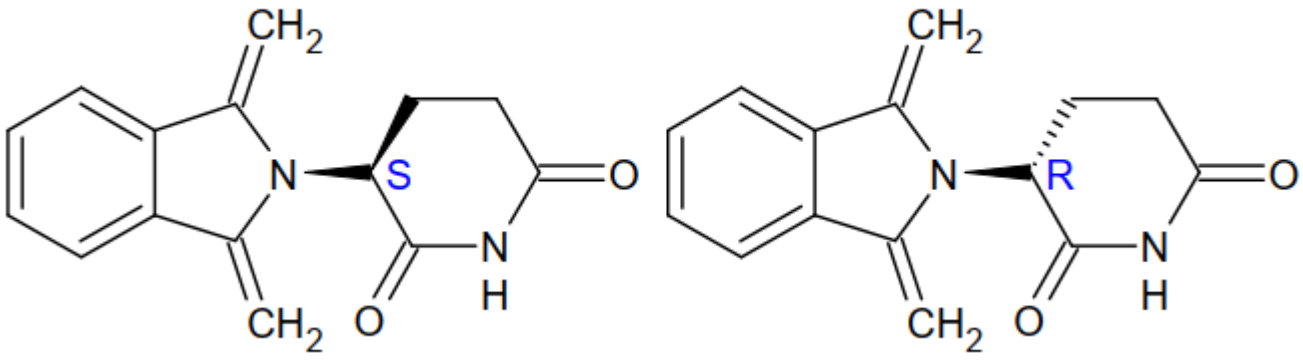
Guest Author – Ryan Splitstone, Product Marketing Manager

Chemical isomers originate from the same chemical formula but differ in the spatial arrangement of their chemical functional groups or bond order of the molecule. In the case of chiral compounds or enantiomers, they are isomers (stereoisomers) that are mirrored images of one another and therefore, non-superimposable but have the same physical and chemical properties. Although the different enantiomers might have the same physical and chemical properties, their molecular interaction and complexing potential are different. For example, thalidomide is a terrible instance of the potential interactions difference between enantiomers of a chemical compound.

When thalidomide was initially developed, it demonstrated anti-nausea and sedative effects as a result of the R enantiomer and was prescribed to pregnant mothers (Figure 1). However, unbeknownst to the industry, the S-enantiomer resulted in thousands of birth defects and became a notorious example of how chiral compounds and their interactions could have potentially unforeseen consequences.

Figure 1. Indicated R- and S-enantiomers of Thalidomide



Chiral Compounds & Chromatography

In modern-day analytical science, chromatography has become the primary means to both separate and quantify enantiomers. Silica backbone particles that are functionalized with enantioselective polysaccharide chains have become the primary type of column used because they deliver both diverse enantioselectivities with high chromatographic performance. In addition, the immobilized polysaccharide phases provide wide solvent compatibility allowing flexibility in the modes the column can operate in, including reversed phase, normal phase, and SFC mode. Because of the silica backbone's mechanical strength, the columns are backpressure stable and can withstand pressures of 300 bar and be prepared in bulk media form when needed.

How do Polysaccharide Columns Work?

