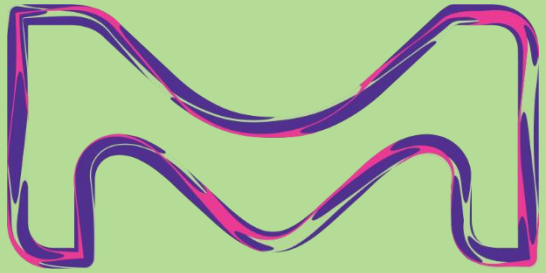
Lopinavir Assay Method

European Pharmacopeia (EP10.2) Monograph

March 2020



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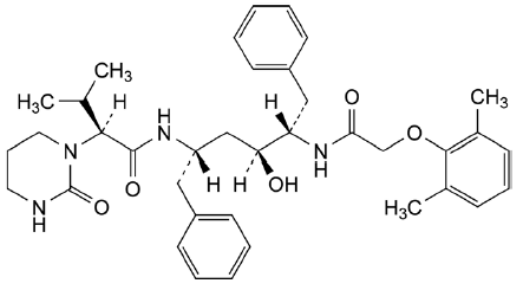
Lopinavir – Assay Method (EP)

Introduction

This paper illustrates how it is possible to set-up the assay method for Lopinavir testing following the current European pharmacopeia guidelines (10.2). The monograph assay method calls for a column with l = 0.25 m, Ø = 4.6 mm end-capped octadecylsilyl silica gel for chromatography with 4 µm particle size. No particular HPLC column is referenced in the EP knowledge database for assay method, and the method is of isocratic nature.

This gives a chance to replace the monograph column geometry/particle size with a shorter and faster alternative column (up to 70% reduction in length) packed with smaller particles (up to 50% reduction). This can save valuable time, and at the same time you can benefit from improved separation efficiency, which typically translates into better method performance and sensitivity. In this study, the limit of detection (LOD) is better than 1 ppm using HPLC-UV detection.



Lopinavir
((2S)-N-[(1S,3S,4S)-1-Benzyl-4-[[2-(2,6-dimethylphenoxy)-acetyl]amino]-3-hydroxyl-5-phenylpentyl]-3-methyl-2-[2-oxo-tetrahydropyrimidin-1(2H)-yl]butanamide

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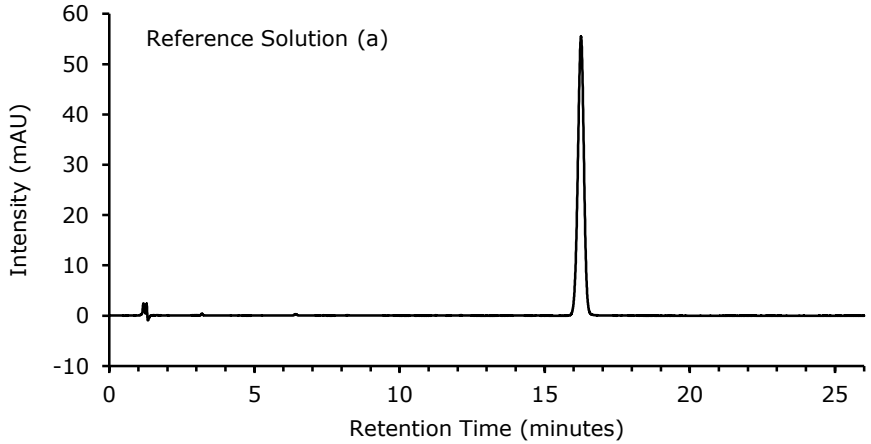
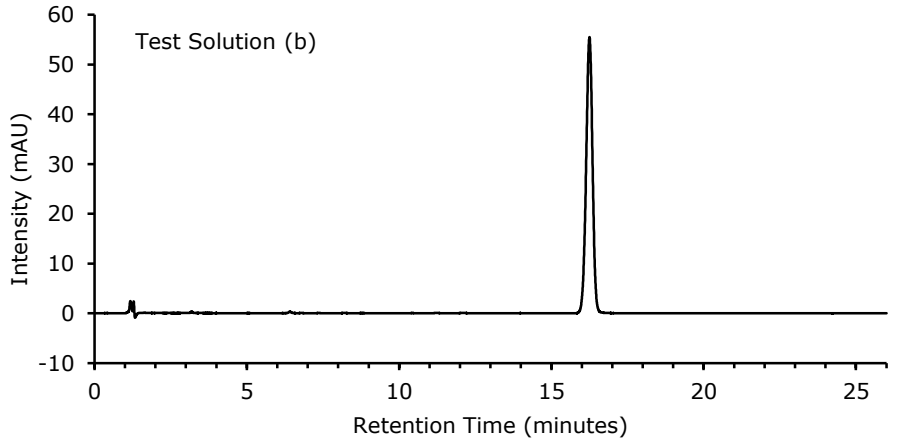
Experimental Conditions			
Column	Ascentis® Express C18 (2.7µm) 150x4.6 mm	Injection:	12 µL
Detection	UV = 215 nm (micro flow cell; 1.4 µL/7mm)	Flow Rate:	1.0 mL/min
Mobile phase A	Acetonitrile/phosphate buffer solution 45/55 (v/v)	Temperature:	50 °C
Phosphate buffer	Dissolve 0.9 g of dipotassium hydrogen phosphate and 2.7 g of potassium dihydrogen phosphate in 900 mL of water and mix well. Adjust to pH 6.0 with phosphoric acid, dilute to 1000 mL with water and filter.	Pressure Drop:	153 bar (2219 psi)
Solvent mixture	Acetonitrile/water 50/50 (v/v)		
Test solution (a)	Dissolve 50.0 mg of the substance to be examines in the solvent mixture and dilute to 100 mL with the solvent mixture.		
Test solution (b)	Dilute 5.0 mL of the test solution (a) to 100 mL with the solvent mixture.		
Reference solution (a)	Dissolve 50.0 mg of Lopinavir CRS in the solvent mixture and dilute to 100 mL with the solvent mixture. Dilute 5 mL of this solution to 100 mL with the solvent mixture.		





