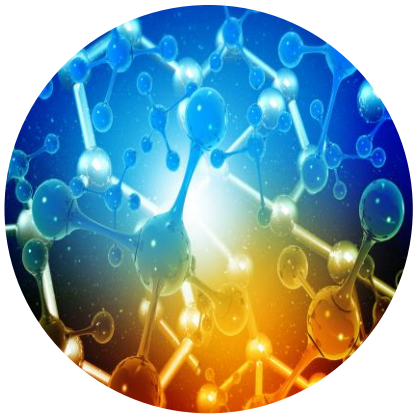

Ph. Eur. Monograph 2743: Zoledronic Acid Related Substances on Luna™ 5 µm Phenyl-Hexyl Column

Janja Vasilić[1], Amra Perva Uzunalić, PhD[1], Dirk Hansen, PhD[2], Heiko Behr, PhD[2], and Bryan Tackett, PhD[3]
[1]University of Maribor, Faculty of Chemistry and Chemical Engineering Group for Separation Analysis, Smetanova 17, 2000 Maribor, Slovenia
[2]Phenomenex Ltd. Deutschland, Zeppelinstr. 5, 63741 Aschaffenburg, Germany
[3]Phenomenex Inc., 411 Madrid Ave., Torrance, CA 90501 USA

Overview

Zoledronic acid belongs to the bisphosphonate family of drugs and is used to treat several different bone diseases.

In this application note we show the separation of Zoledronic acid from its related substances following Ph. Eur. monograph 2743. The Luna 5 µm Phenyl-Hexyl column used for this study met the system suitability criteria of a $R_S \geq 1.5$ between Zoledronic acid and Impurity A and of a $R_S \geq 1.5$ between Nitrate and Impurity B.

All reference solutions were prepared as indicated in Ph. Eur. monograph 2743 for Zoledronic acid. 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):

- Y0002020, Zoledronic acid monohydrate CRS
- Y0002011, Zoledronic acid impurity A CRS
- Y0002027, Zoledronic acid impurity B CRS

Experimental Preparation

The following procedure has been used to precondition the system and column before each test series.
The instrument was rinsed without column for 20 min at a flow rate of 5 mL/min with a 25% solution of Acetic acid. Then it was rinsed with water for 2h at a flow rate of 5 mL/min. The column was rinsed with the **Mobile Phase** at a flow rate of 0.6 mL/min for ≅ 1h. During this time, the **Test Solution** was injected 15 times, applying a run time of 3 min for each injection.

LC-UV Conditions

Columns: Luna 5 µm Phenyl-Hexyl (00G-4257-E0)
Dimension: 250 x 4.6 mm
Mobile Phase: **Mobile Phase** (**Table 1**)
Flow Rate: 0.6 mL/min
Injection: 10 µL
Temperature: 20 °C
Detector: UV @ 215 nm
System: Agilent® 1260 Infinity I

Table 1. Preparation of Test and Reference Solutions

Solution	Composition
Mobile Phase	Transfer 10.8 g of Sodium octane sulfonate and 37.0 mg of Sodium edetate into a 1000 mL flask, then dissolve in 500 mL of water. Add 10.0 mL of Perchloric acid and 2.0 mL of Phosphoric acid and dilute to 1000 mL with water.
Solvent	**Mobile Phase**
Test Solution	Weigh 40.0 mg of the reference substance Zoledronic acid monohydrate CRS into a 20.0 mL flask, fill up to ¾ volume with **Mobile Phase**, sonicate for 30 min, allow to come to room temperature and then dilute to 20.0 mL with **Mobile Phase**.
Reference Solution (a)	Dissolve 2.0 mg of Zoledronic acid impurity A CRS, 5.0 mg of Zoledronic acid impurity B CRS, and 2.0 mg of Sodium nitrate in the **Mobile Phase** and dilute to 50.0 mL with the **Mobile Phase**. Transfer 1.0 mL of this solution into a 20.0 mL flask, add 7.0 mL of the **Mobile Phase** and dilute to 20.0 mL with the **Test Solution**.
Reference Solution (b)	Transfer 1.0 mL of **Test Solution** into a 100 mL volumetric flask, fill up to volume with **Mobile Phase** and mix well. Transfer 1.0 mL of this solution into a 10.0 mL volumetric flask, fill up to volume with **Mobile Phase** and mix well.

