APPLICATIONS

Chromatographic Enantioseparation of Racemic Herbicide Agents using Lux® Polysaccharide-Based Chiral Stationary Phases

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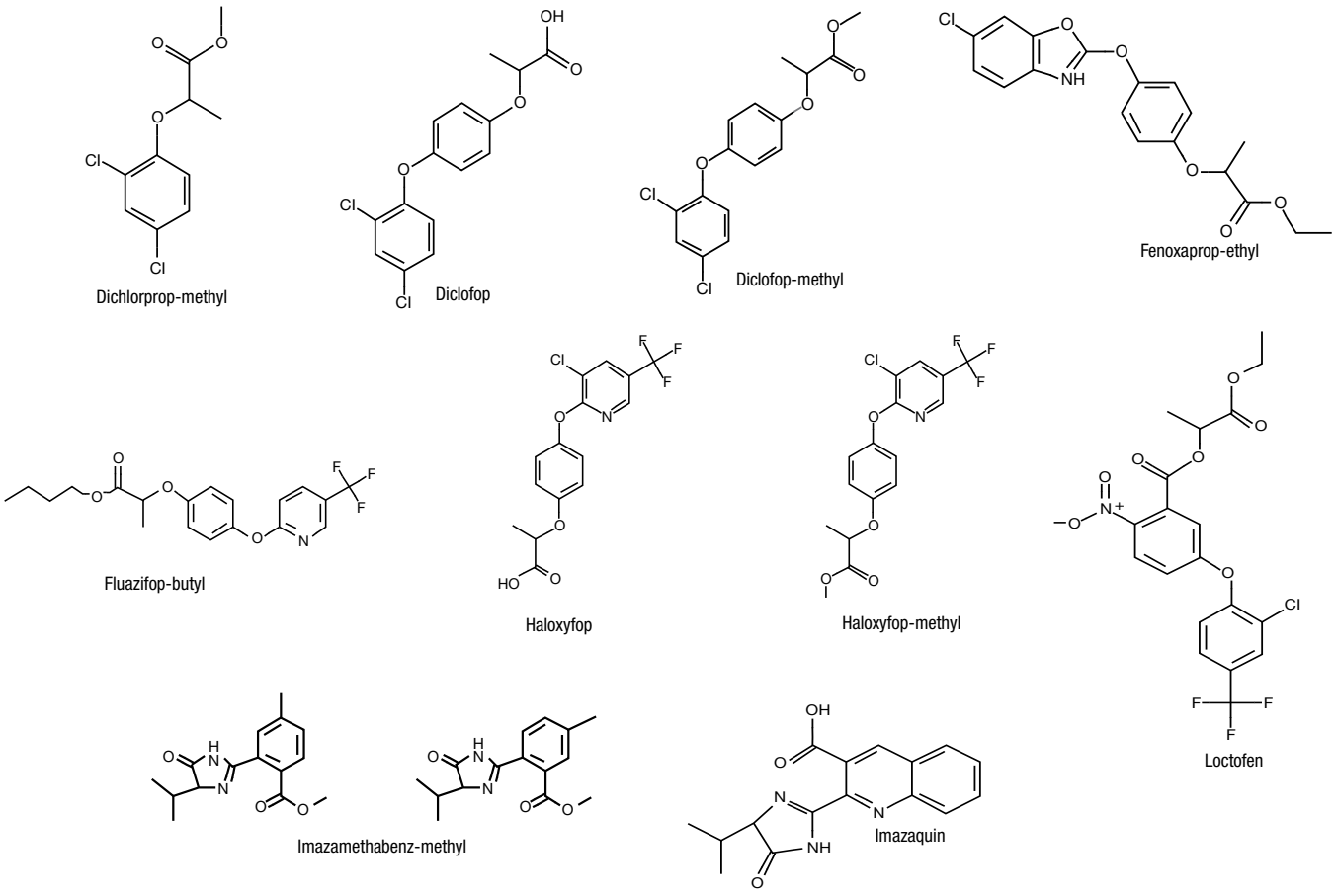
In this technical note, we report the chiral chromatographic separation of various herbicide agents using Lux polysaccharide-based chiral stationary phases. The reported enantioseparations are the results of a systematic screening of five different Lux phases in normal phase and reversed phase separation modes.

