

Ph. Eur. Monograph 0923: Labetalol Hydrochloride Assay and Related Substances on Gemini™ 3 μm NX-C18 Column

Zeshan Aqeel¹, Bryan Tackett, PhD¹, and Heiko Behr, PhD²

¹Phenomenex Inc., 411 Madrid Ave., Torrance, CA 90501 USA

²Phenomenex Ltd. Deutschland, Zeppelinstr. 5, 63741 Aschaffenburg, Germany

Overview

Labetalol Hydrochloride is an alpha and beta blocker medication that is used to lower blood pressure by relaxing blood vessels and slowing heart rate.

In this application note we show the separation of Labetalol Hydrochloride from its related substances following Ph. Eur. Monograph 0923. We used a Gemini 3 μm NX-C18 column and compared it to the Waters XBridge 3.5 μm C18 column, originally used in the monograph. 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.

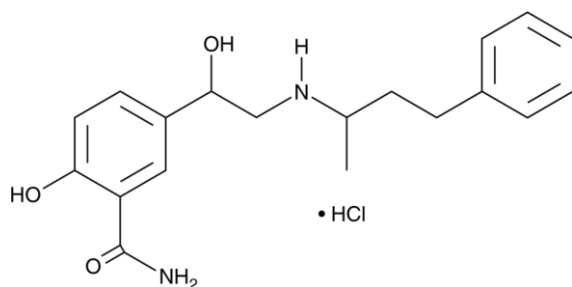
The use of the 3 μm particle size of the Gemini NX-C18 column is a new allowable adjustment (active starting July 1st, 2022) in a gradient method since the L/d_p ratio ($150/3 = 50,000$) is within the allowable range of -25 to +50 % of the L/d_p ratio ($150/3.5 = 42,900$) for the original 3.5 μm column used to elucidate the assay method.

The Gemini NX-C18 column showed no retention of the peak under Test Solution (b) and Reference Solution (c) for the Assay analysis of Labetalol Hydrochloride. According to Chapter 2.2.46, the Mobile Phase can be adjusted $\pm 30\%$ relative to the composition. We can increase Mobile Phase A to a maximum of 58.5 %. Under these adjusted conditions, the peak for Labetalol Hydrochloride was resolved 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 Structure



LC-UV Conditions – Related Substances

Columns: Gemini 3 μm NX-C18 ([00G-4453-E0](#))
Waters® XBridge® 3.5 μm C18

Dimensions: 150 x 4.6 mm

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

Gradient:	Time (min)	%B
	0	0
	5	0
	40	100
	45	100
	45.1	0
	55	0

Flow Rate: 1.5 mL/min

Injection: 20 μL

Temperature: 40 °C

Detector: UV @ 230 nm

System: Agilent® 1290

LC-UV Conditions – Assay

Columns: Gemini 3 μm NX-C18 ([00G-4453-E0](#))
Waters XBridge 3.5 μm C18

Dimensions: 150 x 4.6 mm

Mobile Phase: **Mobile Phase A** / **Mobile Phase B** (45:55, v/v)
Mobile Phase A / **Mobile Phase B** (58.5:41.5, v/v) (NX-C18)

Flow Rate: 1.5 mL/min (Isocratic)

Injection: 20 μL

Temperature: 40 °C

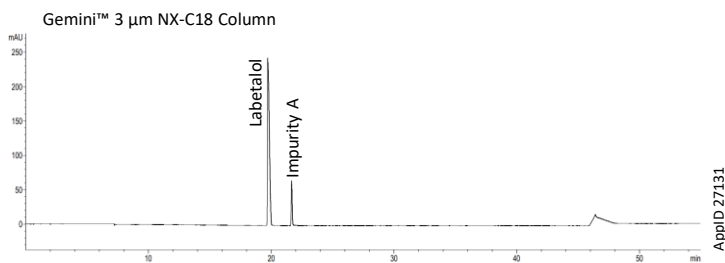
Detector: UV @ 230 nm

System: Agilent 1290

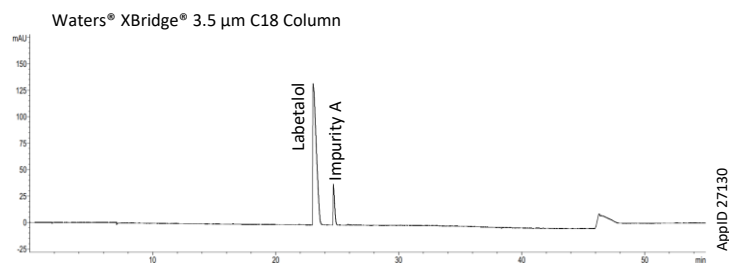
Table 1. Preparation of Test and Reference Solutions

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) .

