No Chromophore? No Problem! Universal HPLC Detection

Topics

- Key concepts behind Thermo Scientific™ Charged Aerosol Detection (CAD)
  - Universal Detection and Uniform Response
- How does CAD work?
- Recent Advances
- Use of Thermo Scientific™ Vanquish™ Duo and CAD for Relative Quantitation

Key Concepts – Universal Detection

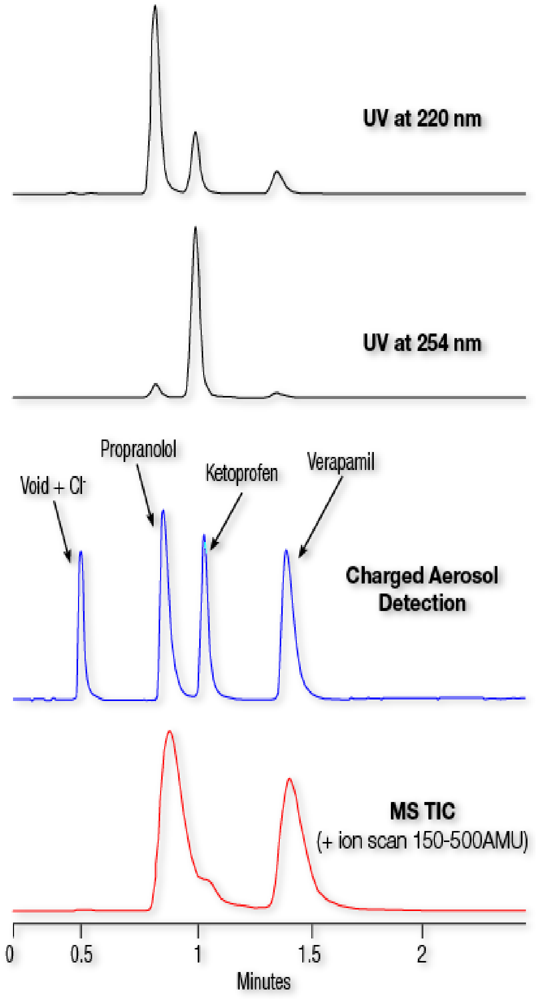
Response for every component within eluent (except mobile phase)

Discriminator is volatility (similar to ELSD)

- Eluents need to be volatile
- Detects all species that form stable (non-volatiles) or semi-stable (semi-volatiles) aerosol particles

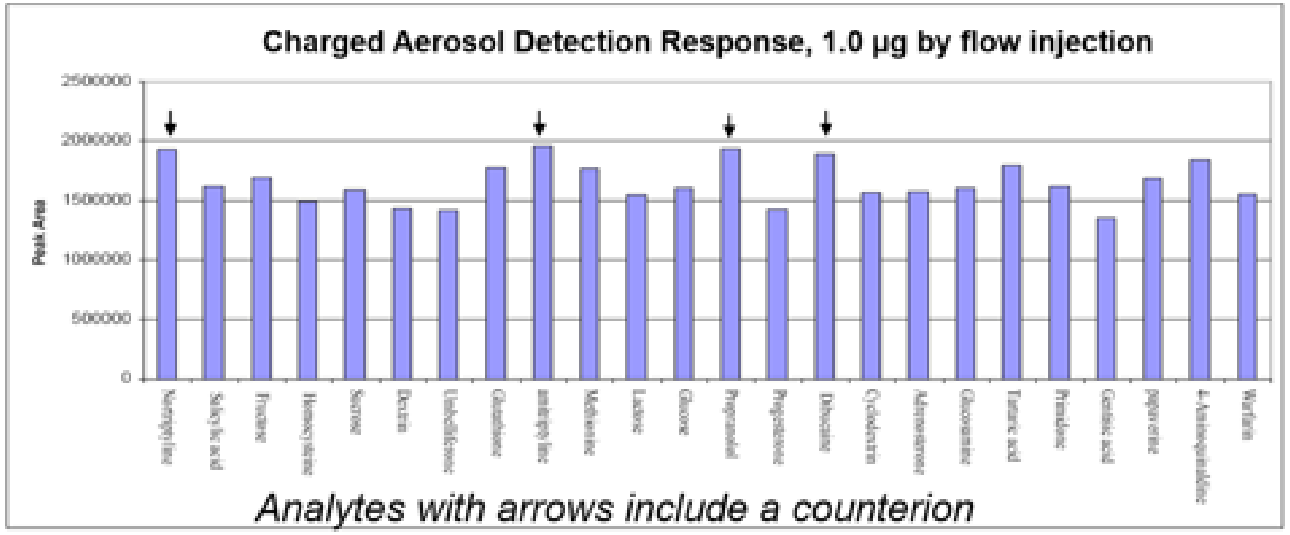
Does not rely on a chromophore or ability to form a gas phase ion (Unlike UV or MS)

Enables a more comprehensive view of sample composition



Key Concepts – Uniform Response

- Same response for all components – independent of chemical structure
- Very desirable for many applications (i.e., when use of individual standards is not feasible)
  - Drug libraries, synthetic mixtures
  - Impurities and degradants
  - Herbal medicines
  - Natural products
  - Polymers and surfactants
  - Lipids
- Allows estimation of quantity

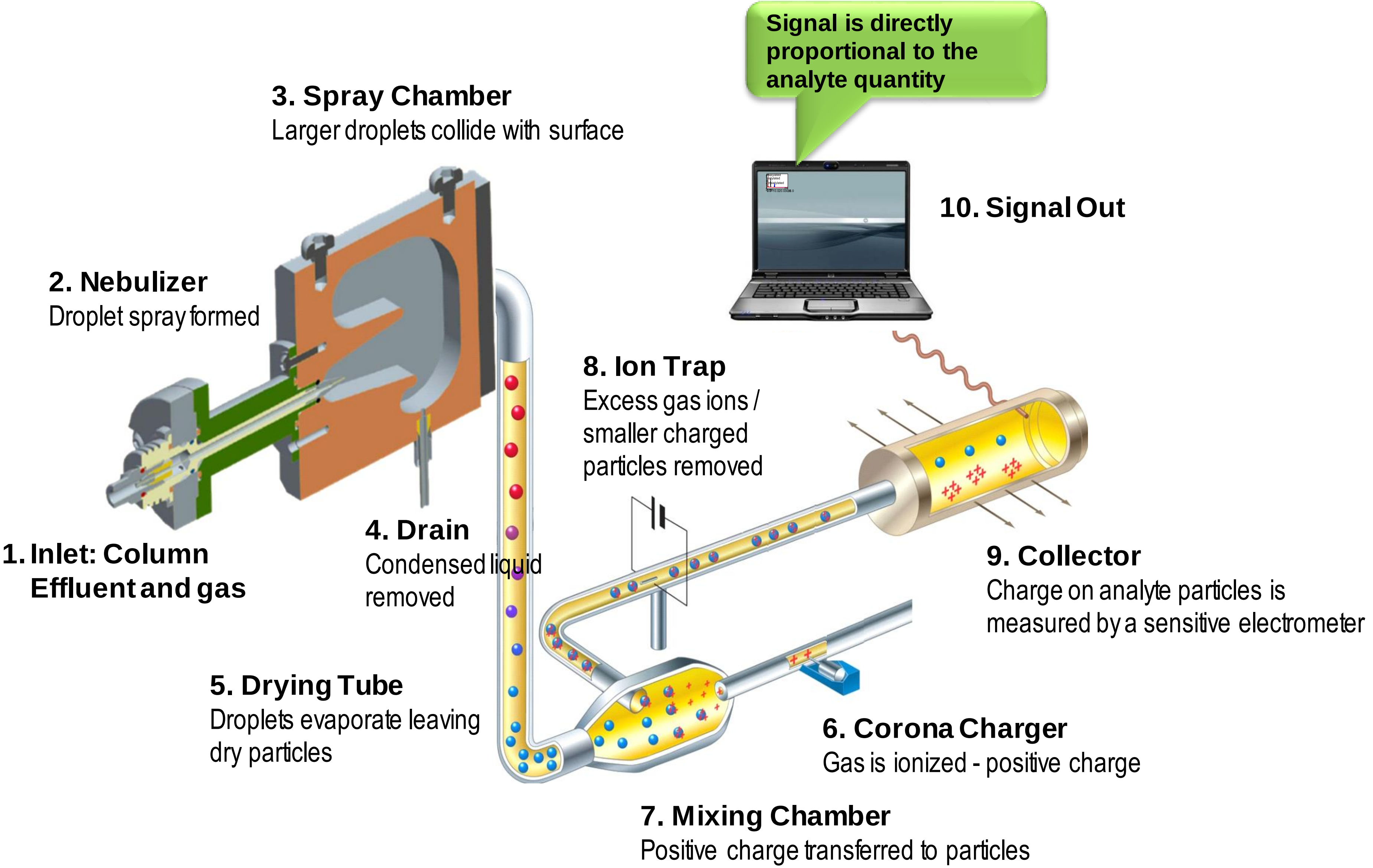


10.7% RSD variation in CAD response among a wide diversity of non-volatile analytes

