Ivermectin and Related Substances: Ph. Eur. Monograph 1336

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Introduction

Ivermectin is an active substance used for the oral treatment of parasitic diseases and is a member of the avermectin group. The action of ivermectin is to bind to chloride channels, which leads to paralysis and death of the parasites. Ivermectin is a mixture of the two ivermectin components H2B1a and H2B1b. The two molecules differ structurally only by a methylene group.

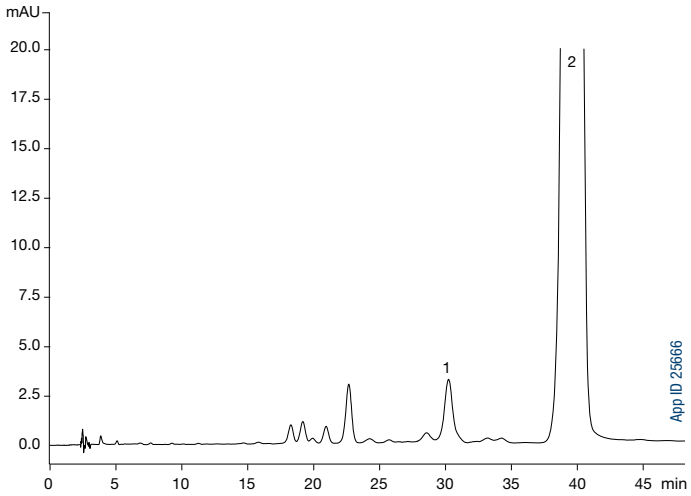
The related substances test of the European Pharmacopoeia (Ph. Eur.) Monograph 1336 outlines the separation of components H2B1a and H2B1b. This method was studied, and improvements were made to provide a faster separation time whilst maintaining the resolution (RS) required in the system suitability test, at the same time remaining within the allowable adjustments laid out by the European Pharmacopoeia 10.

Ph. Eur. Monograph 1336 Details	
Test Solution:	Dissolve 40.0 mg of the substance to be examined in methanol R and dilute to 50.0 mL with the same solvent.
Reference Solution (a):	Dissolve 40.0 mg of Ivermectin CRS* in methanol R and dilute with 50.0 mL with the same solvent.
Column:	
Size:	L = 250 mm, ID = 4.6 mm
Stationary Phase:	Octadecylsilyl silica gel for chromatography R (5 µm)
Mobile Phase:	Water R, methanol R, acetonitrile R (15:34:51 v/v/v)
Flow Rate:	1 mL/min
Detection:	Spectrophotometer @ 254 nm
Injection:	20 µL
Temperature:	25 ° C
Elution Order:	1. Component H2B1b 2. Component H2B1a
System Suitability:	Reference Solution (a): Resolution: Minimum 3.0 between the 1st peak (Component H2B1b) and 2nd peak (Component H2B1a) Symmetry factor: maximum 2.5 for the principle peak in the chromatogram

* Ivermectin CRS (I8000010), batch 2.0 was purchased from European Directorate for the Quality of Medicines & HealthCare (EDQM) – Council of Europe; Postal address: 7 Allee Kastner CS 30026 F - 67081 STRASBOURG (France).

Original Method as Described in the Monograph

Reference solution (a)



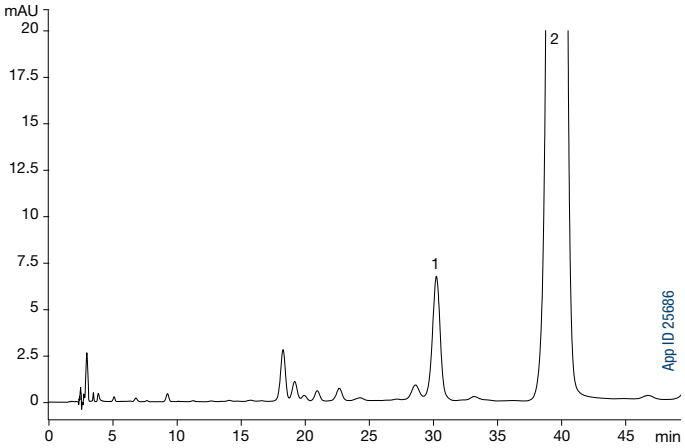
Peak	Name	Retention time, min	Symmetry	Resolution
1	Component H2B1b	30.28		
2	Component H2B1a	39.54	1.1	7.4

HPLC Conditions

Column:	Luna® 5 µm C18(2)
Dimension:	250 x 4.6 mm
Part No.:	00G-4252-E0
HPLC System:	Shimadzu® Nexera® XR
Mobile Phase:	Water, methanol, acetonitrile (15:34:51 v/v/v)
Flow Rate:	1.0 mL/min
Injection Volume:	20 µL
Temperature:	25 ° C
Detection:	UV @ 254 nm

