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A review of fraction collection technology for supercritical fluid chromatography

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ABSTRACT

Separation and purification of complex mixtures are a necessity and a challenge in many industrial fields. Preparative chromatography is a technique used to separate, purify, and isolate the different components found in these complex mixtures. Supercritical fluid chromatography (SFC) is an attractive technique in the industry due to its analysis speed, low analysis cost, green nature, and application space. As such, SFC has been appropriately scaled to match the industry's needs. An examination of preparative SFC is discussed, with this review focusing on the development and application of fraction collection. The various devices used to assist in the separation of the carbon dioxide mobile phase from the analyte and the modifier are described, as well as the techniques to control the fractionation. Applications of fraction collection are also discussed, with two-dimensional chromatography and supercritical fluid extraction coupled with SFC highlighted.

1. Introduction

Supercritical fluid chromatography (SFC) is a separation technique which has recently increased in popularity due to its similarity to high performance liquid chromatography (HPLC), using nearly identical instrumentation and software [1,2]. The main component of the mobile phase is a high density carbon dioxide (CO_2). It creates a mobile phase with an increased diffusivity, a viscosity an order of magnitude lower relative to traditional liquids, and better solubility relative to purely gaseous phases [3,4]. Thus, using high-density CO_2 allows SFC to be faster than HPLC with less pressure drop across the column, allowing the use of longer columns and smaller particle sizes [5]. The application space of SFC ranges across most of the combined application space of HPLC and gas chromatography (GC) allowing the separation of a wide range of polar and hydrophobic analytes [2,6,7]. While SFC was first reported in 1962, modern packed column SFC, more amenable to polar solutes, did not exist until 1982 when Hewlett Packard presented a series of papers using modern 3 μm totally porous particles, with modifier gradients, UV detection, and fixed outlet pressures [8–10].

Recently, research into SFC has increased, as more instrumentation has become commercialized and improved, along with an increase in the availability of a wide range of packed column stationary phase chemistries [3]. It is worthy to conjecture that the range of available stationary phase chemistries has increased over the years somewhat due to the development of alternate HPLC separation modes, especially hydrophilic interaction liquid chromatography (HILIC), which features the use of a range of polar stationary phases [11]. Interest in SFC has also increased due to its inherent advantages, including its more environmentally friendly nature, its versatility as a separation technique, and its lower operational costs [3].

Around 1980, regulators began to require that the pharmacological effects of the enantiomers of any new small drug-like molecule needed to be individually characterized. Eventually, this led to the practice of producing pure enantiomer drugs for various treatments. The first chiral separations *via* SFC were reported in 1985 [12]. Since then, it was gradually understood that SFC could be significantly faster than the notoriously slow chiral HPLC.

SFC helped alleviate many of the issues that prep HPLC faced. SFC is usually practiced as a normal phase technique, with the advantage that the toxic, highly flammable, expensive hexane/

Abbreviations: SFC, supercritical fluid chromatography; HPLC, high performance liquid chromatography; GLS, gas liquid separator; GC, gas chromatography; HILIC, hydrophilic interaction liquid chromatography; prep, preparative; SFE, supercritical fluid extraction; SMB, simulated moving bed.

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heptane is replaced by non-toxic, non-flammable, inexpensive CO_2 [5–7]. In addition, the SFC mobile phase diffusivity and viscosity allowed longer columns with smaller particles, shorter analysis times, and faster cycle times [13]. SFC practitioners began using 5 μm particles, while HPLC was still using 10 μm or larger particles. Effectively, an SFC column has at least the same throughput as an HPLC column with twice the inner diameter. This has been especially helpful as 9 out of 10 drug candidates fail upon testing. SFC enabled drug developers to quickly eliminate failed compounds. Overall, SFC has gained a foothold in drug development for its enantiomeric separations, automated purification of drug libraries, and detection of trace impurities [14–18].

When preparative SFC is used, appropriate fraction collection instrumentation is needed. An advantage and disadvantage when collecting fractions from SFC, is that the pressurized CO_2 mobile phase converts to a gas when it is no longer under pressure [13]. This allows the fractions to have less liquid, causing a faster dry-down once finished [5]. The solubility of the analytes also decreases as the CO_2 loses pressure, allowing a more rapid and complete collection of the fractions produced during the separation [13]. These advantages allow preparative SFC to be complementary to preparative LC [19]. A disadvantage and challenge is that the rapid expansion of CO_2 after depressurization can make it difficult to collect fractions without loss of material. At depressurization, the CO_2 expands, at the speed of sound, up to 500 times its original volume. The fraction collectors' purpose is to slow down that expansion, making it less violent and more controllable. The design of appropriate fraction collection interfaces has been key to the usefulness of preparative SFC.

