

# Resurgence in Fluorinated Chiral Polysaccharide Phases for Supercritical Fluid Chromatography

Christine M. Aurigemma<sup>1</sup>, William P. Farrell<sup>1</sup>, Jeff R. Elleraas<sup>1</sup> and Matthew Przybycic<sup>2</sup>

1. Pfizer Worldwide Research and Development, Oncology Medicinal Chemistry, La Jolla Laboratories, 10770 Science Center Drive, San Diego, CA 92121 USA

2. ES Industries, Inc, West Berlin, NJ 08091 USA

The incorporation of fluorine within biologically active molecules in pharmaceutical drug discovery has resulted in chiral recognition challenges using existing chiral stationary phases (CSPs). Building upon decades-old research, several new phases were created to explore fluorophilic retention mechanisms towards enantioseparation of fluorinated compounds. While the concept of aromatic halogenated substituents within polysaccharide CSPs is not new, the availability of these types of phases with fluorine substituents is limited. The initial set of fluorinated phases, incorporating either 4-fluoro-3-methyl phenylcarbamate or 2-fluoro-5-methyl phenylcarbamate, were prepared on cellulose and evaluated by SFC using drug-like compounds with fluorinated functional groups. During the evaluation, it was discovered that these prototype CSPs also separated other halogenated compounds, but with less overall retention and solvent consumption. Based on these results, modifications were made, and two new iterations of fluorinated phases were created, and 3 of the 4 prototypes were later commercialised. The separation of racemic compounds achieved using these fluorinated phases using SFC will be presented, as well as comparative separations using other halogenated stationary phases.

## Introduction

Chiral SFC is the primary tool for the separation of enantiomers in support of drug discovery efforts. SFC uses a simple mobile phase system consisting of non-toxic CO<sub>2</sub> and an organic modifier, typically an alcohol such as methanol [1]. The lower toxicity of solvents and reduced overall waste generation of SFC make this a greener alternative to normal phase HPLC, and is thus ideal for preparative scale applications including chiral separations [2-6]. Not only is SFC considered a green technique, but high diffusivity and low viscosity of supercritical CO<sub>2</sub> enable higher flow rates and shorter run times, which leads to faster and more efficient separations relative to HPLC [7]. The most frequently used chiral selectors for SFC are the polysaccharides phases, which are substituted phenyl carbamates and benzoates bonded to either amylose or cellulose. These coated cellulose and amylose phases do have some solvent restrictions, and are often limited to the use of alcohol modifiers, while some of the newer immobilised phases such as the Chiralcel IA, IB and IC (Chiral Technologies, Inc, West Chester, PA USA) have expanded solvent compatibility [8]. Other examples of CSPs include the Pirkle-type, such as the Regis Whelk-O1 (Regis Technologies, Inc, Chicago, IL USA) and macrocyclic glycoprotein phases exemplified by Astec® Chirobiotic™ V, T,

and R-series (Sigma-Aldrich, St. Louis, MO, USA). While the Pirkle-type and peptide macrocyclic glycoprotein phases have a broader solvent compatibility range than coated polysaccharide derivatives and are usually applied in reverse phase (aqueous/organic) or in polar organic mode (e.g. 20mM ammonium acetate in methanol), the diversity of the polysaccharide-based phases and their variable chiral recognition abilities make them more versatile across a broad range of compounds. This is especially true for the substituted phenyl carbamates and benzoates of these polysaccharides, whose chiral recognition abilities have been recognised in a number of publications over the years [9-14].

The recognition of the importance of 3D geometry of lead molecules is leading to the synthesis of novel molecular entities [15] which are creating enantioseparation challenges for which an increasing number of chiral compounds are not separating on any traditional polysaccharide CSPs. In addition, compounds with low molecular weight (e.g. MW < 200 Da), and those with limited complexity/functionality near the chiral centre contribute to diminished chiral recognition ability with these phases. While gas chromatography is probably best suited for separating these compounds, it is inconsistent with the goals of isolating pure enantiomers at a satisfactory scale for medicinal chemistry support. In Figure

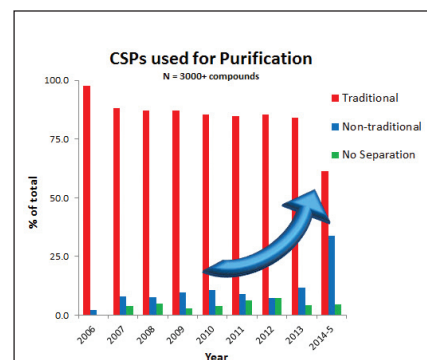


Figure 1. Traditional polysaccharide CSP usage over the last 10 years compiled from purification methods of internal compounds.

1, a review of over 3000 internal chiral compounds over several years and the methods used to resolve them indicates an exponential shift from traditional phases towards non-traditional CSPs. Interestingly, this data also coincides with the continued trend in the increased incorporation of halogens (in particular, fluorine) in medicinal chemical designs.

