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New Capabilities for Analysis of Biogenic Amines using Ion Chromatography

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Outline

- **Properties of biogenic amines**
  - Alkyl, unsaturated/aromatic, ion-pair formers
  - Low-to-moderate water solubility
  - Cationic at low pH
- **Achieving retention and separation**
  - Cation exchange columns with mixed selectivities
- **Options for detection**
  - Electrical conductivity
  - ESI-MS/MS
- **Capabilities of Capillary IC-MS/MS**
- **High Ionic Matrix Challenges**

Biogenic amines

- **Involved in**
  - Cellular growth, regulation, repair and breakdown
  - Protein and nucleic acid synthesis
- **Implicated in**
  - Allergic responses
- **Properties of biogenic amines**
  - Chains of aminopropyl units with pkas above physiological pH
  - Alkyl, unsaturated/aromatic, ion-pair formers
  - Other amine structures with biological activity
  - Low-to-moderate water solubility
  - Cationic at low pH

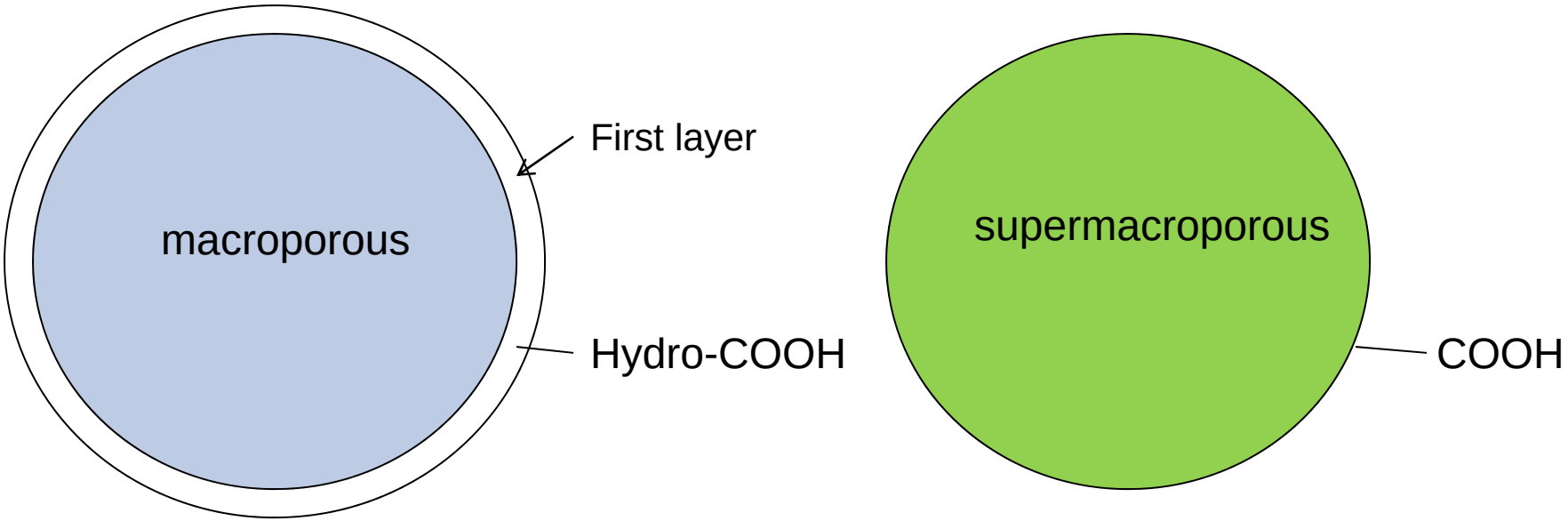
Cation exchange selectivity

- Weak cation exchange sites have high selectivity for H^+
  - allows control of charge density and capacity with pH,
  - eliminates the need for a divalent eluent cation
  - simplifies use of eluent suppressor
- Substrate particles are formulated with varying surface area, pore size and hydrophilicity
- Cation exchange capacity can be increased to allow
  - better peak resolution
  - wider sample pH range,
  - higher matrix ionic strength
  - more diverse analyte concentrations
- Column cleanup is easy

Comparison of IonPac CS17 and IonPac CS19

	CS17	CS19	Comment
Efficiency	<	50% Higher	
Capacity	<	65% Higher	
Selectivity	more hydrophilic phase		Selectivity differences for hydrophobic analytes
Solvent compatibility	100%	100%	Acetone, Acetonitrile, IPA

