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Recent developments in preparative-scale supercritical fluid- and liquid chromatography for chiral separations

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Abstract

This review covers preparative-scale chromatography for enantioseparations in both supercritical fluid and liquid chromatography published between 2016 and 2020. When pertinent, some fundamental publications from outside this time window are cited. The recent developments in instrumentation, novelties in method development and chiral stationary- and mobile phases are placed in context, while throughput, scale-up and sample issues are presented for supercritical fluid chromatography, and large-batch techniques together with computer-assisted method development, are described for liquid chromatography. Preparative-scale applications are reported for natural products, pharmaceutical compounds and pesticides, as well some other small molecules. Finally, a section is devoted to reports comparing supercritical fluid- and liquid chromatography and the strategies of various commercial users are briefly described. The goal of this review is to present current practices in the field of medium to large-scale chiral separations and offer indications as to how the best approach may be chosen for a given application.

53

54 **Abbreviations**

55 AcN acetonitrile

56 API active pharmaceutical ingredient

57 CAMP chiral additive at the mobile phase

58 CPME cyclopentylmethylether

59 CSP chiral stationary phase

60 DCM dichloromethane

61 DEA diethylamine

62 EA ethyl acetate

63 EtOH ethanol

64 iPrOH isopropanol

65 MeOH methanol

66 MTBE methyl tert-butylether

67 *n*-Hept *n*-Heptane

68 *n*-Hex *n*-Hexane

69 TFA trifluoroacetic acid

70 TFE trifluorethanol

71 THF tetrahydrofuran

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109 **1. Introduction**

110

111 Why did evolution favor L-amino acids and D-sugars as the homochiral building blocks of proteins
112 and nucleic acids in all living species? While its origins remain obscure, chirality exerts a profound
113 influence on biology and defines many of the key mechanisms of life. Chiral molecules consist of at
114 least two forms which cannot be superimposed on their mirror image, known as enantiomers or
115 optical isomers, and different enantiomers can have very different pharmacological effects. The
116 global market for chiral chemicals was valued at USD 39.79 billion in 2015 and is projected to
117 expand at a compound annual growth rate of 13.67% to be USD 96.89 billion by 2023. Within this
118 market are flavors and fragrances, agrochemicals such as herbicides, pesticides, plant growth
119 regulators and fungicides (30% of registered pesticides are chiral compounds [1]), and lastly
120 pharmaceuticals within which chiral compounds accounted for 72.5% of the global market in 2015.
121 Among the top-selling drugs on the market, most of the pharmaceutical blockbusters are pure
122 stereoisomers, clearly showing that methods which separate enantiomers are needed for biological
123 testing [2].

124 Among the different methods developed for enantioseparation, chromatographic resolution of the
125 racemic mixture is the most widely used technology (for more on the available methods, the reader
126 is referred to a comprehensive review from 2018 detailing both analytical- and preparative-scale
127 enantioseparation by chromatography and related methods [3]). Two approaches can be pursued:
128 the first, “indirect” one forms true diastereoisomers, through derivatization of the compound using a
129 chiral agent (CDA), which can then be subsequently separated in an achiral environment; the
130 second, “direct” one, forms transient diastereoisomers between the solute and either a chiral
131 stationary phase (CSP) or a chiral additive to the mobile phase (CAMP). While the CAMP method
132 is classically employed in capillary electrophoresis (CE), this direct approach is not very popular in
133 either supercritical fluid chromatography (SFC) or high-performance liquid chromatography
134 (HPLC), mainly because it is only suitable for analytical-scale and not preparative-scale

135 separations. However CE presents many advantages over chromatography, primarily derived from
136 the small dimensions of the silica capillary. These include high flexibility and separation power,
137 short migration times, low consumption of analyte and chemicals, and a wealth of available chiral
138 selector types, while the use of capillaries results in very high plate numbers. The first CE chiral
139 separation was reported in 1985 by Gassman *et al.* [4] on the resolution of amino acids using a
140 ligand-exchange separation mechanism. Three years later, Snopek *et al.* achieved the separation of
141 pseudoephedrine enantiomers using β -cyclodextrin and heptakis(2,6-di-O-methyl)- β -cyclodextrin as
142 additives to the leading electrolyte in isotachopheresis [5]. Recently, the major contributions to
143 chiral resolution by CE have been reported by the groups such of Fanali, Chankvetadze, Fillet and
144 Scriba, among others [6-9].

145 While HPLC with single and multi-column processes has been used for many years [10-12], SFC is
146 starting to carve out a niche [13] thanks, in particular, to the work of Berger and colleagues in the
147 mid-1990s [14], and has found its main field of application in chiral separation at the preparative
148 scale [15; 16].

149 This review will report the recent developments in preparative-scale chromatographic separations,
150 covering preparative SFC and HPLC publications over the last five years and highlighting the
151 emerging trends at the dawn of the 2020s. Readers will find a summary table at the end of the
152 manuscript summarizing the publications cited herein and one at the end of each of the three
153 applications sections (Section 4) summarizing the methods used.

154 **2. Recent developments in instrumentation**

155 **2.1 Supercritical fluid chromatography**

156 The first application of a supercritical fluid as a mobile phase for chromatography was reported by
157 Klesper *et al.* [17] in 1962. However, because of poor availability of accurate and reliable
158 commercial devices, use of supercritical fluid chromatography was confined for many years to
159 academic research [18] with home-made devices. It was not until the early 1990s that a first
160 generation of SFC devices was commercialized by several suppliers such as Gilson or Hewlett-

161 Packard (HP) then by Berger, Jasco, Pic Solution or Novasep, which permitted rapid development
162 of this technique in industry and especially in pharmaceutical discovery. The first devices dedicated
163 to purification by SFC were essentially of the same design and enabled purification of a few
164 milligrams for the semi-preparative systems, up to several kilograms of mixtures consisting of 2, 3
165 or 4 compounds for the pilot, preparative-scale devices. They were all equipped with a binary
166 solvent delivery pump, an injector suitable for multiple injection of one feed solution, a UV or
167 diode array detector, a Back-Pressure Regulator (BPR), a Gas Liquid Separator (GLS) and a limited
168 set of fraction collectors (from 3 to 8). The choice of preparative SFC systems is growing but is still
169 limited to 6 main suppliers providing equipment which accept columns with dimension from 1cm to
170 80cm internal diameter (ID).

171 Most of the research investments and innovations in SFC have been made by these suppliers to
172 reduce the extra column volumes and/or to enhance detector sensitivity. Nowadays, the
173 performance of analytical SFC devices is close to UHPLC systems and SFC is now suitable for
174 different applications like metabolite identification, metabolomic, pharmacokinetic, or
175 environmental research. However, the suppliers of preparative SFC devices have innovated too.
176 Recently, thanks to the development of a new generation of GLS preventing the formation of
177 aerosols and allowing fraction collection at atmospheric pressure, open bed collectors are now
178 available. The commercialization of such devices (by Jasco, PIC Solution, Sepiatec, Waters or
179 recently by Shimadzu) enables higher throughput and represents a step forward for the development
180 of the preparative SFC. Meanwhile, the coupling of preparative SFC apparatuses to mass
181 spectrometers by suppliers like Sepiatec, Waters or PIC Solution has extended the range of
182 applications for this technology. Indeed, the combination of the open bed collector and the mass
183 spectrometry-directed collection is a configuration suitable for both the purification of libraries
184 (achiral chromatography) and the separation of a set of racemic mixtures (chiral chromatography).
185 Until now, racemic mixtures have usually been purified one-by-one due to the limited number of
186 fractions collectable by compatible apparatus, resulting in a relative low turn around despite the

187 numerous advantages of this technique. Thanks to the development of these new devices,
188 preparative SFC-MS is becoming a real alternative for the separation of a series of compounds. This
189 type of device, equipped with an autosampler and a mass detector, is dedicated to the separation of
190 small amount of compounds (< 50 mg) requiring one or two injection per sample, however some of
191 them have additional features to perform stacked injections and thereby increase yield to several
192 grams.

193 It is worth mentioning that Shimadzu has recently released a new preparative-scale SFC system.
194 This is the result of a collaboration with the Enabling Technologies Consortium™ (ETC), a “forum
195 for pharmaceutical and biotechnology companies to discuss ideas, share information, and
196 collaborate on the development of new enabling technologies”. Like the previously mentioned
197 preparative SFC-MS systems, this device is suitable for research and for yields of up to a few
198 grams.

199 However, the price of SFC devices remains high compared to preparative HPLC devices and SFC is
200 not suitable for all compounds, often making the acquisition of both HPLC and SFC preparative-
201 scale devices necessary. Hence, hybrid systems have been developed to do both analytical scale and
202 preparative scale SFC on the same device (Waters, PIC Solution or Jasco), or else dual systems to
203 perform both HPLC and SFC on one system (Agilent, Shimadzu). In addition, ABSys [19-21] has
204 developed several features to economically and efficiently set up a preparative SFC device from an
205 LC device.

206 **2.2 Liquid chromatography**

207 Much has changed since the first complete separation of the enantiomers of a racemate by ligand-
208 exchange liquid chromatography was reported by Rogozhin and Davankov in the late 1960s [22].
209 Preparative-scale chromatography technologies implemented the most frequently today are batch-
210 HPLC, steady state recycling (SSR) and simulated moving bed (SMB). From a practical point of
211 view, batch HPLC involves a single column process, while SSR is semi-continuous

212 chromatography on a single column and SMB is a continuous, multicolumn chromatographic
213 approach.

214 From an equipment point of view, large-batch HPLC systems mirror their smaller-scale laboratory
215 counterparts but use large diameter, pre-packed columns of 20, 30 or 50 mm I.D (or larger).

216 SSR liquid chromatography is used to support production of early-stage drug development. The
217 principle is that only the purified fractions are collected: the non-separated portion of the
218 chromatogram is recycled at the column inlet and a new injection of racemate is performed while
219 the unresolved portion is recycled. The steady state is reached when the amounts of product injected
220 and collected are equal and the productivity will not decrease with the number of cycles. From an
221 instrumental point of view, an SSR device is merely a Batch HPLC with a recycling loop and a 4-
222 port manifold which allows the recycling and the injection of new racemate, along with two
223 collectors for the pure enantiomers.

224 Chiral separation using the SMB principle employs a series of columns connected in a loop to
225 continuously collect two fractions, each containing a pure enantiomer, from a continuously-eluting
226 racemic mixture. The remaining mixture of enantiomers that has not been separated is recycled
227 through the columns while the racemic mixture is newly injected. The advantage here is that there is
228 no need to have a complete separation since only a fraction of the enantiomer is collected as a
229 “pure” fraction and the unresolved mixture remains in the loop. An SMB device is equipped with 6
230 to 8 columns in series, with a recycling loop and 2 inlet (for feed and eluent) and 2 outlet (for
231 extract and raffinate) streams connected between the columns. The countercurrent movement of the
232 solid is simulated by moving the inlet and outlet ports in the direction of the liquid flow. This
233 approach has been optimized by Novasep, and their Varicol® device allows the use of 5 or 6
234 columns with a claimed 30% increase in productivity over conventional systems.

