

BioBasic[®] Strong Cation Exchange HPLC Columns

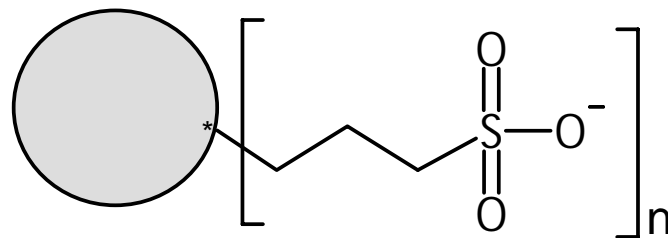
Overview

- Ion Exchange Chromatography
 - *Physical Properties of BioBasic SCX*
 - *Chromatographic behavior*
 - Key chromatographic properties
- Applications
 - *Biomolecules*
 - *Small Molecules*
- BioBasic SCX for 2D LC/MS
 - *Protein and Peptide fractionation*

Physical Properties – BioBasic SCX

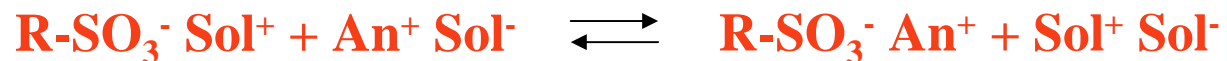
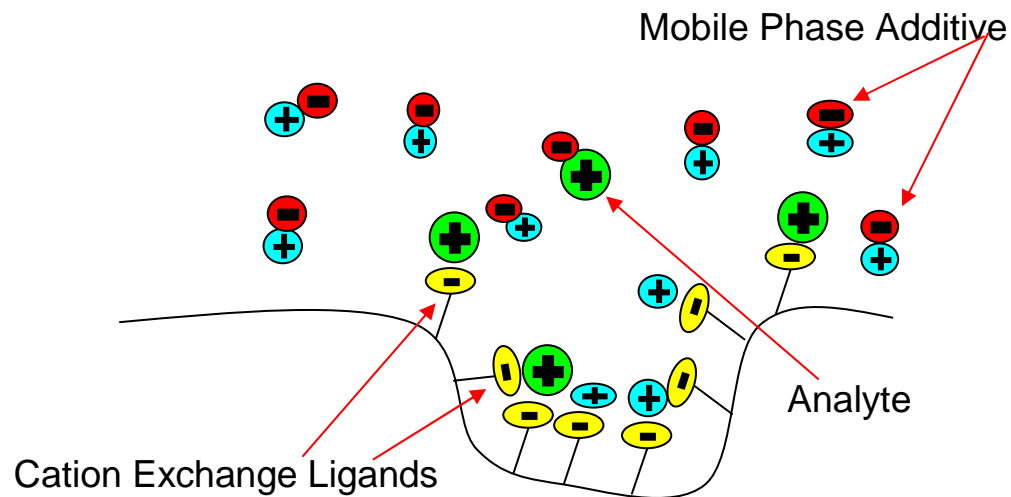
- Silica based cation exchange stationary phase
 - *Very pure 5 μ m, 300Å pore spherical silica substrate*
- Sulfonic acid cation-based exchange ligand
- Ligand covalently bound to proprietary, protein friendly, polymer coated silica

Sulfonic Acid Structure



Cation Exchange Mechanism

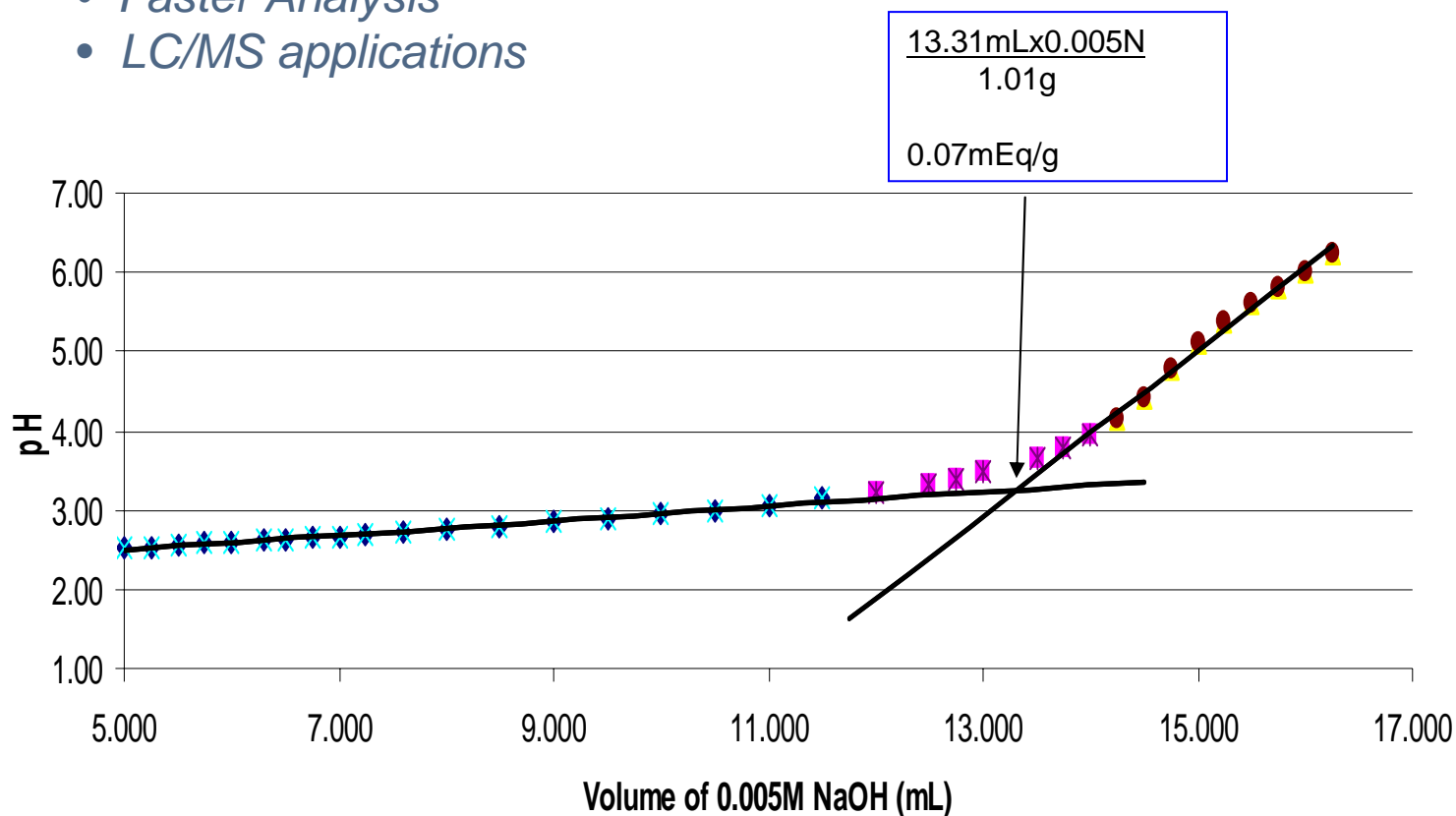
- Retention is based on the ion exchange mechanism between the sulfonic acid ligand and the ionized analyte
- Retention is affected by pH, ionic strength, and “counter-ion” character of the mobile phase additive as well as net charge of the analyte