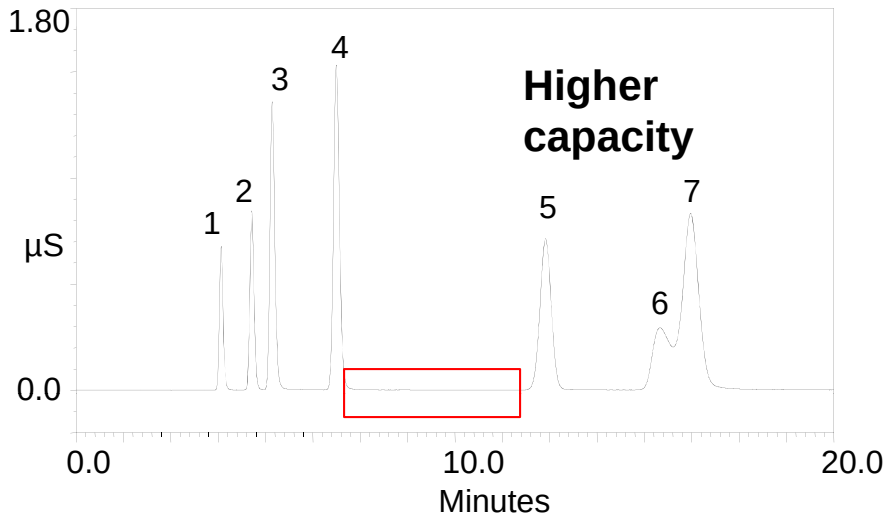
Comparison of two cation exchange phases



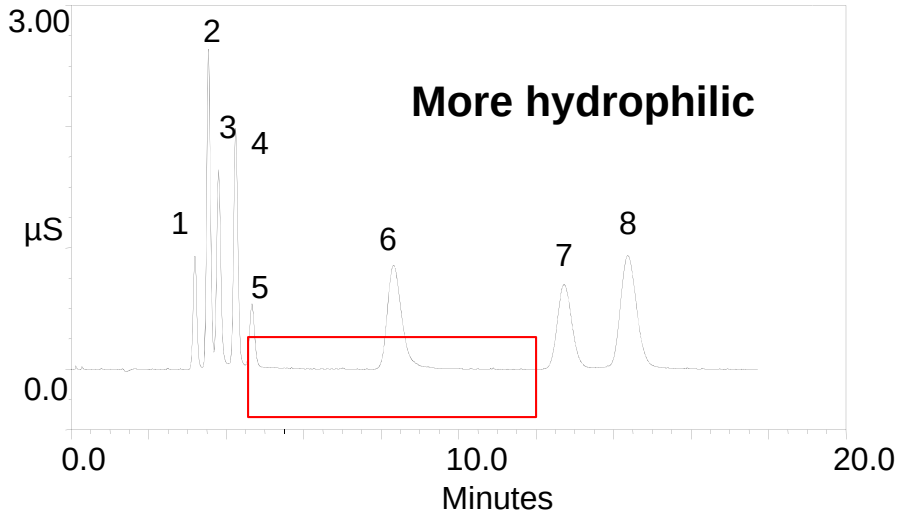
CS17
More hydrophilic

CS19
Higher capacity

Selectivity comparison – IonPac CS17 eluent and standard



Column: IonPac CS19, 2x250 mm
Eluent: 6 mM MSA at 0.25 mL/min
Temperature: 30 °C
Peaks:
1. Li^+
2. Na^+
3. NH_4^+
4. K^+ and Dimethylamine
5. Mg^{2+}
6. Triethylamine
7. Ca^{2+}



Column: IonPac CS17, 2x250 mm
Eluent: 6 mM MSA at 0.25 mL/min
Temperature: 30 °C
Peaks:
1. Li^+
2. Na^+
3. NH_4^+
4. K^+
5. Dimethylamine
6. Triethylamine
7. Mg^{2+}
8. Ca^{2+}

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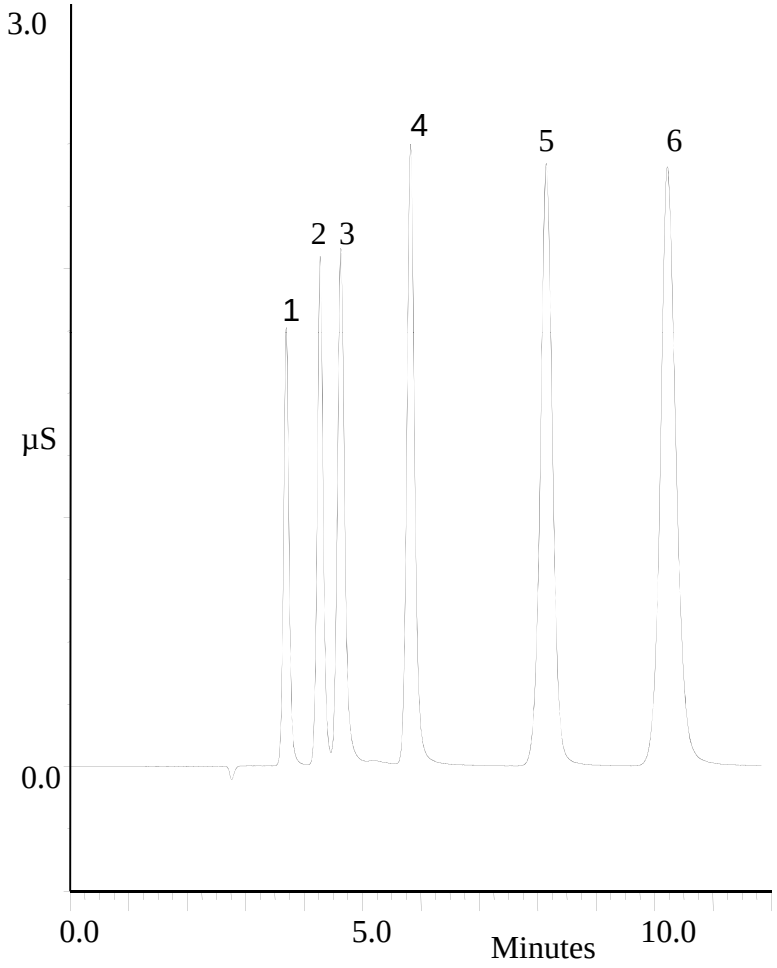
Mono/divalent cation exchange selectivity of the IonPac CS19



