

*Chiral Separation of Basic  
Compounds in HPLC Using Acidic  
Additives*

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## *Additives for Acids in Chiral HPLC*

- In HPLC acid additives are required for elution of acid analytes from polysaccharide CSPs
- Mechanism thought to be simple ion suppression
- Recent work showed not all acids are equal, suggesting ion-pair mechanism



## *Acidic Additives in Chiral HPLC*

It was observed that amino acid esters showed retention and selectivity increases with ethanesulfonic acid (ESA) – although not required for elution

- Strong memory effect
- Mechanism thought to involve additional hydrogen bonding site of ESA bound to CSP
- ESA is not localized nor stoichiometric – a localized pH effect

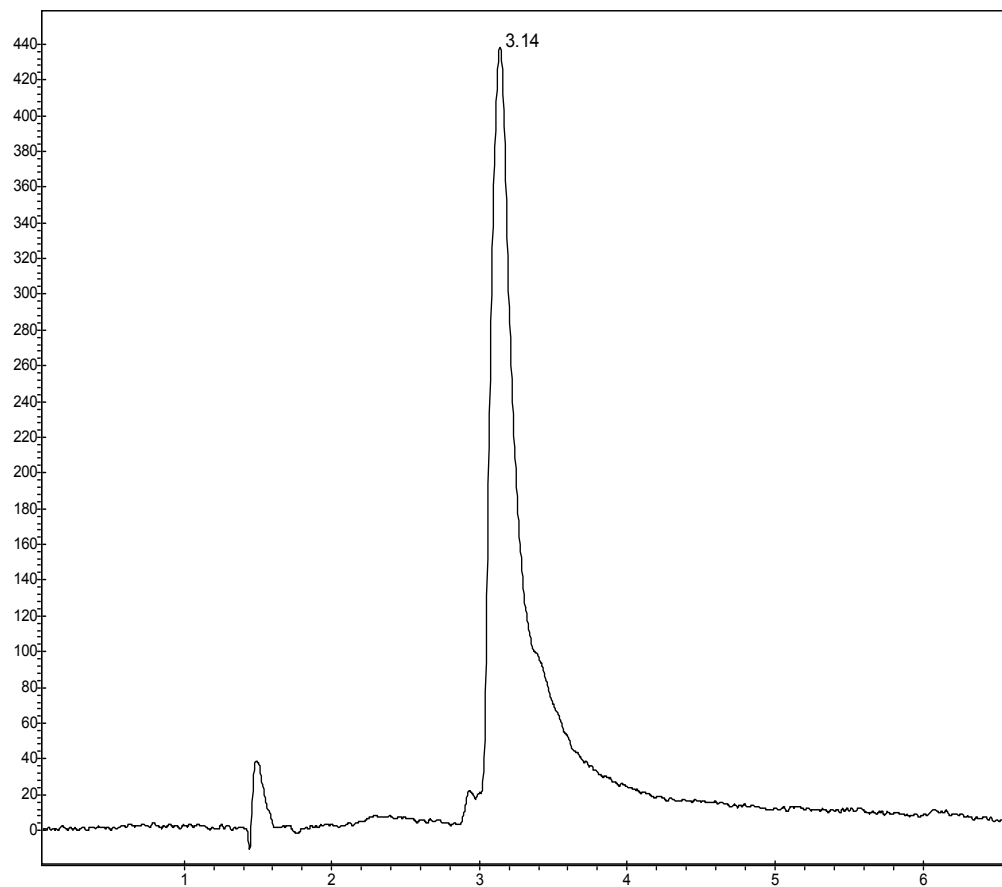


## *Acidic Additives in Chiral SFC*

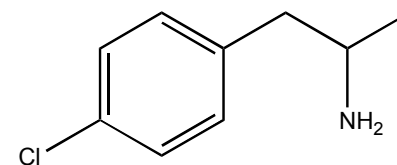
- Acids not required to elute acid analytes
- ESA effect observed for amino acid esters
- ESA allowed separation of a wide variety of basic analytes not previously separated in SFC
- Dilution with ESA required → eluting as “salt”
- No memory effect



