CHIRAL SEPARATIONS USING A MODIFIED WATER STATIONARY PHASE IN SUPERCRITICAL FLUID CHROMATOGRAPHY

by

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ABSTRACT

A novel means of achieving chiral separations in supercritical fluid chromatography (SFC) using a water stationary phase is presented. By adding various chiral selectors to the phase, different chiral analytes can be readily separated using neat CO_2 as a mobile phase. For example, by adding β-cyclodextrin it is found that certain flavanone enantiomers can be separated, while using the antibiotic vancomycin as a selector provides separation of some chiral phenoxypropionic acids. Other additives such as sodium chloride and triethylamine are also explored and found to enhance certain separations when also present in the water phase. While column pressure has a moderate impact on chiral analyte retention and separation in this SFC method, column temperature has a comparatively larger influence. In particular, relatively cooler temperatures below about 5 ^{o}C are found to markedly increase resolution and selectivity. For instance, notably large resolution of 4.7 is achieved for a phenoxypropionic acid pair at 0 ^{o}C and 150 atm CO_2. Since the method does not require modifier to elute such polar species, it is also readily compatible with FID detection and does not generate organic waste. Therefore, results indicate that this approach could be a potentially simple and flexible means of achieving chiral separations in SFC.

Keywords: supercritical fluid; chromatography; chiral; water; stationary phase

INTRODUCTION

Supercritical Fluid Chromatography (SFC) is a useful separation technique that is complimentary to conventional Gas (GC) and Liquid (LC) Chromatography [1, 2]. The most commonly used mobile phase in SFC is supercritical CO_2, owing to its environmental compatibility, modest critical point, available purity, low cost, and non-toxicity [1]. As well, it is readily compatible with the versatile Flame Ionization Detector (FID), which is widely sought for its universal sensitive response towards organic analytes [3]. Accordingly, SFC-FID has been employed in a variety of applications and can greatly facilitate the analysis of molecules that do not respond in conventional UV absorbance or light scattering detectors [4]. However, supercritical CO_2 is non-polar and often ineffective at eluting polar analytes in SFC [1]. One route to address this is adding a polar organic modifier (e.g. methanol) to the mobile phase to improve the solubility and separation efficiency of such analytes [5]. While this greatly facilitates the resulting peak shape and SFC performance, it reduces the environmental advantage of the technique and disables usage of the FID in such separations due to the high background response of the modifier, even at low concentrations [5].

Recently we introduced a novel capillary SFC method that employs water as a stationary phase and CO_2 as a mobile phase [6]. The technique establishes a water stationary phase on the inner wall of an otherwise uncoated stainless steel (SS) capillary, and provides stable system operation over the wide range of temperatures and pressures used in SFC [6]. The method produces partition-based separations that exhibit a normal-phase retention pattern, and yields good peak shapes for polar analytes using only CO_2 as the mobile phase [6, 7]. This results in a greener, FID-compatible SFC approach to such separations. As well, the system provides useful efficiency and selectivity for polar analytes over non-polar analytes that can lead to highly

simplified analyses [6–10]. Given its unique attributes, further development of this water stationary phase SFC method would be useful.

Chiral chromatography is an important tool in analyzing optical isomers that is of great interest to many research areas including pharmaceuticals [11], flavor and aroma [12], and agrochemicals [13]. Selectivity in such separations normally relies on interactions with a chiral selector that can discriminate between enantiomers [14]. Since an enormous range of applications rely upon such separations, numerous chiral stationary phases have been developed for chromatography using different selectors [15]. For example, due to their natural chirality, various cyclodextrins are often used as selectors in separations [16, 17].

In SFC, packed columns composed of chiral selectors bonded to stationary phase support particles (e.g. silica) are commonly employed in this area [18], and chiral SFC is well known to provide beneficial properties of a CO_2-based mobile phase in such separations [18]. However, most of these SFC endeavours still require using moderate to high proportions of organic modifier to improve separations and overcome deleterious analyte interactions with silica supports [19]. Beyond this, selector choice and method development in chiral separations is also generally a cumbersome trial-and-error process, where such phases are often expensive, difficult to manufacture, and/or fragile in use [15, 18, 20]. Thus, novel SFC methods that can help address these issues are needed.

In this regard, it would be interesting if the water stationary phase SFC method could be developed for chiral separations through adding a chiral selector to the aqueous stationary phase. While this has not yet been investigated, such additions to mobile phases are well known to enable chiral selectivity [21], and some analogous stationary phase examples have also been reported. For example, cyclodextrins embedded in polysiloxane coatings have conventionally

provided chiral separations in capillary GC [22]. Likewise, cyclodextrins dissolved in liquid film coatings on different stationary phase particles have been used in packed GC and LC separations [23–25]. Thus, such an approach could potentially be beneficial in SFC. This paper investigates the use of chiral additives to a water stationary phase in capillary SFC for the separation of enantiomeric analytes. The effect of chiral selector, additives, and various operating conditions on enantioselective separations are presented, and the general characteristics and flexibility of this approach in SFC chiral method development are discussed.

EXPERIMENTAL

Instrumentation and Operation

The apparatus used has previously been described in detail [6]. Briefly, CO_2 was introduced to the system using an ISCO model 260D syringe pump (Teledyne ISCO, Lincoln, NE, USA) operated in constant pressure mode. The pump outlet was connected to 1.7 m of a SS preheating coil (1/16 in. O.D. x 0.010 in. I.D.; VICI Valco, Brockville, Canada) situated in an HP5890A GC oven (Agilent Technologies, Mississauga, Canada) used to control temperature. This was then led outside the wall where it connected to a model C74H internal sample injector fitted with a 20 nL sample loop (VICI Valco).