Column: IonPac CS19, 2 x 250 mm
Eluent: 8 mM MSA at 0.25 mL/min
Temperature: 30ºC
Detection: Suppressed Conductivity
Injection volume: 5 µL

Peaks:

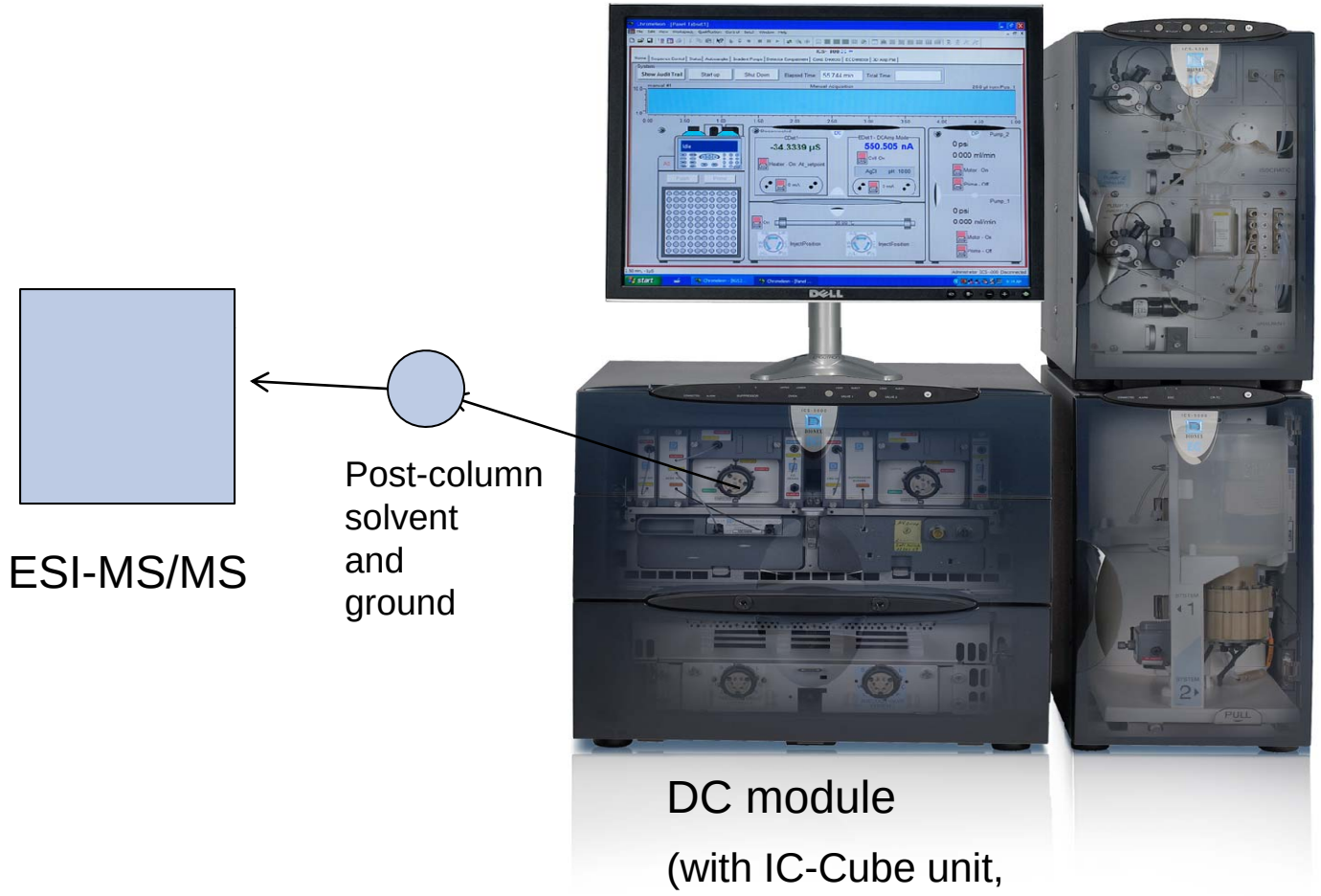
1. Li^+ 0.25 mg/L
2. Na^+ 1.00
3. NH_4^+ 1.25
4. K^+ 2.5
5. Mg^{2+} 1.25
6. Ca^{2+} 2.5

NOTE: Concentrations are approximate.