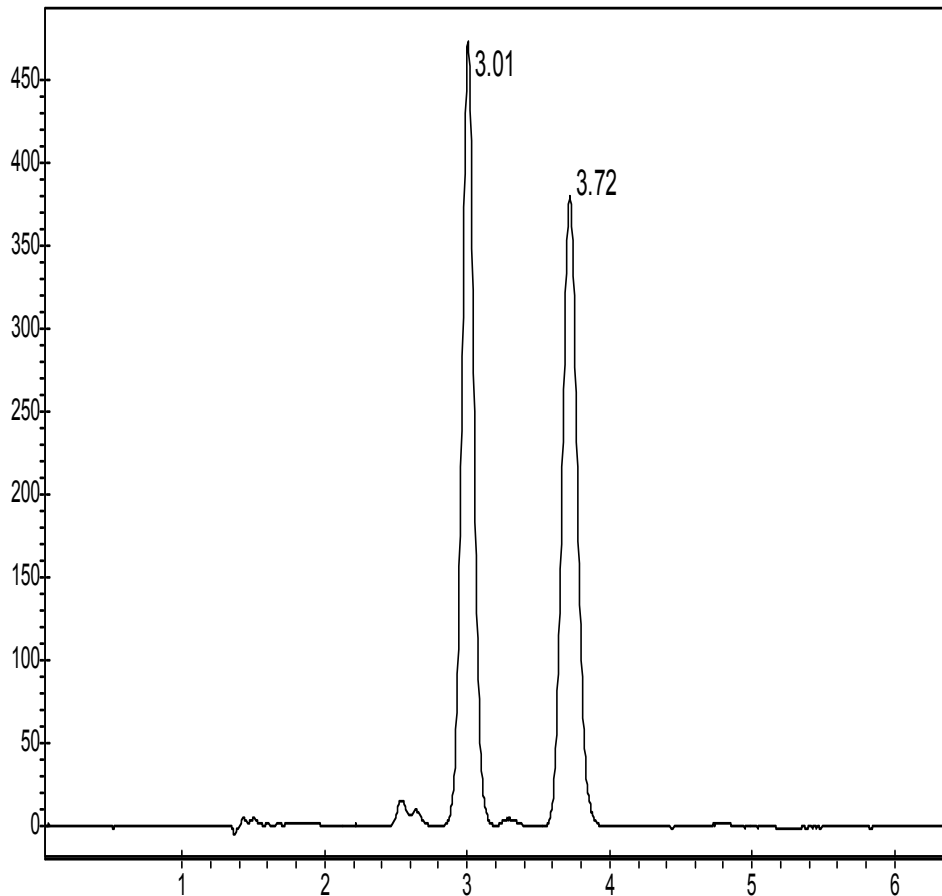
# *Chiral SFC of Chloramphenamine*



Chloramphenamine  
20% EtOH  
HCl salt in EtOH  
AD-H

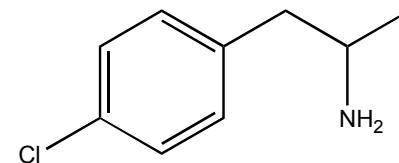


# Chiral SFC of Chloramphetamine with ESA

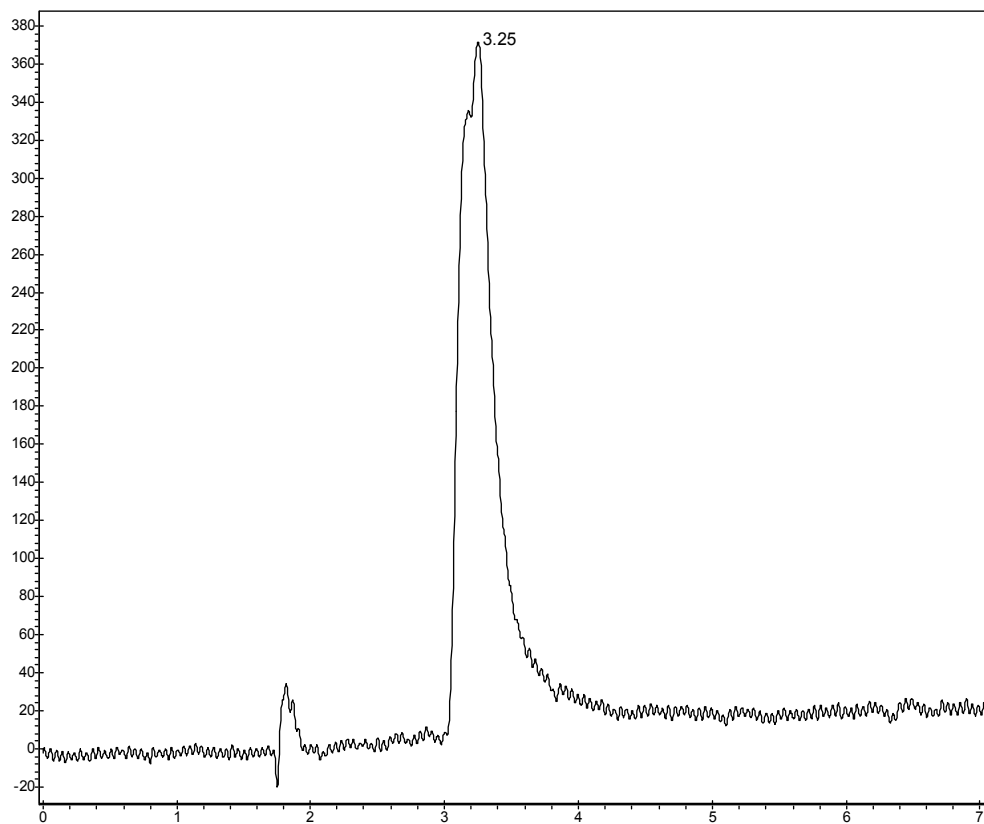


Chloramphetamine  
20% EtOH (0.1%ESA)  
HCl salt in EtOH/ESA  
AD-H

$\alpha = 1.47, R_s = 3.99$



# *Chiral SFC of an amino acid ester*

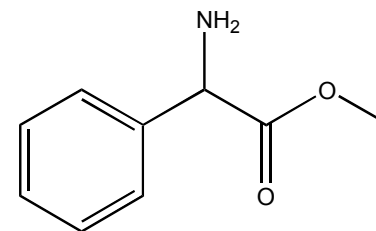


Phenylalanine-methyl ester

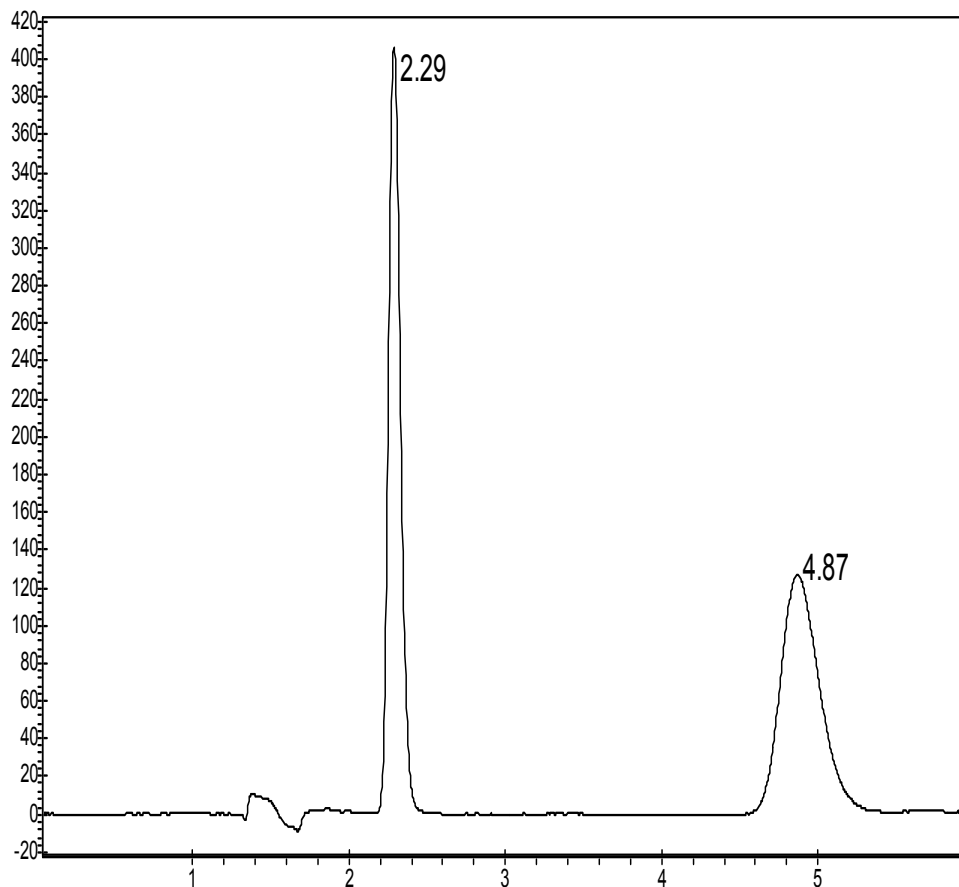
20% EtOH

HCl salt in EtOH

AD-H

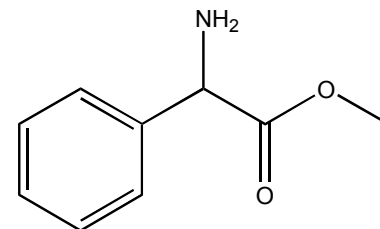


# Chiral SFC of an amino acid ester with ESA



Phenylalanine-methyl ester  
20% EtOH (0.1%ESA)  
HCl salt in EtOH/ESA  
AD-H

$$\alpha = 4.26, R_s = 8.64$$

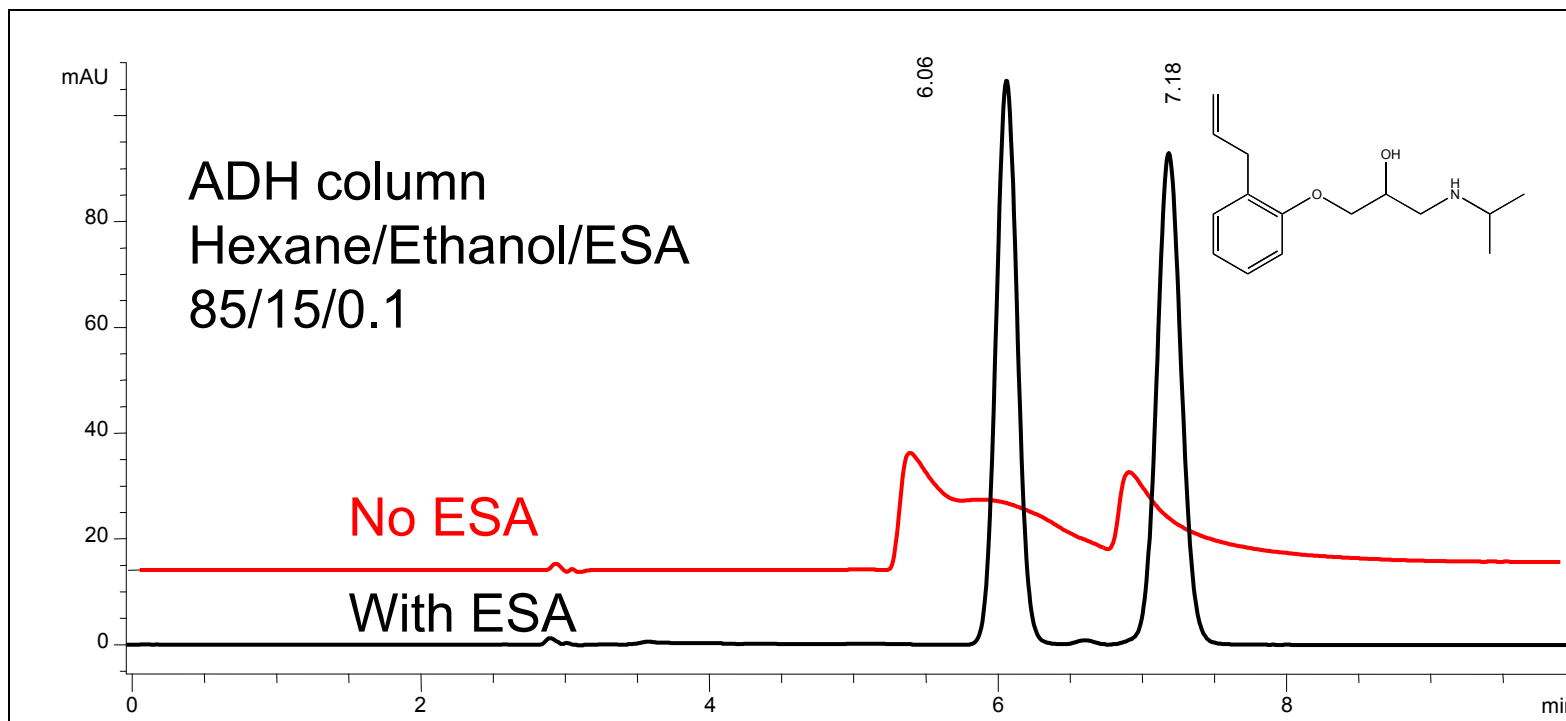


## *Evaluation of ESA for Chiral HPLC*

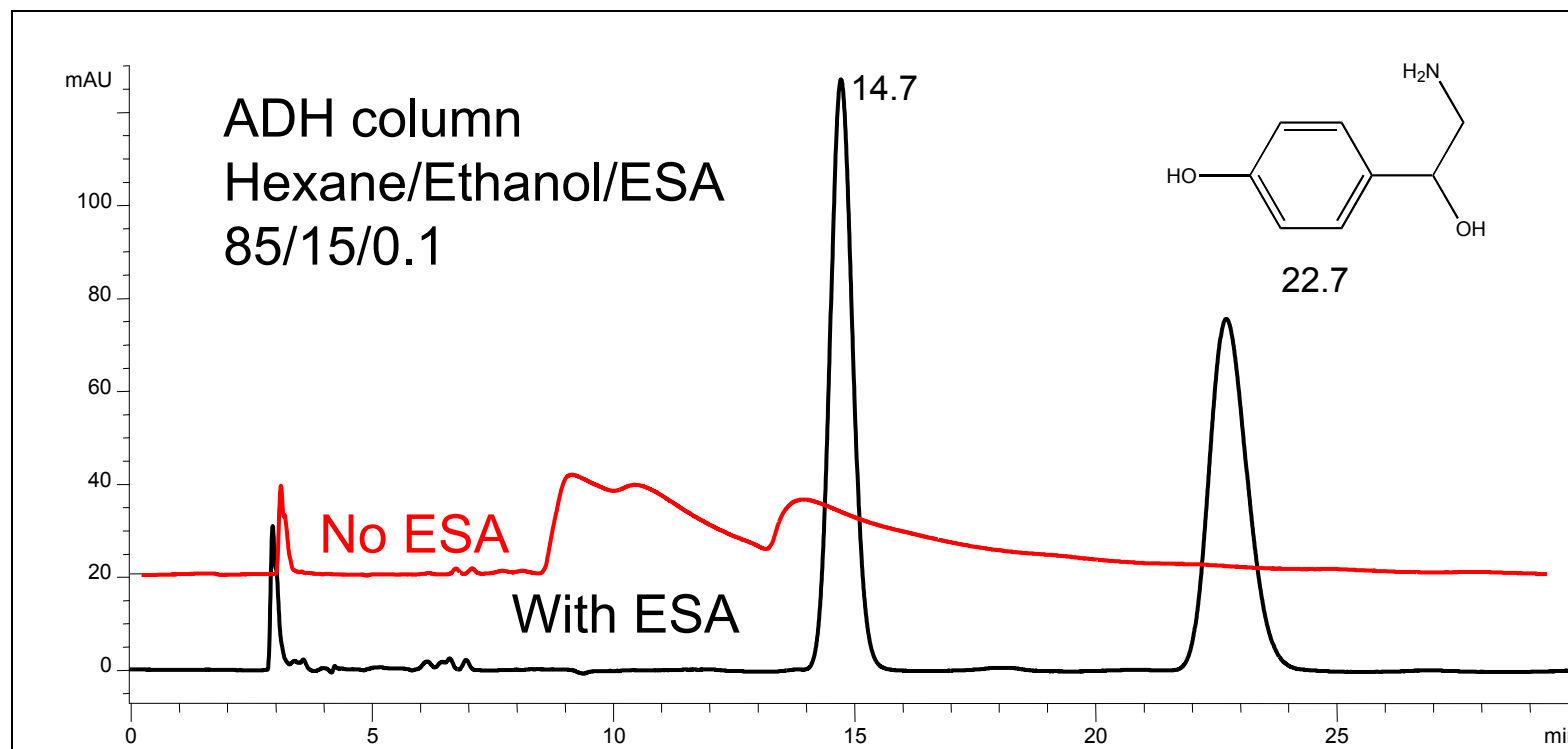
- Screening experiments performed on a variety of basic analytes (~50)
- Conditions: AD-H column, 85/15/0.1 hexane/ethanol /ESA, sample in m.p.
- Initial success rate ~ 55%
- Success across range of sample types



