Technical Guide

Charged Aerosol Detection

See what other universal detectors are missing

Thermo SCIENTIFIC

Charged Aerosol Detection

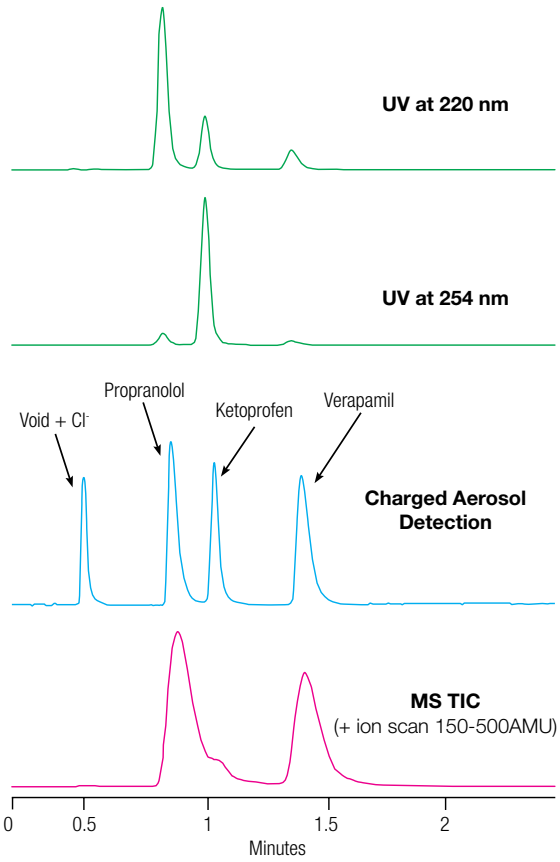
No single liquid chromatography (LC) detector delivers ideal results. Often, one analyte responds more strongly than another, or may not respond at all. UV absorbance requires that the molecule contain a chromophore. Inter-analyte response can vary greatly depending upon the nature of the chromophore present.

Refractive index cannot be used with gradient elution and is temperature sensitive.

Mass spectrometry can only measure compounds that will form gas-phase ions.

Evaporative light scattering shows varied inter-analyte response and complex, non-linear calibration curves.

What is most desired in a universal detector is the ability to accurately measure a wide range of analytes with consistent response. Charged aerosol detection can measure any non-volatile and many semi-volatile analytes at sub-nanogram levels and does not require a compound to contain a chromophore or be able to ionize. Variance in inter-analyte relative response is minimal whether analyzing small molecules or proteins. The technique is fully gradient compatible.



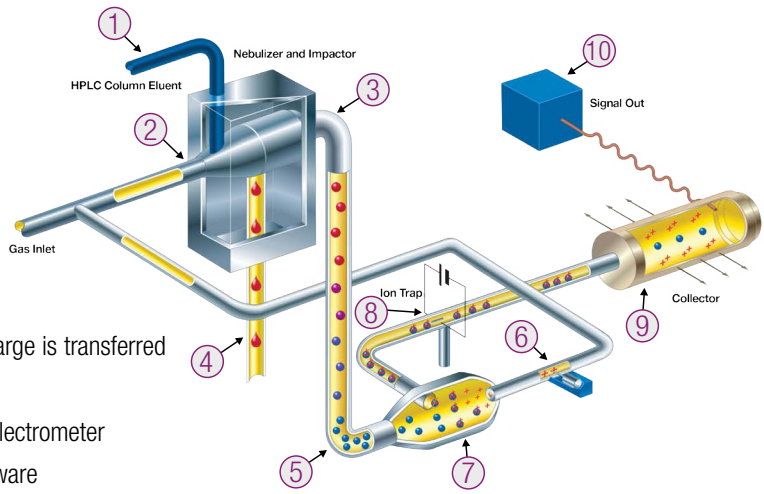
▲ Comparison of Charged Aerosol Detection to UV and MS

How the Technology Works

Charged aerosol detection first nebulizes eluent from the column. Large droplets exit the detector to waste. Selected smaller droplets enter the drying tube and form particles once the solvent is evaporated. Particles then enter a reaction chamber where they collide with ionized gas formed when nitrogen is passed over a corona wire. Charge is transferred from the ionized gas to the analyte particles. Once unreacted ionized gas is removed by an ion trap, the charge on the particle is measured by a sensitive electrometer. The response of the detector is directly related to the mass of the analyte entering the detector. An increase in the amount of an analyte eluting from the column leads to an increase in the size of the particles being formed. Larger particles can accommodate more charge, resulting in a higher response from the detector. As long as the analyte will form a particle it will be measured by charged aerosol detection, independent of its chemical structure.

Charged aerosol detection delivers predictable results without the need for complex detector optimization and has the flexibility to measure a broad range of analytes in many different matrices. The Thermo Scientific™ Dionex™ Corona™ Veo™ charged aerosol detector is HPLC/UHPLC compatible and with its extended flow rate range, can be used with capillary, microbore and analytical scale columns. The detector improves on all the benefits of charged aerosol detection in a design perfectly matched to your laboratory's needs.

