Journal of Pharmaceutical and Biomedical Analysis 69 (2012) 77–92

Contents lists available at SciVerse ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Review

Supercritical fluid chromatography for the enantioseparation of pharmaceuticals

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ARTICLE INFO

Article history:
Received 1 December 2011
Received in revised form 18 January 2012
Accepted 19 January 2012
Available online 28 January 2012

Keywords:
Supercritical fluid chromatography (SFC)
Chiral stationary phase
Enantioseparation
Pharmaceuticals
Screening strategies

ABSTRACT

Chirality has a significant impact on drug discovery and development processes in the pharmaceutical industry. As the number of enantiopure drugs launched onto the market is yearly increasing, the need for fast and performant enantioseparation methods with minimal costs is becoming more compelling. In this context, sub- and supercritical fluid chromatography (SFC), being applicable at an analytical, as well as at a preparative scale is gaining more interest. In this review a practical overview is given of the different parameters that are important in supercritical fluid chromatographic separations. A comparison is made between the applicability for chiral separations of SFC and conventional high-performance liquid chromatography (HPLC), and illustrated by means of examples. Different aspects of method development and the upscaling feasibility in SFC are discussed. This review aims to give the reader a practical insight in the use of supercritical fluid chromatography for the chiral separation of pharmaceutical compounds.

Contents

1. Introduction

Chiral centres are common in the amino-acid and carbohydrate building blocks that constitute proteins, sugars and nucleic acids. As a result, chirality is a fundamental characteristic of all living organisms, and all essential physiological processes display enantioselectivity [1–3]. In other words: one enantiomer may interact stronger with a certain target site than the other, due to the difference in spatial configuration [4]. The isomer which generates a therapeutic response by interacting with the target site is called the eutomer. The isomer which binds less strong to the target site is referred to as the distomer. It can display no activity, less activity, an antagonistic activity, another activity through interaction with other target sites, or even toxic effects [5]. This applies for endogenous as well as exogenous substances (active drug substances).

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doi:10.1016/j.jpba.2012.01.021

Although early insights proved this potential and substantial pharmacological difference between enantiomers, it was not until the 1960s, after the thalidomide-disaster, that interest and research activity was increased in the field of chirality [6,7].

Nowadays, the European, USA and Japanese regulatory authorities therefore impose strict guidelines for the commercialization of chiral drug substances. Enantioselective identification and quantification methods should be developed for each active pharmaceutical ingredient with chiral properties. In addition pharmacokinetic and toxicological assays should be executed with both pure enantiomers and with the racemate [8–11]. These requirements made chiral separations a well-recognized and extensively studied topic in today's drug development [7]. Accumulating evidence that show medicinal advantage of using pure enantiomers over racemates of active drug substances, have boosted the sale numbers of so called single-enantiomer drugs [4–7,12].

Undoubtedly it is clear that the resolution of racemates on analytical level for drug development as well as on preparative level for drug commercialization, is vital. While different approaches, such as asymmetric synthesis, enantioselective crystallisation and kinetic resolution procedures can be used to obtain pure enantiomers, chromatographic resolution remains the most favored and cost-effective approach, especially in early drug development [5,13–15]. Chromatographic enantioseparations can be done with gas chromatography, thin layer chromatography, capillary electrophoresis, capillary electrochromatography, supercritical fluid chromatography and high-pressure liquid chromatography [16–27]. The easy applicability, plethora of reported applications, advanced instrumentation and extensive knowledge favored HPLC as most used separation technique in the pharmaceutical industry [14,16–18,20,22,23,26,27]. However, several limitations can be encountered during the development of chiral HPLC-methods, such as rather long equilibration- and analysis times and the need of using toxic and flammable solvents. As diffusion processes in HPLC can be relatively slow, significant peak broadening may occur and affect the quality of the separation, resulting in low efficiencies. This is a significant drawback for enantiomeric purity determinations, when peak overlap occurs between the main enantiomer and the enantiomeric impurity. High efficiencies are also important at a preparative level, since upscaling methods tends to decrease efficiency among others due to the larger particles used [28,29].

These issues raised the interest in alternative separation techniques. This review focuses on one of these alternatives for enantioseparations, namely SFC. A practical overview is given of the different parameters that are important in supercritical fluid chromatographic separations. Different types of chiral stationary phases (CSPs), used in SFC, are described and relevant applications are cited. A comparison is made between the applicability for chiral separations of SFC and conventional HPLC, and illustrated by means of examples. Different aspects of method development and the upscaling feasibility in SFC are discussed.

2. Supercritical fluid chromatography

2.1. Supercritical fluids

Klesper et al. [30] were the first to propose the use of supercritical fluids as eluents for chromatographic separations in 1962. A supercritical fluid is a physico-chemical state of a substance that occurs when temperature and pressure are elevated at or above its thermodynamic critical point, as shown in Fig. 1 [13,29]. A supercritical fluid possesses unique characteristics compared to the liquid or gas state. Its density is higher than the gas state, implying that the supercritical fluid has a higher solvating power; its viscosity is lower and diffusivity is higher than the liquid state, implying that it induces a lower pressure across a column and migrates faster [29,31,32]. Supercritical fluid chromatography was developed to take advantage of the aforementioned characteristics.

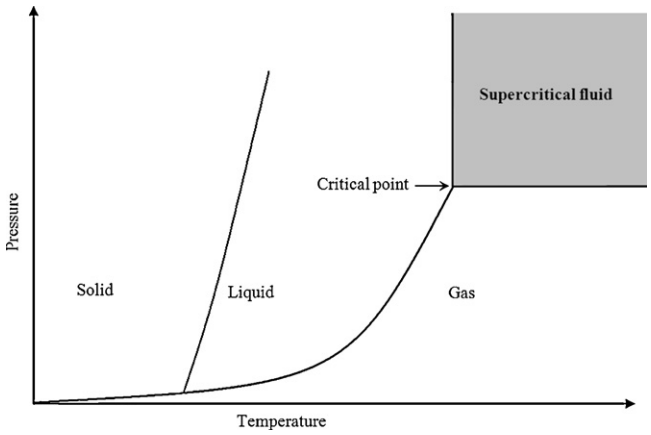


Fig. 1. Phase diagram of a pure substance [31].

Strictly spoken, the supercritical state only occurs above both the critical pressure and temperature. However, there is no discontinuous transition between the liquid and supercritical states. In addition, mixing the primary eluent of the mobile phase (*e.g.* CO_2) with modifiers (*e.g.* methanol) and additives (*e.g.* an amine) has an (unknown) influence on the critical point. In practice this implies that SFC is often performed in the subcritical region. This has no consequences since similar characteristics as for the supercritical state apply in this region. For this reason, in this review no distinction will be made between subcritical and supercritical fluid chromatography [33].

Table 1 shows the critical temperatures and pressures of some selected substances [31,34]. In SFC, for multiple reasons, carbon dioxide (CO_2) is used almost exclusively as eluent. Its critical temperature and pressure are relatively low, thus only minimal instrumental requirements are necessary to form supercritical CO_2. The low critical temperature allows analyzing thermo-labile compounds. Reducing the pressure to atmospheric pressure converts the CO_2 to a gaseous state, which is easy to remove after analysis. This implies a very significant reduction in waste generation and -disposal. In addition carbon dioxide, can be purified and recycled after analysis for re-use on a preparative scale, resulting in significant cost reductions. Finally, carbon dioxide is inert and safe to use since it is harmless for humans. As a result SFC is seen as a green technology. The solvent is also rather inexpensive compared to organic solvents. The aforementioned characteristics indicate why carbon dioxide is the most favored eluent for supercritical fluid chromatography [19,29,35,36].

2.2. Chiral stationary phases

The number of reported CSPs in the literature exceeds 1500 and is still increasing. For chiral SFC most columns from HPLC applications can be adopted directly. A classification into three groups

Table 1
The physical parameters critical temperature and pressure of some selected substances [31].

	Critical temperature (°C)	Critical pressure (atm)
CO_2	31	73
N_2O	37	72
NH_3	133	111
H_2O	374	218

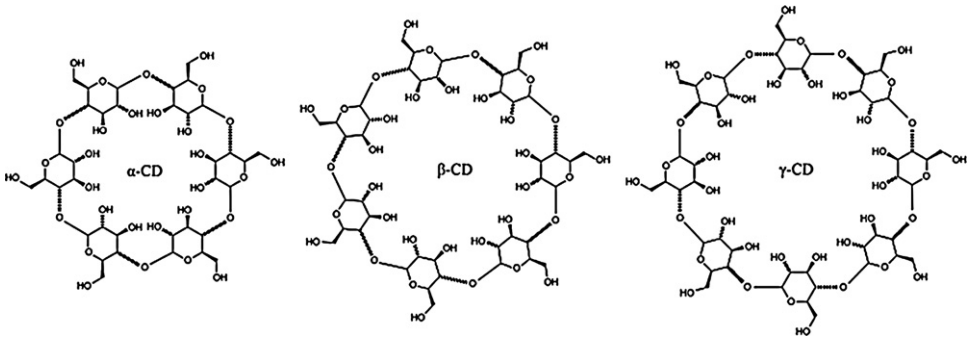


Fig. 2. General structure of the underivatized cyclodextrins (CD) commonly used as chiral selectors [37].

of the CSPs used in practice for SFC can be made: (i) CSPs with macrocyclic selectors, as cyclodextrins and macrocyclic antibiotics, (ii) CSPs with low-molecular-weight selectors, such as Pirkle-type CSPs, and (iii) CSPs with macromolecular selectors, as proteins, derivatized polysaccharides and synthetic polymers [29,37]. For the majority of the chiral SFC separations, polysaccharide-based, Pirkle-type and macrocyclic antibiotic-based CSPs are used.

2.2.1. Cyclodextrins and macrocyclic antibiotics

Cyclodextrins are cyclic structures, composed of glucopyranose units. Depending on the number of these units, 6, 7 or 8, an α-, β- or γ-cyclodextrin, respectively, is formed (Fig. 2) [37]. Cyclodextrins have the shape of a truncated cone with a relatively hydrophobic interior chiral cavity and a hydrophilic exterior surface surrounded by hydroxyl groups. The presence of the hydrophobic cavity enables the entrapment of hydrophobic (parts of) molecules. The secondary hydroxyl groups on the outside allow the selector to interact with analytes *via* hydrogen-bonding and/or dipole–dipole interactions, leading to enantioselective separations. Derivatization of the hydroxyl groups with ionic functional groups, *e.g.* sulphate groups, alters the enantioselectivity. Although this type of chiral selector is extensively used in chiral capillary electrophoresis as mobile phase additive, and in chiral gas chromatography coated on the capillary wall, successful applications in SFC using this selector coated on a CSP have also been reported (Table 2) [38–42]. However the formation of enantioselective inclusion complexes might be hindered by the apolar carbon dioxide, competing also for the hydrophobic cavity of the cyclodextrins [37,42,43].

CSPs based on macrocyclic antibiotics, *e.g.* vancomycin (V), teicoplanin (T) and ristocetin (R), are more widely used in SFC. Due to the multiple active chiral interaction sites within their macromolecular structure, these stationary phases display a broad enantioselectivity. They consist of a similar aglycone part containing fused macrocyclic glycopeptide rings and linked carbohydrate moieties (Fig. 3) [44]. These stationary phases show a good stability because this type of selectors is always bonded on the silica, but long equilibration times have been reported as a disadvantage [43,45]. The selectors contain ionisable groups such as amines,

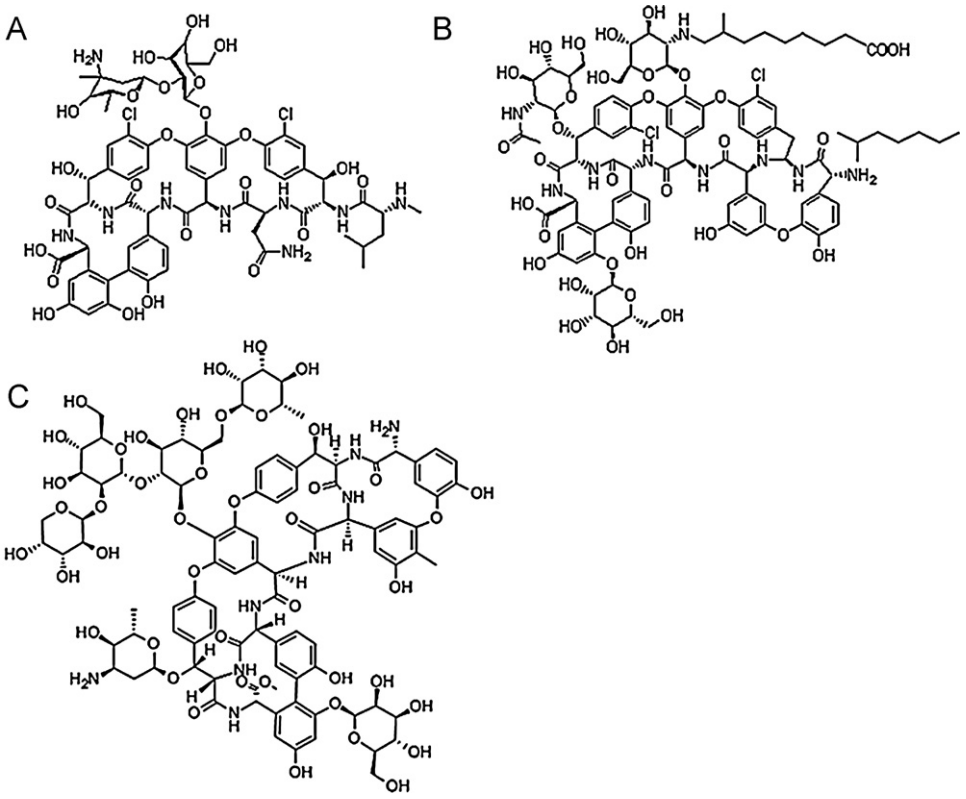


Fig. 3. Structures of macrocyclic glycopeptides that are used as chiral selectors: (A) vancomycin, (B) teicoplanin, and (C) ristocetin [44].

