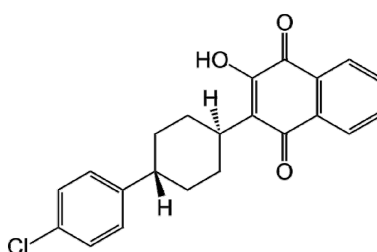


Atovaquone Oral Suspension (USP)



Atovaquone belongs to the class of naphthoquinones; a hydroxy-1,4-naphthoquinone, an analog of ubiquinone, with antipneumocystic activity. It is manufactured in the US in the liquid form, or oral suspension, under the brand name Mepron.

The current USP monograph for Atovaquone oral suspension specifies the use of a L1 (RP-18) column with 125x4.6 mm geometry (no particle size mentioned), used at a flow rate of 3.0 mL/min.

At this flow rate, with normal particulate columns (5 μ m), the back pressure over the HPLC system will be very high on a fresh column, and only after some analyses it goes beyond the sustainable backpressure limit and causes failure in analysis.

Of these reasons we have transferred the current method to a 100x4.6 mm id monolithic column, i.e. a Chromolith® HighResolution RP-18 endcapped column, where the back pressure is only around 100 bar (1440 psi) under given experimental conditions. A pressure that any HPLC system easily can accommodate, and thereby provide robust and reliable results over time.

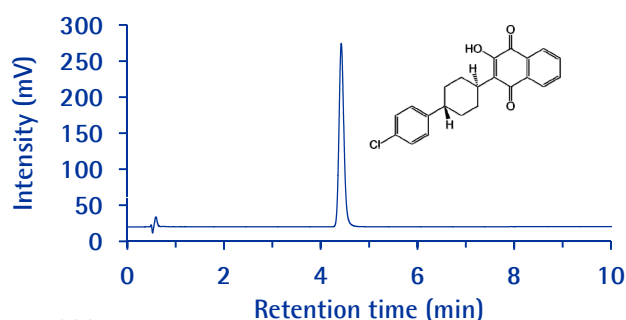
The method meet all acceptance criteria with good reproducibility for the specified sample solution per current monograph, and will not require a complete re-validation for acceptance.

Atovaquone Oral Suspension (USP)

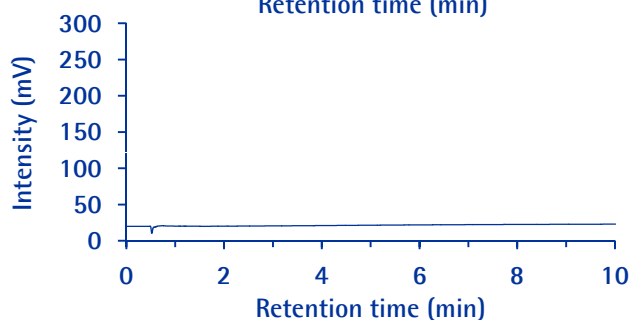
Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped, 100x4.6 mm	1.52022.0001
Injection:	20 µL	
Detection:	UV 220 nm	
Cell:	10 µL	
Flow Rate:	3.0 mL/min	
Mobile Phase:	Acetonitrile, methanol, water, and phosphoric acid; 480:160:360:5 (v/v/v/v)	
Temperature:	30°C	
Diluent:	0.1 M methanolic sodium hydroxide	
SST Solution:	Nominally 3 mg/mL from a known volume of well-mixed oral suspension	
Standard Solution:	Not less than (NLT) 750 mg of atovaquone prepared as following procedure: In an appropriately sized, low-actinic volumetric flask, add 20% volume of water, swirl for 5 min, add 60% volume of 0.1 M methanolic sodium hydroxide, and sonicate for 15 min. Allow to cool, and dilute with 0.1 M methanolic sodium hydroxide to volume. Immediately filter a 20-ml portion, discarding the first 5 mL of the filtrate.	
Sample solution:	0.09 mg/mL of atovaquone from the clear filtrate of the Sample stock solution. Transfer to an appropriately sized, low-actinic volumetric flask, and dilute with a mixture of methanol and water (1:1) to volume. Minimize exposure of this solution to light.	
Pressure Drop:	104 Bar (1510 psi)	



Sample solution



Blank

Suitability
requirement:
NMT 1.5

Chromatographic Data :

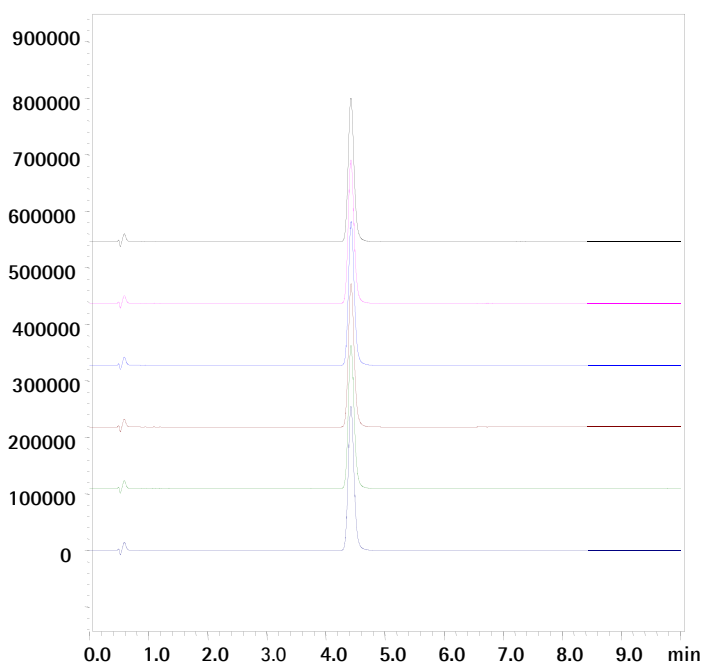
No.	Compound	Retention Time (min)	Tailing factor	Theoretical Plate
1	Atovaquone	4.4	1.2	9466

Atovaquone Oral Suspension (USP)

Chromolith® HighResolution RP-18 endcapped

Repeatability Data

The relative standard deviation (RSD) for six repeated analyses of the sample solution (0.09 mg/mL of atovaquone) were excellent with far better precision than the specified suitability requirement (NMT 2.0%).



Injection Number	Retention time	Area
1	4.42	1786281
2	4.43	1784126
3	4.43	1784598
4	4.42	1786092
5	4.42	1785619
6	4.43	1788002
Average	4.43	1785786
Standard Deviation	0.00	1374
RSD (%)	0.04	0.08

Recommended chemicals and reagents to reproduce this alternative method

Product	Name of the Chemical	Article Number
1	ortho-Phosphoric acid 85% for analysis EMSURE® ACS, ISO, Reag. Ph Eur	1.00573
2	Sodium hydroxide pellets for analysis (max. 0.02% K) EMSURE® ACS, Reag. Ph Eur	1.06469
3	Acetonitrile gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur	1.00030
4	Methanol gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur	1.06007