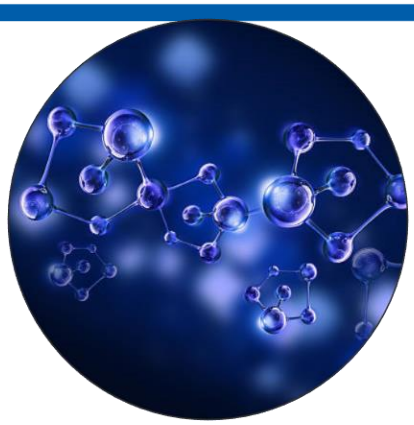

Ph. Eur. Monograph 1028: Metoprolol Tartrate and Related Substances with Ph. Eur. Method Modernization

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Overview

Metoprolol Tartrate is an immediate-release cardio selective β-1-adrenergic receptor inhibitor used to treat angina and hypertension. The European Pharmacopoeia (Ph. Eur.) monograph method for the LC-UV determination of related compounds for Metoprolol Tartrate is based on an end-capped octadecylsilyl silica gel column run under isocratic mobile phase conditions. This application note demonstrates the potential method improvements that can be achieved within the allowed adjustments of chromatographic conditions. A Luna™ C18(2) and a Kinetex ™C18 column were used for evaluating the related substances method, within the new allowable adjustments published by European Pharmacopoeia, Chapter 2.2.46.

System suitability per Ph. Eur. Monograph 1028 for Metoprolol Tartrate Related Substances is a minimum resolution of 6.0 between the peaks due to Impurity A and Metoprolol Tartrate. The results in the two methods show that the system suitability criteria were met. The use of a Luna 5 µm C18(2), 150 x 4.6 mm or a Kinetex 2.6 µm, 100 x 4.6 mm column constitute an allowed adjustment to the original column dimensions with the flow rate scaled accordingly to accommodate the adjustments to column length (L), internal diameter (ID), and particle size (dp). With the Kinetex 2.6 µm C18, 100 x 4.6 mm column, we demonstrated a reduction in total analysis time over the Luna 5 µm C18(2), 150 x 4.6 mm column. The monograph requires a run time equal to 3 times the retention of Metoprolol Tartrate, for Luna 5 µm C18(2) 150 x 4.6 mm this equates to 39 minutes, while for the Kinetex 2.6 µm C18 100 x 4.6 mm it is 9.9 minutes. Utilizing the Kinetex column, a time saving of 29.1 minutes per run can be realized.

All the reference solutions were prepared as indicated in Ph. Eur. monograph 1028 for Metoprolol Tartrate. The following certified reference standards (CRS) were purchased from the European Directorate for the Quality of Medicines & HealthCare (EDQM) – Council of Europe; Postal address: 7 Allee Kastner CS 30026 F - 67081 Strasbourg (France):

- M1830000, Metoprolol Tartrate CRS
- Y0000145, Metoprolol Impurity A CRS

Figure 1. Metoprolol Tartrate Structure.

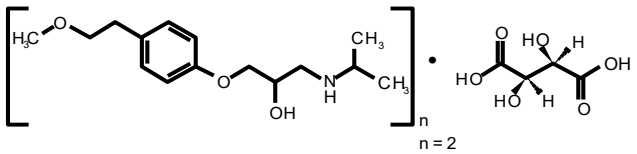


Table 1. Ph. Eur. Adjustments of Chromatographic Conditions and Method Comparison.

Method Parameter	Allowable Adjustments (Isocratic)	Monograph Method	Method 1	Method 2
Stationary Phase	No change of the identity of the substituent permitted	End-capped Octadecylsilyl Silica Gel for Chromatography R (C18)	As Specified	As Specified
Column Length (L), Column Particle Size (dp), L/dp	The particle size and/or length of the column may be modified provided that the ratio of L/dp remains constant or in the range of -25 % to +50 % of the prescribed ratio.	Length = 150 mm Particle Size = 5 µm L/dp = 30	Length = 150 mm Particle Size = 5 µm L/dp = 30 (As Specified)	Length = 100 mm Particle Size = 2.6 µm L/dp = 38.5 Deviation = +28.3 % (Allowed)
Column Internal Diameter (dc)	In the absence of a change in particle size and/or length of the column, the internal diameter of the column may be adjusted.	3.9 mm	4.6 mm (Allowed)	4.6 mm (Allowed)
Flow Rate (F)	Flow rate is adjusted for changes in column diameter and particle size using the following equation: $F_2 = F_1 x \left(\frac{dc_2^2 x\ dp_1}{dc_1^2 x\ dp_2}\right)$ F_1 = flow rate indicated in the monograph, in mL/min F_2 = adjusted flow rate, in mL/min dc_1 = internal diameter of the column indicated in the monograph, in mm dc_2 = internal diameter of the column used, in mm dp_1 = particle size indicated in the monograph, in µm dp_2 = particle size of the column used, in µm After an adjustment due to a change in column dimensions, an additional change in flow rate of ± 50 per cent is permitted.	1 mL/min	1.39 mL/min (Allowed) Adjusted back to 1.0 mL/min Deviation = -28.1 % (Allowed)	2.68 mL/min (Allowed) Adjusted back to 1.5 mL/min Deviation = -44.0 % (Allowed)
Column Temperature	± 10 °C	20-25 °C	As Specified	As Specified
Composition of Mobile Phase	The amount of the minor solvent components may be adjusted by ± 30 % relative; no component is altered by more than 10 % absolute.	See **Mobile Phase** in Table 2	As Specified	As Specified
Detection Wavelength	No adjustment permitted	280 nm	As Specified	As Specified
Injection Volume	When column dimensions are changed, it may be adjusted with the equation: $V_{inj2} = V_{inj1} x \left(\frac{L_2 x\ dc_2^2}{L_1 x\ dc_1^2}\right)$ V_{inj1} = injection volume indicated in the monograph, in µL V_{inj2} = adjusted injection volume, in µL dc_1 = internal diameter of the column indicated in the monograph, in mm dc_2 = internal diameter of the column used, in mm L_1 = column length indicated in the monograph, in mm L_2 = new column internal diameter, in mm	20 µL	28 µL (Allowed) Not changed from 20 µL	19 µL (Allowed) Not changed from 20 µL

