Enantiomeric and Diastereoisomeric Resolutions of Chiral Triazole Fungicides using Lux® Polysaccharide-Based Chiral Stationary Phases

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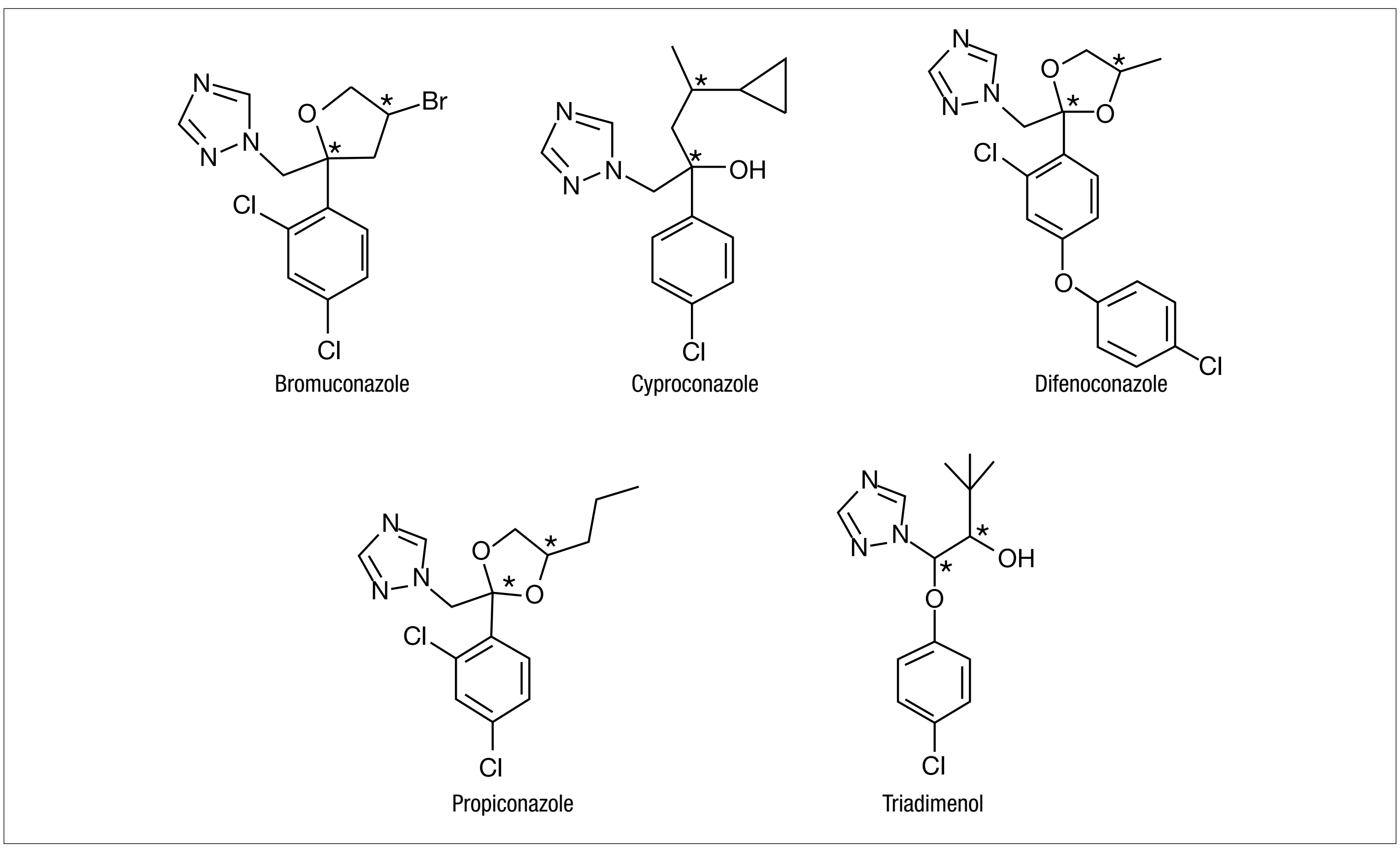
In this technical note, we report the enantiomeric and diastereoisomeric separations of five fungicides containing two stereogenic centers using Lux polysaccharide-based chiral stationary phases. The reported separations are the results of a systematic screening of five different Lux phases in normal phase and reversed phase separation modes. For each compound screened, baseline resolution of the four different stereoisomers is provided with a run time below 25 minutes.