While many recent reviews discuss preparative SFC, very few published articles have focused on the nature of fraction collection itself [13,20–26]. This review focuses on the recent history and implementation of fraction collection for SFC. Discussed first is preparative SFC with a focus on theory and the techniques used. Next, the evolution of the fraction collector is discussed, from the beginning of an open outlet to the modern gas-liquid separator. Then, select applications of SFC using fraction collection are discussed, including two-dimensional chromatography and supercritical fluid extraction (SFE) coupled to SFC. This review is centered foremost on fraction collector technology; we do not provide a comprehensive review of all applications of this technology and apologize if coverage of some relevant material is omitted.

2. Scale and techniques for preparative supercritical fluid chromatography

The scale of chromatographic separations is often categorized as one of three types: analytical, semi-preparative, or preparative scale. Table 1 provides exemplary operating conditions and hardware common to each scale when referring to SFC. From this, we can see that the use of preparative SFC is a misnomer, as operations on the scale of true preparative SFC do not yet exist. The most useful scale for larger-scale purification by SFC is the semi-preparative scale, with a column diameter of 20 mm and a flow rate around 100 mL/min. In this work, the moniker prep-SFC will be used to refer to work that was mostly performed at the semi-preparative scale.

Since the inception of SFC, the potential of its use for preparative work was seen [14]. Indeed, prep-SFC has been the main application of SFC over the years [13]. Of course, the technology and techniques associated with prep-SFC have evolved with its use.

Different injection modes for efficient loading of sample on the column have been developed and tested over the years. Mixed stream injection is when the sample is dissolved in the mobile phase. For SFC, since the sample cannot be dissolved in the high pressure CO_2, the analytes are usually dissolved in the mobile phase modifier. Since this causes a change in the composition of the mobile phase when the sample plug is introduced to the column, peak distortion and broadening occurred [22]. Modifier stream injection was created to try to counteract the peak distortion caused by mixed stream injection [27]. First introduced in prep-SFC, this technique involves injecting the sample directly into the modifier stream, before the modifier mixes with the CO_2. Using this technique, there is no change in modifier concentration after injection. When comparing modifier stream injection to mixed stream injection, modifier stream injection was shown to provide better peak shape for most compounds. Peaks were broadened, but only by the time it took for the pump to flush the sample loop. The sharper peaks often exhibited significant improvements in throughput, provided that the pumping system used a reasonable compressibility compensation.

Chiral separations can involve separations with as few as two peaks to separate. It is often true that there are long delays after injection, with a flat baseline, before peaks emerge and resolve. Stacked injections were created, first using prep-SFC, to allow multiple injections on-column at the same time [28]. This technique involves injecting the sample before the previous run has finished eluting. If timed correctly, more samples can be injected in the same time, without any consequences on the separation. This allowed the "empty spaces" to be filled with peaks, causing a near continuous elution of enantiomers, as seen in Fig. 1 [29]. Up to several grams of a pure enantiomer could be produced per hour, greatly increasing throughput.

Fraction collection itself is often separated into two main categories [30]. Preparative batch SFC is the traditional method, where large volumes of analytes are loaded on to the system followed by a series of identical injections, and collected as a few fractions. Stacked injection may be used in batch methods to increase the throughput of the analysis. Simulated moving bed (SMB) is the other type of prep-SFC process [30–32]. With this technique, the sample is continuously injected and the fractions are continuously collected. While SMB has been performed, it has failed to be incorporated as a mainstream technique due to the expensive columns needed [33]. Because SFC requires a backpressure of 100 bar or higher, the columns need to be more robust than in prep-HPLC, as the column head pressure is often close to 350 bar. These columns usually also have 50 cm inner diameters, causing an immense amount of force on the column head.

Recycling of the CO_2 has been utilized, but a challenge to recycling CO_2 is product recovery and CO_2 purity [5,13]. When recycling, 3–4% of the modifier will be retained in the CO_2. If one were to pump the recycled CO_2 directly into the instrumentation, the retention time of the analytes would change. Patented techniques are used to recycle the CO_2 and not every system will have the capability [21]. Recycling CO_2 is often seen as unnecessary, as bulk cryogenic CO_2 from a dewar is used to supply the CO_2 for prep-SFC. The cost of the CO_2 tank is around $0.05/L which is much less than the cost of the energy required to recycle the CO_2 used.

Finally, an understanding of method development and how it relates to fraction collection has also evolved. It has been reported that there can be degradation of compounds that have been fractionated [34–37]. When collected, solutes become concentrated in the small fractions, and many different reactions can occur. This effect is increased as the high pressure CO_2 mobile phase's high diffusivity is known to increase reaction rates. Many different solutions have been created to combat this issue, including nitrogen purging and the addition of a base to combat CO_2's acidity, however this could ultimately compromise the purity of the fraction and/or require further purification.

