

*Multi-Mode Scout Kits that
Utilize Different Selectivities
as a Key Component in
Choice of Analytical or
Process HPLC Columns*

Leslie Brown, MicroSolv Technology
Corporation, Bill Ciccone

Topics Covered in this Presentation

- Why use Multi-Phase HPLC Columns?
- Which Size HPLC Column is Optimal for Your Separation Objective?
- Mini-Scout Rapid Method Development Kits.

Mini-Scout Kits™.

75mm x 4.6mm ID

Rapid Method Development Kit

50mm x 3.0mm ID

LC-MS Methods Kit™

20mm x 4.0mm ID

Mini LC-MS Kit™

20mm x 2.0mm ID

Micro-Scout Kit™

Mini-Scout Kits™

- Each kit contains:



5 Tested HPLC Column (with C-grams)

2 Super-Link End Fittings

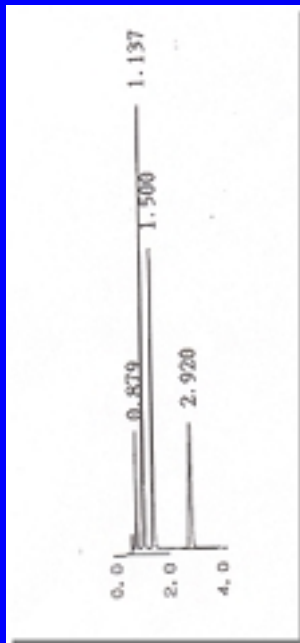
1 Super-Link Column Coupler

1 Instruction Manual

Mini-Scout Kits™

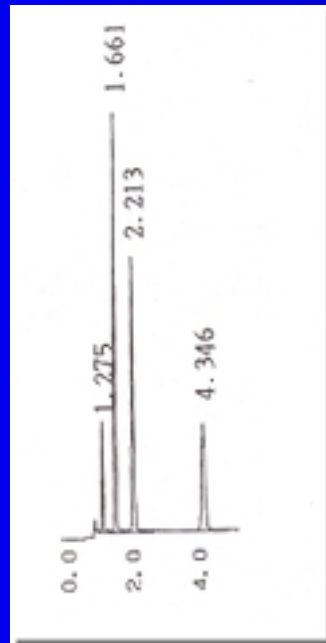
- Why use a 75mm x 4.6mm ID format?

50mm x 4.6



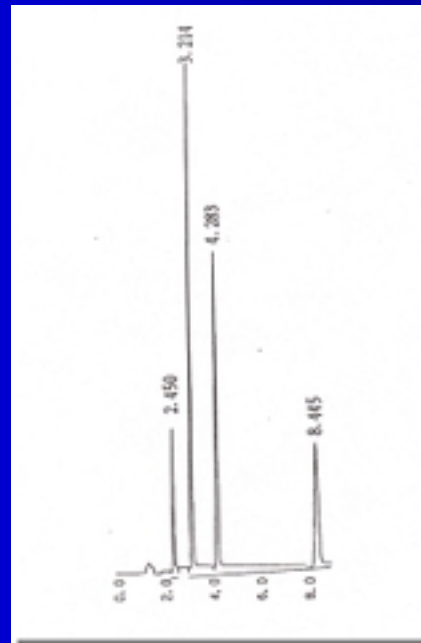
4,675 N/Column
464 psi

75mm x 4.6



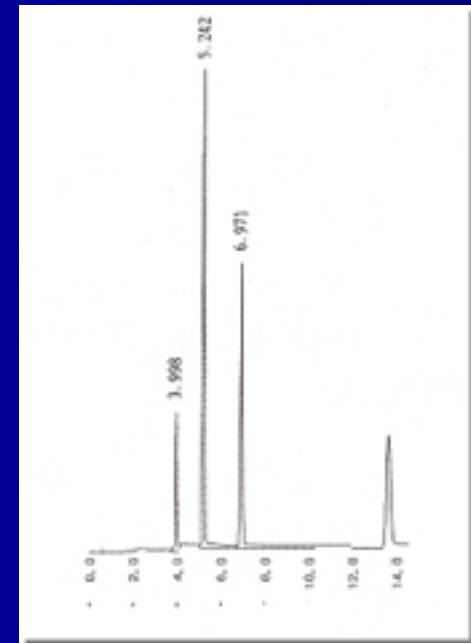
7,433 N/Column
580 psi

150mm x 4.6



14,521 N/Column
957 psi

250mm x 4.6

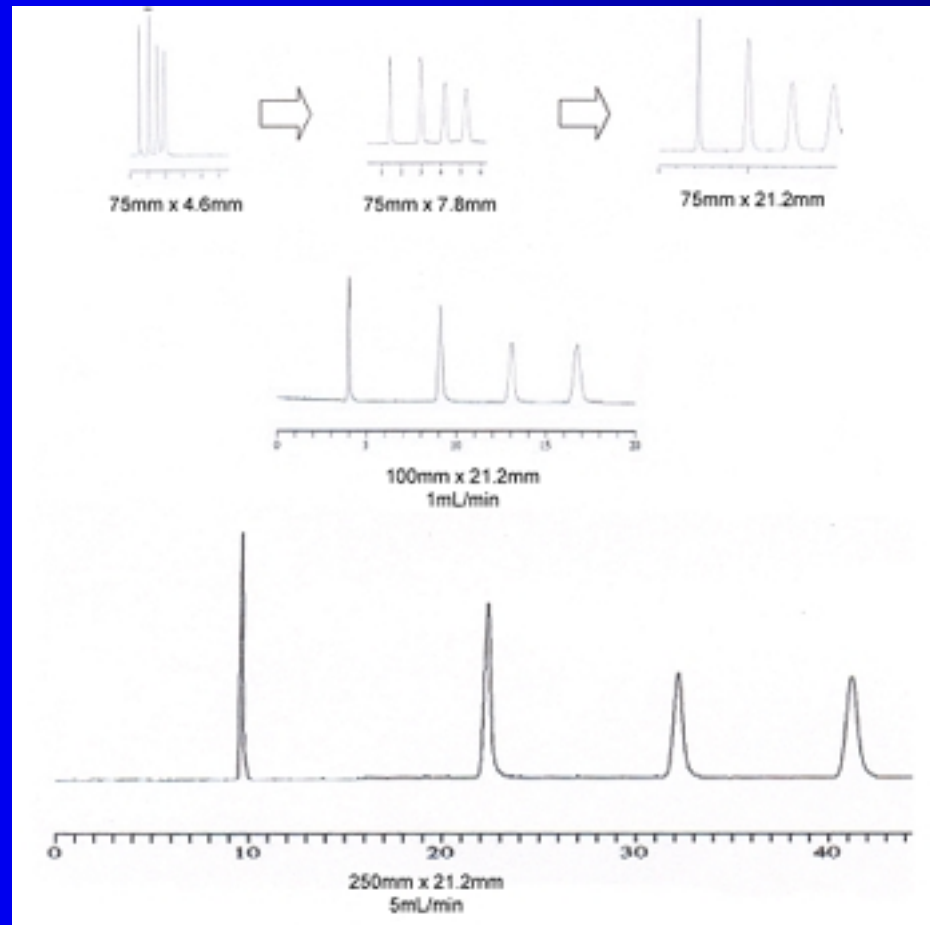


22,505 N/Column
1,377 psi

Flow Rate = 1mL/minute, Ambient

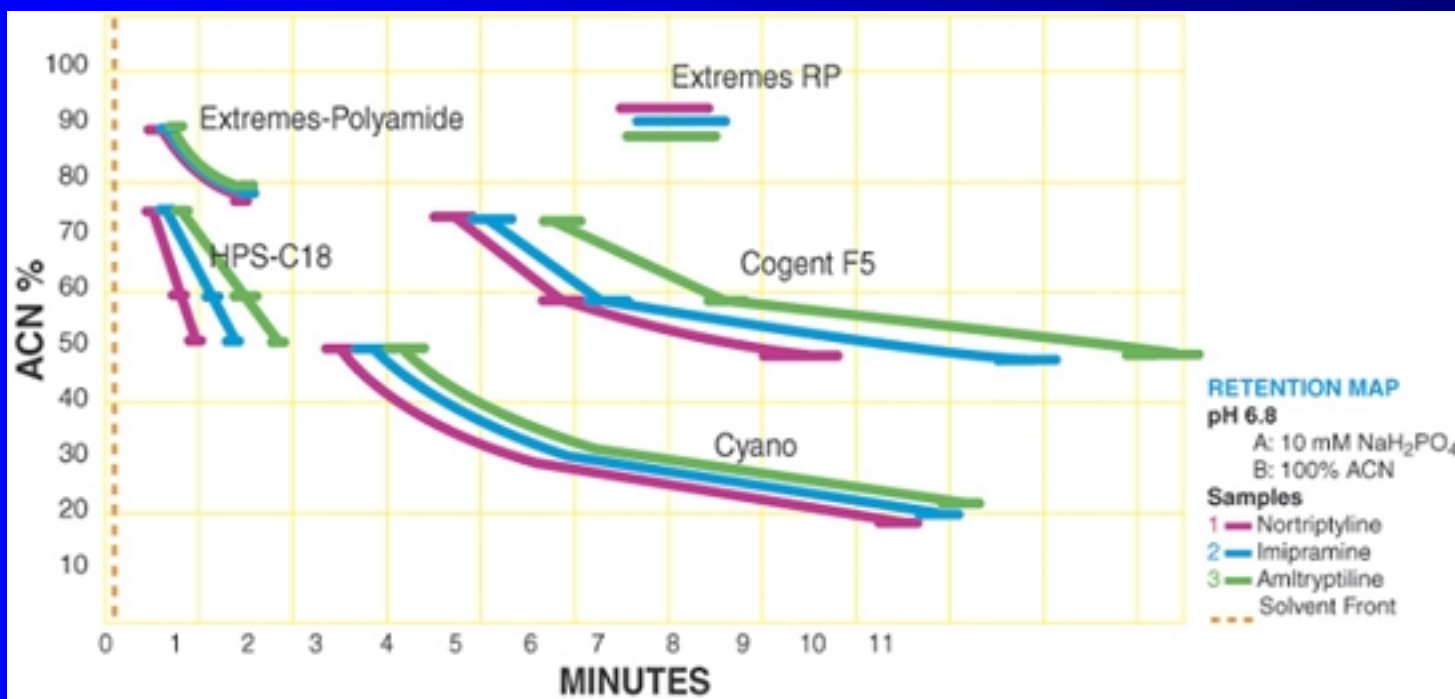
Mini-Scout Kits™

- Use the Mini-Scout Kits to predict Both Optimal Analytical and Preparative HPLC Column Sizes.



