APPLICATIONS

Chromatographic Considerations for LC-MS/MS Analysis of Amphetamine in the Presence of Gabapentin using Kinetex® Core-Shell LC Columns

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Overview

Since gabapentin is unmetabolized and excreted renally, it is found in high concentrations in urine. These high concentrations can make analysis of other compounds, specifically amphetamine, difficult due to overloading the LC column and saturating the source of the mass spectrometer[1]. This work aims to examine the most commonly used drug research panel method conditions and evaluate modifications that can be made to alleviate these issues for robust analysis of amphetamine in a high concentration gabapentin positive sample.

Materials

Reference standards and deuterated internal standards were purchased from Cerilliant® Corporation (Round Rock, TX).

Ammonium formate and formic acid were purchased from Sigma-Aldrich® (St. Louis, MO).

HPLC-grade methanol and acetonitrile were purchased from Honeywell® (Morris Plains, NJ).

Purified water was obtained using a Sartorius® arium® comfort II filtration system (Göttingen, Germany).

Sample Preparation

A standard comprised of amphetamine, pregabalin, gabapentin, morphine, norhydrocodone, hydromorphone, oxymorphone, and noroxycodone was prepared at 50 ng/mL. A second standard was prepared with all compounds at 50 ng/mL except gabapentin which was 25 µg/mL.

Development of LC-MS/MS Method Conditions

Columns: Kinetex® 2.6 µm Biphenyl
Kinetex 2.6 µm C18
Kinetex 2.6 µm Phenyl-Hexyl

Dimensions: 50 x 3.0 mm

Part Nos.: 00B-4622-Y0 (Biphenyl)
00B-4462-Y0 (C18)
00B-4495-Y0 (Phenyl-Hexyl)

Mobile Phase: A: 0.1 % Formic acid in Water, 10 mM Ammonium formate, 0.1 % Acetic acid, or Ammonium acetate
B: Methanol or Acetonitrile

Gradient:

Time (min)	% B
0	10
5	90
7	90
7.1	10
10	10

Flow Rate: 0.5 mL/min

Injection: 10 µL

Temperature: 25 °C

Detection: MS/MS

Detector: SCIEX Triple Quad™ 4500

Backpressure: 160 bar

Final LC-MS/MS Method Conditions

Column: Kinetex 2.6 µm Phenyl-Hexyl

Dimensions: 50 x 3.0 mm

Part No.: 00B-4495-Y0

Mobile Phase: A: 10 mM Ammonium formate
B: 0.1 % Formic acid in Methanol

Gradient:

Time (min)	% B
0	5
4	95
4.5	95
4.51	5
6	5

Flow Rate: 0.5 mL/min

Injection: 10 µL

Temperature: 30 °C

Detection: MS/MS

Detector: SCIEX Triple Quad™ 4500

Backpressure: 158 bar

Revision: 0
PHEN-RUO-00084

Figure 1.
Chromatogram of panel with regular vs. overloaded gabapentin peak

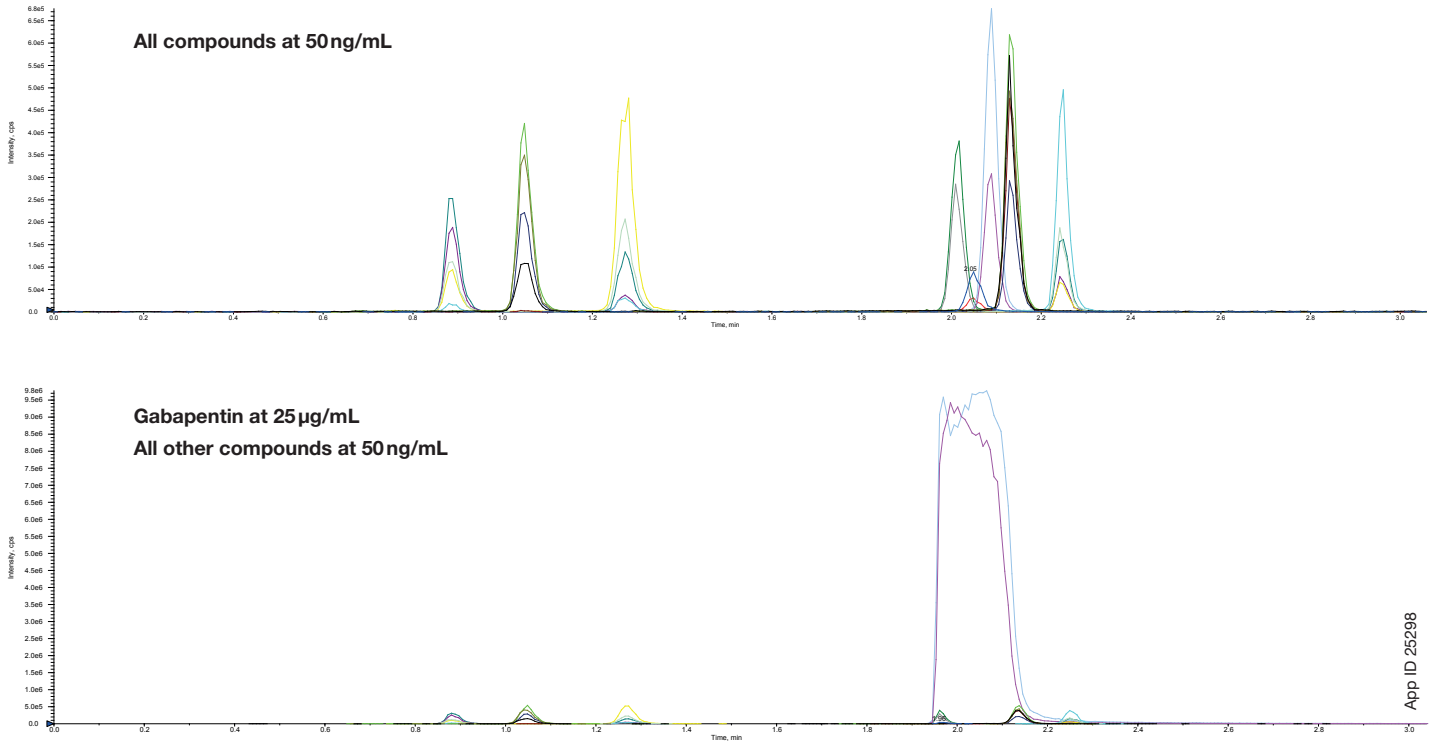
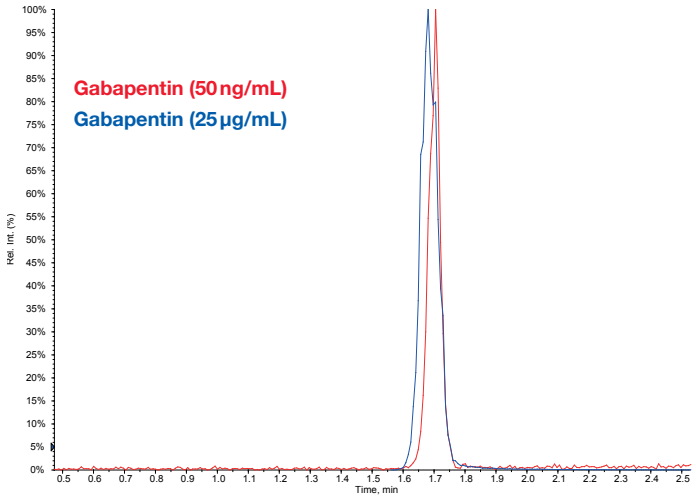
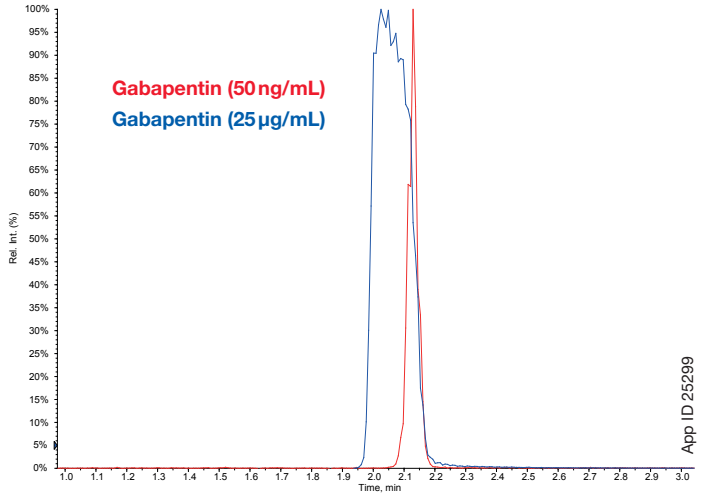


