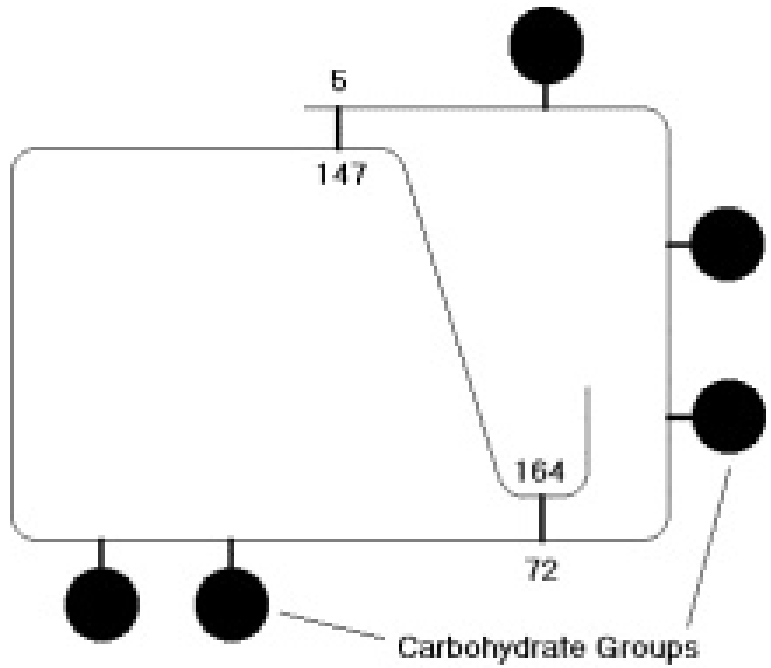
Chiral AGP

ChromTech

Schematic drawing of α_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**

- **Uncharged modifier**
  - nature
  - concentration

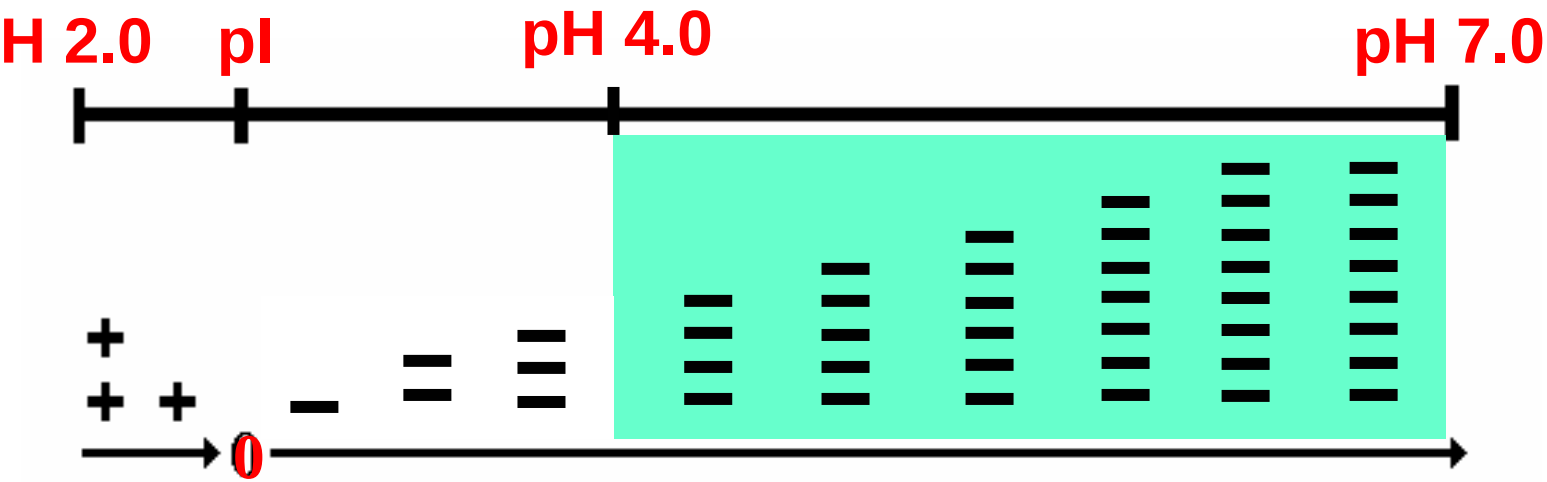
- **Buffer**
  - concentration
  - nature

- **Charged modifier**
  - nature
  - concentration

Most important tool in method development

pH

Net charge of AGP at different pH



Increasing net negative charge of AGP at higher pH

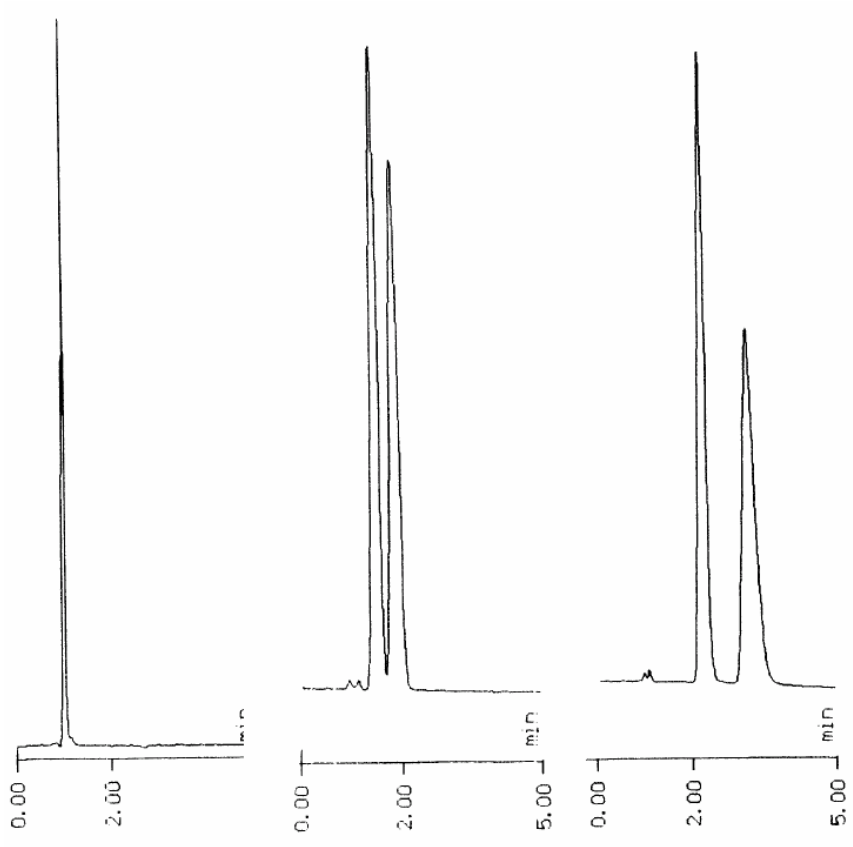
= pH-range used in chromatography

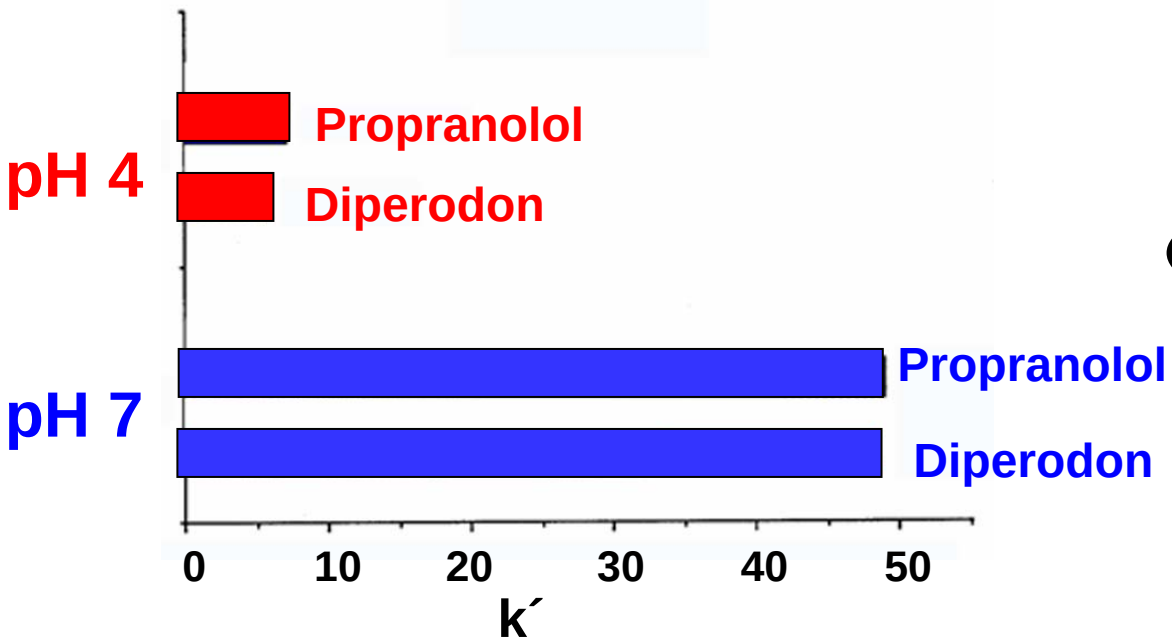
pI = isoelectric point of AGP, i.e. the pH(2.7) where the protein has a net charge of zero.

