

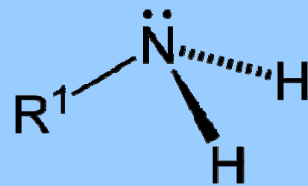
# Amines by Supercritical Fluid Chromatography

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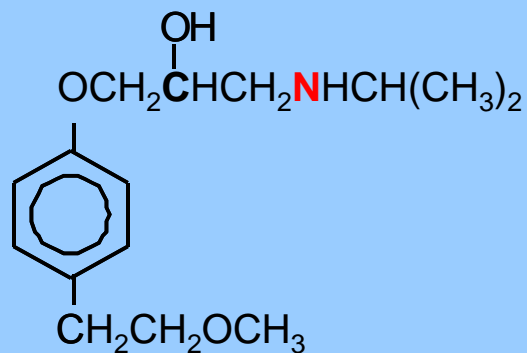


# Amines by Supercritical Fluid Chromatography

- Introduction, metoprolol
- Capillary columns (with FID)
- Packed columns
- Additives & Without
- Non silica support;  
& CMPA
- NMR
- Artefact
- Water sample
- Summing up

# Introduction (amines, metoprolol)

- Amines are common
- With CO<sub>2</sub> – amines will react
  - Well .....
- Metoprolol:



Related compounds available

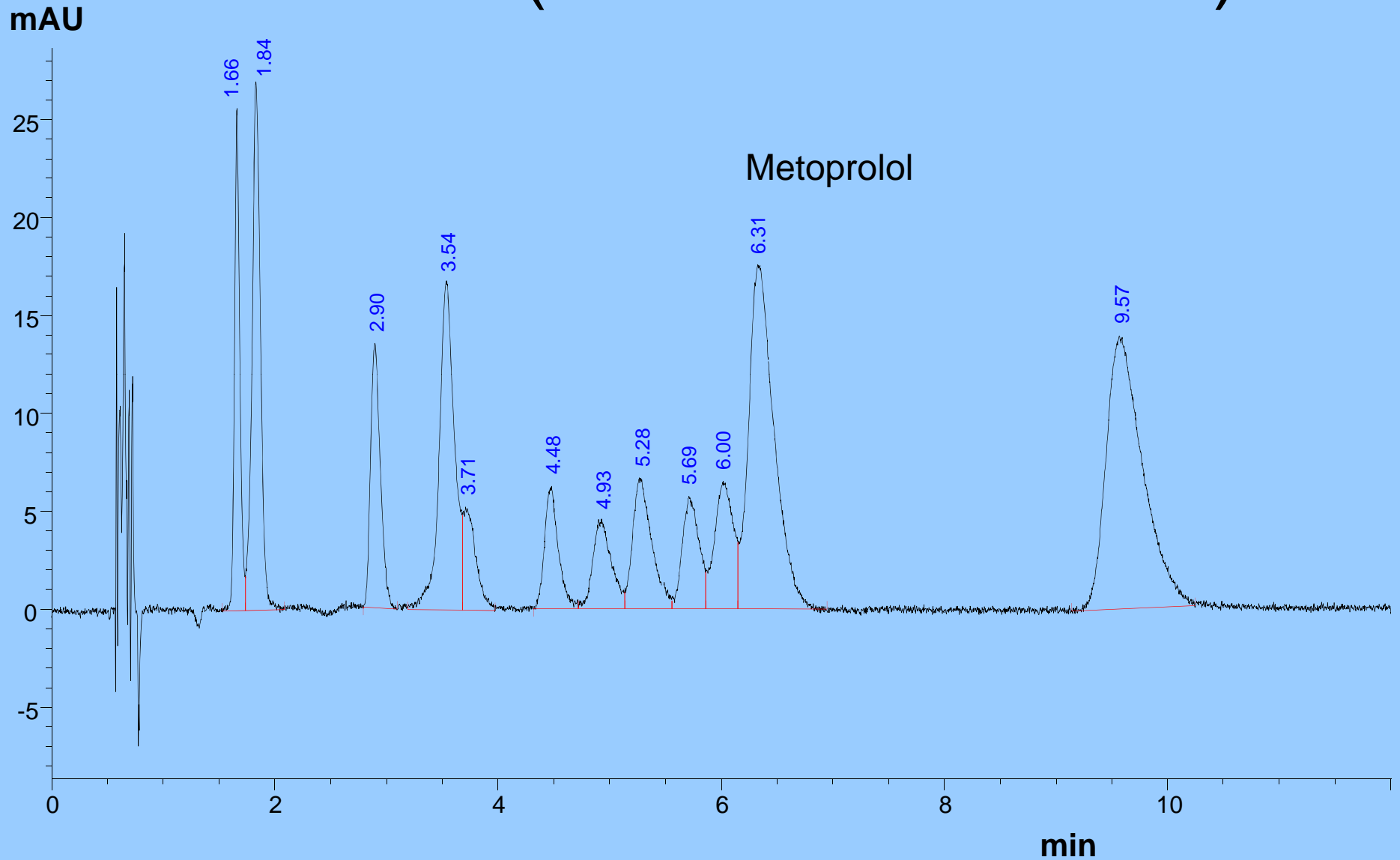
# Capillary columns (with FID)

- Neat supercritical fluids can be used
  - Detection with FID attractive
- CO<sub>2</sub>
- N<sub>2</sub>O
- But .....

# Capillary vs packed columns

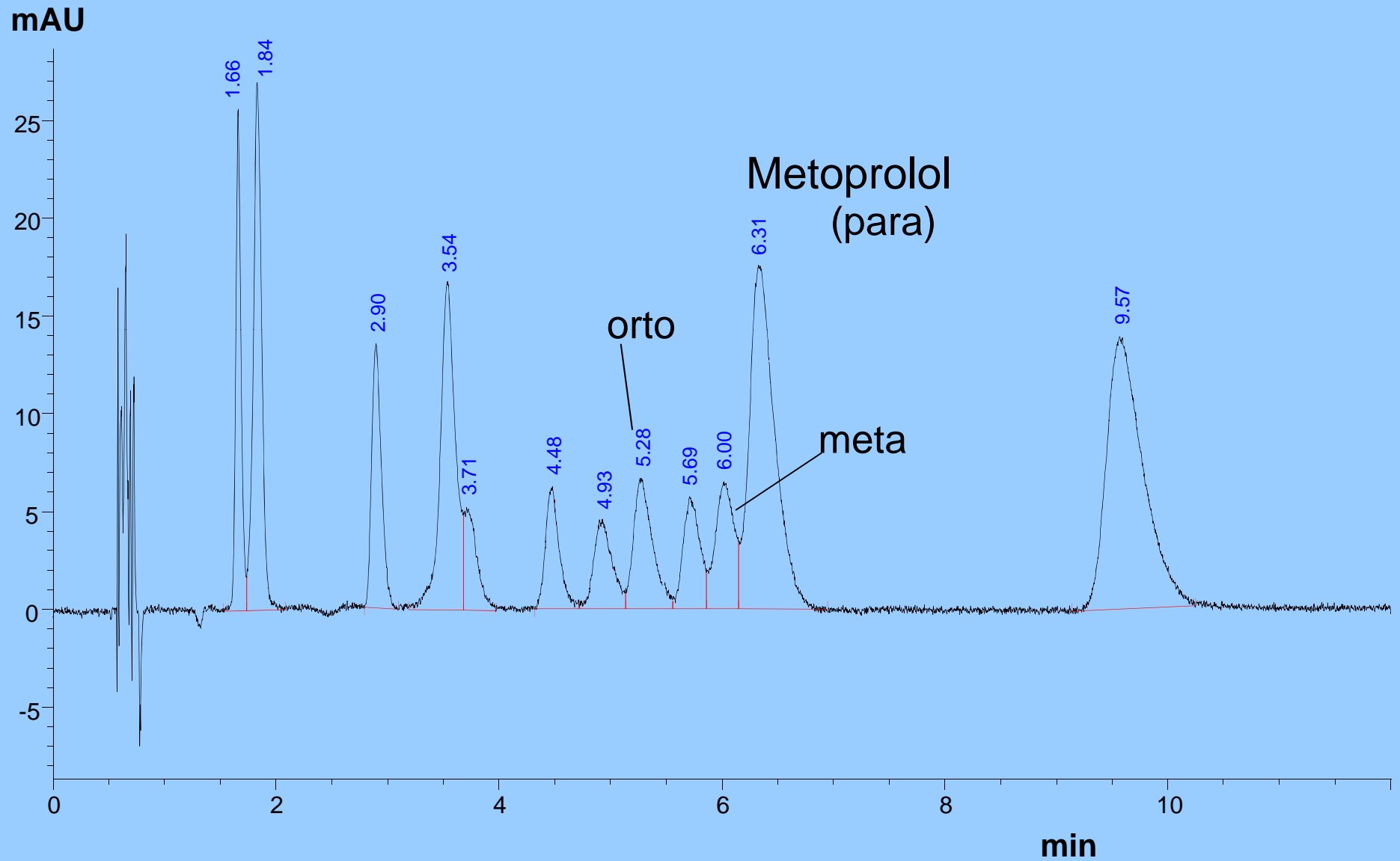
- Capillary SFC
  - Takes time
  - Sample intro
  - Sample capacity
  - Solubility
  - Column dimensions
    - **FID**
- Packed SFC
  - Faster
  - Choice of columns
  - Low pressure drop
  - Flow-rate
  - Loadability
    - **UVD**

