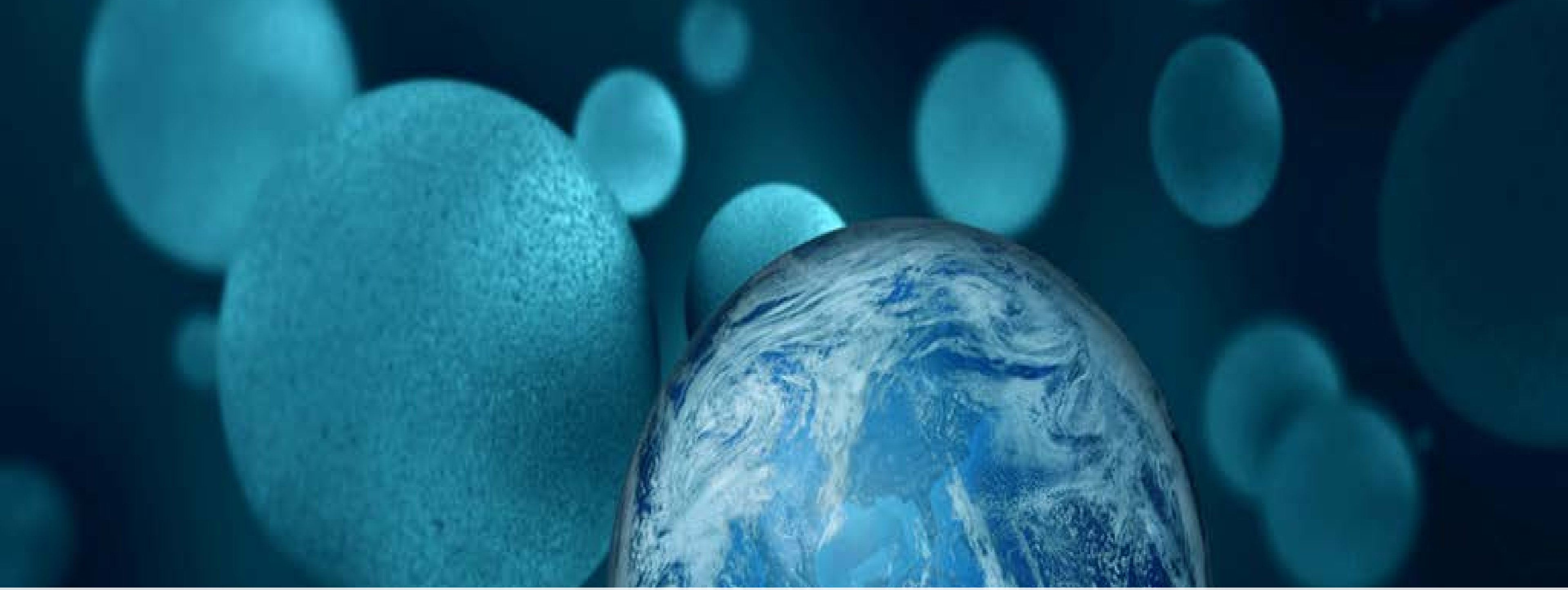


ThermoFisher SCIENTIFIC

Charged Aerosol Detection 101

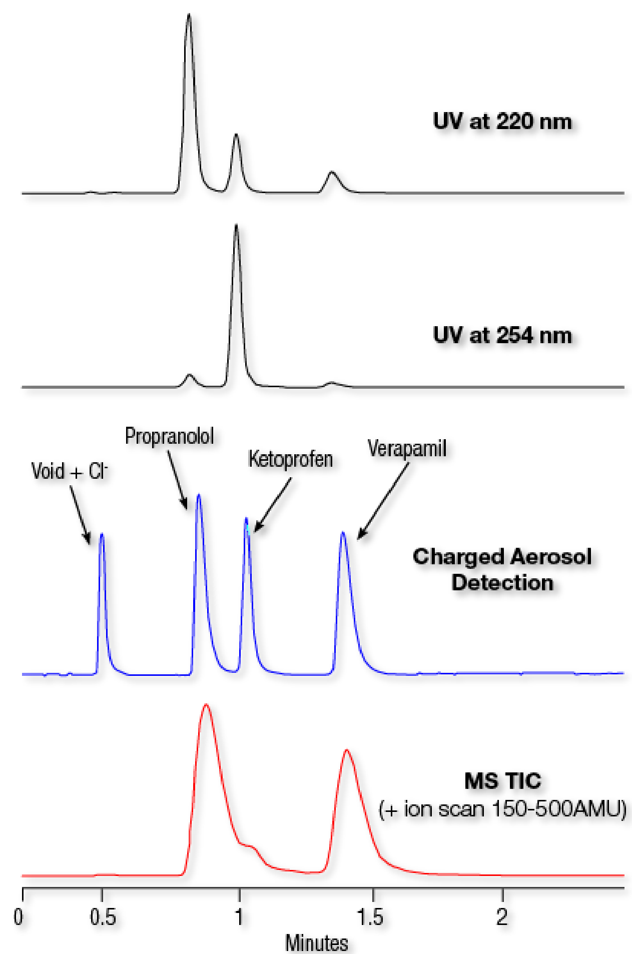
Dr. Alexander Schwahn
European Sales Support Expert for Biopharma Industry
Thermo Fisher Scientific, Reinach, Switzerland

Outline

- Introduction to charged aerosol detection (CAD)
- How charged aerosol technology works
- Comparison of CAD to ELSD
- CAD product evolution
- Example applications
- Summary

Introduction to Charged Aerosol Detection (CAD)

- Used to quantitate any non-volatile and many semi-volatile analytes with LC
- Provides consistent analyte response independent of chemical structure and molecule size
- Neither a chromophore, nor the ability to ionize, is required for detection.
- Dynamic range up to four orders of magnitude from a single injection (*sub-ng to µg quantities on column*)
- Mass sensitive detection – CAD provides relative quantification without the need for reference standards
- Compatible with gradient conditions for HPLC, UHPLC, and micro LC



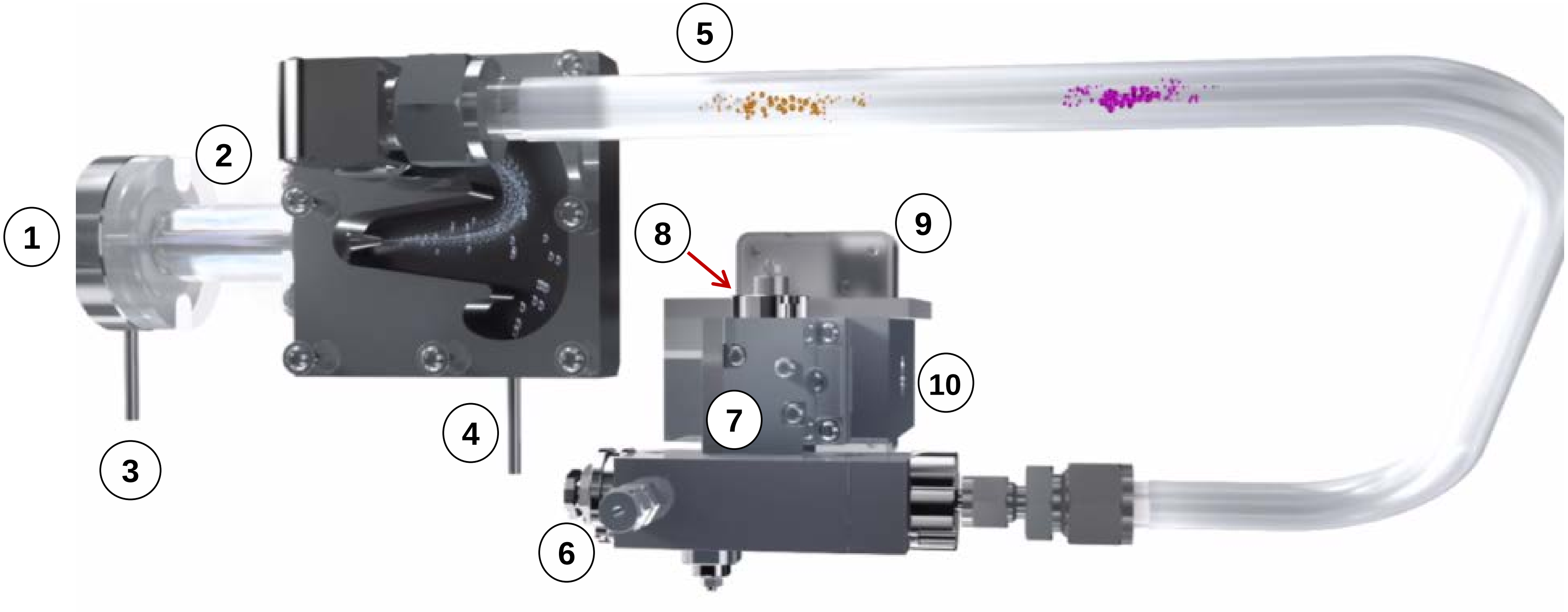
Comparison of charged aerosol detection to UV and MS

ThermoFisher SCIENTIFIC

Charged Aerosol Detection – How It Works

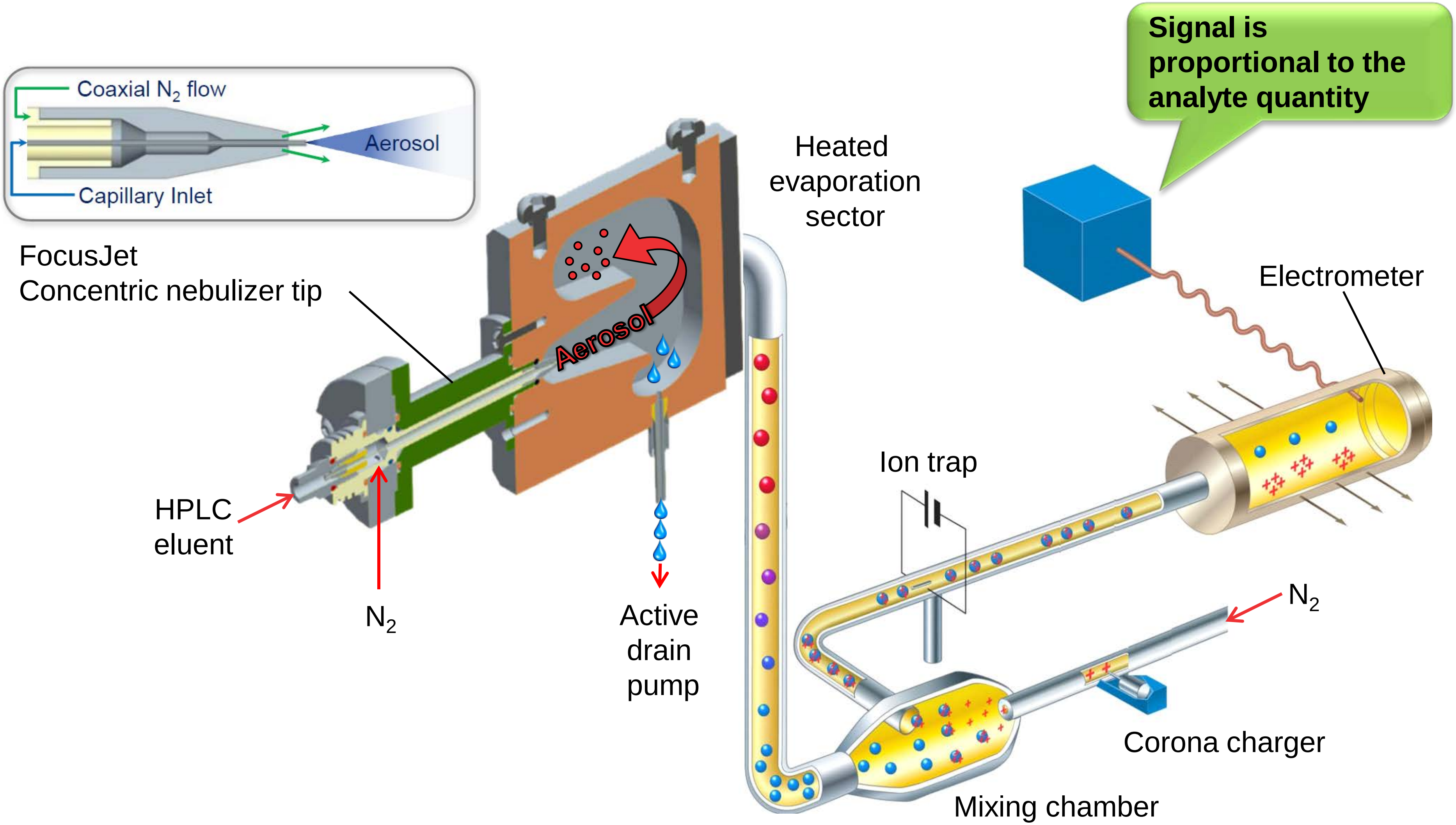
Flow path through a Thermo Scientific™ Dionex™ Corona™ Veo™ charged aerosol detector

