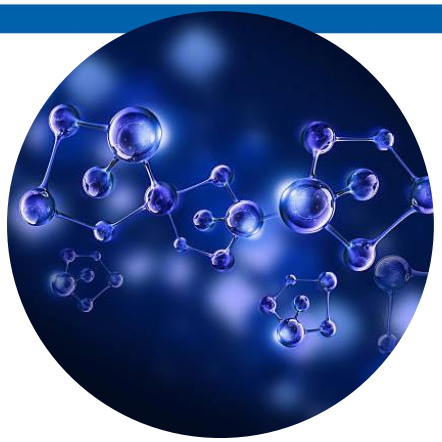

TN-1327

Modernization of USP and Ph. Eur. Method – Pantoprazole Sodium Sesquihydrate Organic Impurities

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Introduction

Pantoprazole is a proton pump inhibitor used for the treatment of stomach ulcers and short-term treatment of erosive esophagitis due to gastroesophageal reflux disease (GERD). This study shows the effective separation of Pantoprazole from its organic impurities and related substances according to Ph. Eur. Monograph 2296 and USP Monograph for Pantoprazole Sodium Sesquihydrate Organic Impurities Test 2. Further, as part of the modernization of the chromatographic method to improve the performance and reduce the time of analysis using smaller particles and shorter columns within the allowable adjustments of chromatographic conditions as per USP General Chapter <621> and Ph. Eur. Liquid Chromatography 2.2.46 general chapter guidelines. All the chromatographic conditions for the analysis mentioned in both Ph. Eur. and USP are the same except the concentration of the standard solution and the system suitability criteria as indicated below.

In this technical note, the fully porous Luna™ 5 µm C18(2), 125 x 4.0 mm (specified dimensions as per the monograph) column was used for the analysis of Pantoprazole. Further, modernization of this method was carried out on a fully porous, thermally modified, Luna Omega 3 µm C18, 100 x 3.0 mm column and a fully porous organo-silica Gemini™ 3 µm NX-C18, 100 x 3 mm column by scaling down the method to current column dimensions and the method has been verified with adjusted flow rate (rounding off to single decimal point) and gradient within the adjustments of chromatographic conditions allowed per USP and Ph. Eur. for gradient methods (**Table 1**).

System suitability per USP Monograph for Pantoprazole Sodium Sesquihydrate Organic Impurities Test 2 is resolution no less than (NLT) 1.5 between Pantoprazole related compound E and Pantoprazole related compounds D and F (which are observed to co-elute), a symmetry factor no more than (NMT) 2.0, and relative standard deviation (%RSD) NMT 5.0 %. System suitability per Ph. Eur. Monograph 2296 for Pantoprazole Sodium Sesquihydrate Related Substances is a minimum resolution of 1.5 between the peaks due to impurities E and D and F, and the chromatogram obtained is similar to the chromatogram supplied with Pantoprazole for System Suitability CRS (**Figure 2**).

All reference solutions were prepared as indicated in Ph. Eur. monograph 2296 for Pantoprazole Sodium Sesquihydrate. 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 30026F - 67081 Strasbourg (France):

- Y0000835, Pantoprazole Sodium Sesquihydrate CRS
- Y0001001, Pantoprazole for System Suitability CRS

Figure 1. Pantoprazole Sodium Sesquihydrate

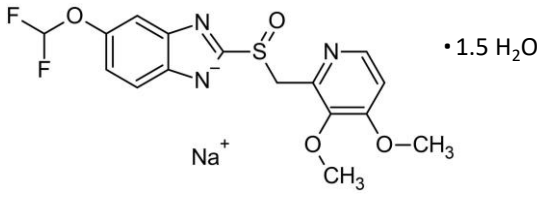


Table 1. Ph. Eur. and USP Adjustments of Chromatographic Conditions and Method Comparison.