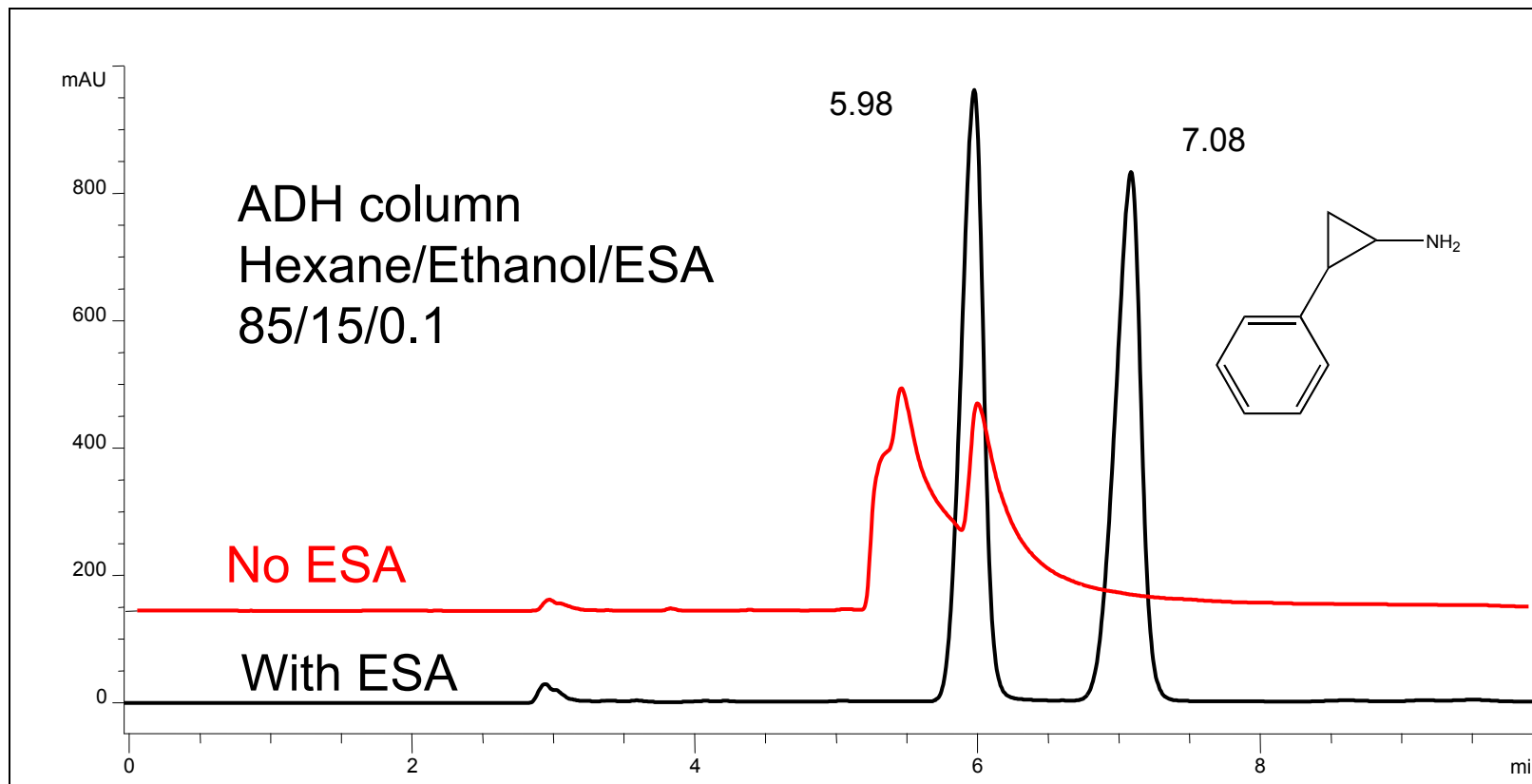
# *HPLC with ESA: Alprenolol ( $\beta$ -blocker)*



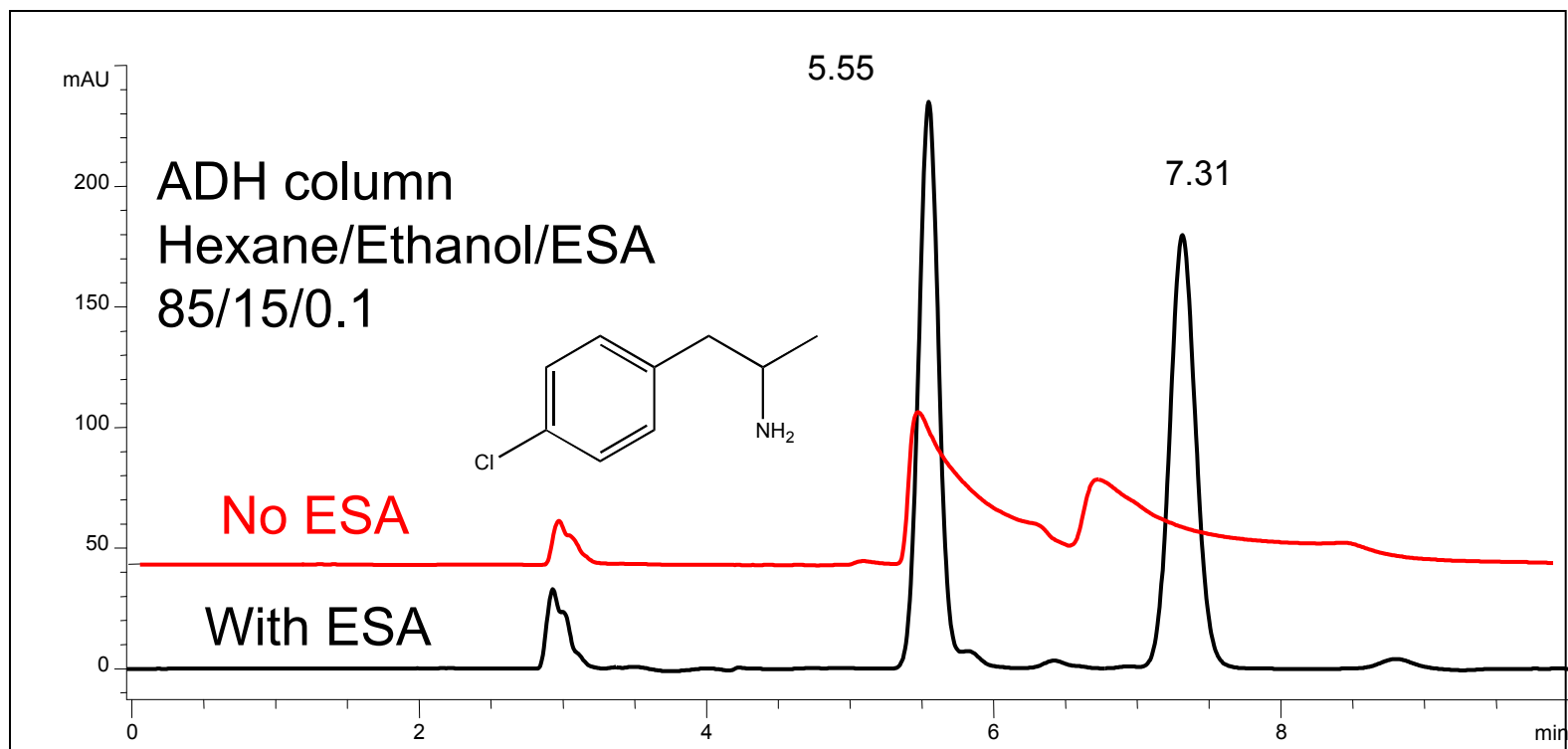
# *HPLC with ESA: Octopamine (1° amine)*



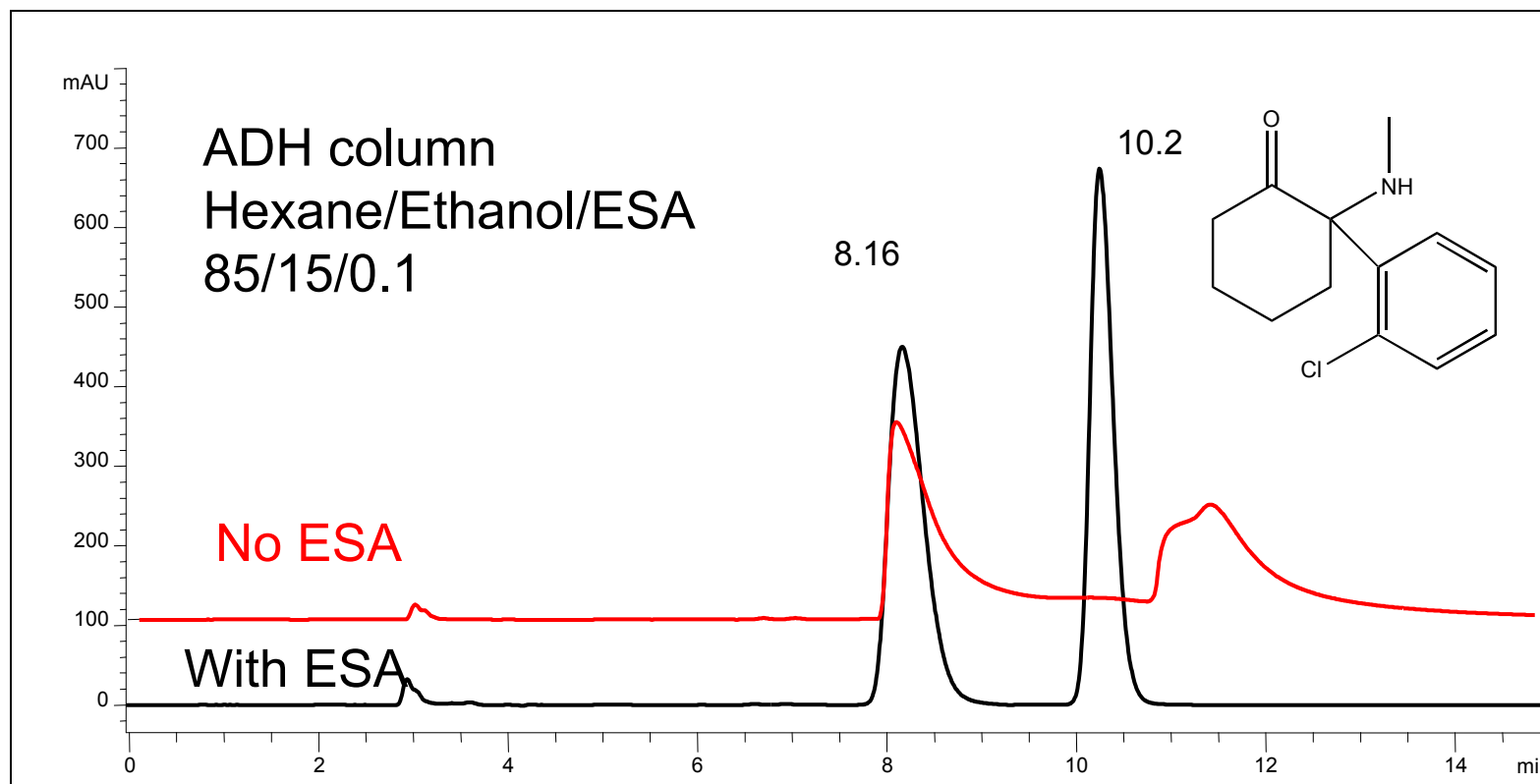
# HPLC with ESA: Tranylcypromine (1° amine)



