Fluoro-methylphenylcarbamates of Cellulose as Chiral Stationary Phases for Supercritical Fluid Chromatography

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ABSTRACT

The use of aromatic halogenated substituents within carbohydrate chiral stationary phases is not a new concept, with early work dating back to the mid to late 1990's[1,2]. Chloro-, bromo-, and fluoro-methylphenylcarbamates all showed chiral recognition, and there are several chloro-phenyl substituted versions available commercially. However, with the resurgence of fluorinated chemistry being used for drug development, the need for phases suitable for chiral recognition of fluorinated compounds is facilitated by re-introduction of these types of halogenated phases. Introduction of a fluorophilic retention mechanism can be particularly useful in support of medicinal chemistry drug discovery efforts, where more than a third of newly approved small molecule drugs contain fluorine[3,4].

Two new fluorinated phases, 4-fluoro-3-methylphenylcarbamate and 2-fluoro-5-methylphenylcarbamate, were prepared on cellulose and evaluated for use with drug-like compounds containing fluorine constituents. Separations of a wide variety of these compounds will be presented as well as comparative separations using other halogenated stationary phases.

Introduction

Traditional Phases

- Chiral SFC is the primary tool for separation of enantiomers for medicinal chemistry support. Among the chiral selectors are the popular polysaccharides phases, which are substituted phenyl moieties bonded to either amylose or cellulose.

Column	Column type	Chiral Selector	Column	Column type	Chiral Selector
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 Pirkle type	
Chiralcel OD	Coated - Cellulose		ES Industries CC4*	Coated - Cellulose	

Figure 1: Common phases used for chiral separations

- Typical mobile phases used are limited to alcohols combined with hydrocarbons (e.g. hexanes or heptane in HPLC) or carbon dioxide (SFC).
- Additional phases commonly used include Pirkle-type (such as the Whelk-O1) and peptide macrocyclic phases. These have a broader solvent compatibility range, but are usually applied in reverse phase mode (aqueous/organic) or in polar organic mode (e.g. 20mM ammonium acetate in methanol), as well as in SFC.

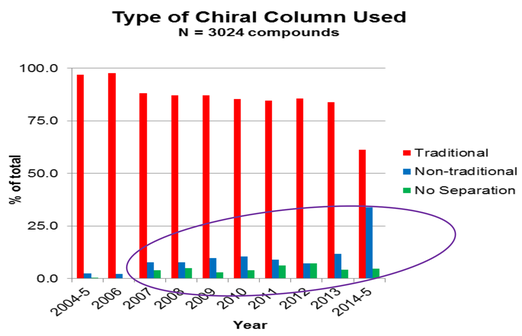


Figure 2: Chiral phases used in purification support of medicinal chemistry

- As shown in Figure 2, an increasing number of chiral compounds achieve separation on any of these traditional phases, especially those compounds with low molecular weight (i.e. MW < 200 Da) or have limited complexity/functionality near the chiral center. Gas chromatography is probably best suited for these compounds; however, it is inconsistent with the goals of isolating pure enantiomers at any workable scale for medicinal chemistry support. Ideally, successful separations of the enantiomers would have a selectivity of >1.1 and a minimum resolution >1.5.

Prototype Phases – 1st Generation

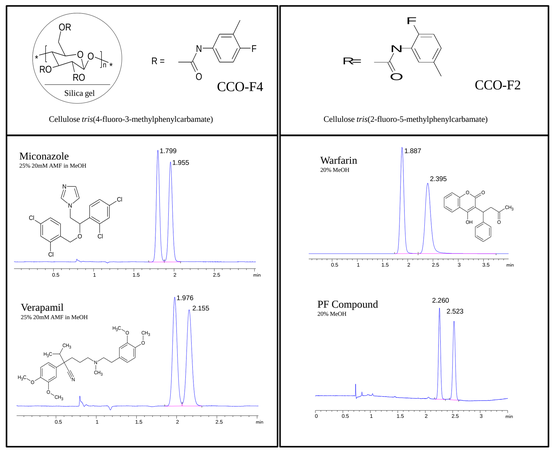


Figure 3: Example separations using 1st Generation fluorinated phases[5]

- Expecting to capitalize on fluorophillic interactions, two prototype phases were made which demonstrate versatility against a broad selection of compounds.

