Chiral AGP

ChromTech
Schematic drawing of $\alpha_1$-acid glycoprotein (AGP)

**Characteristics**

- Peptide chain: 183 aa
- Carbohydrate content: 45%
- Molecular weight: 40000
- Isoelectric point (pI): 2.7
The AGP column has a unique property!

The chiral bonding properties of the stationary phase can be changed dynamically.

Enantioselectivity can be induced and improved by simple changes of the mobile phase composition.
Optimization of enantioselectivity and retention

- **pH**
- **Buffer**
  - concentration
  - nature
- **Uncharged modifier**
  - nature
  - concentration
- **Charged modifier**
  - nature
  - concentration
Most important tool in method development

pH
Net charge of AGP at different pH

pH 2.0  pl  pH 4.0  pH 7.0

Increasing net negative charge of AGP at higher pH

= pH-range used in chromatography

pl = isoelectric point of AGP, i.e. the pH(2.7) where the protein has a net charge of zero.
pH effects - strong acids

Separation of 2-phenoxypropionic acid at different pH

pH 7.0  pH 6.0  pH 5.0
Separation of basic drugs at low pH on CHIRAL-AGP 100x4.0 mm

Remoxipride

Procyclidine

Nefopam

Pheniramine
Separation of tricyclic basic drugs at low pH on CHIRAL-AGP 100x4.0 mm

Alimemazine

Trimipramine

Dixyrazine

Cyamamezine
Another important tool in method development on CHIRAL-AGP

Nature and concentration of uncharged organic modifier

2-propanol, 1-propanol

acetonitrile

ethanol, methanol etc.
Influence of uncharged modifier concentration on retention and enantioselectivity

Analyte: Methylphenylcyanoacetic acid ethyl ester

Enantioselectivity

Retention

Decreasing modifier concentration

Increasing enantioselectivity

Increasing retention
Effect of organic modifier character on enantioselectivity

Modifier conc. (M)

- 2-propanol
- 1-propanol
- Acetonitrile

Separation factor ($\alpha$)

Methylphenobarbital
Influence of the type of organic modifier on the enantioselective retention of clevidipine

<table>
<thead>
<tr>
<th>Organic Modifier</th>
<th>Enantiomer Retention</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-propanol (20%)</td>
<td>(R,S)</td>
</tr>
<tr>
<td>Methanol (36%)</td>
<td>(S)</td>
</tr>
<tr>
<td>1-propanol (16%)</td>
<td>(R)</td>
</tr>
<tr>
<td>Acetonitrile (20%)</td>
<td>(S)</td>
</tr>
<tr>
<td>Dimethylsulphoxide (15%)</td>
<td>(R)</td>
</tr>
</tbody>
</table>

Column: CHIRAL-AGP 150x4.0 mm
Mobile phase: Organic modifier in 25 mM phosphate buffer pH 7.0

Another important tool in method development on CHIRAL-AGP:

Nature and concentration of buffer

Acetate
Phosphate
Citrate
Tris
Formate etc.
Influence of the buffer conc. on resolution and retention of the enantiomers of naproxen

0.01 M Sodium phosph. buffer pH 7.0

0.05 M Sodium phosph. buffer pH 7.0
Influence of acetate concentration on retention and enantioselectivity of propranolol

Column: CHIRAL-AGP 100x4.0 mm
Mobile phase: 0.5 % 2-propanol in acetate buffer pH 4.1

<table>
<thead>
<tr>
<th>Acetate mM</th>
<th>$k'_1$</th>
<th>$k'_2$</th>
<th>$\alpha$</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>5.73</td>
<td>7.29</td>
<td>1.27</td>
</tr>
<tr>
<td>25</td>
<td>6.54</td>
<td>8.79</td>
<td>1.34</td>
</tr>
<tr>
<td>96</td>
<td>7.04</td>
<td>10.7</td>
<td>1.52</td>
</tr>
</tbody>
</table>
LC/MS

The type and concentration of buffer is important when developing methods for MS-detection.

Methods based on phosphate buffers or other nonvolatile buffers can easily be transformed to MS compatible methods by changing to ammonium acetate or ammonium formate buffers.
Fast chiral separation suitable for MS detection

Desmetylsibutramine

CHIRAL-AGP 50x4.0 mm

Mobile phase: 5% CH3CN in 10 mM ammonium acetate buffer pH 4.1
Rapid separation of acidic compounds using MS-compatible conditions.

Etodolac: 15% CH₃CN in 10 mM amm.acetate.

Proglumide: 9% CH₃CN in 10 mM amm.acetate. Column: CHIRAL-AGP 50x2.0 mm.

Naproxen: 1% CH₃CN in 10 mM amm.acetate.
Charged organic modifiers can be an important tool in method development. They have the most dramatic effects on the enantioselectivity and the retention.

