



This is part two of a two part series to 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. In the first part of this series we reviewed mobile phase characteristics, mobile phase modifiers/additives and basic column characteristics. In this second part we will cover the role of the stationary phase, column selection and sample considerations.

### Characteristics of a SFC Stationary Phase

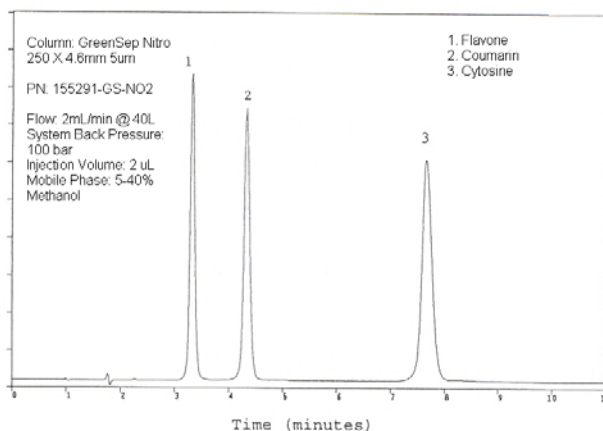
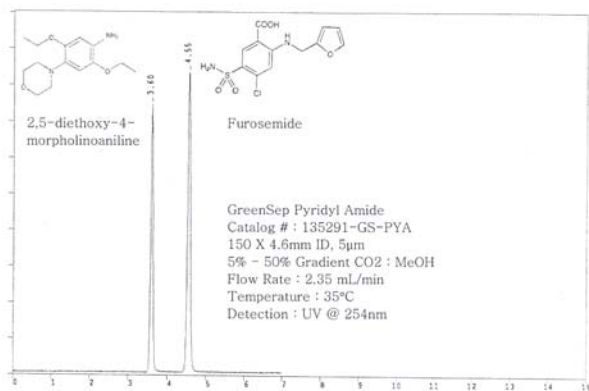
SFC chromatography is an excellent orthogonal technique to reversed-phase HPLC because of its robustness and its relationship to normal phase LC. When used with polar-bonded stationary phases SFC is normal phase chromatography minus many of the problems inherent in normal phase LC such as retention time changes depending on very small amounts of polar compounds in the mobile phase. Some of the key characteristics for a SFC stationary phase include selectivity, interaction with analytes to elute symmetrical peaks. Unfortunately, many SFC separations have been forced to utilize older types of stationary phases from "normal phase" HPLC such as unmodified silica, diol, amino and cyano. These phases are poorly adapted to SFC and present a number of limitations for SFC separations. Limitations include: low capacity, poor selectivity, and poor peak shape for SFC separations. To overcome these limitations we have developed a new line specifically engineered SFC stationary phases called GreenSep SFC. GreenSep stationary phases have proven to be superior to conventional stationary phases (such as diol, cyano etc...) in the areas of separation selectivity, peak shape and loading capacity. The chromatograms shown below are prime examples of the superior peak shape performance, selectivity and loading capacity obtainable with the GreenSep with SFC columns.

### Column Selection

The development of new GreenSep SFC stationary phases has been a key area of development for ES Industries. ES Industries is pleased to present a list of our GreenSep SFC column chemistries that have shown unique selectivity. All of these materials are available in sub-2micron, 5um, 7um, 10um, and 15um particle size. Columns from 1mmID (various analytical sizes) to 100mmID (various preparative sizes) are available. Bulk packing of 7um or higher is also available.

**GreenSep Ethyl Pyridine** - SFC separation of amines would normally require the addition of an amine to the mobile phase; however **GreenSep Ethyl Pyridine** does not require the addition of these peak shape modifiers. Mobile phase composition and fraction collection is greatly simplified without the use of amino additives. **GreenSep Ethyl Pyridine** is the SFC column of choice for the retention and rapid separation of chemical containing strong amine groups.

