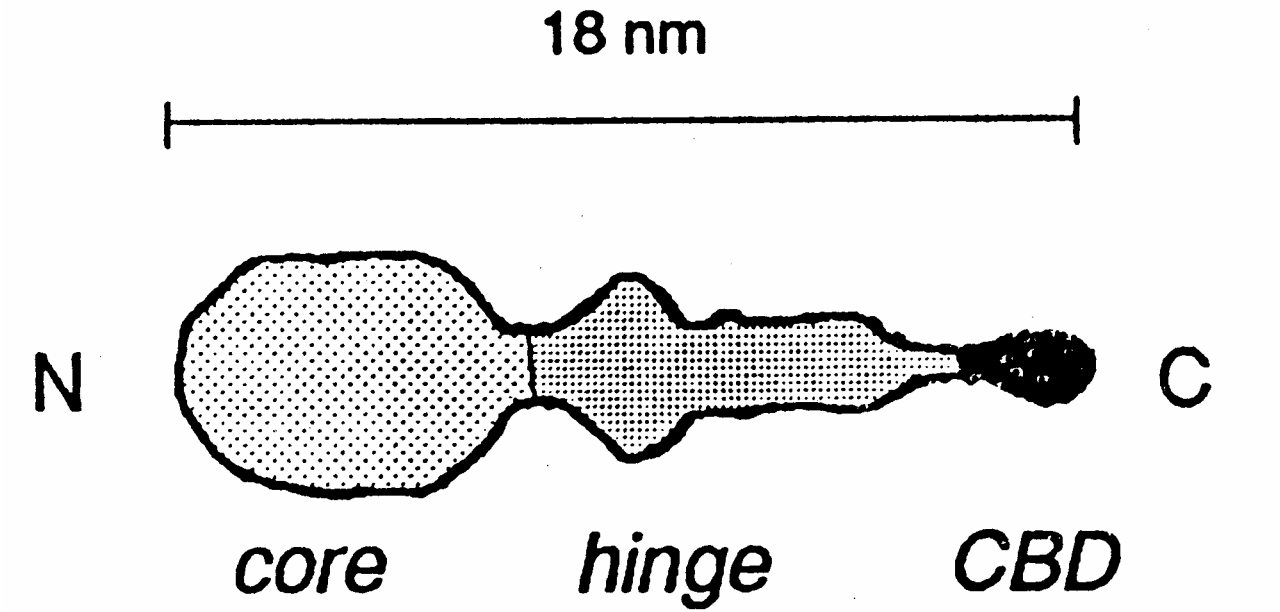
Chiral CBH

ChromTech

Low resolution structure of CBH 1

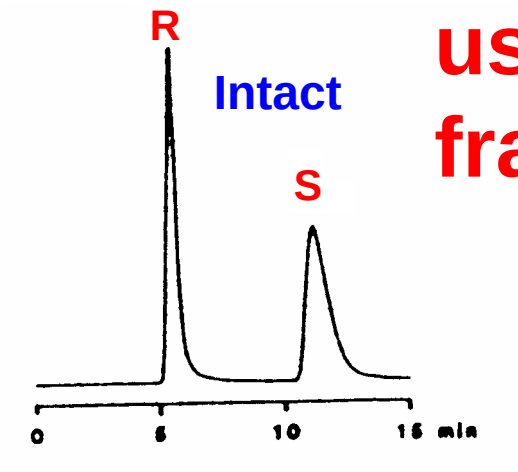
Calculated from small angle X-ray scatering analysis

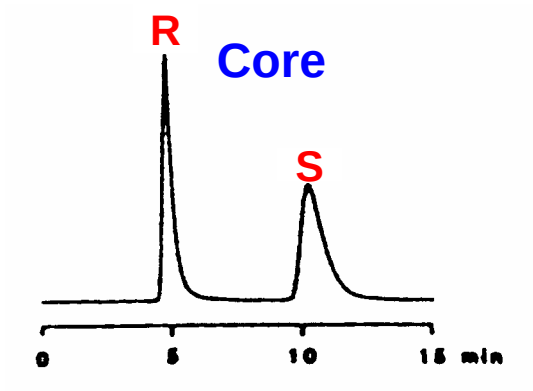


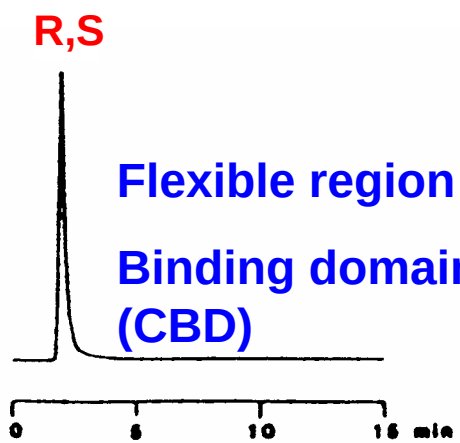
Characteristics of CBH 1

Molecular weight	64 000
Amino acid residues	497
Isoelectric point	3.9

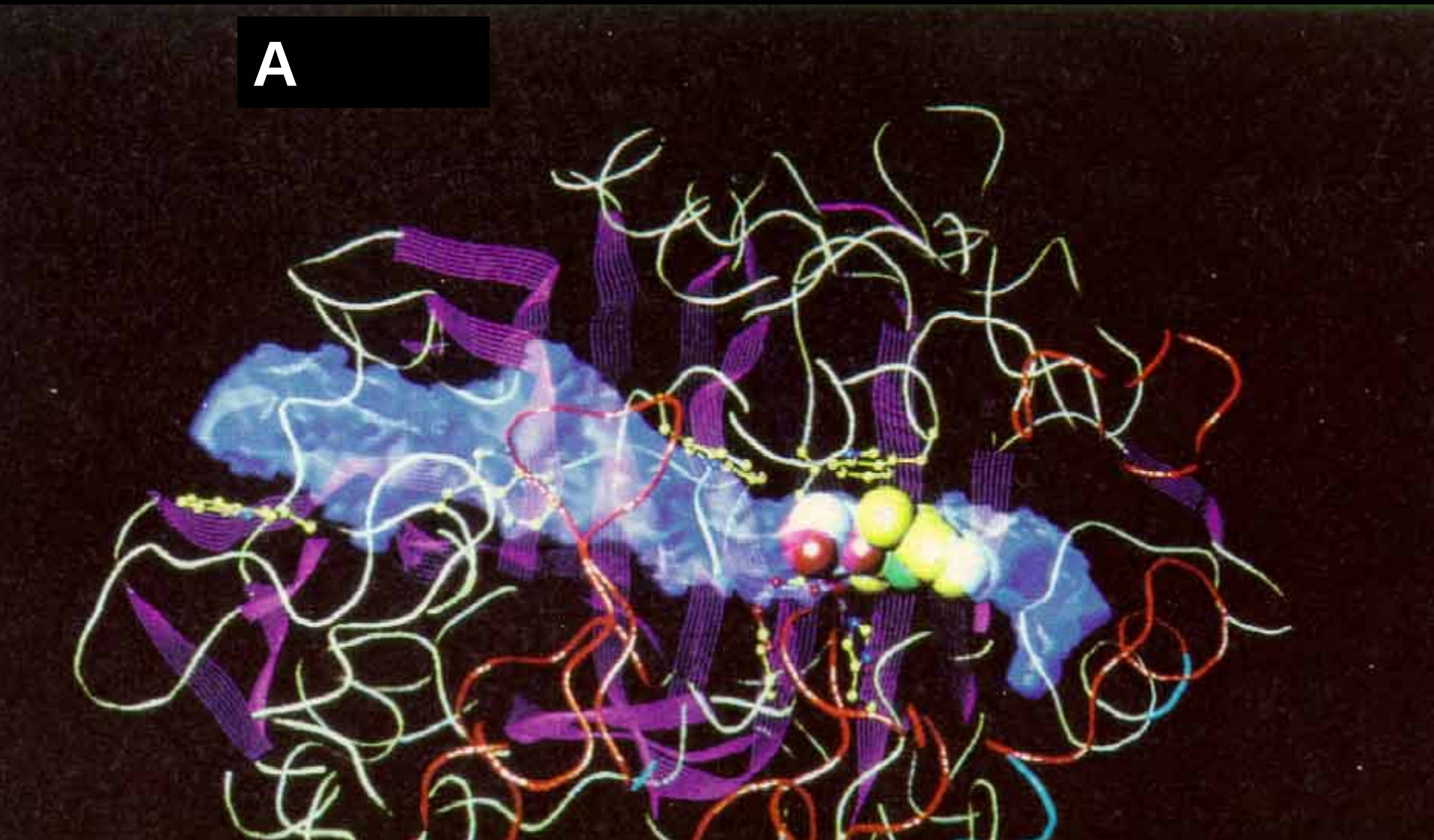
Chromatography of propranolol using the intact protein and the fragments of CBH 1