Dual microbore/capillary ion chromatograph

- **ICS-5000 RFIC™ System**
  - Capable of performing both conventional and capillary-scale IC separations
  - Includes a Dual-Pump module (DP), an Eluent Generator (EG), and a Detector/Chromatography module (DC)
  - Modular design
  - Dual-channel capillary RFIC system,
  - Dual-channel conventional RFIC system,
  - Dual-channel hybrid RFIC system

IC-CD-ESI-MS/MS System



ICS-5000 IC Cube™

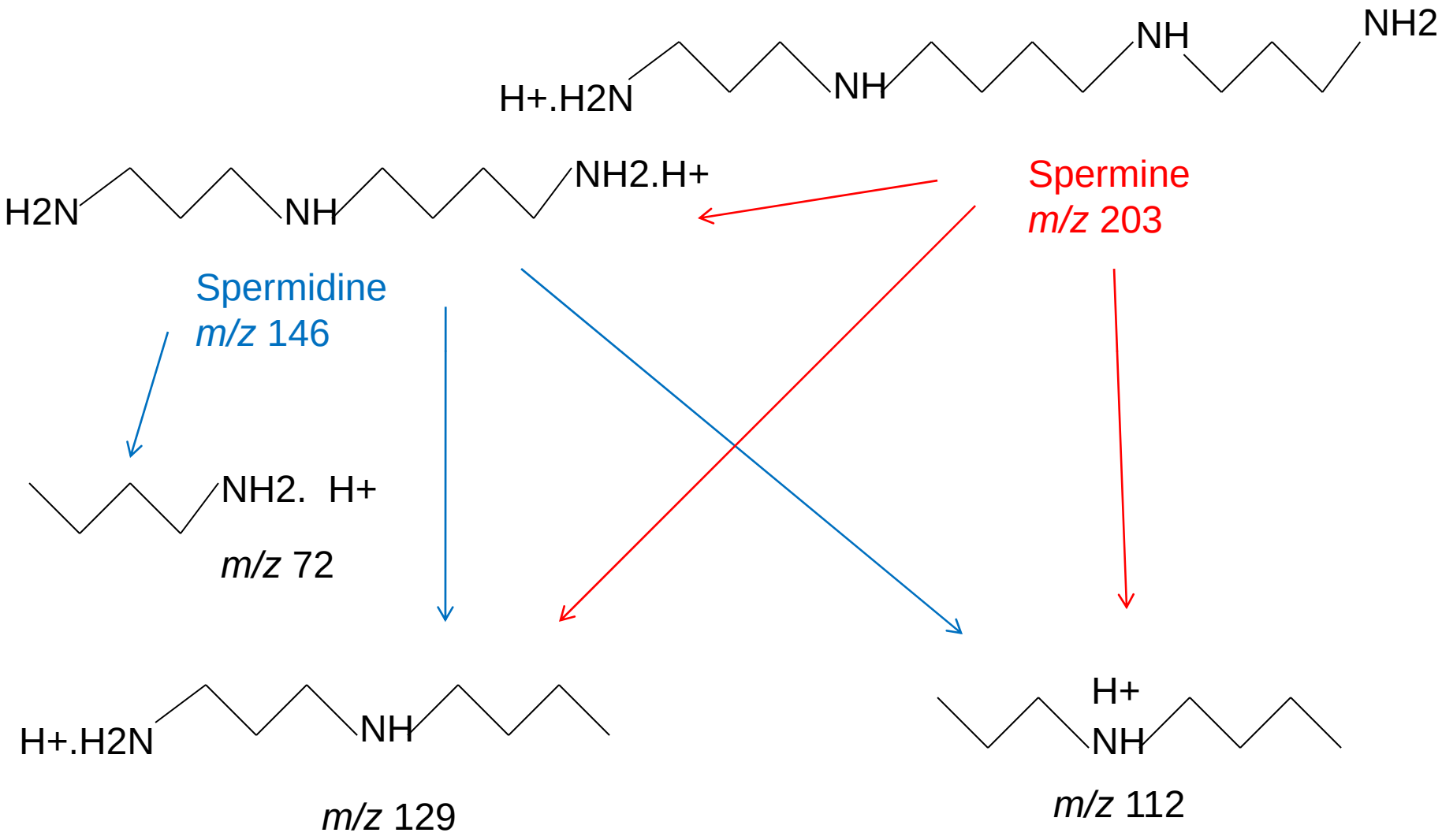
- Small housing that resides in the DC
- Holds an EG degas cartridge, capillary column cartridge, injection valve, capillary suppressor cartridge, optional Carbonate Removal Device
- Unique plumbing reduces the number of operator-made fluidic connections by 50%

