SFC Without Additives - An Imidazole Based Stationary Phase Designed for the Task

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Introduction

Preparative supercritical fluid chromatography (SFC) is a powerful tool for the purification/isolation of both chiral and achiral compounds. Many chemicals can potentially be used as a supercritical mobile phase in SFC, however virtual all current practitioners of SFC use CO2 which offers several advantages, particularly when compared to preparative liquid chromatography. CO2 has the potential to act as both a weak Lewis acid and Lewis base, and it can participate in conventional or nonconventional hydrogen bonding interactions. In addition, it is miscible with a wide range of organic solvents, nonflammable, and has little UV absorbance at lower wavelengths. CO2-based SFC is particular well suited to the area of preparative chromatography where it can be easily removed after fractionation, enabling the rapid recovery of isolated, pure compounds. In addition, any residual amounts of CO2 in isolated products are considered to be nontoxic.

One of the drawbacks of using CO2as a mobile phase in SFC is that it is relatively non-polar even though it has been described as a quadrupolar solvent because of its significant quadrupole moment. In order to modify the elution strength of CO2 to allow wider use with molecules of increased polarity, organic solvent modifiers are mixed into the CO2 stream using a second high pressure HPLC pump. Commonly used modifier solvents are methanol or ethanol, but other organic solvents and solvent mixtures are also used. Because of the non-polar behavior of CO2 the stationary phase plays a very key role in SFC separations. In addition, many SFC separations require the use of additives such triethylamine in order to diminish peak tailing and maintain acceptable retention factors, particular when separating amines. Additives are difficult to remove and potentially alter the chemical properties for compounds being purified and isolated using SFC. As a result of these concerns, the use of additives is discouraged when the purifying and isolating compounds using SFC. In recent years SFC optimized stationary phases have been developed to avoid the use of mobile phase additives while delivered desired chromatographic performance of ionizable compounds.

Results

We have developed an imdazole based stationary phase GreenSep™ Basic – imidazole based stationary phase.

These examples were chromatographed using the newly introduced Shimadzu Nexera UC SFC system. The Shimadzu Nexera UC system had a fixed wavelength UV detector and a 5 µL fixed injection loop. The operating conditions for the examples are contained in the figures.

Structure for GreenSep Basic

The first example is a comparative example to illustrate the chromatographic selectivity of GreenSep Basic for the separation of closely related caffeine analogs (Figure 1).

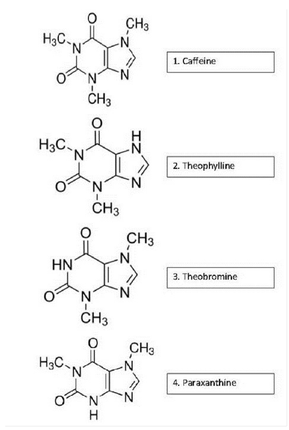


Figure 1 – Caffeine Analog Mixture Structures

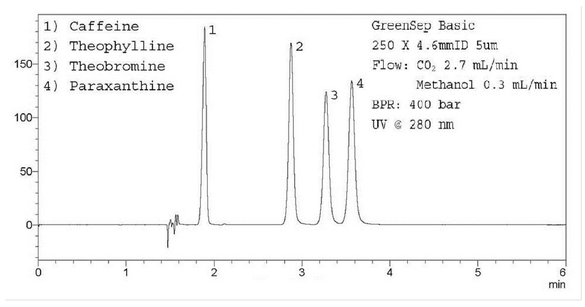


Figure 2 shows the excellent peak shape for a strong amine containing compound. Amitriptyline – a strong amine that is sensitive to silanol activity. Used extensively in HPLC to measure silanol activity.

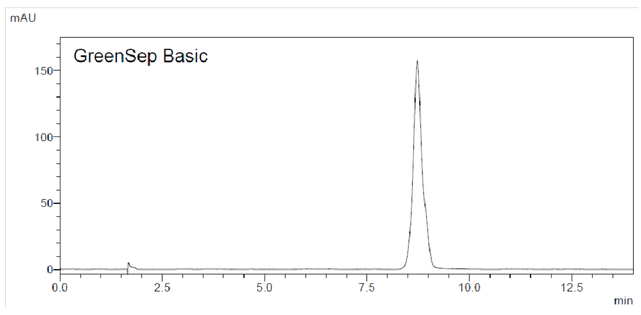


Figure 3 shows the excellent peak shape without additives for a wide variety of strong amine containing compounds.

Peak #	Name	Structure	USP Tailing	Half width peak efficiency per column
1	N-[(E)-phenylmethylidene]naphthalen-1-amine		1.2	11981
2	Noscapine		1.2	11281
3	Trimipramine		3.0	4432
4	Amitriptyline		2.8	4932
5	Clomipramine		3.3	2713
6	1-aminoisoquinoline		1.5	9364
7	Nortriptyline		1.7	7297

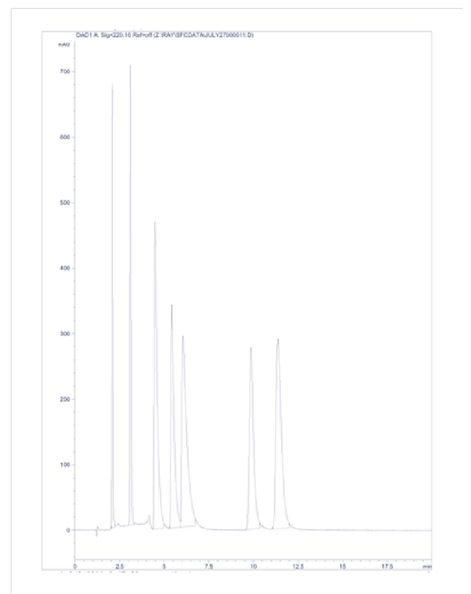
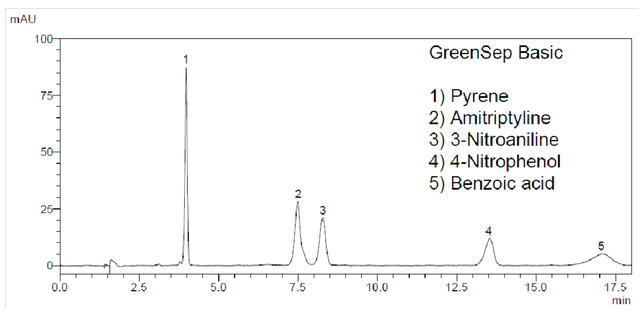


Figure 4 shows a mixture of amines and other funtionalized compounds.



Conclusions

We have developed an imdazole based stationary phase that provides excellent peak shape for amine containing compounds without mobile phase additives while providing superior selectivity.