235 Compared to batch HPLC, since no complete separation is needed, the racemate concentration in
236 SSR or SMB (and hence the productivity) can be substantially higher than with a standard batch
237 process, and the volume of solvent consumed substantially lower. While SMB technology allows

238 the separation of 100s of grams, even kilograms of racemate, it requires several days of
239 development before production and a standard batch process remains the most suitable when only a
240 few grams are to be separated.

241 Obviously liquid chromatography (LC) at the preparative scale is a mature technology and
242 noteworthy advances in recent years are few. Further complicating the compilation of a review such
243 as this is the commercial and proprietary nature of many technologies. Publicity from one of the
244 biggest manufacturers in this sector cites preparative LC installations still in operation after 40
245 years, but few specific details of instrumentation are available and little mention made of
246 enantioseparation. The Prochrom® package of products and services and the Hipersep® pilot are
247 two such products.

248 While Mattrey *et al.* [23] do not deal exclusively with chiral nor preparative LC in their
249 comprehensive and wide-ranging review, their authoritative *tour d'horizon* of computer-assisted
250 method development deserves a mention. They report on the increasing potential, accessibility –
251 and perhaps the inevitability – of this technology as an important tool in screening for all aspects of
252 chromatographic method. They note that chiral analyses, and in particular the sometimes-
253 unpredictable performance of CSPs in their early days, contributed much to drive what is fast-
254 becoming an industry in its own right: Bennett *et al.* [24] make the case for one such product (see
255 section 3.2).

256 **3. Recent method developments**

257 **3.1 Supercritical fluid chromatography**

258 3.1.1 Chiral stationary phases and mobiles phases

259 The choice of a preparative chiral stationary phase (CSP), in both SFC and HPLC, is based upon
260 several criteria: the CSP must be universal to separate different types of compounds with a high
261 resolution; the utilization of the CSP in preparative chromatography requires a high loading
262 capacity; and finally, it is best to select robust CSPs to handle many purifications – indeed, the only
263 thing all CSPs have in common is their high cost, making it expensive to change them often. A few

264 evolutions in CSPs have been observed recently and will be discussed briefly here, as more details
265 are available in a recent review by West focusing on the analytical scale [25].

266 In addition to the introduction of chlorinated CSPs providing different selectivity [26], and the
267 development of the generic polysaccharide based CSPs with the same chiral selector but from
268 several suppliers, the main improvement in recent years is the development of immobilized
269 polysaccharide-based stationary phases. Polysaccharide-based stationary phases are increasingly
270 recognized as a powerful media for the separation of enantiomers, however the first generation of
271 these stationary phases consisted of polysaccharide coatings on the silica support, leading to
272 compatibility issues with some solvents like dichloromethane or THF. Indeed, as the polysaccharide
273 is soluble in these solvents, there is a major risk of bleeding of the phase which is not covalently
274 linked to the silica. A new generation of these phases, with the polysaccharide immobilized on the
275 silica, is extending their application range. The first preparation of an immobilized type CSP was
276 described more than 30 years ago by Okamoto *et al.* [27], but initial results showed a progressive
277 degradation of enantioselectivity. Different immobilization processes have been investigated to
278 obtain new CSPs with an enantiomeric recognition capacity close to the original coated phases. The
279 first immobilized polysaccharide-based CSPs, commercialized in 2005, were Chiralpak IA, based
280 on the 3,5 dimethylphenylcarbamate of amylose [28], followed by Chiralpak IB (3,5
281 dimethylphenylcarbamate of cellulose). Both are immobilized versions of well-known coated
282 phases (Chiralpak AD and Chiralcel OD, respectively). Immobilized versions of amylose tris (S)- α -
283 methylbenzylcarbamate, amylose tris (3-chloro-4-methylphenylcarbamate) and tris (4-
284 methylbenzoate) have been commercialized very recently. Immobilized CSPs with chiral selectors,
285 unavailable in coated versions, were also developed such as Chiralpak IC or Lux-I cellulose-5 or
286 Chiral ART cellulose SC or reflect i-cellulose C (tris-3,5 dichlorophenylcarbamate of cellulose),
287 Chiralpak ID (tris-3 chlorophenylcarbamate of amylose), Chiralpak IE (tris-3,5
288 dichlorophenylcarbamate of amylose) and, the newest, Chiralpak IG or Lux-I amylose-3 (tris-3
289 chloro 5 methylphenyl carbamate) commercialized in 2016 [29]. These immobilized-

290 polysaccharide-based CSPs provide different selectivities and are now commonly used in the
291 screening approach for preparative-scale enantiomeric separations [30]. This new type of chiral
292 stationary phase also represents an opportunity to explore new organic solvents as modifiers such as
293 DCM, THF or MTBE (all miscible with scCO₂) in a quest for novel enantioselectivities [28; 31;
294 32]. In addition, these “exotic” solvents may also enhance productivity in preparative
295 chromatography thanks to the higher solubility of the racemates [33], and recently published papers
296 on this aspect will be presented in section 4.1 [34-36]. These solvents are now frequently used in
297 preparative SFC, even if alcohols are still more commonly used.

298 In addition to the polysaccharide-based CSPs, specific CSPs have been recently developed and used
299 in SFC: (i) the anion exchange phases Chiralpak QD-AX and QN-AX [37] provide a good
300 separation of chiral acids; (ii) the zwitterionic CSPs Chiralpak ZWIX(+) and Chiralpak ZWIX(-)
301 dedicated to the separations of amino-acids [38]; (iii) Chirobiotic T2 for the separation of aromatic
302 amino acids [39] and (iv) cyclofructan CSPs for enantiomeric separations of α -aryl-ketone.
303 However, no paper showing the utilization of these CSPs in preparative SFC has yet been
304 published.

305 Regarding the mobile phase, it is widely acknowledged that SFC is perhaps better described as
306 Subcritical rather than Supercritical fluid chromatography: indeed, the supercritical state only
307 occurs above the critical pressure and temperature and the addition of a modifier to the scCO₂
308 influences these critical points. However, as there is no discontinuous transition between liquid and
309 supercritical states, the consequence on the physical properties of the mobile phase are negligible.
310 For more polar compounds the proportion of modifier can reach 50% while for highly hydrophilic
311 analytes (peptides, sugars or nucleobases), the addition of water to the mobile phase is needed to
312 achieve reasonable retention. A recent example used a mobile phase containing a high percentage of
313 modifier (above 50%), including 10% water, to separate chiral aromatic amino acids on a
314 Chirobiotic T column [39]. However, the proportion of water is limited due to miscibility limits and
315 increases in detection noise. Some applications using a percentage of modifier above 50% mixed

316 with liquid CO₂ and a high percentage of water were reported by Olesik [40]. This approach, known
317 as Enhanced fluidity liquid chromatography (EFLC), was used by Bennett *et al.* in the first
318 application of EFLC for preparative-scale separation of hydrophilic analytes. They report that
319 HILIC-type columns can be used on SFC instrumentation with EFLC methods for both analysis and
320 purification of very polar and hydrophilic compounds [41]. No chiral separation has so far been
321 reported using EFLC, however the works of Bennett demonstrates that a specific class of
322 compounds may be explored with this technique.

323 3.1.2 Increasing throughput and scale-up

324 There is a need for fast chiral separation in early-stage drug discovery and rapid separation of the
325 pure enantiomer. To reduce the process time, several options or strategies can be developed: the
326 first is to reduce the screening time. To do this, both column dimension and particle size have
327 reduced, year-on-year, from 250 × 4.6mm with 10µm particle size to 50 × 3mm packed with 1.6µm
328 particle size [23], to achieve run times of less than 3 minutes. The run time in some cases has been
329 more drastically reduced with chiral separation reported by T. Berger of less than 30 seconds (7.5s
330 for trans-stilbene oxide enantiomer separation) [42; 43]. In this race to ultrafast separation, the
331 interesting concept of a segmented chiral-achiral liquid chromatography column, introduced in 2018
332 by Chankvetadze *et al.*, is of particular note with baseline separation of enantiomers achieved in a
333 time of between 1-2 s [44].

334 This last, extreme strategy requires the modification of the devices to reduce dead volumes to a
335 minimum and cannot be used routinely. However, Zawatzki *et al.* [45] showed that a very short run
336 time is a way to set up a Multiple Injection in a Single Experimental Run (MISER). Some analytical
337 SFC devices are designed for this and permit the injection of many samples of the same mixture in
338 one run. MISER can be used to monitor enantiomerisation or to analyze several fractions from a
339 preparative separation. Zawatzki and colleagues report the analysis of 96 samples of Tröger's base
340 on a Chiralpak AD (4 × 10mm) in 33 minutes. This high throughput is also essential for rapid
341 screening of the numerous and diverse CSPs available.

342 A second option to enhance throughput in SFC is the introduction of open-access both for analytical
343 screening and preparative scale enantioseparation [46]. A platform with both an analytical system,
344 consisting of only four chiral columns for screening plus one modifier, and a preparative-scale,
345 mass-triggered SFC system was developed. About 70% of the separation can be carried out thanks
346 to this process. The use of a preparative SFC-MS platform with autosampler and open-bed
347 collection is also an opportunity to increase the throughput of specialized chiral separation
348 laboratories (full service platform). Indeed, with the standard batch preparative SFC system,
349 compounds are handled one by one, the number of fractions it is possible to collect is limited, and
350 the whole system requires flushing between purifications. With an SFC-MS prep' platform there is
351 no limit to the number of fractions collectable, and the target is tracked by its molecular weight and
352 purification can thus be performed in a continuous flow.

353 Another way to increase productivity was reported by Da Silva *et al.* [47]. They showed that the use
354 of high linear velocities in preparative SFC (above 10mm/s) is efficient for the separation of the
355 enantiomers of the Tröger's base on the Chiralpak AD. Productivity was similar when using a 2cm
356 ID column with 20 μ m particles and high linear velocity when compared with a 3cm ID column
357 with 5 μ m particles. In addition, solvent consumption was lower with the smaller column.

358 In these examples we have been talking about batch purification, meaning one column was used to
359 separate the isomers, but continuous mode or Simulated moving bed (SMB) is also used in SFC.
360 The SMB process has been already described for HPLC in section 2.2, and SMB-SFC also requires
361 the use of several columns (usually 6 or 8). A comparison of these approaches in SFC was made in
362 2007 by Peper *et al.* [48]; more recently, Johannsen *et al.* [49] compared productivity them using
363 stacked injections, concluding that the choice of technique depended on the separation required.
364 While the solvent consumption with SMB is usually lower, an economic comparison of these
365 approaches for the separation of two mixtures showed that, in spite of a better productivity with
366 SMB-SFC, the batch process remained more economic.

367 The scale-up in SFC is more complex than that of HPLC due to the compressibility of the mobile
368 phase which alters its density and hence the pressure or temperature along the column. According to
369 Tarafder *et al.* [50], the strategy for reliable and accurate SFC scale-up is to maintain equivalent
370 mobile phase density in both the analytical and the preparative systems by keeping column length
371 and particle size, eluent composition, flow-rate, temperature and pressure the same. However, as
372 noted by Enmark *et al.* [51], scale-up in SFC is complicated by differences in instrumentation: the
373 analytical SFC units use a volume flowmeter while the preparative systems use a mass flowmeter.
374 An accurate measurement of the volumetric fraction of modifier inside the column is therefore
375 required to reliably scale-up in SFC.