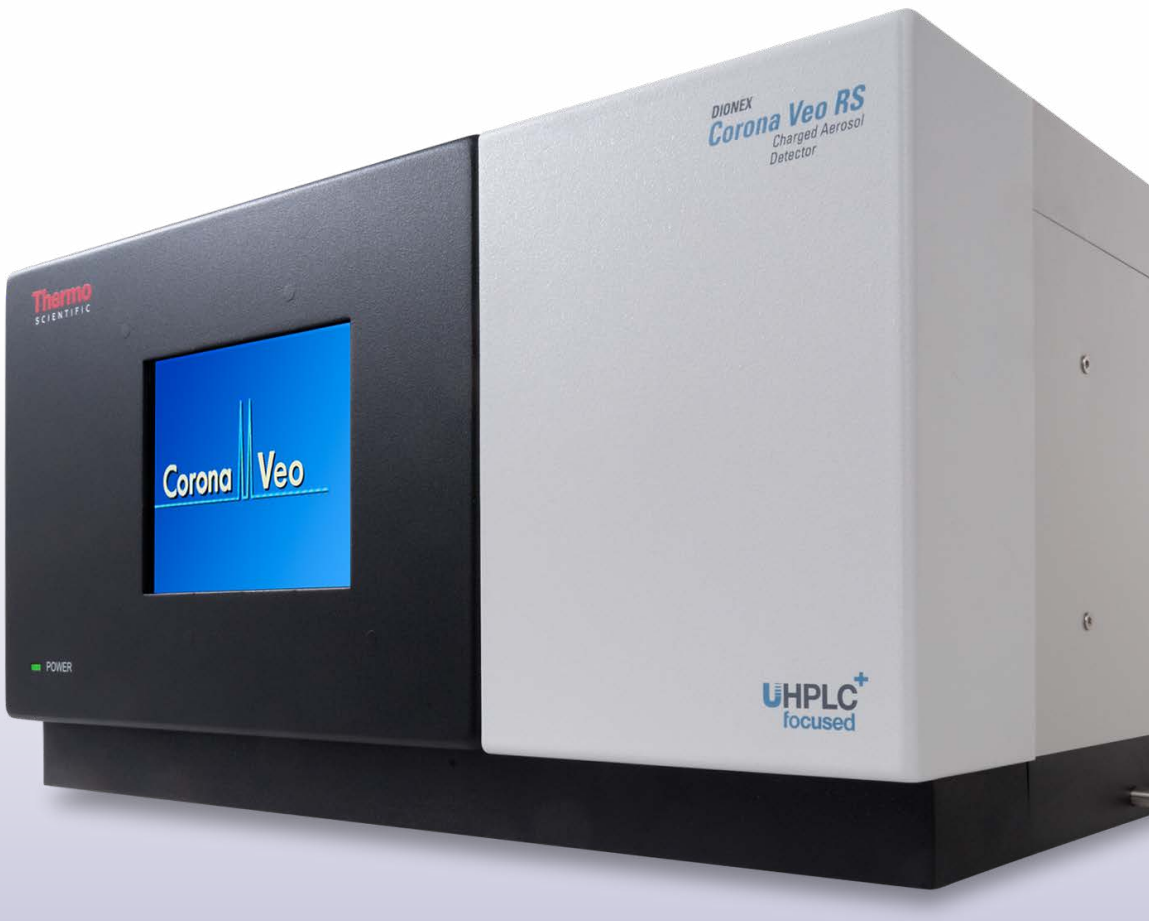
1. Liquid eluent enters from HPLC system
2. Pneumatic nebulization occurs
3. Small droplets enter drying tube
4. Large droplets exit to drain
5. Dried particles enter mixing chamber
6. Gas stream passes over corona needle
7. Charged gas collides with particles and charge is transferred
8. High mobility species are removed
8. Charge is measured by a highly sensitive electrometer
9. Signal transferred to chromatographic software



Schematic of engine and operating principles.

