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Review Article

SUPERCRITICAL FLUID CHROMATOGRAPHY (SFC) - AN ALTERNATIVE METHOD TO TRADITIONAL CHROMATOGRAPHIC METHODS: A REVIEW

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Abstract:

This review is intended to provide an update of the supercritical fluid technology in experimental, pharmaceutical and industrial processing systems employing dense carbon dioxide, in its supercritical, near-critical or liquid state, as a solvent. Many separations by this method are now carried out with supercritical carbon dioxide as the mobile phase and packed liquid-chromatography columns as the stationary phase. Although carbon dioxide has many practical advantages, including its near-ambient critical temperature and minimal interference with spectrometric detection, the use of other supercritical fluids or addition of modifiers to carbon dioxide may extend the applications of this technique.

Keywords: Supercritical fluid chromatography (SFC), Supercritical fluid extraction (SFE), Supercritical fluids (SF).

Introduction:

Supercritical fluid chromatography (SFC) is a separation technique in which the mobile phase is fluid and relatively close to its critical temperature and chromatographic pressure. In Supercritical Fluid Chromatography (SFC), the sample is carried through a separating column by a supercritical fluid (typically carbon dioxide) where the mixture is divided into unique bands based on the amount of interaction between the individual analytes and the stationary phase in the column. As these bands leave the column their identities and quantities are determined by a detector. Supercritical fluid chromatography (SFC) is a relatively recent chromatographic technique, having been commercially available since only about 1982. As a result there is a large amount of

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research currently underway both in SFC method development and in hardware development. Supercritical Fluid Extraction (SFE) designed to perform a variety of extractions in supercritical fluid to meet the day-to-day rigors of the research lab. It reveals information about the Chemical nature of certain components and makes identification easier but its use is limited to molecules with chromophores. The wide variety of applicable detectors makes it useful as a tool for the exploration of complex product mixtures. Owing to the good transparency of pure and modified Carbon dioxide, UV detection is the most used method in packed column Supercritical fluid chromatography. On the other hand, the compounds lacking chromophores requires universal detection such as FID. ¹

Advantages:

Supercritical fluid chromatography has several main advantages over other conventional chromatographic techniques (GC and HPLC). Compared with HPLC, SFC provides rapid separations without the use of organic solvents. With the desire for environmentally conscious technology, the use of organic chemicals as used in HPLC could be reduced with the use of SFC. Because SFC generally uses carbon dioxide collected as a by-product of other chemical reactions or is collected directly from the atmosphere, it contributes no new chemicals to the environment. In addition, SFC separations can be done faster than HPLC separations because the diffusion of solutes in supercritical fluids is about ten times greater than that in liquids (and about three times less than in gases). This results in a decrease in resistance to mass transfer in the column and allows for fast high resolution separations. Compared with GC, capillary SFC can provide high resolution chromatography at much lower temperatures. This allows fast analysis of thermolabile compounds. Compared with HPLC, SFC provides rapid separations without the use of organic solvents. This allows fast analysis of thermolabile compounds. Supercritical fluid extraction minimizes use of organic solvents. Fast, efficient extraction of a variety of materials may be achieved using supercritical carbon dioxide.^[2] A wide range of operations pressures up to 10,000 psi (69 MPa) and temperatures ambient to 200oC. Flow control automation without the need for an external cooling bath. Flow rates from 0.01 to 24.0 ml/min, good resolution and sensitivity, rapid separations and analysis of complex glycan mixtures.^{2,3} The advantages of supercritical fluid extraction (compared with liquid extraction) are that it is relatively rapid because of the low viscosities and high diffusivities associated

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with supercritical fluids. The extraction can be selective to some extent by controlling the density of the medium and the extracted material is easily recovered by simply depressurizing, allowing the supercritical fluid to return to gas phase and evaporate leaving no or little solvent residues.^{4,5,6}

It can be used with non-volatile and thermally labile analytes (unlike GC) and can be used with the universal flame ionization detector (unlike HPLC), as well as producing narrower peaks due to rapid diffusion. It can be used with non-volatile and thermally labile analytes (unlike GC) and producing narrower peaks due to rapid diffusion.

Instrumentation:

Principle of Supercritical fluid chromatography:

Supercritical fluid chromatography can most easily be described as an adaptation of either high performance liquid chromatography (HPLC) or gas chromatography (GC) where the major modification is the replacement of either the liquid or gas mobile phase with a supercritical fluid mobile phase.

In general there are two hardware setups used:

- 1) An HPLC like setup with two reciprocating pumps designed to provide a mixed mobile phase with a packed analytical column placed in an oven followed by an optical detector in which the pressure and flow rates can be independently controlled, 2) A GC like setup with a syringe pump followed by a capillary column in a GC oven with a restrictor followed by a flame ionization detector, where the pressure is controlled by the flow rate of the pump.
- 2) In SFC the mobile phase is initially pumped as a liquid and is brought into the supercritical region by heating it above its supercritical temperature before it enters the analytical column. It passes through an injection valve where the sample is introduced into the supercritical stream and then into the analytical column. It is maintained supercritical as it passes through the column and into the detector by a pressure restrictor placed either after the detector or at the end of the column. The restrictor is a vital component as it keeps the mobile phase supercritical throughout the separation and often must be heated to prevent clogging; both variable and fixed restrictors are available.