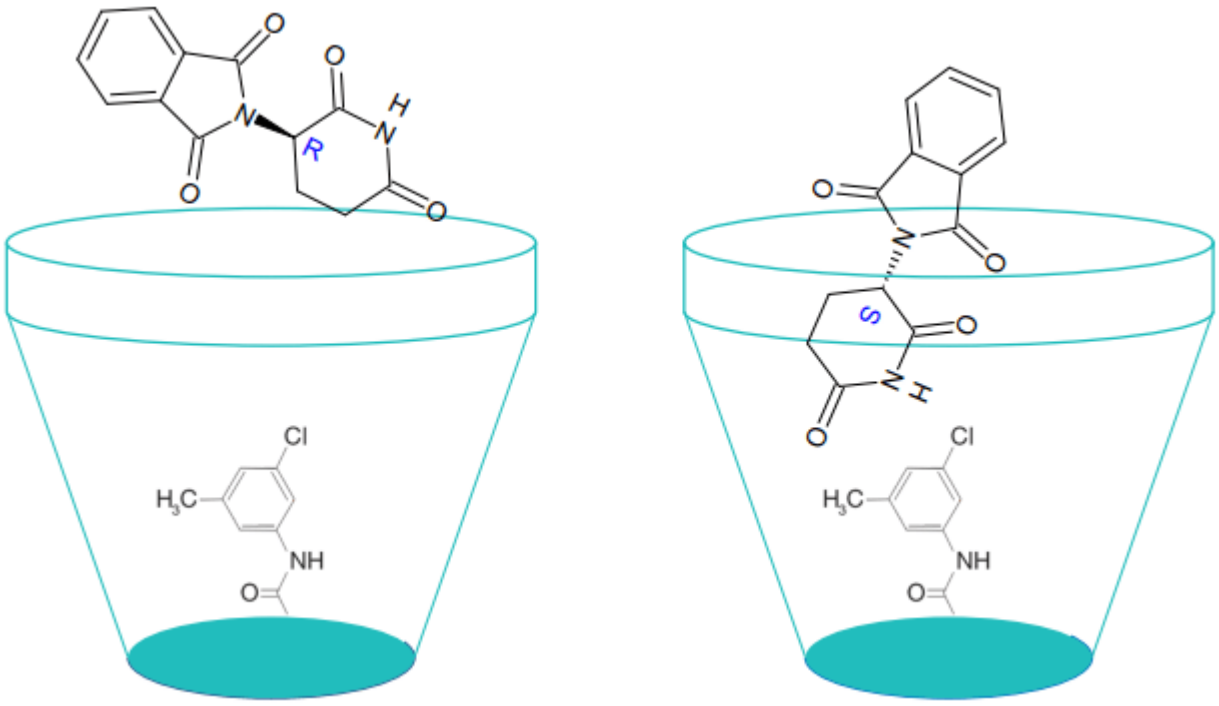
The polysaccharide complex forms a semi-inclusive, three-dimensional polysaccharide complex that is functionalized with a chiral selector that exhibits diverse and specific

chromatographic selectivity. Therefore, overall enantioselectivity is a combination of inclusion complexing, chiral selector, and the selected mobile phase convention.