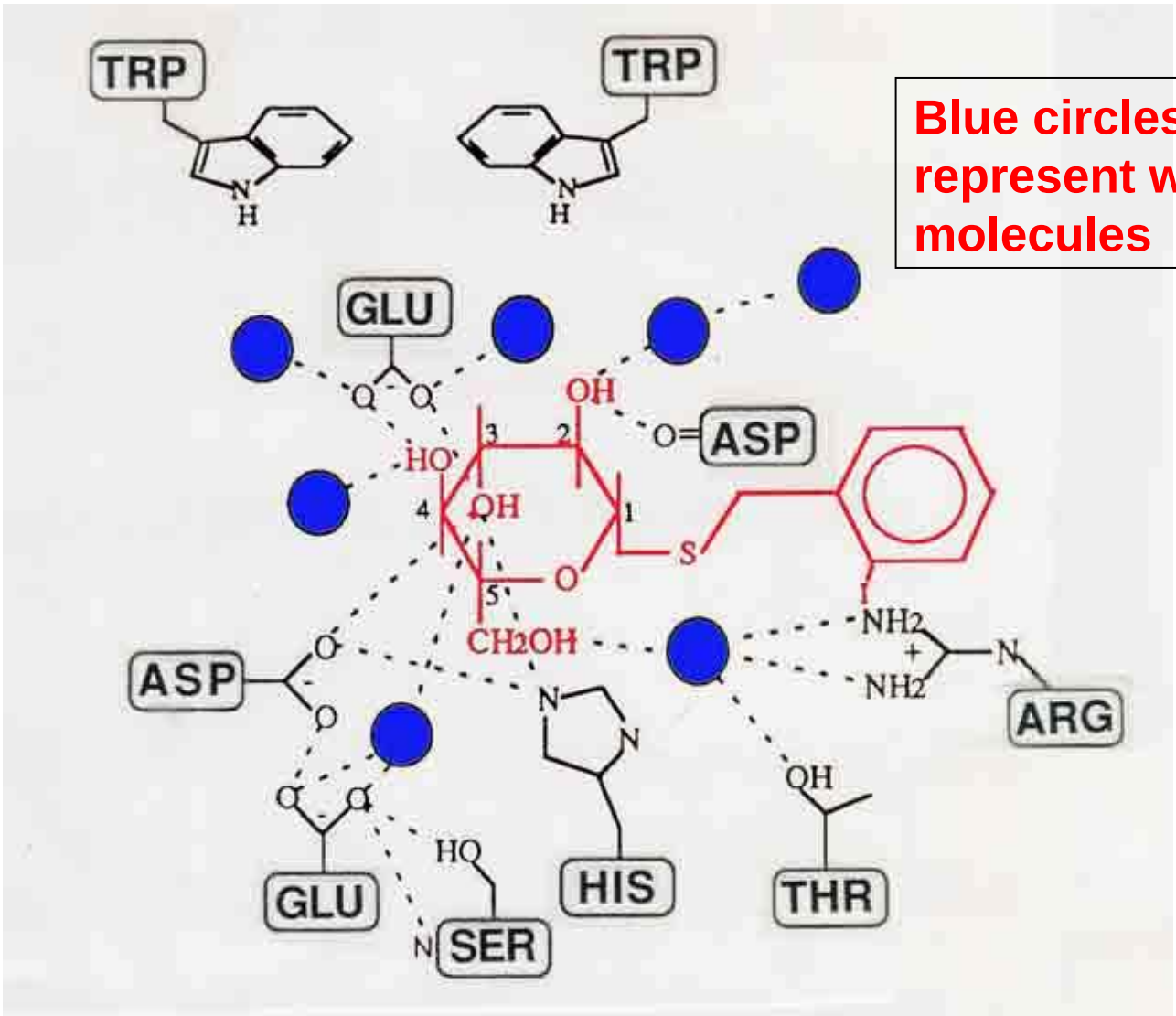
Core unit of CBH 1 (X-ray studies) orthogonal to the active site(the blue shaded tunnel)



Core unit of CBH 1 (X-ray studies) seen
the active site (the blue shaded tunn

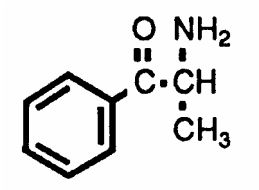
B

Schematic representation of the network of hydrogen bonds involved in the binding of the iodo-benzyl glycoside to the binding site of the core unit