1 Inlet from column
2 FocusJet™ concentric nebulizer
3 Gas inlet
4 Micro drain pump
5 Evaporation tube
6 Corona charger
7 Mixing chamber
8 Ion trap
9 Electrometer
10 Gas exhaust



Charged Aerosol Detection – How It Works

Flow path through a Thermo Scientific™ Dionex™ Corona™ Veo™ charged aerosol detector



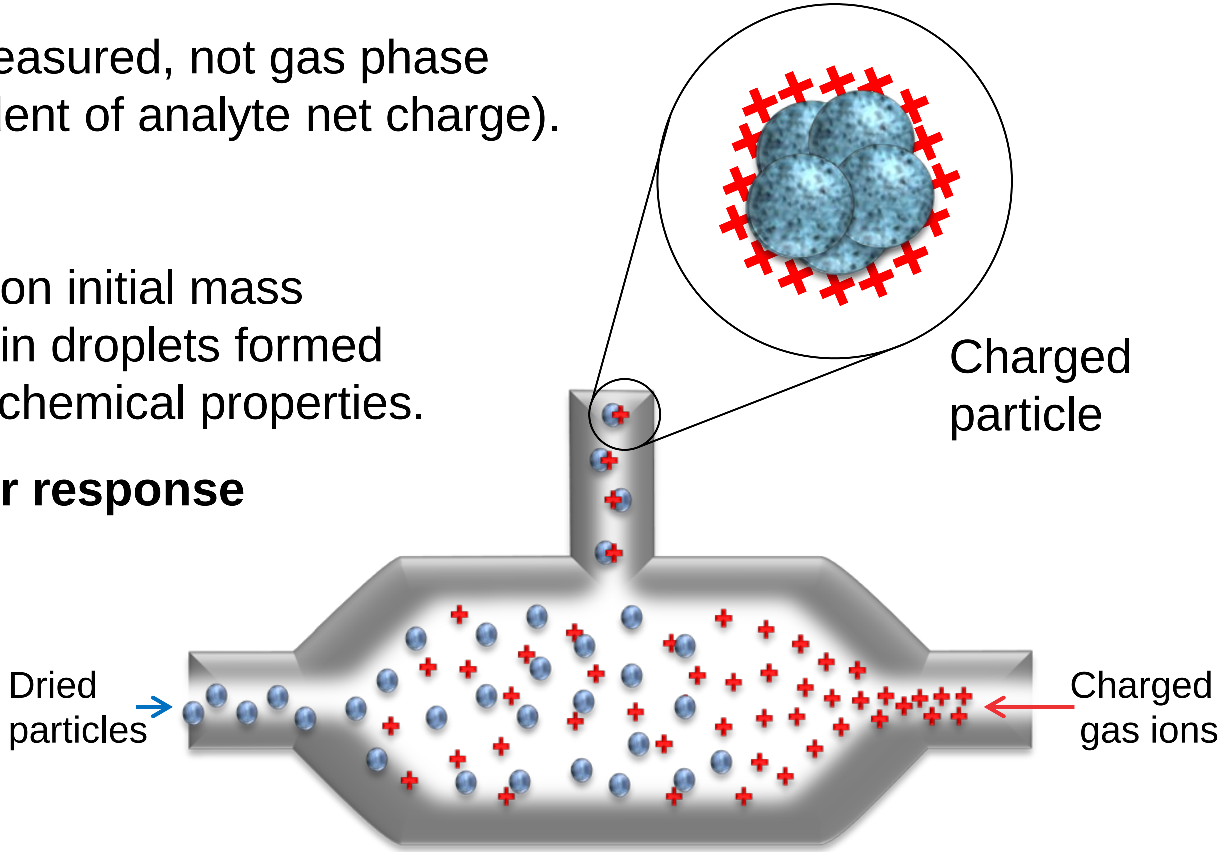
Particle Charging for Charged Aerosol Detection

- Particle size proportional to mass of analyte
- Charge per particle proportional to particle size
- Charged particles are measured, not gas phase ions as in MS (Independent of analyte net charge).

➡ CAD response depends on initial mass concentration of analyte in droplets formed but is independent of its chemical properties.

➡ **Nearly uniform detector response**

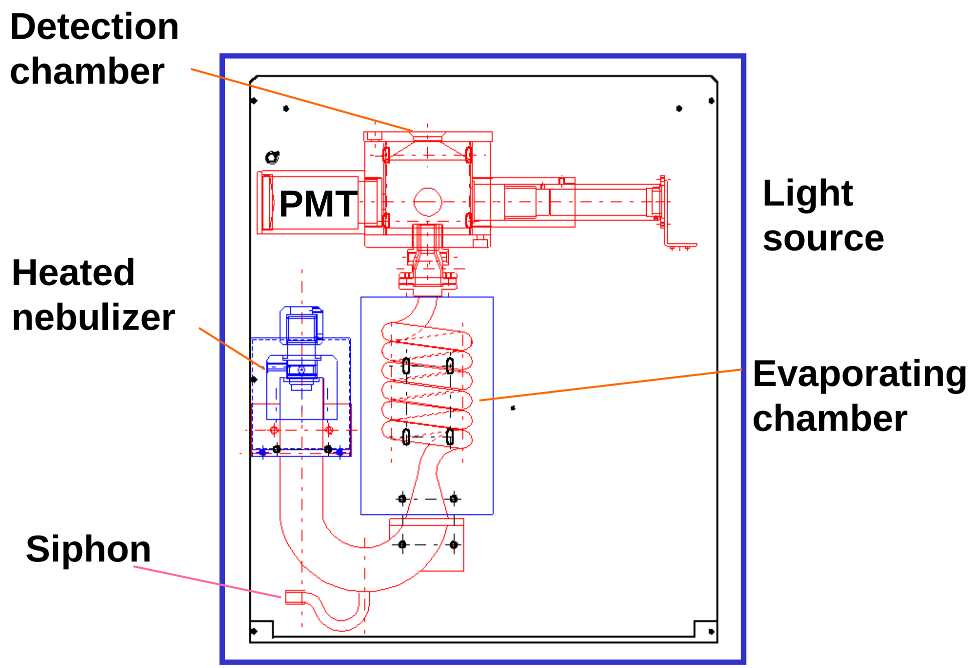
However,
sample needs to be non-volatile or at least only semi-volatile.



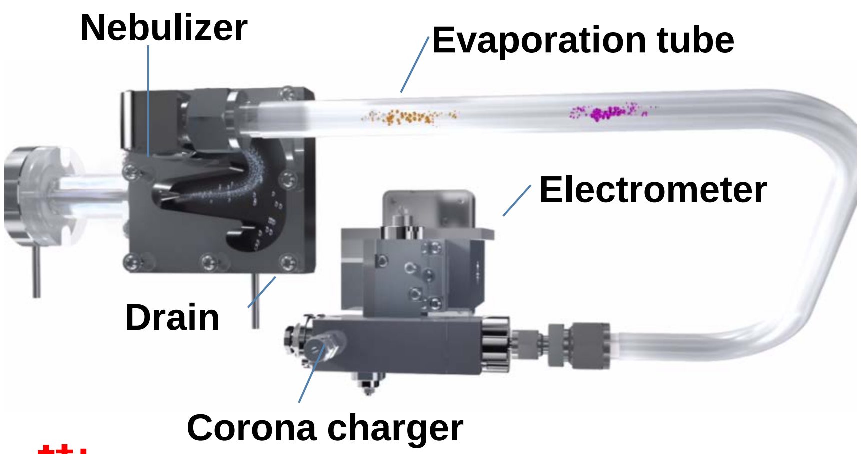
Diffusional charging of aerosol in mixing chamber

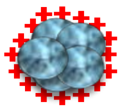
ThermoFisher SCIENTIFIC

Evaporative light scattering detector (ELSD)



