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Study on the Variation in Optimal Conditions for Chiral SFC Separations Using Different Polysaccharide Based Stationary Phases

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Introduction

Chromatographic analysis and purification of optically active compounds are fields of increased importance within the pharmaceutical industry. In the analytical field, robust chiral separation methods are needed for purity analysis of pharmaceutical products and intermediates. Supercritical fluid chromatography (SFC) has emerged as a fast and effective technique within chiral chromatography. This poster outlines a study on variation of the chromatographic conditions for chiral SFC separations.

Experimental

This study was conducted by separating a series of different chiral samples mainly pharmaceuticals on a number of chiral columns all packed with amylose coated chiral stationary phases (CSP). The CSPs included in this study are either commercially available or in-house Kromasil prototypes. The chiral separations were conducted with three different alcohols as mobile phase modifiers and the differences in chiral selectivity evaluated. The chromatographic parameters varied in this study are visualized in figure 1 below.

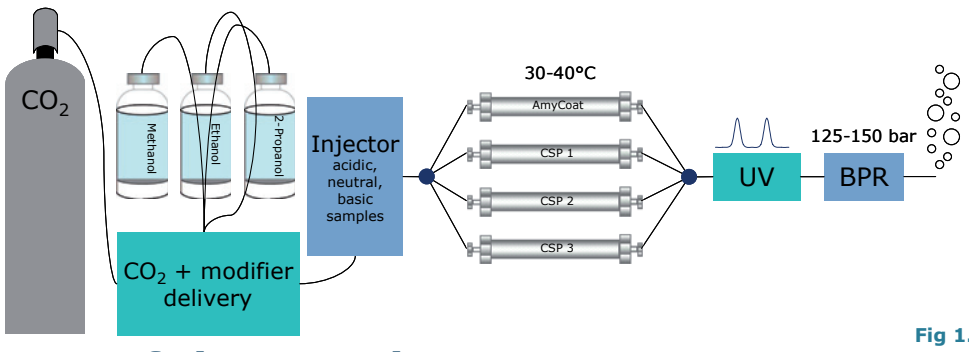


Fig 1.

Nature of the sample

This study in optimization of chiral SFC applications includes a series of acidic, neutral and basic racemic samples. The chiral samples were chosen to resemble a wide variation of chemistries of small chiral molecules. Figure 2 show several examples of chiral separations of acidic, neutral and basic compounds using Kromasil 3-AmyCoat in SFC mode.

Assorted chiral SFC separations of acidic, neutral and basic compounds all performed using Kromasil 3-AmyCoat 4.6x150mm

