Separation of Doxapram Hydrochloride and its Organic Impurities per USP Monograph

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Overview

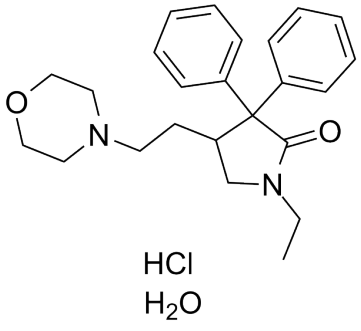
Doxapram Hydrochloride is a respiratory stimulant that has an inhibitory effect on myocardial IK1 potassium channels. The development of a quick and efficient analysis of Doxapram Hydrochloride and its related organic impurities is of interest for drug manufacturers. In this application note, we report the separation of Doxapram Hydrochloride and its related organic impurities using a Luna Omega 3 µm PS C18 column and a Kinetex 2.6 µm PS C18 column according to the USP monograph for Doxapram Hydrochloride.

System suitability per USP Monograph for the Doxapram Hydrochloride Assay is a tailing factor no more than (NMT) 2.0 for Doxapram Hydrochloride and a percent relative standard deviation (%RSD) of NMT 0.73 % for Doxapram Hydrochloride. All system suitability requirements for Doxapram Hydrochloride Assay were met with the Waters XSelect CSH C18 and the Luna Omega PS C18 columns. The Kinetex PS C18 column met the requirement of %RSD NMT 0.73 % but did not meet the requirement for tailing factor NMT than 2.0 for system suitability (**Figure 2**).

System suitability per USP Monograph for the Doxapram Hydrochloride Organic Impurities is a resolution no less than 5.0 between Doxapram Hydrochloride and Doxapram Related Compound B, as well as a %RSD NMT 5.0 %. These requirements for System Suitability for Organic Impurities were met for each of the columns tested (**Figure 3** and **4**).

All solutions were prepared as indicated in the USP Monograph for Doxapram Hydrochloride. USP Doxapram Hydrochloride RS (Catalog No. 1225000) and USP Doxapram Related Compound B RS (Catalog No. 1225022) were purchased from USP.

Figure 1. Doxapram Hydrochloride



LC-UV Conditions

Column: Luna™ Omega 3 µm PS C18 (00B-4758-E0)
Kinetex™ 2.6 µm PS C18 (00B-4780-E0)
Waters® 2.5 µm XSelect® CSH C18

Dimension: 50 X 4.6 mm

Mobile Phase: A: 0.01 % Trifluoroacetic Acid in Water
B: 0.01 % Trifluoroacetic Acid in Acetonitrile

Flow Rate: 1 mL/min

Gradient:

Time (min)	%B
0	10
20	50
25	50
25.1	10
30	10

Injection Volume: 5 µL

Temperature: 35 °C

Detector: UV @ 220 nm

System: Agilent® 1260 Binary UHPLC

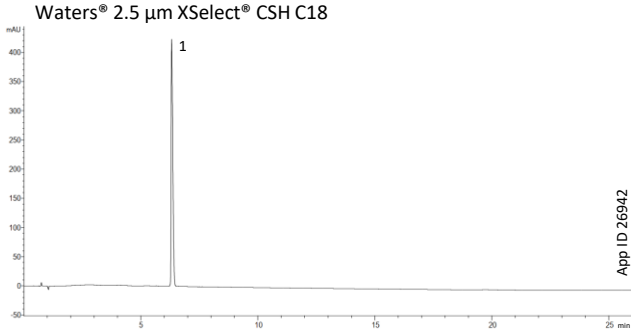
NOTE: The forthcoming revision to USP General Chapter <621> will become official December 1st, 2022, and adjustments to column dimensions for gradient methods will be allowed provided that the L/dp ratio remains constant or within the range between -25 % to +50 % of the prescribed L/dp ratio indicated in the monograph. In this monograph, the indicated column length was 50 mm, and the particle size was 2.5 µm; therefore, the Luna Omega 3 µm and Kinetex 2.6 µm columns used here would be an allowable adjustment.