Working of Supercritical fluid chromatography:-

The detailed instrumentation of supercritical fluid chromatography is described below supercritical fluid chromatography consist of following components as shown in figure-1

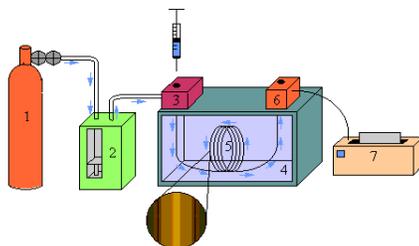


Fig 1: Instrumentation of Super Critical Fluid Chromatography.

Where,

1. Gas Supply or mobile phase.
2. High Pressure Pump
3. Injector
4. Oven
5. Analytical Column
6. Detector
7. Chromatograms

Gas supply or Mobile Phase:

There are a number of possible fluids which may be used in SFC as the mobile phase. However, based on its low cost, low interference with chromatographic detectors, and good physical properties (nontoxic, nonflammable, low critical values) carbon dioxide is the standard. The main disadvantage of carbon dioxide is its inability to elute very polar or ionic compounds. This can be overcome by adding a small portion of a second fluid called a modifier fluid. This is generally an organic fluid which is completely miscible with carbon dioxide (alcohols, cyclic ethers) but can be almost any liquid including water. The addition of the modifier fluid improves the solvating ability of the supercritical fluid and sometimes enhances selectivity of the separation. It

can also help improve separation efficiency by blocking some of the highly active sites on the stationary phase.

Modifier fluids are commonly used, especially in packed column SFC.

Table 1:-Comparison of transport property of mobile phases.

Solvent Phase	Density (g _m . ₃)	Viscosity (centipoise)	Diffusivity (cm ² sec ⁻¹)
Gas	~0.001	0.005 - 0.035	0.01 - 1.0
SCF	0.2 - 0.9	0.02 - 0.1	0.1x10 ⁻⁴ - 3.3x10 ⁻⁴
Liquid	0.8 - 1.0	0.3 - 2.4	0.5x10 ⁻⁵ - 2.0x10 ⁻⁵

Supercritical Fluids:

For every substance there is a temperature above which it can no longer exist as a liquid, no matter how much pressure is applied. Likewise, there is a pressure above which the substance can no longer exist as a gas no matter how high the temperature is raised. These points are called the supercritical temperature and supercritical pressure respectively and are the defining boundaries on a phase diagram for a pure substance. Beyond which the substance has properties intermediate between a liquid and a gas and is called a supercritical fluid. In this region the fluid has good solvating power and high diffusivity, which make it a good choice as a mobile phase in chromatography. Part of the theory of separation in SFC is based on the density of the supercritical fluid which corresponds to solvating power. As the pressure in the system is increased, the supercritical fluid density increases and correspondingly its solvating power increases. Therefore, as the density of the supercritical fluid mobile phase is increased, components retained in the column can be made to elute. This is similar to temperature programming in GC or using a solvent gradient in HPLC. There are a number of possible fluids that may be used in SFC as the mobile phase. However, based on its low cost, low interference with chromatographic detectors, and good physical properties (nontoxic, nonflammable, low critical values) carbon dioxide is the standard. The main disadvantage of carbon dioxide is its inability to elute very polar or ionic compounds. Pure supercritical carbon dioxide is a relatively non-polar solvent, but has some limited affinity with polar molecules due to its large molecular quadrupole. Modifiers can often be added to improve the solubility of polar molecules (ex. Methanol, Ethanol, water, amines). Both isocratic and gradients can be run in SFC. The addition of the modifier fluid improves the solvating ability of the supercritical fluid and sometimes enhances selectivity of the separation. It can also help improve separation efficiency by blocking some of the

highly active sites on the stationary phase. Modifier fluids are commonly used, especially in packed column SFC. Co-solvents generally the co-solvent or modifier is a material added to the supercritical solvent at low percentage, which makes a significant change to the solvent properties. For example the selectivity (α) of 2-naphthol with respect to anthracene can be enhanced greatly by adding methanol to supercritical CO₂. Unmodified CO₂ will give a selectivity of about 7. It rises to 30 if 3.5% methanol is added as a co solvent. Predicting co solvent effects is difficult-dealing with exchange between surfaces and mixed solvent.

Properties of Supercritical Fluids:

1. There are drastic changes in some important properties of a pure liquid as its temperature and pressure is increased approaching the thermodynamic critical point. For example, under thermodynamic equilibrium conditions, the visual distinction between liquid and gas phases, as well as the difference between the liquid and gas densities, disappear at and above the critical point. Similar drastic changes exist in properties of a liquid mixture as it approaches the thermodynamic critical loci of the mixture.
2. Other properties of a liquid fuel that change widely near the critical region are thermal conductivity, surface tension, constant-pressure heat capacity and viscosity. In comparing a liquid sample with a supercritical fluid sample of the same fuel both possessing the same density, thermal conductivity and diffusivity of a SF are higher than the liquid, its viscosity is much lower, while its surface tension and heat of vaporization have completely disappeared. These drastic changes make a supercritical fuel appreciably preferred over that of a liquid fuel with the same density. Further, it is expected that the combustion phenomena resulting from that of a supercritical fuel will be quite different from that of a liquid fuel.

