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# OILS: EXTRACTION BY SOLVENT BASED METHODS

*See* **III / FATS / Extraction by Solvent Based Methods**

# OLIGOMERS: THIN-LAYER (PLANAR) CHROMATOGRAPHY

*See* **III / SYNTHETIC POLYMERS / Thin-Layer (Planar) Chromatography**

# ON-LINE SAMPLE PREPARATION: SUPERCRITICAL FLUID EXTRACTION

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## Introduction

Over the past few years, there have been several advances in the use of new sample-preparation strategies prior to chromatographic analyses. These include supercritical fluid extraction (SFE), solid-phase microextraction (SPME) and accelerated solvent extraction (ASE). Each of these techniques is relatively new and will be used in more analytical strategies. SPME, for example, stands out in the realm of sample preparation in that the technique is solventless. ASE is also particularly exciting since the technique represents a modern version of long-established Soxhlet extractions. Therefore, by elevating temperatures and pressures to keep the liquid solvent from vaporizing, ASE approaches can be thought of as a 'universal' sample-preparation tools. The advantage of SFE is the fact that a supercritical fluid (i.e. carbon dioxide) is utilized with its blend of liquid and gaseous properties to achieve selective extraction of target analytes without major interference (depending on the sample). Of these three techniques, only SPME and SFE can be considered selective tools and they are also the only ones that can be interfaced directly to a chromatograph. The discussion in this article will focus on the use of SFE as a viable and selective strategy for sample preparation.

SFE continues to evolve as it is applied to a more and more diverse range of sample matrices. In the early years, much emphasis was placed on using SFE for environmental methods but, this has now blossomed into the wide application of SFE for food and agricultural analyses, polymer characterization, and pharmaceutical assays.

One of the distinct advantages of SFE (besides the physical properties of liquid-like density, gas-like viscosity, no surface tension and intermediate diffusivity) is the ability to directly couple the extraction effluent from a sample matrix to an analytical chromatograph for quantitative or qualitative determination.

In analytical chemistry, sample preparation is often the most error-prone step, requiring arduous and sometimes lengthy procedures before the actual sample can be analysed. SFE directly addresses the problem and provides analysts with the option of directly coupling the sample preparation procedure to the various forms of column chromatography to effectively achieve the analytical objectives. An added advantage is that the nature of the online interfacing of SFE does not exclusively limit the use of the chromatographic instrument to only SFE sample introduction. The flexibility exists whereby the analyst can use SFE in an offline collection mode as well as online. Offline SFE gives the analyst the most capability for manipulation in method development and analytical characterization, since the extracted effluent can be collected and then taken to any analytical instrument (i.e. GC, LC, SFC, MS, NMR, IR, UV).

This chapter will describe the use of online SFE/GC and SFE/SFC in terms of theory of operation, interface mechanics, instrumentation and application examples. In addition, some examples of selectivity enhancements in SFE will be described.

## On-line Interfacing Mechanics

A generalized scheme of online SFE is shown in **Figure 1.** A SFE system delivery pump compresses the primary extraction fluid (usually carbon dioxide) and solubilizes the analytes from a matrix which is contained in a heated extraction vessel. These solubilized analytes are then transferred online to an analytical chromatograph (i.e. GC, SFC or HPLC). The transfer line is used to control the volume of supercritical fluid that is flowing through the sample matrix. Depending on the analytical need, there is considerable flexibility obtainable when interfacing SFE online to a capillary GC. For many determinations, flame ionization and mass spectrometric detectors have been employed. However, it is also possible to utilize the more selective and sensitive detectors such as nitrogen–phosphorus and electron-capture detectors, depending on the application. In all of these cases, the detectors have a very low response to $CO_2$, depending for the most part on the impurities present in the commercial supply of $CO_2$. Most of the published online SFE/GC applications have utilized capillary columns ranging from 0.20-mm internal diameter to 0.53-mm internal diameter and have encompassed the full range of GC stationary phase coatings. To date, there have been no reports of SFE fluids stripping off capillary column stationary phase coatings after online interfacing.

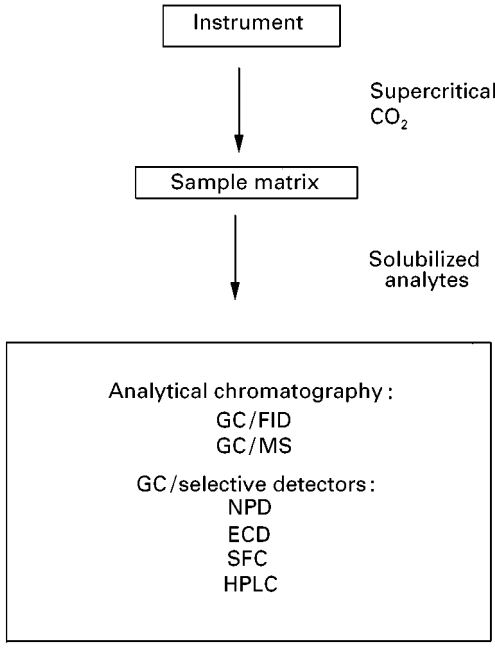


**Figure 1** Generalized scheme for online SFE.

As a means of sample introduction to GC, online SFE presents itself as an alternative to other means of sample introduction such as headspace, purge and trap, thermal desorption, pyrolysis, and even conventional syringe injection. **Figure 2** shows a comparison of online SFE/GC with conventional syringe injection using eucalyptus leaves and a fuel-contaminated sediment sample. The two modes of online SFE/GC, namely, split and on-column, were utilized. As can be seen, the GC peak shapes and amplitudes were comparable for online SFE and conventional syringe injection. A noticeable difference in the chromatograms is the absence of a solvent peak from the flame ionization detector. In comparison to headspace, purge and trap and thermal desorption, SFE has the potential to encompass a wide range of volatile to non-volatile analytes, depending on the sample and the extraction conditions that solubilize the entire sample (e.g. certain polymer matrices).

