Ph. Eur. Monograph 2280: Fexofenadine Hydrochloride Related Substances and Assay

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Overview

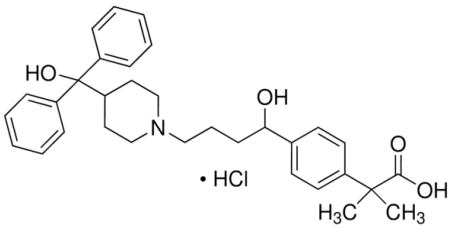
Fexofenadine Hydrochloride is a selective peripheral H1 blocker and is classified as a second-generation antihistamine used in the treatment of allergy symptoms, such as hay fever and urticaria. This study for Fexofenadine Hydrochloride and its related substances is based on the Ph. Eur. monograph 2280 where a phenylsilyl silica gel stationary phase is used under isocratic conditions. In this application note, we demonstrate the separation of Fexofenadine Hydrochloride from its related substances per the Ph. Eur. monograph using Kinetex™ Biphenyl and Phenyl-Hexyl columns in comparison with the ZORBAX® SB-Phenyl column used in the elucidation of the monograph.

System suitability per Ph. Eur. Monograph 2280 for Fexofenadine Hydrochloride for Related Substances is a minimum resolution of 10 between the peaks due to Fexofenadine Hydrochloride and impurity A. System suitability per Ph. Eur. Monograph 2280 for Fexofenadine Hydrochloride for Assay is a maximum % Relative Standard Deviation (%RSD) of 0.85 for 6 injections and a symmetry factor of the peak for Fexofenadine Hydrochloride of 0.8-1.8. All three columns met the system suitability requirement for resolution between Fexofenadine Hydrochloride and Fexofenadine Impurity A. This demonstrates that the Kinetex Biphenyl and Phenyl-Hexyl columns are suitable alternatives to the ZORBAX SB-Phenyl column for Fexofenadine Hydrochloride related substances.

All the reference solutions were prepared as indicated in Ph. Eur. monograph 2280 for Fexofenadine Hydrochloride. The following certified reference standards (CRS) were purchased from the European Directorate for the Quality of Medicines & HealthCare (EDQM) – Council of Europe; Postal address: 7 Allee Kastner CS 30026 F - 67081 Strasbourg (France):

- Y0000789, Fexofenadine Hydrochloride CRS
- Y0000751, Fexofenadine Impurity A CRS
- Y0000753, Fexofenadine Impurity C CRS

Figure 1. Fexofenadine Hydrochloride Structure.



LC-UV Conditions

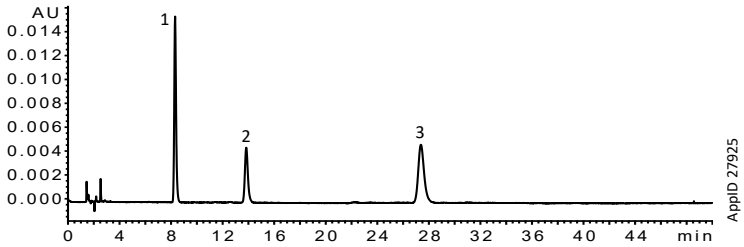
Columns: Kinetex 5 µm Biphenyl (00G-4627-E0)
Kinetex 5 µm Phenyl-Hexyl (00G-4603-E0)
ZORBAX 5 µm SB-Phenyl
Dimensions: 250 x 4.6 mm
Mobile Phase: Acetonitrile R / **Buffer** / Triethylamine R (350:650:3, v/v/v)
Buffer: Dissolve 6.64 g/L of Sodium Dihydrogen Phosphate Monohydrate R and 0.84 g/L of Sodium Perchlorate R in Water for Chromatography R, adjust to pH 2.0 ± 0.1 with Orthophosphoric Acid.
Flow Rate: 1.5 mL/min (Isocratic)
Injection: 20 µL
Temperature: 25 °C
Detector: UV @ 220 nm
System: Waters® ACQUITY Arc® HPLC

Table 1. Preparation of Test and Reference Solutions

Solution	Composition
Solvent Mixture	Mix equal volumes of Acetonitrile R and **Buffer** solution.
Test Solution (a)	Dissolve 25.0 mg of *Fexofenadine Hydrochloride CRS* in 25 mL of the **Solvent Mixture**.
Test Solution (b)	Dilute 3.0 mL of **Test Solution (a)** to 50.0 mL with Mobile Phase.
Reference Solution (a)	Same as Test Solution (b).
Reference Solution (b)	Dilute 1.0 mL of **Test Solution (a)** to 100.0 mL with the Mobile Phase. Dilute 1.0 mL of this solution to 10.0 mL with the Mobile Phase.
Reference Solution (c)	Dissolve 1 mg each of *Fexofenadine Impurity A CRS* and *Fexofenadine Impurity C CRS* in 20 mL of **Reference Solution (a)** and dilute to 200.0 mL with the Mobile Phase.