Table 2
Selected examples of chiral SFC separations with CSPs based on cyclodextrin selectors.

Commercial name	Chiral selector	Mobile phase	Substances analyzed	
Sumichiral® OA-7500	Heptakis(2,3,6-tri-O-methyl)-beta-cyclodextrin	CO_2/2-propranol (98/2) (v/v)	Alpha-tetralol, 2-phenylpropionic acid, 1-phenylethylamine	[38]
β-Cyclose®-OH T	Mono-2-O-pentenyl-β-cyclodextrin	CO_2 + variable % (methanol + diethylamine)	Aminogluthetimide, thalidomide	[39]
β-Cyclose®-6-OH T	Mono-6-O-pentenyl-β-cyclodextrin			
β-Cyclose®-2-OH	Oxidized mono-2-O-pentenyl-β-cyclodextrin			
β-Cyclose®-6-OH	Oxidized mono-6-O-pentenyl-β-cyclodextrin			
MPCCD®	Mono-6-(3-methylimidazolium)-6-deoxyperphenylcarbamoyl-β-cyclodextrin chloride	CO_2/2-propranol (97/3) (v/v)	1-(p-Fluorophenyl)ethane, 1-(p-chlorophenyl)ethanol, 1-(p-bromophenyl)ethanol, 1-(p-iodophenyl)ethanol,1-(p-methoxyphenyl)ethanol, 1-(p-chlorophenyl)-3-butene-1-ol, 1-(p-chlorophenyl)-3-butene-1-ol, 1-(p-phenylphenyl)-3-butene-1-ol, 1-(p-trifluoromethylphenyl)-3-butene-1-ol, 1-(p-bromophenyl)-3-butene-1-ol, 1-(m-fluorophenyl)-3-butene-1-ol, 1-(m-chlorophenyl)-3-butene-1-ol, 1-p-fluorophenyl-1-phenyl-methanol	[40]
MDPCCD®	Mono-6-(3-methylimidazolium)-6-deoxyper(3,5-dimethylphenylcarbamoyl)-β-cyclodextrin chloride			
OPCCD®	Mono-6-(3-octylimidazolium)-6-deoxyperphenylcarbamoyl-β-cyclodextrin chloride			
ODPCCD®	Mono-6-(3-octylimidazolium)-6-deoxyper(3,5-dimethylphenylcarbamoyl)-β-cyclodextrin chloride			
Cyclobond® I 2000 RN	(R)-Naphthylethylcarbamoylated-β-Cyclodextrin	CO_2/methanol variable % (95–70/5–30) (v/v)	34 substances: *e.g.* alanine ethyl ester, alanine methyl ester, leucine methyl ester, valine methyl ester, phenylalanine methyl ester, 1-cyclohexylethylamine, α-methylbenzylamine, ancymidol, bendroflumethiazide, cromakalin, ibuprofen, mephenytoin, piperoxan, tolperisone, tropicamide, verapamil, suprofen, amongst others	[41]
Cyclobond® I 2000SN	(S)-Naphthylethylcarbamoylated-β-cyclodextrin			

Table 3
Selected examples of chiral SFC-separations with CSPs based on macrocyclic antibiotics.

Commercial name	Chiral selector	Mobile phase	Substances analyzed	
Chirobiotic T	Teicoplanin	Gradient from 5% to 30% (methanol + 0.1% diethylamine or trifluoroacetic acid) over 5 min	Acebutolol, alprenolol, althiazide, amlodipine, atropine, bendrotlumethizide, clenbuterol, cyclopenthiazide, cyclothiazide, dyspyramide, ephedrine, felodipine, fenoprofen, fenoterol, flurbiprofen, guaifenesine, hexobarbital, indapamide, ibuprofen, ketamine, ketoprofen, lormethazepam, mandelic acid, medetomindine, mephobarbital, metoprolol, nadolol, naproxen, oxazepam, oxprenolol, 2-phenyl cyclohexanone, pindolol, polythiazide, promethazine, propranolol, salmeterol, spironolactone, tetramisole, tiaprofenic acid, *trans*-stilbene oxide, trifluoro-anthranylethanol, tropic acid, verapamil, warfarin	[45]
Chirobiotic V	Vancomycin			
Chirobiotic® R	Ristocetin	CO_2/methanol variable % 70–75/30–25 (v/v)	Dichlorprop, ketoprofen, warfarin, coumachlor, thalidomide	[48]
Chirobiotic® R	Ristocetin	CO_2/(methanol + 0.1–0.5% triethylamine or trifluoroacetic acid) variable % 93–33/7–67 (v/v)	111 substances including alanine, asparagine, serine, tyrosine, lysine, chlorthalidon, flurbiprofen, norgestrel, prolactone, fenoprofen, indoprofen, ibuprofen, atenolol, oxprenolol, metoprolol, alprenolol, acebutolol	[49]
Chirobiotic® T	Teicoplanin			
Chirobiotic® TAG	Teicoplanin aglycon			
Chirobiotic® R	Ristocetin	CO_2/(methanol + 0.1–0.5% triethylamine or acetic acid) 75/15 (v/v)	Warfarin, thalidomide, efavirenz	[50]
Chirobiotic® R	Ristocetin	CO_2/methanol variable % 98–80/2–20 (v/v)	24 dihydrofuroangelicins, dihydrofuropsoralens and other coumarine derivatives	[51]
Chirobiotic® T	Teicoplanin			
Chirobiotic® TAG	Teicoplanin aglycon			
In-house made	Vancomycin	CO_2/(methanol + 1% triethylamine or acetic acid) variable % 90–50/10–50 (v/v)	Alprenolol, atenolol, benzoin, binaphtol, bipivacain, dichlorprop, ethotoin, etidocaine, fendilin, ketoprofen, mepivacaine, methaqualone, methyprylon, metixene, metoprolol, norverpamil, *o*-chlorprop, *p*-chlorprop, pentivacaine, phensuximide, prilocaine, practolol, propranolol, ropivacaine, thalidomide, warfarin, verapamil	[52]

that are thought to play an important role in the chiral recognition of analytes. Furthermore, enantioselective recognition also arises from different interactions, such as hydrogen bonding, hydrophobic inclusion in cavities, dipole stacking, steric repulsion and π–π interactions. Under specific conditions, it is also possible for enantiomers to form inclusion complexes with the aglycone part of the selector [37,43,44,46]. Vancomycin-based stationary phases perform especially well for the separation of basic compounds, teicoplanin is applicable for the enantioseparation of both basic and acidic compounds and ristocetin is most appropriate for the separation of acidic compounds. However, this is not a fixed rule of thumb, since chiral recognition on these CSPs arises from very complex mechanisms that are not only influenced by characteristics of the analyte, but also by the mobile phase composition, analysis conditions, *etc.* [47]. Table 3 shows on overview of some exemplary separations that were obtained in SFC on this type of CSPs [45,48–52].

2.2.2. Pirkle-type

Brush-type or Pirkle-type stationary phases were the first chiral stationary phases reported in the literature [53,54]. These stationary phases are rationally designed to target specific chiral interactions with an analyte. Structurally they consist of single strands with either π-donor or π-acceptor aromatic fragments, as well as hydrogen bonding agents and dipole-stacking inducing functional groups that are covalently bonded onto a silica surface. Enantioselectivity on these CSPs arises from a three-point interaction between the analyte and chiral selector which forms a labile diastereomeric complex with one enantiomer, while the other enantiomer forms a diastereomeric complex through a two-point interaction (Fig. 4). This will result in different complex stabilities, different retentions and, hence enantioseparation [55].

This type of CSPs has been extensively studied for application in chiral SFC and shows the advantage of having an excellent physical stability under relatively extreme pH conditions. The main drawback arises from the strong residual silanol activity that is not shielded by the covalent bonding of the chiral selectors onto the silica matrix. This drawback has somewhat limited the success of these CSPs in SFC [29,56]. In Table 4 an overview is given of some chiral separations on these CSPs in SFC [57–60].

2.2.3. Polysaccharides and synthetic polymers

Polysaccharide-based stationary phases are the most successful CSPs and have taken a dominant position in chiral SFC because of their easy accessibility and broad enantioselectivity. Underivatized polysaccharides, such as cellulose and amylose, only show limited enantioselectivity, since their helical structure is too dense to allow inclusion and enantiorecognition of many molecules [37,61,62]. In 1973, the use of derivatized polysaccharide-based selectors was first reported by Hesse and Hagel [63]. They performed an esterification of the hydroxyl groups of cellulose with acidic acid and found a derivative (cellulose triacetate) with a broad enantiorecognition ability. Later, Okamoto et al. [64] developed and commercialized various kinds of CSPs which used cellulose esters as selectors. Among all polysaccharide-derivatives, the carbamate and benzoate esters show the best performance. Numerous polysaccharide-based stationary phases are commercially available nowadays (Table 5). Since the selectors are coated and not bonded on the silica matrix of these CSPs, they have a limited solvent compatibility. Solvents that dissolve the selector coating around the particles are therefore incompatible *e.g.* aceton or tetrahydrofuran [65]. To overcome this problem immobilized CSPs were prepared with a covalently bonded chiral selector on the silica matrix, *e.g.* Chiralpak® IA, IB, IC and ID (Table 5) [66,67].

Due to their multiple binding sites, the chiral recognition mechanisms of derivatized polysaccharides are rather complex and often not fully elucidated. Chiral discrimination can arise from inclusion interactions inside the polysaccharide helical structure, aromatic functional groups may undergo π–π interactions with the selector, and hydrogen bindings may add to the enantioselectivity. Important to note is that enantiodiscrimination again has to arise from a three-point interaction between the chiral selector and the analyte (Fig. 4) [55].

Introduction of electron-withdrawing groups like halogens, or electron-donating substituents such as alkyl groups in the structure enhances the enantioselective interactions. Based on this knowledge, chlorinated and methylated polysaccharide derivatives were developed as chiral selectors by Chankvetadze et al. [37,61,68–70] *e.g.* cellulose tris(3-chloro-4-methylphenylcarbamate) (Table 5). There is an abundance of chiral SFC applications in the literature that use polysaccharide-based stationary phases and they are undoubtedly the most used CSPs. Given the high number of applications, it would have little value to cite only a few examples. In addition, this review aims to give an overview of different aspects of chiral SFC, instead of summarizing all applications.

Synthetic polymer-based stationary phases have also been used in chiral SFC. This type of CSPs is developed by the polymerization of single chiral monomers to a three-dimensional polymer network. Although the recognition mechanisms on these stationary phases are not fully elucidated to date, the main interaction is assumed to be chiral inclusion into the three-dimensional network, supported by the formation of hydrogen-bonds. The limited stability of these CSPs under high pressure makes them more suited for SFC than for HPLC applications. However, even then only a limited number of CSPs with these selector types are commercialized and their reported use in SFC is rather limited. For instance, the enantiomers of ibuprofen and naproxen were separated in SFC using Kromasil® CSPs consisting of O,O′-bis(3,5-dimethylbenzoyl)-N,N′-diallyl-L-tartar diamide or O,O′-bis(4-tert-butylbenzoyl)-N,N′-diallyl-L-tartar diamide as chiral selectors [71,72].

2.3. Mobile phase

Carbon dioxide is the main eluent used in SFC, because of its multiple advantages over other eluents, as mentioned above. However, the polarity of CO_2 can be compared to that of heptane and hexane, which necessitates the use of modifiers to increase the elution strength of the mobile phase [28,35,72,73]. In practice modifier concentrations are kept below 50%. One reason is that using higher concentrations of modifier might alter the critical point of the mixture with carbon dioxide too much and a situation might occur in which the mobile phases no longer exists in a sub- or supercritical state but in a liquid–vapor state, that no longer displays the supercritical-fluid characteristics [73–75]. A second reason is that small concentrations of polar modifiers already can dramatically increase the solvent strength [35,73].