Method Parameter	Allowable Adjustments	Ph. Eur. and USP Monograph Method	Proposed Modernization Method
Column Length (L) in mm	Not Specified	125 mm	100 mm
Column Diameter (dc) in mm	Not Specified	4.0 mm	3.0 mm
Column Particle Size (dp) in µm	Not Specified	5 µm	3 µm
L/dp	L/dp remains constant or between the -25 % to +50 % of prescribed ratio.	25,000 (125 mm / 5 µm)	33,333 (100 mm / 3 µm) (+33.33 %)
Flow Rate (F) in mL/min	Adjustment is permitted to maintain linear velocity when changing column dimensions.	1.0 mL/min	0.9 mL/min (Customized flow rounding off)
Gradient	%B 20 80 20 20	Time 0 40 45 60	Time 0 19.2 21.6 28.8
Column Temperature	± 5 %	40 °C	As Specified
Mobile Phase pH	± 0.2	7.0	As Specified
Injection Volume	Can be reduced so long as precision and detection limits are met.	20 µL	9 µL
Wavelength	None	290 nm and 305 nm	As Specified
Buffer Concentration	± 10 %	10 mM	As Specified

LC Conditions

Column: Luna 5 µm C18(2), 125 x 4.0 mm (00E-4252-D0)
Luna Omega 3 µm C18, 100 x 3.0 mm (00D-4784-Y0)
Gemini 3 µm NX-C18, 100 x 3.0 mm (00D-4453-Y0)

Mobile Phase: Mobile Phase (**Table 2**)

Gradient:

Monograph Method		Modernized Method	
Time (min)	**%B**	**Time (min)**	**%B**
0	20	0	20
40	80	19.2	80
45	20	21.6	20
60	20	28.8	20

Flow Rate: 1.0 mL/min – Monograph Method
0.9 mL/min – Modernized Method

Injection Volume: 20 µL – Monograph Method
9 µL - Modernized Method

Temperature: 40 °C

Detector: UV @ 290 nm and 305 nm

System: Waters® Arc HPLC

Table 2. Preparation of Test and Reference Solutions

Solution	Composition
Mobile Phase	A: 1.74 g/L of Dipotassium Hydrogen Phosphate, pH adjusted to 7.00 ± 0.05 with 330 g/L solution of Phosphoric Acid. B: Acetonitrile
Solvent Mixture – Ph. Eur.	Acetonitrile / 40 mg/L solution of Sodium Hydroxide (0.001 N Sodium Hydroxide in Water), (50:50, v/v).
Diluent - USP	
Test Solution	Dissolve 23 mg of Pantoprazole Sodium Sesquihydrate CRS in **Solvent Mixture** and dilute to 50.0 mL with **Solvent Mixture**.
Reference Solution (a) – Ph. Eur.	Dilute 1.0 mL of **Test Solution** to 100 mL with **Solvent Mixture**. Dilute 1.0 mL of this solution to 10 mL with **Solvent Mixture**.
Reference Solution (b) – Ph. Eur. **System Suitability Solution – USP**	Dissolve 2.5 mg of Pantoprazole for System Suitability CRS (containing impurities A, B, C, D, and E) in **Solvent Mixture** and dilute to 5.0 mL with **Solvent Mixture**.
Standard Solution – USP	0.03 mg/mL of Pantoprazole Sodium Sesquihydrate CRS in **Diluent**.

Figure 2. Reference Chromatogram for Pantoprazole Sodium Sesquihydrate from EDQM.