pH effects - strong acids

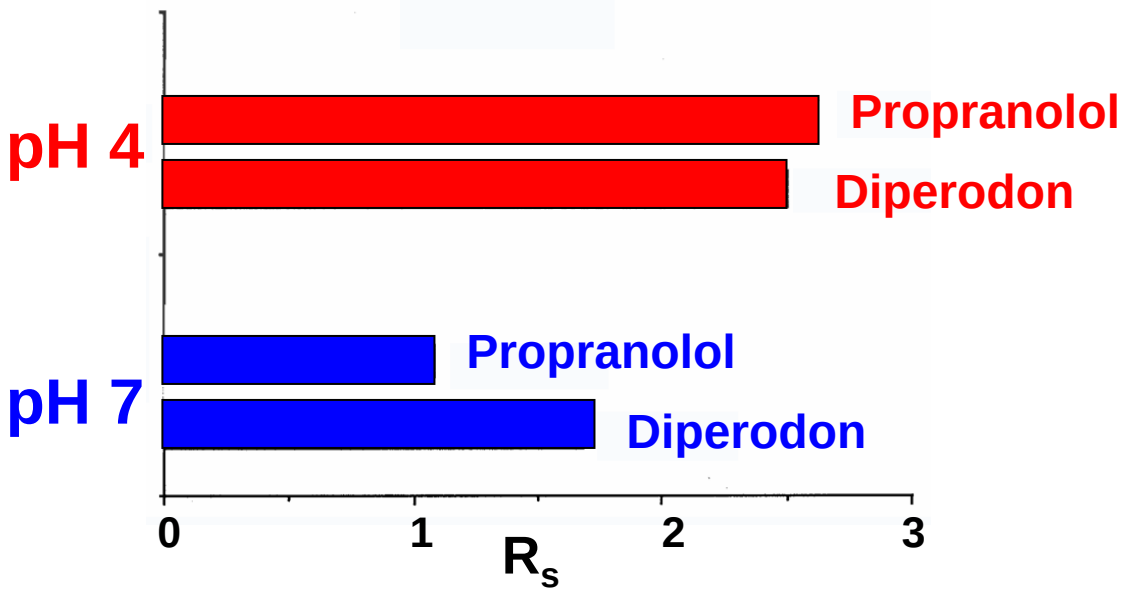
Separation of 2-phenoxypropionic acid at different pH

pH 7.0 pH 6.0 pH 5.0



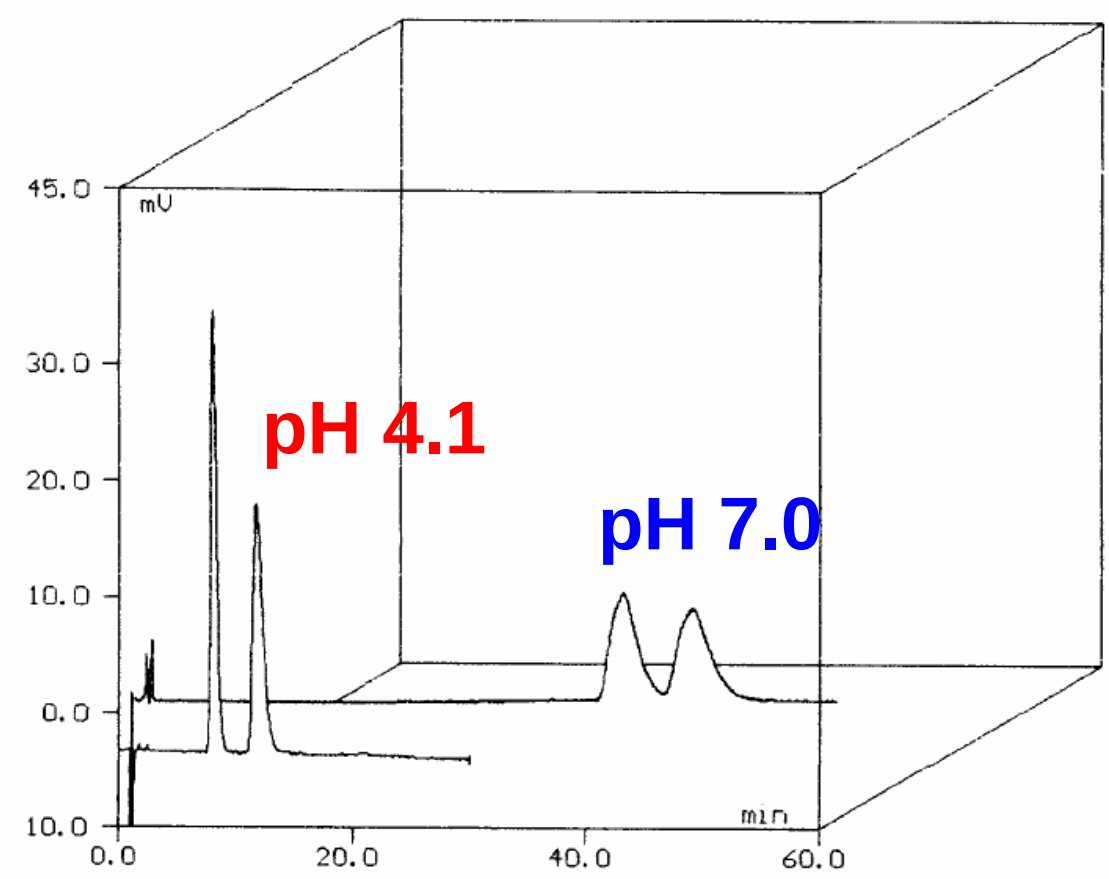


Retention

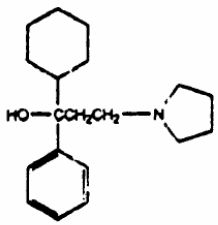


Resolution

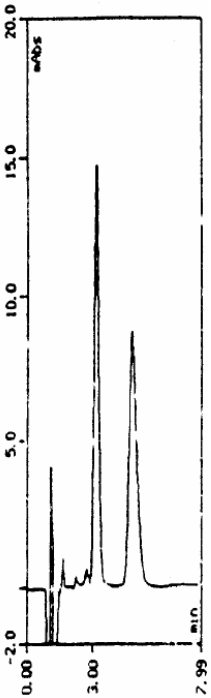
pH effects - propranolol



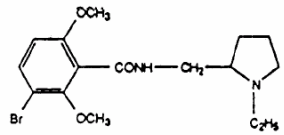
Separation of basic drugs at low pH on CHIRAL-AGP 100x4.0 mm

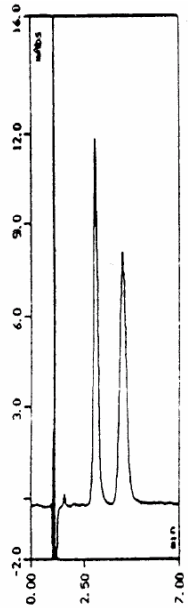


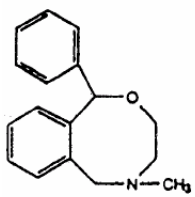
Procyclidine



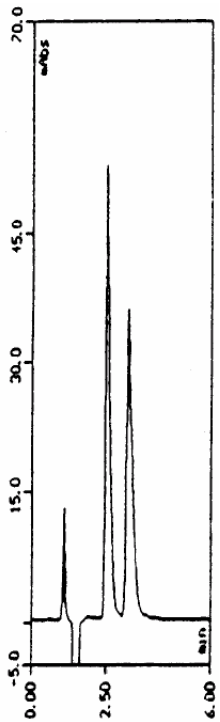
Remoxipride

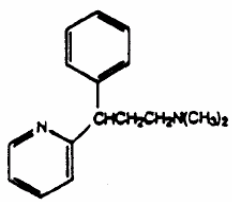




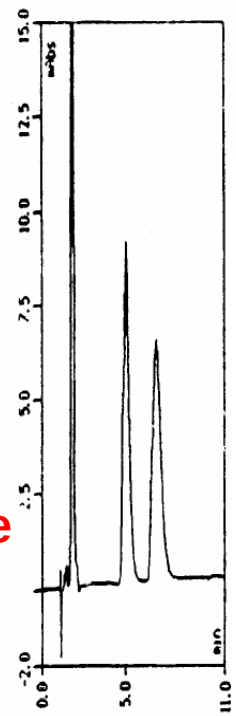


Nefopam

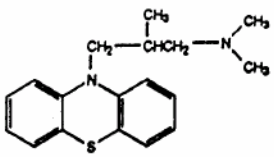


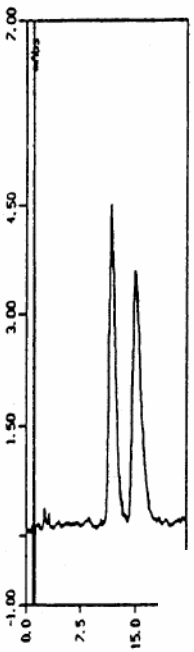


Pheniramine



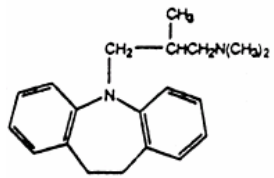
Separation of tricyclic basic drugs at low pH on CHIRAL-AGP 100x4.0 mm