Various polar organic solvents can be used as modifier in the supercritical mobile phase since they are nearly all miscible with CO_2; most frequently used are methanol, ethanol and isopropranol. Occasionally, the use of acetonitrile is also reported [35,72,76]. Because of the competition that occurs between the modifier and analytes for interaction sites on the stationary phase, a decrease in retention is observed when increasing the modifier content in the mobile phase. The modifier not only affects the mobile phase strength and consequently the retention, but also has an impact on the enantioselectivity. Chiral recognition is a process arising from multiple and complex interactions between the analyte and the stationary phase. Modifiers can influence these enantioselective interactions in several ways, *e.g.* the organic modifier may alter the sterical positioning of the chiral selector chains or affect the structure of analytes. By carefully choosing the type of modifier,

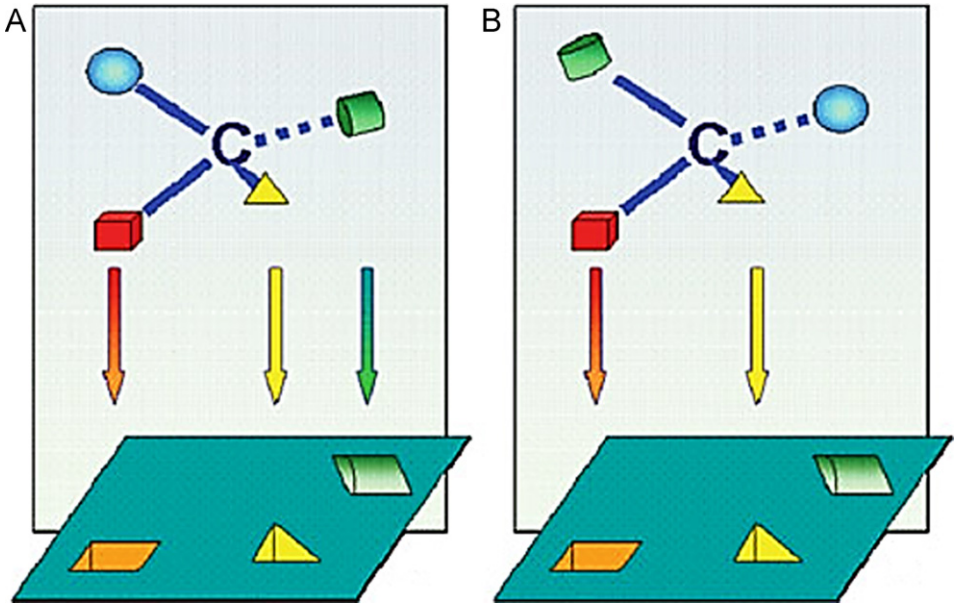


Fig. 4. The three-point interaction model: (A) the interaction of the enantiomer were three groups match exactly with three sites of the selector, (B) the mirror image enantiomer of A which can interact only with maximum two sites of the selector.
Reproduced with permission from Ref. [55].

the desired separation can be obtained by masking or enhancing particular interactions [76–78].

The addition of a polar organic modifier to the mobile phase might not be sufficient to achieve the desired separation. Many pharmaceutical compounds contain acid (carboxylic) and/or basic (amine) functional groups that may interact too strongly with the silica-matrix of the stationary phase. As a result, they may either fail to elute or elute with distorted peak shapes, resulting in low chromatographic efficiencies. To overcome this problem, polar additives are dissolved in the mobile phase, usually at concentrations between 0.1 and 2.0% [79–87].

Basic additives are commonly used when analysing basic compounds and acid additives for acidic compounds. Consequently, the ionization of analytes is suppressed, maximizing the interaction with the neutral polysaccharide-based selectors and facilitating the adsorption and desorption processes [80]. However, additives exert effects through multiple mechanisms. Basic additives, mainly amine derivatives, are used to decrease non-stereospecific interactions by shielding residual silanol groups and other non-specific binding sites, which favors enantioselective interactions as retention mechanism [21,80,84].

Acidic additives, *e.g.* trifluoroacetic acid or formic acid, protonate amino functions, which improves the enantioselectivity by enhancing hydrogen bonding interactions formed with the carbamate groups of the chiral selectors [87]. These additives can also form neutral salts with basic compounds, which can interact better with the stationary phases and which can be separated intact [84].

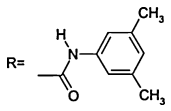
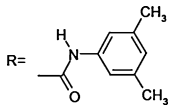
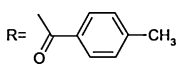
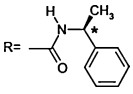
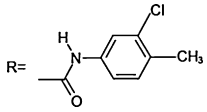
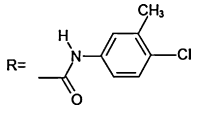
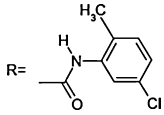
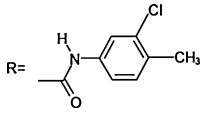
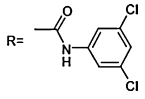
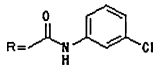
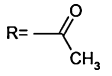
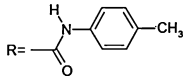
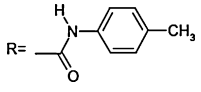
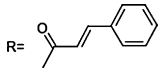
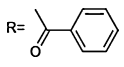
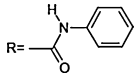
The presence of carbon dioxide in the mobile phase complicates the possible interactions even more. In the presence of protic modifiers, carbon dioxide displays an acidic character. As a result, transient complexes can be formed between the carbon dioxide and amine groups of analytes or with basic additives. However the impact of this complex formation on (enantioselective) interactions is unclear [84,85].

Table 4
Selected examples of chiral SFC separations with Pirkle-type chiral stationary phases.

Commercial name	Chiral selector	Mobile phase	Substances analyzed	
Chirex® 3005	(R)-1-Naphthylglycine and 3,5-dinitrobenzoic acid	CO_2/methanol, 55/45, (v/v)	Ketoprofen	[57]
Whelk® O1 (S,S)	1-(3,5-Dinitrobenzamido)-1,2,3,4,-tetrahydrophenanthrene	CO_2/organic modifier[a], variable % 80–60/20–40, (v/v)	2-Phenyl glutaric anhydride, isradipine, felodipine, 2,2,2-trifluoro-*N*-(1-phenyl-ethyl)-*N*-(tosylmethyl)acetamide, benzoin, 1,2,3,4-tetrahydro-2,2,4,7-tetramethylquinoline, flavanone, fluoxetine, flurbiprofen, propranolol, sulpiride, warfarin	[58]
Whelk® O1 (S,S)	1-(3,5-Dinitrobenzamido)-1,2,3,4,-tetrahydrophenanthrene	CO_2/(organic modifier[b] + 0.1% diethylamine), variable % 80–60/20–40, (v/v)	*N*-benzyl-α-methylbenzylamine, acebutolol, fluoxetine, norephedrine, isoproterenol, 1-(chlorobenzhydryl)-piperazine, phenylalaninol, propranolol, pindolol, tyrosine, 2-(diphenylhydroxymethyl)-pyrrolidine	[59]
Chirex® 3005	(R)-1-Naphthylglycine and 3,5-dinitrobenzoic acid	CO_2/methanol, 55/45, (v/v)	Ketoprofen	[57]
ChyRoSine®-A	3,5-Dinitrobenzoyl tyrosine	CO_2/ethanol, 92/8, (v/v)	Oxazepam	[60]

[a] Organic modifiers: methanol, ethanol, isopropanol and 2,2,2-trifluoroethanol.
[b] Organic modifiers: methanol, ethanol, isopropanol, isobutanol and acetonitrile.

Table 5
Commercially available polysaccharide-based CSPs.

Chiral selector	Structure	Commercial name(s)	Manufacturer
			
Amylose tris(3,5-dimethylphenylcarbamate)		Chiralpak® AD/Chiralpak® IA Amycoat® RegisPack®	Chiral Technologies Kromasil Regis Technologies
Cellulose tris(3,5-dimethylphenylcarbamate)		Chiralcel® OD/Chiralpak® IB Lux® Cellulose-1 Cellucoat® Astec Cellulose DMP RegisCell®	Chiral Technologies Phenomenex Kromasil Sigma–Aldrich Regis Technologies
Cellulose tris(4-methylbenzoate)		Chiralcel® OJ Lux® Cellulose-3	Chiral Technologies Phenomenex
Amylose tris((S)-α-methylbenzylcarbamate)		Chiralpak® AS	Chiral Technologies
Cellulose tris(3-chloro-4-methylphenylcarbamate)		Chiralcel® OZ Lux® Cellulose-2	Chiral Technologies Phenomenex
Cellulose tris(4-chloro-3-methylphenylcarbamate)		Lux® Cellulose-4	Phenomenex
Amylose tris(5-chloro-2-methylphenylcarbamate)		Chiralpak® AY Lux® Amylose-2 RegisPack® CLA-1	Chiral Technologies Phenomenex Regis Technologies
Amylose tris(3-chloro-4-methylphenylcarbamate)		Chiralpak® AZ	Chiral Technologies
Cellulose tris(3,5-dichlorophenylcarbamate)		Sepapak®-5 Chiralpak® IC	(Sepaserve) Phenomenex Chiral Technologies
Amylose tris (3-chlorophenylcarbamate)		Chiralpak® ID	Chiral Technologies
Cellulose triacetate		Chiralcel® OA	Chiral Technologies
Cellulose tris(4-chlorophenylcarbamate)		Chiralcel® OF	Chiral Technologies
Cellulose tris(4-methylphenylcarbamte)		Chiralcel® OG	Chiral Technologies
Cellulose tricinnamate		Chiralcel® OK	Chiral Technologies
Cellulose tribenzoate		Chiralcel® OB	Chiral Technologies
Cellulose tris(phenylcarbamate)		Chiralcel® OC	Chiral Technologies

Blackwell et al. [83] studied the effect of ten different mobile phase additives (acidic, basic and neutral) on chiral selectivity, efficiency, and retention in supercritical fluid chromatography. All additives improved efficiency by sterically blocking the access to the surface silanol groups. Another observation is that additives adsorb and aggregate to a greater extent on the stationary phase surface than methanol, which was used as modifier. Depending on the nature of the adsorbed additives (acidic, basic or neutral), the chemical properties of the stationary phase surface change. This implies that additives also affect the enantioselective interactions between analyte and stationary phase. The effect of an additive on the retention and chiral selectivity is highly dependent on the analyte nature. Also, the retention is influenced to a much greater extent for analytes that display a strong interaction with stationary phase adsorption sites.

The effects of isopropylamine or triethylamine as additives in chiral SFC were investigated by Phinney and Sander [80], using 12 pharmaceutical racemates and two polysaccharide-based CSPs. They found results that corresponded with those of Blackwell et al. [83]: small increases in additive concentration may dramatically shift retention times by influencing the solvent strength of the mobile phase, this effect is dependent on the nature of the analyte and CSP.

Interesting to note is that for the chiral separation of acidic analytes, the addition of an additive to the mobile phase might not be necessary to achieve satisfying chromatographic results. This is explained by the fact that carbon dioxide displays a relative "acidic" character in presence of protic modifiers such as methanol [84,85].

Uses of other additives are also reported. Stringham [84] used ethanesulfonic acid as additive in ethanol, methanol and isopropanol for the chiral analysis of basic amines on polysaccharide-based CSPs. The strong acid was found to act as a counter-ion, which forms stable ion-pairs with the amines and which results in successful separations.

Our research group recently investigated the combined use of isopropylamine (IPA) and trifluoroacetic acid (TFA) in methanol-containing mobile phases for the separation of basic, acidic, neutral and amphoteric pharmaceuticals on polysaccharide-based CSPs [88]. It was found that with a combination of IPA and TFA in the mobile phase different and broader enantioselectivities are obtained compared to the separate use of IPA for basic analytes or TFA for acidic analytes. The combination of these two additives resulted in the separation of compounds that could not be resolved when only one additive was used (Fig. 5) [88,89].

When using additives in the mobile phase it is important to take into account potential *memory effects*. This is a phenomenon where additives, after their removal from the mobile phase, leave a trace on the chiral selector of the CSP, altering its initial properties [17]. For HPLC this memory effect has been extensively studied and different rinsing procedures and additive uses were proposed [90–95]. The effect is believed to last longer in apolar mobile phases (as is the case in SFC) than when polar eluents are used. A combined use of acidic and basic additives is claimed to reduce the memory effect in HPLC [17,100,101]. However, up till now there are no reports in the literature on the investigation of the memory effects in SFC.

2.4. Other parameters

The mobile phase density, which partially determines its solvent strength, is dependent on the temperature and pressure. In other words, pressure and temperature are important parameters to optimize SFC separations.

In general, the applied pressure has a larger effect on retention times and the resulting resolutions than on enantioselective recognition [28,73]. As the pressure increases, the mobile phase viscosity increases and the separation efficiency decreases. Since small variations in pressure can lead to significant density changes, it is thus important to consider the influence of the applied pressure in method development [73].

The effect of the temperature on SFC separations is more complex. Increasing the temperature will reduce the mobile phase density, which will result in lower retention factors. This implies that the resolution of chiral separations generally improves at lower temperatures. As the diffusivity in supercritical fluids stays high, even at lower temperatures, only minimum efficiency losses are seen when lowering the temperature. However, the temperature effect is not always so straightforward, since it can also affect the analyte affinity for the stationary phase hereby influencing the enantioselectivity. This is explained by the fact that enantioselectivity is the result of a difference in enantiomeric binding enthalpy and disruptive entropic effects of two enantiomers. When the temperature increases, a decrease in binding enthalpy difference between enantiomers is seen, leading to a decrease in enantioselectivity. At a certain temperature, the iso-elution temperature, the binding enthalpy of both enantiomers will be equal and they will co-elute. Confirmation of the iso-elution temperature in chiral SFC separations was made by Stringham and Blackwell [96]. When the temperature is above this iso-elution point, chiral separations will no longer be driven by a difference in binding enthalpy between two enantiomers, but the difference in disruptive entropy effects will control the separation. The main advantage of working above the iso-elution temperature is that the selectivity and efficiency of separations improve when the temperature increases, enabling to control and optimize chiral separations in a straightforward manner [96,97].