Mini-Scout Kits™

- Why Use 5 **Different** Phases in Method Development?



Mini-Scout Kits™

- Why have the Option of **Only** 5 Different Phases?
*Why not have **More** Options?*

UPHOLDC27

Extremes C18

HPS Amino

HPS C8

hQ C18

Aclarity C18

Axis Amino

Cogent Phenyl

Axis C8

Extremes RP

Cogent F5

HPS C18

HPS Cyano

hQ C8

Aclarity C8

Axis Cyano

Axis C18

Aegis C18 (Coming Soon)

Mini-Scout Kits™

- Advance Your Lab's Output with Pre-Mapped Phases.

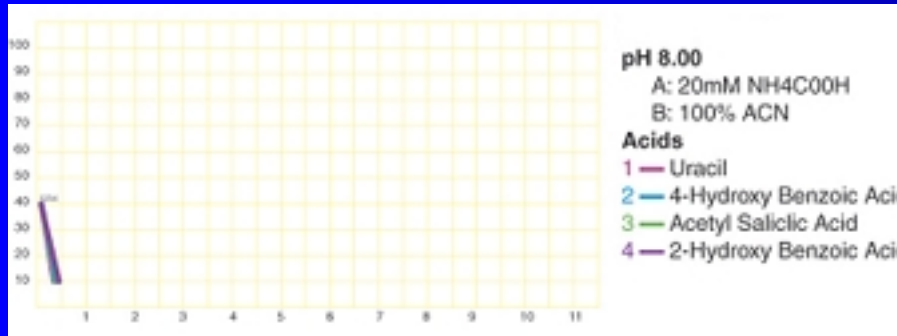
Acidic Compounds

Basic Compounds

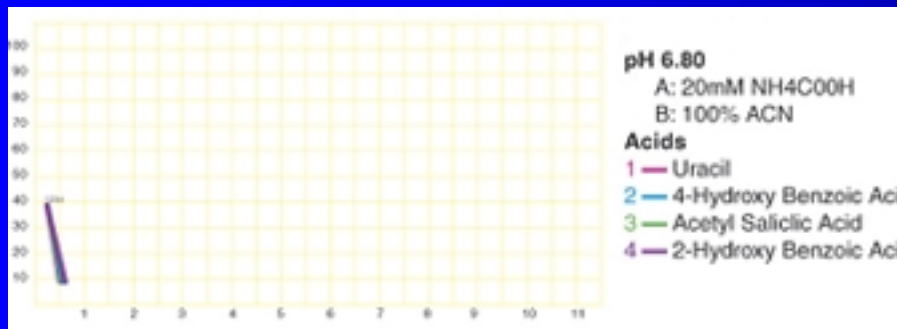
Neutral Compounds

SELECTIVITY

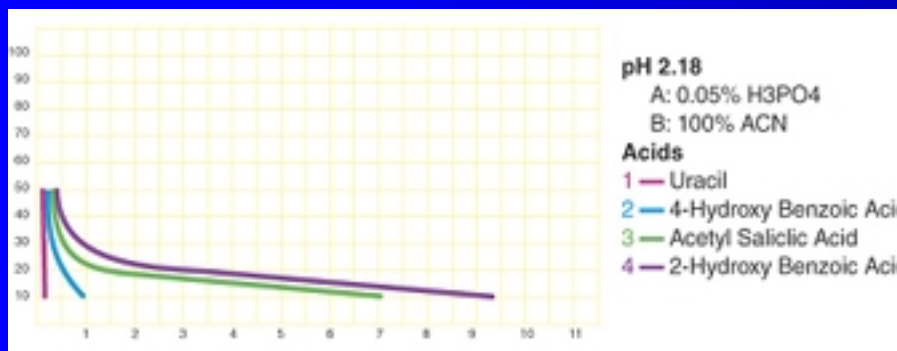
Acidic Compounds



- pH 8.00
20mM NH₄COOH + NH₄OH



- pH 6.80
20mM NH₄COOH + NH₄OH

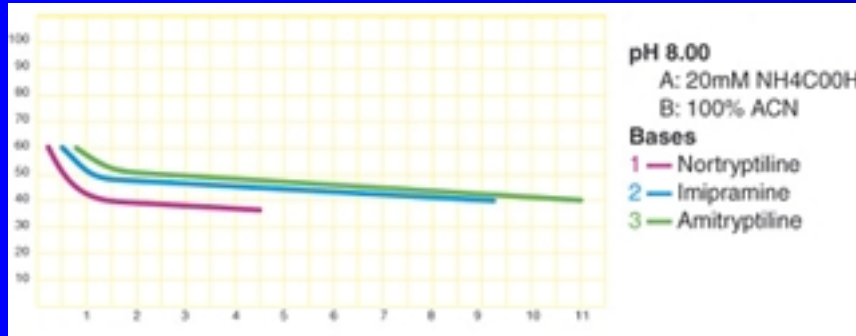


- pH 2.18
0.05% v/u Phosphoric Acid/DW

Flow Rate = 3mL/min

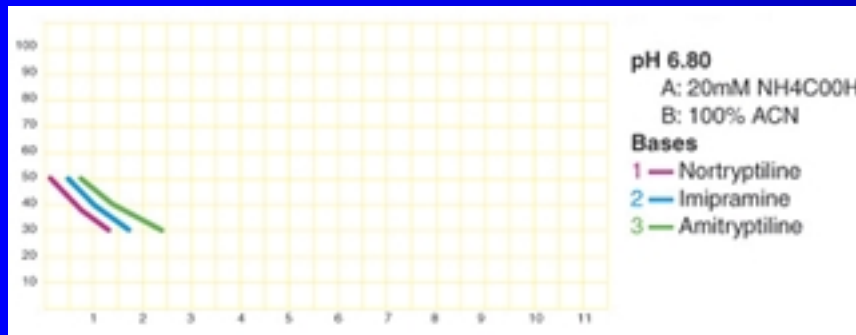
Note typical shape of retention map.

Basic Compounds



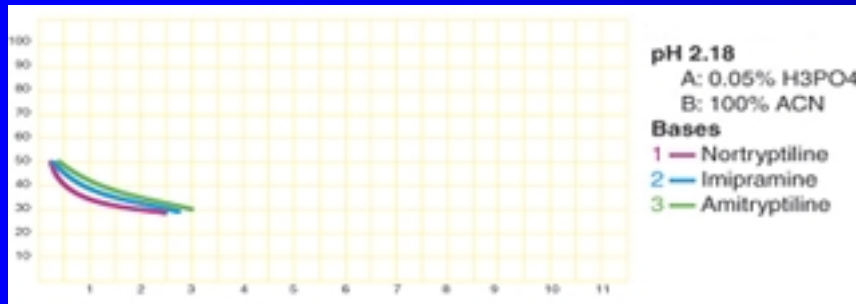
● pH 8.00

20mM NH₄COOH + NH₄OH



● pH 6.80

20mM NH₄COOH + NH₄OH



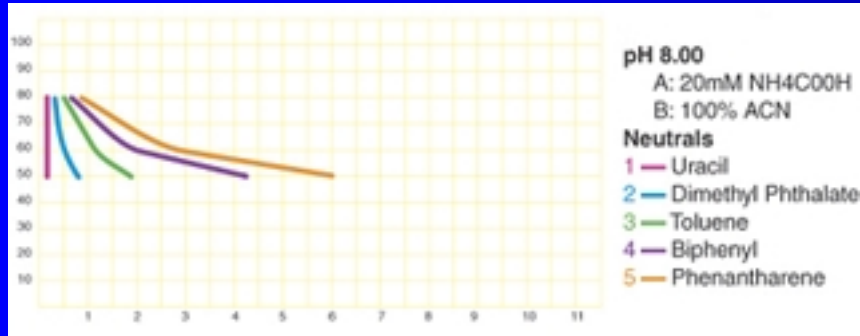
● pH 2.18

0.05% v/u Phosphoric Acid/DW

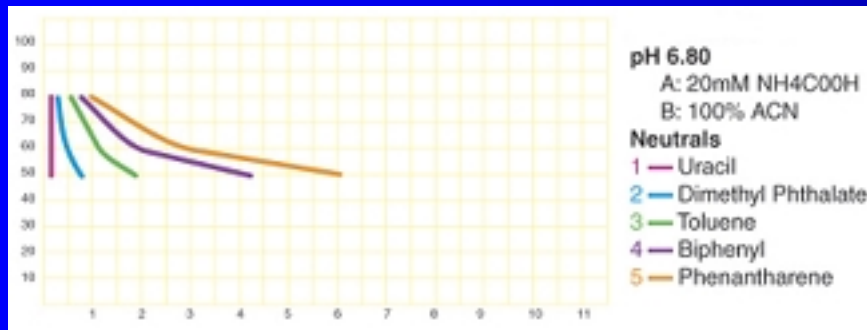
Flow Rate = 3mL/min

Note typical shape of retention map.