Table 1
A table showing the operating conditions when using SFC and 5 μm particles. The max pump pressure would be < 350 bar.

I.D. (mm)	Flow, mL/min	Injection Volume (mL)	Mass per Injection (mg)	Users	Scale
4.6	5	0.01–0.05	<10	Many	Analytical
10	20	0.1–0.5	50	Some	Semi-Prep
20	75–150	1.0–2.0	<200	Hundreds	Semi-Prep
30	150–200	2.0–5.0	<400	Few	Prep
50	500	10	<1200	Few	Prep

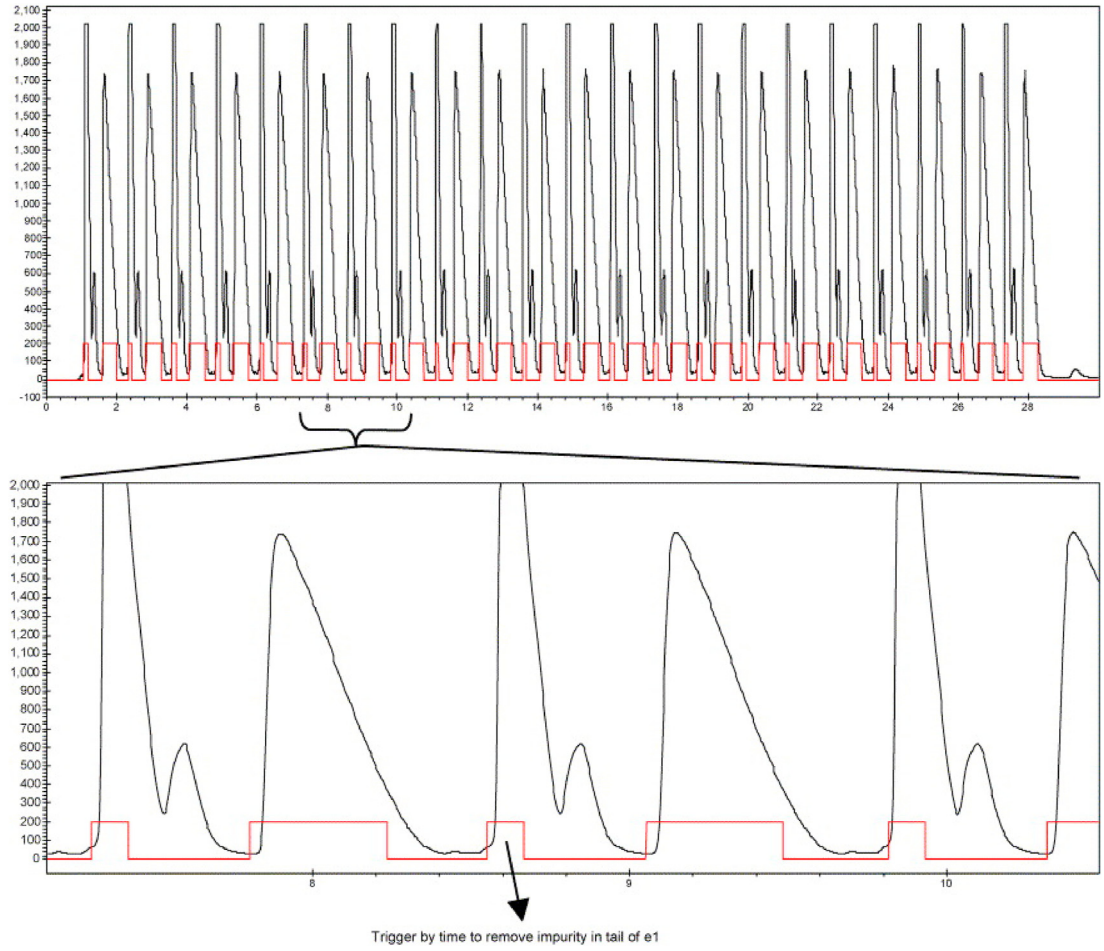

Fig. 1. An example of stacked injections, showing a chiral separation. Twenty-two stacked injections were performed using a 25 cm × 20.0 mm AD column with a particle size of 10 μm and a flow rate of 70 mL/min [29]. Reproduced with permission from Elsevier (copyright 2005).

3. SFC fraction collection

Preparative supercritical fluid chromatography instrumentation is similar to instrumentation in high pressure liquid chromatography except for one key difference – the fraction collector [4]. While most SFC hardware can be adapted from HPLC instrumentation easily with only minor adjustments, the fraction collector needs to be completely changed due to the expansion of the CO_2. In 1996, it was assessed that commercial preparative SFC instrumentation was not advanced enough to perform preparative work at the milligram or gram scale [4,21,38]. Ten years later, the technology developed to a point where preparative instruments were successful and widely used, especially in the pharmaceutical and fine chemical industries [23,39]. A summary and timeline of the main technological advancements is given in Table 2.

