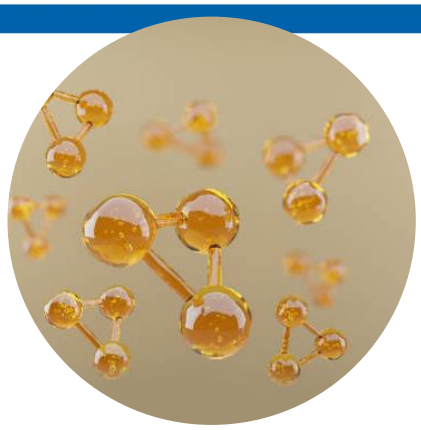

Ph. Eur. Monograph 1425: Fosfomycin Trometamol Assay and Related Substances on Luna™ Omega 3 µm SUGAR Column

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Overview

Fosfomycin Trometamol is an antibiotic used for bladder infections. In this application note we show the separation of Fosfomycin Trometamol from its related substances following Ph. Eur. Monograph 1425. We used a Luna Omega 3 µm SUGAR column in two different dimensions (250 x 4.6 mm and 150 x 4.6 mm) and compared them to the ZORBAX 5 µm NH_2, 250 x 4.6 mm column originally used in the monograph.

System suitability per Ph. Eur. Monograph for Fosfomycin Trometamol is the same for both Assay and Related Substances: Minimum resolution of 1.5 between the peaks due to Impurity A and Fosfomycin, and minimum peak-to-valley ratio of 1.5 between the peak due to Impurity C and the peak due to Impurity B for reference solution (c). The peak-to-valley ratio is defined as H_p/H_v, where H_p = height above the baseline of the peak due to Impurity C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to Impurity B.

The ZORBAX 5 µm NH_2 column described in the Ph. Eur. monograph for Fosfomycin Trometamol presents challenges when evaluating the peak-to-valley criteria between Impurity B and Impurity C. Specifically, the noisy baseline from unresolved sample compromises the consistent evaluation of the peak-to-valley parameter. The resulting measurement is then subjective from one System Suitability Standard to the next.

The Luna Omega 3 µm SUGAR columns underwent only about 20 minutes of mobile phase equilibration prior to triplicate injections of the blank and displayed stable baselines across these blank injections (data not shown). Complete baseline resolution between Impurity B and Impurity C was achieved using both the prescribed dimensions (250 x 4.6 mm) and the shorter dimensions (150 x 4.6 mm) of the Luna Omega 3 µm SUGAR columns. The longer 250 mm column also afforded improved resolution between Fosfomycin and Impurity A, while preserving the expected run time. While using a smaller 3 µm particle size would be expected to generate higher pressures, the use of a shorter 150 mm column, which maintains the L/dp ratio, alleviates these concerns. The resolution between Fosfomycin and Impurity A meets suitability requirements on the shorter Luna Omega 3 µm SUGAR column but may not afford as much flexibility when accounting for prolonged use of the column during routine sample analysis.

All reference solutions were prepared as indicated in Ph. Eur. monograph 1425 for Fosfomycin Trometamol. The following certified reference standards (CRS) were purchased from the European Directorate for the Quality of Medicines & HealthCare (EDQM) – Council of Europe; Postal address: Allee Kastner CS 30026 F - 67081 Strasbourg (France):

- F0400000, Fosfomycin Trometamol CRS

LC-UV Conditions

Columns: Luna Omega 3 µm SUGAR, 250 x 4.6 mm (00G-4775-E0)
Luna Omega 3 µm SUGAR, 150 x 4.6 mm (00F-4775-E0)
ZORBAX® 5 µm NH_2, 250 x 4.6 mm
Mobile Phase: Mobile Phase (**Table 1**)
Flow Rate: 1.0 mL/min (Isocratic)
Injection: 5 µL
Temperature: Ambient with column in column heater, off
Detector: Refractive Index Detector at 35 °C
System: Agilent® 1260

Table 1. Preparation of Test and Reference Solutions

Solution	Composition
Mobile Phase	10.89 g/L of Potassium Dihydrogen Phosphate in HPLC Water.
Test Solution	Same as Reference Solution (a)
Reference Solution (a)	Dissolve 0.600 g of Fosfomycin Trometamol CRS in the Mobile Phase and dilute to 5.0 mL with the Mobile Phase.
Reference Solution (b)	Dilute 1.0 mL of Reference Solution (a) to 100.0 mL with the Mobile Phase. Dilute 3.0 mL of this solution to 10.0 mL with the Mobile Phase.
Reference Solution (c)	Wet 0.3 g of Fosfomycin Trometamol CRS with 60 µL of Water and heat in an oven (Shimadzu GC-MS) at 60 °C for 24 hours. Dissolve the residue in the Mobile Phase and dilute to 20.0 mL with the Mobile Phase (Solution A). Dissolve 0.6 g of Fosfomycin Trometamol CRS in Solution A and dilute to 5.0 mL with the same solution (*in situ* degradation to obtain impurities A, B, C, and D).