# Diol silica (HAc/TEA additives)

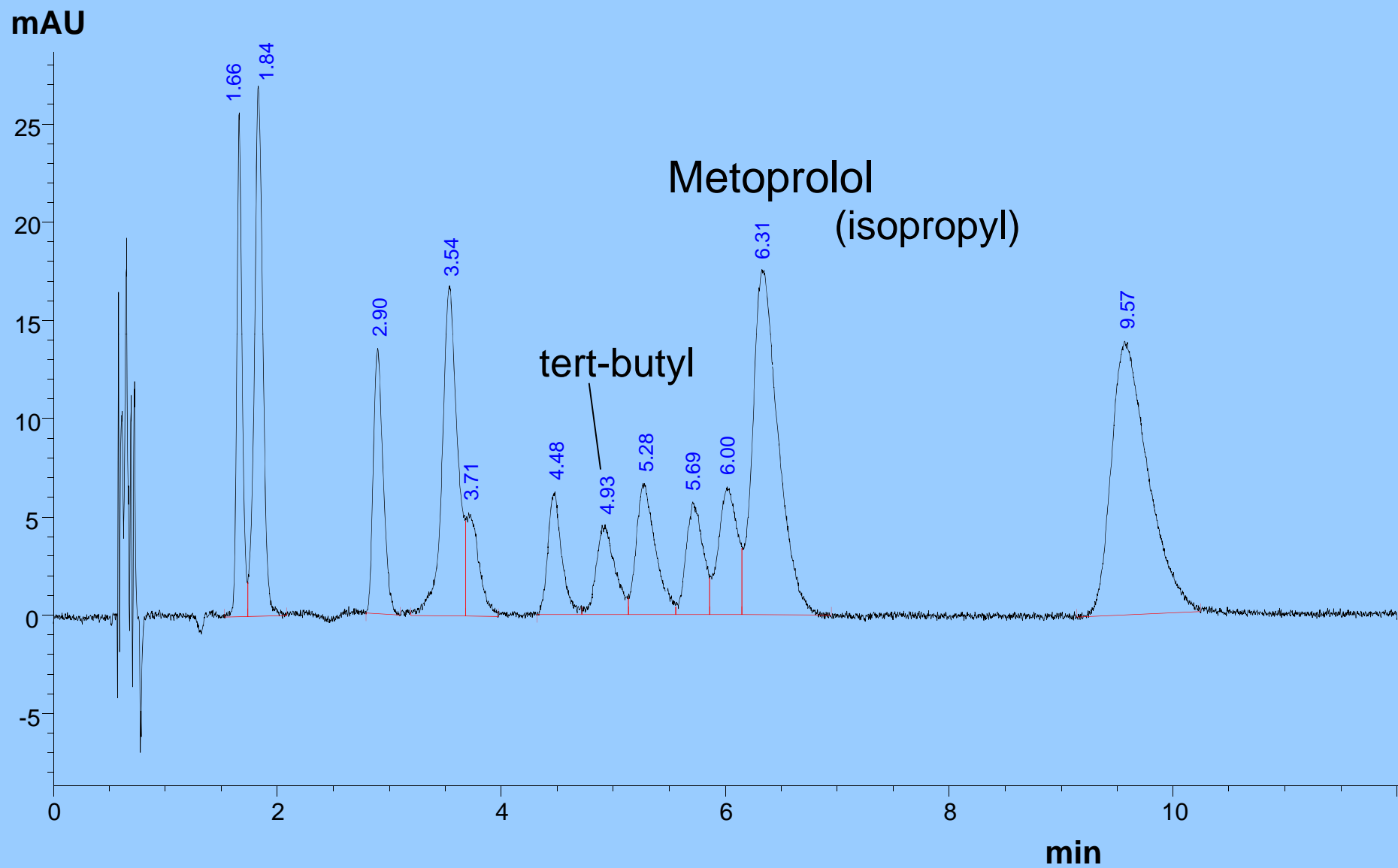


10 % MeOH, 2.0 ml/min, /150 bar, 40°C, 273 nm, 125x4 mm ID LiChrospher diol, 35/7 mM