UHPLC+ focused

See a Broad Range of Analytes in Many Different Matrices

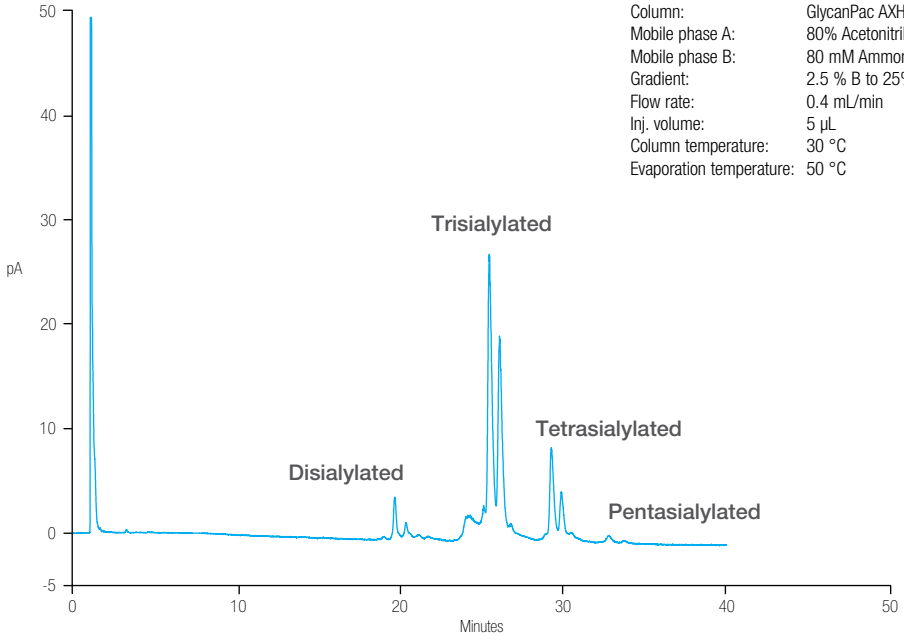


Native Glycans

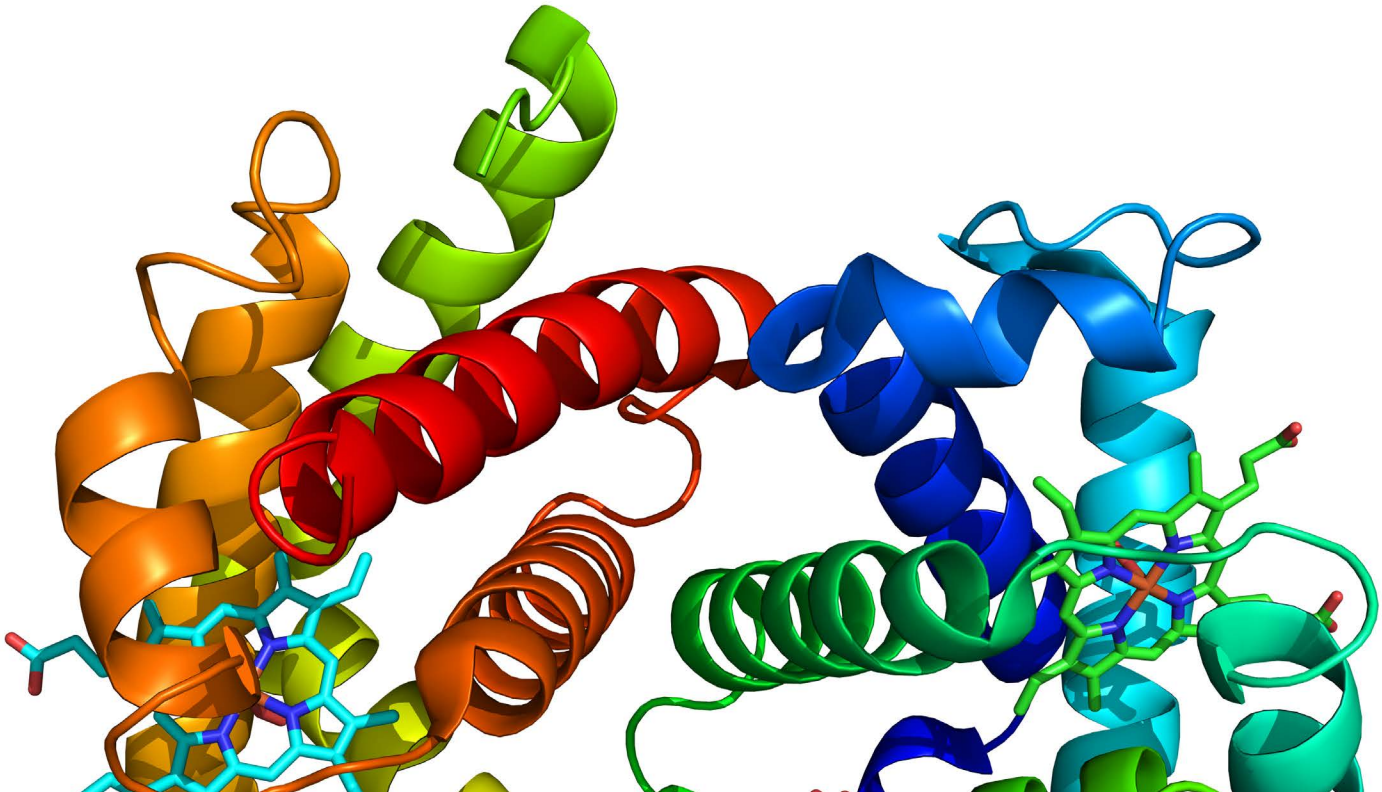
Glycans, the oligosaccharide component of glycoproteins, have many important physiological roles including their involvement in cell-cell interactions and correct protein folding. Oligosaccharides lack a strong chromophore so their measurement by UV absorbance detection is challenging.

In this example, sialylated N-linked alditols liberated from bovine fetuin were separated by HPLC using the new Thermo Scientific™ GlycanPac™ AXH-1 column and measured directly using charged aerosol detection.

Column:	GlycanPac AXH 1.9 µm, 2.1 × 150 mm
Mobile phase A:	80% Acetonitrile
Mobile phase B:	80 mM Ammonium formate, pH 4.4
Gradient:	2.5 % B to 25% B from 1 to 40 min
Flow rate:	0.4 mL/min
Inj. volume:	5 µL
Column temperature:	30 °C
Evaporation temperature:	50 °C



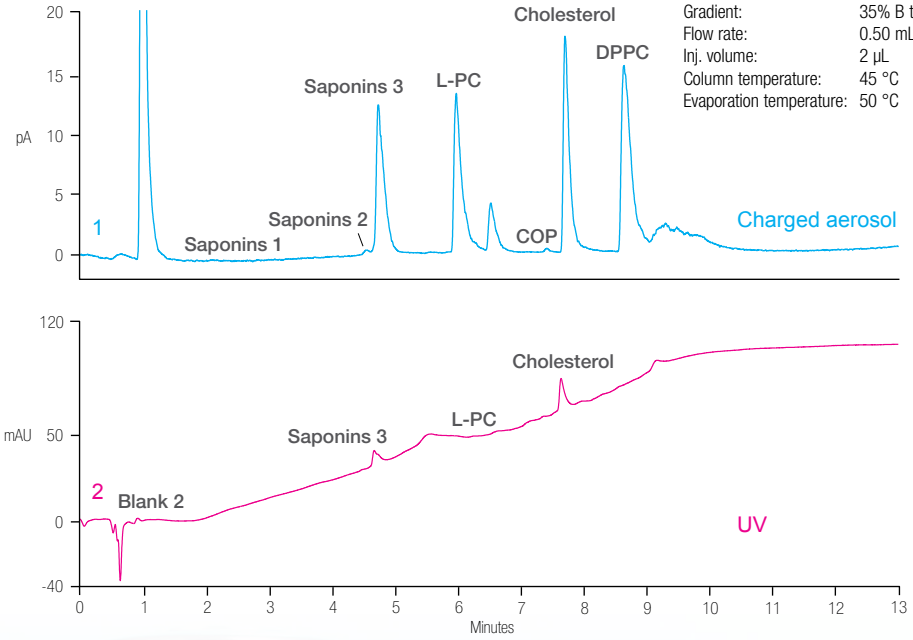
▲ Determination of Native Glycans (100 pmol/µL)



Adjuvants

Adjuvants are agents added to a vaccine designed to enhance the host's immunological response. AbISCO-100® is a suspension of purified saponins from *Quillaja saponaria*, cholesterol from sheep wool and egg phosphatidyl choline in phosphate buffered saline. As seen in these chromatograms, standards and a synthetic mixture representing these components elute within 12 minutes from the Thermo Scientific™ Hypersil GOLD™ PFP column with good resolution. All components and several degradation products were detected by the Corona Veo detector, whereas some, such as the phosphatidyl cholines, showed poor response by UV detection.