Separation columns were made using 316 SS tubing (1/16 in. O.D. x 0.01 in I.D.; VICI Valco). Various lengths (30 to 100 ft) were examined, and 50 ft was most often used. The column inlet was connected to the injector and then coiled inside of the GC oven in normal use. For certain sub-ambient separations, the column and preheating coil were instead led into a Polyscience model 1162 refrigerated circulating bath (VWR International, Edmonton, Canada) adjacent to the GC. A 1/16 in. zero dead volume SS union (VICI Valco) and PEEK sleeve

connected the column outlet to a fused silica capillary restrictor (20 cm x 50 µm I.D.; Polymicro Technologies, Phoenix, AZ, USA) that was used to maintain system pressure. The restrictor normally had a 2 cm taper fabricated on the outlet and under typical conditions (e.g. 30 ^{o}C and 80 atm of CO_2) it yielded an average column flow rate near 75 µL min^{-1} or 2.5 cm s^{-1}. This enabled several hours of stable system operation without any bleeding of the chiral selector into the FID. Occasionally, the restrictors would plug due to erosion of the fused silica from contact with water at elevated detector temperatures and would be replaced as necessary. This could also be avoided by using stainless steel restrictors as well. Where possible in direct comparisons, restrictors yielding similar flows were used. The restrictor outlet was situated inside the FID jet about 1 cm below the burner surface. The FID gas flow rates used were 210 mL min^{-1} of medical-grade air (Praxair, Calgary, Canada) and 40 mL min^{-1} of hydrogen (Praxair). The detector block temperature was held constant at 350 °C.

Stationary Phase Preparation

Chiral selectors were used as received and weighed to prepare stock solutions in HPLC-grade water. Typical concentrations were 8-16mM for the cyclodextrins (1-2% w/v) and 20-30mM for vancomycin as discussed in the text. Sodium chloride was normally added (0.1-1 M) to these solutions. In some trials as noted, 72mM (1% v/v) triethylamine (made in equimolar HCl for neutral pH) was also added. These solutions were then used to coat the SS capillary column as described previously [6] and it was mounted inside the GC oven or circulating bath for use.

Chemicals and Reagents

Instrument grade CO_2 (99.99%; Praxair) and HPLC grade water (Honeywell Burdick & Jackson, Muskegon, MI, USA) were used. Standard analyte solutions (20-30mg mL^{-1}) were prepared in acetone (≥99.5%), ethanol (≥99.5%) or 1-propanol (≥99%; all Merck, Dermstadt,

Germany). Hexanes (≥98.5%; Merck) were often added at 1% v/v as a void marker. Native β-cyclodextrin (≥97%), 2-(hydroxypropyl)-β-cyclodextrin (0.7 molar substitution ratio), methyl-β-cyclodextrin (1.6-2.0 molar substitution ratio) and vancomycin hydrochloride (CRM 92.7%; all Sigma, Oakville, Canada), triethylamine (99%; BDH, Toronto, Canada) and sodium chloride (≥99%; Merck) were used as additives. Racemic mixtures of 2'-hydroxyflavanone (≥98%), flavanone (≥97.5%), 2-phenoxypropionic acid (≥98%) and 2-(3-chlorophenoxy)propionic acid (≥98%; all Sigma) were used as test analytes. As an application, 2'hydroxyflavanone was spiked (20 mg mL^{-1}) into a commercial bee propolis sample purchased locally. The sample was diluted 3-fold in ethanol to reduce viscosity for injection. In another application, 2-(3-chlorophenoxy)propionic acid was added (20% v/v, 40mg mL^{-1}) to a commercial apple juice sample obtained from a local grocery store. All other details are given in the text.

RESULTS AND DISCUSSION

Chiral Selector and Stationary Phase Optimization

Given their notable versatility as chiral selectors, cyclodextrins were first explored in the water stationary phase SFC system. Different chiral flavanone test analytes were also employed, since they have been separated previously using cyclodextrins [26, 27]. Initially, various beta-cyclodextrin (βCD) derivatives were surveyed for their ability to provide chiral selectivity for 2'hydroxyflavanone (2'HF) isomers. As seen from the typical results shown in figure 1, when using only water as the stationary phase (figure 1a), this analyte was modestly retained in the SFC system consistent with its limited water solubility, but the isomers were not separated. When a methylated βCD derivative was added to the phase as a selector (figure 1b), the 2'HF analyte was found to be significantly more retained, but still no separation of the enantiomers

was observed. Using a 2-hydroxypropyl βCD derivative as a selector (figure 1c) was found to retain 2'HF less so than the methylated analog, but it did provide some modest chiral selectivity. Finally, similar results were also found for an unmodified native βCD (figure 1d), but a slightly smaller retention was noted. Given this, the latter two selectors were further examined.

In an effort to improve the separations, the addition of sodium chloride to the water phase was next examined, since salt has shown significant effects on native βCD complexation of solutes [28]. This was initially probed with low concentrations (150 mM) of sodium chloride in the water phase, as others have used [24]. Surprisingly, salt had very little effect on analyte peak shape and separation for the 2-hydroxypropyl βCD selector. In fact, efforts to further increase the concentration of salt or selector also failed to improve the separation shown in figure 1c. Therefore this selector was not pursued in subsequent trials.