Inclusion Complexing and Amylose vs. Cellulose

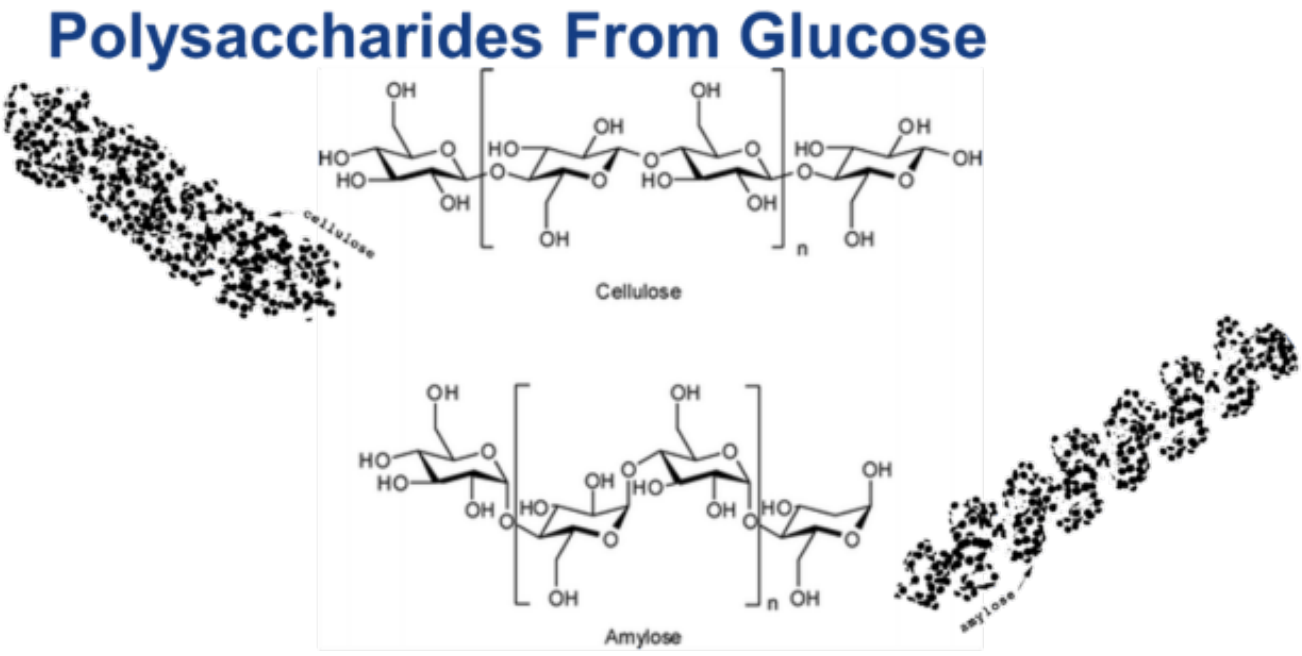
Especially in the case of reversed phase mode, inclusion complexing significantly impacts chiral distinction. This chiral distinction is driven by the ability of two chiral compounds' ability to diffuse into the 'inclusion' space within the three-dimensional polysaccharide complex (Figure 2). Because of the structural differences between amylose and cellulose, they both have different inclusive complexing potential or selectivity.

Figure 2. Inclusion Complexing



Therefore, the position and properties of substituents determine the chromatographic elution order of the chiral compounds. For example, a chiral compound that is aromatic will have a related elution order relative to the substituent position of its functional groups. This typically follows the increased retention time pattern of meta < ortho < para. The 'bulkier' nature of the meta and ortho limits the potential compound 'inclusion' and results in it being excluded slightly more than the more linear structure. The properties of the substituent also affect the selectivity and is highest, in general, when strong hydrogen bonding groups are present. Cellulose- or amylose-based polysaccharides columns are the most common chiral stationary phases developed from glucose. Cellulose and amylose provide different selectivity in chiral separations as cellulose is generally considered 'flatter' versus the three-dimensional helical structure of amylose (Figure 3).