Column:	Hypersil GOLD PFP 1.9 µm, 2.1 × 100 mm
Mobile phase A:	0.1% Formic acid in water
Mobile phase B:	0.1% Formic acid in 10:90 acetonitrile:reagent alcohol
Gradient:	35% B to 83%B in 6 min to 90% B in 10 min
Flow rate:	0.50 mL/min
Inj. volume:	2 µL
Column temperature:	45 °C
Evaporation temperature:	50 °C

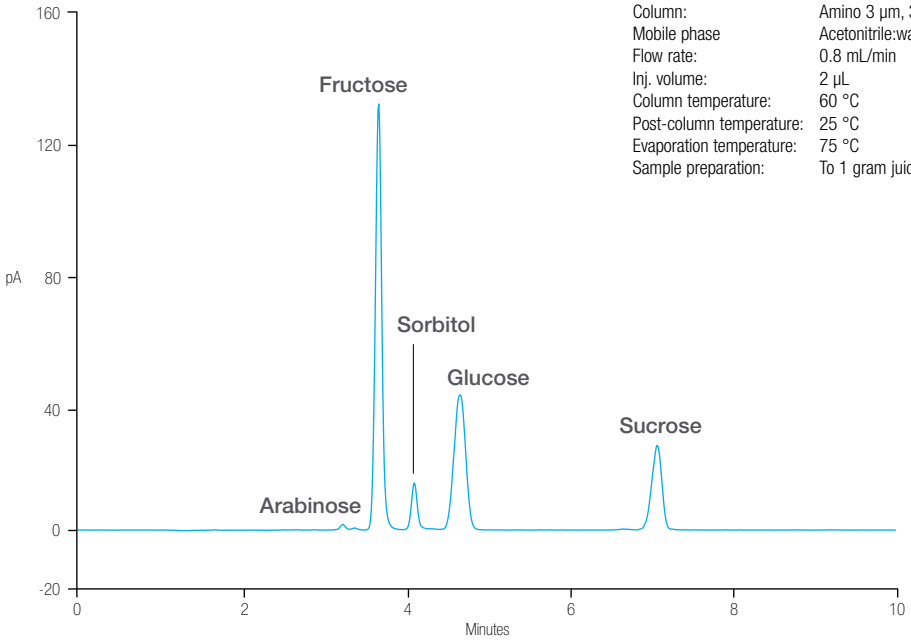


▲ **Adjuvant components:**

Saponins	64 µg/mL
Cholesterol	160
DPPC	160
Lyso-PC (L-PC)	200

Carbohydrates

Most simple carbohydrates possess weak chromophores so require derivatization prior to measurement by UV absorbance detection. Presented here is a simple direct method for the measurement of monosaccharides and disaccharides in fruit juice. Carbohydrates were resolved using HILIC conditions and were detected with the Corona Veo detector.



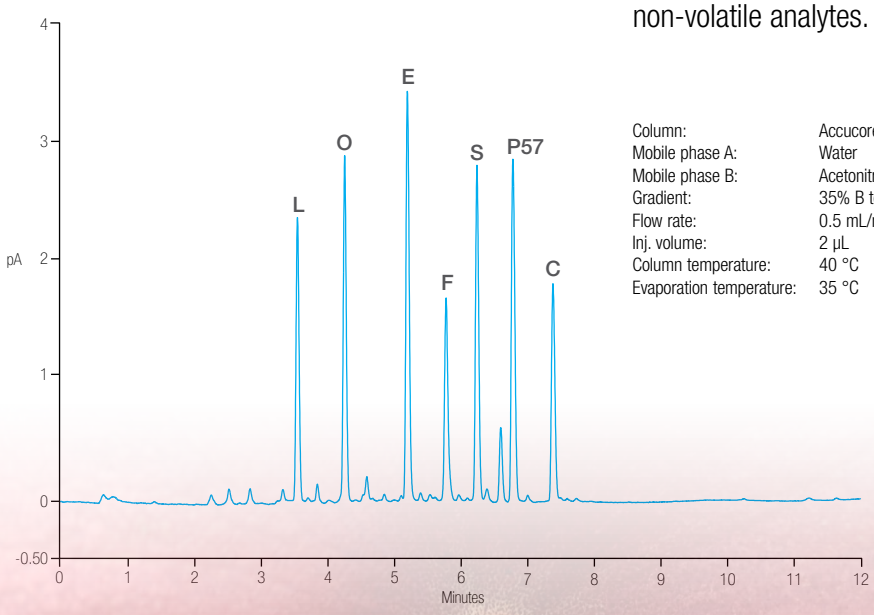
Column:	Amino 3 µm, 3.0 × 250 mm
Mobile phase	Acetonitrile:water (92:8)
Flow rate:	0.8 mL/min
Inj. volume:	2 µL
Column temperature:	60 °C
Post-column temperature:	25 °C
Evaporation temperature:	75 °C
Sample preparation:	To 1 gram juice sample add 20 mL of 85% acetonitrile