**Figure 3** shows a generalized schematic diagram of a typical online SFE/GC. A typical procedure for performing online SFE/GC involves first loading (usually weighing out) a small sample into an extraction vessel, depending on analyte sensitivities and analytical objectives. After weighing out a sample, the end caps of the vessel are tightened and the extraction cell is placed in the extraction oven. After pressure and thermal equilibration for the charged extraction vessel in the static mode (closed outlet of vessel to $CO_2$ flow), an electronic high-pressure switching valve changes position and shifts the extraction to the dynamic mode (opened outlet of the vessel for flow) transferring the extraction effluent through a heated

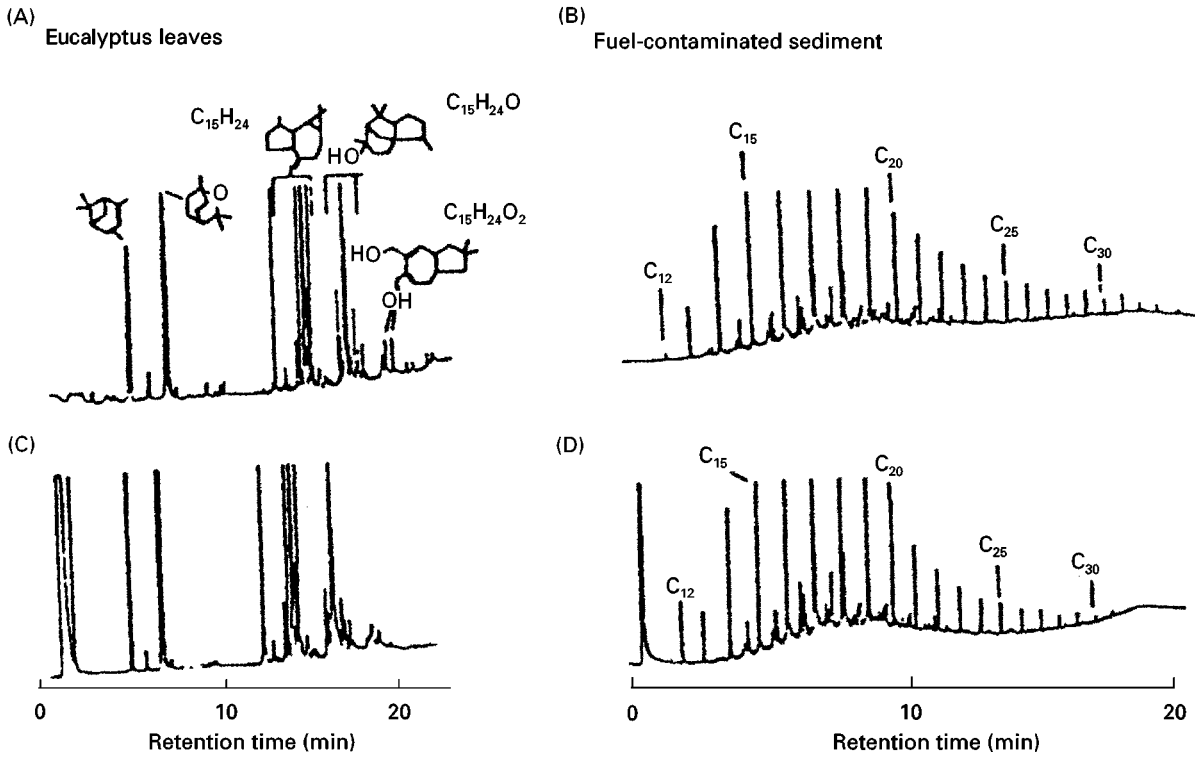


**Figure 2** Comparison of chromatographic peak shapes obtained using (A) on-column SFE/GC and (B) split SFE/GC with (C) conventional on-column and (D) split GC injections of methylene chloride extracts. Reproduced with permission from Hawthorne SB *et al.* (1989) *Journal of Chromatographic Science* 27: 347–354 and Hawthorne SB *et al.* (1990) *Journal of Chromatographic Science* 28: 2–8.

transfer line (made of fused silica or stainless steel) directly into a capillary GC injection port as shown in **Figure 3**.

The flow through the transfer line is regulated by restricting (crimping) the stainless-steel line or by using small inner-diameter fused silica. The decompressed $CO_2$, gaseous flow typically ranges from 35 to 300 mL $min^{-1}$ depending on the extraction vessel void volume (i.e., sample size). To achieve highly efficient extractions, three to five void volumes of supercritical fluid need to be flushed through the charged extraction vessel. The decompressed gas flow into the GC needs to be set at each extraction pressure setpoint to achieve efficient extractions.

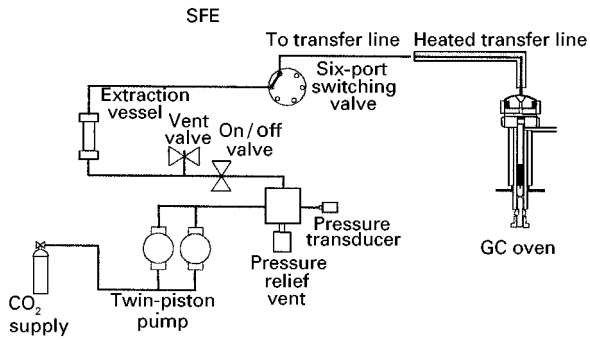


**Figure 3** Generalized online SFE/GC interface: schematic diagram.

Two modes of online SFE/GC exist, namely split SFE/GC and on-column SFE/GC. **Figure 4** is a pictorial representation of what is occurring during SFE introduction into GC. During split SFE/GC, the solubilized analytes exit the extraction vessel through a stainless steel (1/32 in × 0.007 in internal diameter) transfer line which is inserted directly through the septum and septum cap of an unmodified split/splitless capillary injection port. The supercritical fluid state is maintained until it reaches the tip of the transfer line (i.e. restrictor) and decompresses directly inside the heated injection port. So therefore, in theory, the analytes are purposely not allowed to fall out of solution until they are completely transferred to the GC injection port. The heat of the injection port aids in minimizing the expansive cooling of the supercritical fluid upon decompression. After decompression, the analytes vaporize inside the heated injection port, mix with the GC carrier gas, are homogenized inside the existing glass split-injection port liner, and decompressed gaseous $CO_2$ (and