Table 2:- Typical supercritical solvents.

Compound	T _c ° C	P _c atm	d [*]
CO ₂	31.3	72.9	0.96
C ₂ H ₄	9.9	50.5	---
N ₂ O	36.5	72.5	0.94
NH ₃	132.5	112.5	0.40
n-C ₅	196.6	33.3	0.51
n-C ₄	152.0	37.5	0.50
CCl ₂ F ₂	111.8	40.7	1.12
CHF ₃	25.9	46.9	----
H ₂ O	374.1	218.3	----

Pumps:

In general, the type of high pressure pump used in SFC is determined by the column type.

a) For packed columns reciprocating pumps are generally used. Reciprocating pumps allow easier mixing of the mobile phase or introduction of modifier fluids.

b) while for capillary SFC syringe pumps are used. Syringe pumps provide consistent pressure for a neat mobile phase. In capillary SFC small sample volumes must be quickly injected into the column and therefore pneumatically driven valves are used.

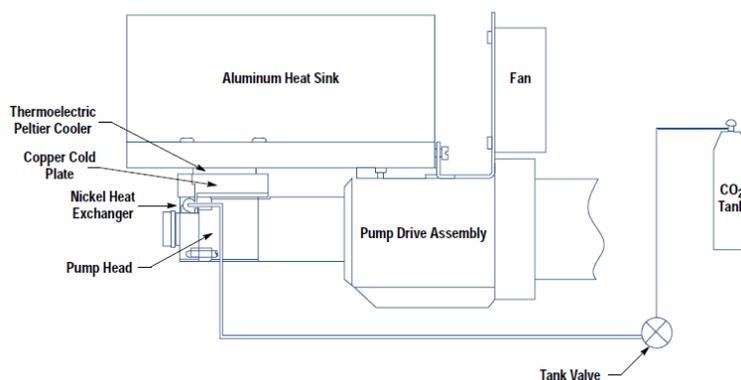


Fig 2:-sketch of SFC pump showing major components and thermoelectric device.¹¹

Commercially available Supercritical fluid chromatography pumps:**1. PU-2080-CO₂ Supercritical Carbon Dioxide Pump (Analytical Range)**

This carbon dioxide transfer pump is for analysis applications. It employs Slow Suction-Quick Delivery (SSQD), recognized for low pulsation as its pumping mechanism, thereby enabling stable transfer. It enables transfer at a stable compositional ratio when using it along with a modifier pump, thereby achieving separation and extraction with high reproducibility. The PU-2080-CO₂ also makes it possible to build an extremely compact analysis system without requiring any external cooling equipment.

2. PU-2086-CO₂ Supercritical Carbon Dioxide Pump (Semi-Preparative)

This dual-head supercritical carbon dioxide pump is for applications ranging from analysis to preparative isolation. It employs a specially shaped cam to achieve stable pumping performance up to a high flow rate. A single system can be used to analyze batches and perform purity inspections. It also conserves space by combining the PU-2086 with a compact cooling head.

- 3. PU-2088-CO₂ Supercritical carbon dioxide pumps (Preparative)** This dual-head supercritical carbon dioxide pump is specifically designed for preparative isolation. Due to its ability to transfer carbon dioxide at a stable rate of 10 to 120 mL/min, it can process large volumes of samples up to about 30 mm of column internal diameter for preparative SFC applications and up to 1L of vessel volume for extraction applications.

Modifier Delivery Pumps:

- 1. PU-2085/PU-2080/PU-2089/PU-2086/PU-2088**

Supercritical fluid systems that use carbon dioxide add a modifier solvent when boosting the degree of extraction freedom. Achieving a stabilized compositional ratio (0.1 to 50%) is crucial to performing separation analysis, requiring a pump that performs stable fluid transfer. The PU-2080, PU-2085, PU-2086, PU-2088, and PU-2089 transfer pumps renowned for the use in HPLC, are the optimal choice for pumping modifier solvent.

- 2. PU-2085/2080 Semi-micro/Analytical HPLC pump**

These pumps are capable of low-pulse pumping due to optimizations of the pump head, check valve, plunger, and other parts, thereby delivering a stable baseline. JASCO's proprietary SSQD pumps deliver co-solvent at a stable rate with low pulsation. The PU-2085 is particularly efficient at pumping in the low flow range. The PU-2085 and PU-2080 are optimal for pumping 0.1 to 3 mL/min and 0.3 to 7 mL/min, respectively.

- 3. PU-2089 4-solvent low pressure gradient pump**

The PU-2089 is an all-in-one quaternary pump that delivers all the functionality of the PU-2080 HPLC pump, the LG-2080-04 four-solvent low-pressure gradient unit, and the DG-2080-54 four-solvent degasser. The PU-2089 can switch between four different modifier solvents.