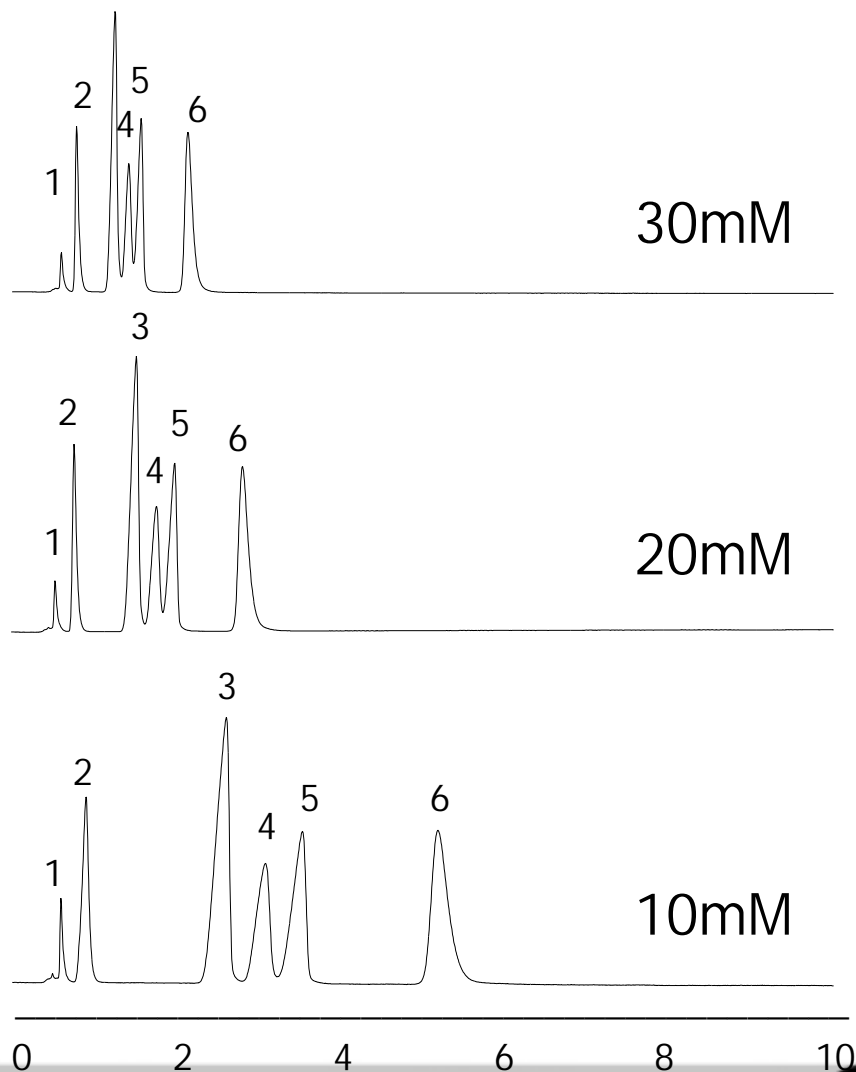
Ion Exchange Capacity

Ion exchange capacity of BioBasic SCX facilitates the use of lower additive concentrations