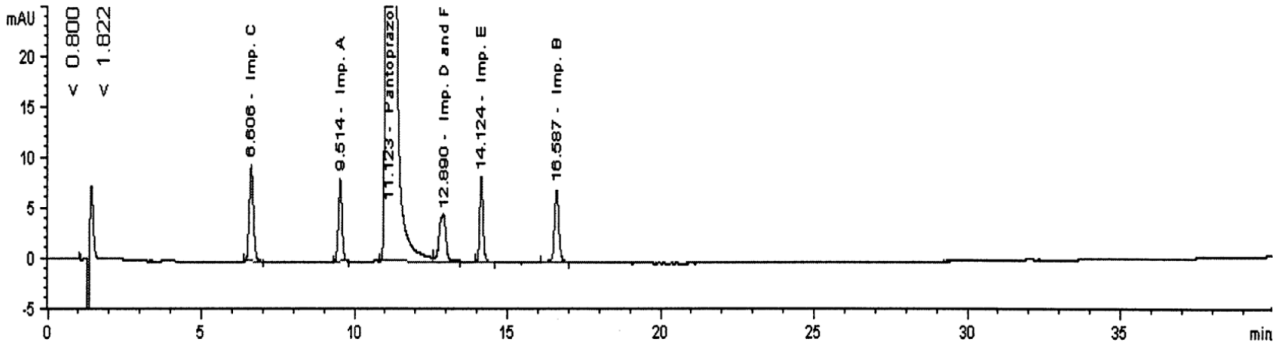
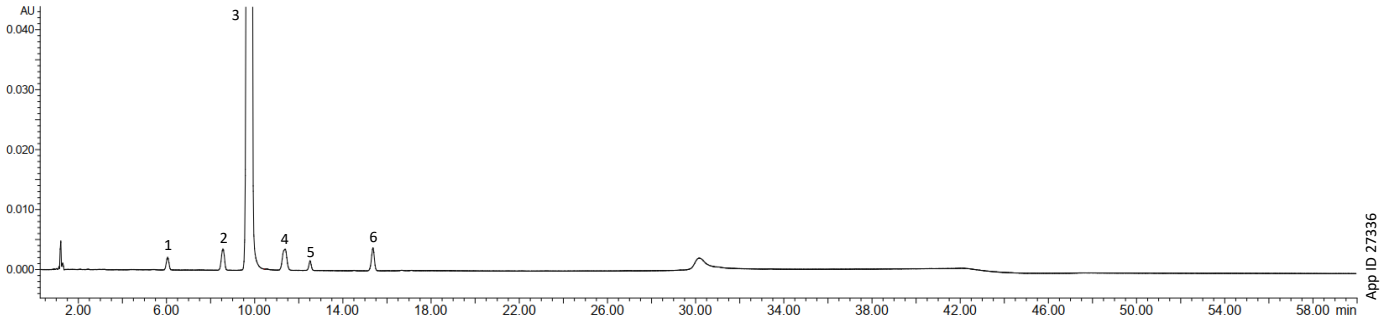
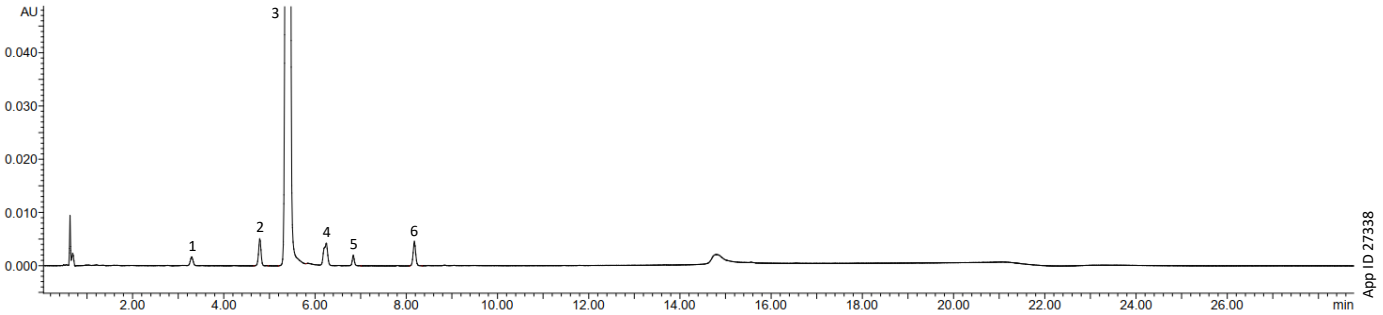


Figure 3. System Suitability Solution (Reference Solution (b)) for Monograph Method on a Luna™ 5 µm C18(2), 125 x 4.0 mm Column.



