The Promise of Supercritical Fluid Extraction and Chromatography:

What You Need to Know to Advance Your Product Research

A EUROFINS WHITE PAPER

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Supercritical fluid extraction (SFE) coupled with supercritical fluid chromatography (SFC) provides several unique capabilities and offers notable advantages over other extraction and chromatography methods.

To help researchers better understand these procedures and their role in analyzing vitamins in supplements or detecting common contaminants, this white paper covers the theory and the history of supercritical fluids, the methods and instrumentation, sample preparation and the benefits observed in testing fat soluble vitamins, such as D2, D3 and K1 in supplements, and detecting trace amounts of patulin and aflatoxin M1 in consumer food products.

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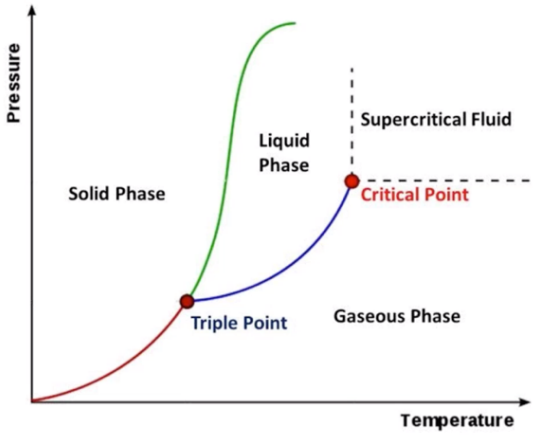
Supercritical Fluid: A Transition Point

The solid, liquid and gaseous phases of a substance can be manipulated by adjusting temperature and pressure. When a substance is heated above its critical temperature and compressed above its critical pressure, it is called a supercritical fluid, which can be described as a very dense gas.



Supercritical fluid has unique properties, sharing elements from both the gas state and liquid state. For example, a supercritical fluid is very diffuse like a gas, making it an attractive extraction characteristic. Similar to a fluid, it has liquid-like solvation powers. By adjusting the temperature and pressure, the physical properties of a supercritical fluid can change, affecting its solvating powers and chromatographic abilities.

The History of Supercritical Fluids and Current Status

Supercritical fluids were first identified and characterized in the 1822 by Cagniard de la Tour, who was initially studying acoustics by rolling a marble into a "steam digester" device, which boiled and pressurized liquid in its barrel1. When the digester heated the liquid beyond the boiling point, Cagniard de la Tour noted that the marble no longer splashed within the liquid but seemed to bounce in the barrel of the digester. He went on to perform additional experiments to better define these observed phenomena with supercritical fluids.

Many other scientists performed various high-pressure experiments as well. 1891, Louis-Paul Cailletet create a manometer on the Eiffel Tower with a long tube of mercury extending 300 meters to generate immense pressures towards the bottom of the tube, pressure that could force a substance in the gaseous phase into the supercritical phase.

Fast forward past these early supercritical fluid devices and experiments and into the 1960s. Here, the idea of using supercritical fluids and packed columns was proposed by Jim Lovelock, the scientist who designed the gas chromatographic unit that was put into orbit to analyze the atmosphere of Mars in the 1970s for the Viking missions.

Unfortunately, the solvating strength of carbon dioxide was estimated as being more polar than it really was; this estimation stalled the technology from gaining widespread acceptance in the following decades. Supercritical fluid was still studied and utilized in the 1980s and 1990s, when open tubular column systems gave way to packed column systems, but pressure control was an issue. This resulted in a lack of sensitivity because the carbon dioxide could not be kept consistently in the supercritical state; researchers observed high background noise using optical detectors with the state of the technology at that time.

Despite the setbacks, a few pioneers continued to study the technology and make improvements on the pumping of the carbon dioxide and also developed improved back pressure control, which led to significantly improved performance.