No.	Compound	Retention Time (min)	Tailing Factor
1	t_0 void volume	1.1	
2	Lopinavir CRS	16.2	0.97

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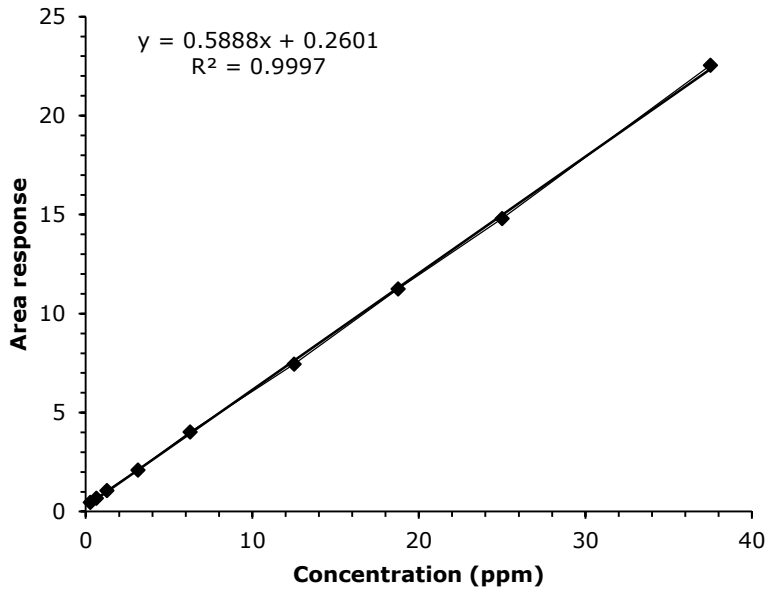
1. Specificity: Inject reference solution (a) and determine the retention time and the content of desired analyte				
		Retention Time (min)	Area (%)	Tailing Factor
1	Lopinavir CRS	16.3	96.3	0.95

2. Standard Repeatability (25 ppm)	
STD 1	14.79
STD 2	14.82
STD 3	14.86
STD 4	14.90
STD 5	14.93
Mean	14.86
Standard Deviation	0.06
(%) RSD	0.4

3. LOD & LOQ	
Conc. (ppm)	Mean Area (mAU*min)
0.25	0.46
0.63	0.67
1.25	1.06
3.13	2.10
6.25	4.02
12.50	7.44
18.75	11.24
25.00	14.86
37.50	22.54
STEYEX	0.1348
Slope	0.5888
LOD (ppm)	0.8
LOQ (ppm)	2.3



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Product list	PN
Ascentis® Express C18 (2.7µm) 150x4.6 mm	53829-U
Acetonitrile gradient grade for liquid chromatography LiChrosolv®	1.00030
Water for chromatography (LC-MS grade) LiChrosolv®	1.15333
or tap fresh from an appropriate Milli-Q® water purification systems	
Millex® syringe filter units, disposable, Durapore® PVDF, pore size 0.45 µm, non-sterile	SLHVX13NK
Ortho-phosphoric acid EMSURE®	1.00573
Potassium di-hydrogen phosphate EMSURE®	1.04873
Di-Potassium hydrogen phosphate LiChropur®	1.05104
Lopinavir CRS	Y0001498
Lopinavir for system suitability CRS	Y0001505
Lopinavir for peak identification CRS	Y0001506