

*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

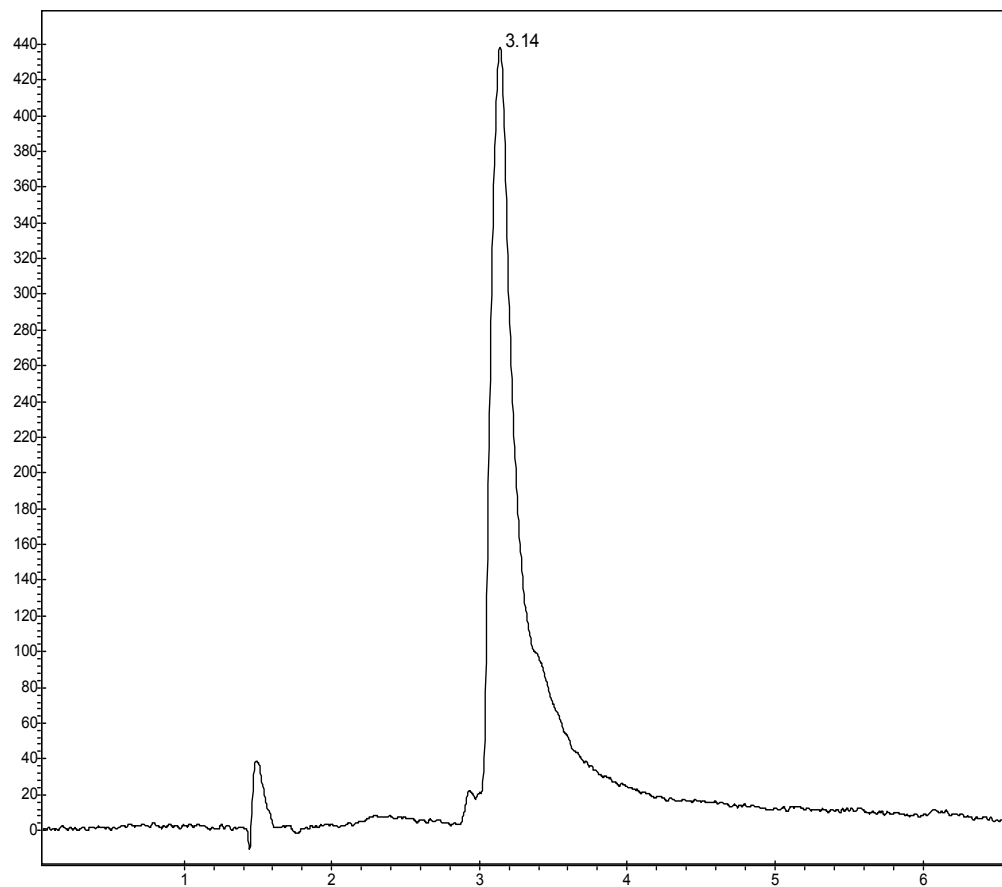


Acidic Additives in Chiral SFC

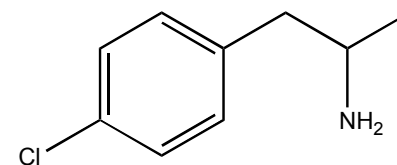
- Acids not required to elute acid analytes
- ESA effect observed for amino acid esters
- ESA allowed separation of a wide variety of basic analytes not previously separated in SFC
- Dilution with ESA required → eluting as “salt”
- No memory effect



