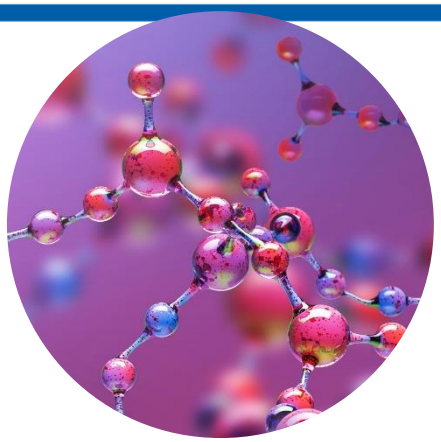

TN-1303

Assay of Doxepin Hydrochloride According to USP Monograph Using Three Different HPLC Columns

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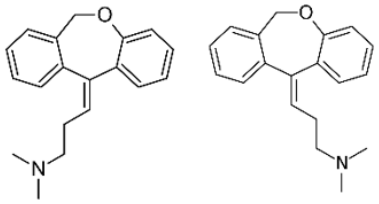
Introduction

Doxepin Hydrochloride belongs to a class of drugs referred to as tricyclic antidepressants (TCA) which are used to treat a broad range of psychotic depressive disorders. Doxepin Hydrochloride drug substance is characterized as a small molecule that is marketed as a geometric isomeric mixture which contains approximately 15 % of the (Z)-isomer and 85 % of the (E)-isomer. Although having the same chemical structures, most stereoisomers of drugs exhibit notable differences in biological activity. As a result, the pharmaceutical industry and regulatory bodies promote and require that the stereoisomeric composition of a drug be studied, and its effect well-characterized. A high-performance liquid chromatographic (HPLC) method is an accurate, reliable, and popular method for the determination and analysis of isomers in drug substances, and is also simple and highly suitable for routine analysis.

In this technical note, we study the performance of three HPLC columns for the separation of the Doxepin Hydrochloride isomers using the method defined by the United States Pharmacopeia (USP) monograph for Doxepin Hydrochloride. To meet system suitability, the USP monograph for the assay of Doxepin Hydrocholoride requires that the resolution between the (E)- and (Z)-isomers be not less than (NLT) 1.5, the symmetry factor for each isomer not more than (NMT) 2.0 and a percent relative standard deviation (RSD) NMT 2.0 %. The results from this study showed that all columns were able to meet system suitability, confirming the capability of the columns to successfully resolve the (E-) and (Z-) isomers of Doxepin Hydrochloride.

Figure 1.
Structures of (E)-Doxepin and (Z)-Doxepin Stereoisomers



(E)- Doxepin (Z)- Doxepin

Experimental Conditions

Materials and Methods

All reference standards were obtained from USP and all solutions were prepared as indicated in the USP monograph for Doxepin Hydrochloride. The columns used in this study were the fully porous Luna® 5 µm C8(2), 150 x 4.6 mm, the superficially porous core-shell Kinetex® 5 µm C8, 150 x 4.6 mm, and the Kinetex 2.6 µm C8 75 x 4.6 mm, which were all obtained from Phenomenex (Torrance, California, USA), and analyses were performed using an Agilent® 1290 HPLC system (Agilent Technologies Santa Clara, California, USA)

The Standard solution was prepared by diluting USP Doxepin Hydrochloride RS in mobile phase to a concentration of 0.1 mg/mL, as directed in the USP monograph for Doxepin Hydrochloride.

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The USP monograph for Doxepin Hydrochloride calls for a 5 µm L7 (C8) column in a 125 x 4.0 mm dimension run under isocratic conditions at a flow rate of 1.0 mL/min. USP General Chapter <621> allows for adjustments to the column dimensions (length (L), internal diameter (ID), and particle size (d_p)) within specific allowable limits. A column with the same length and particle size, but with a different internal diameter (ID) can be used as long as the volumetric flow rate (mL/min) is adjusted to maintain the same linear velocity. For the Luna® 5 µm C8(2) and Kinetex® 5 µm C8 columns used in 150 x 4.6 mm dimensions, the flow rate was adjusted accordingly to 1.32 mL/min.