Injectors or samplers:

For packed SFC a typical LC injection valve is commonly used. In capillary SFC small sample volumes must be quickly injected into the column and therefore pneumatically driven valves are used.

Autosampler for SFC:

The AS-2059-SF autosampler is used to measure multiple-specimen samples. When using supercritical carbon dioxide as a mobile phase, it replaces the content of the sample loop with supercritical carbon dioxide with each injection.

A continuous introduction of samples requires the aspiration of the next sample after safely discharging the carbon dioxide in the sample loop and fully replacing it with an organic solvent. The AS-2059-SF can safely discharge carbon dioxide in the sample loop and continuously inject samples. The injection method can be selected as either a fixed injection volume that fills the entire sample loop or a variable injection volume that introduces the sample in a portion of the sample loop. The variable injection volume method is also capable of full injection with zero sample loss.

Ovens or column ovens:

The ovens used in SFC are generally conventional GC or LC ovens. Density changes due to changes in temperature have a major impact on the separation and extraction process on supercritical fluid systems. Achieving stable extraction efficiency and peak retention times requires the use of an oven to precisely control extraction vessels and regulate column temperature. JASCO offers the CO-2060, which is capable of temperature control at or below room temperature and the CO-2065 and SFC-Sro. The SFC-Sro is designed for temperature control for cup-shaped, high-pressure vessels and it includes a mixing function.

CO-2060/2065 Column Oven:

The CO-2060/2065 ovens are capable of temperature control with a precision of $\pm 0.1^{\circ}\text{C}$ with excellent reproducibility. The CO-2060 is for heating and cooling, while the CO-2065 is for heating only. Both models detect abnormal temperatures and solvent leaks and include a function for shutting off the heat. A manual indicator, preheating coil, reaction coil, and other such devices can be installed on both the CO-2060 and the CO-2065.

Furthermore, the CO-2060 can easily perform not only cooling (used for optical isomer separation columns), but also temperature increases and decreases by the use of a time program.

Analytical columns:

Once the sample is injected into the supercritical stream it is carried into the analytical column. The column contains a highly viscous liquid (called a stationary phase) into which the analytes can be temporarily adsorbed and then released based on their chemical nature. This temporary retention causes some analytes to remain longer in the column and is what allows the separation of the mixture. Different types of stationary phases are available with varying compositions and polarities.

There are two types of analytical columns used in SFC,

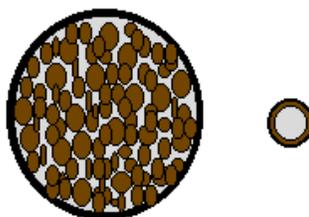
a) packed columns

b) Capillary columns.

a) Packed columns:- contain small deactivated particles to which the stationary phase adheres. The columns are conventionally stainless steel.

b) Capillary columns:- Capillary columns are open tubular columns of narrow internal diameter made of fused silica, with the stationary phase bonded to the wall of the column.

Column Crosssections



Typical Dimensions

	ID (mm)	Length (m)	
Packed	2.0- 4.6	0.03-0.25	3-10 μ m particle diameter
Capillary	0.025-0.1	1-35	0.1-3 μ m film thickness

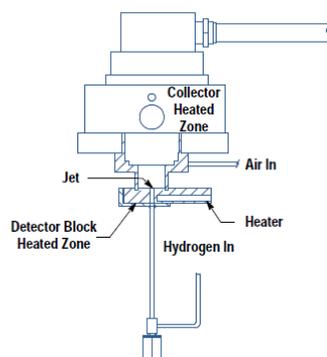
Fig 3:- Column Cross Sections.

Detectors:

SFC is compatible with both HPLC and GC detectors. As a result, optical detectors, flame detectors, and spectroscopic detectors can be used. However, the mobile phase composition, column type, and flow rate must be taken into account when the detector is selected as they will determine which detector is able to be used. Some care must also be taken such that the detector components are capable of withstanding the high pressures of SFC.

Table 3:-Comparison of SFC and GC flame ionization detectors.¹¹

SR.NO.	PARAMETERS	SFC DETECTOR	GC DETECTORS
1	Physical size	6mm thick, compact with jet permanently attached	25mm thick, with jet separate part
2	Temperature gradient	1°c from sensor to jet tip.	17°c from sensor to jet tip.
3	Problem area	No spiking or flame out	Spiking and flame out
4	Collector	Heated collector zone	No heated collector

**Fig 4:-sketch of SFC flame ionization detector.¹¹**

Evaluating optical characteristics under a supercritical state requires a detector and a high pressure resistant cell. Monitoring samples in supercritical fluid is possible by replacing the flow cell section of JASCO's liquid chromatography detector with a high-pressure resistant cell.

1. UV-2070/2075 UV/Vis Detector:-

The UV-2070/2075 UV/Vis detector combines high performance, multifunctionality, and superior operability for most standard detection techniques in chromatography.