Once refined, this unique chemistry proved to be highly valuable in the pharmaceutical field. Supercritical fluid chromatography has a unique ability to readily resolve optical isomers. Since the differing optical isomers of drugs may be pharmaceutically beneficial on one hand or toxic on the other, the identification of these isomers is critical early in the drug development process. More importantly, supercritical fluid can help purify and selectively collect the desired optimal isomer on an industrial scale.

Today, our industry has witnessed a resurgence of a supercritical fluid chromatography. With many applications in the consumer product testing, the SFC-SFE platform provides laboratories with a reliable, innovative means of performing crucial analysis.

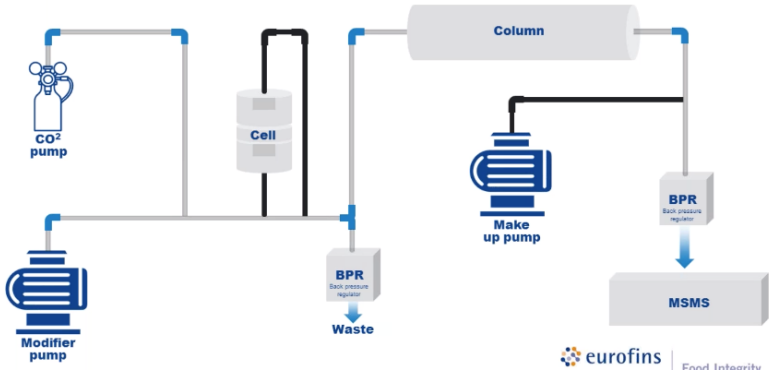
Instrumentation for SFE-SFC

Nearly two hundred years after the initial discovery of a supercritical fluid in the barrel of the "steam digester," instrumentation now offers a wide range of capabilities to support SFE-SFC. Eurofins uses the Shimadzu Nexera unified chromatography SFE-SFC instrument that can load 5 or 0.2 mL sample cells, depending on the capacity needed. The instrument is versatile, serving as an extractor- chromatographic unit, but can be used alone as a supercritical or liquid chromatographic unit. For detection, the system includes a tandem mass spectrometer and a diode array as currently configured.

At a high level, the system has several essential components. These include a carbon dioxide pump, a modifier pump, a makeup pump, two backpressure regulators and a column compartment

As carbon dioxide is delivered by the supercritical fluid pump, the modifier pump helps improve the solvating abilities of carbon dioxide with a flow of solvent. Modification is necessary because, by itself, carbon dioxide in the supercritical state is very non-polar and not a particularly good solvating reagent. To extract and chromatograph samples with increased speed and reliability, the carbon dioxide is altered by adding a modifier solution, such as an alcohol, water, acid or base to enhance the extraction or the chromatography of the analyte.

The first stage of the analysis process is the static extraction. In the static extraction process, the flow of the supercritical fluid and modifier will fill and pressurize the cell containing the sample. As the cell pressurizes, analytes in the sample matrix will be solubilized in the supercritical fluid.



Dynamic Extraction and the Role of Back Pressure Regulators

When the static extraction portion of the analysis is complete, the SFE unit switches to a mode called dynamic extraction. In this action, a valve is activated downstream from the cell allowing the flow of supercritical fluid and extracted analytes through the cell, to the analytical column. As an analogy, the static extraction can be compared to steeping a teabag in hot water, and the dynamic extraction can be compared to preparing coffee with a Keurig machine.

The two back pressure regulators are a crucial element of this system, not only for maintaining a supercritical state in the system, but also for setting sample dilution rates. The back pressure regulators positioned before and after the column can vary the load placed on the column. An increase in pressure of the regulator post column forces more of the sample extract to the waste path plumbed before the column. This setting is useful for samples containing a high concentration of analyte. Conversely, a lower post column back pressure regulator setting will allow more analyte to flow to the column. This setting would be appropriate for a trace level analysis.

Although the system is heavily automated, chemists still must apply their expertise to successfully set up conditions to ensure strong retention of the analyte onto the column during the dynamic extraction. If there is any elution of the compound during the extraction, the resulting chromatography will have broad or misshaped peak, leading to inaccurate quantitation. Therefore, chemists must determine a correct stationary phase and a correct content of modifier to apply during the extraction.