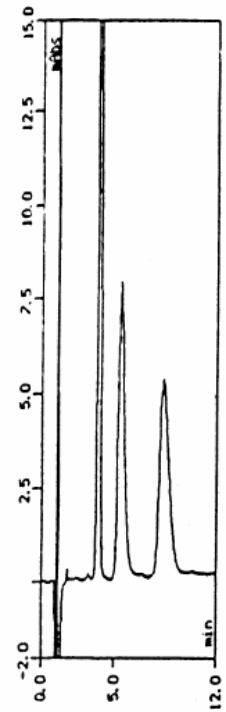


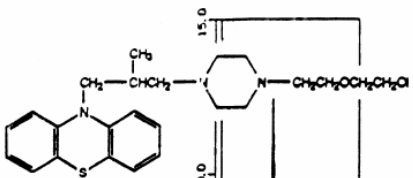


Alimemazine

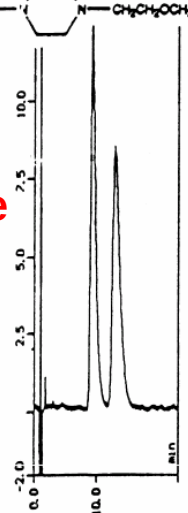
Trimipramine



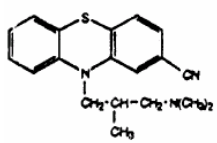


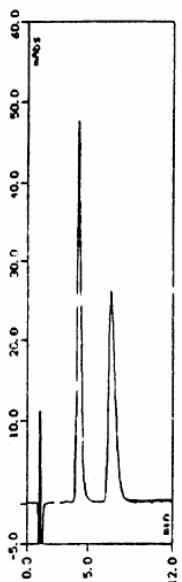


Dixyrazine



Cyamamezine





Another important tool in method development on CHIRAL-AGP

Nature and concentration of uncharged organic modifier

2-propanol, 1-propanol

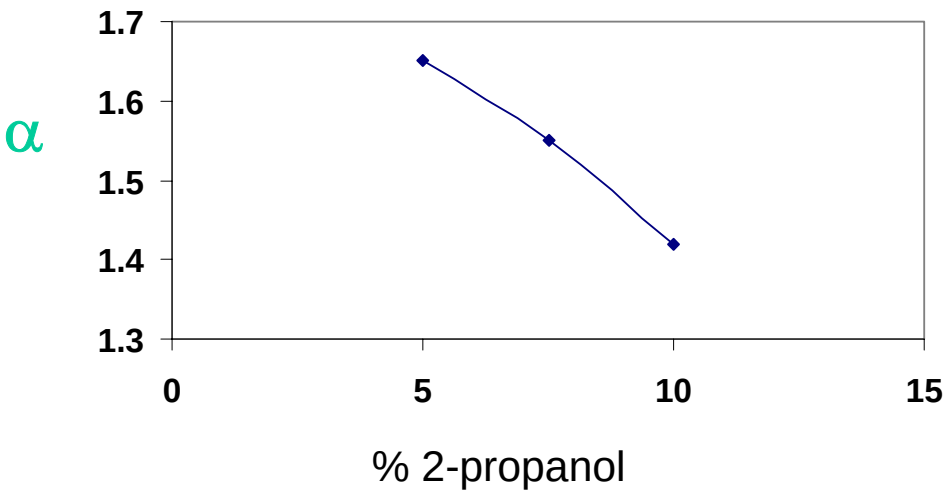
acetonitrile

ethanol, methanol etc.

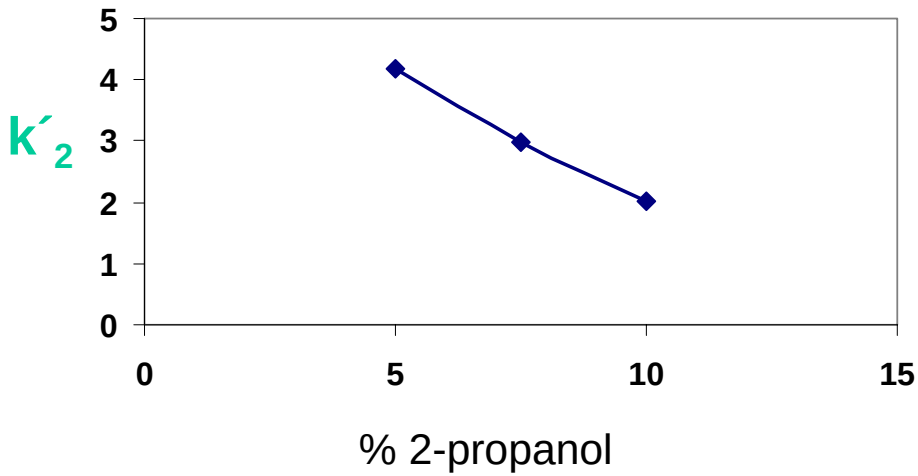
Influence of uncharged modifier concentration on retention and enantioselectivity

Analyte: Methylphenylcyanoacetic acid ethyl ester

Enantioselectivity



Retention

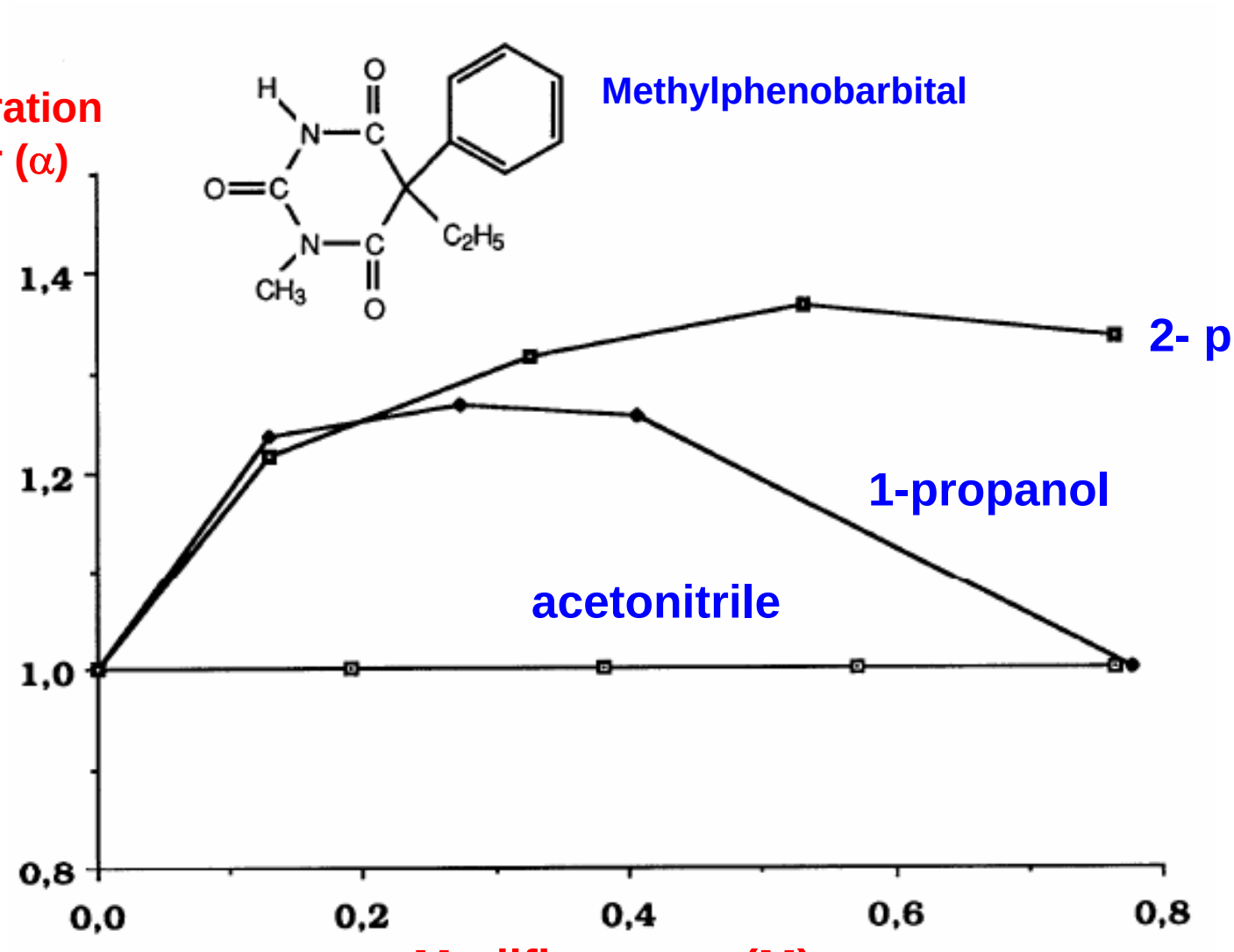


Decreasing modifier concentration

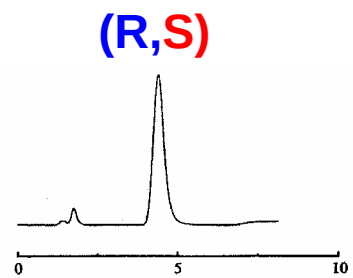
Increasing enantioselectivity

Increasing retention

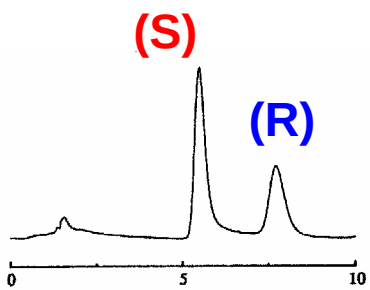
Effect of organic modifier character on enantioselectivity



