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Separation of Pharmaceutical Enantiomers using Supercritical Fluid Technology

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ABSTRACT: The use of supercritical fluids as chromatographic mobile phases provides rapid separations with high efficiency, favoring their use in enantioselective separations. Supercritical fluid technology (SFC) is a versatile tool in the purification, enantioseparation and large-scale production of enantiomers of pharmaceuticals over comparable liquid chromatographic methods and is gaining popularity in pharmaceutical industry. The addition of organic modifiers and additives to the supercritical carbon dioxide mobile phase extends the utility of packed column SFC to polar and even ionic compounds. SFC/MS is employed for detection of impurity and confirmation / identification of enantiomers.
Keywords: Supercritical fluid chromatography, supercritical fluid extraction, pharmaceuticals, enantioseparation.

INTRODUCTION

Reformulations in single enantiomeric form of drugs previously approved as racemates resulted in racemic switches and the development of single enantiomer drugs has gained considerable attention in the pharmaceutical industry [1]. Further, the growing emphasis on single enantiomer drugs has been fuelled by regulations governing chiral drugs and the recognition that enantiomers of a chiral drug substance may have dramatically different pharmacological activities and different pharmacokinetic and pharmacodynamic properties [2]. Chiral separation, also called chiral resolution, is a procedure used to separate the two isomers of a racemic compound in pharmaceutical industry as well as in clinical analysis. Supercritical fluids such as carbon dioxide and water provide an excellent tunable environment for materials processing, and their applications include pharmaceutical applications.

Method of separation of racemates via partial or total diastereomeric salt (complex) formation and crystallization from a proper solvent has a lot of disadvantages: i) complete enantiomer separation can not be achieved in one step, ii) a small amount of the wanted form is retained in the mother liquor, iii) a large amount of active resolution agent is needed, iv) the widely used organic solvents cause impurities in the product [3]. Separation by supercritical fluid extraction (SFE) using carbon dioxide can eliminate such problems [4]. SFE is a selective and convenient technique for sample preparation and has been already used in enantiomeric analysis [5, 6].

The basic idea of the applicability of SFE for resolution is that after the partial diastereomer formation of the racemate, only the free enantiomeric mixture is soluble in supercritical carbon dioxide [2, 7]. Separation of salt pairs may be accomplished by selective extraction at designated pressures due to the differences in their phase behavior in CO_2 although they exhibited nearly identical solubility in carbon dioxide. Because formation of ion pair complexes occurred readily in media of low polarity, supercritical carbon dioxide offered an attractive alternative to traditional organic media [8].

SFE proved to be an efficient method for the resolution of racemic N-methylamphetamine enantiomers using O, O'-di-p-toluoyl-(2R, 3R)-tartaric acid as a resolution agent. After the partial diastereomeric salt formation, the unreacted enantiomers were extracted

with supercritical carbon dioxide and the maximal chiral separation was obtained at quarter of an equivalent resolution agent molar ratio [9]. A combination of SFE to a liquid chromatography-circular dichroism (LC-CD) system has been applied to the determination of isomers of menthone using unmodified carbon dioxide. The results suggested the usefulness of SFELC-CD systems for chiral analysis when complex samples are involved [10].

Chromatographic techniques such as thin layer chromatography, gas chromatography (GC), supercritical fluid chromatography (SFC), and high-performance liquid chromatography (HPLC) have been used for enantiomer separation. SFC is a separation technique that uses super/subcritical fluid CO_2 and polar organic modifiers such as alcohols as mobile phases and columns packed with a wide variety of stationary phases. The racemic sample is introduced by injection through a sample loop and is carried by the mobile phase onto the column where the separation takes place [11]. SFC has become the choice of chromatography for separating stereoisomers owing to its speed, efficiency and cost effectiveness [12] and its applications on (E)-(Z) isomers were also reported[13]. (*E*)-2-benzylidene-3-(cyclohexylamino)-2,3-dihydro-1*H*-inden-1-one (BCI) is a synthetic molecule which contains an asymmetrical carbon and a sp^2-hybridized C-C double bond resulting in both optical and (E)-(Z) isomerism. The rapid simultaneous resolution of all four isomers of BCI, both chiral and (E)-(Z) isomeric, by SFC has been reported [14].