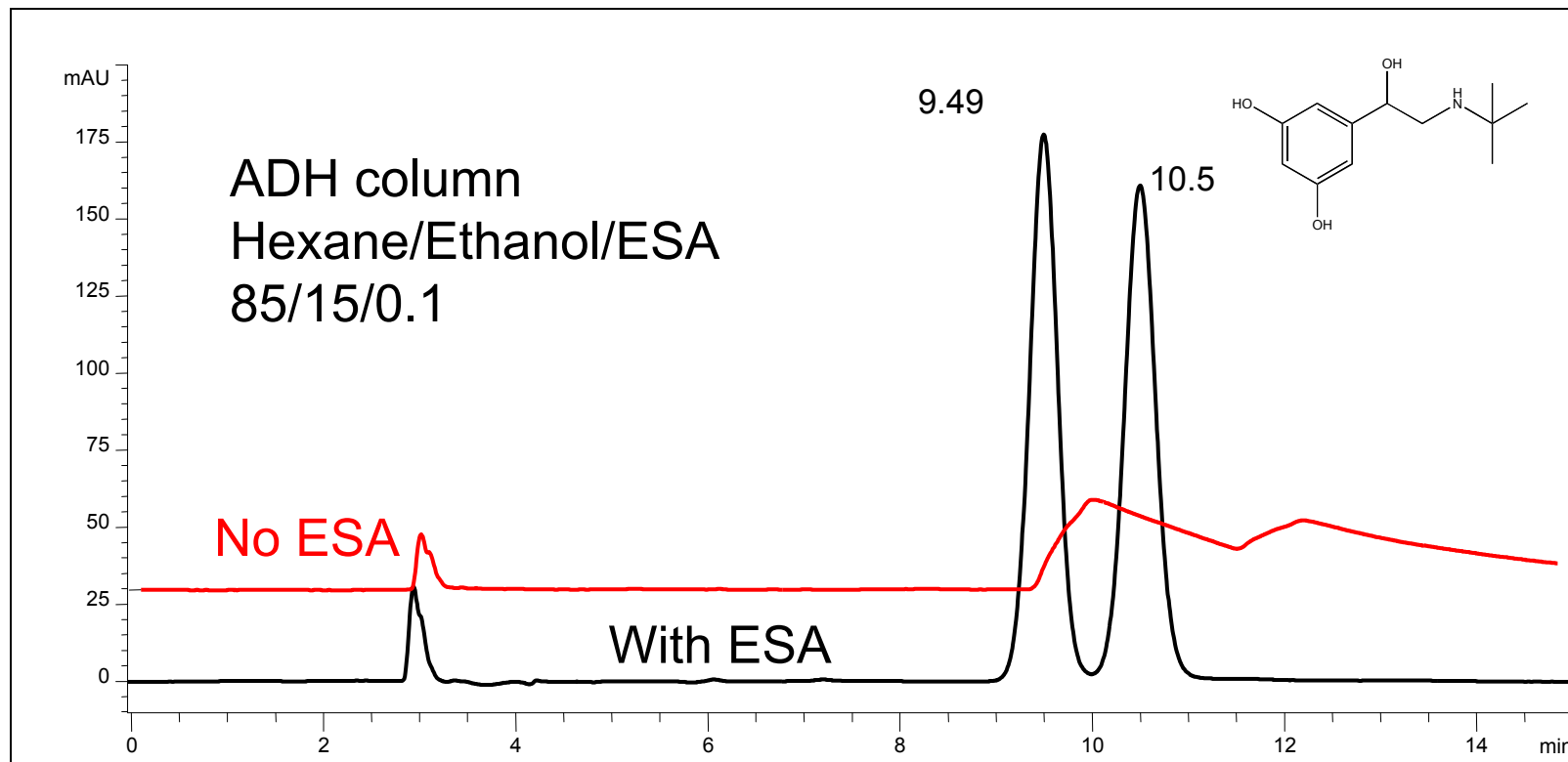
# HPLC with ESA: Chloramphenicol (1° amine)



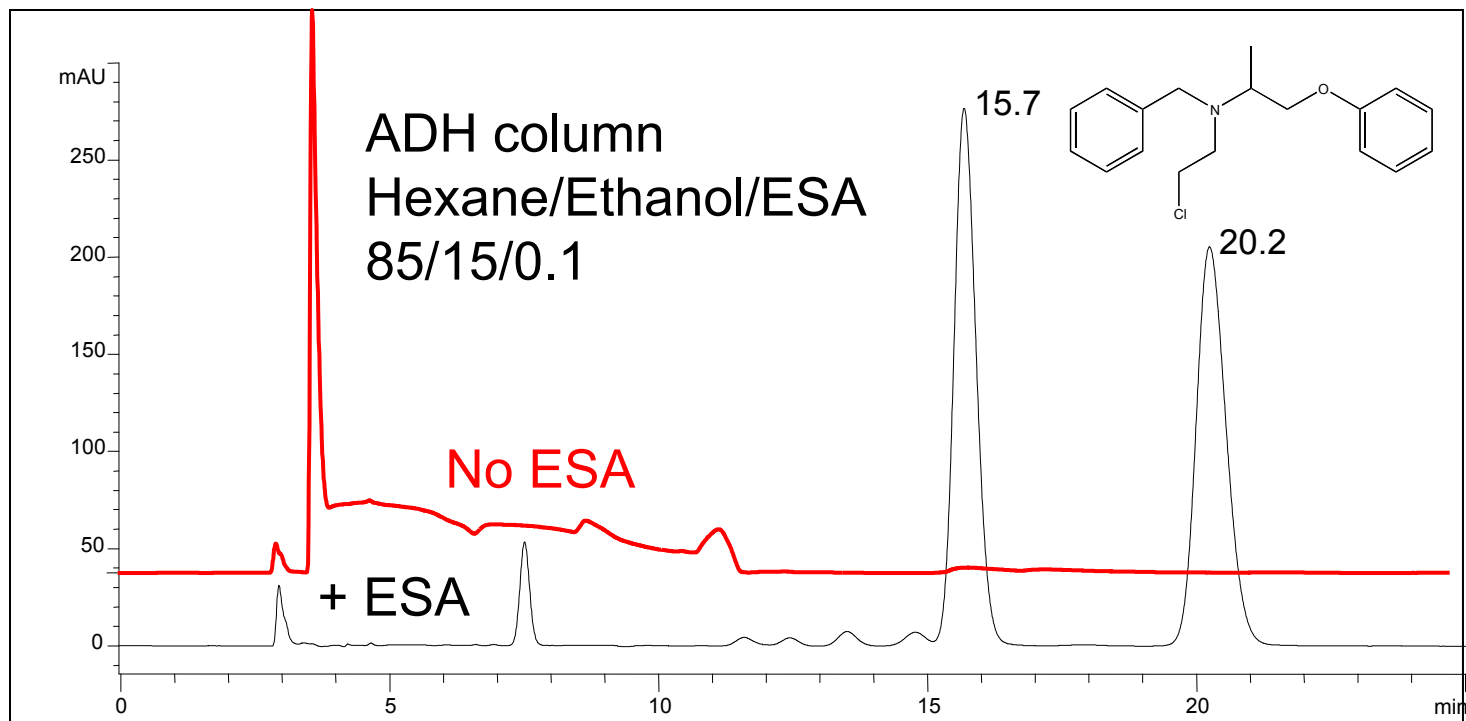
# HPLC with ESA: Ketamine (2° amine)



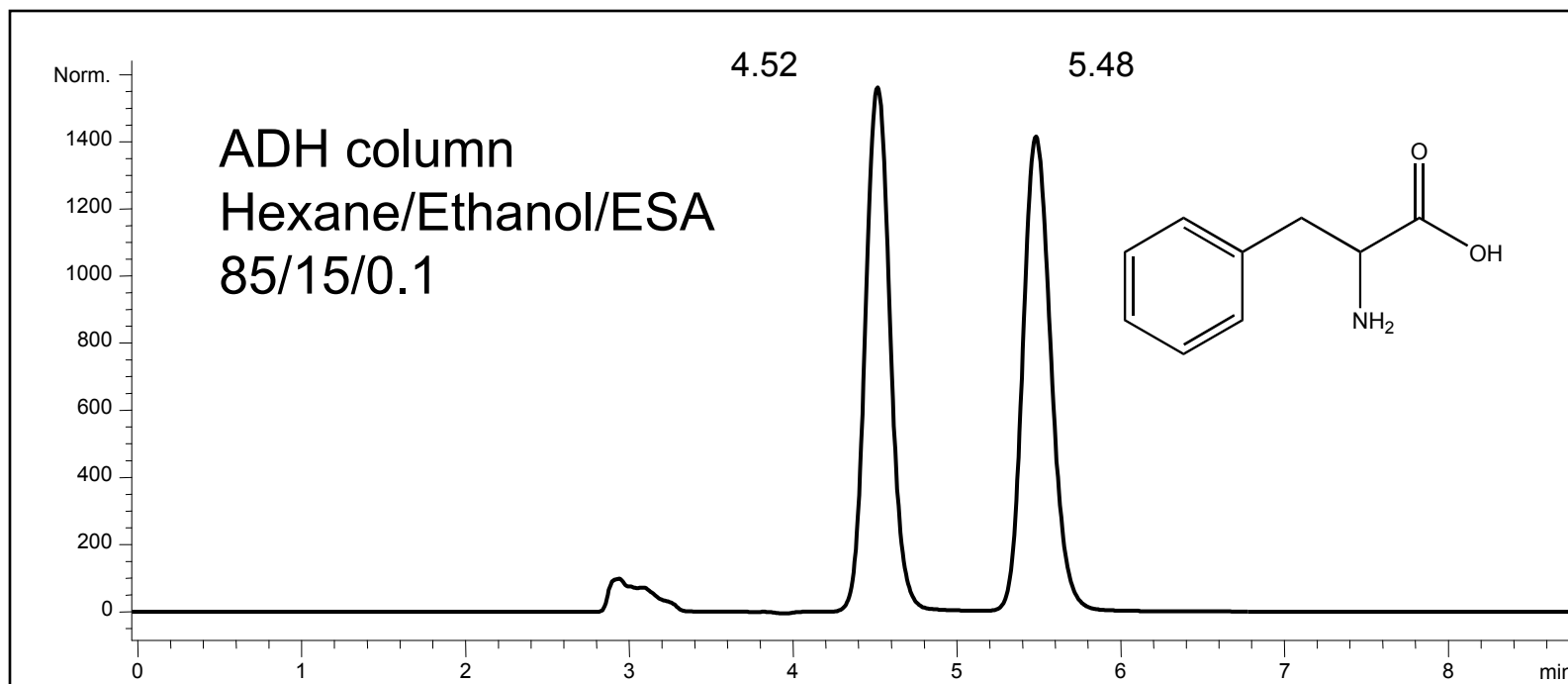
# *HPLC with ESA: Terbutaline* *(2° amine)*