Original Method as Described in the Monograph

Test solution



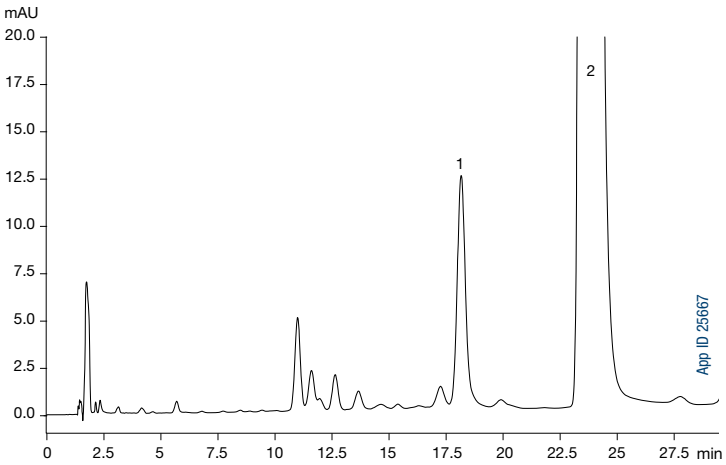
Peak	Name	Retention time, min	Symmetry	Resolution
1	Component H2B1b	30.28		
2	Component H2B1a	39.57	1.1	7.5

HPLC Conditions

Column:	Luna® 5 µm C18(2)
Dimension:	250 x 4.6 mm
Part No.:	00G-4252-E0
HPLC System:	Shimadzu® Nexera® XR
Mobile Phase:	Water, methanol, acetonitrile (15:34:51 v/v/v)
Flow Rate:	1.0 mL/min
Injection Volume:	20 µL
Temperature:	25 ° C
Detection:	UV @ 254 nm

Faster Method Within Allowable Adjustments

Test solution



Peak	Name	Retention time, min	Symmetry	Resolution
1	Component H2B1b	18.19		
2	Component H2B1a	23.67	1.5	7.1

HPLC Conditions

Column:	Luna® 3 µm C18(2)
Dimension:	150 x 4.6 mm
Part No.:	00F-4251-E0
HPLC System:	Shimadzu® Nexera® XR
Mobile Phase:	Water, methanol, acetonitrile (15:34:51 v/v/v)
Flow Rate:	1.0 mL/min
Injection Volume:	20 µL
Temperature:	25 ° C
Detection:	UV @ 254 nm

Adjustments for Meeting System Suitability
(European Pharmacopeia 9.0, Chapter 2.2.46. Chromatographic Separation Techniques)

Method Parameter	Allowed Adjustments (isocratic elution)	Method 1	Method 2
Mobile Phase pH	± 0.2 units	Not specified	No change
Concentration of Salts in Buffer	± 10 %	Not specified	No change
Composition of the Mobile Phase	± 30 % of the minor solvent component relative or 2 % absolute, whichever is the larger. No other component is altered by more than 10 % absolute.	As specified in Monograph 1336 Details Table	No change
Wavelength of Detector	no deviations permitted	254 nm (as specified)	No change
Injection Volume	May be decreased, provided detection and repeatability of the peak(s) to be determined are satisfactory.	20 µL (as specified)	
Column Temperature	± 10 °C	Ambient (as specified)	No change
Stationary Phase	No change of the identity of the substituent permitted (e.g. no replacement of C18 by C8)	octadecylsilyl silica gel for chromatography (as specified)	No change
Column Length	± 70 %	250 mm (as specified)	150 mm (- 40 %)
Column Internal Diameter	± 25 %	4.6 mm	No change
Particle Size	- 50 %	5 µm (as specified)	3 µm (- 40 %)
Flow Rate	± 50 %	1.0 mL/min (as specified)	No change

Ordering Information

3 µm MidBore™ and Analytical Columns (mm)									SecurityGuard™ Cartridges (mm)	
Phases	**30 x 3.0**	**50 x 3.0**	**150 x 3.0**	**30 x 4.6**	**50 x 4.6**	**75 x 4.6**	**100 x 4.6**	**150 x 4.6**	**4 x 2.0***	**4 x 3.0***
									/10pk	/10pk
C18(2)	00A-4251-Y0	00B-4251-Y0	00F-4251-Y0	00A-4251-E0	00B-4251-E0	00C-4251-E0	00D-4251-E0	00F-4251-E0	AJ0-4286	AJ0-4287
									for ID: 2.0-3.0 mm	3.2-8.0 mm

5 µm Analytical and Semi-Prep Columns (mm)					SecurityGuard™ Cartridges (mm)	
Phases	**100 x 4.6**	**150 x 4.6**	**250 x 4.6**	**250 x 10**	**4 x 3.0***	**10 x 10‡**
					/10pk	/3pk
C18(2)	00D-4252-E0	00F-4252-E0	00G-4252-E0	00G-4252-N0	AJ0-4287	AJ0-7221
					for ID: 3.2-8.0 mm	9-16 mm

*SecurityGuard™ Analytical Cartridges require holder, Part No.: KJ0-4282
‡SemiPrep SecurityGuard™ Cartridges require holder, Part No.: AJ0-9281

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