▲ Analysis of Apple Juice

Hoodia

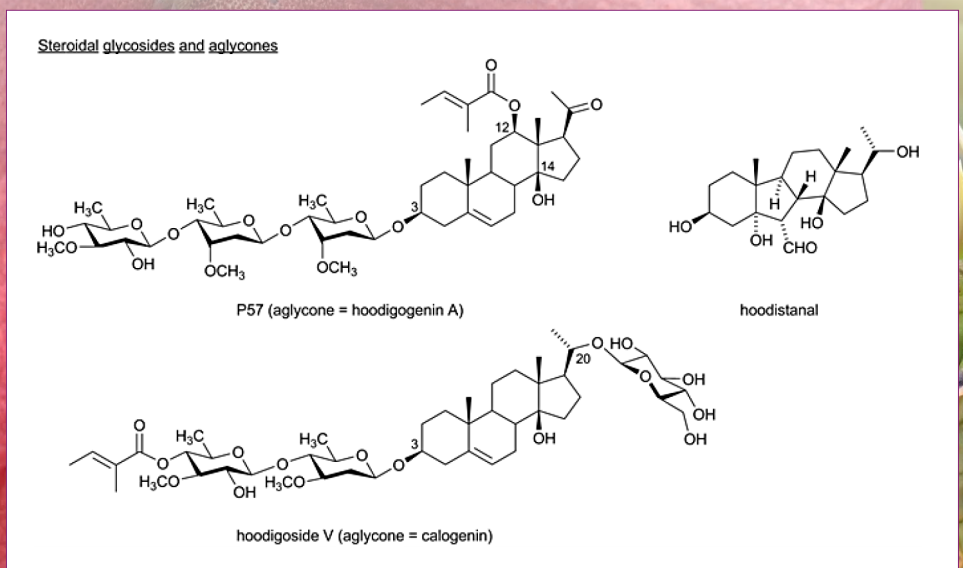
Hoodigosides are oxypregnane steroidal glycosides abundant in *Hoodia gordonii* and related plants native to the deserts of southwestern Africa. This plant is used traditionally to ease hunger during long hunting expeditions and enjoys wide use today in dietary supplements purported to aid in appetite suppression and weight loss.

This application highlights the superior sensitivity of HPLC with charged aerosol detection for determination of hoodigosides in plant extracts. Eight hoodigosides isolated from dried plant material are separated within 15 min on a Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC system paired with an Thermo Scientific™ Accucore™ C18 analytical column. The Accucore column delivers superb resolution with low backpressure and the charged aerosol detector provides sensitive detection of all non-volatile analytes.



Column:	Accucore C18 2.6 µm, 2.1 × 150 mm
Mobile phase A:	Water
Mobile phase B:	Acetonitrile
Gradient:	35% B to 90% B in 10 min
Flow rate:	0.5 mL/min
Inj. volume:	2 µL
Column temperature:	40 °C
Evaporation temperature:	35 °C

▲ Analysis of *Hoodia gordonii* hoodigosides in a Supplement (10 ng/µL)

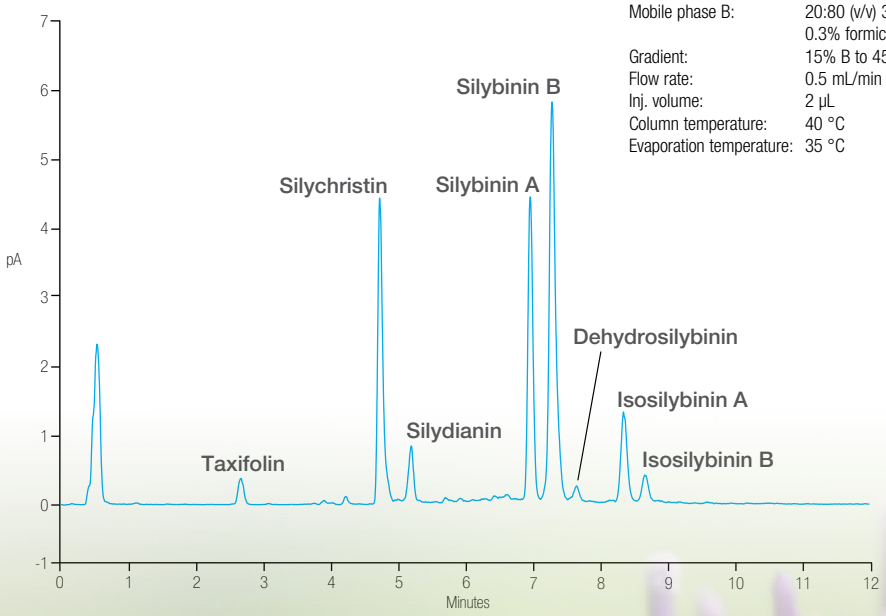


Milk Thistle

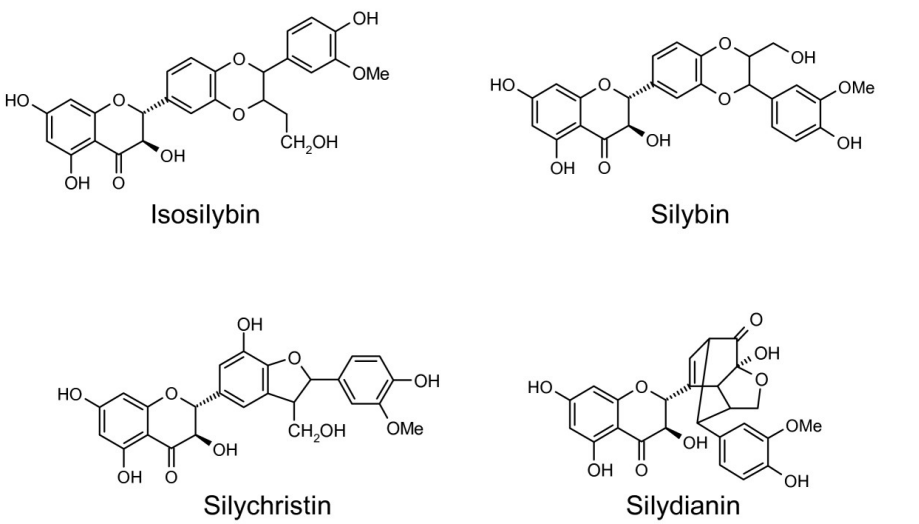
The milk thistle plant is native to Mediterranean Europe. *Silybum marianium* has long been used as an herbal remedy to promote liver health. The seeds, root, and milky sap of the milk thistle contain an antioxidant flavonolignan complex known as the silymarin group.