376 Comparing SMB-SFC and SMB-LC, it is clear that the first approach presents more technical
377 challenges, notably controlling the flow-rate and proportioning the flow between the outlets and the
378 following column. However both the solvent usage and time spent evaporating the collected
379 fractions are generally lower in SMB-SFC than in SMB-LC, while higher productivities are also
380 reported for SFC-SMB relative to SMB-LC.

381 3.1.3 Sample integrity before, during and after purification

382 The wider range of solvents usable with the newer CSPs has gone some way to aid the preparative-
383 scale separation of poorly-soluble compounds: it is nevertheless still necessary to avoid sample
384 precipitation and the resultant blockage of the inlet or any impact on the concentration of the feed
385 solution.

386 Leek *et al.* [52] modified their injection system with the replacement of the sample loop by an
387 extraction vessel, containing the sample adsorbed onto silica or celite[®], and the quantity of
388 compound injected depends on the volume of mobile phase passing through this vessel. This
389 example shows that the injection is a key step in preparative-scale purification. Indeed, the sample
390 concentration is usually high to maximize productivity, and several studies have been carried out on
391 the impact on enantioresolution of the injection mode and the modifier- and mixed streams [53].

392 Two recent papers described the impact of the nature of the solvent used to dissolve the sample:

393 Desfontaines *et al.* [54] showed that MTBE, DCM, AcN or CPME were for the most part well-
394 adapted to large volume injections, while MeOH was the poorest in this regard. They also reported
395 that the choice of the solvent depended on the stationary phase used. Meanwhile, Shalliker *et al.*
396 [54] described the impact of the viscosity of the solvent on the peak shape.

397 The solubility of the sample is a key point, however another key issue in preparative SFC is sample
398 stability: preventing its degradation, epimerization, racemization or interconversion is essential in
399 preparative chiral separation. These phenomena usually occur in the column during the separation,
400 in the collection flask, or during the subsequent evaporation step, but the modifier may also play a
401 role.

402 As explained previously, the nature of the modifier impacts the retention, the selectivity and the
403 solubility of the racemates but this modifier can also induce the degradation of some categories of
404 compounds such as esters, anhydrides, phenol esters and thiol esters. An approach developed by
405 Byrne *et al.* [56] is the replacement of the standard alcohol by 2,2,2 trifluoroethanol (TFE). They
406 report that the presence of the fluoro- substituent strongly reduces the nucleophilic reactivity of the
407 oxygen of the hydroxyl group. This strategy was also used by Asokan *et al.* [57] for the purification
408 of boronate ester. Boronic acid picanol esters were successfully separated without degradation or
409 epimerization using TFE as modifier. It is worth noting that thanks to its stronger eluting strength
410 compared with MeOH, the percentage of TFE in the mobile phase was lower and the stack injection
411 cycle time reduced, with sharper peaks leading to higher loading *per* injection and hence higher
412 throughput.

413 Another potential source of degradation is linked to the acidity of the mobile phase. The combined
414 presence of CO₂ and MeOH results in the formation of methyl carbonic acid which may favor
415 degradation either inside the column or in the fraction collector. As described in a second paper,
416 Asokan *et al.* [58] report that the concentration of CO₂ in the fraction collector depends on the
417 system and the pressure used in the phase separator. For the compounds potentially degraded by
418 methyl carbonic acid, and purified using a phase separator requiring a pressure around 50bar,

419 degradation can be avoided by replacing MeOH with another solvent, by bubbling nitrogen into the
420 fraction collector to chase CO₂ from the MeOH, or finally by adding a basic additive to neutralize
421 the solvent in the mobile phase or collected fraction.

422 The choice of the modifier is thus key to ensure the integrity of the analyte during purification. In
423 addition, it is essential to control the kinetics of the degradation after separation. For instance,
424 Bajpai *et al.* [59] developed a process to prevent the degradation of one of their internal
425 compounds: purification required diethylamine (DEA) in the mobile phase, however a degradation
426 of the target was observed during the post-purification process due to this basic additive. To
427 neutralize the base, a post column make-up introduced TFA (and, at the same time, DEA was
428 substituted by ammonium hydroxide to avoid the formation of TFA-DEA adducts in the final
429 compound). Another separation of atropisomers posed particular challenges due to the potential
430 interconversion of the isomers depending on their rotational energy barriers. The R&D team of
431 Bristol-Mayer-Squibb succeeded in separating and isolating atropisomers by SFC, with good purity,
432 thanks to simple ice baths added at the fraction collection reservoir to slow this interconversion.

433 **3.2 Liquid chromatography**

434 3.2.1 HPLC, SMB and CCC

435 The maturity of HPLC technologies and the commercial nature of preparative-scale methods for
436 chiral separations means few publications describe in any detail the recent advances in this sector.

437 *Mode of injection*

438 In the field of natural products, the advantages of at-column dilution in preparative-scale
439 separations of highly polar, water-soluble compounds were presented by Jaffuel *et al.* [60], while
440 Ferrera-Queiroz *et al.* [60] investigated the use of a dry load injection method for poorly water-
441 soluble compounds. While neither study was strictly concerned with chiral compounds, and the dry
442 load injection method is perhaps too involved (as described) at anything beyond the semi-
443 preparative scale, they merit inclusion to illustrate the advantages of creative thinking with respect
444 to sample introduction. Innovative methods for injecting samples have been a staple of preparative-

445 scale chromatography for some time, and Taheri *et al.* [62] described the optimization of a stacked-
446 injection method for the semi-preparative enantioseparation of tramadol by chiral LC. The isomers
447 of this poorly soluble molecule were separated with acceptable throughput by means of saturated
448 injections of the racemate, and their study offers a model for enantioseparation of other poorly
449 soluble molecules.

450 *Simulation*

451 In 2018, Forssén and Fornsted [63] published a paper including both chiral and non-chiral
452 possibilities from a comprehensive modelling study of the impact of LC column and chiral
453 stationary phase type on maximal productivity (P_{R-MAX}) of enantioseparations. Using Monte Carlo
454 simulations, they concluded that column efficiencies should be at least 500 theoretical plates but
455 that nothing is to be gained by increasing separation efficiency above this limit. While increases in
456 P_{R-MAX} can be achieved by increases in selectivity if $P_{R-MAX} < 2$, increasing selectivity if it is already
457 > 3 will have the opposite effect. Similarly, they report that substantial gains in P_{R-MAX} can be
458 achieved by increasing relative saturation of the enantioselective site if this is $< ca. 40\%$, but will
459 yield only moderate productivity gains if it is above this limit. Perhaps most pertinent to this
460 review, they reported that predicting the productivity of up-scaling of analytical-scale injections on
461 CSPs is not straightforward – good analytical performance, they warn, does not necessarily predict
462 good productivity at the preparative scale.

463 *Chiral stationary phases*

464 In one of the most comprehensive recent reviews of monolithic CSPs, Guo *et al.* [64] extensively
465 reviewed the preparation of this class of stationary phases for enantioseparation covering
466 cyclodextrin-, polysaccharide-, protein- and antibiotic-functionalized CSPs on various supports, as
467 well as ligand-, ion-exchange- and brush-type CSPs. Superficially porous silica as a carrier for
468 polysaccharide CSP development can be found in a recent work by Chankvetadze *et al.* [65]. These
469 two papers are necessarily focussed upon analytical-scale LC, given the relative youth of the field,
470 but they deserve a mention here since these CSPs display low flow resistance and good selectivity,

471 while the speed of progress among non-chiral, monolithic stationary phases of this type potentially
472 promises a rapid evolution of this sector for chiral applications.

473 In an innovative study aimed at achieving genuinely preparative-scale chiral LC at reasonable cost,
474 Malik *et al.* [66] describe what a cookery book might describe as a ‘store cupboard recipe’ for a
475 robust, reusable CSP consisting of bovine serum albumin (BSA) bonded to silica gel. Bemoaning
476 the fact that over 200 CSPs had been produced but that few (if any) had been successfully used for
477 “large-scale preparations in real terms”, the authors noted that many CSPs developed for LC
478 contain proteins. They rightly point out that many are also expensive (particularly true at the
479 preparative scale) and that they are often constrained in terms of percentage of organic modifier,
480 loading capacity, pressure limits and robustness. While not trivial, Malik *et al.* [66] described the
481 preparation of a CSP where BSA is covalently bonded to silica gel using ingredients found in many
482 laboratories’ cupboards. They used this CSP in an open column set-up for the direct
483 enantioresolution of 30mg of propranolol and atenolol and 3g of phenylalanine with >97% isomeric
484 purity.

485 Xie *et al.* [67] also cite the high cost of CSPs as one of the motivations for their review of less well-
486 known CSPs for LC. Focussing on stationary phases based on chitosan- and cyclofructan
487 derivatives and chiral porous materials, their review covers mostly analytical-scale methods; they
488 nonetheless cite the cyclofructan-based stationary phases, in particular the functionalised, six-
489 membered (CF6) CSPs, as having potential for preparative-scale LC owing to their stability and
490 high loading capacity. While the native CF6 possesses only limited chiral recognition capability,
491 aliphatic- and aromatic-functionalized CF6 are able to separate enantiomers of primary amines and
492 other racemic compounds such as acids, secondary- and tertiary amines and alcohols.

493 *Minor hardware adaptations*

494 Building on work outside the period of this review (*ie.* published before 2016), Wrzosek *et al.* [68]
495 sought to overcome the inherent “50% maximum yield” of chiral chromatography where the
496 preferred enantiomer is selected from the racemate by LC and the undesired isomer discarded. The

497 authors described a proof-of-concept study combining preparative, chiral LC in series with an
498 enzymatic reactor for racemization of the unwanted isomer. Using mandelic acid as their model
499 compound, the authors also modelled their method to increase the potential transfer of this
500 technique to other applications. In simple terms, the model's key elements consisted of just two
501 parameters – the absorption isotherms of the enantiomers on the LC column and the rate of
502 racemization in the enzymatic reactor.

503 Simulated Moving Bed chromatography

504 The conventional SMB system is considered as the gold standard for separations in the range of
505 hundreds of kilograms to hundreds of tons and is currently used commercially to produce Union
506 Chimique Belge (UCB)'s antiepileptic active pharmaceutical ingredient (API) Keppra, Lundbeck
507 A/S's Lexapro/Cipralext, Pfizer's Zoloft and Cephalon's Armodafinil, among others.

508 Chen *et al.* [69] reported considerable solvent savings when they compared preparative SMB and
509 LC to separate metalaxyl enantiomers from the racemate; they achieved excellent purity of the two
510 isomers (>99%) from a concentrated racemate solution (15 mg/mL) and consumption of mobile
511 phase was 9-fold lower using SMB. Cunha *et al.* [70] investigated SMB to separate the L and D
512 forms of praziquantel at high purity (100%) and semi-preparative scale (> 0.250 kkd). They built
513 several versions of their SMB unit around a cellulose *tris* CSP (Chiralcel OZ) and found that, in all
514 the scenarios investigated, at least one of the outlet streams produced one enantiomer at high purity
515 (often 100% optical purity). They compared their results with previous studies and reported at least
516 a doubling of productivity with much shorter run times, opening the way for studies with the
517 individual enantiomers of this drug.

518 Counter-Current Chromatography

519 This technique is considered as having a low environmental impact as it uses liquid-liquid
520 partitioning to separate different compounds with minimal flow, and thus consumes less solvent. In
521 their short review on the topic, Wu *et al.* [71] only briefly mention preparative-scale counter-current

522 chromatography (CCC) for enantioseparation, concluding that its future in this field of application
523 may rest primarily as a complement to HPLC.

