

Tactics addressing challenging flash chromatography purifications



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TELEDYNE ISCO

A Teledyne Technologies Company

Tactics addressing challenging flash chromatography purifications



Difficult purification situations:

- ▶ Basic compounds
- ▶ Polar compounds
- ▶ Tight separations
- ▶ Natural products



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About Teledyne Isco



C-18 Reverse phase

Amine

Basic Alumina

Neutral Alumina

Acidic Alumina

Cyano

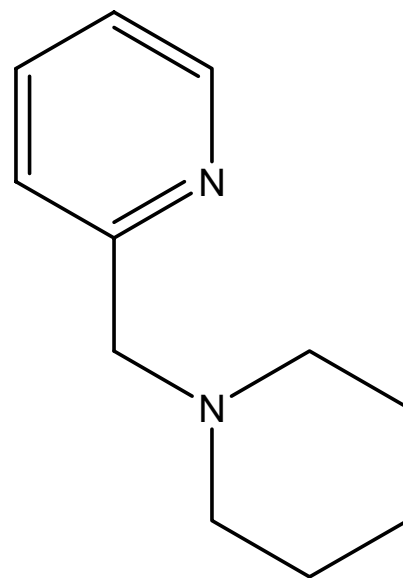
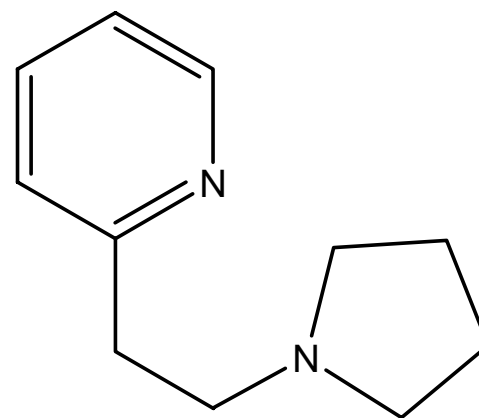
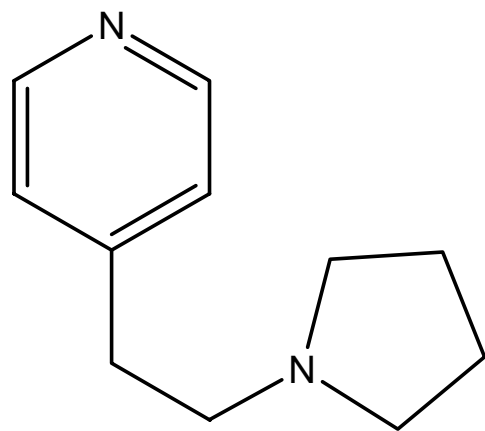
Florisil

SAX

SCX



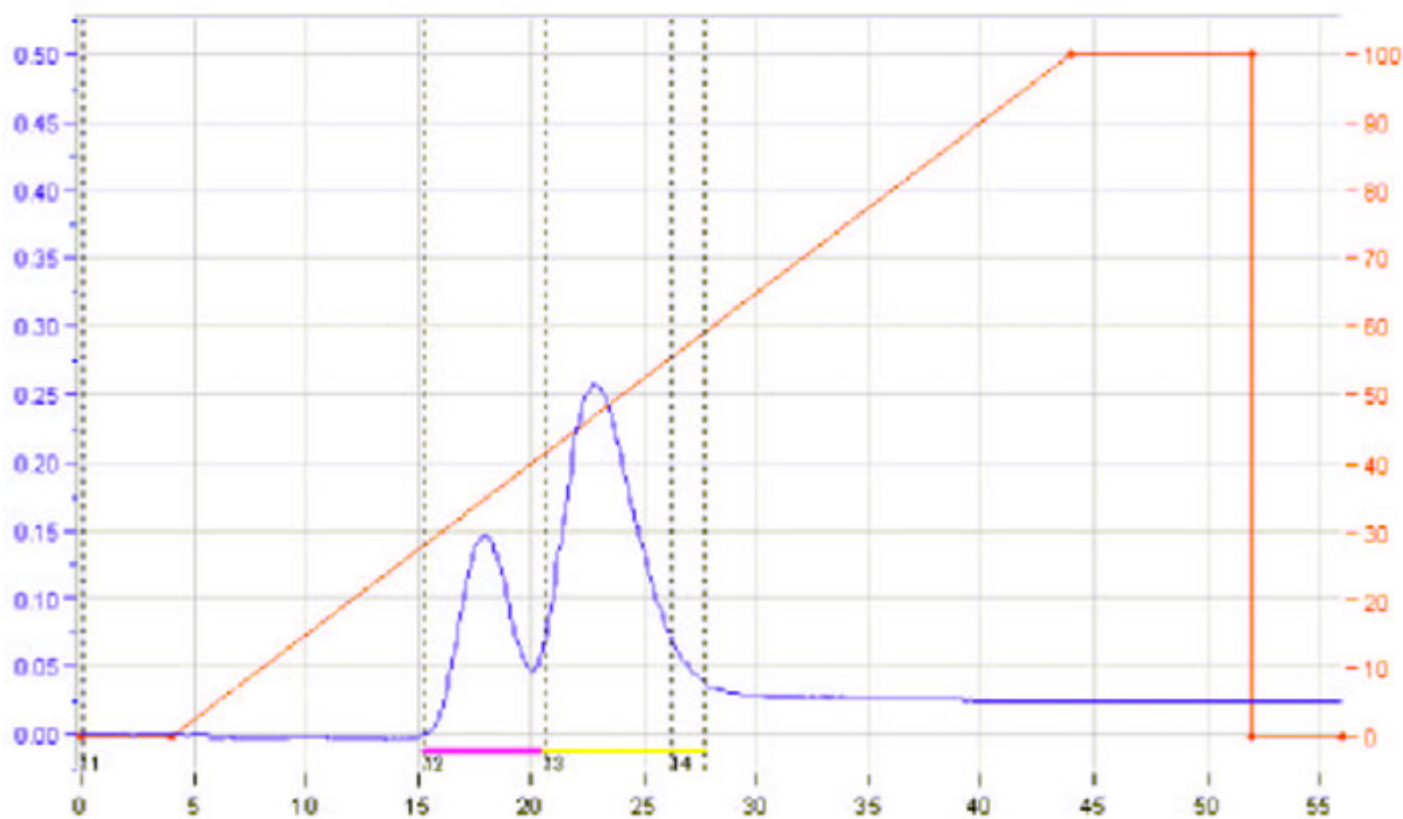
Application 1: Separation of Pyridine Derivatives



