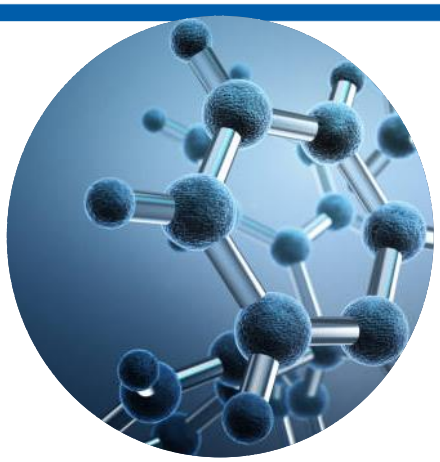

TN-1102

Enantiomeric Separation of Proton Pump Inhibitors Including Rabeprazole and Pantoprazole Using Lux™ Polysaccharide-Based Chiral Stationary Phases in Reversed Phase Conditions

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Introduction

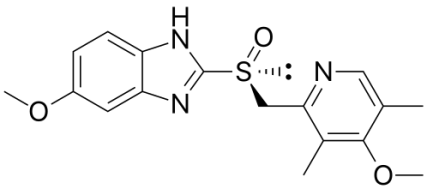
Proton pump inhibitors (PPI) are widely used globally to reduce acid secretion in the stomach, and to treat the symptoms associated with gastroesophageal reflux disease (GRD). Common PPI drugs include Esomeprazole (Nexium®), the S-enantiomer of the racemic drug Omeprazole (Prilosec®), Dexlansoprazole (Dexilant®), and the R-enantiomer of the racemic drug Lansoprazole (Prevacid®). Other racemic PPI drugs include Pantoprazole (Protonix®) and Rabeprazole (AcipHex®).

In this technical note, a chiral screen was performed on Lux polysaccharide-based columns to identify chiral stationary phases (CSP) for possible preparative scale separation of the enantiomers of four benzimidazoles: Omeprazole, Lansoprazole, Rabeprazole, and Pantoprazole under conditions suitable for mass spectroscopy (MS) detection. Optimization of the chromatographic conditions with respect to retention, enantio-separation, and resolution was achieved by variation of the mobile phase constituents at room temperature.

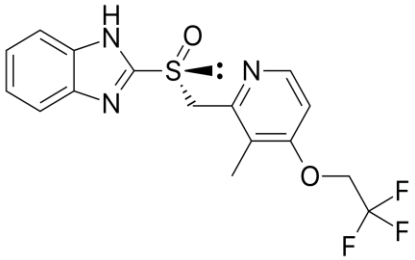
LC Conditions

Columns: Lux 5 µm Cellulose-1 (00G-4459-E0)
Lux 5 µm Cellulose-2 (00G-4457-E0)
Lux 5 µm Cellulose-3 (00G-4493-E0)
Lux 5 µm Cellulose-4 (00G-4491-E0)
Dimensions: 250 x 4.6 mm
Mobile Phase: See **Figures**
Flow Rate: 1.0 mL/min (Isocratic)
Injection Volume: See **Figures**
Temperature: Ambient
LC System: Agilent® 1100
Detection: UV @ 220 nm

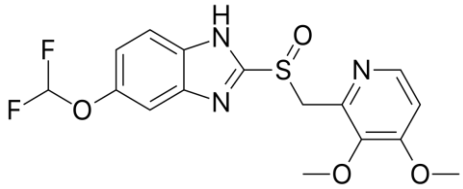
Figure 1 . Structures of Analytes.



