Ivermectin Injection Solution

From Particulate to Monolithic Column

As per the USP36 –NF31 monograph method for Ivermectin solution, the liquid chromatograph should be equipped with 245 nm detector and a 250x4.6 mm column that contains 5 µm packing L1 (RP-18). The Performance criteria to be met are:

- A relative retention time of about 1.3 to 1.5 to that of the principal peak is found
- The resolution between the first peak (H2B1b) and the second peak (H2B1a) is not less than 3.0

Within the scope of allowed monograph method changes, see page 8, and only to perform partial revalidation, the method can be changed by:

- Reduction of particle size to maximum 2.5 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.

Two different columns for the Ivermectin solution monograph method were used.
a) Purospher® STAR RP-18 endcapped (5µm) Hibar® 250x4.6 mm
b)Chromolith® HighResolution RP-18 endcapped 100x4.6 mm

The column alternative a) has the exact specification of the monograph method procedure and would only require a partial revalidation to prove that LOD, linearity, and performance criteria are met. Alternative b) the Chromolith® HighResolution RP-18 endcapped 100x4.6 mm column is a monolithic column (having no particle size), and thus would require a complete method revalidation and discussion/submission to auditor for acceptance.

Three reasons why we recommend changing to column alternative b) despite complete revalidation is required:

1. **The method will run three times faster** (Time-saving: 29 minutes per sample)
   (yes...the column length is 60% shorter, thus one reason for the method run-time reduction, but the shorter diffusion distances in a monolithic column gives advantages of particles).
2. **Higher chromatographic resolution between the two target molecules**
   (Chromolith® HighResolution provide performance corresponding to sub-3 µm particle packed columns)
3. **The method will run at 50% lower column backpressure**
   (No need to change instrument and still have high efficiency separation, and with low backpressure you also, as an added value get instrument safety at no extra cost. Less maintenance, less wear on pumps etc)

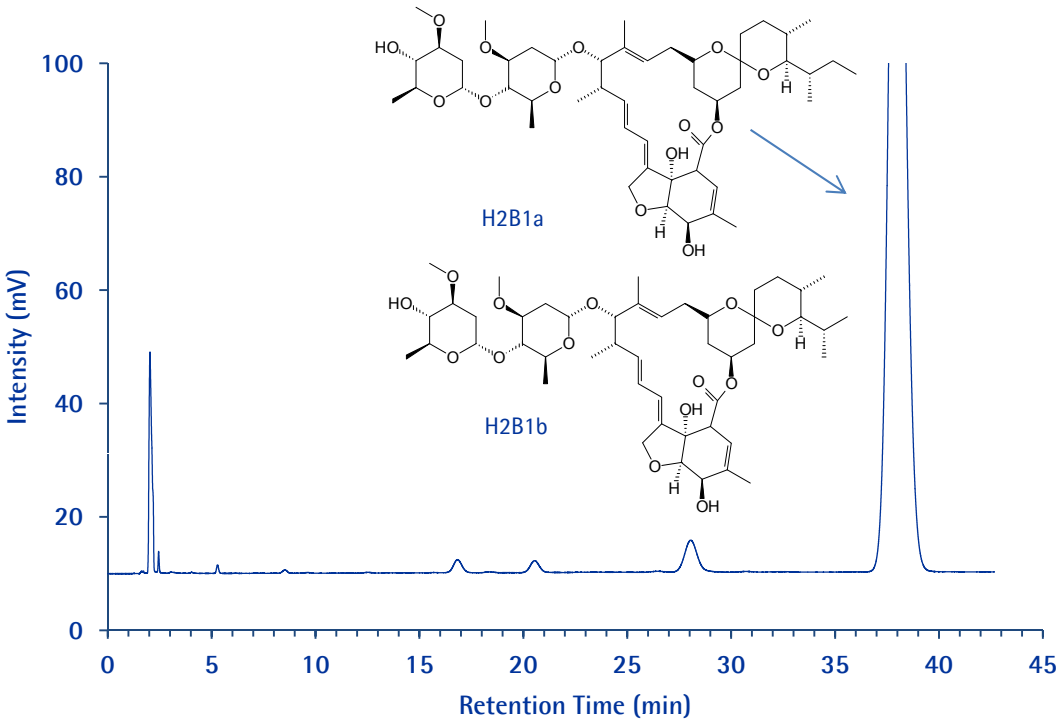
Ivermectin Injection Solution (USP)

