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Column Selection for LC-MS/MS Analysis of a 14 Analyte Antipsychotic Panel

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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 14 Antipsychotics 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 Antipsychotics drug class can be found in TN-0163. Briefly, 500 µL of human serum was spiked with an Anticonvulsants standard mix (1 ng/mL) and extracted using the Strata-X Method Development plate under Neutral, Basic, or Acid loading buffer, followed with Neutral, Basic, or Acidic elution buffer. The best results were obtained using the Strata-X-CW 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 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	20	0	45
	1	40	0.5	45
	2	80	1.5	85
	3	95	3	95
	3.5	95	3.5	95
	3.5	20	3.51	45
	5	20	5	45

Flow Rate:	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:	350° C
GS1:	55 psi
GS2:	60 psi
CUR:	35 psi
IS:	2500 V

Table 1. MS Transitions.

Analyte	Q1 Mass (Da)	Q3 Mass (Da)
Olanzapine	313.1	256.1
Norclozapine	313	192.1
Clozapine	327	270.1
9-OH-Risperidone	427.2	207.2
Haloperidol	376.1	165
Risperidone	411.2	191.1
Promethazine	285	86.1
Quetiapine	384.1	253.1
Ziprasidone	413	194
Dehydroariprazole	447	286.1
Chlorpromazine	319	86
Fluphenazine	439.2	171
Ariprazole	448	285.1
Lurasidone	493.2	166.1

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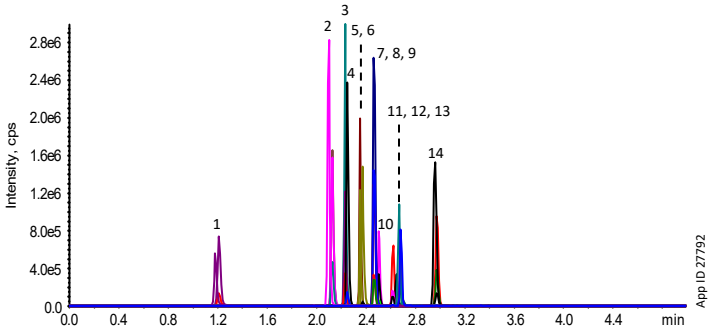
Results and Discussion

The logP (1.8 to 4.9) and pKa (7.6 to 9.2) range of the 14 antipsychotics represents a panel of analytes with moderate to high hydrophobicity and basic functionality. A mix of Antipsychotics 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 critical isobaric pair of Olanzapine and Norclozapine (**Figure 1b**). To evaluate the chromatography from a matrix-matched sample, 14 antipsychotics 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-CW sorbent with the acidic load and basic elution solvents were found to be the most successful 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** and **2b**).

The Antipsychotics standards mix was also analyzed with the Luna™ Omega 3 µm Polar C18 column. Analytes were separated with good peak shape, however the isobaric analytes Norclozapine and Olanzapine failed to show complete separation (**Figures 3a** and **3b**). Because of the lack of separation of this isobaric pair, spiked serum was not analyzed on the Luna Omega 3 µm Polar C18 column.

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



Peak No.	Analyte	Retention Time (min)
1	Olanzapine	1.2
2	Norclozapine	2.11
3	Clozapine	2.2
4	9-OH-Risperidone	2.3
5	Haloperidol	2.4
6	Risperidone	2.4
7	Promethazine	2.5
8	Quetiapine	2.5
9	Ziprasidone	2.5
10	Dehydroariprazole	2.6
11	Chlorpromazine	2.7
12	Fluphenazine	2.7
13	Ariprazole	2.7
14	Lurasidone	3

Figure 1b. Separation of Isobaric Norclozapine and Olanzapine Antipsychotics on a Kinetex 2.6 µm Biphenyl Column.

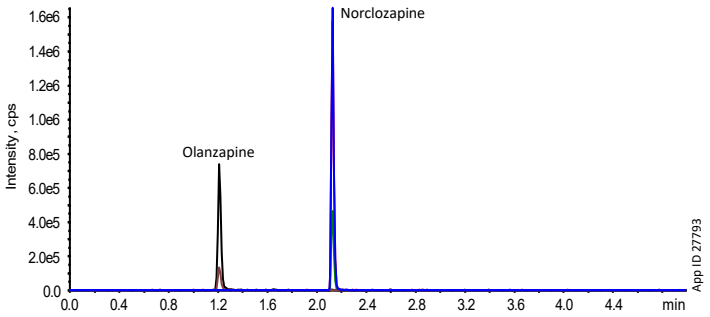
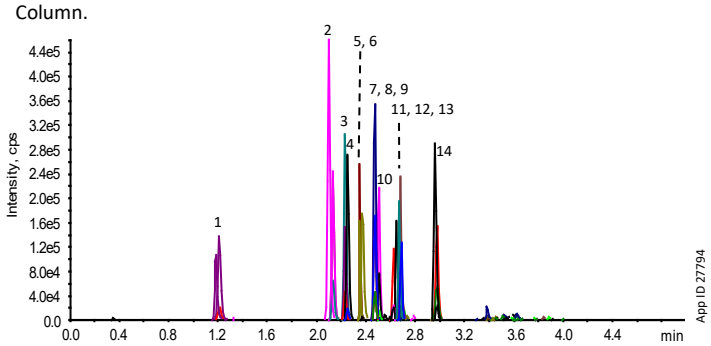


Figure 2 a . Analysis of Antipsychotics Extracted from Serum Using Strata™-X-CW, Under Acidic Load and Basic Elution, on a Kinetex™ 2.6 µm Biphenyl Column.