While the 3,5-dimethylphenylcarbamates of cellulose and amylose (Chiralcel OD and Chiralpak AD, respectively) stationary phases (Chiral Technologies, Inc) remain an integral component of our chiral SFC screening protocol, reduced success with the other amylose and cellulose-based CSPs has led to an expansion of our screening set to incorporate newer phases to potentially provide alternative mechanisms for chiral

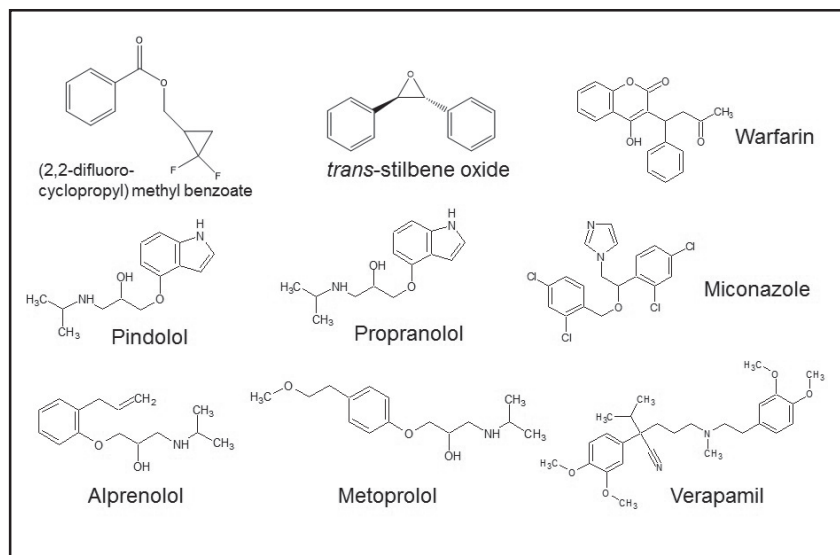


Figure 2. Compounds used for evaluation of the chiral stationary phases.

recognition. Studies have shown that changes in chiral recognition are driven by modifications to the structure of the polysaccharide phase, and the addition of electron-donating/electron-withdrawing substituents to specific positions on the benzylic ligand has resulted in several new phases [16]. For instance, an electron-withdrawing chlorine substituent on the polysaccharide CSP has demonstrated better chiral recognition abilities than without chlorine in both HPLC and SFC. [17–21] Research on halogenated substituents of polysaccharide CSPs

dates back to the 1980's, where chloro-, bromo- and fluoro-substituted methylphenylcarbamates demonstrated enhanced chiral recognition for halogenated compounds [22,23]. As a result, several chloro-phenyl substituted CSPs have become commercially available over the last decade, such as the Lux Cellulose-2 (cellulose tris (3-chloro-4-methylphenylcarbamate) and the Lux Amylose-2 (amylose tris (5-chloro-2-methylphenylcarbamate) phases (Phenomenex, Torrance, CA, USA).

The driving force for this study,

however, is the continued resurgence in fluorinated compounds required to support drug discovery campaigns. Fluorine is widely used in medicinal chemistry to enhance metabolic stability, and greater than one-third of newly approved drugs contain fluorine [24–26]. One compound of interest, (2,2-difluorocyclopropyl) methyl benzoate, shown in Figure 2, is lipophilic, has low molecular weight, and possesses limited diversity at the chiral centre. The compound exhibited limited retention and negligible separation on nearly every CSP in Table 1, with only a partial separation achieved on the Chiralcel OJ column using normal phase HPLC. Speculating that a fluorophilic mechanism could potentially enhance selectivity and retention of this compound, we sought to create several fluorinated chiral phases since the few that were commercially available were unsuccessful. In this paper, we demonstrate the unique enantioselectivities of these prototype fluorinated polysaccharide CSPs for SFC not only for compounds containing fluorine but for other halogen-containing compounds. We also compare differences in chiral recognition abilities between these fluorinated phases and other commercially available halogenated CSPs.

## Experimental

### Chemicals and Materials

The following were purchased from Sigma-Aldrich (St. Louis, MO, USA): 4-fluoro-3-methylphenyl isocyanate, 2-fluoro-5-methylphenyl isocyanate, 4-fluoro-3-(trifluoromethyl) benzoyl chloride and 3,4-difluorobenzoyl chloride. These monomers were bonded to cellulose by ES Industries, Inc (West Berlin, NJ, USA) to form tri-substituted carbamates or benzoates, and then coated over silica gel with 5- $\mu\text{m}$  particle using a proprietary process. Each phase was packed into 4.6mm I.D. x 250mm columns. The first generation prototypes created were the CCO-F4 (cellulose tris(4-fluoro-3-methylphenylcarbamate) and the CCO-F2 (cellulose tris(2-fluoro-5-methylphenylcarbamate). A second set of prototype columns were made after the initial evaluation of first generation phases, and these were the CCO-F4-CF3 (cellulose tris(4-fluoro-3-trifluoromethylphenylbenzoate) and the CCO-DiF (cellulose tris(3,4-difluoromethylphenylbenzoate). The

Table 1. Representative structures of prototypes and widely used commercial CSPs.