LC-UV Conditions

Columns: Luna™ 5 µm C18(2), 150 x 4.6 mm (00F-4252-E0) – Method 1
Kinetex™ 2.6 µm C18, 100 x 4.6 mm (00D-4462-E0) – Method 2

Mobile Phase: Mobile Phase (**Table 1**)

Flow Rate: 1.0 mL/min (Isocratic) – Method 1
1.5 mL/min (Isocratic) – Method 2

Injection: 20 µL

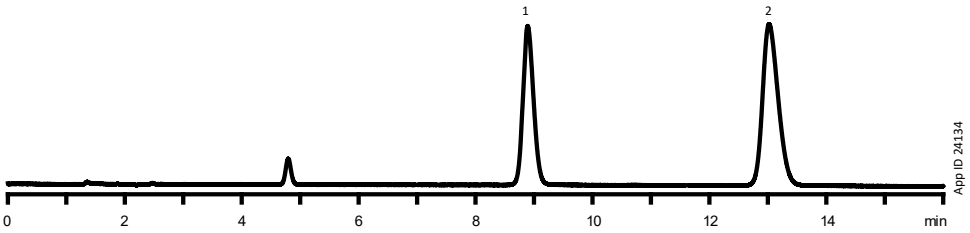
Detector: UV @ 280 nm

Table 1. Preparation of Test and Reference Solutions

Solution	Composition
Mobile Phase	Dissolve 3.9 g of Ammonium Acetate R in 810 mL of Water R, add 2.0 mL of Trimethylamine R, 10.0 mL of glacial Acetic Acid R, 3.0 mL of Phosphoric Acid R and 146 mL of Acetonitrile R and mix.
Test Solution	Dissolve 20.0 mg of *Metoprolol Tartrate CRS* in Mobile Phase and dilute to 100 mL with Mobile Phase.
Reference Solution (a)	Dilute 1.5 mg of *Metoprolol Impurity A CRS* and 2.5 mg of *Metoprolol Tartrate CRS* in the Mobile Phase and dilute to 50.0 mL with the Mobile Phase.
Reference Solution (b)	Dilute 1.0 mL of the Test Solution to 20.0 mL with the Mobile Phase. Dilute 1.0 mL of this solution to 50.0 mL with the Mobile Phase.

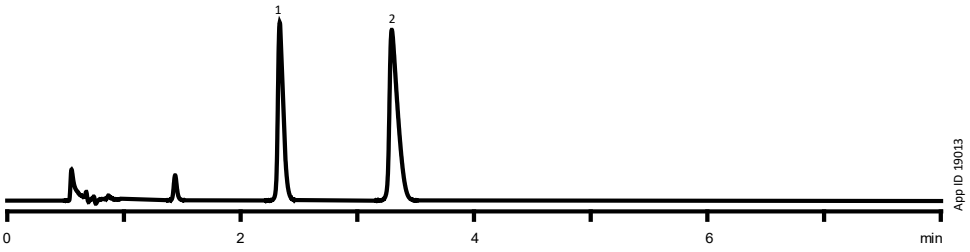
Figure 2. System Suitability Test for Related Substances Using Reference Solution (a).

Method 1 on Luna 5 µm C18(2) , 150 x 4.6 mm Column



Peak No.	Analyte	Retention Time (min)	Resolution
1	Impurity A	8.9	10.10
2	Metoprolol Tartrate	13.0	

Method 2 on Kinetex 2.6 µm C18 , 100 x 4.6 mm Column



Peak No.	Analyte	Retention Time (min)	Resolution
1	Impurity A	2.3	9.21
2	Metoprolol Tartrate	3.3	

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