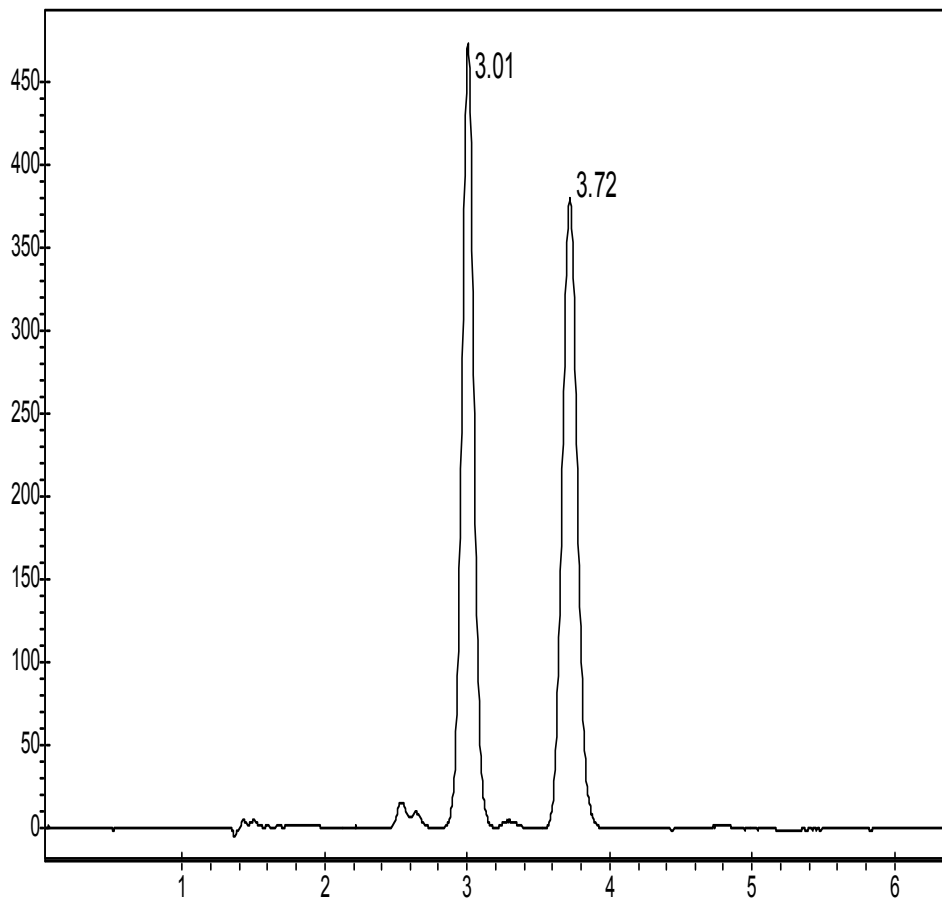
Chiral SFC of Chloramphenamine



Chloramphenamine
20% EtOH
HCl salt in EtOH
AD-H

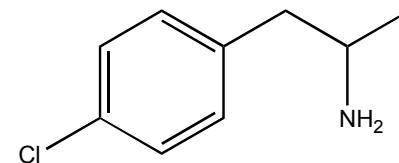


Chiral SFC of Chloramphetamine with ESA

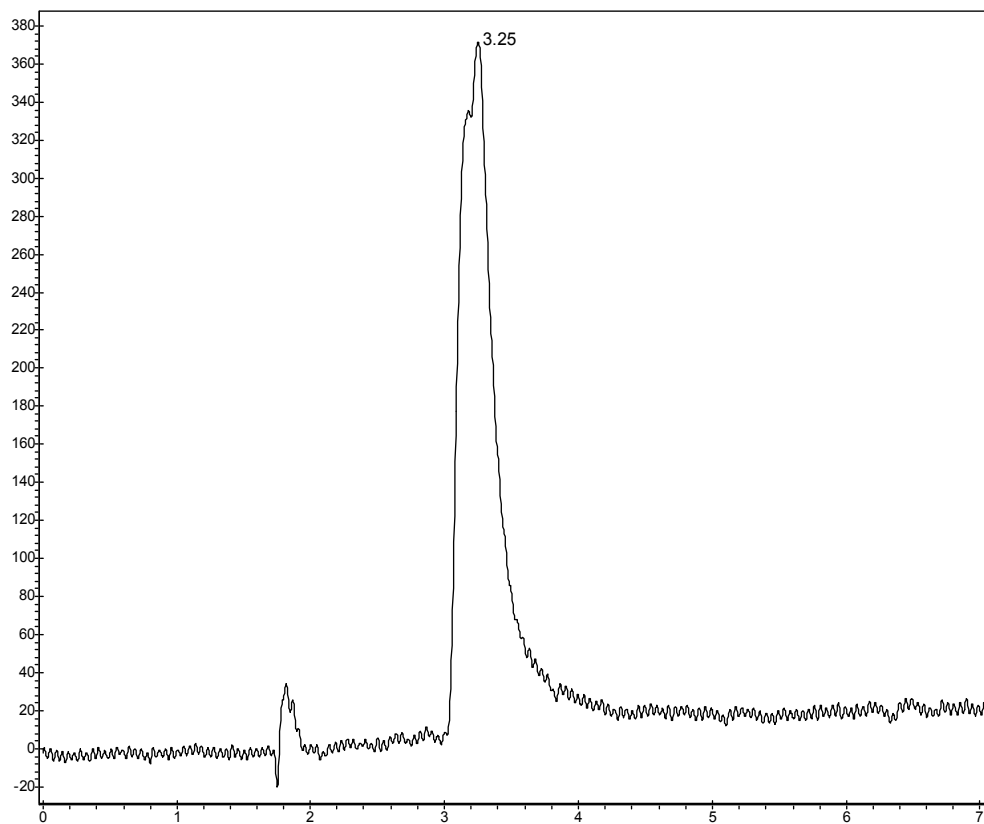


Chloramphetamine
20% EtOH (0.1%ESA)
HCl salt in EtOH/ESA
AD-H

$\alpha = 1.47$, $R_s = 3.99$



Chiral SFC of an amino acid ester

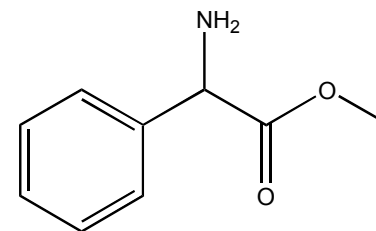


Phenylalanine-methyl ester

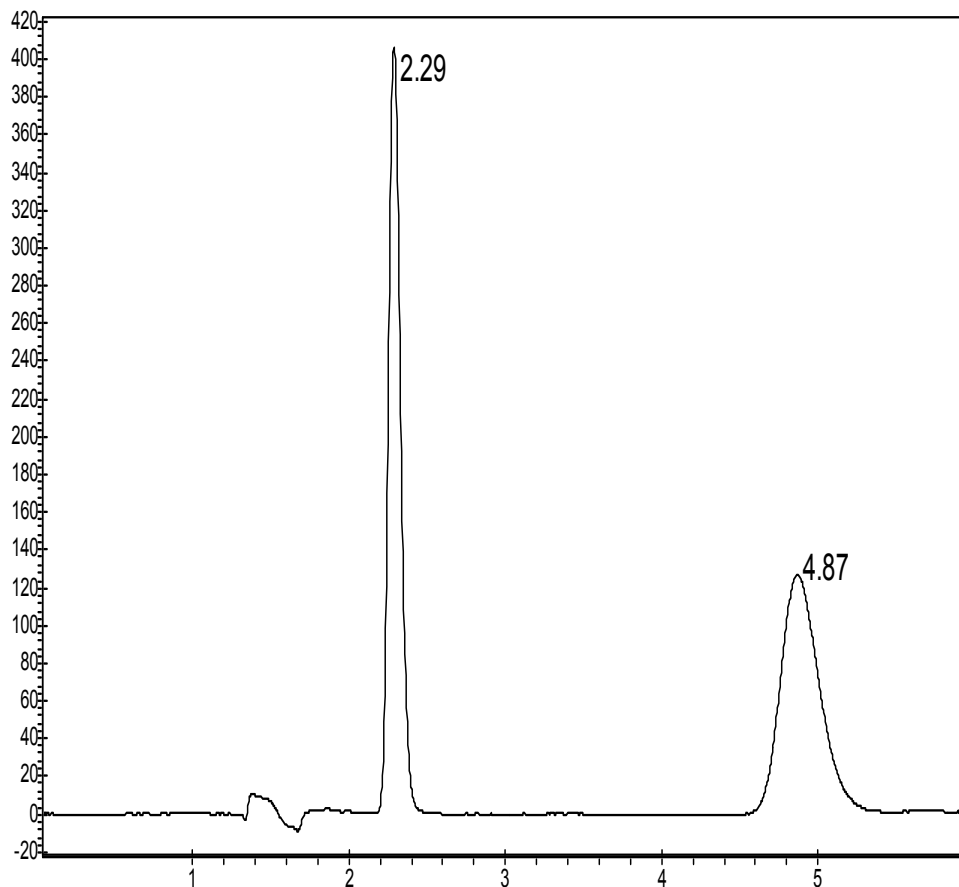
20% EtOH

HCl salt in EtOH

AD-H

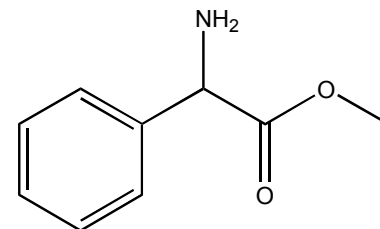


Chiral SFC of an amino acid ester with ESA



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

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

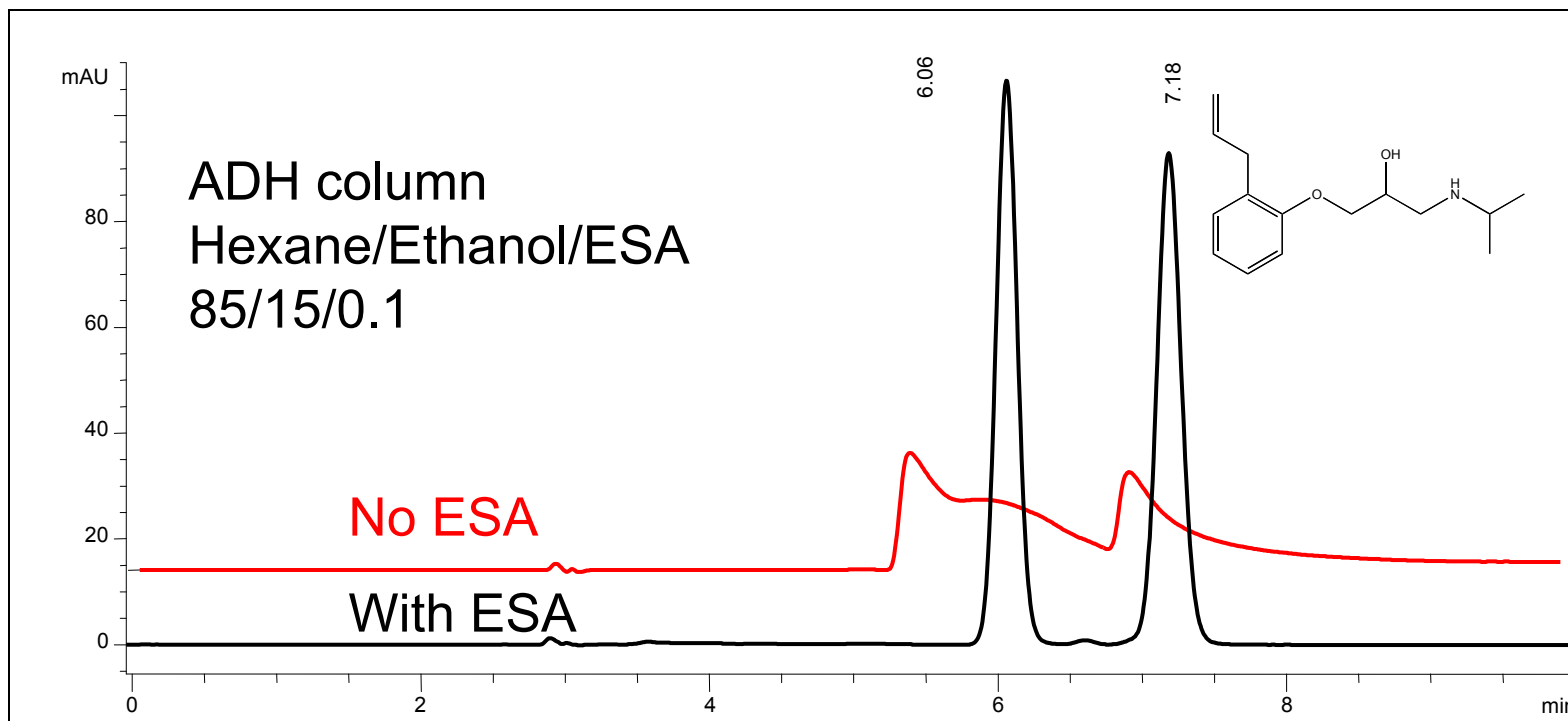


Evaluation of ESA for Chiral HPLC

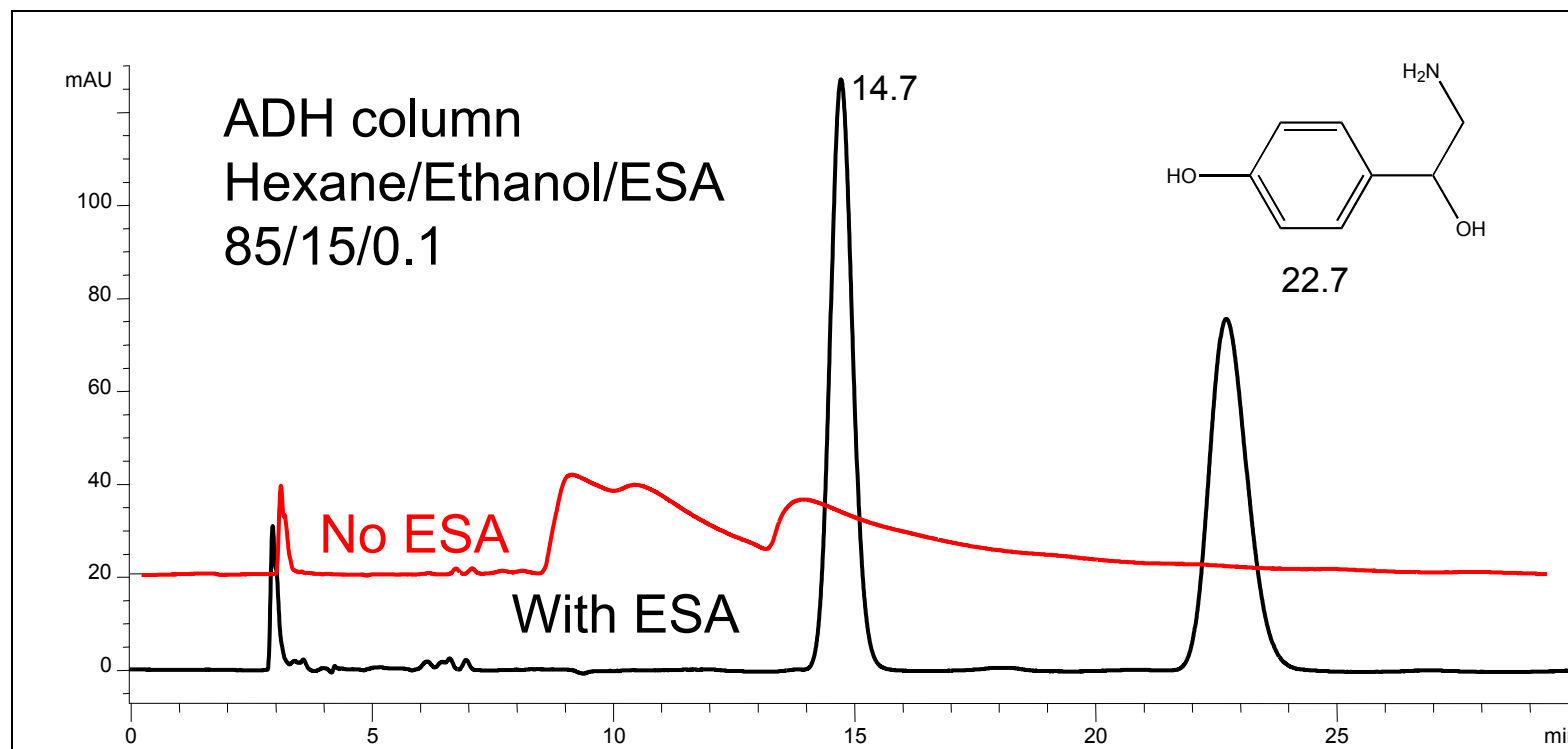
- Screening experiments performed on a variety of basic analytes (~50)
- Conditions: AD-H column, 85/15/0.1 hexane/ethanol /ESA, sample in m.p.
- Initial success rate ~ 55%
- Success across range of sample types