Charged aerosol detector



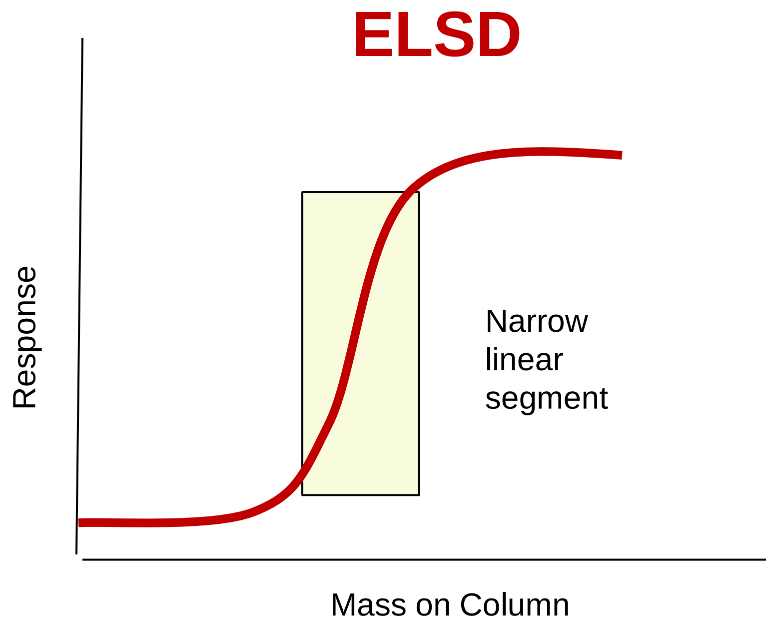


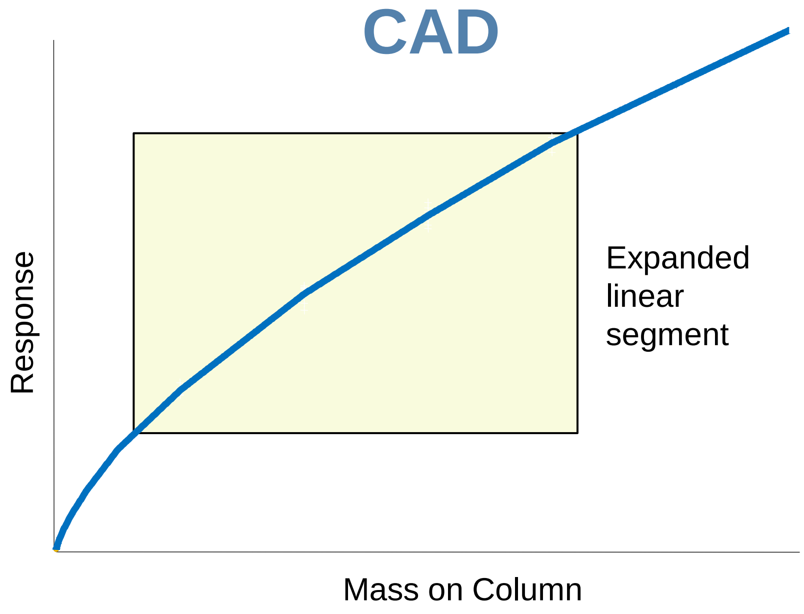
- Particle size is proportional to mass of analyte + background residue

Measures the **optical reflection** of solute particles after the sample has been passed through a nebulizer

Measures **charged particles** by an electrometer generating a signal that is proportional to particle size (Mass of analyte) after nebulization

Detector Response Characteristics



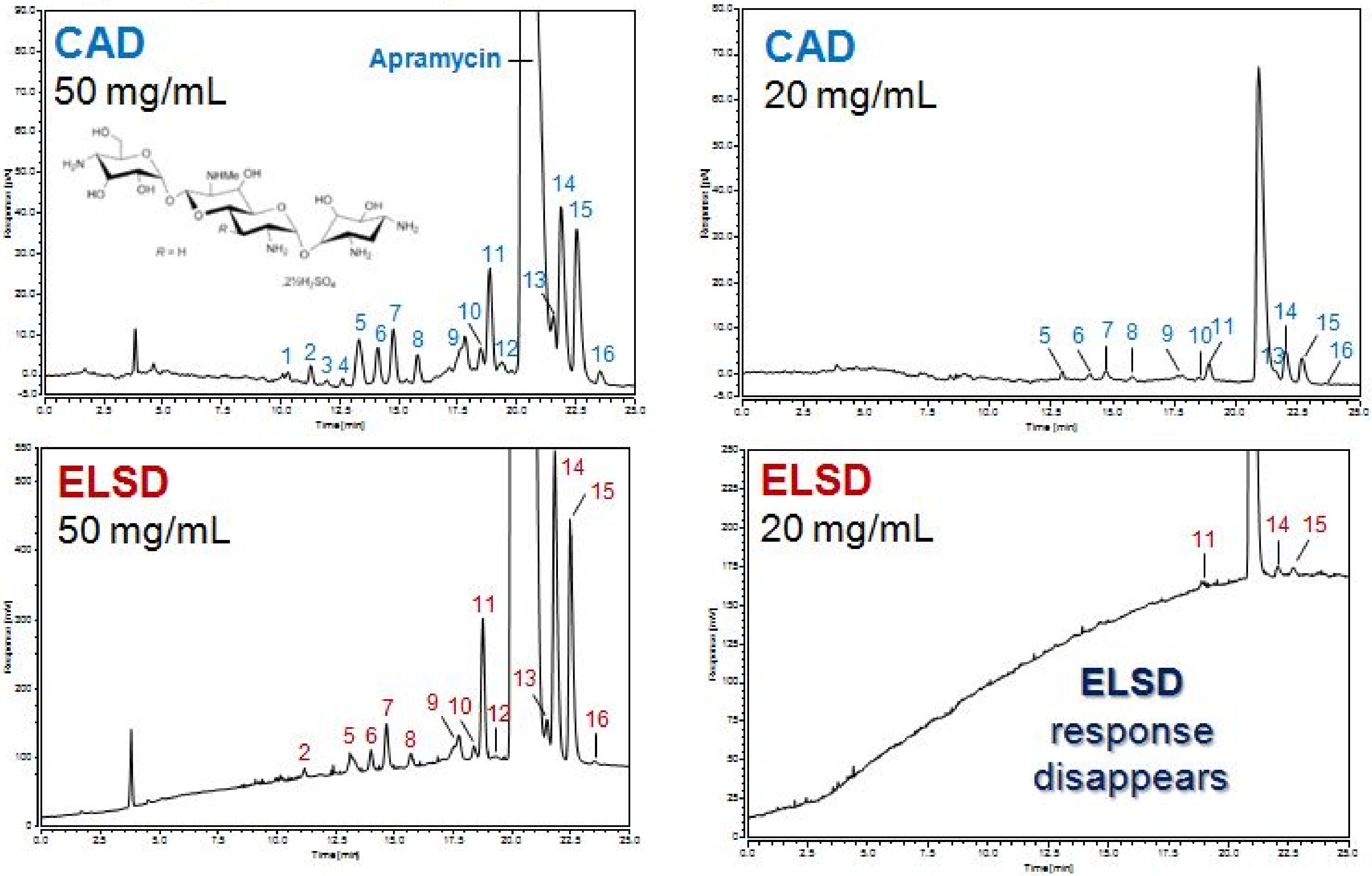


- For Rayleigh scattering: b = 2
- For Mie scattering: b = 1⅓
- For Refraction and reflection scattering: b = ⅔

- Nonvolatiles - Decreasing slope with increasing mass (b ~ ⅔)

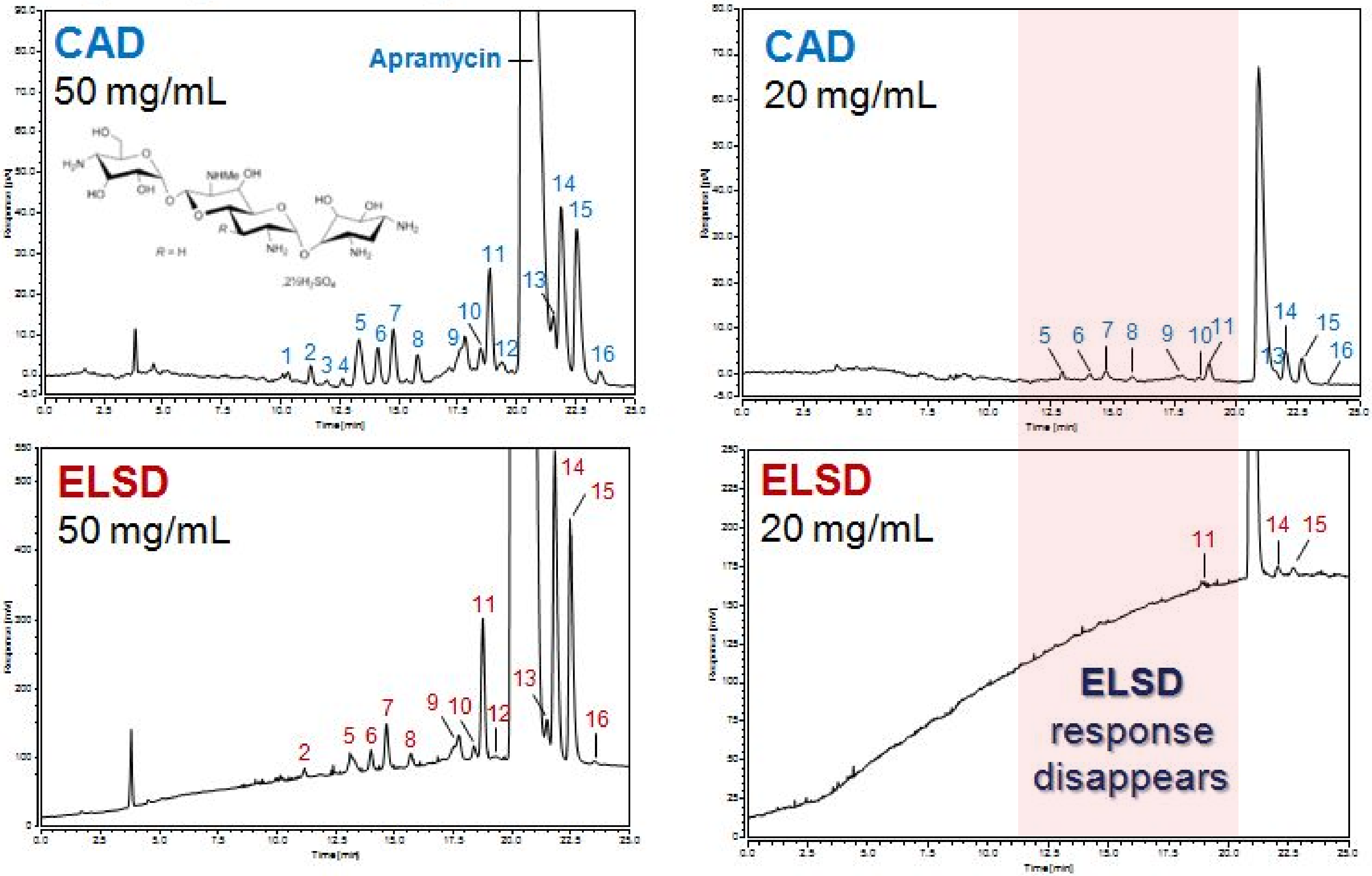
ELSD exhibits a narrower linear calibration range than CAD.

Apramycin and impurities



ThermoFisher SCIENTIFIC

Apramycin and impurities



Thermo Fisher
SCIENTIFIC

Calibration Consideration with Using Universal Detectors

- Over short ranges, both ELSD and charged aerosol detector can offer linear response.
- All aerosol-based detectors exhibit a non-linear response over large concentration ranges.
- Several calibration curve approaches are available:
  - Selections –

ELSD	CAD
Sigmoidal response behavior	Parabolic response behavior
Log-log	Log-log
Point to point	Quadratic
	Power function

The most appropriate approach depends upon the data.

Comparison Review

Feature	Evaporative light scattering	Charged aerosol
Response	Sigmoidal	Curvilinear
Dynamic range	*2*–3 orders	>4 orders
LoQ and LoD	LoQ and LoD often higher (Worse) than estimated by SNR	LoQ and LoD often lower (Better) than estimated by SNR
Sensitivity (LoD)	>10 ng	<1 ng
Semi-volatility range	Similar	Similar
Analyte response	Variable - Dependent on compound	Independent of structure
Flow rate range (0.2 – 2 mL/min)	Possibly several nebulizers	One nebulizer
Ease of operation	Can be complex	Simple

Evolution of Charged Aerosol Detectors

2015 — Thermo Scientific™ Vanquish™ charged aerosol detector — Full integration with Thermo Scientific™ Vanquish™ UHPLC platform, slide-in module design, reduced flow path for optimum operation

2013 — Corona Veo RS CAD — Extended micro flow rate range; total redesign with concentric nebulization and optimized spray chamber for enhanced sensitivity, heated evaporation and electronic gas regulation

Dionex Corp. acquired by Thermo Fisher Scientific Inc.