- *Faster Analysis*
- *LC/MS applications*



Effect of Additive Concentration on Retention



BioBasic SCX, 5 μ m, 50x4.6mm

Eluent: A: NH₄ acetate, pH 4.5

B: Acetonitrile

60% A / 40% B

Flow: 1.0 mL/min

Detector: UV @ 254

Sample: 1. Caffeine

2. Pyridine

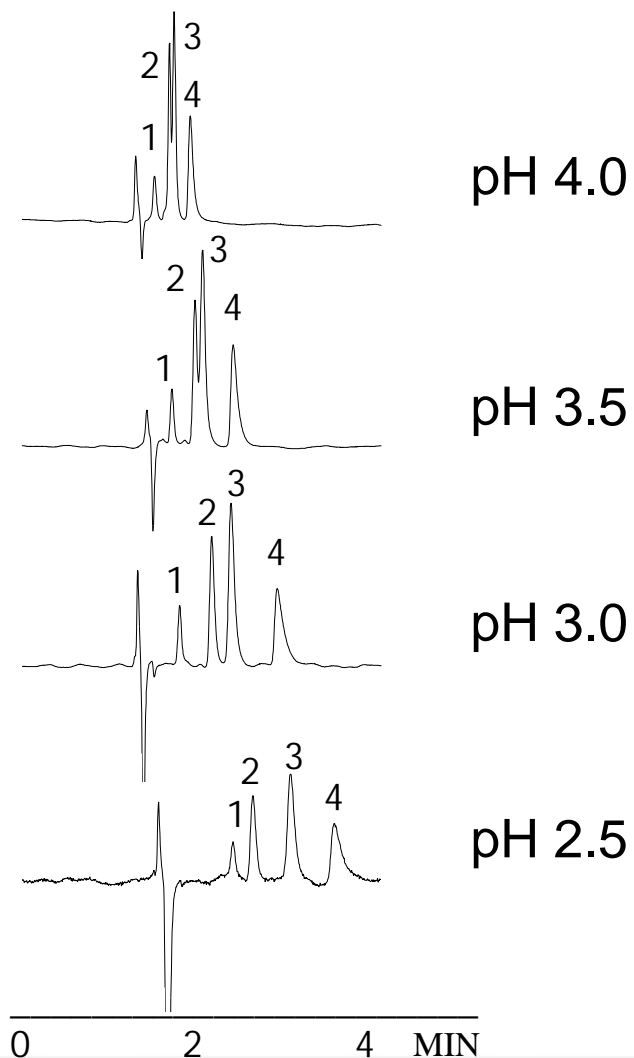
3. Diphenhydramine

4. Dextromethorphan

5. Pseudoephedrine

6. Nicotine

Effect of pH on Retention



BioBasic SCX, 5 μ m, 150x4.6mm

Eluent: A: 5mM NH₄ formate

B: A + 1M NaCl

95% A / 5% B

Flow: 1.0 mL/min

Detector: UV @ 210

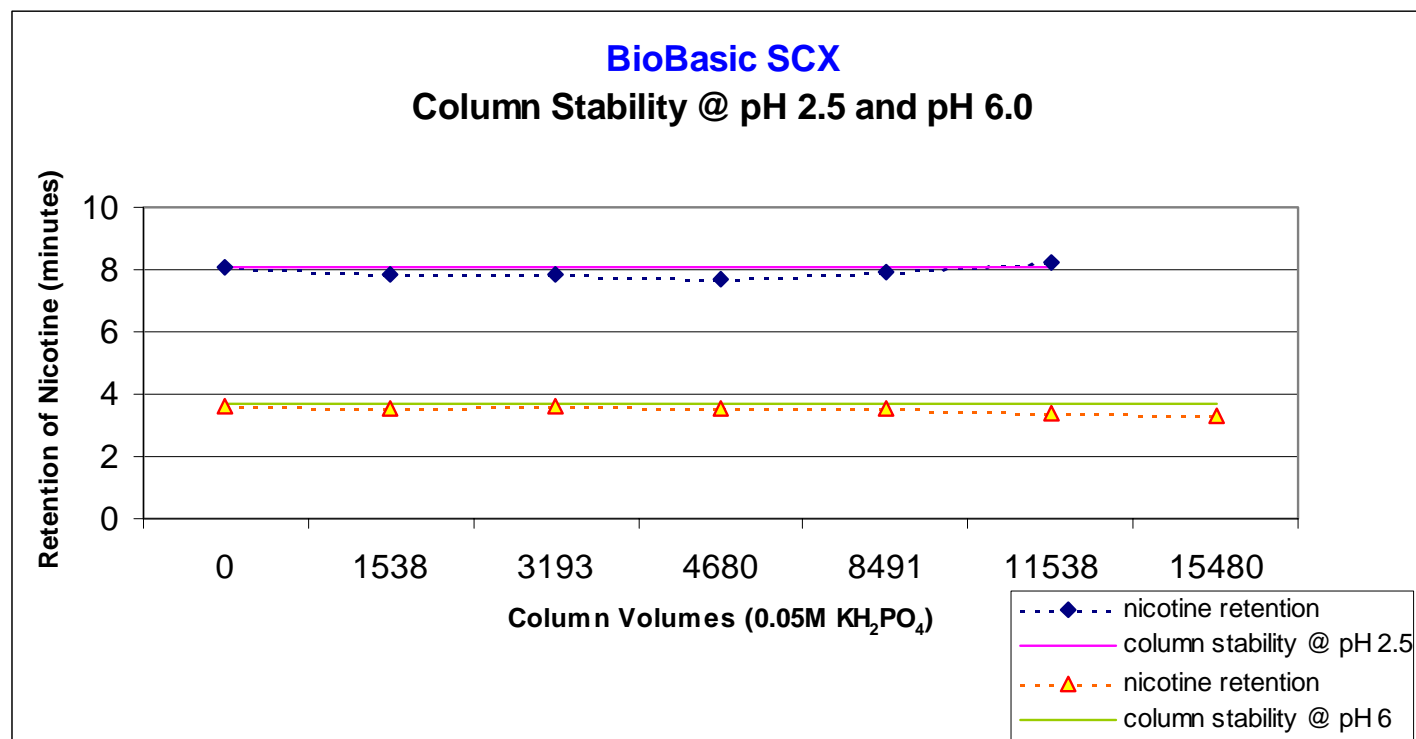
Sample: 1. Gly-Tyr

2. Val-Tyr-Val

3. Methionine Enkephalin

4. Leucine Enkephalin

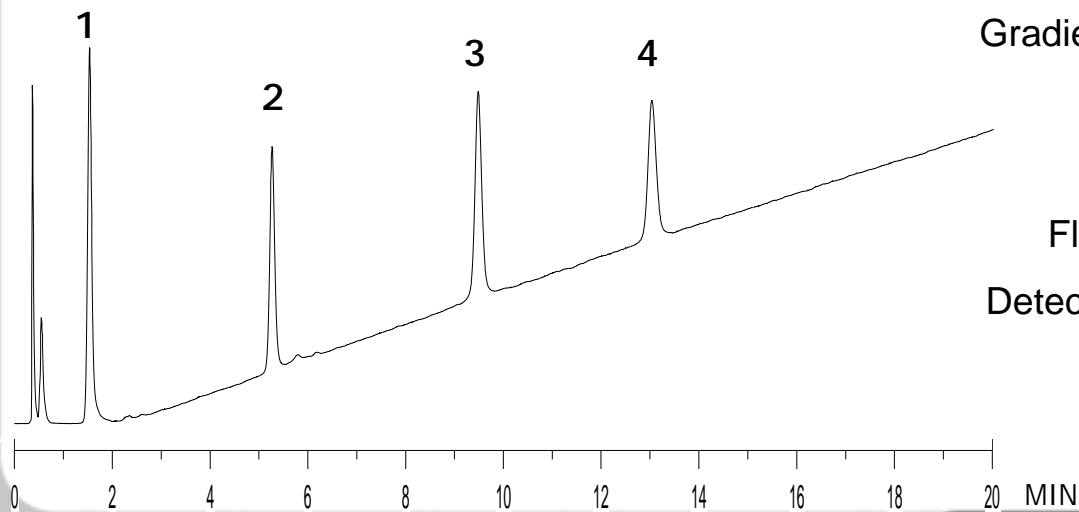
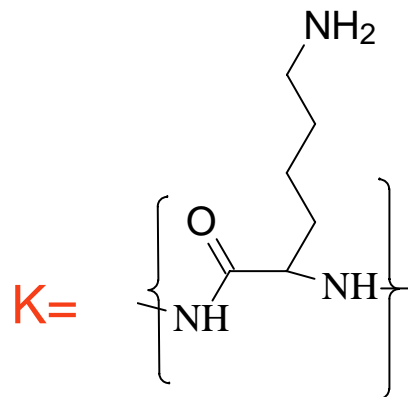
Stability of BioBasic SCX



Nicotine retention for stability study at pH 6 is significantly less due to use of NH_4 formate buffer (pH 3.1) as opposed to NH_4 acetate buffer (pH 4.5) in the mobile phase.