Introduction

Fungicides have many positive uses such as increasing food production, decreasing damage to crops, reducing plant diseases, and more, but they also pose risks to humans and the environment. Of the 1693 pesticides listed in a recent review,[1] 482 (28 %) are chiral (chemical compounds containing one or more centers of asymmetry) of which 104 are classified as fungicides. The degradation of those chiral fungicides by soil microbes is stereoselective and each stereoisomer will be eliminated from the environment following a different pathway.[2,3] The degradation difference of chiral fungicides, combined with possible stereospecific toxicity can affect not only efficacy, but also exposure and risk to humans and environment.[3] In the pharmaceutical industry, mainly due to the potential stereospecific toxicity, chiral drugs are routinely tested for chiral purity, whereas pesticides generally are not.

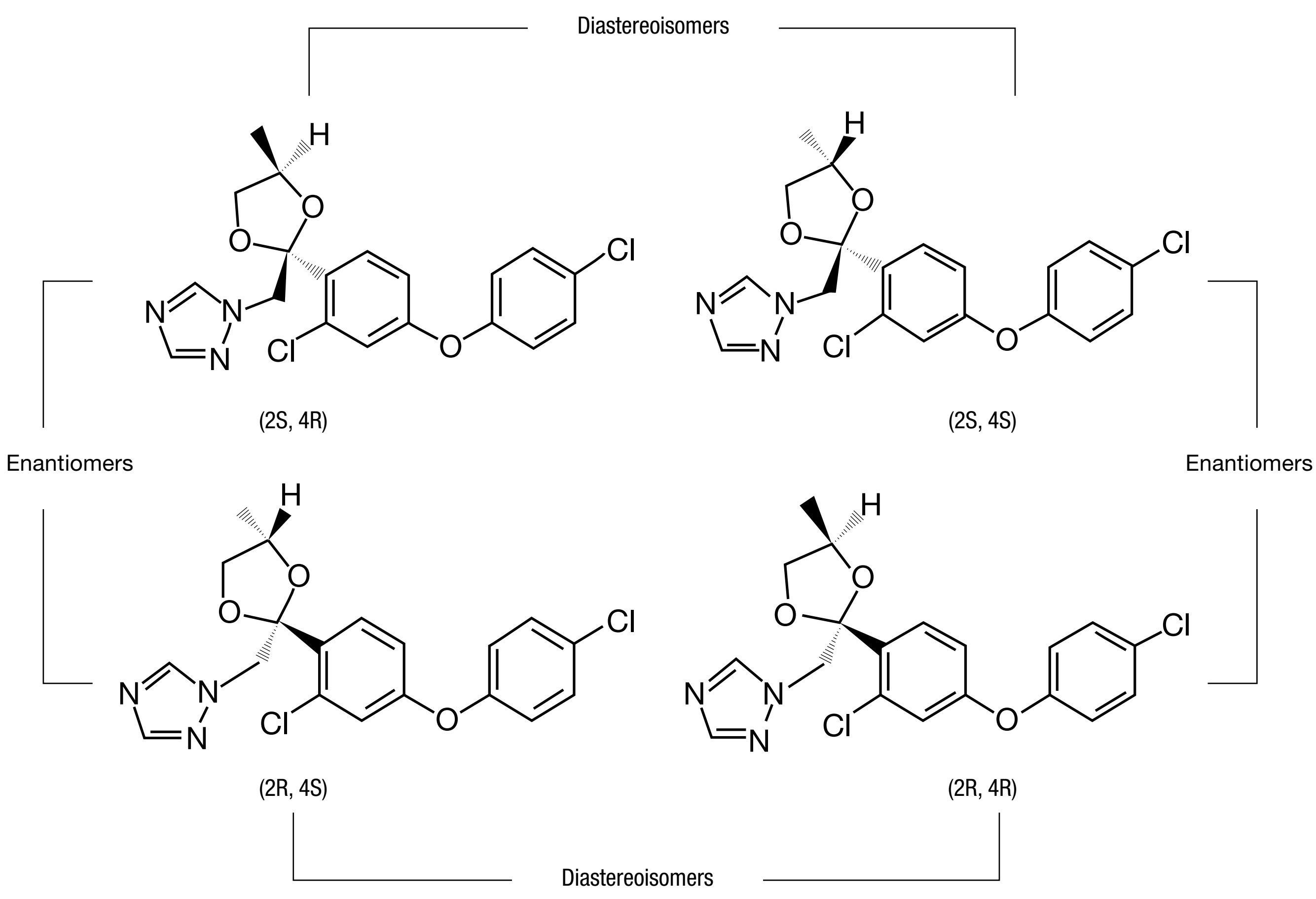
In this application note, we present the enantiomeric and diastereoisomeric separations of five triazole fungicides: Bromuconazole, Cyproconazole, Difenoconazole, Propiconazole, and Triadimenol. The chemical structure for each fungicide is represented in **Figure 1**.