Column	Column Type	Chiral Selector - R	Column	Column Type	Chiral Selector - R
Chiralpak AD	Coated-Amylose		Lux Cellulose-2	Coated-Cellulose	
Chiralpak AS	Coated-Amylose		Lux Cellulose-4	Coated-Cellulose	
Chiralpak IC	Immobilized-Cellulose		Lux Amylose-2	Coated-Amylose	
Chiralcel OJ	Coated-Cellulose		Whelk-O1	Bonded Pirle-type	
Chiralcel OD	Coated-Cellulose		CC4	Coated-Cellulose	
CCO-F4	Coated-Cellulose		CCO-DiF	Coated-Cellulose	
CCO-F2	Coated-Cellulose		CCO-F4-CF3	Coated-Cellulose	

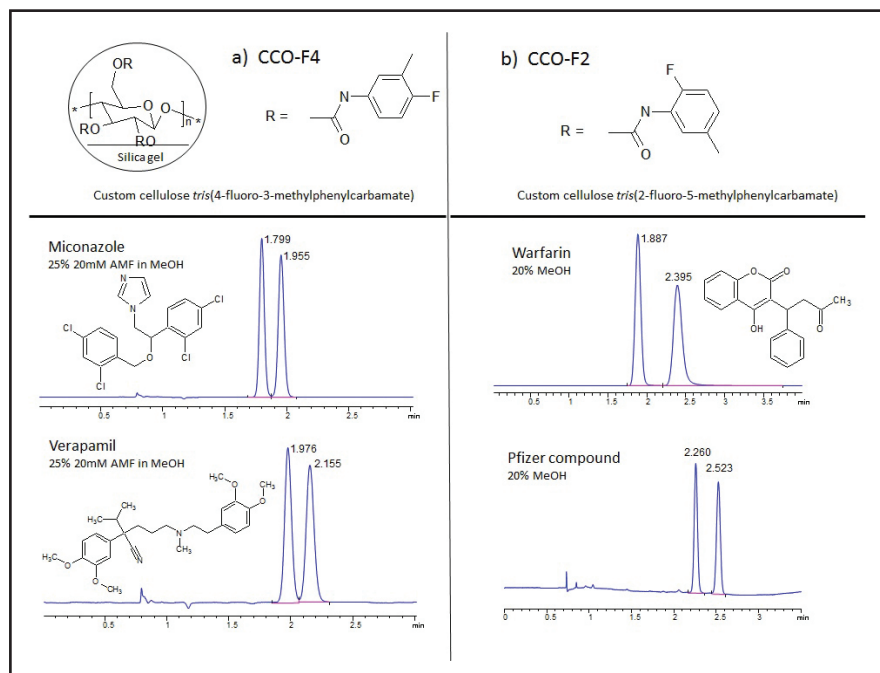


Figure 3. Example of separations using the a) CCO-F4 and b) CCO-F2 fluorinated phases. Each column is 4.6mm I.D. x 250mm length containing 5- $\mu$ m particles maintained at 25°C. The mobile phase consists of CO<sub>2</sub> and percentage co-solvent listed in each chromatogram delivered at a flow rate of 3.0mL/min with 160 bar outlet pressure.

ChromegaChiral CC4 column (4-chloro-3-methylphenyl carbamate), was also purchased from ES Industries, Inc. in the same dimensions and particle size to be used for comparative purposes.

All compounds used for this study (Figure 2) were also purchased from Sigma-Aldrich except for (2,2-difluorocyclopropyl) methyl benzoate (PubChem CID #101586031), and a single Pfizer proprietary compound, both of which were

synthesised in-house.

HPLC grade methanol, acetonitrile, and isopropanol (J.T. Baker, Phillipsburg, NJ, USA); ammonium formate and ammonia (Fisher Scientific, Pittsburgh, PA, USA); and bulk grade carbon dioxide (AirGas West (Escondido, CA, USA) were used in this study. The CO<sub>2</sub> was purified and pressurised to 1500 psig using a custom booster and purifier system from Va-Tran Systems, Inc. (Chula Vista, CA, USA).

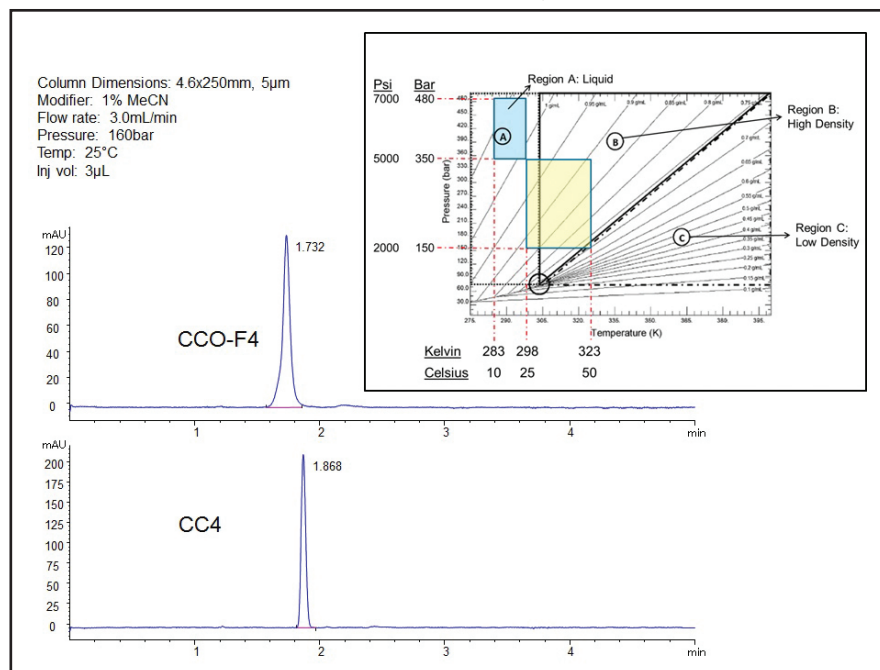


Figure 4. Chromatograms of (2,2-difluorocyclopropyl) methyl benzoate using standard SFC conditions with 1% acetonitrile as modifier on the CCO-F4 (top) and CC4 (bottom) phases. Inset: Isopycnic plot for carbon dioxide indicating the density profile at various temperatures and pressures. Yellow region represents normal operating conditions in SFC. Blue region represents rarely used region in SFC. Adapted from Tarafder [29].