Ion Exchange Behavior

- Sample:
1. Ac-G-G-G-L-G-G-A-G-G-L-K-amide
 2. Ac-K-Y-G-L-G-G-A-G-G-L-K-amide
 3. Ac-G-G-A-L-K-A-L-K-G-L-K-amide
 4. Ac-K-Y-A-L-K-A-L-K-G-L-K-amide



BioBasic SCX, 5 μ m, 50x4.6mm

Gradient: A: 10mM KH₂PO₄ , pH 4.8 / ACN (75:25)

B: A + 0.5M NaCl

0 to 50% B in 20 minutes

Flow: 1.0 mL/min

Detector: UV @ 210

Batch to Batch Reproducibility

BioBasic SCX, 5 μ m, 50x4.6mm ID

Gradient: A: 10mM KH₂PO₄, pH 4.8 / ACN (75:25)

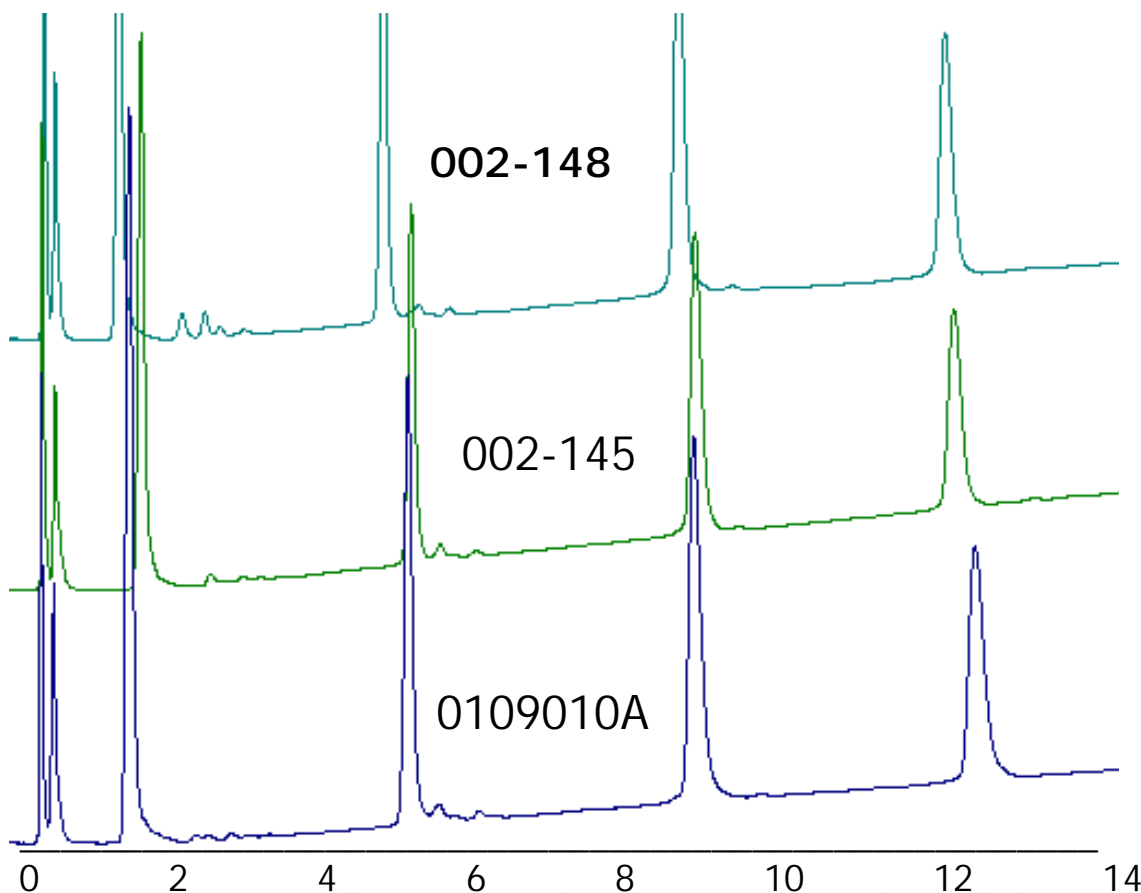
B: A + 0.5M NaCl

0 to 50%B in 20 min

Flow: 1.0 mL/min

Detector: UV @ 210

Sample: Eichrome CX Peptide mix



Broad Range of Applicability

- Small Molecules
 - *Basic Pharmaceuticals*
 - *Organic Amines*
- Biomolecules
 - *Proteins*
 - *Peptides*
 - *Proteomics (2D LC/MS/MS)*
 - Fractionation column
- Cation Exchange LC/MS Methods
 - *Ability to use lower concentrations of volatile buffers*

Application - Vitamins

BioBasic SCX, 5 μ m, 150x4.6mm

Gradient: A: 50mM KH₂PO₄, pH 3.0

B: 0.5M KH₂PO₄, pH 3.0

0% B for 6 minutes,

100% B at 6.1 minutes

Flow: 1.0 mL/min

Detector: UV @ 254

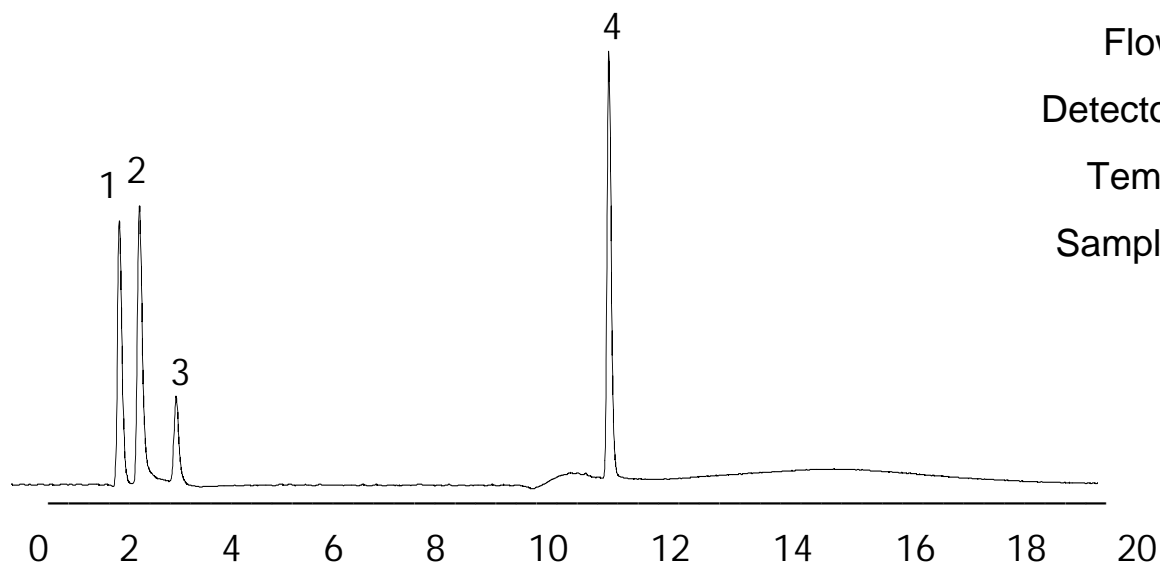
Temp: 50°C

Sample: 1. Cyanocobalamin (B12)