Once a sample has been extracted with the static and dynamic stages and analytes have been focused onto the column, the next step is analysis. In this state, the back pressure regulator prior to the column is set very high, forcing the entirety of the flow coming from the pumps to the column. The analytes are now eluted off of the column with an increasing concentration of alcohol from the modifier pump.

The Role of the Make-Up Pump

The make-up pump supplies a solvent flow introduced to the system downstream from the analytical column. This device would not be necessary when using an optical detector such as a Diode Array detector, since this detector can be plumbed within the supercritical flow path before the back pressure regulator.

However, if using a tandem mass spectrometer, it is impossible to keep the carbon dioxide in a supercritical state given that the detector operates in a strong vacuum. Therefore, once the supercritical fluid leaves the terminal end of the back pressure regulator, the carbon dioxide will return to the gaseous state where it will not be able to solvate the analyte and transfer it to the tandem mass spectrometer. The makeup pump is there strictly to supply a solvent to carry the analytes onto the mass spectrometer for analysis. This is also a good opportunity for adding modifiers to enhance ionization either with acidic components, basic components or a buffer.

The Speed of Sample Analysis

A typical analysis procedure for fat soluble vitamin testing with legacy methods would typically require at least two hours of hands-on time for steps such as weighing, saponification, solvent additions, physical extractions, partitions, reconstitutions and filtration. Once the completed sample extraction is loaded on a chromatography system, the instrument cycle time is typically 10-30 minutes per injection. The total time spent between weighing of the sample and the calculation of final results can easily be in excess of 24 hours.

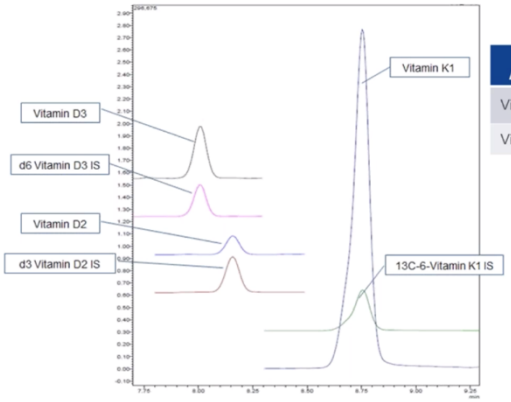
In contrast, SFE-SFC vastly simplifies the hands-on process to only about four minutes per sample. Here, the sample is weighed, hydrated with water, internal standards and alcohol then mixed with water-absorbent material. This mixture is transferred to the extraction cell and loaded on the instrument for a cycle time of about 15 minutes. From sample weighing to collecting a chromatogram, the total time is less than 20 minutes.

A Green Chemistry

Beyond the rapid analysis advantages, supercritical extraction also has an environmental benefit. The use of supercritical carbon dioxide is referred to as a "green" chemistry, since the use of hazardous and environmentally damaging solvents is eliminated. The analysis of compounds that are suitable for SFE-SFC by legacy methods require the use of solvents such as hexane, dichloromethane, ethers, toluene, THF and DMSO. There are inherent safety and environmental risks associated with the transport, usage, disposal and storage of these chemicals. When using SFE-SFC, these solvents are replaced by supercritical carbon dioxide, which leaves no waste: after extraction and analysis, the carbon dioxide is vented to a laboratory exhaust system. For modifiers, only the relatively benign alcohols are used.

Evaluating Vitamins, Patulin and Aflatoxin with SFE-SFC

SFE-SFC allows for highly sensitive analysis with small sample amounts. We evaluated this technology and performed in-house, single laboratory validation of vitamin D and vitamin K in supplements. Our team at Eurofins determined that the SFE-SFC-MSMS method produced results within the certified/reference value and range of certified reference material, achieving the same results as traditional AOAC method. In addition, a number of supplements were procured and analyzed via SFE-SFC-MSMS and legacy routine AOAC based methods. The results of the two techniques showed good correlation between average determined results and reproducibility.