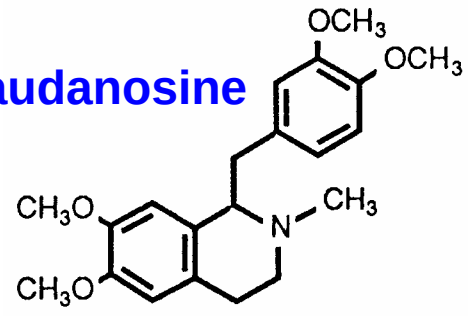


Examples of compounds resolved on the CHIRAL-CBH column

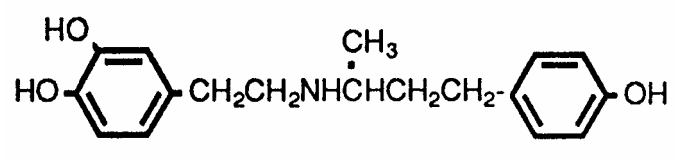
Cathinone



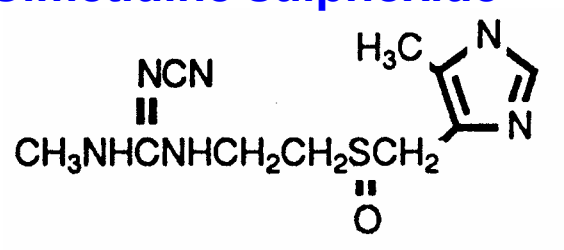
Laudanosine



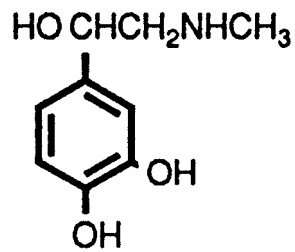
Dobutamine



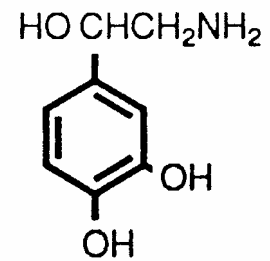
Cimetidine sulphoxide



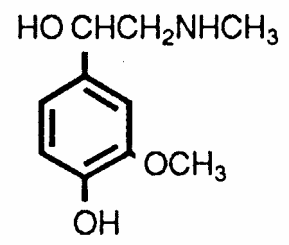
Epinephrine



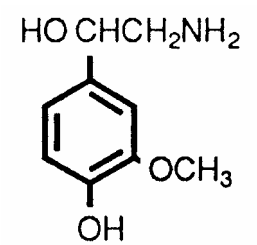
Norepinephrine



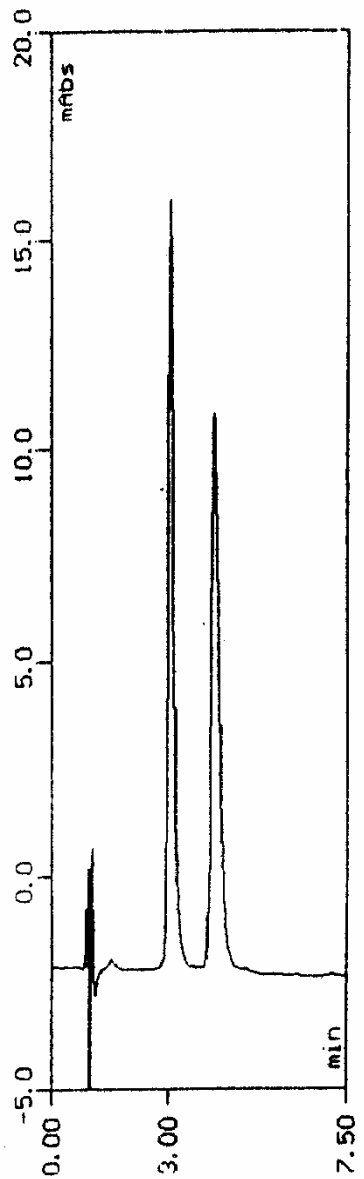
Metanephrine

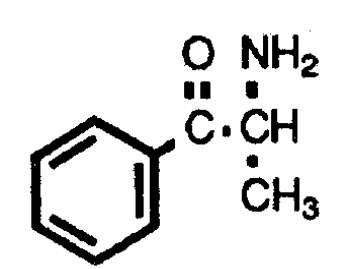


Normetanephrine



Separation of the enantiomers of cathinone using CHIRAL-CBH



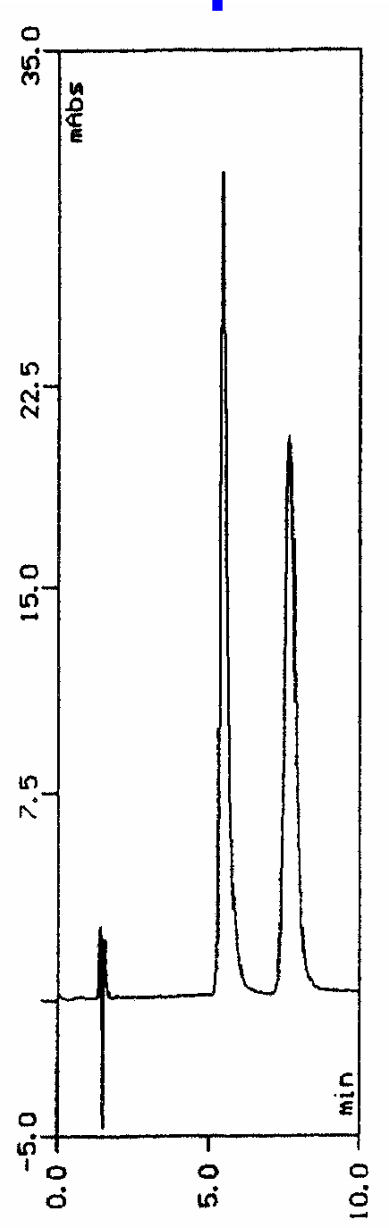


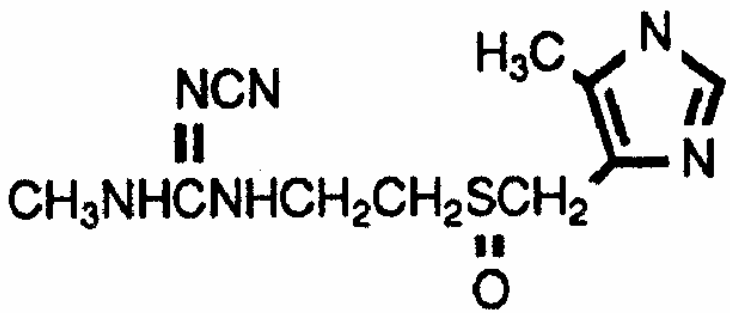
Column: **CHIRAL-CBH 100 x 4.0 mm**

Mobile phase: **5% acetonitrile in 50 mM sod. ph. b. pH 7.0 + 50 μM disodium EDTA**