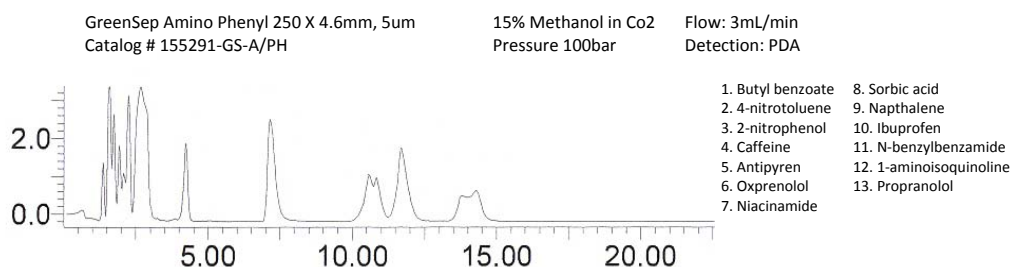
**GreenSep Pyridyl Amide** – Can separate compounds functionalized with both amine bases and acidic groups. Mobile phase composition and fraction collection is greatly simplified without the use of amino or TFA additives.



**GreenSep Nitro** - Specifically designed for the separation of geometrical isomers as well as diastereomers. It is the column of choice in separating compounds that contain aromatic group, polarizable electrons and conjugate systems.

**GreenSep PFP** - Specifically designed for the separation of geometrical isomers as well as diastereomers. It is the column of choice in separating compounds that contain aromatic group, polarizable electrons and conjugate systems. In addition, **GreenSep PFP** is useful for the separation of halogenated compounds. In many cases **GreenSep PFP** provides orthogonal separations when compared to **GreenSep Nitro**.

**GreenSep Amino Phenyl** - Specifically designed for the separation of amines, alcohols and acids by SFC without the use of additives.

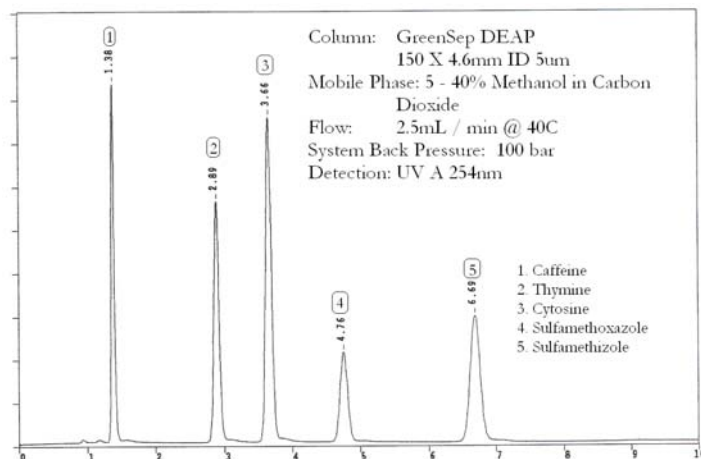


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**GreenSep DEAP** - Many SFC separations require the addition of amine modifiers to the mobile phase to improve peak shape however, DEAP does not require the addition of these peak shape modifiers. Mobile phase composition and fraction collection is greatly simplified without the use of amino additives. It has greater retention for amines than **GreenSep Ethyl Pyridine**.



**GreenSep Silica** - This is a 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 Silica is available for analytical and preparative column formats in particle sizes from 1.8um ^ 20 um.

**GreenSep Basic** - A phase based on imidazole chemistry providing a highly basic character for this stationary phase. GreenSep Basic is the SFC column is ideally suited for the retention and rapid separation of chemicals containing amine groups. GreenSep Basic has less retention than GreenSep DEAP but more than GreenSep Ethyl Pyridine.

### Sample Considerations

Many of polar compounds show poor retention by reverse phase HPLC, however many of these small polar molecules are an excellent match for separation via SFC. The range of molecules separated using SFC is rapidly increasing including peptides and proteins. SFC is an excellent preparative separation technique. Preparative separations can be performed quickly and the carbon dioxide mobile phase is easily removed. An important consideration for SFC is sample solubility. Care should be taken with large injection volumes particular for preparative SFC. In these situations the injected sample can “crash out” plugging the column and shutting down the chromatographic system. There are several possible ways of avoiding “crash out” including the use of a mobile phase co-solvent such as acetonitrile or methanol, mixing the injected sample with solvent that is more compatible with carbon dioxide.

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