2. Riboflavin (B2)

3. Pyridoxine (B6)

4. Thiamine (B1)



Application – Organic Amines

Eluent: A: 20mM NH₄ acetate, pH 4.5

B: Acetonitrile

60% A / 40% B

Flow: 1.0 mL/min

Detector: UV @ 254

Sample: 1. Caffeine

2. Pyridine

3. Diphenhydramine

4. Dextromethorphan

5. Pseudoephedrine

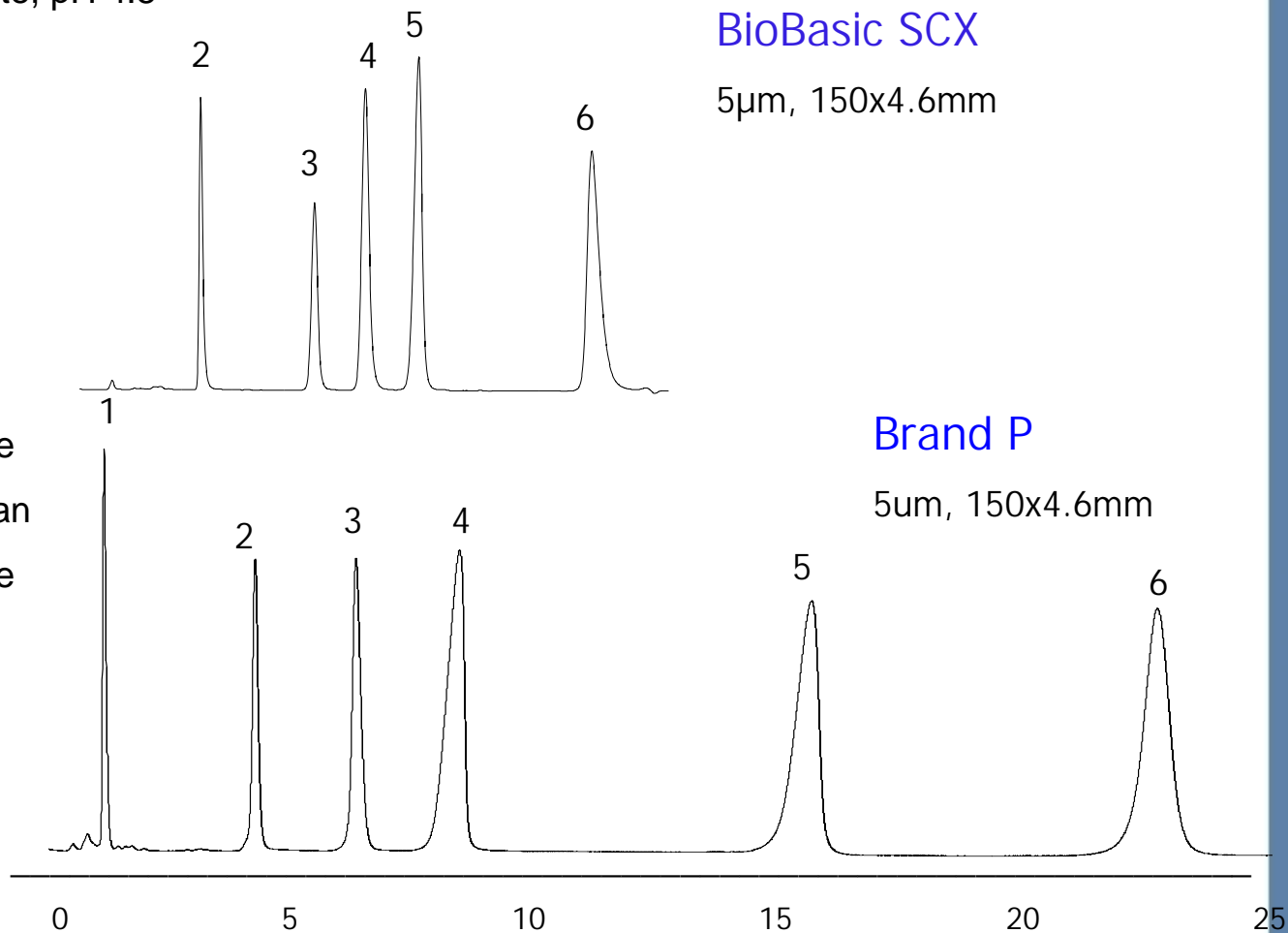
6. Nicotine

BioBasic SCX

5µm, 150x4.6mm

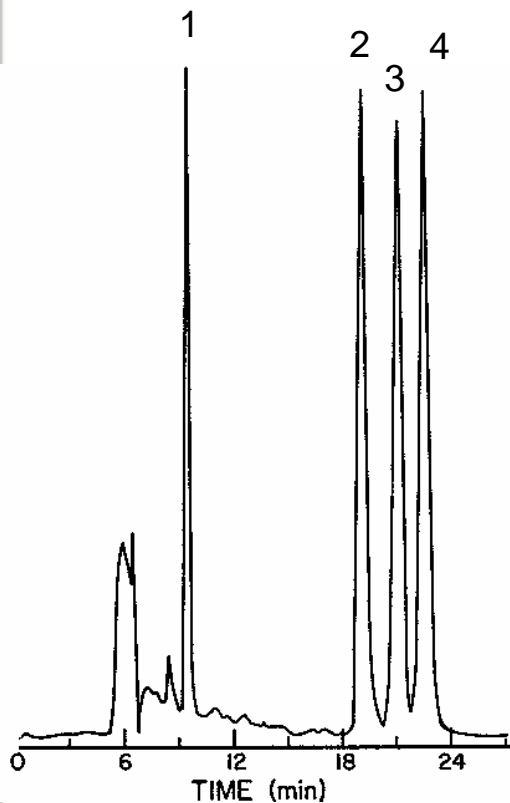
Brand P

5µm, 150x4.6mm



Application – Neurotransmitters