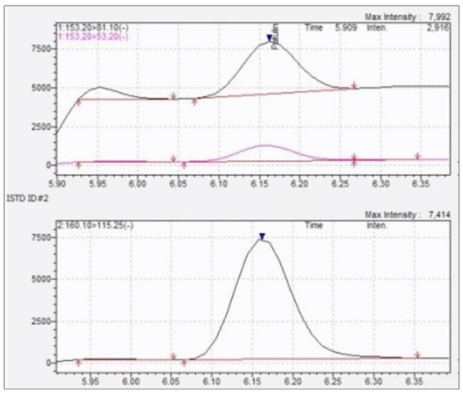


NIST 3280 Vitamin Tablet

Analyte	SFE-SFC-MSMS average result (µg/g)	Certified /Reference value and range (µg/g)	RSD
Vitamin D2	8.28	8.60 (6.0-11.2)	6.8
Vitamin K1	22.8	22.8 (20.6-25.0)	2.1

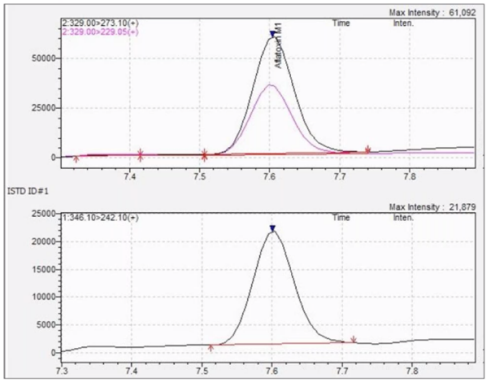
To further evaluate the potential of this technology, our team also examined SFE-SFC-MSMS analysis of two mycotoxins with unique analytical challenges.

Patulin is a mycotoxin found in rancid fruits, most notably in apples. This compound is an intriguing target analyte for SFE-SFC-MSMS analysis for several reasons. It is relatively polar, being readily soluble in water. Typically, polar compounds are not suitable for analysis by supercritical fluid methods. Also, the Patulin is a low molecular weight compound that ionizes predominantly with a negative mass spectrometer source; in our experience, this attribute creates challenges in detection and quantitation at trace levels. For our evaluation, we tested samples of apple juice that were fortified at the FDA action level and the European Union (EU) action level. At 50 parts per billion (ppb), we observed a very good recovery and a very good precision. In the case of the EU action level at 10 ppb, a good average recovery is observed, but the precision is less than desired. A possible solution to improve the precision would be an adjustment of the extract load placed on column with manipulation of backpressure regulator settings.



Fortification level	Average recovery	RSD
50 ppb	96.0	6.3
10 ppb	101	17.5

Testing aflatoxin M1 is another interesting application of SFE-SFC-MSMS as the action levels of this mycotoxin are very low at 0.5 ppb with the FDA and 0.025 ppb with the EU. In our experiment, aliquots of whole milk were fortified with aflatoxin M1 at the FDA and EU action levels. The results of this experiment were similar to patulin. The method accuracy and precision was quite good at the FDA action level, but not as reliable at the EU action level. Initial method modification to improve the performance at the EU action level would be the same as for patulin: manipulate the instrument to load more sample extract onto the analytical column.



Fortification level	Average recovery	RSD
0.5 ppb	91.2	1.8
0.025 ppb	96.2	19.7

The Promise of SFE-SFC

SFE-SFC serves as an efficient and environmentally-friendly technique to perform analytical separations and offers a resolution that can be difficult to achieve with gas chromatography or liquid chromatography. This innovative testing solution reduces the complexity of testing non-polar compounds and helps sponsors advance their products by offering a rapid turnaround time.

References:

[1] Berche, et al. Critical phenomena: 150 years since Cagniard de la Tour. J. Phys. Studies 13 (2009) 3201
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