The iso-elution temperature of a racemic mixture is not only dependent on the analyte, but also on the applied chromatographic conditions (with emphasis on the mobile phase composition). The main drawback is that iso-elution temperatures are mostly far above the allowable temperature working range, which is related to the column stability. Therefore, for most compounds it is unfeasible to work in a temperature range above the iso-elution point. For instance, for polysaccharide-based CSPs, the mostly used phases in chiral SFC, the temperature limit is around 50 °C, while most iso-elution temperatures are above 100 °C [65,98–100].

The influence of the temperature on the chiral SFC separation of omeprazole and other related benzimidazoles with the polysaccharide-based CSPs Chiralpak® AD-H, was studied by Del Nozal et al. [101]. A decrease in retention was found when increasing the temperature, indicating that temperatures were below the iso-elution point. When ethanol was used as modifier in the mobile phase, the opposite effect was seen. Iso-elution temperatures of several compounds at different chromatographic conditions were calculated and confirmed that the iso-elution temperatures were almost always considerably above the column's temperature working range, except for lansoprazole and rabeprazole when 15% ethanol and omeprazole when 20% ethanol was used. Hence, lower temperatures gave in general better separations. Selditz et al. [102] examined the influence of temperature on the SFC separation of structurally related 2-amidotetralins with a Pirkle type CSP, namely Welk® O-1. In analogy with Del Nozal et al. [101], they observed improvements of the resolution at lower temperatures.

2.5. Detection techniques

Conventional detectors from both liquid- and gas chromatography have been successfully applied in SFC, *e.g.* ultraviolet (UV), fluorescence, flame ionization, ... Most theoretical and method development studies in SFC are carried out with a UV spectrophotometric detector [103,104]. The principal restriction on the use of this detector type is the pressure limit of the spectrometer flow cell. The back-pressure regulator, which generates a constant (high)

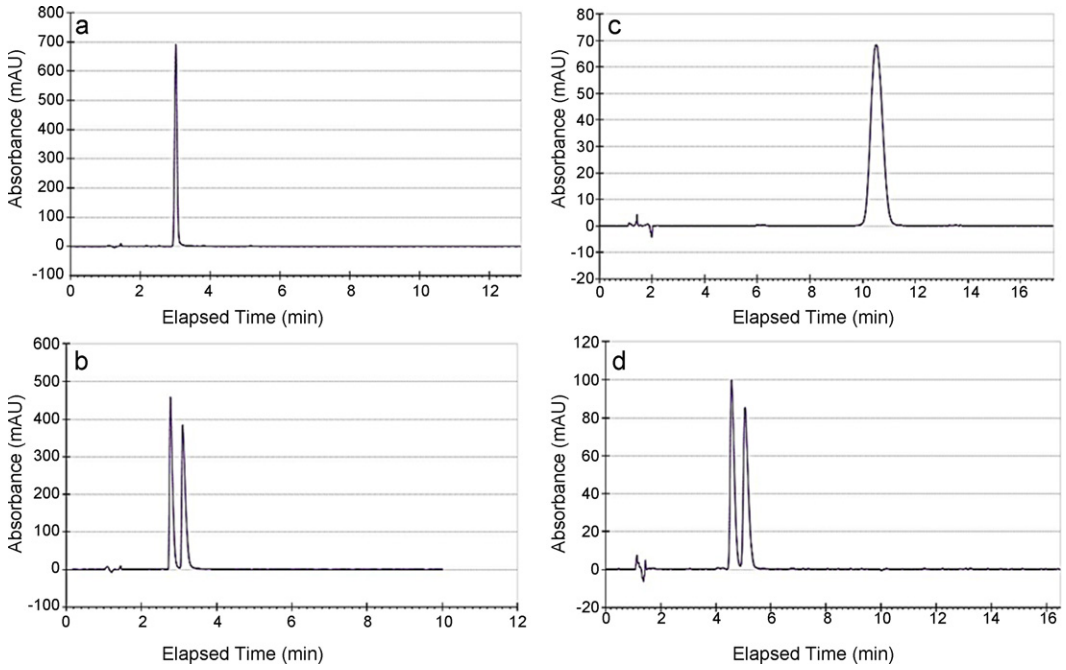


Fig. 5. Chromatogram of tetramisol on Chiralcel® OD-H with mobile phase (a) 80/20 (v/v) CO_2/(MeOH + 0.5%IPA) and (b) 80/20 (v/v) CO_2/(MeOH + 0.1%IPA + 0.1%TFA), and chromatogram of methadone on Chiralcel® OZ-H with mobile phase (c) 80/20 (v/v) CO_2/(MeOH + 0.1%IPA) and (d) 80/20 (v/v) CO_2/(MeOH + 0.1%IPA + 0.1%TFA), flow rate 3.0 ml/min, 30 °C, backpressure 150 bar and detection at 220 nm.
Adapted with permission from Ref. [88].

pressure, is placed after the UV detector to ensure that the mobile phase is still in the supercritical state when passing through the flow cell. Otherwise the mobile phase would partially convert into the gaseous state and this would lead to a significant increase of the detector noise, having a detrimental effect on the chromatographic result. For this reason UV detectors used for SFC are equipped with high-pressure flow cells [103,105].

However, similar to other chromatographic techniques there is also a tendency to couple SFC with a mass spectrometer (MS) for more sensitivity and selectivity, especially when dealing with complex mixtures or for enantiomeric purity determinations. The higher volatility of the mobile phase in SFC, makes it easy to interface SFC with MS. Carbon dioxide, used as main eluent in SFC, is inert and can therefore be heated and evaporated in a mass spectrometer ion chamber [106]. This makes SFC actually more appropriate than HPLC to couple with electrospray ionization (ESI), atmospheric pressure photoionization (APPI) or atmospheric pressure chemical ionization (APCI) interfaces [107,108]. A proton-donating organic modifier, such as methanol, is usually required to enhance and obtain ionization of the analytes. However, as nearly all SFC methods require polar organic modifiers in order to achieve satisfying chromatographic results this does not impose practical issues [105]. Because of its lower surface tension, resulting in better ionization efficiency and sensitivity, methanol is often preferred over isopropanol or ethanol [109].

Several successful SFC-MS applications are reported in the literature. Coe et al. [110] developed and validated an SFC-MS/MS method for the chiral analysis of R- and S-warfarin in human plasma samples. For this purpose a Chiralpak® AD-H column was used in combination with a guard column and a mobile phase containing 30% ethanol. The column effluent was coupled to an MS with an APCI interface. The APCI interface was preferred over an APPI or ESI because of the superior signal intensity, stability and day-to-day reproducibility. The developed and validated method was then applied successfully for routine analysis of clinical samples with a throughput of 460 samples within two days.

Zhao et al. [109] developed a novel automated chiral method development strategy using SFC-MS. Four CSPs (Chiralpak® AD and AS, and Chiralcel® OD and OJ) are screened at eight different methanol concentrations all containing 0.2% isopropylamine. Each screening cycle takes 25 min and results are evaluated in an automated way. This approach was applied on a mixture of six unidentified pharmaceutical racemates and resulted in the successful separation of all enantiomers. In addition, the lower detection limits of MS over UV and the shorter analysis times of SFC over NPLC or RPLC are emphasized.

Bolaños et al. [107] reported several SFC-MS applications, including chiral separations, that were used in drug discovery at Pfizer. SFC-MS is exploited for high-throughput quality control analysis and purification of samples. In this context SFC was coupled to an APCI interface and was found to be an excellent ionization source in combination with SFC. As high flow rates can be used, analysis times are shortened and instrument efficiencies are maximized. A baseline separation of two unidentified enantiomers using Chiralpak® AD-H and APCI SFC-MS is reported.

3. Supercritical fluid chromatography *vs.* liquid chromatography

As noted earlier, HPLC remains the most applied chromatographic technique for enantioseparations in the pharmaceutical industry. However, SFC also has potential for routine applications and its advantages over HPLC arise from the characteristics of supercritical fluids.

Since the diffusivity in supercritical fluids is higher than in liquids, SFC separations can be performed at higher flow rates compared with HPLC, without compromising the efficiency. This can be explained by the Van Deemter equation (1), which describes the

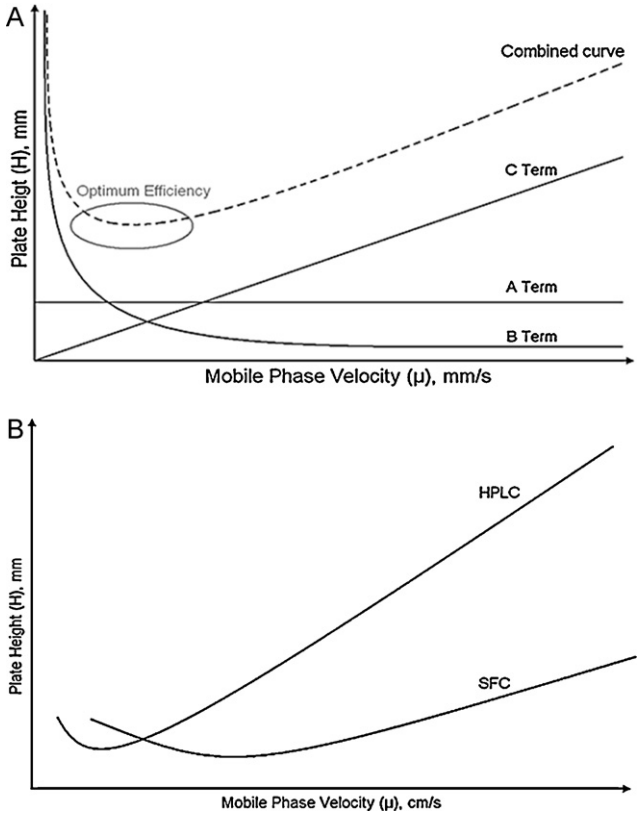


Fig. 6. (a) General Van Deemter curve; (b) comparison of the Van Deemter curves for HPLC and SFC.

different factors contributing to the plate height (H) and thus the efficiency of a separation [111].

$$H = A + \frac{B}{\mu} + C\mu \quad (1)$$

The A term represents the coefficient accounting for the band broadening caused by Eddy diffusion, the B term is the coefficient for the longitudinal diffusion, μ is the linear velocity of the mobile phase and C is the coefficient for the mass transfer kinetics of the analyte between the mobile and stationary phase. The combined curve of all influencing terms shows the relation between the theoretical plate height and the mobile phase velocity (Fig. 6a). It can be seen that under normal conditions the C-term contributes most to the plate height. The curve of the C-term is less steep in SFC than in HPLC, implying that the band broadening caused by the mass transfer kinetics increases less as a function of the mobile phase velocity in SFC because of the higher diffusivity of the analyte in the supercritical mobile phase. In Fig. 6b the influence of the mobile phase velocity (flow rate) on the plate height is compared for HPLC and SFC. For HPLC, the plate height increases, and thus efficiency decreases very fast when the flow rate increases. For SFC, this increase is less steep, due to the flatter C-term contribution, implying that higher flow rates can be used while maintaining the efficiency of the separation [29,31,111–115]. These higher flow rates also result in shorter column equilibration-, and analysis times than in HPLC, reducing the method development time.

A second advantage of supercritical fluids over regular liquids arises from their lower viscosity. This reduced viscosity decreases the pressure drop across the column and permits the use of higher flow rates, longer columns and/or column coupling [28,116]. As the pressure build-up in SFC is much lower than in HPLC, the same particles sizes can be used at an analytical and preparative scale, making the upscaling of methods more straightforward than in HPLC [29].

As final advantage, the cost reduction for solvents and waste removal can be mentioned. Since SFC replaces the liquid and more toxic eluents used in HPLC, it is seen as a green technology [28].

Borman et al. [117] compared the performance of 66 HPLC and seven SFC systems for the separation of a chemically diverse sample set of 100 racemates. In this study HPLC yielded the highest separation rates, partly because of the variety of selectors and separation modes. Nevertheless, the potential advantages of SFC in terms of speed and compound solubility are acknowledged. In addition a certain degree of complementarity between both techniques was seen: HPLC generated 14 and SFC 18 unique separations. However, as most compounds were resolved with more than one technique, the choice for a given technique can depend on other factors such as availability and experience in the lab.

Williams et al. [118] compared chiral HPLC to SFC by screening 21 compounds with three chiral selectors, *i.e.* a Pirkle type (Chirex® 3022), a polysaccharide-based (Chiralcel® OD-H) and a cyclodextrin-derivative (Cyclobond I 2000 RN and SN). Results showed that column equilibration times and method optimizations were faster in SFC than in HPLC. For many racemates an increased resolution for SFC separations was found compared to HPLC, but analysis times were not always lower in SFC.

White [119] compared chiral SFC to HPLC using appropriate gradient screenings for each technique. In SFC, enantioresolution and the subsequent method optimization was found to be faster compared to HPLC. Therefore SFC was selected as default technique for chiral method development and purification. HPLC was only chosen as second technique for samples that failed to be separated by SFC.