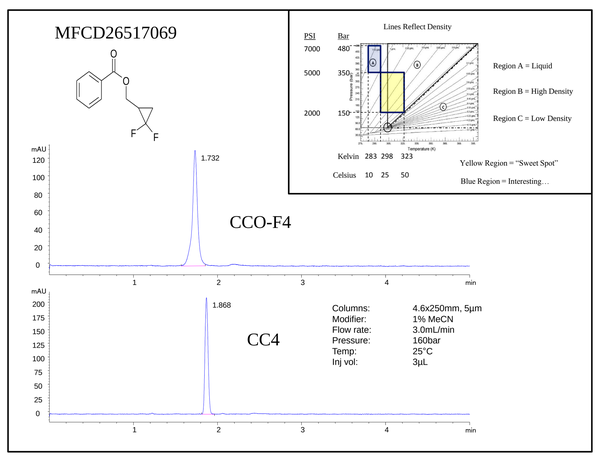


Figure 4: Example chromatograms of MFCD26517069 using standard SFC conditions with 1 % acetonitrile as modifier. Inset: Yellow region represents normal operating conditions in SFC. Blue region represents rarely used region in SFC.

- Using MFCD26517069 as a probe compound, no separation of enantiomers was initially obtained using any traditional phases in SFC mode. In addition, the prototype fluorinated phases also appeared to be ineffective.
- Since the compound is very non-polar, mobile phases of hydrocarbons (HPLC) and carbon dioxide (SFC) were also explored.

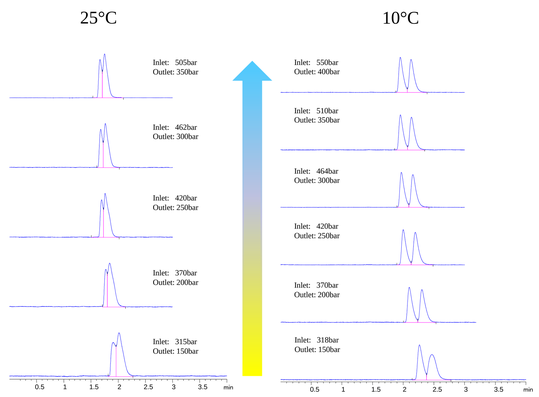


Figure 5: Chromatograms of MFCD26517069 at various pressure and temperature settings

- Borrowing from some the partial success using HPLC (see Figure 6), modification of the density of the mobile phase in SFC mode did result in enantiomeric separation. Figure 5 shows the effects of temperature and pressure on the separation as the conditions move from the yellow to blue regions indicated in the Figure 4 inset.

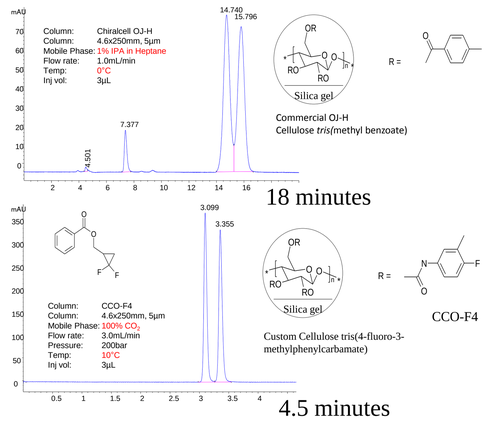


Figure 6: Analytical chromatograms of the separation of MFCD26517069 using HPLC (top) and SFC (bottom) conditions.

- A non-ideal separation was achieved using HPLC conditions and a traditional phase (top), but was extremely sensitive to temperature and thus difficult to scale for purification.
- Using a fluorinated phase, complete separation was achieved using SFC conditions. Significant savings in solvent and time were achieved upon scale-up to purification. Only a small percentage of methanol was added post-column to facilitate collection.

Prototype Phases – 2nd Generation

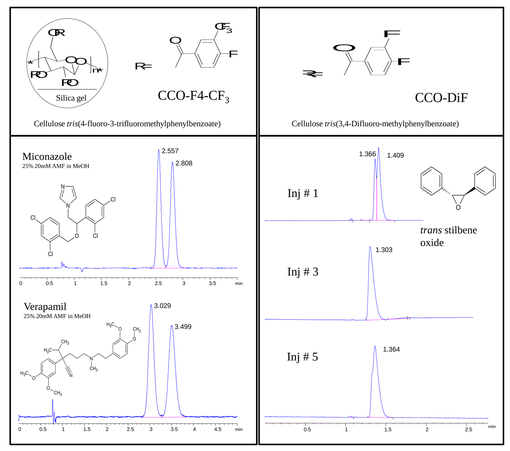


Figure 7: Example separations on the 2nd Generation prototype fluorinated phases[5]

- Following on the success of the CCO-F4 phase, two additional phases were designed to probe different dipole interactions, of which only one demonstrated versatility against a broad selection of compounds. The difluorinated column showed significant bleed and loss of selectivity even with low percentages of solvent (<5%).