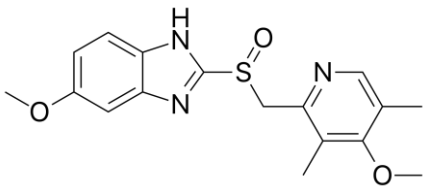
Esomeprazole (Nexium)



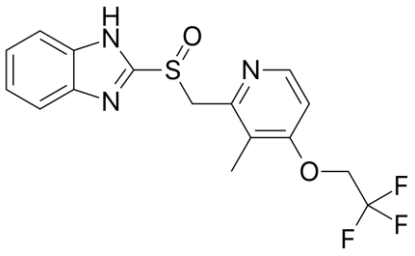
Dexlansoprazole (Dexilant)



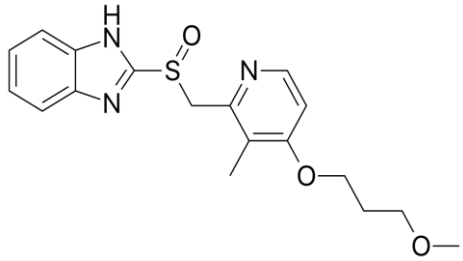
Pantoprazole (Protonix)



Omeprazole (Prilosec)



Lansoprazole (Prevacid)



Rabeprazole (AcipHex)

Results and Discussion

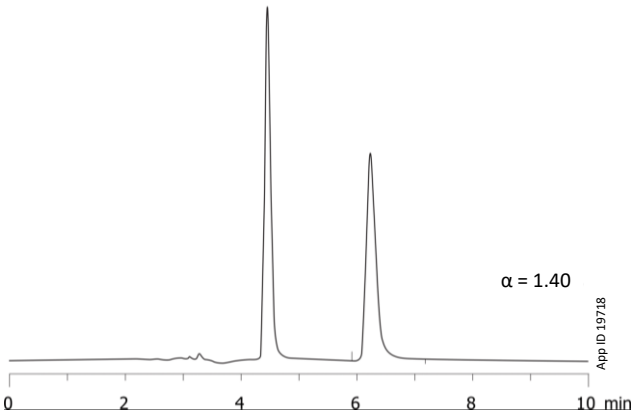
Four different polysaccharide-based chiral stationary phases (Lux™ Cellulose-1, Lux Cellulose-2, Lux Cellulose-3, and Lux Cellulose-4) were explored in the reversed phase elution mode for the enantio-separation of Omeprazole, Lansoprazole, Rabeprazole, and Pantoprazole using mobile phases consistent with LC-MS detection.

In order to reduce the volume of solvents used, the screening procedure was initially done on Lux 5 µm columns with dimensions of 150 x 4.6 mm. After the screening identified the best Lux polysaccharide phases, mobile phase conditions were further optimized on 250 mm length columns of the same particle size and column internal diameter. The best results are shown in Figures 2-5; Rabeprazole and Lansoprazole show optimal resolution on Lux Cellulose-4 whereas Omeprazole and Pantoprazole are best resolved on Lux Cellulose-2 phase.

Basic or acidic mobile phase additives are often required for improving resolution and peak shapes of ionizable analytes. Aqueous mobile phase buffer at higher pH with Ammonium salts such as Acetate or Bicarbonate (with Ammonia) can be effective in the chiral separation of basic racemic compounds. Ammonium salts are thermolabile, hence fully compatible with MS detectors, and even amenable to preparative purifications (as they can be easily removed from the final product).

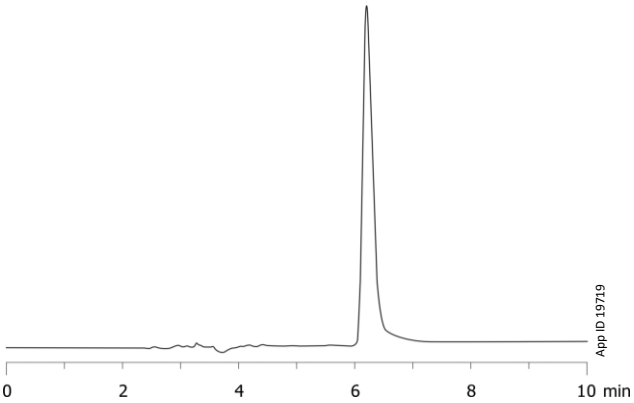
The chromatogram for the racemic Lansoprazole is shown in **Figure 2a**; while **Figure 2b** confirms the identity of the single enantiomer of Lansoprazole as Dexlansoprazole. Likewise, the chromatogram in **Figure 3b** confirms the identity of the single enantiomer of Omeprazole as Esomeprazole. The chromatogram for the racemic Omeprazole is shown in **Figure 3a**. Pantoprazole and Rabeprazole chromatograms shown in **Figures 4** and **5** are racemic mixes. The Lux Cellulose-2 and Lux Cellulose-4 columns, respectively, provide more than enough enantioselectivity to allow for chromatographic separation. This suggests that it would be possible to isolate enantiomerically pure compounds using Lux polysaccharide-based chiral columns.