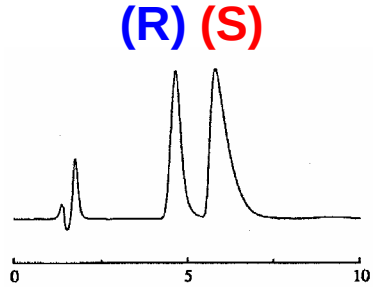
Influence of the type of organic modifier on the enantioselective retention of clevidipine



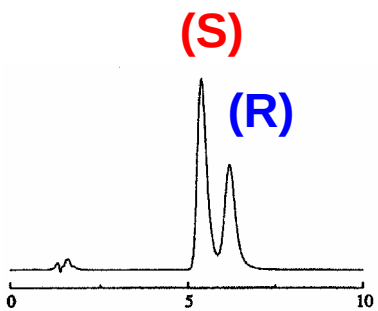
2-propanol (20%)



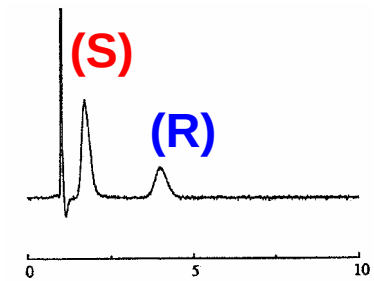
Methanol (36%)



1-propanol (16%)



Acetonitrile (20%)



Dimethylsulphoxide (15%)

Column: CHIRAL-AGP 150x4.0 mm

Mobile phase: Organic modifier in 25 mM phosphate buffer pH 7.0

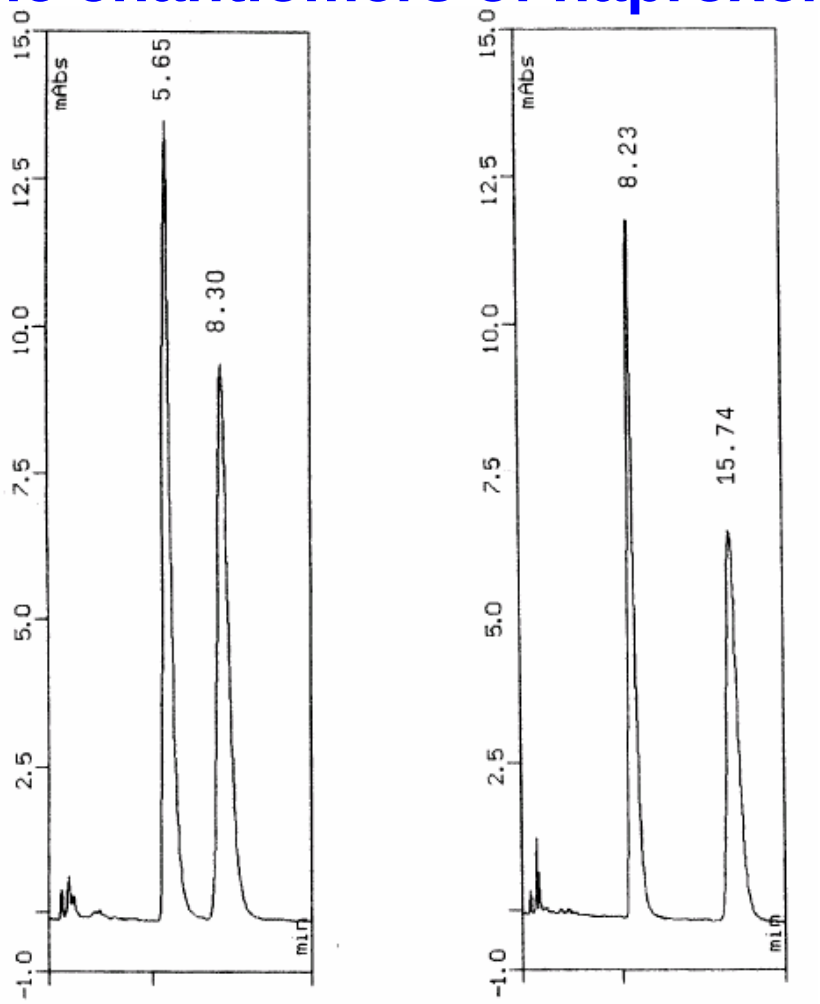
From A. Karlsson et al in Chromatographia, vol.53 (2001) 135-139

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

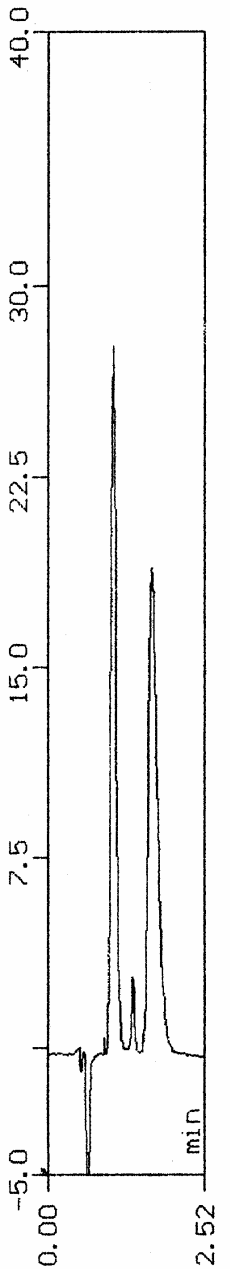
Column: CHIRAL-AGP 100x4.0 mm
Mobile phase: 0.5 % 2-propanol in acetate buffer pH 4.1

Acetate mM	k'_1	k'_2	α
12	5.73	7.29	1.27
25	6.54	8.79	1.34
96	7.04	10.7	1.52

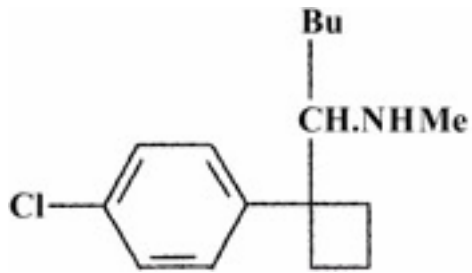
LC/MS

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



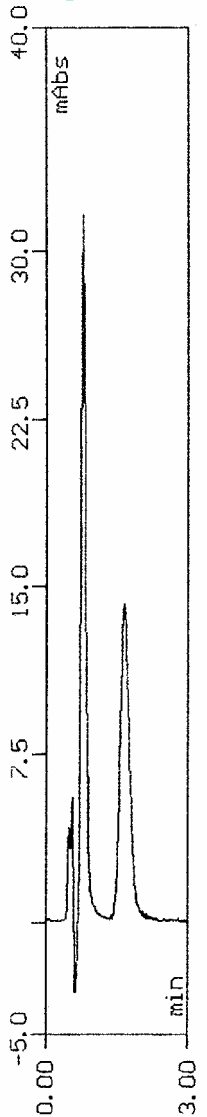
Desmetylsibutramine

CHIRAL-AGP 50x4.0 mm

Mobile phase: 5% CH3CN in 10 mM ammonium acetate buffer pH 4.1

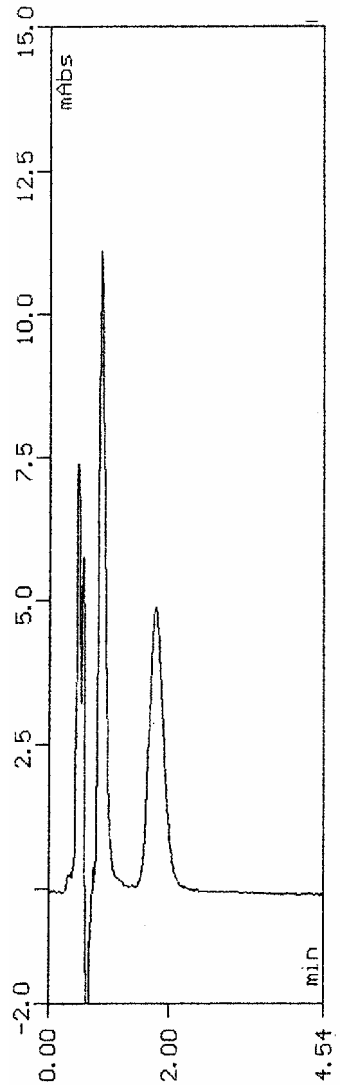
Rapid separation of acidic compounds using MS-compatible conditions

Etodolac



15% CH_3CN in
10 mM amm.acetate

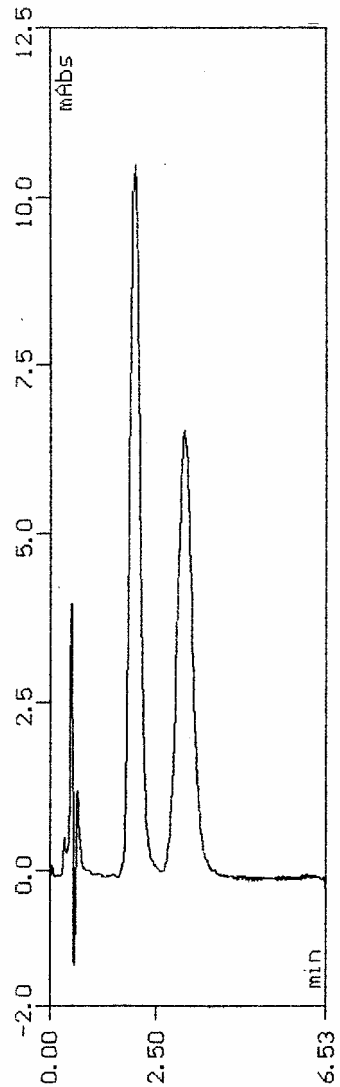
Proglumide



9% CH_3CN in
10 mM amm.acetate

Column: CHIRAL-AGP 50x2.0 mm

Naproxen



1% CH_3CN 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.