Topics

- Key concepts behind Charged Aerosol Detection (CAD)
  - Universal Detection and Uniform Response
- How does CAD work?
- Recent Advances
- Use of Vanquish Duo and CAD for Relative Quantitation

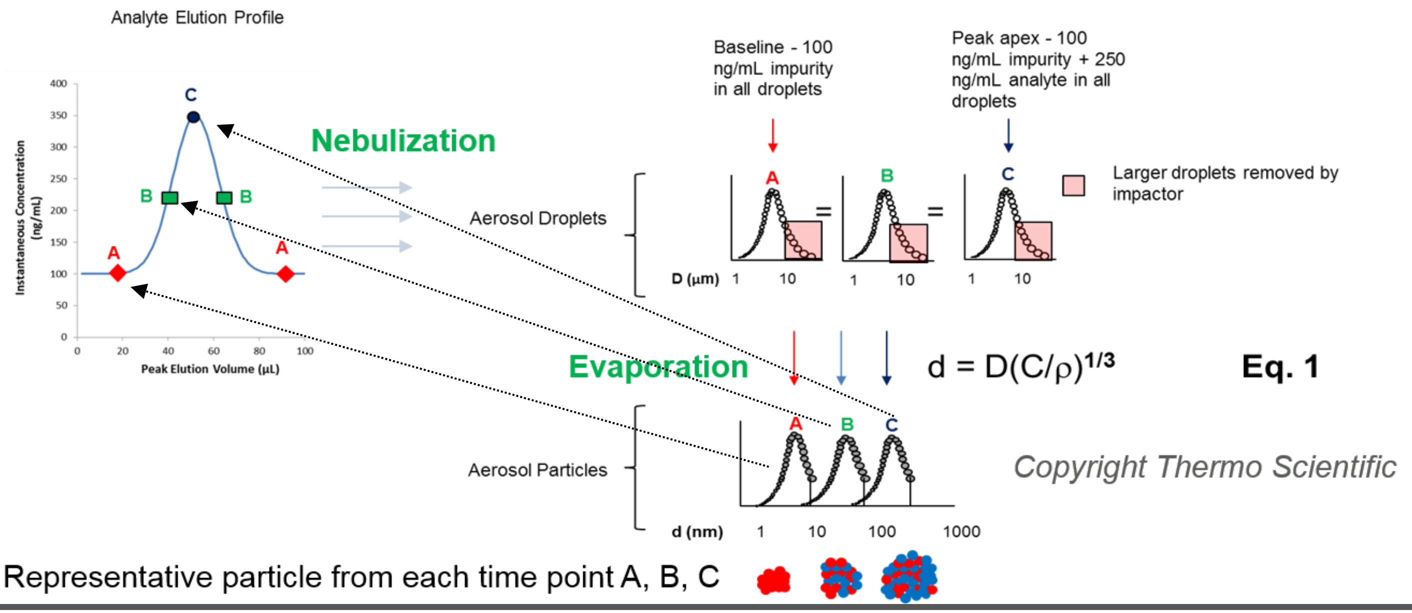
How Charged Aerosol Detection Works



ThermoFisher SCIENTIFIC

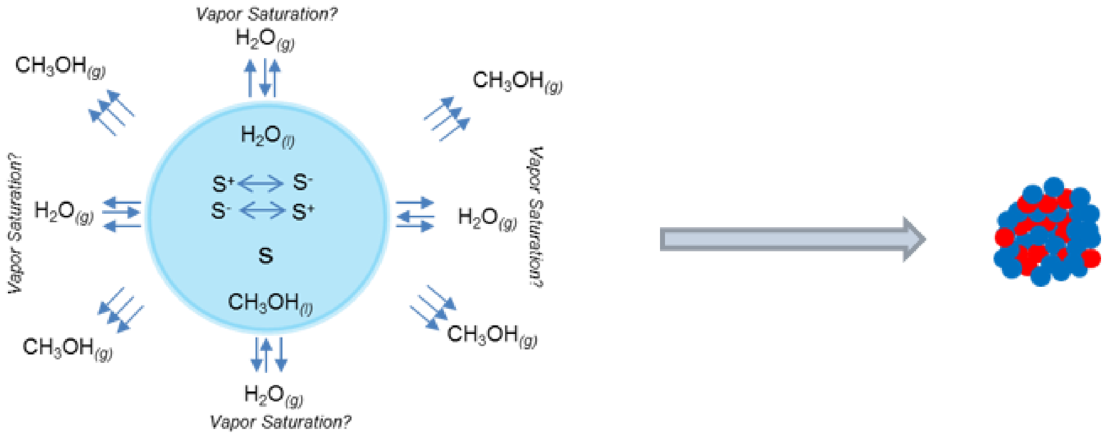
Evaporative Aerosol Detection

- Pneumatic nebulizers create wide droplet size distribution
  - Isocratic – size distribution ~ constant throughout separation
  - Solvent gradient – size distribution changes with eluent viscosity and surface tension
- With less volatile eluents (e.g., aqueous) - **largest droplets must be removed** (‘size cut’)
- Evaporation from remaining droplets produces a steady stream of dried particles
- Size of each particle depends on nonvolatile solute mass concentration in initial droplet