Figure 1. Fosfomycin Trometamol

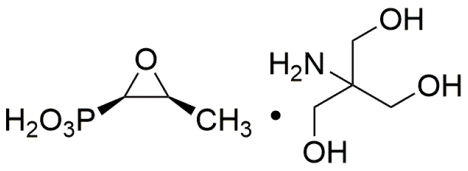
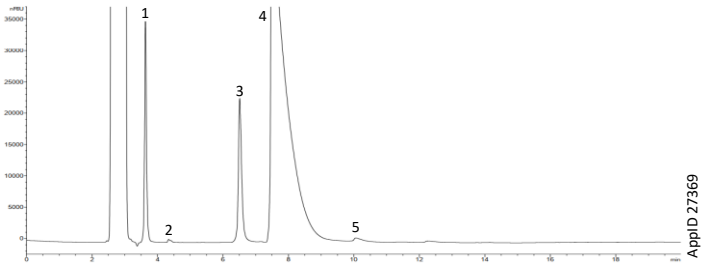


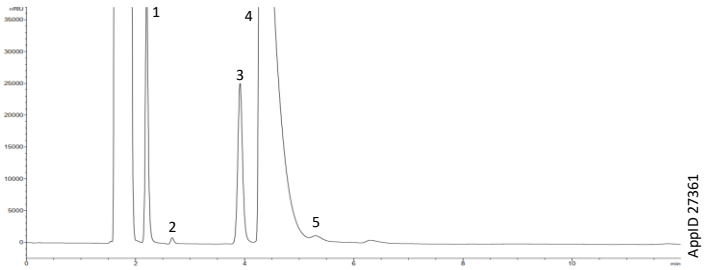
Figure 2. System Suitability Test for Assay and Related Substances Using Reference Solution (c)

Luna™ Omega 3 µm SUGAR Column, 250 x 4.6 mmm



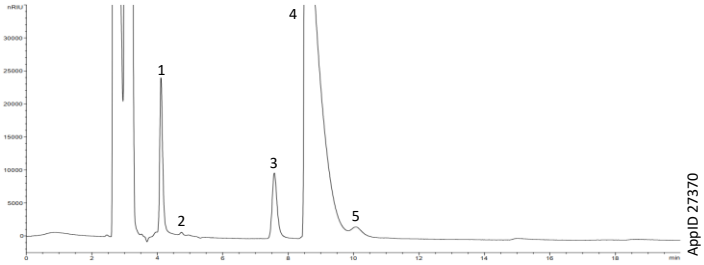
Peak No.	Analyte	Retention Time (min)	Area	Symmetry Factor	Resolution	H_p/H_v
1	Impurity B	3.63	150034.2	1.19	4.77	N/A
2	Impurity C	4.34	3136.2	1.87		
3	Impurity A	6.51	166991.2	1.29	2.24	-
4	Fosfomycin Trometamol	7.51	1689662.9	6.29		-
5	Impurity D	10.06	16475.3	1.54	-	-

Luna Omega 3 µm SUGAR Column, 150 x 4.6 mm



Peak No.	Analyte	Retention Time (min)	Area	Symmetry Factor	Resolution	H_p/H_v
1	Impurity B	2.20	155082.6	1.17	4.84	N/A
2	Impurity C	2.67	4154.3	1.29		
3	Impurity A	3.92	153497.9	1.09	1.67	-
4	Fosfomycin Trometamol	4.30	1693915	4.26		-
5	Impurity D	5.30	18765.9	1.98	-	-

ZORBAX® 5 µm NH_2 Column, 250 x 4.6 mm



Peak No.	Analyte	Retention Time (min)	Area	Symmetry Factor	Resolution	H_p/H_v
1	Impurity B	4.12	173158.6	1.33	-	1.9
2	Impurity C	4.74	7445.1	1.24	-	
3	Impurity A	7.58	107955.4	1.26	2.02	-
4	Fosfomycin Trometamol	8.54	1735789.3	5.81		-
5	Impurity D	10.08	44138.7	1.57	-	-

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