By contrast, the same salt addition noticeably improved peak shape and separations for the native βCD selector. However, the results were inconsistent and varied considerably. To address this, salt concentrations up to 1M were examined and they greatly improved peak shape and resolution reproducibility, with retention times that varied only 1% RSD from run to run. In exploring other salts (e.g. magnesium, calcium, or nitrate) little difference was observed, and levels above 1M did not show further benefits. Thus, 1M sodium chloride was typically added to the water phase in subsequent explorations with the native βCD selector. The concentration of native βCD present was also examined up to a 2% w/v load. It was confirmed that 2'HF enantiomeric resolution improves for higher selector loads by providing greater retention with similar selectivity. As such, 2% w/v native βCD yielded the best chiral separation and was used subsequently. Larger levels could not be probed due to its limited water solubility.

Therefore, by exploring the various selectors and stationary phase conditions above,

chiral resolution of the 2'HF pair could indeed be achieved with the water stationary phase SFC system, as demonstrated in figure 2a. This highlights a flexibility of this approach, which allows a range of selectors and other additives to be screened for their separation properties quickly and inexpensively, without need for organic solvents or lengthy cross-linking procedures.

Operating Characteristics

In addition to stationary phase effects, SFC system conditions were also probed for their influence on the 2'HF separation. For instance capillary lengths from 30 to 100 feet were evaluated since this is easily done given the relatively low cost of the SS tubing used. While the 100 foot column gave the best resolution, it also yielded the longest analysis time. Since a 50 foot length offered a reasonable compromise in speed and efficiency it was used going forward.

The effect of mobile phase pressure on separation was also confirmed. Under the typical mild SFC conditions used (e.g. 30 ^{o}C and 80 atm) even small pressure increases resulted in significant loss of retention and resolution mainly due to the increased mobile phase density. For example, increasing the pressure from 80 to 90 atm at 30 ^{o}C produced about a 20% loss in retention and resolution of the 2'HF chiral pair. This is expected in the highly compressible region near the CO_2 critical point and is useful for controlling retention.

Varying temperature also yielded a similar effect near the critical region (i.e. 25-50 ^{o}C). For example, reducing the temperature by 10 ^{o}C at 80–90 atm similarly decreased analyte retention due to the increased mobile phase density, and reduced resolution by 30-40%. Conversely, the greater resolution and retention found at higher temperatures could also be offset by higher pressures. Thus, similar separations were realized at 30 ^{o}C and 80 atm (figure 2a) and 50 ^{o}C and 110 atm (figure 2b). As seen in each, the analytes are resolved in about 25 minutes, which compares well with chiral LC methods using βCD selectors for 2'HF [29].

Incidentally, notable differences were found at much lower temperatures. In particular, below about 15 °C analyte retention stopped reducing and began to increase again, so much so that it warranted higher pressures for shorter analysis times. This is likely due to an increase in complexation strength between the analyte and cyclodextrin selector, which has been observed with low temperature mobile phases containing native βCD [30]. Even more, after changing relatively little over the temperatures explored, chiral selectivity also unexpectedly increased dramatically below about 5 °C. Of note, selectivity for the 2'HF chiral pair increased from 1.3 near 25 ^{o}C to 2.5 at 0 °C. The Van't Hoff plot for the separations (figure 3a) illustrates this and clearly shows that selectivity sharply increases at cooler temperatures, which may indicate a different mechanism contributing toward enantioselectivity of the phase under such conditions. Figure 3b illustrates how retention changes over the same range for comparison and again reflects this selectivity increase. A 2'HF separation at 5 ^{o}C is demonstrated in figure 4a, which shows good selectivity and retention for these enantiomers despite using a larger mobile phase pressure.

Interestingly, low temperatures with the native βCD selector had an even greater effect on the separation of flavanone, a related chiral pair that normally showed very little retention on the water phase. For instance, it separated at 35 ^{o}C and 80 atm, but higher temperatures eroded this result while lower temperatures down to about 10 ^{o}C yielded no analyte retention. Remarkably though, below about 5 ^{o}C analyte retention and separation was greatly restored, such that higher pressures were used to offset this. Further, as with 2'HF, increasing selectivity was also seen.

Table 1 compares the selectivity of the flavanone pair at different temperatures. As seen, while moderate selectivity of 1.2 was observed at 35 ^{o}C, none was obtained below this temperature until about 5 ^{o}C, where it sharply increased to 2.8 and then continued to 3.6 at -8 ^{o}C.

Initially at -10 ^{o}C the water phase was observed to freeze, and so 2M salt was temporarily used to prevent this. Under these conditions, selectivity further increased to 4.1, which is quite high for chiral separations, especially employing a native βCD selector. Although potentially interesting, even lower temperatures were not further explored here. Figures 4b and c show typical flavanone separations at 35 and -8 ^{o}C respectively. As seen, a significantly improved separation is achieved at -8 ^{o}C, where the analytes separate in under 20 minutes with a resolution near 2. Incidentally, increasing the flow rate at a temperature of -5 ^{o}C was observed to separate the analytes in under 10 minutes, as illustrated in figure 4d. Therefore, reasonably fast and efficient flavanone separations may be achieved at these low temperatures.

The benefits of low temperatures with a native βCD loaded water stationary phase are somewhat unexpected since this selector usually displays quite modest selectivity compared to derivatives, and is predicted to be ineffective in cool aqueous solution due to its low solubility [31]. Still, it has been reported as a chiral additive in such phases and, while an unusual discontinuity in separation selectivity was not noted, some enhanced analyte complexation was [25, 30]. Certainly, the analyte interaction with native βCD at these low temperatures appears to be fairly unusual. Of note, selectivity plots like that of figure 3a are often linear for chiral separations [18]. In fact, the sharp increases observed here for the flavanones are rarely seen [32], and to our knowledge have never been reported for cyclodextrins. It might be wondered if this is because chiral SFC perhaps does not often encompass such wide temperature ranges or utilize such low temperatures. However, several chiral SFC reports have looked at much wider ranges and/or lower temperatures than those explored here and found no increase in selectivity at the cooler settings [33–35]. In fact, if any deviation was observed, it usually resulted from the selectivity levelling off at lower temperatures. Thus, the findings in figure 3 do seem uncommon.