IC Cube™

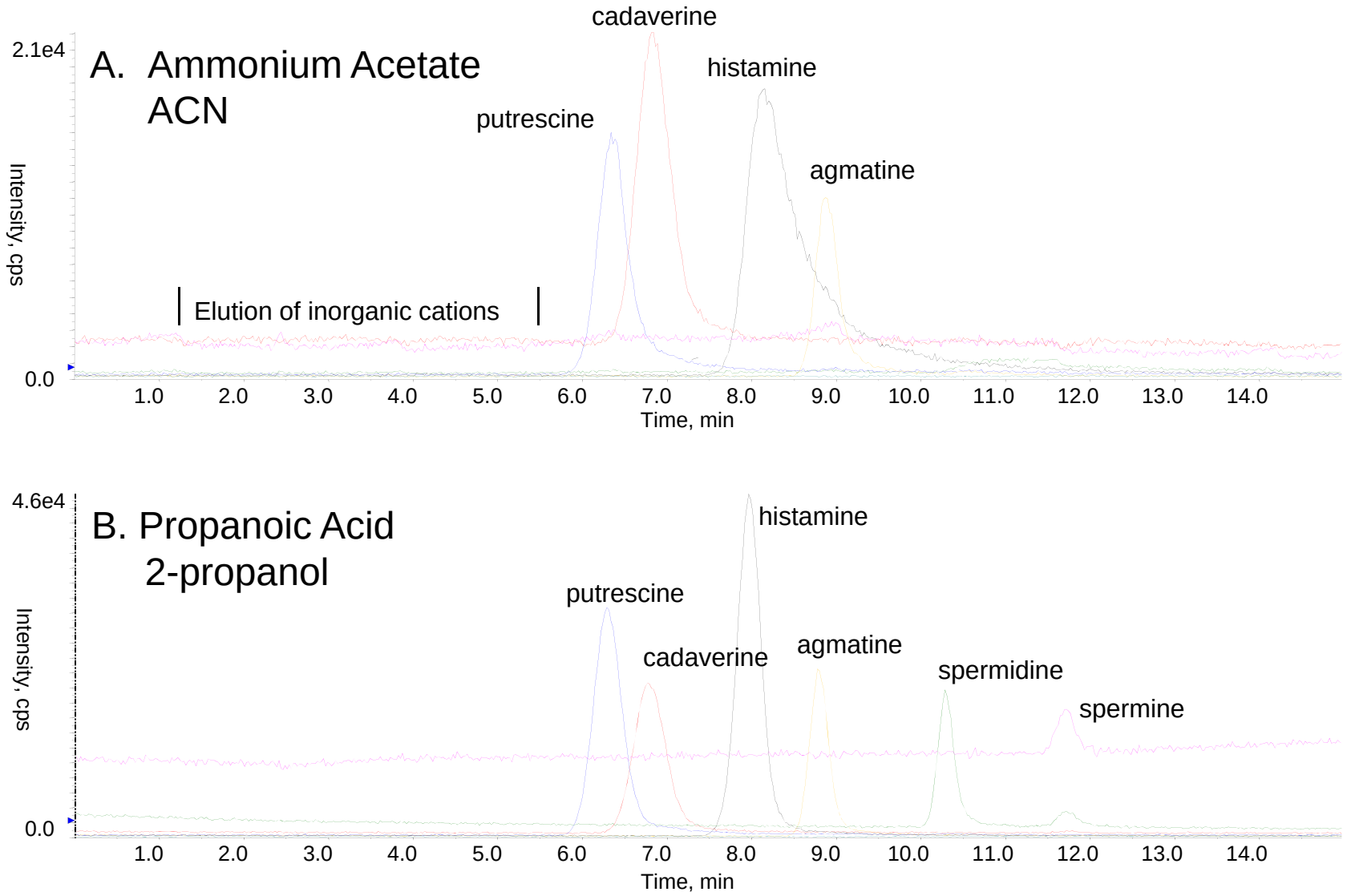
ESI-MS/MS detection of biogenic amines

Name	Formula	Q1/Q3	pK_a
Putrescine	C4H12N2 aliphatic	89.1/72.1	9.35
Cadaverine	C5H14N2 aliphatic	103.1/86.1	10.05
Histamine	C5H9N3 aromatic	112/95	9.75
Agmatine	C5H14N4 decarboxylated arginine	131/72.1	12.5, 9.07
Phenethylamine	C8H11N	122/105	9.8
Spermidine	C7H19N3 aliphatic	146.1/72.1	10.88, 9.81, 8.34
Spermine	C10H26N4 aliphatic	203.1/129.1 203.1/112	10.02, 9.21, 4.42