Brand S, 100x2.1mm

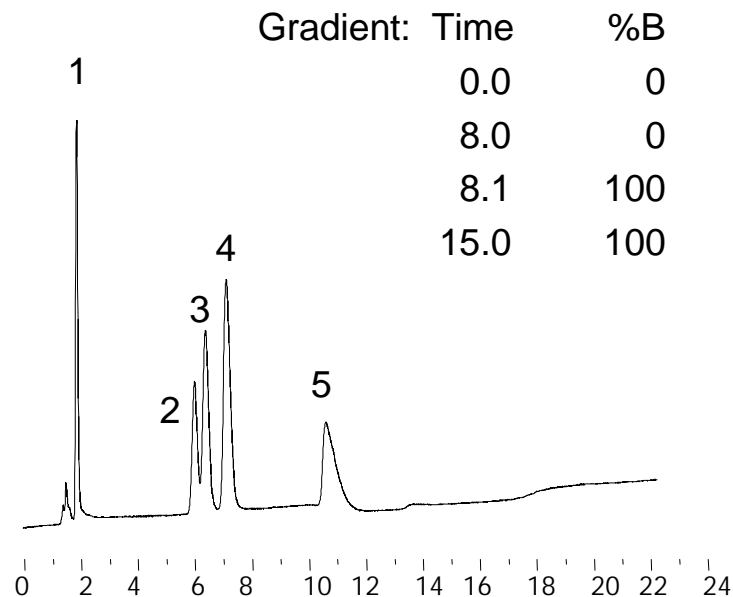


Eluent: 0.35M KH_2PO_4 , pH 5.94
 Flow: 0.4 mL/min
 Detector: UV @ 254

Sample:

1. L-Dopa
2. Epinephrine
3. Norepinephrine
4. Dopamine
5. Ephedrine

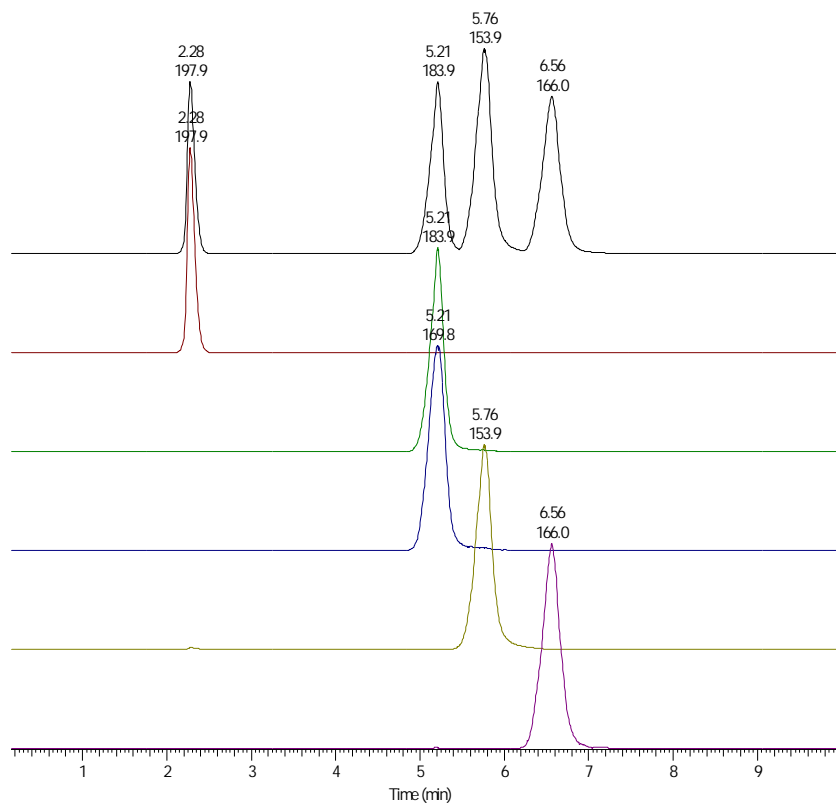
BioBasic SCX 5 μm , 150x4.6mm



Gradient:	Time	%B
	0.0	0
	8.0	0
	8.1	100
	15.0	100

Eluent: A: 20mM NH_4 acetate (pH 4.8 with CH_3COOH)
 B: 0.1M NH_4 acetate (pH 4.8 with CH_3COOH)
 Flow: 1.0 mL/min
 Detector: UV @ 210nm
 Temp.: 40°C