Most recently, interest has expanded in using SFC with chiral stationary phases. SFC is rapidly replacing HPLC in many pharmaceutical as the standard screening and method development tool for chiral compounds. In general, SFC can potentially separate any compound soluble in methanol or a less polar solvent. SFC has some significant advantages over standard HPLC methods such as more flexible in solvent selection, less pressure drop across the columns, faster column equilibration, faster method development, higher efficiency separations and significantly less generation of hazardous waste, improving selectivity and solubility**,** having polar modifiers direct mixing with CO_2 compared to HPLC. The major advantage that SFC holds over GC is the ability to separate thermally labile compounds. At present, SFC has become an attractive alternative for chiral drug separation which has assumed its role as a complimentary method to both HPLC and GC [15]. Mechanistically, SFC plays a unique role, acting as a bridge between gas and liquid chromatography. SFCs also presents advantages in the quality control analysis of chiral drugs using packed SFC chiral columns with CO_2 as mobile phase and this separation may be done faster using SFC than with either HPLC or GC, with the necessary resolution [16].

The use of SFC for the separation of enantiomers has been one of its most successful applications due to the inherent advantages of using liquid carbon dioxide in the mobile phase, resulting from its high diffusivity and low viscosity [17]. The SFC screen is considered the first try for chiral separations of new compounds and utilized a narrow combination of only four columns (Chirlapak AD and AS, and Chiralcel OD and OJ) and two solvent modifiers (methanol and isopropanol) for rapid enantiomer resolution [18]. Until recently, SFC has found the greatest success in small preparative scale chiral separations in drug discovery laboratories. However, various studies demonstrated that there is significant potential for extending SFC to achiral column screening and method development [19]. The benefits of SFC using reverse phase columns as an alternative technique to RP-HPLC has been reported [20].

The separation and purification of immunosuppressant agent cyclosporine A was achieved by SFC using dense CO_2 mixed with light alcohols to meet the requirements of US Pharmacopoeia. The results indicated that normal phase silica could be used and supercritical carbon dioxide modified with 2-propanol was the most suitable mobile phase. Further, cryogenic chiral separation of aromatase inhibitor finrozole was also reported [21].

Major emphasis in SFC today concerns packed columns although several studies using open tubular columns continue to appear each year. Packed column SFC tends to obtain higher column efficiency than normal-phase HPLC [22]. Packed column SFC coupled to mass spectrometry (pSFC-MS) appears more and more as a complementary technique for high-throughput analysis [23].

Albendazole is an anthelmintic agent used both in veterinary and human practice. Its main metabolites are albendazole-sulfoxide which is believed to be further converted to albendazole sulfone. The enantioseparation of albendazole sulfoxide by chiral SFC was achieved on Chiralpak AD (with 2-propanol as modifier) and Chiralcel OD (with methanol as modifier [24]. The enantioseparation of 123 clinically used racemic drugs by SFC on commercial chiral stationary phases has been reported [25].

Preparative, chiral SFC is drawing extensive attention for separation of enantiomers due to the green characteristics of supercritical fluids. The use of SFC

for preparative enantioseparation has enjoyed significant attention [26-28]. With preparative SFC, the product is recovered in a more concentrated form relative to HPLC, greatly reducing the amount of solvent that must be evaporated, and resulted in considerable savings in labour [29]. CHIRAL TECHNOLOGIES EUROPE launched the new series of 3 µm CHIRALPAK® and CHIRALCEL® DAICEL chiral stationary phases for the analytical separation of enantiomers. These new chiral columns will allow faster enantioselective analytical separations, rapid SFC high throughput screening as well as higher efficiency analytical separations. A high-productivity preparative SFC separation of the enantiomers of flurbiprofen (an anti-inflammatory and an analgesic drug) has been accomplished on a semipreparative SFC column without acidic additives [30].

The semipreparative chiral separation of lansoprazole and two related compounds (pantoprazole and rabeprazole) using SFC has been reported [31]. Chiral SFC was used to separate the enantiomers of racemic mixtures of pharmaceutical compounds and cryogenic temperatures substantially enhanced the separation efficiency of chiral SFC [32]. Several chiral sulfoxides enantiomers belonging to the family of the substituted benzimidazoles , including omeprazole, lansoprazole, pantoprazole, rabeprazole, oxfendazole and ricobendazole were separated on Chiralpak AD column using SFC [33].
A method was developed to separate the enantiomers of a non-steroidal anti-inflammatory drug ibuprofen and flurbiprofen on a Whelk-O 1 stationary phase using various modifiers in SFC. Out of several modifiers used for analysis, one combination of 5% isopropanol and 5% ethanol modifier (total *v/v* 10%) achieved the best peak resolution of 1.39 at 35°C [34]. A SFC method was developed for chiral separation of ibuprofen enantiomers [35].