2. MD-2010/2015 Diode Array Detector

Multichannel detectors are currently used in the same manner as general-purpose detectors. The MD-2010 and 2015 deliver high resolution and high sensitivity as a result of an optimized optical system and

Ashok Panday**et al.* /*International Journal of Pharmacy & Technology* redesigned data processing circuits. They also can be used as stand-alone detectors, even without a data station. These detectors are equipped with a four-channel analog output terminal. Both time and wavelength accumulation can be set and samples can be measured in the optimal state for the desired sensitivity and resolution.

3. CD-2095 Circular Dichroism Chiral Detector

This detector measures optical isomers with circular dichromatic absorption between 220 and 420 nm with high sensitivity and excellent selectivity. The CD-2095 is generally 10 to 100 times more sensitive than an optical rotation detector. It can measure both CD and UV chromatograms as well as g-factor (CD/UV) chromatograms. Since g-factor in particular has a proportional relationship with the compositional ratio of optical isomer samples, the CD-2095 can perform compositional measurements and high purity fractionation for non-separated peaks. In addition, spectral measurements for CD and UV are possible by stopped-flow spectrometry.

Chromatograms:

The results of a run in SFC are a chromatogram; the printout of the detector signal versus time.

Applications of supercritical fluid chromatography:

Pharmaceutical applications:

Nanoparticle formation using supercritical fluids:

SFE were used in a number of different circumstances for the preparation of micro-nanodispersed organic systems of industrial use are their special solvent properties and the technical feasibility of large temperature gradients. The generation of a sufficiently high super saturation for the initiation of a precipitation reaction with conventional solvents is on the one hand limited by the generally low dependency of the solubility on temperature, on the other by the difficulty to realize technically the necessary rapid heat exchange. With this in mind one process in which liquid CO₂ is used as cooling agent deserves attention. In this so-called contact cooling process the active compound solution at -78 °C is sprayed into a CO₂ stream and particle formation is induced in the spray droplets by crystallization. From the viewpoint of toxicological acceptability, the non-combustibility, and the favorable critical data of CO₂ (P_c=74 bar, T_c=31°C) the so-called RESS process (rapid

Ashok Panday*et al. /International Journal of Pharmacy & Technology expansion of supercritical solutions) appears particularly attractive, especially because extra process steps to remove residual solvent may no longer be required. However, supercritical fluid carbon-dioxide can act as an oxidizing agent with oxidation sensitive compounds such as β -carotene and is thus ruled out as a precipitation medium. Both in the GAS process (gas antisolvent process) and in the PCA process, supercritical fluid carbon-dioxide instead of water serves as the precipitation medium from organic solvents. In the physically related SEDS the organic active-compound solution and the supercritical fluid carbon-dioxide are brought into contact in a coaxial mixing nozzle, and thus a rapid extraction of the solvent (e.g. acetone) is possible. However, to date there have only been reports of particle formation in the micrometer range.^{23, 24}

Generation of pharmaceutical co-crystals:

Supercritical fluids act as a new media for the generation of novel crystalline forms of APIs (Active Pharmaceutical Ingredients) named as pharmaceutical co-crystals. Supercritical fluid technology offers a new platform that allows a single-step generation of particles that are difficult or even impossible to obtain by traditional techniques. The generation of pure and dried new co-crystals (crystalline molecular complexes comprising the API and one or more conformers in the crystal lattice) can be achieved due to unique properties of SCFs by using different supercritical fluid properties: supercritical CO₂ solvent power, anti-solvent effect and its atomization enhancement.^{7, 8}

Supercritical fluid extraction:

The advantages of supercritical fluid extraction (compared with liquid extraction) are that it is relatively rapid because of the low viscosities and high diffusivities associated with supercritical fluids. The extraction can be selective to some extent by controlling the density of the medium and the extracted material is easily recovered by simply depressurizing, allowing the supercritical fluid to return to gas phase and evaporate leaving no or little solvent residues. Carbon dioxide is the most common supercritical solvent. It is used on a large scale for the production of essential oils and pharmaceutical products from plants.^{4, 5, 6}

Enrichment of vitamin E from natural sources:

SFE offers several advantages for the enrichment of tocopherols over conventional techniques such as vacuum distillation, in particular a lower operating temperature. As starting material one can use various edible