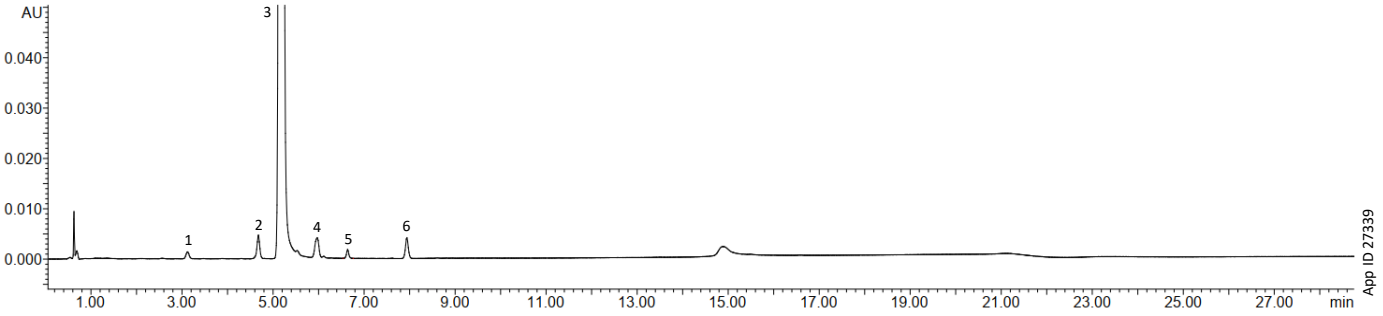
Peak No.	Analyte	Retention Time (min)	Relative Retention Time	Resolution (NLT 1.5)	Symmetry Factor
1	Related Impurity C	6.05	0.62	-	1.02
2	Related Impurity A	8.56	0.88	-	0.95
3	Pantoprazole Sodium Sesquihydrate	9.77	1.0	-	0.92
4	Related Impurities D and F	11.38	1.17	4.19	0.90
5	Related Impurity E	12.51	1.28		1.02
6	Related Impurity B	15.36	1.57	-	0.94

Figure 4. System Suitability Solution (Reference Solution (b)) for Modernized Method on a Luna™ Omega 3 µm C18, 100 x 3.0 mm Column.



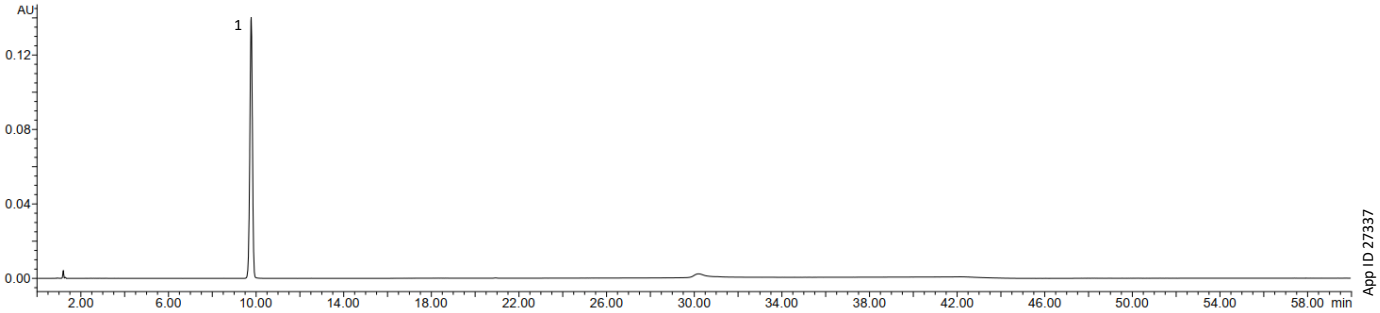
Peak No.	Analyte	Retention Time (min)	Relative Retention Time	Resolution (NLT 1.5)	Symmetry Factor
1	Related Impurity C	3.30	0.61	-	1.05
2	Related Impurity A	4.79	0.89	-	0.98
3	Pantoprazole Sodium Sesquihydrate	5.41	1	-	0.99
4	Related Impurities D and F	6.25	1.16	5.15	0.85
5	Related Impurity E	6.84	1.27		1.16
6	Related Impurity B	8.18	1.51	-	1.04

Figure 5. System Suitability Solution (Reference Solution (b)) for Modernized Method on a Gemini™ 3 µm NX-C18, 100 x 3.0 mm Column.