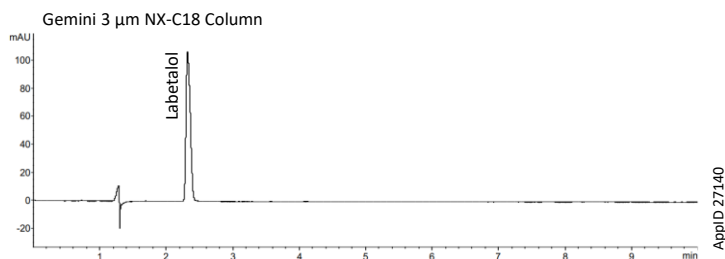


Figure 2. System Suitability Test for Related Substances Using Reference Solution (b)

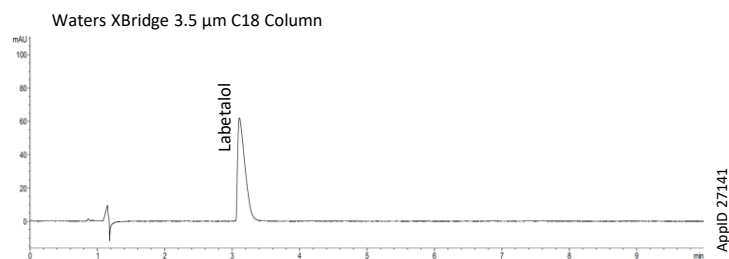
Inj. No.	Labetalol		Impurity A		R _s Labetalol/ Impurity A
	t _R	Area	t _R	Area	
1	19.712	2630.3	21.660	342.3	8.89
2	19.678	2634.5	21.635	358.4	8.83
3	19.616	2636.1	21.58	335.5	9.02
Average					8.91
% RSD					1.09



Inj. No.	Labetalol		Impurity A		R _s Labetalol/ Impurity A
	t _R	Area	t _R	Area	
1	23.039	2612.4	24.689	318.8	4.36
2	23.072	2611.7	24.723	330.4	4.35
3	23.094	2604.6	24.753	320	4.36
Average					4.36
% RSD					0.13

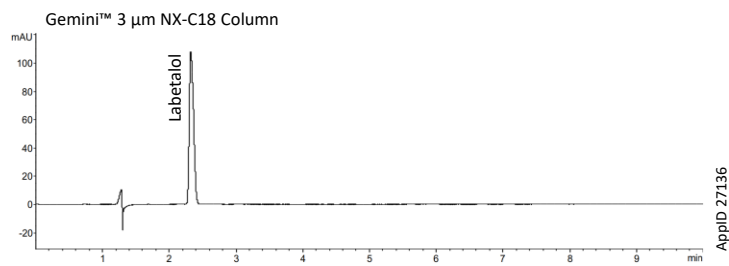
Figure 3. System Suitability Test for Assay Using Test Solution (b)

Inj. No.	Labetalol				
	t _R	Area	Height	Width	Symmetry Factor
1	2.139	476.2	106.9	0.073	1.50
2	2.322	474.8	107.2	0.075	1.48
3	2.32	475.3	107.4	0.076	1.48
Average	2.32	475.43	107.17	0.07	1.49
% RSD	0.07	0.15	0.23	2.05	0.58