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oils or their distillates. Most promising feed materials are CPO and SODD. CPO contains several tocotrienols and tocopherols at a total concentration of approximately 500 ppm. SODD may contain (after several conventional concentration steps) about 50% tocopherols. Both materials can be used for the production of enriched fractions of tocochromanols although it is possible to recover tocochromanols directly from CPO, it is better to produce esters of the triglycerides in order to be able to more easily separate these compounds from the tocochromanols. In this method, the triglycerides are subject to an esterification with methanol to form fatty acid methyl esters, which are easily extractable with CO₂. That means that the tocochromanols, together with other unsaponifiable matter such as squalene and sterol are enriched in the bottom phase of an extraction column. This attempt is described in more detail by. For a discussion of enriching tocochromanols, phase equilibrium data have to be considered first. FFA and tocochromanols exhibit a much higher solubility in CO₂ than the triglycerides. Hence, these components are enriched in the gaseous phase, expressed by a distribution coefficient being higher than one. The distribution coefficient of the triglycerides is smaller than one, whereas that for the carotenes is much smaller than one, meaning that these components stay in the liquid oil phase. Thus, tocochromanols can be extracted as the top phase product in a separation column, whereas carotenes remain in the bottom phase product together with triglycerides. For recovering the carotenes together with the tocochromanols the above mentioned esterification to volatile (CO₂ soluble) methyl esters makes possible to recover tocochromanols and carotenes (together with squalene and sterols) as bottom product from this natural source. When the glycerides (in case of the esterification) or the FFAs from deodorizer distillates have been removed, then there is a feed material available for obtaining enriched fractions of tocochromanols and carotenes of much higher concentration. In this feed material, tocochromanols and carotenes (in case of palm oil) are the main components and have to be separated from other unsaponifiable substances present, such as squalene and sterols. Of these compounds, squalene has the highest solubility in supercritical fluid carbon-dioxide, all phytosterols have a rather low solubility in CO₂ (and remain in the oil phase), and tocochromanols exhibit an intermediate solubility between the two. In a second separation step tocochromanols are separated from phytosterols. A further purification of these compounds is possible, e.g. with adsorptive or chromatographic techniques, again using supercritical fluids.^{14, 15}

Natural products:

Capillary supercritical fluid chromatography (SFC) is uniquely suited for the separation of complex mixtures of natural product shaving nonpolar to moderately polar components with a molecular weight range of 100-1000 Daltons.¹⁰

Special applications of supercritical fluids to food processing:

As mentioned before carbon dioxide is the most common supercritical fluid in the food industry. Due to the non-toxicity and low critical temperature, it can be used to extract thermally labile food components and the product is not contaminated with residual solvent. Further, the extract's color, composition, odor, texture are controllable and extraction by supercritical fluid carbon dioxide retains the aroma of the product. Supercritical fluid extraction provides a distinct advantage not only in the replacement but also extracts oils that are lower in iron and free fatty acid. Some application of SFE in food is mentioned below:

Removal of fat from foods:

Edible oils and their components has been the target of supercritical fluid processing since the early 70s. Although triacylglycerides are only fairly soluble in supercritical fluid carbon dioxide, the advantages of organic solvent-free processing have stimulated research and development in various areas. One of these is the removal of fat from food. The process has been fully designed for commercial application, using the aforementioned standard design. The process has the advantage of producing fat-free or fat-reduced potato chips. According to the expected taste the amount of remaining fat in the potato chips can easily be controlled.^{12, 13.}

Removal of alcohol from wine and beer, and related applications:

De-alcoholized wine or beer is achieved by removing ethanol from water. Distillation is well known for this purpose with the disadvantage that aroma compounds will also be removed. New techniques like membrane separation (pervaporation) emerge, and in between these is SFE with CO₂ Starting from an aqueous solution with about 10% (w/w) ethanol, ethanol can be removed by supercritical carbon-dioxide in a stripping column. The rate of ethanol removal depends strongly on temperature. Reducing the alcohol content to values well below 0.5% (w/w) requires about 2.5h at 45°C under non-optimized conditions. Much shorter times for the

ethanol removal can be obtained if flow rates and mass transfer equipment are carefully selected. With the information available in the literature, for instance from, a column for dealcoholizing aqueous solutions can be designed. Recovery of aroma compounds is achieved by a side column in which a separation from ethanol is carried out. A related process that can be mentioned is the recovery of absolute alcohol. Many studies were carried out at conditions of complete miscibility of ethanol and CO₂ in order to get a high solubility of ethanol in the vapor phase. At these conditions, anhydrous ethanol cannot be produced. However, ethanol can be concentrated above azeotropic composition whenever the pressure in the ternary mixture CO₂ + ethanol + water is below the critical pressure of the binary mixture CO₂ + ethanol.^{14, 16}

Encapsulation of liquids for engineering solid products:

A liquid product can be entrapped by adsorption onto solid particles (liquid at the outside of solid particles), by agglomeration (liquid in the free volumes between the solid particles), or by impregnation (liquid within the pore system of the solid particles). Microspheres or larger capsules can be formed, totally encapsulating the liquid. The solid material provides a coating for the liquid inside. Such particulate products can be achieved by means of supercritical fluid processing. An example is the so called concentrated powder form-process, wherein CO₂ is mixed (dissolved) in the liquid feed by static mixing. The CO₂-liquid feed mixture is then sprayed into a spray chamber at ambient conditions together with the substrate material. The CO₂ is suddenly released from the liquid, and the liquid forms small droplets. During the spraying process, solid substrate and liquid droplets are intensively mixed and combined to a solid particulate product of the type described above. The product is finally removed from the chamber as a free flowing powder and separated from the outgoing gas stream by a cyclone. With this type of process, a wide variety of solid substrates can be applied to uptake liquids of different kind and up to about 90%. As advantages can be claimed the easier handling and storage, prevention of oxidation processes, and easier dosage. Solid products can also be formed under high pressure conditions. As an example for such a type of process, the encapsulation or adsorption of tocopherol acetate on silica gel. Here, about 50% of tocopherol acetate can be incorporated onto the silica gel without apparent change of morphology and flow properties of the powder. The powder with 50% loading is still free flowing. The amount which can be adsorbed at high pressures is comparable to that of normal pressure. Only at very high densities, the equilibrium

