CHIRAL SEPARATIONS BY CHROMATOGRAPHY

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This book is dedicated to the pioneers, authors, and column manufacturers in chiral separations, who have helped to move this field of science at a rapid pace.

PREFACE

This book has been planned for use by scientists interested in separations of isomeric compounds, especially those compounds that are chiral in nature—that is, are optically active. Information is provided on stereochemistry, separation methods (TLC, GLC, HPLC, and CE), and detection methods. Detailed discussion is given on HPLC methods. This includes derivatization, mobile-phase additives, ligand exchange, ion pairing, inclusion, and various chiral stationary phases (CSPs)—namely, brush-type, cellulosic, cyclodextrins, and protein-based. Some insight is provided into modern methods used in the design of CSPs, with special emphasis on target-directed designing methods. Detailed discussion includes method development and the choice of the right method and CSP. The importance of preparative separations for chiral compounds is discussed. Regulations and requirements are covered as they apply to development of chiral or enantiomeric compounds.

Stereoisomerism can result from a variety of sources aside from the single asymmetric carbon (chiral or stereogenic center). A molecule with a stereogenic axis can also be chiral. As a matter of fact, stereoisomerism can result from various sources (see chapter 3).

As discussed in chapter 1, enantiomers have identical physical properties except for the rotation of the plane of polarized light—that is, they have a different sign for optical rotation (plus or minus). Optically active molecules have attracted great attention because living systems are chiral. Proteins, nucleic acids, and polysaccharides possess chirally characteristic structures that are closely related to their functions. Because of chirality, living organisms usually show different biological responses to one of a pair of enantiomers (optical isomers) in drugs, pesticides, food, and waste compounds.

The importance of determining the stereoisomeric composition of chemical

compounds, especially those of pharmaceutical significance, cannot be overemphasized, as covered in chapter 2. The differences in pharmacologic activity is exemplified by dextromethorphan, which is an over-the-counter antitussive (cough suppressant), whereas levomethorphan is a controlled narcotic. Less dramatic examples abound in that about 60% of the most prescribed drugs possess one or more asymmetric centers in the drug molecule. A fairly large percentage of the commonly used drugs are used in either racemic or diastereomeric forms. The differences in physiologic properties between the enantiomers of these drugs have not yet been examined in many cases, mainly because of the difficulties of obtaining both enantiomers in optically pure forms.

The primary focus of this book is on the methods used most commonly, namely, chromatographic methods:

- Thin-layer chromatography
- Gas chromatography
- High pressure liquid chromatography
- Supercritical fluid chromatography
- Capillary electrophoresis
- Membrane separations

Discussed at length are the first three methods; information is also provided on the emerging supercritical methods. High pressure liquid chromatography has been discussed extensively because it has greater versatility than the other chromatographic methods. The methods covered also include usage of formation of diastereomers where these reactions favor separation or simplify it in terms of utilization of materials. The last two methods listed above, which are not strictly chromatographic, provide interesting avenues for separations.

Discussion of the desirable features of CSPs and understanding chiral chromatography in chapters 6 and 7 should be of great help in method development, which is covered in chapter 8. Assistance with the most daunting task of method selection is covered at length in chapter 10, and this chapter also provides selected applications to help the readers solve their individual problems.

This book is based on an American Chemical Society short course organized by me and taught jointly with Professor William Pirkle and Dr. Christopher Welch. Somehow or other I got volunteered into writing this book as I bought into their inputs that their schedules would not permit this hard commitment. I will always cherish their contributions to this course and the camaraderie developed over the time we worked together. The book tries to fill the gap of the current textbooks in the field of chiral separations by chromatography, notwithstanding an excellent contribution to this field by Stig Allenmark in 1991.

I would like to thank Bill Pirkle and Chris Welch for their contribution to the ACS copyrighted course. Many thanks to my wife, Fay, for being continuously helpful in numerous ways.

Calabash, North Carolina S. A.
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An Overview

Molecules that relate to each other as an object and its nonsuperimposable mirror image are chiral (from the Greek word *cheir*, meaning hand: they are like a pair of hands). The chiral molecules are also called enantiomers. A pair of enantiomers is possible for all molecules containing a single asymmetric carbon atom (one with four different groups attached). The asymmetric carbon has also been called the chiral center or stereogenic center (see definitions, at the end of this chapter). The chiral molecules are stereoisomeric in nature—that is, they are isomeric molecules with identical constitution but a different spatial arrangement of atoms. The symmetry factor classifies stereoisomers as either chiral or achiral molecules.

Diastereoisomers, or more simply diastereomers, are basically stereoisomers that have more than one stereogenic center and are not enantiomers of each other. Although a molecule may have only one enantiomer, it can have several diastereomers or none at all. It is important to remember that two stereoisomers cannot be both enantiomers and diastereomers of each other simultaneously.

