Ramipril and Related Substances

From HPLC to UHPLC

The benefit of scaling from HPLC to UHPLC is illustrated with the USP36 –NF31 monograph method for ramipril related compounds, where the liquid chromatograph should be equipped with 210 nm detector and a 250x4.0 mm column that contains 3 µm packing L1 (RP-18) and is maintained at a temperature of 65°C.

Within the scope of allowed monograph method changes, and only to perform partial revalidation, this method can be changed by:

- Reduction of particle size to maximum 1.5 µm (50%)
- Shortening the column to a length of 75 mm (70%)
- Reduction of inner diameter if linear velocity is kept constant
- Reduction of injection volume as long as limit of detection (LOD) and linearity is OK.

Using the same mobile phases and gradient program as per monograph, this method was first finalized on a 250x4.6 mm Purospher® STAR RP-18 endcapped column with 5 µm packing, page 20, and thereafter scaled to a 100x2.1 mm Purospher® STAR RP-18 endcapped column with 2µm packing, see page 21. The UHPLC application is an allowed monograph modification per USP guidelines but the application using the larger HPLC column is not allowed. It is possible to reduce particle size, by maximum 50 %, but no increase.

Performance criteria:
Chromatograph the Resolution solution, and record the peak responses as directed for Procedure: the **resolution, R, between ramipril related compound A and ramipril is not less than 3.0.** Similarly chromatograph the Test solution, and record the peak responses as directed for procedure: **the retention time for ramipril is between 16 and 19 minutes; and the tailing factor for the ramipril peak is between 0.8 and 2.0.** Chromatograph the Standard solution, and record the peak responses as directed for Procedure: the relative standard deviation for replicate injections is not more than 5.0%. [**The relative retention times are about 0.8 for ramipril related compound A, 1.0 for ramipril, 1.3 for ramipril related compound B,** 1.5 for ramipril related compound C, and 1.6 for ramipril related compound D.]

NOTE: no Ramipril related compound C and D were available at time of developing this application.
Thus reason why it is not marked as an USP method, despite it follow the monograph experimental conditions.

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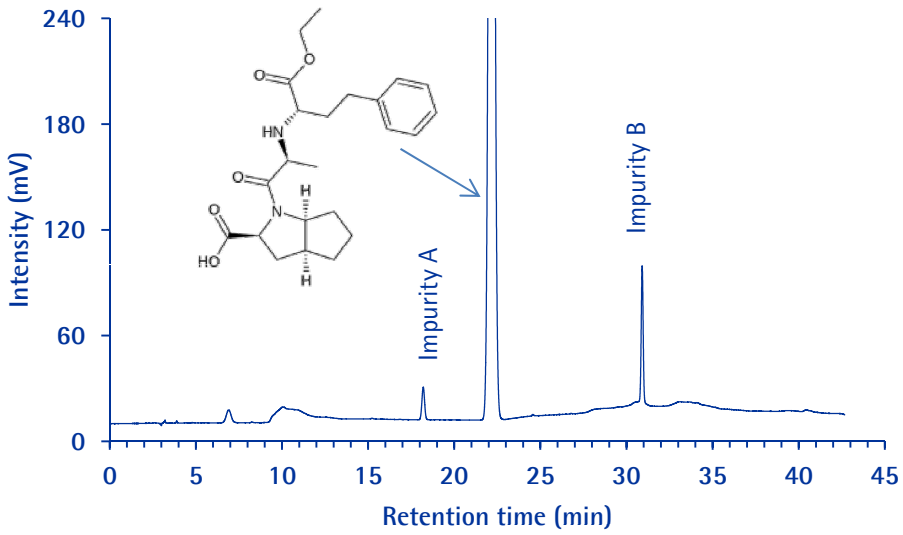
Purospher® STAR RP-18 endcapped (HPLC)

Chromatographic Conditions

Column: Purospher® STAR RP-18 endcapped (5µm) Hibar® RT 250x4.6 mm 1.51456.0001
Injection: 10 µL
Detection: UV 210 nm
Cell: 10 µL
Flow Rate: 1.0 mL/min
Mobile Phase: A: Dissolve 2.0 g of sodium perchlorate in a mixture of 800 mL of Milli-Q water and 0.5 ml of triethylamine. Adjust pH to 3.6 with phosphoric acid. Add 200 mL and acetonitrile and mix.
B: Dissolve 2.0 g of sodium perchlorate in a mixture of 300 mL of Milli-Q water and 0.5 ml of triethylamine. Adjust pH to 2.6 with phosphoric acid. Add 700 mL acetonitrile and mix.
Gradient: See table
Temperature: 65 °C
Diluent Solution A
Sample: Dissolve 25 mg of sample in diluent and dilute to 25 ml with same solvent.
Pressure Drop: 61 to 74 Bar (884 to 1073 psi)



Time (min)	% A	% B
0.0	90	10
6.0	90	10
7.0	75	25
20.0	65	35
30.0	25	75
40.0	25	75
45.0	90	10
55.0	90	10

Chromatographic Data

No.	Compound	Retention Time (min)	RRT	Asymmetry
1	Ramipril RS A	18.2	0.82	1.0
2	Ramipril	22.2	1.00	1.0
3	Ramipril RS B	30.9	1.39	1.0

