TN-1356

Column Considerations for LC-MS/MS Analysis of a Panel of 22 Antidepressants

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Introduction

Liquid chromatography with tandem mass spectrometry (LC-MS/MS) methods are considered the gold standard for therapeutic drug monitoring (TDM) of medications. LC-MS/MS provides several advantages for TDM, including high sensitivity, selectivity, and the ability to analyze multiple drugs in a single sample. Typically, serum or whole blood is used as the standard matrix for therapeutic drug monitoring, with serum preferred due to its ease of handling compared to whole blood. However, serum is a complex biological matrix containing various endogenous compounds that can interfere with analysis. These matrix effects can affect the accuracy and precision of drug concentration measurements, often requiring the use of complex sample preparation methods.

In this technical note, we demonstrate a fast and accurate method for the LC-MS/MS analysis of 22 Antidepressants using a Strata™-X Method Development 96-well plate that is packed with 4 different polymeric Solid Phase Extraction (SPE) sorbents. This is combined with a fast LC method using a Kinetex 2.6 µm Biphenyl LC column or a Luna Omega 3 µm Polar C18 LC column to resolve all target analytes.

Sample Preparation

A 1 ng/mL standard mix was used for initial LC-MS/MS analysis. A more detailed explanation of the use of the Strata-X Method Development 96-well plate (Part No.: KS0-8209) to determine the best sample extraction protocol for the Antidepressants drug class can be found in TN-0163. Briefly, 500 µL of human serum was spiked with an Antidepressants standard mix (1 ng/mL) and extracted using the Strata-X Method Development plate under Neutral, Basic, or Acid loading buffer, followed by Neutral, Basic, or Acidic elution buffer. The best results were obtained using the Strata-X-C sorbent chemistry and the Acid load/Basic elution extraction conditions. After dry-down, samples were reconstituted in 500 µL of initial mobile phase and spiked with 5 ng/mL internal standards. 5 µL of sample was injected onto columns for analysis.

LC Conditions

Column:	Kinetex™ 2.6 µm Biphenyl	Luna™ Omega 3.0 µm Polar C18
Dimensions:	50 x 3.0 mm	50 x 3.0 mm
Part No.:	00B-4622-Y0	00B-4760-Y0
Mobile Phase:	A: 0.1 % Formic acid in Water B: 0.1 % Formic acid in Methanol	A: 2 mM Ammonium Acetate B: 2 mM Ammonium Acetate in Methanol

Gradient:	**Time (min)**	**% B**	**Time (min)**	**% B**
	0	40	0	45
	3	55	0.5	45
	5	55	2.5	95
	5.5	95	3.5	95
	6	95	3.51	45
	6.1	40	5	45
	8	40		

Flow Rate:	0.8 mL/min	0.7 mL/min
Injection Volume:	5 µL	
Temperature:	40 °C	
LC System:	Agilent® 1260 Infinity	
Detection:	MS/MS	
Detector:	SCIEX® 6500 Triple Quad™	

MS/MS Conditions

Ion Source:	ESI
Polarity:	Positive
Source Temperature:	450° C
GS1:	55 psi
GS2:	60 psi
CUR:	35 psi
IS:	2500 V

Table 1. MS Transitions.

Analyte	Q1 Mass (Da)	Q3 Mass (Da)	Analyte	Q1 Mass (Da)	Q3 Mass (Da)
Selegiline	188	91	Amoxapine	314	271
Hydroxybupropion	256.2	238	Desipramine	267.1	72
Bupropion	240.3	184	Imipramine	281.1	86
Venlafaxine	278.1	121	Duloxetine	298.1	44
Mirtazapine	266.1	195.2	Nortriptyline	264.1	233.1
Citalopram	325.1	109	Paroxetine	330	192.1
N-Desmethyl-Doxepin	266.1	107	Amitriptyline	278.1	233.1
Doxepin	280.1	107	Trimipramine	295.1	100.1
Trazodone	372.2	176	N-Desmethyl-Clomipramine	301.1	72
Norfluoxetine	296.1	134.1	Clomipramine	315.1	86.1
Fluoxetine	310.1	44	Sertraline	306.1	159