Figure 2a. Lansoprazole (Prevacid®).



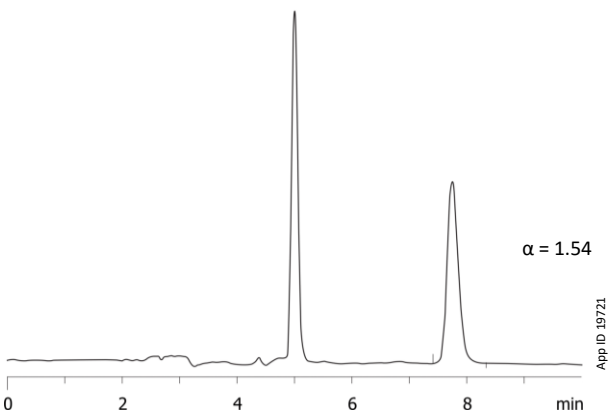
Column: Lux 5 µm Cellulose-4
Mobile Phase: Methanol / 20 mM Ammonium Bicarbonate / Diethyl Amine (90:10:0.1, v/v/v)
Injection Volume: 1 µL
Concentration: 1 mg/mL

Figure 2b. Dexlansoprazole (Dexilant®).



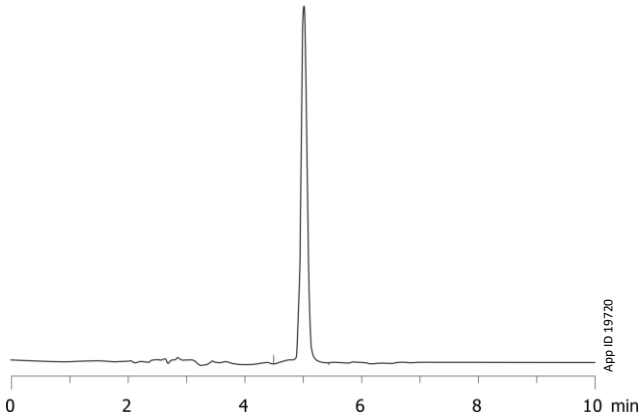
Column: Lux 5 µm Cellulose-4
Mobile Phase: Methanol / 20 mM Ammonium Bicarbonate / Diethyl Amine (90:10:0.1, v/v/v)
Injection Volume: 0.15 µL
Concentration: 3.75 mg/mL

Figure 3a. Omeprazole (Prilosec®).



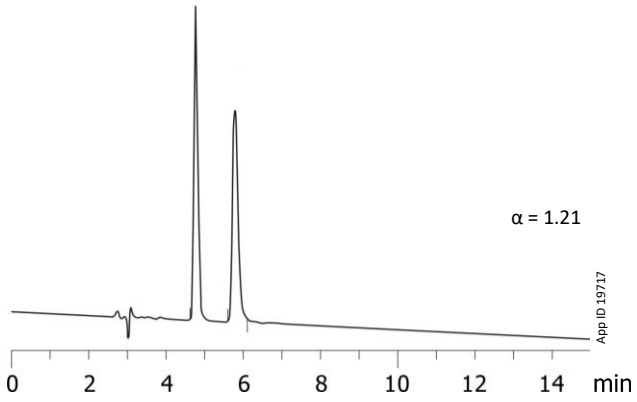
Column: Lux™ 5 µm Cellulose-2
Mobile Phase: Acetonitrile / 20 mM Ammonium Bicarbonate / Diethyl Amine (80:20:0.1, v/v/v)
Injection Volume: 1 µL
Concentration: 1 mg/mL

Figure 3b. Esomeprazole (Nexium®).