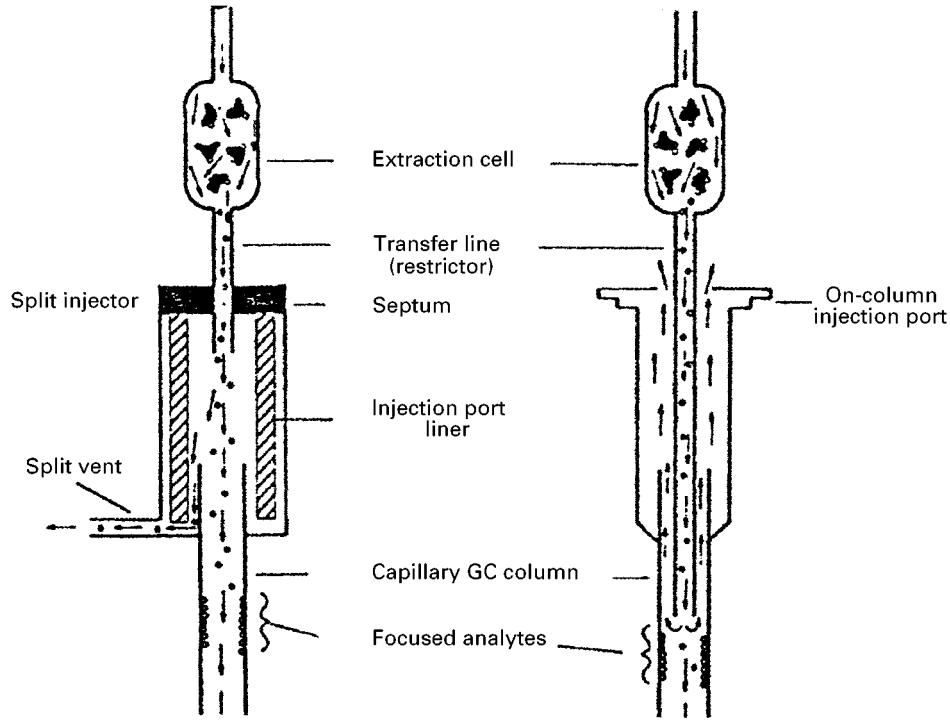


**Figure 4** Pictorial representation of online SFE/GC interfacing. Left, split SFE/GC; right, on-column SFE/GC.

analytes) flows out of the split vent during the dynamic extraction transfer mode. This is reproducible and potentially quantitative since the split ratio does not change from run to run. The development of temperature-programmable injection ports has allowed even further advances in focusing techniques after SFE deposition.

During on-column SFE/GC, the solubilized analytes exit the extraction vessel through a fused silica transfer line (10–50 μm internal diameter) which is inserted directly into an on-column capillary injection port. All of the solubilized analytes and decompressed gaseous $CO_2$ enter the GC capillary column, maximizing the sensitivity of an analysis (analogous to on-column syringe GC injection). For this reason, however, the fused silica transfer line needs to be physically removed after the dynamic extraction transfer mode since the decompressed $CO_2$ would essentially becomes the GC carrier gas and possibly extinguish a flame ionization detector. In split SFE/GC, the transfer line normally remains inserted in the injection port during the entire analytical run. The majority of published applications have been accomplished using online split SFE/GC. In general, split SFE/GC is better suited for generalized method development and characterization of a variety of different samples.

For both online split and on-column SFE/GC, the stationary phase of the GC capillary column is responsible for focusing the extracted analytes. Depending on the volatility range of the analytes, additional cooling (i.e. a cooled injection port) may be necessary to achieve sharp chromatographic peak shapes. This can be accomplished with a temperature-programmable injection port or by cooling the entire GC oven. An example of this is shown in **Figure 5**, demonstrating the effect of the cryogenic trapping temperature on the SFE/GC characterization of BTEX and n-alkanes from Tenax-TA. During the extraction, the GC capillary column was maintained at $-50$, $-25$, 5 or 25°C. After each extraction the GC oven was heated to 40°C at 50°C $min^{-1}$ and then at 8°C $min^{-1}$ to 300°C. The lower the setting of the GC oven temperature, sharper chromatographic peak shapes were obtained for the earlier eluting (more volatile) species. Maintaining the GC oven temperature at $-50$°C yielded the best chromatographic performance. In practice, at temperatures below $-50$°C, plugging of the transfer line occurs because of the freezing of the decompressed $CO_2$ inside the capillary column. The duration of the dynamic extraction transfer mode is usually the same as the duration of the initial (cryogenically cooled) temperature of the GC oven. After the dynamic transfer, normal GC temperature programming is performed and analytical GC results are obtained. This same routine can be applied if a temperature-programmable injection port is available.