Figure 2.
Gabapentin peak shape under different mobile phase conditions (peak intensity normalized)



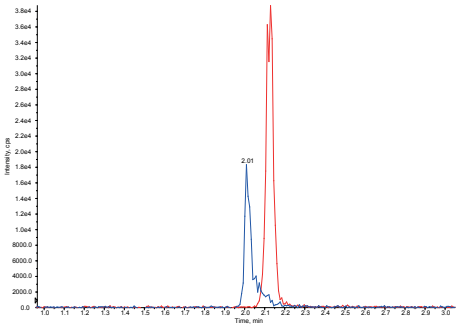
Column: Kinetex® Phenyl-Hexyl
Mobile Phase: 10 mM Ammonium formate and Methanol



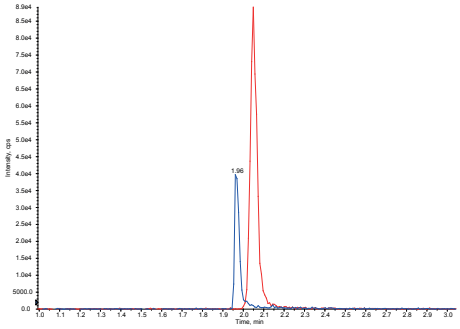
Column: Kinetex Phenyl-Hexyl
Mobile Phase: 0.1 % Formic acid and Methanol

Figure 3.
Amphetamine peak comparison, Mobile Phase A (0.1 % Formic acid), Mobile Phase B (Methanol vs. Acetonitrile)

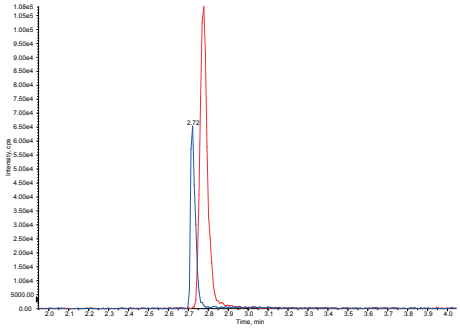
Amphetamine peak in sample with: Gabapentin (50 ng/mL), Gabapentin (25 µg/mL)



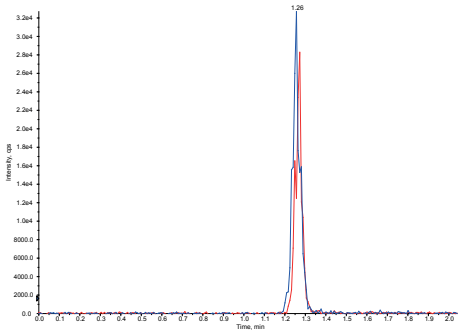
Column: Kinetex® Phenyl-Hexyl
Mobile Phase: 0.1 % Formic acid and Methanol



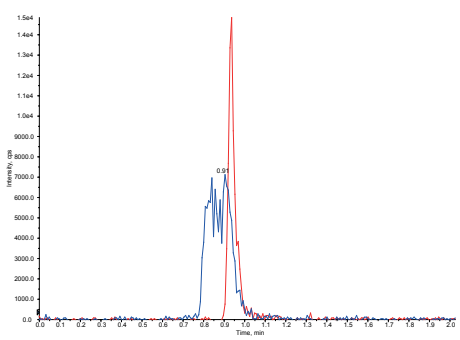
Column: Kinetex C18
Mobile Phase: Phase: 0.1 % Formic acid and Methanol



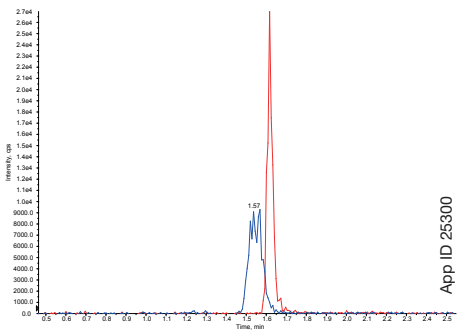
Column: Kinetex Biphenyl
Mobile Phase: 0.1 % Formic acid and Methanol