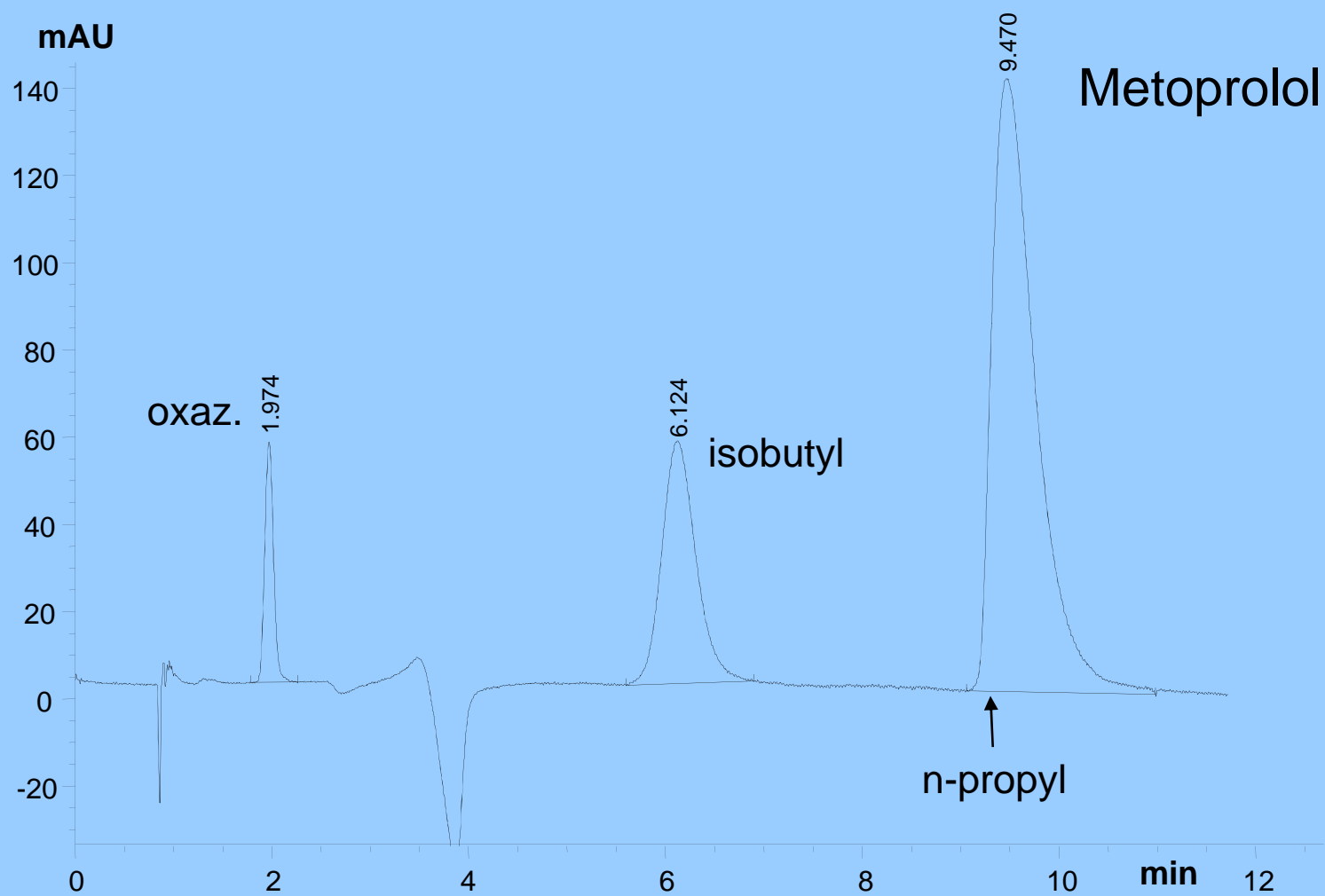
# Positional isomers



# Bulky alkylsubstituent

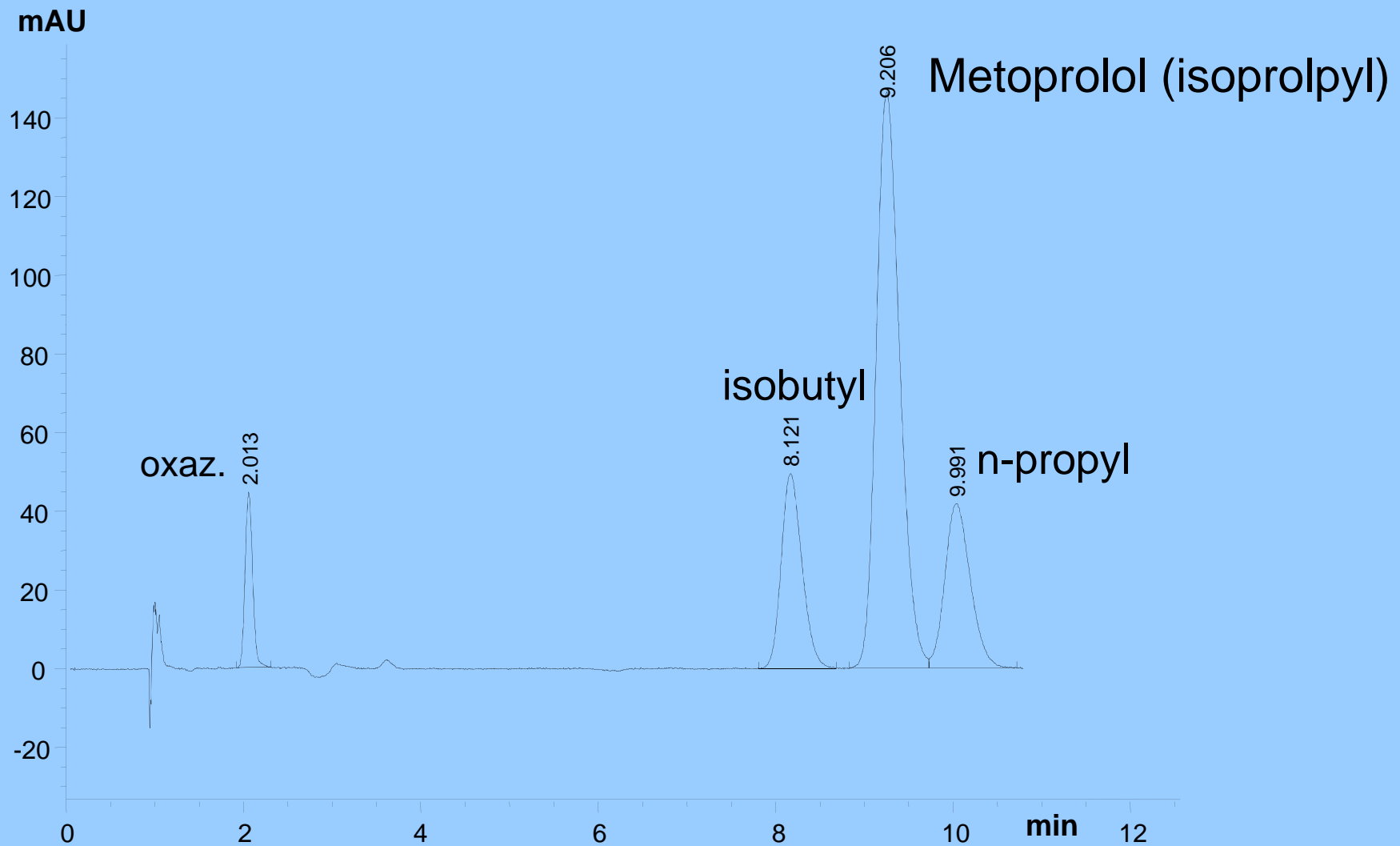


# Ethanesulfonic acid / TEA



10 % MeOH with 18 mM TEA, 1.5 ml/min, /150 bar, 40°C, 254 nm, 125x4 mm ID diol

# Ethanesulfonic acid / TEA

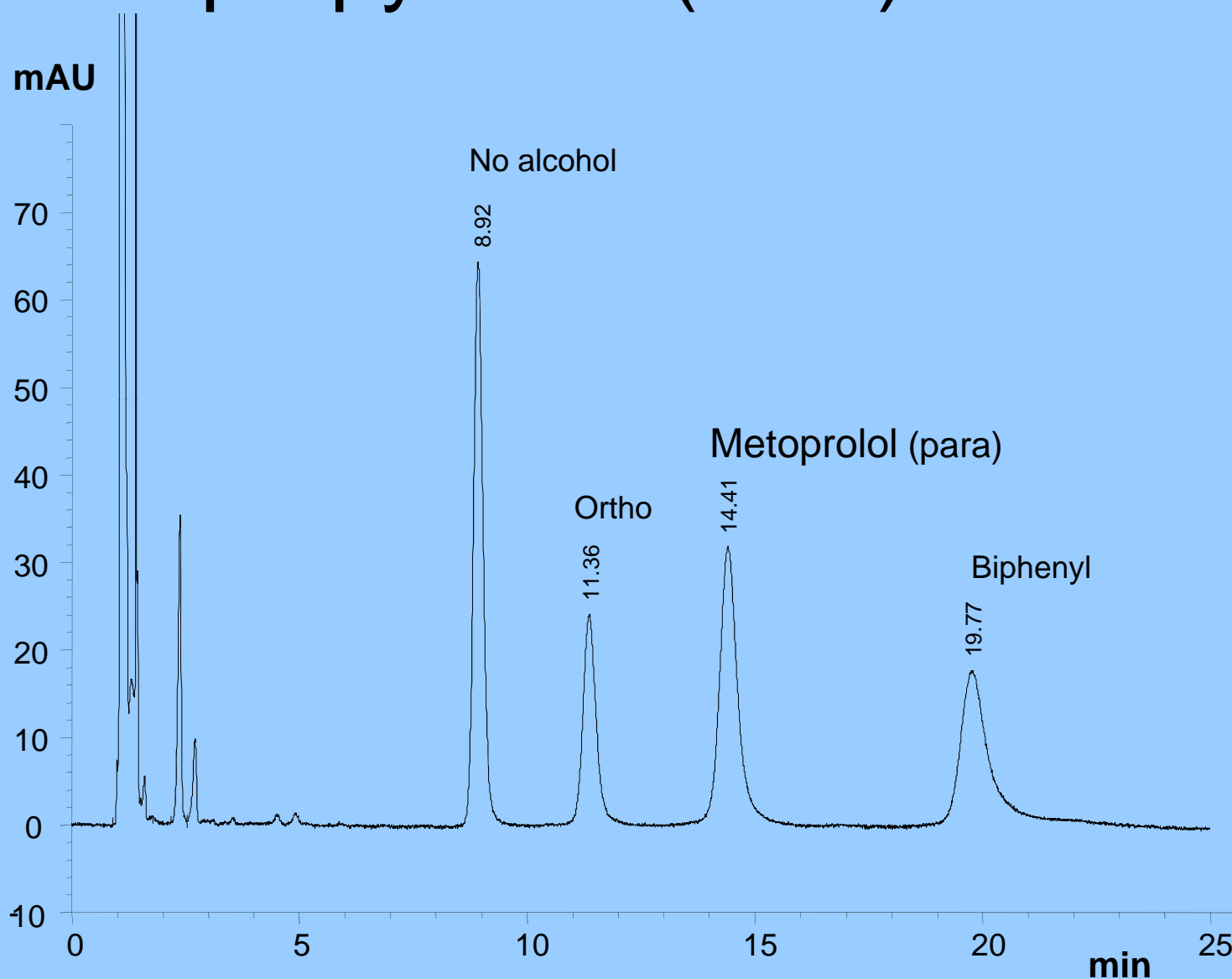


10 % MeOH with 24/18 mM, 1.5 ml/min, /150 bar, 40°C, 254 nm, 125x4 mm ID diol