Figure 1.
Chemical structure of chiral fungicides



All triazole fungicides evaluated in this application contain two stereogenic centers and therefore can have four stereoisomers as depicted in **Figure 2** for the example of Difenoconazole. The stereoisomers that are mirror images are also called enantiomers (SS/RR and SR/RS). Enantiomers can be separated from each other by chiral chromatography using chiral stationary phases (CSPs) in high performance liquid chromatography (HPLC). HPLC is recognized as the most popular and reliable tool for both analytical and preparative separation of chiral compounds.[4] As a matter of fact, 76 % of the analytical chiral separations reported in the recent chiral pesticides review[1] were performed by HPLC; gas chromatography (GC) was second with 18 % of the separations reported. Polysaccharide-based CSPs such as Lux® are the most widely used phases for the chromatographic separation of enantiomers.[4,5] Those CSPs show excellent success rate for chiral separation of a broad range of chiral compounds, as well as high loading ability for preparative applications under both, HPLC[6] and supercritical fluid chromatography (SFC)[7].

Figure 2.
Structure of stereoisomeres for Difenoconazole



Material and Methods

All HPLC analyses were performed using an Agilent® 1100 series LC system (Agilent Technologies, Inc., Palo Alto, CA, USA) equipped with quaternary pump, in-line degasser, multi-wavelength UV detector, and autosampler. Lux® columns used for analysis were obtained from Phenomenex (Torrance, CA, USA). The HPLC column dimensions were 250 x 4.6 mm ID and all columns were packed with 5 µm particles. The flow rate was 1.0 mL/min and temperature was ambient. Standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). All solvents were purchased from EMD (San Diego, CA, USA).

Results and Discussion

The five triazole fungicides depicted in **Figure 1** were analyzed on Lux polysaccharide-based CSPs (Cellulose-1, Cellulose-2, Cellulose-3, Cellulose-4, and Amylose-2) in normal phase (NP) and reversed phase (RP) separation modes. After performing a systematic screening, the separations that showed optimum resolution between all the peak were selected, even though in most of the cases, alternative separation was obtained with other Lux phases and/or modes. The separation results as well as the selectivity between each stereoisomer are summarized in **Table 1**. For each fungicide screened, we provide the chemical identification number (CID). This unique number can be linked to The PubChem Project website for further research regarding each compound's pharmaceutical properties. Additionally, the Lux phases used, the retention time of the first and last stereoisomers, as well as the isocratic conditions used for each compound are listed in **Table 1**. As expected, polysaccharide-based Lux columns are quite successful at resolving chiral compounds of this type. For each fungicide tested, all the stereoisomers are separated with selectivity greater or equal to 1.1 between adjacent peaks. In the last column of the **Table 1**, the corresponding Phenomenex application number is provided. Those applications are easily accessible on our website (www.phenomenex.com) and can be searched by application number, structure, CID, or compound name.

Table 1.
Enantiomeric and diastereoisomeric separations of fungicides using Lux polysaccharide-based CSPs

Analyte	CID	CSPs	Mobile Phase	Rt_1 (min)	Rt_4 (min)	$\alpha_{(1,2)}$	$\alpha_{(2,3)}$	$\alpha_{(3,4)}$	App ID
Bromuconazole	3444	Lux Cellulose-2	ACN/20mM NH_4HCO_3 (60:40) DEA (0.1 %)	14.63	23.18	1.36	1.14	1.11	21751
Cyproconazole	86132	Lux Cellulose-4	ACN/20mM NH_4HCO_3 (60:40) DEA (0.1 %)	6.44	9.16	1.20	1.18	1.25	21755
Difenoconazole	86173	Lux Cellulose-3	Hexane/EtOH (85:15) DEA (0.1 %)	11.07	15.10	1.07	1.07	1.29	21681
Propiconazole	43234	Lux Cellulose-1	Hexane/IPA (80:20) DEA (0.1 %)	6.83	10.16	1.33	1.21	1.12	21726
Triadimenol	41368	Lux Cellulose-2	Hexane/IPA (80:20) DEA (0.1 %)	5.06	7.58	1.28	1.36	1.19	21739

