

## Enantiomeric Separation of Proton Pump Inhibitors Including Rabeprazole and Pantoprazole Using Lux<sup>®</sup> Polysaccharide-Based Chiral Stationary Phases in Reversed Phase Conditions

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### Abstract

We demonstrate in this technical note the successful separation of proton pump inhibitors (PPI) Omeprazole, Lansoprazole, Rabeprazole and Pantoprazole using Lux polysaccharide-based columns. These chiral separations indicate the potential to prepare enantiomerically pure forms of racemic active ingredients Pantoprazole (Protonix) and Rabeprazole (Aciphex) using chiral chromatography.

### Introduction

The competition for market share between PPI is fierce. Among therapeutic agents, PPI accounted for \$13.6 billion of the total \$300.3 billion in sales for the year 2009<sup>1</sup>. In terms of total United States prescription revenues, the PPI Nexium<sup>2</sup> is second only to Lipitor, a member of the drug class known as statins, used for lowering blood cholesterol.

Esomeprazole **1**, the active ingredient for the drug Nexium is the S enantiomer form of Omeprazole **2**, the active ingredient for the drug Prilosec. The patent for Prilosec, which posted sales of \$5.6 billion in 2001, expired in 2002. Generic Omeprazole may have eroded the market share of Prilosec significantly had it not been for the chiral separation, subsequent asymmetric synthesis, and timely marketing of Esomeprazole. By the end of 2002 combined sales of Nexium and Prilosec were nearly \$6.6 billion.

This marketing patent-loss pattern has also been demonstrated with the introduction of Dexlansoprazole **3** (Kapidex) which is the R enantiomer of Lansoprazole **4** (Prevacid) and which was approved by the FDA in 2009 corresponding to the expiration of patent protection for the drug Prevacid.

A chiral screen was performed on Phenomenex Lux polysaccharide-based columns to identify chiral stationary phases (CSP) for possible preparative scale separation of the enantiomers of four benzimidazoles: Omeprazole **2**, Lansoprazole **4**, Rabeprazole **5** and Pantoprazole **6** under conditions suitable for mass spectroscopy (MS) detection. Optimization of the chromatographic conditions with respect to retention, enantioseparation, and resolution was achieved by variation of the mobile phase constituents at room temperature. The structures of the four analytes are depicted in **Figure 1**.

### Materials and Methods

All Analyses were performed using an HPLC Agilent<sup>®</sup> 1100 series (Agilent Technologies, Palo Alto, CA, USA).

Chiral chromatographic separations followed by UV detection were performed using Lux Cellulose-1, Lux Cellulose-2, Lux Cellulose-3, Lux Cellulose-4, and Lux Amylose-2 HPLC columns with dimensions 250 mm x 4.6 mm ID packed with 5  $\mu$ m particles (Phenomenex, Torrance CA USA).

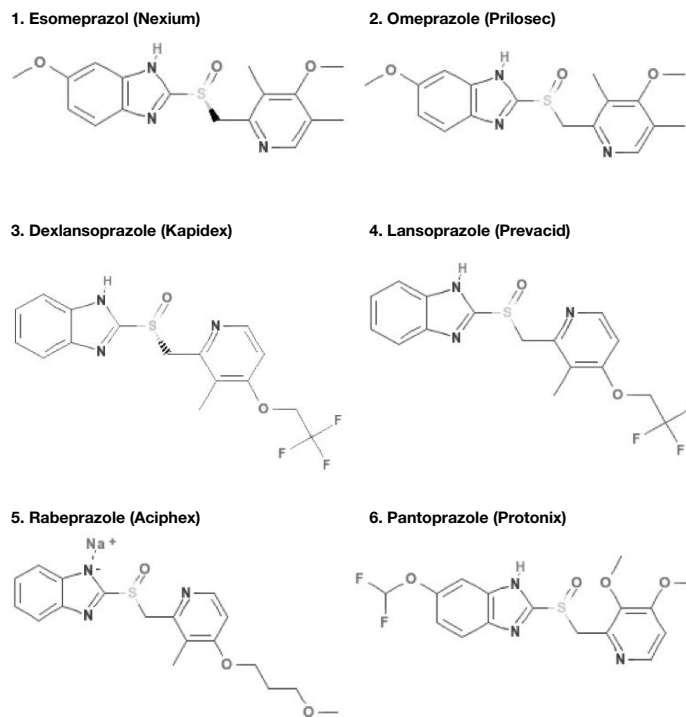
The system flow rate was set to 1 mL/min and the column temperature was ambient. The mobile phases consisted of acetonitrile or methanol with 0.1% diethyl amine (DEA) (solvent A) and 20 mMolL ammonium bicarbonate with 0.1% DEA (solvent B).

