Chiral Analysis in Drug Discovery and Development

With the number of single-enantiomer drugs expected to increase rapidly in the coming years, it is crucial to have state-of-the-art tools available for chiral analysis to keep up with the demands of high-throughput screening in the current R&D environment.

Since Louis Pasteur discovered chirality in 1847, it has become an integral and important part of life. Chiral drugs have molecular structures that lack an internal plane of symmetry and are non-superimposable with their mirror images. This property of a molecule is also called ‘handedness’. A chiral molecule and its mirror image are called a pair of enantiomers. Most molecules found in nature – such as amino acids, sugars, proteins, DNAs and RNAs – are chiral and usually only one of the two enantiomers is formed naturally. For example, virtually all the amino acids found in nature are L-(left-handed). In the pharmaceutical industry, currently more than two thirds of the drugs on the global market are chiral drugs serving in myriads of therapeutic areas such as anxiety, indigestion, heartburn, arthritis, AIDS, cancer and allergies (1). Several top-selling blockbuster drugs – such as Lipitor, Nexium and Plavix – are all chiral. With the recent rapid developments in biotechnology, chiral drugs will play an even more important role in saving patients’ lives because almost all biopharmaceuticals are chiral.

Originally, pharmaceutical companies were allowed by the US FDA to test and develop both enantiomers together (racemate) for a chiral drug (2). It was subsequently discovered, however, that often only one of the two enantiomers was active and the other one was either non-active or even harmful. This is because the interaction between a drug molecule and its target is very much dependent on the 3D environment around them. For a pair of enantiomers, their interactions with the target in the 3D environment are different. The most famous example is the thalidomide molecule sold in the 1950s; the drug was introduced as a racemic mixture for use as a sedative, but was later withdrawn from the market following the occurrence of birth defects in the children of mothers who took it to treat morning sickness. It was later found that the inactive enantiomer was the cause of the teratogenicity.

In the US in 1992, the FDA recommended using stereochmically pure drugs; this required that the drug labelling should include a unique, established name and a chemical name with the appropriate stereochemical descriptors that should specify identity, strength, quality and purity from a stereochemical viewpoint (3). Since then, for pharmaceutical companies, single enantiomer drugs have become the standard when working with compounds featuring asymmetric centres.

Shortening timelines for chiral drug discovery and development usually depends on the efficiency of asymmetric synthesis, enantiomeric separation and determination of absolute configuration (AC). Therefore, the availability of powerful tools for chiral analysis is crucial in streamlining the discovery and development of a chiral drug. This article will review the tools available for chiral analysis, including techniques for generating single enantiomers and techniques for their AC determination.
Chiral Salt Resolution
Chiral salt resolution (or diastereomeric crystallisation) is the classical method for the separation of racemic mixtures by seeding with a pure enantiomer or a chiral resolving agent during the crystallisation of the racemic mixture. When successful, this is the best method for the manufacture of single enantiomers in bulk. For example, Merck has used this with great success in the manufacture of \(\alpha\)-methyl DOPA. Unfortunately, the method has very limited application because it can only be applied to a conglomerate (a mixture of crystals of the two enantiomers), and these account for only less than 20 per cent of all known racemates (4).

Chiral Chromatography
Currently, the most popular technique for the separation of a racemic mixture is either high performance liquid chromatography (HPLC) or supercritical fluid chromatography (SFC). Chiral chromatography uses columns coated with polysaccharide-based chiral compounds as the stationary phase that has different affinities for a pair of enantiomers in the mobile phase running through the column. Therefore, the two enantiomers will elute from the column at different times and be separated. The introduction of automated SFC instrumentation with stacked injection produces much faster separations per unit time, as well as fractions with higher purities compared with HPLC. Most of the enantiomer separations in the drug discovery stage are carried out using chiral chromatography (5).

Asymmetric Synthesis
This technique introduces chirality during the synthetic sequence, and requires either asymmetric catalysis or auxiliary chiral synthesis. Ideally asymmetric synthesis should be the most cost-effective method for producing single-enantiomer products because all the precursors are converted to the desired enantiomer. However, in practice the implementation of this approach can be limited by several factors such as the efficiency of the catalyst, the availability of the catalyst and the reaction conditions (a very low temperature or high pressure, and reaction kinetics) (6).