524 More recently, Huang *et al.* [72] provided a comprehensive review of chiral separation by CCC,
525 citing its loading capacity, low cost, scalability and the complete recoverability of both enantiomers,
526 as well as the chiral selector, as the principal advantages of the technique. While they acknowledge
527 that separation efficiency remains below that of HPLC, its potential usefulness at the preparative
528 scale makes it a worthy complement to both SFC and LC. They cite several studies from within the
529 period covered by the present review which employed β -cyclodextrin derivatives as a chiral selector
530 in high-speed CCC (HSCCC) enantioseparations of ibuprofen, 2-(substituted phenyl)propanoic
531 acids, naringenin and pheniramine (respectively: Rong *et al.* [73], Tong *et al.* [74], Wang *et al.* [75]
532 and Xu *et al.* [76]).

533 Recycling elution mode was used by Rong *et al.* [73], with hydroxypropyl- β -cyclodextrin (HP- β -
534 CD) dissolved in the lower phase, in an HSCCC separation of ibuprofen enantiomers. The relatively
535 low number of theoretical plates and very similar *K*-values of the (*R*)- and (*S*)-ibuprofen
536 enantiomers made recycling necessary, and the authors reported a high enantiopurity (>97.5%) and
537 recovery (>82%) at a multi-milligram production scale for both isomers.

538 Tong *et al.* [74] also used HP- β -CD as a chiral selector, in a continuation of their previous work on
539 2-phenyl propionic acid derivatives, for the enantioseparation of ten 2-(substituted
540 phenyl)propanoic acid racemates. While not strictly preparative in scale (only 1 mmol/L solutions
541 of racemate mixtures were investigated), they nevertheless offer a proof-of-concept for this class of
542 chiral molecules, achieving enantiopurity >97% and recovery of each enantiomer of *ca.*80%, whilst
543 providing a detailed investigation of optimum separation conditions.

544 Wang *et al.* [75] also employed HP- β -CD as a chiral ligand, but exploited the synergy between it
545 and a Cu(II)-complexed amino acid ionic liquid (Cu(II)-[1-butyl-3-methylimidazolium][L-Pro]) for
546 the enantioseparation of intractable naringenin racemic mixtures. Achieving enantiomeric purity of
547 98% at a multi-milligram production scale, they used UV-vis and NMR spectra to elucidate the role

548 of thermodynamic stability of the quaternary complexes amongst the metal ion, the ionic liquid,
549 HP- β -CD and the racemate in this enantioseparation.

550 Again at the multi-milligram scale of production, Xu *et al.* [76] described a method for the almost
551 complete separation of enantiomers of pheniramine using carboxymethyl- β -cyclodextrin (CM- β -
552 CD): they obtained 99% enantiopurity and a recovery of 85-90% from 20mg of racemate.

553 3.2.2 Computer-assisted Method Development

554 Despite the analytical scale of many publications dealing with this topic, the supported scalability of
555 some of these studies is of particular interest to this review – as Fornstedt *et al.* [77] observe in their
556 excellent primer on the topic, the complexity of preparative-scale enantioseparations can be too
557 expensive to optimize without computer simulations. Both Bennett *et al.* [24] and Tsay *et al.* [78]
558 describe the use of a simulation package from ACD/Labs to manage scale-up from analytical- to
559 preparative scales, covering several key techniques and obstacles to this process. While not directly
560 concerned with enantioseparation, their studies reflect the growing use of this aspect of LC method
561 development and are the more pertinent because trial-and-error method development for
562 preparative-scale chiral LC is often unfeasible – as remarked by Mattrey *et al.* [23], in many
563 laboratories it is more a case of “when” and not “if” this technology will be used.

564 **4. Recent applications at the preparative-scale from 2016 to 2020**

565 The present article covers the published works from 2016 to 2020, but the reader may refer to a
566 review by Speybrouck and Lipka for studies published before 2016 [16].

567 **4.1 Supercritical fluid chromatography**

568 All the experimental conditions in the following articles are summarized in Table 1. As SFC is
569 considered an eco-friendly method, some sort of ecological metric is increasingly reported in
570 published articles in order to evaluate the environmental impact of the chromatographic method. At
571 the preparative scale, productivity is also a parameter of key importance.

572 The environmental factor (E-Factor) is defined as a waste-to-product ratio for any chromatographic
573 procedure. Solvent usage corresponds to the volume of solvent consumed to purify a known amount

574 of racemate (expressed in L/g). Productivity is defined as the amount of product separated *per* unit
575 time *per* amount of stationary phase, and is expressed in kg racemate *per* kg CSP per day (kkd),
576 while production rate is defined as the amount of product separated *per* unit time (kg product/day).
577 In early-stage development, productivity is often very low (0.1 kkd or even poorer), while with a
578 good separation productivity is generally in the range of 1 kkd and an exceptional separation might
579 have a productivity greater than 10 kkd [79]: these values can be considered as useful guidelines
580 when assessing productivity.

581 Natural products

582 In 2017, Xin *et al.* reported the separation and the purification of four lignan diastereoisomers from
583 *Piper kadsura* by chiral SFC: (-)-Galbelgin (compound **1**), (-)-Ganschisandrin (compound **2**),
584 Galgravin (compound **3**) and (-)-Veraguensin (compound **4**) [36]. These four lignans are known for
585 their anti-neuroinflammatory activity and the four diastereoisomers are present together in the *Piper*
586 *kadsura* extract. In this work, chiral SFC was tried because successive achiral-HPLC and achiral-
587 SFC methods had failed to resolve the diastereoisomers. Thus a three-step method was carried-out,
588 separating two pairs of diastereoisomers (compounds **1** and **2** from compounds **3** and **4**) on a first
589 CSP (CSP-1) then, in two subsequent steps, separating compound **1** from compound **2** and then
590 compound **3** from compound **4** on a second CSP (CSP-2).

591 Step 1 was performed on CSP-1 column, injecting 90mg.

592 Thanks to stacked injections, the extract mixture could be injected four times in one 30min cycle,
593 and the productivity increased by 277%; indeed, while a single injection was completed in 22.5 min
594 four were completed in 32.5 min using stacked injections. The four diastereoisomers were collected
595 into two parts (Fraction 1 and Fraction 2) weighing 150 mg and 470 mg, respectively, with four
596 injections implemented twice.

597 Step 2 was performed on CSP-2 injecting 12mg using stacked injections. The cycle time was
598 reduced, with the productivity increased by 291% as one injection was completed in 7.7 min while
599 fourteen injections were completed in 37 min. Following those conditions, a 150 mg mixture was

600 purified and 103.1 mg of compound **1** and 10.0 mg of compound **2** were respectively obtained in 37
601 min.

602 Step 3 was also performed on the CSP-2 column, injecting 45mg, and once again stacked injections
603 were performed. The cycle time was reduced, and productivity increased by 235% as one injection
604 was completed in 8 min while ten injections were completed in 34 min. Following those conditions,
605 a 470 mg mixture was purified and 152.3 mg of compound **3** and 178.6 mg of compound **4** were
606 respectively obtained in 34 min. The four lignans were obtained with a purity >98%.

607 Though not particularly original, the novelty of this work resides in its use of the “exotic” solvent
608 DCM to increase sample solubility.

609 In 2019, Lin *et al.* published the semi-preparative separation of enantiomers of dihydromyricetin
610 [80]. Dihydromyricetin is a natural dihydroflavonoid compound mainly isolated from traditional
611 Chinese medicines (TMC), namely, *Ampelopsis grossedentata* and *Hovenia dulcis*. This
612 dihydroflavonoid compound exhibits many beneficial pharmacological activities, such as anti-
613 oxidation, anti-inflammation, hepatoprotection and anti-hypertensive activities. A productivity of
614 0.07 kkd was achieved through stacked injections of 40mg per 12.8 min cycle. The solvent use was
615 5.86 L/g. The productivity was improved to 0.17 kkd through stacked injections of 80mg per 11.4
616 min cycle and solvent use was reduced to 2.75 L/g. Lastly a productivity of 0.27 kkd was achieved
617 through stacked injections of 120mg per 10.5 min cycle and solvent use further reduced to 1.76 L/g.
618 While this study is a good example of how productivity can be improved by injecting more of the
619 racemate, this increase in productivity (and decrease in MeOH consumption) nevertheless came at a
620 cost: the enantiomeric purities of two enantiomers were 98.5% and 98.4% at 40mg injected, falling
621 slightly to 98.5% and 97.3% at 80mg injected, but were both less than 95% when 120mg was
622 injected.

623 Small pharmaceutical molecules or intermediates

624 E. Francotte reported the preparative separation of 50 g of racemic 1-benzyl-3-methyl-2-piperidone,
625 a drug intermediate [81]. Stacked injections were again implemented with a 1.8 min cycle time and

626 240 injections of 208 mg each were performed. The method demonstrated a high efficiency, leading
627 to a productivity up to 2.3 kkd. The enantiomeric excess of the separated enantiomer was greater
628 than 99%. This work also reported the preparative separation of two iodo-aryl regioisomers and
629 highlights the ability of chiral stationary phase to separate regioisomers.

630 In 2016, Wu *et al.* [26] reported the separation of three synthetic intermediates. The first was a non-
631 polar, linear hydrocarbon, carboxylic acid (A) containing two chiral centers. The enantiomeric ratio
632 of the active trans-target to the undesired trans-isomer was 5:1. A productivity of 0.15 kkd was
633 achieved thanks to stacked injections (6.5 min per cycle) with a recovery >80%. Interestingly this is
634 the first report of the use of a mixture of carbone dioxide and *n*-heptane (and *i*PrOH) as a mobile
635 phase. The second example was a two-step preparative separation to obtain a chirally pure drug
636 intermediate. Indeed, the studied compound (B) contain two chiral centers and among the four
637 diastereoisomers (B1 to B4) only one was desired (B2). The optimized conditions led to
638 unsatisfactory loading and productivity when the four isomers were tested on the Chiralpak IC, so
639 another four-step approach was tried. The first step was synthesis of a pair of B3 and B4 isomers; a
640 second step was chiral SFC of the B3 and B4 mixture to produce pure B3; a third step was
641 epimerization of B3 to the mixture containing target B2 and B3 isomers, and the final step was
642 chiral SFC of the B2 and B3 mixture to obtain pure B2. A productivity rate of 204 g/day was
643 achieved with a high recovery through stacked injections of 440mg per 3.1 min cycle. In total, 1kg
644 was separated in one week, and the targeted B2 isomer was recovered with an enantiomer excess
645 >99%. At the end of the process, the undesired B3 isomer was epimerized in a B2 and B3 mixture
646 for recycling. The third compound described by Wu *et al.* was a polar, chiral API (C). A
647 productivity rate of 22.8 g/day was achieved with a high recovery. In this particular case, although
648 the loading capacity per injection cycle could have been increased, it was not investigated as the
649 target enantiomer eluted second.

650 Interestingly, this is the first time that 5% DCM has been successfully used on a coated CSP. The
651 authors stated that no performance deterioration was observed on the column after extended runs

652 with this ternary, co-solvent system. In addition, this work underlines the complementarity between
653 chlorinated and non-chlorinated CSPs, as chlorinated phases permitted the authors to successfully
654 resolve several structurally diverse compounds by chiral SFC which were not well resolved on the
655 non-chlorinated CSPs.