Application 1: Separation of Pyridine Derivatives

52 mg on celite run on 4g RediSep Basic Alumina column

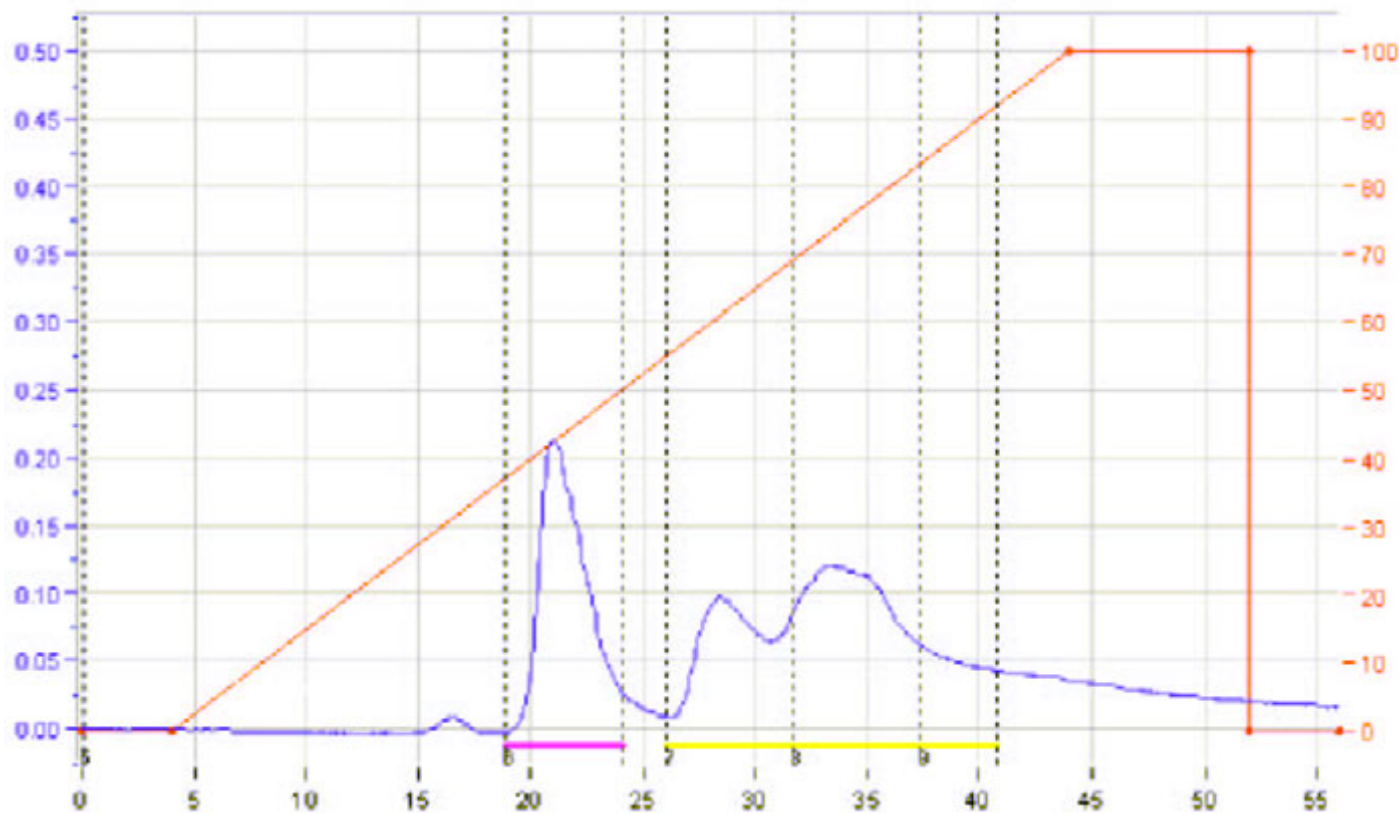
Solvents: hexane, ethyl acetate



Application 1: Separation of Pyridine Derivatives

52 mg on celite run on 4g RediSep Amine column

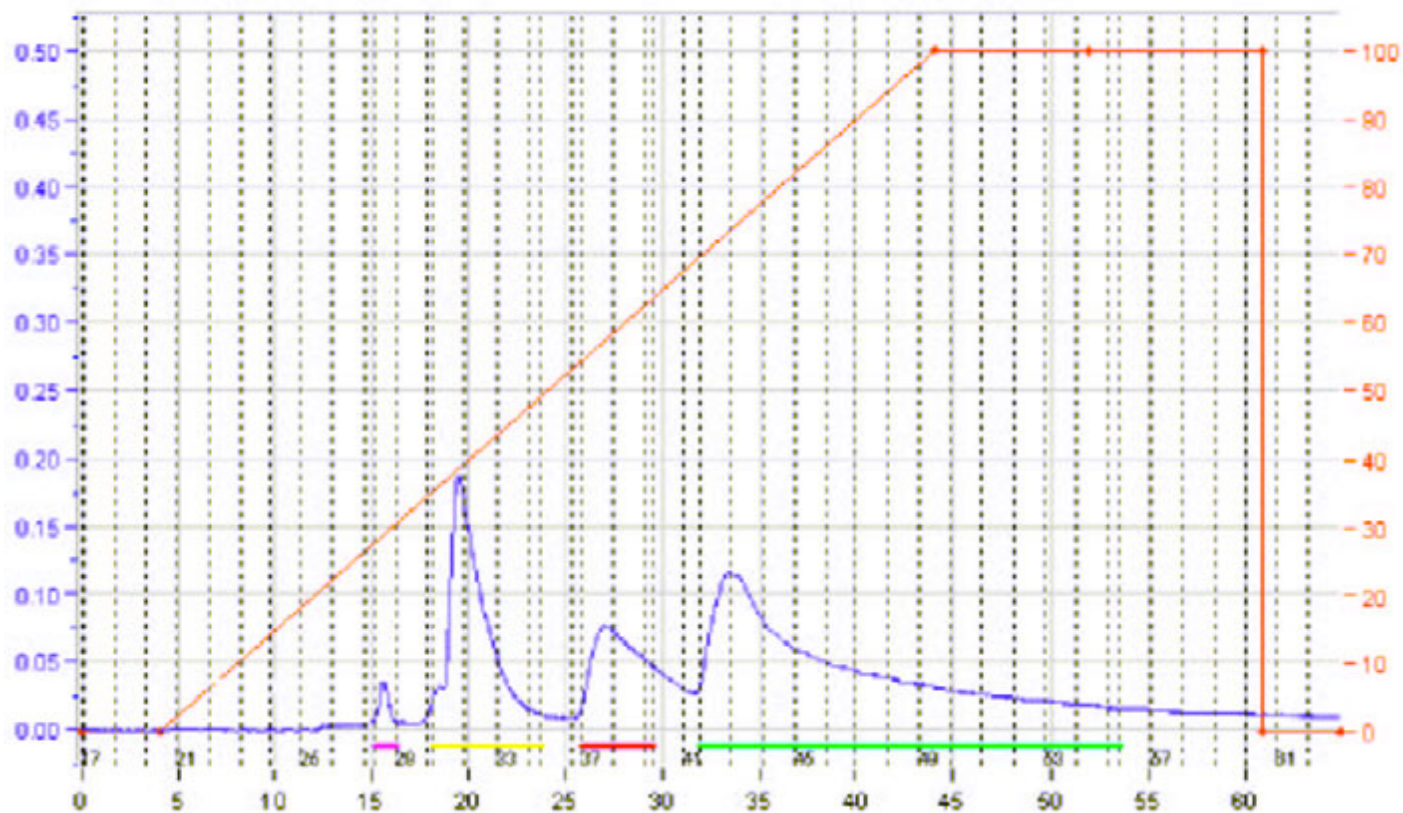
Solvents: hexane, ethyl acetate



Application 1: Separation of Pyridine Derivatives

157 mg on celite run on 2 stacked 14g RediSep Amine column

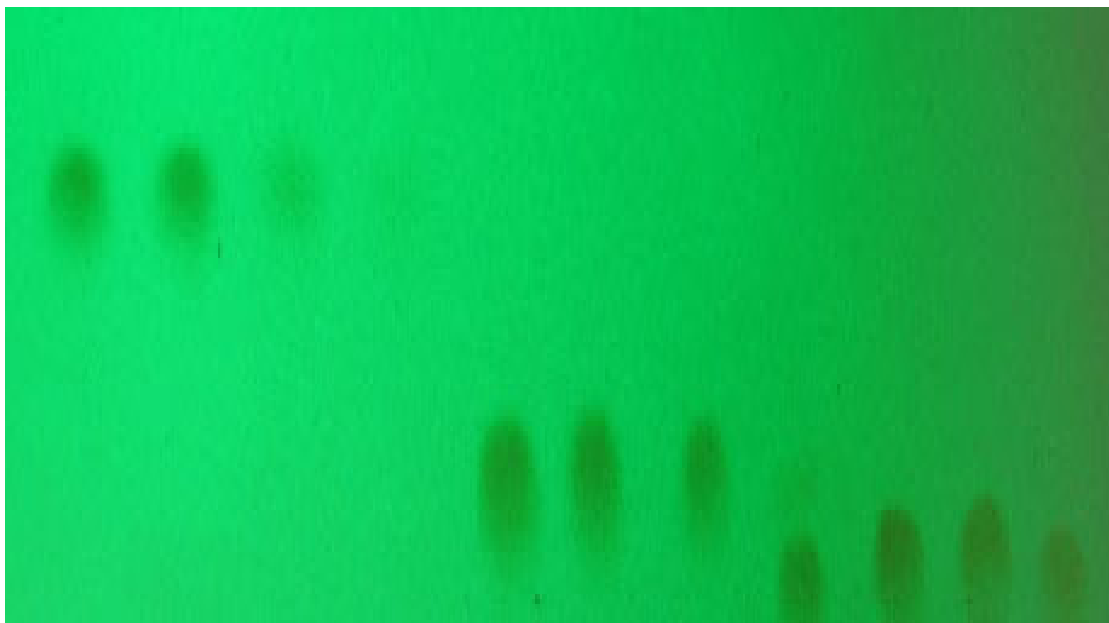
Solvents: hexane, ethyl acetate



Application 1: Separation of Pyridine Derivatives