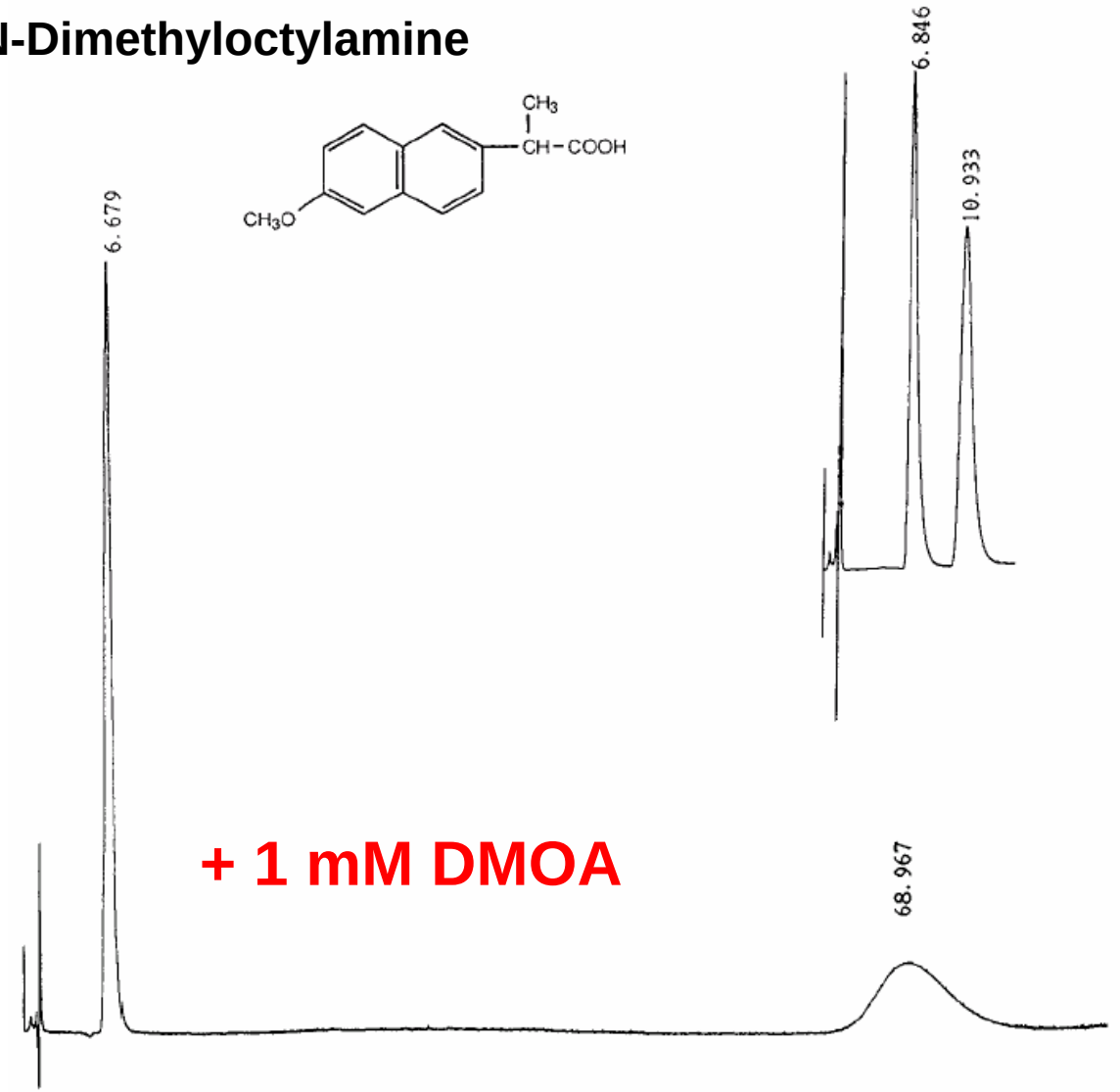
Examples of modifiers:

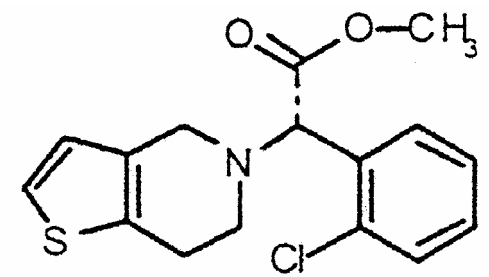
Cationic: N,N-dimethyloctylamine (DMOA) and other amines

Anionic: Hexanoic- and octanoic acid

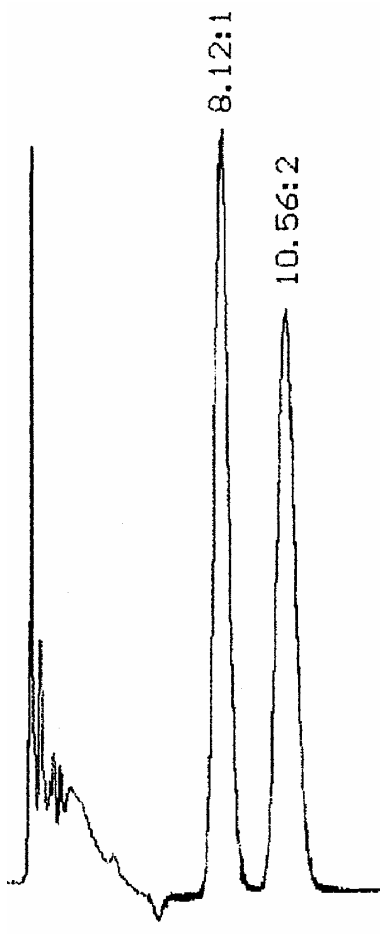
Influence of DMOA concentration on the enantioselectivity of naproxen