	Original column 5 µm C8 125 x 4.0 mm	Luna 5 µm C8(2) 150 x 4.6 mm	Kinetex 5 µm C8 150 x 4.6 mm	Kinetex 2.6 µm C8 75 x 4.6 mm
Flow Rate	1.0 mL/min	1.32 mL/min	1.32 mL/min	1.32 mL/min
L/d_p (-25% to +50%)	25,000	30,000	30,000	28,846

LC Conditions

Column: Luna 5 µm C8(2) (150 x 4.6 mm)
Kinetex 5 µm C8 (150 x 4.6 mm)
Kinetex 2.6 µm C8 (75 x 4.6 mm)

Part No.: 00F-4249-E0 (Luna 5 µm C8(2))
00F-4608-E0 (Kinetex 5 µm C8)
00C-4497-E0 (Kinetex 2.6 µm C8)

Pressure (bar): 261 bar (Luna 5 µm C8(2))
273 bar (Kinetex 5 µm C8)
349 bar (Kinetex 2.6 µm C8)

Mobile Phase (30:70) Methanol/ 27.6 g/L monobasic sodium phosphate, pH 2.5

Flow Rate 1.32 mL/min

Injection Volume: 20 µL

Temperature 50 °C

Detection UV @ 254 nm

Instrument Agilent® 1290

Results and Discussion

The first column evaluated was the Luna 5 µm C8(2). Separation of the peaks was achieved with a resolution of 2.84, easily meeting the system suitability requirement of NLT 1.5. The recorded symmetry factor for each of the (E)- and (Z)-isomer peaks were under the required threshold of NMT 2.0.

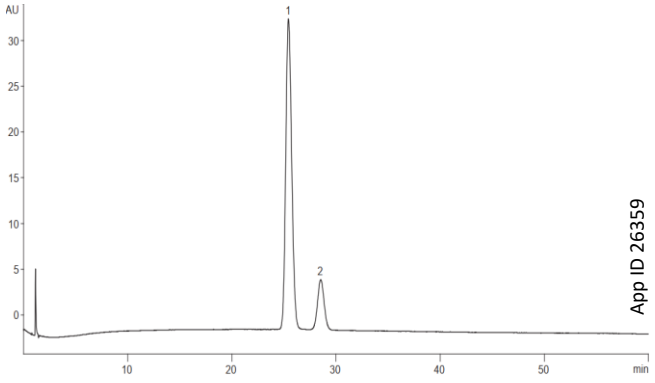
A very low RSD was also calculated for 5 replicate injections under the same conditions, meeting the NMT 2.0 % requirement for %RSD (**Figure 2**).

The Kinetex 5 µm C8 column also resolved the peaks corresponding to the (E)- and (Z)-isomers with a resolution value of 3.78. The symmetry factor and %RSD observed for each of the isomer peaks met the system suitability requirements (**Figure 3**).

The Kinetex 2.6 µm C8 column provided excellent resolution, fulfilling all system suitability requirements. From the results we can see that the Kinetex 2.6 µm C8 was able to separate the structurally similar molecules at high resolution while also resulting in a reduced analysis time (**Figure 4**). In addition, all columns generally provided good signal-to-noise ratio (S/N) values for each isomer peak. The Kinetex 2.6 µm C8 column provided higher S/N values, indicating higher sensitivity, resulting from the higher efficiency expressed by the smaller 2.6 µm core-shell particle. This is an important consideration for the analysis of samples at low concentrations.

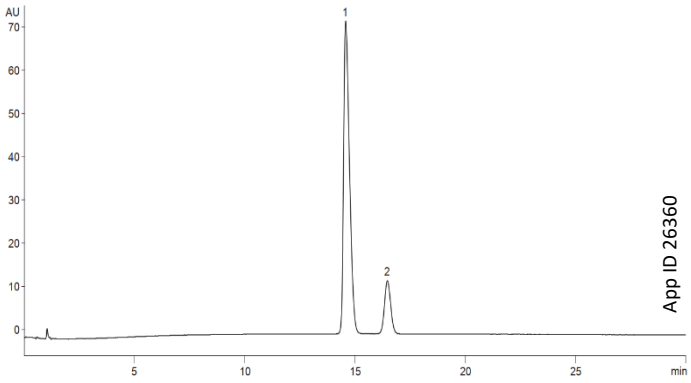
The core-shell morphology of the Kinetex column, coupled with a smaller particle size and shorter column length produced suitable results in this study.