Application – Neurotransmitters by LC-MS



BioBasic SCX, 5 μ m, 150x2.1mm

Eluent: A - 20mM NH₄ formate, pH=4

B - Acetonitrile

95%A / 5%B

Flow: 200 μ L/min

Detector: APCI +

Thermo Finnigan LCQ^{DUO}

Vaporizer Temp: 400 °C

Cap Temp: 150 °C

Spray Voltage: 6 kV

Source Voltage: 27 V

Sheath Gas: 50 au

Aux Gas: 0 au

Sample: 1. L-Dopa

2. Epinephrine

3. Norepinephrine

4. Dopamine

5. Ephedrine

Application - Proteins

BioBasic SCX, 5 μ m, 150x4.6 mm

Eluent: A: 0.02M TRIS, pH 6

B: A + 1.0M Na acetate, pH 6

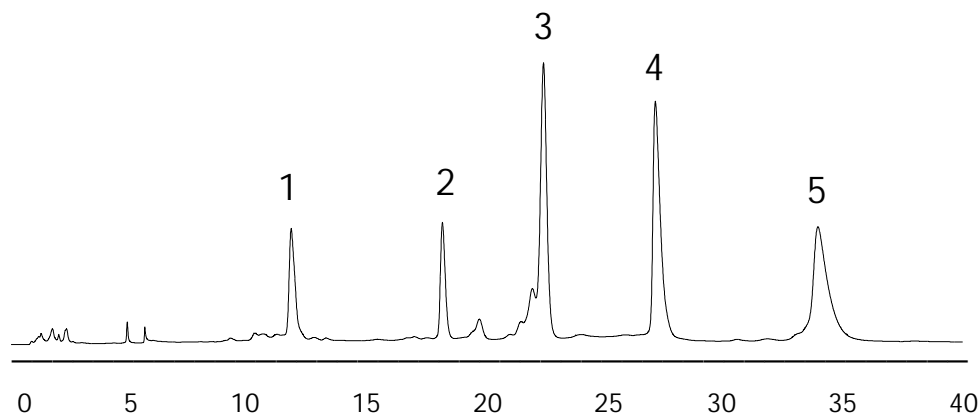
0 to 100% B in 60 minutes

Flow: 1.0 mL/min

Detector: UV @ 280

Sample: Protein Mix

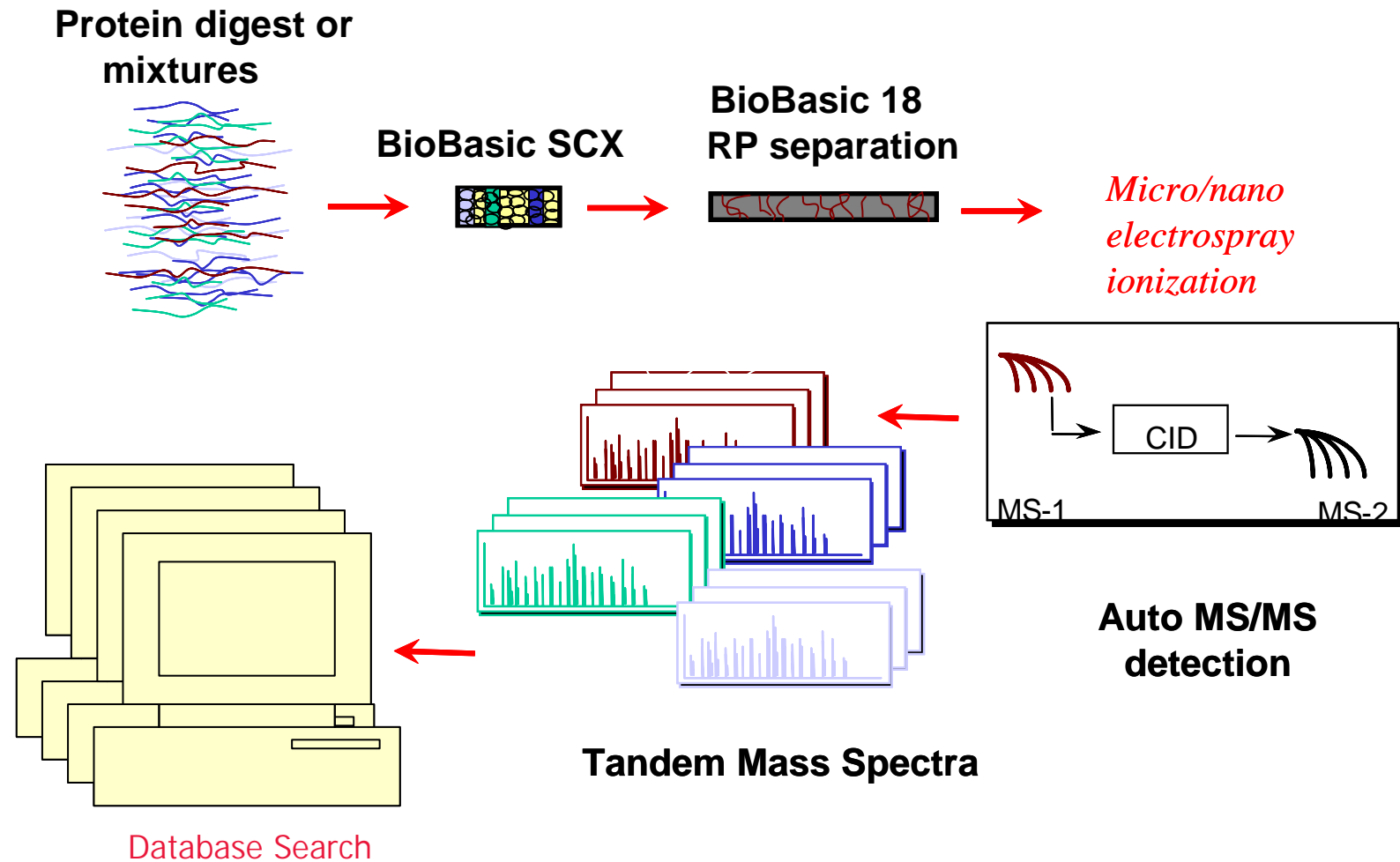
1. Trypsinogen
2. Ribonuclease A
3. Chymotrypsinogen A
4. Cytochrome C
5. Lysozyme



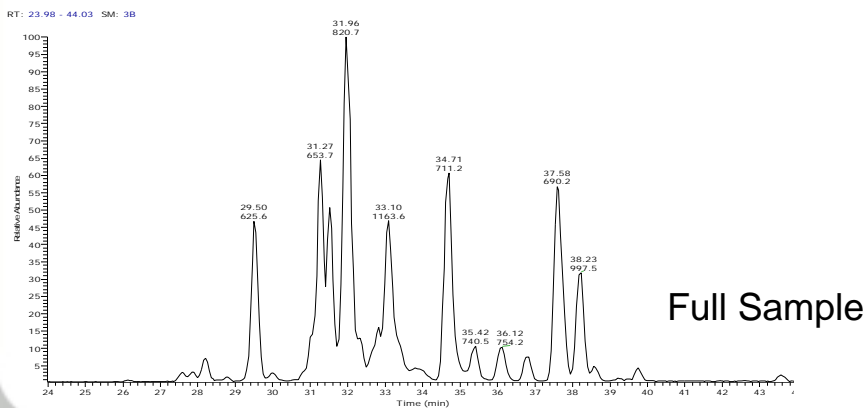
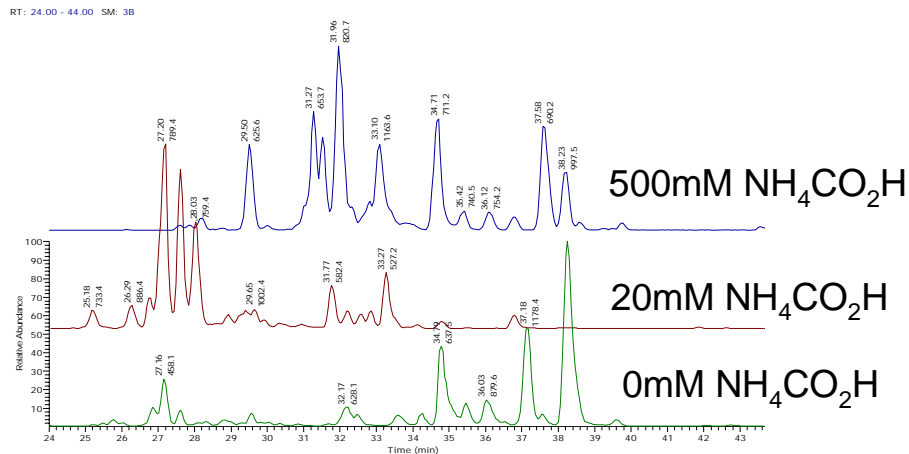
BioBasic SCX for 2D LC/MS

- Protein Identification
- DeNovo Sequencing
- Peptide Mapping
- Post-Translational Modifications

Overview 2D LC/MS



2D Chromatography of BSA Digest



Fractionation from BioBasic® SCX

BioBasic SCX 5 μm , 100x0.32mm ID

Inj. volume: 3 μL (1500 fmol total digest on column)