TLC of resulting fractions

► Fully separated products

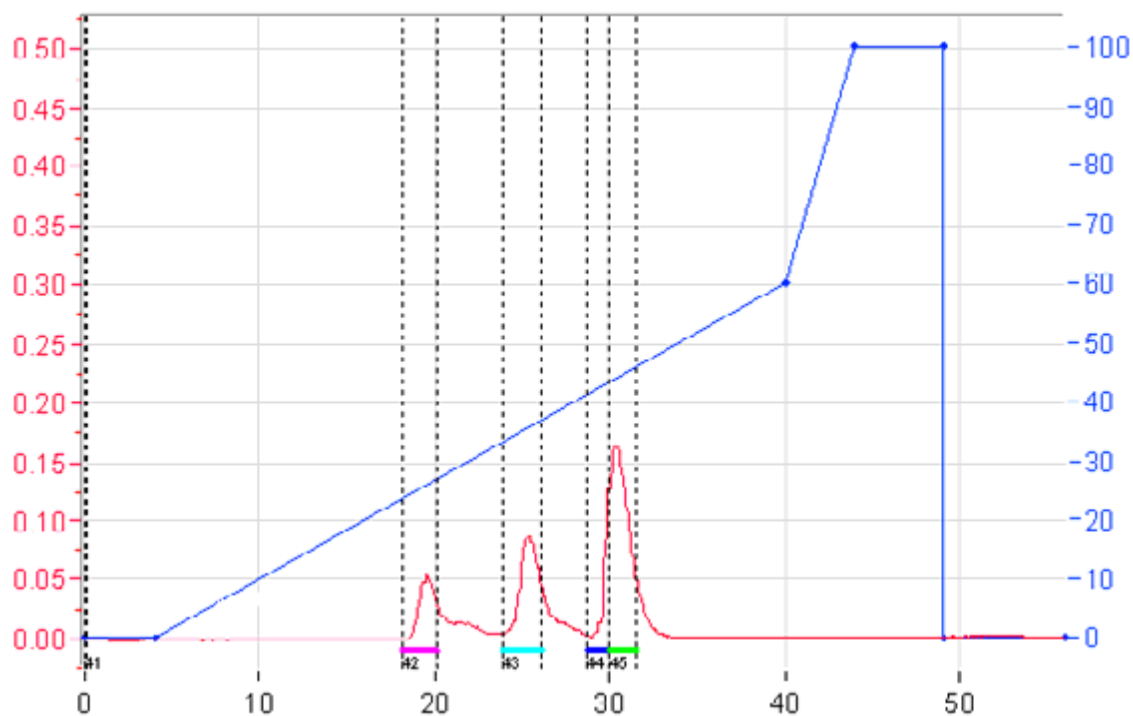
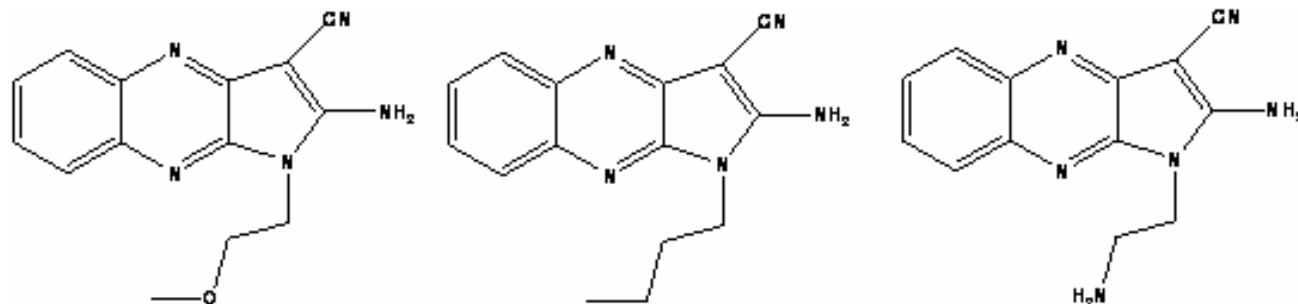


Amine TLC plate
In EtOAc



Application 2: Separation of polar compounds

Low solubility
polar heterocycles:



7mg preloaded on C-18
reversed phase powder

4.3g C-18 reversed phase
RediSep column

A: H₂O + 0.1% TFA
B: MeCN + 0.1% TFA

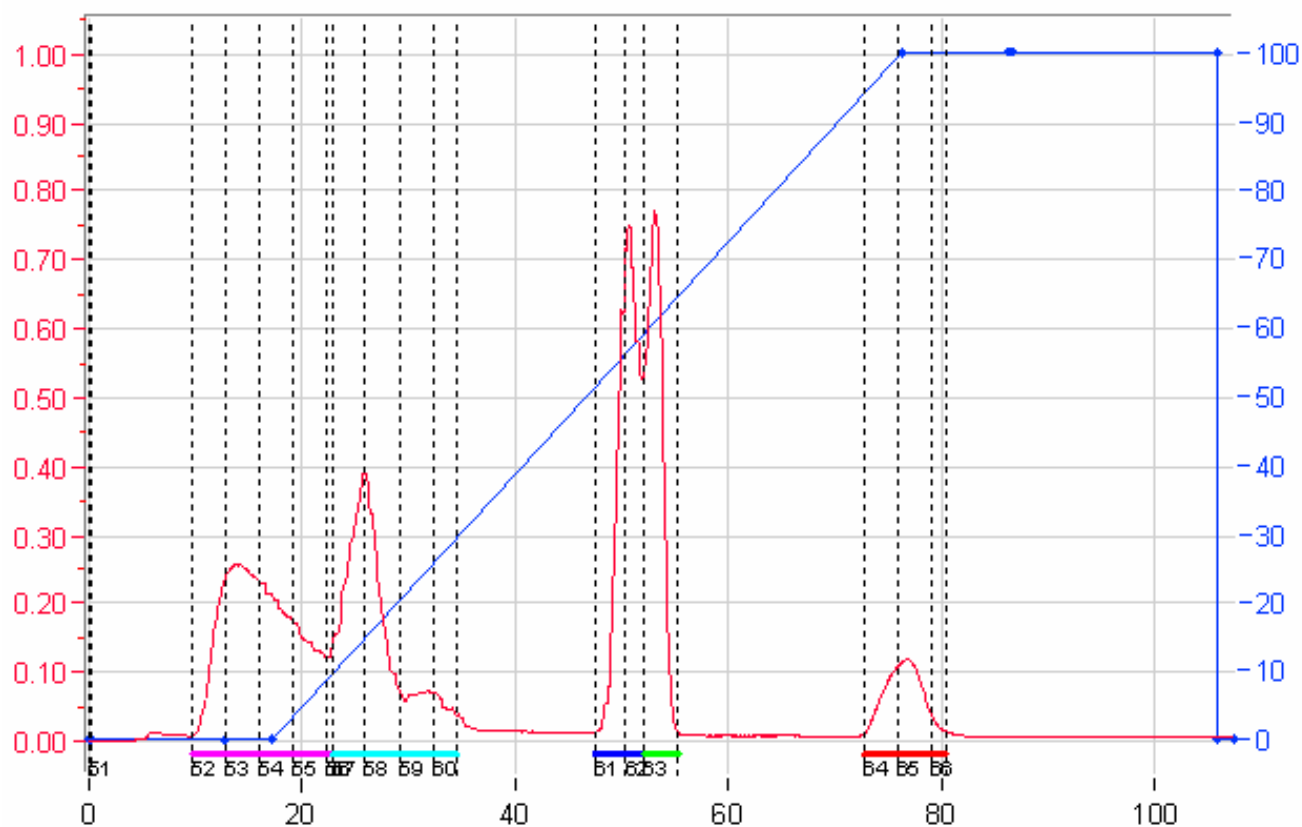
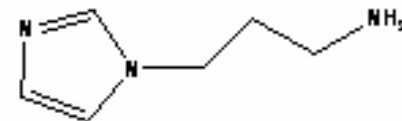
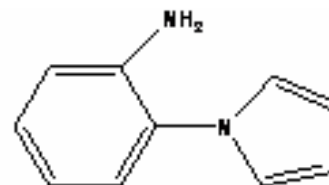
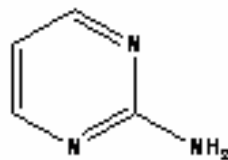
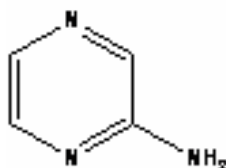
12ml/min