## Instrumentation

Analyses were performed using an Agilent 1260 SFC/MS system consisting of a binary pump, SFC control module, UV/DAD detector, and column compartment with an internal 2-position, 6-port valve, and 6120 MSD with an APCI source (Agilent, Inc, Santa Clara, CA, USA). A CTC HTS PAL autosampler (Leap Technologies, NC, USA) was equipped with a 10  $\mu$ L syringe and a 5  $\mu$ L fixed loop with a control method to vent CO<sub>2</sub> from the loop prior to sample introduction. The effluent of the SFC is split to the MSD using a 3-way tee (Valco, Houston, TX, USA) and a 50 cm long, 50  $\mu$ m I.D. PEEKsil capillary tubing (Upchurch Scientific, Oak Harbor, WA, USA). All data were acquired using Agilent 64-bit ChemStation (Version C.01.05).

## Analysis Conditions

The mobile phase consisted of CO<sub>2</sub> and the percentage co-solvent listed in each chromatogram, delivered at a flow rate of 3.0mL/min with 160 bar outlet pressure and the column temperature maintained at 25°C.

## Results

While fluorinated CSPs have been researched and reported to demonstrate higher chiral recognition abilities than traditional polysaccharide CSPs, their evaluation for selectivity was limited to a small number of chiral probes and then used only in HPLC [22]. This research attempts to determine whether fluorinated phases are suitable for chiral SFC applications with the goal of addressing separation inefficiencies for halogenated compounds. Using several different standards, we were able to show that the CCO-F4 and CCO-F2 phases could achieve chiral recognition, and the structures of these prototype phases are shown in Table 1. In Figure 3 both columns appear to demonstrate versatility against the range of compounds used confirming the finding of Yashima and Chankvetadze, who have extensively studied similar column substrates [19,20,23]. The separations of miconazole and the racemic Pfizer compound (both of which are halogenated), provided some initial encouragement because the phases, while different from those previously reported, have demonstrated favourable preliminary results.

Evaluating the columns against our target compound, (2,2-difluorocyclopropyl) methyl benzoate, we were initially discouraged that no separation was achieved using standard conditions and a range of modifier solvents. In Figure 4 with acetonitrile as the co-solvent,

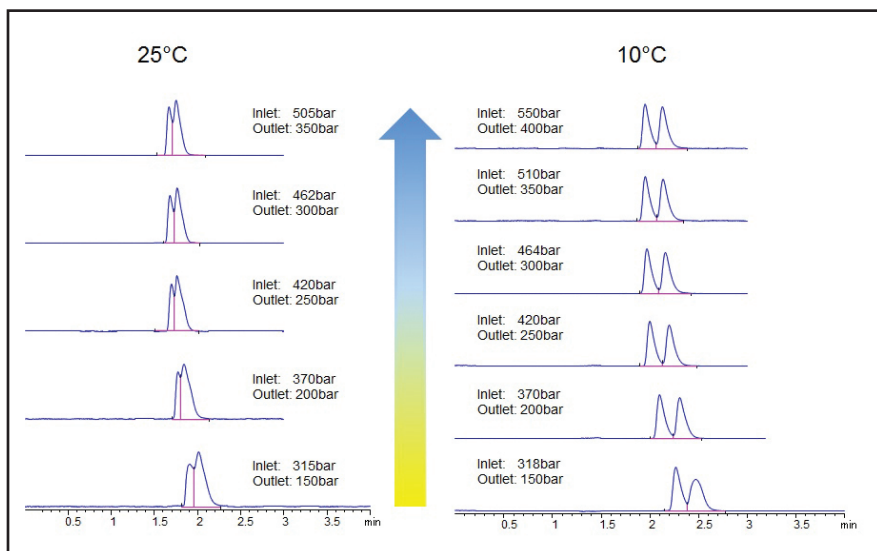


Figure 5. Chromatograms depicting the effects of temperature and pressure on the separation of (2,2-difluorocyclopropyl) methyl benzoate on the CCO-F4 phase as the conditions move from the yellow to blue regions indicated in the Figure 4 inset.

