Development of novel stationary phases for Supercritical Fluid Chromatography (SFC)

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Abstract

The introduction of bonded phases such as the 2-ethylpyridine phase has expanded the use of supercritical fluid chromatography (SFC) for achiral applications with the Pharmaceutical Industry. Its application in SFC separations for basic solutes can be achieved without utilizing a basic additive (e.g. triethylamine) to reduce the peak tailing and retention times of polar, basic solutes. This translates to a simplified mobile phase solvent system consisting of CO₂ and alcohol(s) which can minimize ultraviolet (UV) detector noise and enhances mass spectrometric (MS) detection. However, the 2-ethylpyridine phase does not exhibit the same compatibility with acidic solutes, which separate better on bare silica or (1, 2)-propanediol phases.

In order to examine the effects the phase has on selectivity, this paper describes the preparation of several novel phases: 5-Hydroxy-3-pyridinyl, 3-Hydroxyphenyl, (3, 4)-Dihydroxypyranyln, and 2-ethylpyrazinyl and presents a comparison of small molecule selectivity to that of similarly prepared 2-ethylpyridine and (1, 2)-propanediol phases.

Experimental

Sample Preparation

Each commercial compound was dissolved in methanol to approximately 1-mg/mL. The Library Database, version 12.01 (Advance Chemical Development, Toronto, Canada).

Physical chemical data for the compounds and phases was calculated using ACD Labs PhysChem 300.

Figure 1: Separation of the 3 acidic components on each phase.

Figure 2: Separation of the 3 basic components on each phase using a methanol gradient 20%-50% @ 0.5/min

Discussion

Since the most problematic separations in SFC usually involve the interaction of a charged functional group on the analyte with the stationary phase of the chromatographic system, each column was evaluated against both acidic and basic compounds. In Figure 1, the separation of 3 acidic compounds is demonstrated, with the best overall separation occurring on the 3-hydroxy- and 3,4-dihydroxy-phenyl phases. There also appears to be slightly higher selectivity of flurbiprofen (pKa = 4.14) and ketoprofen (pKa = 4.23) on the pyridine phase relative to the pyridyl phase. This may be due to difference in pKa’s of most basic nitrogen atoms of the pyridine (pKa = 3.6) and pyrazine (pKa = 0.76, 2.05) rings as calculated using ACD Labs software.

Conclusions

Chromatographic selectivity and performance can be enhanced for acidic and basic compounds by modifying the column chemistry under the sample mobile phase composition. This approach will enable separations while eliminating the need for mobile phase additives which can complicate purification, interfere with detector functionality or reduce sensitivity.