CASE STUDY

Ph. Eur. Labetalol Hydrochloride Assay and Related Substances

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Introduction

Labetalol Hydrochloride is an α- and β-blocker medication that is used to lower blood pressure by relaxing blood vessels and slowing heart rate. The development of a quick and efficient analysis of Labetalol Hydrochloride and its related substances has a potential application for generic drug manufacturers. The Ph. Eur. Monograph method uses the Waters® 3.5 µm XBridge® C18 150 x 4.6 mm column for both Assay (Isocratic) and Related Substances (Gradient), and also specifies the column as "end-capped ethylene bridged C18 hybrid." The recent revision (official as of July 1, 2022) which harmonized the Ph. Eur. and USP allowable adjustments for liquid chromatography methods allows for the use of the Gemini™ 3 µm NX-C18 as an alternative to the Waters 3.5 µm XBridge C18 column referenced in the Labetalol Hydrochloride monograph.

In this case study we show the separation of Labetalol Hydrochloride from its related substances following Ph. Eur. Monograph 0923 using a Gemini 3 µm NX-C18 column and compared it to the Waters XBridge 3.5 µm C18 column originally used in the monograph. We also ran a Luna™ Omega 3 µm C18 column (data not shown), but because the monograph requires an "end-capped ethylene bridged C18 hybrid" column, it's use would have been outside the allowable adjustments as it does not meet this description. The Gemini 3 µm NX-C18 column used for this study met the system suitability criteria for Related Substances analysis of a minimum resolution (R_S) of 4.5 between the peaks due to Impurity A and Labetalol Hydrochloride in the chromatogram obtained with Reference Solution (b). The Waters XBridge column did not meet the system suitability criteria.

Key Concepts:

- Making the correct adjustments to gradient methods when the particle size is changed will yield equivalent results and allow for meeting system suitability requirements.
- Understand how to work within the allowed adjustments for Pharmacopoeia methods.

The use of the 3 µm particle size of the Gemini NX-C18 column is a new allowable adjustment in a gradient method since the L/dp ratio (150/3 = 50,000) is within the allowable range of -25 to +50 % of the L/dp ratio (150/3.5 = 42,900) for the original 3.5 µm column used to elucidate the assay method. When the particle size is changed, the flow rate requires adjustment because smaller-particle columns will require higher linear velocities to provide the same performance. The flow rate is adjusted for particle size using the following equation:

$$F_2 = F_1 \, x \, \frac{dc_2^{\,2} \, x \, dp_1}{dc_1^{\,2} \, x \, dp_2}$$

F_1 = flow rate indicated in the monograph (mL/min)
F_2 = adjusted flow rate (mL/min)
dc_1 = internal diameter of the column indicated in the monograph (mm)
dc_2 = internal diameter of the column used (mm)
dp_1 = particle size indicated in the monograph (µm)
dp_2 = particle size of the column used (µm)

The adjusted flow rate for the Gemini NX-C18 3 µm column would be:

$$F_2 = 1.5 \, x \, \frac{4.6^2 \, x \, 3.5}{4.6^2 \, x \, 3.0}$$

$$F_2 = 1.75$$

A change in column dimensions, and thus in column volume, impacts the gradient volume which controls selectivity. In this case the column dimensions remain unchanged, but because the flow rate has been adjusted this would impact the gradient. As such the gradient table needs to be modified to maintain the original gradient slope and hence the same selectivity. Gradients are adjusted to the column volume by changing the gradient volume in proportion to the column volume. This applies to every gradient segment volume. The new gradient time for each gradient segment can be calculated using the following equation:

$$t_{G2} = t_{G1} x \left(\frac{F_1}{F_2}\right) x \left(\frac{L_2 x dc_2^2}{L_1 x dc_1^2}\right)$$

t_{G1} = particle size indicated in the monograph (min)
t_{G2} = particle size of the column used (min)
F_1 = flow rate indicated in the monograph (mL/min)
F_2 = adjusted flow rate (mL/min)
L_1 = column length indicated in the monograph (mm)
L_2 = new column length (mm)
dc_1 = internal diameter of the column indicated in the monograph (mm)
dc_2 = internal diameter of the column used (mm)