While the reason for this is unclear, it is possible at lower temperatures that cyclodextrin adopts a favored geometry for enantiomer discrimination, which is known to have multiple competing mechanisms [26, 36]. For example, it could be that the water stationary phase is increasing in rigidity as the temperature lowers, similar to reports using ice particles for separations [37], and enhancing enantioselectivity [25]. Granted, bulk properties of the thin water layer observed in the former system were not deemed a major factor in retention, while they do remain very important here in allowing analyte partitioning and separation. Still, the rigidity of the chiral water stationary phase could be altering as it cools and play a role in the results obtained here. Nonetheless, these relatively simple flavanone separations with the water stationary phase SFC system compare well with other methods, producing greater selectivity, resolution, and/or speed relative to reports using native βCD [26] and modified βCD selectors [27, 29].

Other Chiral Selectors and Additives

As anticipated, not all analytes separated well on these cyclodextrin loaded phases. For example, phenoxypropionic acids are an important group of polar chiral analytes whose enantiomers often vary in herbicidal activity [38] and environmental persistence [39], and they have been separated using cyclodextrin phases [40]. However, while the test analytes 2-phenoxypropionic acid (2PPA) and 2-(3-chlorophenoxy)propionic acid (3CPA) were highly retained by all of the cyclodextrins used here, no chiral selectivity was observed for any of them. While this may be due to analyte deprotonation in the water stationary phase, which can impact complexation [41], it remained necessary to examine other selectors.

The most successful of these was vancomycin, a macrocyclic antibiotic that has also found use as a chiral selector. For instance, vancomycin has more diverse functionality than

native βCD, including two amine groups, one of which is believed to dominantly contribute to chiral selectivity for acidic analytes [42]. As well, its heptapeptide 'basket' structure is more open than a βCD cavity, and it promotes a relatively broader analyte selectivity [43]. Previously, vancomycin required specialized bonding to retain its native selectivity for acidic analytes in chromatographic separations [33]. Thus, since vancomycin can be used in the native form directly in the coated water phase, it was examined here also.

When exploring separations of 2PPA and 3CPA with 20 mM vancomycin in the water stationary phase, lower temperatures were again generally preferable. For example, little separation occurred at 15 ^{o}C but much better results were evident at 0 ^{o}C. While further reductions to -5 ^{o}C were attempted, the separations became worse and retention was prolonged. Therefore, similar to the flavanones, cooler temperatures were also primarily utilized.

Due to its relatively high water solubility, the vancomycin load was also increased and it notably improved separations. For example, increasing the concentration from 20 to 30mM enhanced selectivity by about 50% for both test analytes. This had even greater impact than in the βCD separations, since 30mM vancomycin alone nearly resolved the 3CPA enantiomers. Also similar to the βCD trials, sodium chloride had a positive influence on peak shape for the acids, however the optimum level was lower at 0.25M due to an apparent ionic character of the selective interaction. Figure 5a and b demonstrate this with the separation of 2PPA under optimum conditions using 0.50 and 0.25M sodium chloride respectively. As seen, the lower salt loading has a notable effect on both retention and separation of the chiral analytes, which are much better resolved for the latter condition.

Another water phase additive explored here was triethylamine (TEA), which has been shown to increase retention but reduce separation selectivity for acidic chiral analytes when

present in SFC mobile phases [33]. Remarkably, through adding only 1% TEA to the water phase, both retention and selectivity of the acidic analytes greatly increased by nearly 40%. For example, as seen in figures 5c and d, selectivity of the 3CPA chiral pair increased by about 0.6 and yielded a large resolution of 4.7 from TEA addition. These separations also reproduced well with analyte retention having RSD values of less than 0.5% from run to run.

Therefore, these acidic analytes can also be well separated by again varying the above parameters. For instance, in doing so the 3CPA selectivity increased from 1.5 to 3.2. While further optimization could yield more gains, it should be noted that all the additives are not always needed for satisfactory separation. For example, near baseline separation of 3CPA was also achieved in under 20 minutes using 0.50M salt and no TEA. Thus, the tunable nature of the water stationary phase method can add versatility and aid in chiral SFC method development.

In general, these results compare quite favorably with other chiral separations of such analytes employing vancomycin [33, 44, 45]. Of note, modest selectivity was reported for phenoxypropionic acid separations in SFC using vancomycin bonded phases, but the FID could not be used since modified CO_2 was required [33]. Therefore, it may be interesting to further explore the performance of vancomycin in the water phase with these and other analytes given its high solubility and flexibility in optimizing chiral separations.

Mixed Selectors

Another inquiry examined if both 3CPA and 2PPA could be separated together on the vancomycin loaded water stationary phase. However, under conditions that enabled their individual separation, near complete overlap was observed between the second eluting 3CPA and the first eluting 2PPA enantiomers (figure 6a). Since cyclodextrins are known to alter achiral separation selectivity [46], and native βCD earlier caused both of the acids here to be highly

retained (though unseparated), it was wondered if adding it to the vancomycin water phase could increase analyte retention and facilitate separation. In doing so, this was indeed the case, however it also reduced chiral selectivity of the vancomycin present. Ultimately by optimizing these opposing trends in favor of separation, it was determined that just 0.5% native βCD added to the vancomycin water phase improved the selectivity and resolution of the 3CPA and 2PPA chiral pairs such that each enantiomer was easily visible in this joint separation (figure 6b). While more experimentation would further improve the resolution observed, it is interesting that simple addition of a second selector to the water phase can add versatility to this approach.