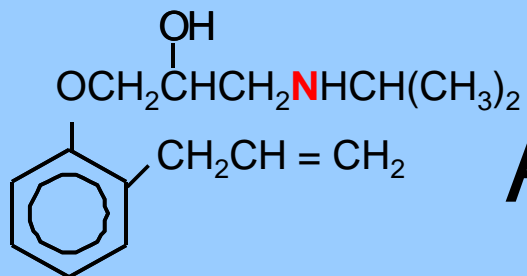
# SFC on silica (with UVD)

- Different selectivity compared with RP-LC
- Alternative columns
- Purity of API (metoprolol bulk substance)
- Amine additive; or
- Acid + amine additive

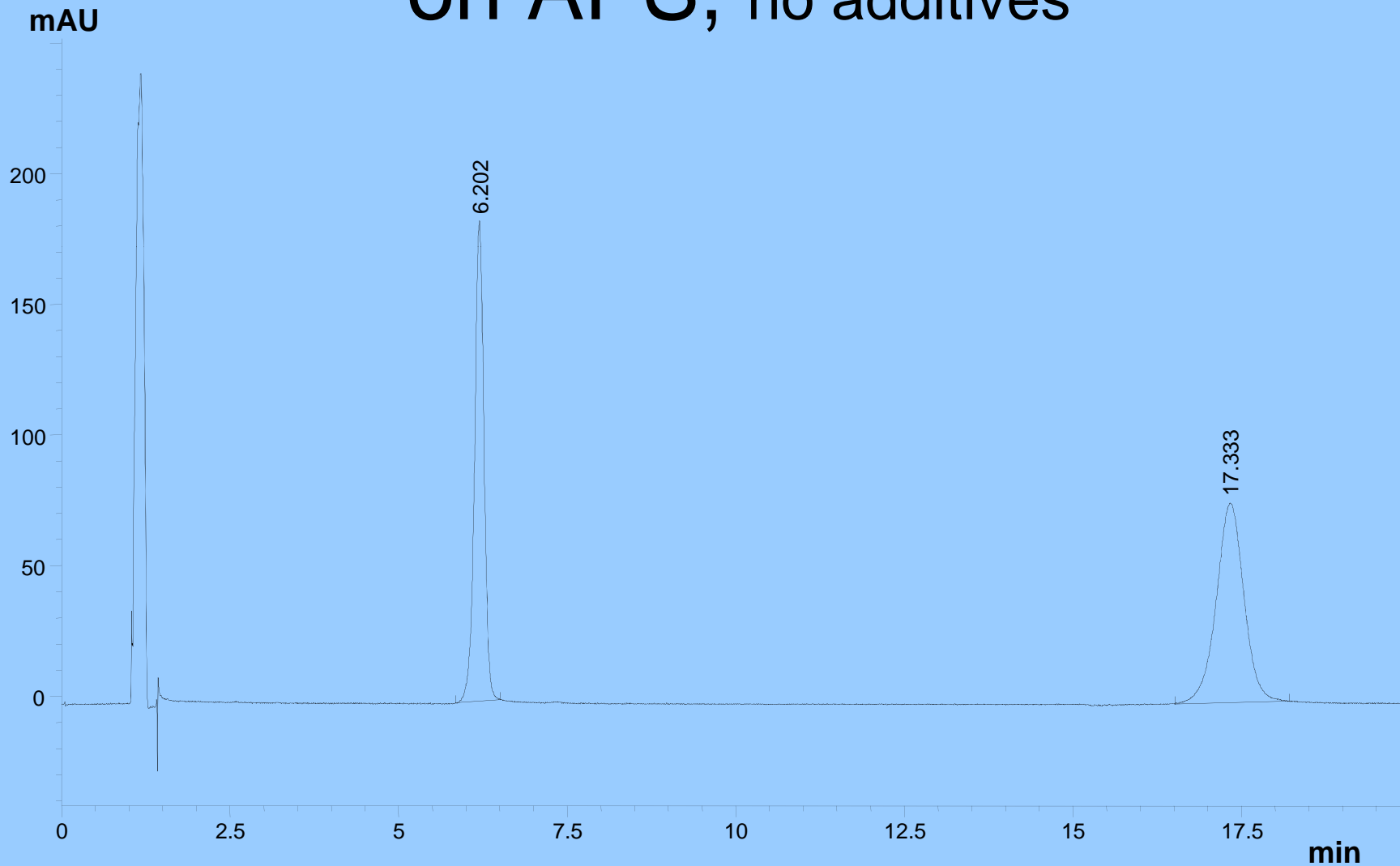
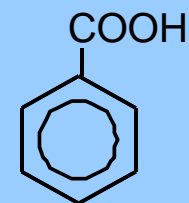
# Aminopropylsilica (APS) - no additive(s)