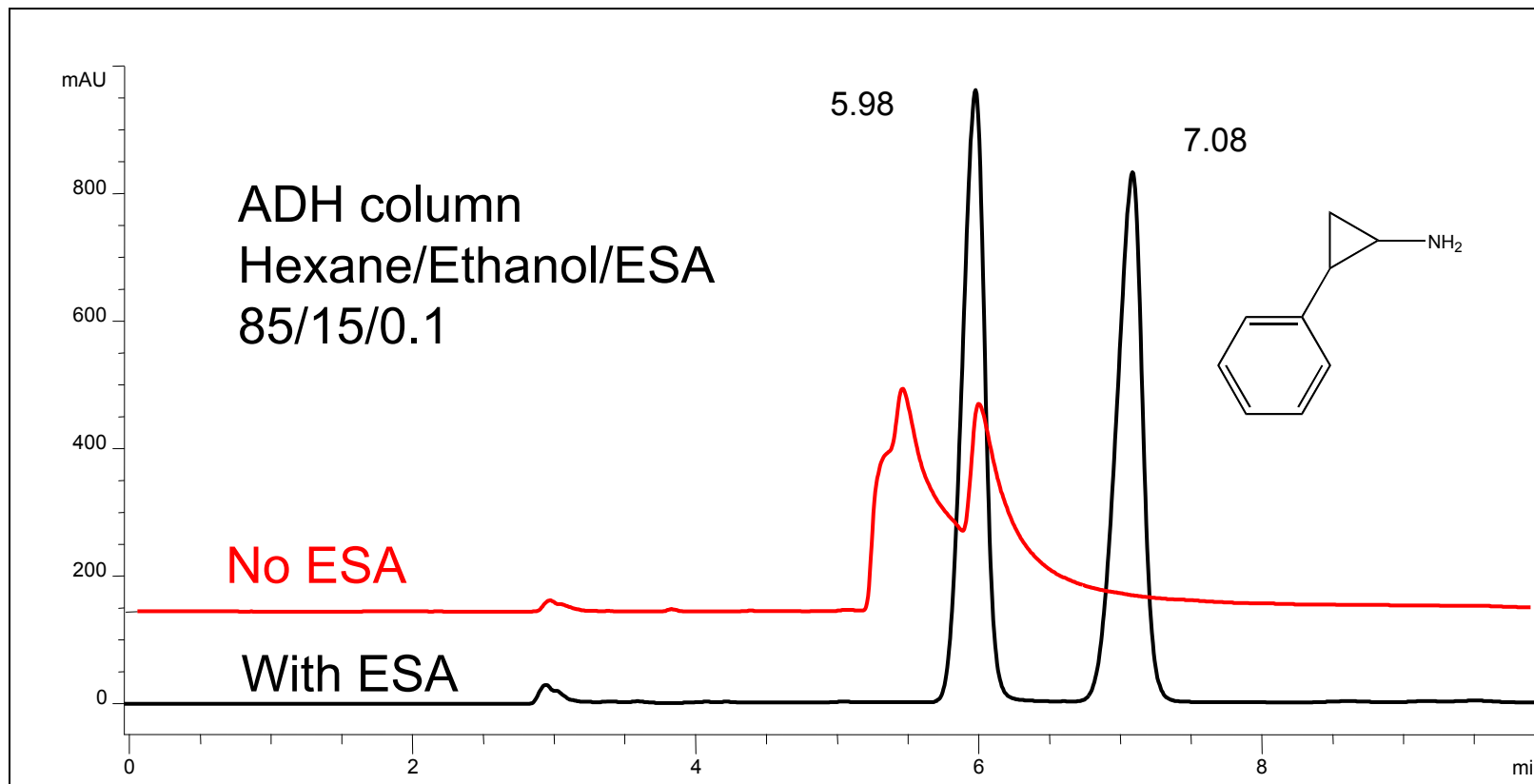
HPLC with ESA: Alprenolol (β -blocker)



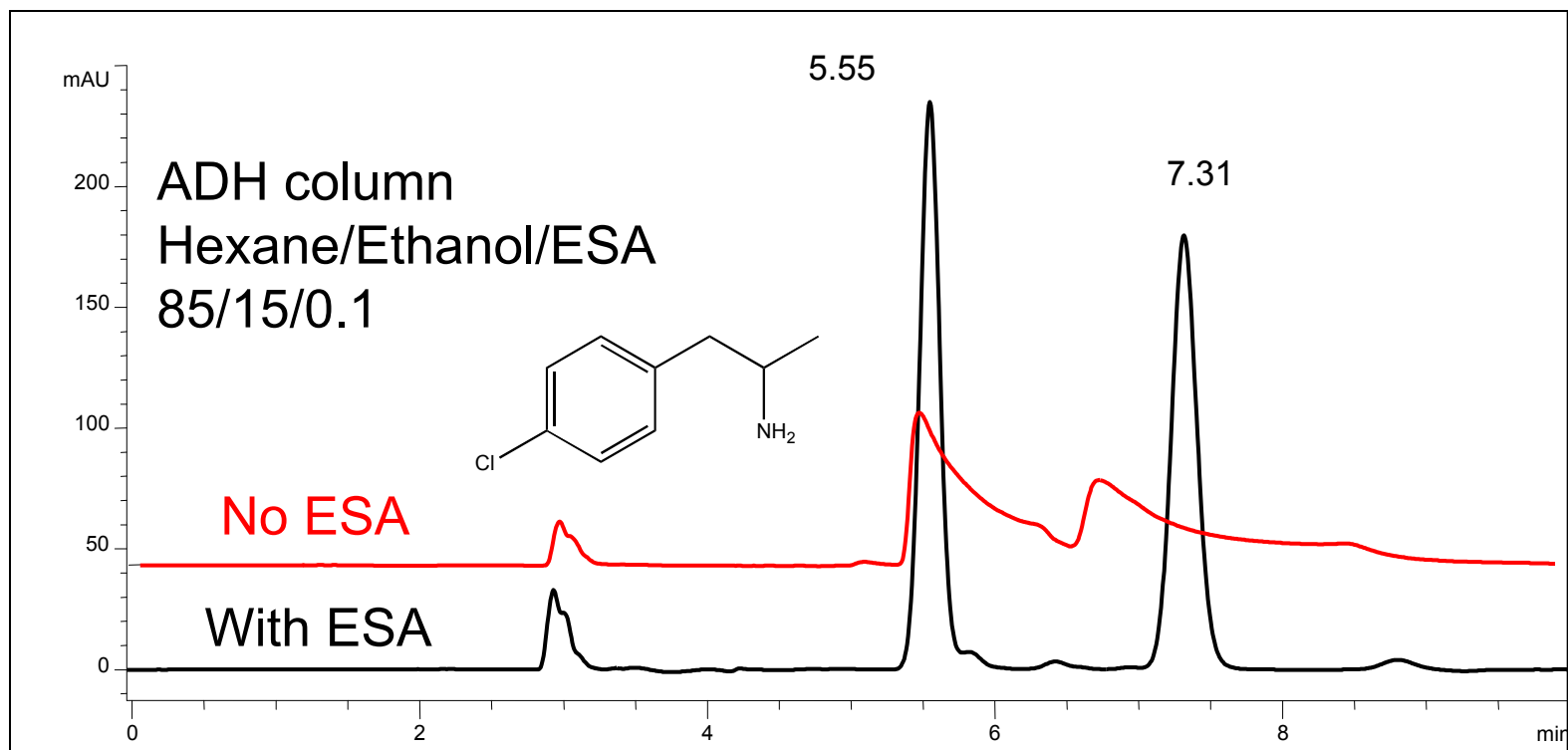
HPLC with ESA: Octopamine (1° amine)



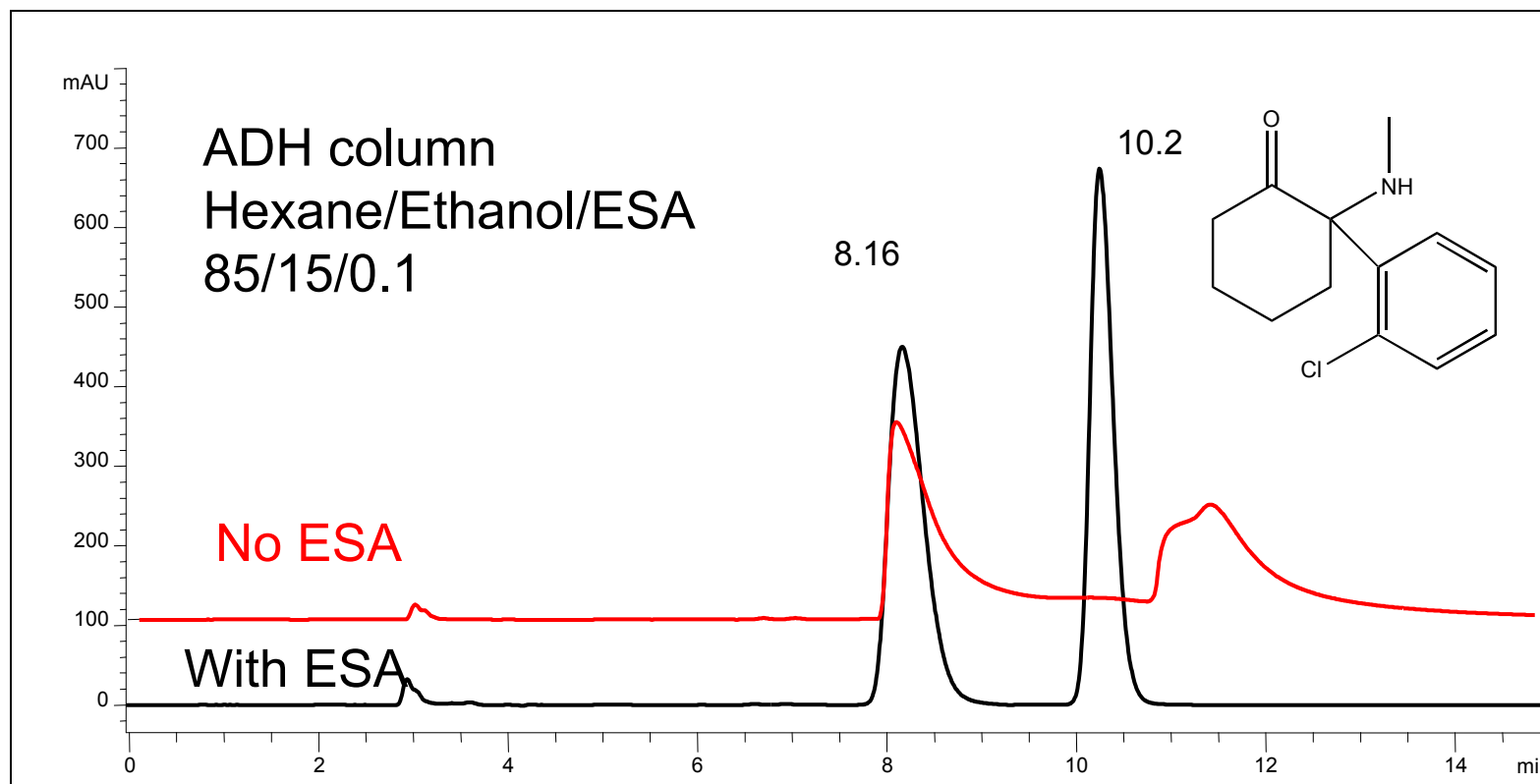
HPLC with ESA: Tranylcypromine (1° amine)



