Introduction

CHIRAL-AGP, CHIRAL-HSA and CHIRAL-CBH are protein-based chromatographic supports with very broad application domain for enantiomer separation at analytical level [1]. A great number of publications have been devoted to them since early 1980s.

When getting in Daicel hands, two main objectives were targeted for method development:

1) Simplify the approaches for generic screening and straightforward separation optimisation for chiral compounds of diverse chemical nature.

2) Develop methods being LC-MS compatible.

An exhaustive investigation was undertaken in our laboratories with the main objective of developing a straightforward screening strategy with the three columns[2].

Chiral selectors and application scope

| CHIRAL-AGP | α-C Acid glycoprotein (orosomucoid) |
| CHIRAL-HSA | Human serum albumin |
| CHIRAL-CBH | Cellobiohydrolase |

All these selectors are chemically bonded onto 5μm spherical silica gel. The three columns operate solely under RP conditions. Among them, CHIRAL-AGP is the most versatile and suitable for enantiomer resolution of all kinds of compounds, whilst CHIRAL-HSA is more specific for acidic chiral species and CHIRAL-CBH is a good alternative for basic chiral analytes. The broadest applicability of CHIRAL-AGP determines its dominant position in the column choice to start the method development with no need of preliminary analysis of the sample nature.

Main chromatographic parameters

Several chromatographic parameters may influence the results of the enantiomer resolution:

- pH value of the buffer
- the nature and concentration of the buffer
- the nature (IPA, EtOH, MeOH...) and the percentage (0-15%) of the organic modifier
- the charged additives (≤10mM) in the mobile phase
- the temperature (≤30°C)

Among all these parameters, the pH value of the buffer is the most important. It determines the ionic interaction between the chiral selector and the analyte molecules, regulates the enantioselectivity and impacts the sample retention.

As a rule of thumb, three pH values (4.5 — 5.8 — 7.0) would be enough for efficient regulation of the retention time.

Predictable effect of pH on retention

The proteins are negatively charged in the recommended pH range. The higher the pH value, the more negatively charged the proteins. As a consequence, higher pH will increase the retention of basic compounds and reduce the one of acidic analytes. In contrast, the variation of pH value should normally have no influence on the retention of neutral molecules.

Initial conditions and method optimisation

| Column: | CHIRAL-AGP |
| Buffer: | 10mM ammonium acetate |
| Organic modifier: | 5% IPA (2-propanol) |

The initial conditions represent the best compromise for all kinds of compounds, for analysis time, for enantioselectivity and for the LC-MS compatibility of the method.

Examples of one-hit separations with the starting condition

In our study, the initial screening leads to successful enantiomer resolution on CHIRAL-AGP for 25% of the 76 racemic compounds tested without any further optimization. The success rate reaches 85% by following the chart below for method optimisation.

Method optimisation by varying the organic modifier content

Method optimisation by changing the organic modifier

Chiral separation on CHIRAL-CBH

CHIRAL-CBH is a good alternative for enantiomer separation of basic compounds. Being complementary to CHIRAL-AGP in this domain, it can significantly enhance the success rate of chiral separation for basic species. The scheme for method development on CHIRAL-CBH is essentially the same as for CHIRAL-AGP.

Separations on CHIRAL-CBH with the starting condition

Method development on CHIRAL-HSA

CHIRAL-HSA is versatile for chiral resolution of acidic compounds. Due to the structural characteristics of the chiral selector, the strategy for method development differs from CHIRAL-AGP and CHIRAL-CBH.

Starting conditions:

| Buffer: | 10mM ammonium acetate |
| Organic modifier: | 15% IPA (2-propanol) |

Optimisation steps:

- Use charged additives (e.g. Heparin/mannuronic acid, Orotic acid)
- Adjust the modifier percentage
- Replace IPA with other organic modifiers (ACN, EtOH MeOH)

Examples of specific separations on CHIRAL-HSA

References