SFC coupled with mass spectrometry (SFC-MS) has had a great advance in productivity; due to increase in reliability and robustness of both SFC and MS systems and the role of SFC-MS analysis in drug discovery process have been reported [36].

A novel process for the resolution of enantiomers of mandelic acid by simultaneous reaction of the enantiomers with *R*(+)-α-methylbenzylamine as the chiral agent, and precipitation of the formed diastereomeric salts in a supercritical carbon dioxide environment has been reported. The highest resolution efficiency (e.e = 63%) was achieved when a partial diastereomeric salt formation was performed simultaneously with the precipitation, at 8 MPa and 328 K [37].

Scientists have reported the enantiomeric separation of cetirizine and oxfendazole on a Chiralpak AD column using SFC. The enantioseparation of cetirizine was only feasible when 2-propanol was used as a modifier, obtaining better results in presence of the additives triethylamine (TEA) and trifluoroacetic acid (TFAA). On the other hand, the best results for oxfendazole enantioresolutions in terms of high resolution and short analysis time were obtained with ethanol [38]. A comparative study of the enantiomeric separation of several antiulcer drugs such as omeprazole, lansoprazole, rabeprazole and pantoprazole using HPLC and SFC on the Chrialpak AD column showed that only two compounds (omeprazole and pantoprazole) could be enantiomerically resolved using HPLC, on the contrary SFC allowed the enantiomeric separation of all the compounds studied with higher resolutions and lower analysis times [39]. Using the columns based on polysaccharide derivatives, HPLC and SFC enantiomeric separation of several compounds, including an antifungal drug and several of its precursors has been reported. The results showed that most of the separations obtained by SFC are better, in terms of high resolution and short analysis time, than those obtained by HPLC [40].

Bifonazole, 1- (1, 1'-biphenyl)-4-ylphenylmethyl-1H-imidazole, belongs to a group of antimycotics, imidazole derivatives with a broad spectrum of activity. It is applied for skin and nail infections with the fungus *Malassezia furfur* and *Candida spp.* and also expresses *in vitro* antibacterial action against some gram-positive cocci [41]. The enantiomeric separation of bifonazole by SFC on Chiralpak AD in the presence of methanol as modifier has been reported [42]. An isocratic supercritical/subcritical fluid chromatography method for the separation of naproxen enantiomers on the Kromasil CHI-TBB column using supercritical CO_2 as mobile phase with 2-propanol as modifier was reported. The experimental conditions were temperature 293 K–323 K, pressure 9.4 MPa–21.3 MPa, and 2-propanol concentration 6%–15% (by mass), respectively. The enthalpy contribution to the overall enantiomer transfer energy was more important than the entropy contribution in the temperature range examined. The preferred operation conditions were found to be 293 K, 9.4 MPa, and the concentration of 2-propanol in the mobile phase 11% (by mass) [43].

A packed column SFC method for the separation of ibuprofen enantiomers on a chiral stationary phase and CO_2 with modifier as mobile phase has been developed and among 11 different stationary phases, the Kromasil CHI-TBB phase showed by far the best separation properties [44].

Packed column SFC was used to resolve the enantiomers of two nonsteroidal anti-inflammatories ibuprofen and flurbiprofen on a Chiralpak AD column with a binary mobile phase of carbon dioxide/methanol to achieve resolution. The effects of composition, pressure, temperature, and flow on ibuprofen enantiomer separation were examined and temperature afforded the greatest change in resolution followed by pressure and composition. Baseline resolution of ibuprofen was achieved in less than 7 min and fluibiprofen baseline resolved in less than 4 min**.**

Inhibition of the MDM2–p53 interaction can stabilize the p53 protein and offer a novel strategy for cancer therapy. Researchers have explored various enantiomeric separation approaches to resolve the Nutlin-3 (small molecule antagonist of the MDM2–p53 interaction) enantiomers using chiral SFC [45].
Scientists studied combined chiral and achiral separations of a preoxysome proliferation receptor agonist drug, using SFC for both separation of enantiomers and separation of active drug from process impurities [46]. The separation of the enantiomers of flurbiprofen on an amylose-derived chiral stationary phase, Chiralpak AD-H, by SFC under both linear and non-linear conditions has been reported. The number of theoretical plates greater than 5000 indicated high efficiency of SFC [47].
A simple SFC chiral assay to determine the possibility of interconversion of the desired R and less active S isomers of a drug candidate was reported [48]. Factors influencing the enantioseparation of racemic ibuprofen via partial diastereomeric salt formation with *R*-(+)-phenylethylamine and subsequent SFE of the unreacted enantiomers were reported [49].