Collection of fractions started at the inception of supercritical fluid chromatography [40]. The physico-chemical properties of supercritical fluids allow them to be easier with which to work relative to gases in regular preparative gas chromatography, while also avoiding fraction-eluent separation problems exhibited by preparative liquid chromatography. One of the challenges of collecting the fractions in SFC compared to HPLC is handling the depressurization of the mobile phase post backpressure regulator [13]. While depressurization provides the benefit of an easy method of separating the product from the mobile phase, the CO_2 can expand to about 500 times the volume it was in the subcritical state [25]. The expansion of the mobile phase can also cause an aerosol to form, which could cause a loss of 20–30% of the product, contaminating the area, as well as potentially harming the chemist. This aerosol can also cause a remixing of the compounds and could

lead to a collected fraction which was less pure than when it was originally detected [41].

While other strategies were originally used, the first major step in the development of fraction collection technology was the use of a cyclone, which is still in use today [40]. One of the first uses of a cyclone was by Perrut in 1984 [42]. The cyclone was the beginning of a standardization for fraction collection of SFC; it obsoleted some of the various methods that were used beforehand. A cyclone uses centrifugal force to separate droplets of the modifier [25]. It achieves this by directing flow tangentially towards a wall, which will create a circular motion, from which the cyclone gets its name. A cyclone was the first major way to fractionate the analytes of interest without creating an aerosol. Cyclones separate the two phases of the mobile phase by increasing the temperature or decreasing the pressure. Once the CO_2 expands, it no longer solvates the system, and most of the product will remain behind in the liquid modifier. Then, when the effluent impacts the wall, the heavier droplets of the modifier containing the analyte fall to the bottom and are collected, while the CO_2 gas escapes, rising to the top of the device. One of the major problems with cyclones are the costs, as they are large devices made from thick stainless steel. Cyclones also have a very large surface area, which require significant cleaning in between different runs; this involves disassembling the unit. Analyte adsorption to the walls of the device can also be a significant source of material loss.

One of the problems with cyclones for prep-SFC was the fact that fractions needed to be collected at high pressure due to the high pressure CO_2 mobile phase. In an effort to fix this issue, a backpressure regulator was developed that had a low internal volume and could collect the analytes without causing the remixing of the compounds [43]. Another method was the use of a recycle-SFC column trap [44]. That system worked by having two columns, where the additional column is placed between a switching valve and an in-line pump. This allowed the mobile phase to circulate without causing any pressure fluctuations. The additional column was meant to retain compounds that had weaker retention, and the system allowed components to be retained in the first column and better separated the unwanted compounds from the analytes of interest. Another technique, which was developed to overcome issues with cyclones, used two cartesian style robot arms, one of which would collect one fraction while the other arm was washed in preparation for collection of the next one [45]. An additional system attempted to use a collection solvent which would contain the fraction but had the downside of using a large amount of solvent which could reduce the concentration of the fraction, as well as potentially degrade the sample [46,47].

The Berger separator was introduced in 2002 as the next major evolution of the cyclone. A Berger separator is different than a cyclone, in the fact that it is operated at atmospheric pressure, without the formation of aerosols [46,47]. The atmospheric pressures allow multiple vessels or open beds to be used to collect the fractions. There is also virtually no carryover, which allows multiple samples to be run together, without any steps in between. The Berger separator is self-cleaning when compared to the heavy metal cyclones; with the metal cyclones, a new cyclone would need to be used for each fraction, as effluent covers the metal walls, with gravity slowly collecting the fractions. With the Berger separator, separate cassette banks are used to collect the fractions with multiple chambers to collect and store the fractions. Liners are included to simplify transport, as well as to reduce cross-contamination. The only cleaning required is between the transfer lines, which connect the SFC instrument to the Berger separator. The apparatus decompresses the CO_2 slowly after the backpressure regulator while creating a film of modifier containing the analyte on the capillary walls. This was a great step towards the evolution of preparative SFC instrumentation, as it helped fix many problems associated with use of cyclone technology for this purpose. Traditional cyclones can not be used in a self-cleaning system run every 5 min, while the Berger separator can [39]. This separator was first used in the purification of combinatorial chemistry libraries [15]. A limitation of the Berger separator is the limited number of fractions one can obtain, due to the use of selection valves. To increase the number of fractions, valves in series can be used, but that complicates the instrumentation greatly.