ACN = Acetonitrile, IPA = Isopropanol, EtOH = Ethanol, DEA = Diethylamine, NH_4HCO_3 = Ammonium bicarbonate

The enantiomeric and diastereoisomeric separations for the stereoisomers of Bromuconazole, Cyproconazole, Difenoconazole, Propiconazole and Triadimenol are respectively shown in **Figure 3, 4, 5, 6** and **7**.

Figure 3.
Stereoselective HPLC analysis on the stereoisomers mix of Bromuconazole

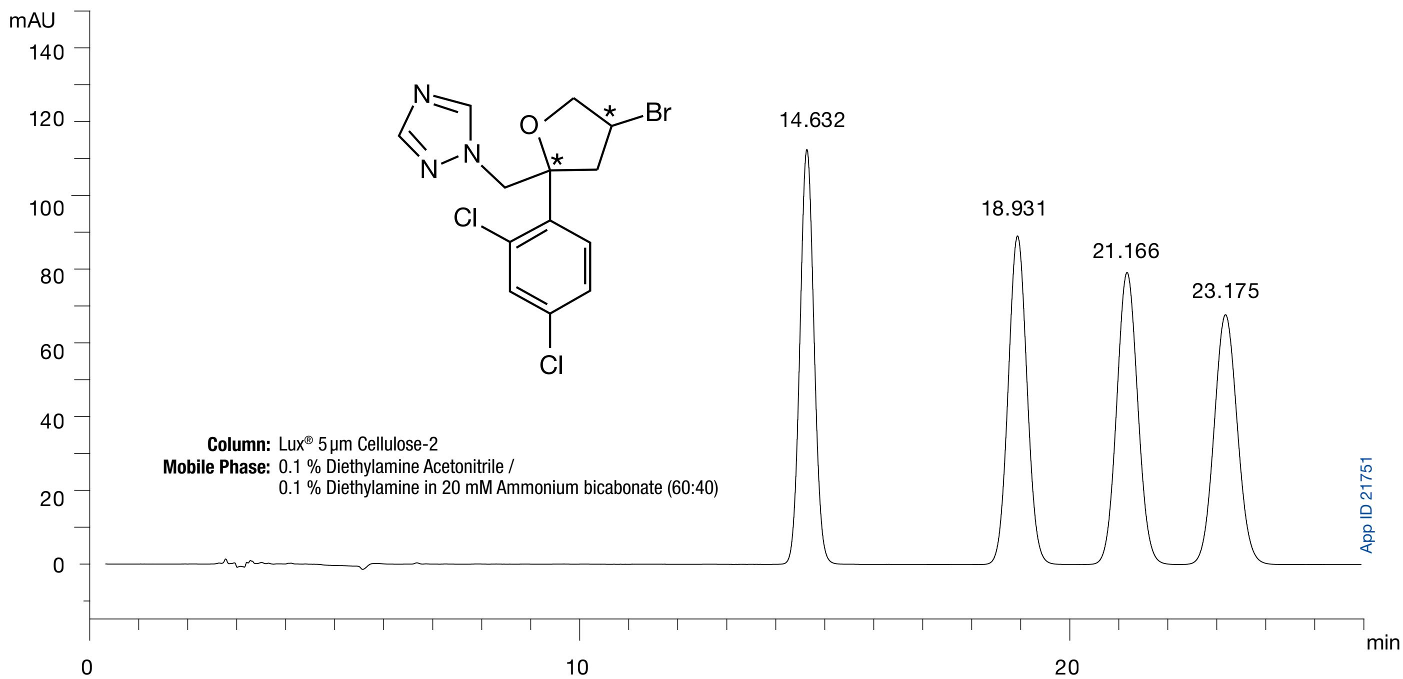


Figure 4.
Stereoselective HPLC analysis on the stereoisomers mix of Cyproconazole

