compounds that are hard to resolve or elute early on C18 and other phenyl chemistries.
- Limits ionization suppression and allows simple, MS-friendly mobile phases.
- Part of Restek's Raptor LC column line featuring 1.8, 2.7, and 5 µm SPP core-shell silica.

Product Name	Units	Cat.#
Raptor Biphenyl, 2.7 µm, 50 x 2.1 mm HPLC Column	ea.	9309A52
Raptor Biphenyl, 2.7 µm, 100 x 2.1 mm HPLC Column	ea.	9309A12

Raptor Biphenyl EXP Guard Column Cartridge

- Patented titanium hybrid ferrules can be installed repeatedly without compromising high-pressure seal.
- Auto-adjusting design provides ZDV (zero dead volume) connection to any 10-32 female port.
- Guard column cartridges require EXP direct connect holder (cat.# 25808).
- Pair with EXP hand-tight fitting (cat.# 25937–25938) for tool-free installation.
- Great with any Raptor column to get ultimate protection from particulates and matrix contamination, especially when using dilute-and-shoot or other minimal sample preparation techniques.

Product Name	Units	Cat.#
EXP Hand-Tight Fitting (Nut w/Ferrule)	ea.	9309A0252

EXP Direct Connect Holder

Product Name	Units	Cat.#
EXP Direct Connect Holder for EXP Guard Cartridges, Includes Fitting & Ferrules	ea.	25808

EXP Hand-Tight Fitting (Nut w/Ferrule)

Product Name	Units	Cat.#
EXP Hand-Tight Fitting (Nut w/Ferrule)	ea.	25937

Short-Cap Vial with Grad Marking Spot

Product Name	Units	Cat.#
Short-Cap Vial with Grad Marking Spot, 9-425 Screw-Thread, 2.0 mL, 9 mm, 12 x 32 (vial only), Amber	100-pk.	21142

Vial Inserts

Product Name	Units	Cat.#
Vial Inserts, Glass, Big Mouth w/Bottom Spring, 250 µL	100-pk.	21776

Vial Caps

Product Name	Units	Cat.#
Short Screw Cap, Polypropylene, Screw-Thread, PTFE/Silicone/PTFE Septa, Blue, Preassembled, 2.0 mL, 9 mm	100-pk.	24497