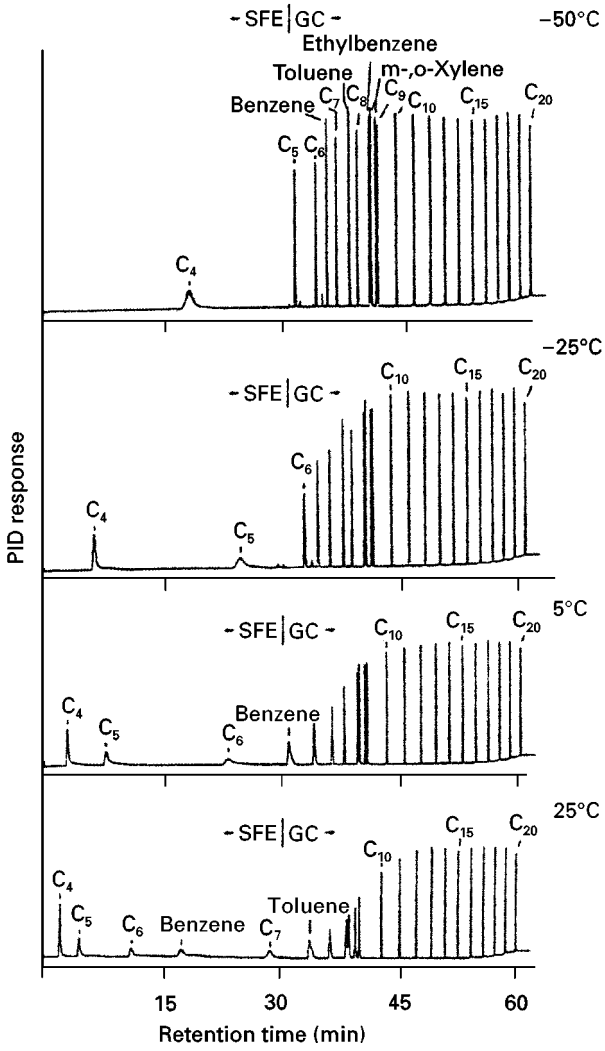


**Figure 5** Effect of cryogenic trapping temperature on the online SFE/GC/FID analysis of rosemary. Reproduced with permission from Burford MD and Hawthorne SB (1994) *Journal of Chromatography A* 685: 79–94.

**Figures 6–8** represent several example applications displaying the advantages of utilizing online SFE/GC. In each of these cases, flame ionization detection (FID) was utilized for the target analyte characterizations. In **Figure 6**, a rapid field survey was conducted using online SFE/GC to determine the carbon number range for total petroleum hydrocarbons (TPH) in soil. A fouled isocracker catalyst from a refinery was analysed and the aromatic component contamination was determined using online SFE/GC-MS, shown in **Figure 7**. In **Figure 8**, orange oil was spiked into an extraction vessel filled with hydromatrix (i.e., pelletilized diatomaceous earth) and rapidly extracted. In this, online SFE with $CO_2$ as the extraction fluid was invaluable since early eluting components in the orange oil were not overwhelmed by the response from classical liquid solvents.

## Selectivity in On-line SFE/GC

Compared to conventional liquid extractions like Soxhlet or ASE, a distinct advantage of SFE is the ability to tune the operational extraction parameters to achieve the selective extraction of certain analytes from a complex sample. An obvious approach in controlling SFE selectivity is by varying extraction temperatures and pressures. These parameters directly control extraction densities which in turn affect the threshold solubilities or mobilities of specific analytes. Certain classes of compounds, in theory, have distinct threshold solubilities. For example, a qualitative SFE/GC characterization of polynuclear aromatic hydrocarbons (PAHs) at different extraction pressures has been performed. In going from low to high SFE pressures, a noticeable difference, despite some overlap, was experienced in the distribution of the chromatograms by retention time and peak amplitude, indicating the potential for online SFE class fractionation. At 80 atm, two-ring, alkylated two-ring, three-ring, and lower alkylated three-ring PAHs are extracted. At 125 atm, the extracted fraction consists of alkylated three-ring, four-ring and some alkylated four-ring PAHs. At the highest pressure, 200 atm, alkylated four-ring and larger PAHs are extracted. The ability to tune selectivities by varying SFE densities is not only dependent on the target analytes of interest but also on the sample matrix. This is due to the contributions of other SFE mechanisms besides solubility, namely diffusion and adsorption effects.

Another means of enhancing SFE selectivities and efficiencies is by the use of modifiers such as methanol. These modifiers enhance extraction efficiencies by affecting solubilities, diffusion rates or surface adsorption, depending on the sample matrix and the target analytes. In SFE, modifiers can be added to the primary supercritical fluid by using dual-supply pumps or by uniquely adding a specific volume of modifier directly to the extraction vessel with the sample. In online SFE/GC, depending on the modifier identity, the modifier may elute in the GC as a discrete peak together with the extracted analytes. Depending on the modifier concentration, a retention gap or thick-film capillary column may be needed to separate the large (solvent-like) modifier peak and focus the target analyte peaks. Some modifiers such as formic acid, do not have an appreciable response with conventional flame ionization detectors. **Figure 9** shows the results for the online SFE/GC–FID characterization of sucrose esters in ground tobacco with and without BSTFA (*N*,*O*-bis(trimethylsilyl)trifluoroacetamide) as a derivatizing agent. In this case, BSTFA was added for derivatization during the

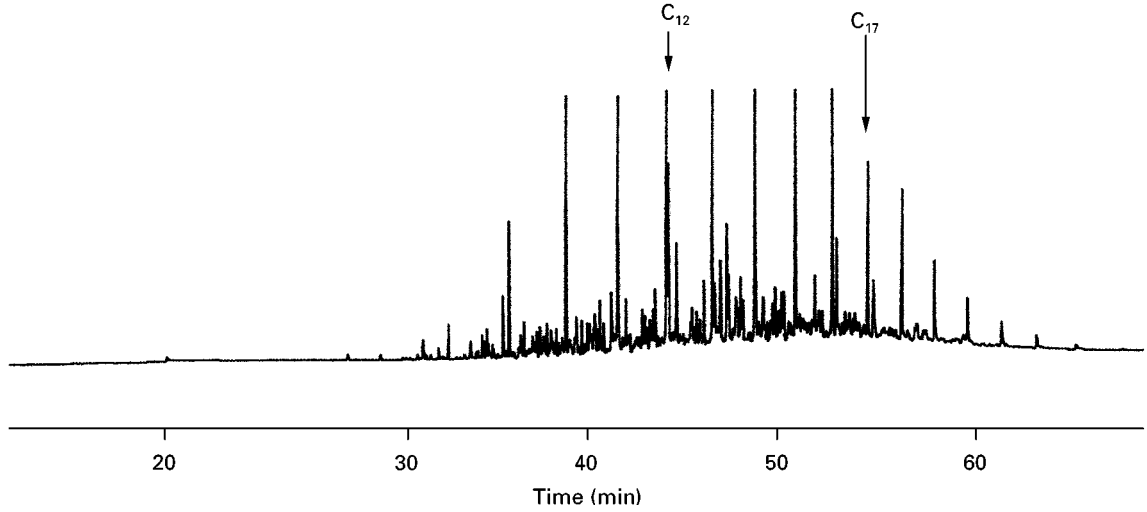


**Figure 6** Online SFE/GC determination of total petroleum hydrocarbons in soil (diesel fuel range).

extraction but also functioned as a modifier as well. It is clearly evident when comparing the two chromatograms that the BSTFA derivatized the sucrose esters during the SFE step, and therefore improved the detectability of these compounds. **Table 1** lists another example of the use of modifiers in online SFE/GC to enhance the extraction efficiency of selected aromatics analytes from a petroleum residue. The percentage recoveries for the selected aromatics were low when using only supercritical $CO_2$ for SFE and were distinctly enhanced when different modifiers were added to the residue in the extraction vessel. A period of static equilibration was required under the outlined extraction conditions, for the full modifier effect. Moreover, by varying only the modifier identities (keeping the modifier concentrations constant at 7%) obvious differences in percentage recoveries were obtained. Propylene carbonate and benzene achieved comparable efficiencies as opposed to the lower efficiencies obtained with methanol for this particular sample matrix and analyte.