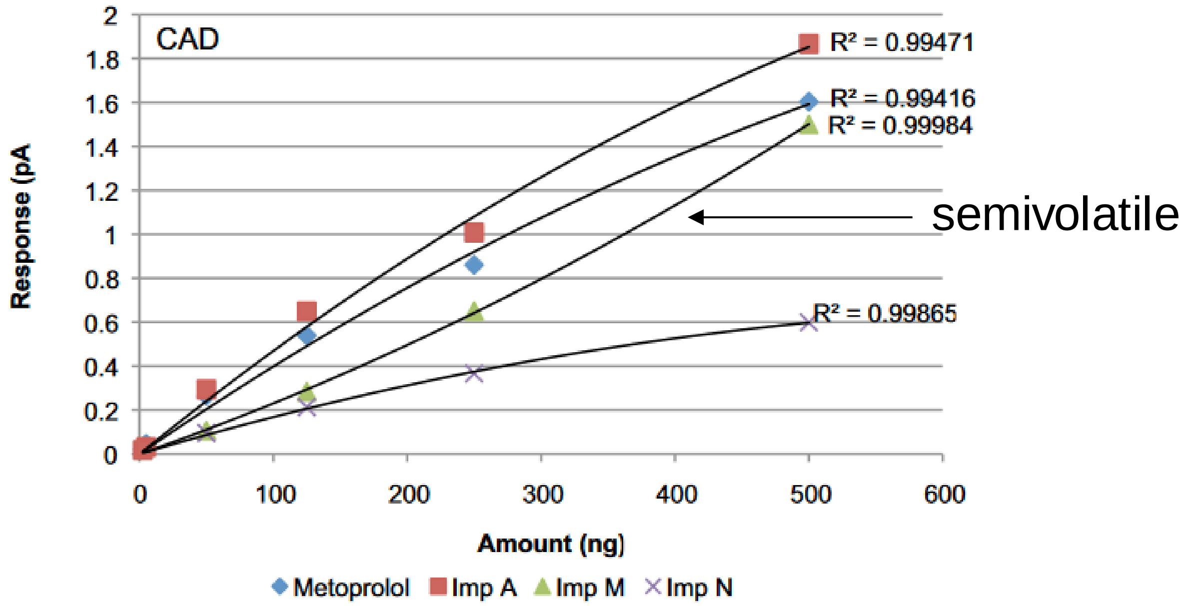
Droplet Evaporation

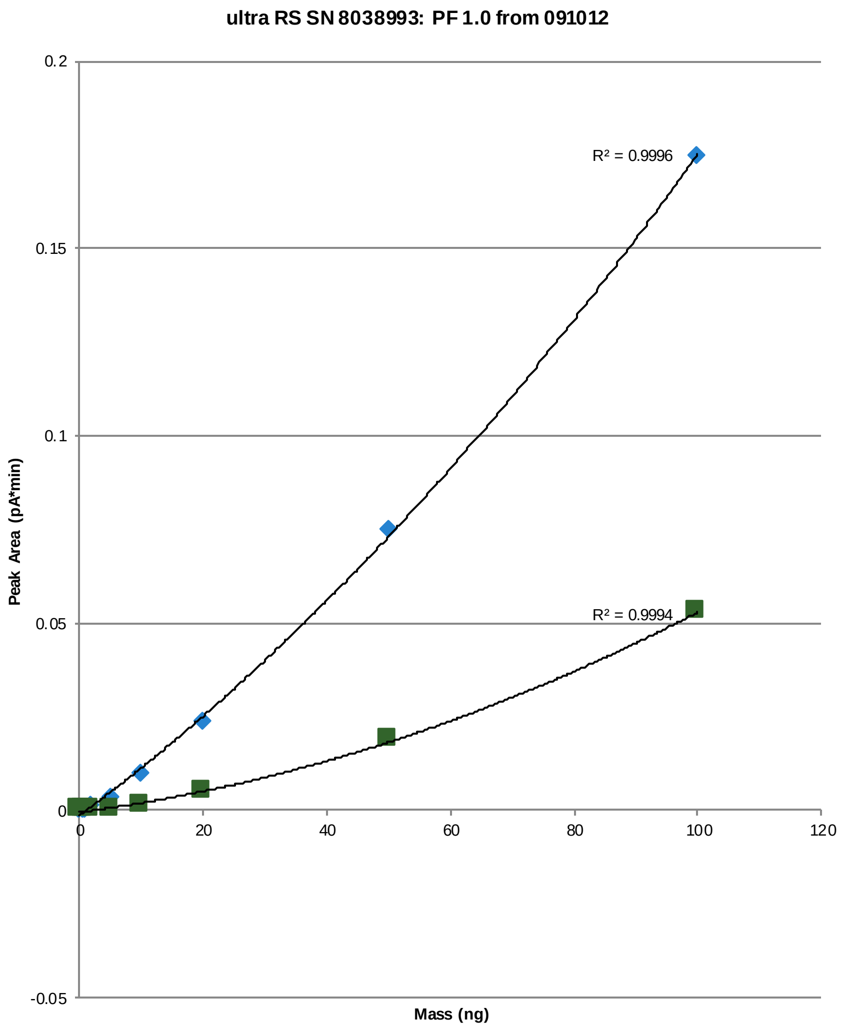
- Solutes that form stable particles - behave as nonvolatiles and should have **similarly-shaped response curves**
- Solutes that form less stable particles - behave as semivolatiles
  - Lower overall response, non-linear drop at low end = **shape of response curve is distinct from that of nonvolatiles**
- Not fully predicted by boiling point, vapor pressure, etc. – more complex
  - Important to consider possible ionic interactions
- ↑ Evap T - more analytes behave as semivolatiles – less uniform response



Response Curve Shape

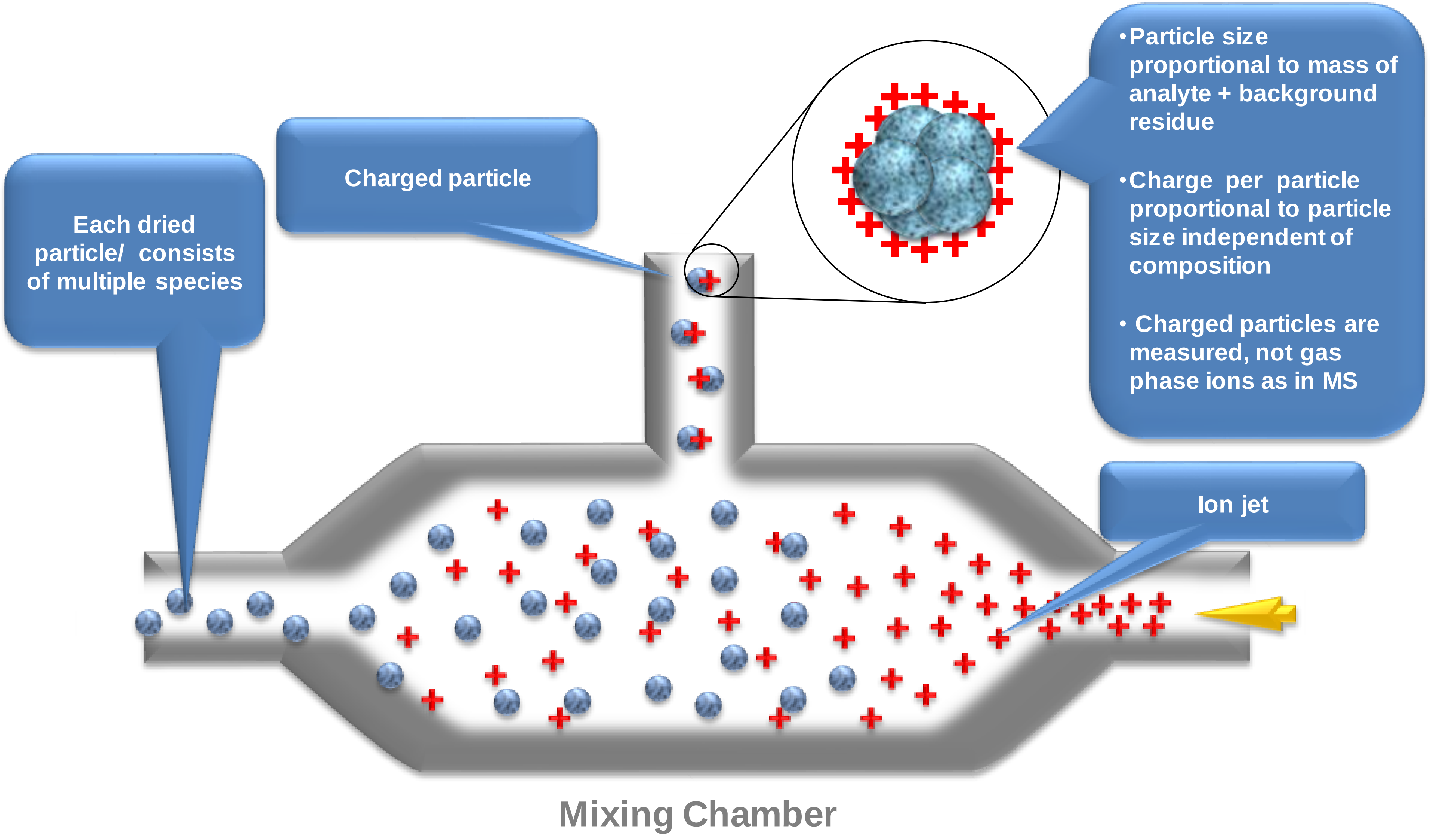
- Nonvolatiles - similarly-shaped response curves
- Semi-volatiles
  - Higher exponent (b) → supralinear, most pronounced at low end
    - Smaller particles evaporate more quickly
  - ↑ variability / imprecision
  - Higher Evap T – more analytes behave as semivolatiles