Introduction

Herbicides 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 1,693 pesticides listed in a recent review,[1] 482 (28 %) are chiral (chemical compounds containing one or more centers of asymmetry) of which 150 are classified as herbicides. The mode of action for many herbicides is to interfere with chiral plant hormones controlling growth and, therefore, the configuration of the herbicides plays a role in efficacy. As a result, some of those herbicides, such as dichlorprop-methyl, diclofop-methyl, fenoxaprop-ethyl, and haloxyfop-methyl are produced as single or enriched stereoisomer formulation. Additionally, the degradation of those chiral herbicides by soil microbes is enantioselective[2] and each enantiomer will be eliminated from the environment following a different pathway. The degradation difference of chiral herbicides, combined with possible enantiospecific toxicity can affect not only efficacy, but also exposure and risk to humans and environment. In the pharmaceutical industry, mainly due to the potential enantiospecific toxicity, chiral drugs are routinely tested for chiral purity, whereas pesticides generally are not.

Separations of chiral compounds can be performed 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. As a matter of fact, 76 % of the analytical chiral separations reported in the recent chiral pesticides review[1] were performed by HPLC, and 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.[3,4] 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. The various herbicide agents analyzed in this study are depicted in **Figure 1**.

Figure 1. Chemical structure of herbicides agents racemic mixtures separation modes



Material and Methods

All 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

Eleven racemic herbicide agents depicted in **Figure 1** were analyzed on five different 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 with various mobile phases, the best separation was selected, even though in most of the cases, alternative separation was obtained with other Lux phases and/or modes. The racemic herbicide agents separated in this study are listed in **Table 1**. For each compound tested we provide the chemical identification number (CID) of the racemate. This unique number can be linked to The PubChem Project website for further research regarding each compound's pharmaceutical properties. The table summarizes the Lux phases used, the selectivity, the retention time of the first enantiomer, as well as the isocratic conditions used for each compound.

Lux columns are quite successful at resolving chiral compounds of this type. All the herbicides agents tested are separated with selectivity greater than 1.1. In the last column, the corresponding Phenomenex application number is provided. Those applications are easily accessible on our website (www.phenomenex.com/ChiralAppSearch) and can be searched by application number, structure, CID, or compound name. The chiral separations reported in **Table 1** are baseline resolved with a resolution greater than 1.5. The retention time for the first enantiomer is between 5 and 19 min and all the separations are completed in less than 21 min. With basic and neutral herbicides derivatives, 0.1 % of diethylamine (DEA) was used as an additive whereas with acidic derivatives 0.1 % of formic acid (FA) was used as the additive. The presence of DEA favors dissociation of the amino group and improves peak shape. A similar effect is observed with formic acid as the additive with acidic pain reliever such as Dichlofop and Haloxyfop.

Table 1. Chiral separations of herbicides agents using Lux polysaccharide-based CSPs