254 nm



Application 2: Separation of polar compounds

Primary amines:



30mg preloaded on C-18
reversed phase RediSep
prepacked cartridge

4.3g C-18 reversed phase
RediSep column

A:H₂O
B:MeCN

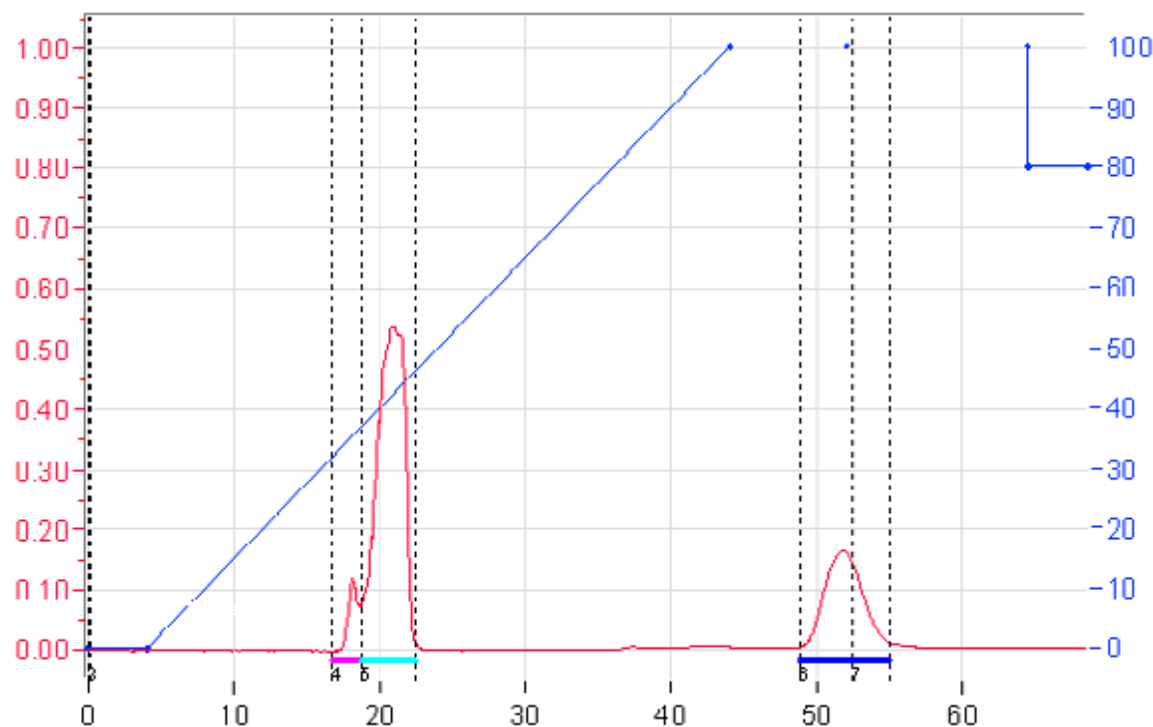
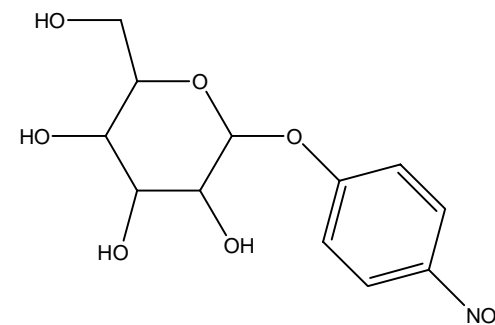
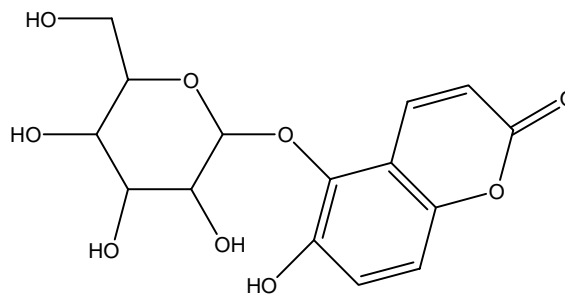
12ml/min

220 nm



Application 2: Separation of polar compounds

Carbohydrates:



22mg preloaded on C-18
reversed phase RediSep
prepacked cartridge

4.3g C-18 reversed phase
RediSep column

A:H₂O
B:Acetonitrile

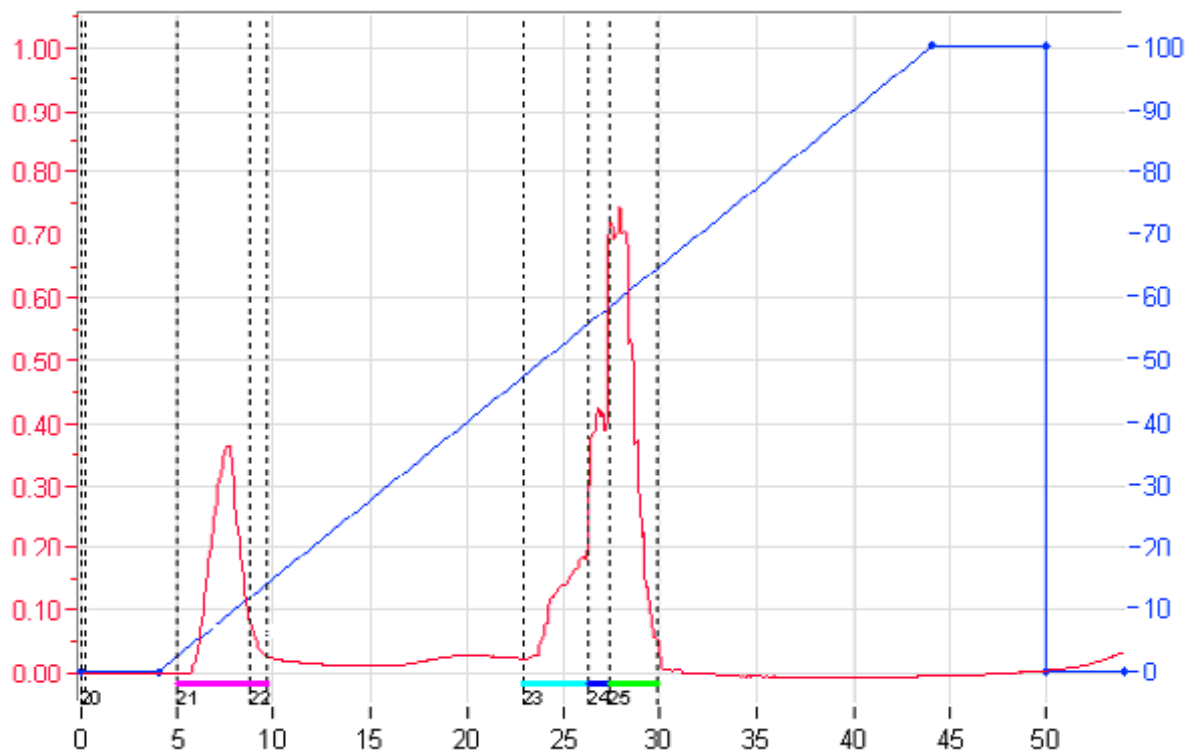
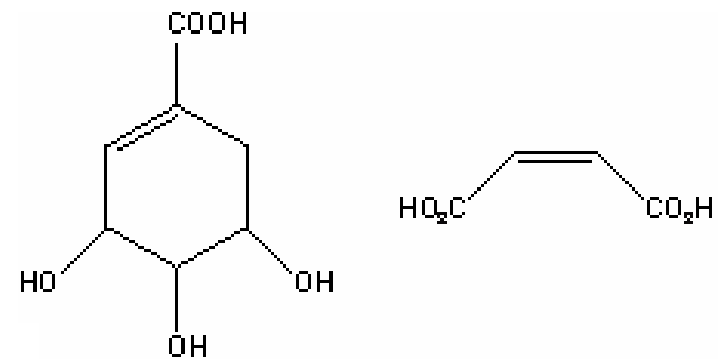
20ml/min