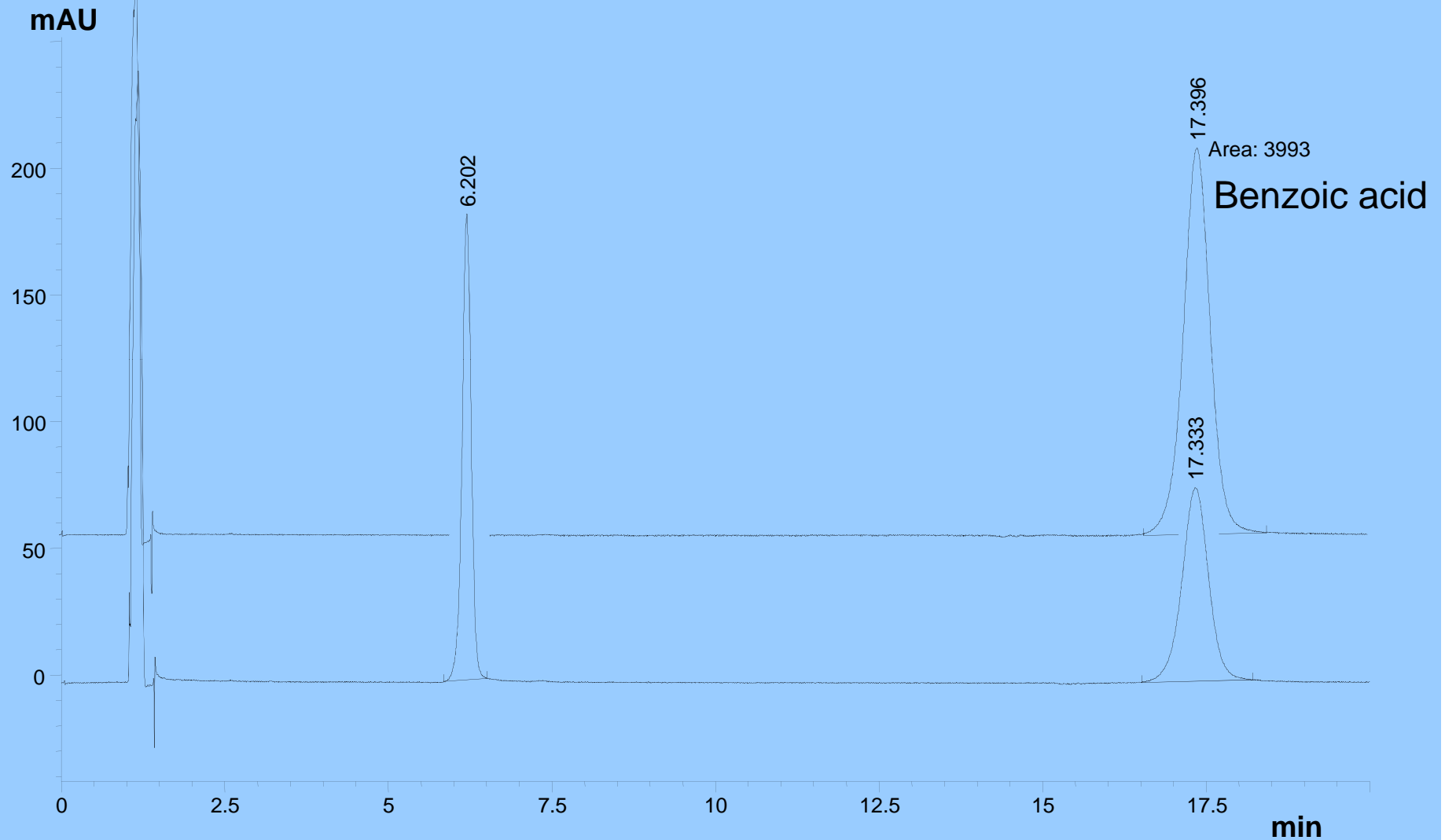
8 % MeOH, 1.5 ml/min, /250 bar, 60°C, 254 nm, 125x4.6 mm ID aminopropylsilica



# Alprenolol benzoate on APS, no additives



# Alprenolol benzoate

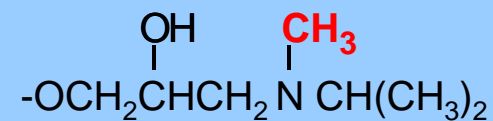
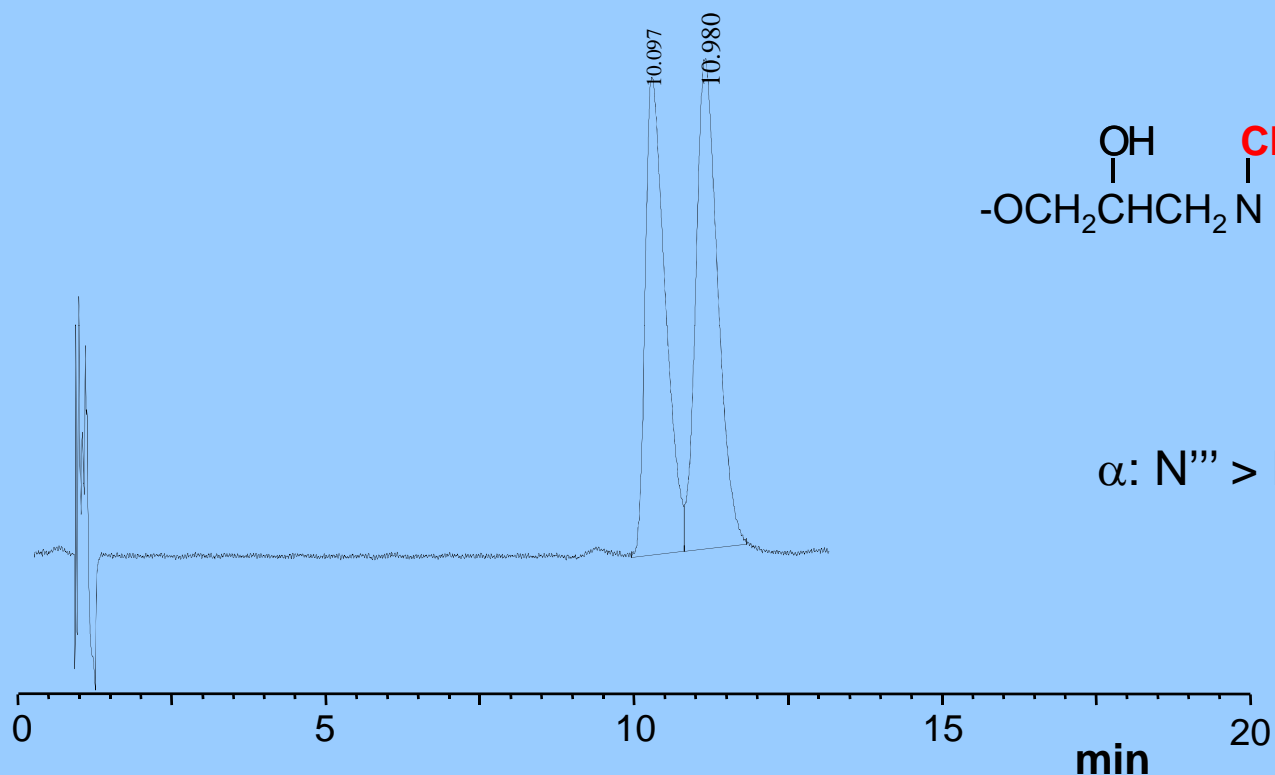


150 x 4.6, 1.5 ml/min, 10 % MeOH, /150 bar, 30°C

# Hypercarb

- Robust support (porous graphitic carbon)
- Higher additive concentration required; ~ 5x
- Different selectivity
- Adsorptive ..... (cleaning can be necessary)
- CMPA (e.g. L-(+)-tartaric acid)

