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 CO_2 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)-Dihydroxyphenyl, and 2-ethylpyrazinyl and presents a comparison of small molecule selectivity to that of similarly prepared 2-ethylpyridine and (1, 2)-propanediol phases.

Introduction

Highly polar compounds (e.g. acidic or basic compounds) usually require a modifier additive to improve elution in SFC, which can also potentially reduce the stability of samples or complicate the interfacing to MS detectors that utilize spray type inlets. However, if the stationary phase contains an appropriate functional group, the modifier additive is no longer needed. In many cases, the presence of an acidic or basic center on the phase tends to affect peak shape caused by excessive interaction with the phase. The goal of this study is to study several novel phases suitability for acids and bases selectivity, while maintaining acceptable peak shape and utilizing a simple mobile phase.

Experimental

Sample Preparation

Each commercial compound was dissolved in methanol to approximately 1-mg/mL. The Library compounds used were originally prepared to 30mM in DMSO and subsequently diluted in methanol to approximately 250 μ M.

Instrumentation

The SFC/MS system used in this experiment is a customized supercritical fluid chromatograph, configured from instruments obtained from several manufacturers. The mass spectrometer used is a single quadrupole LC/MSD with an APCI source (Agilent, Palo-Alto, CA). A Berger analytical supercritical fluid chromatograph consisting of a dual pump fluid control module (FCM-1200), thermal control module (TCM-2000), and an Agilent 1100 DAD (Thar Instruments, Pittsburgh, PA) was used. The SFC system is also connected to a CTC HTS PAL autosampler (Leap Technologies, NC). The CTC HTS PAL was equipped with a 25 µL syringe and 20 µL fixed loop and the control method includes a routine to vent the loop of liquid CO_2 prior to sample introduction to prevent sample loss.

All data were acquired using Agilent 32-bit ChemStation™ (version B.03.01 [317]) in combination with Berger SFC Massware MSD™ software (version 5.4) from Thar Instruments Inc. The effluent of the SFC is split to the MSD using a tee (Valco, Houston, TX) and PEEKsil capillary tubing (Upchurch Scientific, Oak Harbor, WA); 50 cm long with an internal i.d. of 50 µm.

Physical chemical data for the compounds and phases was calculated using ACD Labs PhysChem Database, version 12.01 (Advance Chemical Development, Toronto, Canada).

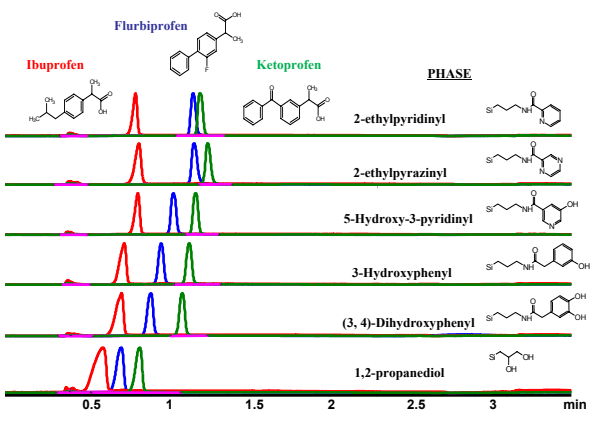
Analysis Conditions

Analysis was performed using 4.6mm x 150mm prototype COSMOSIL (Nacalai USA, Inc., San Diego) and a ZymorSPHER 1,2-propanediol SFC columns (Zymor, Inc., Wayne, NJ) with 5 µ particle and 100Å pore sizes. The flow rate was 5.6 mL/min with the outlet backpressure set to 140 bar. The injection volume was 15 uL. The mobile phase composition was varied from 5% to 50% @ 18%/minute and the column temperature was at ambient, unless otherwise noted.