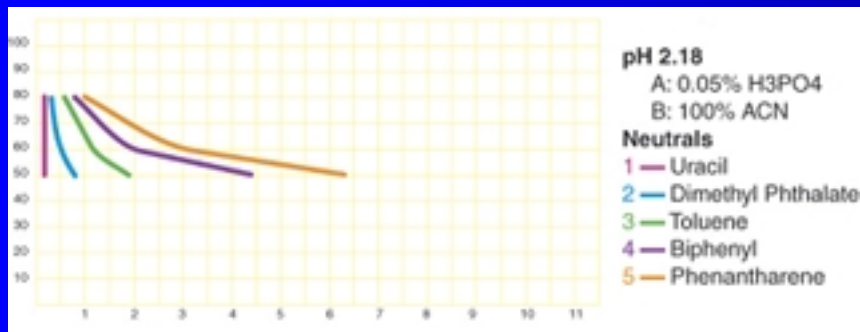
Neutral Compounds



- pH 8.00
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20mM NH₄COOH + NH₄OH



- pH 2.18
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Flow Rate = 3mL/min

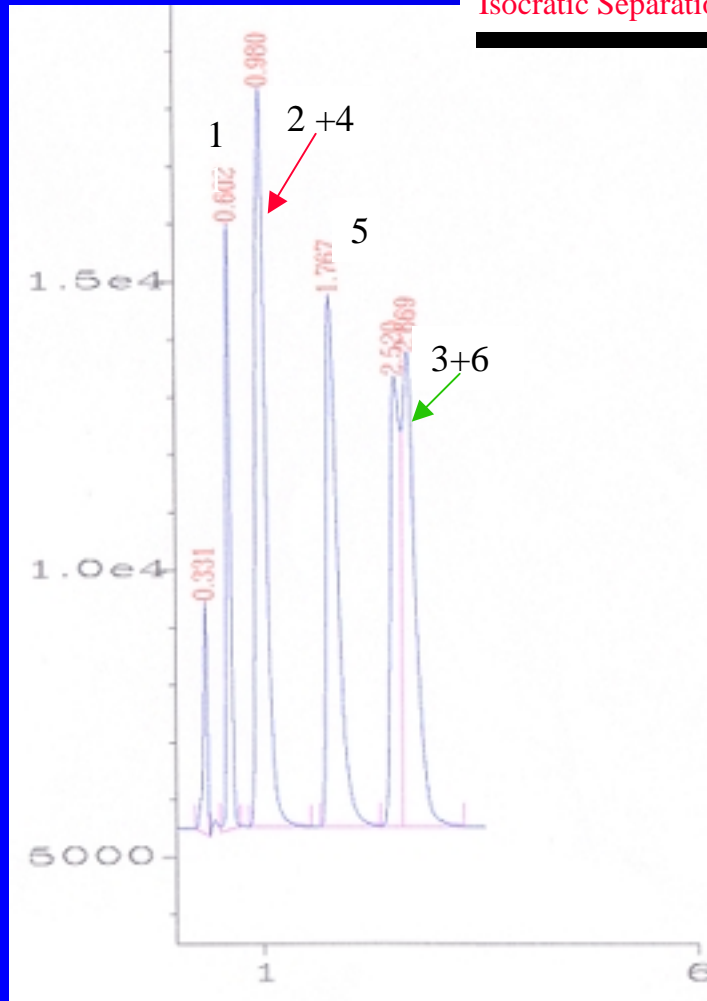
Note typical shape of retention map.

Significance of Mapping to “Multi-Mode” Generic Method Development.

