

APPLICATIONS

Ph. Eur. Monograph 401: Levothyroxine Sodium on Luna[®] C18(2) and Luna Omega C18

Amra Perva Uzunalic¹, Dirk Hansen² and Heiko Behr²

¹University of Maribor, Faculty of Chemistry and Chemical Engineering Group for Separation Analysis, Smetanova 17, 2000 Maribor, Slovenia

²Phenomenex Ltd Deutschland, Zeppelinstraße 5, 63741 Aschaffenburg, Germany

Overview

Levothyroxine is a synthetic form of the thyroid hormone thyroxine (T4). The pharmaceutical is used to treat thyroid hormone deficiency.

In this application note we show the suitability of two Phenomenex reversed phase HPLC columns for the analysis of related substances according to Ph. Eur. monograph 401. We also show the effect of adapting the column ID and the flow rate according to the allowable adjustments of chapter 2.2.46 of the European Pharmacopoeia.

Standards and Solutions

Levothyroxine sodium CRS, Liothyronine sodium CRS and Levothyroxine for peak identification CRS have been purchased from EDQM (Strasbourg, France).

Table 1: Test solutions

Name	Step 1	Step 2	Step 3
Solvent Mixture	Mix mobile phase A with ethanol (96%) (1:2 V/V)		
Ref. a	Dissolve 2.5 mg of levothyroxine sodium CRS and 2.5 mg liothyronine sodium CRS (impurity A) in the Solvent Mixture .	Dilute to 25.0 mL with the Solvent Mixture .	Dilute 1.0 mL of the solution to 50.0 mL with the Solvent Mixture .
Ref. b	Dilute 1.0 mL of Ref. a to 10.0 mL with the Solvent Mixture .		
Ref. c	Dissolve 25.0 mg levothyroxine sodium CRS in the Solvent Mixture .	Dilute to 50.0 mL with the Solvent Mixture .	Dilute 10.0 mL of the solution to 25.0 mL with the Solvent Mixture .
Ref. d	Dissolve 2.0 mg of levothyroxine for peak identification CRS (containing impurities F and G) in 10.0 mL of the Solvent Mixture .	Sonicate for 10 min.	

HPLC Conditions

Columns: Luna 3 µm C18(2), Luna Omega 3 µm C18

Dimension: 150 x 4.0 mm, 150 x 4.6 mm

Part Nos.: [00F-4251-DO](#), [00F-4874-EQ](#)

Mobile Phase: (A) 1.97 g phosphoric acid in 2 L water
(B) 1.97 g phosphoric acid in 2 L acetonitrile