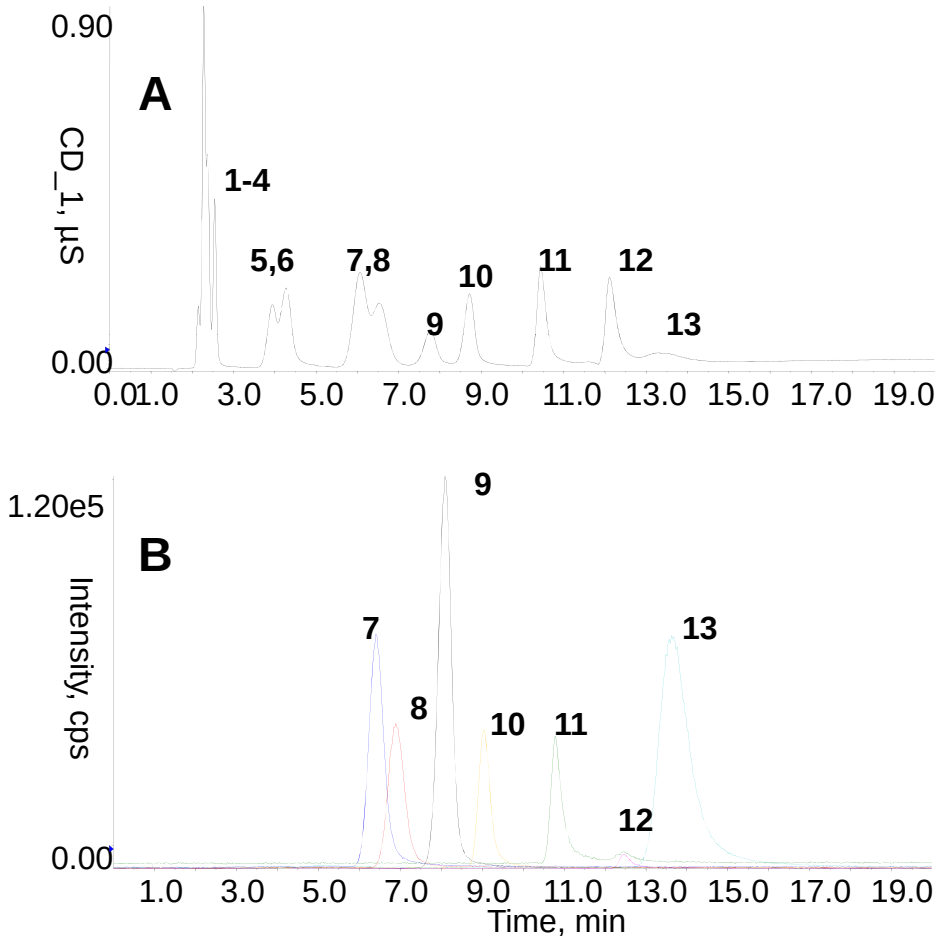
Main fragments of spermine and spermidine



Effect of post-column solvent compositions on ESI



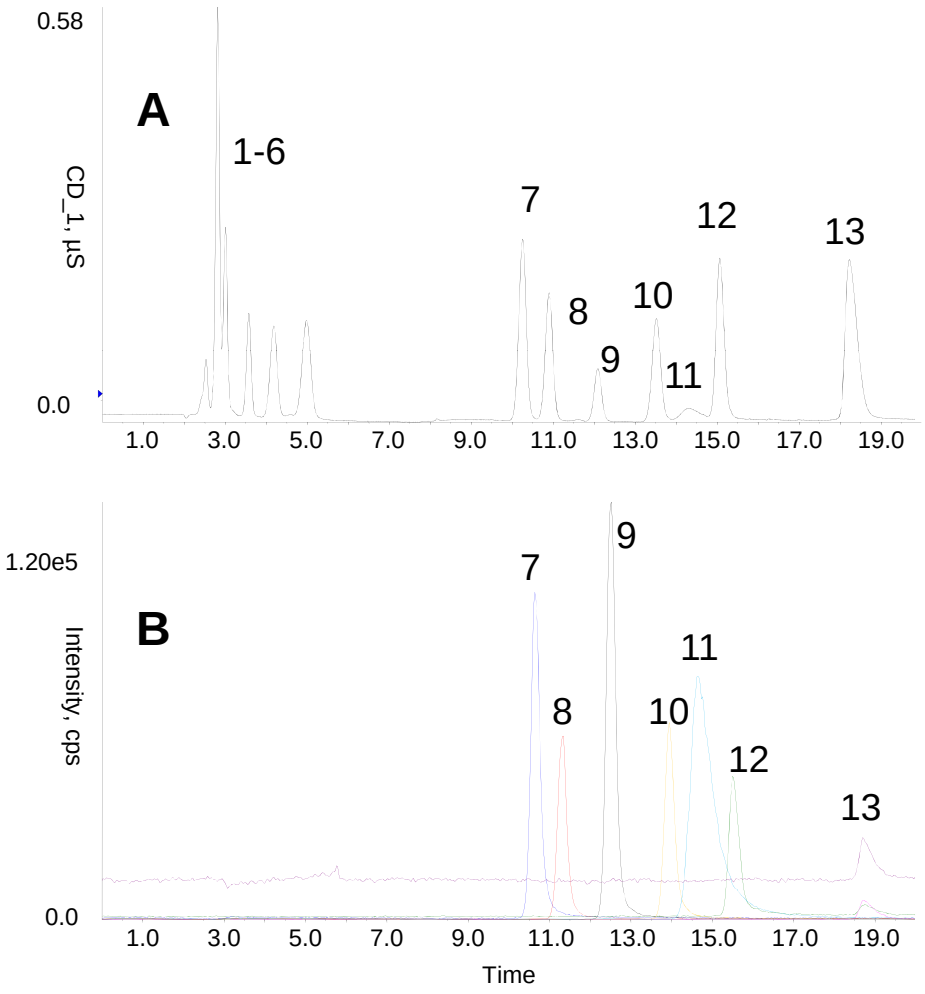
Fast determination of biogenic amines using CS17 and CapIC-MS/MS



Column: Prototype Capillary CS17, 0.4 x 250 mm
Suppressor: CCES
Flow rate: 15 µL/min
Gradient: 10-55 mM MSA
Postcolumn:
10% propanoic acid/25% IPA/65% water
PC flow rate: 10 µL/min
Detection: A,Conductivity; B, ESI-MS/MS, API2000
Injection volume: 0.4 µL