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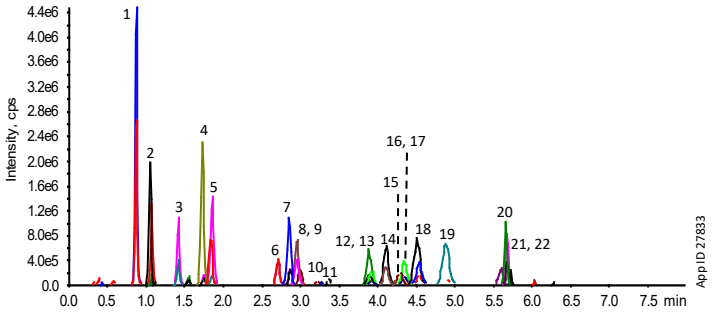
Results and Discussion

The 22 antidepressants represent a panel of weak bases (pKa of 7 to 10.5) with moderate to high hydrophobicity (logP of 2.2 to 5.1). The mix of Antidepressants standards was analyzed with the Kinetex™ 2.6 µm Biphenyl column showing good peak shape and separation (**Figure 1a**). It also demonstrated great selectivity towards the separation of the two critical isobaric pairs: Venlafaxine and Amitriptyline, and Mirtazapine and N-Desmethyl-Doxepin (**Figures 1b** and **1c**). To evaluate the chromatography from a matrix-matched sample, 22 Antidepressants were spiked into serum and extracted using the Strata™-X Method Development plate before being analyzed on a Kinetex 2.6 µm Biphenyl column. The Strata-X-C sorbent with the acidic load and basic elution solvents were found to be a sufficient extraction method for these analytes. For more information on the SPE protocol, please see TN-0163. The same degree of separation of analytes was observed in the extracted sample as the neat standard (**Figures 2a**, **2b** and **2c**).

The Antidepressants standards mix was also analyzed using the Luna™ Omega 3 µm Polar C18 column. Analytes were separated with good peak shape (**Figure 3a**) and demonstrated selectivity towards the separation of the two critical isobaric pairs: Venlafaxine and Amitriptyline, and Mirtazapine and N-Desmethyl-Doxepin (**Figures 3b** and **3c**). The serum spiked with the 22 Antidepressants and extracted using the Strata-X Method Development plate was also analyzed on a Luna Omega 3 µm Polar C18 column. The same degree of separation of analytes was observed in the extracted serum sample as compared to the Antidepressants standard mix (**Figures 4a**, **4b**, and **4c**).

Figure 1a. Analysis of Antidepressants Standards on a Kinetex 2.6 µm Biphenyl Column.



Peak No.	Analyte	Retention Time (min)	Peak No.	Analyte	Retention Time (min)
1	Selegiline	0.9	12	Amoxapine	3.9
2	Hydroxybupropion	1.1	13	Desipramine	3.9
3	Bupropion	1.4	14	Imipramine	4.1
4	Venlafaxine	1.7	15	Duloxetine	4.2
5	Mirtazapine	1.9	16	Nortriptyline	4.3
6	Citalopram	2.7	17	Paroxetine	4.3
7	N-Desmethyl-Doxepin	2.8	18	Amitriptyline	4.5
8	Doxepin	2.9	19	Trimipramine	4.9
9	Trazodone	2.9	20	N-Desmethyl-Clomipramine	5.6
10	Norfluoxetine	3.3	21	Clomipramine	5.7
11	Fluoxetine	3.4	22	Sertraline	5.7

Figure 1b. Separation of Isobaric Venlafaxine and Amitriptyline Antidepressants on a Kinetex 2.6 µm Biphenyl Column.