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

Separation of the enantiomers of cimetidine sulphoxide using CHIRAL-CBH





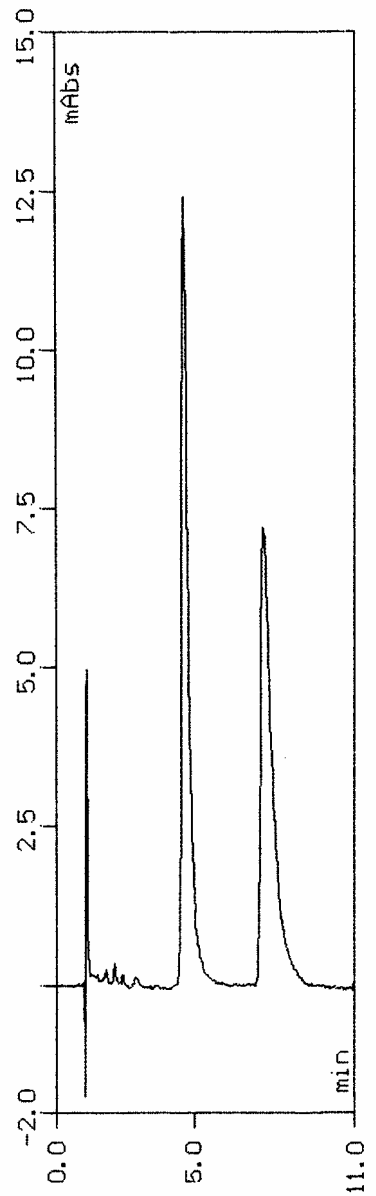
Column: CHIRAL-CBH 150 x 4.0 mm

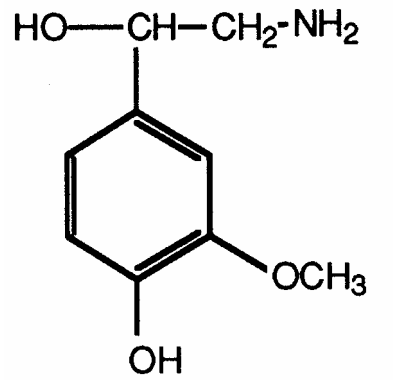
Mobile phase: 10 mM sod. ph. b. pH 6.0

+ 50 μM disodium EDTA

Sample conc.: 0.03 mg/ml

Separation of the enantiomers of normethanephrine using CHIRAL-CBH

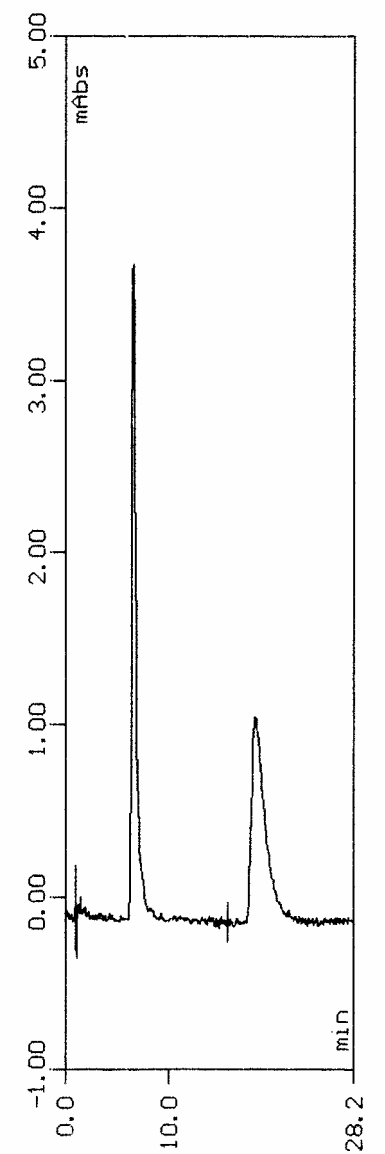


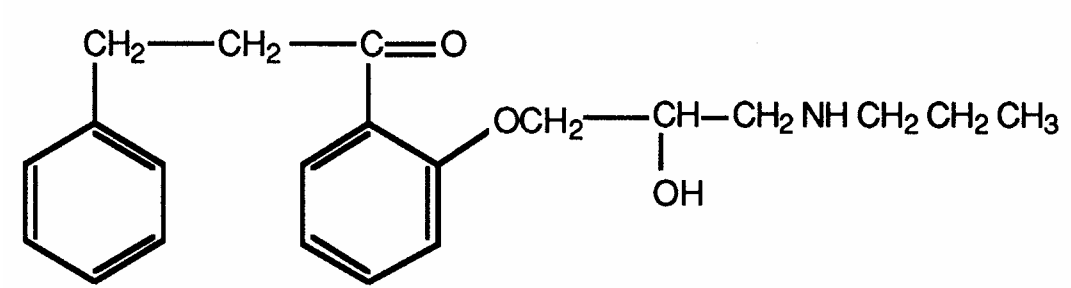


Column: **CHIRAL-CBH 100 x 4.0 mm**

Mobile phase: **0.5% 2-propanol in 10 mM sod. ph. b. pH 6.8**

Separation of the enantiomers of propafenone using CHIRAL-CBH



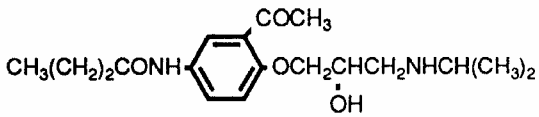


Column: **CHIRAL-CBH 100 x 4.0 mm**

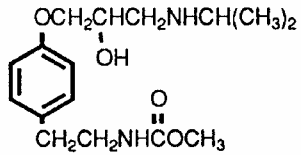
Mobile phase: **5% 2-propanol in 10 mM sod. ph. b. pH 6.0**

A selection of beta-blockers that have been separated by the CHIRAL-CBH column

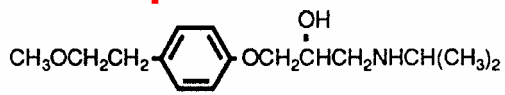
Acebutolol



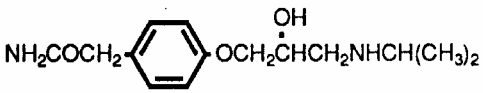
Pamatolol



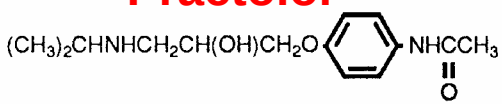
Metolprolol



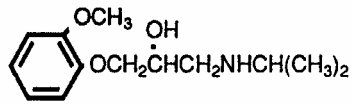
Atenolol



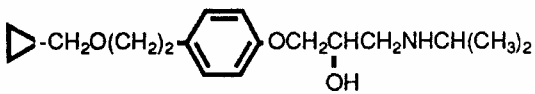
Practolol



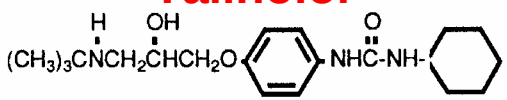
Moprolol



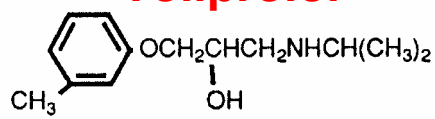
Betaxolol



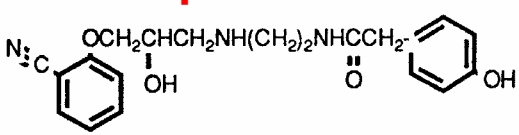
Talinolol



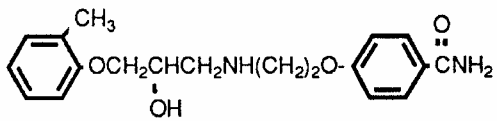
Toliprolol



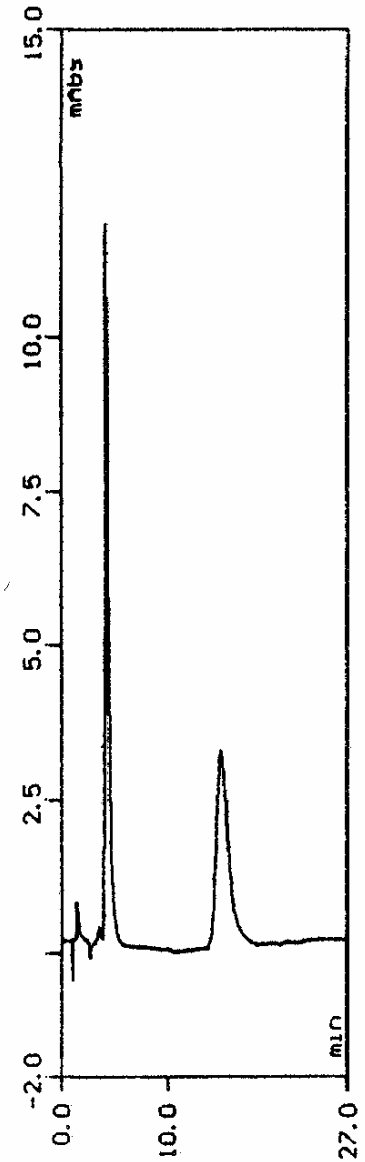
Epanolol

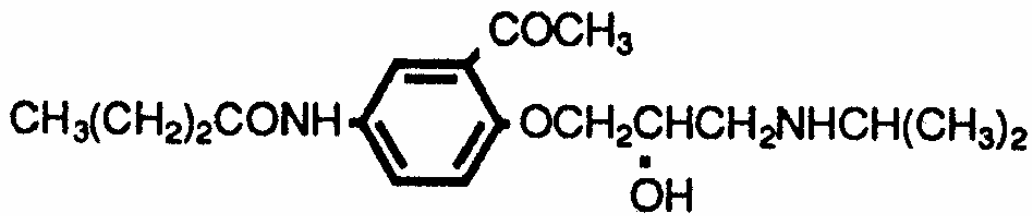


Tolamolol



Separation of the enantiomers of acebutolol using CHIRAL-CBH



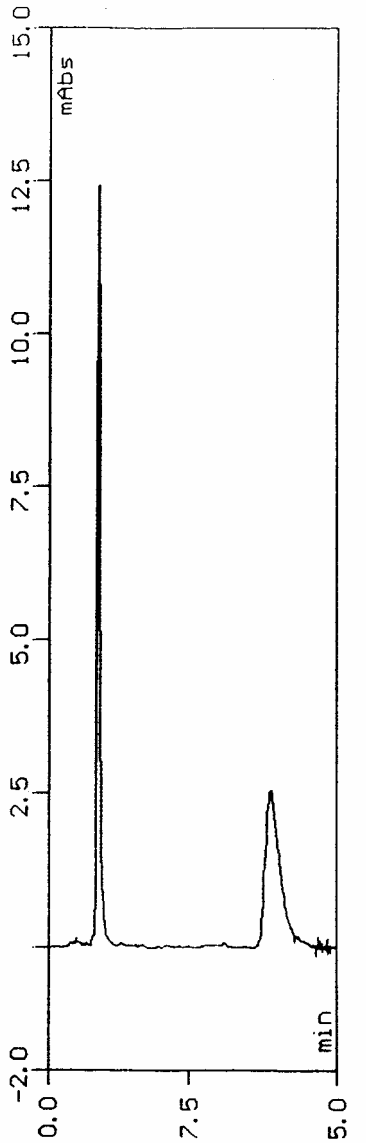


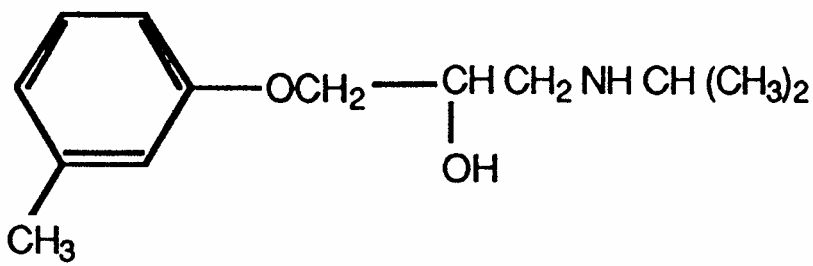
Column: CHIRAL-CBH 100 x 4.0 mm

Mobile phase: 5% 2-propanol in 10 mM sod. Ac. B. pH 5.5 + 50 μM disodium EDTA

Sample conc.: 0.03 mg/ml

Separation of the enantiomers of toliprolol using CHIRAL-CBH





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

Mobile phase: **5% 2-propanol in 10 mM sod. Ph. b. pH 6.0 + 50 μM disodium EDTA**