ThermoFisher SCIENTIFIC

Particle Charging for Charged Aerosol Detection

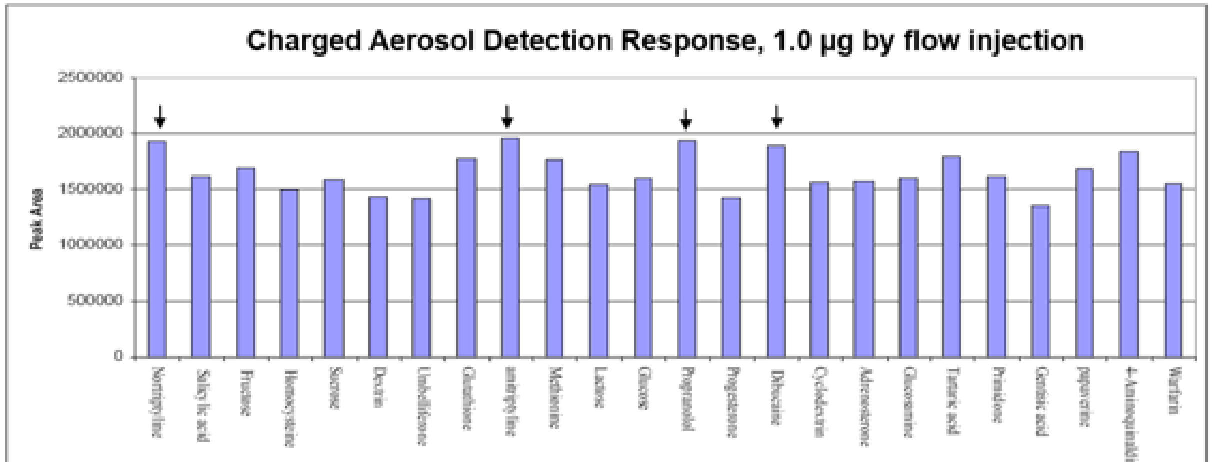


ThermoFisher SCIENTIFIC

- Dependency of Response on Analyte Properties*
  - ELSD
    - Refractive index (RI), which may vary > 2-fold for analytes within expected RI range of 1.4 – 1.6
    - Absorbance and fluorescence: mainly when using a monochromatic (e.g., laser diode) light source
  - CAD
    - **Only a minor dependence** on particle dielectric constant (ε): 3 to 5% difference between NaCl (ε = 3.3) and Ag (ε = infinity)
  - CNLSD
    - Particle ‘wettability’ and ‘solubility’ with respect to the condensing fluid, usually water

Several comparison studies* - CAD has been consistently shown to provide more analyte-independent response than ELSD

Direct comparison: Similar experiments (to right)
% RSD - CAD – **8.0**; CNLSD – **39.0**

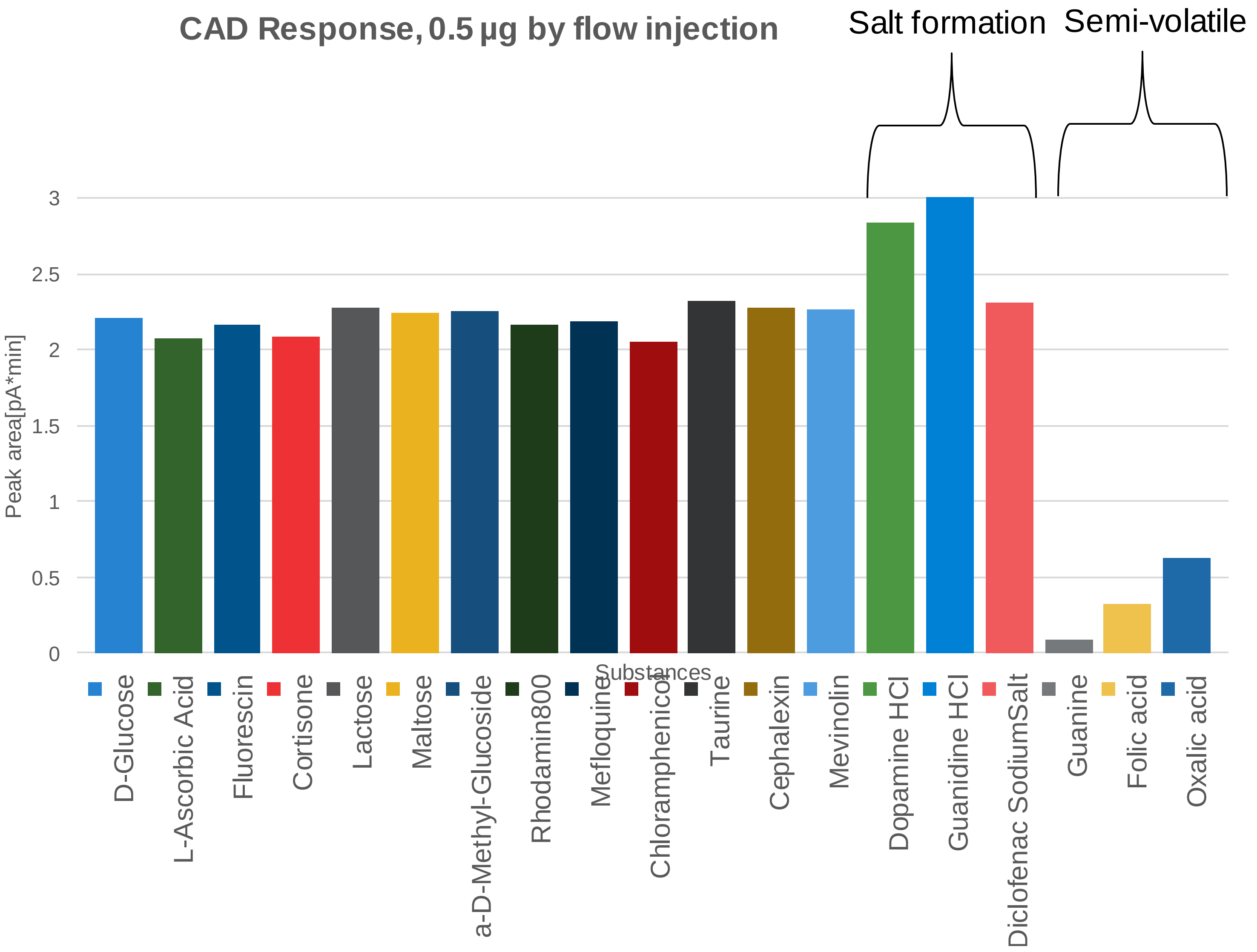


Analytes with arrows include a counterion

** Gamache PH, editor. Charged Aerosol Detection for Liquid Chromatography and Related Separation Techniques. John Wiley & Sons; 2017 May 8*

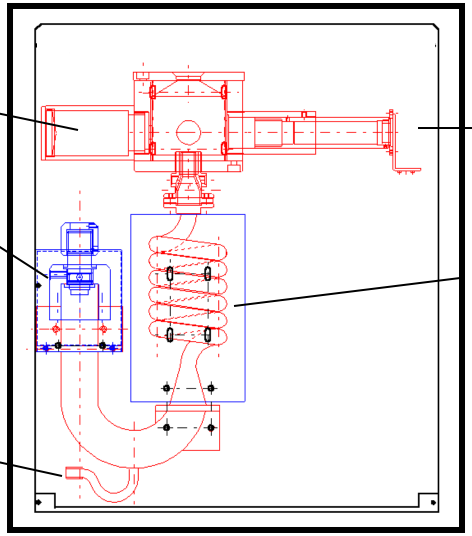
CAD – Unipolar Diffusion Charging of Aerosol Particles

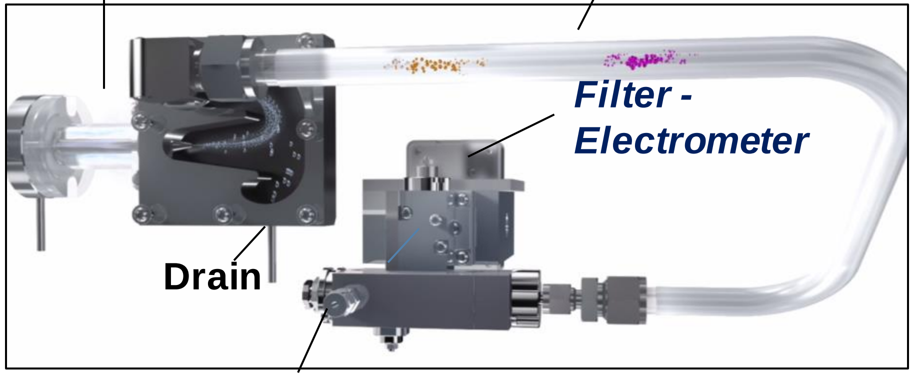
- Size-dependent charge, effectively independent of particle (chemical) composition
- Uniform response for nonvolatiles in isocratic conditions
- Ionic analytes + mobile phase additives = larger particles, higher response
- Semivolatiles – lower response, preferential loss of smaller particles