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
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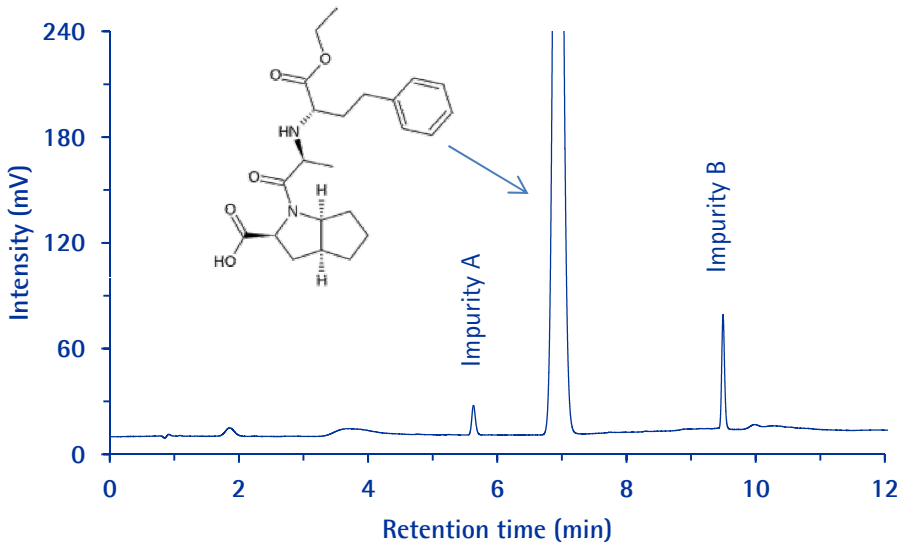
Ramipril and Related Substances

Purospher® STAR RP-18 endcapped (UHPLC)

Chromatographic Conditions

Column:	Purospher® STAR RP-18 endcapped (2µm) Hibar® HR 100x2.1 mm	1.50648.0001
Injection:	2 µL	
Detection:	UV 210 nm	
Cell:	2.5 µL (Use 0.1 mm tubing)	
Flow Rate:	0.3 mL/min	
Mobile Phase:	A: Dissolve 2.0 g of sodium perchlorate in a mixture of 800 mL of Milli-Q water and 0.5 ml of triethyl-amine. Adjust pH to 3.6 with phosphoric acid. Add 200 mL and acetonitrile and mix. B: Dissolve 2.0 g of sodium perchlorate in a mixture of 300 mL of Milli-Q water and 0.5 ml of triethyl-amine. Adjust pH to 2.6 with phosphoric acid. Add 700 mL acetonitrile and mix	
Gradient:	See table	
Temperature:	65 °C	
Diluent	Solution A	
Sample:	Dissolve 25 mg of sample in diluent and dilute to 25 ml with same solvent.	
Pressure Drop:	196 to 164 Bar (2827 to 2378 psi)	



Time (min)	% A	% B
0.0	90	10
1.66	90	10
1.93	75	25
5.54	65	35
8.31	25	75
11.08	25	75
12.46	90	10
15.23	90	10

'does not need full revalidation'

Chromatographic Data

No.	Compound	Retention Time (min)	RRT	Asymmetry
1	Ramipril RS A	5.6	0.81	1.1
2	Ramipril	6.9	1.00	1.1
3	Ramipril RS B	9.5	1.38	1.1

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As can be seen on page 20 and 21, both columns meet the performance criteria in terms of:

a) The resolution, R, between ramipril related compound A and ramipril (not less than 3.0)
b) The relative retention time between ramipril related compound A (ramipril RS A), ramipril and ramipril related compound B (ramipril RS B)
c) The tailing factor for the ramipril peak (between 0.8 and 2.0).

d) The application using HPLC conditions also meet the retention time requirement for ramipril

The UHPLC column - Purospher® STAR RP-18 endcapped (2µm) 100x2.1 mm thus seem to meet monograph and the customer would benefit from:

1. **Faster method** (Time-saving: 40 minutes per sample or 360%)
   (yes...the column length is 60% shorter and this provide 60% time saving but the real gain is to scale the method to a column with smaller particle size and not having to keep same linear velocity).

2. **Higher chromatographic resolution and efficiency**

....but this is not true. The retention time requirement for ramipril is NOT between 16 and 19 minutes. In addition, the flow rate has not been scaled to maintain same linear velocity. Monograph method is documented at 1.0 mL/min on 4.6 mm column and thus the flow rate should be reduced by a factor or 4.8 for the 2.1 mm i.d. UHPLC column, calculations see page 18. A flow rate of 0.2 mL/min should have been used instead of 0.3 mL/min. With the current experimental conditions, this would give comments from an auditor and very likely a request for method change.

The larger Purospher® STAR RP-18 endcapped (5µm) 250x4.6 mm column can definitely not be used. The particle size is larger than monograph method and would require complete revalidation and discussion with auditor and authorities. Most likely it would not be an accepted method.

More information about Merck Millipore UHPLC columns and how to appropriately scale methods can be found at www.merckmillipore.com/chromatography; in Chrombook and the 2013 application guide – UHPLC[2].