Stereoisomerism can result from a variety of sources aside from the single chiral carbon or chiral center already mentioned (see also chapter 3). The point to remember is that it is not necessary for a molecule to have a chiral carbon in order to exist in enantiomeric forms, but it is necessary for a molecule as a whole to be chiral.

Chirality and Biological Activity

Enantiomers have identical physical and chemical properties except for the rotation of the plane of polarized light—that is, they have a different sign for optical rotation (plus or minus). The molecules with (+) optical rotation are called dextro-

rotatory (e.g., d-amphetamine), and those with (−) rotation are called levorotatory (e.g., l-amphetamine). Optically active molecules have attracted great attention because living systems are chiral. Proteins, nucleic acids, and polysaccharides possess chiral characteristic structures that are closely related to their functions. Because of chirality, living organisms usually show different biological responses to one of a pair of enantiomers (optical isomers) in drugs, food, pesticides, and waste compounds. For example, depending on the taster, sodium L-(+) glutamate tastes good, whereas its mirror image, D-(−)glutamate, tastes bitter or flat. To obtain a better understanding of D and L, as well as *R*, *S* nomenclatures, the reader may refer to the Appendix of this chapter or the discussion in chapter 3.

A mixture consisting of equal amounts of enantiomers—that is, a racemate—is obtained experimentally by chemical reactions carried out in an achiral environment. To obtain an optically pure species, the separation of an enantiomeric mixture, or optical resolution, is necessary. Furthermore, an accurate assessment of the isomeric purity of a substance is critical since isomeric impurities may have unwanted toxicologic, pharmacologic, or other effects. These impurities may be carried through the synthesis and preferentially act at one or more steps, yielding an undesirable level of another impurity.[1] It is not uncommon for one isomer of a series to produce the desired effect, while another may be inactive or even produce undesired effects. Some examples of activity differences between chiral molecules are given in Table 1.1. It is noteworthy that d-amphetamine is a potent central nervous system stimulant, whereas the l-isomer has little, if any, effect. Furthermore, biological activity does not always favor the d-isomer—for example, see epineph-

Table 1.1 Biological Activities of Some Isomeric Compounds

Category/Name	Activity
Drugs	
Amphetamine	d-Isomer is a potent central nervous system stimulant, whereas the l-isomer has little, if any, effect.
Epinephrine	l-Isomer is 10 times more active as a vasoconstrictor than the d-isomer.
Propanolol	Racemic compound is used as a drug; however; only (*S*)-(−)-isomer has the desired adrenergic activity.
Food	
Asparagine	d-Asparagine tastes sweet, while l-enantiomer tastes bitter.
Carvone	(*S*)-(+)-Carvone smells like caraway, while (*R*)-(−)-carvone smells like spearmint.
Insecticide	
Bermethrine	The d-isomer is much more toxic than the l-isomer.
Vitamin	
Ascorbic acid	(+)-Isomer is a good antiascorbutic, while (−)-isomer has no such properties.

Source: Reproduced from S. Ahuja, *Chiral Separations: Applications and Technology*, American Chemical Society, Washington, D.C., 1997. Copyright American Chemical Society 1997.

rine. Large differences in activity between enantiomers point out the need to accurately assess isomeric purity of pharmaceutical, agricultural, food, and other chemical entities.

The importance of determining the stereoisomeric composition of chemical compounds, especially those of pharmaceutical importance, cannot be overemphasized.[2] The most often quoted dramatic, though moot, example in this area is thalidomide—the teratogenic activity that leads to deformation of children has been claimed to be due exclusively to the (S)-enantiomer. The differences in pharmacological activity are better exemplified by dextromethorphan, which is an over-the-counter antitussive (cough suppressant), whereas levomethorphan is a controlled narcotic. Less dramatic examples can be found in that about 60% of the top prescribed drugs possess one or more asymmetric centers in the drug molecule. A significant percentage of the commonly used drugs are used in either the racemic or the diastereomeric form. The differences in physiologic properties between the enantiomers of these drugs has not been fully examined in many cases because of the difficulties in obtaining both enantiomers in optically pure forms. The patient may be taking a useless, or even undesirable, enantiomer when ingesting a racemic mixture, because, as mentioned, the enantiomers can exhibit different biological properties.

The world market for enantiomeric drugs exceeded $96 billion in 1998 (Table 1.2) and is expected to surpass well over $100 billion in the year 2000. This increase is the result of our interest in finding drugs with greater therapeutic activity and low toxicity. It is not uncommon for one of the enantiomers to be significantly active or toxic.

To ensure the safety and pharmacological effects of currently used and newly developing drugs, it is important to isolate and examine the enantiomers separately. Furthermore, it is necessary to measure and control the stereochemical composition of drugs because of the potential for specific problems:

Table 1.2 Sales of Enantiopure Drugs

Type	Sales (billion $)
Antibiotics	23.2
Anticancer	7.6
Antiviral	6.2
Cardiovascular	21.1
Central nervous system	7.8
Gastrointestinal	1.4
Hematologic	6.2
Hormones	11.6
Respiratory	4.2
Miscellaneous	6.9
Total	96.2

Source: *Chem. Eng. News*, Oct. 1999.

