Separation of Ezetimibe and its Organic Impurities per USP Monograph

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Overview

Ezetimibe is a cholesterol absorption inhibitor used to treat hyperlipidemia. The development of a quick and efficient analysis of Ezetimibe and its related organic impurities is of interest for generic drug manufacturers. In this application note, we report the separation of Ezetimibe and its related organic impurities per the proposed revision to the USP monograph for Ezetimibe. The proposed revision (PF 49(2), March 2023) to the Assay and Organic Impurities, Procedure 1, for Ezetimibe was validated using a Kinetex 5 µm F5 (L43, Pentafluorophenyl) column with dimensions of 150 x 4.6 mm.

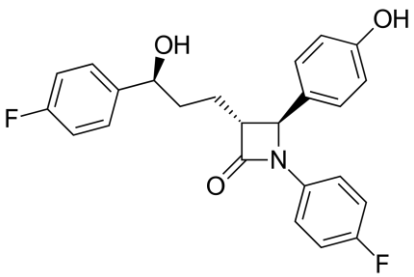
System suitability per USP Monograph for Ezetimibe Assay is a symmetry factor no more than (NMT) 1.5 for Ezetimibe and a percent relative standard deviation (%RSD) NMT 0.73 % for Ezetimibe.

System suitability per USP Monograph for Ezetimibe Organic Impurities is resolution no less than (NLT) 1.5 between Ezetimibe and O-Fluorobenzene Isomer, a symmetry factor NMT 1.5 for Ezetimibe, and a %RSD NMT 10 % for Ezetimibe.

All system suitability requirements for Ezetimibe Assay and Organic Impurities were met by the Kinetex 5 µm F5 column.

All solutions were prepared as indicated in the USP Monograph for Ezetimibe. USP Ezetimibe RS (Catalog No. 1269028) and USP Ezetimibe System Suitability Mixture RS (Catalog No. 1269039) were purchased from USP.

Figure 1. Ezetimibe Structure.



LC-UV Conditions

Column: Kinetex™ 5 µm F5
Dimensions: 150 x 4.6 mm
Part No.: 00F-4724-E0
Mobile Phase: A: Water
B: Acetonitrile
C: Methanol

Gradient:

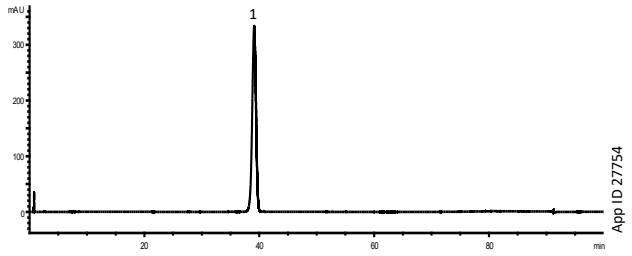
Time (min)	%A	%B	%C
0	66	24	10
37	66	24	10
60	40	50	10
70	40	50	10
80	10	80	10
90	10	80	10
90.1	66	24	10
100	66	24	10

Flow Rate: 2.0 mL/min
Injection Volume: 60 µL
Temperature: Ambient
Backpressure: 373 bar
Detector: UV @ 215 nm (0-5 min)
UV @ 248 nm (5-100 min)
LC System: Agilent® 1260 Infinity

Table 1. Preparation of Solutions.

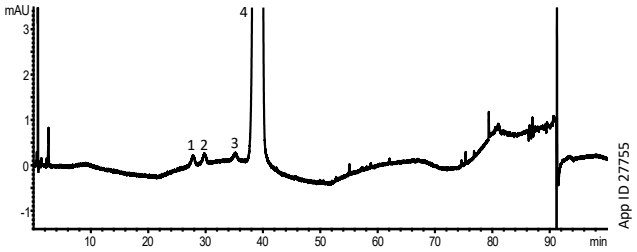
Solution	Composition
Diluent	Acetonitrile / Methanol / Water (27:10:63, v/v/v). Add 1.0 mL of Glacial Acetic Acid per liter of the mixture.
Standard Solution – Assay and Organic Impurities	0.25 mg/mL of *USP Ezetimibe RS* prepared as follows: Transfer a suitable amount of USP Ezetimibe RS to a suitable volumetric flask. Dissolve in about 1 % - 2 % of the flask volume of Acetonitrile and dilute with **Diluent** to volume.
Sample Solution – Assay and Organic Impurities	Same as **Standard Solution**.
Sensitivity Solution – Organic Impurities	0.125 µg/mL of *USP Ezetimibe RS* from the **Standard Solution** in **Diluent**.

Figure 2. Standard Solution – Assay.



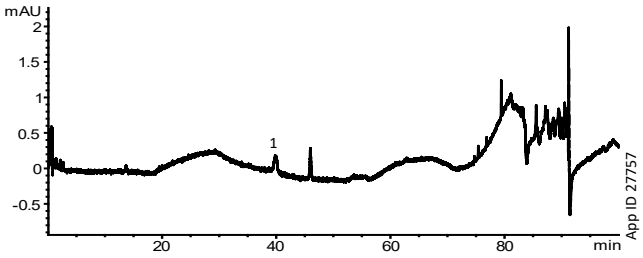
Peak No.	Analyte	Retention Time (min)	Area	Area %RSD	Symmetry Factor
1	Ezetimibe	39.15	15305.48	0.09	0.94
N = 6 Injections					

Figure 3. Standard Solution – Organic Impurities.



Peak No.	Analyte	Retention Time (min)	Area	Height	Resolution
1	Desfluoroaniline Analog	27.79	10.3	2.3E-1	-
2	Ezetimibe Diastereomers	29.75	9.4	2.2E-1	-
3	O-Fluorobenzene Isomer	35.05	9.2	2.0E-1	3.51
4	Ezetimibe	39.11	15366.7	333.5	
N = 6 Injections					

Figure 4. Sensitivity Solution – Organic Impurities.



Peak No.	Analyte	Retention Time (min)	Area	Area %RSD
1	Ezetimibe	39.85	10.93	4.81
N = 6 Injections				

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