Table 1. Preparation of Solutions

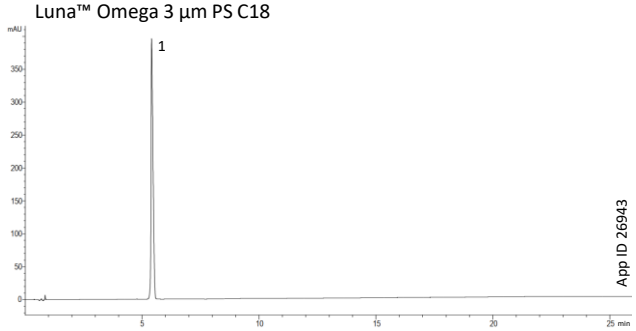
Solution	Composition
Diluent	Acetonitrile/Water (30:70, v/v)
Standard Solution – Assay	0.2 mg/mL of USP Doxapram Hydrochloride RS in Diluent
Standard Solution – Organic Impurities	0.004 mg/mL each of USP Doxapram Hydrochloride RS and USP Doxapram Related Compound B RS in Diluent
System Suitability Solution – Organic Impurities	2 mg/mL of USP Doxapram Hydrochloride RS and 0.04 mg/mL of USP Doxapram Related Compound B RS in Diluent

Figure 2. Standard Solution – Assay

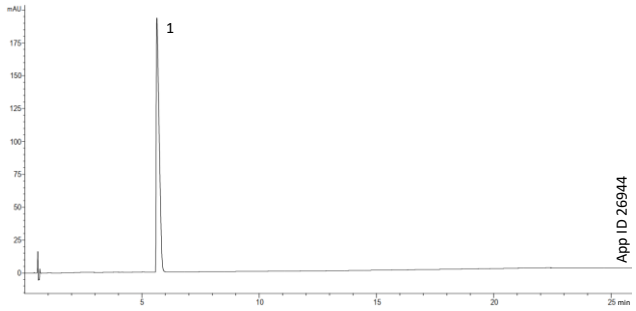
Waters® 2.5 µm XSelect® CSH C18



Luna™ Omega 3 µm PS C18

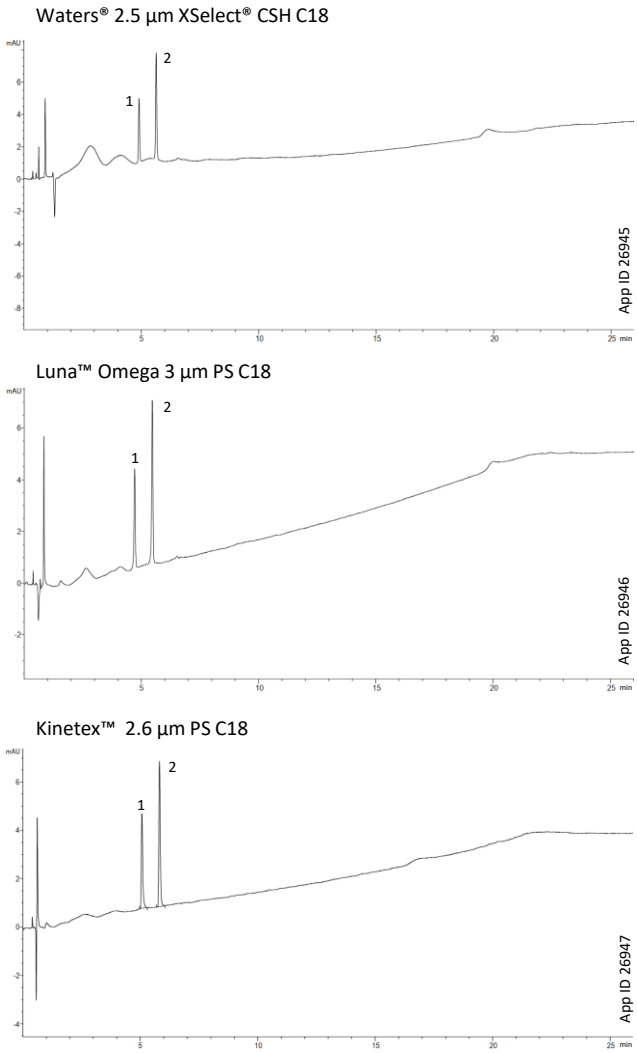


Kinetex™ 2.6 µm PS C18



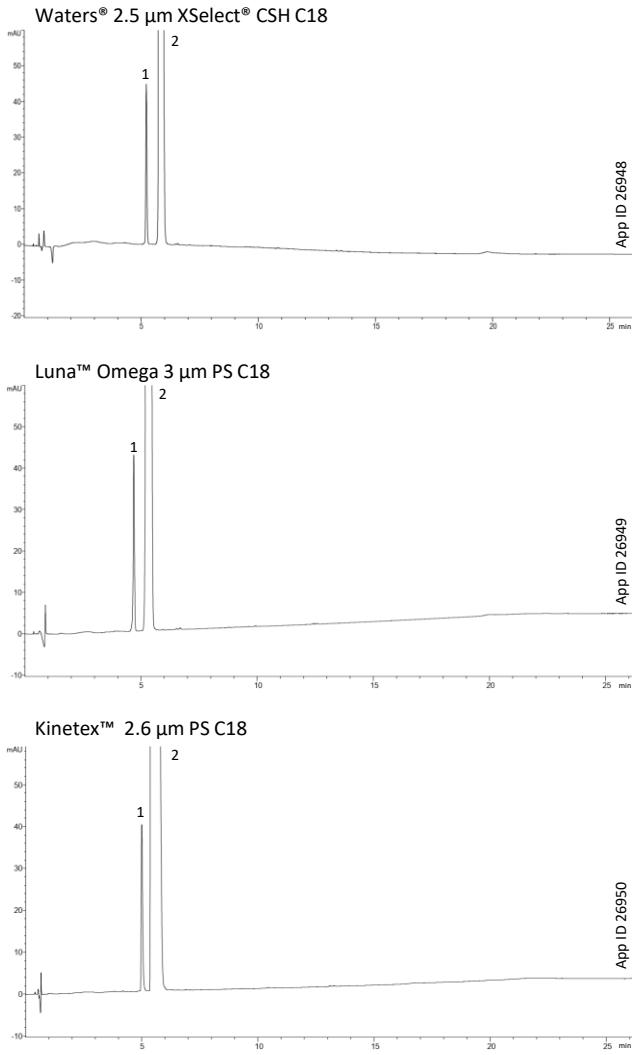
		Waters 2.5 µm XSelect CSH C18		Luna Omega 3 µm PS C18		Kinetex 2.6 µm PS C18	
Peak No.	Analyte	Tailing Factor	Area %RSD	Tailing Factor	Area %RSD	Tailing Factor	Area %RSD
1	Doxapram Hydrochloride	1.769	0.166	1.569	0.026	2.849	0.097
N = 5 Injections							

Figure 3. Standard Solution – Organic Impurities



		Waters 2.5 µm XSelect CSH C18	Luna Omega 3 µm PS C18	Kinetex 2.6 µm PS C18
Peak No.	Analyte	Area %RSD	Area %RSD	Area %RSD
1	Doxapram Related Compound B	0.974	0.739	0.431
2	Doxapram Hydrochloride	0.272	0.475	0.758
N = 6 Injections				

Figure 4. System Suitability Solution – Organic Impurities



		Waters 2.5 µm XSelect CSH C18	Luna Omega 3 µm PS C18	Kinetex 2.6 µm PS C18
Peak No.	Analyte	Resolution	Resolution	Resolution
1	Doxapram Related Compound B	3.63	3.30	1.54
2	Doxapram Hydrochloride			
N = 3 Injections				

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