Figure 1. System Suitability Test using Reference Solution (a) on Luna™ 5 µm Phenyl-Hexyl Column

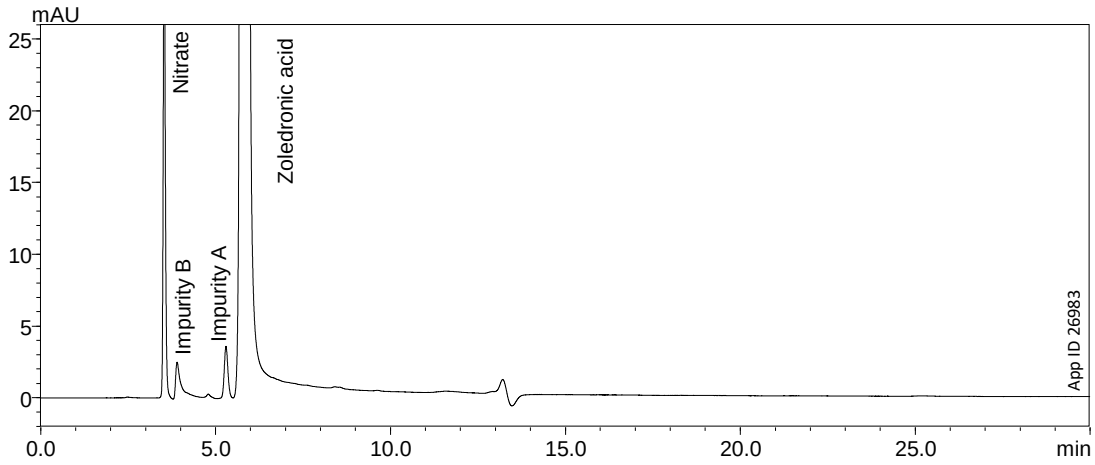

Table 2. System Suitability Test using Reference Solution (a) on Luna 5 µm Phenyl-Hexyl Column

	Nitrate		Impurity B		R_S Nitrate/ Impurity B	Impurity A		Zoledronic Acid			R_S Zoledronic acid/ Impurity A
Inj. No.	t_R	Area	t_R	Area		t_R	Area	t_R	Area	Tailing Factor	
1	3.534	147015	3.904	27266	2.261	5.297	25940	5.809	13285766	1.122	2.682
2	3.535	147245	3.904	28174	2.256	5.297	25958	5.810	13286267	1.123	2.681
3	3.535	147026	3.904	28079	2.264	5.298	25941	5.810	13294622	1.123	2.682
4	3.535	147178	3.903	29160	2.267	5.298	25954	5.811	13296135	1.122	2.684
5	3.535	146997	3.903	28438	2.271	5.297	25941	5.810	13291767	1.122	2.680
6	3.535	146992	3.902	28789	2.273	5.297	25967	5.810	13295290	1.122	2.684
Average	3.535	147076	3.903	28318	2.265	5.297	25950	5.810	13291641	1.122	2.682
% RSD	0.010	0.074	0.02	2.31	0.29	0.01	0.04	0.01	0.04	0.04	0.06