Figure 3. Cellulose and Amylose Structure



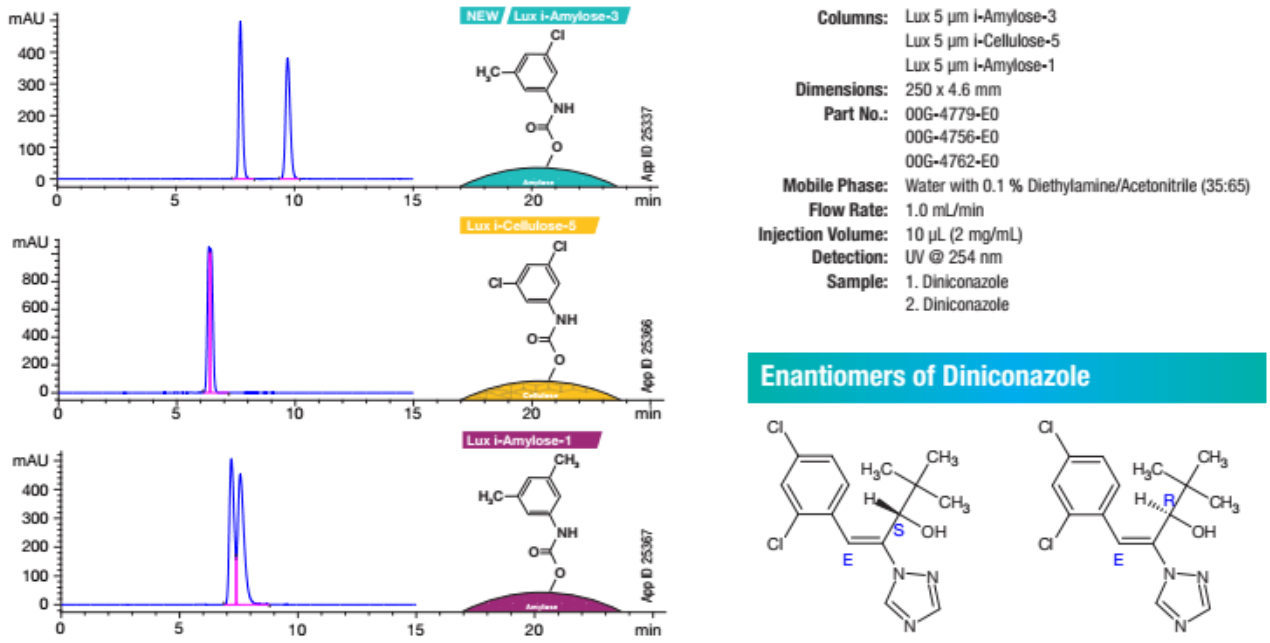
Phenomenex Lux® polysaccharide-based chiral stationary phases come in both amylose- and cellulose-based phases, which includes three immobilized phases that create a complementary yet distinctive set of chiral tools. For example, these phases give scientists complementary selectivity while reversing the elution order of enantiomers. In some cases, the order of elution can be reversed with the same stationary phase but have varying compositions of the mobile phase.

Chiral Column and Mobile Phase Selectivity

Column and mobile phase selectivity typically affect relative resolution greater than any other chromatographic parameter and is often the quickest way to substantially change the observed separation. The mobile phase can also impact resolution, as can factors such as temperature; however, those parameters primarily impact peak performance over selectivity.

In the development of a chiral separation, the Lux immobilized phases can be used in various separation modes and have a wide solvent stability allowing them to be versatile and cost-effective separation tools. We recommend starting your initial chiral screen with our three immobilized chiral selectivity that exhibit distinct enantioselectivity with the same strong solvent compatibility (Figure 4).

Figure 4. Comparison of Lux Immobilized Selectivity



Advantages of Immobilized Polysaccharide Columns

The term ‘immobilized’ is used to describe chiral columns with polysaccharide chains that are covalently bonded to the base silica particle rather than held in place by adhesion forces. The

chiral columns that rely on adhesion force are generally termed ‘coated’ phases and are more restrictive in their solvent compatibility. Because of the chemically crosslinked covalent bond stability, the chiral phase allows the expanded use of strong solvents like tetrahydrofuran, dichloromethane, dimethyl sulfoxide, methyl tert-butyl ether, ethyl acetate, and others. This advantage economically translates into broader solvent flexibility and an increase in enantioselectivity potential from one phase. Furthermore, the immobilization allows the sample to stay dissolved in strong organic solvents without the need to perform a time-consuming solvent switch.

Conclusion

In conclusion, chiral compounds are non-superimposable isomers and certain compounds can have unforeseen interactions. Because of potential adverse effects, it is important to be able to analytically separate these chiral compounds. However, because of the inherent difficulties in predicting differences in chiral compounds’ interactions, using LC columns with broad enantioselectivity and wide solvent compatibility offers the best chance of successful separations. With the addition of the Lux i-Amylose-3, the Lux column portfolio includes three immobilized stationary phases and six coated phases. Altogether, these novel chiral columns deliver a wide and complementary range of enantioselectivity under normal phase, reversed phase, polar organic, polar ionic, or SFC separation modes.

For more information regarding the Phenomenex Lux Column Portfolio, visit: www.phenomenex.com/Lux.

Have any questions regarding the above information or any other technical inquires? Reach out to our technical specialists today through Chat Now – our free online service that can help with method optimization, chromatographic tips, product recommendations, and provide quotes for an easier buying process.

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