In a more challenging application, a vancomycin/native βCD loaded water stationary phase was explored for its ability to separate enantiomers of both flavanone and 3CPA simultaneously. This too was met with certain obstacles, since there was no interaction seen between vancomycin and flavanone, while native βCD greatly increased 3CPA retention as shown above. Thus by promoting the separation of one pair while minimizing the loss in resolution of the other, optimum mutual separations were realized using high selector loadings, low salt concentrations, a TEA additive, and a pressure program to help further control analyte retention. As seen from the example shown in figure 7, this enabled baseline separation of the flavanone chiral pair while maintaining moderate 3CPA selectivity. It is also evident that the large native βCD concentration adds significant retention to the 3CPA chiral pair, even when using a pressure program to offset this. Consequently the analysis is long, making it more difficult to identify these peaks, which are also shown on an expanded scale for clarity. Nonetheless, even though continued optimization would be beneficial, this shows that simple exploration of various additives can readily lead to such mutual chiral analyte separations. Thus,

348 since using two different chiral selectors at once for this purpose is uncommon in conventional
349 methods [47], the ability to customize the water stationary phase in this way is interesting.

350 **Applications**

351 Since the water stationary phase has previously shown beneficial selectivity in analyzing
352 various samples [6–10] some applications of the developed chiral method were also briefly
353 explored. First was the analysis of a commercial honey bee propolis, which is a natural resin
354 from beehives prized for its anti-oxidant, anti-inflammatory and anti-bacterial properties, to
355 name a few [48]. Among the compounds attributed to this are flavanones, and due to the role that
356 chirality plays in their pharmacological effects, the analysis of these chiral molecules in such
357 complex matrices is of increasing interest [49]. To gauge the utility of the water stationary phase
358 for such an analysis, a propolis sample containing the chiral flavanone 2'HF was diluted in
359 ethanol to reduce its viscosity and it was directly injected into the system under optimal
360 conditions. As seen in figure 8a, when using native βCD as a selector, the 2'HF analyte pair is
361 well resolved and efficiently separated from the early eluting non-polar components of the
362 propolis sample, which are likely related to the beeswax residues present.

363 Another application was the analysis of a chiral phenoxypropionic acid in apple juice.
364 Due to the differing herbicidal and environmental behavior of these enantiomers and their
365 potential impact on food safety, their analysis in apple juice and other matrices is of great interest
366 [38, 39, 50]. Using a vancomycin loaded water stationary phase, the analysis of a commercial
367 apple juice containing 3CPA was examined under optimal conditions. As shown in figure 8b, the
368 chiral pair is again well separated and does not show any major interference from sugars or other
369 polar matrix components present, even though the juice was directly injected as a neat sample. It
370 should be noted that the spiked concentrations used above were larger than typical for such

samples in order to compensate for the significantly lower injection volume used here (i.e. 20 nL) relative to most conventional comparison methods (e.g. 20 µL) and the resultant analyte mass delivered to the detector. Still, some flavanones are found in comparably high natural abundance for certain samples [51]. None the less, given the large signal to noise ratios obtained in figure 8 and the high sensitivity of the FID, optimizing injection volumes should also readily allow for detection of such analytes at lower levels.

Overall then, the water stationary phase can be useful in such separations since it also has the potential to reduce interference from various sample matrix components [6–10]. For instance, many charged and/or highly polar species tend to heavily partition into the water phase and are strongly retained, while many non-polar compounds often elute rapidly. As a result, this can greatly reduce the overlap of many target analytes with matrix components and simplify sample preparations [6–10]. For example, it could alleviate the need for conventional solid-phase extraction clean-up steps that are commonly employed for such complex samples shown above [50] and save time in method development.

CONCLUSIONS

By modifying a water stationary phase in SFC with optimized selectors and additives, chiral separations can be readily achieved. The method presented demonstrates a wide range of flexibility in customizing the stationary phase for target analyte separations. Additionally, it is relatively simple and inexpensive and does not rely upon special bonded phases or organic modifiers to elute polar chiral analytes, which increases environmental compatibility and enables FID usage. Finally, given the unique selectivity of the water stationary phase itself, it can also greatly reduce sample preparation requirements and facilitate method development. Therefore,

further exploration of this novel chiral SFC approach in analytical or even preparative capacity could be potentially beneficial. Further, while the narrow bore capillaries used here would not be very suitable for preparative use, a stainless steel packed particle bed format demonstrated earlier could be [52].

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Table 1: Effect of temperature on flavanone separation selectivity.

Temperature (^{o}C)	Selectivity
35[a]	1.2
10	1.0
5	2.8
0	3.1
-8	3.6
-10[b]	4.1

Conditions are 2% native βCD chiral selector, 1 M NaCl, and 150 atm CO_2, except for a) at 80 atm CO_2, and b) at 2M NaCl.

FIGURE CAPTIONS

Figure 1: Effect of different chiral selectors in the water stationary phase on the separation of 2'HF enantiomers (*) at 30 ^{o}C and 80 atm CO_2. Selectors are a) none, b) methylated βCD, c) 2-hydroxypropyl βCD, and d) native βCD. The acetone solvent elutes first in each trial, except for d, where the ethanol solvent elutes last. The structure of 2'HF is shown inset in a).

Figure 2: Separation of 2'HF enantiomers (*) at a) 30 ^{o}C and 80 atm CO_2, and b) 50 ^{o}C and 110 atm CO_2. Water stationary phase contains 2% native βCD as the chiral selector and 1M NaCl. The solvent elutes first in each.