# 0.25 mM CMPA (L-(+)-tartaric acid)

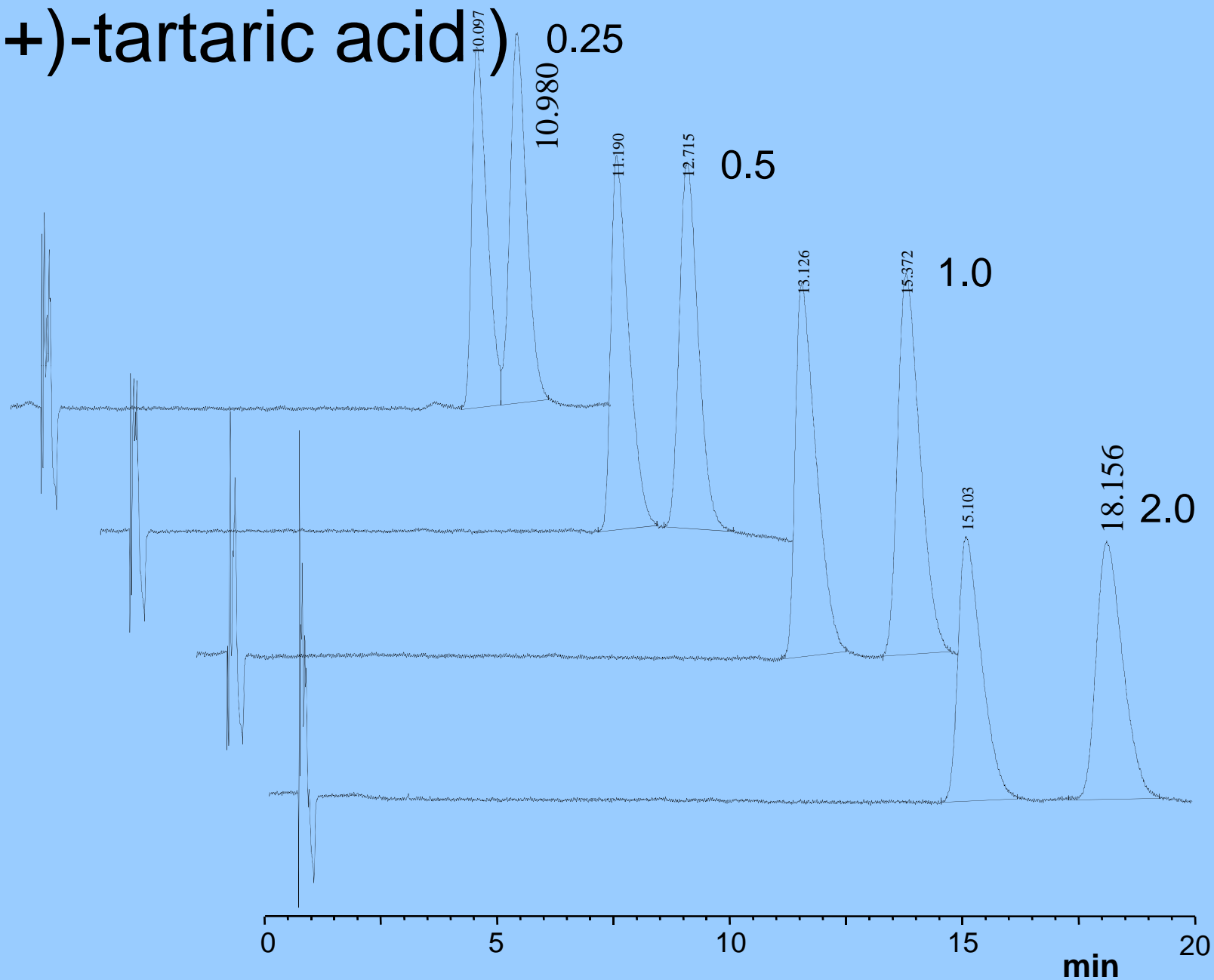


$\alpha: \text{N}''' > \text{N}''$

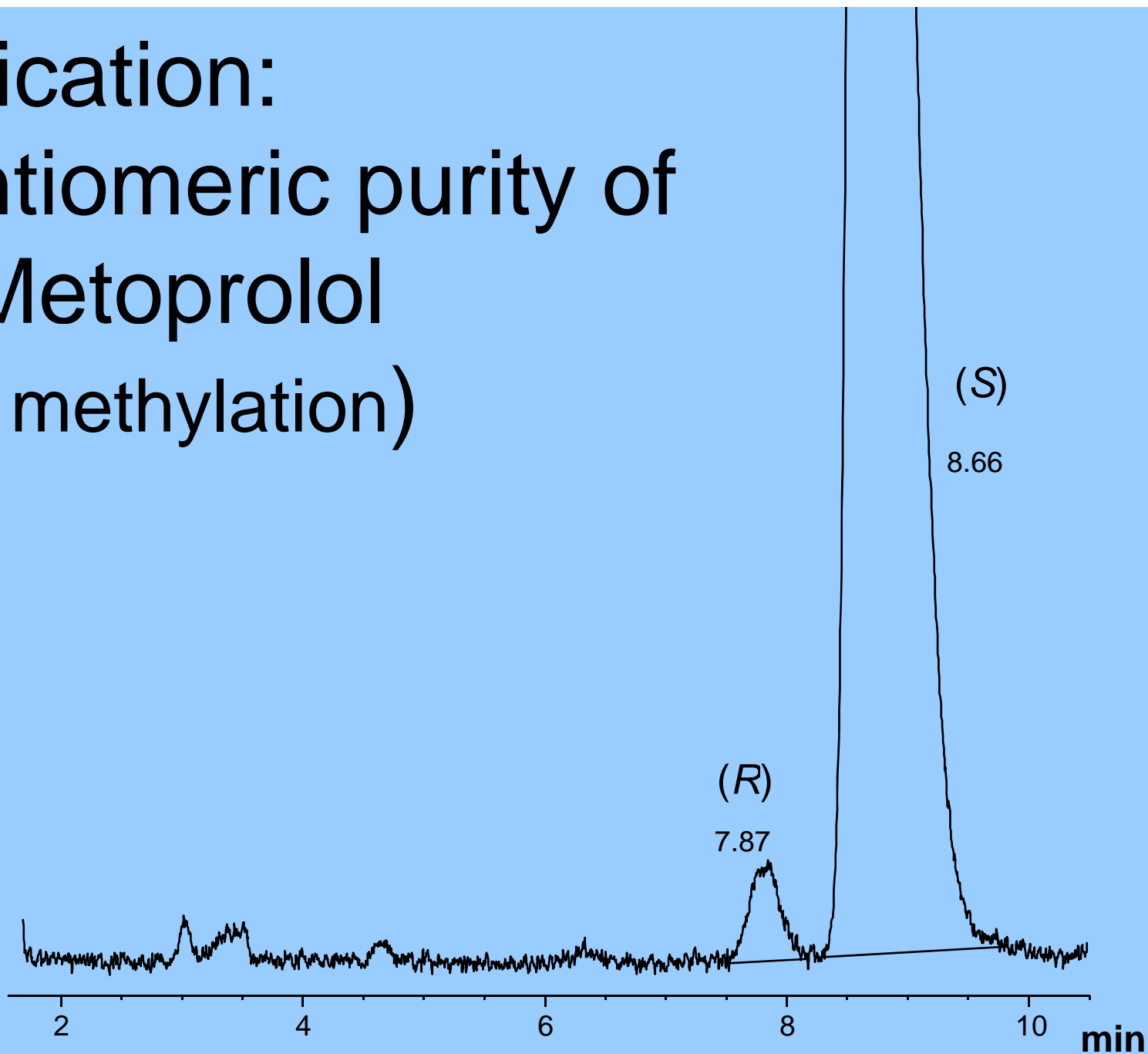
Hypercarb 100 x 3 mm ID  
1.0 ml/min 10 % MeOH  
24 mM DMOA  
/150 bar, 273 nM

Hypersil: mute (increased  $k$  only)