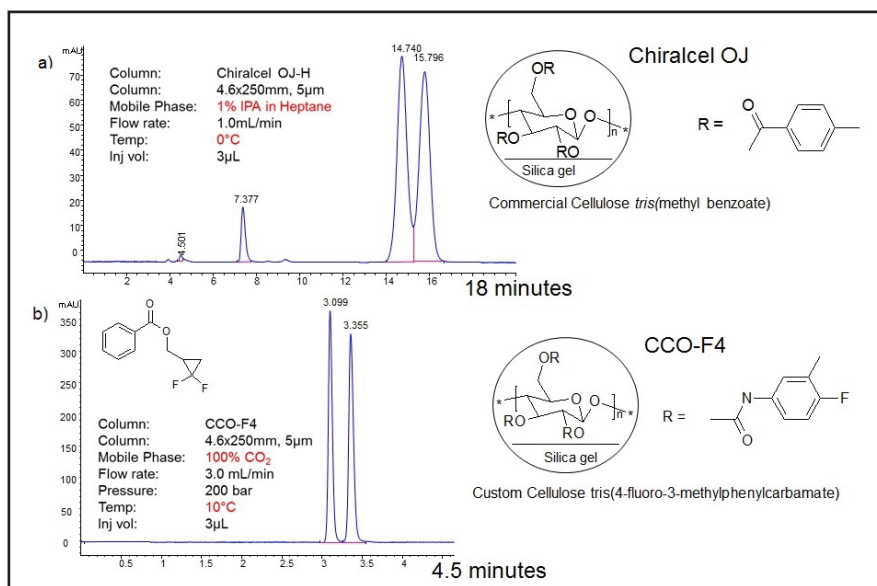


Figure 6. Analytical chromatograms of the separation of (2,2-difluorocyclopropyl) methyl benzoate using a) HPLC with a Chiralcel OJ-H column; and b) SFC with a CCO-F4 column.

no separation was achieved even after reducing the solvent percentage to 1% on either the CCO-F4 or CC4 phases despite using the full range of the typical polar protic solvent (alcohols) or aprotic solvents normally used in SFC. Realising that the compound may be too lipophilic for use with the addition of any organic solvent, the use of pure CO<sub>2</sub> as the modifier was also explored.

Elimination of the co-solvent and lowering the temperature of the mobile phase to 10°C resulted in a discrete separation of the enantiomers. As shown in Figure 5, further optimisation through variation of the temperature and pressure parameters enabled us to change the density of the CO<sub>2</sub> to mimic a weakly polar solvent and thus enable successful chiral recognition by

SFC. Based on these results, we determined that a condition of 10°C and 200 bar outlet pressure provided the best separation of the enantiomers.

The basis for this phenomenon can be explained using the isopycnic plot developed by Tarafdar and Guichon and shown in the inset of Figure 4 [29,30]. This plot demonstrates the density variations with changes in pressure and temperature. The typical region of operation for SFC is highlighted in yellow, which represents a modestly dense fluid (0.8 – 0.95 g/mL). By moving towards the blue region, the density of CO<sub>2</sub> approaches that of a liquid (> 1.0 g/mL), which in this case enables sufficient solubility of the compound to induce interactions with the CSP.

Continuing our experiments with the (2,2-difluorocyclopropyl) methyl benzoate, the near-optimal HPLC separation using a traditional CSP as well as the optimised SFC conditions with the CCO-C4 phase is compared in Figure 6.

Only a partial separation was achieved using HPLC, and it was difficult to optimise further since the separation degraded with minute changes in temperature and solvent composition. Therefore, the separation was hard to scale for purification especially at the low column temperature. Using SFC with the fluorinated phase, not only did we achieve complete enantioseparation using pure CO<sub>2</sub> conditions, but the separation was 4 times faster than the best conditions developed using HPLC. Since only a small percentage of methanol was added post-column to facilitate fraction collection, a significant savings in solvent and time were realised upon scale-up to SFC purification using the CCO-F4 phase.

Driven by the success of the first two prototype CCO-F4 and CCO-F2 phases, we decided to replace the methyl moiety of the CCO-F4 phase with trifluoromethyl groups thus enhancing the fluorine content of the CSP. Okamoto et al. reported that chiral recognition is dependent on the position, type and the number of substituents on the aromatic ligand of the polysaccharide CSP.

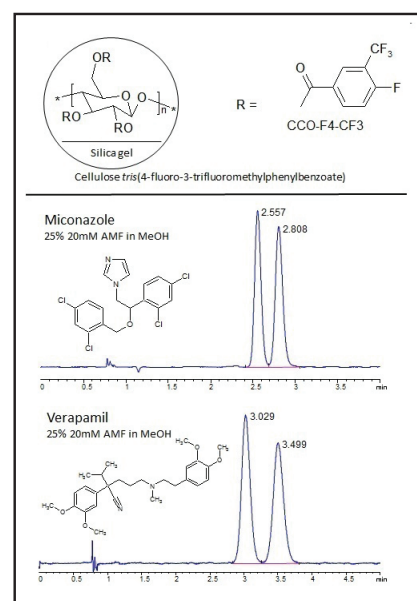


Figure 7. Separations of miconazole and verapamil using the CCO-F4-CF3 fluorinated phase. Column dimension is 4.6mm I.D. x 250mm length containing 5-µm particles maintained at 25°C. The mobile phase consists of CO<sub>2</sub> and percentage co-solvent listed in each chromatogram delivered at a flow rate of 3.0mL/min with 160 bar outlet pressure.