- *Unique Separation Opportunities:*

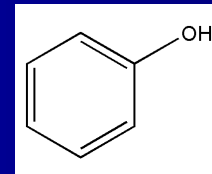
Cogent HPS C18

Isocratic Separation

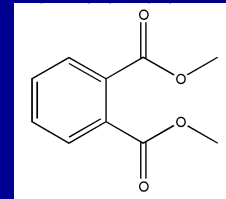


3mL/min 20mM NH_4COOH &
 NH_4OH pH 8.00

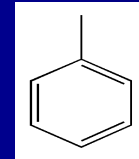
(1) *Phenol*



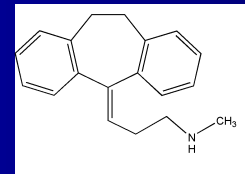
(2) *Dimethyl Phthalate*



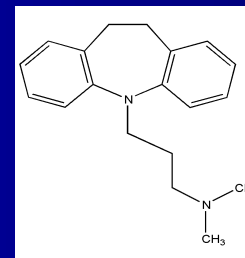
(3) *Toluene*



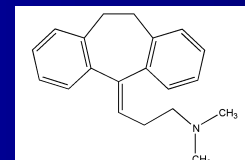
(4) *Nortriptyline*



(5) *Imipramine*

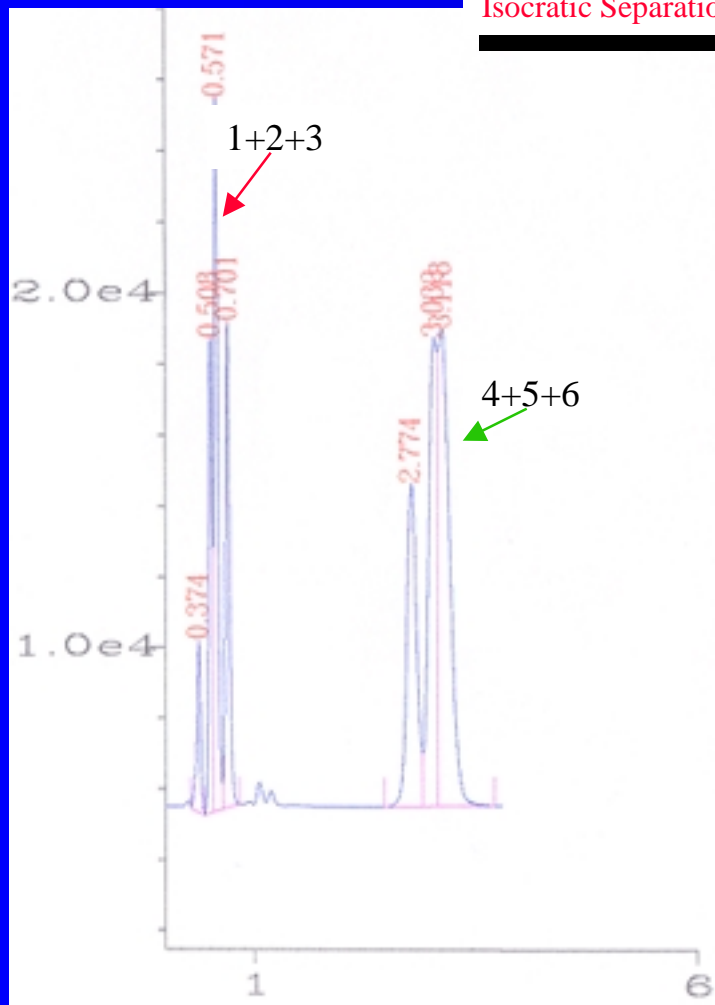


(6) *Amitriptyline*



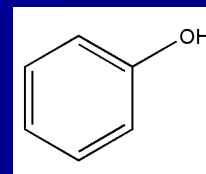
Cogent HPS Cyano

Isocratic Separation

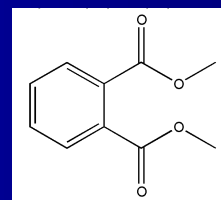


3mL/min 20mM NH_4COOH &
 NH_4OH pH 8.00

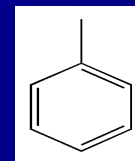
(1) Phenol



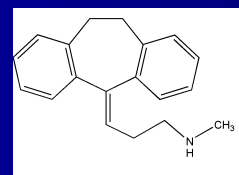
(2) Dimethyl Phthalate



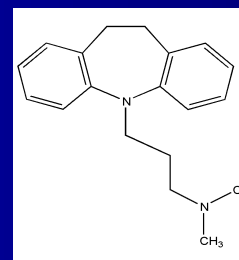
(3) Toluene



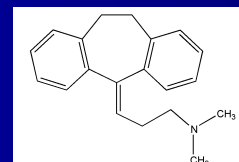
(4) Nortriptyline



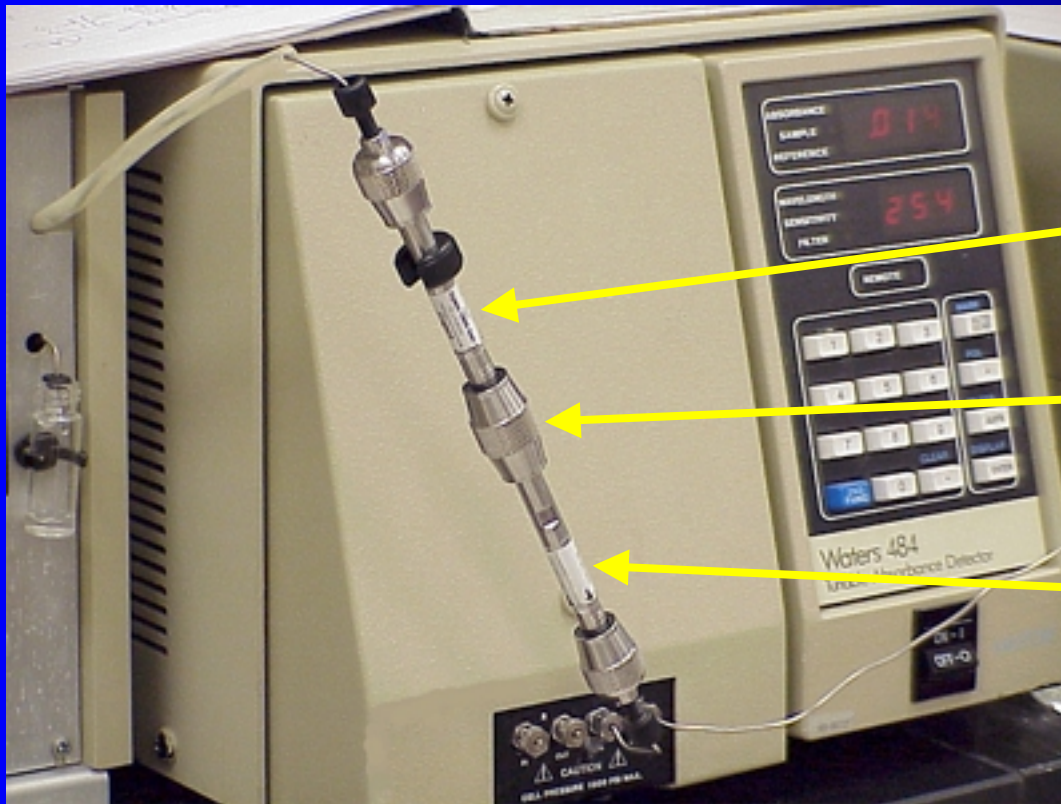
(5) Imipramine



(6) Amitriptyline



Multi-Mode Chromatography



HPS C18

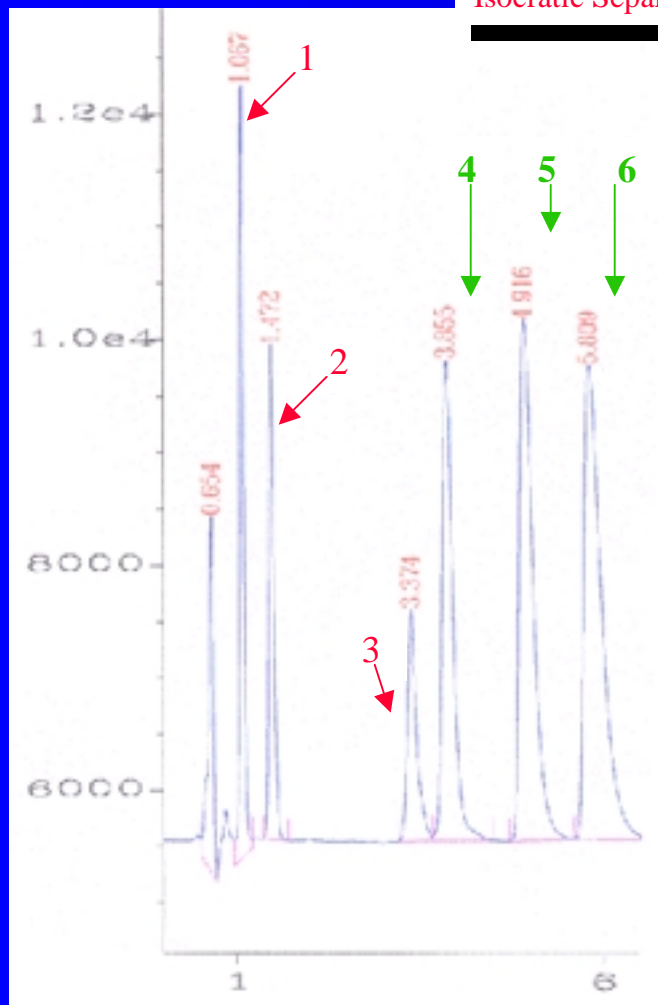
Coupler

HPS Cyano



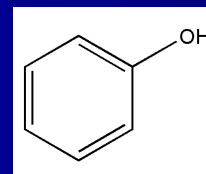
Multi-Mode w/ HPS C18 and Cyano

Isocratic Separation

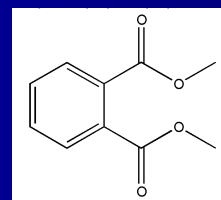


3mL/min 20mM NH_4COOH &
 NH_4OH pH 8.00

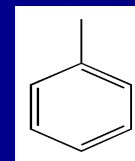
(1) Phenol



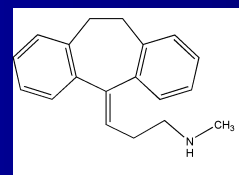
(2) Dimethyl Phthalate



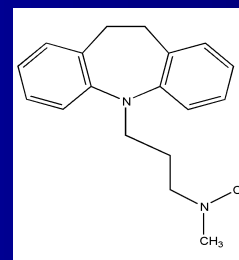
(3) Toluene



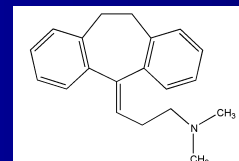
(4) Nortriptyline



(5) Imipramine



(6) Amitriptyline



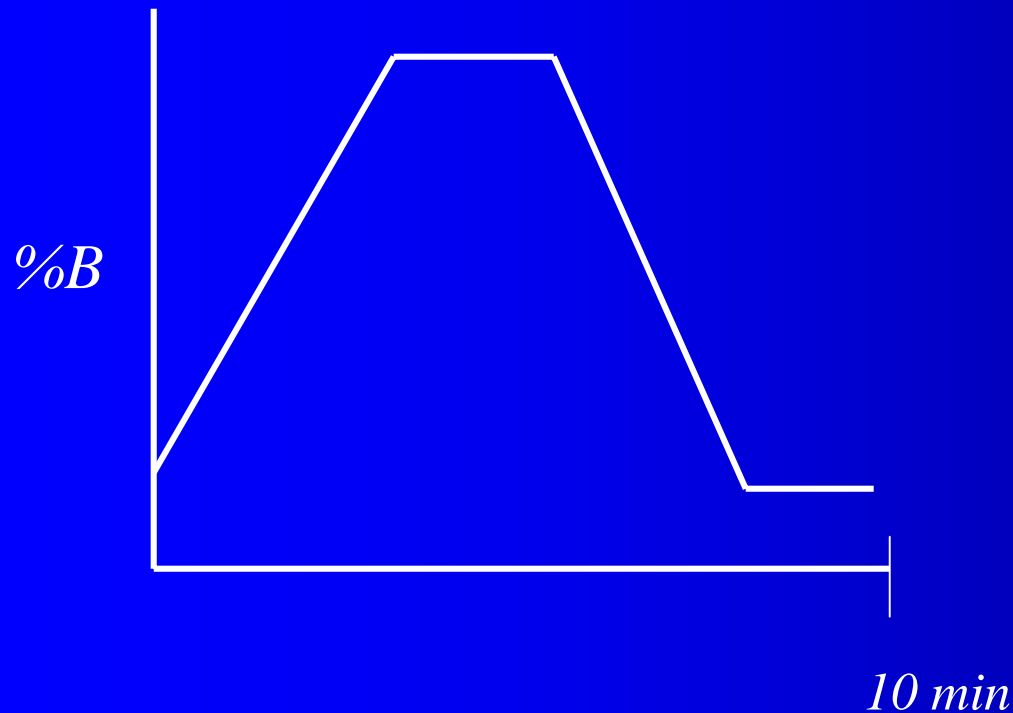
Multi-Mode Chromatography

- Unlike Mixed Mode Chromatography
- Multi-Mode is reproducible...every time

Significance of Mapping to Mini-Scout Generic Gradient Strategy

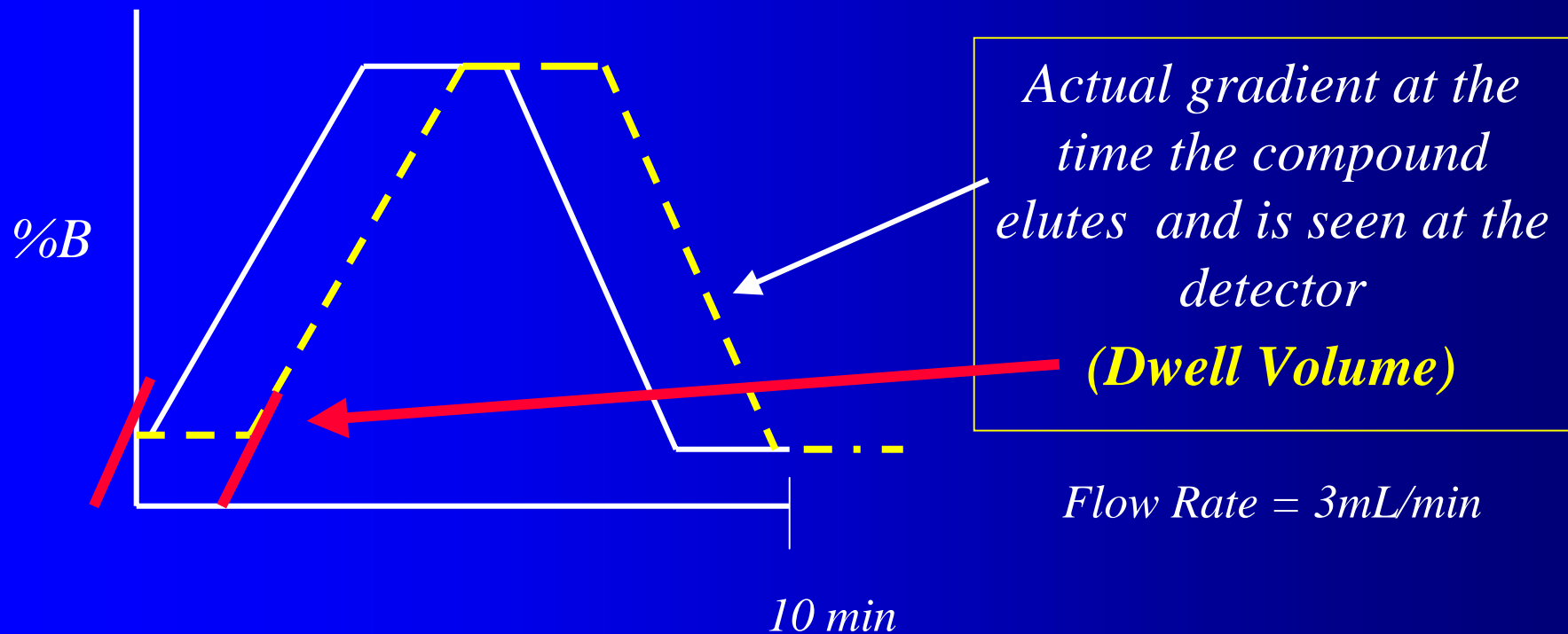
Significance of Mapping to Mini-Scout Generic Gradient Strategy

Programmed Gradient



*Start % Depends on the
solubility of the least soluble
component.*

Significance of Mapping to Mini-Scout Generic Gradient Strategy



Mini-Scout Format Columns Allow for Rapid Analyses

- Run 75mm x 4.6mm ID Columns at 3mL/min
- Run **FIVE** or More Different Selectivities in the **SAME TIME** as a Standard Search Gradient run at 1mL/min with a 250mm x 4.6mm ID Column