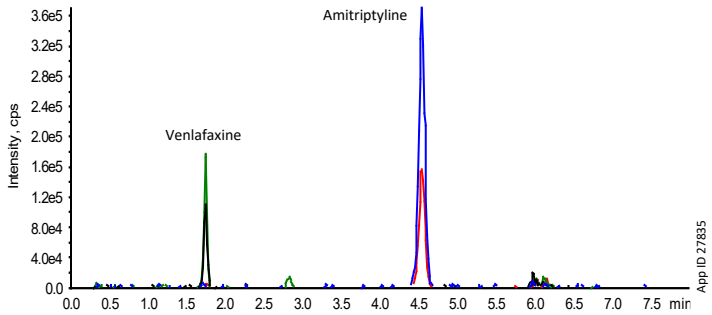


Figure 1c. Separation of Isobaric Mirtazapine and N-Desmethyl-Doxepin Antidepressants on a Kinetex 2.6 µm Biphenyl Column.

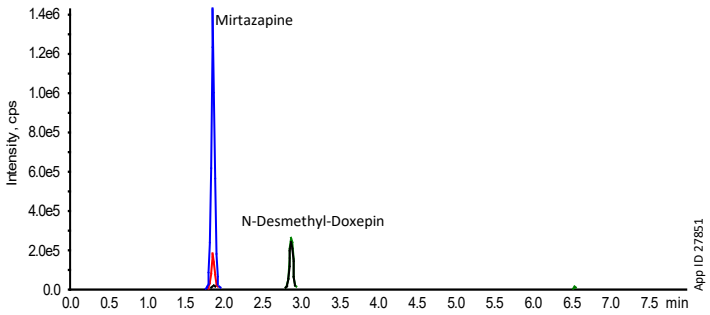
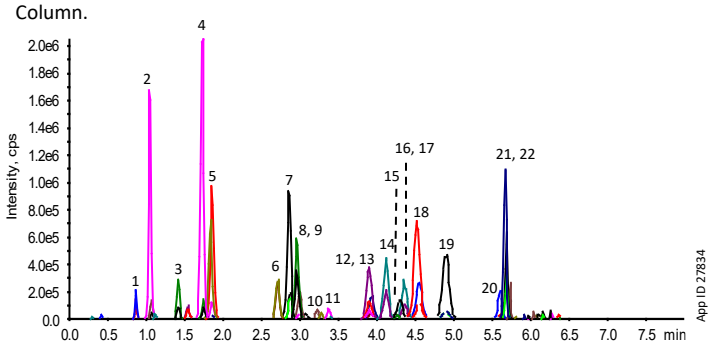


Figure 2a. Analysis of Antidepressants Extracted from Serum Using Strata™-X-C, Under Acidic Load and Basic Elution, on a Kinetex™ 2.6 µm Biphenyl Column.



Peak No.	Analyte	Retention Time (min)	Peak No.	Analyte	Retention Time (min)
1	Selegiline	0.9	12	Amoxapine	3.9
2	Hydroxybupropion	1.1	13	Desipramine	3.9
3	Bupropion	1.4	14	Imipramine	4.1
4	Venlafaxine	1.7	15	Duloxetine	4.2
5	Mirtazapine	1.9	16	Nortriptyline	4.3
6	Citalopram	2.7	17	Paroxetine	4.3
7	N-Desmethyl-Doxepin	2.8	18	Amitriptyline	4.5
8	Doxepin	2.9	19	Trimipramine	4.9
9	Trazodone	2.9	20	N-Desmethyl-Clomipramine	5.6
10	Norfluoxetine	3.3	21	Clomipramine	5.7
11	Fluoxetine	3.4	22	Sertraline	5.7

Figure 2b. Separation of Venlafaxine and Amitriptyline Antidepressants Extracted from Serum Using Strata-X-C, Under Acidic Load and Basic Elution, on a Kinetex 2.6 µm Biphenyl Column.

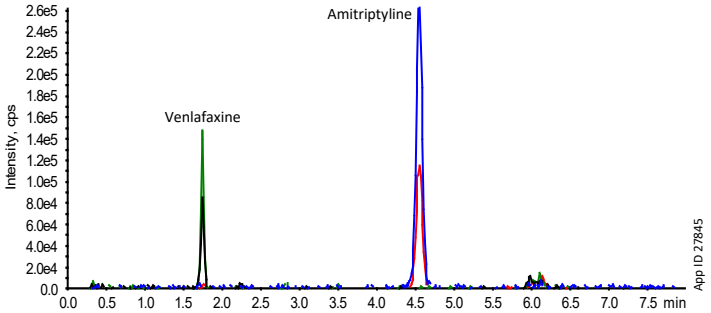


Figure 2c. Separation of Mirtazapine and N-Desmethyl-Doxepin Antidepressants Extracted from Serum Using Strata-X-C, Under Acidic Load and Basic Elution, on a Kinetex 2.6 µm Biphenyl Column.