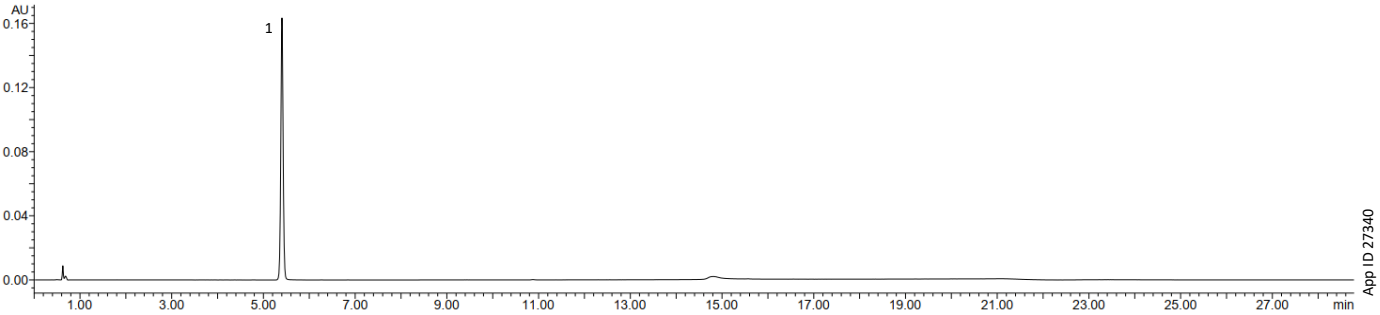
Peak No.	Analyte	Retention Time (min)	Relative Retention Time	Resolution (NLT 1.5)	Symmetry Factor
1	Related Impurity C	3.12	0.60	-	1.06
2	Related Impurity A	4.68	0.90	-	0.90
3	Pantoprazole Sodium Sesquihydrate	5.18	1	-	1.00
4	Related Impurities D and F	5.97	1.15	5.51	0.88
5	Related Impurity E	6.64	1.28		1.10
6	Related Impurity B	7.94	1.53	-	1.03

Figure 6. Standard Solution for Monograph Method on a Luna™ 5 µm C18(2), 125 x 4.0 mm Column.



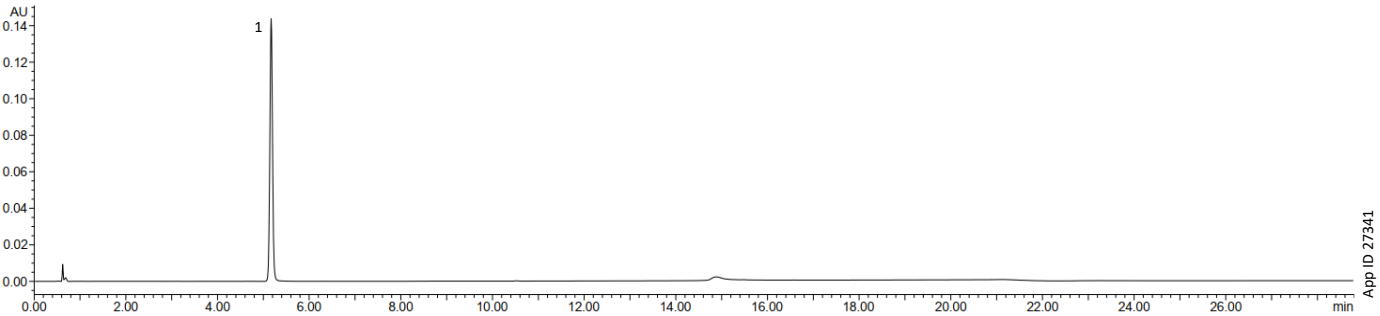
Peak No.	Analyte	Retention Time (min)	Area	Area %RSD	Symmetry Factor
1	Pantoprazole Sodium Sesquihydrate	9.78	1155049	0.2	0.94
N = 6 Injections					

Figure 7. Standard Solution for Modernized Method on a Luna Omega 3 µm C18, 100 x 3.0 mm Column.