Examples of modifiers:

Cationic: N,N-dimethyloctylamine (DMOA) and other amines

Anionic: Hexanoic- and octanoic acid
Influence of DMOA concentration on the enantioselectivity of naproxen

DMOA = N,N-Dimethyloctylamine

+ 1 mM DMOA
Clopidogrel

Column: CHIRAL-AGP 100x4.0 mm

Mobile ph. 16% acetonitrile and 1 mM N,N- dimethyloctylamine (DMOA) In 10 mM ammonium acetate pH 5.5
Influence of the concentration of an anionic modifier (octanoic acid) on the enantioselectivity of atropine

Separation factor ($\alpha$)

Octanoic acid conc. (M)
Simple method development strategy
Method development CHIRAL-AGP

Characterize your sample

<table>
<thead>
<tr>
<th>Amine</th>
<th>Acid</th>
<th>Nonprotolyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Hydrophobic</td>
<td>- Strong</td>
<td></td>
</tr>
<tr>
<td>- Hydrophilic</td>
<td>- Weak</td>
<td></td>
</tr>
</tbody>
</table>

Choose the appropriate method development scheme where you will find the starting mobile phase
<table>
<thead>
<tr>
<th>Compound type</th>
<th>Starting mobile phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrophobic amine</td>
<td>10 mM ammonium or sodium acetate buffer pH 4.5</td>
</tr>
<tr>
<td>Hydrophilic amine</td>
<td>5% 2-propanol in 10 mM sodium phosphate buffer pH 7.0</td>
</tr>
<tr>
<td>Weak acid or non-protolyte</td>
<td>5% 2-propanol in 10 mM sodium phosphate buffer pH 7.0</td>
</tr>
<tr>
<td>Strong acid</td>
<td>10 mM sodium phosphate buffer pH 7.0</td>
</tr>
</tbody>
</table>
If you have characterized your compound as a hydrophobic amine follow the scheme below:

Start with 10 mM ammonium or sodium acetate buffer pH 4.5

- **Retention and enantioselectivity**

  - **Optimize with pH and/or uncharged modifiers**
  - Test another uncharged modifier: acetonitrile, methanol, 1-propanol, ethanol
  - Test low conc. of a charged modifier*:
    - octanoic acid 1-20 mM
    - hexanoic or heptanoic acid 1-20 mM
    - tetraethyl- and tetrapropyl-ammonium bromide 1-5 mM

- **No or low enantioselectivity and low retention**

  - Increase pH stepwise and adjust retention with 2-propanol (lower conc. gives higher enantioselectivity)
  - Test another uncharged modifier: acetonitrile, methanol, 1-propanol, ethanol

- **Enantioselectivity and too high retention**

  - Decrease pH to 4 and/or add 2-propanol
  - Test another uncharged modifier: acetonitrile, methanol, 1-propanol, ethanol

- **Too high retention. No enantioselectivity**

  - Test different uncharged modifiers: 2-propanol, acetonitrile, methanol, 1-propanol, ethanol
Separation of the calcium channel blocking agent Isradipine

**Column:** CHIRAL-AGP 100 x 4.0 mm

**Mobile phase:** 15% 2-propanol in 30 mM phosphate buffer pH=6.8

**Detection:** UV 225 nm

**Sample conc.:** 0.02 mg/ml
Roxifiban

Column: CHIRAL-AGP 100x4.0 mm
Mobile ph.: 5% 2-propanol in 10 mM phosph. buffer pH 7.0

Separation of the enantiomers of the antiulcer drug omeprazole

Column: CHIRAL-AGP 100 x 4.0 mm

Mobile phase: 10% acetonitrile in 10 mM sodium phosphate buffer pH=6.5

Detection: UV 210 nm

Sample conc.: 0.02 mg/ml
Separation of the enantiomers of the anticholinergic drug proglumide

**Column:** CHIRAL-AGP 100 x 4.0 mm

**Mobile phase:** 5% 2-propanol in 10 mM sodium phosphate buffer pH=6.0

**Detection:** UV 225 nm

**Sample conc.:** 0.02 mg/ml
Separation of the enantiomeres of clenbuterol

**Column:** CHIRAL-AGP 100 x 4.0 mm

**Mobile phase:** 1% 2-propanol in 10 mM sodium acetate buffer pH=5.0 (total acetate concentration 15 mM)

**Detection:** UV 225 nm

**Sample:** 0.02 mg/ml
Separation of the enantiomers of remoxipride

**Column:** CHIRAL-AGP 100 x 4.0 mm

**Mobile phase:** 30 mM sodium acetate buffer pH=4.0 (total acetate concentration 170 mM)

**Detection:** UV 210 nm

**Sample:** 0.02 mg/ml
Stability study of the CHIRAL-AGP column

Sample: Bumadizon

Mobile phase: 10% 2-propanol in 10 mM ph. b. pH 6.0

30.5 liters of mobile phase

2030 samples have been injected during the study

Guard column exchanged after 7.5 liters of mobile phase, corresponding to 147500 column volumes.
Determination of enantiomeric purity of disopyramide using the CHIRAL-AGP column

R-disop. 0.12%

S-disop. 0.92%

Column: CHIRAL-AGP 100 x 4.0 mm

Mobile phase: 10 % 2-propanol in 0.01 M Na phosph. b.pH 7.0

Sample conc.: 0.7 mg/ml
Purity determination of (+) - mepivacaine

Mepivacain
Chiral analysis of methadone enantiomers in patient plasma

Column: CHIRAL-AGP 100 x 4.0 mm  
Mobile phase: 16% acetonitrile in 10 mM potassium phosphate buffer pH=6.6  
Flow rate: 0.7 ml/min  
Detection: UV 212 nm
Conclusions

- The AGP column most likely has the broadest applicability of all chiral columns available. It separates amines, acids, non-protolytes.

- **Solute retention by:**
  - ionic bonding
  - hydrophobic interaction
  - hydrogen bonding

- The enantioselectivity and the retention can be regulated in many different ways:
  a) pH
  b) **Buffer** (nature and concentration)
  c) **Uncharged modifier** (nature and concentration)
  d) **Charged modifier** (nature and concentration)

- **Simple method development**