### Results and Discussion

Five different polysaccharide-based chiral stationary phases Lux Cellulose-1, Lux Cellulose-2, Lux Cellulose-3, Lux Cellulose-4, and Lux Amylose-2 were explored in the reversed phase elution mode for the enantioseparation of Omeprazole **2**, Lansoprazole **4**, Rabeprazole **5** and Pantoprazole **6** 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  $\mu$ m columns with dimension 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 **5** and Lansoprazole **4** show optimal resolution on Lux Cellulose 4 whereas Omeprazole **2** and Pantoprazole **6** are best resolved on Lux Cellulose 2 phase.

**Figure 1. Structures of Analytes**



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Basic or acidic mobile phase additives are often required for improving resolution and peak shapes of ionisable analytes. Aqueous mobile phase buffer at higher pH with ammonium salts such as acetate or hydrogencarbonate (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 **4** 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**.

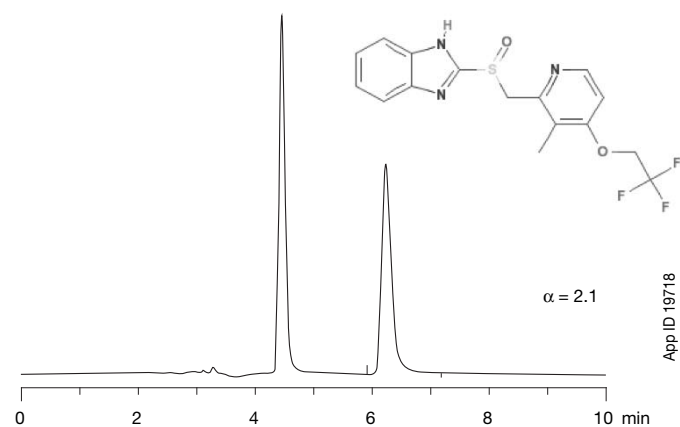
Pantoprazole and Rabeprazole chromatograms shown in **Figures 4** and **5** are racemic mixes. The Lux Cellulose-2 and Lux Cellulose-4 columns, respectively, provide more than enough enantioselectivity to allow for chromatographic separation. This suggests that it would be possible to isolate enantiomerically pure compounds using Lux polysaccharide-based chiral columns.

### Conclusion

The HPLC analysis of the four benzimidazoles Omeprazole, Lansoprazole, Rabeprazole and Pantoprazole allows for fast and accurate identification of their enantiomers. The proven success of marketing enantiomerically pure pharmaceutical ingredients (such as Esomeprazole and Dexlansoprazole) after expiration for the patent of their racemic formulations suggests that chiral purification of Pantoprazole and Rabeprazole will lead to similar success.

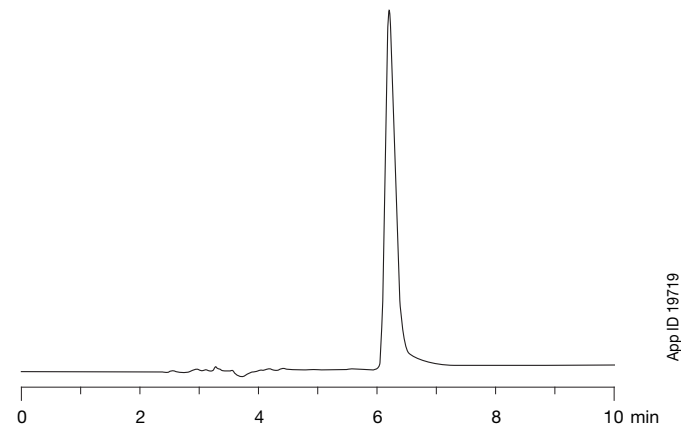
In this technical note, we described the successful separation under reversed phase conditions of both Pantoprazole and Rabeprazole. Based on previous work done at Phenomenex, the separation of Pantoprazole and Rabeprazole can be achieved without the use of base additives such as DEA<sup>3</sup>. Finally, 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.

