LC-MS COMPATIBLE REVERSED-PHASE SCREENING STRATEGIES ON DAICEL PROTEIN-BASED COLUMNS: CHIRAL-AGP, CHIRAL-HSA AND CHIRAL-CBH

Tong ZHANG, Takashi MICHISHITA, Pilar FRANCO
Chiral Technologies Europe, 67400 Illkirch, France

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.

They were initially marketed by ChromTech and have been integrated to the portfolio of the chiral columns from Daicel Chemical Industries, Ltd.

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

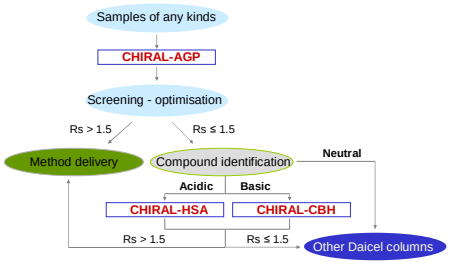
CHIRAL-AGP	α_1-Acid glycoprotein (orosomucoid)
CHIRAL-HSA	Human serum albumin
CHIRAL-CBH	Cellobiohydrolase

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, ACN…) 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.

Protein/Column	Isoelectrical point	Recommended pH range
AGP	2.7	4.0 – 7.0
CBH	3.9	4.0 – 7.0
HSA	4.7	5.0 – 7.0

All the columns in this study are sized 4.0x100mm, tested at T=20°C and at a flow rate of 0.9ml/min

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.

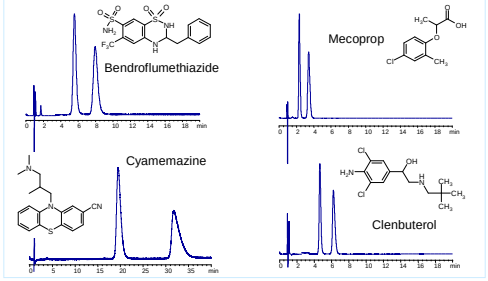
pH	low	high	*retention*
Neutral			Constant to pH
Acidic			Higher pH, lower retention
Basic			Higher pH, higher retention

Initial conditions and method optimisation

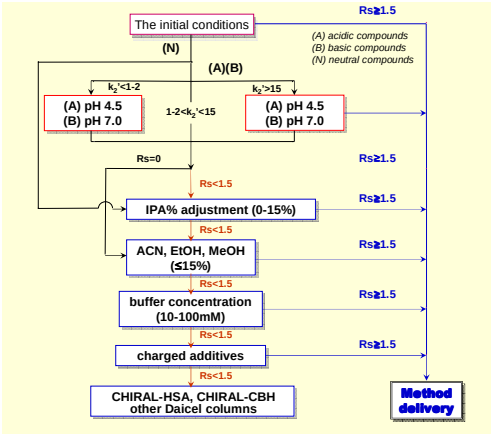
Column:	**CHIRAL-AGP**
pH:	**5.8**
Buffer:	**10mM ammonium acetate**
Organic modifier:	**5% IPA (2-propanol)**

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.

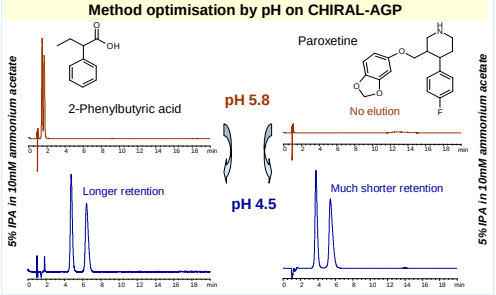
Examples of one-hit separations with the starting condition



In our study, the initial screening leads to successful enantiomer resolution on CHIRAL-AGP for 20% of the 76 racemic compounds tested without any further optimization. The success rate reaches 85% by following the chart below for method optimisation.

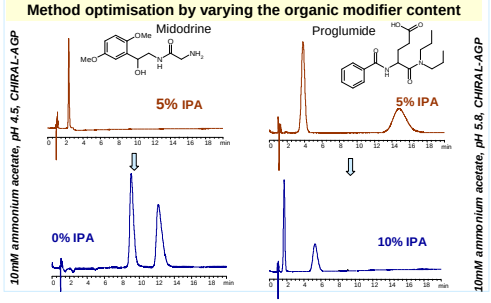


Method optimisation by pH on CHIRAL-AGP

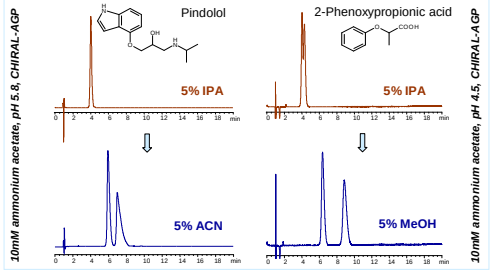


As a rule of thumb, three pH values (4.5 – 5.8 – 7.0) would be enough for efficient regulation of the retention time.

Method optimisation by varying the organic modifier content



Method optimisation by changing the organic modifier

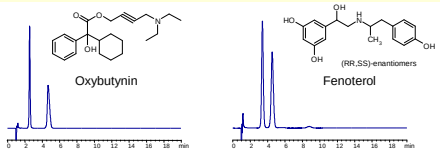


Chiral separation on CHIRAL-CBH

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



Buffer: 10mM ammonium acetate pH: 5.8; Organic modifier: 5% IPA

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.

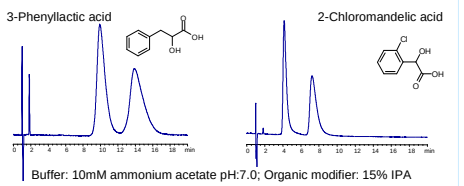
Starting conditions:

pH:	7.0
Buffer:	10mM ammonium acetate
Organic modifier:	15% IPA (2-propanol)

Optimisation steps:

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

Examples of specific separations on CHIRAL-HSA



Buffer: 10mM ammonium acetate pH:7.0; Organic modifier: 15% IPA

CHIRAL-AGP, CHIRAL-CBH and HSA constitute a powerful tool box for resolution of enantiomers under RP conditions. They allow the routine application of screening sequences for samples from biological and other media with various structural nature.

References
[1] J. Haginaka, J. Chromatogr. A, 906 (2001) 253-273.
[2] T. Michishita, T. Zhang, P. Franco, submitted for publication.