Lamivudine Related Substances Method per IP Monograph

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Overview

Lamivudine is an antiretroviral medication that belongs to a class of drugs known as nucleoside reverse transcriptase inhibitors (NRTIs) and plays a crucial role in combination therapy for managing the human immunodeficiency virus (HIV) infection. Additionally, Lamivudine is also utilized in the treatment of chronic hepatitis B virus (HBV) infection. The development of a quick and efficient analysis of Lamivudine and its related substances is of interest for India generic drug manufacturers. In this application note, we report the separation of Lamivudine and its related substances using a Luna Omega 5 µm C18 column and a Kinetex 5 µm C18 column according to the Indian Pharmacopoeia (IP) Monograph for Lamivudine, which references use of a 25 cm x 4.6 mm stainless-steel column packed with octadecylsilane bonded to porous silica (5 µm).

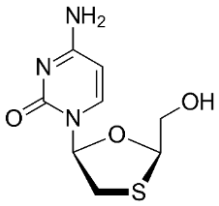
System suitability per IP Monograph for the Lamivudine Related Substances is resolution of not less than (NLT) 10.0 between Lamivudine and Salicylic Acid, theoretical plates NLT 5000, and a tailing factor not more than 1.5 for the peak due to Lamivudine.

The results clearly show that the system suitability criteria met and surpassed the minimum requirement. This illustrates that the Luna Omega 5 µm C18 and Kinetex 5 µm C18 columns meet all requirements for system suitability as outlined in the Indian Pharmacopoeia monograph for Lamivudine. The core-shell Kinetex 5 µm C18 column yielded a faster run time (>25 %) with comparable peak shape, while maintaining the resolution achieved with the fully porous Luna Omega C18 column.

All solutions were prepared as indicated in the IP Monograph for Lamivudine. Salicylic Acid was purchased from Sigma-Aldrich®. 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):

- Y0000425, Lamivudine CRS

Figure 1. Lamivudine



LC-UV Conditions

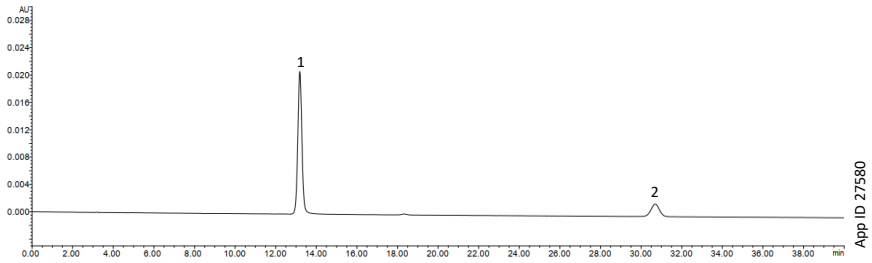
Column: Luna™ Omega 5 µm C18
Kinetex™ 5 µm C18
Dimensions: 250 x 4.6 mm
Part No.: 00G-4785-E0 (Luna Omega)
00G-4601-E0 (Kinetex)
Mobile Phase: Mobile Phase Table 1
Flow Rate: 1 mL/min (Isocratic)
Injection Volume: 10 µL
Temperature: 35 °C
Detector: UV @ 277 nm
System: Waters® ACQUITY Arc® HPLC

Table 1. Preparation of Solutions

Solution	Composition
Mobile Phase	Methanol / Buffer (5:95, v/v) Buffer: 0.19 % w/v of Ammonium Acetate adjusted to pH 3.8 with Glacial Acetic Acid.
Test Solution	Dissolve 50 mg of *Lamivudine CRS* in 70 mL of Mobile Phase and dilute to 100 mL with Mobile Phase.
Reference Solution (a)	Dilute 1 mL of the Test Solution to 100 mL with Mobile Phase. Dilute 1 mL of this solution to 10 mL with Mobile Phase.
Reference Solution (b)	Dissolve 5 mg of Salicylic Acid in Mobile Phase and dilute to 100 mL with Mobile Phase. Dilute 1 mL of this solution to 100 mL with Mobile Phase.
Reference Solution (c)	A solution containing 0.001 per cent w/v each of *Lamivudine CRS* and Salicylic Acid in Mobile Phase.

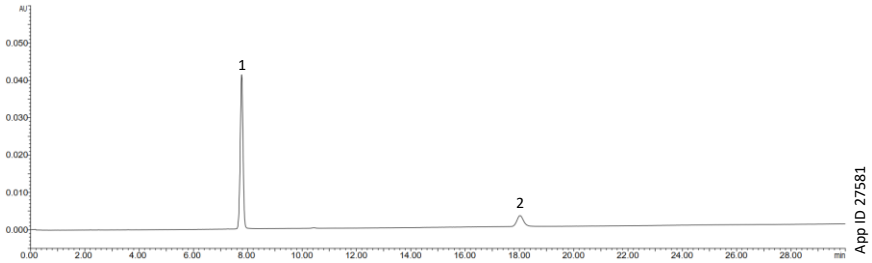
Figure 2. Reference Solution (c)

Luna™ Omega 5 µm C18 Column



Peak No.	Analyte	Retention Time (min)	Area	Area %RSD	Tailing Factor	Theoretical Plates	Resolution
1	Lamivudine	13.14	292683	0.51	1.05	20536	30.81
2	Salicylic Acid	30.55	52125	0.65	0.99	25621	
N = 6 Injections							

Kinetex™ 5 µm C18 Column



Peak No.	Analyte	Retention Time (min)	Area	Area %RSD	Tailing Factor	Theoretical Plates	Resolution
1	Lamivudine	7.81	302193	0.75	0.98	25787	31.83
2	Salicylic Acid	18.14	49676	0.72	1.10	25491	
N = 6 Injections							

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