Peak No.	Analyte	Retention Time (min)	Area	Area %RSD	Symmetry Factor
1	Pantoprazole Sodium Sesquihydrate	5.40	573814	0.1	1.03
N = 6 Injections					

Figure 8. Standard Solution for Modernized Method on a Gemini™ 3 µm NX-C18, 100 x 3.0 mm Column.



Peak No.	Analyte	Retention Time (min)	Area	Area %RSD	Symmetry Factor
1	Pantoprazole Sodium Sesquihydrate	5.17	576581	0.1	1.04
N = 6 Injections					

Conclusions

Pantoprazole Sodium Sesquihydrate Ph. Eur. monograph 2296 and Pantoprazole Sodium USP monograph methods were successfully analyzed on a Luna™ 5 µm C18(2), 125 x 4.0 mm column, run per the monograph conditions, and all system suitability requirements were met. The Gemini™ 3 µm NX-C18, 100 x 3.0 mm column is an ethylene-bridged organo-silica hybrid with an extended pH stability to pH 12. Since the Test Solution is strongly basic (Acetonitrile / 40 mg/L solution of Sodium Hydroxide (0.001 N Sodium Hydroxide in Water), (50:50, v/v), this is a preferred solution for a longer lifetime of the column. Modernization of analytical methods, as demonstrated here, helps to improve the performance and reduce the time of analysis (runtime was reduced by 50 % in the current method of analysis) using smaller particles and shorter columns. These method parameter changes met the allowable adjustments within the Adjustments of Chromatographic Conditions Criteria as per USP General Chapter <621> and Ph. Eur. Liquid chromatography 2.2.46 general chapter guidelines.

All requirements for system suitability were met for both the Pantoprazole Sodium Sesquihydrate Ph. Eur. monograph 2296 and the Pantoprazole Sodium USP monograph using a Luna Omega 3 µm C18, 100 x 3.0 mm column and a Gemini 3 µm NX-C18, 100 x 3.0 mm column under the modernized method shown here, demonstrating that either of these columns would be suitable for improving method performance and reducing run times.

Luna Omega Ordering Information

3 µm MidBore™ Columns (mm)				SecurityGuard™ Cartridges (mm)
Phases	**50 x 3.0**	**100 x 3.0**	**150 x 3.0**	**4 x 2.0* /10 pk**
Polar C18	00B-4760-Y0	00D-4760-Y0	00F-4760-Y0	AJ0-7600
PS C18	00B-4758-Y0	00D-4758-Y0	00F-4758-Y0	AJ0-7605
C18	00B-4784-Y0	00D-4784-Y0	00F-4784-Y0	AJ0-7611
SUGAR	—	—	00F-4775-Y0	AJ0-4496

For ID: 2.0-3.0 mm

Gemini Ordering Information

3 µm Microbore, Minibore, and MidBore Columns (mm)										SecurityGuard Cartridges
Phases	**50 x 1.0**	**20 x 2.0**	**30 x 2.0**	**50 x 2.0**	**100 x 2.0**	**150 x 2.0**	**50 x 3.0**	**100 x 3.0**	**150 x 3.0**	**4 x 2.0* /10pk**
C18	00B-4439-A0	00M-4439-B0	00A-4439-B0	00B-4439-B0	00D-4439-B0	00F-4439-B0	00B-4439-Y0	00D-4439-Y0	00F-4439-Y0	AJ0-7596
C6-Phenyl	—	—	—	00B-4443-B0	00D-4443-B0	00F-4443-B0	00B-4443-Y0	00D-4443-Y0	00F-4443-Y0	AJ0-7914
NX-C18	00B-4453-A0	00M-4453-B0	00A-4453-B0	00B-4453-B0	00D-4453-B0	00F-4453-B0	00B-4453-Y0	00D-4453-Y0	00F-4453-Y0	AJ0-8367

for ID: 2.0-3.0 mm

*SecurityGuard Analytical Cartridges require holder, Part No.: KJ0-4282

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