214 nm



Application 2: Separation of polar compounds

Carboxylic acids:



15mg preloaded on C-18
reversed phase RediSep
prepacked cartridge

4.3g C-18 reversed phase
RediSep column

A: H₂O + 0.1% TFA
B: MeCN + 0.1% TFA

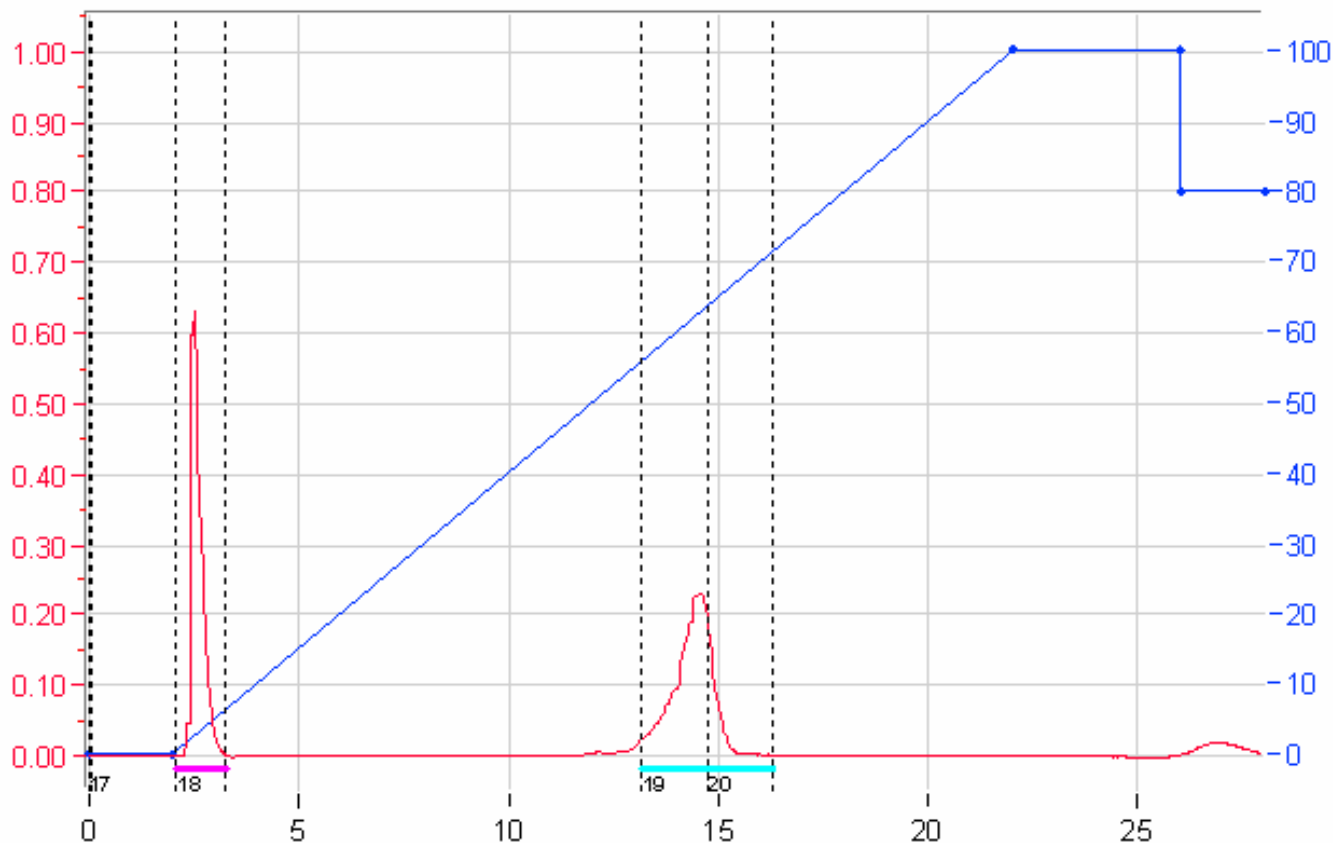
15ml/min

214 nm



Application 2: Separation of polar compounds

Peptides: Gly-Pro-Ala Val-Tyr-Val



67mg preloaded on C-18 reversed phase RediSep prepacked cartridge

13g C-18 reversed phase RediSep column

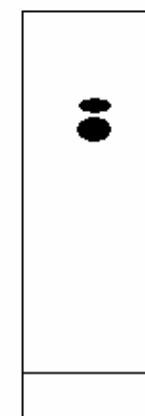
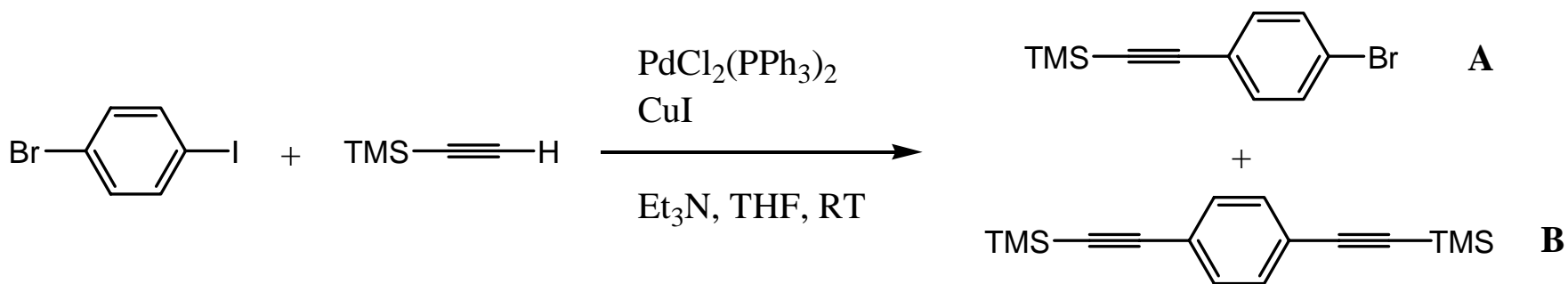
A: H₂O + 0.1% TFA
B: MeCN + 0.1% TFA