For the second gradient segment, the adjusted gradient time would be:

$$t_{G2} = 5 x \left(\frac{1.5}{1.75}\right) x \left(\frac{150 x 4.6^2}{150 x 4.6^2}\right)$$

$$t_{G2} = 4.29$$

A gradient adjustment factor can be calculated and used to determine the new gradient segment times using:

$$Gradient\ adjustment\ factor = \left(\frac{t_{G2}}{t_{G1}}\right)$$

$$Gradient\ adjustment\ factor = 0.86$$

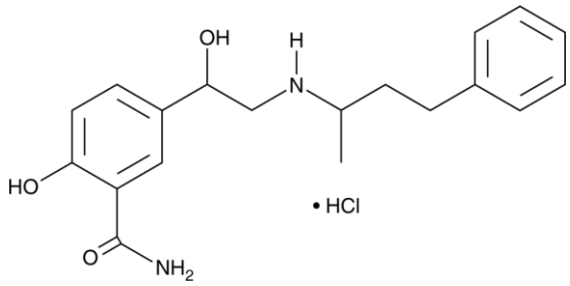
The new gradient timetable is shown in the Application Methods.

The Gemini NX-C18 column showed no retention of the peak under Test Solution (b) / Reference Solution (c) for the Assay of Labetalol Hydrochloride. According to Chapter 2.2.46, the minor component of the Mobile Phase can be adjusted ± 30 % relative to the composition. Therefore, we can increase Mobile Phase A to a maximum of 58.5 %, but we settled on 55 %. Under these adjusted conditions, the peak for Labetalol Hydrochloride was retained using the Gemini NX-C18 column.

All reference solutions were prepared as indicated in Ph. Eur. monograph 0923 for Labetalol Hydrochloride. The following certified reference standards (CRS) were purchased from the European Directorate for the Quality of Medicines & HealthCare (EDQM) – Council of Europe; Postal address: Allee Kastner CS 30026 F - 67081 Strasbourg (France):

- L0050000, Labetalol Hydrochloride CRS
- Y0001548, Labetalol Impurity A CRS

Figure 1. Labetalol Hydrochloride



Application Methods

LC-UV Conditions – Related Substances

Columns: Gemini™ 3 µm NX-C18 (00F-4453-E0)
XBridge® 3.5 µm C18
Dimensions: 150 x 4.6 mm
Mobile Phase: Mobile Phase (**Table 1**)

Gradient:

Time (min)	Adjusted Time (min)	%B
0	0	0
5	4.29	0
40	34.29	100
45	38.57	100
45.01	38.58	0
55	47.14	0

Flow Rate: 1.5 mL/min (XBridge)
1.75 mL/min (Gemini)
Injection Volume: 20 µL
Temperature: 50 °C
Detector: UV @ 230 nm
System: Agilent® 1290

LC-UV Conditions – Assay

Columns: Gemini 3 µm NX-C18 (00G-4453-E0)
XBridge 3.5 µm C18
Dimensions: 150 x 4.6 mm
Mobile Phase: A / B (45:55, v/v)
A / B (55:45, v/v) (Gemini)
Flow Rate: 1.5 mL/min (Isocratic) (XBridge)
1.75 mL/min (Isocratic) (Gemini)
Injection Volume: 20 µL
Temperature: 40 °C
Detector: UV @ 230 nm
System: Agilent 1290

Table 1. Preparation of Solutions

Solution	Composition
Mobile Phase	A: Phosphoric Acid / Water (0.1:99.9, v/v) B: Acetonitrile / Mobile Phase A (50:50, v/v)
Test Solution (a)	Dissolve 25.0 mg of Labetalol Hydrochloride CRS in Mobile Phase A, and dilute to 10.0 mL with Mobile Phase A.
Test Solution (b)	Dilute 1.0 mL of Test Solution (a) to 50.0 mL with Mobile Phase A.
Reference Solution (a)	Dilute 1.0 mL of Test Solution (a) to 100.0 mL with Mobile Phase A. Dilute 1.0 mL of this solution to 10.0 mL with Mobile Phase A.
Reference Solution (b)	Dilute 2 mL of Test Solution (a) to 100 mL with Mobile Phase A. Dissolve 5 mg of Labetalol Impurity A CRS in 50 mL of Mobile Phase B and dilute to 100 mL with Mobile Phase A.
Reference Solution (c)	Same as Test Solution (b).

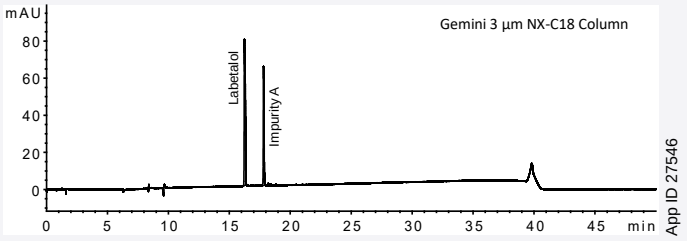
Results and Discussion

Related Substances

The use of the 3 µm particle size of the Gemini™ NX-C18 column is a new allowable adjustment in a gradient method since the L/dp ratio (150/3 = 50,000) is within the allowable range of -25 to +50 % of the L/dp ratio (150/3.5 = 42,900) for the original 3.5 µm column used to elucidate the assay method. This allows for increased flexibility with gradient monograph methods for laboratories, as long as they make the necessary adjustments to flow rate and gradient time.

