Biotin and Related Substances: Ph. Eur. Monograph 1073

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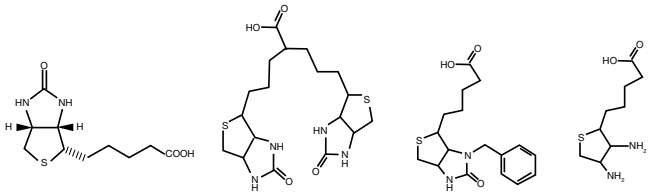


Biotin, also called vitamin B7 and formerly known as vitamin H or coenzyme R, is a water-soluble B vitamin. It is involved in a wide range of metabolic processes, both in humans and in other organisms, primarily related to the utilization of fats, carbohydrates, and amino acids

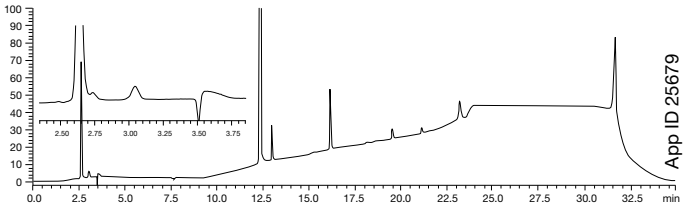
The related substances test of the European Pharmacopoeia (Ph. Eur.) Monograph 1073 outlines the separation of all relevant impurities from Biotin. This method was reviewed, and improvements were made to provide higher resolution (Rs) between the critical pairs within the adjustments allowable under the European Pharmacopoeia 9.0.

Ph. Eur. Monograph 1073 Details	
Solvent Mixture	Water R, acetonitrile R (50:50 V/V)
Reference Solution (b)	Dissolve 5.0 mg of biotin for system suitability CRS* (containing impurities A, C and E) in the solvent mixture and dilute to 5.0 mL with the solvent mixture.
Column	
Size	L = 0.25 m, ID = 4.6 mm
Stationary Phase	End-capped octadecylsilyl silica gel for chromatography R (5 µm)
Temperature	30° C
Mobile Phase	A: Methanesulfonic acid R, acetonitrile R1, water for chromatography R (1:25:1000 v/v/v)
	B: Methanesulfonic acid R, water for chromatography R, acetonitrile R1 (1:25:1000 v/v/v)
Gradient	B (%)
0 – 5 min	5
5 – 20 min	5 to 100
20 – 28 min	100
Flow Rate	1 mL/min
Detection	Spectrophotometer @ 200 nm from 0 to 5 min and @ 210 nm from 5 to 28 min
Injection	10 µL
Relative Retention with Reference to Biotin (about 12 min)	
Impurity C	about 0.2
Impurity A	about 1.1
Impurity E	about 1.3
System Suitability	Reference Solution (b):Resolution: Minimum 1.5 between peaks due to biotin and impurity A.

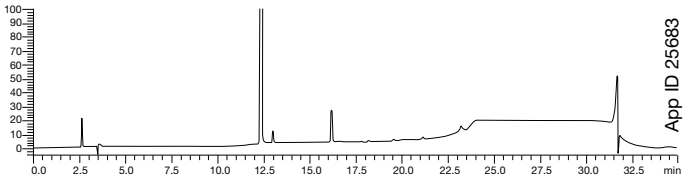
* Biotin for system suitability CRS (Y0001978), batch 1.0 was purchased from European Directorate for the Quality of Medicines & HealthCare (EDQM) – Council of Europe; Postal address: 7 Allee Kastner CS 30026 F - 67081 STRASBOURG (France).

Original Method as Described in the Monograph with Improved Resolution

Reference solution (b) at 200 nm UV



Reference solution (b) at 210 nm UV



Column: Luna® 5 µm C18(2)
Dimensions: 250 x 4.6 mm
Part No.: 00G-4252-E0
Mobile Phase: A: Methanesulfonic acid, acetonitrile, water (1:25:1000 v/v/v)
B: Methanesulfonic acid, water, acetonitrile (1:25:1000 v/v/v)

Gradient:

Time (min)	% B
0	5
5	5
20	100
28	100

Flow Rate: 1.0 mL/min
Injection Volume: 10 µL
Temperature: 30 °C
Detection: UV @ 200 nm from 0 to 5 min
UV @ 210 nm from 5 to 28 min
Sample: 1. Bromide
2. Impurity C
3. Biotin
4. Impurity A
5. Impurity E

Peak	Name	Retention time, min	Relative retention	Resolution
1	Bromide	2.638		
2	Impurity C	3.048	0.25	
3	Biotin	12.354	---	5.2
4	Impurity A	13.007	1.05	7.1
5	Impurity E	16.180	1.31	

APPLICATIONS

Adjustments for Meeting System Suitability (European Pharmacopeia 9.0, Chapter 2.2.46. Chromatographic separation techniques)

Method Parameter	Allowed Adjustments (gradient elution)	Method 1	Method 2
Mobile Phase pH	No adjustment permitted	Not specified	No change
Concentration of Salts in Buffer	No adjustment permitted	Not specified	No change
Composition of the Mobile Phase	Minor adjustments of the composition of the mobile phase and the gradient are acceptable, if the system suitability requirements are met, the principle peak(s) elute(s) within ± 15 % of the indicated retention time(s) and the final elution power of the mobile phase is not weaker in elution power than the prescribed composition	As specified in Monograph 1073 Details Table	No change
Wavelength of Detector	No deviations permitted	200 nm from 0 to 5 min and 210 nm from 5 to 28 min (as specified)	No change
Injection Volume	May be decreased, provided detection and repeatability of the peak(s) to be determined are satisfactory.	10 µL (as specified)	No change
Column Temperature	± 5 °C	30 °C (as specified)	No change
Stationary Phase	No change of the identity of the substituent permitted	End-capped octadecylsilyl silica gel for chromatography (as specified)	No change
Column Length	May be adjusted ± 70 %	250 mm (as specified)	No change
Column Internal Diameter	May be adjusted ± 25%	4.6 mm (as specified)	No change
Particle Size	No adjustment permitted	5 µm (as specified)	No change
Flow Rate	Acceptable when changing the column dimensions	1 mL/min (as specified)	No change

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