DETERMINATION OF ABSOLUTE CONFIGURATION

Techniques that are prevalent in the pharmaceutical industry for determination of the AC of chiral molecules include X-ray crystallography, nuclear magnetic resonance (NMR) methods (Mosher’s method, a chiral liquid crystal NMR technique) and vibrational circular dichroism (VCD).

X-ray Crystallography
X-ray is still considered the most reliable technique, but usually requires at least one heavy atom (atomic number greater than 23) and a single crystal. The amount of material required for testing different crystallisation conditions is significant because, at discovery stage, usually only a small amount of compound is synthesised and it has to be used for multiple tests. The turnaround time (growth of single crystal plus AC determination) is usually slow on the discovery time scale. Very often, when the X-ray result is finally given to the chemist who synthesised the compound, he or she is no longer interested because other test results that have come back in the mean time show that the compound is of very little, if any, potency. Therefore X-ray crystallography is typically used on a potential drug candidate at the late discovery or early development stage (5).

NMR Methods
NMR methods are used routinely by structure elucidation teams in pharmaceutical R&D departments to resolve the relative stereochemistry of compounds. For
AC determination, regular NMR experiments would not give the answer. However, with Mosher’s NMR method, one can obtain derivatives of the unknown enantiomers with Mosher’s reagent, which has a known chirality, and then determine the AC of the two enantiomers by examining the different changes in chemical shifts in the two derivatives. Mosher’s method is used primarily on secondary alcohols, amines and acids. The chiral liquid crystal NMR method uses chiral liquid crystals as an instrument optimised at both enantiomers; 4-hour collection for both sample and solvent; instrument optimised at 1,400 cm⁻¹. Solvent-subtracted IR and VCD spectra are shown.

Vibrational Circular Dichroism (VCD)

VCD is one of the two forms of vibrational optical activity (VOA). VOA is the differential response of a chiral molecule to left versus right circularly polarised infrared radiation during a vibrational transition. The other form of VOA is Raman optical activity (ROA). The IR spectra of a pair of enantiomers are the same, while their VCD spectra are equal in intensity but opposite in sign (mirror images of each other about the zero baseline), as shown in Figure 1 for (1R)-(+)- and (1S)-(-)-Camphor. The VCD spectrum of a chiral molecule can be calculated using density functional theory (DFT). The AC of a chiral molecule can be determined by comparing the measured VCD spectrum with the calculated spectrum. The confidence level of such a comparison can be obtained by the VOACompare algorithm recently developed by BioTools.

The VCD technique was discovered and developed in the 1970s and has become a powerful tool for the AC determination of small chiral molecules in the solution state. The first commercial VCD instrument was brought to market by BioTools in 1997, and since then many pharmaceutical companies and academic research groups have adopted this technique for their AC determination needs. VCD measurements require only five to 10 mg of pure sample in solution form. The fast development of computer processors and memories and the implementation of VCD calculations into Gaussian programs have made it possible to carry out DFT-level VCD calculations on PCs. An example...
of the AC determination of (S)-Binaphthol is shown in Figure 2. Determining the AC by VCD analysis is typically more rapid for small chiral molecules (less than 60 atoms) when compared with analysis by X-ray crystallography.

Due to its relatively low sample requirement and short analysis time, VCD is especially useful for AC determination in the early exploratory and discovery stages when a project is engaged in structure-activity relationship (SAR) and/or structure-property relationship (SPR) studies. SAR and SPR studies usually involve synthesising a series of analogs of the lead compounds; the analogs are then tested for their biological activities and other important properties such as solubility, stability and permeability. The results of the SAR/SPR studies then direct the synthesis towards more potent activity, greater target selectivity and improved physicochemical properties of the lead compounds. Therefore, VCD analysis in the exploratory and discovery stages usually involves analysing a series of analogs with the same core structure but with different substituents. Such series of analogs often exhibit similar observed and calculated VCD features as shown in Figure 3 for phenylglycidal compounds (9). Since the rate-limiting step in VCD analysis is most often the VCD calculations, it would be beneficial to carry out VCD calculations for only one compound in a series of analogs and use it as a reference for AC determination of the other analogs in the same series by comparing the observed VCD of the other compounds with that of the reference. This would significantly reduce the analysis time for AC determination by VCD for analogs.

**CONCLUSION**

In drug discovery and development, chiral analysis will become more and more important. Over the past two decades, the number of single-enantiomer drugs has been increasing steadily – approaching 70 per cent of all APIs – and is expected to increase more rapidly in the coming years.

**References**


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