The standard method for determination of silymarins in milk thistle fruit and powdered extracts, Institute for Nutraceutical Advancement (INA) Method 115. 00, was improved by using a higher efficiency Thermo Scientific™ Acclaim™ RSLC 120 C18 2.2 µm column. The Corona Veo detector provided more uniform response for all nonvolatile analytes than did an UV absorbance detector.

Column:	Acclaim 120 C18 2.2 µm, 2.1 × 100 mm
Mobile phase A:	80:20 (v/v) 3 mM Ammonium formate, 0.3% formic acid:methanol
Mobile phase B:	20:80 (v/v) 3 mM Ammonium formate, 0.3% formic acid:methanol
Gradient:	15% B to 45% B in 4.4 min
Flow rate:	0.5 mL/min
Inj. volume:	2 µL
Column temperature:	40 °C
Evaporation temperature:	35 °C



▲ Analysis of Milk Thistle Supplement (560 ng Silymarin/µL)



Algal Oil

Biofuels are viable, renewable alternates to traditional fuels and include biodiesel (synthesized from vegetable oil) and algal oil. Algal oil can be transformed into multiple fuel types, including diesel, kerosene, and gasoline. Unlike soy and corn-based fuels, algal fuels do not require the use of food crops, is easy to grow, and does not require vast amounts of land to produce useful quantities of oil. The little solid waste that is produced can be used in other products.

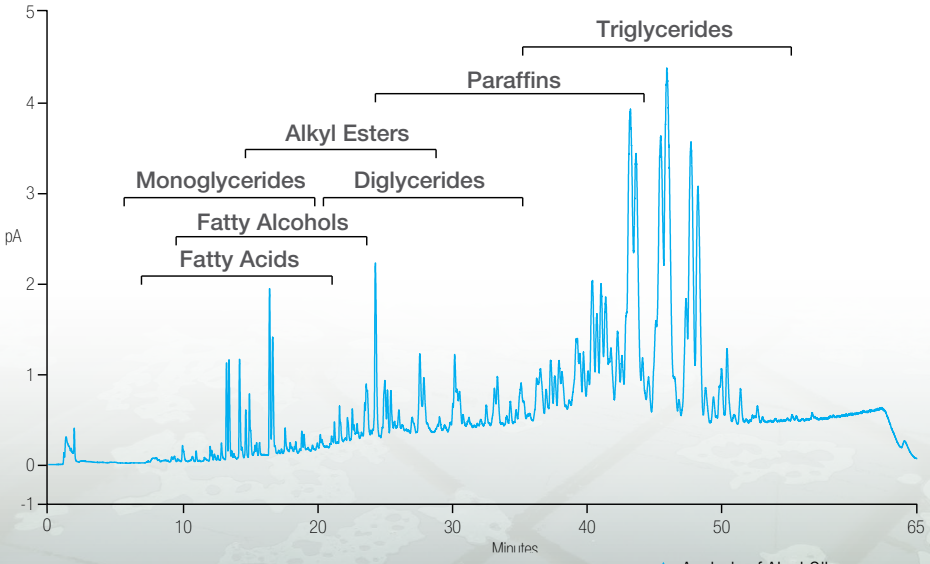
This approach can provide useful information about the components that are found within crude algae oil and can assist in the development of processing technology. This method uses gradient reversed-phase HPLC to separate the crude algae oil into its various components.

Column:	Accucore C18 2.6 µm, 3.0 × 150 mm
Mobile phase A:	Methanol:water:acetic acid (600:400:4)
Mobile phase B:	Tetrahydrofuran:acetonitrile (50:950)
Mobile phase C:	Acetone:acetonitrile (900:100)

Gradient:

Time (min)	Flow Rate (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:	1.0–1.5 mL/min
Inj. volume:	2 µL
Column temperature:	40 °C
Evaporation temperature:	40 °C
Sample preperation	Algal oil (100 µL) was diluted in 1:1 methanol:chloroform (900 µL)