# HPLC with ESA: Phenoxybenzamine (3<sup>o</sup> amine)



# *HPLC with ESA: Phenylalanine (amino acid)*

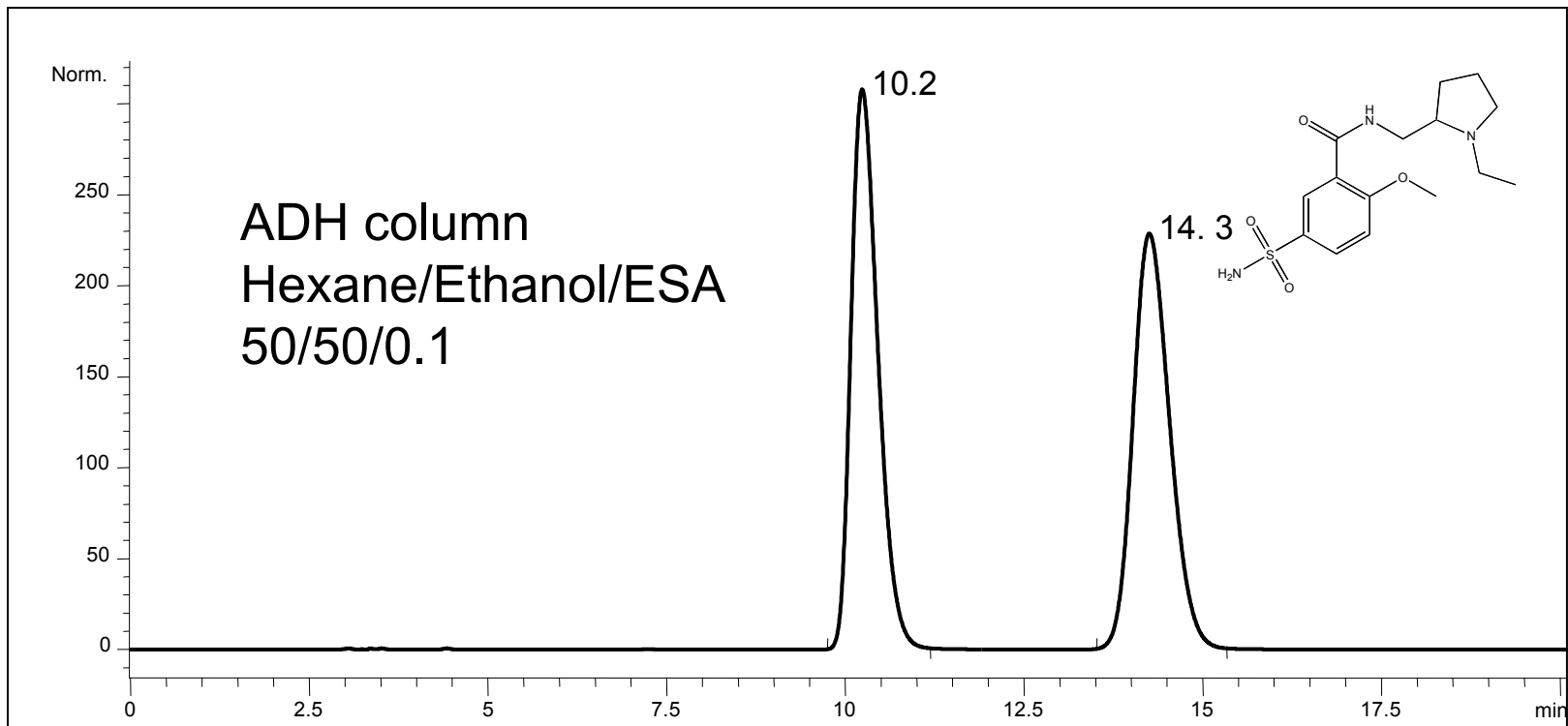


# *HPLC with ESA: Ethanol Level*

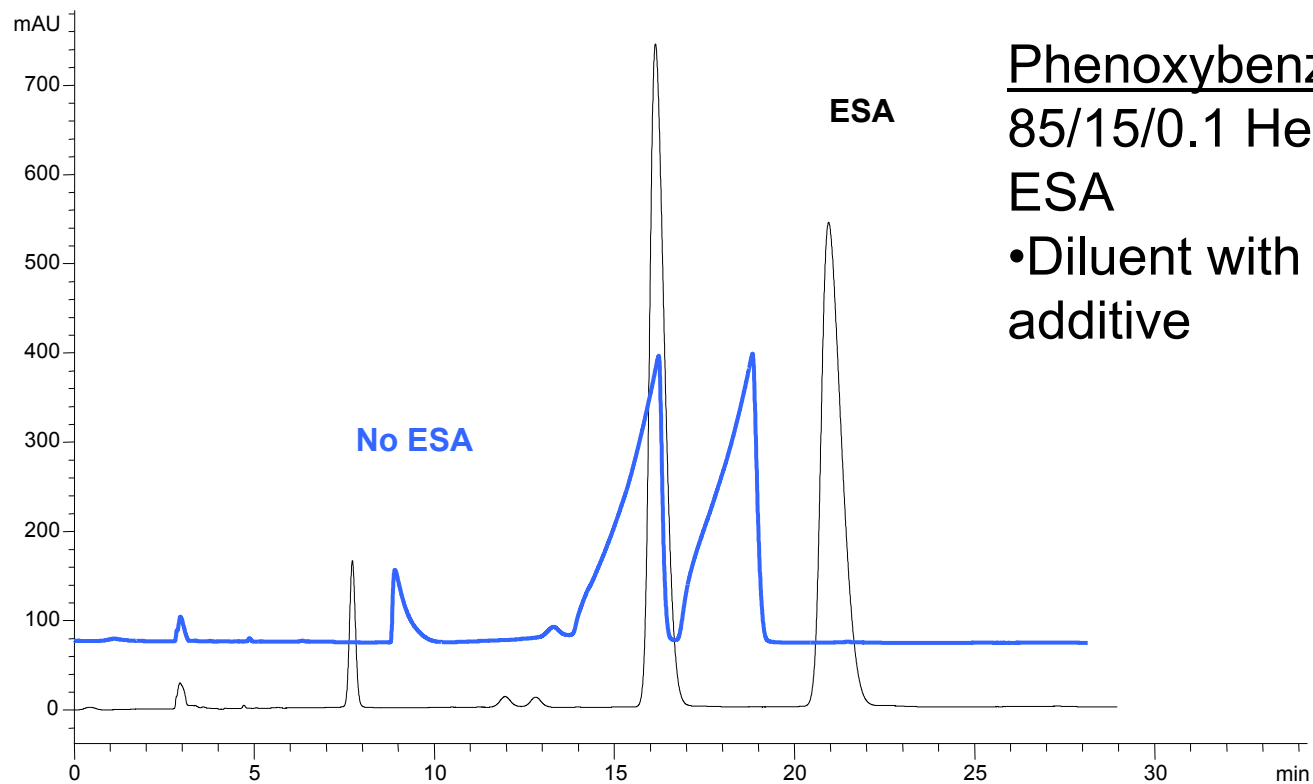
- Several observations of no elution or overlong retention
- Increased ethanol content decreased retention as expected giving some separations not seen before
- Selectivity held constant for about half of compounds (decreased for other half)
- Neat ethanol with ESA → no retention



# *HPLC with ESA: Sulpiride (3<sup>o</sup> amine)*



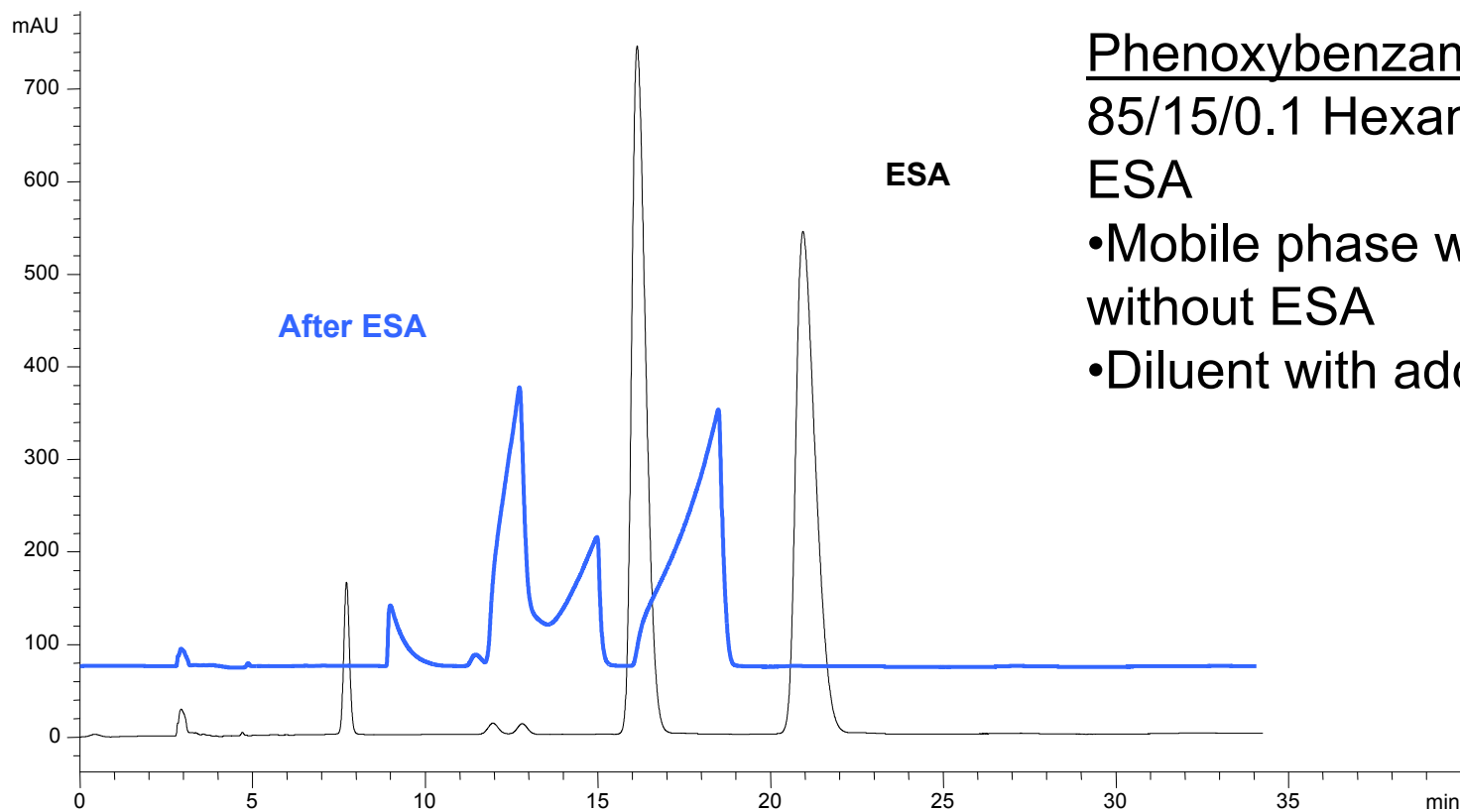
# *Effect of Additive in Diluent*



Phenoxybenzamine  
85/15/0.1 Hexane/Ethanol/  
ESA  
•Diluent with or without  
additive