Peaks:
1-4. Li, Na, NH_4, K
5,6. Mg, Ca
7. Putrescine, 1mg/L
8. Cadaverine, 1 mg/L
9. Histamine, 3 mg/L
10. Agmatine, 1 mg/L
11. Spermidine, 2 mg/L
12. Spermine, 4 mg/L
13. Phenethylamine, 5 mg/L

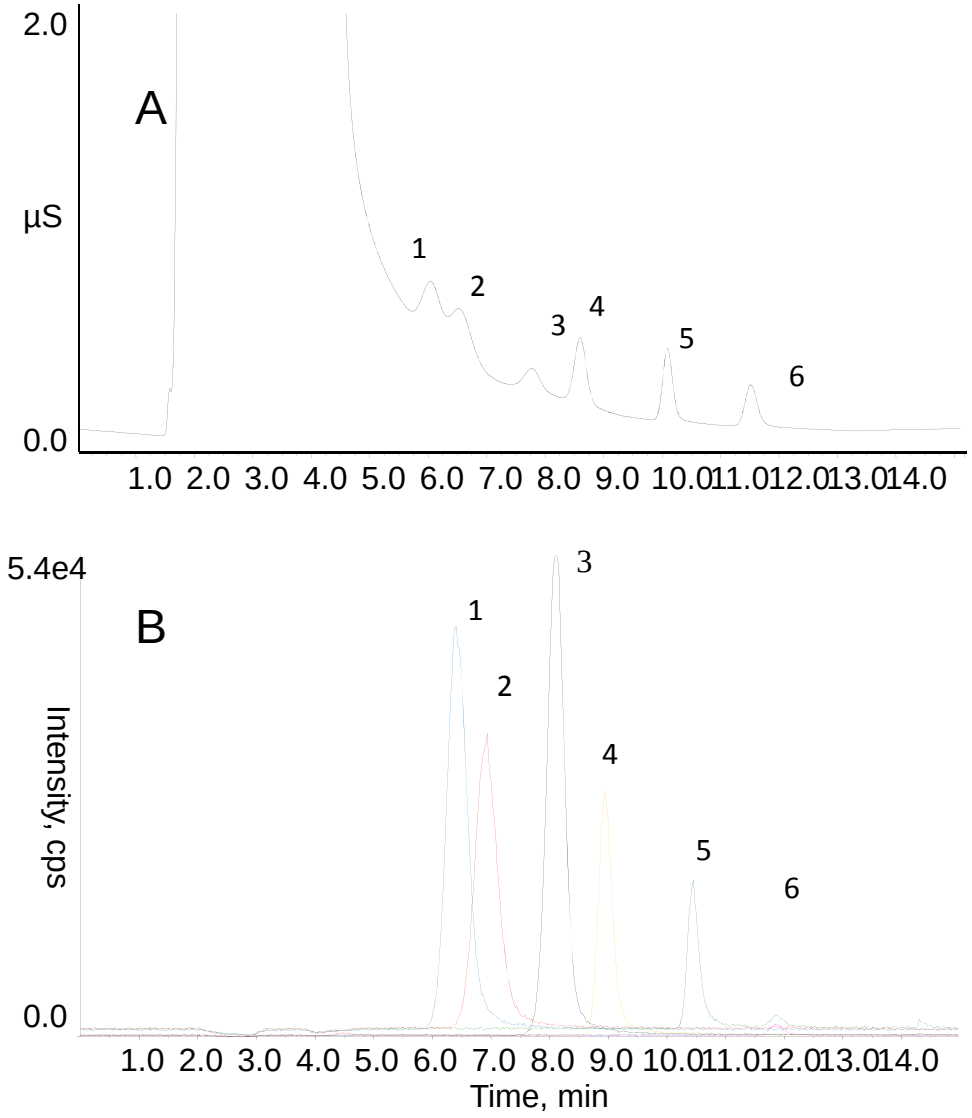
Fast determination of biogenic amines using CS19 and CapIC-MS/MS



Column: Prototype Capillary CS19, 0.4 x 250 mm
Suppressor: CCES
Flow rate: 15 µL/min
Gradient: 10-55 mM MSA
Temp.: 30 °C
Postcolumn:
10% propanoic acid/25% IPA/65% water
PC flow rate: 10 µL/min
Detection: A,Conductivity; B, ESI-MS/MS, API2000
Injection volume: 0.4 µL

Peaks:
1-6. Li, Na, NH_4, K, Mg, Ca
7. Putrescine, 1mg/L
8. Cadaverine, 1 mg/L
9. Histamine, 3 mg/L
10. Agmatine, 1 mg/L
11. Phenethylamine, 5 mg/L
12. Spermidine, 2 mg/L
13. Spermine, 4 mg/L

Detection of biogenic amines in 10% seawater using capillary IC-CD-ESI-MS/MS



Column: Prototype Capillary CS17, 0.4 x 250 mm
Suppressor: CCES
Flow rate: 15 µL/min
Gradient: 10-55 mM MSA
Postcolumn:
10% propanoic acid/25% IPA/65% water
PC flow rate: 10 µL/min
Detection: A, Conductivity
B, ESI-MS/MS, API2000
Injection volume: 400 nL
Sample: San Francisco Seawater diluted 1/10