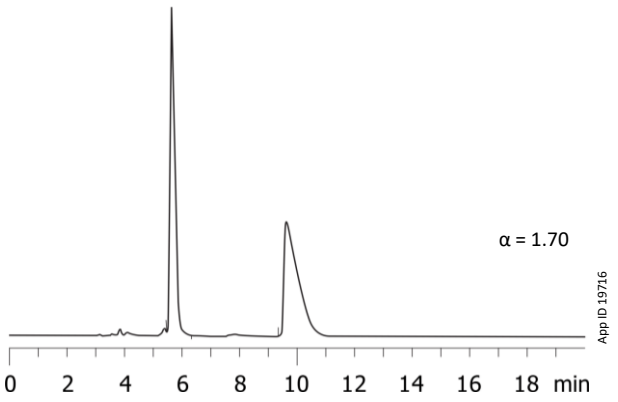
Column: Lux 5 µm Cellulose-2
Mobile Phase: Acetonitrile / 20 mM Ammonium Bicarbonate / Diethyl Amine (80:20:0.1, v/v/v)
Injection Volume: 0.5 µL
Concentration: 2.5 mg/mL

Figure 4. Pantoprazole (Protonix®).



Column: Lux 5 µm Cellulose-2
Mobile Phase: Acetonitrile / 10 mM Ammonium Bicarbonate / Diethyl Amine (60:40:0.1, v/v/v)
Injection Volume: 10 µL
Concentration: 0.5 mg/mL

Figure 5. Rabeprazole (AcipHex®).



Column: Lux 5 µm Cellulose-4
Mobile Phase: Methanol / 10 mM Ammonium Bicarbonate / Diethyl Amine (80:20:0.1, v/v/v)
Injection Volume: 10 µL
Concentration: 0.5 mg/mL

Conclusions

The HPLC analysis of the four benzimidazoles (Omeprazole, Lansoprazole, Rabeprazole, and Pantoprazole) allows for fast and accurate identification of their enantiomers. In this technical note, we described the successful separation under reversed phase conditions of both Pantoprazole and Rabeprazole. Based on previous work, the separation of Pantoprazole and Rabeprazole can be achieved without the use of base additives such as DEA. Finally, these analytical reversed phase conditions can be developed and scaled-up for the preparative chiral purification of enantiomerically pure forms of racemic active ingredients Pantoprazole and Rabeprazole.

Lux™ Ordering Information

5 µm Minibore and Analytical Columns (mm)						SecurityGuard™ Cartridges (mm)	
Phases	**50 x 2.0**	**50 x 4.6**	**100 x 4.6**	**150 x 4.6**	**250 x 4.6**	**4 x 2.0***	**4 x 3.0***
						/10pk	/10pk
i-Amylose-1	00B-4762-B0	00B-4762-E0	00D-4762-E0	00F-4762-E0	00G-4762-E0	AJ0-8640	AJ0-8641
i-Amylose-3	—	00B-4779-E0	00D-4779-E0	00F-4779-E0	00G-4779-E0	AJ0-8651	AJ0-8650
i-Cellulose-5	—	00B-4756-E0	00D-4756-E0	00F-4756-E0	00G-4756-E0	AJ0-8631	AJ0-8632
Cellulose-1	—	00B-4459-E0	00D-4459-E0	00F-4459-E0	00G-4459-E0	AJ0-8402	AJ0-8403
Cellulose-2	00B-4457-B0	00B-4457-E0	00D-4457-E0	00F-4457-E0	00G-4457-E0	AJ0-8398	AJ0-8366
Cellulose-3	—	00B-4493-E0	00D-4493-E0	00F-4493-E0	00G-4493-E0	AJ0-8621	AJ0-8622
Cellulose-4	—	—	00D-4491-E0	00F-4491-E0	00G-4491-E0	AJ0-8626	AJ0-8627
Amylose-1	00B-4732-B0	—	00D-4732-E0	00F-4732-E0	00G-4732-E0	AJ0-9337	AJ0-9336
					for ID:	2.0-3.0 mm	3.2-8.0 mm

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

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