# Higher $\alpha$ with higher mM (L-(+)-tartaric acid)

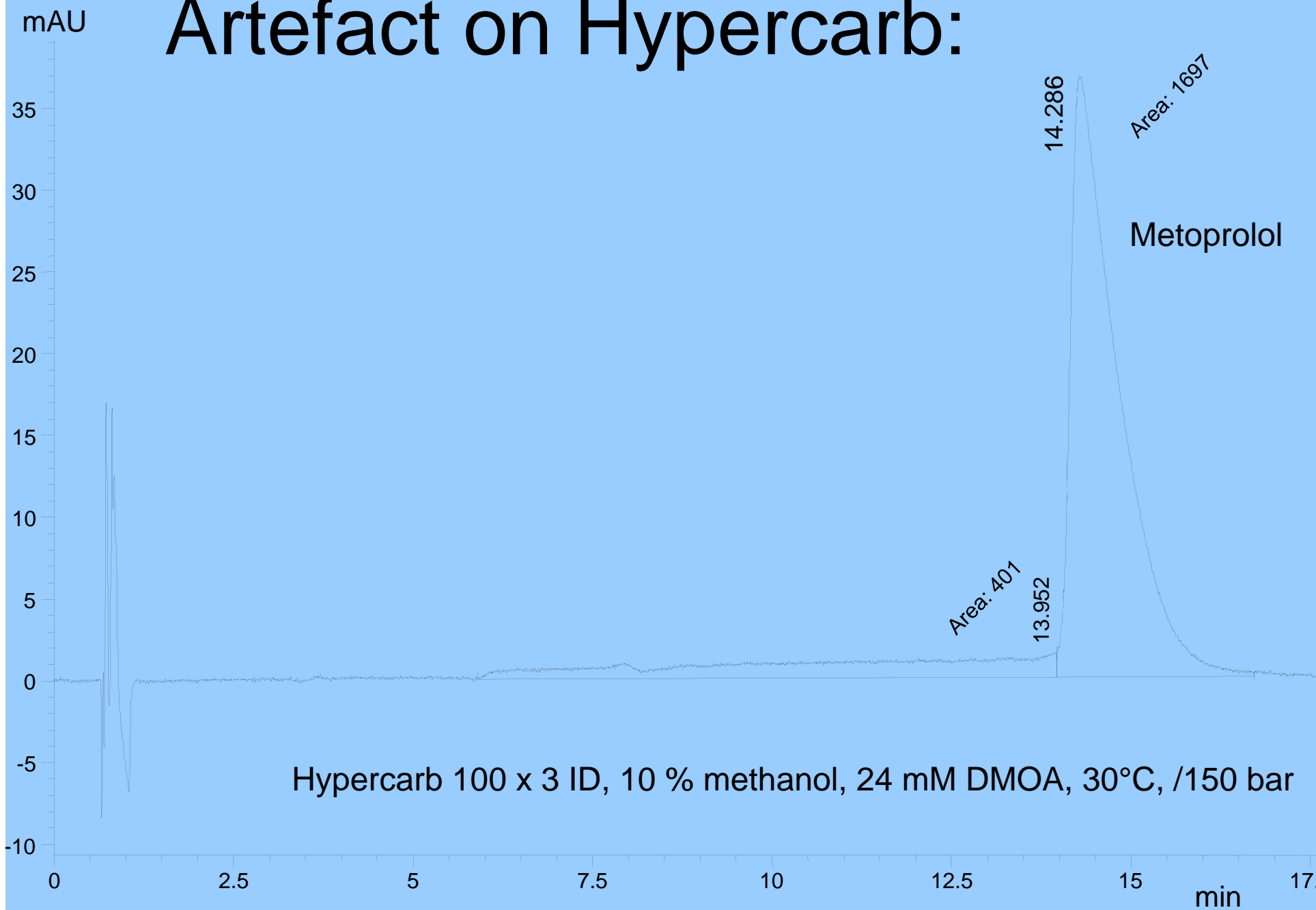


# Application: enantiomeric purity of (*S*)-Metoprolol (after methylation)

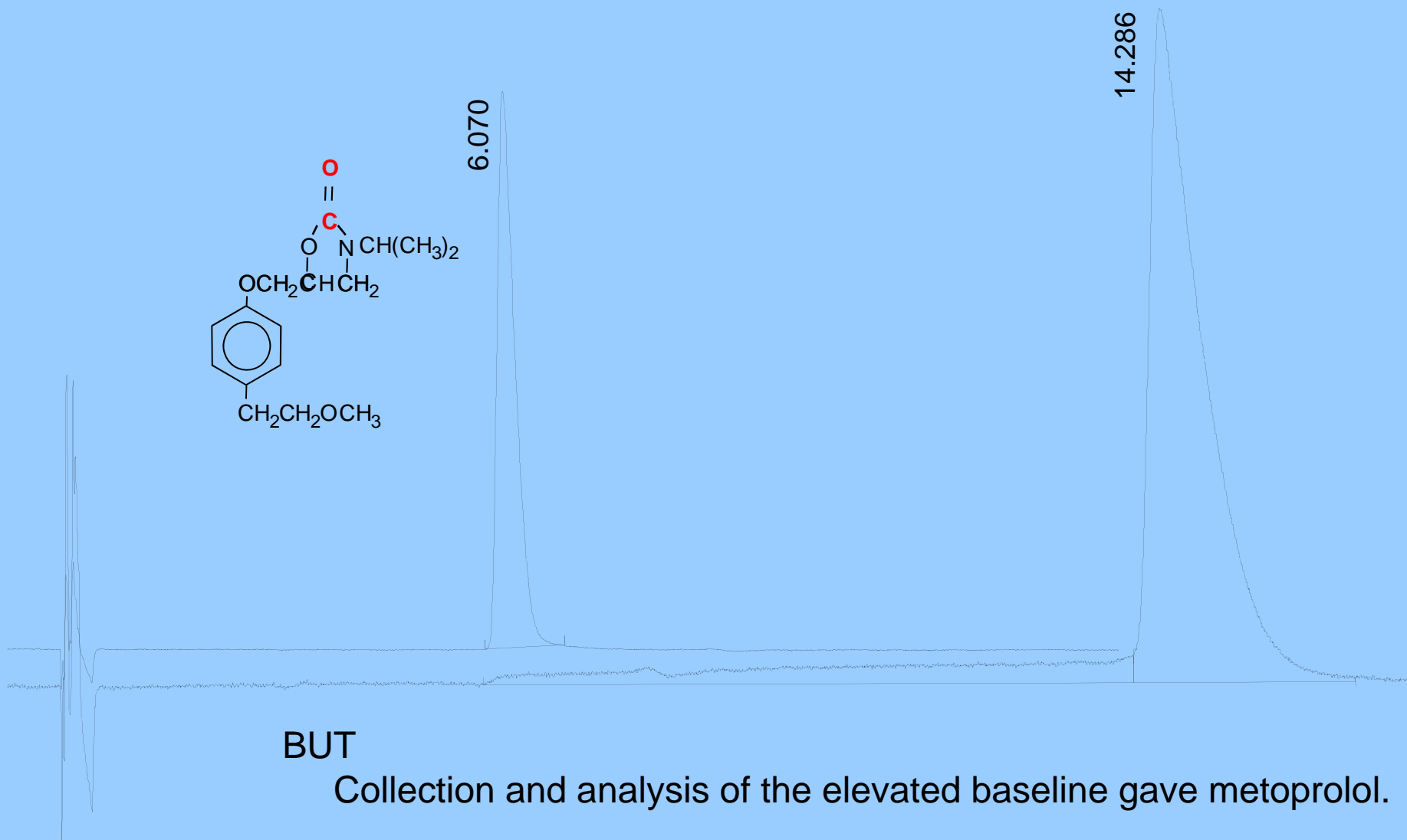


100 x 3 mm ID, 30°C, 2.0 ml/min CO<sub>2</sub> with 8 % MeOH (240 mM DMOA, 5 mM TA)

# Artefact on Hypercarb:



*The elevated baseline starts when corresponding oxazolidinone-2-one elutes.....*



**BUT**

Collection and analysis of the elevated baseline gave metoprolol.