Peaks (spiked):
1. Putrescine, 1mg/L
2. Cadaverine, 1 mg/L
3. Histamine, 3 mg/L
4. Agmatine, 1 mg/L
5. Spermidine, 2 mg/L
6. Spermine, 4 mg/L

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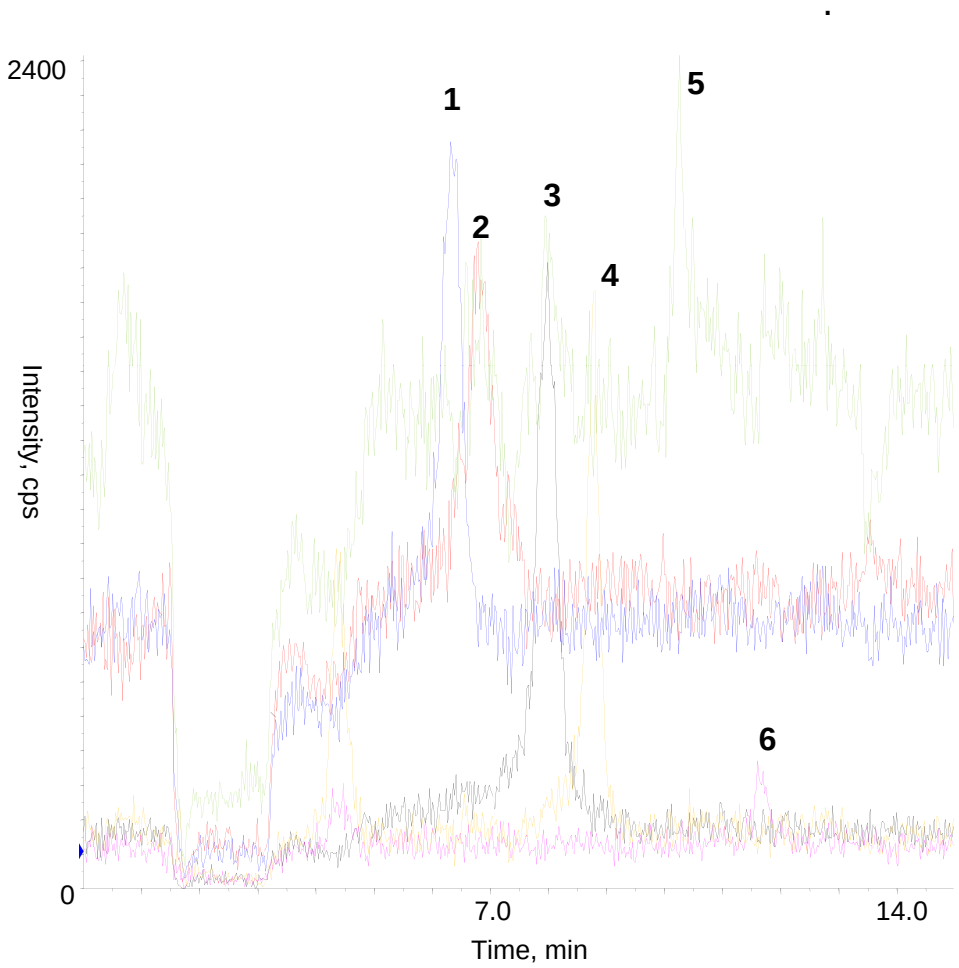
Signal suppression in ESI-MS/MS from very high ionic strength



Column: Prototype Capillary CS17, 0.4 x 250 mm
Suppressor: CCES
Flow rate: 15 µL/min
Gradient: 10-55 mM MSA
Postcolumn:
10% propanoic acid/25% IPA/65% water
PC flow rate: 10 µL/min
Detection: A, Conductivity
B, ESI-MS/MS, API2000
Injection volume: 400 nL
Sample matrix: Seawater, undiluted
Peaks (spiked):
1. Putrescine, 1mg/L
2. Cadaverine, 1 mg/L
3. Histamine, 3 mg/L
4. Agmatine, 1 mg/L
5. Spermidine, 2 mg/L
6. Spermine, 4 mg/L

Retention time stability* in high ionic strength matrix

	Retention Time in Matrix (minutes)		
Amine	**Deionized Water**	**1/10 Seawater**	**Neat Seawater**
Putrescine	6.29	6.27	6.32
Cadaverine	6.78	6.76	6.78
Histamine	7.97	7.98	7.99
Agmatine	8.79	8.82	8.79
Spermidine	10.29	10.26	10.28
Spermine	11.71	11.68	11.60

* Capillary CS17 using gradient elution

Summary

- **Weak cation exchange phases** can be built with varying hydrophobicity, capacity and selectivity in order to better target cations, Including amines at low pH, from matrix cations as well as from each other. The IonPac CS17 and CS19 phases can separate inorganic cations from biogenic amines. The CS19 phase has higher efficiency, higher hydrophobicity and higher capacity than the CS17 phase.
- The **capillary format** of ion chromatography requires only a 400 nL injection volume and avoids matrix diversion or ESI-MS overload for high ionic matrices.
- **ESI-MS/MS detection** of biogenic amines is optimized by the addition of a propionic acid/2-propanol post-column solvent.
- **Future work** will focus on improvements recovery/detection of higher valency cations including spermine.