DMOA= N,N-Dimethyloctylamine





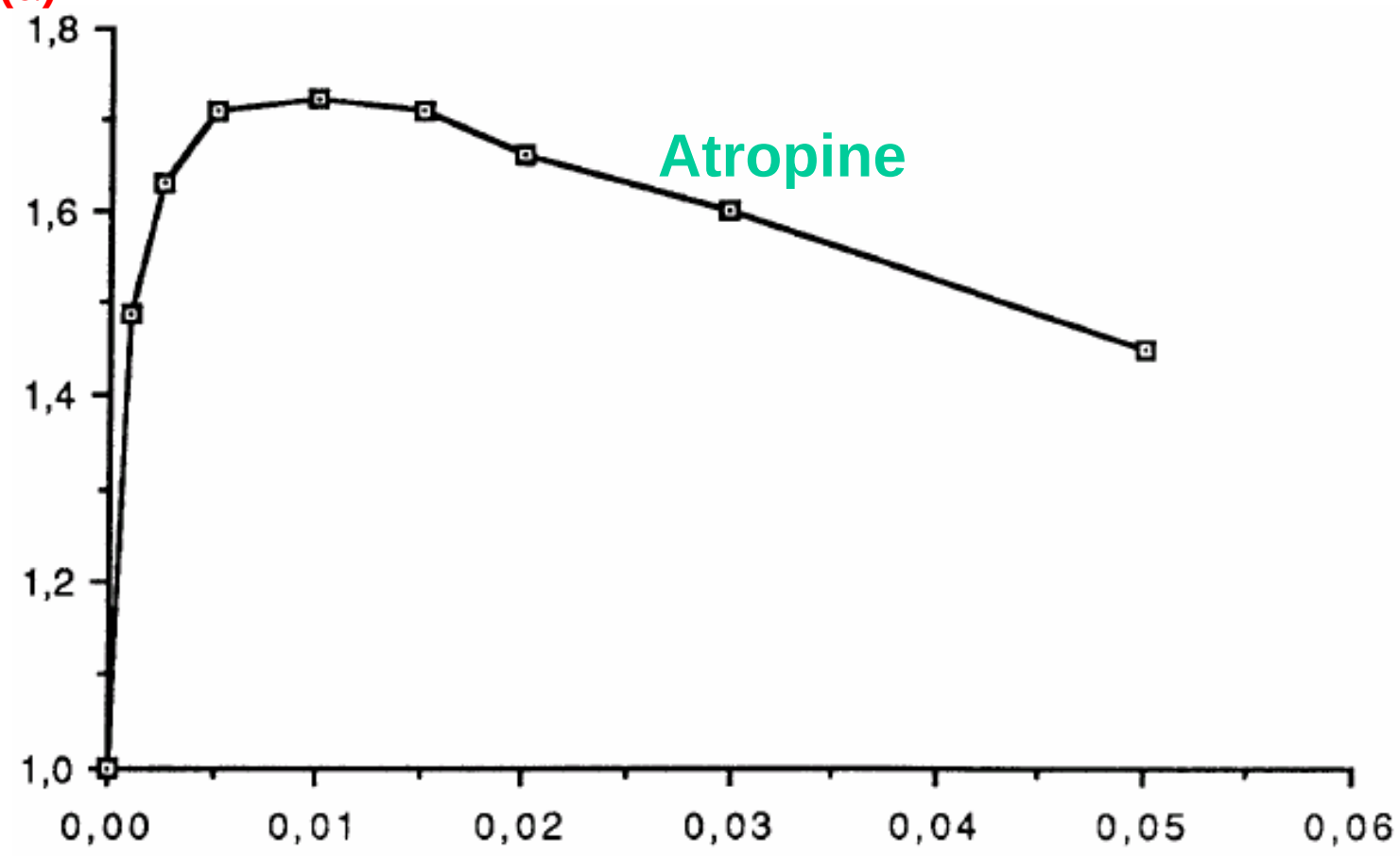
Clopidogrel



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



Simple method development strategy

Method development CHIRAL-AGP

Characterize your sample

Amine	Acid	Nonprotolyte
- Hydrophobic	- Strong	
- Hydrophilic	- Weak	

Choose the appropriate method development scheme where you will find the starting mobile phase

Compound type	Starting mobile phase
Hydrophobic amine	10 mM ammonium or sodium acetate buffer pH 4.5
Hydrophilic amine	5% 2-propanol in 10 mM sodium phosphate buffer pH 7.0
Weak acid or non-protolyte	5% 2-propanol in 10 mM sodium phosphate buffer pH 7.0
Strong acid	10 mM sodium phosphate buffer pH 7.0

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 enantio-selectivity

→ Optimize with pH and/ or uncharged modifiers

No or low enantioselec-tivity and low retention

→ Increase pH stepwise and adjust retention with 2-propanol (lower conc. gives higher enantiose-lectivity

→ 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

Enantioselectivity and too high retention.

→ Decrease pH to 4 and/or add 2-propanol

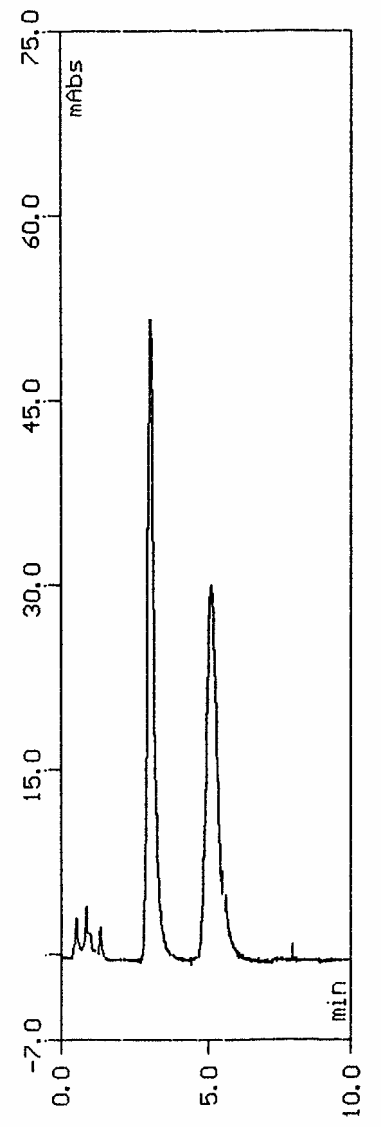
→ Test another uncharged modifier, acetonitrile, methanol, 1-propanol ethanol

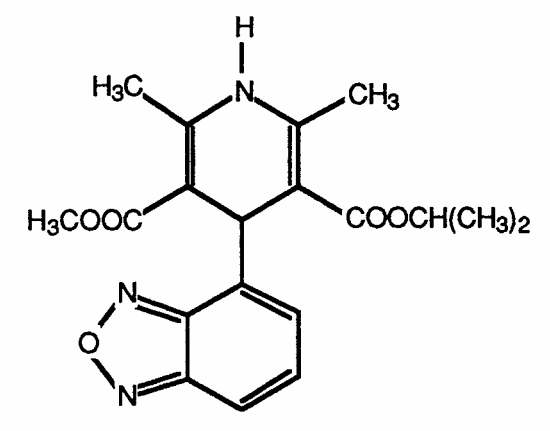
Too high retention. No enantioselectivity.

→ Test different un-charged modifiers: 2-propanol, acetonitrile, methanol, 1-propanol, ethanol

→ Test low conc. of a charged modifier*:
- oct. acid 1-20 mM
- hex. or hept. acid 1-20 mM
- tetraethyl- and tetra-propylammonium bromide 1-5 mM

Separation of the calcium channel blocking agent Isradipine





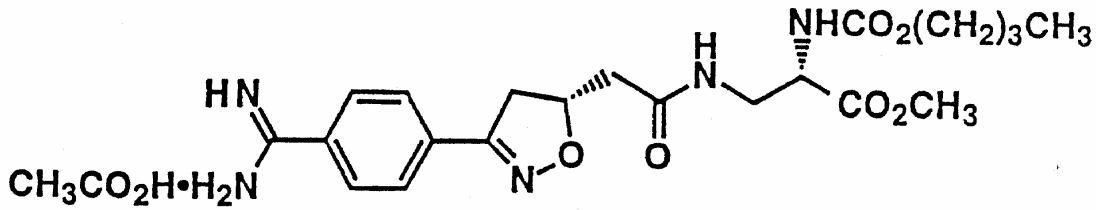
Column: CHIRAL-AGP 100 x 4.0 mm

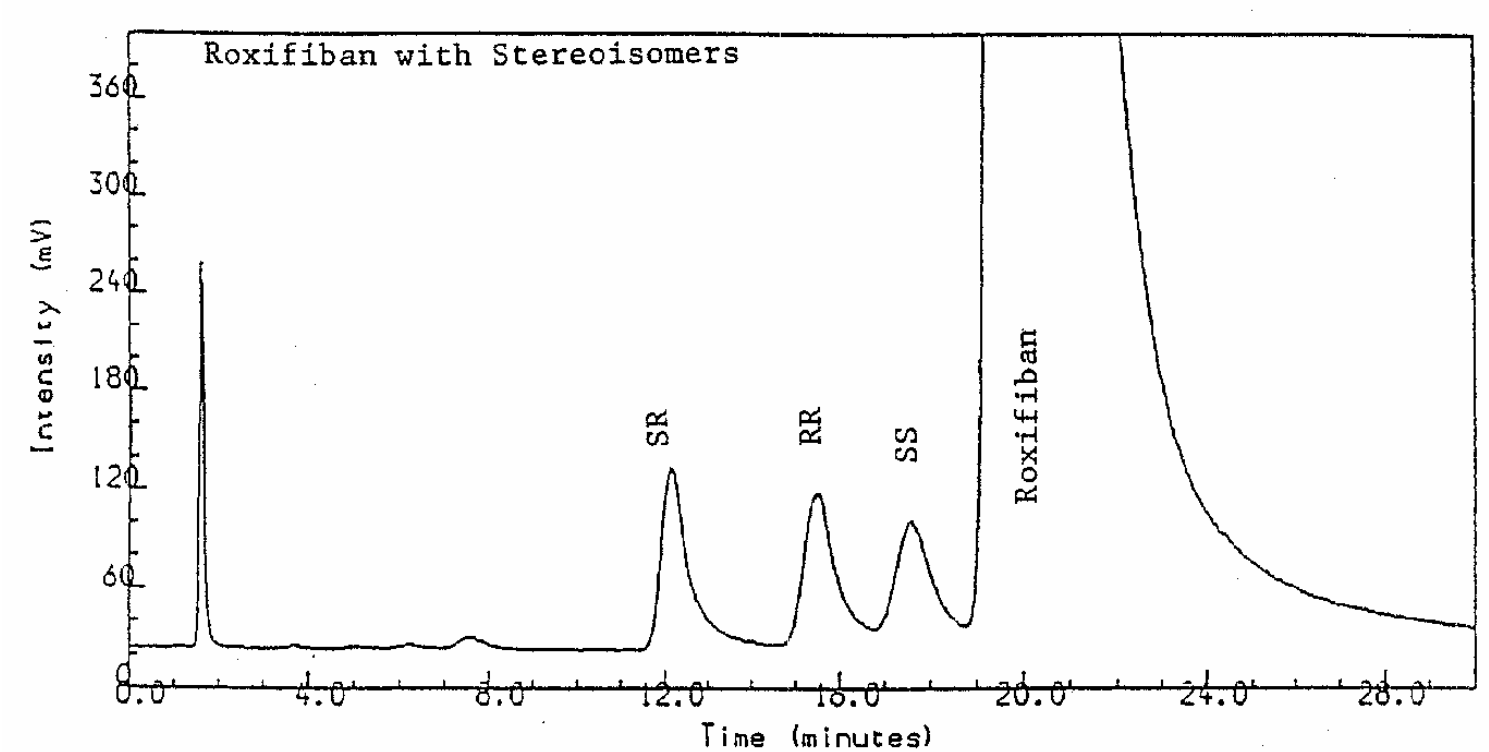
Mobile phase: 15% 2-propanol in 30 mM phosphate buffer pH=6.8