# *Effect of Additive in Mobile Phase*



Phenoxybenzamine  
85/15/0.1 Hexane/Ethanol/  
ESA

- Mobile phase with or without ESA
- Diluent with additive



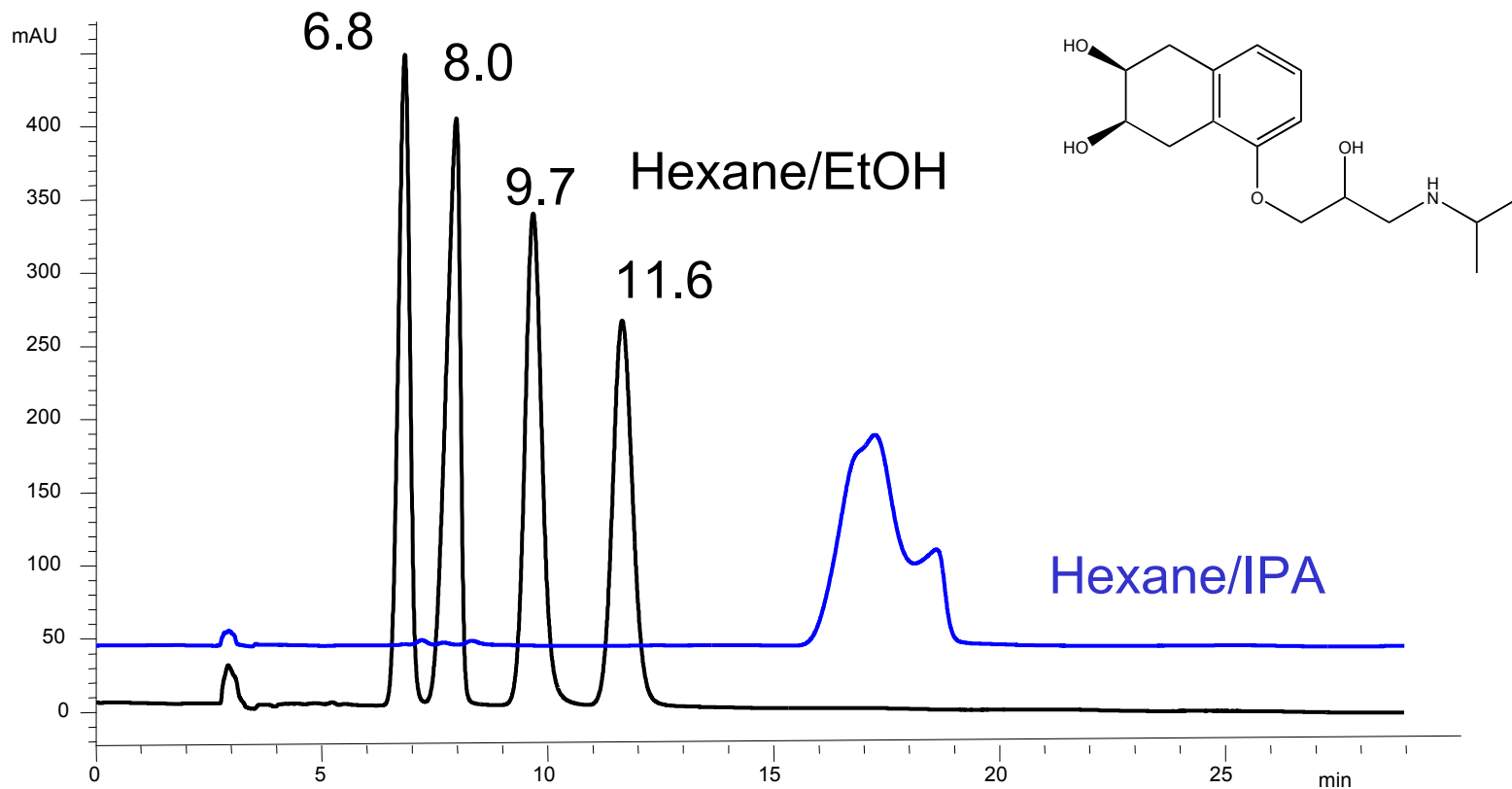
# *HPLC with ESA: Effect of Modifier*

- With polysaccharide CSPs changing ethanol to isopropanol may have dramatic effects
- 26 compounds screened with 80/20/0.1 hexane/isopropanol/ESA
- IPA best for ~ half (EtOH best for ~ half)
- Recommend screening with both