656 The same year, the same research group reported on the preparative separations of 20 propanoic
657 acids [82], developed as novel modulators of the glucocorticoid receptor. Among the 20 propanoic
658 acids, the preparative-scale resolution of compounds **4** and **14** were reported. Preparative separation
659 of compound **4** (without TFA) had a productivity rate of 67.2 g/day achieved through stacked
660 injections of 330mg per 7 min cycle. The solvent use was 0.76 L/g. Preparative separation of the
661 weakly basic compound **14** had a productivity of 0.312 kkd and a production rate of 111 g/day
662 achieved through stacked injections of 247mg per 8 min cycle. The flow-rate was equal to 100
663 mL/min and the solvent use was 0.65 L/g. Further scale-up on a 5 cm internal diameter column was
664 calculated and would have resulted in a production rate higher than 120g/day, considering a scale-
665 up factor of 2.78.

666 In order to improve selectivity (previously 2.0), a mobile phase consisting of 30% iPrOH and 0.1%
667 DEA was tried and a selectivity of 3.3 was achieved. These conditions were scaled-up to the
668 Chiralcel OJ-H column (250 × 50 mm; 5 μm). The sample was dissolved at 62 mg/mL in
669 iPrOH/DEA leading to a productivity of 0.209 kkd and a production rate of 81.1 g/day achieved
670 through stacked injections of 620mg per 11 min cycle. The flow-rate was equal to 220 mL/min and
671 the solvent use was 1.17 L/g. Despite an improved resolution, the productivity was nevertheless
672 lower and solvent consumption higher with this alternative mobile phase. This is mainly due to the
673 peak tailing of the 2nd eluting enantiomer and the large injected volume (10 mL) required by the low
674 solubility of the sample in the iPrOH/DEA mixture. This example illustrates once again the
675 importance of the sample solubility (*i.e* the feed concentration) in productivity.

676 In 2017, Dai *et al.* reported on the resolution at the preparative scale of atropisomers [83]. These
677 atropisomeric compounds contain two chiral axes, resulting in four atropisomers to be separated,

678 and are pharmaceutical inhibitors of Bruton's tyrosine kinase (BTK) which plays a critical role in
679 multiple cell types and is implicated in numerous autoimmune diseases. They found that isolated
680 fractions were unstable and racemized to give two peaks. Thus, a second preparative SFC was then
681 carried out on a Chiralpak AD column to which ice baths were added at the fraction collection
682 reservoirs to slow the interconversion.

683 In 2017, Zehani *et al.* published a small-scale chiral separation of four isoxazole derivatives [84].
684 These four 3-carboxamido-5-aryl isoxazole analogues are novel potential ligands of sub-type 2
685 cannabinoid receptors (CB2), designed as treatments for Inflammatory Bowel Diseases (IBD).

686 Preparative separation of compound 1:

687 A productivity of 0.40 kkd and a production rate of 0.70 mg/min (1 g/day) were achieved. The
688 solvent use was 0.87 L/g and the E-Factor was calculated as being 4.82.

689 Preparative separation of compound 2:

690 A productivity of 0.23 kkd and a production rate of 0.39 mg/min (0.56 g/day) were achieved. The
691 solvent use was 1.01 L/g and the E-Factor was calculated to be 7.86.

692 Preparative separation of compound 3:

693 A productivity of 0.34 kkd and a production rate of 0.58 mg/min (0.84 g/day) were achieved. The
694 solvent use was 0.48 L/g and the E-Factor was 5.28.

695 Preparative separation of compound 4:

696 A productivity of 0.24 kkd and a production rate of 0.40 mg/min (0.58 g/day) were achieved. The
697 solvent used was 0.98 L/g and the E-Factor was 7.55.

698 While in this article the E-Factors are calculated, these calculations remain scarce in the literature.

699 In 2019, Yip *et al.* published the evaluation and comparison of two possible approaches to large
700 scale SFC separation of a key intermediate in the synthesis of two S1P₁-active pharmaceutical
701 ingredients, namely BMS-986166 and compound 2 [35]. Recently, the S1P₁ (sphingosine-1-
702 phosphate-subtype 1) receptor has been highlighted as an attractive drug target in treatment of
703 autoimmune diseases. The authors describe the development of an efficient chiral SFC for purifying

704 kilogram-quantities of a shared, key intermediate in preparation of the two S1P₁ drug candidates.
705 Figure 1 in Supplementary Information presents their two possible routes to prepare the
706 enantiomerically pure alcohol (Int 2A):

707 Route 1) SFC of methyl esters (Int 1A and 1B) followed by conversion of Int 1A to the alcohol (Int
708 2A) and route 2) conversion of the methyl esters to alcohols (Int 2A and 2B) followed by SFC to
709 obtain the desired Int 2A and the two desired APIs.

710 Comparing the two routes, separation of Int 1A and 1B led to a throughput of 94.08 g/day (0.72
711 kkd) as compared with 1.7 g/h (0.31 kkd) for Int 2A and 2B. The solvent consumption in SFC of Int
712 1A and 1B was 1.43 L/g, which was 63% of 2.27 L/g in Int 2A and 2B. Thus, route 1 was chosen
713 and around 1 kg of the ester intermediate was purified by SFC on the 3 cm Chiralpak IC column in
714 less than twelve days, with a high recovery and a diastereoisomeric excess superior to 99% of the
715 desired Int 1A isomer. Hundreds of grams of the enantiomerically pure Int 1A were converted to the
716 Int 2A alcohol and then carried through subsequent steps to synthesize both BMS-986166 and
717 compound 2.

718 Pesticides

719 In 2017, Yan *et al.* reported the preparative separation of four β -cypermethrin stereoisomers [85].

720 Synthetic pyrethroids are a family of important chiral pesticides containing multiple stereoisomers,
721 and β -cypermethrin in particular is one of the most commonly used pesticides due to its persistence,
722 photostability, high toxicity for insects, and strong endocrine disruptive ability for aquatic
723 organisms.

724 A three-steps approach was developed: the four isomers of β -cypermethrin were separated in a first
725 step leading to two peaks, each containing two stereoisomeric pairs. β -Cypermethrin was injected in
726 10 stacked injection volumes of 2 mL with a 5 min cycle time. Two stereoisomeric pairs (labeled as
727 P1 and P2) were collected separately and P1 was subsequently separated. The mobile phase was
728 20% EtOH in CO₂ and the P1 sample was injected in 10 stacked 2 mL injections with 5 min cycle
729 time leading to two enantiopure products. The P2 was also separated and injected in 10 stacked 2

730 mL injections with a 2 min cycle time leading to two enantiopure products. The four isomer
731 configurations were determined according to the literature and were denoted 1R-cis- α S, 1R-trans-
732 α S, 1S-cis- α R, and 1S-trans- α R based on their elution order from the chiral column. Their
733 recoveries were about 94.5, 97.7, 96.5, and 95.6% and their enantiomeric purities were about 97.39,
734 97.53, 92.19, and 95.61%, respectively.

735 **4.2 Liquid chromatography**

736 All the experimental conditions depicted in the following articles are summarized in Table 2.

737 Natural products

738 In 2017, Gao *et al.* published their work on the separation of the isborneol enantiomers [86].
739 Isorneol is a monoterpene compound with good biological activity as an anti-inflammatory,
740 antibacterial, and analgesic.

741 In 2020, Tanno *et al.* reported the semi-preparative separation of α -tocopherol [87]. Tocopherol is a
742 lipophilic antioxidant and oxidation products of α -Toc, such as 8 α -hydroperoxy- α -tocopherone
743 (Toc-OOH) exert an antioxidative effect, but accumulation of such products can alter food quality.
744 Toc-OOH possesses chiral centers at the 2-,4- and 8-carbon along with the hydroperoxide binding
745 8 α -carbon, thus constituting two stereoisomeric forms. In order to optimize and validate the LC-
746 MS/MS quantification method of the stereoisomers in extra-virgin olive oil (marketed or oxidized),
747 standards of these Toc-OOH isomers were needed in significant quantities but were not
748 commercially available. The separation of the 8 α (S)-hydroperoxy- α -tocopherone (8 α (S)-Toc-OOH)
749 and 8 α (R)-hydroperoxy- α -tocopherone (8 α (R)-Toc-OOH) stereoisomers was performed by
750 connecting two columns in series, which is rarely reported in LC owing to the increase in back-
751 pressure it creates. This purification was repeated several times to provide sufficient quantities of
752 each standard.

753 Small pharmaceutical molecules or intermediates

754 In 2016, Procopiou *et al.* reported the preparative separation of enantiomers of N-CBZ-3-
755 fluoropyrrolidine-3-MeOH [88]. This compound is a building block for synthesis of $\alpha_v\beta_6$ integrin

756 inhibitors used in the treatment of idiopathic pulmonary fibrosis. The injection mass was 38
757 g/injection, and the run-time was 24 min. Their enantiomeric purities were 99.5 and 99.8%
758 respectively. Thus, to purify 5 kg, 132 injections were carried-out in 52.8 hours, consuming 1626 L
759 of EtOH and 6505 L of *n*-heptane for elution, underlying high consumption of alkane, requiring
760 high amounts of energy to evaporate.

761 In 2016, Kurka *et al.* published the semipreparative separation of cis-itraconazole [89]. Itraconazole
762 displays anticancer and antiangiogenic properties but is also an antifungal drug, administered as a
763 racemate, and the molecule presents three chiral centers resulting in eight stereoisomers. However,
764 for clinical purposes only the cis-racemate mixture is used, containing four stereoisomers: (+)-
765 2R,4S,2'R; (+)-2R,4S,2'S; (-)-2S,4S,2'R; (-)-2S,4R,2'S. The chiral separation of these four
766 stereoisomers is required in order to comply with current legislation and describe their individual
767 biological properties. A four-step HPLC approach was developed for the semi-preparative
768 purification of four stereoisomers (Fig. 2 in Supplementary Information). The elution order
769 observed on Lux Cellulose-1 column was: the two (+)-2R,4S stereoisomers eluted first in one peak
770 (Fraction 1), followed by the two (-)-2S,4R stereoisomers separated into two peaks. The first step
771 consisted in the collection of Fraction 1 and the two levorotary stereoisomers. Subsequently,
772 Fraction 1 was separated and the (+)-2R,4S,2'R and (+)-2R,4S,2'S isomers were collected in step
773 two. In the case of the Cellulose-3 column, the two 2'R stereoisomers co-eluted first (Fraction 1'),
774 followed by the two 2'S stereoisomers, again separated into two peaks, such that three peaks were
775 collected in step 3. Finally, Fraction 1' containing the (+)-2R,4S,2'R and (-)-2S,4R,2'R isomers was
776 separated and the two isomers collected (step 4).

777 During these steps, triple mobile phase recycling was used leading to a 50% reduction of mobile
778 phase consumption. The chiral purity of each of the four isomers was >97%.