20ml/min

214 nm



Application 3: Column stacking

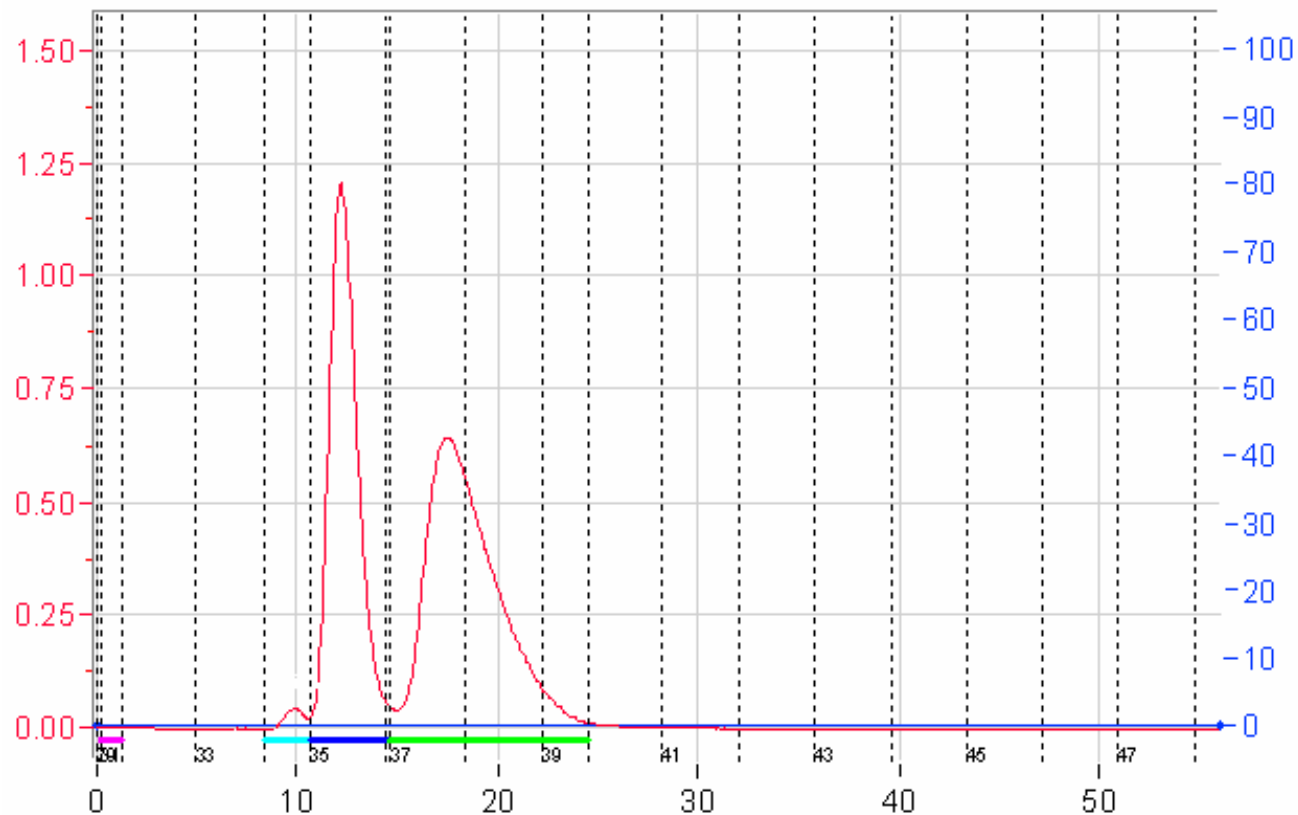


Hexane



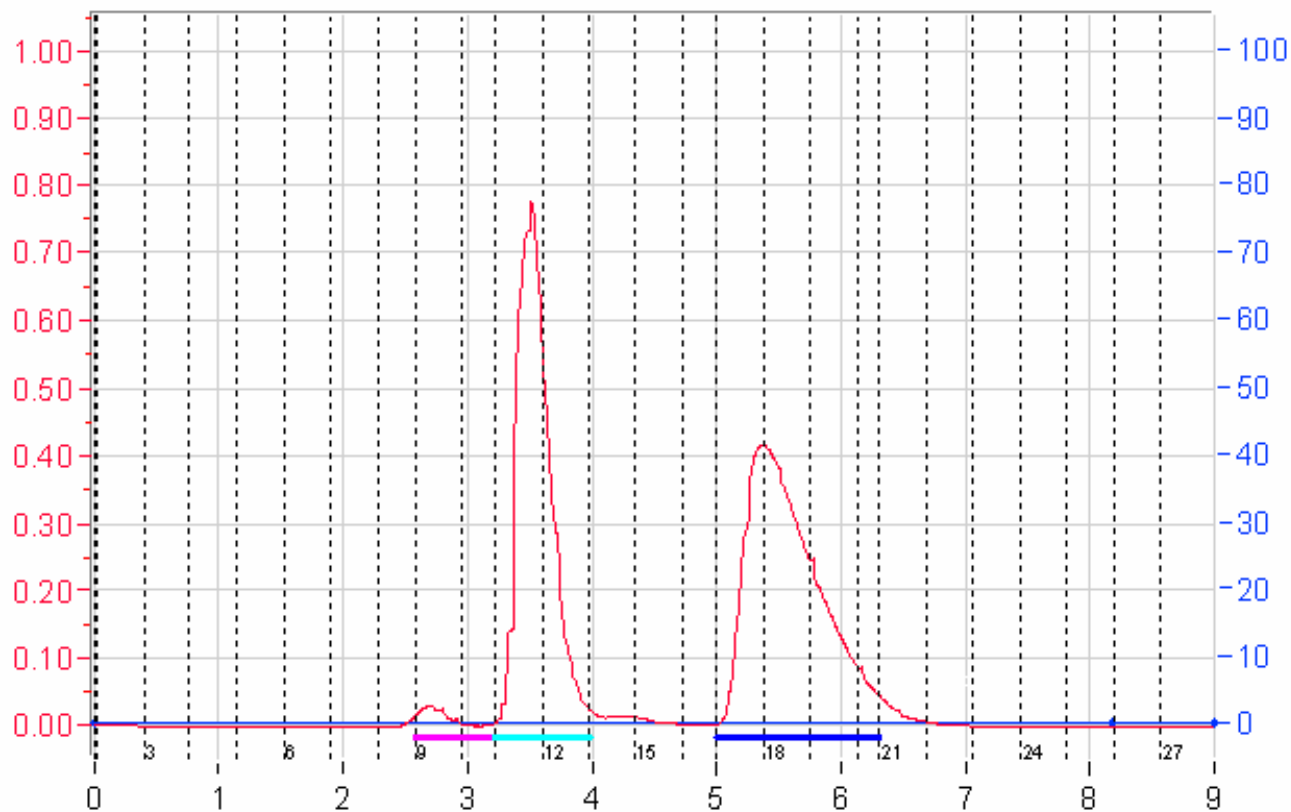
Application 3: Column stacking

1 normal phase silica gel 4g RediSep column



Application 3: Column stacking

6 Stacked normal phase silica gel 4g RediSep columns



Application 4: Natural Product Purification

Scutellaria Baicalensis

- ▶ Also known as Chinese Scullcap or Baikal Scullcap
- ▶ Traditional Chinese Medicine treatment for inflammatory skin conditions, allergies, high cholesterol and triglycerides
- ▶ Contains Flavonoid substance: Baicalin



Application 4: Natural Product Purification

Scutellaria Baicalensis

- ▶ Step 1: General analytical information collection
- ▶ Step 2: Full batch separation
- ▶ Step 3: Separation of resulting unresolved peaks under refined conditions



Application 4: Natural Product Purification

Scutellaria Baicalensis

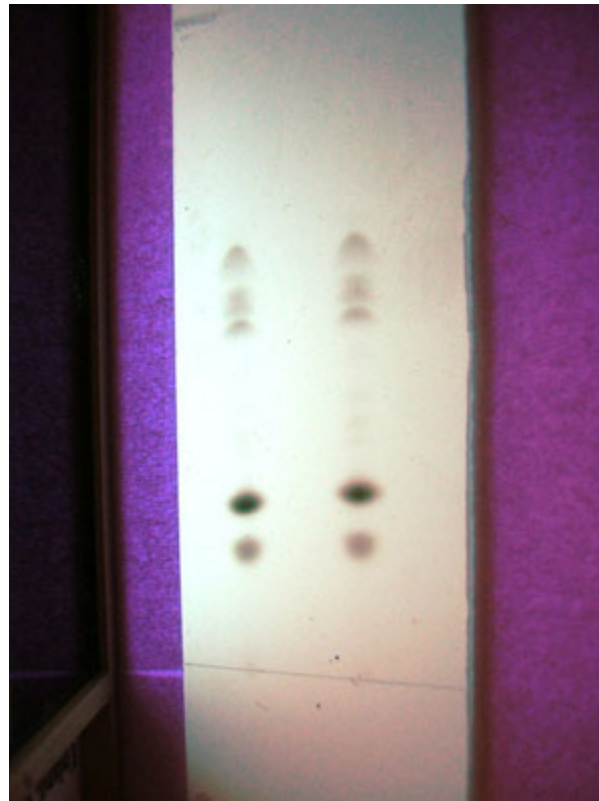
- ▶ Step 1: General analytical information collection
 - GC analysis: contains 6 compounds
 - UV spectrophotometer: $\lambda_{\max} = 277$ nm