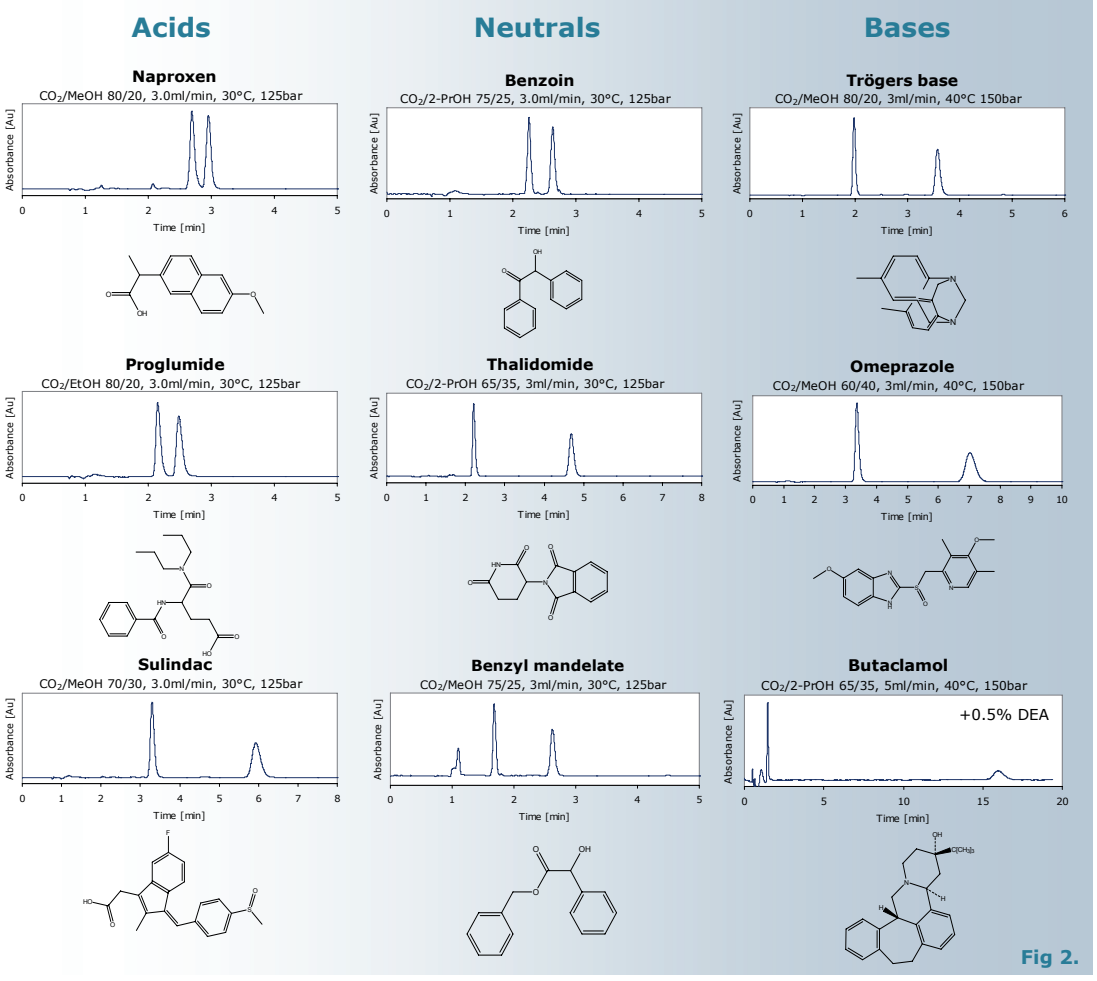


Fig 2.

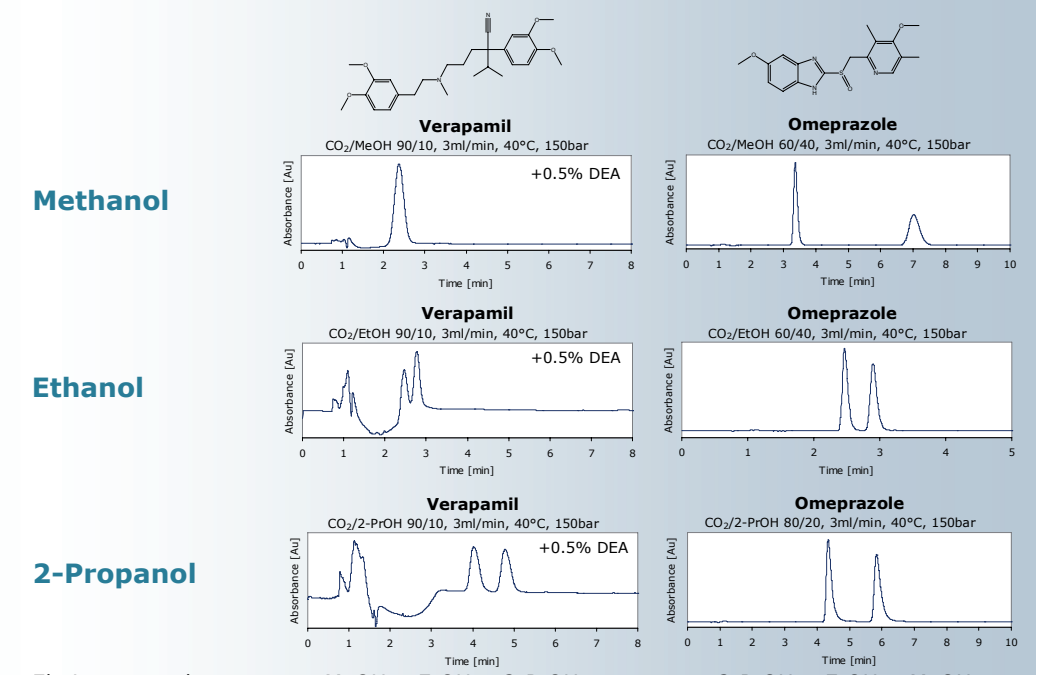
It has been reported in the literature that some basic compounds specifically amines do not separate well in chiral SFC.[1)] This was partially confirmed in this study. Neutral and acidic chiral samples generally separate well in SFC mode. Some variation between the selectivity for acidic samples were through observed for the tested CSPs.