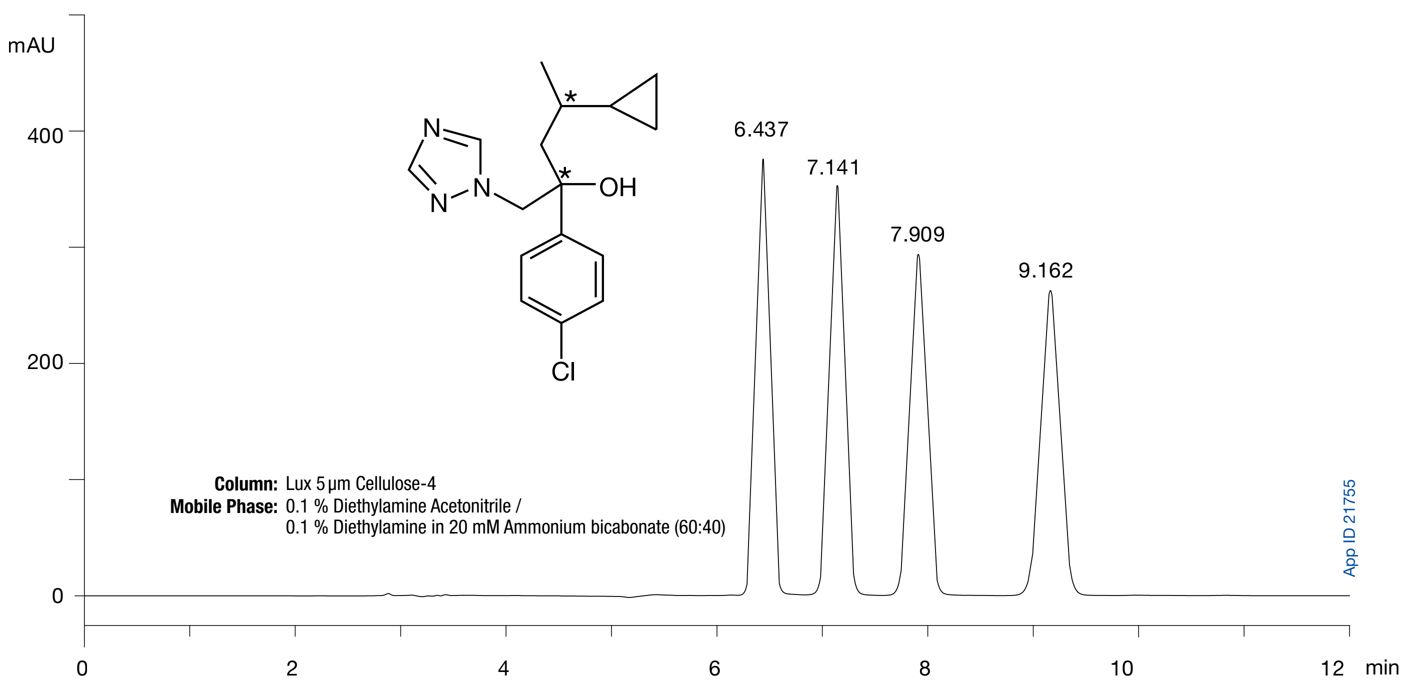


Figure 5.
Stereoselective HPLC analysis on the stereoisomers mix of Difenoconazole