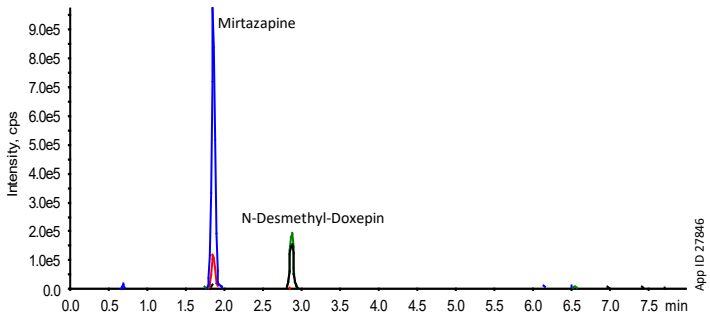
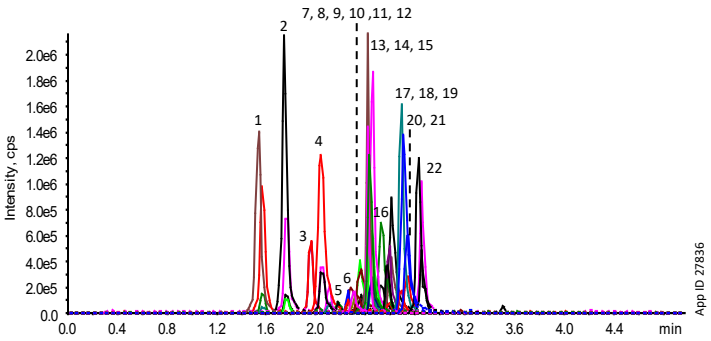


Figure 3a. Analysis of Antidepressants Standards on a Luna™ Omega 3 µm Polar C18 Column.



Peak No.	Analyte	Retention Time (min)	Peak No.	Analyte	Retention Time (min)
1	Hydroxybupropion	1.5	12	Paroxetine	2.3
2	Venlafaxine	1.7	13	Mirtazapine	2.4
3	Citalopram	1.9	14	Trazodone	2.4
4	N-Desmethyl-Doxepin	2.0	15	Nortriptyline	2.4
5	Bupropion	2.1	16	Amoxapine	2.5
6	Selegiline	2.2	17	Imipramine	2.6
7	Doxepin	2.3	18	N-Desmethyl-Clomipramine	2.6
8	Norfluoxetine	2.3	19	Sertraline	2.6
9	Fluoxetine	2.3	20	Amitriptyline	2.7
10	Desipramine	2.3	21	Trimipramine	2.7
11	Duloxetine	2.3	22	Clomipramine	2.9

Figure 3b. Separation of Isobaric Venlafaxine and Amitriptyline Antidepressants on a Luna Omega 3 µm Polar C18 Column.

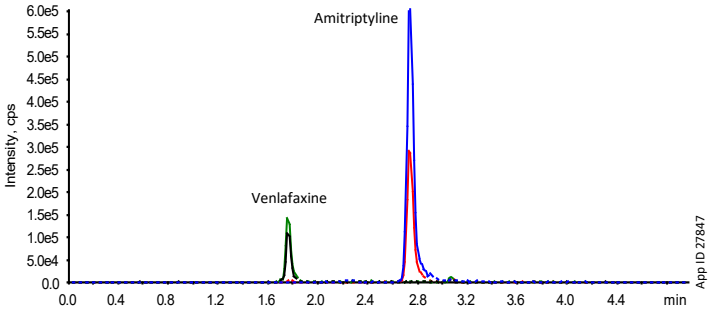
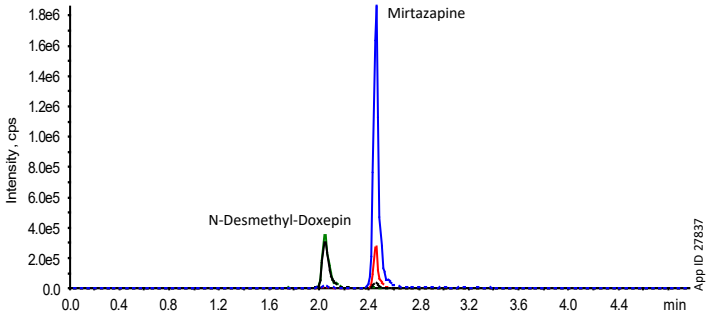