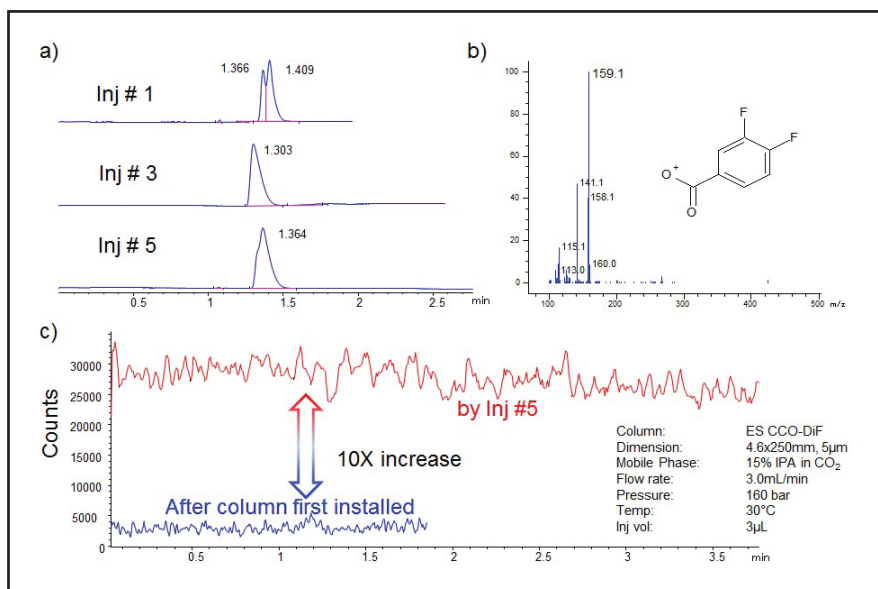


Figure 8. Preliminary results for the CCO-DiF column indicating significant instability of the phase. a) SFC/UV chromatograms of replicate injections of trans-stilbene oxide using 15% isopropanol as a modifier; b) Mass spectra of the column effluent indicating a possible identity of the noise; c) Total ion chromatograms of the CCO-DiF column taken shortly after first installation (Blue) and after the 5<sup>th</sup> injection (Red).

More specifically, improvements in chiral recognition can be achieved by adding substituents to the 3- or 4-position [9,11-12]. Following this logic, we chose to incorporate readily available trifluoromethyl and fluorine on the 3-position of the benzylic ring, utilising the corresponding benzoyl chlorides to create the second generation phases: cellulose tris(4-fluoro-3-trifluoromethylphenylbenzoate), or CCO-F4-CF<sub>3</sub>; and cellulose tris(3,4-difluoro-methylphenylbenzoate) or CCO-DiF.

Although we chose to use the benzoate linker to reduce costs, there is precedence that the chiral discrimination between the resulting benzoates and carbamates is consistently preserved [31]. Figure 7 demonstrates that the prescribed changes did not diminish the selectivity of miconazole or verapamil enantiomers even with the change in linker and the addition of the trifluoromethyl substituent. The preliminary data suggests that the chiral discrimination of the CCO-F4 and CCO-F4-CF<sub>3</sub> columns are similar.

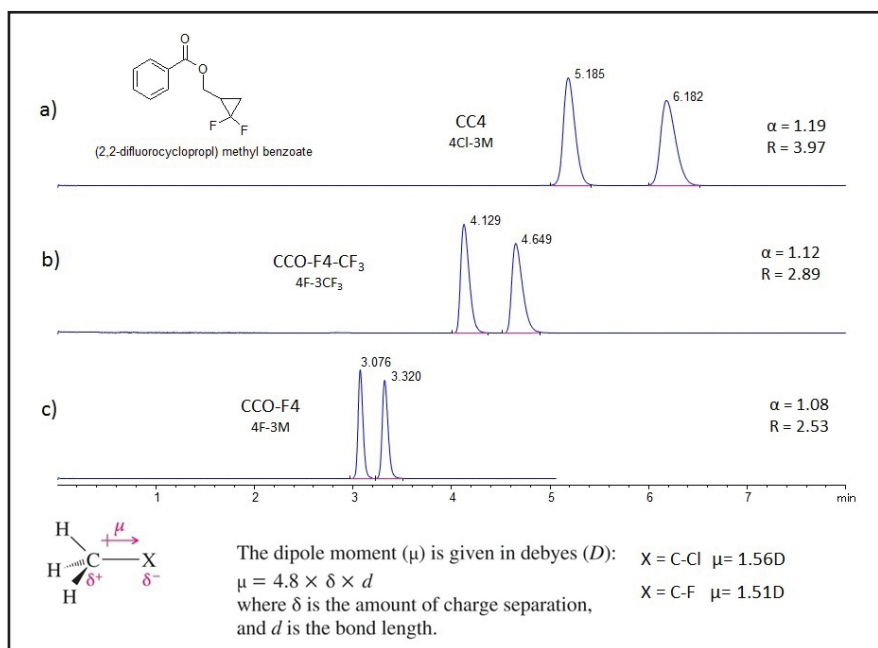


Figure 9. Example chromatograms of (2,2-difluorocyclopropyl) methyl benzoate on a) CC4; b) CCO-F4-CF<sub>3</sub>; and c) CCO-F4 phases demonstrating the possible effect of stationary phase dipole moments on each separation. Each column is 4.6mm I.D. x 250mm length containing 5-µm particles maintained at 10°C. The mobile phase consists of 100% CO<sub>2</sub> delivered at a flow rate of 3.0mL/min with 200 bar outlet pressure. Dipole moment calculations reflect the magnitude differences in an ideal system and were not measured for each specific phase. Adapted from Crab [36].  $R$  and  $\alpha$  were calculated using Agilent Chemstation software.

The difluoromethyl substitution phase (CCO-DiF), on the other hand, exhibited significant instability to solvent percentages above ~5% v/v, such that subsequent injections of the trans-stilbene oxide marker resulted in loss of selectivity (Figure 8a). In addition, the m/z found in the mass spectral trace (8b) indicates the presence of significant bleed of the benzylic moiety (8c).

This was observed across several columns and numerous modifier combinations, and use was only determined to be acceptable if the solvent percentage was kept very low (<5% v/v). This effect was not observed on any of the other phases tested (data not shown). Further studies with this column were halted pending assessment of the stability issues.