Column: Kinetex Phenyl-Hexyl
Mobile Phase: 0.1 % Formic acid and Acetonitrile



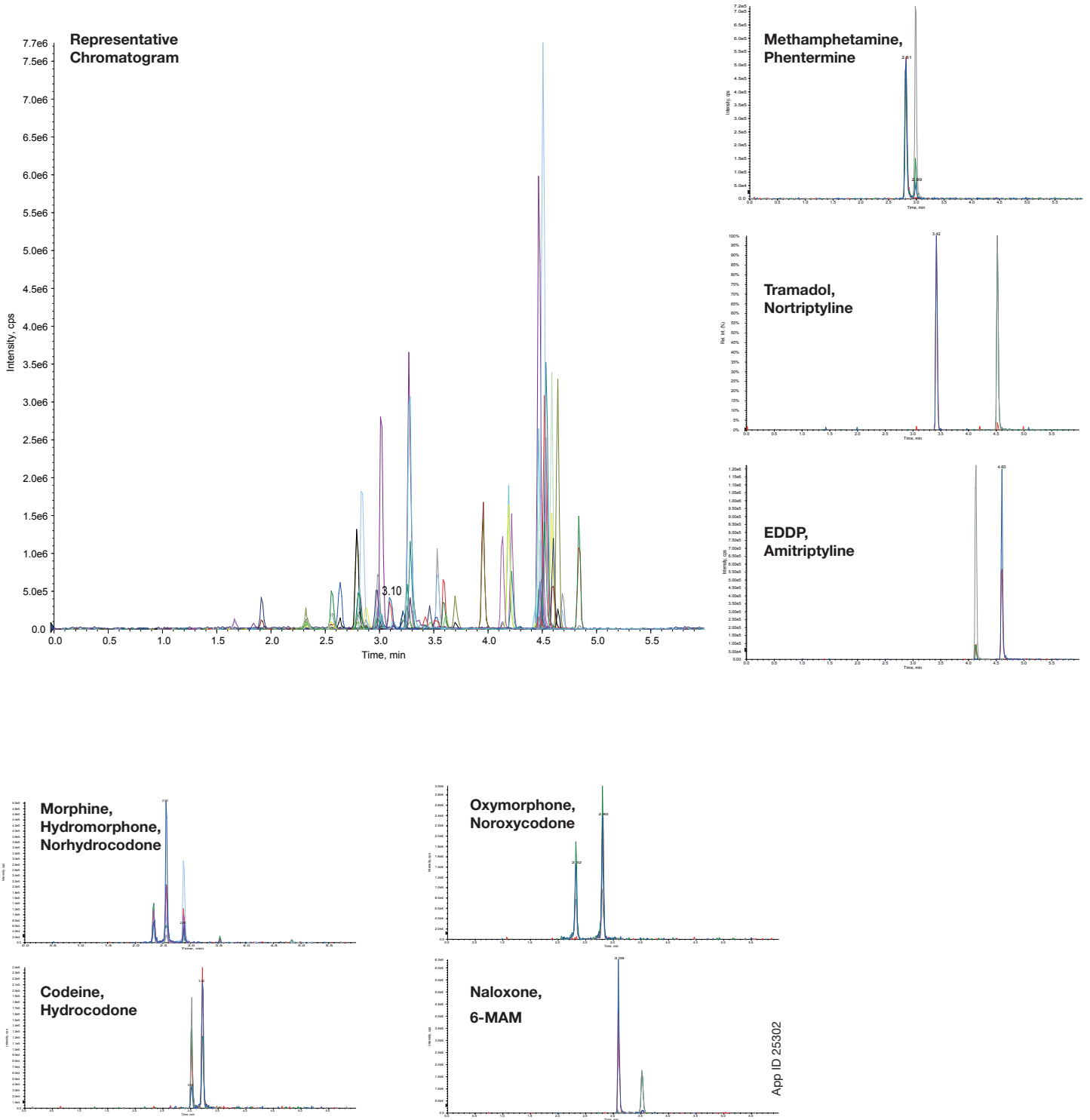
Column: Kinetex C18
Mobile Phase: 0.1 % Acetic acid and Acetonitrile