Figure 2. Luna 5 µm C8(2), 150 x 4.6 mm



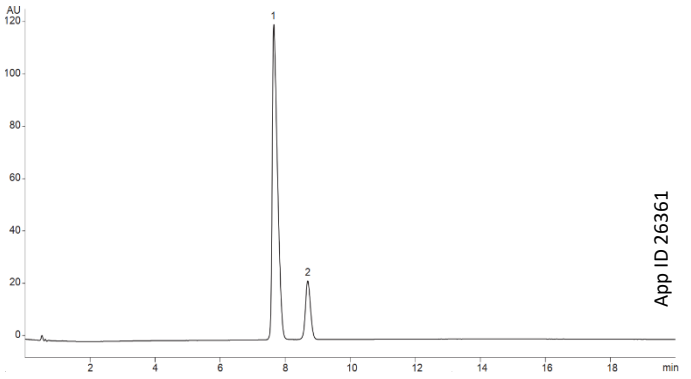
Peak #	Time	Analyte	Area	Height	Width	S/N	Symmetry Factor	Resolution	% RSD (n=5)
1	25.4	(E)-isomer	1340	34	0.62	722	1.15	2.84	0.22
2	28.5	(Z)-isomer	238	5.5	0.59	93	1.05		0.67

Figure 3. Kinetex® 5 µm C8, 150 x 4.6 mm



Peak #	Analyte	Time	Area	Height	Width	S/N	Symmetry Factor	Resolution	% RSD (n=6)
1	(E)-isomer	14.6	1346	72.4	0.28	2414	1.49	3.78	0.18
2	(Z) - isomer	16.5	241	12.3	0.30	344	1.11		0.24

Figure 4. Kinetex 2.6 µm C8, 75 x 4.6 mm



Peak #	Analyte	Time	Area	Height	Width	S/N	Symmetry Factor	Resolution	% RSD (n=6)
1	(E)-isomer	7.7	1350	120.6	0.16	4027	1.71	3.64	0.86
2	(Z) - isomer	8.7	240	22.4	0.16	367	1.09		0.74

Conclusions

As shown above, the Luna® 5 µm C8(2), Kinetex® 5 µm C8, and Kinetex 2.6 µm C8 columns were all able to resolve the (E)- and (Z)-isomers of Doxepin Hydrochloride.

When comparing the fully porous Luna versus the superficially porous core-shell Kinetex, which are of the same column dimension, we see that the Kinetex produced a superior peak shape for both the (E)- and (Z)-isomers, resulting in increased sensitivity. Kinetex also gave a S/N that was 3x higher and resolution value was higher by 30 %. In addition to the benefit of the higher resolution, the total analysis time was lower by approximately 50 %. This is due to the lower surface area associated with the core-shell morphology of the Kinetex particle.

Comparing the Kinetex columns, we notice that the shorter Kinetex column (Kinetex 2.6 µm C8, 75 x 4.6 mm), produced taller and sharper peaks and also afforded the fastest analysis time, with retention times half the time compared to the longer 5 µm Kinetex column. Shorter column length and smaller particle size aided in the short retention time and overall better peak shape without sacrificing resolution.

Column	Peaks	RT (min)	S/N	Resolution
Luna 5 µm C8(2) 150 x 4.6 mm	(E)-isomer	25.4	722	2.84
	(Z)-isomer	20.8	93	
Kinetex 5 µm C8 150 x 4.6 mm	(E)-isomer	14.6	2414	3.78
	(Z)-isomer	16.5	344	
Kinetex 2.6 µm C8 75 x 4.6 mm	(E)-isomer	2.9	4027	3.64
	(Z)-isomer	5.0	367	

Overall, all three columns met the system suitability requirement for resolution, symmetry factor, and %RSD for the assay portion of the USP monograph for Doxepin Hydrochloride.