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

Examples of drugs resolved on the CHIRAL-CBH column

Substance	k'_1	α	R_s
Acebutolol	3.77	4.2	7.3
Atenolol	1.69	2.3	4.2
Betaxolol	3.82	3.6	6.5
Bisoprolol	3.21	4.0	7.8
Cathinone	2.36	1.5	3.3
Cimetidine sulphoxide	3.04	1.5	3.3
Dobutamine	5.62	1.8	3.4
Dropropizine	4.16	1.5	2.9
Epanolol	9.52	2.2	3.6
Epinephrine	4.26	1.7	4.0
Laudanosine	2.82	2.0	3.0
Metanephrine	8.52	1.4	2.7
Metoprolol	3.59	3.2	7.2
Moprolol	4.72	1.9	4.2
Norepinephrine	3.40	2.1	5.0

Examples of drugs resolved on the CHIRAL-CBH column

Substance	k'_1	α	R_s
Normetanephrine	3.28	2.0	4.8
Octopamine	3.03	2.5	6.8
Oxybutynine	5.20	2.1	3.4
Pamatolol	4.01	2.3	5.3
Phenylethanolamine	4.62	1.5	3.3
Practolol	3.22	1.4	2.3
Prilocaine	3.16	1.5	2.9
Propafenone	7.06	2.0	4.1
Proxyphylline	1.22	1.6	2.5
Talinolol	3.12	1.8	3.1
Tetrahydropapaveroline	3.17	1.8	3.5
Timolol	1.55	4.1	5.3
Tetramisole	2.45	1.6	3.3
Tolamolol	3.46	2.1	3.7
Toliprolol	3.95	6.1	10.8

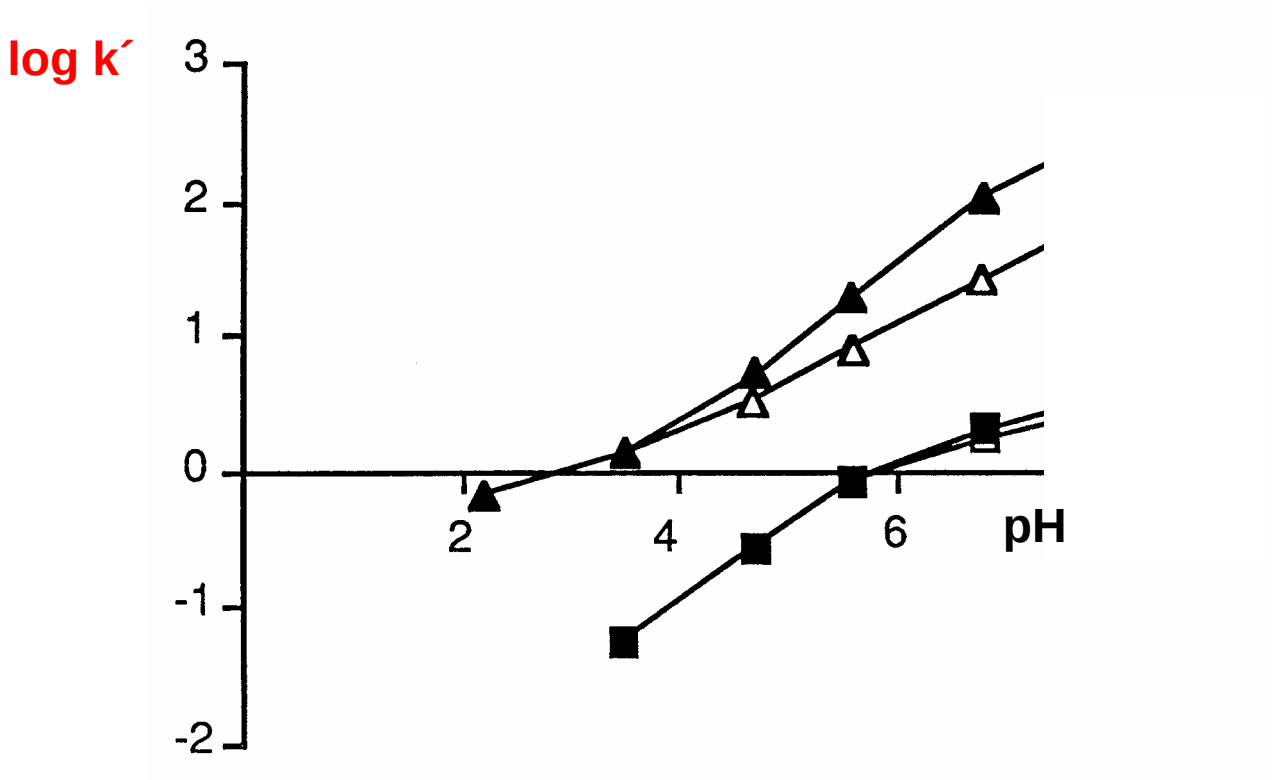
Optimization of enantioselectivity and retention

- **pH**

- **Uncharged modifier**
  - nature
  - concentration

- **Buffer**
  - concentration
  - nature

Influence of pH on the retention of amines on the CHIRAL-CBH column



Effect of pH changes on basic compounds

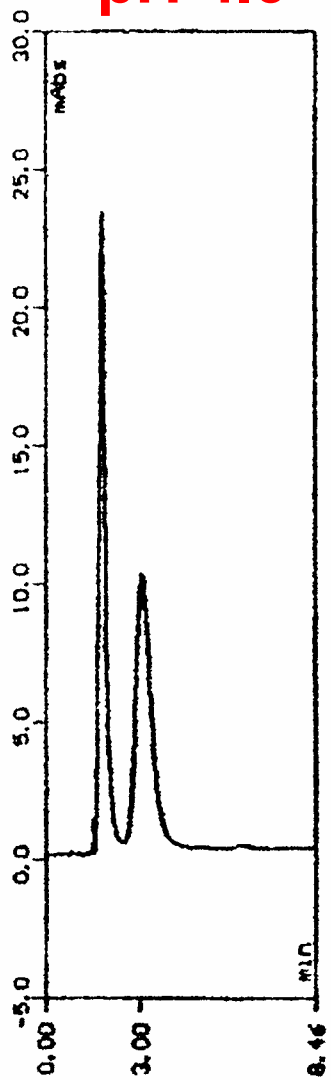
Column: CHIRAL-CBH

100 x 4.0 mm

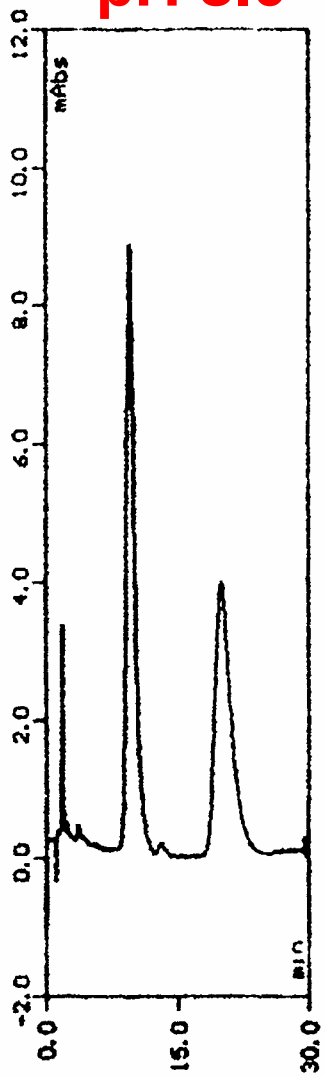
Mobile phase: 5% 2-propanol in 10 mM sodium acetate buffer + 50 μM disodium EDTA