Zhang et al. [120] compared chiral SFC to HPLC by screening three neonicotinoid insecticides on three polysaccharide-based CSPs (Chiralcel® OD-H, Chiralpak® AD-H and Chiralpak® IB). Similar results were found for HPLC and SFC in terms of enantioselectivities, but retention times were shorter for SFC.

Toribio et al. [121] compared HPLC to SFC for the chiral separation of a series of four anti-ulcer drugs (omeprazole, pantoprazole, rabeprazole and lansoprazole) on Chiralpak® AD-H. Rabeprazole and lansoprazole could not be separated with HPLC, while all compounds were successfully separated with SFC. In addition, resolutions were higher and analysis times were significantly shorter for the SFC methods.

Similar results were seen by Bernal et al. [122] who compared HPLC to SFC for the chiral separation of four antifungal drugs. For certain compounds they could not reach a separation in HPLC, while SFC provided satisfactory results. Moreover, the separations generated in HPLC showed broader peaks and longer analysis times.

Matthijs et al. [20] compared two modes of HPLC (polar organic solvent chromatography (POSC) and normal-phase liquid chromatography (NPLC)) to SFC for the enantioseparation of 25 compounds. Generally SFC gave the best separations, although a certain degree of complementarity with POSC and NPLC was seen (*e.g.* promethazine and oxprenolol were better separated with NPLC and compound BXC was uniquely separated with POSC). Efficiency and analysis speed were comparable in SFC and POSC.

Summarized, it can be stated that chiral recognition mechanisms in SFC generally resemble those in HPLC, resulting in rather similar enantioselectivities in both techniques. Thus, no separation technique is superior over the other in terms of enantioresolution. However, specific cases, where HPLC proved to yield better results, as well as separations where SFC yielded better results are reported in the literature. Advantages of SFC mostly arise from the characteristics of the supercritical fluids used in the mobile phase [72,111,118]. Although SFC is traditionally compared with

normal-phase liquid chromatography, separations realised in RPLC have also been achieved with SFC [118].

4. Method development for chiral SFC

As can be deduced from the above, a very broad range of parameters may be varied to obtain an optimal chiral SFC separation. The process of establishing the most appropriate combination of chiral stationary phase, mobile phase modifier, additives, temperature and pressure can be very time-consuming and labor intensive. In modern practice it is common to use chiral screenings to evaluate enantioselectivity towards given racemates. Considering the fact that enantioselectivity remains complex and unpredictable, chiral screenings are the quickest and most efficient way to select a system for further method development. In such approach, a racemate is injected onto some chiral systems, in order to find adequate separation conditions that can be further optimized. The chromatographic systems evaluated in the screening step have two main requisites, *i.e.* they have to display a broad enantioselectivity and they have to be complementary. Additionally, a screening should have a high-throughput capacity to limit the method development time needed [18,30]. Nowadays, SFC has become the preferred technique for chiral method development in many laboratories, especially in early stages of drug development [18,27,28,119,123]. As mentioned earlier, polysaccharide-based CSPs are undoubtedly the most commonly used in SFC and thus also in chiral SFC screenings. However, chiral SFC separation strategies (combining both screening and optimization steps) are rather scarce, compared to the number of chiral HPLC screenings reported in the literature.

4.1. Screening strategies

Maftouh et al. [18] reported a screening approach for chiral pharmaceuticals using polysaccharide-based stationary phases. After evaluating several types of chiral selectors, four CSPs based on polysaccharide-derivatives (Chiralpak® AD-H and AS-H, and Chiralcel® OJ-H and OD-H) were selected for the screening. Two modifiers (methanol and isopropanol) were evaluated at 10 and 20%, respectively. For acidic compounds, 0.5% trifluoroacetic acid and for basic, neutral and amphoteric compounds 0.5% isopropylamine were added to the mobile phase, in order to improve peak shapes and chromatographic efficiency. After applying this screening approach on a test set of 40 pharmaceuticals, a success rate of 38 separated compounds (of which 28 were baseline separated) was reached. New methanol-based mobile phases and new polysaccharide-based chiral stationary phases were tested in our group to assess their applicability in an updated version of the screening strategy originally defined by Maftouh et al. [18,88,89]. Combining isopropylamine and trifluoroacetic acid in one mobile phase with 20% methanol as modifier leads to higher success rates within 30 min. In addition, the initial screening approach is simplified and has an improved throughput capacity [89].

Hamman et al. [124] developed a high-throughput screening approach for SFC, using six columns in the following sequence: Chiralpak® AD-H, Lux® Cellulose-1, Chiralcel® OJ-H, Chiralpak® IC, Chiralpak® AS-H and Lux® Cellulose-4. For screening, a 2.5 min gradient is used, starting with a linear increase from 10% to 55% modifier over 1.5 min and maintaining 55% modifier for one min. As modifiers methanol, ethanol and isopropanol were selected, with 0.1% diethylamine or triethylamine as additive, when analyzing basic compounds. The 46 chiral compounds of the test set were all separated with this gradient screening.

Welch et al. [125] developed a tool for tandem column SFC screenings, which allows to screen 10 individual columns. This is achieved by serial coupling of two groups of five columns. Per run, one column is selected from each group, resulting in 25 possible tandem column arrangements. A screening gradient was proposed starting at 4% methanol for 4 min, increasing to 40% methanol over the next 18 min and finally maintaining this mobile phase composition during 7 min. However, the focus of this work was to develop a practical screening instrument thus no evaluation was made of the enantioselective performance of different stationary phases.

Pirzada et al. [126] used a modified 15 min screening derived from the gradient screening of Welch et al. [125] to evaluate the enantioselectivity of eight CSPs (Chiralpak® AD-H and IC, Chiralcel® OD-H, RegisCell®, Sepapak®-2, 3 and 4 and Kromasil® Cellucoat). The gradient starts with 4% methanol containing 25 mM isobutylamine, for 4 min and then ramps at 4% per min to 40%, which is maintained for 2 min. Using a test set of 48 racemates, a selection of the best performing columns was made based on the highest number of baseline and unique separations. Chiralpak® AD-H, Chiralcel® OD-H, RegisCell®, Chiralpak® IC, and Sepapak®-4 were selected as most successful and orthogonal CSPs. Finally, a resolution map was composed in which the enantioselective performance of Chiralcel® OD-H and Regiscell® were compared in terms of resolution. This illustrated that Regiscell® often provided better results than Chiralcel® OD-H, although both columns use the same chiral selector.

White [119] developed a 2.5 min gradient screening for chiral SFC using a set of commercially available standard racemates. Four polysaccharide-based CSPs are screened: Chiralpak® AD-H and AS-H, Chiralcel® OD-H and OJ-H with three modifiers: methanol, ethanol and isopropanol, all containing 0.4% diethylamine as additive to improve peak shapes. The gradient starts at 5% modifier during 0.5 min. Then the modifier content increases to 50% over 1 min. After this, the modifier is decreased instantaneously to 5% and kept at this composition for 1 min. No screening sequence for the columns or mobile phases is given, nor are there any success rates reported.

Zeng et al. [127] designed a parallel four-channel SFC/MS system to execute chiral screenings and method optimization in a fully automated way. Samples are screened with two groups of four columns. The first group contains Chiralpak® AD-H and AS-H and Chiralcel® OD-H and OJ-H and the second group Chirobiotic® V, -R, -T and -Tag. The first tested modifiers are methanol, ethanol and isopropanol with 10 mM ammonium acetate, the next group of modifiers consists of methanol with 0.1% ethylsulfonic acid, methanol with 0.1% ethylsulfonic acid and 0.1% ammonium acetate, and methanol with 0.1% methylsulfonic acid. Parallel screening of the four CSPs of one group is done, using a four-way splitter to control the mobile phase flow onto the columns. An automated column switching valve controls the group of screened CSPs. Further method optimization is conducted in a fully automated way, depending on the achieved screening results, using an in-house developed software, named IPOCSS. Although it is reported that more than 100 racemates were tested with this strategy, the results of this screening are not presented in the paper.

4.2. Column coupling

The relatively low pressure drop over the column enables to couple columns in SFC and to modify the selectivity as well as the number of theoretical plates. This can be advantageous since a single CSP may not provide the desired selectivity, especially when dealing with a mixture of different chiral racemates or chiral samples in a complex achiral matrix. Because, when coupling columns in SFC, the experienced backpressure depends on their position in the instrument, the chromatographic behavior can differ significantly when switching the column order [125].

Phinney et al. [128] reported several applications with coupled columns in SFC, which proved the potential benefits. A mixture

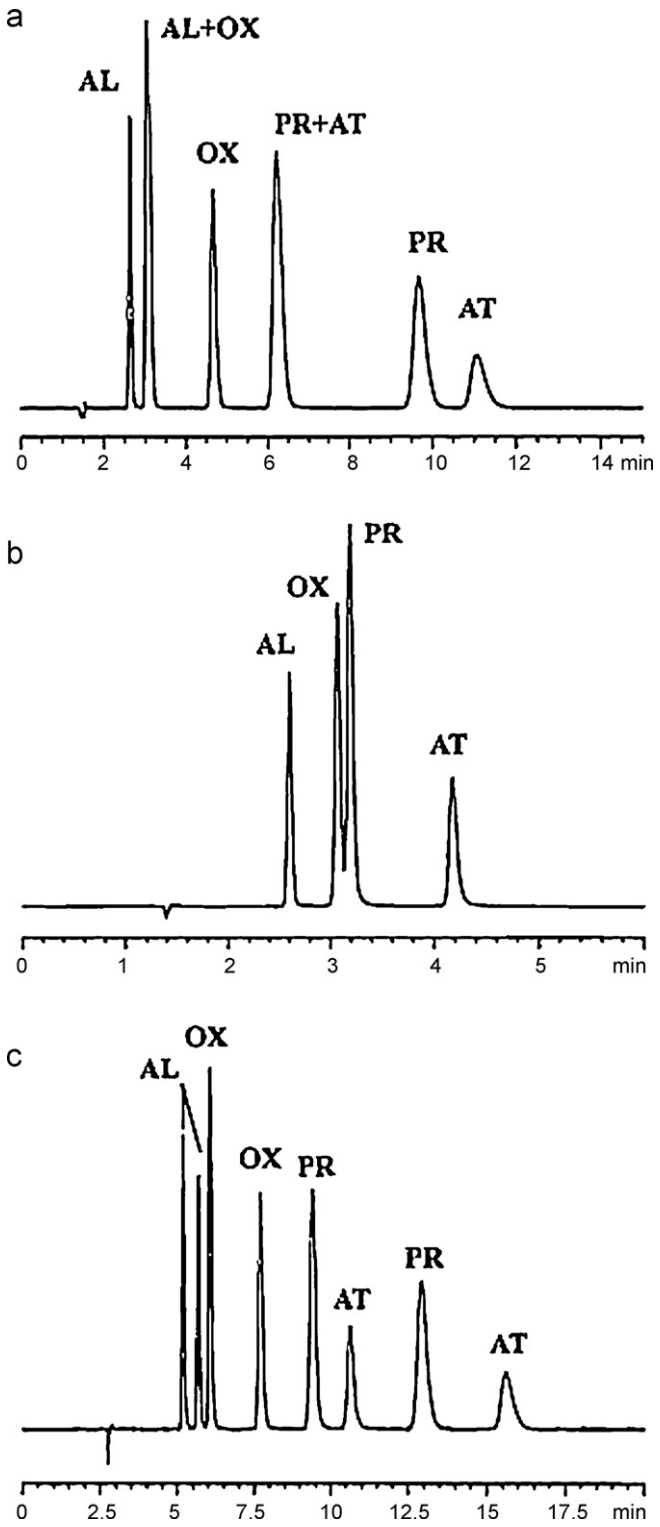


Fig. 7. Separation of four β-blockers on Chiralcel® OD-H (a), on the achiral cyano-bonded phase (b), and the coupled cyano/Chiralcel® OD-H system (c). Chromatographic conditions: 80/20 CO_2/(methanol + 0.5% isopropylamine) (v/v), 2.0 ml/min, 150 bar, 30 °C, λ = 280 nm. AL = alprenolol, OX = oxprenolol, PR = propranolol, AT = atenolol.

Adapted with permission from Ref. [77].

of four chiral β-blockers (alprenolol, atenolol, oxprenolol and propranolol) was separated on Chiralcel® OD-H, using with a mobile phase containing 20% methanol and 0.5% isopropylamine as additive. Peak overlap of alprenolol and oxprenolol, as well as overlaps of atenolol and propranolol occurred (Fig. 7a). Different achiral columns were tested in combination with the same mobile phase as the one used in combination with Chiralcel® OD-H in order to allow easy column coupling. A cyano-bonded phase was selected since it allowed achiral resolution of all four β-blockers (Fig. 7b). Serial coupling of the cyano and Chiralcel® column provided full separation of all eight enantiomers of the four β-blockers (Fig. 7c). The authors also presented the successful separation of a mixture of eight benzodiazepines, of which four were chiral racemates, by coupling an amino-based achiral column with Chiralcel® OD-H, using 10% ethanol with 0.5% isopropylamine as modifier. Lastly, they developed a method for the chiral separation of guaifenesin and phenylpropanolamine in a cough syrup. Coupling an achiral cyano-column and Chiralpak® AD-H resulted in separation of both enantiomer couples of the matrix components. As mobile phase for this separation, 90% carbon dioxide with 10% methanol containing 0.5% isopropylamine was used.