**Fig. 2a Lansoprazole (Prevacid)**



**Column:** Lux<sup>®</sup> 5  $\mu$ m Cellulose-4  
**Dimensions:** 250 x 4.6 mm  
**Part No.:** 00G-4491-E0  
**Injection Volume:** 1  $\mu$ L  
**Concentration:** 1 mg/mL  
**Mobile Phase:** 90/10/0.1  
Methanol/20 mMol of  $\text{NH}_4\text{HCO}_2$ /DEA

**Fig. 2b Dexlansoprazole (Kapidex)**

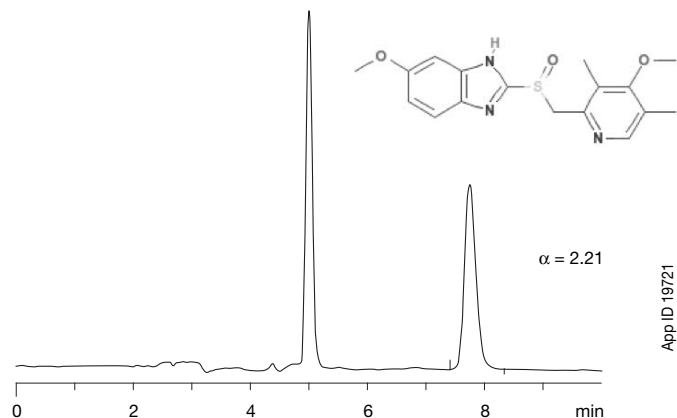


**Column:** Lux<sup>®</sup> 5  $\mu$ m Cellulose-4  
**Dimensions:** 250 x 4.6 mm  
**Part No.:** 00G-4491-E0  
**Injection Volume:** 0.15  $\mu$ L  
**Concentration:** 3.75 mg/mL  
**Mobile Phase:** 90/10/0.1  
Methanol/20 mMol of  $\text{NH}_4\text{HCO}_2$ /DEA

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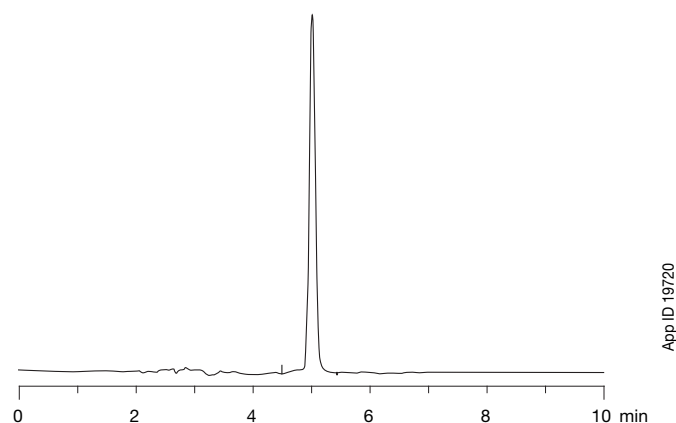
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**Fig. 3a Omeprazole (Prilosec)**



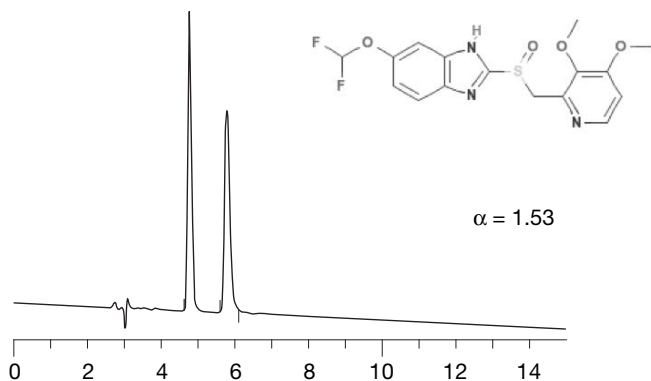
**Column:** Lux<sup>®</sup> 5 μm Cellulose-2  
**Dimensions:** 250 x 4.6 mm  
**Part No.:** 00G-4457-E0  
**Injection Volume:** 1 μL  
**Concentration:** 1 mg/mL  
**Mobile Phase:** 80/20/0.1  
 Acetonitrile/20 mMol NH<sub>4</sub> HCO<sub>3</sub>/DEA