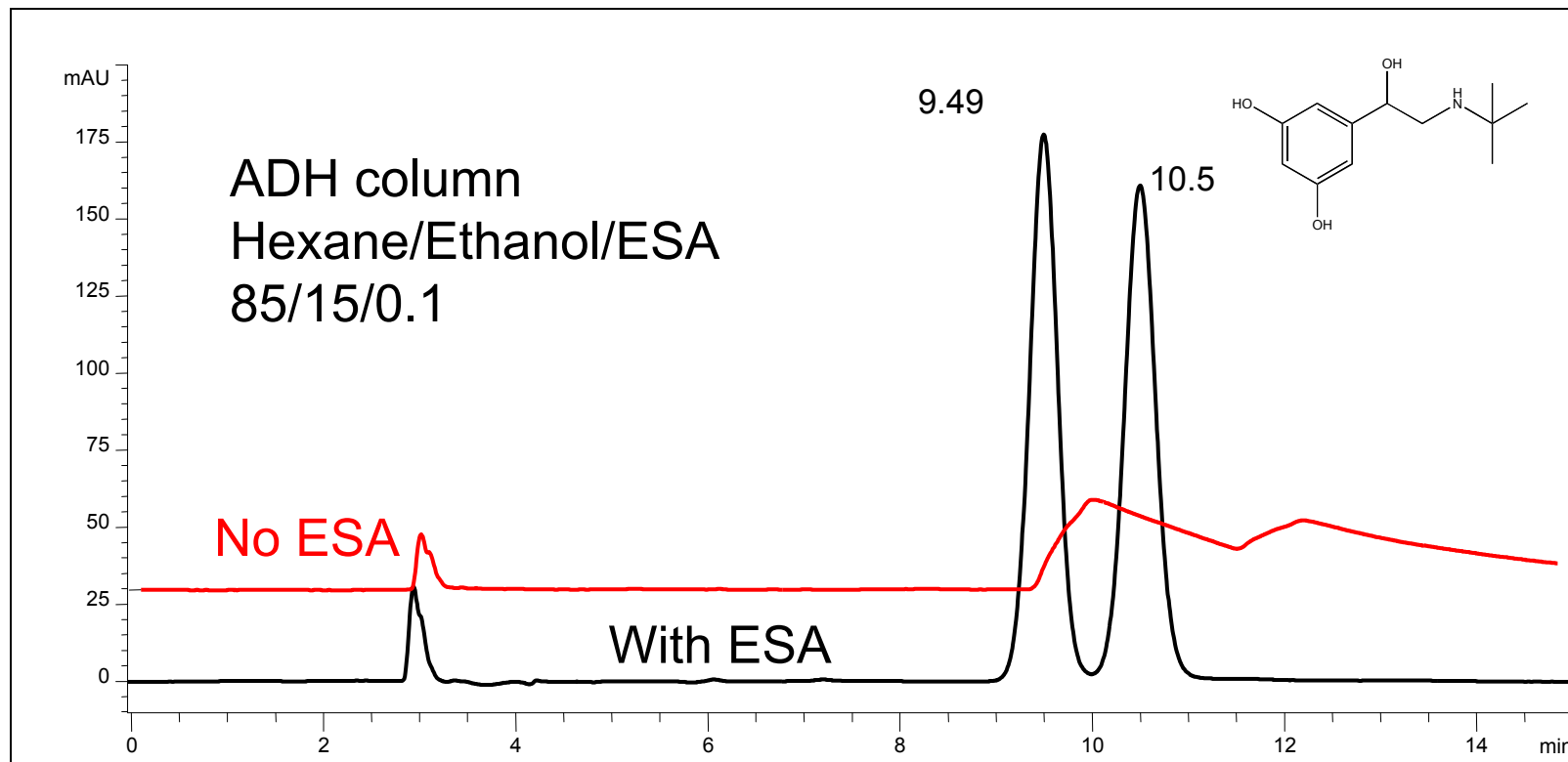
HPLC with ESA: Chloramphenicol (1° amine)



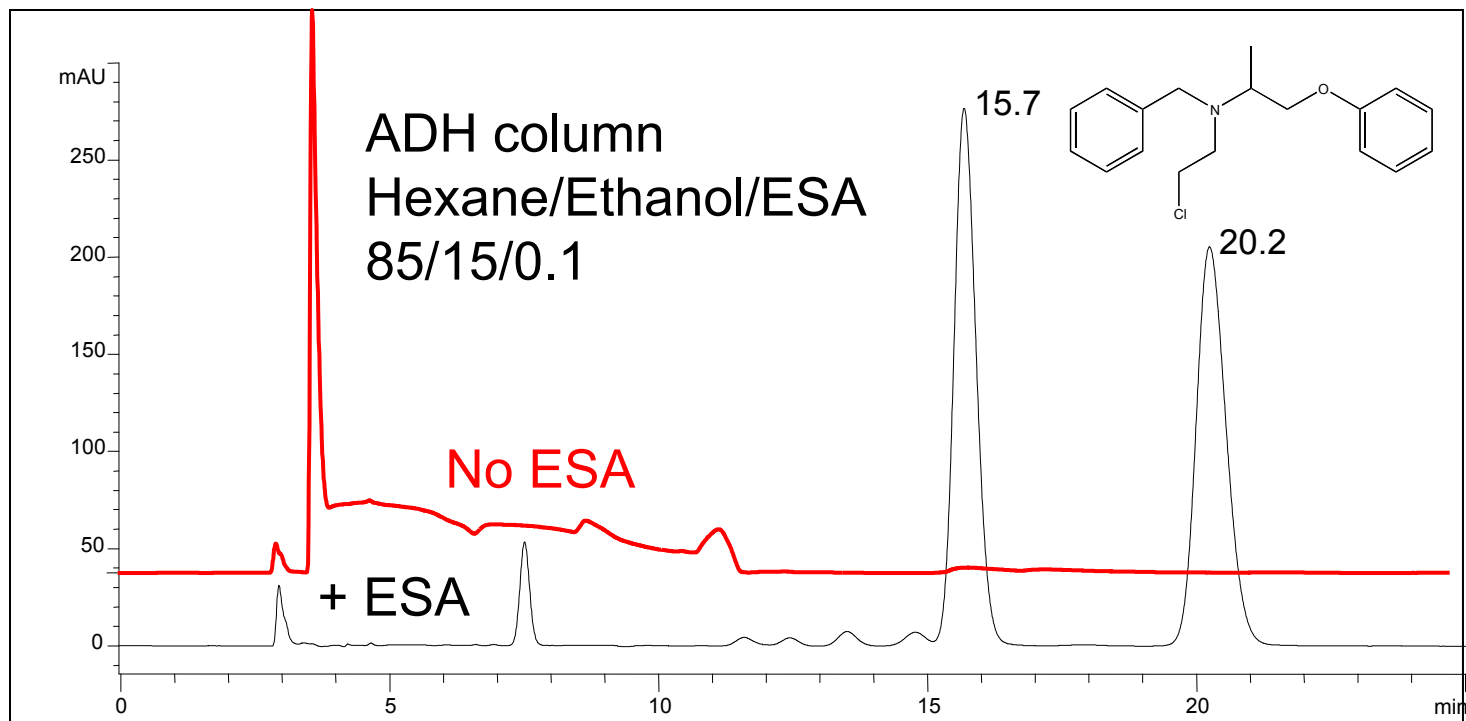
HPLC with ESA: Ketamine (2° amine)



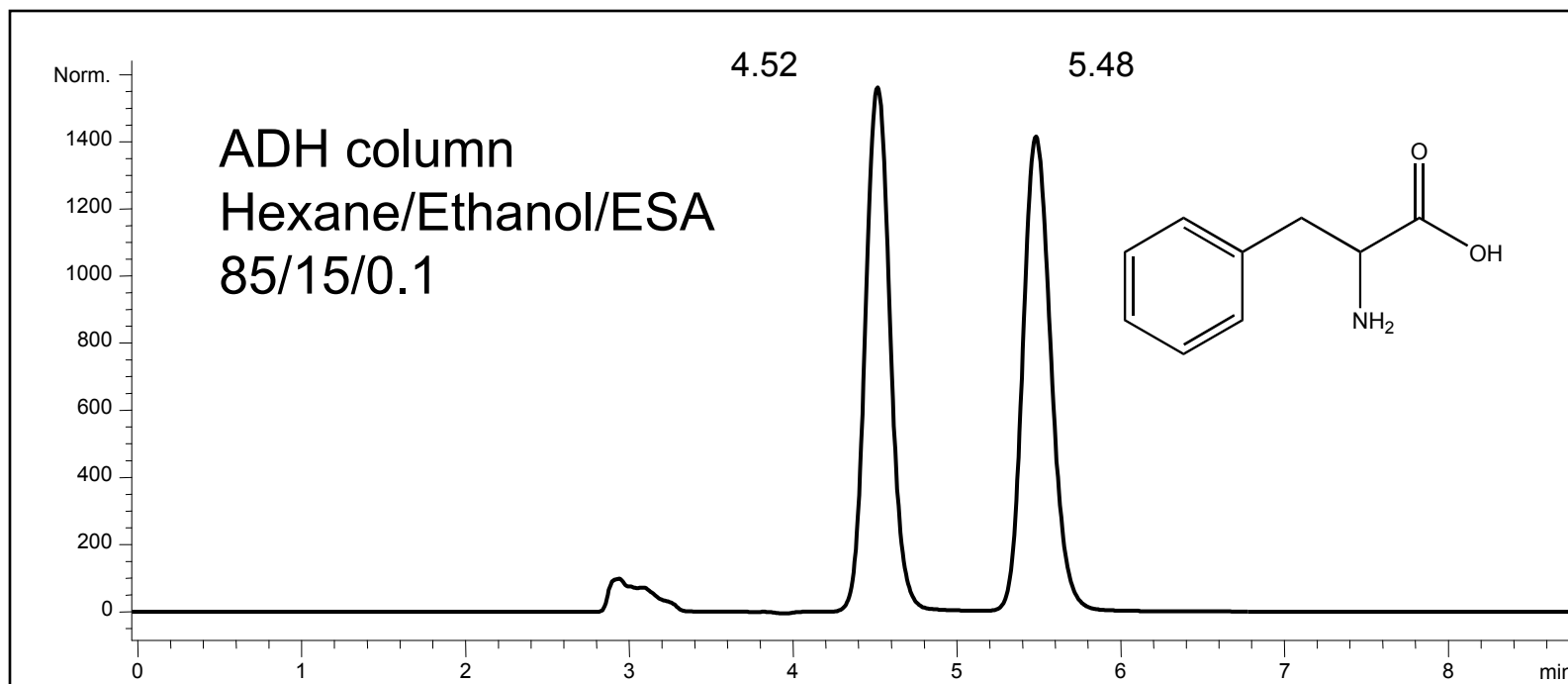
HPLC with ESA: Terbutaline *(2° amine)*



HPLC with ESA: Phenoxybenzamine (3^o amine)