2011 — Corona *ultra* RS CAD — Unified with Dionex™ UltiMate™ 3000 UHPLC+ system, added on-board diagnostics / monitoring, automated flow diversion capability and selection of linearization parameters

ESA Biosciences, Inc. acquired by Dionex Corp.

2009 — Corona *ultra* CAD — UHPLC compatible, stackable design, enhanced sensitivity, touch-screen user interface with real-time chromatogram display, incorporated precision internal gas regulation system

2006 — Corona *Plus* CAD — Expanded solvent compatibility with heated nebulization, software drivers for popular CDS systems and external gas conditioning module for improved precision.

2005 — Corona CAD — Introduction of the first commercial charged aerosol detector for HPLC with full control via front panel interface. Designed for near-universal detection on any HPLC system using isocratic or gradient separations

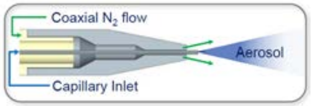
ThermoFisher SCIENTIFIC

Corona Veo and Vanquish Charged Aerosol Detectors

- Concentric nebulization system improves sensitivity and precision.
- Thermally controlled evaporation scheme widens the scope of applications.
- Corona Veo and Vanquish RS model includes low flow capabilities for micro LC, as well as UHPLC.
- Usability and serviceability have been enhanced.
- CDS drivers available for use with all Thermo Scientific and many other vendor systems.

	Corona Veo	VeoRS
Nebulization	FocusJet	FocusJet
Flow rates	0.2 – 2.0 mL/min	0.01 – 2.0 mL/min
Data collection	100 Hz	200 Hz
Evaporation Temp.	35°C or 50°C	RT+5°C – 100°C
Gas pressure control	manual	electronic

Vanquish CAD



FocusJet

Corona Veo RS

Vanquish CAD model	Flow rate range (ml/min)	Data rate (Hz)	Evap temp (°C)	Positioning
Horizon	0.01 – 2.0 (Microflow)	2 – 200	Settable from ambient +5–100	Ideal for R&D and methods development Labs
Flex	0.2 – 2.0	2 – 100	Selectable 35, 50 or 70	Suitable for routine analysis in QC/QA Labs

Pharma and Biopharma Application Areas

- Drug composition
  - Impurity testing
- Formulation
  - Counterions
  - Surfactants / Excipients
- Degradation / Stability testing
- Characterization
  - Glycan analysis
  - Adjuvant analysis
- Excipient raw material analysis and lot-to-lot variability

Pharma and Biopharma Application Areas

- Drug composition
  - Impurity testing
- Formulation
  - Counterions
  - Surfactants / Excipients
- Degradation / Stability testing
- Characterization
  - Glycan analysis
  - Adjuvant analysis
- Excipient raw material analysis and lot-to-lot variability
- Cleaning validation
- Mass balance
- Extractables / Leachables
- PEGylation and antibody-drug conjugates
- siRNA lipid delivery vehicles
- QbD
- MIST (Metabolites in safety testing)

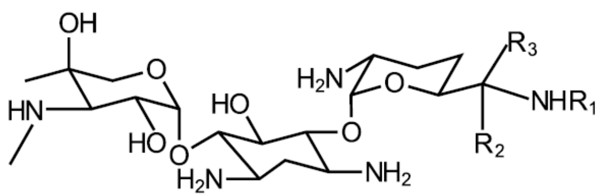
Visit the charged aerosol detection website and the free Thermo Scientific AppsLab library of analytical applications, to see more examples of HPLC-CAD solutions:

www.thermofisher.com/cad

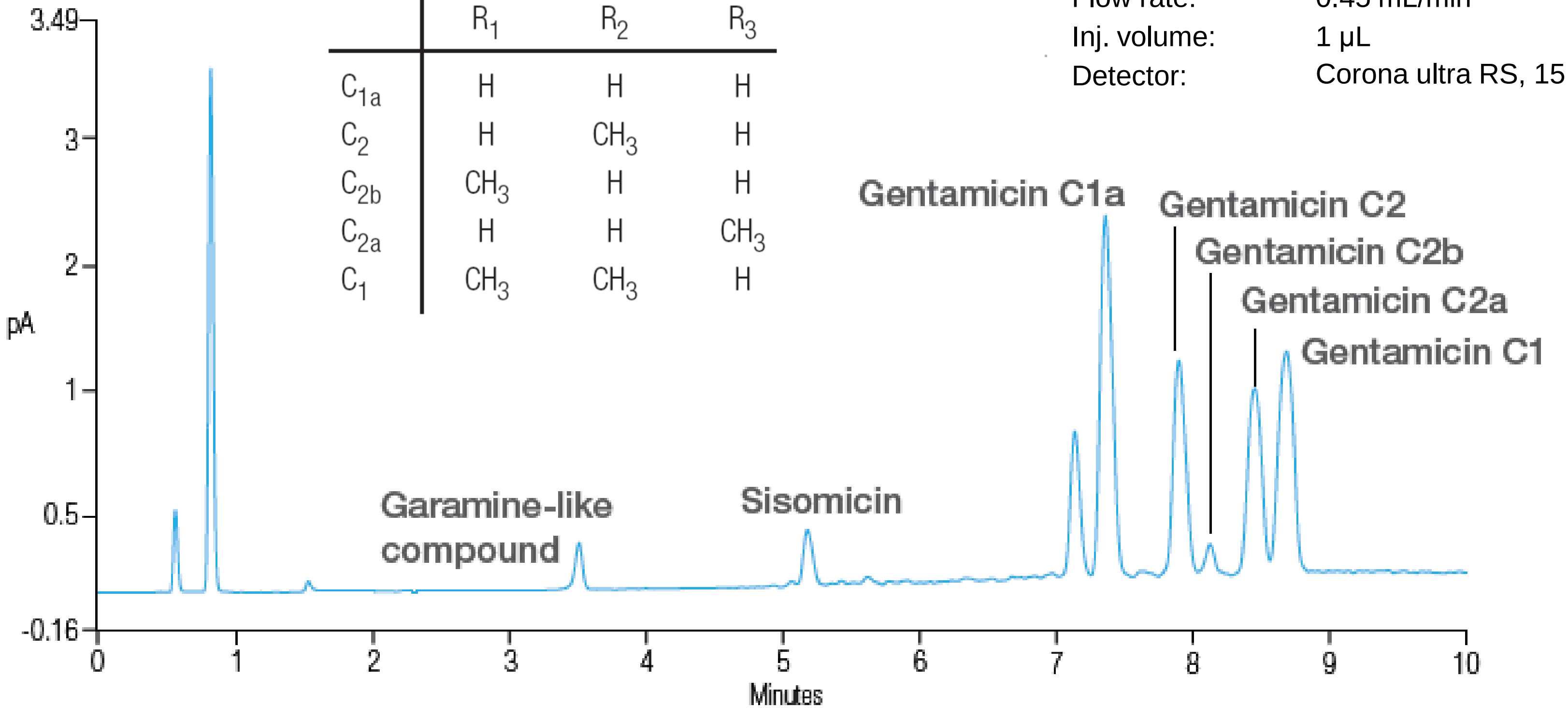
www.thermofisher.com/appslab

Analysis of Gentamicin standard

(200 µg/mL)



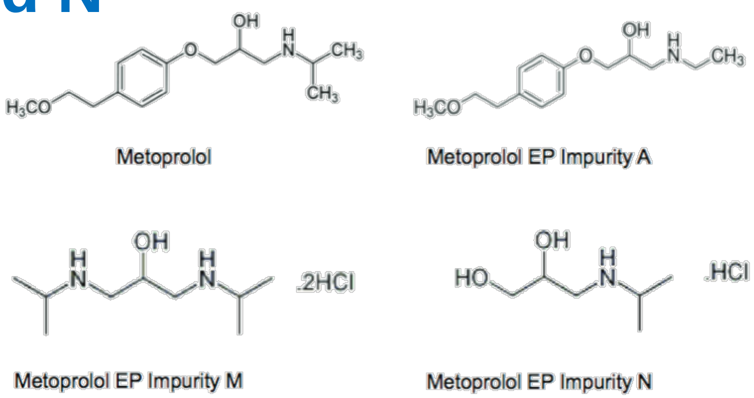
	R_1	R_2	R_3
C_{1a}	H	H	H
C_2	H	CH_3	H
C_{2b}	CH_3	H	H
C_{2a}	H	H	CH_3
C_1	CH_3	CH_3	H