Discussion

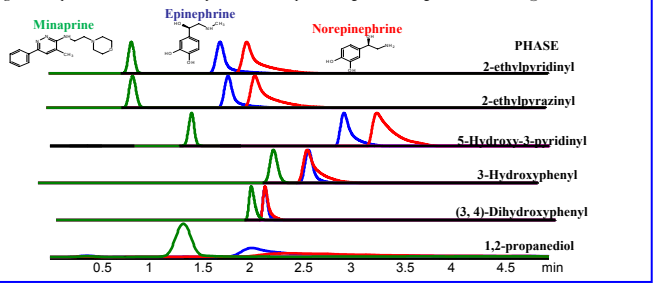
Since the most problematic separations in SFC usually involve the interaction of a charged functional group on the analyte with the stationary phase of the chromatographic system, each column was evaluated against both acidic and basic compounds. In Figure 1, the separation of 3 acidic compounds is demonstrated, 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 fluribiprofen (pKa = 4.14) and ketoprofen (pKa = 4.23) on the pyrazinyl phase relative to the pyridinyl phase. This may be due to difference in pKa's of most basic nitrogen atoms of the pyridinyl (pKa = 3.6) and pyrazinyl (pKa = 0.76, 2.05) rings as calculated using ACD Labs software.

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



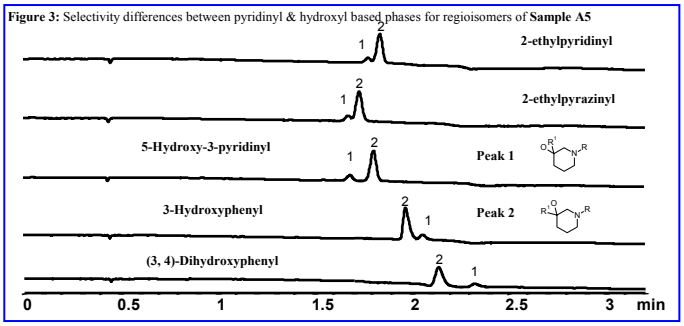
The peak distortion of ibuprofen is likely due to the low retention on all columns and appears lower on the pyridinyl based phases. The basic nature of the phase may contribute to the shielding of residual silanol activity diminishing the contribution of hydrogen bonding on the carboxylate (pKa = 4.41). Interestingly, the 5-Hydroxy-3-pyridinyl phase has acceptable separation of all three acids and the best peak shape for ibuprofen.

Figure 2: Separation of the 3 basic components on each phase using a methanol gradient 20%-50% @7%/min



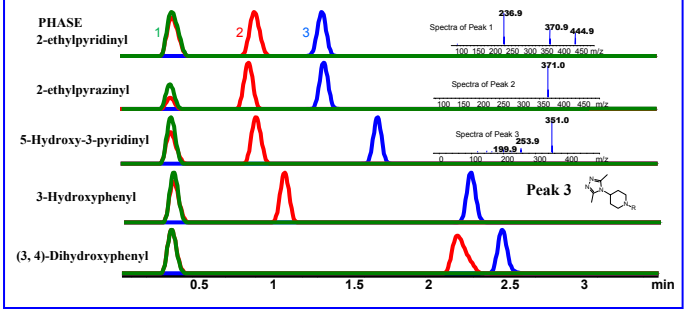
In Figure 2, the separation of 3 basic compounds is demonstrated, with the best overall separation occurring on pyridinyl based phases. The basic compounds continue to exhibit tailing on all columns, but the pyridinyl phases demonstrate suitable selectivity for epinephrine and norepinephrine. The addition of ammonium formate or basic additives to the modifier does improve peak shape (not shown).

Figure 3: Selectivity differences between pyridinyl & hydroxyl based phases for regioisomers of **Sample A5**



Figures 3 & 4 are example chromatograms from the analysis of 64 Library compounds acquired on each column and highlight the selectivity differences between phases under the same conditions. In Figure 3, well A5 shows a peak order reversal between the target component (peak 2) and a major impurity (peak 1) on the pyridinyl and hydroxy-phenyl phases. Figure 4 shows the normalized extracted ion chromatograms (EIC) of the major components of well A11 which shows dramatic retention of the target analyte (peak 3).

Figure 4: Normalized EIC chromatogram of library sample A11 on each phase. **Inset:** mass spectra for each peak



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.