References:
1) Y.K. Ye et. al. Journal of Chromatography A, 1041 (2004) 211-217
2) M. Maftouh et. al. Journal of Chromatography A, 1088 (2005) 67-81

Importance of the modifier

Methanol is by far the most common mobile phase modifier in SFC applications, both chiral and achiral. In this study the appropriateness of this position of methanol as the first choice is studied by performing all separation attempts with methanol, ethanol and 2-propanol as mobile phase modifiers. Figure 3 show the difference in chiral selectivity obtained with a number of amylose based chiral stationary phases between runs performed with each of the three alcohols as modifier. The effect of the modifier on a chiral separation varies greatly with analyte, but as reported by Maftouh et al. 2-propanol does often lead to the best selectivity of two enantiomers.[2)] Though fast equilibration is a key advantage of the SFC technique, extremely long equilibration times were observed for one of the tested CSPs. When switching between modifier alcohol it took up to 30 minutes to obtain reproducible separations.

Effect of the mobile phase modifier on chiral selectivity, all separations performed using Kromasil 3-AmyCoat 4.6x150mm



Elution strength: MeOH > EtOH > 2-PrOH
Selectivity (α): 2-PrOH > EtOH > MeOH

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Effect of the mobile phase modifier on the chiral selectivity of several different CSPs based on amylose coated silica

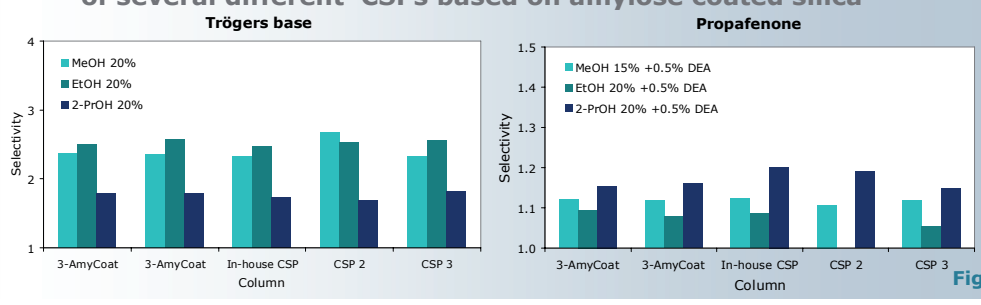

Fig 3.

Additives in the modifier

A general attitude against mobile phase additives exist in the field of chiral chromatography. Our SFC study show that little or no advantage of additive in the mobile phase modifier is observed for separation of acidic and neutral samples. This is explained by the fact that the nature of the carbon dioxide based mobile phase is slightly acidic and that neutral samples in general do not need additives. Some basic racemates on the other hand do need modifier additive to elute and separate in SFC, an example is given in figure 4. Diethylamine (DEA) was used as basic modifier additive.

Separation of propafenone without and with basic mobile phase additive

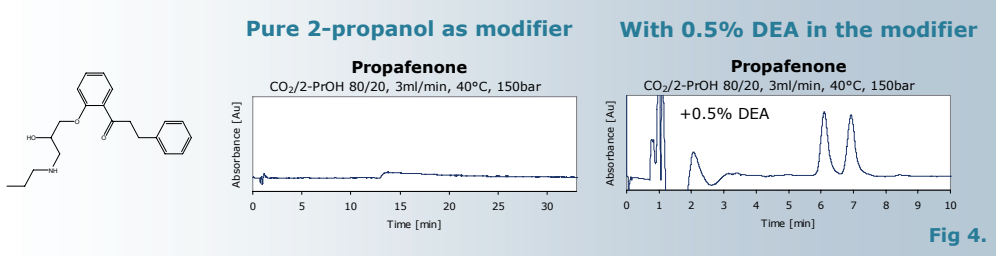

Fig 4.

Conclusion

Kromasil AmyCoat is a versatile CSP suitable for a wide variety of chiral SFC separations. When attempting to solve a chiral separation by way of SFC it is important to screen several mobile phase modifiers to obtain the best possible selectivity and appropriate elution strength. Separation of basic samples may be assisted by a basic modifier additive.