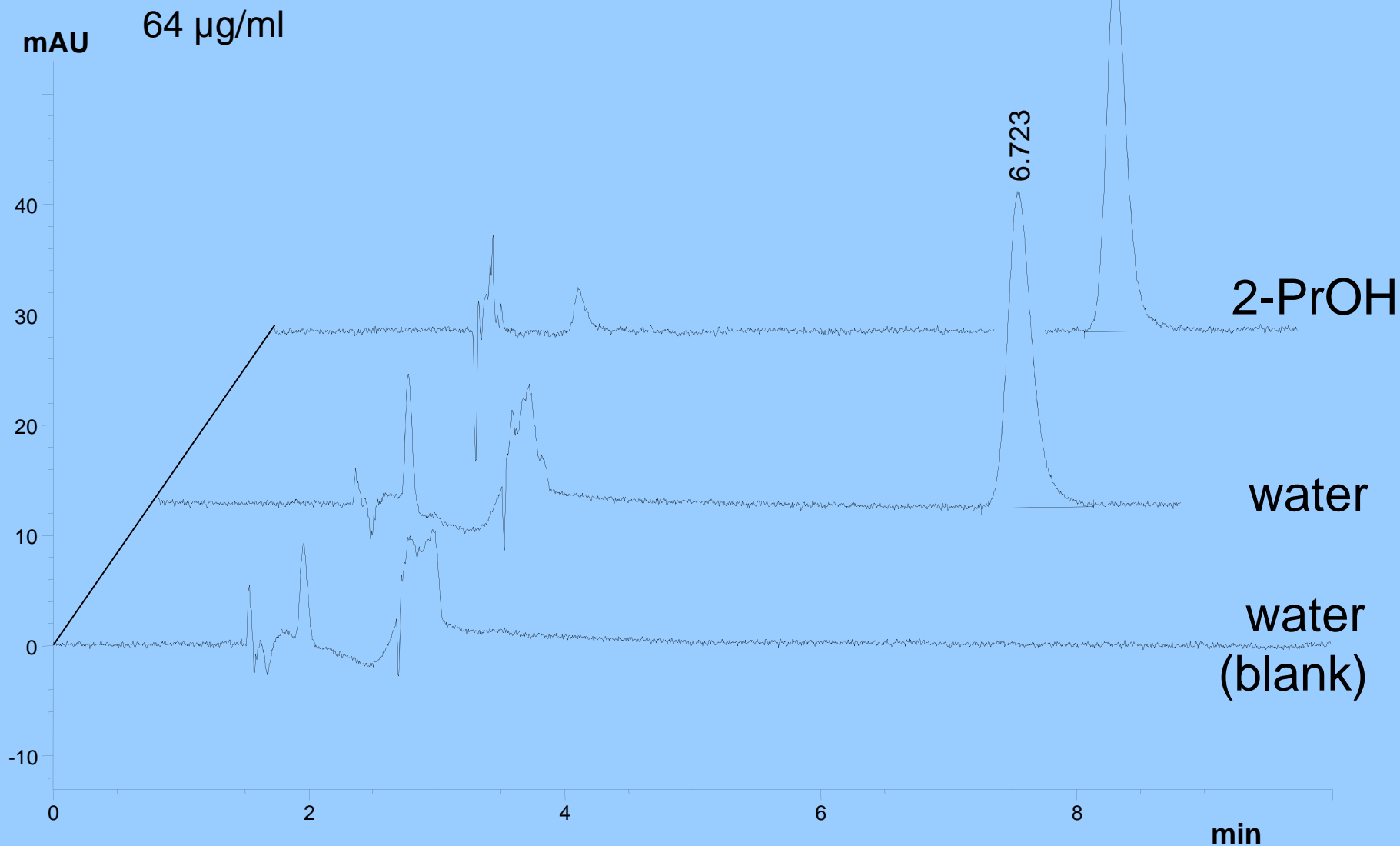
# Steady state NMR Neat CO<sub>2</sub>

- amine + CO<sub>2</sub> may react ?
- Benzylamines; yes !
- Metoprolol appears to be stable.
- Less protected amine analogue not soluble in neat CO<sub>2</sub>

# Samples dissolved in water

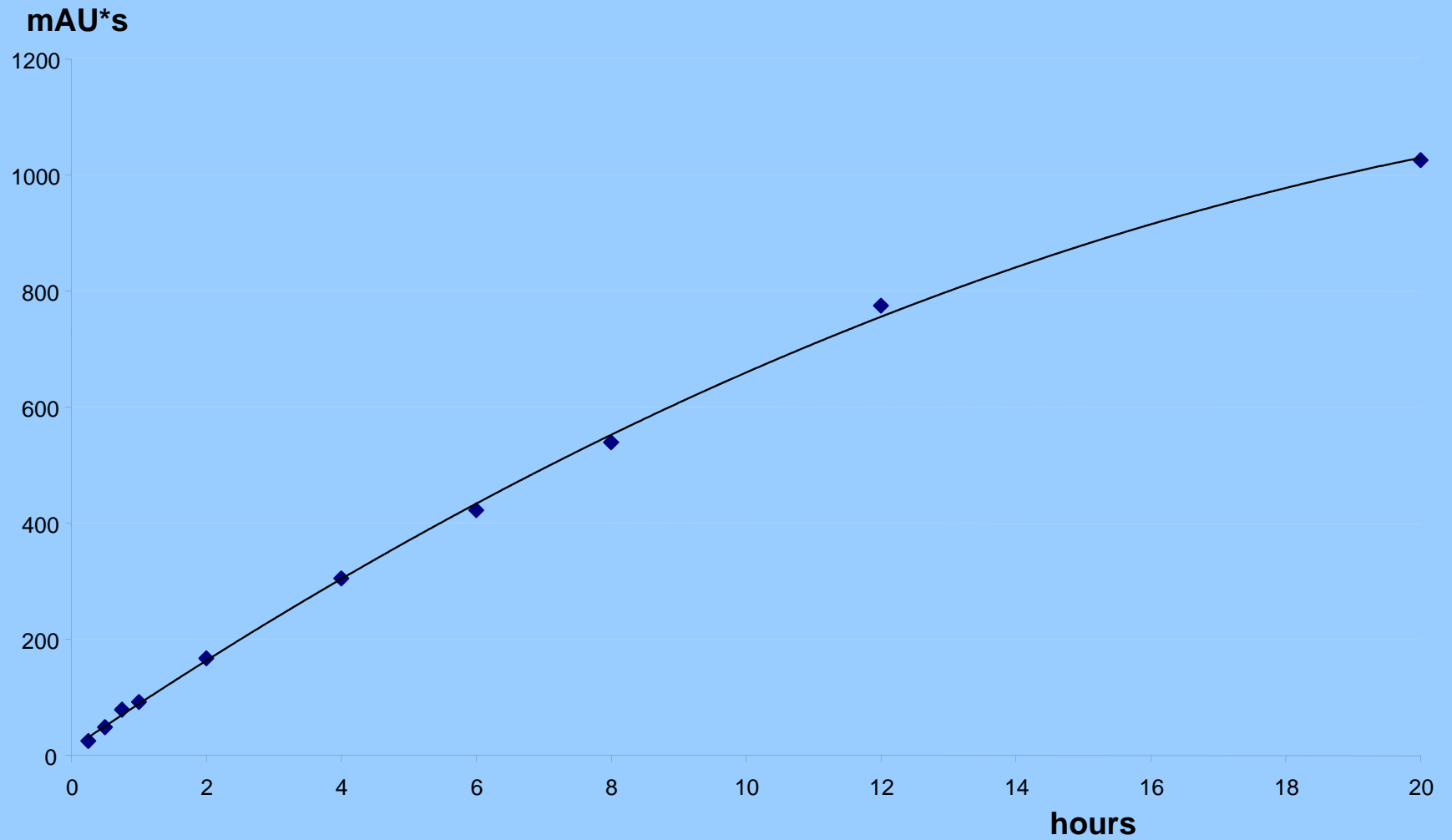
- Can be done by SFC
- 2-propanol as modifier
- Peak compression may occur

# Metoprolol dissolved in ....



30 % 2-PrOH, Hypersil 200 x 4.6 mm ID, 40°C, 1.5 ml/min, /150 bar, 220 nm  
24 mM EtSA and 18 mM TEA additives, 5- $\mu\text{l}$  loop

# From dissolution testing:



# Summing up

- Sample
- Unknowns ?
- Column
- Additives ?

# Acknowledgement

- Anders Karlsson
- Jörgen Vessman
- and many more !



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# Addendum: Further reading (references)

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