As mentioned earlier (see Section 4.1) Welch et al. [125] developed a tool for serial column screening to resolve complex stereoisomeric mixtures. Ten chiral (Chiralpak® AD-H, AS-H, IA, Chiralcel® OD-H, OJ-H, OF, OG, Chiris® AX QD, Chirobiotic® V, and (R,R) Whelko®) and five achiral columns were selected for this purpose (Chromegabond® Diol, amine, nitro and pyridyl amide and Kromasil® Silica). These stationary phases were screened in three arrangements: chiral–achiral, chiral–chiral (same CSP), and chiral–chiral (different CSP). Screening achiral columns had only limited utility to resolve the complex mixtures. Finally, tandem screening of two different chiral columns yielded the best results.

5. Preparative-scale applications of chiral SFC on drug compounds and intermediates

Analytical methods are indispensable throughout the early stages of drug development processes, to asses and assay the enantiomeric contents of a drug substance, to control the enantiomeric purity of starting materials and for batch control. Further in the drug development process, efficient and reliable preparative separation methods which generate enough drug substance for commercialization may become vital. On a preparative scale, other issues of a separation method are emphasized. Enantioselectivity stays a primary requirement, but the loading capacity, robustness, chemical inertness and stability of the CSP become equally important. In addition to these demands, the cost and environmental impact of a separation method must also be taken into account. Solubility of the sample in the mobile phase and removal of this mobile phase after separation are important issues that play a key role in preparative method development, while they are usually rather negligible on analytical scale. The most appropriate preparative chiral separation method for mass purification of a drug product is a final compromise between all these requirements. SFC offers benefits, such as highly efficient and fast separations, easy removal of the mobile phase after separation, and significant reduction of toxic and expensive solvents. An important feature of preparative SFC is the possibility of on-line recycling of the mobile phase. In 1984 Perrut [129] developed and patented a preparative recycling SFC equipment to re-purify the CO_2 of the mobile phase. Nowadays, commercial preparative SFC systems are available with integrated CO_2-recycling devices, which significantly reduces the costs of chiral separation methods [19]. For this reason and due to major instrumental improvements, the interest in SFC on a preparative level is steadily increasing [5,14,27,33,72,119,123,130–132].

Toribio et al. [132] developed a semi-preparative SFC-separation method for omeprazole enantiomers on a 250 mm × 10 mm Chiralpak® AD-H column. A flow rate of 8 ml/min was applied at 35 °C, with a backpressure of 200 bar, and a mobile phase consisting of 75% CO_2 and 25% ethanol. As the peak width in preparative

SFC increases with the load (concentration or volume), the resolution and relative recovery decrease. The highest production rates with purity > 99.9% were achieved with sample concentrations of 10 g/l, which yielded 0.45 mg recovery/min for S-(−)-omeprazole and 0.34 mg recovery/min for R-(+)-omeprazole.

The same research group developed semi-preparative chiral SFC methods for lansoprazole, pantoprazole and rabeprazole [131]. The same general chromatographic conditions as for the separation of the omeprazole enantiomers were used except for the mobile phase compositions. For lansoprazole, the modifier content was 20% methanol, for pantoprazole 25% isopropanol and for rabeprazole best results were achieved using 25% methanol as modifier. The purity of all recovered enantiomers was above 99.9%. The throughput for lansoprazole was found to be 0.025 and 0.090 mg/min, respectively, for the first and second eluting enantiomer, for rabeprazole it was 0.037 and 0.062 mg/min and for pantoprazole throughput was 0.062 and 0.11 mg/min.

Wang et al. [133] developed a semi-preparative supercritical fluid chromatography system interfaced with a mass spectrometer that controlled the fraction collection. They used the equipment for high-throughput purifications of thirteen chiral compounds, such as: warfarin, metoprolol, promethazine, tolbutamide and alprenolol, among others. Chiralpak® AD-H with dimensions 250 mm × 10 mm was used as CSP in combination with a standard linear gradient from 10% to 60% methanol over 5 min and a flow rate of 15 ml/min. Recoveries were in average 77%.

Maftouh et al. [18] scaled up the chiral analytical SFC separation of 3,5-difluoro mandelic acid to a semi-preparative level. The separation was performed on Chiralpak® AD-H with column dimensions of 250 mm × 21 mm, a flow rate of 50 ml/min, and a CO_2-based mobile phase with 3% ethanol containing 0.5% trifluoroacetic acid as additive. The mass recovery rate was 72% in average, with an enantiomeric purity of 100%.

White [119] defined a chiral analytical screening strategy and investigated the transfer of optimized isocratic methods to a preparative scale. Upscaling was found rather straightforward and even enhanced separations were reported on a preparative level due to the extra column length. An exemplary preparative scale separation of an unidentified compound was presented. The chromatogram showed excellent injection-to-injection repeatability. An overall weight recovery of 84.1% with an enantiomeric purity of 97.9% was achieved.

Thus, several published applications but also the pharmaceutical practice have proven the applicability of SFC for (semi-)preparative chiral purifications. The technique delivers high productivity, high reproducibility and reduced costs compared with preparative HPLC methods.

6. Recent developments in the field of SFC

A general trend in HPLC separations is that 3 and 5 μm particles are replaced by sub-2 μm particles, aiming to decrease analysis times while maintaining efficiency. However, minimizing the particle size and increasing the mobile phase velocity, results in a significant increase of the pressure drop across the column in HPLC. SFC seems to be an ideal technique to use columns with sub-2 μm particles since it generates much lower pressure drops. However, no chiral columns have been commercialized to date with particle sizes below 2 μm. Berger [134] investigated the use of 1.8 μm particles in achiral SFC. A separation of four steroids (cortisone, hydrocortisone, prednisone and prednisolone), four sulfonamides (sulfamethoxazole, sulfadimethizole, sulfaquinoxaline and sulfamethizole), four anti-inflammatory drugs (ibuprofen, flurbiprofen, naproxen and ketoprofen), three xanthenes (caffeine, theophylline and theobromine), and four nucleic acids (thymine, uracil, adenine and cytosine) was performed. High efficiencies, very short run times (mostly below 1 min), and only modest pressure drops were observed. This implies that columns with this particle size might be used on a regular SFC equipment, handling pressures of only 400 bar.

However, the development of new columns with smaller particle sizes emerges the need for equipment that minimizes dead volumes, in order to maximize efficiency of these columns. Following the ultra performance HPLC, an ultra performance SFC equipment has been developed and marketed recently [135]. The impurity determination of S-benzyl mandelate in R-benzyl mandelate was achieved on this UPSFC system with Chiralpak® AD-H (150 mm × 4.6 mm, 5 μm). The analysis time was below 1.5 min and the LOQ of S-benzyl mandelate was determined to be 0.07% of the main peak from R-benzyl mandelate [136]. The four stereo-isomers of permethrin were baseline separated with high resolutions in less than 6 min on the UPSFC system using Chiralcel® OJ-H (250 mm × 4.6 mm, 5 μm) as stationary phase [137]. The enantiomers of 1,1′-bi-2-napthol (binol) were separated with a resolution above 2.5 in less than 2 min on the UPSFC system using Chiralpak® AS-H (150 mm × 4.6 mm, 5 μm) as CSP [138]. These results indicate that high detector sensitivities and efficiencies can be obtained with this optimized equipment. These recent instrumental achievements indicate that there is still potential to improve the performance and results of supercritical fluid chromatography. However, for optimal results and routine use in chiral SFC separations, chiral columns with sub-2 μm particles need to be developed and commercialized.

7. Conclusions

Over the past decades, a renewed interest in supercritical fluids as eluent for chromatographic separations has developed. Instrumental improvements, along with the development and commercialization of a broad range of robust chiral stationary phases, have boosted the research again in the field of supercritical fluid chromatography. The green features of SFC give the technique potential benefits over conventional HPLC in terms of solvent cost reduction and waste removal. Meanwhile, instrumental improvements in the field of supercritical fluid chromatography tend to precede with as example the recently introduced ultraperformance SFC. Although the vast majority of chiral separations in SFC are achieved on polysaccharide-based stationary phases, other CSPs such as Pirkle-type and antibiotic-based columns are also applicable. Yielding significantly shorter retention times than conventional HPLC, method development is fast and rather straightforward in SFC, as the mobile phase parameters to be optimized are relatively limited, because only a few organic modifiers are commonly used and pH is not investigated in the CO_2-based mobile phase. On the other hand, temperature and pressure, parameters that are less significant in HPLC, play a primordial role in SFC method development. The low pressures generated through the stationary phase in SFC, allow coupling several columns, in order to obtain the desired selectivity towards more complex mixtures of racemates. Although this approach is not thoroughly investigated to date, some successful separations have been reported. Chiral screening strategies for SFC, that enable fast and efficient method development, are rather scarce up till now. Upscaling analytical SFC-methods to a preparative scale is rather straightforward and successful examples have been reported in the literature.

Summarized, it can be stated that, having proved its benefits and applicability, SFC has conquered a place next to HPLC as preferred

technique for chiral separations in drug discovery and development processes.