Figure 3: a) Van't Hoff plot for the SFC separation of 2'HF using 2% native βCD and 1M NaCl in the water stationary phase. Temperature range shown spans 50-0 ^{o}C. b) Plot showing how retention varies in a) using a similar format.

Figure 4: Separation of a) 2'HF (*) at 5 ^{o}C and 150 atm CO_2, b) flavanone (#) at 35 ^{o}C and 80 atm CO_2, c) flavanone (#) at -8 ^{o}C and 150 atm CO_2, and d) flavanone (#) at -5 ^{o}C and 150 atm CO_2. Stationary phase contains 2% native βCD as the chiral selector and 1M NaCl. The solvent elutes first in each. The mobile phase velocity is c) 2.7 cm s^{-1} and d) 4.0 cm s^{-1}. The structure of flavanone is shown inset in b).

Figure 5: Separation of a) 2PPA (#) using 0.5M NaCl, b) 2PPA (#) using 0.25M NaCl, c) 3CPA (+) using 0.25M NaCl, and d) 3CPA (+) using 0.25M NaCl and 1% TEA. The other conditions are 30mM vancomycin loading, 0 ^{o}C, and 150 atm CO_2. The solvent elutes first in each. The structure of 2PPA and 3CPA are shown inset in a) and c) respectively.

Figure 6: Separation of 3CPA (+) and 2PPA (#) on a 30 mM vancomycin loaded water stationary phase a) without, and b) with 0.5% native βCD present. Other conditions are 0.25M NaCl, 0 ^{o}C, and 150 atm CO_2. The solvent elutes first in each.

Figure 7: Separation of flavanone (*) and 3CPA (+) enantiomers on a 30mM vancomycin and 2% native βCD loaded water stationary phase. Other conditions are 0.25M NaCl, 1% TEA, 0 ^{o}C, and 80 atm CO_2 for 28 min, then 7.5 atm min^{-1} to 250 atm. The ethanol solvent elutes at 35 minutes. An expanded view of the 3CPA enantiomers are shown inset.

Figure 8: Separation of a) 2'HF enantiomers (*) in an ethanol diluted bee propolis sample, and b) 3CPA enantiomers (+) in a neat apple juice sample. Conditions are a) 2% native βCD selector, 1M NaCl, 10 ^{o}C, and 120 atm CO_2, and b) 30mM vancomycin selector, 0.5 M NaCl, 0 ^{o}C, and 150 atm CO_2. The ethanol diluent elutes last in a).

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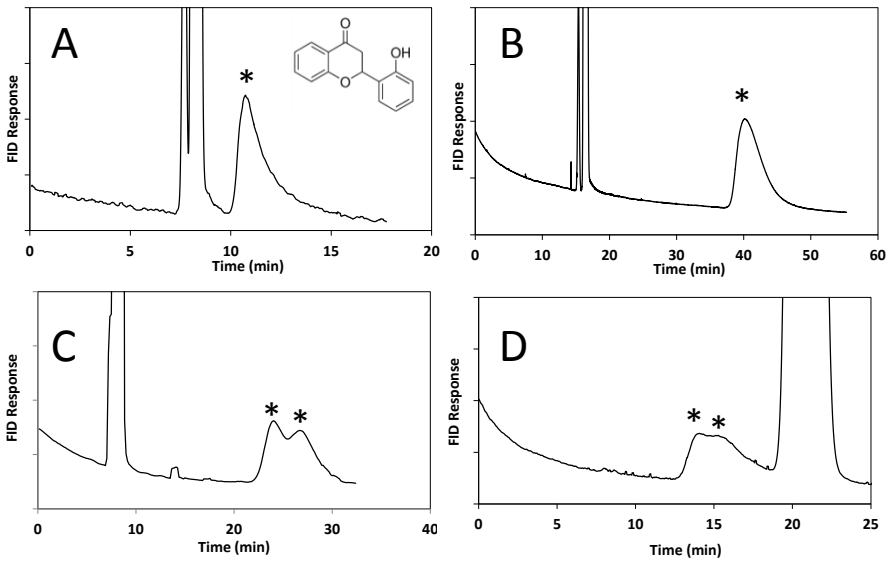

Figure 1

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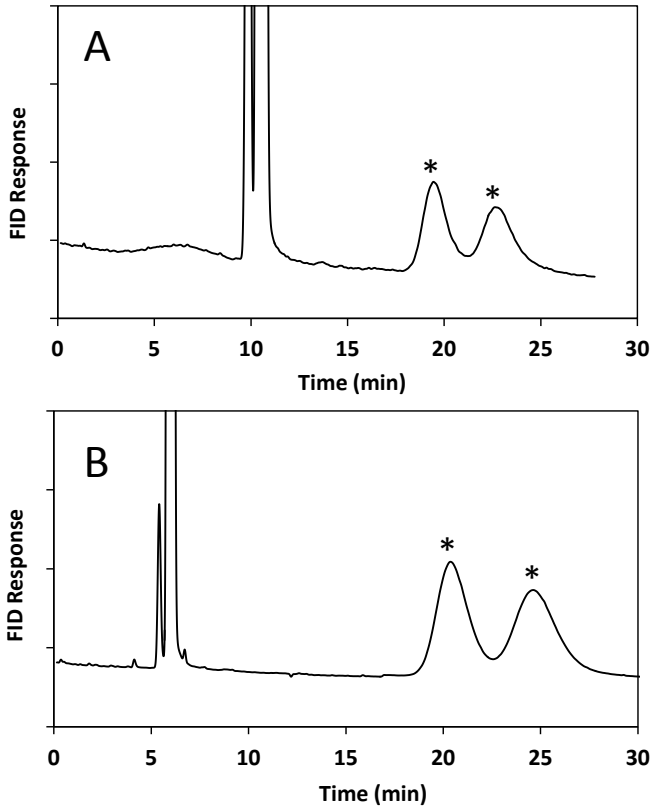