The newest development toward maximizing efficiency for fraction collection with SFC is the use of a gas-liquid separator (GLS). Consistent with other techniques [48-50] the purpose of the device is to assist the expansion of the CO_2 in a controlled way, while collecting the modifier and analyte. Interaction between the gaseous CO_2 and the modifier are minimized, even at atmospheric pressures [51]. In 2006, one of the first gas liquid separators was introduced [52]. Shown in Fig. 2A, this GLS was designed to act like a cyclone; the effluent is directed towards the walls of a device that has an open-top. While before, the gas and liquid phases would be separated by increasing the inner diameter of the capillaries in the flow path, this device uses a splitter which separates the gas and the liquid phase away from the container used to collect the fractions. In this container, the liquid containing the analytes drips down to a separate collection device, while the gas phase escapes through the top. This GLS is suited well for repeated injections of the same analyte in bulk purification, as any sample loss is washed down by the next injection of the same material [51]. This device is not good if there are multiple analytes, as then, cleaning of the device is needed. The GLS can be operated in a parallel or serial configuration [48]. In a parallel configuration, one GLS is in series with one container meant to collect the sample. A serial configuration has one GLS that fractionates the effluent into multiple vessels.

Recently, new GLSs have been invented which aim to improve open stream fraction collection without generating any aerosol. Examples of newer GLSs are shown in Fig. 2B and C. Other recent GLSs

Table 2
A listing showing the main developments of fraction collection technology, when it was first reported, the importance of the invention, problems associated with the invention, and references used in this paper to discuss the invention.

Type	First Reported	Importance	Problems	Reference
Simple Outlet	1962	Established the idea of fractionation	Aerosol formation, Contamination, Loss of product	[8]
Cyclone	1984	First mainstream way to produce fractionation	High pressure Expensive Cleaning is time consuming Sample loss	[42]
Berger Separator	2002	Atmospheric Conditions Self-Cleaning	Limited number of fractions	[46]
Gas-Liquid Separator	2006	Modern technique where separation occurs away from the storage unit	Cleaning	[52]

were designed to allow for self-cleaning, for easier separations using fingers to facilitate dripping, to have more flow channels for the CO_2 to escape, and for more information control using sensors to detect the gas and liquid level in the device [48–50,53].

The device invented by Fogelman and Agilent helped alleviate many of the problems faced with the older GLS technology [48]. The flow of effluent goes through a path that continuously self-cleans to minimize cross-contamination while minimalizing manual rinsing. Another advantage is the collection of fractions at atmospheric pressure, which allows any container size to be used without over pressurization being a concern. These improvements are achieved by using a porous filter which collect droplets of effluent. These droplets then coalesce to become larger and pass through the filter wall into the collection tube. The external surface of the filters is then contained in a spiral housing with a sealed top, which forces the effluent downward. These features combine to create a device, which allows precise differentiation of fraction components with virtually no aerosol generation from the separator.

The devices by Wikfors and Agilent, as well as the device created by Goto and Shimadzu, work by having a robotic arm move the GLS to the appropriate containers [49,53]. These devices also use special geometry to allow a better separation of the gas and liquid. One issue with these devices may be a "last drop" effect where a drop of effluent containing the dilute end of a peak may still adhere to the GLS. Shaking the GLS before moving the arm to a different container can help alleviate this effect by displacing the small drop.

3.1. Directed fractionation

Control of preparative SFC is also of importance when discussing fraction collection. Without computer assisted control, manual collection is needed, which involves human error and is time consuming. One of the most common ways to direct fraction collection, even today, is with UV detection [16]. UV-controlled collection is an older technique as mass-directed SFC fraction collection was harder, and there were not commercially-available solutions initially. This made UV detection much more popular in applications, particularly in high-throughput methodologies [15,54]. Another earlier way to control fraction collection was by using a predictive method [55]. The preparative retention time is predicted from a calibration curve, with fraction windows set so that any analyte will be collected in one of the four positions. While these techniques were functional, mass-directed systems were still preferred and groups tried to create them.

The first mass-directed prep-SFC fractionation was performed in 2001 by Dupont Pharmaceutical [56]. It was a significant step, as when compared to the earlier techniques of direct fractionation, the mass-directed fractionation was a more sensitive and selective technique. Fractionation started when both a threshold was reached, and the product was detected in the mass spectrometer. Unfortunately, there was a loss of sample as aluminum foil was used as the seal on the collection needle. Another mass-directed SFC purification system was introduced in 2006 [57]. This system fixed issues with software compatibility, as well as the interface between the prep-SFC, the mass spectrometer, and the fraction collector. This system enabled real time fractionation of the effluent based on a mass spectrometry signal.

