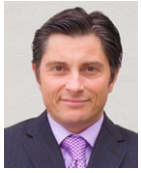
A Q&A

Current Trends in Chiral Separations



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A discussion of the trends, development, and use of chiral stationary phases and separation modes

Chiral compounds such as pharmaceuticals, pesticides, and raw materials are challenging to work with because enantiomeric species can provide varying physiochemical properties. Chromatography products and systems are available for use in a range of separation modes to assist in the accurate separation and analysis of these distinct compound forms. *LCGC* recently sat down with Marc Jacobs, PhD, to discuss trends in the development of chiral columns and his advice on choosing columns and separation modes for chiral screening.

LCGC: How did you initially become interested in the field of chirality?

Jacob: Early in my academic career in France, I was a student of Professor Pierre Duhamel, a pioneer of asymmetric synthesis. I was introduced to the concept of chirality in one of his classes. My PhD thesis was on the topic of asymmetric synthesis of amino acids, which are important chiral natural products. In France, chirality has historical roots from long ago when Louis Pasteur separated tartaric acid using a microscope. I've been involved in the field of chiral analysis for the last 20+ years.

LCGC: Why is there so much emphasis on chiral analysis and the detection of enantiomers in pharmaceutical products?

Jacob: Chiral purity is particularly important in pharmaceuticals. Because pharmaceutical products are developed for use in humans, you must ensure that the purity of the pharmaceutical ingredient is well understood and characterized. Since the 1990s, the FDA has required the analysis of the enantiomers for a chiral drug in development. Many drugs before the 1990s were developed as racemates. Pharmaceutical analysts found that sometimes an enantiomer, also called a distomer, can have no activity, and in some cases, can have toxic activity. The good enantiomer is referred to as the "eutomer."

The case of thalidomide raised the importance of chiral analysis. The drug was used in the 1950s as a sedative and to alleviate morning sickness in pregnant women. One of the enantiomers of the drug, however, possessed some adverse properties, and many children were born with severe deformities. After the discovery of these effects, the FDA required the analysis of chiral drugs for all of the existing enantiomers to determine whether or not toxic properties exist.

LCGC: What recent improvements have been made in the kinds of chiral columns in use today for analytical and preparative separations?

Jacob: Most of the major developments in chiral chromatography took place in the 1980s with the introduction of the polysaccharide-based chiral stationary phase, which is the most useful phase for analytical and preparative separations. In the 1980s, just two different types of phases were used, which were based on a cellulose backbone or an amylose backbone. Now, we have a dozen or more stationary phases. Current developments are focused on generating different chiral selectors on those polysaccharide-based stationary phases.

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About 10 years ago, the immobilized version of polysaccharide-based stationary phases was introduced. These phases were coated on the silica material, which created issues with the solubilization of the chiral selector during a chromatographic analysis. The current immobilized version is robust and opened up opportunities for chiral separations because it allows for the use of more types of organic solvents. With a chiral separation, you cannot predict which phase will separate the two enantiomers. Typically, quite a few columns and mobile phases must be screened to obtain good results.

LCGC: Do you still see a need for both coated and immobilized polysaccharide chiral columns?

Jacob: My visits to customers indicate that coated phases are not disappearing and are useful in terms of improving success. Because you cannot predict the separation, you need a high success rate with the column or columns being used. Analysts continue to use both coated and immobilized columns in their screens, especially in SFC, as well as in normal-phase and reversed-phase chromatography. Immobilized phases are of specific interest because they give you access to a new chiral selector and enhance your chance of finding a successful chiral separation.

LCGC: What separation modes are most widely used for chiral separations?

Jacob: Historically, normal-phase mode has been used, which is a mixture of an alkane and alcohol, for example, hexane and ethanol or hexane and IPA. The polar organic mode (POM), which is a mixture of two organic solvents, has also been used. POM uses methanol and acetonitrile or ethanol and acetonitrile in the mobile phase, which are compatible with coated or immobilized phases. The variety of solvents that could be used was limited originally because primarily coated stationary phases were used.

Approximately 90% of chiral separations are carried out on polysaccharide-based phases. In the last 20 years, supercritical fluid chromatography (SFC) has become prominent because of its speed. With SFC, there is less viscosity from the solvent in the mobile phase, so separations can be run at higher flow rates—almost four times faster—which results in faster screening. SFC mobile phases contain at least 60% carbon dioxide (CO_2), so there is less mobile phase to evaporate, which is important in preparative chromatography. Analytical-scale separation use much less solvent overall, which makes it a "green" technology. Analytical separations are powerful using polysaccharide-based columns in reversed-phased mode. Analysts thought these phases would not be stable, but reversed-phase separations using water/acetonitrile or water/methanol demonstrate good results, and their use is growing.

We did some work on FMOC amino acids, which are an important chiral raw material as a building block for the synthesis of peptides. Reversed-phase chromatography was able to separate all of the natural protected amino acids. With immobilized chiral stationary phases, a variety of solvents can be considered. In the polar organic mode, tetrahydrofuran (THF) can be mixed with ethyl acetate or MTBE with methanol—many combinations are possible. This type of approach is not common, however, and it is expensive to use these organic solvents. Consequently, historically, normal phase has been used most commonly for chiral analysis, with SFC being used for preparative separations and in pharmaceutical analysis, and reversed-phase for analytical methods.

LCGC: What recommendations do you have for stationary phases and modes to start with when undertaking chiral LC screening projects?

Jacob: I will address small molecules because we're discussing chiral separations of pharmaceuticals, which generally have only one chiral center. Three to six chiral stationary phases will yield about a 90% success rate in screenings.

I recommend Lux Amylose-1 and Cellulose-1, which cover the polysaccharide backbones. These phases were developed in the 1980s by Yoshio Okamoto and commercialized. Another option is a chiral selector with an ester, called Cellulose-3, that gives you another type of interaction with your molecule. I would also include a chlorinated phase, Cellulose-4 or Cellulose-2, which were developed by Professor Bezhan Chankvetadze, a collaborator of Phenomenex. I suggest a couple of immobilized phases because these chiral selectors are unique and not available as coated phases, like the i-Cellulose-5. These six phases should give you almost 99% success, but just Amylose-1, Cellulose-1, and Cellulose-3 will give you about 75% success.

Regarding mode, I suggest SFC, if possible, for speed, as well as polar-organic, reversed-phase, and normal-phase modes depending on your equipment. In sum, three to six phases and a couple of separation modes.

LCGC: Have you seen an increase in chiral separations in any industries other than pharma?

Jacob: We see some chiral analysis of basic chemical raw materials such as FMOC amino acids, which are a critical raw material made all over the world. Chiral analysis is also used in the agrochemical industry for chiral pesticides, herbicides, and fungicides. Because these products are used in food, it's critical to determine whether there are adverse effects stemming from their chemistry. Some chemicals used in the electronics industry are chiral and require analysis and classification. Most of the pharmaceutical drugs that are chiral are now being developed as pure enantiomer and require a battery of tests. But it remains to be seen whether the development of small molecule drugs will increase or stay stable in the future as other types of drug are developed in the pharmaceutical industry.