Figure 3c. Separation of Isobaric Mirtazapine and N-Desmethyl-Doxepin Antidepressants on a Luna Omega 3 µm Polar C18 Column.



Have questions or want more details on implementing this method? We would love to help!
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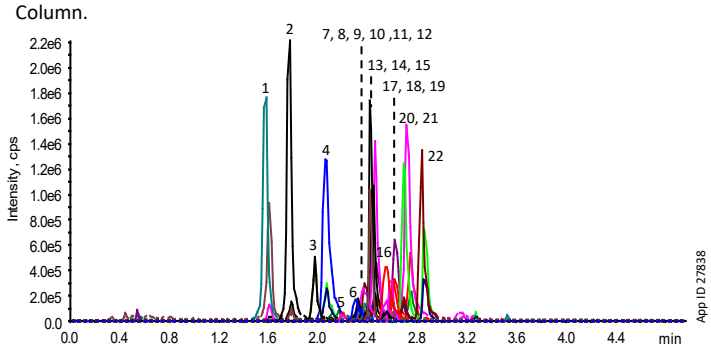
Figure 4a. Analysis of Antidepressants Extracted from Serum Using Strata™-X-C, Under Acidic Load and Basic Elution, on a Luna™ Omega 3 µm Polar C18 Column.



Peak No.	Analyte	Retention Time (min)	Peak No.	Analyte	Retention Time (min)
1	Hydroxybupropion	1.5	12	Paroxetine	2.3
2	Venlafaxine	1.7	13	Mirtazapine	2.4
3	Citalopram	1.9	14	Trazodone	2.4
4	N-Desmethyl-Doxepin	2.0	15	Nortriptyline	2.4
5	Bupropion	2.1	16	Amoxapine	2.5
6	Selegiline	2.2	17	Imipramine	2.6
7	Doxepin	2.3	18	N-Desmethyl-Clomipramine	2.6
8	Norfluoxetine	2.3	19	Sertraline	2.6
9	Fluoxetine	2.3	20	Amitriptyline	2.7
10	Desipramine	2.3	21	Trimipramine	2.7
11	Duloxetine	2.3	22	Clomipramine	2.9

Figure 4b. Separation of Venlafaxine and Amitriptyline Antidepressants Extracted from Serum Using Strata-X-C, Under Acidic Load and Basic Elution, on a Luna Omega 3 µm Polar C18 Column.

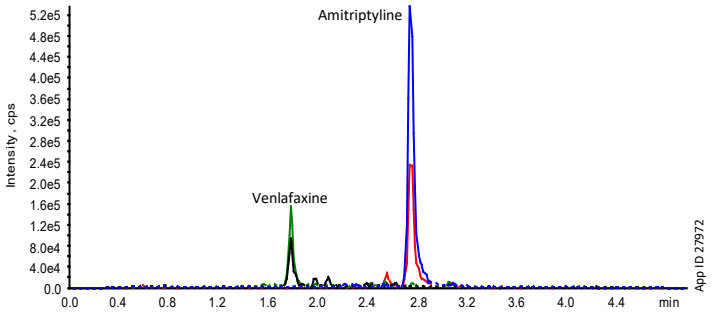
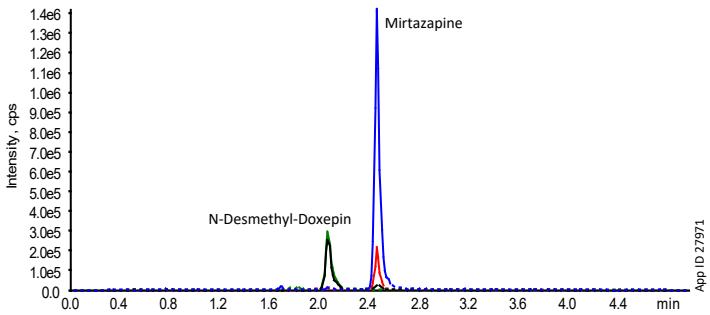