779 It is worth noting that “*artisanal*”, small-scale chromatography is always implemented on analytical
780 columns, illustrating their high loading capacity. In 2016, Lal and Bhushan published the semi-
781 preparative separation of ketorolac (Ket) [90], a nonsteroidal anti-inflammatory drug (NSAID). The

782 therapeutic activity of ketorolac resides almost exclusively in the (S)-(-) enantiomer while the drug
783 is marketed and administered as a racemic mixture. Fractions pertaining to the first-eluting
784 enantiomer (En-I) and the second-eluting enantiomer (En-II) were collected, leading to 150 mg of
785 En-I and 158 mg of En-II. Another example was published in 2017 by Chi *et al.* [91] reporting the
786 semi-preparative resolution of (S)- and (R)-tetrahydro- α -(1-methylethyl)-2-oxo-1(2H)-pyrimidine
787 acetic acids ((S)-TPA and (R)-TPA, respectively), known as useful intermediates in the synthesis of
788 human immunodeficiency virus (HIV) protease inhibitors like lopinavir.

789 In 2016, Sadutto *et al.* [92] reported the semi-preparative separation of bicalutamide, which is a
790 selective nonsteroidal, anti-androgen drug already used for treatment of prostate cancer, but for
791 which only the R-enantiomer presents biological activity. A volume of 2 mL containing 21.6 mg of
792 bicalutamide was purified. The yield was 90% and the throughput was 960 mg per day.

793 In 2016 Silva *et al.* [93] reported the chiral separation of some cathinone derivatives, which are
794 novel psychotropic drugs. In particular, 3,4-methylenedioxypropylamphetamine (MDPV) was purified.
795 Thanks to multiple injections, 100 mg of MDPV were separated, resulting in 45.5 mg of the first-
796 eluting enantiomer and 41.3 mg of the second. In 2018 Silva *et al.* [94] published more of their
797 work on cathinone derivatives in “legal highs”, this time studying the compounds pentedrone and
798 methylone. Over 14 hours, multiple injections of sample solution, totaling 9.80 mg of pentedrone,
799 resulted in 3.53 mg of the first-eluting pentedrone enantiomer (72% recovery) and 3.48 mg of the
800 second (71% recovery). Regarding methylone, 12 mg were injected on the device, resulting in 4.80
801 mg of the first-eluting enantiomer (80% recovery) and 4.74 mg of the second (79% recovery). It is
802 noteworthy that the column was unable to separate the two racemates with the same performance
803 after 14 hours and a washing process was needed to regenerate the column.

804 In 2019, Onyameh *et al.* [95] reported the preparative separation of a ligand with a high affinity for
805 the 5HT₇ receptor: 5-chloro-2-{2-[3,4-dihydroisoquinolin-2(1H)-yl]ethyl}-2-methyl-2,3-dihydro-
806 1H-inden-1-one (SYA40247). This racemic compound needs to be separated as the 5HT₇ receptor is

807 a known target of drug development for the treatment of schizophrenia. Two repeated injections
808 were made and the enantiomeric purity of each evaporated fraction was 100%.

809 **4.3 Comparing supercritical fluid- and liquid chromatography**

810 All the experimental conditions depicted in the following articles are summarized in Table 3.

811 Small pharmaceutical molecules or intermediates

812 In 2016, Leek *et al.* [52] reported the large-scale isolation of enantiomers of compound A, an
813 undisclosed, key intermediate used in AstraZeneca R&D. The preparative SFC separation was
814 implemented through stacked injections of 500mg with a 2 min cycle time. The complete resolution
815 of the 5kg batch was estimated to require 53 hours with a MeOH use of 0.08 L/g and a productivity
816 equal to 6.4 kkd. However, after ten injections a slow increase in the pressure drop was observed
817 and eventually led to the complete obstruction of the tubing from sample loop to the column.
818 Different strategies were tested to circumvent this precipitation of the poorly soluble compound in
819 the carbon-based mobile phase. An apolar solvent (DCM) was added to the modifier to improve
820 solubility of the sample, but no improvement was observed and in addition a decrease in selectivity
821 was observed. A lower concentration of feed solution was tested (*i.e* 100mg/mL instead of 250
822 mg/mL), but this approach was also unsuccessful.

823 Building on the work of Cox and Shaimi, who described injection by extraction to solve this
824 problem of low solubility in the carbon-based mobile phase [96], the authors used an extraction
825 vessel (100 × 10 mm) filled with 4.4 g compound A:silica 1:5 mixture ratio. The principle of this
826 approach is that only the soluble amount in the mobile phase is extracted and injected, thus avoiding
827 precipitation. The same CSP and mobile phase were used, but the flow-rate was increased to 150
828 g/min. With a dynamic mode of injection (40 seconds, 52 mg racemate): a productivity of 0.2 kkd
829 was achievable and 190 days were calculated as being needed to purify the 5 kg batch. Even though
830 precipitation was avoided by the extraction injection approach, it was not possible to further
831 improve the scale-up of the extraction column and an HPLC method was thus evaluated. At a flow-
832 rate of 600mL/min, the authors calculated that with an injected amount of 4.8 g and a cycle time of

833 10 min, the time needed to resolve the 5kg batch would be 174 hours, but productivity of only 0.4
834 kkd was achievable. In this particular case the main issue was not the time required but the solvent
835 consumption: more than 6000 L of mobile phase would have been required, mainly composed of
836 heptane. In this article neither of the two methods were adopted by AstraZeneca and the resolution
837 was eventually performed by crystallization of a diastereomeric salt.

838 In 2016, Rossi *et al.* reported the semi-preparative purification of 20 mg of a novel potent σ_1
839 receptor agonist by SFC and HPLC [97]. The aim was to elucidate its role as a therapeutic target in
840 several neurodegenerative diseases or mood disorders. In small-scale, preparative SFC, 20mg of the
841 racemate were purified in 40 cycles of 10 min each, leading to 9.1 mg of the (S) enantiomer and 8.2
842 mg of the (R) enantiomer at an overall yield of 86.5%, and an enantiomeric excess >99.5%. In
843 small-scale, preparative HPLC 21mg of racemate were separated in 7 cycles of 16 min each,
844 leading to 8.7 mg of the first-eluting enantiomer and 9.1 mg of the second, with an overall yield of
845 88.8 % and an enantiomeric excess >99.9%.

846 In this study, semi-preparative HPLC was found to outperform semi-preparative SFC, as
847 productivities were equal to 0.0270 kkd and 0.0072 kkd respectively, with an average recovery of
848 around 45%. Another method was developed in SFC with other conditions of mobile and stationary
849 phases but the productivity obtained was even lower at 0.0065 kkd.

850 But it should be noted that the comparison in this case is not optimal for two reasons: the CSPs
851 were not chemically identical and the SFC method had not been fully optimized. But in any case,
852 neither of the two SFC methods exhibited productivity greater than the 0.0270 kkd obtained using
853 semi-preparative HPLC.

854 In 2017, Leek and Andersson published three case studies of new candidate drugs developed at
855 AstraZeneca [34]. Case study No.1 dealt with the requirement for 100 g of pure enantiomer from
856 the optimized active pharmaceutical ingredient (API) destined to treat acute myeloid leukemia. The
857 racemic intermediate, compound A, was a weak base with low solubility in alcohol. The
858 preliminary experiments carried-out by SFC showed insufficient loadability due to the low

859 solubility of compound A in the carbon dioxide-based mobile phase and normal-phase LC was thus
860 investigated. The quantity injected was 9.45g with a cycle time of 13 min and a fraction volume of
861 1.8L/injection. The productivity was quite low (0.9 kkd) and the solvent consumption was 830 L/kg
862 racemate. In this example, normal phase LC outperformed SFC which failed to separate the desired
863 compound.

864 Case study No.2 concerned the dextrorotary enantiomer of the racemic API (compound B) for the
865 development of a first-line anti-inflammatory therapy for asthma patients. Preparative SFC was
866 chosen because the desired enantiomer eluted first. The quantity injected was 3.4g using an
867 injection cycle time of 155 s, with a fraction volume of 70mL/injection. The productivity was quite
868 high (5.7 kkd) and the solvent consumption was 70 L/kg racemate. A total of 1.1 kg of the API with
869 an overall yield of 96% and an enantiomeric excess >99.9% was achieved.

870 Case study No.3 aimed to address the shortcomings of case No.2, since it can be seen that, even if
871 successful, the SFC method developed above is uneconomical and wasteful with only half the API
872 being used after the separation step. Thus, a combination of chiral SFC and racemization was
873 investigated in order to evaluate the toxicology of compound C. The quantity injected was 0.5g for
874 each 190 s injection cycle and the productivity remained quite high (4.4 kkd) and solvent
875 consumption lower than with normal-phase LC. The second-eluting and unwanted enantiomer was
876 evaporated to dryness after chiral separation and then dissolved at 30mg/mL in MeOH/TEA 3/1
877 molar equivalent before being left overnight under rotation. More than 95% was thereby racemized,
878 and after 3 cycles of chromatography and racemization, a total yield of 87% was obtained.

879 In 2018, Yan *et al.* reported the preparative separation of enantiomers of lenalidomide [98]. Over
880 the past 10 years, Lenalidomide has been used for the treatment of multiple myeloma, other
881 hematological malignancies and myelodysplastic syndromes, with significantly improved overall
882 survival in myeloma. In this work, two different SFC methods were developed on two different
883 CSPs and compared.

884 Method 1: many conditions were not entirely favorable: the maximum injection mass was about 8
885 mg and the elution time was about 20 min, while the cycle time for the stacked injections was
886 estimated to be at least 14 min. Thus, a second approach was tested on another chiral stationary
887 phase type.

888 Method 2: the injection volume was set at 3 mL leading to a productivity of 0.16 kkd achieved
889 through 10 stacked injections of 30mg per 5 min cycle. The solvent use was 2.6 L/g. The recoveries
890 for each enantiomer were about 81.7 and 79.5%, and their enantiomeric purities were 97.4, and
891 97.5%, respectively. This work was compared to previous publications which used HPLC [99; 100]
892 and many features were better in SFC. In the first HPLC study, Tojo and co-workers who reported a
893 productivity of 0.015 kkd [99]. In the SFC study, the productivity was about ten times higher (0.16
894 g kkd), while consumption of MeOH was only 2.6 L/g, much less than the second HPLC study
895 which reported a solvent use of 17.4 L/g of EA [100], indicating that SFC separation was both more
896 productive and economical than HPLC.

897 In 2019, Cheng *et al.* described the preparative separations of 6-(4-aminophenyl)-5-methyl-4, 5-
898 dihydro-3(2H)-pyridazinone (SKF 93505) [101]. SKF 93505 is a key synthetic intermediate for
899 cardiotonic agent levosimendan which is the levorotatory enantiomer of simendan, a positive
900 inotropic drug and calcium sensitizer for the treatment of short-term, decompensated heart failure.
901 The analysis time of a single injection in SFC and HPLC was about 7.5 and 9 min, respectively.
902 Stacked injection mode was used to increase the productivity with an injection cycle of 5 min,
903 which increased productivity to 33 and 44% in SFC and HPLC, respectively. To separate 1 g of
904 racemate SKF 93505, the SFC technique took about 2.8 h with 4.6 L/g MeOH consumption, and the
905 HPLC technique took about 2.8 h with 3.4 L/g solvent use. The productivity of both modes was
906 equal to 0.171 kkd using a concentration of feed solution equal to 15 mg/mL. The enantiomeric
907 excess values of each enantiomer were 100% for both isomers in SFC, and were 100% (R isomer)
908 and 98.82% (S isomer) in HPLC. In this particular case, then, both techniques enabled fast, low-cost

909 separations of (R) (-) SKF 93505 with high yields (98 % for SFC and 95% for HPLC) and very
910 high enantiopurity.