**Once you have chosen the most appropriate
phase for best selectivity,
a Generic Gradient may be rapidly optimized.**

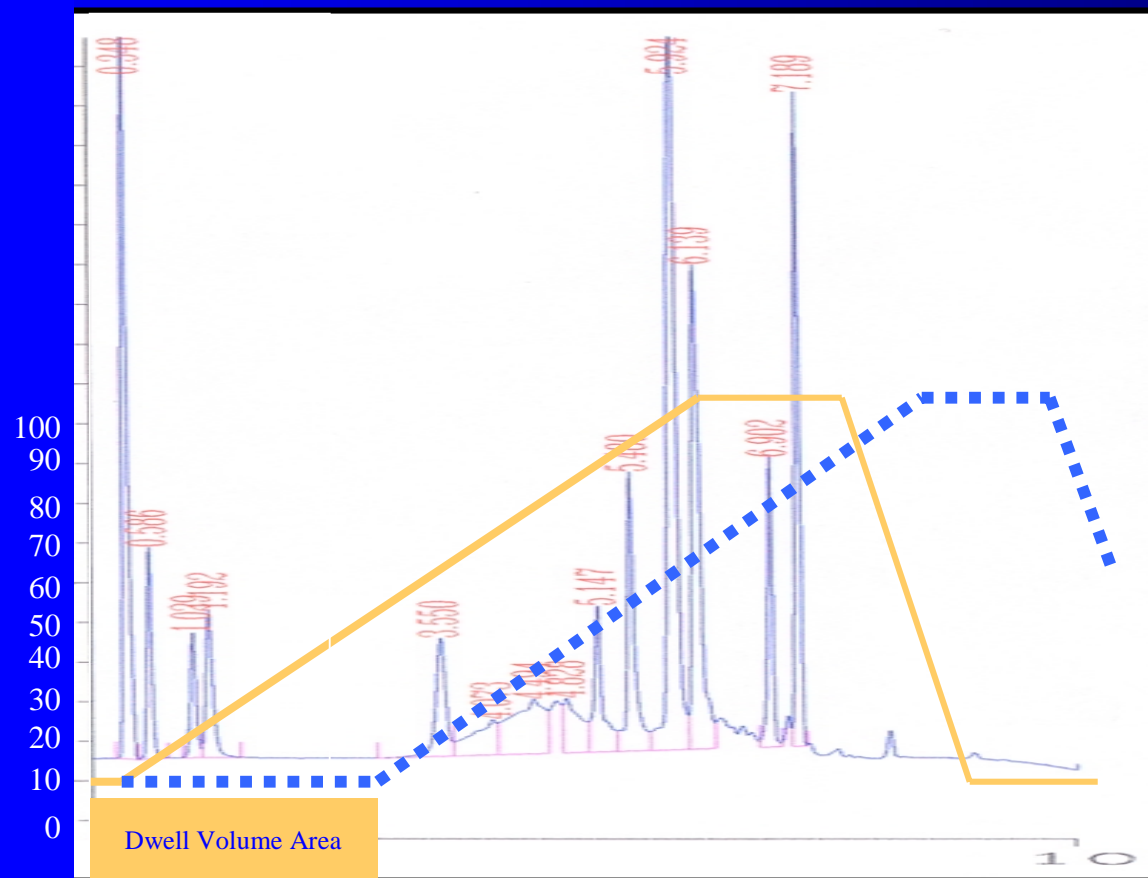
Once you have chosen the most appropriate phase for best selectivity, a Generic Gradient may be rapidly optimized.

*Compounds Tested by Generic Gradient Strategy
in next 3 Slides*

1. Nortriptyline
2. Imipramine
3. Amitriptyline
4. 4-Hydroxy Benzoic Acid
5. Acetyl Salicylic Acid
6. 2-Hydroxy Benzoic Acid
7. Dimethyl Phthalate
8. Toluene
9. Biphenyl
10. Phenanthrene
11. Pyridine
12. Phenol