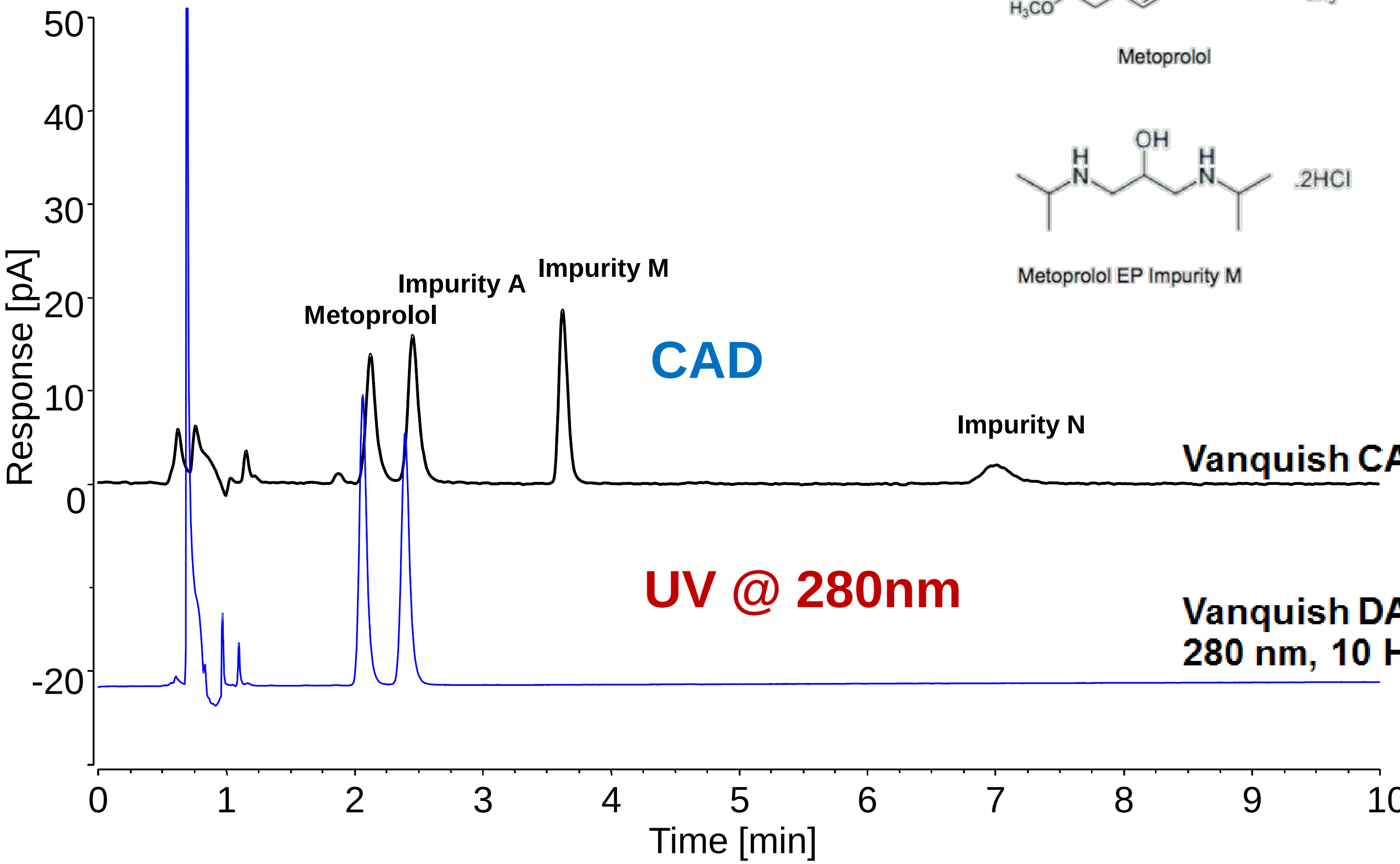


Column:	Thermo Scientific™ Acclaim™ RSLC PolarAdvantage II, 2.2 µm, 2.1 × 100 mm
Mobile phase	A: 0.025:95:5 HFBA:water:acetonitrile
Mobile phase	B: 0.3:95:5 TFA:water:acetonitrile
Gradient:	0 to 1.5min,1 to 10%B 1.5 to 7min,10 to 100% B 7 to 10min,100% B 4 min. pre-injection equilibration
Flow rate:	0.45 mL/min
Inj. volume:	1 µL
Detector:	Corona ultra RS, 15 °C, 60 Hz

Orthogonal and Complimentary Detection with DAD and CAD

Metoprolol and impurities A, M and N

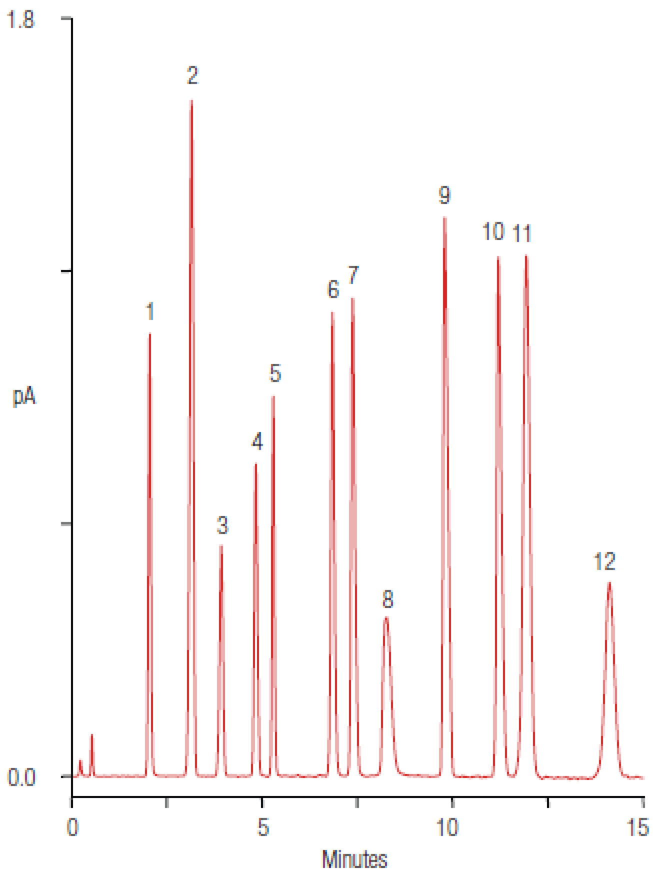




Isocratic HILIC chromatographic method using both UV and charged aerosol detection

Formulation – Counterions

Drug counterions



Instrumentation: Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC system
Column: Acclaim Trinity P2, 3 µm, 3 × 50 mm
Col. temp: 30 ºC
Flow rate: 0.6 mL/min
Inj. volume: 2 µL
Mobile phase A: Water
Mobile phase B: 100 mM ammonium formate, pH 3.65
Gradient:

Time (min)	-8.0	0.0	1.0	11	15
%A	90	90	90	0	0
%B	10	10	10	100	100

Charged aerosol: Corona Veo
RS; 55 ºC, 5 Hz, 2 s, PF 1.5
Sample: 20 to 100 ng/µL each in deionized water

Peaks

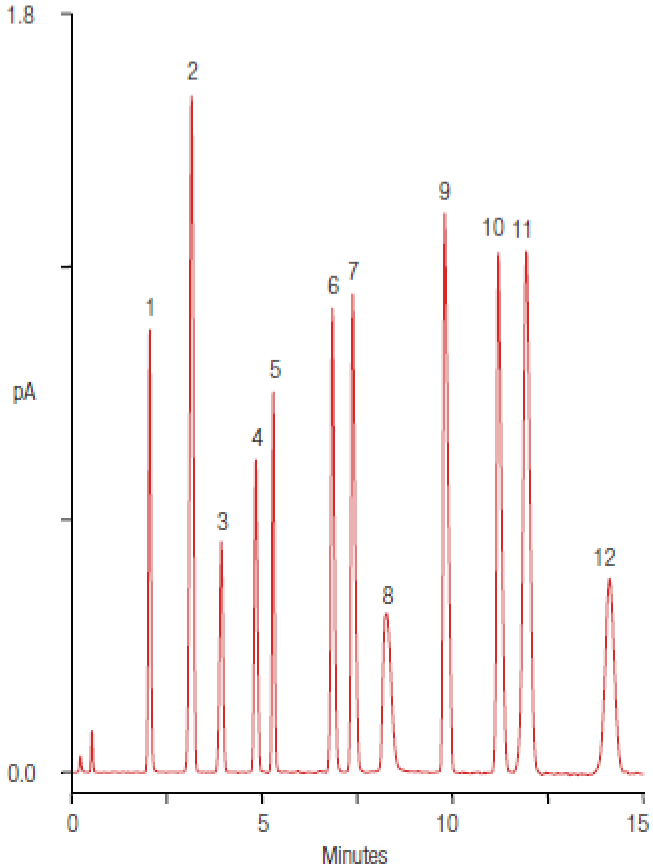
1. Phosphate
2. Sodium
3. Potassium
4. Chloride
5. Malate
6. Bromide
7. Nitrate
8. Citrate
9. Fumarate
10. Sulfate
11. Magnesium
12. Calcium

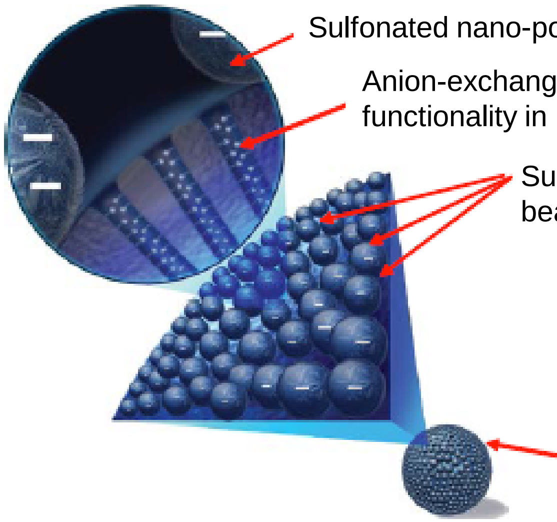
Anions, cations, organic and inorganic ions simultaneously

Drug counterions

Instrumentation: UltiMate 3000 RSLC system

Column: **Acclaim Trinity P2, 3 µm, 3 × 50 mm**





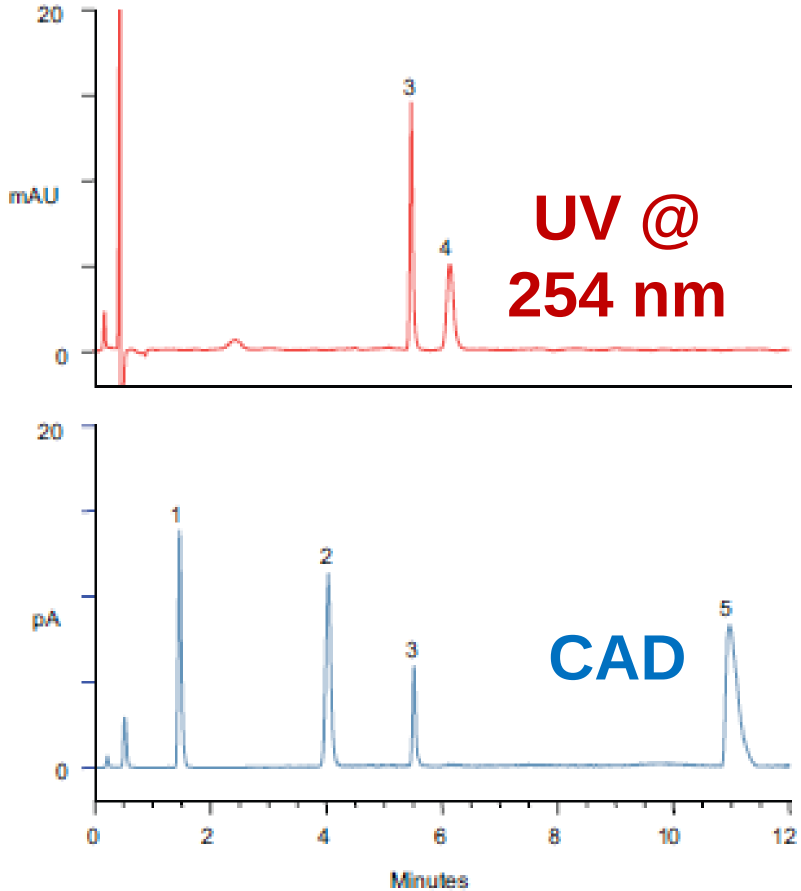
Peaks

1. Phosphate
2. Sodium
3. Potassium
4. Chloride
5. Malate
6. Bromide
7. Nitrate
8. Citrate
9. Fumarate
10. Sulfate
11. Magnesium
12. Calcium

Anions, cations, organic and inorganic ions simultaneously

Formulation – API and Counterions

Adderall® (Shire Pharmaceuticals) and counterions



Instrumentation: UltiMate 3000 RSLC system
Column: Acclaim Trinity P2, 3 µm, 3 × 50 mm
Col. temp: 30 ºC
Flow rate: 0.6 mL/min
Inj. volume: 5 µL
Mobile phases: A: Acetonitrile
B: Water
C: 100 mM ammonium formate, pH 3.65

Gradient:

Time (min)	A	B	C
-8.0	35	59	6
0.0	35	59	6
0.5	35	59	6
5.0	35	0	65
10	20	0	80
12	20	0	80