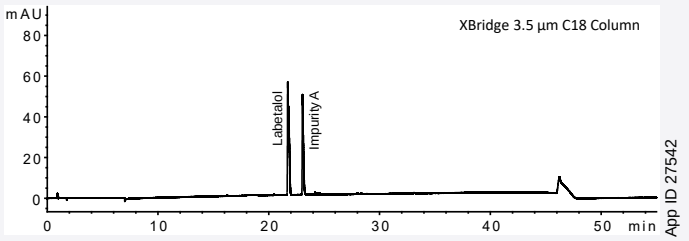
For the gradient Related Substances method, no adjustments to mobile phase composition were required for retention of Labetalol (since the initial mobile phase composition was much weaker than the isocratic mobile phase), but the flow rate and gradient both were adjusted for the Gemini NX-C18 column (**Figure 2**).

Figure 2. System Suitability Test for Related Substances using Reference Solution (b)



	Labetalol		Impurity A		R_S Labetalol / Impurity A
Inj. No.	t_R	Area	t_R	Area	
1	16.24	460.9	17.81	247.0	11.57
2	16.23	460.8	17.80	325.8	11.43
3	16.22	457.2	17.79	219.3	11.69
Average					**11.56**
% RSD					**1.13**

Figure 2 Cont'd. System Suitability Test for Related Substances using Reference Solution (b)



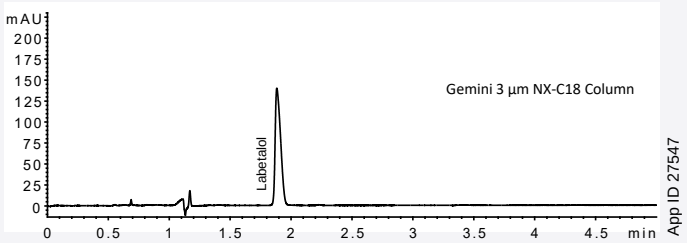
	Labetalol		Impurity A		R_S Labetalol / Impurity A
Inj. No.	t_R	Area	t_R	Area	
1	21.71	530.5	23.04	345.4	5.66
2	21.70	529.9	23.05	257.9	5.96
3	21.70	529.9	23.05	257.9	5.96
Average					**5.86**
% RSD					**2.96**

Assay

For the isocratic Assay method, the Gemini™ NX-C18 column did not give enough retention for Labetalol. Under the monograph mobile phase conditions, Labetalol eluted in the void. Therefore, per the allowable adjustments for isocratic methods, the mobile phase composition was adjusted (**Figure 3**). Specifically, the minor component (A) was increased from 45 % to 55 %; this was an increase of mobile phase A of <30 % relative to the maximum adjustment allowed. This allowed adjustment to the mobile phase composition provided increased retention for Labetalol so that it no longer eluted in the void volume.

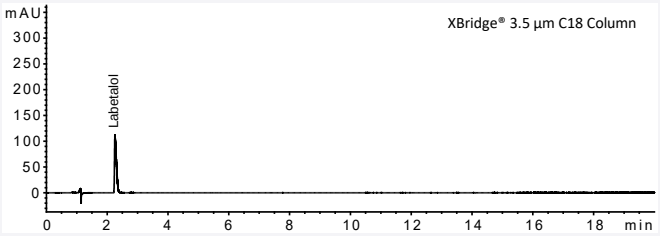
Labetalol retention time on the Gemini NX-C18 was less than the retention time on the Waters® XBridge® C18 (1.88 min vs. 6.95 min). Peak shape (Symmetry) was superior on the Gemini NX-C18 and met the system suitability requirement for peak symmetry.