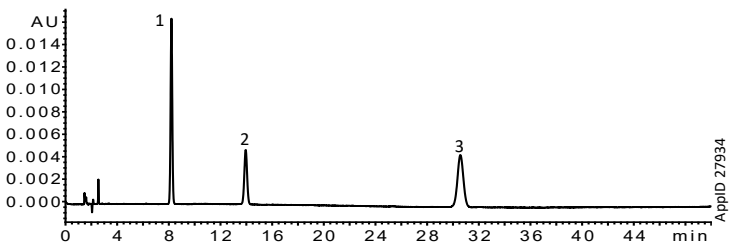
Figure 2. System Suitability Test for Related Substances Using Reference Solution (c).

Kinetex™ 5 µm Biphenyl Column



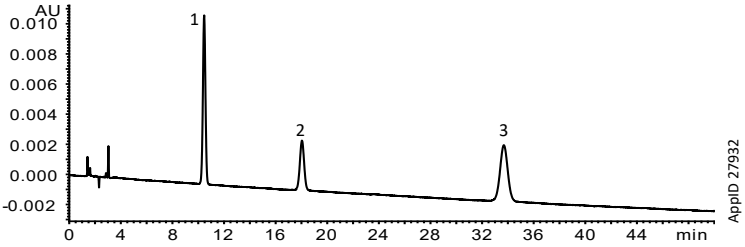
Peak No.	Analyte	Retention Time (min)	RRT	Resolution	RRT per Monograph
1	Fexofenadine Hydrochloride	8.29	-	16.70	1.00
2	Fexofenadine Impurity A	13.80	1.66		1.70
3	Fexofenadine Impurity C	27..32	3.30	-	3.20
N = 6 Injections					

Kinetex 5 µm Phenyl-Hexyl Column



Peak No.	Analyte	Retention Time (min)	RRT	Resolution	RRT per Monograph
1	Fexofenadine Hydrochloride	8.14	-	18.19	1.00
2	Fexofenadine Impurity A	13.86	1.70		1.70
3	Fexofenadine Impurity C	30.39	3.73	-	3.20
N = 6 Injections					

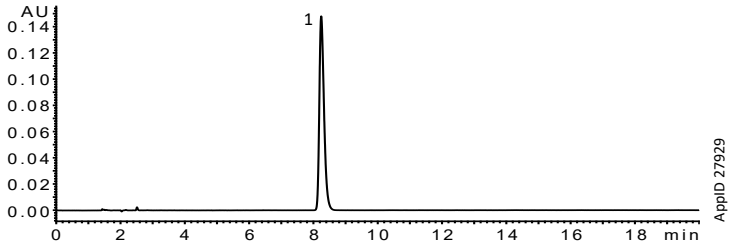
ZORBAX® 5 µm SB-Phenyl Column



Peak No.	Analyte	Retention Time (min)	RRT	Resolution	RRT per Monograph
1	Fexofenadine Hydrochloride	10.72	-	15.62	1.00
2	Fexofenadine Impurity A	18.50	1.72		1.70
3	Fexofenadine Impurity C	34.50	3.22	-	3.20
N = 6 Injections					

Figure 3. System Suitability Test for Assay Using Test Solution (b) / Reference Solution (a).

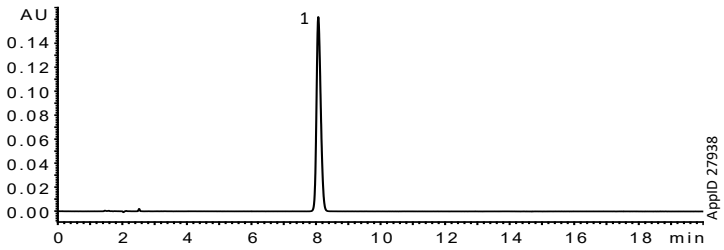
Kinetex™ 5 µm Biphenyl Column



Peak No.	Analyte	Retention Time (min)	Area	%RSD	Symmetry Factor
1	Fexofenadine Hydrochloride	8.23	1495178.60	0.60	1.40

N = 6 Injections

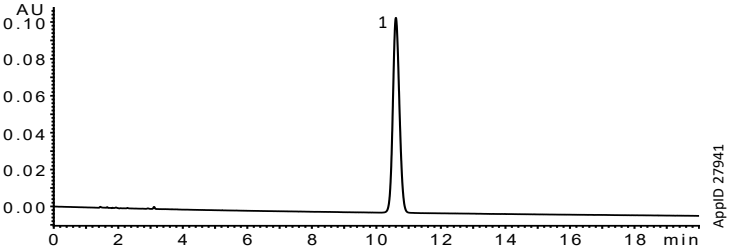
Kinetex 5 µm Phenyl-Hexyl Column



Peak No.	Analyte	Retention Time (min)	Area	%RSD	Symmetry Factor
1	Fexofenadine Hydrochloride	8.07	1493241.42	0.37	1.22

N = 6 Injections

ZORBAX® 5 µm SB-Phenyl Column



Peak No.	Analyte	Retention Time (min)	Area	%RSD	Symmetry Factor
1	Fexofenadine Hydrochloride	10.76	1618406	0.39	1.01

N = 6 Injections

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