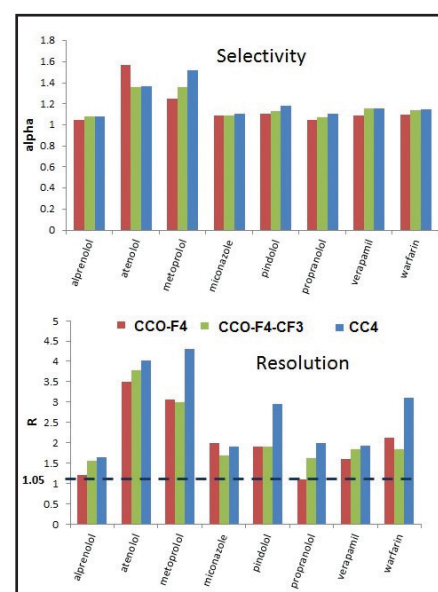


Figure 10. The overall trend in selectivity ( $\alpha$ ) and resolution ( $R$ ) for pharmaceutical compounds supports effectiveness of positional substitution and dipole potential on the stationary phase.

Utilising the optimal conditions determined in Figure 9, separation of the (2,2-difluorocyclopropyl) methyl benzoate enantiomers was achieved on the three stable columns. It is interesting to note that the retention and selectivity (under these conditions) appear to support the assertion that it is both the direction and the magnitude of the electron withdrawing/donating effect that enhances the separation [17,24,32-35]. For instance, the dipole contributions between F and Cl for methane are 1.51D and 1.56D respectively, which could account for the drastic differences in retention and the modest enhancement in selectivity. Since the dipole moment for each specific phase cannot be measured or calculated at this time, these values can only be indicative of the relative differences in magnitude of the dipole effects.

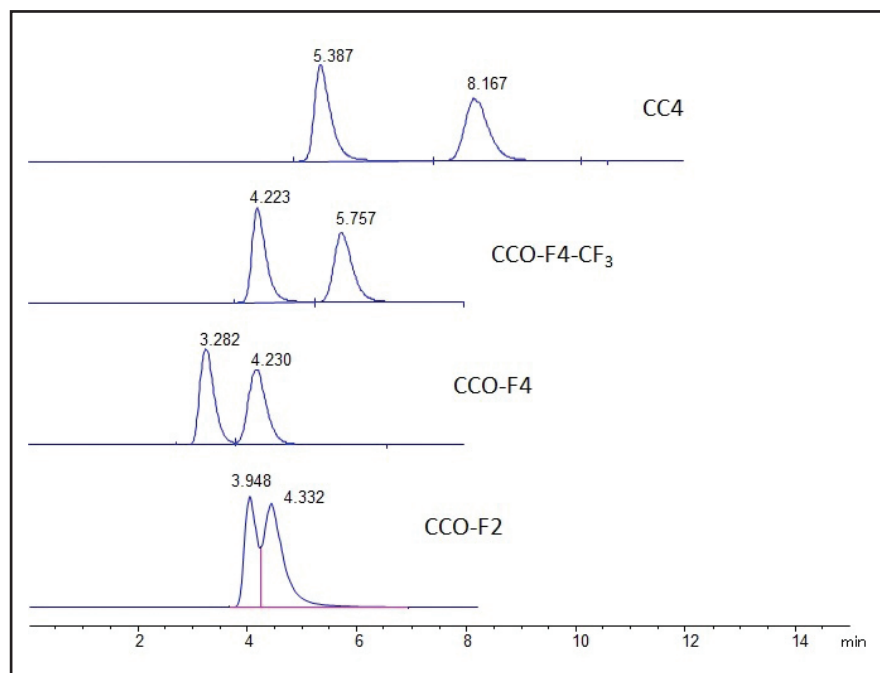


Figure 11. Example chromatograms of metoprolol enantiomers on each of the commercial fluoro-methyl and chloro-methyl phases (CC4, CCO-F4-CF<sub>3</sub>, CCO-F4, and CCO-F2). Each column is 4.6mm I.D. x 250mm length containing 5- $\mu$ m particles maintained at 25°C. The mobile phase consists of CO<sub>2</sub> and 25% 20mM ammonia in isopropanol delivered at a flow rate of 3.0mL/min with 160 bar outlet pressure.

Determining if this trend was a general occurrence, we utilised several more compounds, both halogenated and non-halogenated, under similar conditions for each of the 3 phases. With a few exceptions, the trend in both selectivity and retention remains the same at CCO-F4 < CCO-F4-CF<sub>3</sub> < CC4 (Figure 10).

To further emphasise the effect of the positional substitution differences, selectivity for the metoprolol enantiomers (Figure 11) can be affected by substituting the halogen (4-fluoro to 4-chloro), the methyl group (4-fluoro-3-methyl to 4-fluoro-3-trifluoromethyl), or the relative positions of both the halogen and methyl groups (4-fluoro to 2-fluoro). Although outside the scope of this paper, it would also be interesting to re-evaluate by SFC other halogenated CSPs previously reported using HPLC conditions. [31]

## Conclusion

The use of fluorine as a halogen substituent for chiral polysaccharide stationary phases has demonstrated utility in our preliminary studies and appears comparable to commercial chlorinated phases. Since the CCO-F4, CCO-F4-CF<sub>3</sub> (now CCO-F4-T3) and CCO-F2 are now readily available from ES Industries, they have been incorporated into our routine screening protocol. The magnitude of the selectivity differences from these fluorinated prototype phases appears to derive from potential changes in

the dipole of the benzylic functional groups. Nonetheless, the fluorinated versions offer several advantages in potential savings in organic modifier consumption and shorter retention times for SFC applications. Based on the enhanced selectivity from the CCO-F4 to CCO-F4-CF<sub>3</sub>, the introduction of the trifluoromethyl functionality as a replacement for the methyl group could potentially improve the selectivity of other commercial phases.