Epanolol

pH 4.0

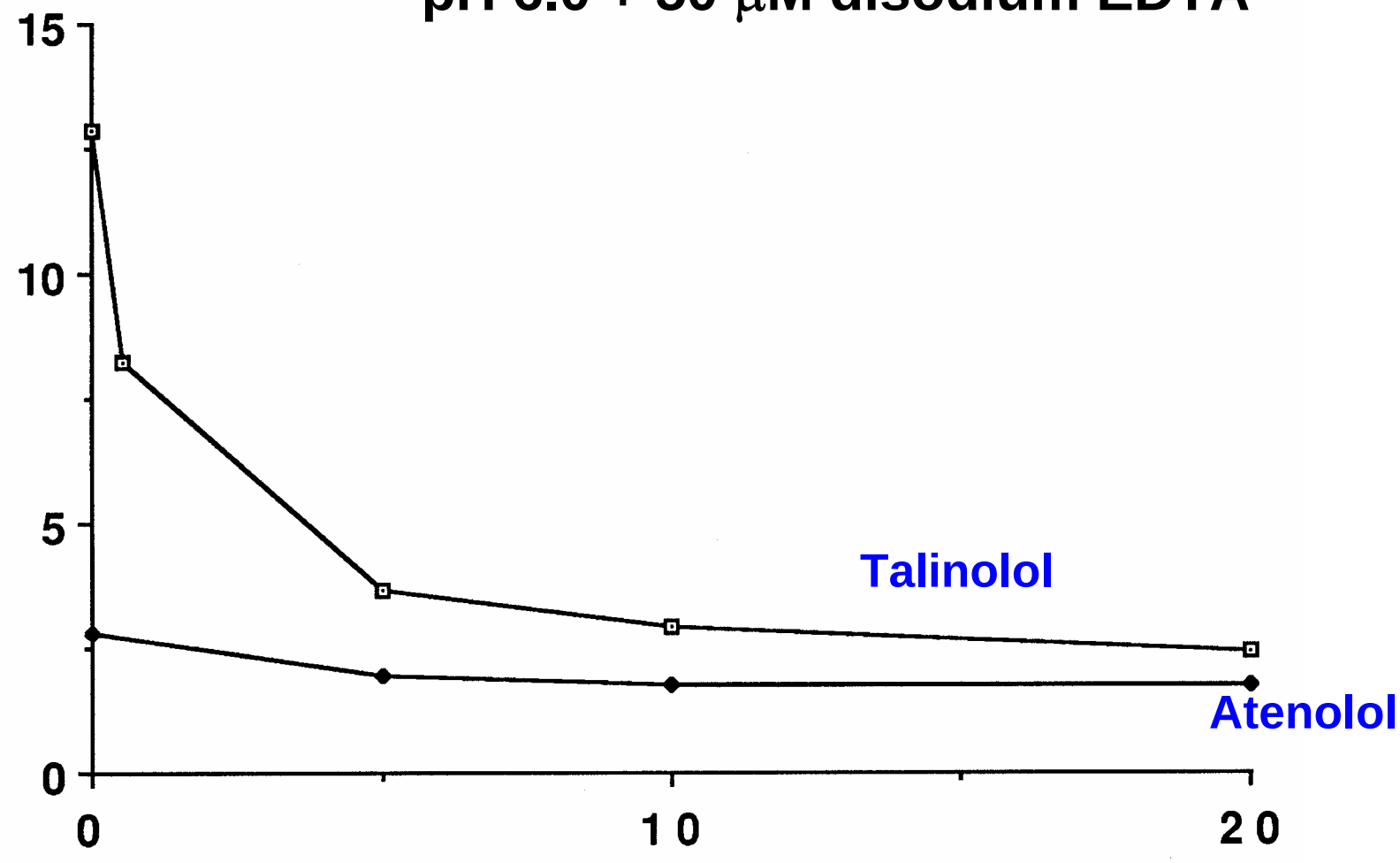


pH 5.0



Retention vs. modifier concentration

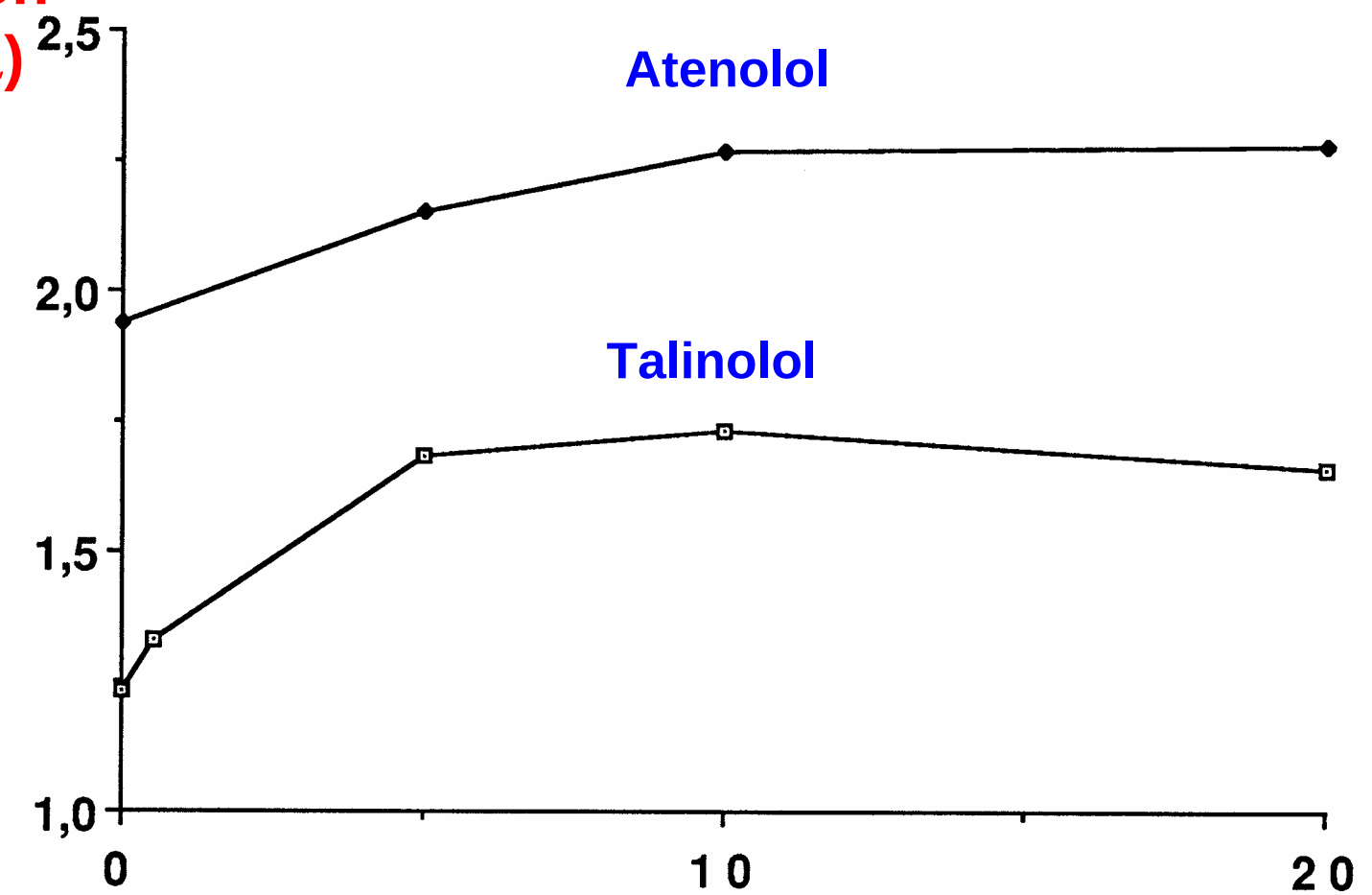
Mobile phase: 10 mM sodium phosphate buffer
pH 6.0 + 50 μM disodium EDTA