UV detector: UV diode array; 254 nm, 5 Hz, 0.5 s
Charged aerosol: Corona Veo RS; 55 ºC, 5 Hz, 2 s, PF 1.5
Peaks:

1	aspartate	24 µg/mL
2	sodium	
3	saccharin	24 µg/mL
4	amphetamine	122 µg/mL
5	sulfate	26 µg/mL

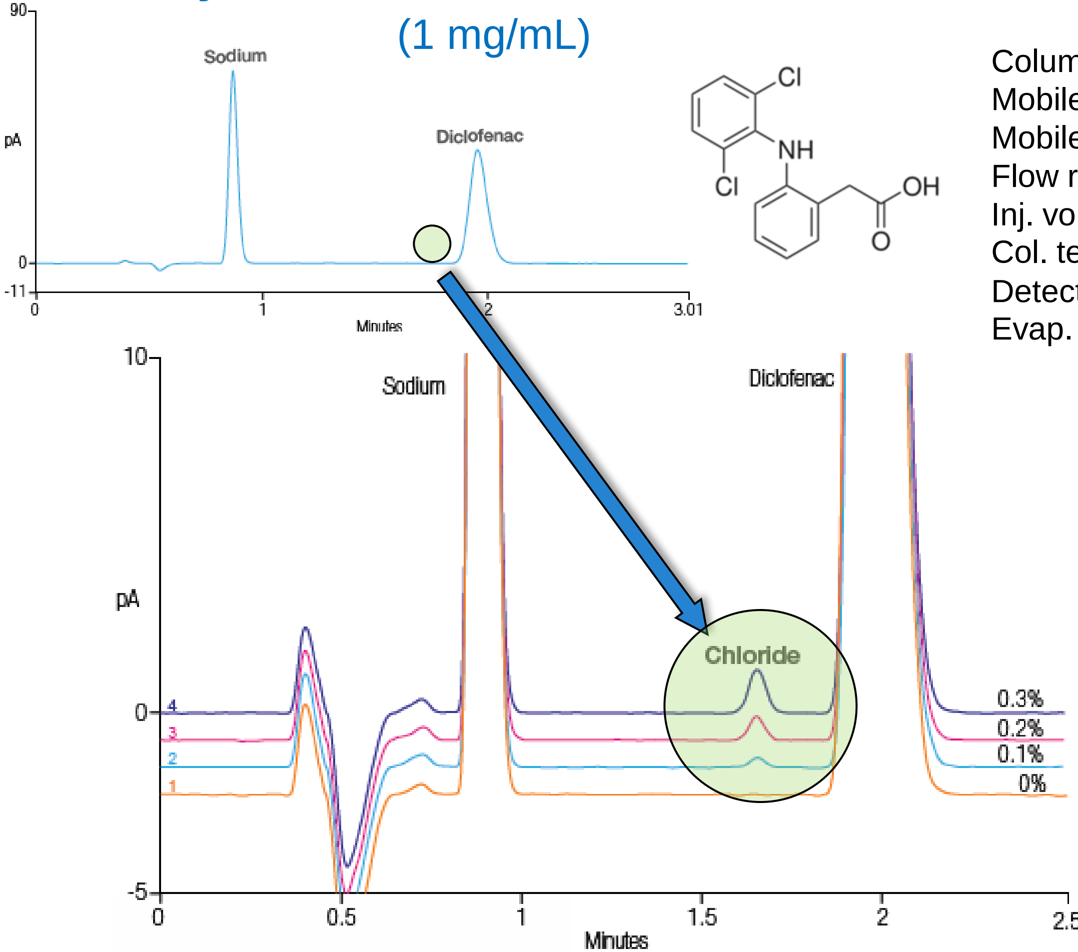
Ref: AN20870

Complimentary detection by CAD and UV/Vis

Formulation – API, Counter-Ions and Impurities

Analysis of diclofenac sodium salt

(1 mg/mL)

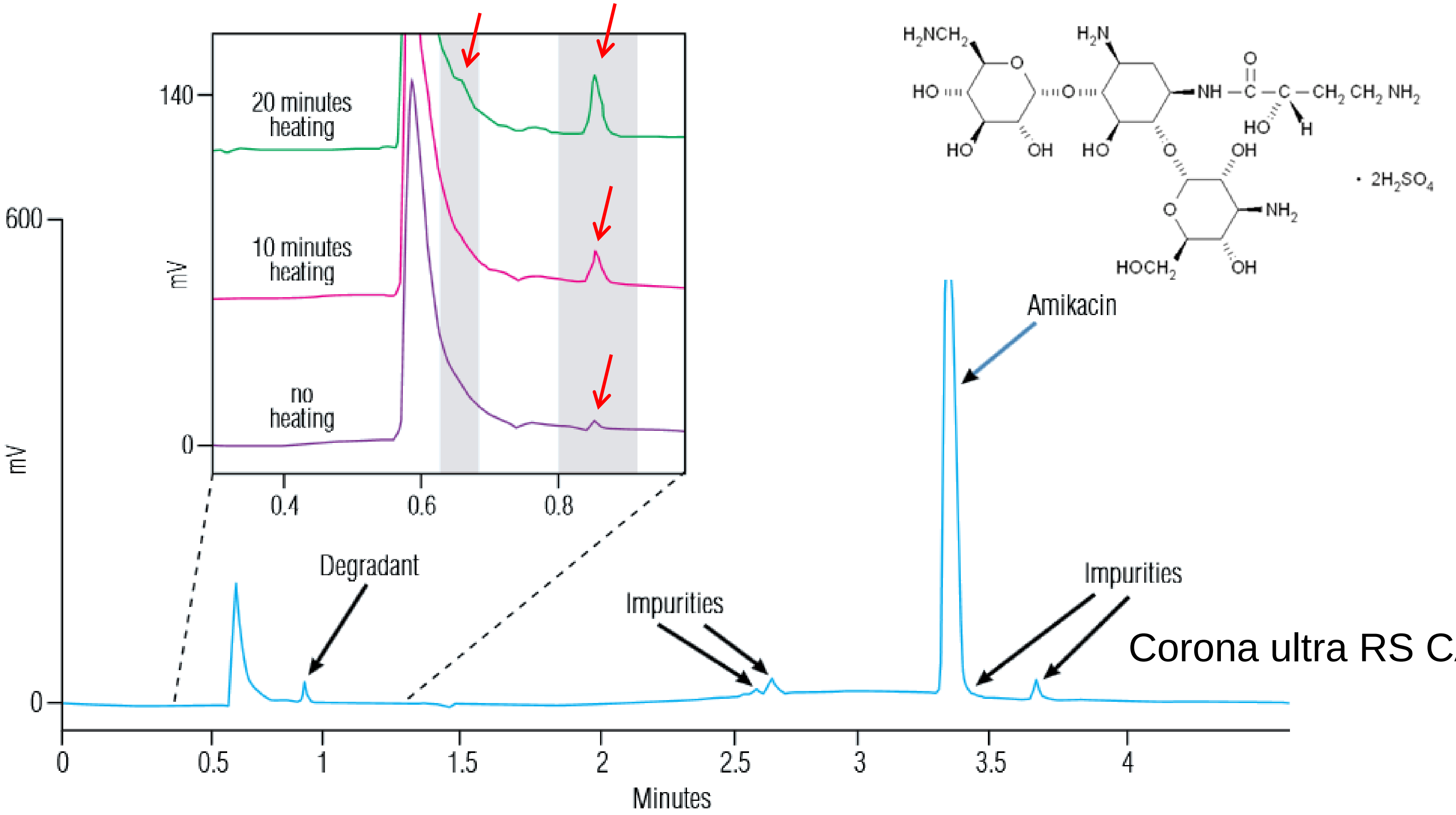


Column:	Acclaim Trinity P1, 3 µm, 3.0 × 50 mm
Mobile phase A:	75% Acetonitrile
Mobile phase B:	25% 200 mM Ammonium acetate pH 4
Flow rate:	0.8 mL/min
Inj. volume:	5 µL
Col. temp:	30 ºC
Detector:	Corona Veo RS CAD
Evap. temp:	60 ºC

Charged aerosol even detects chloride impurity.

ThermoFisher SCIENTIFIC

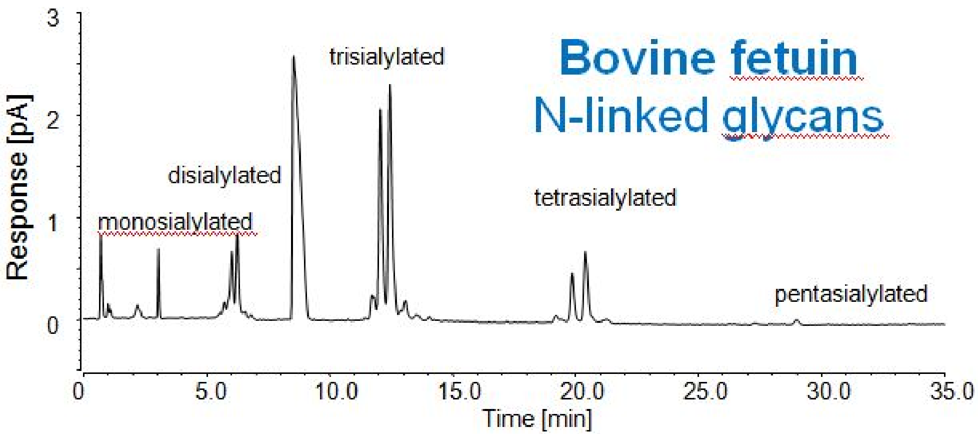
Stability – Forced Degradation



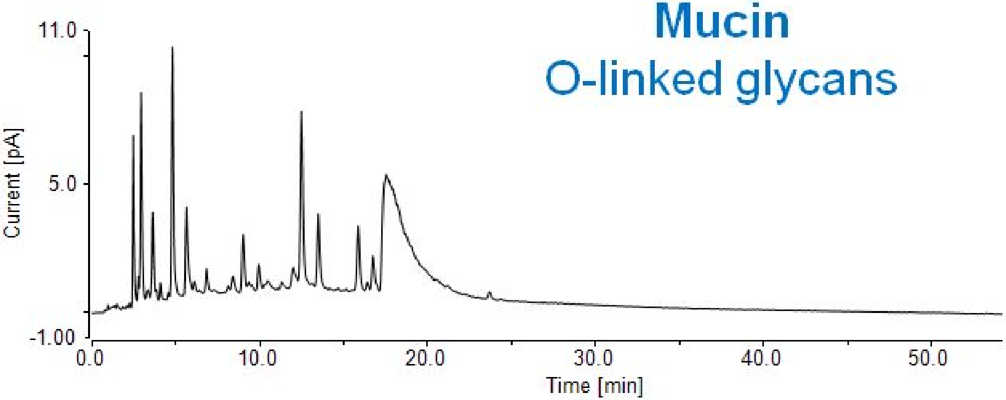
Corona ultra RS CAD

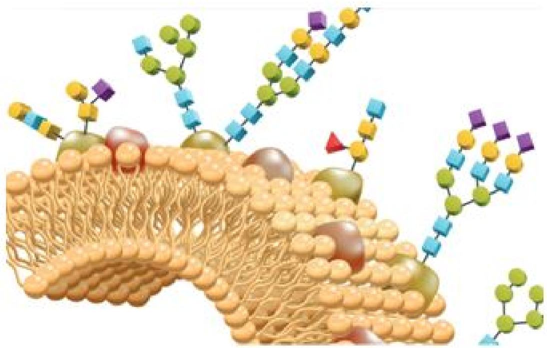
Follow forced degradation of Amikacin sulfate