Fractions eluted with 20 μL NH_4 formate
(concentrations listed, in 0.1% formic acid)
onto BioBasic 18 5 μm 100x0.18mm

Reversed Phase Separation of BSA Digest

BioBasic 18 5 μm , 100x0.18mm ID

Eluent: A: 0.01% Formic Acid

B: ACN + 0.1% Formic Acid

Flow Rate: 2 $\mu\text{L}/\text{min}$

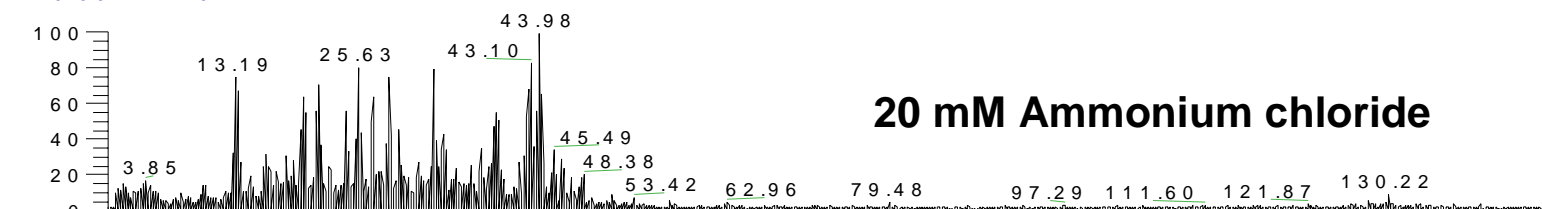
(split 100:1 from 200 $\mu\text{L}/\text{min}$)

Gradient:

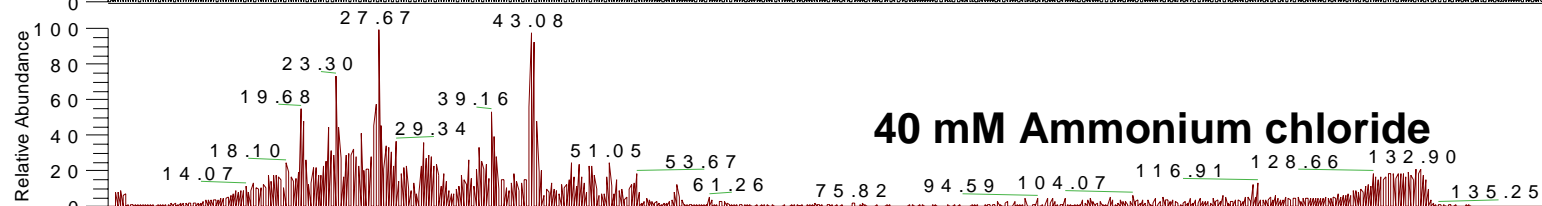
Time	%B
0	0
3	0
65	60

2D LC/MS/MS of Yeast Proteins

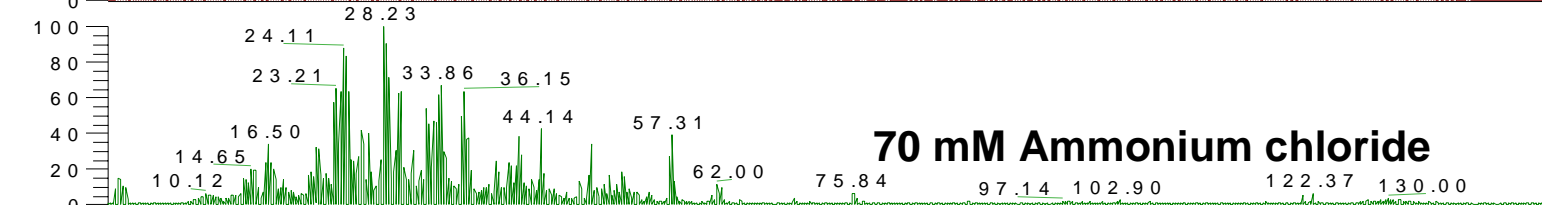
RT: 0.00 - 147.01



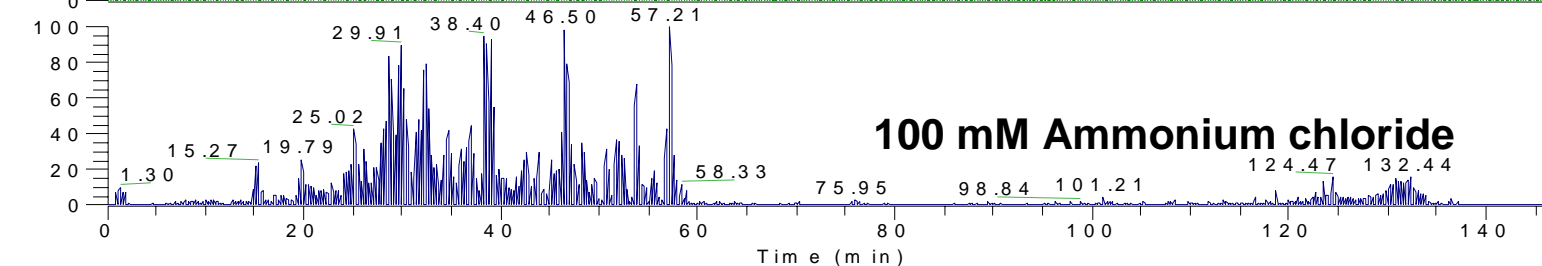
NL:
1.37E9
Base Peak
MS
Yeast_120 m i
ng_01



NL:
4.29E9
Base Peak
MS
yeast_120 m i
ng_02



NL:
2.67E9
Base Peak
MS
yeast_120 m i
ng_03



NL:
4.13E9
Base Peak
MS
yeast_120 m i
ng_04

Summary

- BioBasic SCX elutes analytes with lower concentrations of volatile buffers/salts
 - *Ideal for cation exchange LC-MS*
 - *Faster analysis*
- Excellent peak shapes for both large (proteins) and small (pharmaceuticals) analytes
 - *Protein friendly packing due to hydrophilic polymer coating*
- Efficient component of two dimensional LC/MS methods
 - *Ideal fractionation column*
- Same technology that is fundamental to **Thermo** Electron's ProteomeX Analyzer

For More Information

- Thermo Hypersil-Keystone

Booth #3547

- On the web

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Acknowledgement

- **Thermo** Hypersil-Keystone R&D
 - *Joshua Kline*
 - *Ronald Sherant*
 - *Paul Ross, Technical Director*
- **Thermo** Electron Proteomics Group
 - *Yvonne Lu*
 - *Melissa Chen*
 - *Billy Wu*
 - *Paul Shieh, Applications Lab Director*