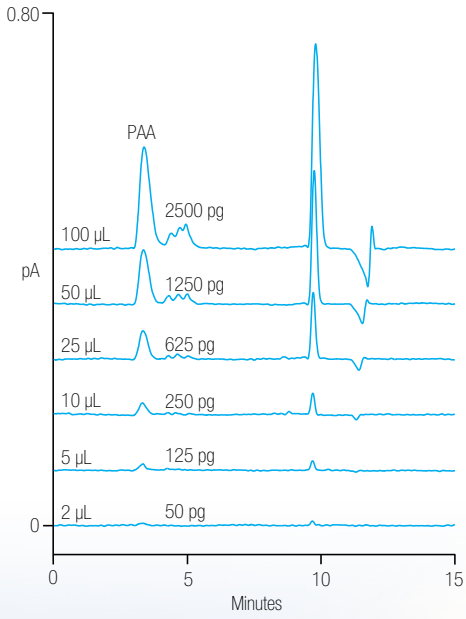


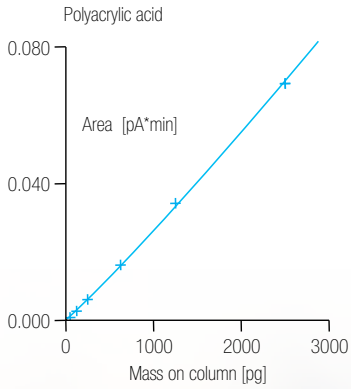
▲ Analysis of Algal Oil

Descalants

Scale-related issues are major problems for the generation of electrical power whether using conventional steam generators or nuclear power. Scale formation causes progressive lowering of the boiler efficiency due to heat retardation as it acts as an insulator. Boiler deposits can also cause plugging leading to decreased operating efficiency, boiler damage, unscheduled boiler outages, and the need for costly cleaning procedures. Polyacrylic acid (PAA) is widely used as an anti-scaling additive. Typical use rates are 1–1000 µg/L, depending on the load of metals in the boiler water. PAA levels need to be measured for process control and for wastewater discharge. Since PAA is the only macromolecule normally present in boiler water, size-exclusion chromatography is appropriate. PAA has a weak UV chromophore at 200 nm, so UV absorbance detection can only be used for more concentrated samples. The increased sensitivity of the Corona Veo detector permits direct measurement of PAA below 10 µg/L.

Column:	Acclaim SEC-300, 5 µm, 4.6 × 300 mm
Mobile phase A:	Acetonitrile
Mobile phase B:	Water
Isocratic:	10:90
Flow rate:	0.35 mL/min
Inj. volume:	2–100 µL
Column temperature:	30 °C
Evaporation temperature:	55 °C





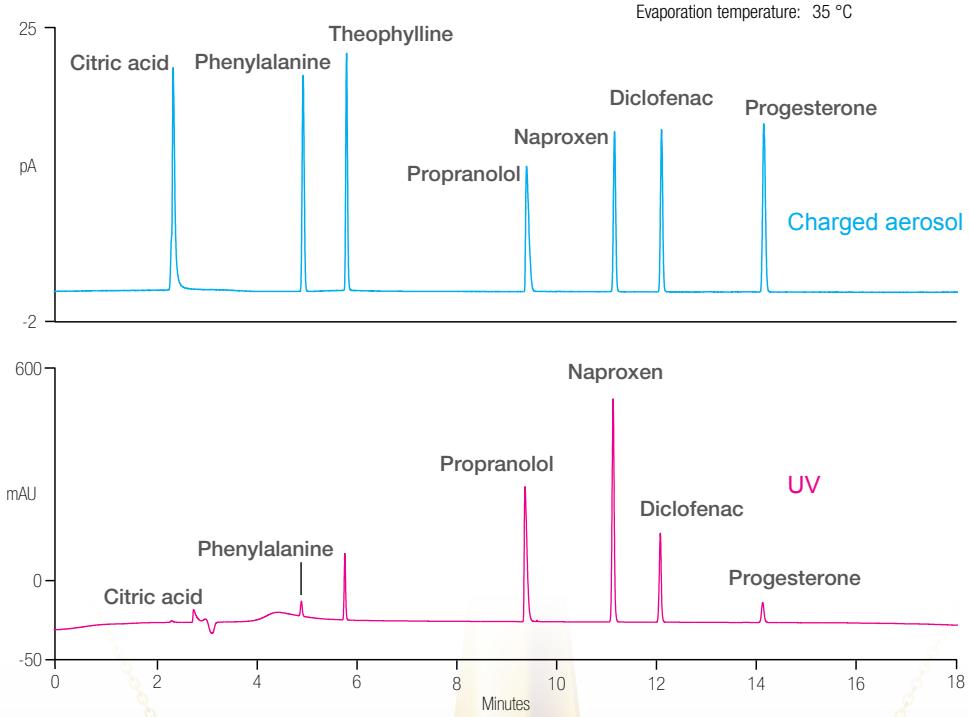
▲ Measurement of Polyacrylic Acid

Mass Balance

Although UV absorbance detection is a common universal technique, an analyte's response is dependent on the amount of analyte present, the strength of its chromophore and the absorbance wavelength chosen. For mass balance studies, where many of the related substances are not available, the use of UV absorbance detection alone can lead to critical errors in estimation. For example, is a small peak in the chromatogram the result of a high abundance of an analyte with a weak chromophore, or a low level of an analyte with a strong chromophore. What about degradation products that totally lack a chromophore? Charged aerosol detection overcomes these problems as all non-volatile and many semi-volatile analytes give a similar response independent of chemical structure.

To illustrate this, six pharmaceutical agents and one excipient were resolved using reversed-phase HPLC and their responses by UV and charged aerosol detection were compared.

Column:	Acclaim 300 C18 3 µm, 4.6 × 150 mm
Mobile phase A:	20 mM Ammonium acetate, pH 4.5
Mobile phase B:	Acetonitrile
Gradient:	2% B to 98% B in 30 min, Inverse Gradient
Flow rate:	0.8 mL/min
Inj. volume:	2 µL
Column temperature:	30 °C
Evaporation temperature:	35 °C