Summary - Evaporative Aerosol Detectors

- Response of all EADs depends on upstream 'spray-drying' process:
  - Relative volatility / partitioning into condensed phase
  - Solvent (gradients) – change in mass transport to downstream detector
  - Salt formation between ionizable analyte and additives
- Downstream aerosol detector of CAD has least dependency on dried particle composition enabling the most uniform response

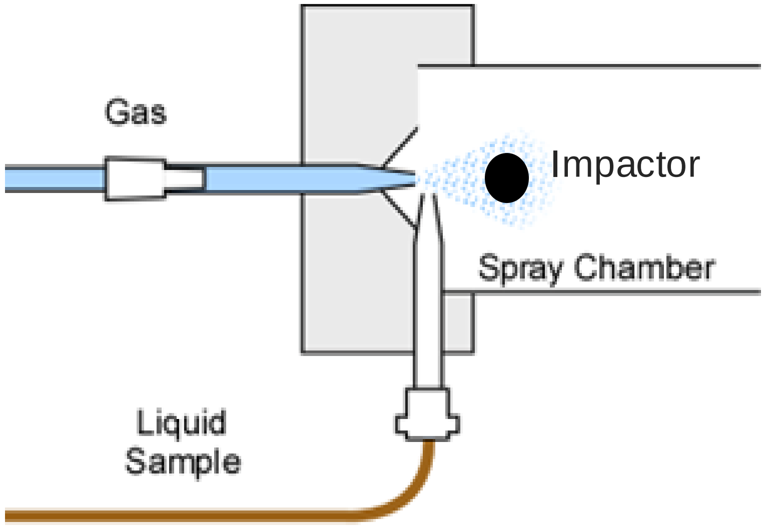




Topics

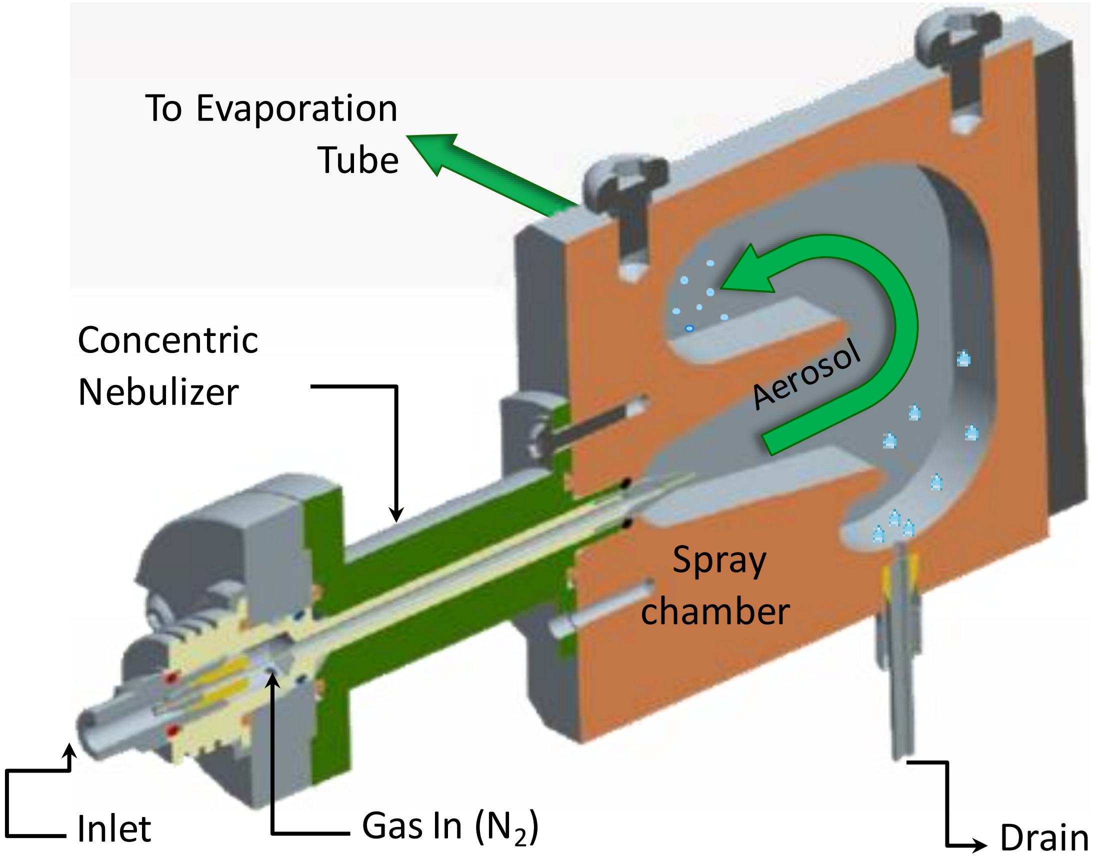
- Key concepts behind Charged Aerosol Detection (CAD)
  - Universal Detection and Uniform Response
- How does CAD work?
- Recent Advances
- Use of Vanquish Duo and CAD for Relative Quantitation

Thermo Scientific™ Dionex™ Corona™ ultra RS™ Charged Aerosol Detector Corona ultra RS



Cross-flow Nebulizer

Thermo Scientific™ Veo and Vanquish Model CADs



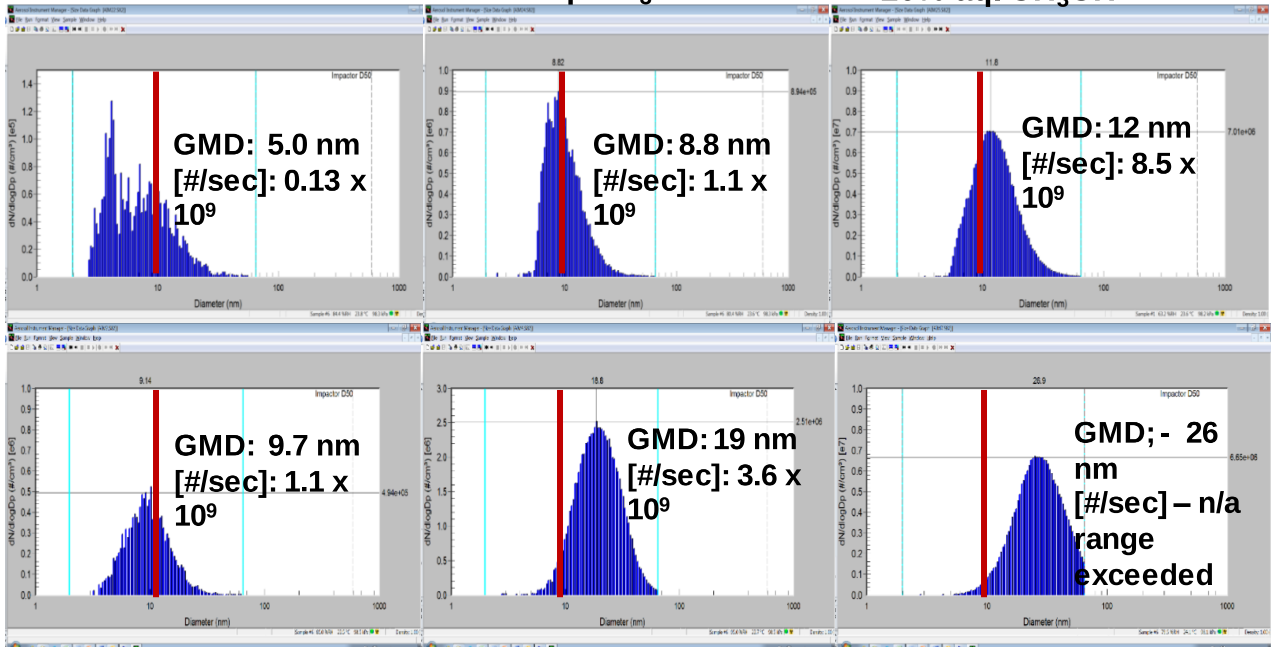
Particle Size Distributions – Comparison of Designs