Figure 2. Reference Solution (b) on Luna™ 5 µm Phenyl-Hexyl Column

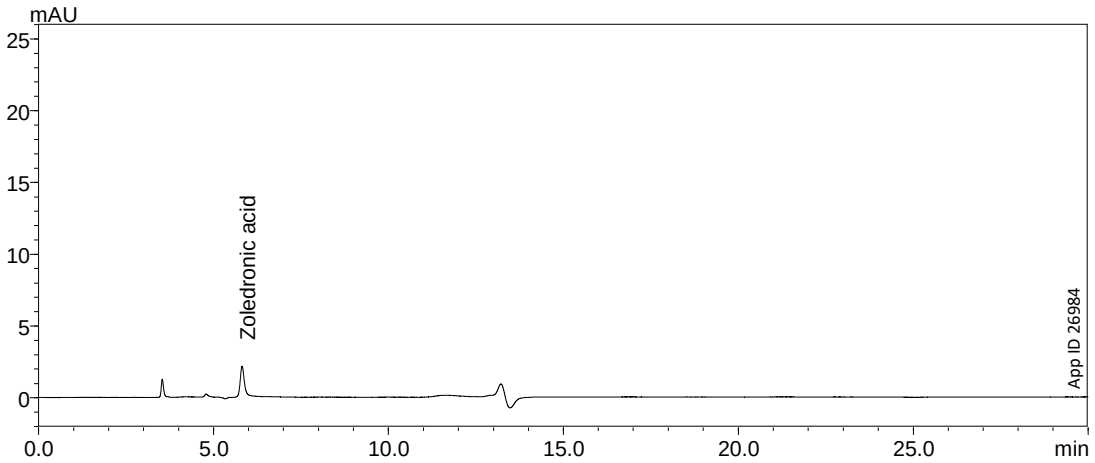

Table 3. Reference Solution (b) on Luna 5 µm Phenyl-Hexyl Column

	Zoledronic acid		
Inj. No.	**t_R**	**Area**	**Tailing Factor**
1	23.485	13837607	1.26
2	23.506	13829646	1.25
3	23.490	13822786	1.26
4	23.430	13802206	1.26
5	23.452	13799546	1.26
6	23.547	13796236	1.26
Average	23.485	13814671	1.26
% RSD	0.18	0.13	0.12

Figure 3. Test Solution on Luna™ 5 µm Phenyl-Hexyl Column

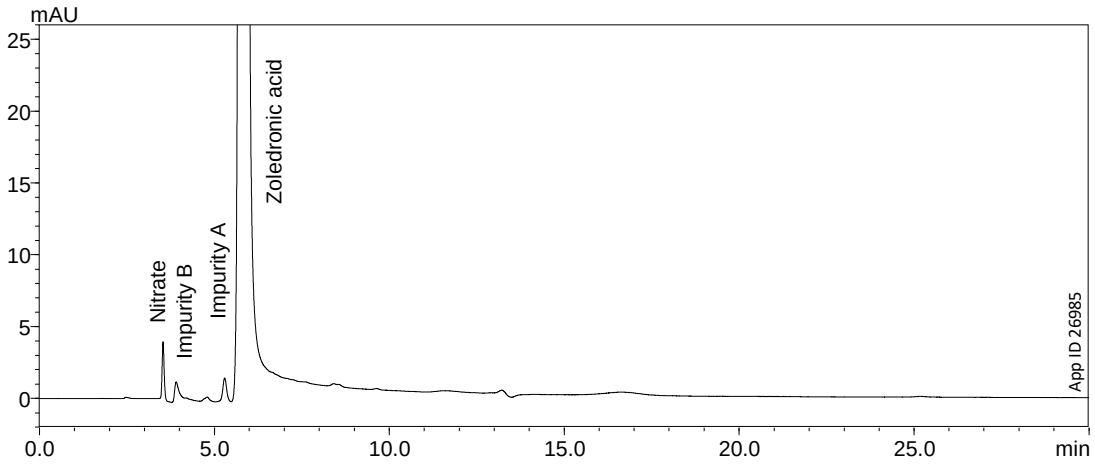

Table 4. Test Solution on Luna 5 µm Phenyl-Hexyl Column

	Nitrate		Impurity B		R_S Nitrate/ Impurity B	Impurity A		Zoledronic Acid			R_S Zoledronic acid/ Impurity A
Inj. No.	t_R	Area	t_R	Area		t_R	Area	t_R	Area	Tailing Factor	
1	3.535	16731	3.911	10946	2.367	5.297	12045	5.810	21064649	1.120	2.572
2	3.534	16535	3.910	11008	2.373	5.296	12055	5.809	21035343	1.121	2.570
3	3.534	16649	3.910	11201	2.361	5.297	12039	5.809	21042222	1.119	2.573
4	3.534	16634	3.908	11082	2.373	5.296	12110	5.808	21019982	1.120	2.573
5	3.533	16542	3.907	11278	2.367	5.295	12094	5.807	21041221	1.120	2.570
6	3.533	16629	3.907	11344	2.368	5.294	11997	5.807	21044690	1.119	2.575
Average	3.534	16620	3.909	11143	2.368	5.296	12057	5.808	21041351	1.120	2.572
% RSD	0.018	0.44	0.04	1.41	0.18	0.02	0.34	0.02	0.07	0.05	0.08

Conclusion

The Luna 5 µm Phenyl-Hexyl column used for this study showed good separation of all Zoledronic acid related compounds according to Ph. Eur. monograph 2743. The system suitability criteria for the resolution between impurity A and Zoledronic acid (R_S ≥ 1.5) and the resolution between Nitrate and Impurity B (R_S ≥ 1.5) were achieved (**Table 2**). Therefore, the Luna 5 µm Phenyl-Hexyl 250 x 4.6 mm column is suitable for the analysis of Zoledronic acid and related substances following the Ph. Eur. monograph 2743.

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Australia
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 5010 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: +65 800-852-3944
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
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