From Screening to Purification, Presentation of a Strategic Approach to Solving Chiral Separation Issues by HPLC

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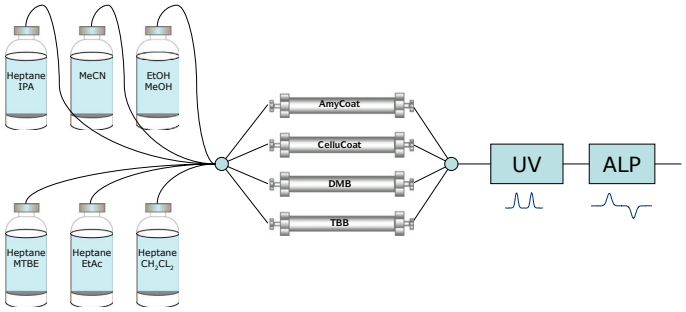
Background

Within the pharmaceutical industry chromatographic analysis and purification of optically active compounds are becoming fields of increased importance. In the analytical field, robust chiral separation methods are needed for purity analysis of pharmaceutical products and intermediates. In addition when enantioselective synthesis fails large scale separation of enantiomers becomes an attractive alternative, This poster outlines the principles of chiral separation solving using Kromasil® chiral stationary phases.

Chiral Screening

When starting chiral HPLC method development the initial screening is typically done using an automated system running a series of experiments combining a number of chiral stationary phases (CSP) with different appropriate mobile phases to test various chromatographic systems. Whether doing screening with the purpose of developing an analytical or a preparative chiral method it is advisable to use a small particle CSP packed in a short or medium length column when initially choosing CSP to achieve high column efficiency and save time. The following diagram shows the principle of a chiral screening system.



Depending on the purpose of the chiral separation method the optimum particle size of the CSP varies. Small particles (3 µm and 5 µm) should be applied in analytical scale work and larger particles used when going to preparative scale. Figure 1 shows the separation of trans-Stilbene oxide on Kromasil CelluCoat 3 µm, 5 µm, and 10 µm.

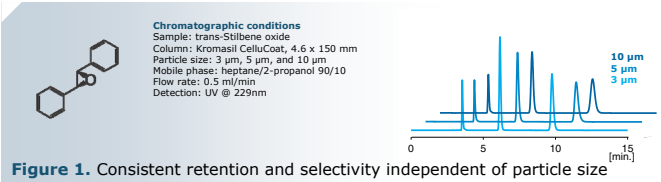


Figure 1. Consistent retention and selectivity independent of particle size

Analytical Chiral Separations

An analytical chromatographic method for purity analysis of product samples should provide robust baseline separation between the product and its enantiomer in a reasonable time. The time factor is especially important when a large number of samples need to be handled. The durability of Kromasil chiral stationary phases allow the columns to be operated at high flow rates. High flow rate combined with small particles and short column length provide very short analysis time.
Our studies have shown that it is important to include mobile phase additives in the screening process not to miss a perfectly fine chiral separation. Based on the chemical structure of the molecule it will interact differently with the CSP. Some of these interactions between analyte and CSP may be strong and in analytical scale can disrupt a good separation if not eliminated by using acidic or basic mobile phase additive.

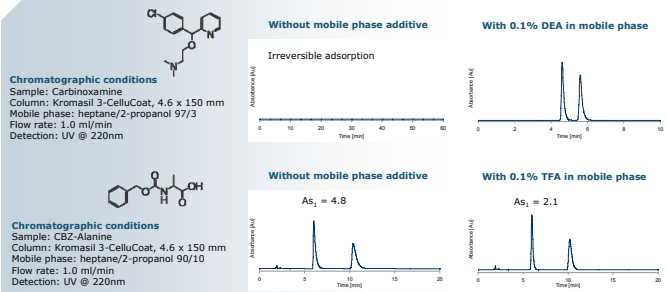


Figure 2.

Figure 2 shows the analytical separation of carbinoxamine and CBZ-alanine on Kromasil CelluCoat 3 µm. As seen from the results presented in figure 2 it is essential for a satisfying analytical separation to use appropriate mobile phase additive when separating acidic or basic racemates. The table below shows suggested mobile phase additives for different analytes.

		Analyte			
		Neutral	**Acidic**	**Basic**	**Strong base**
CSP	**AmyCoat CelluCoat**	No additive	0.1% TFA 0.1% Acetic acid	0.1% DEA	0.1% DEA
	DMB TBB	No additive	0.1% formic acid 0.1% acetic acid	0.1% formic acid 0.1% acetic acid	0.2% formic acid +0.1% TEA

Preparative Chiral Separations

Important aspects in preparative chiral chromatography are loadability, selectivity and solubility which in combination determine productivity. Figure 3 illustrates a preparative application on Kromasil CelluCoat 10 µm. Preparative injections of CBZ-alanine were made on Kromasil CelluCoat 10 µm both with and without TFA in the mobile phase.

As seen from the elution profiles reconstructed from fraction analysis data the mobile phase additive does not provide any advantage in this particular preparative separation, contrary to an analytical separation of the same compound. This is very favorable since all extra mobile phase constituents are disadvantageous for post-HPLC workup.

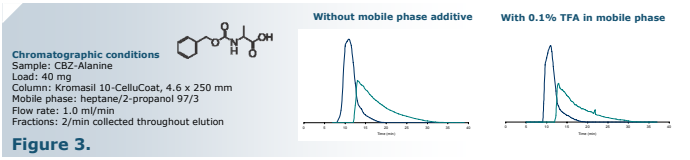


Figure 3.

Additives in the Mobile Phase

A general attitude against mobile phase additives exist in the field of chiral HPLC. The arguments against the use of mobile phase additives are strong and valid, but the advantages must be taken into account. Especially in analytical scale the value of a mobile phase additive must not be underrated, and chiral screening without mobile phase additives should only be done with careful considerations. In preparative chromatography additives are undesirable since they complicate the solvent recovery process and they could also reduce the stability of the enantiomers in the mobile phase, particularly during evaporation. However our small study shows that at overloaded conditions mobile phase additive has no positive effect on the separation of CBZ-alanine as can be seen from the fraction analysis presented in Figure 3. This behavior could be explained by a large amount of analyte being present and a self correction of the system occurs.

General Strategy

The general strategy to be adapted when developing chiral analysis or purification methods can be simplified to the following points.

- Setup and run a screening series using a number of complementary CSP's in combination with mobile phases with appropriate additives, Suggested CSP's are: Kromasil 3-CelluCoat, Kromasil 3-AmyCoat, Kromasil 5-TBB, and Kromasil 5-DMB
- The most promising chromatographic systems with respect to chiral selectivity of the target compound should be investigated further to select the best lead for a new method.
- The chiral HPLC method is finalized by choosing mobile phase strength, column dimension and particle size according to purpose. Mobile phase strength is adjusted to give approximately $1<k'_1<2$, mobile phase additive is often desirable for a robust analytical method, whereas its necessity in preparative scale should be tested under overloaded conditions, and the additive avoided if it does not have a positive effect on the separation, With respect to particle size of the CSP 3 µm and 5 µm particles are recommended for analytical methods and 10 µm particles for preparative use.