



By **James Turner**, *Global Market Manager, Phenomenex*

My earliest memories of chiral molecules date back to my undergraduate studies, sitting on the floor with a set of molecular models desperately trying to figure out whether a molecule was “R” or “S” (Cahn Ingold notation). Yet, when the model was almost complete, adding the last bond (aka a small plastic straw) resulted in the spontaneous decomposition of the model. The issue was compounded by the fact that my roommate had no problem with simply looking at a molecular depiction on paper, rotating it in his mind, and determining the orientation with an unerring degree of accuracy.

### Why Chiral Analysis Matters in Pharmaceutical Development

So, why does all of this matter? Even though many of us struggle to visualize chirality at a cellular level, we are programmed to. This can pose a significant issue when considering chemically synthesized pharmaceutical drugs. Whilst enantiomers of a compound may share the same physical and chemical properties, they can differ in terms of their therapeutic

effect. One enantiomer of an active pharmaceutical product (API) may have a therapeutic effect, while the other may have no effect, or worse, have a toxicological effect. As such, when synthesizing an API drug, companies are required to assess the effects of both enantiomers. They are also required to test produced APIs, recording the levels of each enantiomer. If the alternate enantiomer has a toxicological effect, then it will be necessary to set a limit on the amount of the undesired enantiomer in the final product. It is also necessary to assess the chiral stability of the API, as some drug substances can spontaneously racemize in solution.



The relevance of all of this is that pharmaceutical companies therefore require a way to perform this analysis, which is challenging, given that both enantiomers exhibit the same physical and chemical properties. [Chiral HPLC](#) separations allow for such analysis, providing a chiral environment within which the discrimination of enantiomers can occur. Several different approaches to chiral stationary phases are available, but by far the most popular involves the use of derivatized polysaccharide phases. Such phases are based on cellulose or

amylose, both naturally occurring chiral polymers of glucose. They are typically derivatized from phenyl groups which are substituted with methyl or chlorine groups. Such columns can be used in normal phase, polar organic, reversed phase, or SFC (supercritical fluid chromatography) modes. The selectivity of the columns will vary depending on the mode used.


### How Chiral Stationary Phases Work

The simplest way to understand how these chiral stationary phases work is to consider the following: The cellulose or amylose comprises of strands of glucose, which have spaces or “pockets” within them. Depending on the solvent used as mobile phase the polymers will swell to varying degrees, this in turn alters the size and shape of the “chiral pockets” within the polymer. The phenyl derivatives provide additional interactions and selectivity to the chiral polymer. When analytes are introduced to the column, the aim is that one enantiomer will find it easier to fit into the chiral pockets, interacting with the phenyl groups while the alternate enantiomer will have a lower degree of success in interacting with the derivatized polymer and will, therefore, elute earlier.









### Selecting the Right Column

The question naturally arises: “How do I select the correct column for my desired separation?” The answer is less straightforward. As the interactions between stationary phase and analyte are both complex and highly specific, they are difficult to predict. As such, the screening of stationary phases, together with mobile phase conditions is required. A typical screening will be conducted in normal phase, reversed phase, and polar organic modes. If it is desirable to work with a mass spectrometer as a detector, this will limit the possible separation mode to reversed phase. Whilst this is not a problem, it may necessitate [screening various chiral stationary phases](#) in order to achieve a good (baseline) separation.

# Simplified Chiral HPLC/SFC Column Screening Strategies




## Lux Polysaccharide Chiral Columns

Immobilized			Coated				
Amylose		Cellulose	Amylose	Cellulose			
<p><b>Lux 1-Amylose-1</b> Amylose 10x (3,5-dimethylphenylcarbamate)</p>  <p>Guaranteed Alternative to CHIRALCEL 10<sup>+</sup> and A-2</p>	<p><b>Lux 1-Amylose-3</b> Amylose 10x (3-chloro-5-methylphenylcarbamate)</p>  <p>Guaranteed Alternative to CHIRALCEL 10<sup>+</sup> and G-3</p>	<p><b>Lux 1-Cellulose-5</b> Cellulose 10x (3,5-dichlorophenylcarbamate)</p>  <p>Guaranteed Alternative to CHIRALCEL 10<sup>+</sup> and G-3</p>	<p><b>Lux Amylose-1</b> Amylose 10x (3,5-dimethylphenylcarbamate)</p>  <p>Guaranteed Alternative to CHIRALCEL 10<sup>+</sup>, AD-10<sup>+</sup>, AD-3, AD-10<sup>+</sup>, and AD-3R</p>	<p><b>Lux Cellulose-1</b> Cellulose 10x (3,5-dimethylphenylcarbamate)</p>  <p>Guaranteed Alternative to CHIRALCEL 10<sup>+</sup>, DD-10<sup>+</sup>, DD-3, DD-10<sup>+</sup>, and DD-3R</p>	<p><b>Lux Cellulose-2</b> Cellulose 10x (3-chloro-4-methylphenylcarbamate)</p>  <p>Guaranteed Alternative to CHIRALCEL 10<sup>+</sup>, DD-10<sup>+</sup>, DD-3, DD-10<sup>+</sup>, and DD-3R</p>	<p><b>Lux Cellulose-3</b> Cellulose 10x (3-methylbenzoate)</p>  <p>Guaranteed Alternative to CHIRALCEL 10<sup>+</sup>, DD-10<sup>+</sup>, DD-3, DD-10<sup>+</sup>, and DD-3R</p>	<p><b>Lux Cellulose-4</b> Cellulose 10x (3-chloro-5-methylphenylcarbamate)</p>  <p>Guaranteed Alternative to CHIRALCEL 10<sup>+</sup>, DD-10<sup>+</sup>, DD-3, DD-10<sup>+</sup>, and DD-3R</p>

### Why Choose Lux Chiral Columns?

- Suitable for normal phase, polar organic, SFC, and reversed phase conditions
- 3 µm and 5 µm packed columns, as well as 10 µm and 20 µm bulk media for scale up
- Pressure stable up to 300 bar



- High efficiency and loading capacity
- PhenoLogix Free Chiral Screening!
- Easy scale up to Preparative Axia™ column dimensions

Laboratories with a high throughput of chiral drug candidates may find it convenient to have their own automated chiral screening systems. For those who encounter this requirement less frequently, such an outlay can be cost-prohibitive. As such, our [Chat Support](#) is happy to provide advice on the choice of columns for chiral screening, check our database of existing chiral separations, and offer a [chiral screening service](#) that meet your requirements.

Other resources:

[Lux™ Immobilized and Coated Application eBook](#)

[Webinar: LC Solutions for Improving Chiral Separation Success](#)

[Lux Chiral Columns Make It Easy](#)

[Webinar: Strategies for Simplified Chiral Development](#)

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