Glycoprotein Characterization – Released Glycan Analysis



System:	Thermo Scientific™ Vanquish™ UHPLC system
Column:	Thermo Scientific™ GlycanPac™ AXR-1, 1.9 µm, 2.1 × 150 mm
Mobile phase	A: Deionized water
Mobile phase	B: 1.00 mM Ammonium formate, pH 4.4
Gradient:	4 % B to 39% B in 35 min
Flow rate:	0.4 mL/min
Inj. volume:	2 µL
Col. temp:	30° C
Detector:	Vanquish Charged Aerosol Detector H 50° C, PF 1.0, 10 Hz, 5s





Detection of released glycans – No labeling required

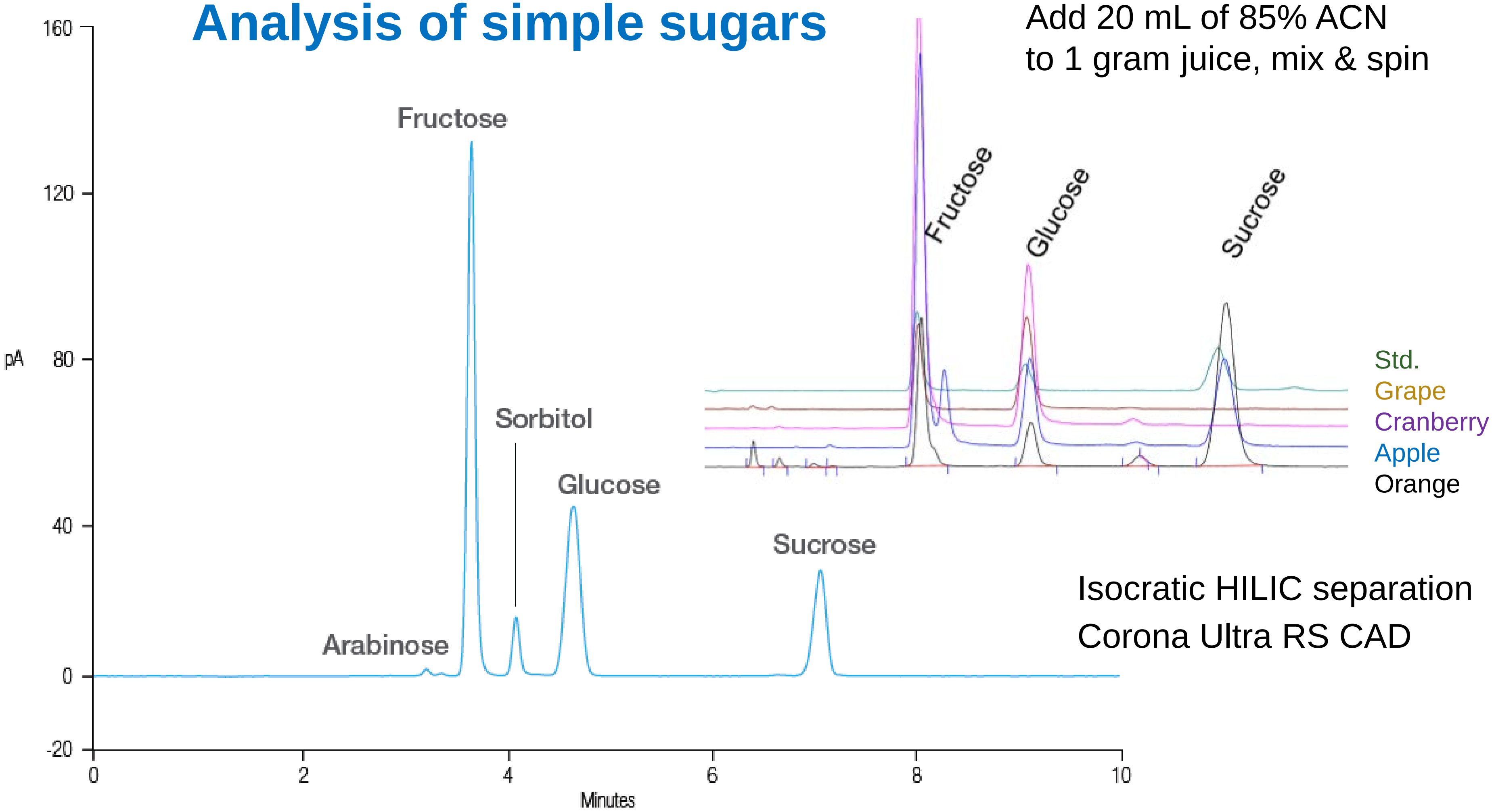
Food and Beverage Application Areas

- Simple carbohydrates
- Lipids
  - Profiling methods
  - Targeted methods
- Artificial sweeteners

Food and Beverage – Simple Carbohydrates

Analysis of simple sugars

Add 20 mL of 85% ACN to 1 gram juice, mix & spin

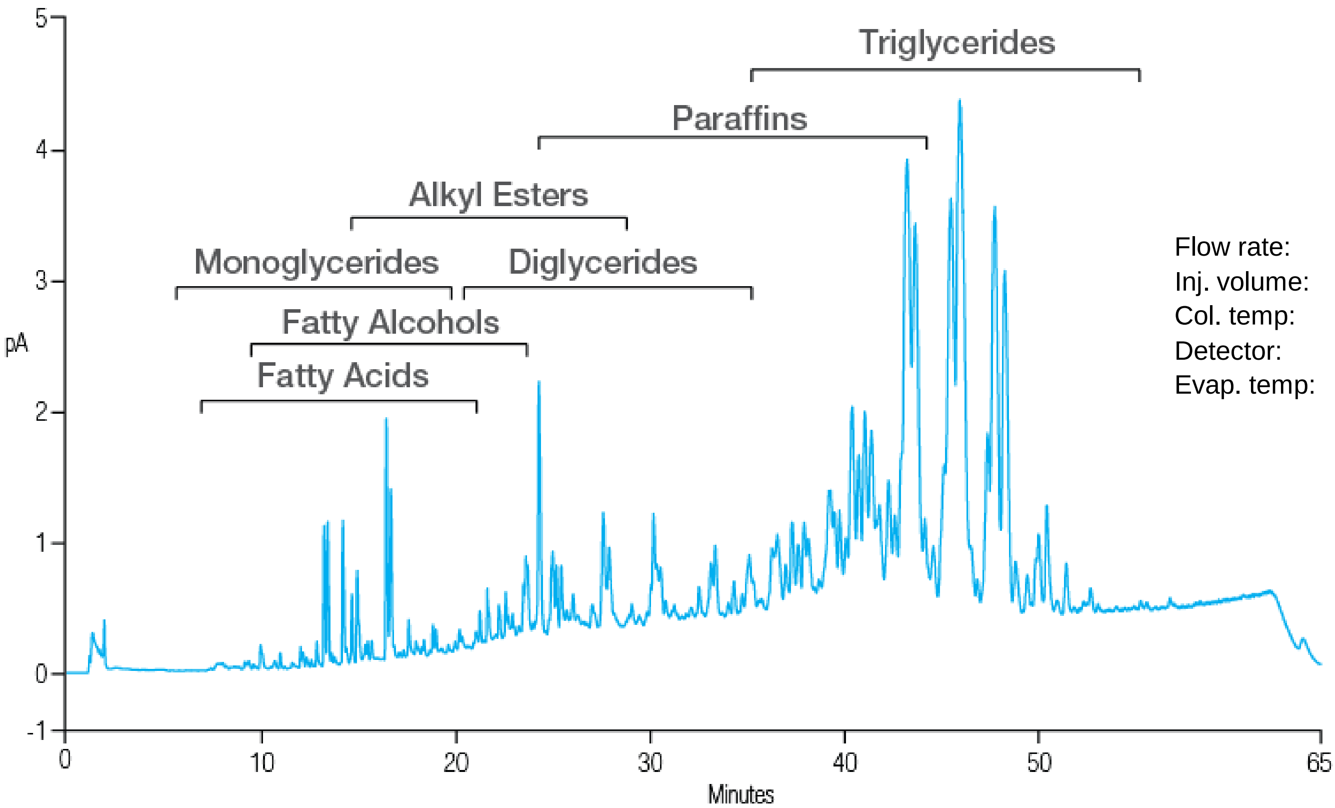


Isocratic HILIC separation
Corona Ultra RS CAD

Simplified sample preparation "Dilute-and-shoot" method

Lipids – Profiling

Global lipids – Algal oils



Column: Thermo Scientific™ Accucore™ C18, 2.6 µm, 3.0x150 mm

Mobile phases:
A: Methanol:water:acetic acid (600:400:4)
B: Tetrahydrofuran:acetonitrile (50:950)
C: Acetone:acetonitrile (900:100)

Gradient:

Time (min)	*FlowRate (mL/min)*	*%A*	*%B*	*%C*
-10.0	1. 00	90	10	0
-0.1	1. 00	90	10	0
0. 0	0. 25	90	10	0
20.0	0. 50	15	85	0
35.0	0. 50	2	78	20
60.0	0. 50	2	3	95
65.0	0. 50	90	10	0

Flow rate: 1.0 mL/min
Inj. volume: 2 µL
Col. temp: 40 ºC
Detector: Corona Veo RS CAD
Evap. temp: 40 ºC

Complex sample – Minimal sample prep

Lipids – Targeted Methods

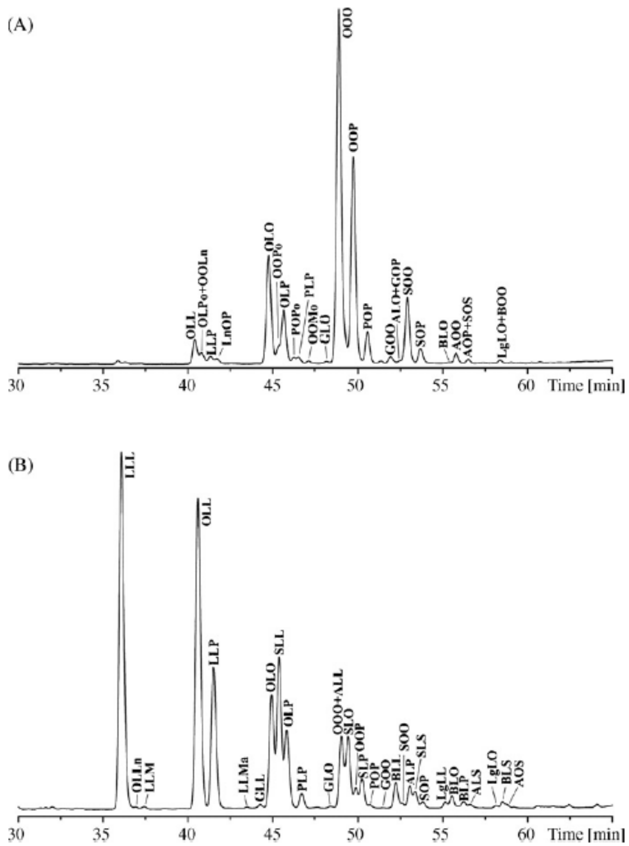


Fig. 3. Representative separations of studied plant oils using charged aerosol detection with mobile phase compensation: (A) olive oil (*Olea europea*) and (B) sunflower oil (*Helianthus annuus*). Conditions: two Hypersil ODS columns (250 mm × 4.6 mm I.D., 5 μm) connected in series. Flow rate: 1 mL/min. Column temperature: 30 °C. Further details in Section 2.