Column: Kinetex Biphenyl
Mobile Phase: 0.1 % Formic acid and Acetonitrile

App ID 25300

Figure 5.
Final method chromatogram and separation of isobars



App ID 25302

Figure 6.
Separation of gabapentin from other analytes

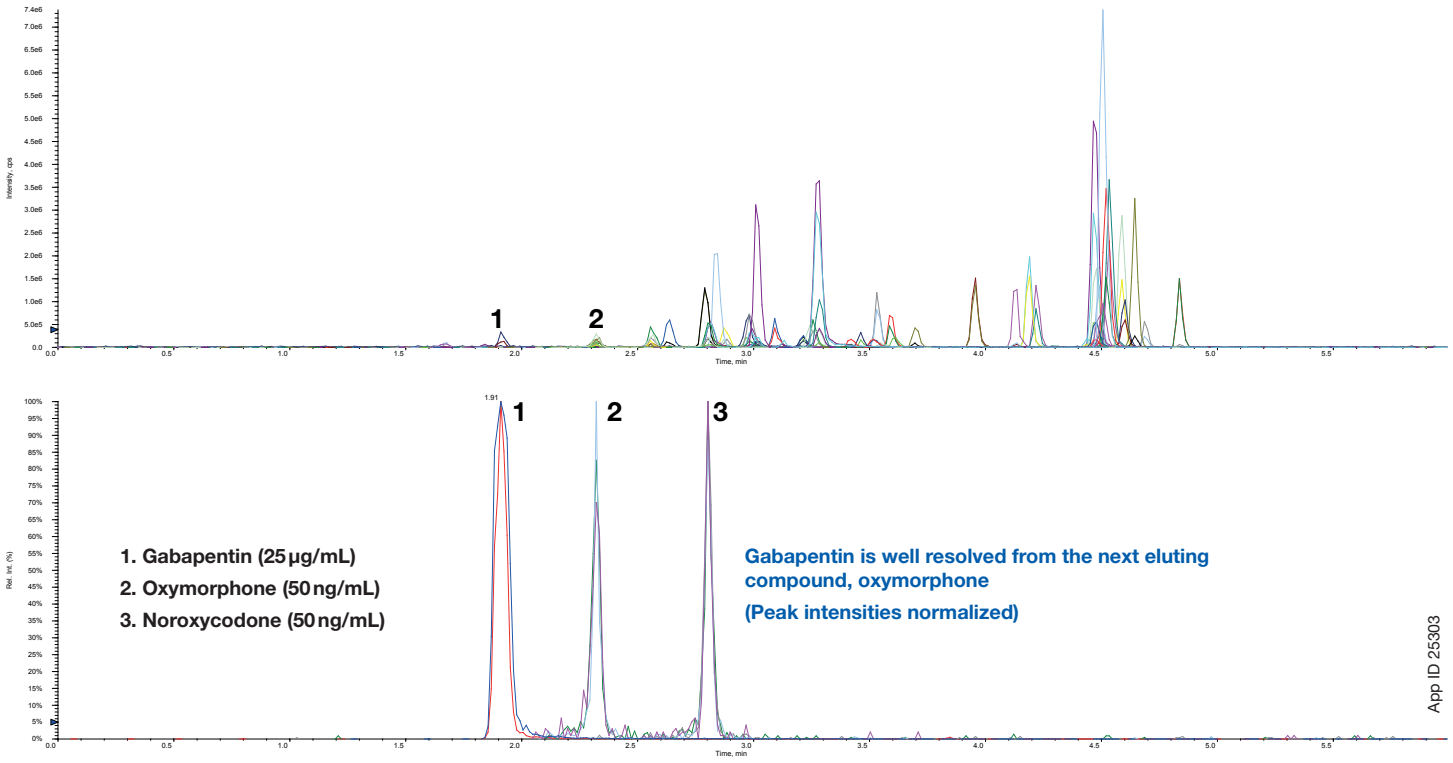
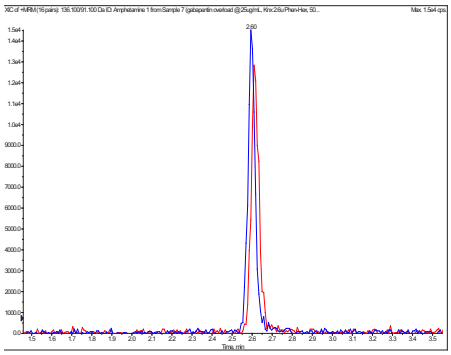
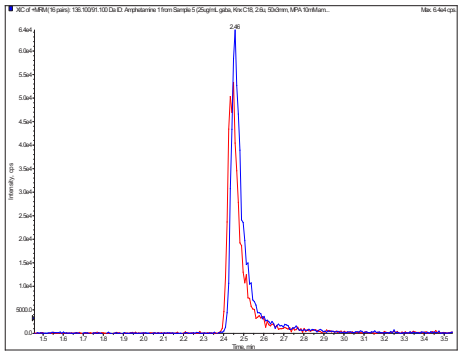