Around 2010, Agilent and Waters started introducing mass-directed SFCs; these were a significant advancement over the earlier technology [39,51]. These instruments started to show the much needed improvement in flow rate, gradient elution, fractionation, and recovery which allowed the technique to become more popular. This allowed SFC instrumentation to see more use in

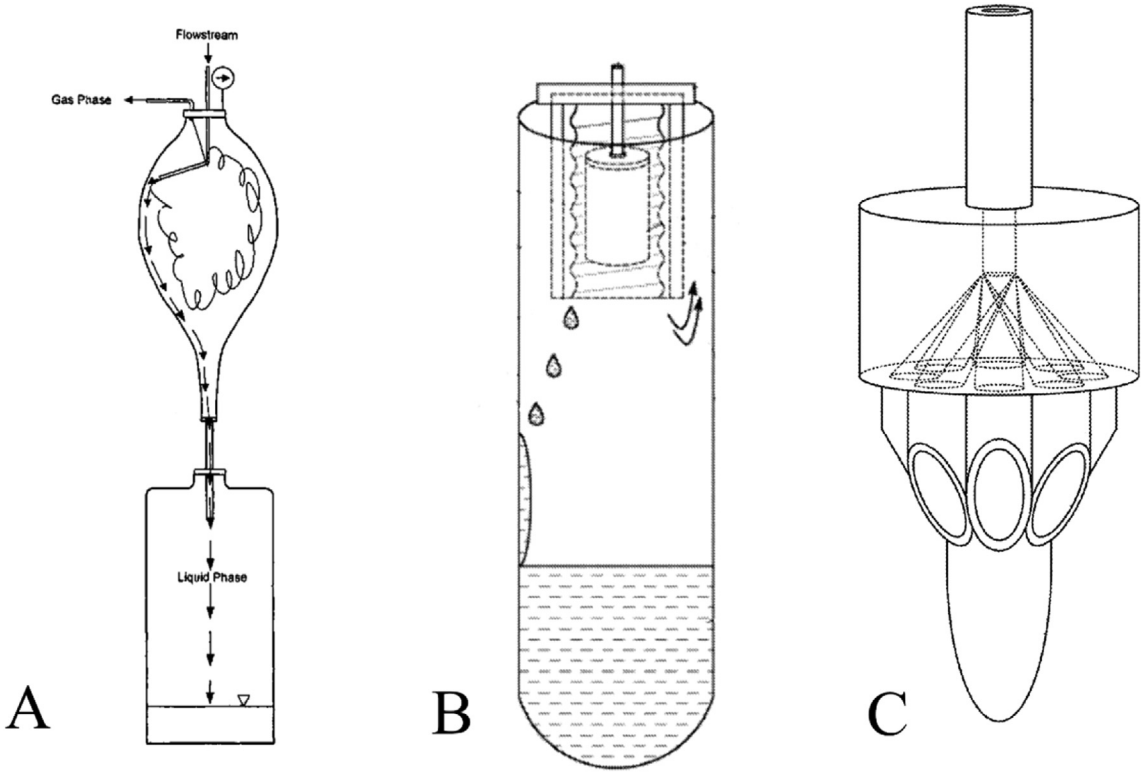

Fig. 2. A gas liquid separator (A) first introduced in 2006. The stream enters the container where the liquid drips on the side and the CO_2 escapes on the top [52]. An example of a GLS using a filter to prevent any aerosol leakage (B) [48]. An example of a modern GLS (C) which has multiple discharge flow channels, recently introduced by Shimadzu [53].

medicinal chemistry for increased throughput purification, due to the advancements of the technology over its predecessor [58]. Recently, mass directed prep-SFC has been utilized for lipidomics allowing absolute quantification for validation, in a clean and "green" way [59].

3.2. Other Considerations for fraction collection

Post-BPR cooling due to the Joule-Thomson effect is a common concern for fraction collection. If the fractions get too cold, they can become supersaturated with CO_2 which could then explode causing liquid to be lost and displaced. This is usually countered by adding heat upstream of the fraction collector. One way of applying heat upstream is at the BPR. Backpressure regulators are normally heated at 40–60 °C. The mass of the BPR is much larger than the mass of the expanding fluid and provides a more than adequate heat capacity. This prevents dry ice from forming in a pure CO_2 environment. In a normal SFC environment where CO_2 is mixed with modifier, dry ice is not a concern. The expansion of the modifier also warms the effluent, counteracting the cooling effect of the expansion of the CO_2. There may be additional ways to heat the effluent, as with the Berger separator. The Berger separator used a low temperature heat exchanger to counteract most of the cooling effects.

Another concern may be collecting fractions at low modifier percentage. At room temperature and atmospheric pressure, approximately three percent of the gas is methanol dissolved in CO_2. When using low modifier concentrations, all the modifier may be vaporized, leaving the sample lost. The sample may be in the aerosol, on the walls of the tubing, or in the collection vessel. This can be counteracted by using a make-up pump, which can be connected post- or pre- BPR. Pre-BRP may be better, as sample may precipitate in the connections between the column and the BPR. Makeup flow should always be reduced, as one of the key advantages of using SFC over other techniques when collecting fractions, is the small volume of the fractions. A reverse gradient can be used regarding the makeup flow, which would allow the modifier concentration in the fractions to be constant. This would allow the same volume to always be collected between fractions, keeping sample dilution consistent. MS directed fraction collectors especially benefit from the incorporation of a reverse gradient of make-up flow, to help accommodate consistent ionization efficiency.