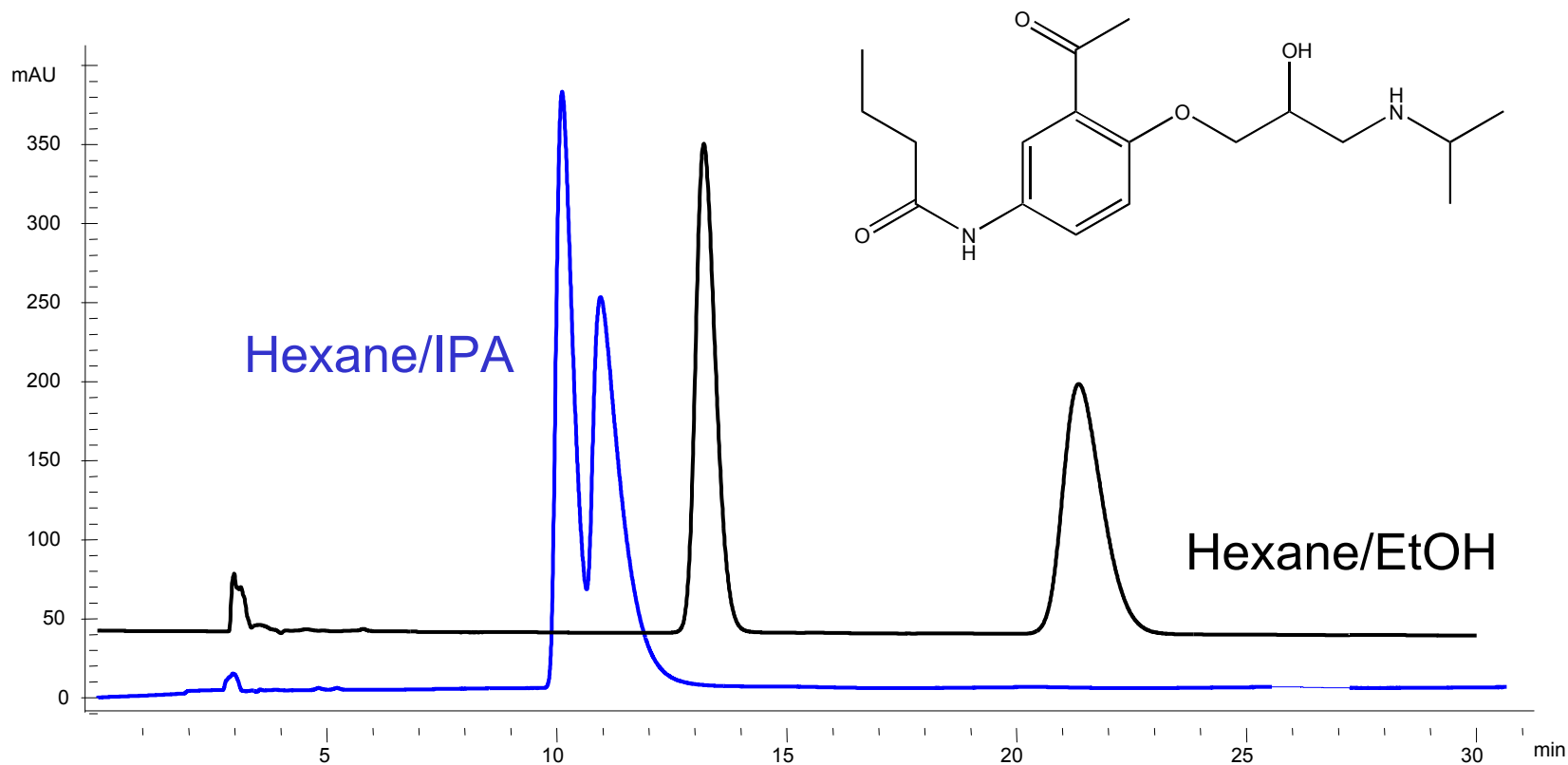
# *HPLC with ESA: Effect of Modifier*

## *Nadolol*



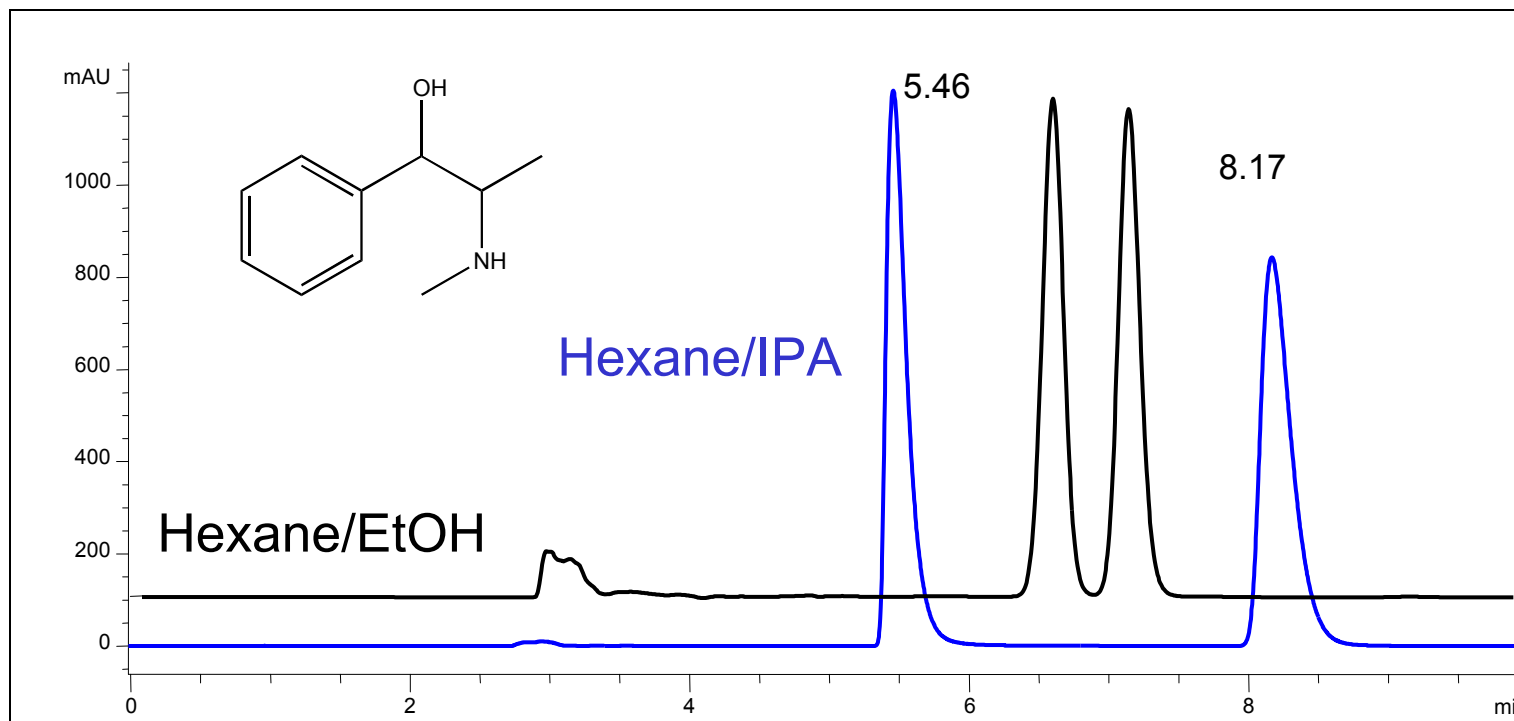
# *HPLC with ESA: Effect of Modifier*

## *Acebutolol*



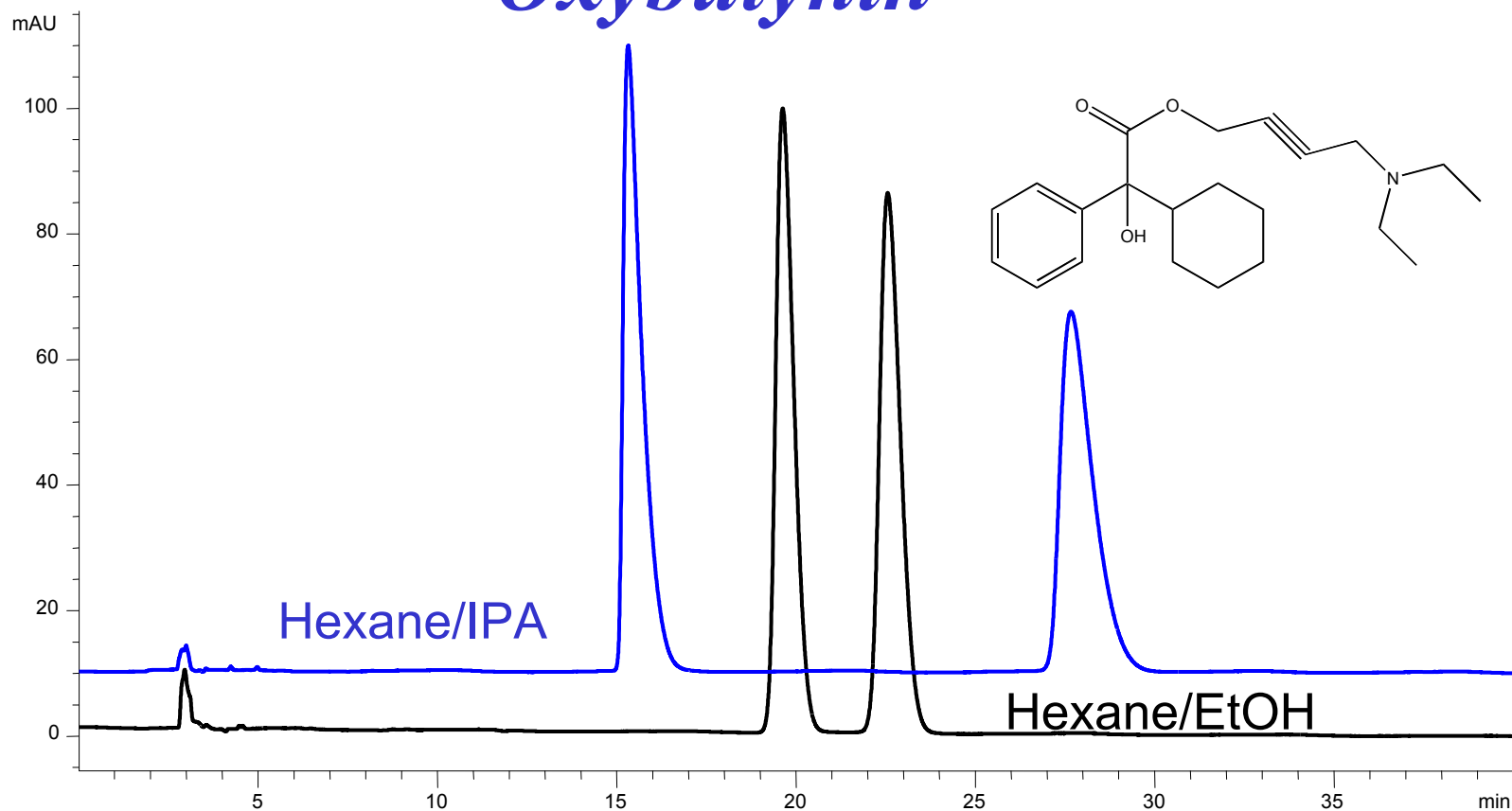
# *HPLC with ESA: Effect of Modifier*

## *Ephedrine*



# *HPLC with ESA: Effect of Modifier*

## *Oxybutynin*



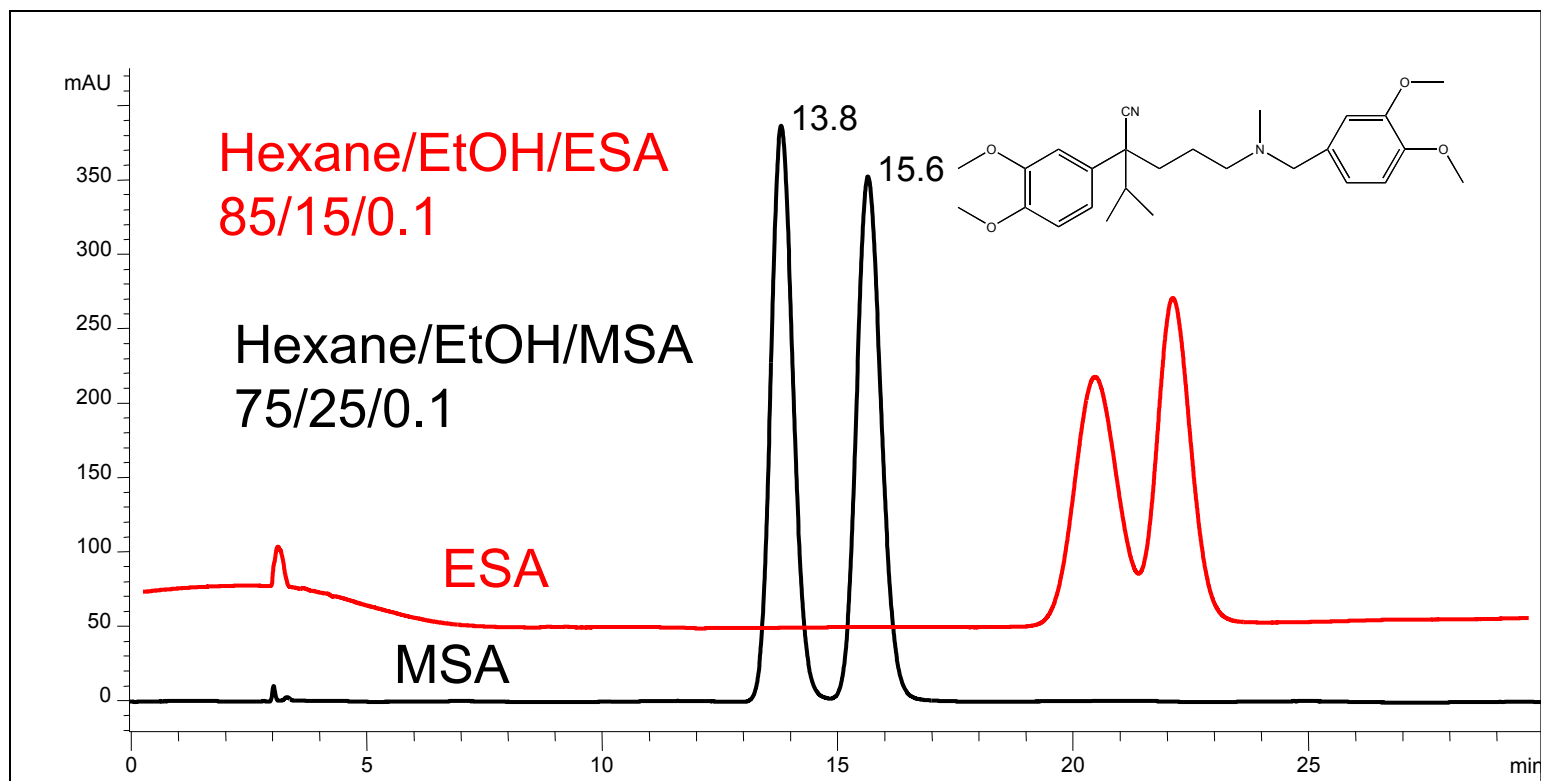
## *HPLC with ESA: Effect of Acid*

- In SFC, methanesulfonic acid (MSA) was occasionally beneficial in lieu of ESA
- 27 compounds screened with 85/15/0.1 hexane/ethanol/MSA
- Retention was higher for MSA ( $pK_a$  effect?)
- MSA often better than ESA, never worse
- Recommend screening with MSA