Gradient:	Time (min)	%A	%B
	0	70	30
	10	70	30
	40	20	80
	50	20	80

Flow Rate: as indicated on the chromatograms

Column Temperature: 25 °C

Injection: 25 µL

System: Shimadzu[®] Nexera[®] XR

Detector: UV @ 225 nm

Figure 1a: Reference a on Luna 3 µm C18(2) with flow rate 1.0 mL/min

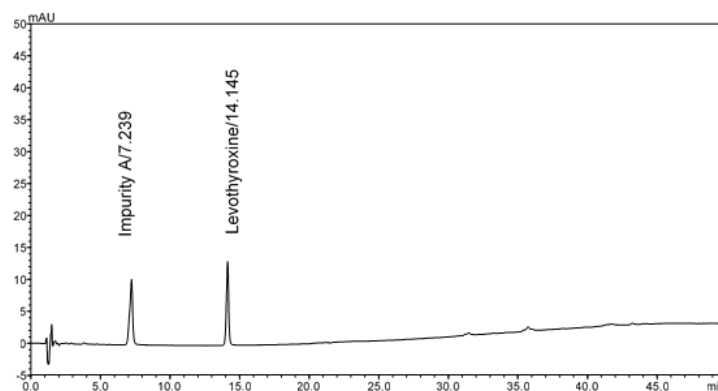


Figure 1b: Reference d on Luna 3 µm C18(2) with flow rate 1.0 mL/min

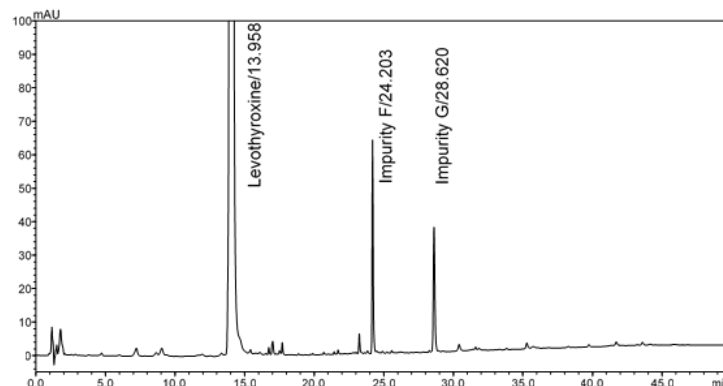


Figure 2a: Reference a on Luna® Omega 3 µm C18 with flow rate 1.0 mL/min

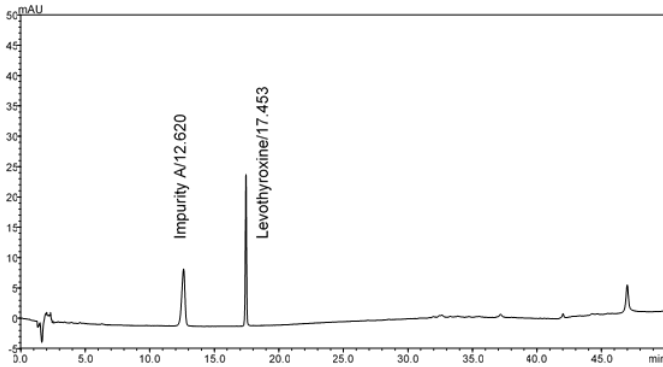


Figure 2b: Reference d on Luna Omega 3 µm C18 with flow rate 1.0 mL/min

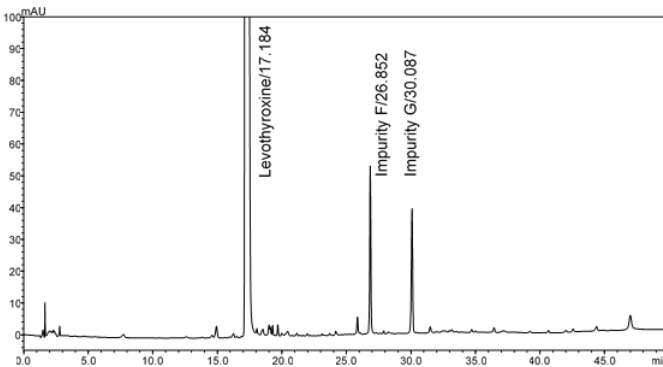


Figure 3a: Reference a on Luna Omega 3 µm C18 with flow rate 1.32 mL/min

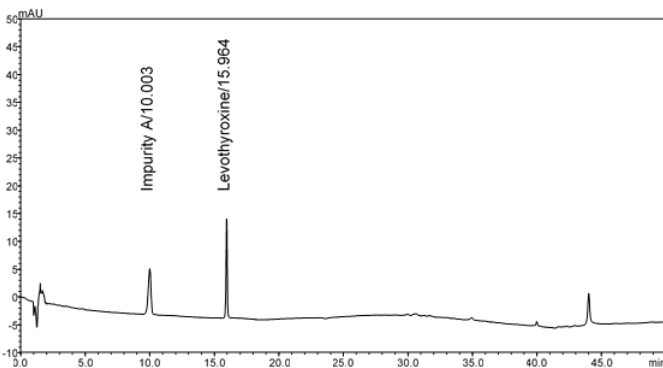


Figure 3b: Reference d on Luna Omega 3 µm C18 with flow rate 1.32 mL/min

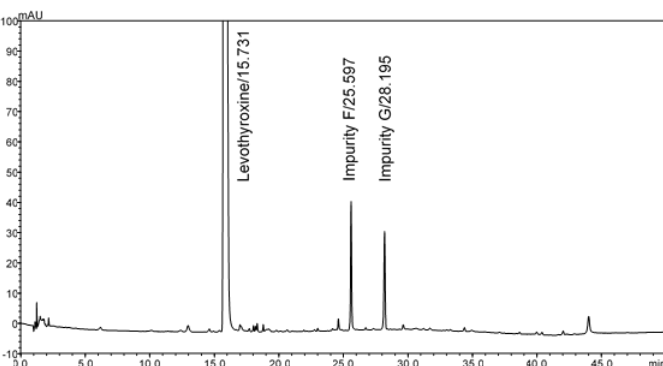


Table 2: Comparison of critical method parameters

Parameter	Luna 3 µm C18(2) 1.0 mL/min	Luna Omega 3 µm C18 1.0 mL/min	Luna Omega 3 µm C18 1.32 mL/min
Imp. A Tailing (Ref. a)	0.833	0.881	0.879
Levothyroxine Tailing (Ref. a)	0.940	0.919	0.932
α Levothyroxine/ Imp. A	21	16	21
R _t Imp. A	7.239 min	12.620 min	10.003 min
R _t Levothyroxine	14.145 min	17.453 min	15.964 min
R _t Imp. F	24.203 min	26.852 min	25.597 min
R _t Imp. G	28.620 min	30.087 min	28.195 min

Conclusion

The Luna 3 µm C18(2) 150 x 4.0 mm and Luna Omega 3 µm C18 150 x 4.6 columns were tested for Ph. Eur. monograph 401. System suitability requires a resolution of greater than 5 for the separation of impurity A and Levothyroxine. This criteria was met even under the original, unscaled flow rate of 1.0 mL for the 4.6 mm ID column. Scaling of the flow rate brought the resolution for the 4.6 mm ID column on par with the 4.0 mm ID column. Therefore, both columns are suitable for this monograph.

APPLICATIONS

Need a different column size or sample preparation format?

No problem! We have a majority of our available dimensions up on www.phenomenex.com, but if you can't find what you need right away, our super helpful Technical Specialists can guide you to the solution via our online chat portal www.phenomenex.com/LiveChat.

Australia

t: +61 (0)2-9428-6444
auiinfo@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

India

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

Ireland

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

Italy

t: +39 051 6327511
italiainfo@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: +65 800-852-3944
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
info@phenomenex.com

🌐 **All other countries/regions**
Corporate Office USA
t: +1 (310) 212-0555
info@phenomenex.com

www.phenomenex.com

Phenomenex products are available worldwide. For the distributor in your country/region, contact Phenomenex USA, International Department at international@phenomenex.com

BE-HAPPY™
guarantee

Your happiness is our mission. Take 45 days to try our products. If you are not happy, we'll make it right.

www.phenomenex.com/behappy

Terms and Conditions

Subject to Phenomenex Standard Terms and Conditions, which may be viewed at www.phenomenex.com/TermsAndConditions.

Trademarks

Luna is a registered trademark of Phenomenex. Shimadzu and Nexera are registered trademarks of Shimadzu Corporation.

Disclaimer

Phenomenex is not affiliated with Shimadzu Corporation.

FOR RESEARCH USE ONLY. Not for use in clinical diagnostic procedures.

© 2020 Phenomenex, Inc. All rights reserved.