Generic Gradient No. 1

% ACN



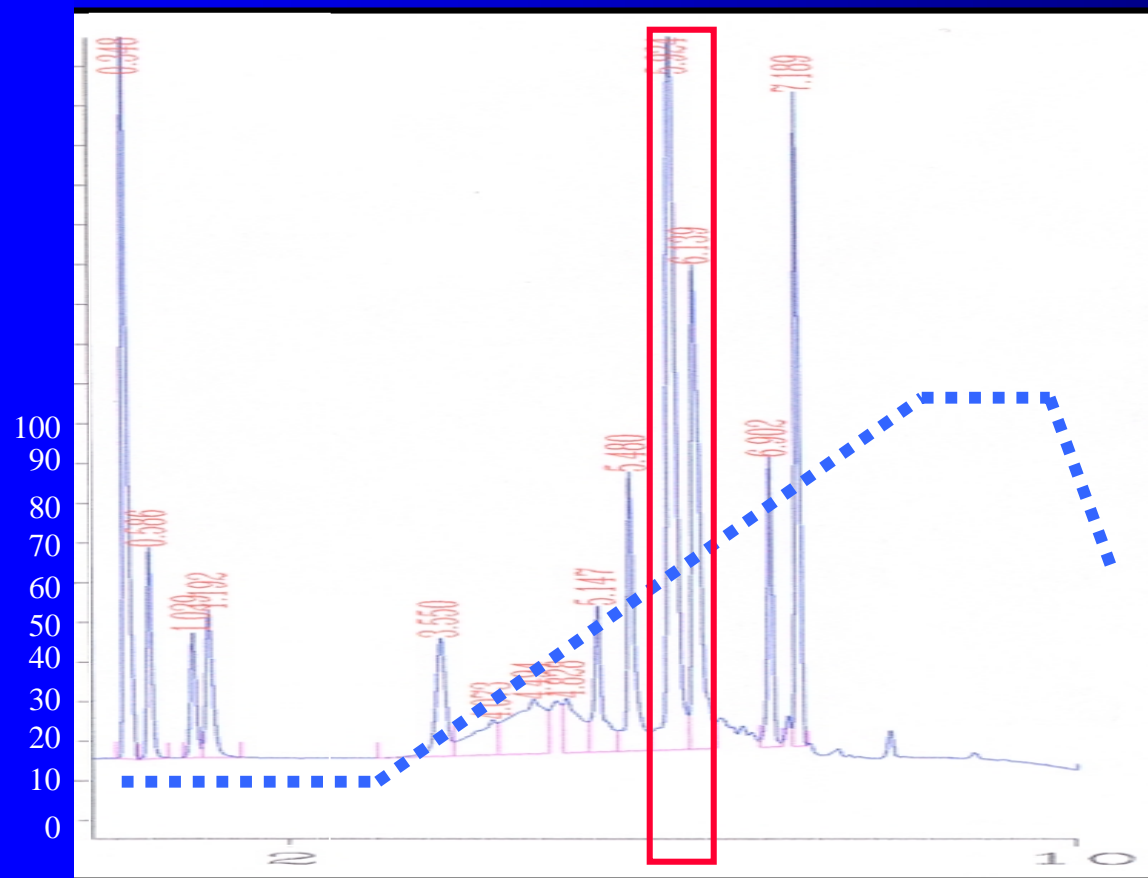
HPS C18
75 x 4.6mm
3mL/Min
1400psig

A= pH 8.00 20mM NH₄COOH & NH₄OH

B= Acetonitrile

Generic Gradient No. 1

% ACN



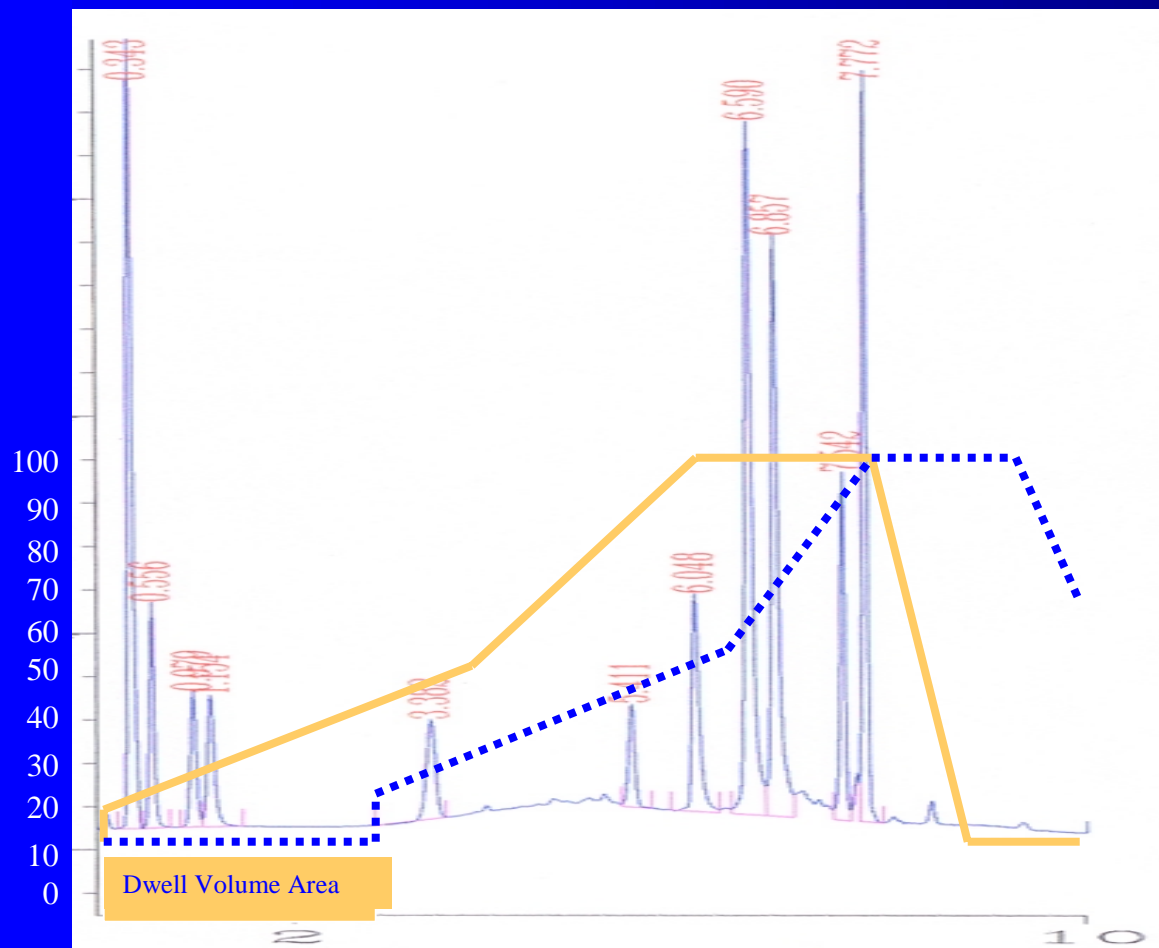
HPS C18
75 x 4.6mm
3mL/Min
1400psig

A= pH 8.00 20mM NH₄COOH & NH₄OH

B= Acetonitrile

First Optimization

% ACN

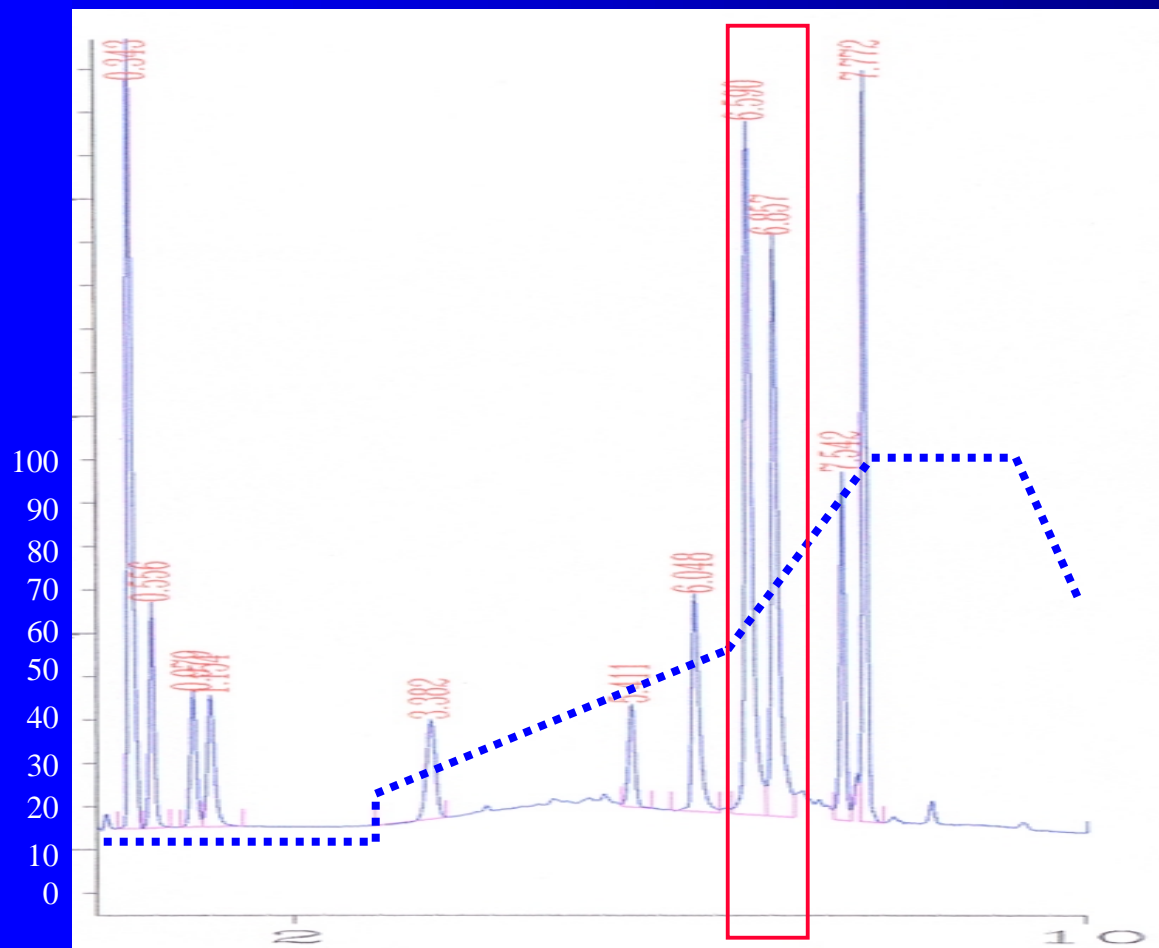


HPS C18
75 x 4.6mm
3mL/Min
1400psig

Note: Due to lag, there is no change in initial group of compounds

First Optimization

% ACN

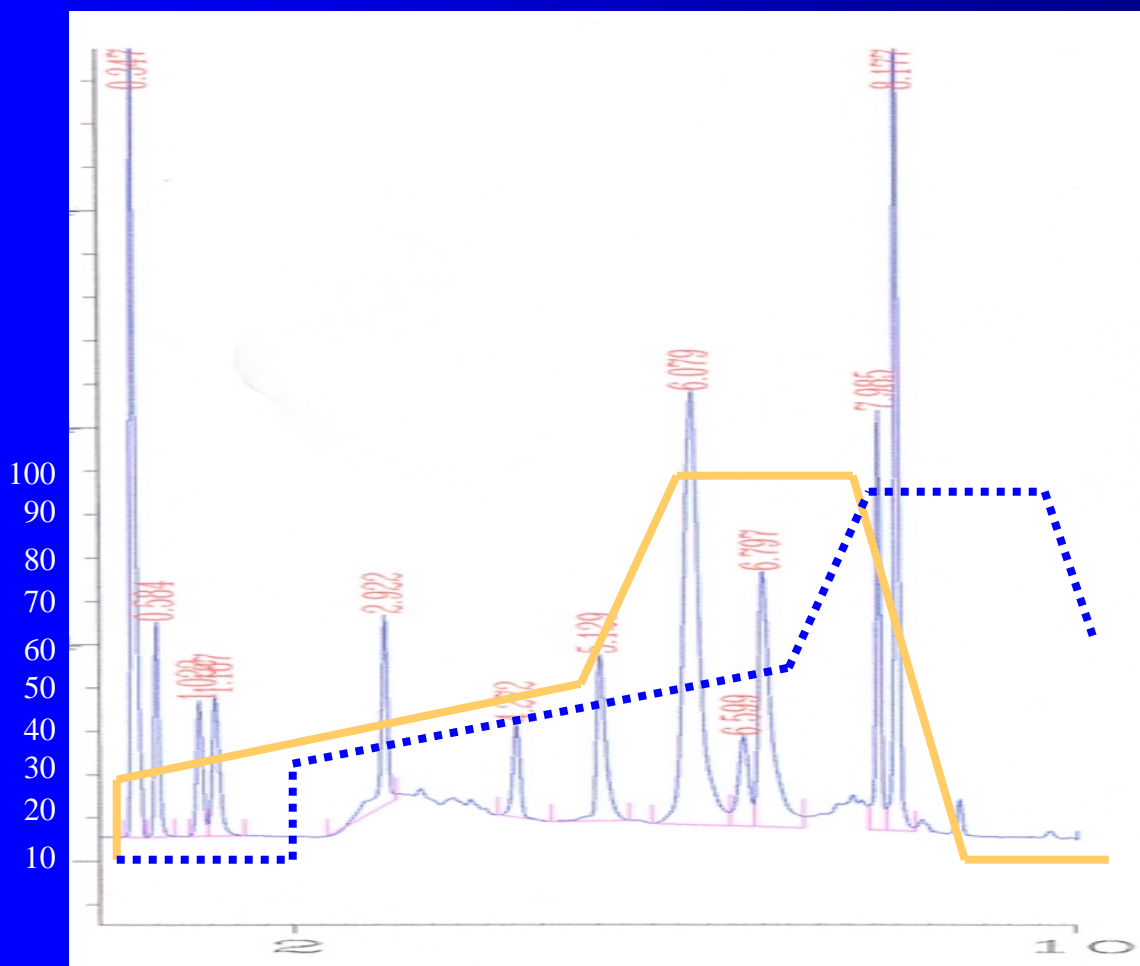


HPS C18
75 x 4.6mm
3mL/Min
1400psig

Note: Due to lag, there is no change in initial group of compounds

Second Optimization

% ACN

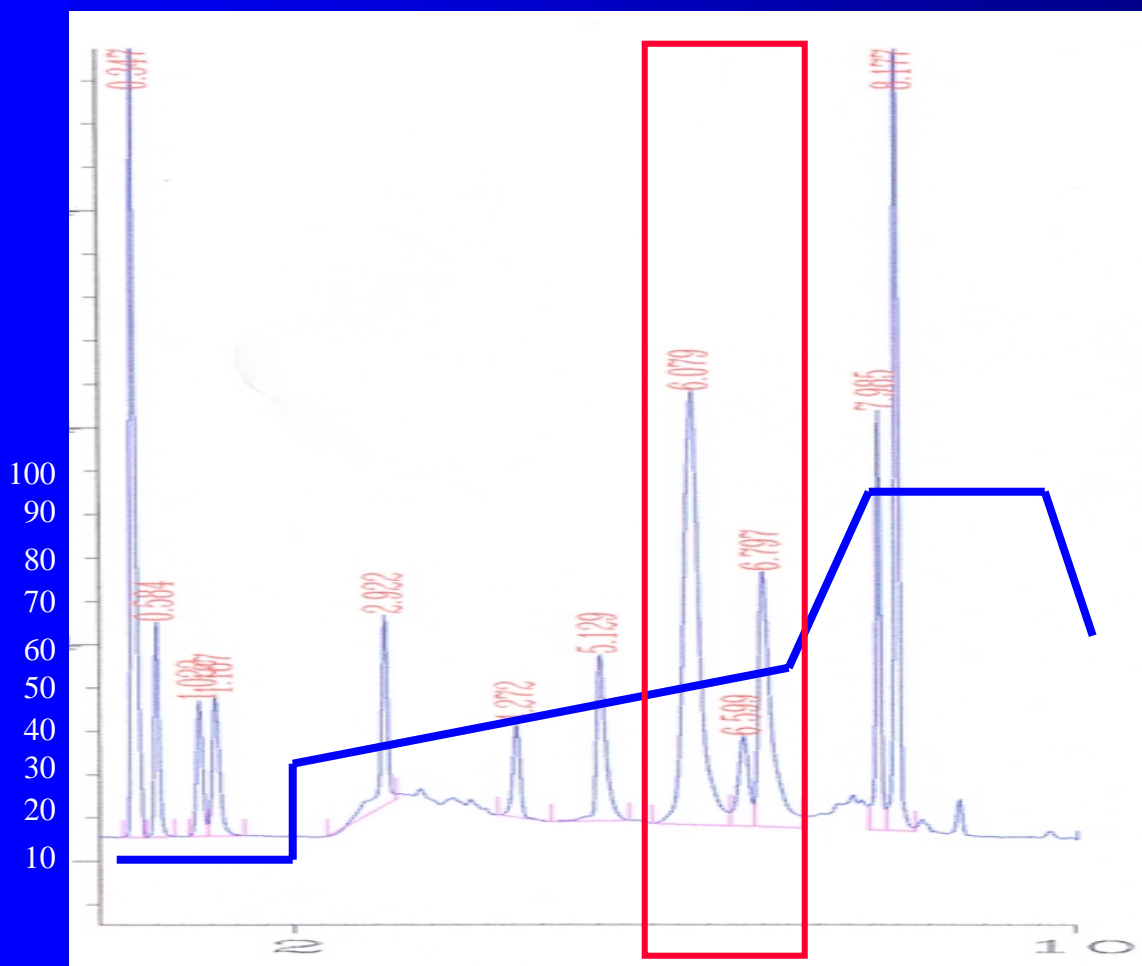


HPS C18
75 x 4.6mm
3mL/Min
1400psig

Note: As initial area, 0-3 Min ok, Step from 10-30% Then flatten to 30-50% in to 5 Min, Then Quick wash-off.