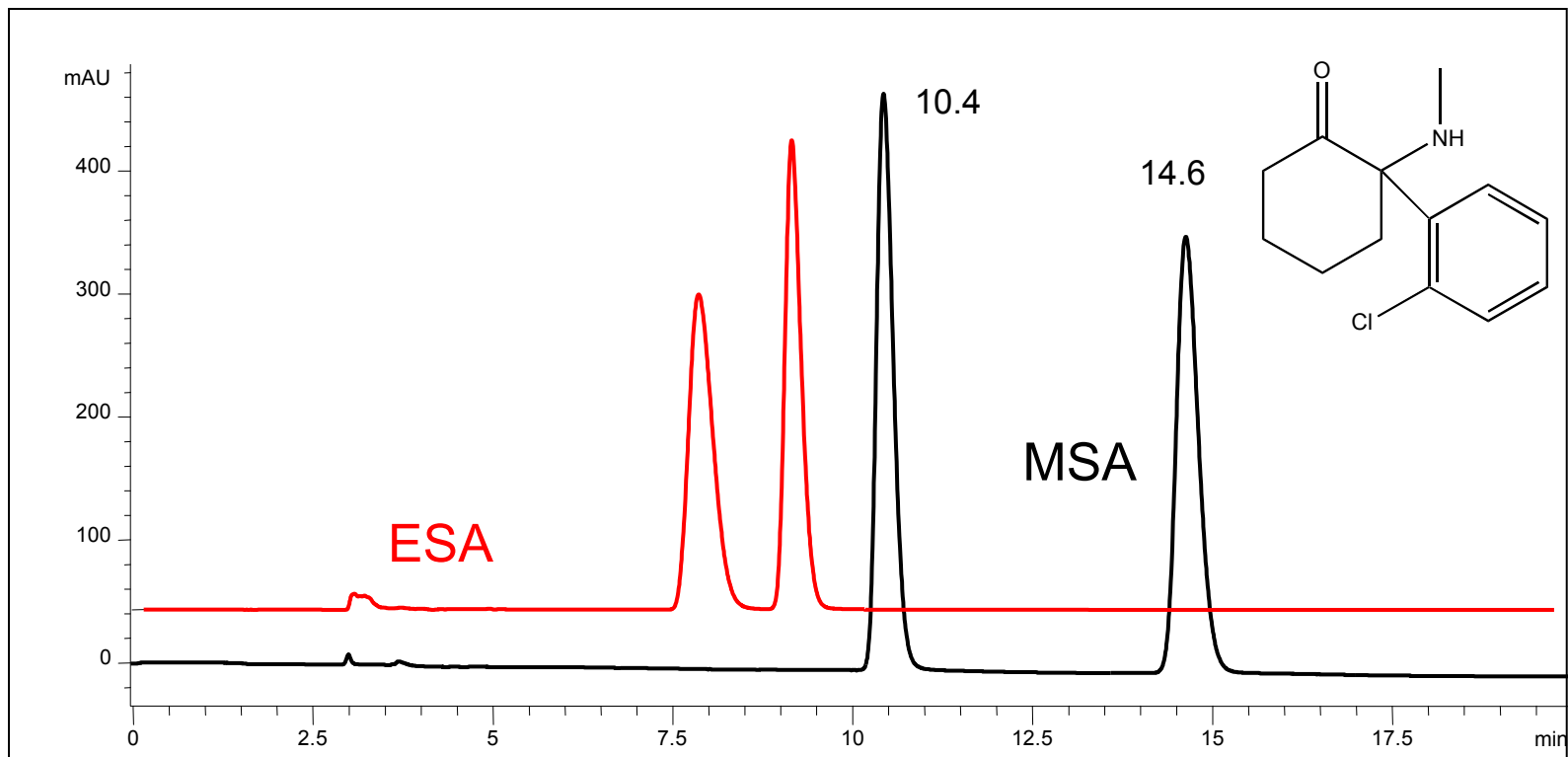


# *HPLC with ESA: Effect of Acid*

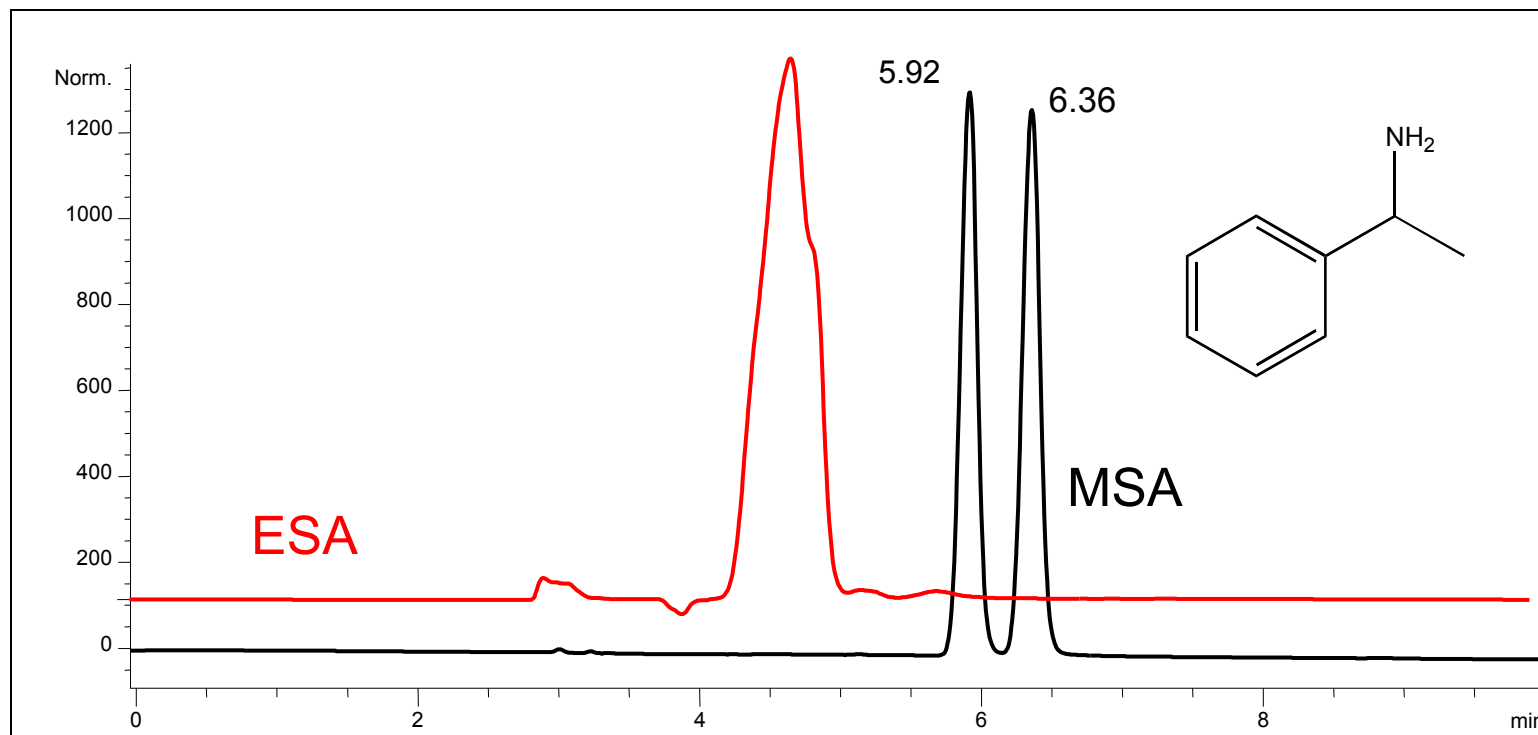
## *Verapamil*



# *HPLC with ESA: Effect of Acid Ketamine*



# *HPLC with ESA: Effect of Acid* *$\alpha$ -methylbenzylamine*



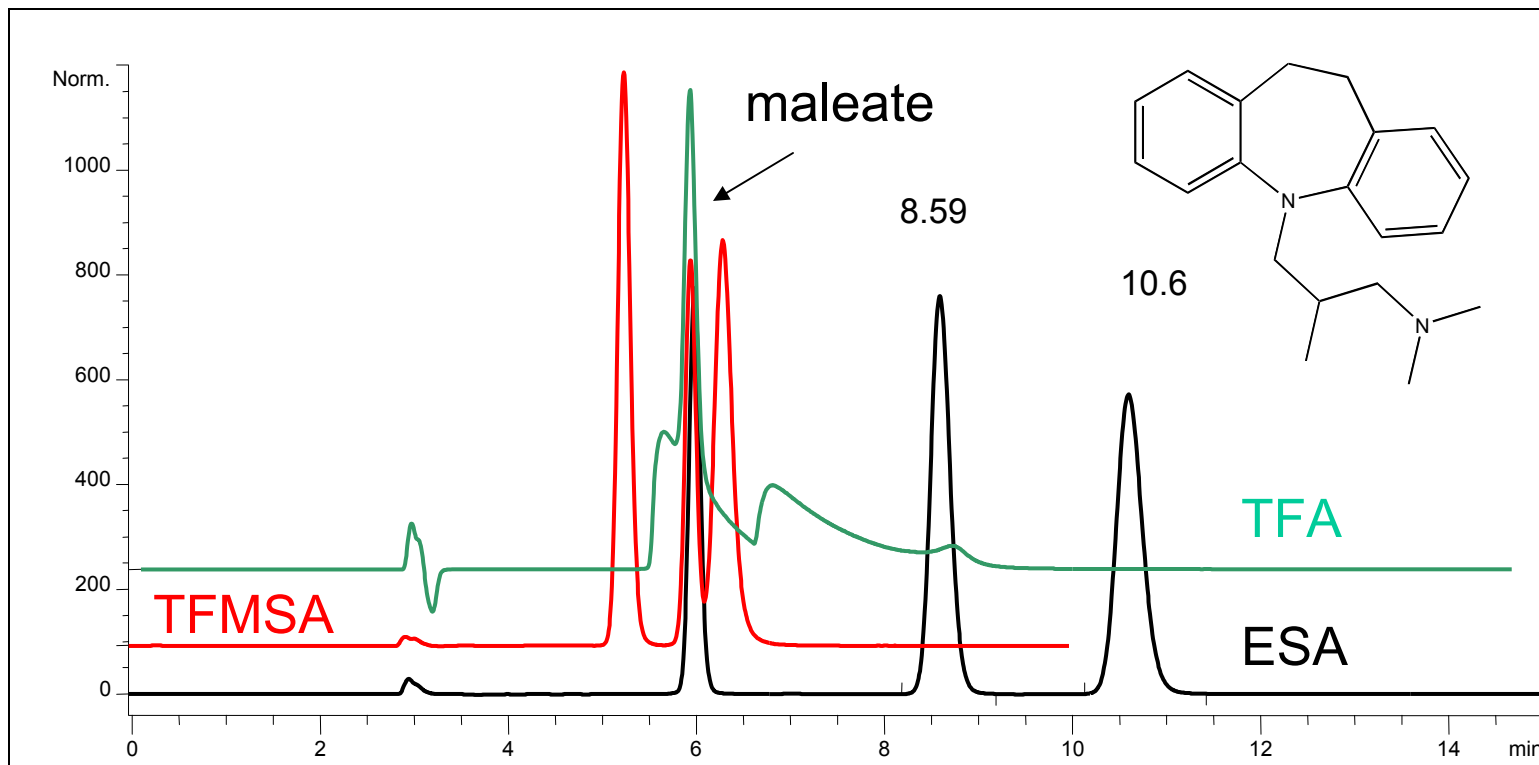
## *HPLC with ESA: Other Acids*

- Trifluoroacetic acid (weaker) gave broad, distorted peaks (unstable ion-pair?)
- Trifluoromethanesulfonic acid (stronger) gave shorter retention (?) often collapsing separation
- Neutral compounds unaffected by acid type



# *HPLC with ESA: Effect of Acid*

## *Trimipramine*



## *HPLC with ESA: Mechanism*

- Mechanism for most basic compounds differs from amino acid esters (localized pH effect)
- Dilution with ESA required → eluting as an ion-pair or salt
- Lack of memory effect supports this concept
- Retention increase with MSA suggests some local pH effect
- Shorter retention with TFMSA suggests that a salt can be too stable to enjoy local effect



## *HPLC with ESA: Conclusions*

- High success rate with a wide variety of basic analytes
- Dilution with ESA required
- Ethanol and isopropanol complementary → screen with both
- MSA better than ESA → screen with MSA
- Acid additives appear complementary to basic additives for basic analytes

**Safety concerns for preparative application!**