Figure 4c. Separation of Mirtazapine and N-Desmethyl-Doxepin Antidepressants Extracted from Serum Using Strata-X-C, Under Acidic Load and Basic Elution, on a Luna Omega 3 µm Polar C18 Column.



Conclusions

These two LC-MS/MS methods successfully separated the 22 Antidepressant drugs and metabolites, including the two isobaric pairs Venlafaxine and Amitriptyline, and Mirtazapine and N-Desmethyl-Doxepin. The Kinetex™ 2.6 µm Biphenyl column had a longer run time (8 mins) and used an easy to prepare mobile phase (Formic Acid in Water and Methanol). The Luna Omega 3 µm Polar C18 column had a shorter run time (5 mins), but the analytes were more condensed in the chromatogram and the separation required a buffered mobile phase (2 mM Ammonium Acetate in Water and Methanol). Both columns provided excellent chromatography, and the separation and selectivity required for LC-MS/MS analysis of these TDM analytes.

SPE Ordering Information

Strata™-X Method Development 96-Well Plate		
Part No.	**Description**	**Unit**
KS0-8209	Strata-X, -X-C, -X-CW, and -X-AW 30 mg/well each	ea

Kinetex™ Ordering Information

2.6 µm Midbore™ Columns (mm)						SecurityGuard™ ULTRA Cartridges (mm)‡
Phases	**30 x 3.0**	**50 x 3.0**	**75 x 3.0**	**100 x 3.0**	**150 x 3.0**	**3/pk**
EVO C18	00A-4725-Y0	00B-4725-Y0	—	00D-4725-Y0	00F-4725-Y0	AJ0-9297
PS C18	00A-4780-Y0	00B-4780-Y0	—	00D-4780-Y0	00F-4780-Y0	AJ0-8950
Polar C18	—	00B-4759-Y0	—	00D-4759-Y0	00F-4759-Y0	AJ0-9531
Biphenyl	—	00B-4622-Y0	—	00D-4622-Y0	00F-4622-Y0	AJ0-9208
XB-C18	00A-4496-Y0	00B-4496-Y0	00C-4496-Y0	00D-4496-Y0	00F-4496-Y0	AJ0-8775
C18	00A-4462-Y0	00B-4462-Y0	00C-4462-Y0	00D-4462-Y0	00F-4462-Y0	AJ0-8775
C8	00A-4497-Y0	00B-4497-Y0	00C-4497-Y0	00D-4497-Y0	00F-4497-Y0	AJ0-8777
HILIC	00A-4461-Y0	—	—	00D-4461-Y0	00F-4461-Y0	AJ0-8779
Phenyl-Hexyl	—	00B-4495-Y0	—	00D-4495-Y0	00F-4495-Y0	AJ0-8781
F5	—	00B-4723-Y0	—	00D-4723-Y0	00F-4723-Y0	AJ0-9321

for 3.0 mm ID

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

Luna™ Omega Ordering Information

3 µm MidBore Columns (mm)				SecurityGuard Cartridges (mm)
Phases	**50 x 3.0**	**100 x 3.0**	**150 x 3.0**	**4 x 2.0*/10pk**
Polar C18	00B-4760-Y0	00D-4760-Y0	00F-4760-Y0	AJ0-7600
PS C18	00B-4758-Y0	00D-4758-Y0	00F-4758-Y0	AJ0-7605
C18	00B-4784-Y0	00D-4784-Y0	00F-4784-Y0	AJ0-7611
SUGAR	—	—	00F-4775-Y0	AJ0-4496

for ID: 2.0 – 3.0 mm

*SecurityGuard Analytical Cartridges require holder, Part No.: KJ0-4282

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