Further selectivities can be obtained in online SFE/GC by the use of alternative supercritical fluids such as sulfur hexafluoride ($SF_6$) and nitrous oxide ($N_2O$). The use of these supercritical fluids in online SFE has been limited compared to $CO_2$, but has demonstrated certain distinct advantages due to their physical properties.

Different adsorbents can also be used to selectively immobilize interfering analytes from a complex sample before analytical determinations. Adsorbents, such as Celite, sodium sulfate, magnesium sulfate,

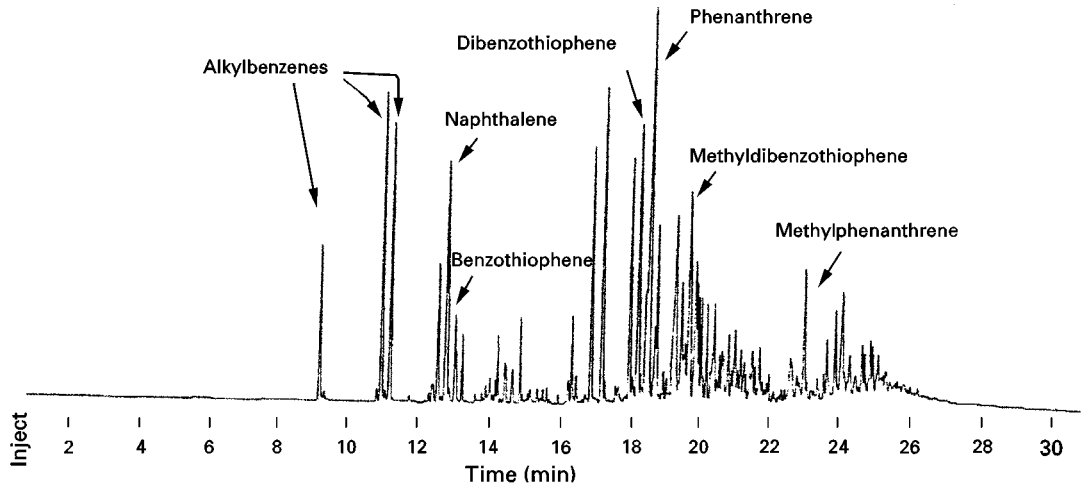


**Figure 7** SFE/GC of a fouled isocracker catalyst. Reproduced from Hawthorne SB.

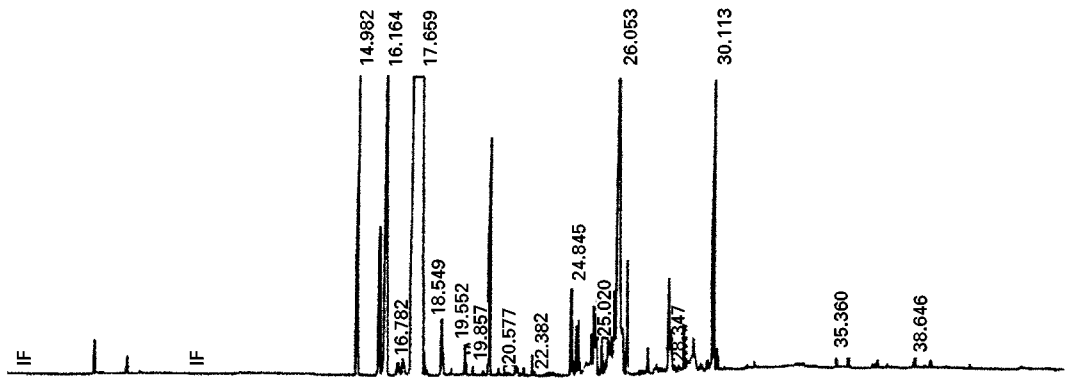


**Figure 8** Online SFE/GC characterization of Orange Oil.

Florisil, alumina, polyurethane foam, and hydromatrix (diatomaceous earth) have been used in online SFE applications to remove interfering analytes and water. An obvious disadvantage could be situations where target analytes are irreversibly bound to the absorbents or where interferences are introduced by the adsorbent. Adsorbents have been utilized by mixing them with proportionate amounts of sample before introduction into an extraction vessel or by utilizing two extraction vessels in series, the first containing the sample, and the second containing adsorbent before the GC.

## On-line SFE/SFC

Supercritical fluid chromatography (SFC) has been used widely as an analytical tool for the separation of relatively nonpolar, thermally unstable and high molecular weight solutes beyond the range of GC. The physical properties of supercritical fluids have unique characteristics to solve many problems, where both GC and high performance liquid chromatography (HPLC) fail. The online modes of SFE/SFC have several distinct advantages that are beyond the scope of either technique when used separately. These