- Larger median size and higher [#] produced by newer CADs
  - **More sensitive to nonvolatile / semivolatile: analyte and impurities**
  - **Semivolatiles – larger particles are more stable**

20% aq. CH_3OH | **1 µg/mL theophylline; 20% aq. CH_3OH** | **10 µg/mL theophylline; 20% aq. CH_3OH**

Corona ultra RS

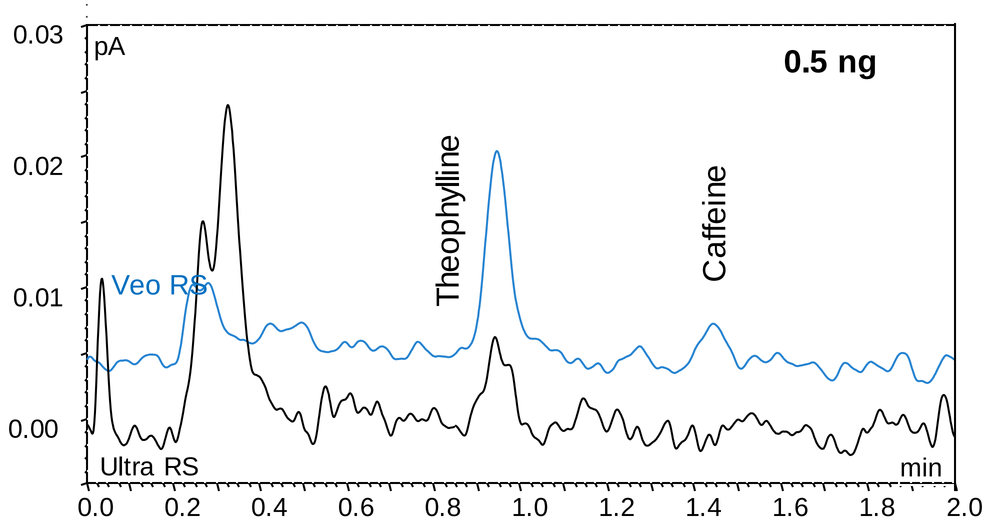
Corona Veo RS

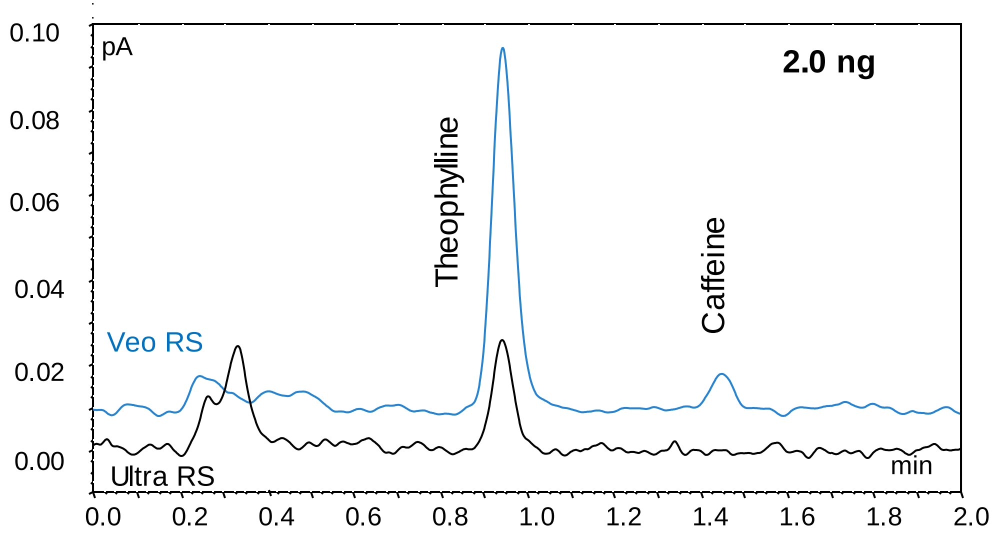


SMPS spectrometer, TSI Model 3938, 1.0 mL/min liquid flow; GMD = geometric mean diameter.

CAD Technology Developments - Improved Sensitivity

- More sensitive detection of:
  - Nonvolatiles (e.g., theophylline)
  - Semi-volatiles (e.g., caffeine) **at higher evap T**
    - (Veo 35 °C, Ultra RS subambient due to evaporative cooling)
  - **Impurities – nonvolatile and semi-volatile**
- Best sensitivity limits with ‘cleanest’ eluents
- Most uniform response with lowest Evap T

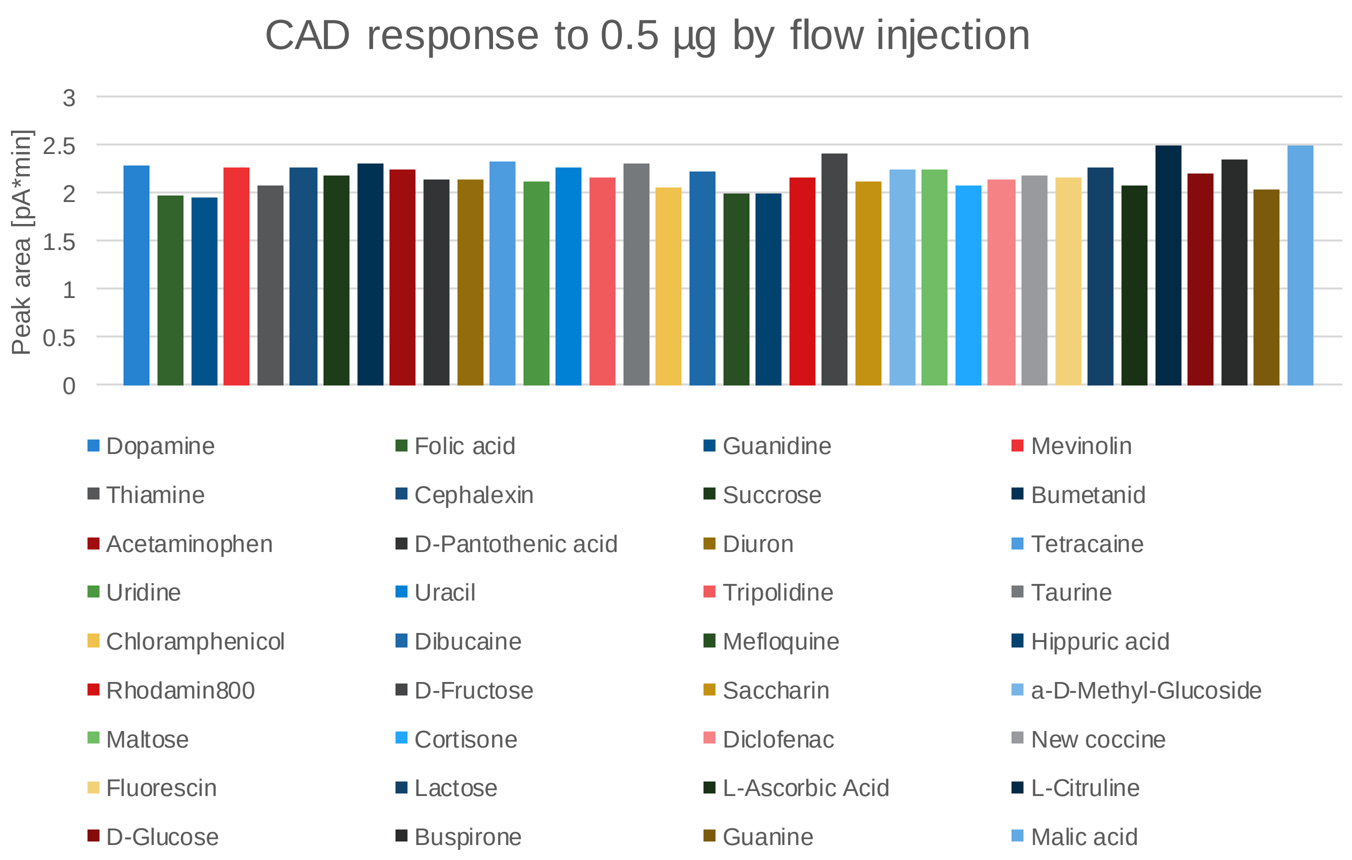




Thermo Fisher SCIENTIFIC

Response Uniformity - CAD

- Flow injection analysis of 36 structurally diverse nonvolatile analytes
- Vanquish CAD, Evap T = 35 °C
- Variance among analytes = 5.8% RSD
- Additional factors?:
  - Purity of starting material (e.g., powder)
  - Changes during preparation (e.g., water absorption)
  - Weighing and dilution errors
  - Analyte degradation, loss on column



