

Ph. Eur. Monograph 0391: Furosemide Related Substances according to on Fully Porous and Core-Shell HPLC Columns

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Overview

Furosemide belongs to the diuretic drugs and can be administered either orally or by injection. It is mainly used for the treatment of edema.

It belongs to the World Health Organization (WHO) List of Essential Medicines, and the World Anti-Doping Agency (WADA) has recently added it to the banned drug list, as there are concerns the drug might be used to mask the use of doping agents.

In this application note we are showing the effective separation of Furosemide from its impurities and related substances according to Ph. Eur. Monograph 0391. Both the fully porous Luna[®] 5 µm C8(2) and the Core-Shell Kinetex[®] 5 µm C8 columns met the system suitability requirements for the separation between Furosemide and Impurity A (≥ 4.0), the signal-to-noise ratio (≥ 40) for reference solution (b), and the relative retention of impurities C (~0.5), A (~0.8), and D (~1.5).

All reference solutions were prepared as indicated in Ph.Eur. monograph 0391 for Furosemide. 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):

- F0700000 Furosemide CRS
- F0702000 Furosemide impurity A CRS
- Y0001493 Furosemide for peak identification CRS

LC-UV Conditions

Columns: Luna 5 µm C8(2)

Kinetex 5 µm C8

Dimension: 250 x 4.6 mm

Part No.: [00G-4249-E0](#)

Part No.: [00G-4608-E0](#)

Mobile Phase: 2.0 g KH₂PO₄ * H₂O + 2.5 g cetrimide R in 700 mL water, pH adjusted to 7.0 with ammonia, 300 mL propanol

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV @ 238 nm

Injection: 20 µL

Table 1: Preparation of test and reference solutions

Solution	Composition
Test solution	Dissolve 50 mg of Furosemide CRS (or the substance to be examined) in the mobile phase and dilute to 50.0 mL with the mobile phase.
Reference solution (a)	Dissolve 2 mg of Furosemide impurity A CRS in the mobile phase, add 2.0 mL of the Test solution and dilute to 20.0 mL with the mobile phase. Dilute 0.5 mL of this solution to 20.0 mL with the mobile phase.
Reference solution (b)	Dilute 1.0 mL of the Test solution 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 2 mg of furosemide for peak identification CRS (containing impurities C and D) in 2.0 mL of the mobile phase.



Chromatographic Results

Figure 1a: Reference solution (a) on Luna® 5 µm C8(2)

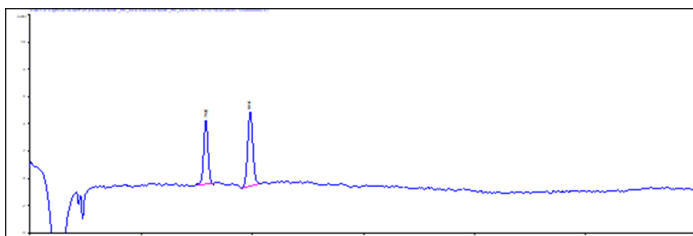


Figure 1b: Reference solution (a) on Kinetex® 5 µm C8

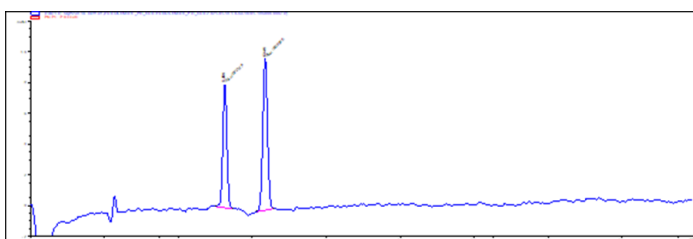


Table 2: Results reference solution (a)

	t_R Impurity A	t_R Furosemide	Resolution
Luna 5 µm C8(2)	7.94	9.94	5.2
Kinetex 5 µm C8	5.25	6.34	4.9

Figure 2a: Reference solution (b) on Luna 5 µm C8(2)

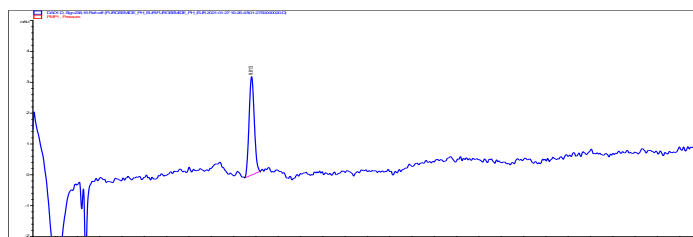


Figure 2b: Reference solution (b) on Kinetex 5 µm C8

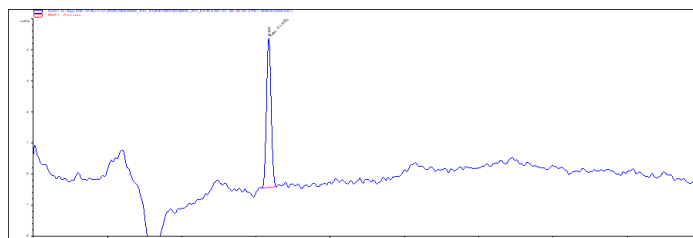


Table 3: Results reference solution (b)

	t_R Furosemide	Signal-to-Noise Ratio
Luna 5 µm C8(2)	9.78	48
Kinetex 5 µm C8	6.40	51

Figure 3a: Reference solution (c) on Luna 5 µm C8(2)

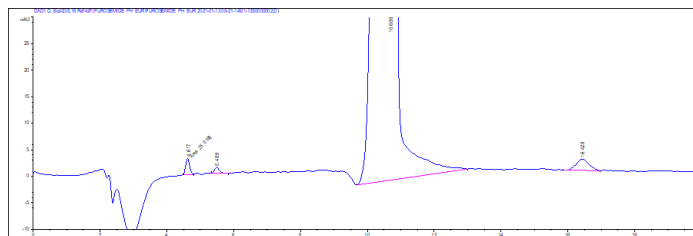


Figure 3b: Reference solution (c) on Kinetex 5 µm C8

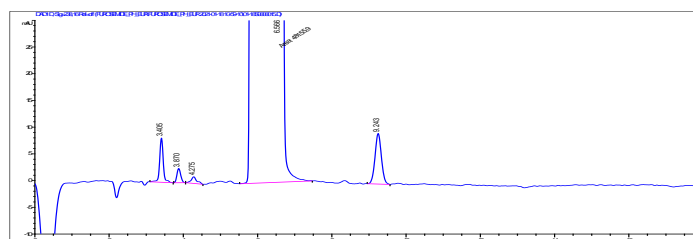


Table 4: Results reference solution (c)

	t_R Impurity C	t_R Furosemide	t_R Impurity D
Luna 5 µm C8(2)	4.61	10.63	16.32
Kinetex 5 µm C8	3.40	6.55	9.21

Order Information

Luna 5µm Analytical Columns (mm)

Phases 250 x 4.6

C8(2) 00G-4249-E0

Kinetex 5µm Analytical Columns (mm)

Phases 250 x 4.6

C8 00G-4608-E0



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