References

[1] W.A. Bonner, Parity violation and the evolution of biomolecular homochirality, Chirality 12 (2000) 114–126.
[2] W.A. Bonner, Chirality and life, Orig. Life Evol. Biosph. 25 (1995) 175–190.
[3] J. Caldwell, Importance of stereospecific bioanalytical monitoring in drug development, J. Chromatogr. A 719 (1996) 3–13.
[4] P.A. Patil, M.A. Kothekar, Development of safer molecules through chirality, Indian J. Med. Sci. 60 (2006) 427–437.
[5] N.M. Maier, P. Franco, W. Lindner, Separation of enantiomers: needs, challenges, perspectives, J. Chromatogr. A 906 (2001) 3–33.
[6] I. Agranat, H. Caner, J. Caldwell, Putting chirality to work: the strategy of chiral switches, Nat. Rev. Drug Discov. 1 (2002) 753–768.
[7] J. Caldwell, Do single enantiomers have something special to offer? Hum. Psychopharmacol. 16 (2001) 67–71.
[8] European Medicines Agency, http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500004339.pdf (accessed 06.11).
[9] European Medicines Agency, http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002816.pdf (accessed 06.11).
[10] R. Shimazawa, N. Nagai, S. Toyoshima, H. Okuda, Present state of new chiral drug development and review in Japan, J. Health Sci. 54 (2008) 23–29.
[11] U.S.Food and Drug Administration, http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm122883.htm (accessed 06.11).
[12] H.J. Federsel, Facing chirality in the 21st century: approaching the challenges in the pharmaceutical industry, Chirality 15 (Suppl.) (2003) 128–142.
[13] J.S. Carey, D. Laffan, C. Thomson, M.T. Williams, Analysis of the reactions used for the preparation of drug candidate molecules, Org. Biomol. Chem. 4 (2006) 2337–2347.
[14] E.R. Francotte, Enantioselective chromatography as a powerful alternative for the preparation of drug enantiomers, J. Chromatogr. A 906 (2001) 379–397.
[15] O. McConnell, Y. He, L. Nogle, A. Sarkahian, Application of chiral technology in a pharmaceutical company. Enantiomeric separation and spectroscopic studies of key asymmetric intermediates using a combination of techniques. Phenylglycidols, Chirality 19 (2007) 716–730.
[16] H. Ates, D. Mangelings, Y. Vander Heyden, Fast generic chiral separation strategies using electrophoretic and liquid chromatographic techniques, J. Pharm. Biomed. Anal. 48 (2008) 288–294.
[17] H. Ates, D. Mangelings, Y. Vander Heyden, High-throughput screening and method development strategies to separate chiral drug compounds in HPLC, SFC and CE, in: S. Ahuja (Ed.), Chiral Separation Methods for Pharmaceutical and Biotechnological Products, John Wiley & Sons, Calabash, NC, USA, 2011, pp. 383–416.
[18] M. Maftouh, C. Granier-Loyaux, E. Chavana, J. Marini, A. Pradines, Y. Vander Heyden, C. Picard, Screening approach for chiral separation of pharmaceuticals. Part III. Supercritical fluid chromatography for analysis and purification in drug discovery, J. Chromatogr. A 1088 (2005) 67–81.
[19] W. Majewski, F. Romelaer, O. Ludemann-Hombourger, http://www.novasep.com/Upload/pdf/Doc-NOVASEP-SFC.pdf (accessed 08.11).
[20] N. Matthijs, M. Maftouh, Y. Vander Heyden, Chiral separation strategy in polar organic solvent chromatography and performance comparison with normal-phase liquid and supercritical–fluid chromatography, J. Sep. Sci. 29 (2006) 1353–1362.
[21] N. Matthijs, M. Maftouh, Y. Vander Heyden, Screening approach for chiral separation of pharmaceuticals. IV. Polar organic solvent chromatography, J. Chromatogr. A 1111 (2006) 48–61.
[22] L. Miller, M. Potter, Preparative chromatographic resolution of racemates using HPLC and SFC in a pharmaceutical discovery environment, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 875 (2008) 230–236.
[23] J.G. Ning, Chiral separations by various techniques, in: S. Ahuja (Ed.), Chiral Separation Methods for Pharmaceutical and Biotechnological Products, John Wiley & Sons, Calabash, NC, USA, 2011, pp. 131–145.
[24] C. Perrin, N. Matthijs, D. Mangelings, C. Granier-Loyaux, M. Maftouh, D.L. Massart, Y. Vander Heyden, Screening approach for chiral separation of pharmaceuticals. Part II. Reversed-phase liquid chromatography, J. Chromatogr. A 966 (2002) 119–134.
[25] C. Perrin, V.A. Vu, N. Matthijs, M. Maftouh, D.L. Massart, Y. Vander Heyden, Screening approach for chiral separation of pharmaceuticals. Part I. Normal-phase liquid chromatography, J. Chromatogr. A 947 (2002) 69–83.
[26] T.J. Ward, K.D. Ward, Chiral separations: fundamental review 2010, Anal. Chem. 82 (2010) 4712–4722.
[27] Y. Zhang, D.R. Wu, D.B. Wang-Iverson, A.A. Tymiak, Enantioselective chromatography in drug discovery, Drug Discov. Today 10 (2005) 571–577.
[28] K.W. Phinney, SFC of drug enantiomers, Anal. Chem. 72 (2000) 204A–211A.
[29] Z. Wang, Separation of chiral compounds by SFC, in: S. Ahuja (Ed.), Chiral Separation Methods for Pharmaceuticals and Biotechnological Products, John Wiley & Sons, Calabash, NC, USA, 2011, pp. 299–329.
[30] E. Klesper, A.H. Corwin, D.A. Turner, High pressure gas chromatography above critical temperatures, J. Org. Chem. 27 (1962) 700–706.
[31] K.D. Bartle, Theory and principles of supercritical fluid chromatography, in: R.M. Smith (Ed.), Supercritical Fluid Chromatography, The Royal Society of Chemistry, Loughborough, UK, 1988, pp. 1–28.
[32] I. Pasquali, R. Bettini, Are pharmaceutics really going supercritical? Int. J. Pharm. 364 (2008) 176–187.
[33] T.L. Chester, J.D. Pinkston, Supercritical fluid and unified chromatography, Anal. Chem. 76 (2004) 4606–4613.
[34] T. Clifford, A single substance as a supercritical fluid, in: T. Clifford (Ed.), Fundamentals of Supercritical Fluids, Oxford University Press, Oxford, UK, 2002, pp. 1–23.
[35] T.A. Berger, Separation of polar solutes by packed column supercritical fluid chromatography, J. Chromatogr. A 785 (1997) 3–33.
[36] R.M. Smith, Supercritical fluids in separation science – the dreams, the reality and the future, J. Chromatogr. A 856 (1999) 83–115.
[37] Y. Vander Heyden, D. Mangelings, N. Matthijs, C. Perrin, Chiral separations, in: S. Ahuja, M.W. Dong (Eds.), Handbook of Pharmaceutical Analysis by HPLC, Elsevier Academic Press, Oxford, UK, 2005, pp. 447–498.
[38] H.F. Kasai, M. Tsubuki, S. Matsuo, T. Honda, Sub- and supercritical chiral separation of racemic compounds on columns with stationary phases having different functional groups, Chem. Pharm. Bull. (Tokyo) 53 (2005) 1270–1276.
[39] R. Duval, H. Leveque, Y. Prigent, H.Y. Aboul-Enein, Enantioseparation of aminoglutethimide and thalidomide by high performance liquid chromatography or supercritical fluid chromatography on mono-2 and mono-6-O-pentenyl-beta-cyclodextrin-based chiral stationary phases, Biomed. Chromatogr. 15 (2001) 202–206.
[40] R.Q. Wang, T.T. Ong, S.C. Ng, Synthesis of cationic beta-cyclodextrin derivatives and their applications as chiral stationary phases for high-performance liquid chromatography and supercritical fluid chromatography, J. Chromatogr. A 1203 (2008) 185–192.
[41] K.L. Williams, L.C. Sander, S.A. Wise, Comparison of liquid and supercritical fluid chromatography using naphthylethylcarbamoylated-B-cyclodextrin chiral stationary phases, J. Chromatogr. A 746 (1996) 91–101.
[42] E. Schneiderman, A.M. Stalcup, Cyclodextrins: a versatile tool in separation science, J. Chromatogr. B: Biomed. Sci. Appl. 745 (2000) 83–102.
[43] B.H. Lingfeng, Chiral recognition mechanism: practical considerations for pharmaceutical analysis of chiral compounds, in: A. Berthod (Ed.), Chiral Recognition in Separation Methods – Mechanisms and Applications, Springer, Lyon, France, 2010, pp. 153–201.
[44] T.E. Beesley, J.T. Lee, A.X. Wang, Method development and optimization of enantiomeric separations using macrocyclic glycopeptide chiral stationary phases, in: G. Subramanian (Ed.), Chiral Separation Techniques – A Practical Approach, Wiley-VCH, Canterbury, UK, 2000, pp. 25–54.
[45] A. Medvedovici, P. Sandra, L. Toribio, F. David, Chiral packed column subcritical fluid chromatography on polysaccharide and macrocyclic antibiotic chiral stationary phases, J. Chromatogr. A 785 (1997) 159–171.
[46] T.J. Ward, A.B. Farris III, Chiral separations using the macrocyclic antibiotics: a review, J. Chromatogr. A 906 (2001) 73–89.
[47] Supelco/Astec, Chirobiotic Handbook: a Guide to Using Macrocyclic Glycopeptide Bonded Phases for Chiral LC Separations, 5th ed., Advanced Separation Technologies, Bellefonte, PA, USA, 2004.
[48] L.A. Svensson, P.K. Owens, Enantioselective supercritical fluid chromatography using ristocetin A chiral stationary phases, Analyst 125 (2000) 1037–1039.
[49] Y. Liu, A. Berthod, C.R. Mitchell, T.L. Xiao, B. Zhang, D.W. Armstrong, Super/subcritical fluid chromatography chiral separations with macrocyclic glycopeptide stationary phases, J. Chromatogr. A 978 (2002) 185–204.
[50] G. Lavison, D. Thiebaut, Evaluation of a ristocetin bonded stationary phase for subcritical fluid chromatography of enantiomers, Chirality 15 (2003) 630–636.
[51] Y. Liu, R.V. Rozhkov, R.C. Larock, T.L. Xiao, D.W. Armstrong, Fast super/subcritical fluid chromatography enantiomeric separations of dihydrofurocoumarin derivatives with macrocyclic glycopeptide stationary phases, Chromatographia 58 (2003) 775–779.
[52] J. Dönnecke, L.A. Svensson, O. Gyllenhaal, K.E. Karlsson, A. Karlsson, J. Vessman, Evaluation of a vancomycin chiral stationary phase in packed capillary supercritical fluid chromatography, J. Microcolumn Sep. 11 (1999) 521–533.
[53] W.H. Pirkle, M.H. Hyun, A. Tsipouras, B.C. Hamper, B. Banks, A rational approach to the design of highly effective chiral stationary phases for the liquid chromatographic separation of enantiomers, J. Pharm. Biomed. Anal. 2 (1984) 173–181.
[54] W.H. Pirkle, W.H. Hyun, B. Bank, A rational approach to the design of highly-effective chiral stationary phases, J. Chromatogr. 316 (1984) 585–604.
[55] A. Berthod, Chiral recognition mechanisms in enantiomers separations – a general view, in: A. Berthod (Ed.), Chiral Recognition in Separation Methods – Mechanisms and Applications, Springer, Lyon, France, 2010, pp. 1–31.
[56] Z. Chen, T. Fuyumuro, K. Watabe, T. Hobo, Influence of spacers and organic modifiers on chromatographic behaviors on chiral diamide stationary phases with N-(3,5-dimethylbenzoyl)-D-phenylglycine, Anal. Chim. Acta 518 (2004) 181–189.
[57] S.H. Hoke, J.D. Pinkston, R.E. Bailey, S.L. Tanguay, T.H. Eichhold, Comparison of packed-column supercritical fluid chromatography–tandem mass spectrometry with liquid chromatography–tandem mass spectrometry for bioanalytical determination of (R)- and (S)-ketoprofen in human plasma following automated 96-well solid-phase extraction, Anal. Chem. 72 (2000) 4235–4241.