The enantioseparation of trans-3-ethoxycarbonyl-4-(4'-fluorophenyl)-1-methyl piperidine-2,6-dione, which is one of the important racemic precursors of trans-(-)-paroxetine, has been investigated using SFC on a Daicel Chiralpak AD column. Supercritical CO_2 modified with methanol, ethanol and 2-propanol were used as mobile phase. Results indicated that among methanol, ethanol and 2-propanol, 2-propanol was proved to be the most favorable modifier, and 9.5% (v/v) of 2-propanol was the preferred concentration at which racemate could be separated with resolution of 15.86 and retention factor of 6.323 [50]. Simulated moving bed (SMB) chromatography is often perceived in the pharmaceutical industry as chromatographic method for separating binary mixtures, like racemates, and racemates and diastereomers [51].
The separation of the mitotane enantiomers on a chiral stationary phase and supercritical fluid CO_2 with modifier, methanol, ethanol or 2-propanol, as mobile phase in semi-preparative scale, and to study the effect of modifier concentration on the separation behavior at constant temperature and pressure was reported [52].
A series of β-blockers enantiomers were directly separated on two chiral stationary phases derived from 3,5-dinitrobenzoyl tyrosine (the commercially available ChyRoSine-A and a recent improved version of this CSP using SFC, and facile separations were achieved within short analysis times. Both amine and hydroxyl protons were found necessary for chiral discrimination to occur. It has been shown that carbon dioxide acted as a complexing agent toward the amino-alcohol by setting up of a bridge with the hydroxyl and the amine protons of the solute, and thus the resulting complex possesses lower acido-basic properties and a higher conformational rigidity, responsible for chiral discrimination [53].

A SFC method using a Chiralpak AD column for the enantiomeric separation of a peroxysome proliferator-activating receptor agonist drug was developed, with the enantiomeric purity determined within 10 min on a 5-cm column. A SFC-tandem mass spectrometry method using a Chiralcel OD-H column was developed for the enantioselective detection of propranolol and pindolol in mouse blood by serial sampling [54]. Simulated moving bed (SMB) chromatography, a continuous multi-column chromatographic process, has become one of the preferred techniques for the separation of the enantiomers of a chiral compound. The SMB technology has found applications both at small and large scales. Scientists have reported the developments, as well as both the fundamentals of the SMB science and technology, and particular emphasis has been placed on the consolidation of the "triangle theory", a design tool that is used both in the academia and industry for the design of SMB processes [55]. Supercritical fluid-simulated moving bed processes under pressure gradient mode offered productivity improvement over the isocratic mode of operation[56]. A tandem-column method using Chiralpak AD-H and Chiralcel OD-H columns was achieved for baseline separation of a mixture of four stereoisomers of chiral pharmaceutical compounds via SFC with a mobile phase consisting of 90% liquid carbon dioxide and 10% ethanol: isopropanol (50:50 v/v) [57]. Purification method development for chiral separation in SFC with the solubilities in supercritical fluid chromatographic mobile phases was reported [58]. A novel strategy for rapid chiral method development has been implemented using sample pooling and SFC-MS on four chiral stationary phases, namely Chiralpak AD and AS, and Chiralcel OJ and OD, and eight different modifier concentrations (5 to 40% methanol-0.2% isopropylamine). In addition, with SFC-MS,

enantiomeric excess was determined with much lower detection limits than UV and much shorter analysis times compared to normal-phase/reversed-phase liquid chromatography [59]. Researchers described an SFC method for the direct analysis and quantitative determination of hydrocortisone in Cortizone 10 Plus crème [60].

CONCLUSIONS

Single enantiomers and stereoisomers have overtaken achiral molecules in the percentage of approved drugs in the pharmaceuticals market. Supercritical fluid chromatography (SFC) is being increasingly used for small and medium scale chiral separations that are mainly encountered during the drug development process. SFC has widespread application in pharmaceutical analysis and enantioseparation and this technique is particularly attractive as it is feasible to access enantiopure substances in a short time while enormously reducing organic solvent consumption. Owing to its high speed, short analysis times, low cost, user-friendliness and limited environmental impact, packed-column SFC is of particular interest, and will become a viable alternative to chiral HPLC to separate chiral drug substances and intermediates.

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