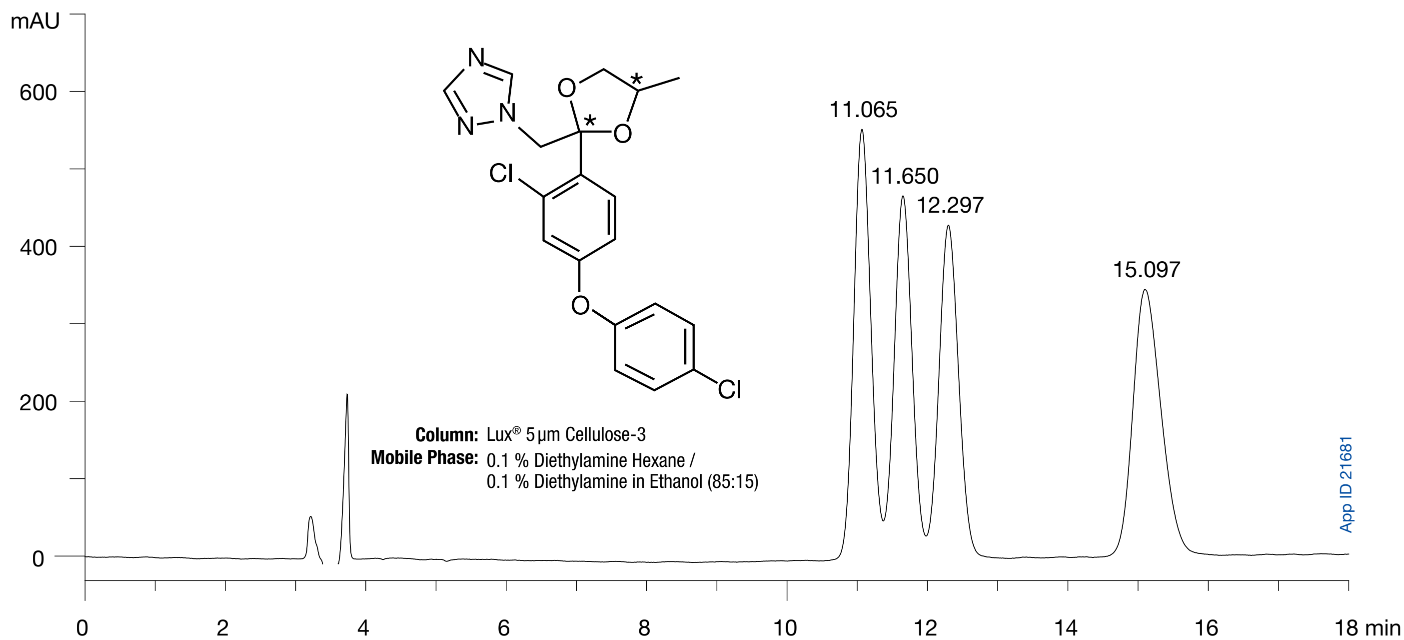


Figure 6.
Stereoselective HPLC analysis on the stereoisomers mix of Propiconazole