Topics

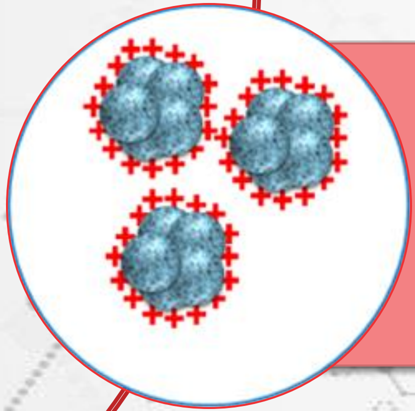
- Key concepts behind Charged Aerosol Detection (CAD)
  - Universal Detection and Uniform Response
- How does CAD work?
- Recent Advances
- Use of Vanquish Duo and CAD for Relative Quantitation

ThermoFisher SCIENTIFIC

Additives

Choice of additive type and concentration – simultaneously consider separation and detection requirements

- pH modifiers (acids and bases)
- Buffers (weak acid + conjugate base or weak base + conjugate acid)
- Ion-pairing reagents (acid or base with hydrophobic tail)



Volatile additives can form less volatile salts with other ionic eluent components

- Analytes, other additives, impurities, sample matrix components

$Cl^- + H^+ \rightarrow HCl$
volatile

$Cl^- + NH_4^+ \rightarrow NH_4Cl$
nonvolatile

- Beneficial when Cl^- is analyte
- Detrimental if impurity/ matrix component

Higher mass transport for solvents with lower viscosity and surface tension

- Analyte - ↑ signal, less uniform response
- Impurities – ↑ baseline signal, noise, drift

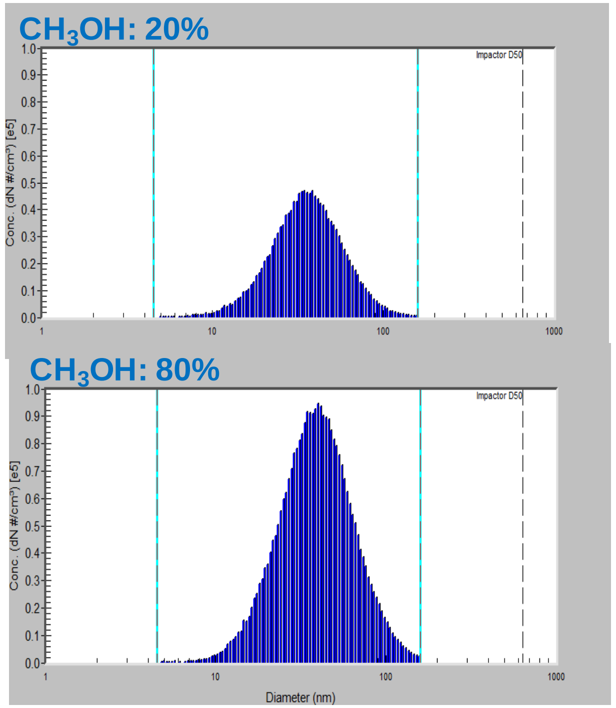
Impurities in weaker solvent (e.g., H_2O) may concentrate on column resulting in

- peaks, artifacts, drift

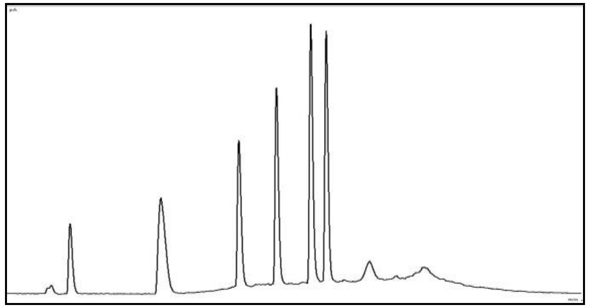
Inverse gradient compensation - more uniform response, less drift

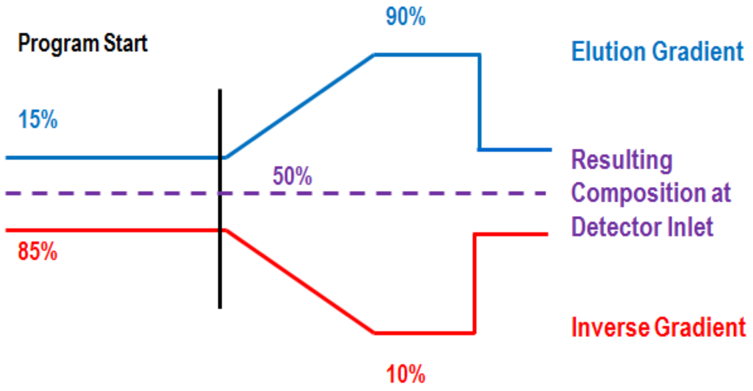
- Column bleed, impurities in weaker solvent still a concern