4. Applications

While method development of fraction collection is important, the main purpose of any method development is to apply it to issues in the real world. For fraction collection, the type of sample dictates what fraction collection method should be utilized. When dealing with a simple mixture, for example a chiral analyte with two peaks, stacked injection should be used with an isocratic mobile phase. Collection should occur before the first peak, during the first peak, in between the peaks, during the second peak, and after the second peak. A GLS is preferred as it is self-cleaning, but a cyclone can be used if it is cleaned well in between different analytes. With regards to a reaction mixture with 10–20 peaks, a mass-directed open bed fraction collector should be used, capable of collecting 1000s of fractions. A single GLS would be preferred, which goes from unit to unit. For collection of trace contaminants, stacked injections should not be used as there does not exist enough resolution in the chromatogram to save time. Therefore, multiple injections should be utilized. Finally, for natural products or extremely complex mixtures, many fractions should be collected, directed by time windows.

4.1. Two-dimensional supercritical fluid chromatography

SFC has been increasingly utilized in 2D chromatography, due to its orthogonal separation nature compared to other techniques [60]. As commercial instrumentation to couple SFC to other chromatography techniques does not exist, offline techniques have been used when SFC is in the first dimension. These offline techniques require fractionation and collection before being injected into another system. While online methodology is preferred, offline approaches have sometimes resulted in better separations and peak capacity [61].

An off-line SFC/SFC/MS system has been utilized for the separation of racemic pharmaceutical compounds [62]. The first dimension was an achiral separation to remove impurities, while the second dimension was a chiral separation to separate the enantiomers. This provided a benefit that is not often seen in traditional liquid chromatography; the SFC mobile phase matched both the achiral and chiral stationary phases.

Fractions of triacylglycerols in fish oil were manually collected before being reconstituted in mobile phase recommended for non-aqueous reverse phase liquid chromatography [61]. This allowed the peak capacity to double, but at the expense of an analysis time twenty times greater than an online method. Fraction collection in the first dimension was also used for simplifying complex biological matrices [63–65]. Fraction collection can also be used when SFC is in the second dimension, to collect the complex mixtures that are normally separated using two dimensional chromatographic techniques [60].

4.2. Preparative supercritical fluid extraction – supercritical fluid chromatography

The coupling of supercritical fluid extraction (SFE) with supercritical fluid chromatography has become increasingly more popular as the instrumentation has become more user friendly and available commercially [66]. The coupling of SFE to SFC allows analyte extraction, separation, and detection to occur on a single system, minimizing analysis time [67]. This means that online SFE-SFC can increase the throughput in sample analysis of complex matrices, such as those from the environment [68]. Another benefit of online supercritical fluid extraction – supercritical fluid chromatography – fraction collection (SFE–SFC–FC) is its ability to concentrate the sample compared to if it was just extracted [69,70]. Online SFE-SFC is more difficult than SFE or SFC by itself, as changing any variable often effects both the extraction and the subsequent separation [66]. Nevertheless, SFE and SFC have been coupled and used in preparative work as early as 1989, due to the benefits that the coupling of SFE and SFC provide [71].

Chiral studies were performed to show preparative SFE-SFC could be more advantageous than traditional prep-LC methods [72]. They found that the SFE-SFC was equivalent or superior in separating all of the chiral compounds they examined, using the Whelk-O1 chiral stationary phase column. They also found that the method development was faster when using the SFE-SFC, compared to HPLC, due to the equilibration time being shorter.

Other experiments show the use of SFE/SFC to obtain lipids [69,73,74]. One study focused on the extraction of oil from corn bran to separate and collect free sterols and ferulate-phytosterol esters; they showed that coupling SFE and SFC provided a four-fold improvement for the free sterols and a ten-fold improvement for the ferulate-phytosterol esters regarding the amount collected when compared to traditional methods [69]. Home-built SFE and SFCs were still primarily used during this time, as commercial SFE-SFC instrumentation in 2002 was not as prominent as it is today. This separation was scaled from an earlier method which used