Peak No.	Analyte	Retention Time (min)
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3	Clozapine	2.2
4	9-OH-Risperidone	2.3
5	Haloperidol	2.4
6	Risperidone	2.4
7	Promethazine	2.5
8	Quetiapine	2.5
9	Ziprasidone	2.5
10	Dehydroariprazole	2.6
11	Chlorpromazine	2.7
12	Fluphenazine	2.7
13	Ariprazole	2.7
14	Lurasidone	3

Figure 2 b . Separation of Isobaric Norclozapine and Olanzapine Antipsychotics Extracted from Serum Using Strata-X-CW, Under Acidic Load and Basic Elution, on a Kinetex 2.6 µm Biphenyl Column.

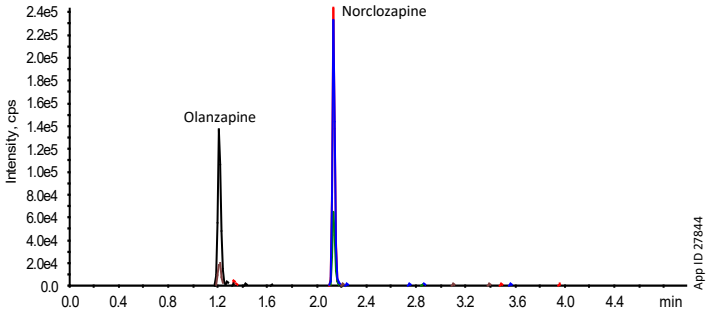
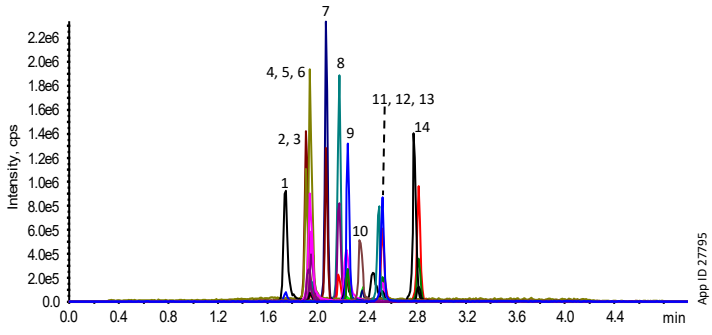
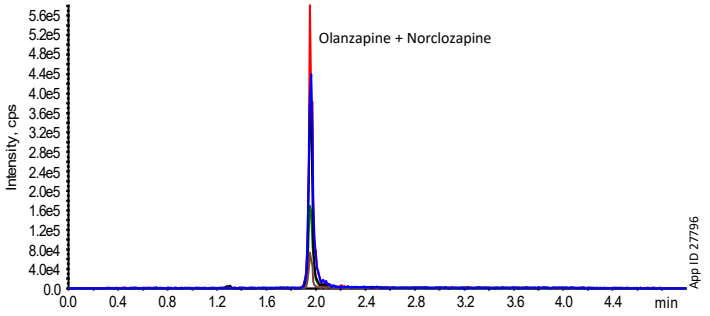


Figure 3 a . Analysis of Antipsychotics Standards on a Luna™ Omega 3 µm Polar C18 Column.



Peak No.	Analyte	Retention Time (min)
1	9-OH-Risperidone	1.7
2	Haloperidol	1.9
3	Risperidone	1.9
4	Olanzapine	2.0
5	Norclozapine	2.0
6	Quetiapine	2.0
7	Clozapine	2.1
8	Promethazine	2.2
9	Ziprasidone	2.3
10	Fluphenazine	2.4
11	Chlorpromazine	2.5
12	Dehydroariprazole	2.5
13	Ariprazole	2.5
14	Lurasidone	2.8

Figure 3 b . Incomplete separation of Isobaric Norclozapine and Olanzapine Antipsychotics 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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Conclusions

The developed LC-MS/MS method utilizing the Strata™-X Method Development plate for sample extraction and the Kinetex™ 2.6 µm Biphenyl column has been found to be the optimal protocol for analysis of the TDM panel of Antipsychotics. A Luna™ Omega 3 µm Polar C18 column was compared to the Kinetex 2.6 µm Biphenyl column but was unable to separate the isobaric pair of Olanzapine and Norclozapine. This suggests that the Kinetex Biphenyl column is a better option for separating Antipsychotic analytes over the Luna Omega Polar C18 column.

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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