Figure 4.
Amphetamine peak comparison, Mobile Phase A (10 mM Ammonium formate), Mobile Phase B (Methanol vs. Acetonitrile)

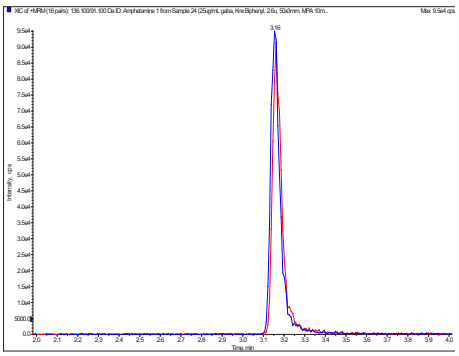
Amphetamine peak in sample with: Gabapentin (50 ng/mL), Gabapentin (25 µg/mL)



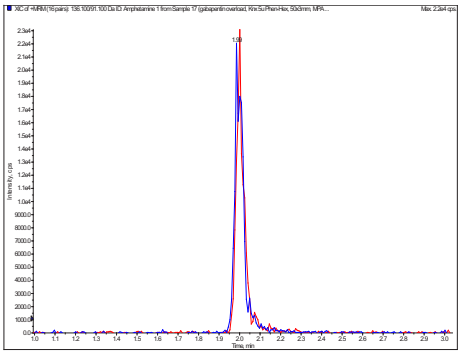
Column: Kinetex® Phenyl-Hexyl
Mobile Phase: 10 mM Ammonium formate and Methanol



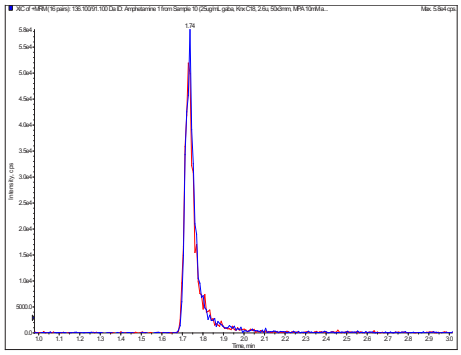
Column: Kinetex C18
Mobile Phase: 10 mM Ammonium formate and Methanol



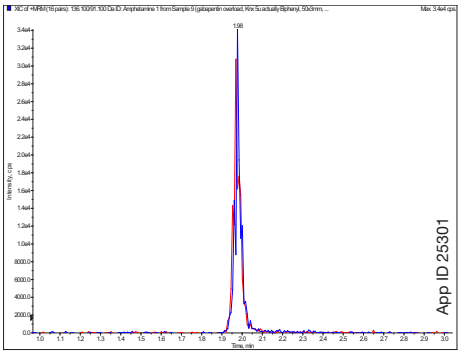
Column: Kinetex Biphenyl
Mobile Phase: 10 mM Ammonium formate and Methanol



Column: Kinetex Phenyl-Hexyl
Mobile Phase: 10 mM Ammonium formate and Acetonitrile



Column: Kinetex C18
Mobile Phase: 10 mM Ammonium formate and Acetonitrile



Column: Kinetex Biphenyl
Mobile Phase: 10 mM Ammonium formate and Acetonitrile

Results and Discussion

Common early eluting drug compounds were selected for initial testing to determine where gabapentin eluted in relation to the rest of the panel (**Figure 1**).

Gabapentin and amphetamine were not chromatographically resolved under conditions that used either 0.1 % formic acid or 0.1 % acetic acid as mobile phase A and methanol as mobile phase B. Switching mobile phase B to acetonitrile provided some resolution, but not enough to separate it from an overloaded gabapentin peak. The gabapentin peak was very wide under the mobile phase conditions that used only an acid modifier (**Figure 2**). The amphetamine peak shape was poor when acetonitrile was used and showed a suppressed signal, with the exception of when it was ran using the Kinetex® Phenyl-Hexyl LC column (**Figure 3**). When methanol was used, the amphetamine peak shape was acceptable, however, amphetamine instead eluted 0.1-0.3 min before the expected retention time (**Figure 3**).

