



APPLICATION NEWS

Basic SFC Primer Part I

In this two part series we provide a Basic SFC Primer for the chromatographer. Supercritical Fluid Chromatography (SFC) has been utilized in various forms in the last 50 years and improvements in instrumentation and column technology have led to the growth of the technique over the last decade. The first part of this series will review the mobile phase characteristics, mobile phase modifiers/additives and basic column characteristics. Part 2 will cover the role of the stationary phase, column selection and sample considerations.

SFC advantages

SFC as a chromatographic technique excels in several areas over LC. One of the main advantages of SFC is the diffusion coefficient of solutes in the SFC mobile phases have been shown to be 3-10 times higher than in normal liquids potentially allowing for very rapid separations. Another SFC advantage is the viscosity of SFC mobile phases is significantly less than LC mobile phases producing much lower pressure drop across the column thus allowing the use of much smaller particles for both analytical and preparative applications. SFC excels particular well in the area of preparative chromatography where the SFC solvents are easily removed enabling the rapid recovery of isolated compounds.

SFC chromatography is an excellent orthogonal technique to reversed-phase HPLC because of its robustness and its relationship to normal phase LC. SFC when used with polar-bonded stationary phases is normal phase chromatography minus many of the problems inherent in normal phase LC. Such as retention time changes depending on very small amounts of polar compounds in the mobile phase.

Mobile Phase Characteristics

The most widely used mobile phase solvents for SFC is carbon dioxide. Super critical carbon dioxide can be easily obtainable at reasonable pressures and temperatures. The typical chromatographic operating pressures for carbon dioxide used in SFC range between 150 – 400 bar. The typical chromatographic operating temperatures are between 40 – 60 C. Carbon dioxide has several other favorable advantages including:

1. **Carbon dioxide has been described as a quadrupolar solvent because of its significant quadrupole moment. An excellent discuss of this topic: "Polar Attributes of Supercritical Carbon Dioxide" P. Raveendran, Y. Ikushima and S. L. Wallen , Accounts of Chemical Research 2005 v38 p478-485**
2. **The potential to act as both a weak Lewis acid and Lewis base, it can participate in conventional or nonconventional hydrogen bonding interactions.**
3. **Its diffusion coefficient of solutes is 3 – 10 times higher than in liquids used routinely in LC.**

4. **Its viscosity of SFC mobile phases is significantly less than LC mobile phases producing much lower pressure drop across the column thus allowing the use of much smaller particles for both analytical and preparative applications.**
5. **Miscible with a wide range of organic solvents.**
6. **Non-flammable.**
7. **Low UV absorbance at low wavelength.**
8. **Carbon dioxide is easily removed enabling the rapid recovery of isolated compounds - plus CO₂ is considered non-toxic regarding any residual amounts.**
9. **Carbon dioxide used for SFC is recovered from the atmosphere making it a “Green” solvent.**

Mobile Phase Modifiers/Additives

A number of different co-solvents or mobile phase modifiers can be added to carbon dioxide in order to enhance separation characteristics. Methanol and ethanol represent some of the more popularly used co-solvents. These types of solvents improve the solvating power of carbon dioxide. Other co-solvents or modifiers that are used with carbon dioxide include acetonitrile, methylene chloride, chloroform and isopropanol. Generally, these types of modifiers are used to improve the solubility of compounds in the carbon dioxide SFC mobile phase.

In many cases acidic, basic and amphoteric compounds don't elute or are eluted with very poor peak shapes even with binary solvent mixtures of carbon dioxide and methanol or ethanol in these cases an additive must be employed. The additive will hopefully improve peak shapes or enable chromatographic elution. Additives that are commonly used include water, acetic acid, ammonium acetate, trifluoroacetic acid (TFA), methyl amine, ammonium hydrogen carbonate and triethylamine. It should be noted that water can be used in small amounts however there is a risk of ice formation at the outlet of the column. Ice formation leading to column plugging is a particularly unsafe condition for preparative SFC applications. Additives are an active area of research for the SFC chromatographer with the rapid introduction of different additives.

SFC Columns/SFC Stationary Phases

Chromatographic column must be specifically designed for use in SFC. The column must be designed to withstand high pressures at both inlet and outlet end because of the use of back pressure regulators to maintain SFC conditions in the chromatographic system. The amount of elastomeric materials in the column should be minimized to guarantee leak free column performance. The hardware used for SFC preparative columns is particularly challenging because pressure on both the inlet and outlet ends. A frit failure or bed failure in a preparative SFC column can project packing material into the preparative instrument. In addition preparative columns can be easily damaged by rapid CO₂ decompression. At ES Industries we pay a lot of attention to hardware quality and packed bed stability for all of our GreenSep SFC columns.

The stationary phase plays a key role in SFC separations and ES Industries is one of the leaders in the development of SFC specific stationary phases with the introduction of the GreenSep SFC columns. One of the more important GreenSep columns developed at ES Industries is GreenSep Basic. GreenSep Basic is based on imidazole chemistry providing a highly basic character for this stationary phase. The chromatogram shown below is a prime example of the superior peak shape performance and separation capacity obtainable with the GreenSep Basic column with SFC. All GreenSep SFC columns have been developed specifically for use in SFC. The GreenSep base silica is metal free ultra high purity chromatographic media that is pressure stable and specifically engineered for high performance SFC separations. The surface is treated to produce maximum SFC separation interactions and loading capacity while maintaining superior peak shape performance for many chemicals. GreenSep Basic is available for analytical and preparative column formats in particle sizes from 1.8 μ m – 20 μ m.

In the next installment we go into the variety of GreenSep polar bonded phases specifically developed for Achiral SFC. In addition, we go into a wide variety of carbohydrate based chiral stationary phases that we have developed at ES Industries for chiral SFC separations. The next installment will also include a discussion on the development of an Achiral SFC column screening kit.