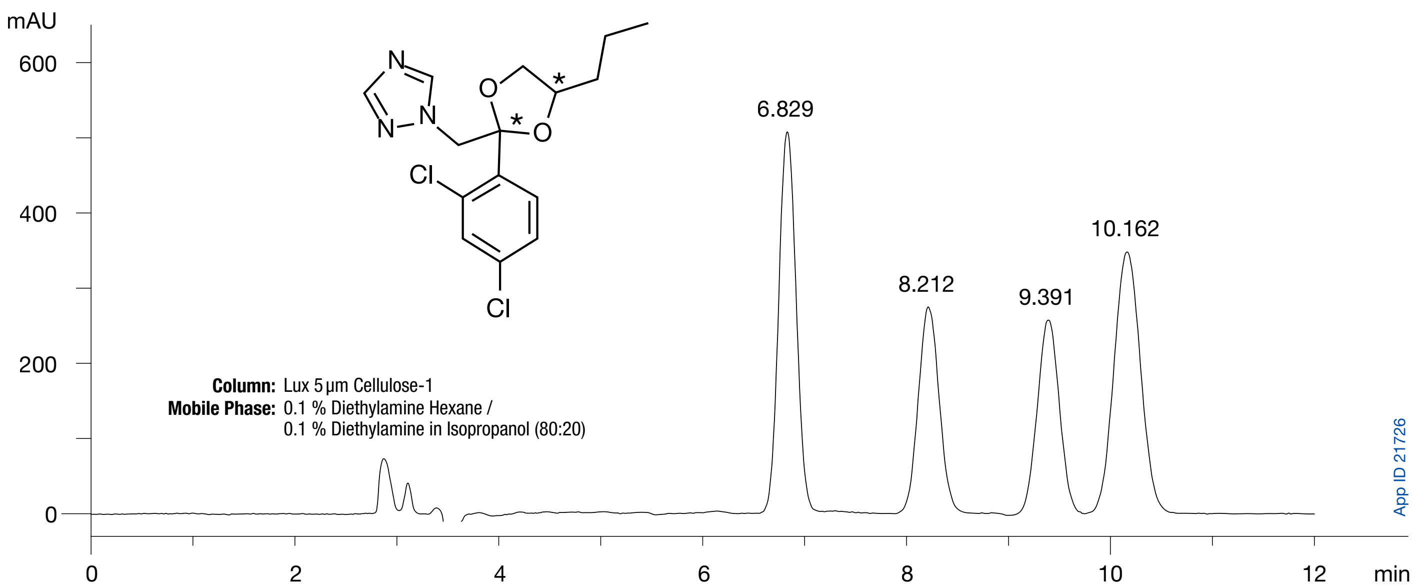
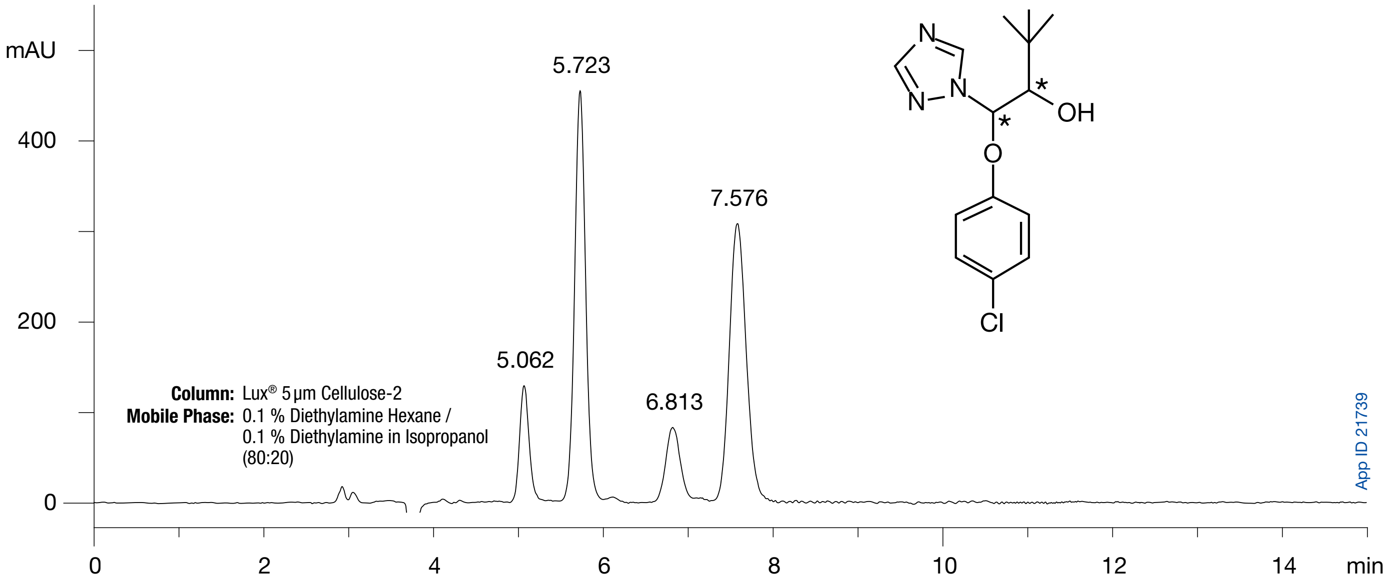


Figure 7.
Stereoselective HPLC analysis on the stereoisomers mix of Triadimenol



Conclusion

In this application note, we described the enantiomeric and diastereoisomeric resolution of five fungicide agents containing 2 stereogenic centers using Lux polysaccharide-based chiral stationary phases. All stereoisomeric separations reported showed baseline resolution between all stereoisomers with run time below 25 min. Those separations can be used not only for analytical but for preparative purposes since our phases are available in various preparative formats such as Axia™ packed preparative columns or bulk media. These analytical and preparative products can also be used under SFC mode for higher throughput.[8]

References

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8. Phenomenex TN-9002.