Second Optimization

% ACN

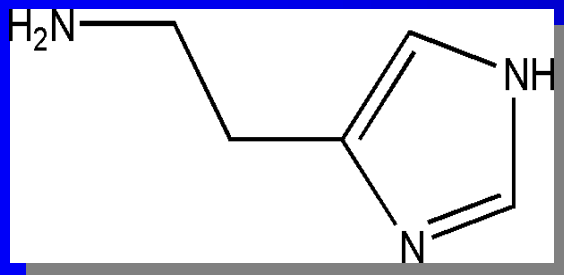


HPS C18
75 x 4.6mm
3mL/Min
1400psig

Note: As initial area, 0-3 Min ok, Step from 10-30% Then flatten to 30-50% in to 5 Min, Then Quick wash-off.

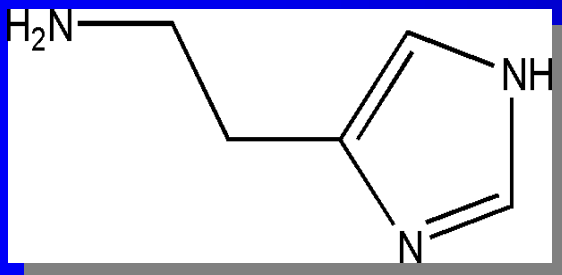
Separation of Histamine using the Mini-Scout™ Strategy

Separation of Histamine using the Mini-Scout™ Strategy



Histamine

Separation of Histamine using the Mini-Scout™ Strategy



Histamine

Phases Tried:

HPS C18

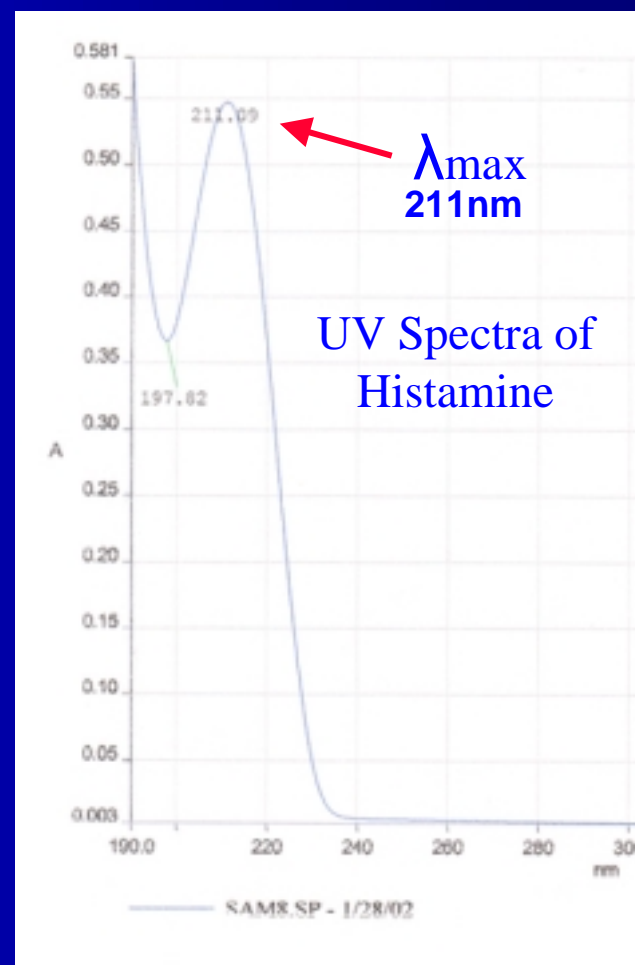
HPS Cyano

Axis Phenyl

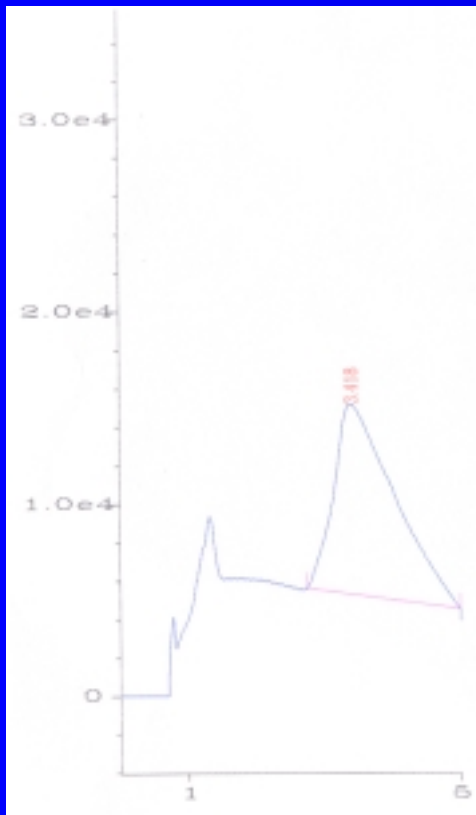
Cogent Extremes RP

Cogent Extremes C18

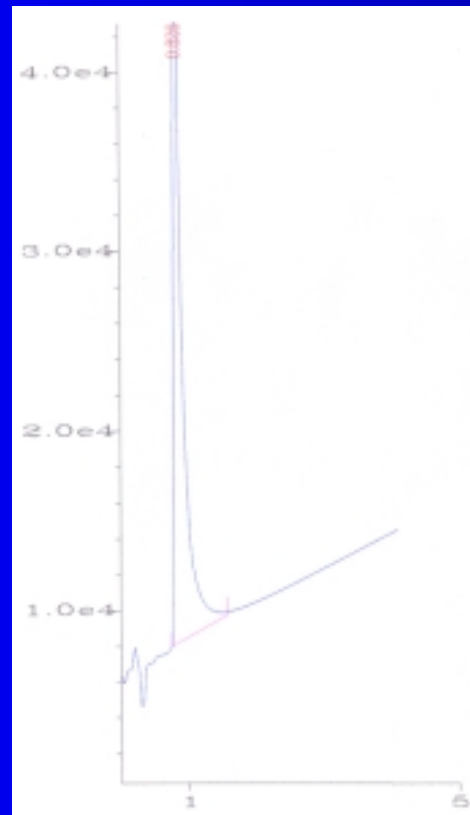
Cogent F5



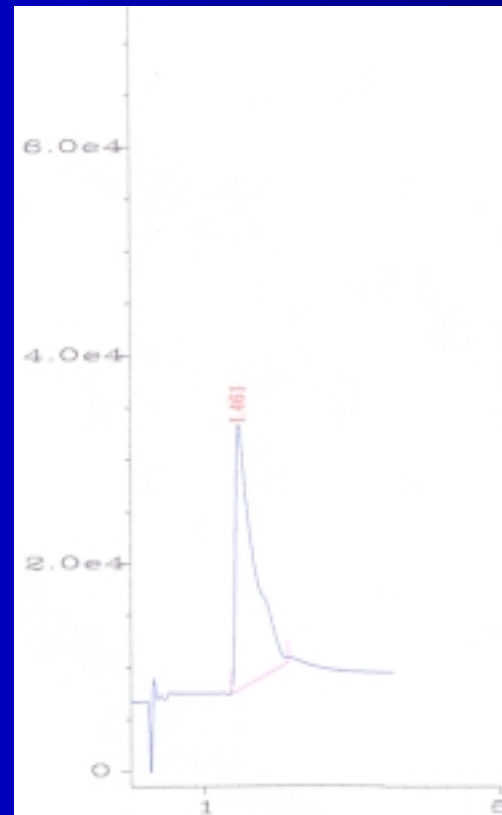
Optimization of Molarity of Ammonium Formate on a Phenyl HPLC Column