Figure 2

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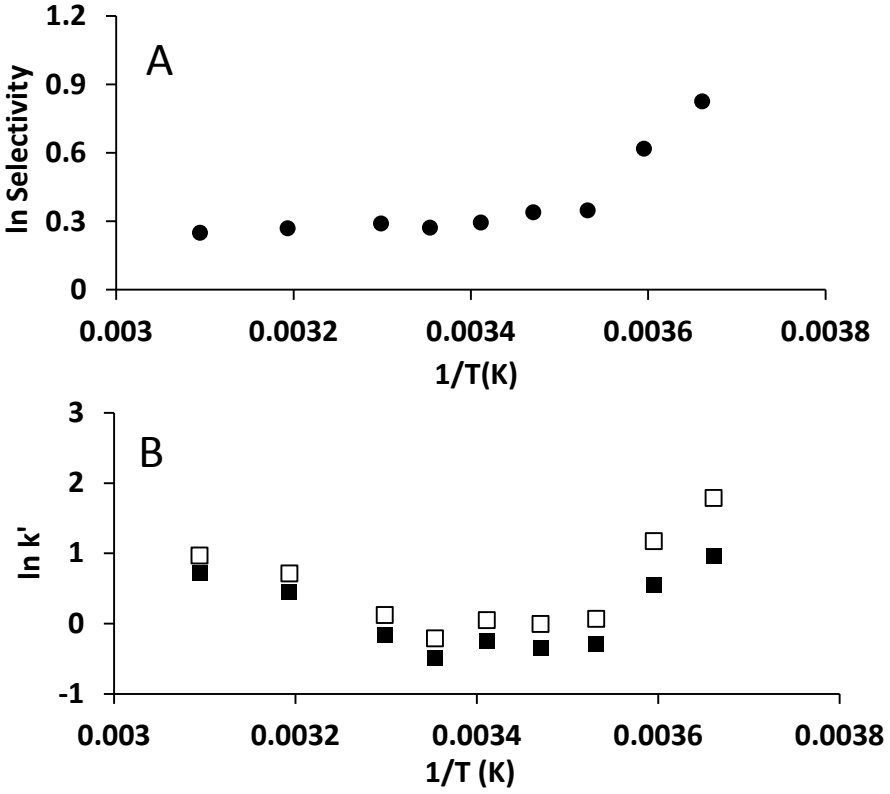

Figure 3

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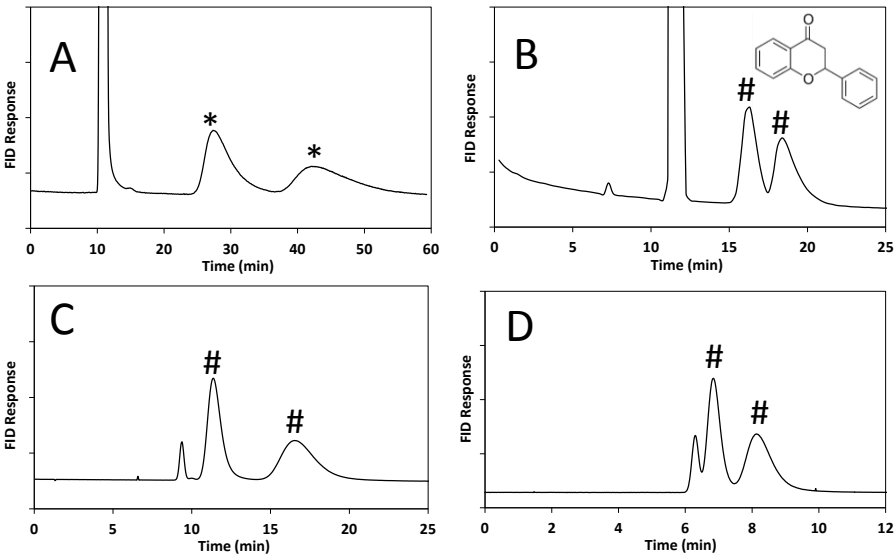

Figure 4

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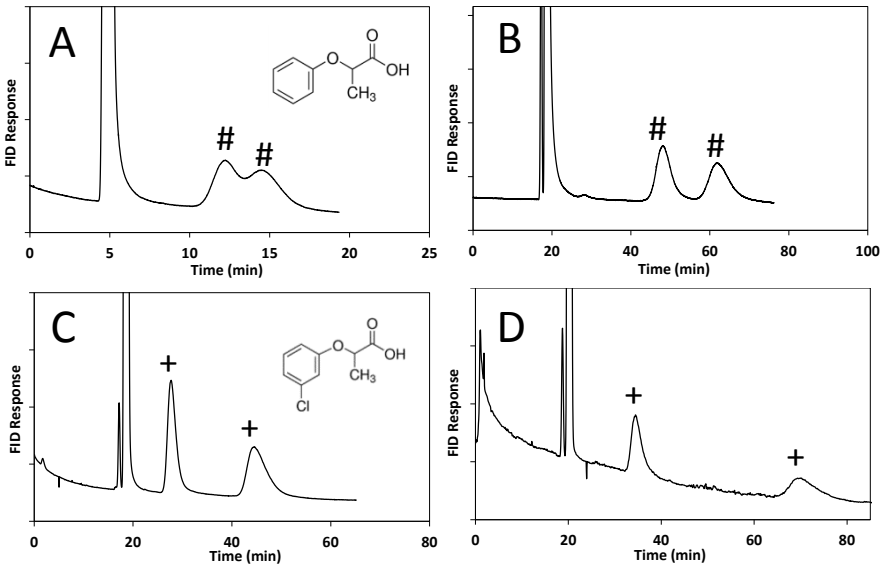