Lux® Ordering Information

3 µm Analytical Columns (mm)							SecurityGuard™ Cartridges (mm)	
Phases	50 x 2.0	150 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
Cellulose-1	00B-4458-B0	00F-4458-B0	00B-4458-E0	00D-4458-E0	00F-4458-E0	00G-4458-E0	AJ0-8402	AJ0-8403
Cellulose-2	00B-4456-B0	00F-4456-B0	00B-4456-E0	00D-4456-E0	00F-4456-E0	00G-4456-E0	AJ0-8398	AJ0-8366
Cellulose-3	00B-4492-B0	00F-4492-B0	00B-4492-E0	00D-4492-E0	00F-4492-E0	00G-4492-E0	AJ0-8621	AJ0-8622
Cellulose-4	00B-4490-B0	00F-4490-B0	00B-4490-E0	00D-4490-E0	00F-4490-E0	00G-4490-E0	AJ0-8626	AJ0-8627
Amylose-2	00B-4471-B0	00F-4471-B0	00B-4471-E0	00D-4471-E0	00F-4471-E0	00G-4471-E0	AJ0-8471	AJ0-8470
						for ID:	2.0–3.0 mm	3.2–8.0 mm

5 µm 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
Cellulose-1	00B-4459-B0	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-B0	00B-4493-E0	00D-4493-E0	00F-4493-E0	00G-4493-E0	AJ0-8621	AJ0-8622
Cellulose-4	00B-4491-B0	00B-4491-E0	00D-4491-E0	00F-4491-E0	00G-4491-E0	AJ0-8626	AJ0-8627
Amylose-2	00B-4472-B0	00B-4472-E0	00D-4472-E0	00F-4472-E0	00G-4472-E0	AJ0-8471	AJ0-8470
					for ID:	2.0–3.0 mm	3.2–8.0 mm

5 µm Semi-Prep Columns (mm)			SecurityGuard Cartridges (mm)
Phases	150 x 10.0	250 x 10.0	10 x 10.0‡
			/3pk
Cellulose-1†	00F-4459-N0	00G-4459-N0	AJ0-8404
Cellulose-2†	00F-4457-N0	00G-4457-N0	AJ0-8399
Cellulose-3	00F-4493-N0	00G-4493-N0	AJ0-8623
Cellulose-4	00F-4491-N0	00G-4491-N0	AJ0-8628
Amylose-2	00F-4472-N0	00G-4472-N0	AJ0-8472
		for ID:	9–16 mm

†Inquire for 10 µm Cellulose-1 and Cellulose-2 columns.

*SecurityGuard Analytical Cartridges require holder, Part No. : KJ0-4282
‡SemiPrep SecurityGuard™ Cartridges require holder, Part No.: AJ0-7220

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APPLICATIONS

Lux® Ordering Information *(cont'd)*

5 µm Axia™ Packed Preparative Columns (mm)					SecurityGuard™ Cartridges (mm)	
Phases	150 x 21.2	250 x 21.2	250 x 30	250 x 50	15 x 21.2**	15 x 30.0♦
					/ea	/ea
Cellulose-1†	00F-4459-P0-AX	00G-4459-P0-AX	00G-4459-U0-AX	00G-4459-V0-AX	AJ0-8405	AJ0-8406
Cellulose-2†	00F-4457-P0-AX	00G-4457-P0-AX	00G-4457-U0-AX	00G-4457-V0-AX	AJ0-8400	AJ0-8401
Cellulose-3	00F-4493-P0-AX	00G-4493-P0-AX	00G-4493-U0-AX	00G-4493-V0-AX	AJ0-8624	AJ0-8625
Cellulose-4	00F-4491-P0-AX	00G-4491-P0-AX	00G-4491-U0-AX	00G-4491-V0-AX	AJ0-8629	AJ0-8630
Amylose-2	00F-4472-P0-AX	00G-4472-P0-AX	00G-4472-U0-AX	00G-4472-V0-AX	AJ0-8473	AJ0-8474
				for ID:	18–29 mm	30–49 mm

†Inquire for Lux 10 µm Cellulose-1 and Cellulose-2 columns

**HPLC PREP SecurityGuard Cartridges require holder, Part No.: AJ0-8223
SFC PREP SecurityGuard Cartridges require holder, Part No.: AJ0-8617
♦ HPLC PREP SecurityGuard Cartridges require holder, Part No.: AJ0-8277
SFC PREP SecurityGuard Cartridges require holder, Part No.: AJ0-8618

Bulk Media		
Phases	100 g	1 kg
10 µm		
Cellulose-1	04G-4501	04K-4501
Cellulose-2	04G-4502	04K-4502
20 µm		
Cellulose-1	04G-4473	04K-4473
Cellulose-2	04G-4464	04K-4464
Cellulose-3	04G-4504	04K-4504
Cellulose-4	04G-4503	04K-4503

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