Application 4: Natural Product Purification

Scutellaria Baicalensis

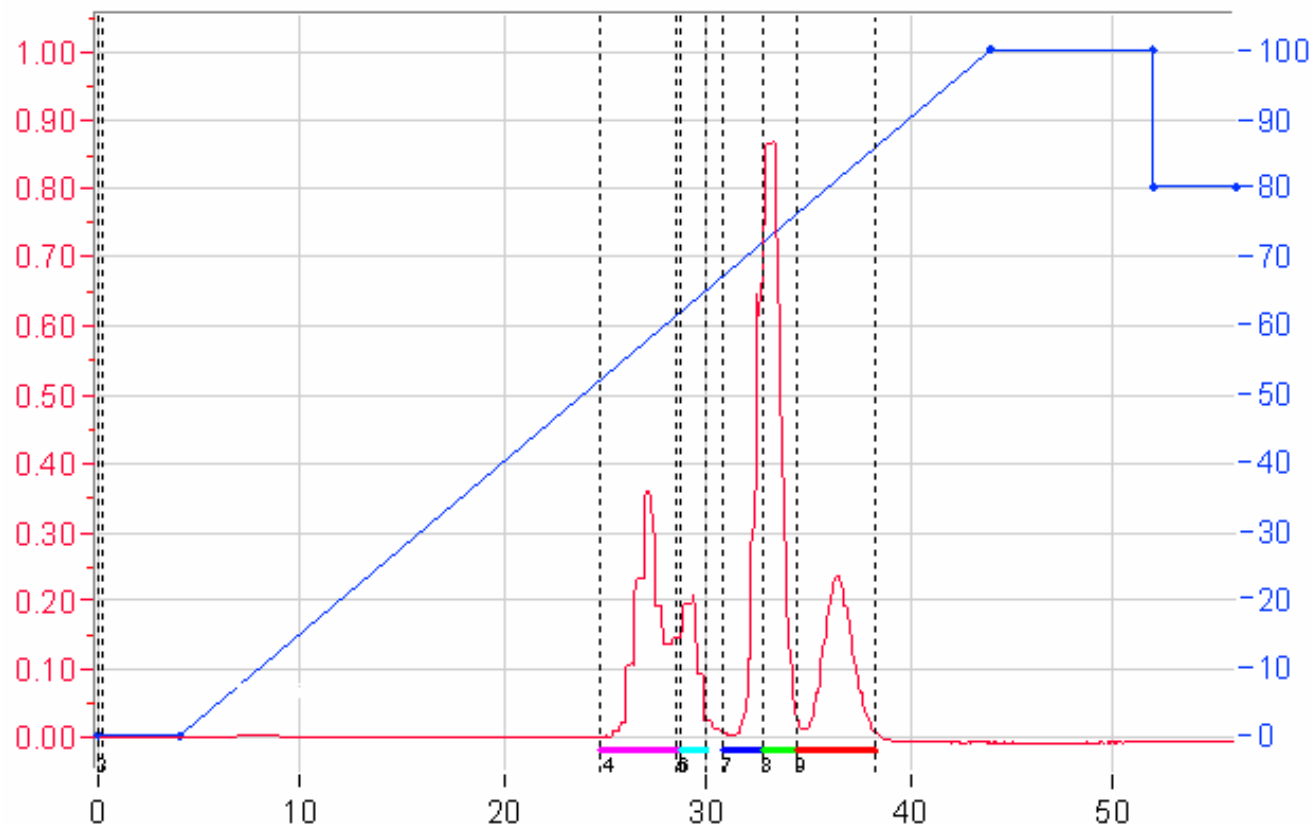
- ▶ Step 1: General analytical information collection
 - C-18 Reversed phase TLC plate (H₂O/MeCN 1:1):



Application 4: Natural Product Purification

Scutellaria Baicalensis

- ▶ Step 1: General analytical information collection
 - Initial natural product chromatogram:



23mg pre-coated on
C-18 reversed phase
Silica powder

4.3g C-18 reversed phase
RediSep column

H₂O/MeCN

12 ml/min

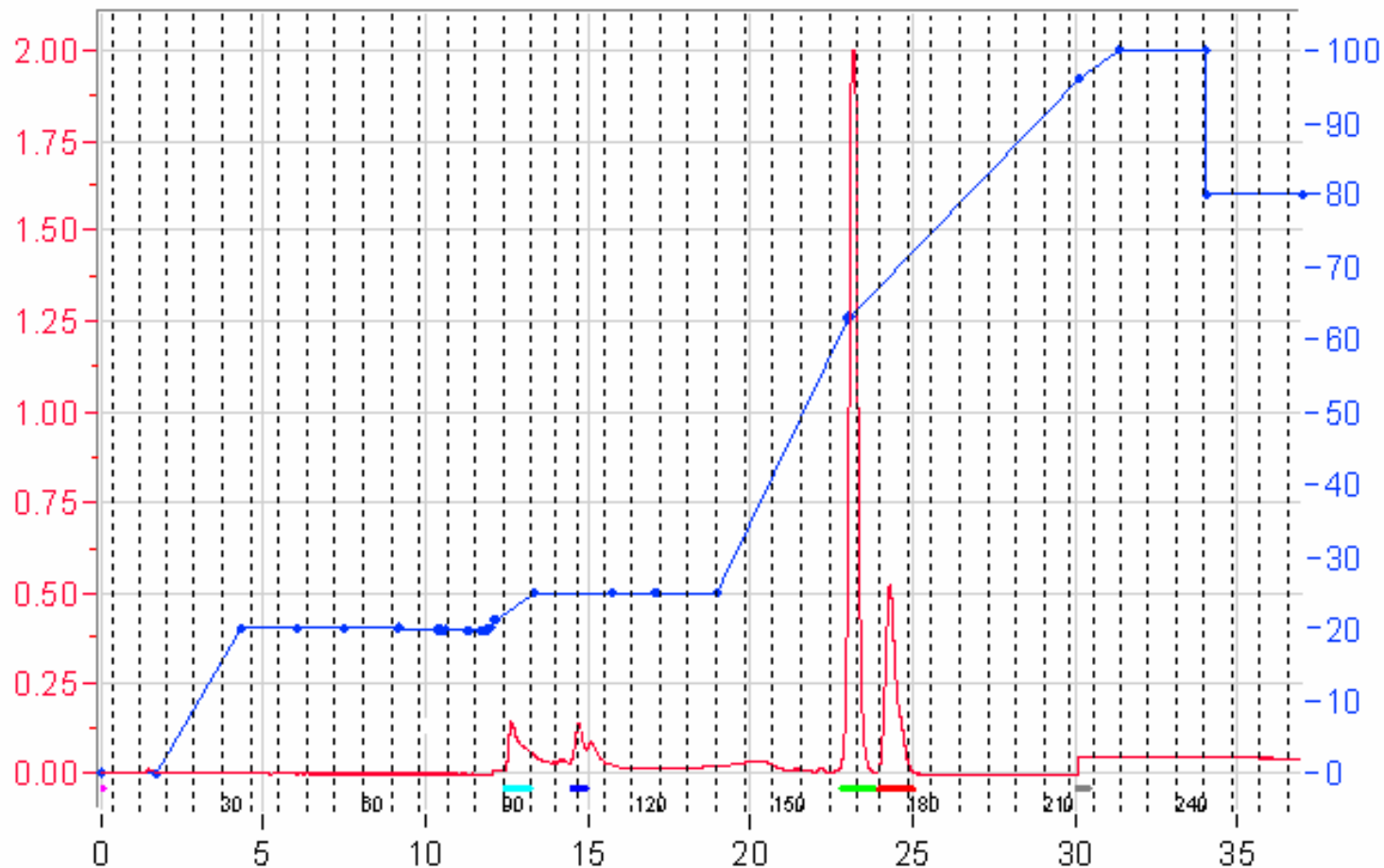
277 nm



Application 4: Natural Product Purification

Scutellaria Baicalensis

► Step 2: Full batch separation



841mg pre-coated
on C-18 reversed
phase Silica
powder

130g C-18
reversed phase
RediSep column

H₂O/MeCN

50ml/min

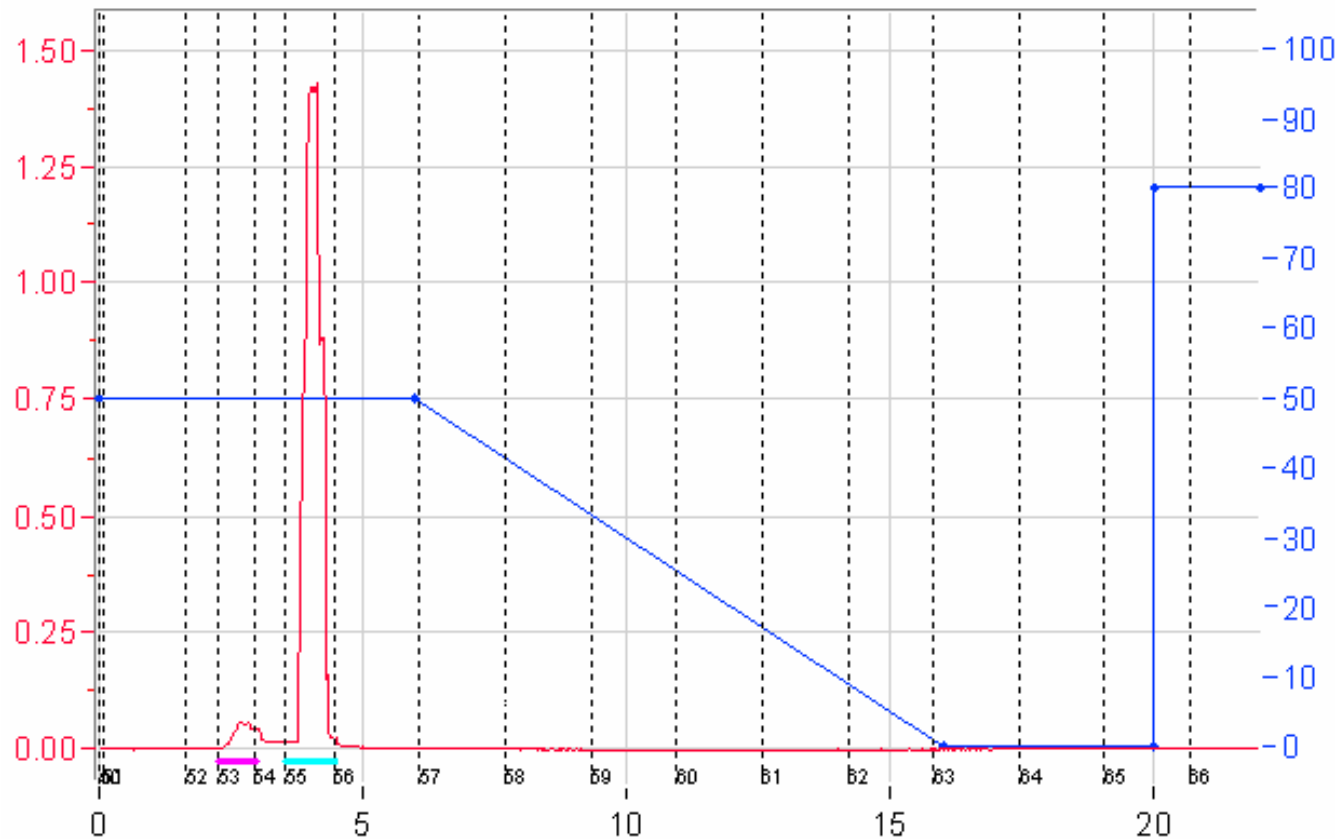
277 nm



Application 4: Natural Product Purification

Scutellaria Baicalensis

- Step 3: Separation of resulting unresolved peaks under refined conditions: Peak group #1



78mg pre-coated on C-18
reversed phase Silica
powder

13g C-18 reversed phase
RediSep column

H₂O/MeCN

15ml/min

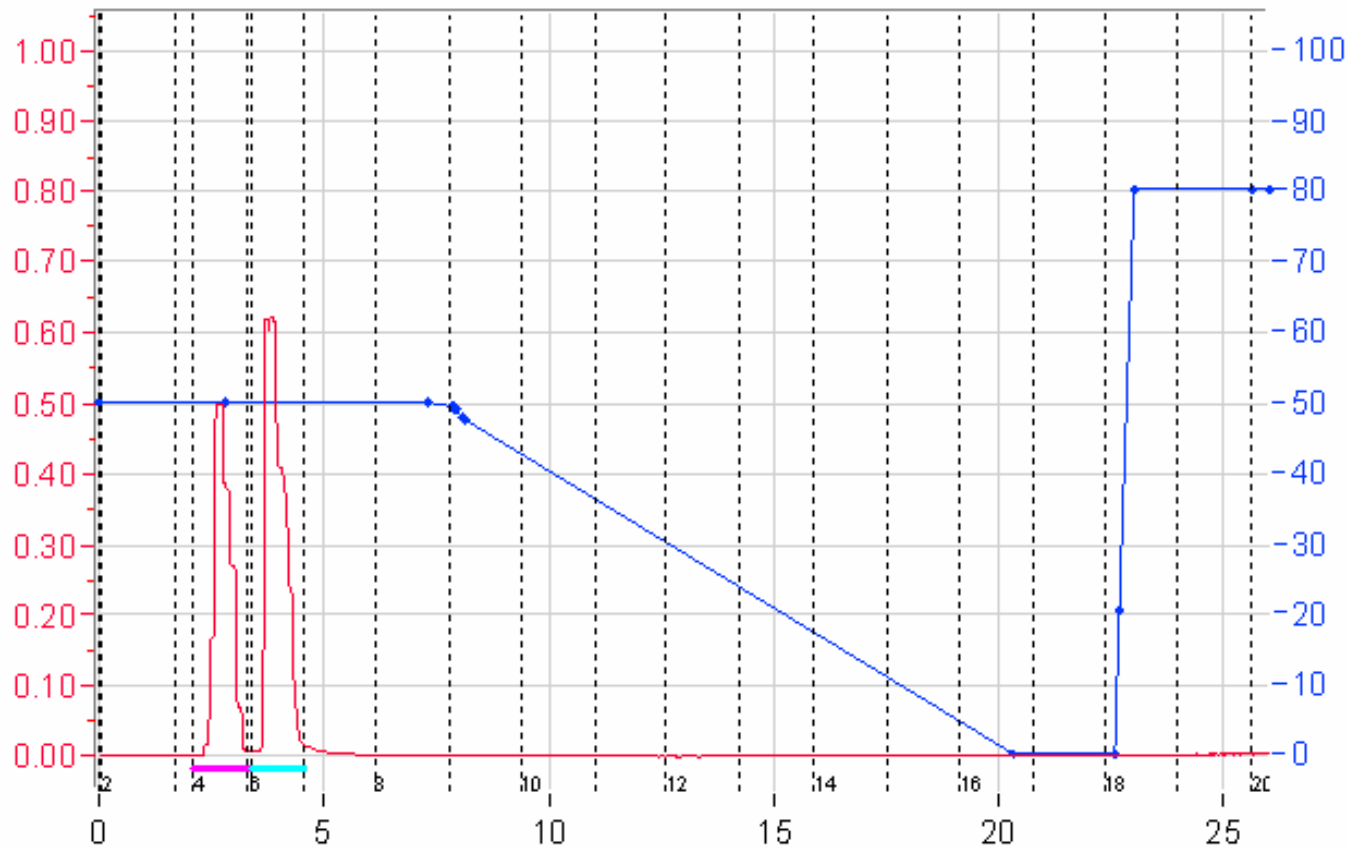
277 nm



Application 4: Natural Product Purification

Scutellaria Baicalensis

- Step 3: Separation of resulting unresolved peaks under refined conditions: Peak group #2



83mg pre-coated on C-18
reversed phase Silica
powder

13g C-18 reversed phase
RediSep column

H₂O/MeCN

15ml/min

277 nm



Purification booklet



Free
www.isco.com/flashguide
flashguide@isco.com

Application notes:
Visit www.isco.com



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Thank you

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