Figure 5

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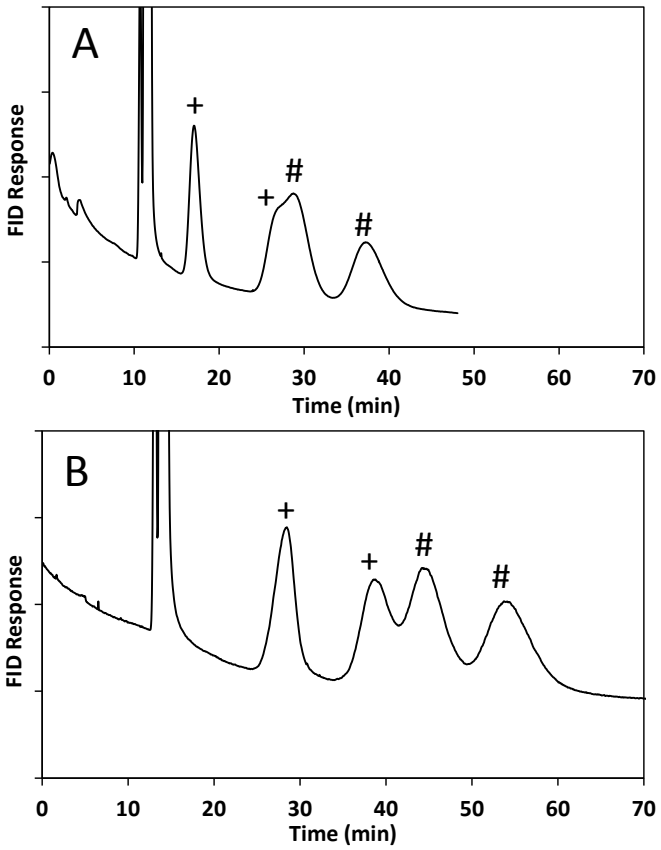

Figure 6

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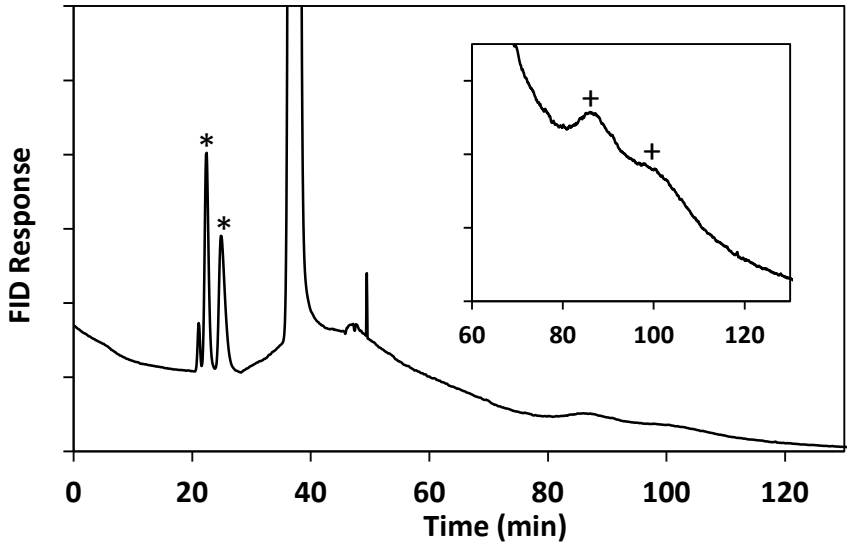

Figure 7

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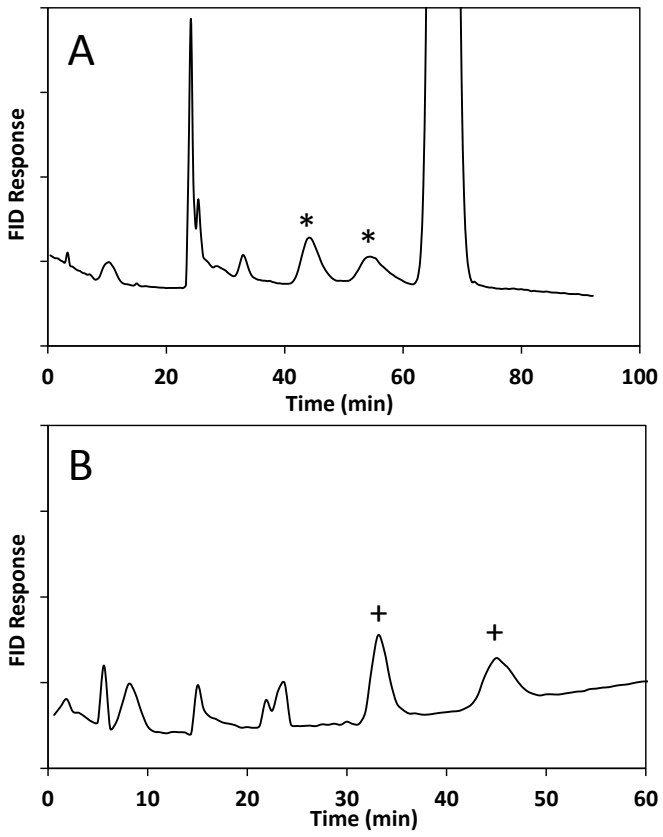
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Figure 8