HPLC with ESA: Phenylalanine (amino acid)

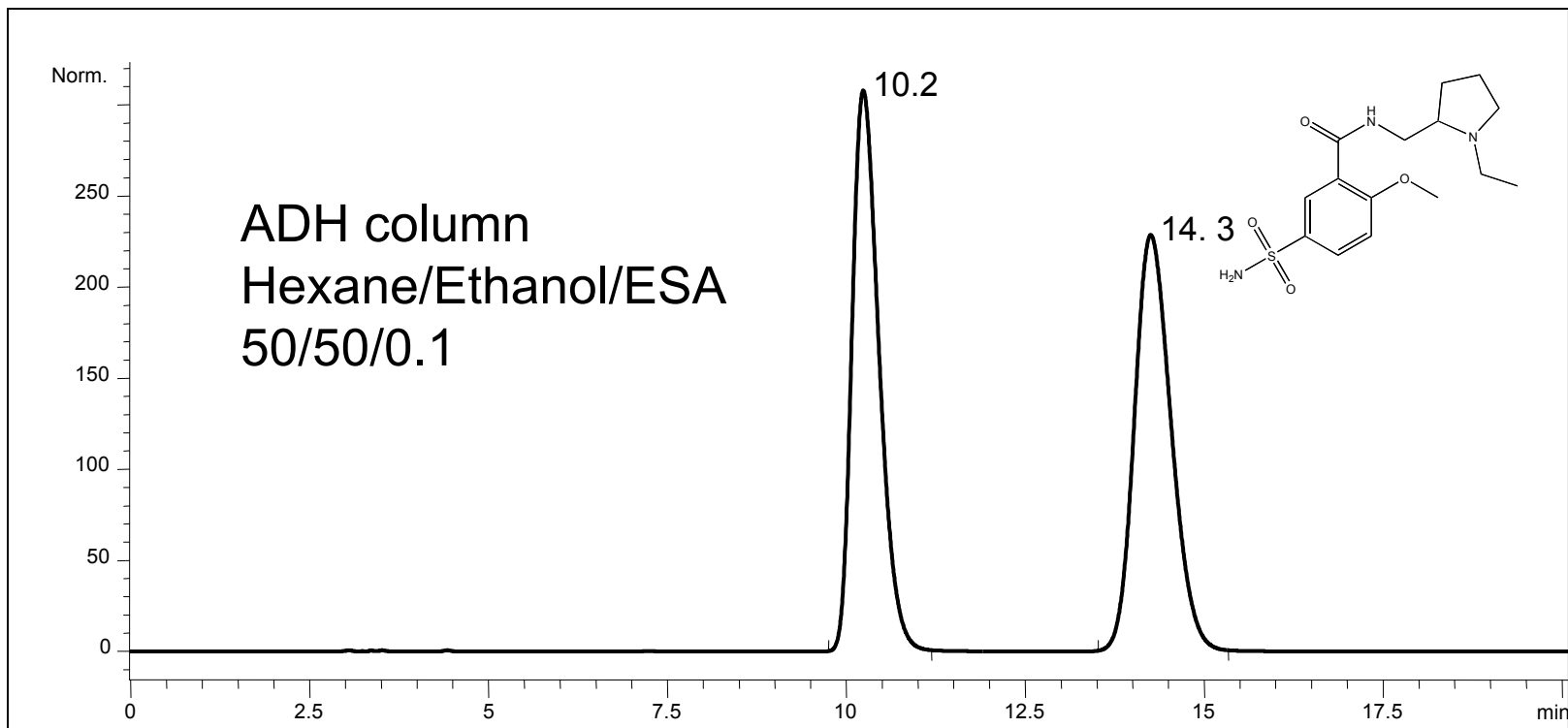


HPLC with ESA: Ethanol Level

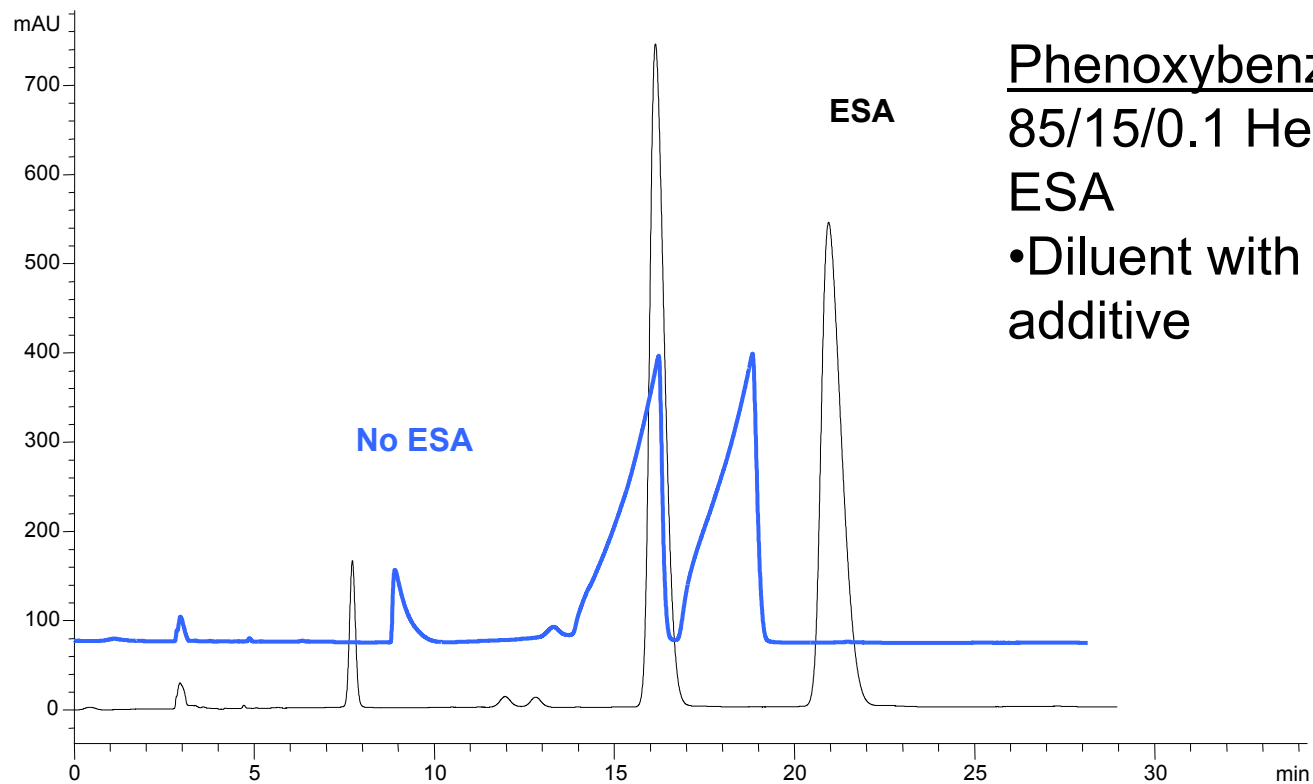
- Several observations of no elution or overlong retention
- Increased ethanol content decreased retention as expected giving some separations not seen before
- Selectivity held constant for about half of compounds (decreased for other half)
- Neat ethanol with ESA → no retention



HPLC with ESA: Sulpiride (3^o amine)