Separation factor vs. modifier concentration

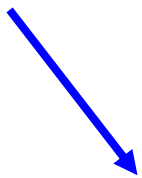
Mobile phase: 10 mM sodium phosphate buffer pH 6.0

50 μM disodium EDTA



Unique feature of the CHIRAL-CBH column
(for many compounds)

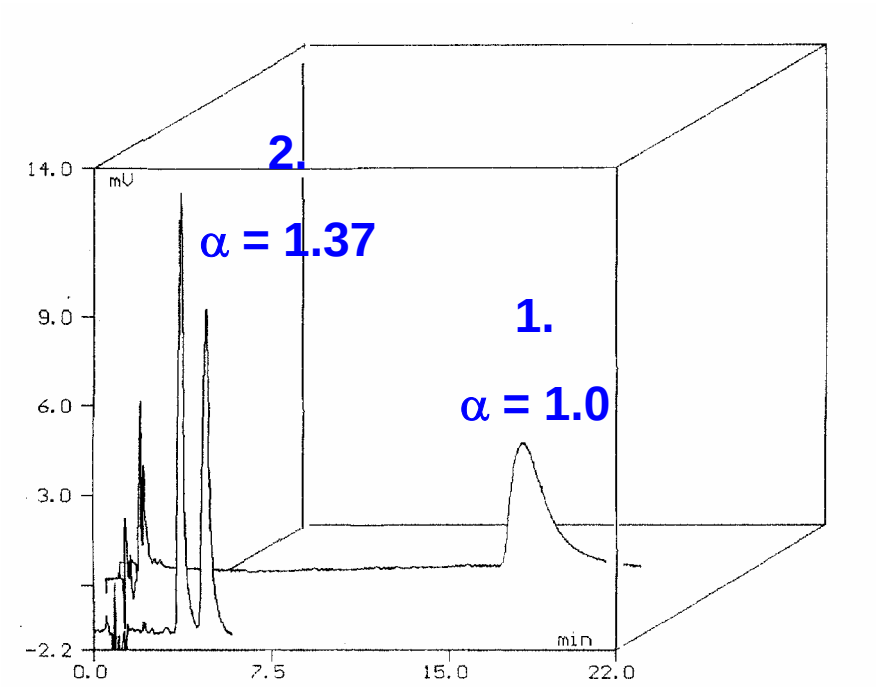
Increasing modifier concentration



Increasing enantioselectivity

Decreasing retention

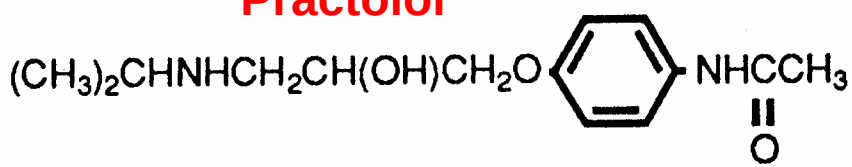
Induction of enantioselectivity by addition of organic modifier



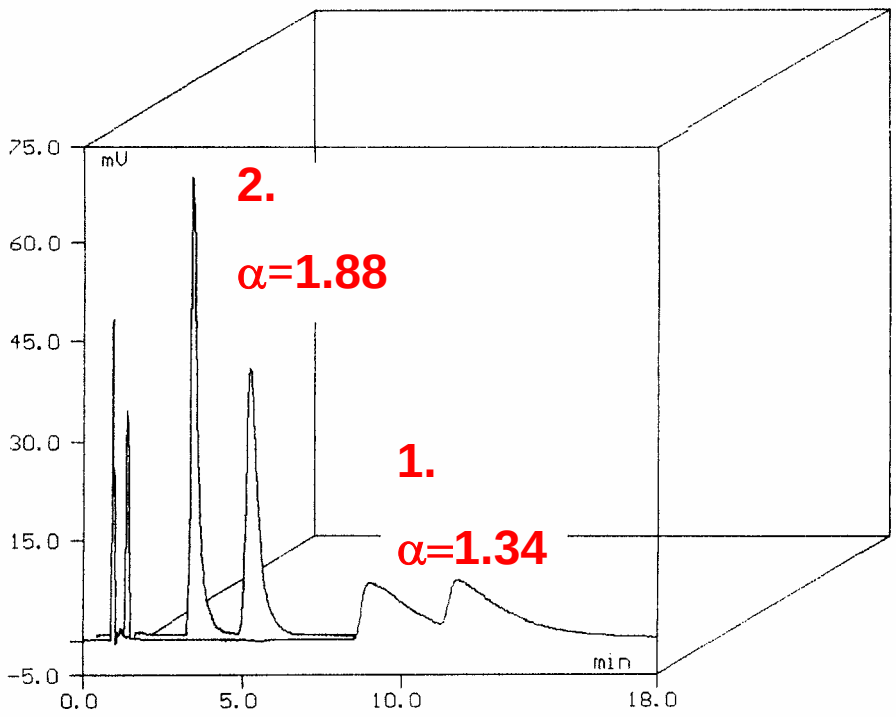
Mobile phases:

1. **10 mM sod. phosph. b. pH 7.0 + 50 μM disodium EDTA**
2. **5% acetonitrile in 50 mM sod.phosph.b. pH 7.0 + 50 μM disodium EDTA**

Practolol



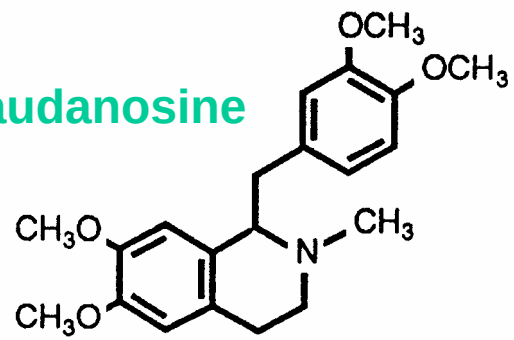
Chromatographic performance vs. 2-propanol concentration



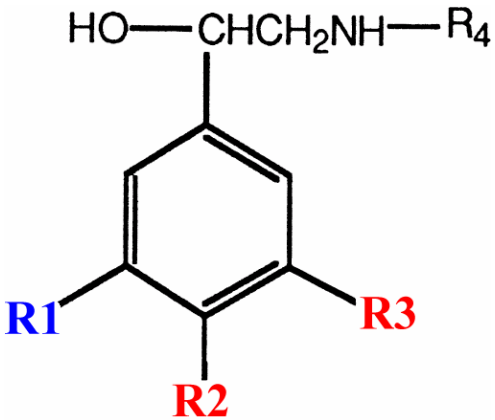
Mobile phases:

1. **10 mM sod.phosph.b. pH 6.0 + 50 μM disodium EDTA**
2. **10% 2-propanol in 10 mM sod.phosph.b. pH 6.0 + 50 μM disodium EDTA**

Laudanosine



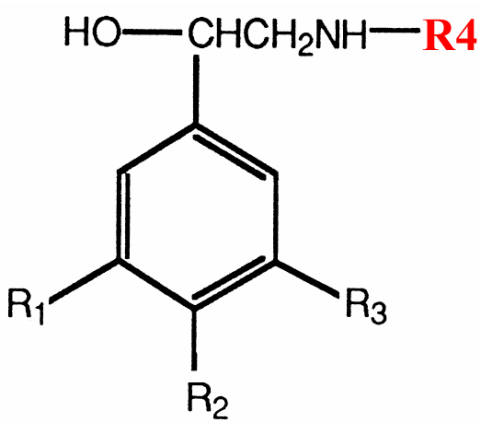
Chromatographic properties of structure analogues to epinephrine on the CBH-column