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loading decreases. In the experiments the autoclave was used to saturate the supercritical carbon-dioxide current with tocopherol acetate, and the density of the solvent was changed in the nozzle where the loaded supercritical carbon-dioxide phase was fed to the absorber. This adsorption at high pressures makes possible the direct product formation in the supercritical fluid, with the advantageous effect that the supercritical solvent can easily be recycled without substantial compression.^{14, 17, 18}

Extraction and characterization of functional compounds:

Nowadays, the growing interest in the so called functional foods has raised the demand of new functional ingredients that can be used by the food industry. These functional ingredients are preferred to have natural origin and to have been obtained using environmentally clean extraction techniques. As expected, the complexity of the natural ingredients with biological activity is very high; this fact has led to the development of new methodologies to extract and characterize them. In order to preserve the activity of such ingredients and to prevent changes in the chemical composition of the functional compounds and/or mixture of compounds, sample preparation techniques based on the use of compressed fluids have been widely developed. SFE has been used to obtain extracts with antioxidant activity from microalgae; by using the combination of SFE and HPLC with both DAD ESIMS Several functional compounds were identified corresponding to different carotenoids along with chlorophyll A and some chlorophyll degradation products. These compounds could be associated to the biological activity of such extracts. Supercritical CO₂ has also been used to extract and characterize antimicrobial compounds and food preservatives from microalgae. Carotenoids are a group of compounds of great importance to human health since they can act, e.g., as potent antioxidants; however, due to their chemical characteristics they are easily degraded by temperature or oxygen, so, the use of SFE has been suggested to minimize risks of activity lost being thus applied to the extraction of carotenoids from different matrices. In this application, a vegetable oil was also used as co-solvent showing an improvement in the extraction yield as well as in the stability of the pigment. In both cases, the use of oils as co-extracting agents presents an important drawback that is the elimination of oil. It helps to improve the extraction but the extract is a mixture of the extracted components of the oil and the “pure” extract. In general terms, the use of SFE allows the analysis of essential oil preserving its integrity, without the formation of off flavors that could interfere in

the characterization of the sample. As it was told before in order to widen the range of application of SFE to relatively polar compounds, small amounts of modifiers ($\leq 15\%$) are added to carbon dioxide allowing the extraction of more polar substances. Other examples of the extraction of valuable compounds from foods using SFE are the isolation of cholesterol from cattle brains and fat-soluble vitamins from Parmigiano regiano cheese. The main problem with cattle brains, as well as many other raw food matrices is its high content in water. Water can interfere in the extraction process in two ways lixiviation or acting as co-solvent thus interfering in the reproducibility of the extraction procedure. In order to avoid this situation, the most common strategy is drying or freeze drying the sample prior to extraction although some authors prefer to mix the sample with a water absorbent inside the extraction cell, for example magnesium sulphate. Another problem related to the extraction of real food samples is their variable fat content which can also interfere in the extraction of the target compounds due to a co-extraction of the fat at the selected extraction conditions. Two main approaches have been used to overcome this problem, the first one uses a fat retainer, mainly basic alumina, neutral alumina, florisil or silica, placed in a separate chamber downstream the extraction thimble, while the other one uses the fat retainer inside the extraction cell.^{17, 19, 20}

Application of SFE in food safety:

At present, food safety includes many different issues such as detection of frauds, adulterations and contaminations. Among these topics, detection of food pollutants is important not only for consumers but also for administrations, control laboratories, and regulatory agencies. In order to protect consumers' health, regulations establish strict limits to the presence of pollutants in foods that must be carefully observed and determined. Generally, the analysis of food pollutants is linked to long extraction and cleanup procedures commonly based on the use of, e.g., soxhlet and/or saponification. These procedures are laborious and time consuming and, besides, usually employ large volumes of toxic organic solvents. With the objective of reducing both, the sample preparation time and the massive use of organic solvents, techniques based on compressed fluids such as SFE have been developed. One of the main areas of application of SFE in the last few years has been in food pollutants analysis, mainly pesticide residues and environmental pollutants. Several methods has been developed for the analysis of multiple pesticides (organochlorine, organophosphorus, organonitrogen and