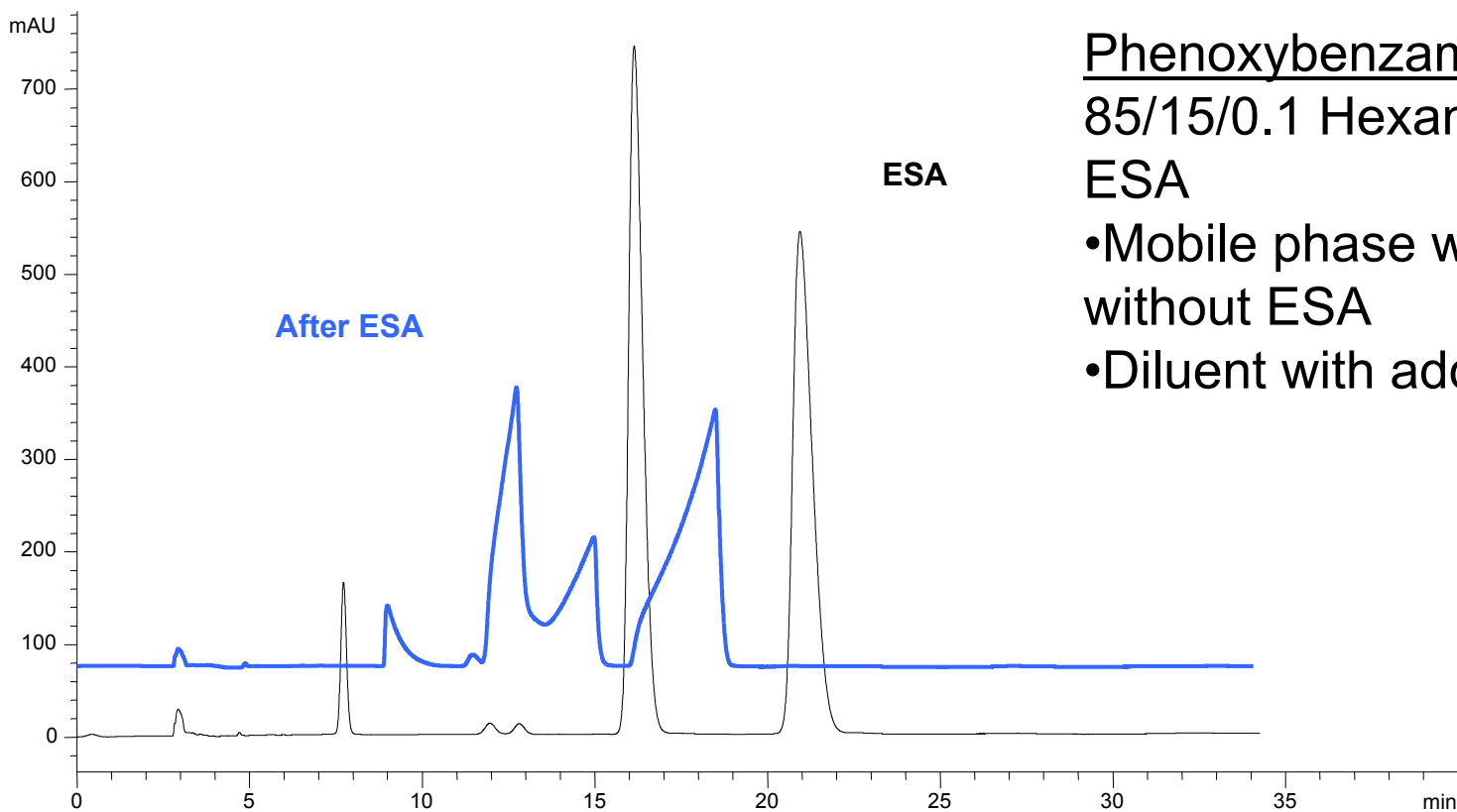
Effect of Additive in Diluent



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



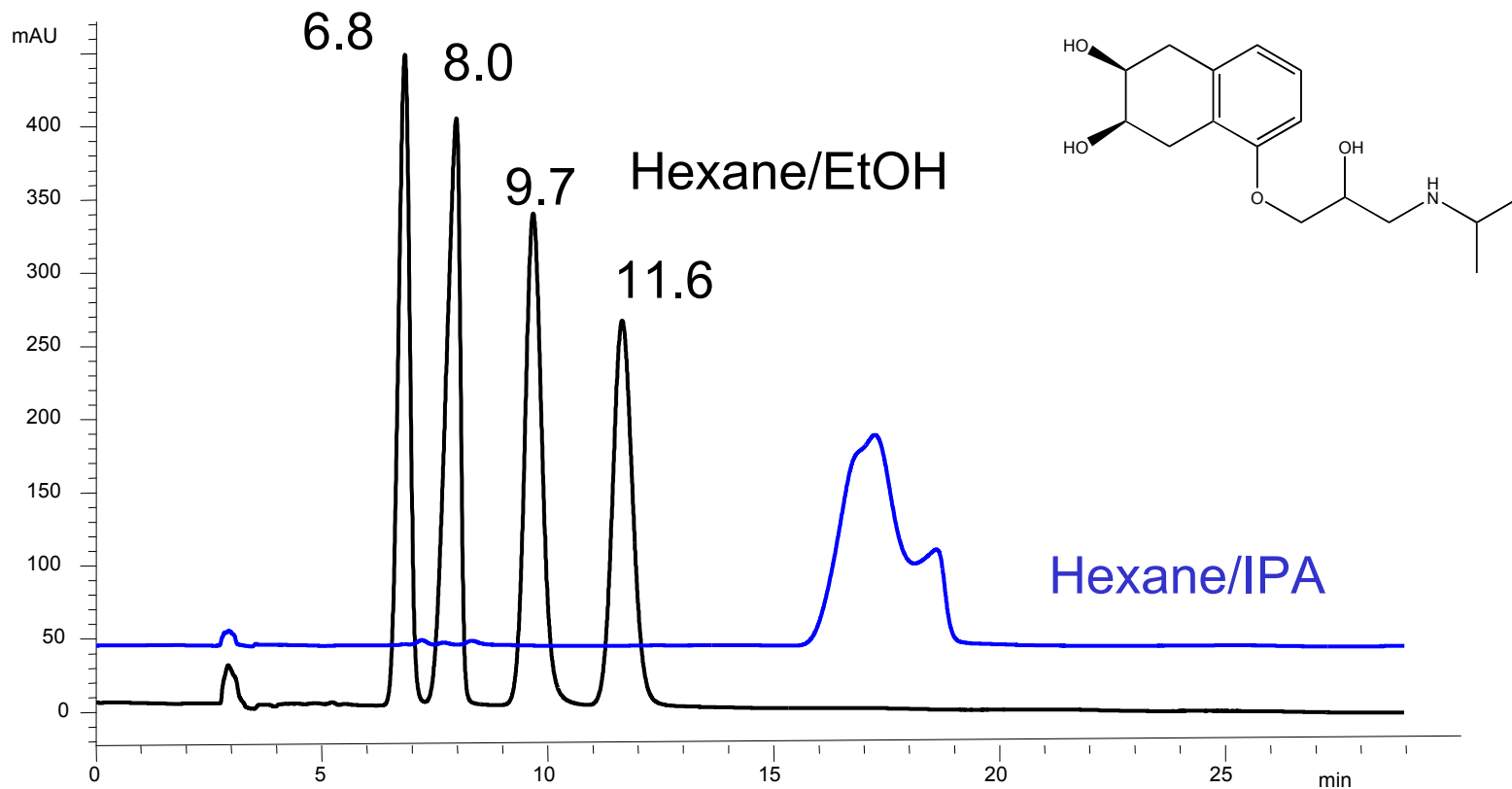
HPLC with ESA: Effect of Modifier

- 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



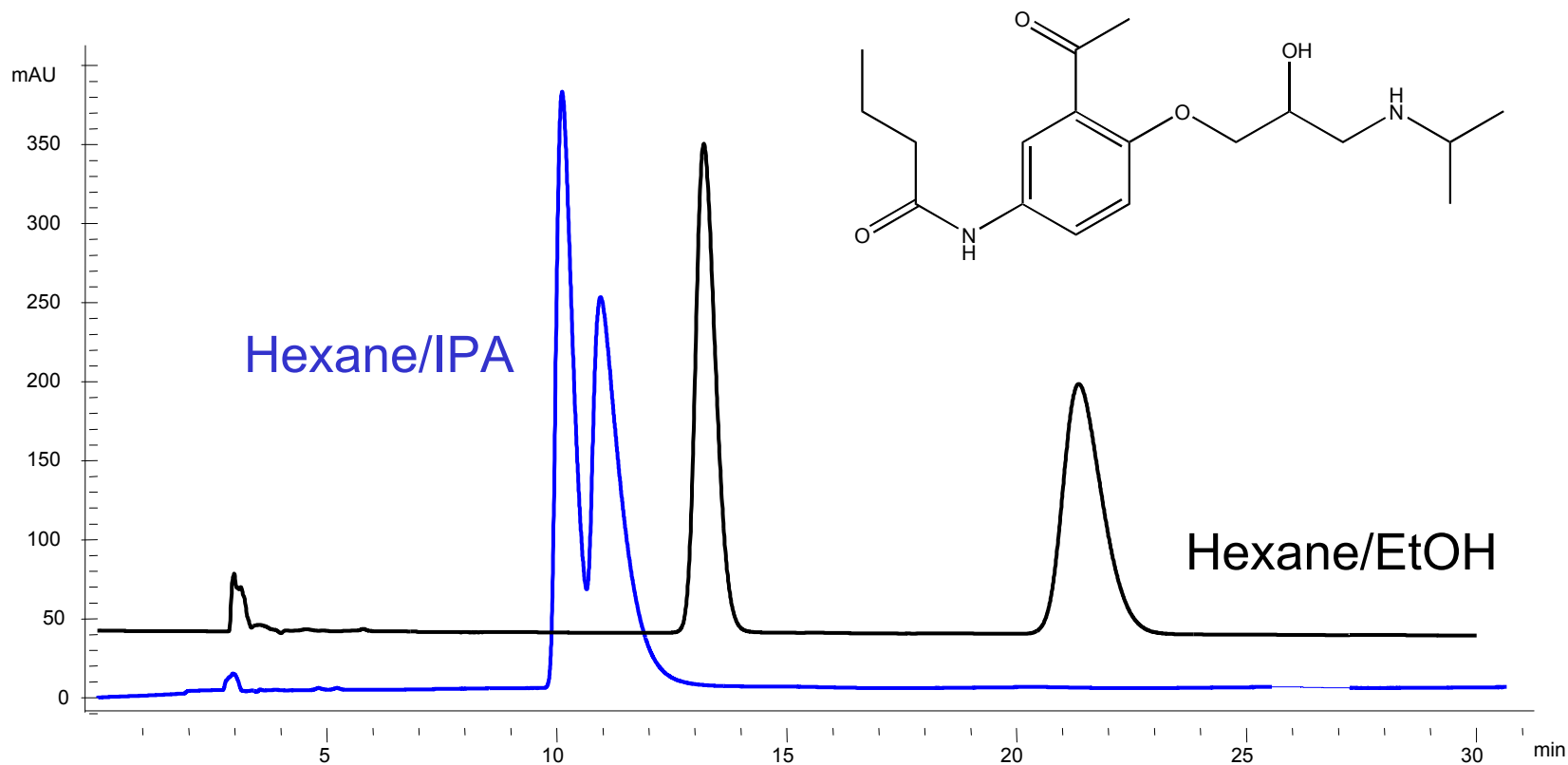
HPLC with ESA: Effect of Modifier

Nadolol