911 Also in 2019, Wang *et al.* reported a comparison of the preparative separation of Corey lactone diol
912 [102]. Purification of Corey lactone diol (CLD) is of utmost importance in the pharmaceutical
913 industry as the levorotary enantiomer has become the key intermediate in the syntheses of
914 prostaglandins (PGs). PGs derivatives are an important class of drugs, involved in the treatment of
915 idiopathic pulmonary hypertension and gastrointestinal tract illnesses. The dextrorotary enantiomer,
916 on the other hand, is a key intermediate in the synthesis of entecavir, a medicine for anti-viral
917 therapy in chronic hepatitis B patients. In both SFC and HPLC, the separation was run on an
918 ADMPC30 column (250 × 20 mm, 5 μm). The preparation of the stationary phase was conducted in
919 house and ADMPC (amylose tris-(3,5-dimethylphenylcarbamate) was coated on 5 μm silica gel.
920 The notation used by the authors of ADMPC-10, ADMPC-20, ADMPC-30, and ADMPC-40
921 corresponds to the ratio of the derivative to the silica gel support (*w/w*) equal to 1:10; 2:10; 3:10,
922 and 4:10, respectively. In SFC the injection was of 130mg and the HPLC the injection quantity was
923 160mg. The analysis time of a single injection in both SFC and HPLC was 3 min. Stacked injection
924 mode was used, with the injection cycle set to 3 min in both SFC and HPLC. To separate 1 g of
925 racemate CLD, the SFC technique took about 23 min with 0.46 L/g MeOH consumption, and the
926 HPLC technique took about 20 min with 0.39 L/g solvent use of AcN. The productivity in SFC was
927 1.248 kkd and 1.536 kkd in HPLC. Concerning the enantiomeric purities, values of each enantiomer
928 in SFC were 99.02% and 94.26% and in HPLC were 98.33% and 99.41%: in this particular case,
929 then, HPLC enabled a higher productivity and a slightly lower solvent consumption.

930 **Conclusion**

931 The new developments and applications in preparative chiral SFC and chiral HPLC have been
932 reviewed in the previous sections and the *pros* and *cons* of each method have been summarized in
933 Figure 1. We have described how the advances in preparative SFC and HPLC are being led by the
934 appearance on the market of new CSPs, able to be used with more nonpolar solvents like DCM

935 while at the same time exhibiting improved robustness and loadability. Innovative injection
936 techniques and intelligent recycling and fraction-collecting approaches have also been important
937 over the last five years, with several offering the potential to avoid the inherently large “carbon
938 footprint” of preparative-scale enantioseparations, both in terms of solvent usage but also in the way
939 that only half the racemate is frequently required (at best) and collected. We have shown that the
940 proportion of preparative enantiomer separations carried out by SFC is growing. The benefits of
941 SFC over HPLC are widely described, Francotte [81] summarizing well the advantages of this
942 technique when writing that in SFC, “the consumption of solvent is reduced by 60-70% and the
943 running cost by 70-80%”. However, in spite of the advantages of SFC, chiral LC remains useful for
944 many applications.

945 One of the motivations for this review concerned the rationale for selecting either SFC or HPLC for
946 a preparative-scale, enantioselective separation. Leek and colleagues from AstraZeneca R&D
947 describe a typical industrial setting, where large-scale chiral separations are performed by SFC for
948 95% of their racemates [52]; a similar observation was made by Miller and co-workers from Amgen
949 [103], who reported a success rate greater than 98% in SFC for their discovery program, in which
950 only SFC is screened and HPLC evaluated only when SFC is unsuccessful. This is also the case at
951 Reachseparations: since January 2020, their split between SFC and HPLC for purification projects
952 is approximatively 80% SFC and 20% HPLC. For Janssen, the ratio of SFC/HPLC depends on the
953 department: in discovery more than 99% of the chiral separations are performed by SFC, while in
954 development about 70% of the chiral separations are handled by HPLC. For other pharmaceutical
955 laboratories, like Eli Lilly, SFC is clearly the future, with the utilization of SFC for almost all
956 batches less than 50g, and huge investment in facilities to handle 1kg batches by SFC. For other
957 users, sample quantity is also a criterion of selection.

958 The selection of the technique is necessarily multifactorial and SFC screening and HPLC screening
959 are sometimes performed in parallel, with the physical properties of the target compound sometimes
960 also leading to an *a priori* choice of either SFC or HPLC. Thus, for hydrophilic compounds, chiral

961 RP-HPLC would be the first option. Some chiral SFC separations reviewed above required up to
962 50% modifier and the advantage of SFC over HPLC regarding solvent consumption is thus largely
963 negated. Solubility is also a criterion – a racemate is often reported as being less soluble in a CO₂-
964 enriched mobile phase, especially at high concentrations, and several users report that it is easier to
965 handle a compound with poor solubility in HPLC than in SFC. Furthermore, it is obvious that the
966 solubility cannot be easily assessed in the mobile phase of SFC with a large proportion of
967 Supercritical fluid CO₂. However, thanks to the development of immobilized polysaccharide CSPs,
968 the use of solvents like DCM, THF or even DMSO is now possible both in SFC and in HPLC,
969 resulting in a wider range of options for increasing the solubility of the racemates. The stability of
970 the compound is also a criterion for selecting either SFC or HPLC, notably the reactivity of CO₂ or
971 acidity of the mobile phase in SFC. However, unlike several industry experts who favor SFC for
972 preparative separation of small quantities, others favor HPLC for small quantities because of the
973 relatively low recovery rate in SFC, while the equipment available in the laboratory also guides the
974 selection of technique, as does the relative expense of each and the familiarity of laboratory
975 personnel with one or the other.

976 To sum up, if the sample stability and solubility is suitable for SFC, most small quantities are
977 handled by SFC. For larger quantities both SFC and HPLC are often screened, with a majority of
978 compounds still separated by SFC if the laboratory has the equipment available to handle large
979 batches with this technique. New CSPs, compatible with a wider range of solvents, are broadening
980 the range of compounds suitable for both techniques while innovative injection, recycling and
981 collection techniques have made significant advances in enantiomeric separations using both SFC
982 and HPLC. With SFC bound to become more accessible, and perhaps more scalable over the
983 coming years, it will be fascinating to see how these two keystone techniques of preparative-scale,
984 chiral separation compare in another 5 years' time.

985 **References**

986

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Pros & Cons of preparative-scale SFC

High **efficiency** due to larger diffusion coefficients of solutes and/or the possibility of longer columns

Faster **flow-rate** and **column equilibration** due to lower viscosity leading to shorter analysis and cycle times, without loss of resolution

Higher column **loadability**, hence productivity, than HPLC because the equilibrium isotherms of compounds between supercritical fluids and the preparative CSPs are flatter

Solubility in supercritical fluid decreases rapidly as the pressure decreases near the critical point, facilitating recovery of the isomer fractions and **increasing yield**

Mobile phase is mainly constituted of carbon dioxide which is **non-flammable** & **less expensive** and **toxic** than common organic solvents, the use of which is also reduced

Instrumentation allows stacked or overlapped injections improving **productivity** and reducing solvent consumption

Pros & Cons of preparative-scale HPLC

When required, **reversed-phase chromatography** is easier to implement in HPLC because of the lack of polar supercritical fluid

From a theoretical point of view, **knowledge** of the physical chemistry of liquid/solid adsorption is widespread and established, whereas it remains lacking for supercritical fluid/solid adsorption

In HPLC, while time-consuming, the **scale-up is straightforward**, based on rules of thumb such as scaling volumetric flow and injection volume to the square of the ratio of the column radius

Although non-toxic, **massive leaks of carbon dioxide** remain a constant risk for the operator together with the storage of CO₂ under pressure

Instrumentation is **simpler** and **cheaper** in HPLC than in SFC, making the technique more accessible

HPLC benefits from a developmental and experiential legacy generating a **huge technical expertise** and **mass of scientific data** available in the literature

Figure 1

Table 1 : Chromatographic conditions for preparative SFC applications

Analyte	Preparative device	Stationary phase	Mobile phase	P _{out}	Flow-rate	Concentration of feed solution	Injected Volume	Reference
• Natural products								
Galbelgin, (-)-Ganschisandrin, Galgravin and (-)-Veraguensin	SFC prep 80 system and six, high-pressure fraction-collection cyclones (Waters Corporation, Milford, MA, USA)	CSP-1 (250×20mm, 5µm)	CO ₂ :MeOH/DCM (1/1) 85:15	150bar	60g/min	50mg/mL 1/1 MeOH/DCM	1.8mL	[35]
		CSP-2 (250×20mm, 5µm)	CO ₂ :MeOH/DCM (1/1) 80:20			15mg/mL 1/1 MeOH/DCM	0.8mL	
		CSP-2 (250×20mm, 5µm)	CO ₂ :MeOH/DCM (1/1) 80:20			30mg/mL 1/1 MeOH/DCM	1.5mL	
Dihydromyricetin	SFC prep 80 system and six, high-pressure fraction-collection cyclones (Waters Corporation, Milford, MA, USA)	EnantioPak AD (250×20mm, 10µm)	CO ₂ :MeOH 60:40	100bar	40g/min	40mg/mL MeOH	-	[79]
• Small pharmaceutical molecules or intermediates								
1-benzyl-3-methyl-2-piperidone	N.R.	Chiralpak AD-H (250×30 mm, 5µm)	CO ₂ :EtOH 95:5	N.R.	120mL/min	N.R.	N.R.	[80]
Iodoaryl regioisomers		Chiralpak IC (250×30 mm, 5µm)	CO ₂ :iPrOH/DEA 75:25/0.25%					
3 Synthetic intermediates Compound A	Berger MGII and Thar SFC Prep 350 device (Waters, Boston, MA, USA)	Lux TM Cellulose-4 (250×30mm; 5µm).	CO ₂ : <i>n</i> -Hept/iPrOH/TFA 88:12 (80/20)/0.10%	100bar	N.R.	29.2mg/mL 8/2 <i>n</i> -Hept/iPrOH	N.R.	[25]
Compound B		Chiralpak IC (250×30 mm, 5µm)	CO ₂ :MeOH 90:10			220mg/mL 1/1 AcN/DCM		
Compound C		Lux TM Amylose-2 (250×30mm; 5µm).	CO ₂ : MeOH/AcN/DCM 50:50 (20/20/10)			50mg/mL 1/2 MeOH/DCM		
2 Propanoic acids Compound 4	Berger MGII and Thar SFC Prep 350 device	Chiralcel OJ-H (250×30mm; 5µm)	CO ₂ :MeOH 70:30	100bar	120mL/min	100mg/mL MeOH	N.R.	[81]
Compound 14		Chiralcel OJ-H (250×30mm; 5µm)	CO ₂ :MeOH 80:20		100mL/min	75mg/mL MeOH		

