



Separation of Ezetimibe and its Chiral 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 chiral impurities is of interest for generic drug manufacturers. In this application note, we report the separation of Ezetimibe and its related chiral impurities using a Lux 5 µm Cellulose-1 column compared with a CHIRALCEL 5 µm OD-RH column according to the USP monograph for Ezetimibe, which specifies a column containing L93 (Cellulose tris(3,5-dimethylphenylcarbamate)) packing and two columns in series with dimensions of 150 x 4.6 mm.

System suitability per USP Monograph for Ezetimibe Chiral Impurities is resolution no less than (NLT) 1.5 between Ezetimibe and R,R,S-Ezetimibe diastereomer, a symmetry factor no more than (NMT) 1.5 for Ezetimibe, and a percent relative standard deviation (%RSD) of NMT 10 % for Ezetimibe. All system suitability requirements for Ezetimibe Chiral Impurities were met by both the Lux Cellulose-1 and CHIRALCEL OD-RH columns. The Lux 5 µm Cellulose-1 column resulted in better resolution and peak symmetry values, as well as lower %RSD values compared to the CHIRAL 5 µm OD-RH 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.

LC-UV Conditions

Columns (2 each, in series): CHIRALCEL® 5 µm OD-RH®
Lux™ 5 µm Cellulose-1 ([00F-4459-E0](#))

Dimensions: 150 x 4.6 mm

Mobile Phase: Acetonitrile / Water (45:55, v/v)

Flow Rate: 0.35 mL/min (Isocratic)

Injection Volume: 10 µL

Temperature: 5 °C

Detector: UV @ 248 nm

LC System: Agilent® 1260 Infinity

Table 1. Preparation of Solutions

| Solution | Composition |
|-----------------------------|---|
| Diluent | 0.1 % Glacial Acetic Acid in Acetonitrile. |
| System Suitability Solution | 0.4 mg/mL of USP Ezetimibe System Suitability Mixture RS in Diluent . |
| Sensitivity Solution | 0.2 µg/mL of USP Ezetimibe RS in Diluent . |
| Sample Solution | 0.4 mg/mL of Ezetimibe in Diluent (can use USP Ezetimibe RS as surrogate for having an actual sample). |

Figure 1. Ezetimibe Structure.

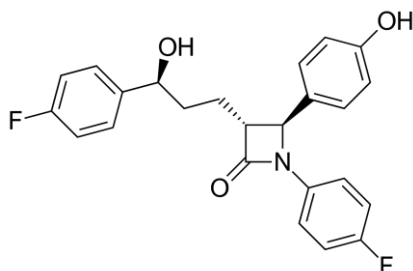
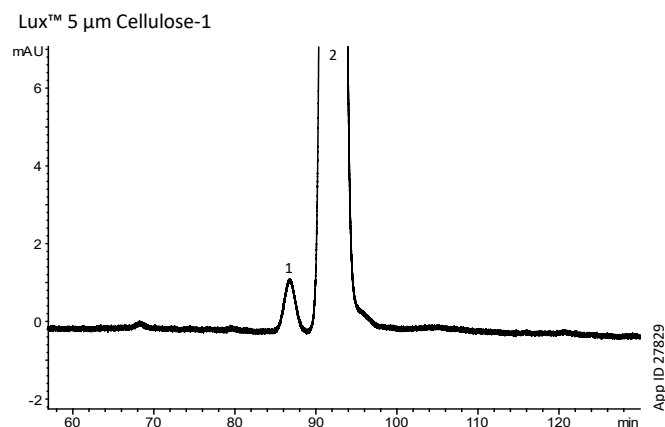
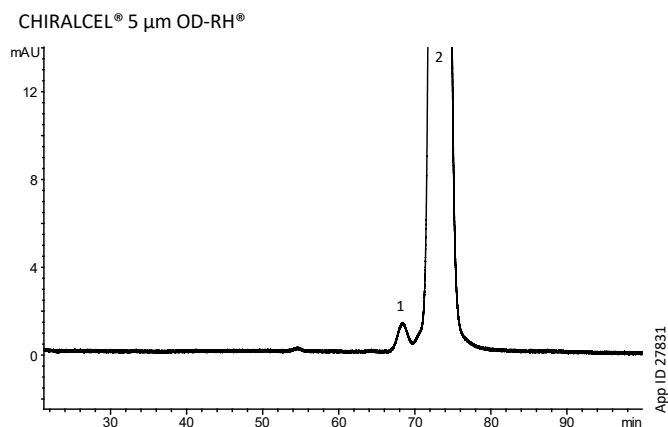
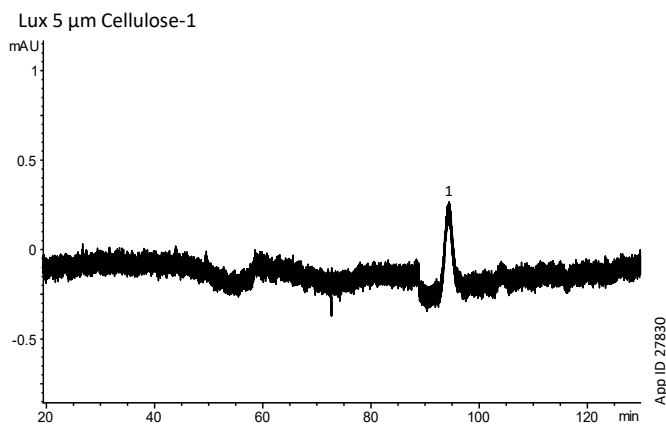