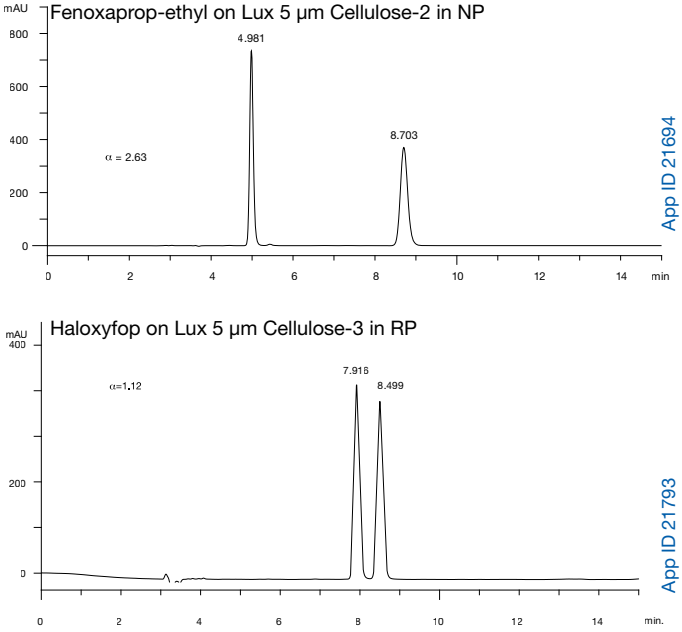
Compound	CID	CSPs	(α)	Rt (min)	Mode	Mobile Phase	App ID*
Dichlorprop-methyl	90988	Lux Amylose-2	1.06	19.22	RP	ACN/20 mM NH_4HCO_3 (40:60) DEA (0.1 %)	21761
Diclofop	38687	Lux Amylose-2	1.25	8.94	NP	Hex/IPA (80:20) FA (0.1 %)	21687
Diclofop-methyl	39985	Lux Cellulose-1	2.51	6.23	NP	Hex/IPA (80:20) DEA (0.1 %)	21688
Fenoxaprop-ethyl	47938	Lux Cellulose-2	2.63	4.98	NP	Hex/IPA (80:20) DEA (0.1 %)	21694
Fluazifop-butyl	50897	Lux Cellulose-3	1.31	7.89	RP	ACN/20 mM NH_4HCO_3 (60:40) DEA (0.1 %)	21786
Haloxyfop	50895	Lux Cellulose-3	1.12	7.92	RP	ACN / H_2O (50:50) FA (0.1 %)	21793
Haloxyfop-methyl	50896	Lux Amylose-2	1.21	6.34	NP	Hex/IPA (80:20) DEA (0.1 %)	21707
Imazamethabenz-methyl	54744	Lux Cellulose-4	1.24	7.75	NP	Hex/IPA (80:20) DEA (0.1 %)	21711
Imazaquin	54739	Lux Cellulose-3	1.38	5.06	NP	Hex/EtOH (60:40) DEA (0.1 %)	21714
Loctofen	62276	Lux Cellulose-2	1.37	7.11	NP	Hex/IPA (80:20) DEA (0.1 %)	21716

ACN = Acetonitrile, IPA = Isopropanol, EtOH = Ethanol, Hex = Hexane, H_2O = Water, FA = Formic acid, DEA = Diethylamine

* To view the full application enter the App ID onto the search field on our website.

All of our Lux® products are pressure stable up to 300 bar. Two examples of chiral separation for Fenoxaprop-ethyl and Haloxyfop are shown in **Figure 2**.

Figure 2.
Representative chromatograms for the chiral separation of herbicides.



Conclusion

In this study, we described the successful chiral separation of a variety of herbicide agents using Lux polysaccharide-based chiral stationary phases. All enantiomeric separations reported showed selectivity greater than 1.1 with the retention time for the first enantiomer below 19 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.

References

1. Ulrich E.M.; Morrison C.N.; Goldsmith M.R.; Foreman W.T. *Reviews of Environmental Contamination and Toxicology*, Springer, New York, NY, **2012,** 217, Chapter 1, 1-74.
2. Müller, M.D.; Buser, H.R. *Environ. Sci*. Technol. **1997**, 31, 1953–1959
3. Chankvetadze, *B. J. Chromatogr. A* **2012**, *1269*, 26-51. (Review).
4. Ikai, T.; Okamoto, Y. *Chem. Rev*. **2009**, 109, 6077-6101.

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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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

Please inquire for 20 µm Lux Amylose-2 media

guarantee

If Lux analytical columns (≤ 4.6 mm ID) do not provide at least an equivalent or better separation as compared to a competing column of the same particle size, similar phase and dimensions, return the column with comparative data within 45 days for a FULL REFUND.

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