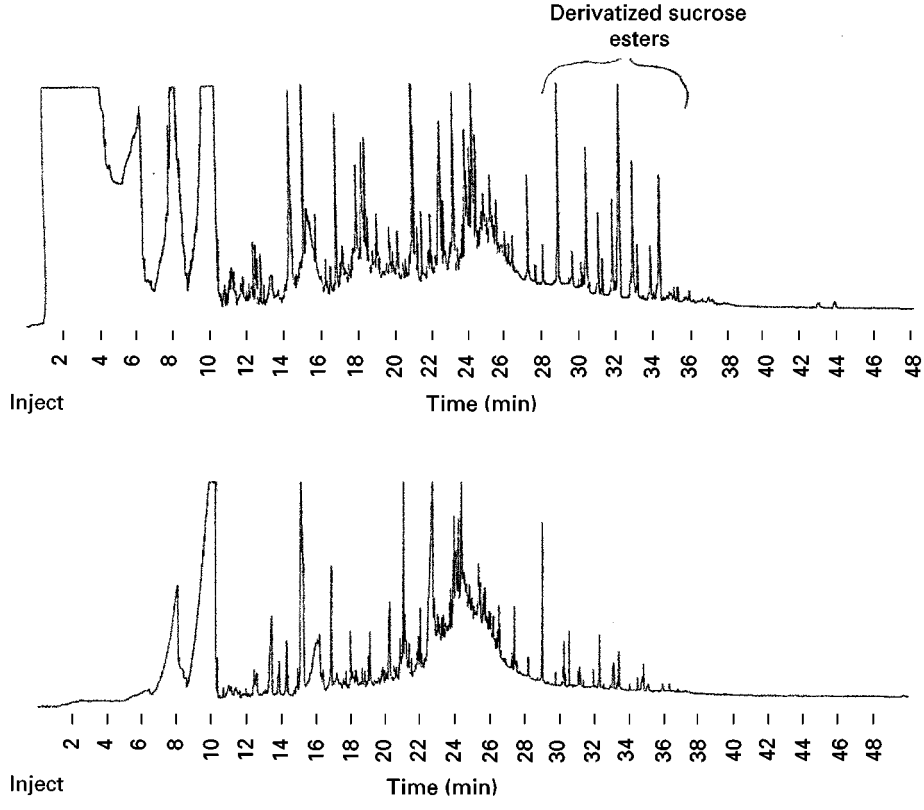


**Figure 9** Online SFE/GC of ground tobacco. Top, BSTFA added; bottom, without BSTFA.

**Table 1** Use of modifiers in online SFE[a]/GC[b]: percentage target analyte yields

| *Compound* | *Modifier* | | | |
|---|---|---|---|---|
| | *Benzene* | *Methanol* | *Propylene carbonate* | *$CO_2$* |
| Ethylbenzene | 101% | 76% | 98% | 45% |
| Cumene | 101% | 86% | 98% | 40% |
| 2-Chloronaphthalene | 100% | 93% | 101% | 73% |
| 1,2,4-Trimethylbenzene | 99% | 87% | 98% | 38% |

[a]SFE: 300 mg of petroleum residue, 425 atm, 65°C, 10 min static, 7 min dynamic.
[b]GC: 30 × 0.25 mm I.D. DB-1, inlet programmed from 0°C to 300°C at 600°C $min^{-1}$, 30°C (7 min) to 325°C at 7°C $min^{-1}$.

advantages are (a) trace analysis capability, (b) preparation with minimal sample contamination, (c) higher reproducibility, (d) increased productivity, and (e) online automation of the sample preparation step with the chromatographic analysis step. In the online mode, a high pressure extraction cell and the extracted components are trapped or focused in a device prior to SFC analysis. SFE/HPLC has been practised by several groups but has been demonstrated in only limited applications. This is largely due to the fact that coupling to GC and SFC is much more straightforward compared to coupling to HPLC. One main limitation is the fact that the LC mobile phase needs to be gas-free since bubbles can cause problems for most LC detectors.

## SFE/SFC Interface Mechanics

The arrangements in these hyphenated techniques usually involved several valves, one or two pumps and ovens. A simple approach has been applied to perform online SFE/SFC in both static (after pressurization of the extraction cell, extraction is allowed without passing any flow of supercritical fluid through the cell) and dynamic (after pressurization of the extraction cell, supercritical fluid continuously flows through the outlet of the cell) modes. Another method which has been used frequently for online SFE/SFC is the use of a second supply pump to pressurize the extraction vessel and the sample while the first supply pump is used solely to obtain chromatographic separation. Applying the second pump for extraction usually creates less technical problems and more freedom since both modes of extraction can be achieved and the chromatographic system is independent of the extraction system.

Other approaches that have been used to perform online SFE/SFC have been systems with multiple switching valves that permit collection of the extracted sample in a cooled adsorbent trap. After extraction and collection, the valves are switched and the cryogenic trap temperature is increased. By switching the valves and heating the trap, the supercritical fluid carries the extracted materials onto the analytical column. **Figure 10** shows an online SFE/SFC system which uses a cryofocusing region to collect the extracted material. In this system, the selector valve is first placed to the column position. During this period the temperature of the cooled region is adjusted to a desired level. Next, the valve is switched to the extraction vessel where the supercritical fluid of the desired density removes the extracted material from the region. During the decompression of $CO_2$, at the tip of the restrictor, the selector valve is switched to the column position and the supercritical fluid moves the analytes from the trap flow to the column. Meanwhile, the trap is also heated to the necessary temperature to help move the analytes onto the column.

Another system which uses a cryofocused trap to perform online high pressure SFE/SFC is shown in **Figure 11.** The system is comprised of three different valves (ten-port/two-position, five-port/four-position and four-port/two-position selector valves) and a zero dead-volume tee. During the extraction period, the mobile phase from the pump enters the tee. Tubing from one outlet of the tee leads the mobile phase to the injector valve for use only in conventional SFC applications. Tubing from the other outlet of the tee goes through the ten-port valve to the extraction vessel and then into the five-port selector valve. From the ten-port valve, the extracted analytes are held at a specified extraction temperature with industrial grade, dry carbon dioxide. All of the extracted material is then collected in the cryofocusing trap. The decompressed $CO_2$ gas from the trap is then vented through the ten-port valve into the oven atmosphere. After completion of the extraction, the pump is equilibrated for SFC. Upon reaching equilibrium, the ten-port and five-port selector valves are switched simul-