Detection: **UV** 225 nm

Sample conc.: 0.02 mg/ml

Roxifiban

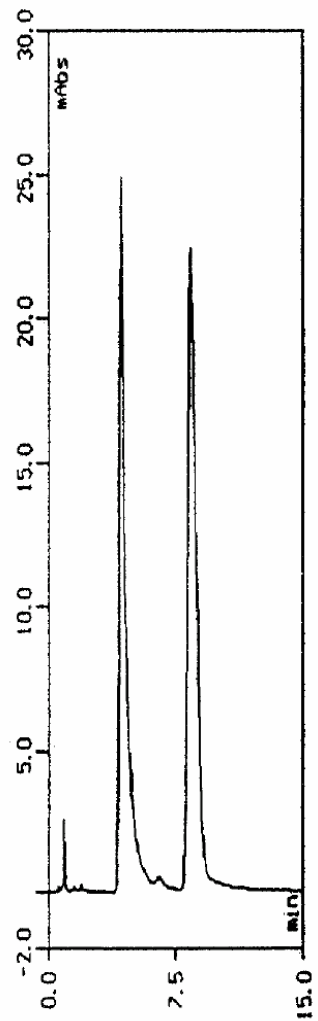


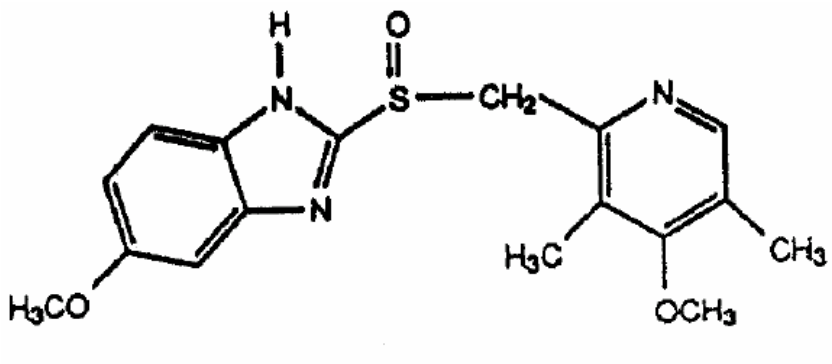


Column: CHIRAL-AGP 100x4.0 mm
Mobile ph.: 5% 2-propanol in 10 mM phosph. buffer pH 7.0

J. Chromatogr., A, 844 , 171 (1999) R.C. Williams et al.

Separation of the enantiomers of the antiulcer drug omeprazole





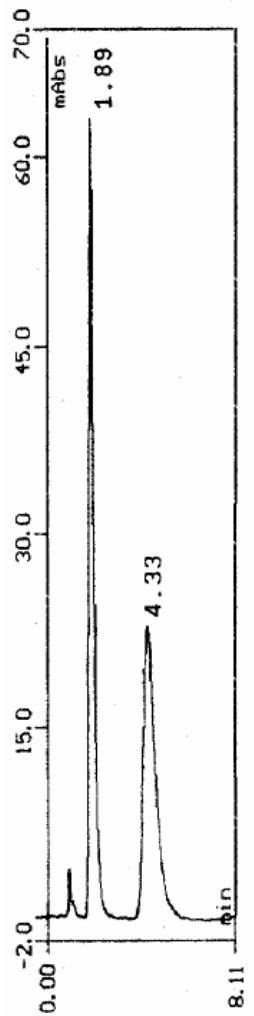
Column: CHIRAL-AGP 100 x 4.0 mm

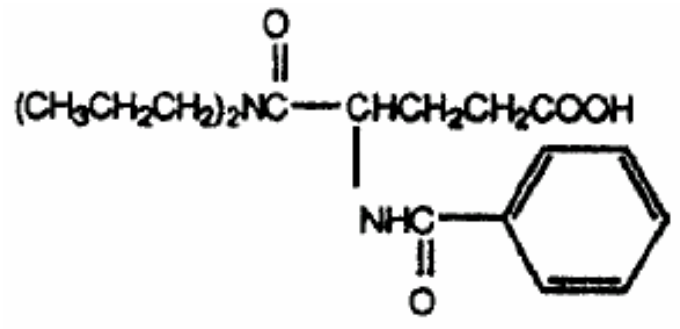
Mobile phase: 10% acetonitrile in 10 mM sodium phosphate buffer pH=6.5

Detection: **UV** 210 nm

Sample conc.: 0.02 mg/ml

Separation of the enantiomers of the anticholinergic drug proglumide





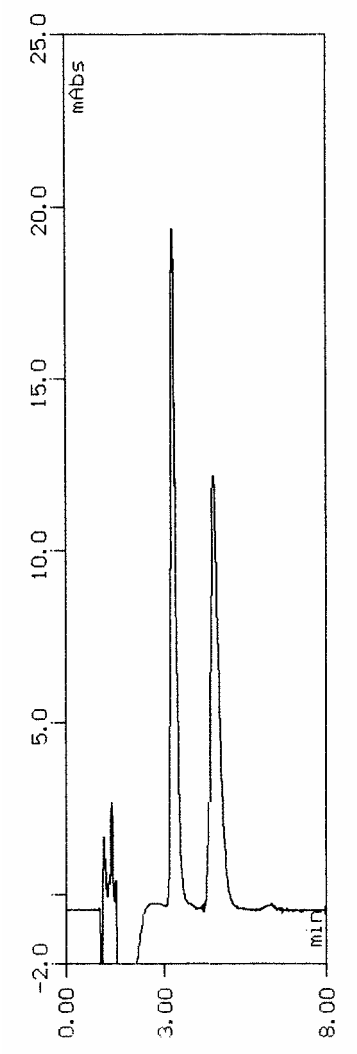
Column: CHIRAL-AGP 100 x 4.0 mm

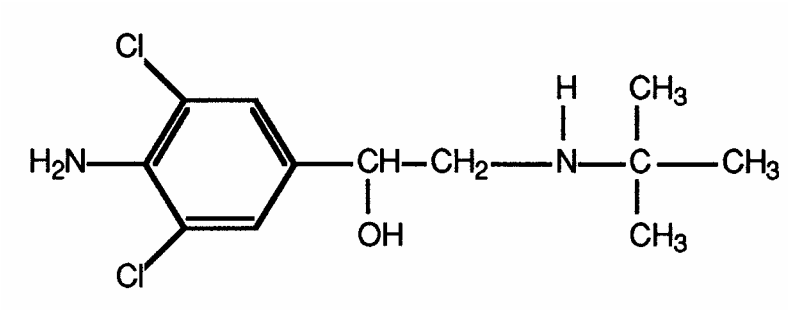
Mobile phase: 5% 2-propanol in 10 mM sodium phosphate buffer pH=6.0

Detection: **UV** 225 nm

Sample conc.: 0.02 mg/ml

Separation of the enantiomers of clenbuterol





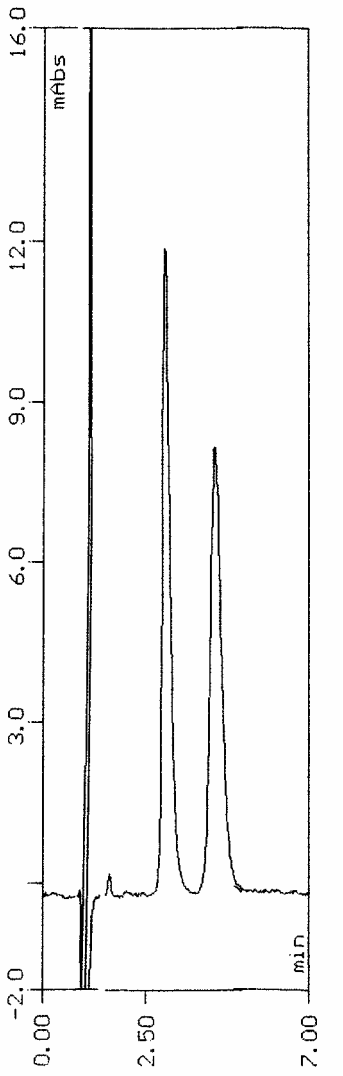
Column: CHIRAL-AGP 100 x 4.0 mm

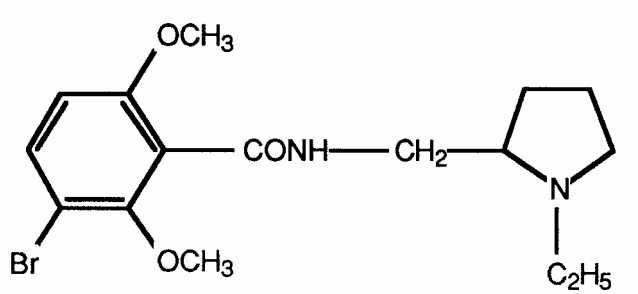
Mobile phase: 1% 2-propanol in 10 mM sodium acetate buffer pH=5.0(total acetate concentration 15 mM)