**Fig. 3b Esomeprazole (Nexium)**



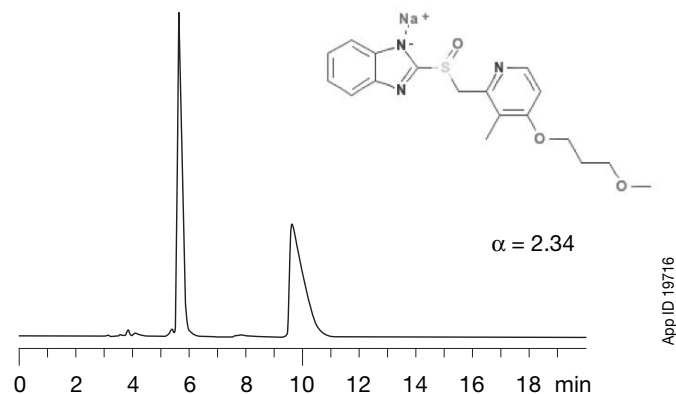
**Column:** Lux<sup>®</sup> 5 μm Cellulose-2  
**Dimensions:** 250 x 4.6 mm  
**Part No.:** 00G-4457-E0  
**Injection Volume:** 0.5 μL  
**Concentration:** 2.5 mg/mL  
**Mobile Phase:** 80/20/0.1  
 Acetonitrile/20 mMol NH<sub>4</sub> HCO<sub>3</sub>/DEA

**Fig. 4 Pantoprazole (Protonic)**



**Column:** Lux<sup>®</sup> 5 μm Cellulose-2  
**Dimensions:** 250 x 4.6 mm  
**Part No.:** 00G-4457-E0  
**Injection Volume:** 10 μL  
**Concentration:** 0.5 mg/mL  
**Mobile Phase:** 60/40/0.1  
 Acetonitrile/10 mMol NH<sub>4</sub> HCO<sub>3</sub>/DEA

**Fig. 5 Rabeprazole (Aciphex)**



**Column:** Lux<sup>®</sup> 5 μm Cellulose-4  
**Dimensions:** 250 x 4.6 mm  
**Part No.:** 00G-4491-E0  
**Injection Volume:** 10 μL  
**Concentration:** 0.5 mg/mL  
**Mobile Phase:** 80/20/0.1  
 Methanol/10 mMol NH<sub>4</sub> HCO<sub>3</sub>/DEA

### References

1. Source IMS Health, IMS National Sales Perspectives; Top Therapeutic Classes by U.S. Sales, 2009
2. Source IMS Health, IMS National Sales Perspectives; Top 15 U.S. Pharmaceutical Products by Sales 2009
3. Phenomenex Technical Note TN-1079: Method Development for Reversed Phase Chiral LC/MS/MS Analysis of Stereoisomeric Pharmaceutical Compounds with Polysaccharide-Based Stationary Phases.

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# APPLICATIONS

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### 3 µm Analytical Columns (mm)