Olive oil

Sunflower oil

Column:	Thermo Scientific™ Hypersil™ ODS C18 5 µm, 4.6 × 250 mm, 2 in series, plus 2 in series for gradient compensation
Mobile phase A:	2-propanol:hexane 1:1 (v/v)
Mobile phase B:	Acetonitrile
Gradient:	see below

Time (min)	*Flow rate (mL/min)*	*%A*	*%B*
0.0	1	20	80
80	1	90	10

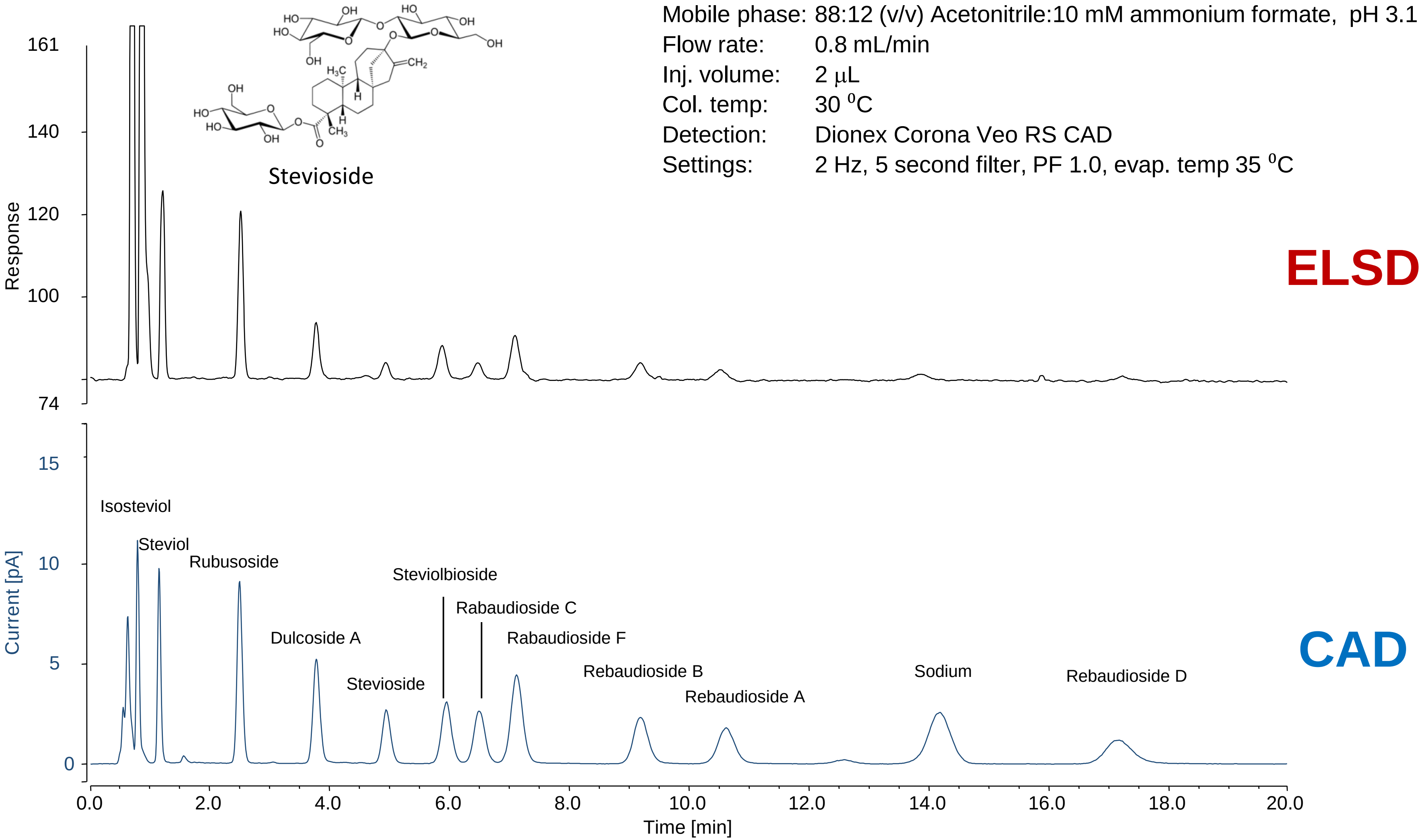
Inverse gradient:	**Yes**
Flow rate:	1.0 mL/min
Inj. volume:	4 µL
Col. temp:	30 ºC
Detector:	Corona CAD 100 pA; 35 psi, low filter
Sample:	B, TG standard GLC#435 and D) GLC#437, dissolved in hexane
Reference:	Sandra et al, J Chromatogr. A 1176 (2007) 135 – 142.

Complex real samples – minimal sample prep

Food Additives: Steviol Glycosides (Sweetener)

Stevioside

Column: Acclaim Trinity P1, 3 µm, 2.1 × 150 mm
Mobile phase: 88:12 (v/v) Acetonitrile:10 mM ammonium formate, pH 3.1
Flow rate: 0.8 mL/min
Inj. volume: 2 µL
Col. temp: 30 °C
Detection: Dionex Corona Veo RS CAD
Settings: 2 Hz, 5 second filter, PF 1.0, evap. temp 35 °C

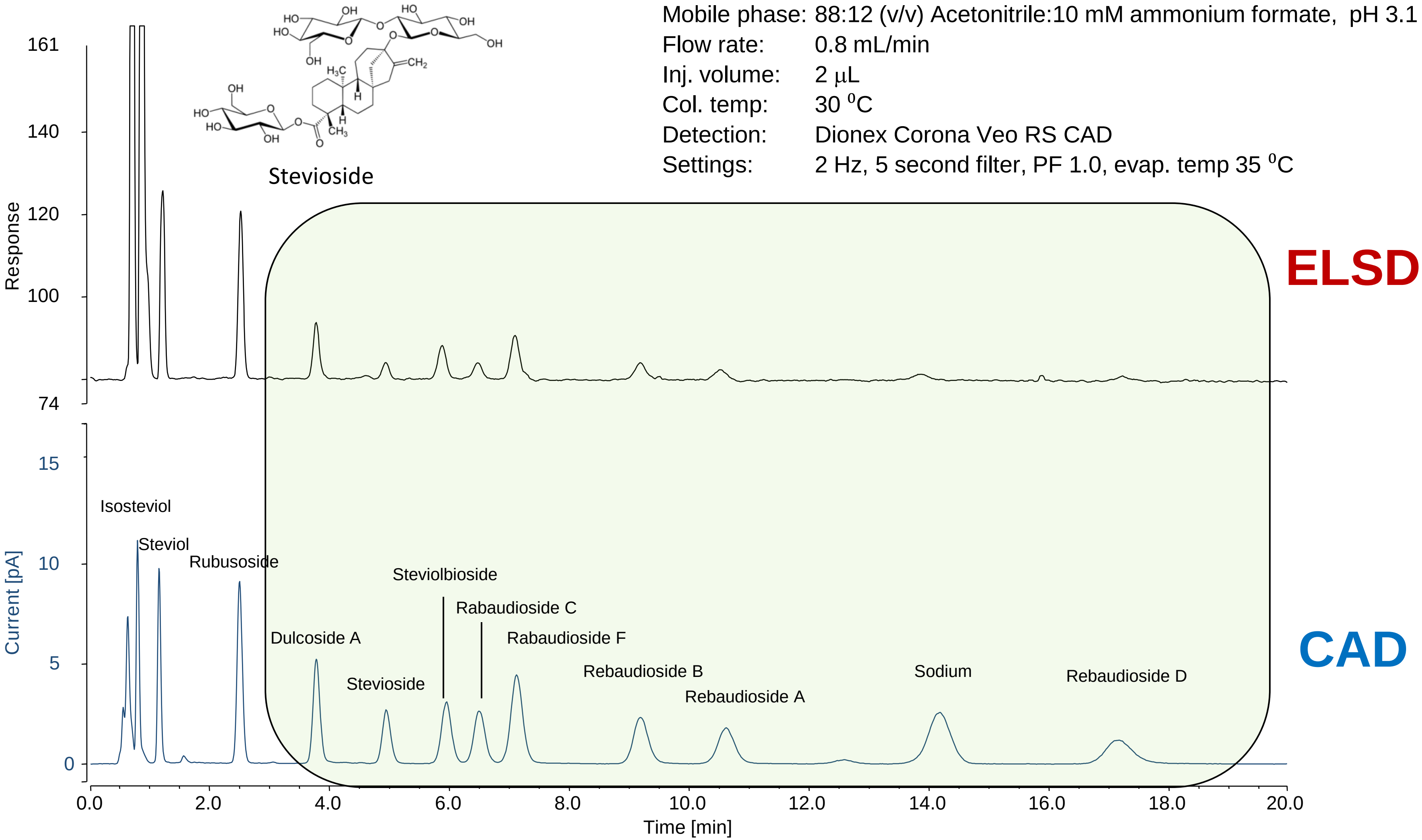


CAD exhibits more uniform response.

ThermoFisher SCIENTIFIC

Artificial Sweeteners: Steviol Glycosides

Column: Acclaim Trinity P1, 3 µm, 2.1 × 150 mm
Mobile phase: 88:12 (v/v) Acetonitrile:10 mM ammonium formate, pH 3.1
Flow rate: 0.8 mL/min
Inj. volume: 2 µL
Col. temp: 30 ^{0}C
Detection: Dionex Corona Veo RS CAD
Settings: 2 Hz, 5 second filter, PF 1.0, evap. temp 35 ^{0}C



CAD exhibits more uniform response.

Summary

- Charged aerosol detection delivers accurate and precise quantification of lipids, carbohydrates, surfactants, amines and counterions that UV/Vis absorbance cannot detect.
- For analytes with chromophores, charged aerosol detection provides uniform response independent of the extinction coefficient.
- Charged aerosol detection provides a good estimate of the amount of unknown impurities and degradation products.
- Charged aerosol detection is superior to ELSD in terms of sensitivity, dynamic range, response uniformity, precision and ease of use.
- More information on charged aerosol detection can be found at www.thermofisher.com/cad
- Bibliography of charged aerosol detector applications can be downloaded from http://analyteguru.com/resources/charged-aerosol-detection-list-of-published-articles/

Thank You Very Much for Your Attention!

Questions?

Do you have additional questions or do you want to talk to an expert from Thermo Fisher Scientific?

Please send an E-Mail to
analyze.eu@thermofisher.com
and we will get back to you.