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

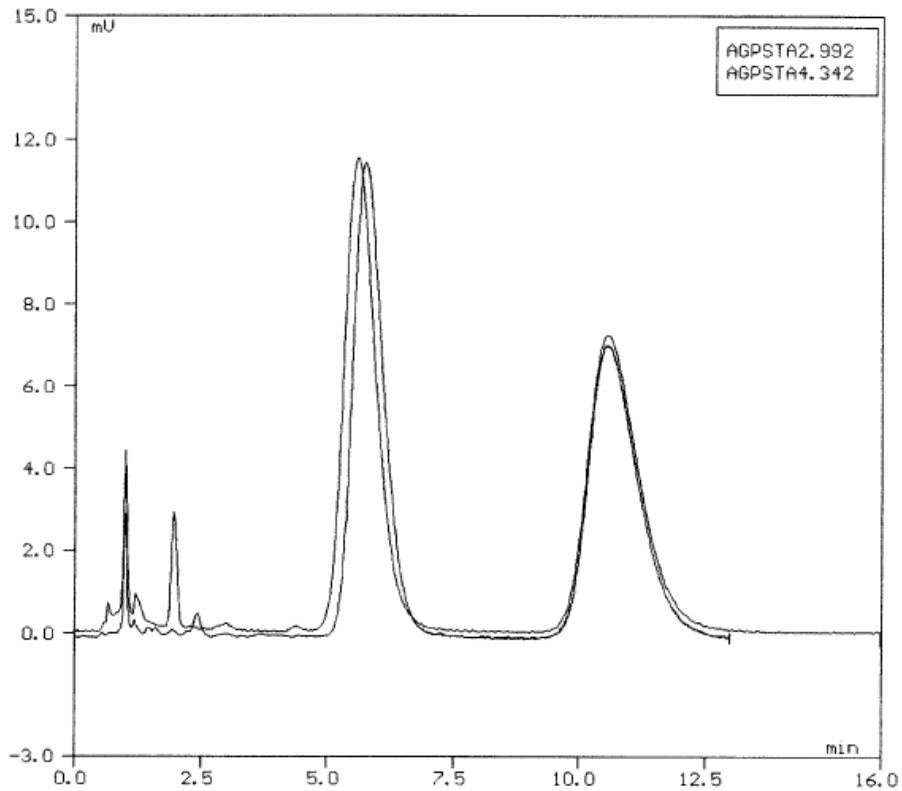
Sample: Bumadizon

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

30.5 liters of mobile phase

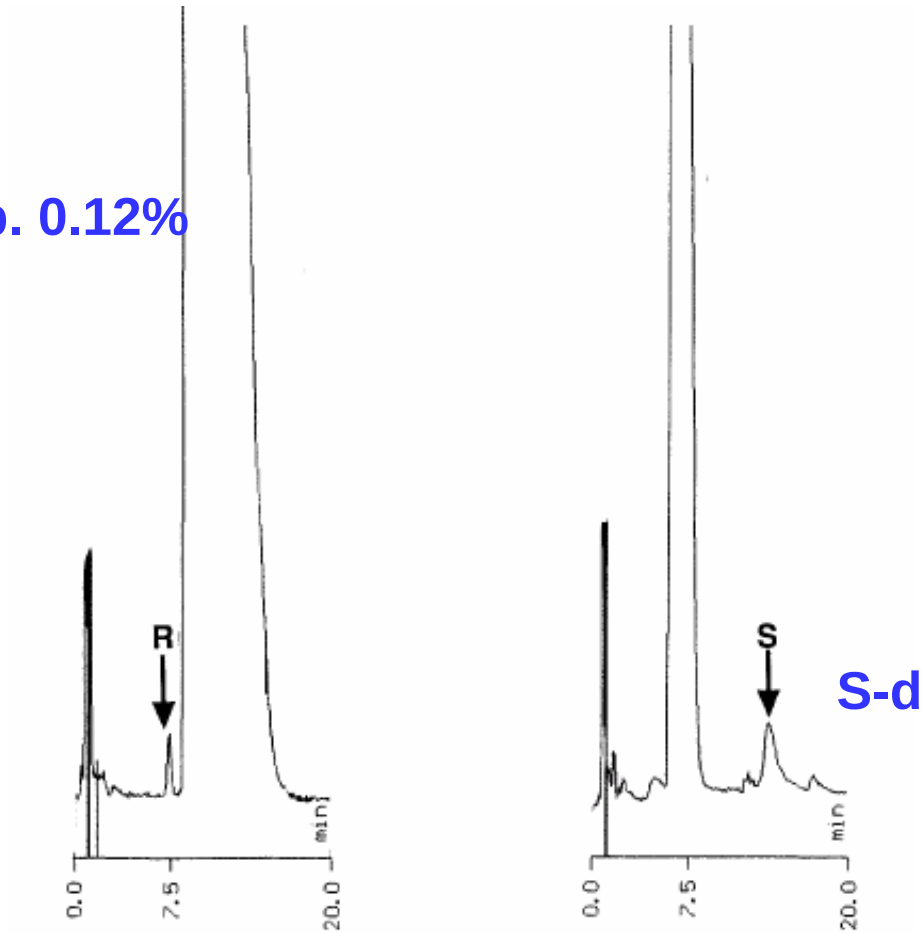
2030 samples have been injected during the study

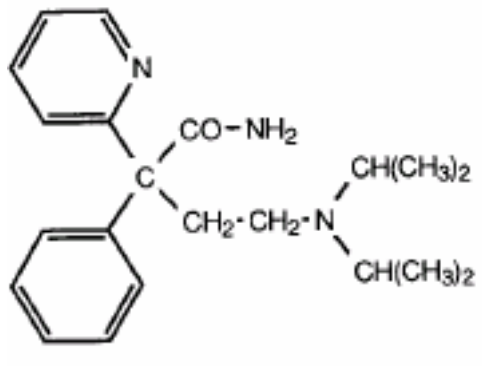
Guard column exchanged after 7.5 liters of mobile phase, corresponding to 147500 column volumes.



Determination of enantiomeric purity of disopyramide using the CHIRAL-AGP column

R-disop. 0.12%





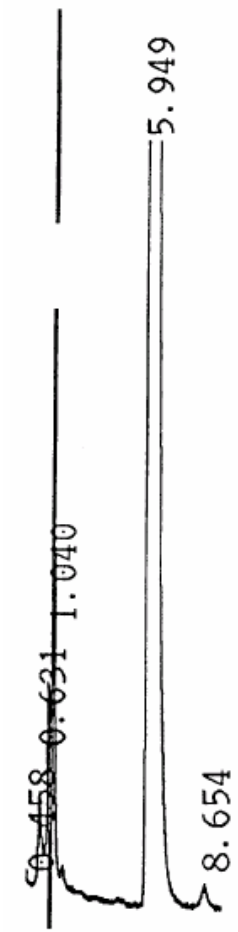
S-disop. 0.92%

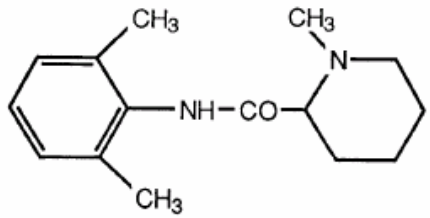
Column: CHIRAL-AGP 100 x 4.0 mm

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

Sample conc.: 0.7 mg/ml

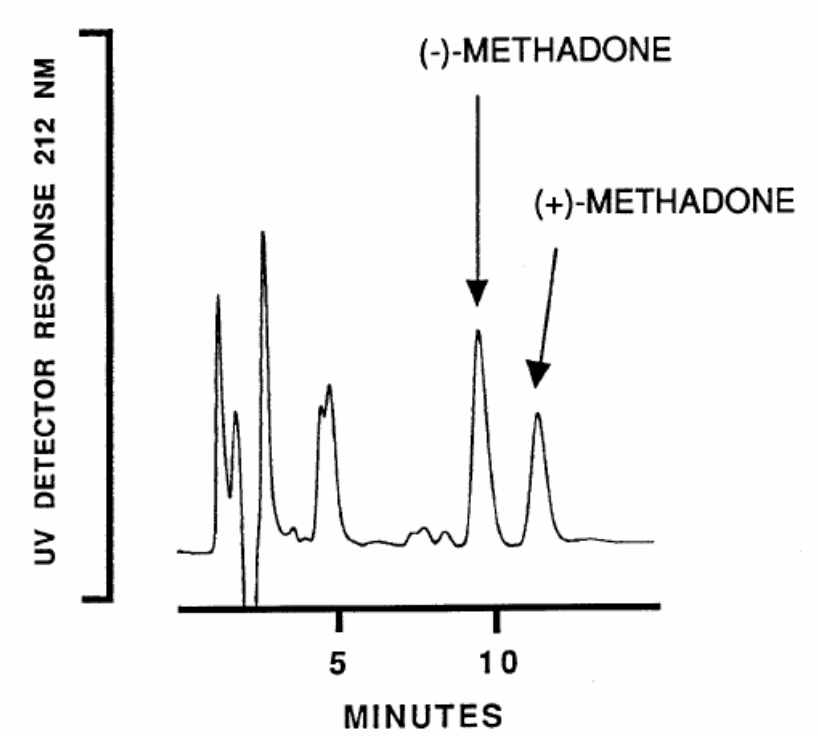
Purity determination of (+) - mepivacine





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 availible. It separates amines, acids, non-protolytes.**

- **Solutes are retained 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**