1. During manufacture, problems of physical separations or preparative-scale separations may arise.
2. During quality control or regulatory analysis, questions of purity and stability predominate.
3. During pharmacologic studies of plasma disposition and drug efficacy, toxicity of metabolites is a primary concern.

Chiral Separations

A large number of approaches can be used to obtain chiral molecules. Since Louis Pasteur reported the first example of optical resolution in 1848, a very large number of compounds have been resolved, mainly by fractional crystallization of the diastereomeric salts. The following methods can be used for separations of chiral molecules:[3]

1. Separation of enantiomers by crystallization
   a. Crystal packing triage
   b. Conglomerates
   c. Preferential crystallization
   d. Preferential crystallization in the presence of additives
   e. Asymmetric transformation of racemates
2. Chemical separation of enantiomers via diastereomers
   a. Formation and separation of diastereomer-resolving agents
   b. Separation via complexes and inclusion compounds
   c. Asymmetric transformation of diastereomers
3. Enantiomeric enrichment
4. Kinetic resolution
5. Enzymatic resolution
6. Partition in heterogeneous solvent mixtures

Discussion on these methods can be found in some recent books.[1, 3–6]. The primary focus of this book is on the methods used most commonly—that is, chromatographic methods—some of which can be related to partition in heterogeneous solvent mixtures. The following chromatographic methods are discussed:

- Thin-layer chromatography
- Gas chromatography
- High pressure liquid chromatography
- Supercritical fluid chromatography
- Capillary electrophoresis
- Membrane separations

The first three methods are discussed at length, and information is also provided on the emerging supercritical methods. High pressure liquid chromatography has been discussed extensively because it is more versatile than the other chromatographic methods. The methods also include using formation of diastereomers when these reactions either favor separation or simplify it in their use of materials.

The last two methods are not strictly chromatographic, but are included in this book to provide interesting avenues for separations.

Chromatographic methods are considered most useful for enantiomeric resolution for a number of reasons, the most important being the ease of separation. It is generally possible to find a chromatographic method that can provide separation in a matter of minutes. This comment does not imply that the knowledge base for identifying such a method already exists or is well categorized. It is meant to highlight the flexibility and efficiency that chromatographic methods offer. This book should provide a better understanding of the available methods so that the reader can design an intelligent strategy that would lead to fast selection of a reliable method for their particular separation.

A historical account of chiral separations by chromatography is given in Table 1.3. It may be noticed that direct resolution of enantiomers by gas chromatography was first reported in 1966, and resolution of enantiomers by liquid chromatography was first achieved via ligand exchange in 1971. Since then, great progress has been made in this field, leading to the development of a number of other approaches for separations, which has in some ways made HPLC the premier technique for separation of chiral compounds. More recently, capillary electrophoresis has offered some unique separations at the analytical scale because of the high efficiency offered by this technique. However, this technique is not very useful for preparative separations. In most cases, HPLC is the technique of choice for preparative separations; membrane separations and supercritical liquid chromatography may provide some other avenues.

Chromatographic methods can be direct or indirect. Indirect methods entail derivatization of a given enantiomeric mixture with a chiral reagent, leading to a pair of diastereomers that can be resolved by a given chromatographic method. In the indirect approach, the enantiomer may be converted into covalent, diastere-

Table 1.3 Historical Account of Chiral Separations by Chromatography

1939	Henderson and Rule: Resolution of racemic camphor derivatives
1952	Dalgliesh: Three-point rule in paper chromatography of amino acids
1966	Gil-Av[a] et al.:[a] Direct resolution of enantiomers by GC
1971	Davankov and Rogozhin: Chiral ligand exchange chromatography
1972	Wulff and Sarhan: Enzyme analogue polymers for chiral LC
1973	Hesse and Hagel: Cellulose triacetate used for chiral resolution
1973	Stewart and Doherty: Agarose-bonded bovine serum albumin (BSA) for chiral resolution
1974	Blaschke: Synthesis of chiral polymers
1975	Cram et al: Chromatography with chiral crown ethers
1979	Pirkle[a] and House:[a] Synthesis of first silica-bonded CSP
1979	Okamoto[a] et al:[a] Synthesis of helical polymers for chiral LC
1982	Allenmark:[a] Use of agarose-bonded BSA in chiral LC
1983	Hermansson: Use of silica-bonded α_1-acid glycoprotein for chiral resolution
1984	Armstrong[a] and DeMond:[a] Use of silica-bonded cyclodextrins

a. For these contributions, see S. Ahuja, *Chiral Separations by Liquid Chromatography*, ACS Symposium Series #471, Washington, D.C., 1991.