Using 10 mM ammonium acetate chromatographically resolved gabapentin and amphetamine, however, amphetamine's peak shape suffered on the Kinetex C18 LC column in particular. Using 10 mM ammonium formate chromatographically resolved gabapentin and amphetamine. Using acetonitrile instead of methanol with the Kinetex Phenyl-Hexyl and Biphenyl phases resulted in a loss of resolution between isomeric species methamphetamine and phentermine, ruling out the use of acetonitrile with the phenyl phases. Peak shape was good for gabapentin and amphetamine under these conditions. The retention time for amphetamine was stable and its signal was not suppressed (**Figure 4**).

A complete method was developed on the Kinetex Phenyl-Hexyl LC column using 10 mM ammonium formate for mobile phase A and 0.1 % formic acid in methanol for mobile phase B (**Figure 5**). Full conditions are listed in the experimental section. Gabapentin is well resolved from the next eluting peak, oxymorphone, and chromatographic resolution of isobaric species was ensured (**Figures 5 and 6**).

Conclusion

Using conditions in which gabapentin elutes before other compounds of interest was most effective at reducing the impact of a high concentration of gabapentin being present in the sample. The effects of gabapentin on amphetamine is presented differently under each set of conditions, highlighting the importance of monitoring all samples for changes in peak shape, retention time, and suppression of internal standard as signs of the presence of an interfering compound. Using 10 mM ammonium formate for mobile phase A and methanol for mobile phase B successfully resolved gabapentin from amphetamine on the Kinetex Biphenyl, Phenyl-Hexyl, and C18 LC columns allowing for reproducible analysis of amphetamine in the presence of a high concentration of gabapentin. The work also presents a complete method using these conditions on a Kinetex Phenyl-Hexyl LC column to analyze amphetamine while maintaining resolution of common isobars.

Acknowledgements

I would like to acknowledge Seyed Sadjadi for his earlier work which inspired this project, and Sean Orlowicz for his insight and encouragement.

Reference

1. Shugarts, Sarah B. Pervasive Gabapentin Interference in the LC-MS/MS Analysis of Amphetamine. The Journal of Applied Laboratory Medicine Jan 2018, 2 (4) 527-534; DOI: 10.1373/jalm.2017.024117

Ordering Information

Kinetex® Core-Shell LC Columns

1.7 µm Minibore Columns (mm)				SecurityGuard™ ULTRA Cartridges‡
Phases	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
Phenyl-Hexyl	00B-4500-AN	00D-4500-AN	00F-4500-AN	AJ0-8788
				for 2.1 mm ID

2.6 µm Minibore Columns (mm)						SecurityGuard ULTRA Cartridges‡
Phases	30 x 2.1	50 x 2.1	75 x 2.1	100 x 2.1	150 x 2.1	3/pk
Phenyl-Hexyl	00A-4495-AN	00B-4495-AN	00C-4495-AN	00D-4495-AN	00F-4495-AN	AJ0-8788
						for 2.1 mm ID

2.6 µm MidBore Columns (mm)				SecurityGuard ULTRA Cartridges‡
Phases	50 x 3.0	100 x 3.0	150 x 3.0	3/pk
Phenyl-Hexyl	00B-4495-Y0	00D-4495-Y0	00F-4495-Y0	AJ0-8781
				for 3.0 mm ID

2.6 µm Analytical Columns (mm)					SecurityGuard ULTRA Cartridges‡
Phases	50 x 4.6	75 x 4.6	100 x 4.6	150 x 4.6	3/pk
Phenyl-Hexyl	00B-4495-E0	00C-4495-E0	00D-4495-E0	00F-4495-E0	AJ0-8774
					for 4.6 mm ID

5 µm Minibore Columns (mm)		SecurityGuard ULTRA Cartridges‡
Phase	50 x 2.1	3/pk
Phenyl-Hexyl	00B-4603-AN	AJ0-8788
		for 2.1 mm ID

5 µm MidBore™ Columns (mm)			SecurityGuard ULTRA Cartridges‡
Phase	50 x 3.0	100 x 3.0	3/pk
Phenyl-Hexyl	00B-4603-Y0	00D-4603-Y0	AJ0-8781
			for 3.0 mm ID

5 µm Analytical Columns (mm)					SecurityGuard ULTRA Cartridges‡
Phase	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	3/pk
Phenyl-Hexyl	00B-4603-E0	00D-4603-E0	00F-4603-E0	00G-4603-E0	AJ0-8774
					for 4.6 mm ID

‡SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000

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