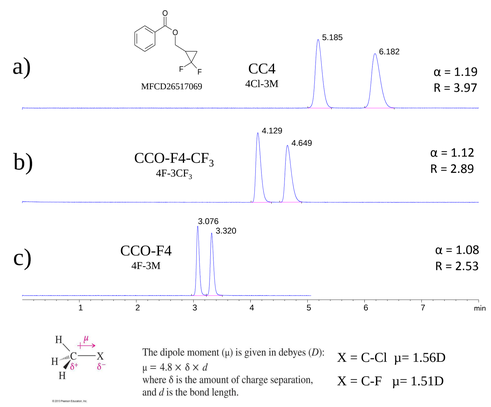


Figure 8: Effect of dipole of moment of stationary phase on the separation of MFCD26517069[5,6]

- Selectivity (α) and resolution (R) values for each separation are included. Dipole moment values provided reflect the magnitude differences in an ideal system and were not measured for each specific phase. a) Cellulose tris-(4-chloro-3-methylphenylcarbamate) phase (CC4). b) Cellulose tris-(4-fluoro-3-(trifluoromethyl)phenylcarbamate (CCO-F4-CF3) phase. c). Cellulose tris-(4-fluoro-3-methylphenylcarbamate) phase (CCO-F4).

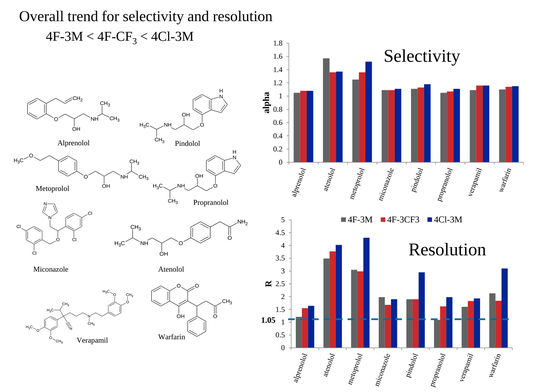
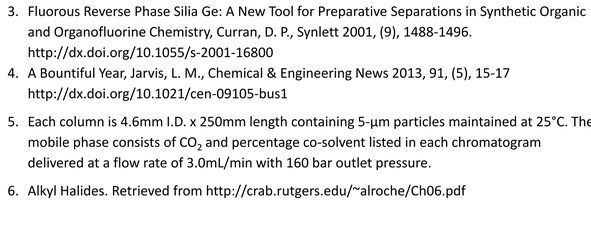


Figure 9: The overall trend in selectivity (α) and resolution (R) for pharmaceutical compounds supports effectiveness of dipole moment manipulation on the stationary phase[5]

- As shown in Figures 8 & 9, the addition of electron-withdrawing functional groups plays a role in the separation mechanism, with the greatest overall effect achieved with chlorine.

- Cellulose tris-(4-chloro-3-methylphenylcarbamate) = 4Cl-3M
- Cellulose tris-(4-fluoro-3-(trifluoromethyl)phenylcarbamate = 4F-CF_3
- Cellulose tris-(4-fluoro-3-methylphenylcarbamate) = 4F-3M



- **Figure 10: Example chromatograms of metoprolol enantiomers on each of the commercial fluoro-methyl and chloro-methyl phases[5]**
- Further demonstrating the effect of the phase dipole, selectivity of metoprolol enantiomers can be enhanced by substituting the halogen (CCO-F4 to CC4), the methyl group (CCO-F4 to CCO-F4-CF_3), or the relative position of both the halogen and methyl groups (CCO-F4 to CCO-F2).

CONCLUSION

The use of fluorine for chiral stationary phases has demonstrated utility and appears comparable to commercial chlorinated phases. The magnitude of the selectivity appears to derive from changes in the dipole of the benzylic functional groups.

Nonetheless, the fluorinated versions offer several advantages in potential savings in solvent consumption and reduced retention time.

The introduction of the CF3 functionality opens a door for further exploration of commercial phases by substituting the methyl group.

Liquid CO_2 can be used to enhance selectivity.

REFERENCES

1. Enantioseparation on fluoro-methylphenylcarbamates of cellulose and amylose as chiral stationary phases for high performance liquid chromatography, Yashima et.al, Polymer Journal, Vol 27, No. 8, pp 856-861 (1995).
2. 3-Fluoro-, 3-chloro- and 3-bromo-5-methylphenyl carbamates of cellulose and amylose as chiral stationary phases for high performance liquid chromatographic enantioseparation, B. Chankvetadze, et al, J. Chrom. A, 787, (1997) 67-77.
3. Fluorous Reverse Phase Silia Ge: A New Tool for Preparative Separations in Synthetic Organic and Organofluorine Chemistry, Curran, D. P., Synlett 2001, (9), 1488-1496. http://dx.doi.org/10.1055/s-2001-16800
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5. Each column is 4.6mm I.D. x 250mm length containing 5-µm particles maintained at 25°C. The mobile phase consists of CO_2 and percentage co-solvent listed in each chromatogram delivered at a flow rate of 3.0mL/min with 160 bar outlet pressure.
6. Alkyl Halides. Retrieved from http://crab.rutgers.edu/~alroche/Ch06.pdf