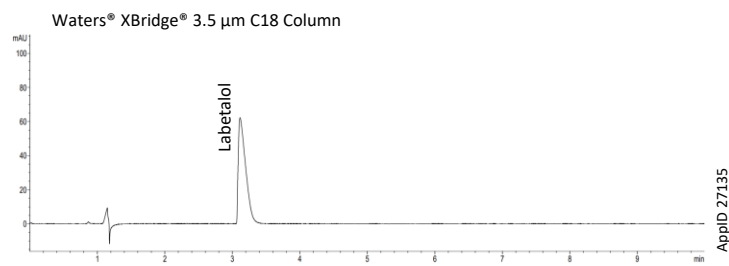


Inj. No.	Labetalol				
	t _R	Area	Height	Width	Symmetry Factor
1	3.107	468.8	62	0.1067	2.69
2	3.109	471	62.2	0.1123	2.64
3	3.112	469.9	62	0.1069	2.66
Average	3.11	469.90	62.07	0.11	2.66
% RSD	0.08	0.23	0.19	2.92	0.83



Figure 4. System Suitability Test for Assay Using Reference Solution (c)

Inj. No.	Labetalol				
	t_R	Area	Height	Width	Symmetry Factor
1	2.322	482.8	107.9	0.073	1.46
2	2.315	479.1	107.2	0.072	1.46
3	2.32	480.7	107.7	0.073	1.47
Average	2.32	480.87	107.6	0.07	1.46
% RSD	0.16	0.39	0.34	0.77	0.33



Inj. No.	Labetalol				
	t_R	Area	Height	Width	Symmetry Factor
1	3.115	474.3	62.2	0.1089	2.64
2	3.113	473.8	62.3	0.1102	2.60
3	3.112	474.9	62.4	0.1083	2.60
Average	3.11	474.33	62.30	0.11	2.61
% RSD	0.05	0.12	0.16	0.89	0.89



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t: +61 (0)2-9428-6444
auinfo@phenomenex.com

Austria

t: +43 (0)1-319-1301
anfrage@phenomenex.com

Belgium

t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
beinfo@phenomenex.com

Canada

t: +1 (800) 543-3681
info@phenomenex.com

China

t: +86 400-606-8099
cninfo@phenomenex.com

Czech Republic

t: +420 272 017 077
cz-info@phenomenex.com

Denmark

t: +45 4824 8048
nordicinfo@phenomenex.com

Finland

t: +358 (0)9 4789 0063
nordicinfo@phenomenex.com

France

t: +33 (0)1 30 09 21 10
franceinfo@phenomenex.com

Germany

t: +49 (0)6021-58830-0
anfrage@phenomenex.com

Hong Kong

t: +852 6012 8162
hkinfo@phenomenex.com

India

t: +91 (0)40-3012 2400
indiainfo@phenomenex.com

Indonesia

t: +62 21 5019 9707
indoinfo@phenomenex.com

Ireland

t: +353 (0)1 247 5405
eireinfo@phenomenex.com

Italy

t: +39 051 6327511
italiainfo@phenomenex.com

Japan

t: +81 (0) 120-149-262
jpinfo@phenomenex.com

Luxembourg

t: +31 (0)30-2418700
nlinfo@phenomenex.com

Mexico

t: 01-800-844-5226
tecnicomx@phenomenex.com

The Netherlands

t: +31 (0)30-2418700
nlinfo@phenomenex.com

New Zealand

t: +64 (0)9-4780951
nzinfo@phenomenex.com

Norway

t: +47 810 02 005
nordicinfo@phenomenex.com

Poland

t: +48 22 104 21 72
pl-info@phenomenex.com

Portugal

t: +351 221 450 488
ptinfo@phenomenex.com

Singapore

t: +65 6559 4364
sginfo@phenomenex.com

Slovakia

t: +420 272 017 077
sk-info@phenomenex.com

Spain

t: +34 91-413-8613
espinfo@phenomenex.com

Sweden

t: +46 (0)8 611 6950
nordicinfo@phenomenex.com

Switzerland

t: +41 (0)61 692 20 20
swissinfo@phenomenex.com

Taiwan

t: +886 (0) 0801-49-1246
twinfo@phenomenex.com

Thailand

t: +66 (0) 2 566 0287
thaiinfo@phenomenex.com

United Kingdom

t: +44 (0)1625-501367
ukinfo@phenomenex.com

USA

t: +1 (310) 212-0555
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