The enantioseparation of our fluorinated target compound was achieved using two different CSP chemistries and two different techniques, HPLC and SFC. The results using the fluorinated phases by SFC suggest that the properties of CO<sub>2</sub>, even as a liquid without the presence of an organic modifier, have clear benefits as a strategy to enhance selectivity. Therefore, SFC is the technique of choice for enantioseparations and has the potential to significantly impact areas where fluorinated and other halogenated chemistry are heavily used.

## Acknowledgments

Special thanks to Professor Bezhan Chankvetadze of Tbilisi State University, Tbilisi, Georgia, for the mechanistic discussion on halogenated chiral stationary phases and to Paul Richardson for his extensive review of this paper.

## References

1. W. Majewski, E. Valery, O. Ludemann-Hombourger, J. Liq. Chromatogr. Relat. Technol., 28 (2005), 1233-1252.
2. L. Miller, J. Chromatogr. A 1250 (2012), 250-255.
3. G. Guiochon, A. Taradfer, J. Chromatogr. A, 1218 (2011), 1037-1114.
4. A. Rajendran, J. Chromatogr. A, 1250 (2012), 227-249.
5. C. Aurigemma, W. Farrell, K. Farrell, Chromatography Today, (Nov/Dec. 2014), 2-5.
6. E. Landagaraya, C. Vaccherb, S. Yousa, E. Lipkac, Journal of Pharmaceutical and Biomedical Analysis, 120 (2016), 297-305.
7. L. Miller, M. Potter, J. Chromatogr. B, 875 (2008), 230-236.
8. M. Lämmerhofer, J. Chromatogr. A, 1217 (2010), 814-856.
9. Y. Okamoto, J. Noguchi, E. Yashima, Reactive & Functional Polymers, 37 (1998), 183-188.
10. T. Ikai, Y. Okamoto, Chem. Rev., 109 (2009), 6077.
11. Y. Okamoto, J. Polym. Sci. A, 47 (2009), 1731.
12. Y. Okamoto, T. Ikai, Chem. Soc. Rev., 37 (2008), 2593.
13. E. Francotte, J. Chromatogr. A, 906 (2001), 379.
14. J. Shen, S. Liu, P. Li, X. Shen, Y. Okamoto, J. Chromatogr. A, 1246 (2012), 137.
15. F. Lovering, J. Bikker, C. Humblet, J. Med. Chem., 52 (2009), 6752-6756.
16. Y. Okamoto, R. Aburatani, K. Hatada, J. Chromatogr. A, 389 (1987), 95.
17. B. Chankvetadze, L. Chankvetadze, Sh. Sidamonidze, E. Yashima, Y. Okamoto, J. Pharm. and Biomed Analysis, 14 (1996), 1295-1303.
18. S. Khater, Y. Zhang, C. West, J. Chromatogr. A, 1363 (2014), 294-310.
19. B. Chankvetadze, E. Yashima, Y. Okamoto, J. Chromatogr. A, 670 (1994), 39.
20. B. Chankvetadze, E. Yashima, Y. Okamoto, J. Chromatogr. A, 694 (1995), 101.
21. Y. Okamoto, R. Aburatani, K. Hatada, Bull. Chem. Soc. Jpn. 63 (1990), 955.
22. B. Chankvetadze, L. Chankvetadze, S. Sidamonidze, E. Kasashima, Y. Okamoto, J. Chrom. A, 787 (1997), 67-77.
23. E. Yashima, C. Yamamoto, Y. Okamoto, Polymer Journal, 27 (1995), 856-861.
24. Y. Okamoto, M. Kawashima, K. Hatada, J. Chromatogr. 363 (1986), 173.
25. Curran, D. P., Synlett., 9 (2001), 1488-1496.
26. Jarvis, L. M., Chemical & Engineering News, 91(2013), 15-17.
27. H. Zhang, Y. Li, Z. Jiang, J. Biomol. Res. Ther., (2012), 1:2.
28. Y. Okamoto, M. Kawashima, K. Hatada, J. Chromatogr., 363 (1986), 173.
29. A. Taradfer, G. Guiochon, J. Chromatogr. A, 1218 (2011), 4576-4585.
30. A. Taradfer, K. Kaczmarek, M. Ranger, D. Poe, G. Guiochon, J. Chromatogr. A, 1238 (2012), 132.
31. B. Chankvetadze, J. Chromatogr. A, 1269 (2012) 26-51.
32. B. Chankvetadze, E. Yashima, Y. Okamoto, Chem. Lett. (1993), 617.
33. B. Chankvetadze, E. Yashima, Y. Okamoto, Chromatogr. A, 670 (1994), 39.
34. B. Chankvetadze, E. Yashima, Y. Okamoto, Chromatogr. A, 694 (1995), 101.
35. C. Yamamoto, Y. Okamoto, Bull. Chem. Soc. Jpn. 77 (2004), 227.
36. <http://crab.rutgers.edu/~alroche/Ch06.pdf>