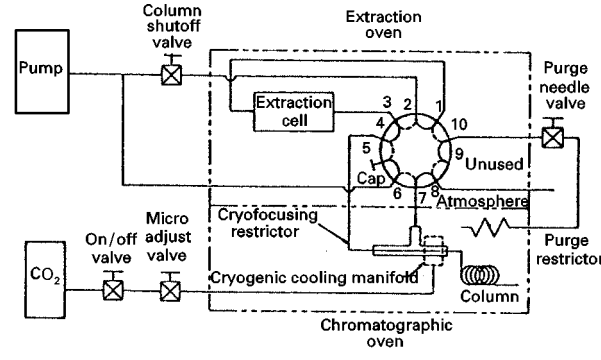


**Figure 10** SFE/SFC system and a cryogenic cooling trap with multiple valves.

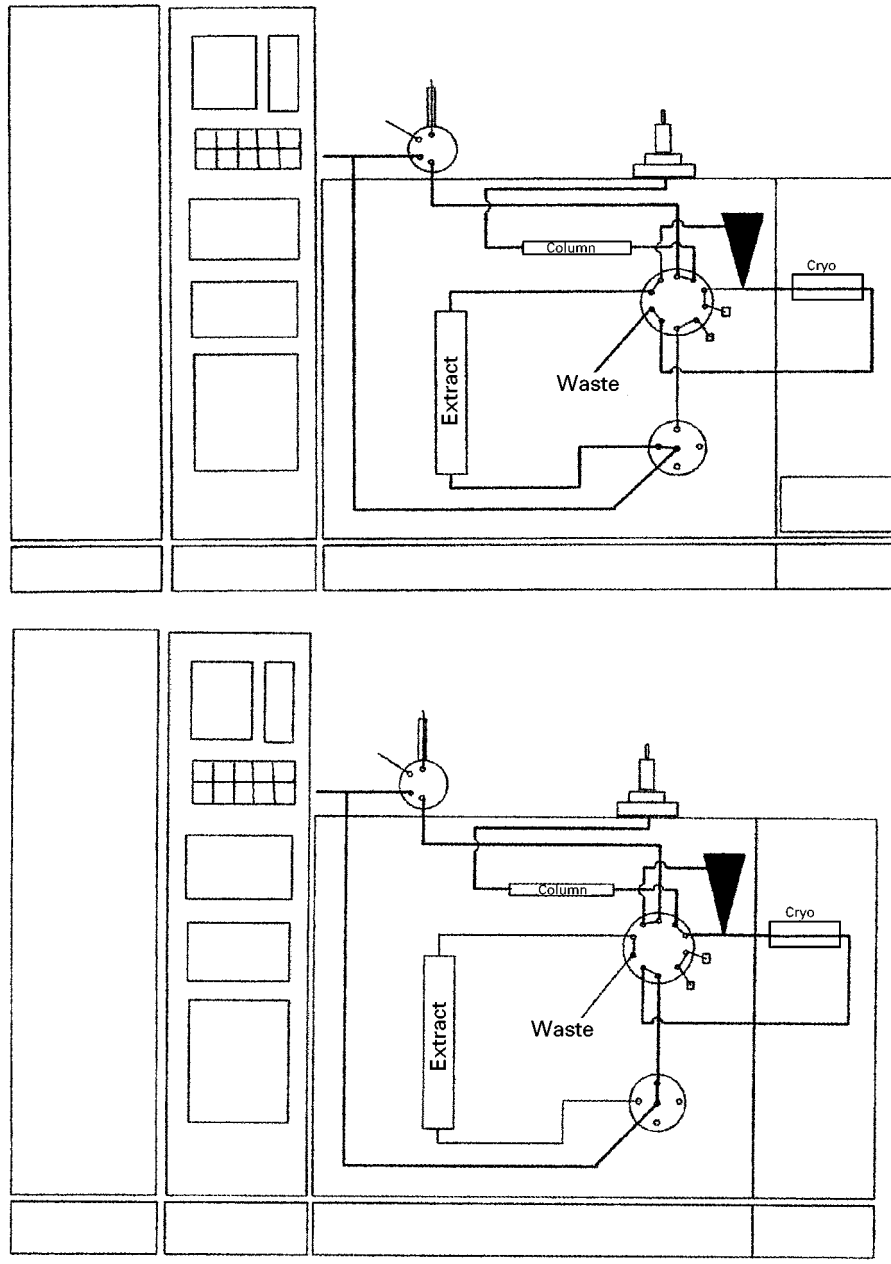


**Figure 11** SFE/SFC system and cryogenic trap with multiple valves in extraction (top) and injection (bottom) modes.

taneously (**Figure 11**). In this configuration, mobile phase passes through the tee, the injection valve, and the ten-port valve into the cryofocused trap, which is then ballistically heated to the specified injection temperature. After backflushing, the mobile phase carries the extracted components from the trap back to the ten-port valve into the chromatographic column. Additional $CO_2$ flow from the other outlet of the tee to the cryofocused trap restrictor prevents backflushing of the extracted material into the restrictor.

Most of the SFE/SFC devices described above are designed to obtain qualitative results. A later design gave quantitative results for different hydrocarbon standards using an online SFE/SFC system. The results showed that the amount of material extracted was directly proportional to the volume of sample placed in the extraction vessel. Later they demonstrated the quantitative analysis for different additives in low-density polyethylene using an FID.

A very elegant use of online SFE/SFC is shown in **Figure 12** which is a chromatogram of five different additives extracted from low-density polyethylene. For this experiment, a polymer was placed in the extraction vessel, extracted (450 atm, 100°C),