	50 x 2.0	150 x 2.0	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	SecurityGuard™ Cartridges (mm)	
Phases							4 x 2.0*	4 x 3.0*
Cellulose-4	00B-4490-B0	00F-4490-B0	00B-4490-E0	00D-4490-E0	00F-4490-E0	00G-4490-E0	AJO-8626	AJO-8627
Cellulose-3	00B-4492-B0	00F-4492-B0	00B-4492-E0	00D-4492-E0	00F-4492-E0	00G-4492-E0	AJO-8621	AJO-8622
Cellulose-2	00B-4456-B0	00F-4456-B0	00B-4456-E0	00D-4456-E0	00F-4456-E0	00G-4456-E0	AJO-8398	AJO-8366
Cellulose-1	00B-4458-B0	00F-4458-B0	00B-4458-E0	00D-4458-E0	00F-4458-E0	00G-4458-E0	AJO-8402	AJO-8403
Amylose-2	00B-4471-B0	00F-4471-B0	00B-4471-E0	00D-4471-E0	00F-4471-E0	00G-4471-E0	AJO-8471	AJO-8470

for ID: 2.0–3.0 mm 3.2–8.0 mm

### 5 µm Analytical Columns (mm)

	50 x 2.0	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	SecurityGuard™ Cartridges (mm)	
Phases						4 x 2.0*	4 x 3.0*
Cellulose-4	00B-4491-B0	00B-4491-E0	00D-4491-E0	00F-4491-E0	00G-4491-E0	AJO-8626	AJO-8627
Cellulose-3	00B-4493-B0	00B-4493-E0	00D-4493-E0	00F-4493-E0	00G-4493-E0	AJO-8621	AJO-8622
Cellulose-2	00B-4457-B0	00B-4457-E0	00D-4457-E0	00F-4457-E0	00G-4457-E0	AJO-8398	AJO-8366
Cellulose-1	00B-4459-B0	00B-4459-E0	00D-4459-E0	00F-4459-E0	00G-4459-E0	AJO-8402	AJO-8403
Amylose-2	00B-4472-B0	00B-4472-E0	00D-4472-E0	00F-4472-E0	00G-4472-E0	AJO-8471	AJO-8470

for ID: 2.0–3.0 mm 3.2–8.0 mm

### 5 µm Semi-Prep Columns (mm)

	150 x 10.0	250 x 10.0	10 x 10.0*
Phases			
Cellulose-4	00F-4491-N0	00G-4491-N0	AJO-8628
Cellulose-3	00F-4493-N0	00G-4493-N0	AJO-8623
Cellulose-2	00F-4457-N0	00G-4457-N0	AJO-8399
Cellulose-1	00F-4459-N0	00G-4459-N0	AJO-8404
Amylose-2	00F-4472-N0	00G-4472-N0	AJO-8472

for ID: 9-16 mm

### SecurityGuard™ Cartridges (mm)

### 20 µm Bulk Media

	100 g	1 kg
Phases		
Cellulose-1	04G-4473	04K-4473
Cellulose-2	04G-4464	04K-4464
Cellulose-3	04G-4504	04K-4504
Cellulose-4	04G-4503	04K-4503

\*Please inquire for 20 µm Lux™ Amylose-2

### 5 µm Axia™ Packed Preparative Columns (mm)

	150 x 21.2	250 x 21.2	250 x 30	250 x 50	SecurityGuard™ Cartridges (mm)	
Phases					15 x 21.2**	15 x 30.0*
Cellulose-4	00F-4491-P0-AX	00G-4491-P0-AX	00G-4491-U0-AX	00G-4491-V0-AX	AJO-8629	AJO-8630
Cellulose-3	00F-4493-P0-AX	00G-4493-P0-AX	00G-4493-U0-AX	00G-4493-V0-AX	AJO-8624	AJO-8625
Cellulose-2	00F-4457-P0-AX	00G-4457-P0-AX	00G-4457-U0-AX	00G-4457-V0-AX	AJO-8400	AJO-8401
Cellulose-1	00F-4459-P0-AX	00G-4459-P0-AX	00G-4459-U0-AX	00G-4459-V0-AX	AJO-8405	AJO-8406
Amylose-2	00F-4472-P0-AX	00G-4472-P0-AX	00G-4472-U0-AX	00G-4472-V0-AX	AJO-8473	AJO-8474

for ID: 18–29 mm 30–49 mm

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

\*\*SemiPrep SecurityGuard Cartridges require holder, Part No. : AJO-7220

\*\*\*PREP SecurityGuard Cartridges require holder, Part No. : AJO-8223

\*PREP SecurityGuard Cartridges require holder, Part No. : AJO-8277



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