Atropisomeric compounds	Waters MGII preparative device (Waters, Boston, MA, USA)	Chiralpak AD-H (250×30mm; 5µm)	CO ₂ :MeOH 70:30	100bar	85mL/min	N.R.	N.R.	[82]
4 Isoxazole derivatives	SFC-PICLAB hybrid 10-20 device (PIC Solution, Avignon, France)	Chiralpak AD-H (250×4.6mm; 5µm)	CO ₂ :EtOH 85:15	150bar	4 mL/min	18mg/mL EtOH	250µL	[83]
			CO ₂ :EtOH 90:10			15.8mg/mL EtOH	250µL	
			CO ₂ :EtOH 93:7			13.2mg/mL EtOH	485µL	
			CO ₂ :EtOH 90:10			13.5mg/mL EtOH	485µL	
S1P ₁ final API	Thar SFC Prep 350 device (Waters, Boston, MA, USA)	Chiralpak IC (250×30mm; 5µm)	CO ₂ :MeOH 48:52	100bar	180mL/min	145mg/mL 1/1 MeOH/DCM	N.R.	[34]
		Chiralpak AD-H (250×4.6mm; 5µm)	CO ₂ :MeOH 50:50		130mL/min	80mg/mL MeOH	1mL	
• Pesticides								
4 β-cypermethrin stereoisomers	SFC prep 80 system and a six high-pressure fraction collection cyclones (Waters Corporation, Milford, MA, USA)	EnantioPak OD (250×20mm; 5µm)	CO ₂ :iPrOH 95:5	100bar	40g/min	10mg/mL iPrOH	2mL	[84]
		EnantioPak AD (250×20mm; 5µm)	CO ₂ :EtOH 80:20					
		EnantioPak AD (250×20mm; 5µm)	CO ₂ :EtOH 85:15					

N.R: Not Reported

Table 2 : Chromatographic conditions for preparative HPLC applications

Analyte	Preparative device	Stationary phase	Mobile phase	Flow-rate	Concentration of feed solution	Injected Volume	Reference
• Natural products							
Isoborneol	Agilent system 1200 series (Palo Alto, CA, USA)	EnantioPak OD (250×10mm; 5µm)	<i>n</i> -Hex:EtOH 99:1	60g/min	50mg/mL <i>n</i> -Hex	N.R.	[85]
α-Tocopherol	2010 A HT series HPLC instrument from Shimadzu Technologies (Kyoto, Japan)	Connected Silica SG120 and Inertsil SIL-100A (250×10 mm; 5µm)	<i>n</i> -Hex:iPrOH 98:2	10mL/min	N.R.	N.R.	[86]
• Small pharmaceutical molecules or intermediates							
N-CBZ-3-fluoropyrrolidine-3-MeOH	N.R.	Chiralpak AD (250×20mm; 10µm)	<i>n</i> -Hept:EtOH 80:20	2567 mL/min	151.5g/mL 6/4 <i>n</i> -Hept/EtOH	250mL	[87]
cis-itraconazole	Smartline HPLC system (Knauer, Berlin, Germany)	Lux™ Cellulose-1 (250×10mm; 5µm)	AcN:MeOH 1:9	5mL/min	830mg/mL 1:9 AcN:MeOH	1mL	[88]
		Lux™ Cellulose-3 (250×10mm; 5µm)	AcN:MeOH 1:14	5mL/min	1000mg/mL 1:14 AcN:MeOH	1mL	
		Lux™ Cellulose-1 (250×10mm; 5µm)	AcN:MeOH 1:10	5mL/min	1500mg/mL 1:10 AcN:MeOH	1mL	
		Lux™ Cellulose-3 (250×10mm; 5µm)	AcN:MeOH 1:5	5mL/min	1500mg/mL 1:5 AcN:MeOH	1mL	
Ketorolac	2010 A HT series HPLC instrument from Shimadzu Technologies (Kyoto, Japan)	Lux™ Amylose-2 (250×4.6 mm; 5µm)	<i>n</i> -Hex:iPrOH/FA 85:15/0.1%	1 mL/min	N.R.	20µL	[89]

Tetrahydro- α -(1-methylethyl)-2-oxo-1(2H)-pyrimidine acetic acids	Hitachi L-2000 device (Tokyo, Japan)	Chiralcel OD-H (250×4.6mm; 5 μ m)	<i>n</i> -Hex:EtOH/AA 920:80/0.2%	N.R.	N.R.	N.R.	[90]
Bicalutamide	200LC Perkin-Elmer (Norwalk, CT, USA)	Chiralpak IA (250×10 mm, 5 μ m)	<i>n</i> -Hex:EA:EtOH 100:30:5	5.5 mL/min	32 g/mL EA	N.R.	[91]
Cathinone derivatives	JASCO 880-PU pump (JASCO, Tokyo, Japan), with a Rheodyne 7125 injector (Rheodyne, Rohnert Park, CA, USA) and a JASCO 880-30 solvent mixer	amylose tris-3,5-dimethylphenylcarbamate (200×70 mm, 7 μ m)	<i>n</i> -Hex:EtOH/TEA 97:3/0.1%	1.5 mL/min	10 mg/mL EtOH	100 μ L	[92]
Cathinone derivatives: pentedrone	Dionex UltiMate instrument (Dionex GmbH, Germany) with a 3000 quaternary pump and a 3000 Automated Fraction Collector LC system	Chiralpak AS (250×10mm; 10 μ m)	<i>n</i> -Hex:iPrOH 97:3	2 mL/min	1 mg/mL 97:30/0.4% <i>n</i> -Hex:iPrOH/DEA	N.R.	[93]
methylone			<i>n</i> -Hex:iPrOH 85:15		1 mg/mL 85:15/0.4% <i>n</i> -Hex:iPrOH/DEA		
5-chloro-2-{2-[3,4-dihydroisoquinolin-2(1H)-yl]ethyl}-2-methyl-2,3-dihydro-1H-inden-1-one	Gilson 306 HPLC system (Gilson, Middleton, WI, USA)	Chiralpak AD-H (250×10mm; 5 μ m)	EtOH: <i>n</i> -Hex/DEA 5:95/0.1%	N.R.	3 mg/mL 5:95/0.1% EtOH: <i>n</i> -Hex/DEA	1 mL	[94]

N.R: Not Reported

Table 3 : Chromatographic conditions for preparative SFC *versus* preparative LC applications

Analyte	Preparative device	Stationary phase	Mobile phase	P _{out}	Flow-rate	Concentration of feed solution	Injected Volume	Reference
<ul style="list-style-type: none"> Small pharmaceutical molecules or intermediates 								
Undisclosed compound A	Thar 70 (Waters, Milford, MA, USA) SuperSep 600 unit (Novasep, Pompey, France)	Chiralpak IC (250×30mm, 5μm)	CO ₂ :MeOH/DEA 85:25/0.1%	80bar	70g/min	250mg/mL MeOH	2mL	[51]
	Hipersep LAB LC110 (Novasep, Pompey, France)	Chiralpak AD (250×110mm, 20μm)	<i>n</i> -Hept:EtOH:MeOH/TEA 80:10:10/0.10%		600mL/min	N.R.	N.R.	
σ ₁ receptor agonist	Analytical/semi-prep device (Waters, Spa, Milan, Italy)	Chiralpak IA (250×10 mm, 5μm)	CO ₂ :(<i>n</i> -Hept:EtOH/DEA) 70:30 (90:10/0.10%)	N.R.	10mL/min	10mg/mL iPrOH	50μL	[96]
	Jasco PU-2089 Plus pump, AS-2055 Plus autosampler and MD-2010 Plus detector (Jasco Europe, Cremella, Italy)	Chiralcel OJ-H (250×10mm; 5μm)	MeOH/DEA 99.9/0.10%		3mL/min	3mg/mL MeOH	1mL	
Case study n°1	Insufficient solubility of compound A in CO ₂ -based mobile phase precluded use of SFC							[33]
API compound A	Hipersep LAB LC110 (Novasep, Pompey, France)	Chiralpak AD (250×110mm, 20μm)	<i>n</i> -Hept:EA/DEA 50:50/0.50%		600mL/min	270mg/mL DCM	N.R.	

Case study n°2 API compound B	SuperSep 600 unit (Novasep, Pompey, France)	Chiralpak IC (250×50mm, 5µm)	CO ₂ :EtOH/FA 80:20/0.50%	N.R.	450g/min	200mg/mL EtOH	N.R.	[33]
Case study n°3 API compound C	SuperSep 600 unit (Novasep, Pompey, France)	Chiralart SA (250×20mm; 5µm)	CO ₂ :iPrOH 60:40	N.R.	80g/min	100mg/mL AcN	N.R.	
Lenalidomide	SFC prep 80 system (Waters, Milford, MA, USA)	Chiralpak AD-H (250×20mm, 5µm)	CO ₂ :MeOH 50:50	100bar	30g/min	2mg/mL MeOH	1, 2, 3 or 4mL	[97]
		(4-chloro- phenylcarbamoylated β-cyclodextrin) (250×20mm, 5µm)	CO ₂ :MeOH 60:40	100bar	50g/min	10mg/mL (25/75) DMSO/MeOH	3mL	
	Bischoff Corp HPD Pump & Lambda 1010	Chiralpak IC (250×20 mm; 5µm)	EA 100		10 mL/min	2.3mg/mL AcN	5mL	[98]
	Agilent 1260 Infinity HPLC system (Agilent Technologies, Waldbronn, Germany).	Chiralpak AD-H (250×4.6mm, 5µm)	MeOH 100		0.8 mL/min	5mg/mL MeOH	40µL	[99]
6-(4- aminophenyl)-5- methyl-4, 5- dihydro-3(2H)- pyridazinone	SFC prep 80 system (Waters, Milford, MA, USA)	Chiralpak AS (250×20 mm, 10µm)	CO ₂ :MeOH 55:45	170bar	60g/min	15mg/mL MeOH	2mL	[100]
	Waters Autopurification LC System (Waters, Milford, MA, USA)	Chiralcel OJ (250×20 mm, 10µm)	MeOH 100		20 mL/min	15mg/mL MeOH	2mL	
Corey lactone diol	SFC prep 80 system (Waters, Milford, MA, USA)	Amylose tris-(3,5- dimethylphenyl carbamate (250×20 mm, 5µm)	CO ₂ :MeOH 80:20	200bar	60g/min	10mg/mL MeOH	N.R.	[101]
	Waters Autopurification LC System (Waters, Milford, MA, USA)		AcN:H ₂ O 93:7		20 mL/min	10mg/mL (93/7) AcN/H ₂ O	N.R.	

N.R: Not Reported

Table 4 : Synopsis of the references by theme.

Field	Preparative SFC references	Preparative HPLC references	Other references
Mobile phase	[32-35] ; [40] ; [52] ; [56 ; 57]		[31] ; [39] ; [53-55]
CSP	[25]		[26-30] ; [36 ; 37] ; [42-44] ; [63-66]
Chiral capillary electrophoresis			[4-8]
Instrumentation	[18-20] ; [58]	[67]	
Reviews	[14 ; 15] ; [17]	[9-11]	[1 ; 2 ; 3] ; [12] ; [22] ; [24]
Processes	[45]	[23] ; [59-61] ; [76 ; 77]	[44]
Theory	[46] ; [49 ; 50]	[62]	[13]
Environmental parameters	[32] ; [78]		
Large-batch	[47 ; 48]	[68-75]	
Applications	[46] ; [78-84] ; [102]	[85-94]	[16] ; [18] ; [21] ; [38] ; [51] ; [95-101]