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pyrethroid) in potatoes, tomatoes, apples, lettuces and honey with a single cleanup step using supercritical CO₂ modified with 10% of acetonitrile. Similar study have been carried out for the analysis of multiresidues of pesticides, using SFE as a cleanup step, in cereals, fish muscle, vegetable canned soups, vegetables or infant and diet foods. A common characteristic of these study is the extremely high selectivity of SFE in the isolation of the low polarity pesticides; this fact makes SFE probably the technique of choice to isolate pesticides from low fat food. As mentioned in the introduction, to correctly asses the concentration of an analyte in a food sample, a quantitative recovery should be obtained that will mostly depend on the recovery of the analytes and not on the extraction itself. To improve the recovery of the pollutants, a common strategy is the use of solid traps. These traps consist on a solid phase compatible with the analyte and are flushed away with a compatible solvent. The trapping step is very important in SFE method development not only because its effect in the quantitative recovery of the analytes but also because an extra selectivity can easily be introduced, especially in the case of solid-phase trapping, avoiding the use of further post-extraction clean up. Supercritical carbon dioxide extraction can advantageously be used to extract non-polar pollutants, such as PAH from foods. Different extraction and cleanup methods have been used, but the extracting conditions turned to be very similar (around 300 bar and 100°C) to optimize the PAH extraction. SFE and analytical uses supercritical carbon-dioxide has been utilized in multiple methods of analysis. It is used as either an extraction medium, as in rapid analyses for fat content, or as a mobile phase, as in supercritical fluid chromatography. Accordingly, the use of supercritical fluids in the detection of fat content, pesticide residues, and supercritical fluid chromatography as well as some analytical applications are highlighted below

Rapid analysis for fat content:

SFE have been used to determine the fat content of numerous products ranging from beef to oil seeds and vegetables. For the analysis of fats content in soybeans, sunflower, safflower, cottonseed, rapeseed and ground beef, it was found that supercritical fluid extraction yielded higher recoveries than those obtained by the AOCS approved methods. The use of an in-line piezoelectric detector is able to measure the change in weight of the sample during the extraction process. This allows for more accurate determination of the final weight of the sample after all of the fat has been extracted (total fat). In addition, it can allow for more rapid determination of

the total fat by determining the point when the steady state mass has been reached without having to re-extract the sample multiple times to confirm that the steady state mass has been reached.^{21, 22}

Rapid analysis for pesticides in foods:

Pesticide residues are a concern among consumers throughout the United States and other countries. Currently the methods of analyzing food products and other substances such as contaminated soil and water involve the use of organic solvents such as hexane and dichloromethane to extract the pesticides from the sample matrix. Once the pesticides have been extracted from the sample matrix, the samples must be “cleaned” to remove any unwanted compounds, such as lipids, which may interfere GC analysis of the sample for any pesticides present. The most common method for cleaning is solid phase extraction. Supercritical fluid extraction provides an alternative to using organic solvents for the extraction of pesticides from their sample matrix. Some of the advantages which supercritical fluid extraction provides over the traditional methods of pesticide extraction are that the extraction can be performed in less time, and utilizes less solvent volume. In addition, supercritical fluid extraction can be tailored to the solute of interest by changing the temperature and pressure of the extraction process. Supercritical fluid extraction can also be tailored for pesticides that contain more polar groups by the addition of polar modifiers to the CO₂ such as methanol.^{21, 22}

Other applications of supercritical fluid chromatography:

Chemical Reactions:

Changing the conditions of the reaction solvent can allow separation of phases for product removal, or single phase for reaction. Rapid diffusion accelerates diffusion controlled reactions. Temperature and pressure can tune the reaction down preferred pathways, e.g., to improve yield of a particular Chiral isomer. There are also significant environmental benefits over conventional organic solvents. In respect to organic compounds it is used for Chiral separations and analysis of high-molecular-weight hydrocarbons.^[9]

Impregnation and dyeing:

Impregnation is, in essence, the converse of extraction. A substance is dissolved in the supercritical fluid, the solution flowed past a solid substrate, and is deposited on or dissolves in the substrate. Dyeing, which is readily carried out on polymer fibers such as polyester using disperse (non-ionic) dyes, is a special case of this. Carbon

dioxide also dissolves in many polymers, considerably swelling and plasticizing them and further accelerating the diffusion process.

Conclusion:

SFC has been suggested as the method of choice for thermo labile compounds. It has been used for developing an ever-expanding niche in the food industry whether it is used as a solvent for extraction or analyses. Now a day, SFE is really needed as advance method which can provide fast, reliable, clean and cheap methods for routine analysis. The successful commercial developments described above share some common characteristics. First, they involve the processing of relatively high-value, low volume products. The decaffeination processes have the unusual advantage of producing a valuable extract (caffeine), as well as a valuable raffinate (decaffeinated coffee beans). Many extraction processes for essential oil/oleoresin-type flavors have had the advantage of being applicable not only to the food industry, but also to highly profitable sectors of the pharmaceutical and fragrance industry. Secondly, these areas have had the benefit of ten years or more of thorough research and development. Thirdly, they consist of relatively simple extraction processes, exploiting the inherent solubility and selectivity characteristics of carbon dioxide, in relation to certain types of lower-molecular-weight organic compounds, in batch or semi-batch systems. According to these criteria, commercial developments in the near future might be expected to continue in the area of food flavors, as well as in the extraction and refining of 'natural' food ingredients, functional foods, alternative medicines and specialty oils. Recent developments in the production of industrial fine powders and thin films, by the rapid nonequilibrium precipitation of non-volatile compounds from dense gas solutions upon expansion, may also find applications in food processing (Matson et al., 1987; Lee et al., 1990; Tom et al., 1993). In rapid expansion of supercritical solution (RESS) processes, a non-volatile solute is dissolved in a supercritical fluid at high pressure, and then precipitated mechanically, rather than thermally, by fast decompression.

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