[58] N. Byrne, E. Hayes-Larson, W.W. Liao, C.M. Kraml, Analysis and purification of alcohol-sensitive chiral compounds using 2,2,2-trifluoroethanol as a modifier in supercritical fluid chromatography, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 875 (2008) 237–242.
[59] C.M. Kraml, D. Zhou, N. Byrne, O. McConnell, Enhanced chromatographic resolution of amine enantiomers as carbobenzyloxy derivatives in high-performance liquid chromatography and supercritical fluid chromatography, J. Chromatogr. A 1100 (2005) 108–115.
[60] L. Siret, N. Bargmann, A. Tambute, M. Caude, Direct enantiomeric separation of beta-blockers on ChyRoSine-A by supercritical fluid chromatography: supercritical carbon dioxide as transient in situ derivatizing agent, Chirality 4 (1992) 252–262.
[61] I. Tomoyuki, Y. Okamoto, Preparation and chiral recognition of polysaccharide-based selectors, in: A. Berthod (Ed.), Chiral Recognition in Separation Methods – Mechanisms and Applications, Springer, Lyon, France, 2010, pp. 33–52.
[62] E. Yashima, Polysaccharide-based chiral stationary phases for high-performance liquid chromatographic enantioseparation, J. Chromatogr. A 906 (2001) 105–125.
[63] G. Hesse, R. Hagel, A complete separation of a racemic mixture by elution chromatography by cellulose triacetate, Chromatographia 6 (1973) 277–280.
[64] Y. Okamoto, M. Kawashima, K. Yamamoto, K. Hatada, Chromatographic resolution 6. Useful packing materials for high-performance liquid chromatographic resolution – cellulose triacetate and tribenzaote coated on macroporous silica–gel, Chem. Lett. (1984) 739–742.
[65] Phenomenex, https://phenomenex.blob.core.windows.net/documents/81069700-fbb9-46e0-95ed-4de49b324e1e.pdf (accessed 08.11).
[66] P. Franco, T. Zhang, Common approaches for efficient method development with immobilised polysacchride-derived chiral stationary phases, J. Chromatogr. B 875 (2008) 48–56.
[67] L. Thunberg, J. Hashemi, S. Andersson, Comparative study of coated and immobilized polysaccharide-based chiral stationary phases and their applicability in the resolution of enantiomers, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 875 (2008) 72–80.
[68] B. Chankvetadze, E. Yashima, Y. Okamoto, Tris(chloro-disubstituted and methyl-disubstituted phenylcarbamates) of cellulose as chiral stationary phases for chromatographic enantioseparation, Chem. Lett. 4 (1993) 617–620.
[69] B. Chankvetadze, E. Yashima, Y. Okamoto, Chloromethylphenylcarbamate derivatives of cellulose as chiral stationary phases for high-performance liquid chromatography, J. Chromatogr. A 670 (1994) 39–49.
[70] B. Chankvetadze, E. Yashima, Y. Okamoto, Methylphenylcarbamates, dichlorophenylcarbamates and chloromethylphenylcarbamates of amylose as chiral stationary phases for high-performance liquid-chromatography, J. Chromatogr. A 694 (1995) 101–109.
[71] M. Johannsen, Separation of enantiomers of ibuprofen on chiral stationary phases by packed column supercritical fluid chromatography, J. Chromatogr. A 937 (2001) 135–138.
[72] K.W. Phinney, Enantioselective separations by packed column subcritical and supercritical fluid chromatography, Anal. Bioanal. Chem. 382 (2005) 639–645.
[73] D. Leyendecker, Selection of conditions for an SFC separation, in: R.M. Smith (Ed.), Supercritical Fluid Chromatography, The Royal Society of Chemistry, Loughborough, UK, 1988, pp. 53–101.
[74] T.L. Chester, The road to unified chromatography: the importance of phase behavior knowledge in supercritical fluid chromatography and related techniques, and a look at unification, Microchem. J. 61 (1999) 12–24.
[75] P.S. Wells, S. Zhou, J.F. Parcher, Unified chromatography with CO_2-based binary mobile phases, Anal. Chem. 75 (2003) 18A–24A.
[76] K.W. Phinney, Sub- and supercritical fluid chromatography for enantiomer separations, in: G. Subramanian (Ed.), Chiral Separation Techniques – A Practical Approach, Wiley-VCH, Canterbury, UK, 2000, pp. 301–315.
[77] J.A. Blackwell, R.W. Stringham, D. Xiang, R.E. Waltermire, Empirical relationship between chiral selectivity and mobile phase modifier properties, J. Chromatogr. A 852 (1999) 383–394.
[78] G.O. Cantrell, R.W. Stringham, J.A. Blackwell, J.D. Weckwerth, P.W. Carr, Effect of various modifiers on selectivity in packed-column subcritical and supercritical fluid chromatography, Anal. Chem. 68 (1996) 3645–3650.
[79] T.A. Berger, J.F. Deye, Role of additives in packed-column supercritical fluid chromatography – suppression of solute ionization, J. Chromatogr. 547 (1991) 377–392.
[80] K.W. Phinney, L.C. Sander, Additive concentration effects on enantioselective separations in supercritical fluid chromatography, Chirality 15 (2003) 287–294.
[81] T.A. Berger, J.F. Deye, Effect of basic additives on peak shapes of strong bases separated by packed-column supercritical fluid chromatography, J. Chromatogr. Sci. 29 (1991) 310–317.
[82] T.A. Berger, Physical chemistry of the stationary phase, in: T.A. Berger (Ed.), Packed Column SFC, The Royal Society of Chemistry, Cambridge, UK, 1995, pp. 72–96.
[83] J.A. Blackwell, R.W. Stringham, J.D. Weckwerth, Effect of mobile phase additives in packed-column subcritical and supercritical fluid chromatography, Anal. Chem. 69 (1997) 409–415.
[84] R.W. Stringham, Chiral separation of amines in subcritical fluid chromatography using polysaccharide stationary phases and acidic additives, J. Chromatogr. A 1070 (2005) 163–170.
[85] Y.K. Ye, K.G. Lynam, R.W. Stringham, Effect of amine mobile phase additives on chiral subcritical fluid chromatography using polysaccharide stationary phases, J. Chromatogr. A 1041 (2004) 211–217.
[86] Y.K. Ye, R.W. Stringham, The effect of acidic and basic additives on the enantioseparation of basic drugs using polysaccharide-based chiral stationary phases, Chirality 18 (2006) 519–530.
[87] Y.K. Ye, R.W. Stringham, M.J. Wirth, Origin of enhanced chiral selectivity by acidic additives for a polysaccharide-based stationary phase, J. Chromatogr. A 1057 (2004) 75–82.
[88] K. De Klerck, D. Mangelings, F. De Boever, D. Clicq, Y. Vander Heyden, Combined use of isopropylamine and trifluoroacetic acid in methanol-containing mobile phases for chiral supercritical fluid chromatography, J. Chromatogr. A, in press.
[89] K. De Klerck, D. Mangelings, Y. Vander Heyden, Enantioselectivity of methanol containing mobile phases on polysaccharide-based chiral stationary phases in supercritical fluid chromatography, in preparation.
[90] J. Putnam, G. Guiochon, A test to determine the nature and presence of the memory effect columns packed with the amylose tris(3,5-dimethylphenylcarbamate) stationary phase, J. Chromatogr. A 1218 (2011) 6302–6307.
[91] J. Putnam, G. Guiochon, The influence of the memory effect on preparative separations using the amylose tris(3,5-dimethylphenylcarbamate) stationary phase, J. Chromatogr. A 1218 (2011) 5157–5165.
[92] J. Putnam, G. Guiochon, The influence of water on the memory effect of the amylose tris (3,5-dimethylphenyl carbamate) stationary phase, J. Chromatogr. A 1217 (2010) 8146–8153.
[93] J. Putnam, G. Guiochon, Characterizing the memory effect on the amylose tris (3,5-dimethylphenyl carbamate) stationary phase, J. Chromatogr. A 1216 (2009) 8488–8495.
[94] R.W. Stringham, K.G. Lynam, B.S. Lord, Memory effect of diethylamine mobile phase additive on chiral separations on polysaccharide stationary phases, Chirality 16 (2004) 493–498.
[95] Y.K. Ye, B. Lord, R.W. Stringham, Memory effect of mobile phase additives in chiral separations on a Chiralpak AD column, J. Chromatogr. A 945 (2002) 139–146.
[96] R.W. Stringham, J.A. Blackwell, Factors that control successful entropically driven chiral separations in SFC and HPLC, Anal. Chem. 69 (1997) 1414–1420.
[97] R.W. Stringham, J.A. Blackwell, "Entropically driven" chiral separations in supercritical fluid chromatography. Confirmation of isoelution temperature and reversal of elution order, Anal. Chem. 68 (1996) 2179–2185.
[98] Akzonobel, http://www.kromasil.com/technical_support/care_and_use_chiralcoat.php (accessed 08.11).
[99] Chiral Technologies Europe, http://www.mz-at.de/pdf/ChiralcelOD.pdf (accessed 08.11).
[100] Chiral Technologies Europe, http://www.mz-at.de/pdf/ChiralcelOJRH.pdf (accessed 08.11).
[101] M.J. Del Nozal, L. Toribio, J.L. Bernal, C. Alonso, J.J. Jimenez, Chiral separation of omeprazole and several related benzimidazoles using supercritical fluid chromatography, J. Sep. Sci. 27 (2004) 1023–1029.
[102] U. Selditz, S. Copinga, C.J. Grol, J.P. Franke, R.A. de Zeeuw, H. Wikstrom, O. Gyllenhaal, Temperature effects on the chromatographic behaviour of racemic 2-amidotetralins on a Whelk-O 1 stationary phase in super- and subcritical fluid chromatography, Pharmazie 54 (1999) 183–191.
[103] A. Pereira, F. David, G. Vanhoenacker, C. Brunelli, P. Sandra, A simple instrumental approach for supercritical fluid chromatography in drug discovery and its consequences for coupling with mass spectrometric and light scatterin detection, LCGC North Am. 29 (2011) 1006–1016.
[104] M.M. Sanagi, R.M. Smith, The emergence and instrumentation of SFC, in: R.M. Smith (Ed.), Supercritical Fluid Chromatography, The Royal Society of Chemistry, Loughborough, UK, 1988, pp. 29–52.
[105] R.M. Smith, O. Chienthavorn, N. Danks, I.D. Wilson, Fluorescence detection in packed-column supercritical fluid chromatographic separations, J. Chromatogr. A 798 (1998) 203–206.
[106] F.B. Li, H.S. Hsieh, Supercritical fluid chromatography-mass spectrometry for chemical analysis, J. Sep. Sci. 31 (2008) 1231–1237.
[107] B. Bolaños, M. Greig, M. Ventura, W. Farrell, C.M. Aurigemma, H. Li, T.L. Quenzer, K. Tivel, J.M. Bylund, P. Tran, C. Pham, D. Philipson, SFC/MS in drug discovery at Pfizer, La Jolla, Int. J. Mass Spectrom. 238 (2004) 85–97.
[108] T.R. Baker, J.D. Pinkston, Development and application of packed-column supercritical fluid chromatography/pneumatically assisted electrospray mass spectrometry, J. Am. Soc. Mass Spectrom. 9 (1998) 498–509.
[109] Y. Zhao, G. Woo, S. Thomas, D. Semin, P. Sandra, Rapid method development for chiral separation in drug discovery using sample pooling and supercritical fluid chromatography–mass spectrometry, J. Chromatogr. A 1003 (2003) 157–166.
[110] R.A. Coe, J.O. Rathe, J.W. Lee, Supercritical fluid chromatography–tandem mass spectrometry for fast bioanalysis of R/S-warfarin in human plasma, J. Pharm. Biomed. Anal. 42 (2006) 573–580.
[111] X. Lou, H.G. Janssen, C.A. Cramers, Temperature and pressure effects on solubility in supercritical carbon dioxide and retention in supercritical fluid chromatography, J. Chromatogr. A 785 (1997) 57–64.
[112] G. Guiochon, A. Tarafder, Fundamental challenges and opportunities for preparative supercritical fluid chromatography, J. Chromatogr. A 1218 (2011) 1037–1114.

[113] P. Jandera, Comparison of various modes and phase systems for analytical HPLC, in: K. Valkó (Ed.), Separations Methods in Drug Synthesis and Purification, Elsevier, Herts, UK, 2000, pp. 1–71.
[114] R.M. Ornaf, M.W. Dong, Key concepts of HPLC in pharmaceutical analysis, in: S. Ahuja, M.W. Dong (Eds.), Handbook of Pharmaceutical Analysis by HPLC, Elsevier Academic Press, Oxford, UK, 2005, pp. 20–45.
[115] R. Tijssen, The mechanisms and importance of zone-spreading, in: E. Katz, R. Eksteen, P. Schoenmakers, N. Miller (Eds.), Handbook of HPLC, Marcel Dekker, New York, USA, 1998, pp. 55–142.
[116] T.A. Berger, Putting packed column supercritical fluid chromatography into perspective, in: T.A. Berger (Ed.), Packed Column SFC, The Royal Society of Chemistry, Cambridge, UK, 1995, pp. 1–21.
[117] P. Borman, B. Boughtflower, K. Cattanach, K. Crane, K. Freebairn, G. Jonas, I. Mutton, A. Patel, M. Sanders, D. Thompson, Comparative performances of selected chiral HPLC, SFC, and CE systems with a chemically diverse sample set, Chirality 15 (Suppl.) (2003) S1–S12.
[118] K.L. Williams, L.C. Sander, S.A. Wise, Comparison of liquid and supercritical fluid chromatography for the separation of enantiomers on chiral stationary phases, J. Pharm. Biomed. Anal. 15 (1997) 1789–1799.
[119] C. White, Integration of supercritical fluid chromatography into drug discovery as a routine support tool. Part I. fast chiral screening and purification, J. Chromatogr. A 1074 (2005) 163–173.
[120] C. Zhang, L. Jin, S. Zhou, Y. Zhang, S. Feng, Q. Zhou, Chiral separation of neonicotinoid insecticides by polysaccharide-type stationary phases using high-performance liquid chromatography and supercritical fluid chromatography, Chirality 23 (2011) 215–221.
[121] L. Toribio, M.J. Del Nozal, J.L. Bernal, C. Alonso, J.J. Jimenez, Comparative study of the enantioselective separation of several antiulcer drugs by high-performance liquid chromatography and supercritical fluid chromatography, J. Chromatogr. A 1091 (2005) 118–123.
[122] J.L. Bernal, L. Toribio, M.J. Del Nozal, E.M. Nieto, M.I. Montequi, Separation of antifungal chiral drugs by SFC and HPLC: a comparative study, J. Biochem. Biophys. Methods 54 (2002) 245–254.
[123] L.T. Taylor, Supercritical fluid chromatography for the 21st century, J. Supercrit. Fluids 47 (2009) 566–573.
[124] C. Hamman, M. Wong, M. Hayes, P. Gibbons, A high throughput approach to purifying chiral molecules using 3 μm analytical chiral stationary phases via supercritical fluid chromatography, J. Chromatogr. A 1218 (2011) 3529–3536.
[125] C.J. Welch, M. Biba, J.R. Gouker, G. Kath, P. Augustine, P. Hosek, Solving multicomponent chiral separation challenges using a new SFC tandem column screening tool, Chirality 19 (2007) 184–189.
[126] Z. Pirzada, M. Personick, M. Biba, X. Gong, L. Zhou, W. Schafer, C. Roussel, C.J. Welch, Systematic evaluation of new chiral stationary phases for supercritical fluid chromatography using a standard racemate library, J. Chromatogr. A 1217 (2010) 1134–1138.
[127] L. Zeng, R. Xu, D.B. Laskar, D.B. Kassel, Parallel supercritical fluid chromatography/mass spectrometry system for high-throughput enantioselective optimization and separation, J. Chromatogr. A 1169 (2007) 193–204.
[128] K.W. Phinney, L.C. Sander, S.A. Wise, Coupled achiral/chiral column techniques in subcritical fluid chromatography for the separation of chiral and nonchiral compounds, Anal. Chem. 70 (1998) 2331–2335.
[129] M. Perrut, Fractionation process for mixtures by elution chromatography with liquid in supercritical state and installation for its operation, US Patent 4478720 (1984).
[130] B. Bolaños, M. Greig, M. Ventura, W. Farrell, C.M. Aurigemma, SFC/MS in drug discovery at Pfizer, La Jolla, Int. J. Mass Spectrom. 238 (2004) 85–97.
[131] L. Toribio, M.J. Del Nozal, Y.L. Bernal, C. Alonso, J.J. Jimenez, Semipreparative chiral supercritical fluid chromatography in the fractionation of lansoprazole and two related antiulcer drugs enantiomers, J. Sep. Sci. 31 (2008) 1307–1313.
[132] L. Toribio, C. Alonso, M.J. Del Nozal, J.L. Bernal, M.T. Martin, Semipreparative enantiomeric separation of omeprazole by supercritical fluid chromatography, J. Chromatogr. A 1137 (2006) 30–35.
[133] T. Wang, M. Barber, I. Hardt, D.B. Kassel, Mass-directed fractionation and isolation of pharmaceutical compounds by packed-column supercritical fluid chromatography/mass spectrometry, Rapid Commun. Mass Spectrom. 15 (2001) 2067–2075.
[134] T.A. Berger, Demonstration of high speeds with low pressure drops using 1.8 μm particles in SFC, Chromatographia 72 (2010) 597–602.
[135] Waters Corporation, Uncharted territory: prepare to be charted. Seminar: Encounter the Green Dimension in Chromatography, in: Introducing the ACQUITY UPSFC, Elewijt, Belgium, 29, April 2011, 2011.
[136] Waters Corporation, http://www.waters.com/waters/library.htm?lid=10206766&cid=511436 (accessed 08.11).
[137] Waters Corporation, http://www.waters.com/waters/library.htm?lid=10206761&cid=511436 (accessed 08.11).
[138] Waters Corporation, http://www.waters.com/waters/library.htm?lid=10206745&cid=511436 (accessed 08.11).