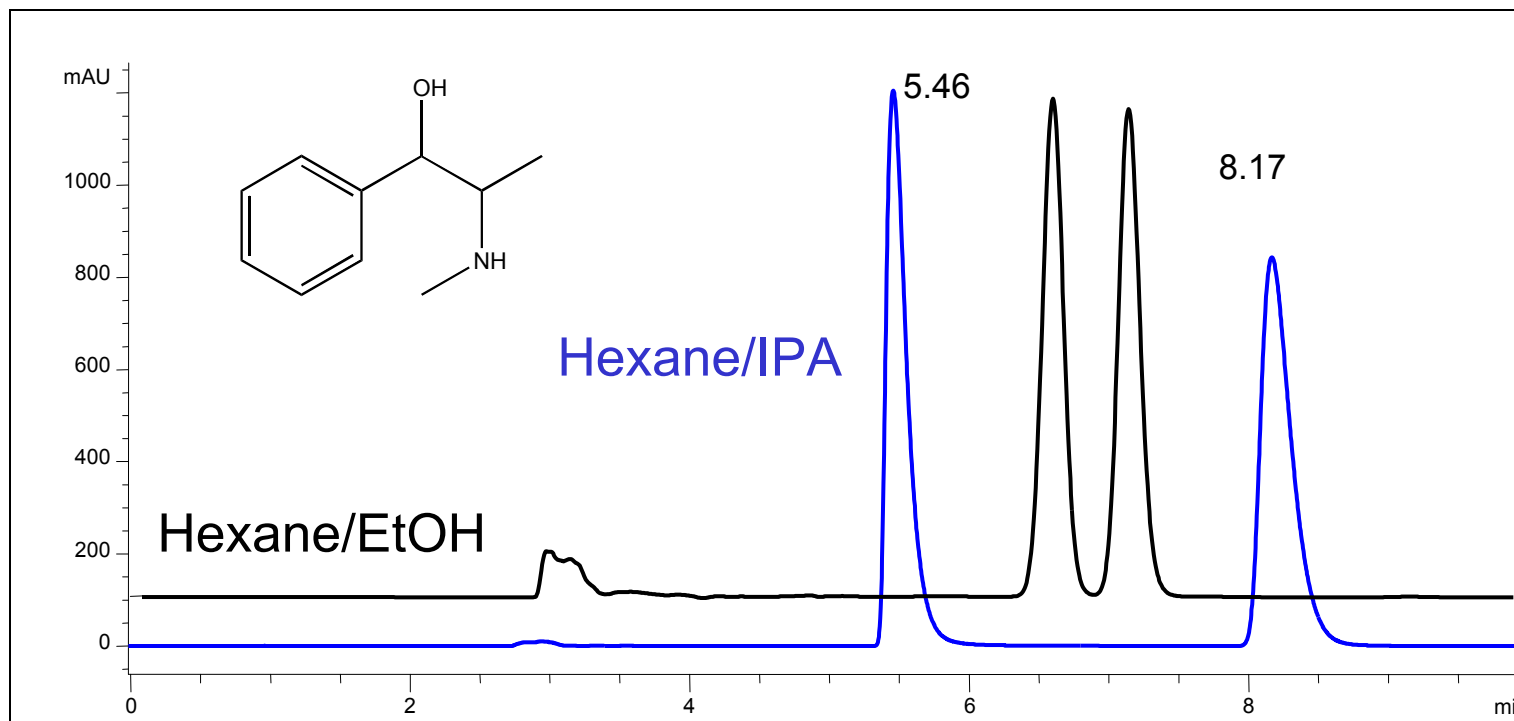
HPLC with ESA: Effect of Modifier

Acebutolol



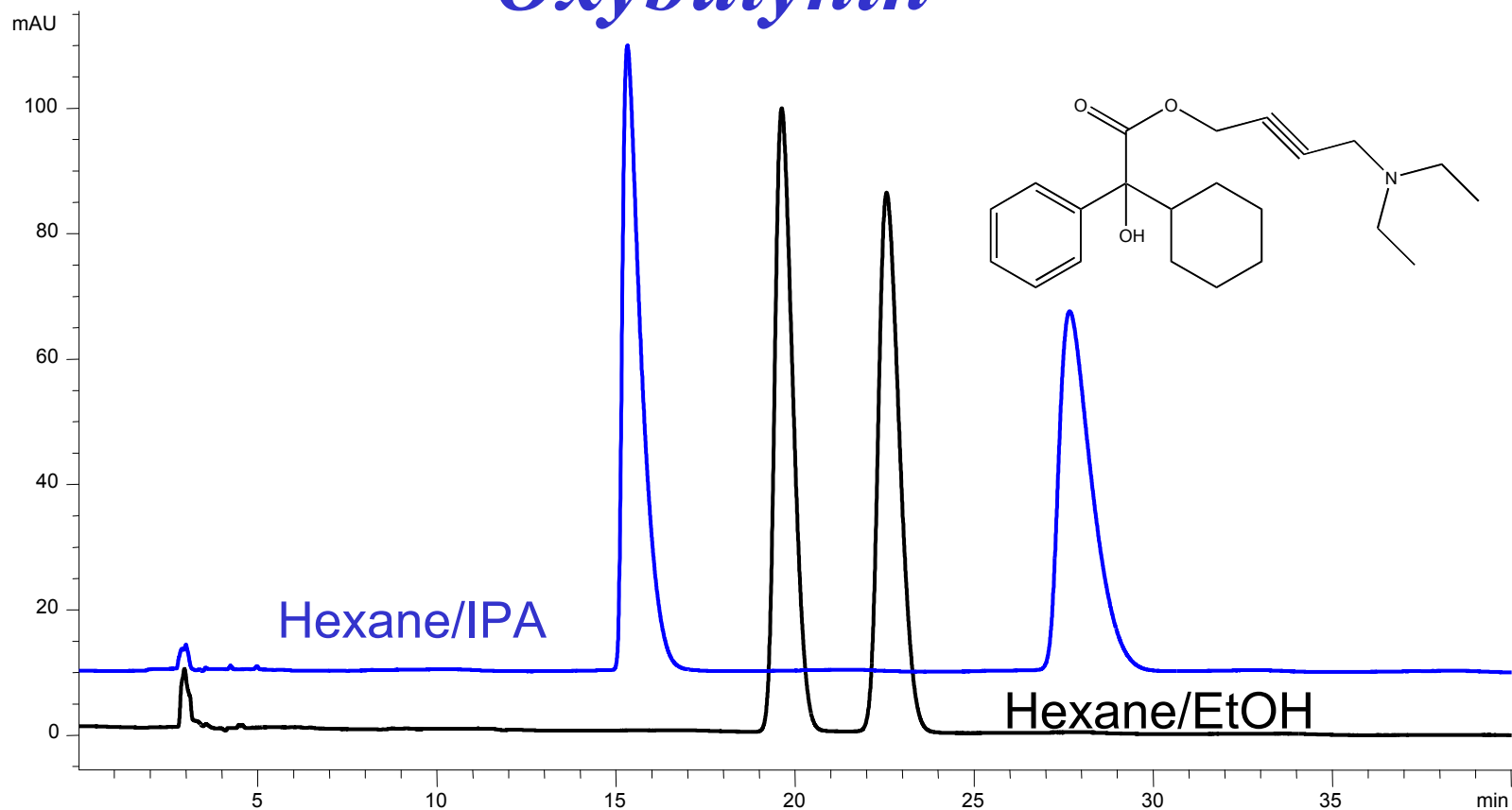
HPLC with ESA: Effect of Modifier

Ephedrine



HPLC with ESA: Effect of Modifier

Oxybutynin



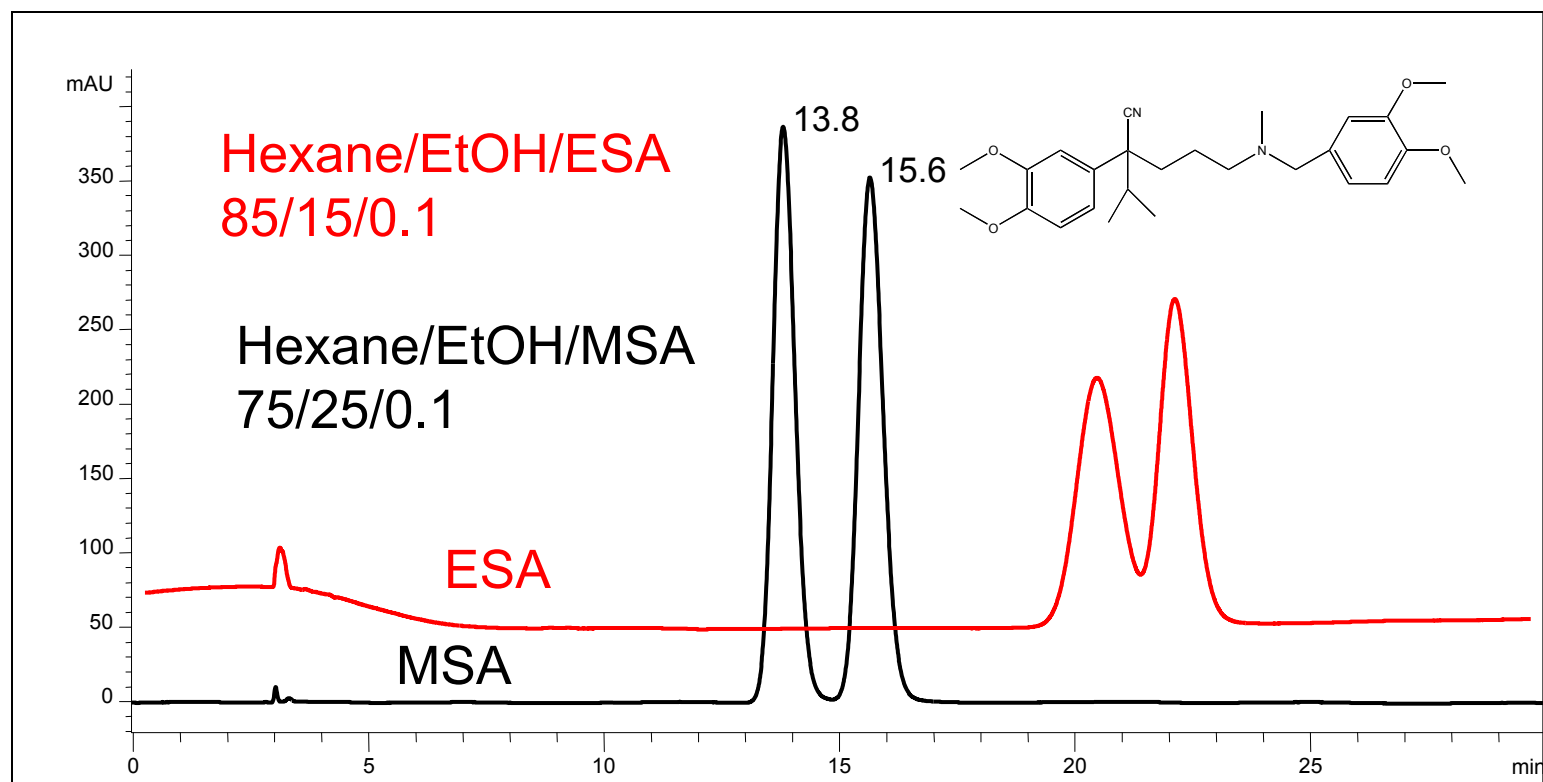
HPLC with ESA: Effect of Acid

- 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