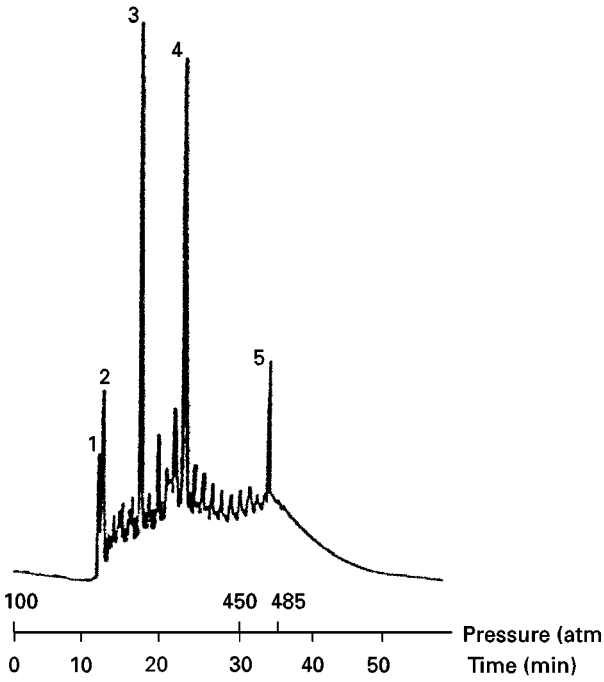


**Figure 12** Online SFE/SFC of different additives from low-density polyethylene. 1, BHT; 2, BHEB; 3, Isonox 129; 4, Irganox 1076; 5, Irganox 1010. (Reproduced from Levy JM and Ashraf-Khorassani M (1992) *Journal of Chromatography* Library Series 53: with permission from Elsevier Science.)

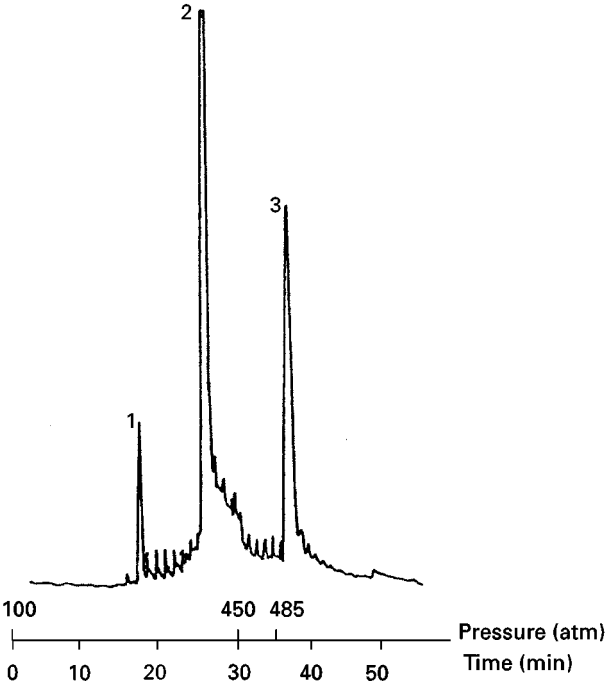


**Figure 13** Online SFE/SFC of additives from polyethylene concentrate. 1, Irgafos 168; 2, Cyasorb 3346; 3, Cyanox 1790. (Reproduced from Levy JM and Ashraf-Khorassani (1992) *Journal of Chromatorgraphy* Library Series 53: with permission from Elsevier Science.)

collected and cryofocused at −25°C. After extraction and collection, the trap was backflushed and the extracted component was flushed into the analytical SFC poly(ethylene glycol) (PEG) column. Each additive was identified and quantitated at the 200–300 ppm level. **Figure 13** shows the extraction and separation of three different additives from another polyethylene sample. Again, after extraction (450 atm, 100°C) and collection (−25°C), the extracted components were backflushed into the SFC octadecyl column, with each additive being determined at the 100–200 ppm level. Besides, polymer applications, online SFE/SFC also has been used for the characterization of caffeine in teas, as shown in **Figure 14.**

## Conclusions

Both directly coupled SFE/GC and SFE/SFC fall into the realm of problem-solving tools that can be

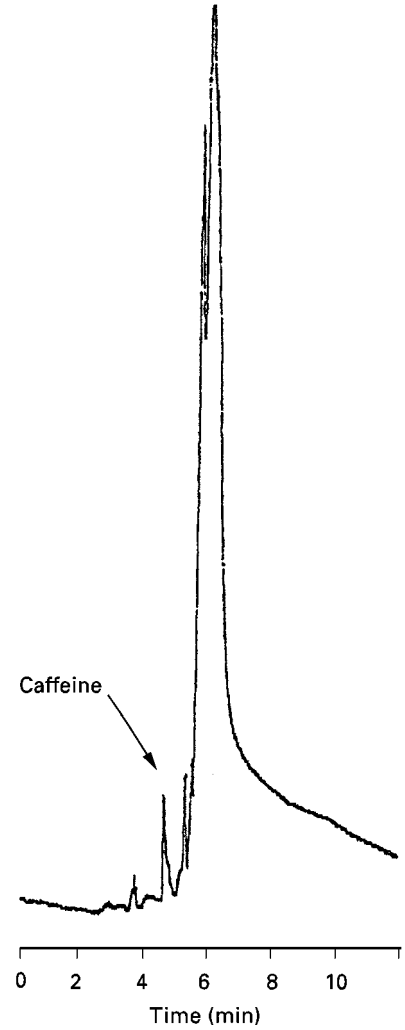


**Figure 14** Online SFE/SFC of tea.

effectively utilized by analytical chemists for qualitative or quantitative characterizations or determinations. For GC, SFE presents itself as a selective sample introduction means for liquid or solid matrices with volatile and nonvolatile analytes. The nature of SFE instrumentation provides an added feature with the potential capability of sample preparation in the field with analytical determinations using an online GC/MS. Online SFE/SFC complements SFE/GC when target analytes are thermally labile or beyond the volatility range of GC. An added feature with SFE/SFC is the wide range of method development capability since SFC can be interfaced to a full array of GC and LC detectors (e.g., flame ionization, ultraviolet absorbance, mass spectrometer, infrared, nitrogen–phosphorus and sulfur chemiluminescence). As SFE technology further evolves, additional capabilities will be added and refined in the area of selectivity enhancement (i.e., modifiers, absorbents, *in situ* derivatization) and operational parameter optimization (what conditions to use for specific analytes and sample matrices).

*See also:* **II/Chromatography: Supercritical Fluid:** Instrumentation. **Extraction:** Supercritical Fluid Extraction. **III/Supercritical Fluid Extraction-Supercritical Fluid Chromatography.**

## Further Reading

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# OPIATES

*See* **III / HEROIN: LIQUID CHROMATOGRAPHY AND CAPILLARY ELECTROPHORESIS**