Kinetex® Ordering Information

5 µm Analytical Columns (mm)					SecurityGuard™ ULTRA Cartridges (mm)†
Phases	**50 x 4.6**	**100 x 4.6**	**150 x 4.6**	**250 x 4.6**	**4 x 3.0* 3 /pk**
EVO C18	00B-4633-E0	00D-4633-E0	00F-4633-E0	00G-4633-E0	AJ0-9296
F5	00B-4724-E0	00D-4724-E0	00F-4724-E0	00G-4724-E0	AJ0-9320
Biphenyl	00B-4627-E0	00D-4627-E0	00F-4627-E0	00G-4627-E0	AJ0-9207
XB-C18	00B-4605-E0	00D-4605-E0	00F-4605-E0	00G-4605-E0	AJ0-8768
C18	00B-4601-E0	00D-4601-E0	00F-4601-E0	00G-4601-E0	AJ0-8768
C8	00B-4608-E0	00D-4608-E0	00F-4608-E0	00G-4608-E0	AJ0-8770
Phenyl-Hexyl	00B-4603-E0	00D-4603-E0	00F-4603-E0	00G-4603-E0	AJ0-8774
HILIC	—	—	00F-4606-E0	00G-4606-E0	AJ0-8772

for 4.6 mm ID

2.6 µm Analytical Columns (mm)					SecurityGuard™ ULTRA Cartridges (mm)†
Phases	**30 x 4.6**	**50 x 4.6**	**75 x 4.6**	**100 x 4.6**	**4 x 3.0* 3/pk**
EVO C18	00A-4725-E0	00B-4725-E0	—	00D-4725-E0	00F-4725-E0
PS C18	00A-4780-E0	00B-4780-E0	—	00D-4780-E0	00F-4780-E0
Polar C18	00A-4759-E0	00B-4759-E0	—	00D-4759-E0	00F-4759-E0
Biphenyl	—	00B-4622-E0	—	00D-4622-E0	00F-4622-E0
XB-C18	—	00B-4496-E0	00C-4496-E0	00D-4496-E0	00F-4496-E0
C18	00A-4462-E0	00B-4462-E0	00C-4462-E0	00D-4462-E0	00F-4462-E0
C8	—	00B-4497-E0	00C-4497-E0	00D-4497-E0	00F-4497-E0
HILIC	—	00B-4461-E0	00C-4461-E0	00D-4461-E0	00F-4461-E0
Phenyl-Hexyl	—	00B-4495-E0	00C-4495-E0	00D-4495-E0	00F-4495-E0
F5	00A-4723-E0	00B-4723-E0	—	00D-4723-E0	00F-4723-E0

for 4.6 mm ID

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

Luna® Ordering Information

5 µm Analytical and Semi-Prep Columns (mm)					SecurityGuard™ Cartridges (mm)	
Phases	**50 x 4.6**	**150 x 4.6**	**250 x 4.6**	**250 x 10**	**4 x 3.0* /10pk**	**10 x 10*** /3pk**
Silica(2)	00D-4274-E0	00F-4274-E0	00G-4274-E0	00G-4274-N0	AJ0-4348	AJ0-7223
C5	00D-4043-E0	00F-4043-E0	00G-4043-E0	00G-4043-N0	AJ0-4293	AJO-7372
C8(2)	00D-4249-E0	00F-4249-E0	00G-4249-E0	00G-4249-N0	AJ0-4290	AJ0-7222
C18(2)	00D-4252-E0	00F-4252-E0	00G-4252-E0	00G-4252-N0	AJ0-4287	AJ0-7221
CN	00D-4255-E0	00F-4255-E0	00G-4255-E0	00G-4255-N0	AJ0-4305	AJ0-7313
Phenyl-Hexyl	00D-4257-E0	00F-4257-E0	00G-4257-E0	00G-4257-N0	AJ0-4351	AJ0-7314
NH_2	00D-4378-E0	00F-4378-E0	00G-4378-E0	00G-4378-N0	AJ0-4302	AJ0-7364
SCX	00D-4398-E0	00F-4398-E0	00G-4398-E0	00G-4398-N0	AJ0-4308	AJ0-7369
HILIC	00D-4450-E0	00F-4450-E0	00G-4450-E0	00G-4450-N0	AJ0-8329	AJ0-8902
PFP(2)	00D-4448-E0	00F-4448-E0	00G-4448-E0	00G-4448-N0	AJ0-8327	AJ0-8376
					for ID: 3.2-8.0 mm	for ID: 9-16 mm

*SecurityGuard™ Analytical Cartridges require holder, Part No.: KJ0-4282
***SemiPrep SecurityGuard Cartridges require holder, Part No.: AJ0-9281

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