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Review article

# Preparative supercritical fluid chromatography: A powerful tool for chiral separations

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ABSTRACT

In 2012, the 4 biggest pharmaceutical blockbusters were pure enantiomers and separating racemic mixtures is now frequently a key step in the development of a new drug. For a long time, preparative liquid chromatography was the technique of choice for the separation of chiral compounds either during the drug discovery process to get up to a hundred grams of a pure enantiomer or during the clinical trial phases needing kilograms of material. However the advent of supercritical Fluid Chromatography (SFC) in the 1990s has changed things. Indeed, the use of carbon dioxide as the mobile phase in SFC offers many advantages including high flow rate, short equilibration time as well as low solvent consumption. Despite some initial teething troubles, SFC is becoming the primary method for preparative chiral chromatography. This article will cover recent developments in preparative SFC for the separation of enantiomers, reviewing several aspects such as instrumentation, chiral stationary phases, mobile phases or purely preparative considerations including overloading, productivity or large scale chromatography.



**Contents**



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## 1. Introduction

"Perhaps looking-glass milk is not good to drink. . . " said Alice to her black kitten in Lewis Carroll's novel, through the looking-glass and what Alice found there. This odd notion provides a great image to convey the concept of chirality in simple terms and foreshadows, in 1871, what can be a consequence of this property.

Why did evolution exclusively selected L-amino acids and D-sugars as the homochiral building blocks of proteins and nucleic acids in all living species, including viruses, bacteria, plants, animals and humans? The origin of chirality in life is still obscure, but it has a profound influence on most biological mechanisms. In the arena of drug discovery, arguably the Thalidomide story provides one of the most dramatic examples of the importance of chirality. Due to the impact of chirality on both the physical and biological properties of molecules, the separation of enantiomers (and stereoisomers in broader terms) has been a hot topic for two centuries. In 1812, Biot discovered optical activity. In 1848, aware of this phenomenon, Pasteur noticed, while squinting down a microscope, that there were two subtly different types of crystal in a sample of tartaric sodium salt, each the mirror image of the other. He very carefully and tediously separated the two types of crystal into separate heaps, re-dissolved each heap, and found that each did indeed rotate light, but in opposite directions. He had separated the two enantiomers of tartaric sodium salt, performing the first resolution of what we now call enantiomers and in so doing discovered molecular chirality. Spontaneous crystallization is thus, one way to separate enantiomers. In general terms, two strategies can be envisaged to obtain the enantiomers of a chiral compound: the first consists in designing an enantioselective synthesis of the desired enantiomer. Two independent syntheses must be developed if both enantiomers are wanted, and typically the enantiomeric excess must be determined for each enantiomer obtained. The second approach relies on the synthesis of a racemic mixture which must be subsequently separated into the corresponding enantiomers.

This separation, or resolution, affording simultaneously both enantiomers, can be achieved using different methods: crystallization, membranes, enzymatic bioconversion, electrophoretic and chromatographic resolution (Fig. 1). Within the domain of analytical chromatography, multiple techniques such as gas chromatography (GC), high performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC) can be used. Upscaling the chromatography to a preparative scale is possible for both HPLC and SFC.

Thanks to further upgrades in instrumentation and chiral column design SFC is now outperforming HPLC, both in an analytical "routine" context and on a preparative scale also, thus taking advantage of its intrinsic properties and green features [1]. In SFC, the mobile phase is constituted by at least 60% of supercritical carbon dioxide ($T = 31\,^{\circ}C$ and $P = 74$ bar). The low viscosity and high diffusivity of the mobile phase together with a lower pressure drop permit a high flow-rate with a reduced influence on efficiency. Carbon dioxide has an elution strength close to that of hexane [2]. Hence a polar modifier such as an alcohol, acetonitrile or even dichloromethane or tetrahydrofuran [3,4] must be added to elute chiral pharmaceutical compounds from the column (immobilized or coated stationary phases (SP)). It is worth noting that the addition of a liquid modifier moves the conditions to subcritical, however the advantages stated previously still exist. Obviously replacing the mobile phase by carbon dioxide will reduce the volume of organic solvent used. After being eluted from the column, $CO_2$ is removed by decreasing the pressure, leaving only a small amount of modifier to evaporate. This reduction in solvent volumes allows for higher product concentrations and reduced time and cost of purification. Last but not least, pure $CO_2$ is not expensive, it is non-toxic and non-flammable. All these reasons have contributed to making SFC a sustainable chromatography technique, which can be characterized by green metrics such as environmental factors. They also make SFC a technique of choice for chiral separations spanning a range from small preparative (a few milligrams to a hundred of grams) up to larger enantioseparations scale (Fig. 2). From a purification point of view, an increased flow-rate, together with an increased sample solubility improves the productivity, which is typically defined as racemate processed per unit mass of stationary phase and per unit time [4]. SFC has found applications in many areas: food related applications, natural products, fossil fuels, polymers, bioactive compounds and achiral pharmaceutics, but the predominant area remains in chiral applications particularly in the pharmaceutical field.

Over the last decade, SFC has been extensively and increasingly documented in different areas, reflecting a renewed interest. A number of important reviews in the field of analytical chiral separation have been published recently. Coming largely from academic groups, they have focused on: i) history of the development of the instrumention [5], ii) separation of pharmaceuticals [6–10], iii)