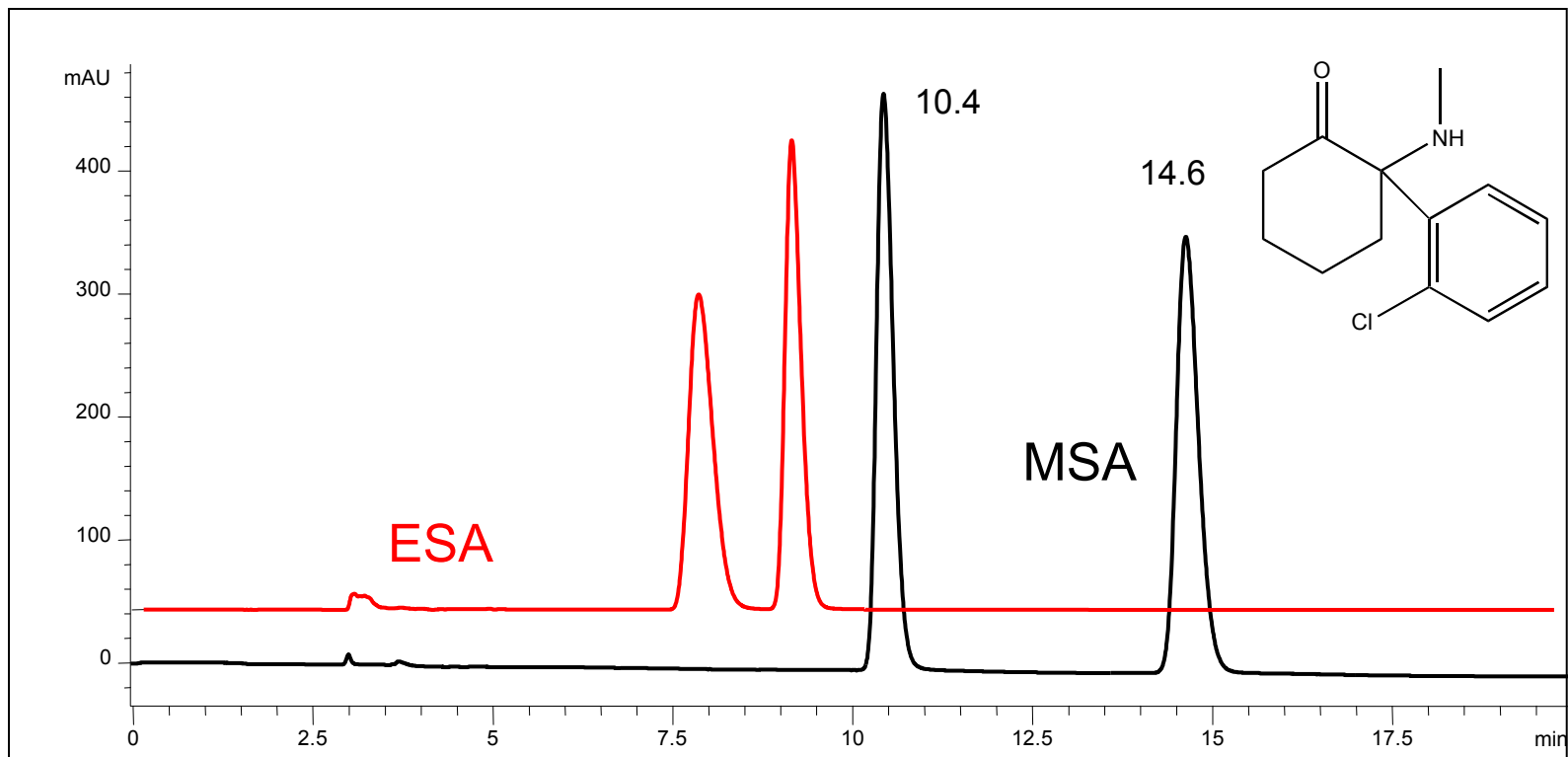


HPLC with ESA: Effect of Acid

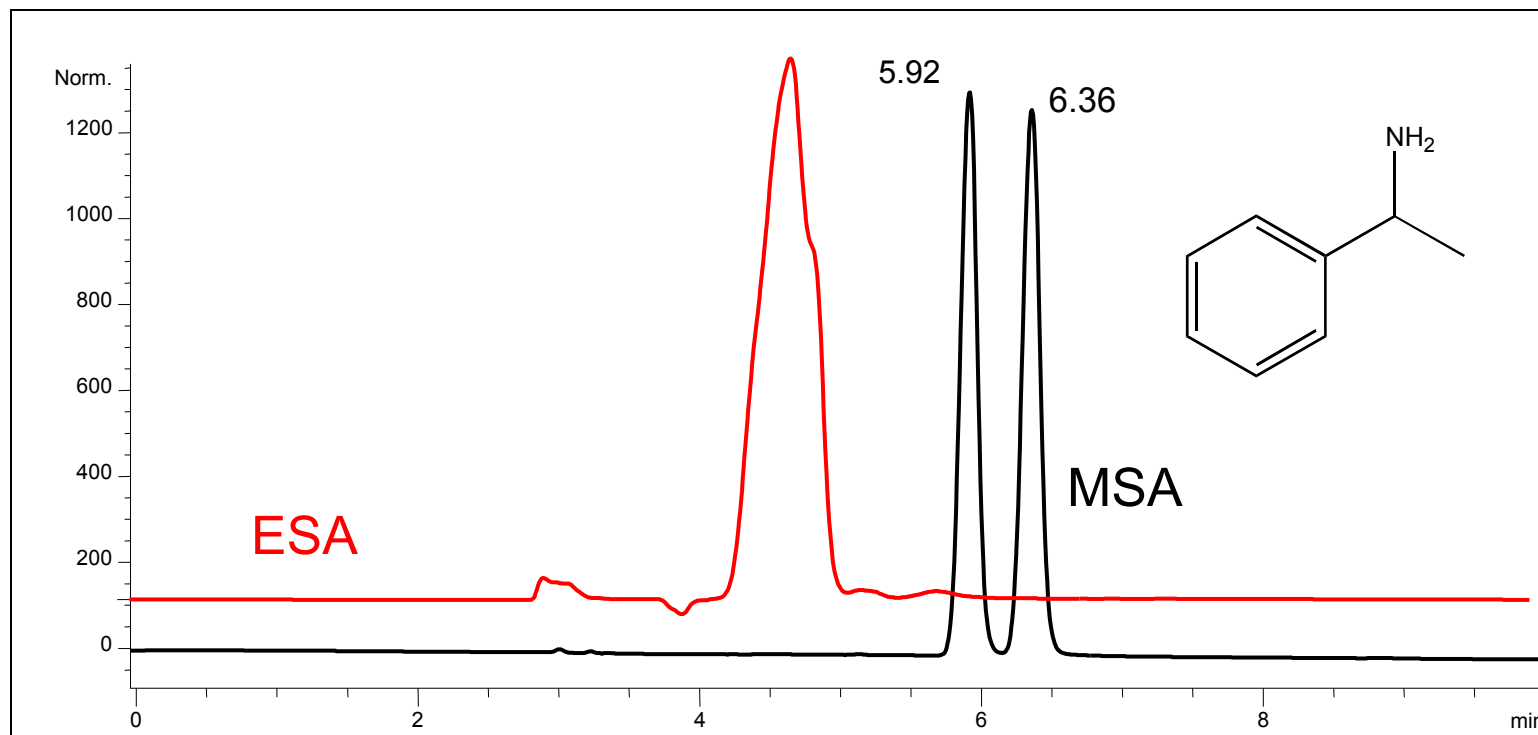
Verapamil



HPLC with ESA: Effect of Acid Ketamine



HPLC with ESA: Effect of Acid *α -methylbenzylamine*



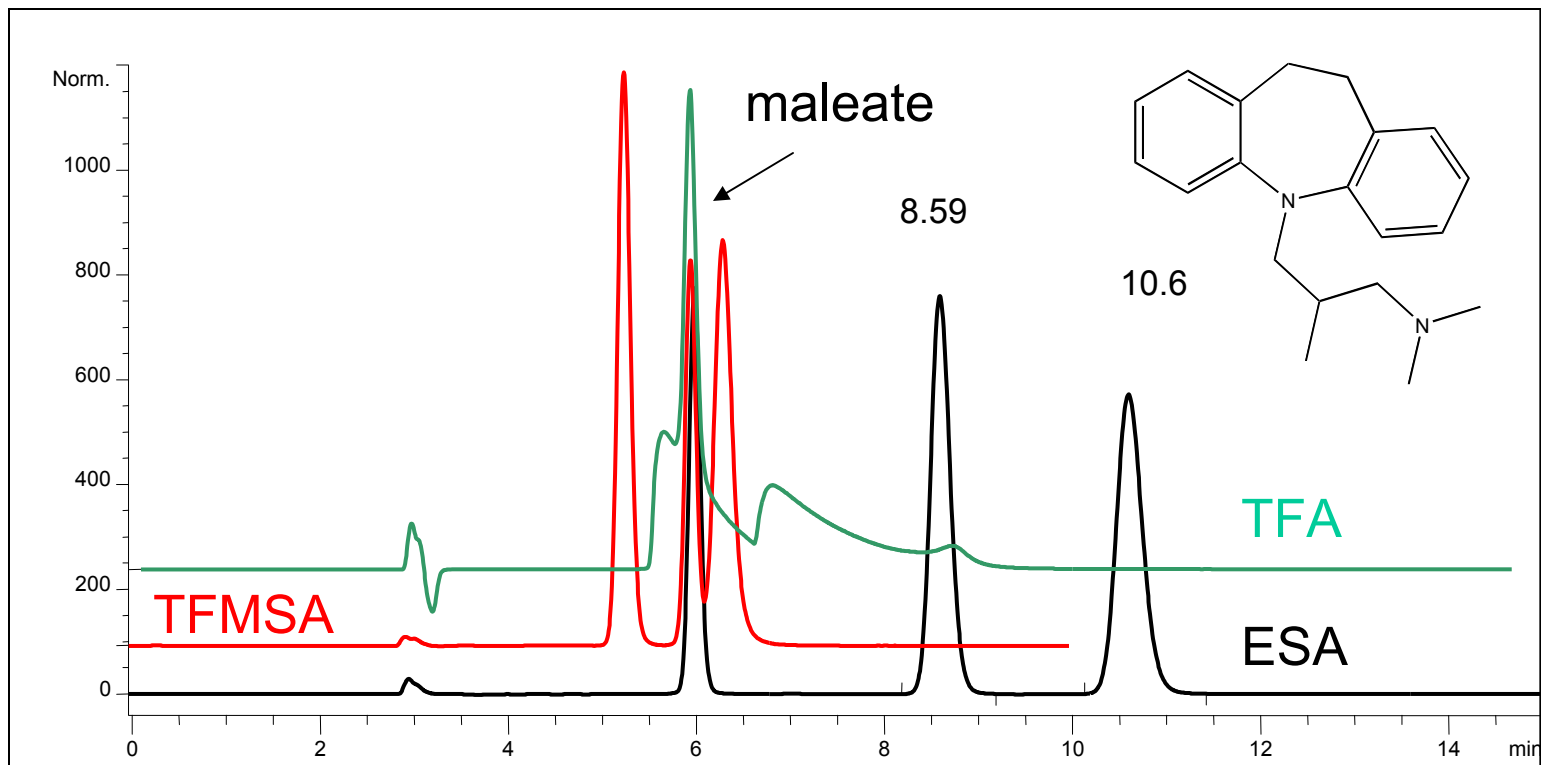
HPLC with ESA: Other Acids

- 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!