10 µg/mL theophylline

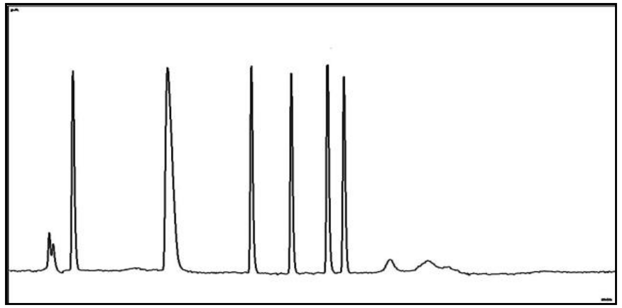


Conventional Gradient Elution



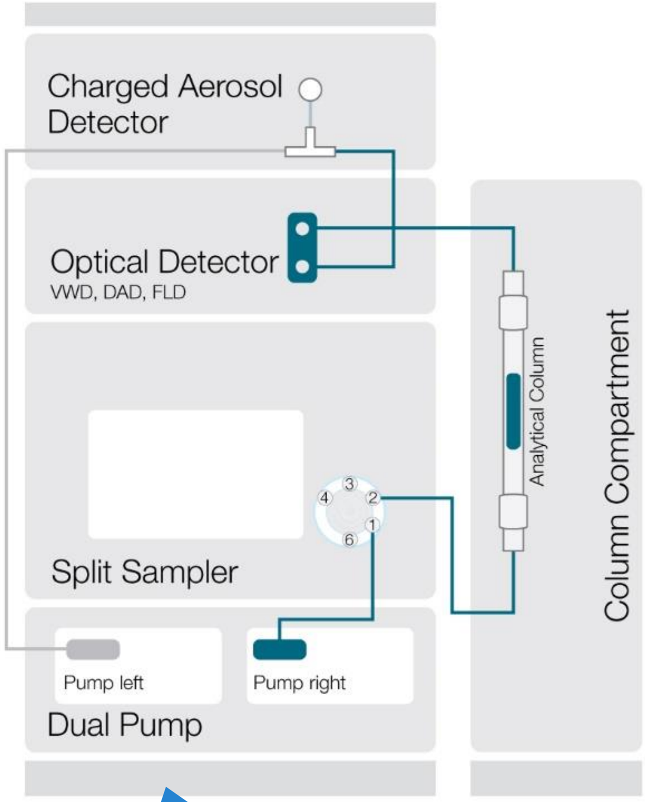


Inverse Gradient Compensation

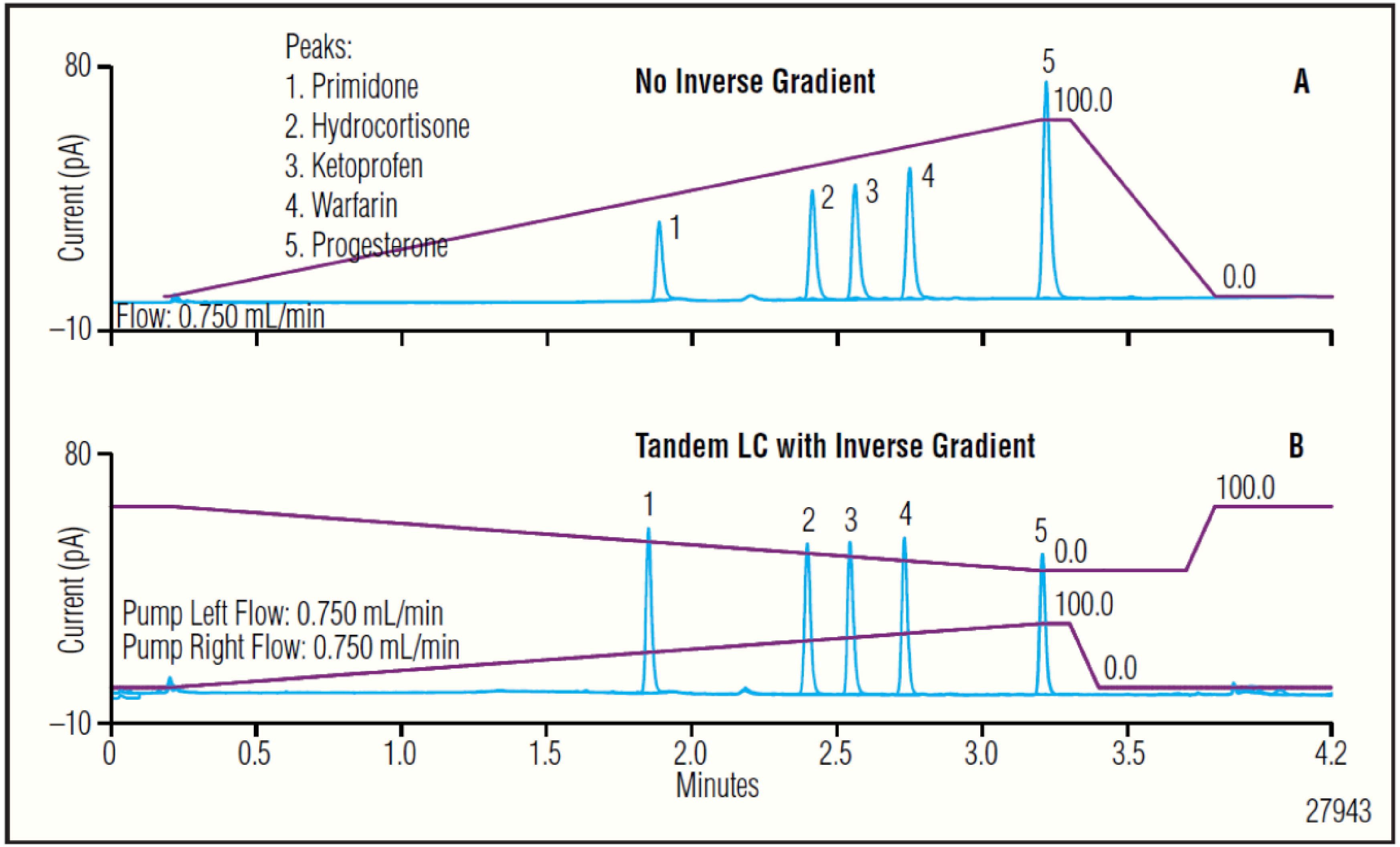


Thermo Fisher SCIENTIFIC

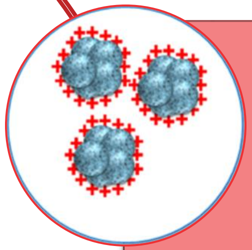
Inverse Gradient Compensation



Vanquish Duo for Inverse Gradient



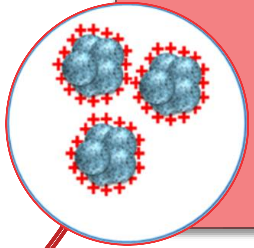
Salt Formation - Influence on Ionic Analyte Response



Volatile / semivolatile

– significant response increase and change in curve shape, toward that of nonvolatiles (decrease in exponent b)

Use lowest practical evaporation T to achieve more uniform response



Nonvolatile ionized analytes

– no change in curve shape, smaller affect on response

Relative quantitation – If extent of ionization and molar mass (M) are known:

$[(\text{Peak Area} \times M_{analyte})/(M_{analyte} + M_{additive})]$

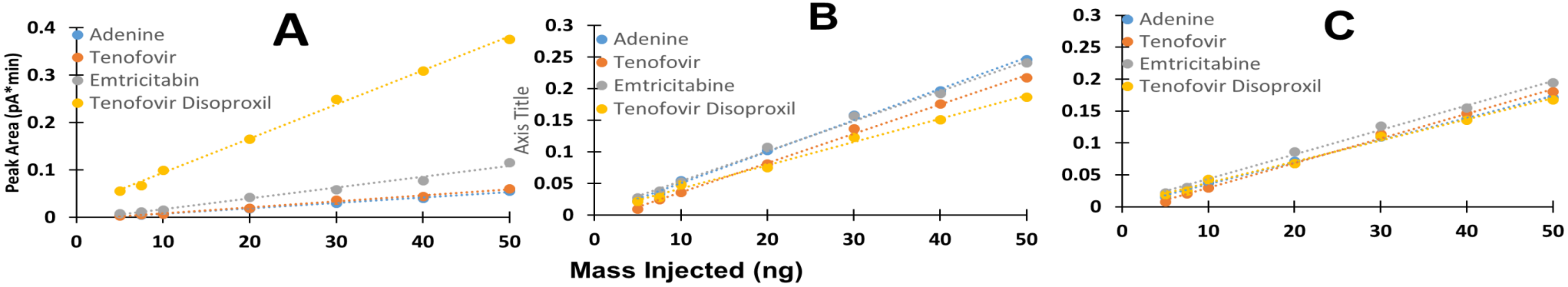
Use low M additives (e.g., formic acid, ammonia) to minimize affect / achieve more uniform response

Estimating quantity in the absence of individual standards

Without inverse gradient compensation

With inverse gradient compensation

With inverse gradient compensation and correction for salt formation*



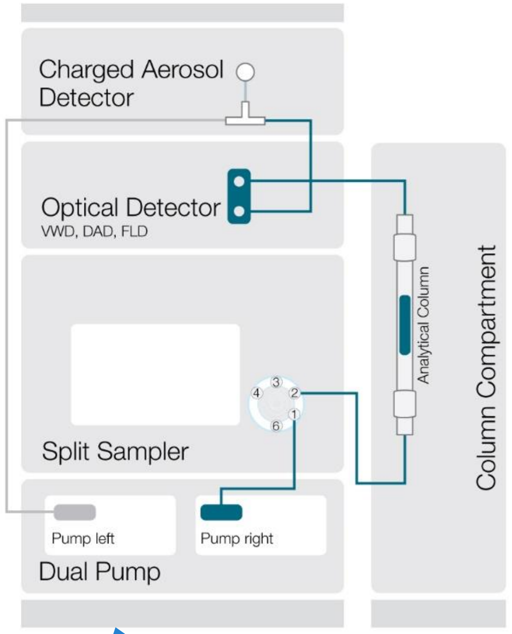
*In 0.1 M acetic acid eluent, each analyte was assumed to have one fully ionized basic functional group

CAD and other EADs have in common:

- Detection scope and eluent requirements
- Response dependency on
  - Solvent composition
  - Analyte volatility
  - Salt formation for ionic solutes

Downstream aerosol detector of CAD enables:

- Better detection limits
- Wider linear and dynamic ranges
- More uniform response



Vanquish Duo for Inverse Gradient

New Technology

- Even better detection limits
- More uniform response
- More flexibility by use of wider range of eluent flow rates and compositions

Uniform response and relative quantitation

- Inverse gradient compensation
- Lowest practical evaporation temperature
- Accounting for effects of salt formation

Join the Fun! *Cache a Chromeleon* Game

- Use your mobile device to complete challenges and earn a Charlie Chromeleon plush toy!
- If you are playing, you have earned points for attending this seminar. Be sure to scan the barcode on the desk outside the door.
- Ask booth staff for more details on how to play.

ThermoFisher SCIENTIFIC

Thank You

Please join me in the

Liquid Chromatography

section of our booth where I'll

address additional comments and questions.