Figure 2. System Suitability Solution.

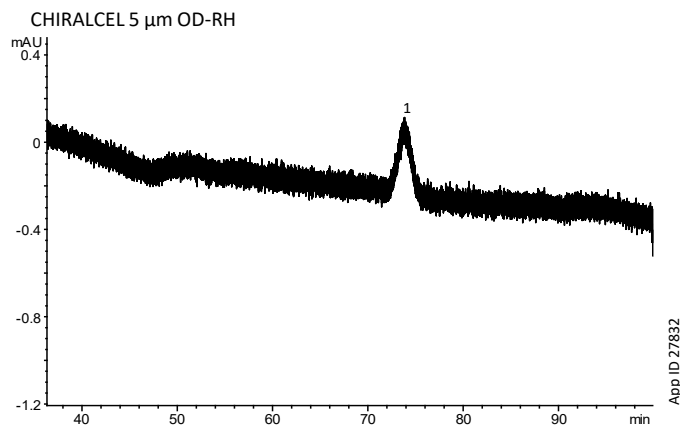
| Peak No. | Analyte | Retention Time (min) | Area | Height | Resolution | USP Tailing Factor |
|------------------|-----------------|----------------------|---------|--------|------------|--------------------|
| 1 | R,R,S-Ezetimibe | 86.51 | 138.1 | 4.42 | 1.99 | - |
| 2 | Ezetimibe | 91.69 | 22878.5 | 225.06 | | 1.09 |
| N = 5 Injections | | | | | | |



| Peak No. | Analyte | Retention Time (min) | Area | Height | Resolution | USP Tailing Factor |
|------------------|-----------------|----------------------|---------|--------|------------|--------------------|
| 1 | R,R,S-Ezetimibe | 68.39 | 93.7 | 1.14 | 1.97 | - |
| 2 | Ezetimibe | 73.18 | 22334.3 | 220.08 | | 1.09 |
| N = 5 Injections | | | | | | |

Figure 3. Sensitivity Solution.

| Peak No. | Analyte | Retention Time (min) | Area | Area %RSD | Height |
|------------------|-----------|----------------------|------|-----------|--------|
| 1 | Ezetimibe | 93.74 | 54.6 | 1.78 | 0.48 |
| N = 5 Injections | | | | | |



| Peak No. | Analyte | Retention Time (min) | Area | Area %RSD | Height |
|------------------|-----------|----------------------|------|-----------|--------|
| 1 | Ezetimibe | 73.50 | 45.4 | 9.2 | 0.43 |
| N = 5 Injections | | | | | |



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