Figure 3. System Suitability Test for Assay using Test Solution (b) / Reference Solution (c)



	Labetalol				
Inj. No.	t_R	Area	Height	Width	Symmetry Factor
1	1.88	434.7	139.8	0.052	1.65
2	1.89	419.6	135.3	0.052	1.61
3	1.89	417.7	135.0	0.052	1.61
Average	**1.88**	**424.0**	**136.7**	**0.05**	**1.63**
% RSD	**0.06**	**2.2**	**2.0**	**0.19**	**1.40**

Figure 3 Cont'd. System Suitability Test for Assay using Test Solution (b) / Reference Solution (c)



	Labetalol				
Inj. No.	t_R	Area	Height	Width	Symmetry Factor
1	2.25	488.5	111.9	0.065	2.35
2	2.25	487.8	112.5	0.066	2.37
3	16.34	20.9	3.8	0.091	2.34
Average	**6.95**	**332.4**	**76.1**	**0.070**	**2.35**
% RSD	**117.05**	**81.2**	**82.3**	**19.980**	**0.52**

Conclusions

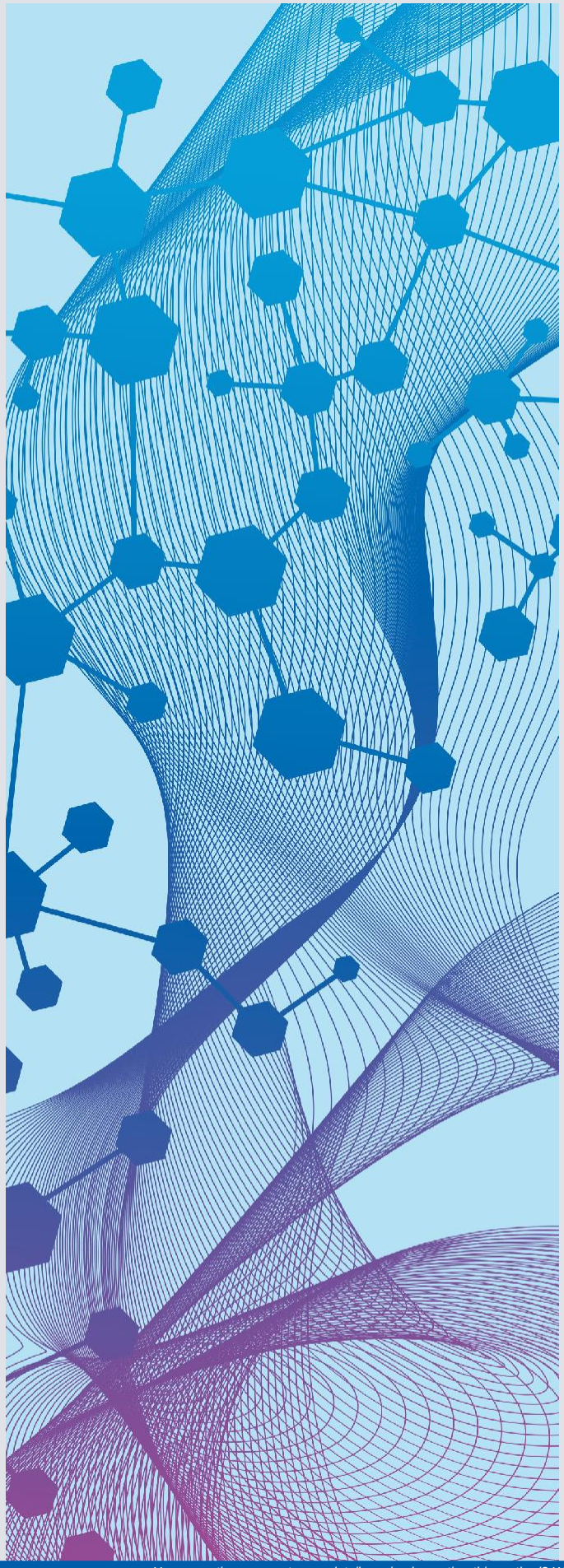
This application was an excellent example highlighting that C18 columns may not be directly interchangeable, but this can be overcome in most cases by using allowable adjustments within the allowable limits to obtain the desired retention and separation.

The Luna™ Omega C18 column gave similar retention as the Waters XBridge C18 column for both Assay and Related Substances, while meeting the system suitability requirements for resolution (minimum 4.5 between Labetalol and Labetalol Impurity A). While using the Luna Omega C18 for this particular monograph would not have been allowed due to the specific description of the column in the monograph, the results for retention under the monograph conditions illustrate the overall retention and performance of the Luna Omega C18 column under low pH mobile phase conditions (0.1 % Phosphoric Acid) is similar to the Waters XBridge C18 column.

Key Learnings:

- C18 columns are not interchangeable, and adjustments may need to be made, within allowed limits, to obtain the desired retention and separation.
- Luna Omega C18 met system suitability requirements although is does not meet the specific column description. As such it could not be utilized in the monograph, however this exercise demonstrates it's overall retention and performance under low pH mobile phase conditions.

CASE STUDY



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