Purospher® STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher® STAR RP-18 endcapped (5µm) Hibar® RT 250x4.6 mm	1.51456.0001
Injection:	20 µL	
Detection:	UV 245 nm	
Cell:	10 µL	
Flow Rate:	1.5 mL/min	
Mobile Phase :	Mixture of acetonitrile, methanol and water; 106:55:39 (v/v)	
Temperature:	Ambient	
Diluent:	Methanol	
Sample:	0.4 mg/mL (400 ppm) of each component in diluent	
Pressure Drop:	118 Bar (1711 psi)	

'does not need full revalidation'



Chromatographic Data

No.	Compound	Time (min)	Relative Retention Time (RRT)	T_{USP}	Resolution
1	H2B1b	28.0	1.0	1.1	–
2	H2B1a	38.0	1.36	1.1	7.9

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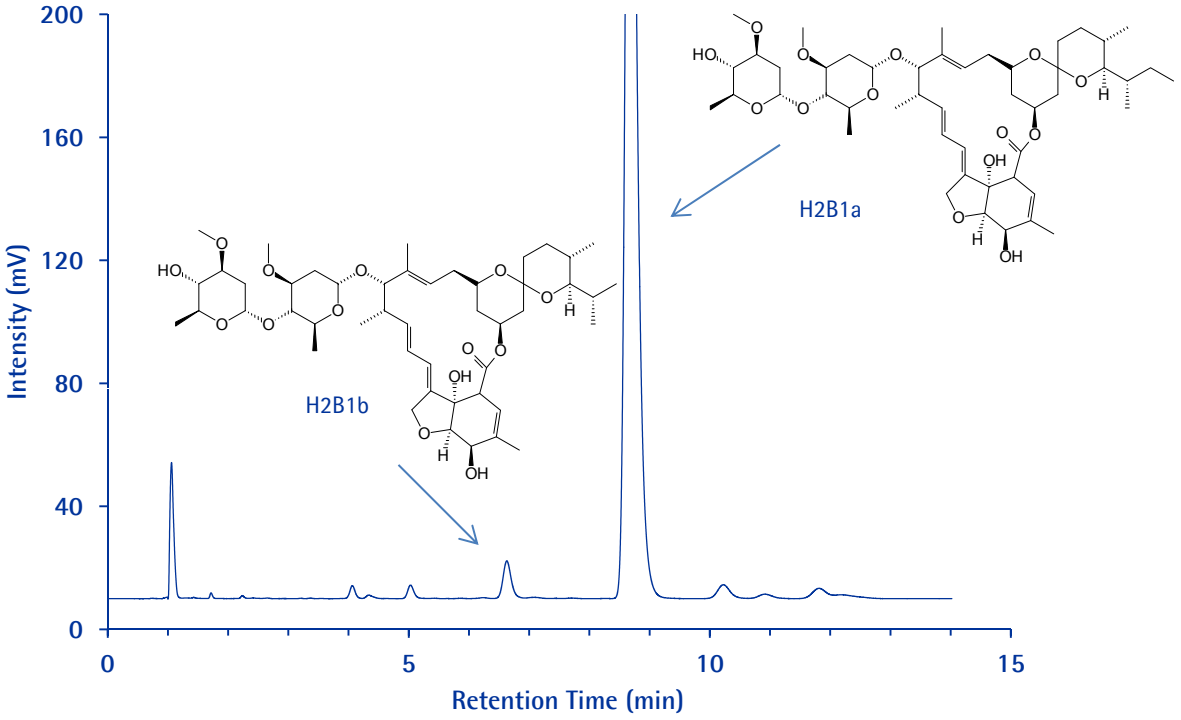
Ivermectin Injection Solution

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped, 100x4.6 mm	1.52022.0001
Injection:	10 µL	
Detection:	UV 245 nm	
Cell:	10 µL	
Flow Rate:	1.5 mL/min	
Mobile Phase:	Mixture of acetonitrile, methanol and water; 106:55:39 (v/v)	
Temperature:	Ambient	
Diluent:	Methanol	
Sample:	0.4 mg/mL (400 ppm) of each component in diluent	
Pressure Drop:	50 Bar (725 psi)	

"needs full validation'



Chromatographic Data

No.	Compound	Time (min)	Relative Retention Time (RRT)	T_{USP}	Resolution
1	H2B1b	6.6	1.0	1.2	–
2	H2B1a	8.7	1.3	1.4	7.9

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