Mobile phase: 5% 2-propanol in 10 mM sod.ph.b. pH 6.0 + 50 μM disodium EDTA

Solute	R1	R2	R3	R4	k´1	α	Rs
Phenylethanolamine	H	H	H	H	1.89	1.41	1.81
Octopamine	H	OH	H	H	2.76	2.54	6.81
Norepinephrine	H	OH	OH	H	3.11	2.17	5.18
Normetanephrine	H	OH	CH_3O	H	3.01	2.06	4.64

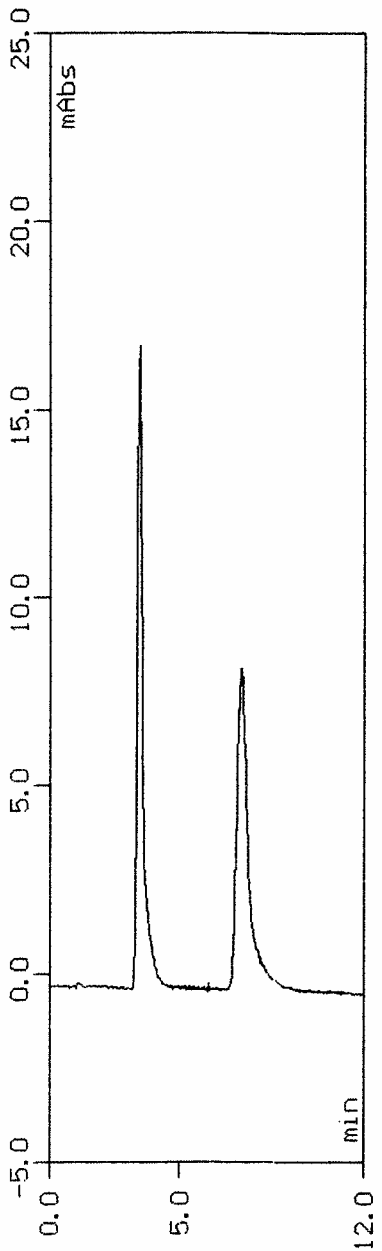
Chromatographic properties of structure analogues to epinephrine on the CBH-column

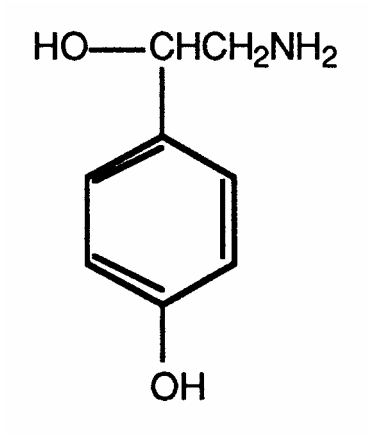


Mobile phase: 5% 2-propanol in 10 mM sod.ph.b. pH 6.0 + 50 μM disodium EDTA

Solute	R1	R2	R3	R4	k'_1	α	Rs
Norepinephrine	H	OH	OH	H	3.11	2.17	5.18
Epinephrine	H	OH	OH	CH_3	1.86	1.50	2.57
Isoprenaline	H	OH	OH	$CH(CH3)_2$	1.55	1.14	0.65

Separation of the enantiomers of octopamine





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

Mobile phase: **5% 2-propanol in 10 mM sod.ph.b. pH 6.0**

Method development CHIRAL-CBH

Hydrophilic and hydrophobic amines

Starting mobile phase: 5% 2-propanol in 10 mM sodium phosphate buffer pH 6.0 with 50 µM disodium EDTA

Results in enantioselectivity and too high retention

↓

Decrease pH and/or increase 2-propanol conc.

↓

No or low enantioselectivity

↓

Try another uncharged modifier as for example acetonitrile or remove the modifier

Results in no or low enantio-selectivity and low retention

↓

Increase pH stepwise to 7 or the buffer conc.

↓

No or low enantioselectivity

↓

Try another uncharged modifier as for example acetonitrile or remove the modifier

No or low enantioselectivity and high retention

↓

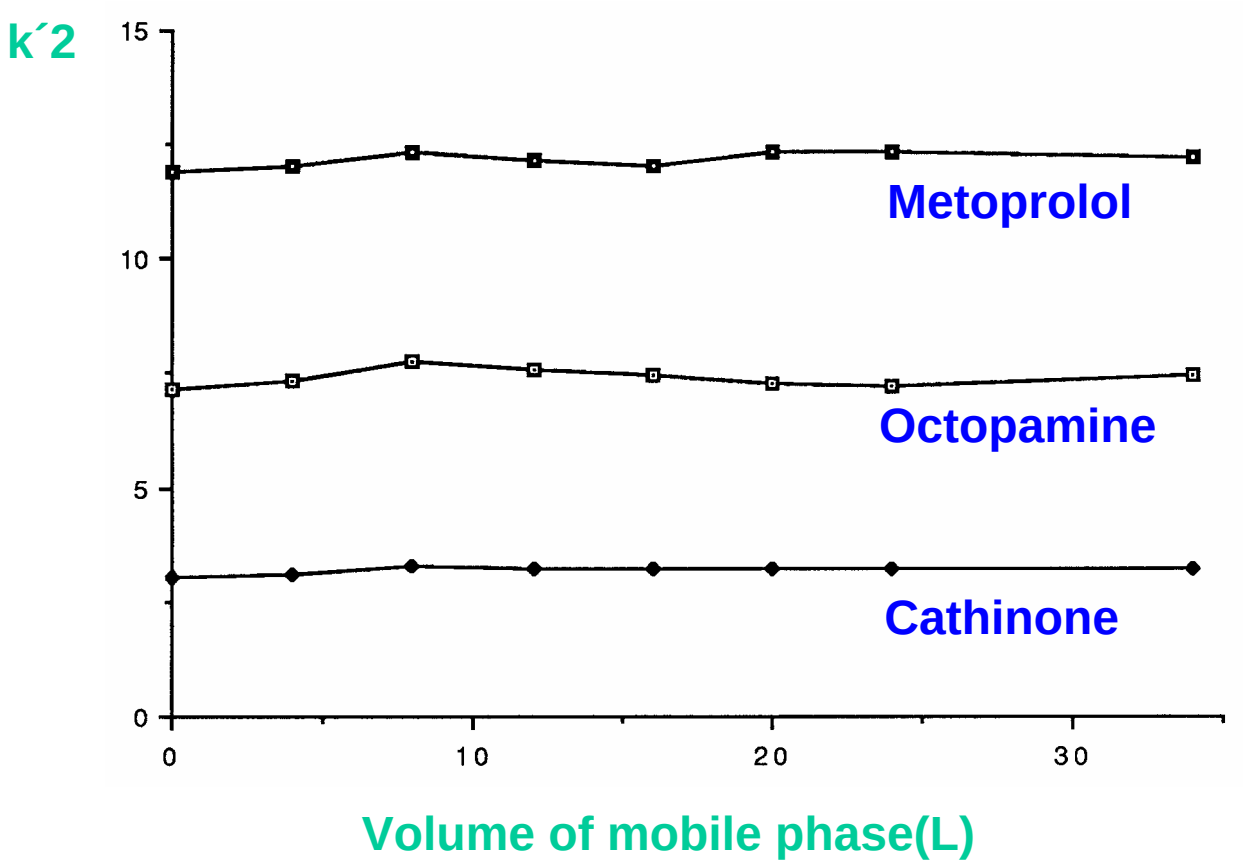
Try another uncharged modifier, for example acetonitrile and/or increase the modifier conc. or the buffer conc.

Stability of the CHIRAL-CBH column

Mobile phase:

5% 2.-propanol in 10 mM sodium phosphate buffer pH 6.0 (+ 50 μM disodium EDTA)

CHIRAL-CBH guard columns (10 x 3.0 mm) where used in the study



Conclusions

- **Simple method development due to the reversed-phase character of the phase**
- **Unique possibilities to induce and improve enantioselectivity by simple changes of the mobile phase composition**
- **Separates basic compounds with very high enantioselectivity and resolution**