20mM



10mM



5mM

Cogent™
Phenyl

75x4.6mm

Flow Rate=
3mL/min

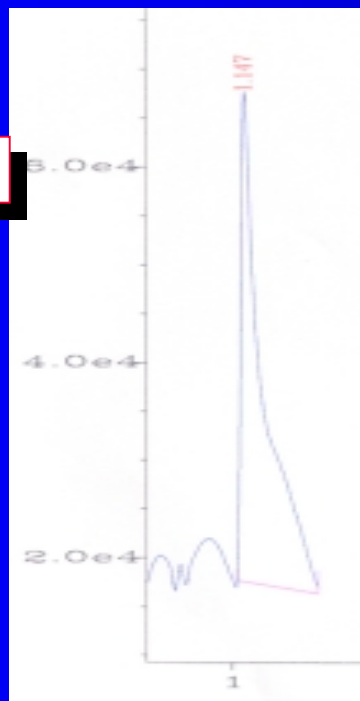
600psig

Optimize for Peak Shape and Retention on a Cogent Phenyl HPLC Column



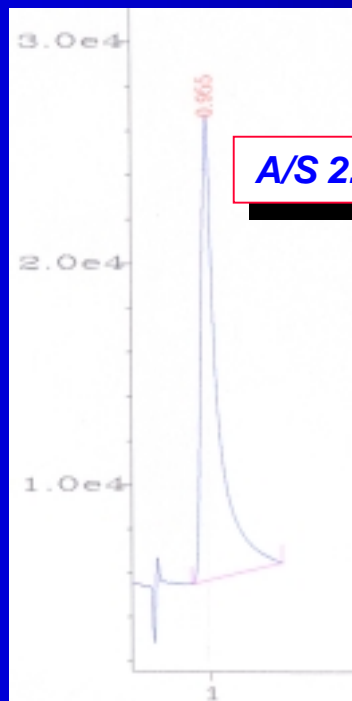
A/S 2.8

0.1M KCL
(pH 8.0)



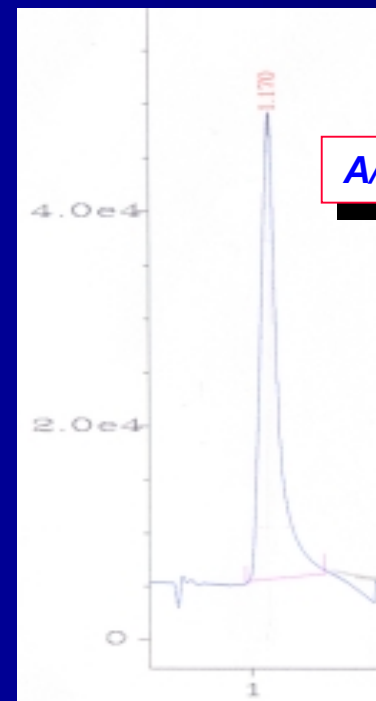
A/S 2.0

0.1M KCL
(pH 8.5)



A/S 1.4

TEA
(pH 8.0)

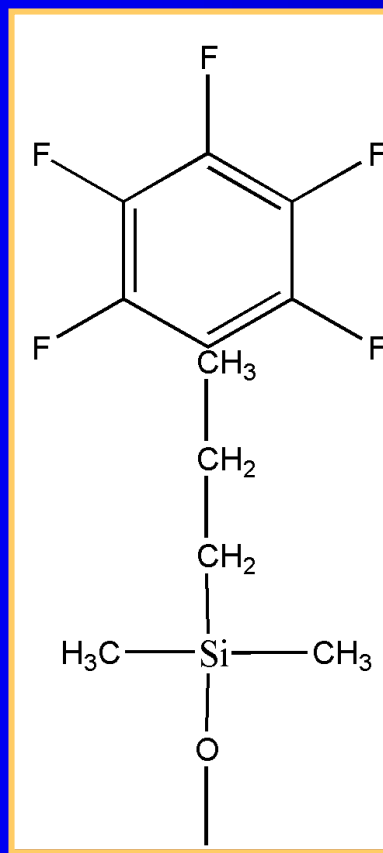


0.1M KCL + 2%ACN
(pH 8.0)

5mM Ammonium Formate (pH 8.0) 3mL/min

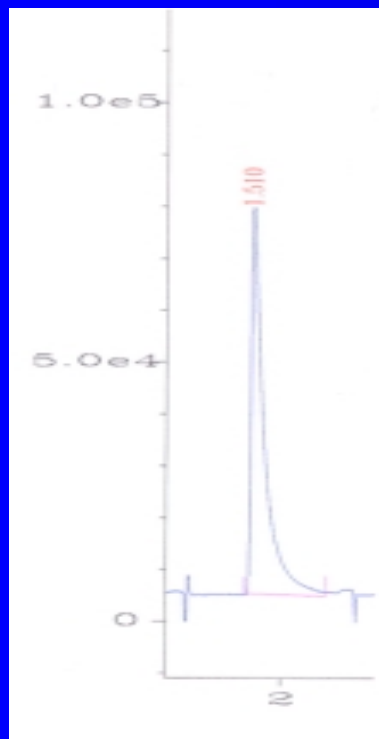
Cogent™ Phenyl Column-75 x 4.6mm ID

Unique Behavior of Histamine on a Cogent F5™

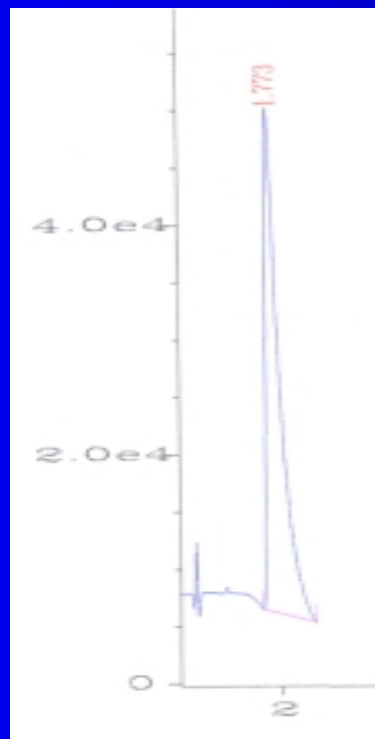


PentaFluoro Phenyl

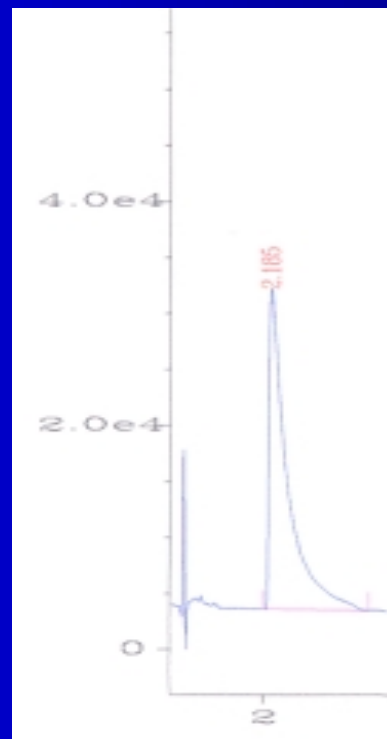
Unique Behavior of Histamine on a Cogent F5™



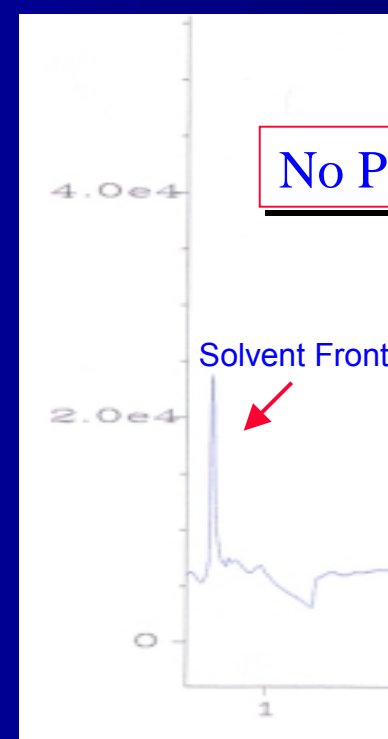
90:10 ACN



30:70 ACN



20:80 ACN



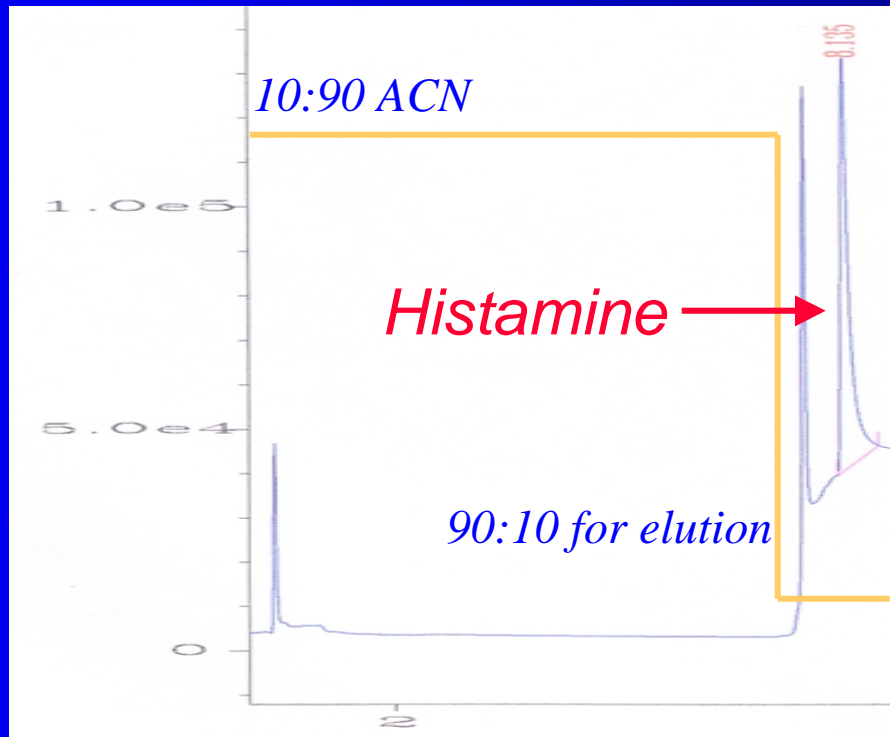
10:90 ACN

75mm x 4.6mm ID

5mM Ammonium Formate & Ammonium Hydroxide pH 8.0

3mL/minute

Unique Behavior of Histamine on a Cogent F5™



Histamine is retained on F5 with 90% ACN for as long as required to remove from other components. Release it from the column a steep drop to 10%.

Mini-Scout Kits Provide:

- Speed
- Selectivity Choices
- Versatility
- Multi-Mode™ Option
- Multiple Column Size Choices for LC-MS

**Advance Your
Lab's Output**

MicroSolv Technology Corporation

- 101 Brighton Avenue
- Long Branch, NJ 07740 USA

Visit our Website for More Information

- www.MicroSolvTech.com