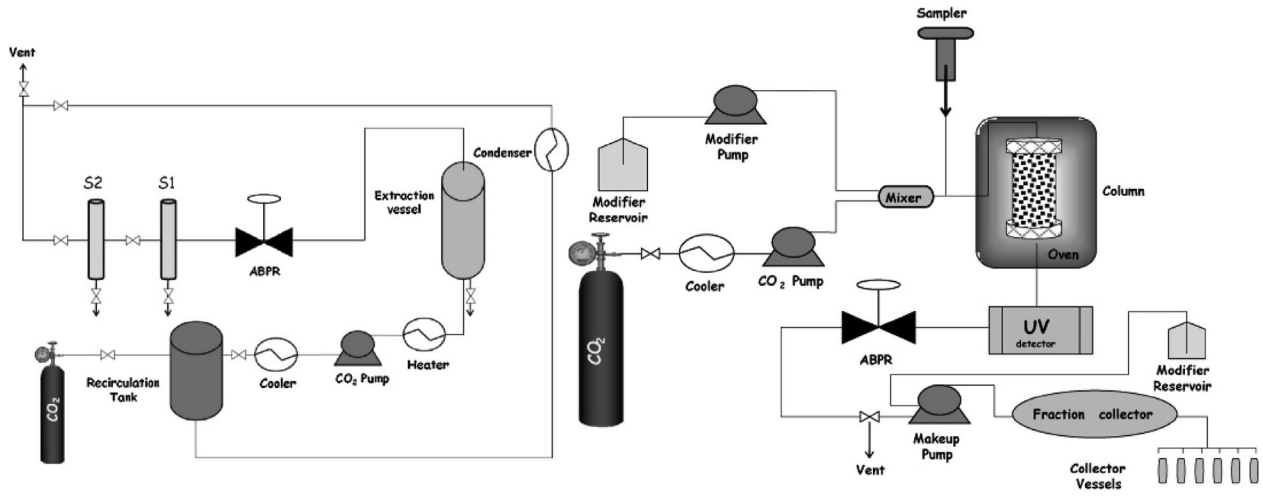

Fig. 3. The schematic diagram an SFE and SFC-FC used to purify thyme extracts. S1 and S2 of the SFE are separators [76]. Reproduced with permission from Elsevier (copyright 2011).

analytical SFC [75]. A similar system was used to examine phospholipids that are found in soyflakes [70]. SFE—SFC—FC again showed an enrichment factor increase of approximately 2- to 20-times depending on the analyte.

In 2011, thyme was extracted, separated, and fractionated for the first time using a semi-preparative SFC used with an SFE shown in Fig. 3 [76]. It was found that there was a two-fold increase of thymol in the fraction when compared to traditional means. More recently, SFE has been coupled to prep-SFC as being a semi-preparative technique was seen as advantageous, and prep-GC could not perform semi-preparative amounts [77]. This technique was used to examine turmeric with some compounds being concentrated more than sixteen times after the SFC, compared to just the extract alone.

5. Conclusion

Supercritical fluid chromatography has emerged as a useful separation technique which complements the instrumentation found in a standard analytical lab. SFC provides many benefits compared to other separation techniques, including its speed of analysis, its more environmentally friendly nature, and its cost. This is especially true in preparative methods, as the scale is much larger, often dealing with milligrams to kilograms of analyte, compared to the nanograms or micrograms in analytical separations. Collection of the fractions itself is also more beneficial in SFC, as most of the CO_2 mobile phase evaporates into the atmosphere, leaving a more concentrated fraction. Special apparatuses must be used to collect the fractions, as the CO_2 and modifier mobile phase create an aerosol when the CO_2 expands. This expansion and aerosol can cause a loss of product, a remixing of your analytes before they are collected, and degradation of your sample. Solutions to this problem evolved from atmospheric conditions, to using cyclones, to the Berger separator, and finally to the most modern gas-liquid separators. A major concern going forward is that current prep-SFC systems do not have proper compressibility compensation. Modern instruments still have issues with reproducibility of fraction collection, due to contamination issues as well as aerosol generation. Some of these issues can be solved; for example, using a robotic arm for the “last drop” effect. Another issue is the collection of fractions at low modifier, although a makeup pump alleviates the issues.

Research into SFC has increased significantly in the recent years. This work is enabling more technological advancements, as well as a deeper understanding of the chromatographic technique. This has led to SFC's understanding as a complementary technique in the lab, as well as its orthogonal separation nature relative to other techniques when performing two-dimensional chromatography. Preparative SFC is already popular, and the improvements to the fraction collection apparatuses should further increase its use. Online supercritical fluid extraction coupled with supercritical fluid chromatography has also seen a rise in popularity due to the newer instrumentation available commercially. The next logical step would be an increase in the coupling of online SFE—SFC—FC, which would provide multiple benefits including quicker analysis and preparation time, while also providing the benefits that fraction collection with supercritical fluids have, especially when analyzing solid samples.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Alexander Kaplitz reports financial support was provided by National Science Foundation. Terry Berger has patent Re: the Berger separator issued to Terry Bereger.

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