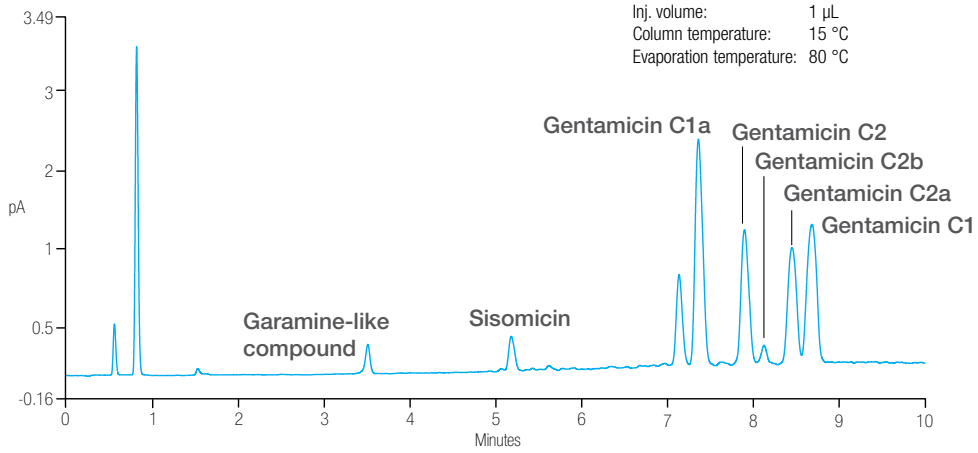


▲ Analysis of a Mixed Standard (100 ng/µL)

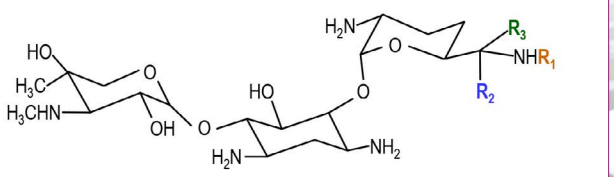
Active Ingredient Composition

Aminoglycoside antibiotics are used to treat gram negative bacterial infections. These antibiotics are typically manufactured by bacterial culture (fermentation) processes and thus can contain a mixture of active compounds, as well as fermentation impurities and degradation products. One example of an antibiotic manufactured by fermentation is gentamicin, an aminoglycoside antibiotic that is produced by Micromonospora echinospora (Micromonospora purpurea). Gentamicin sulfate is a mixture of four major compounds: gentamicins C1, C1a, C2, and C2a. In addition, gentamicin C2b is commonly present as a minor component. These gentamicin congeners are closely related structurally and their analysis is particularly challenging as none of the compounds possess a strong chromophore.

Column:	Acclaim RSLC PA2 2.2 µm, 2.1 × 100 mm
Mobile phase A:	0.025:95:5 Heptafluorobutyric acid:water:acetonitrile
Mobile phase B:	0.3:95:5 Trifluoroacetic acid: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 equilibration time before injection
Flow rate:	0.45 mL/min
Inj. volume:	1 µL
Column temperature:	15 °C
Evaporation temperature:	80 °C

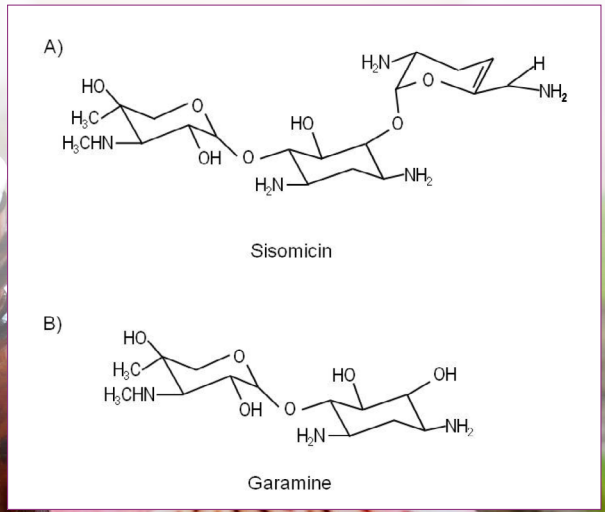


▲ Analysis of a Gentamicin Standard (200 µg/mL)



Gentamicin	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

▼ Structures of A) Sisomicin and B) Garamine.
Note the similarity of sisomicin to gentamicin C_{1a}.

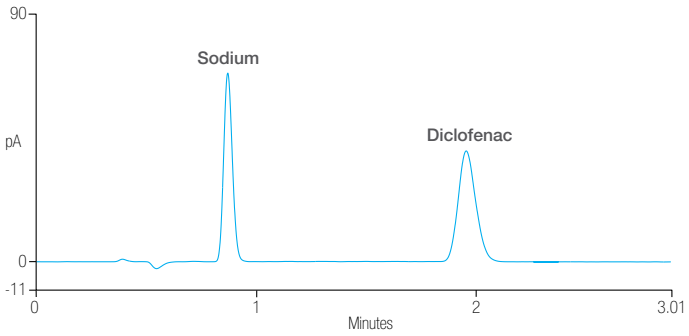


Formulation

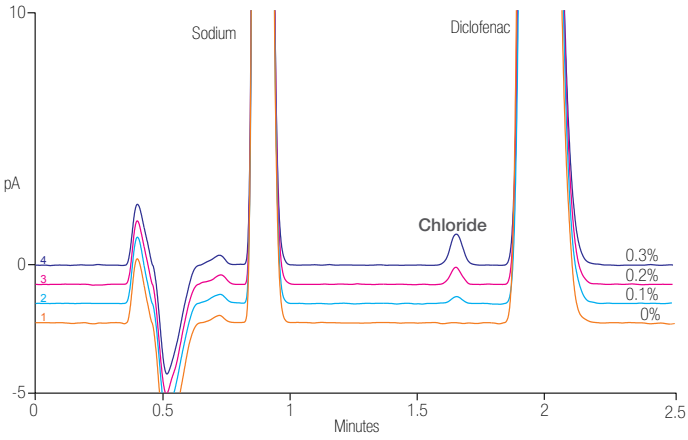
Optimizing a pharmaceutical formulation is a significant aspect of the drug development process. For an active pharmaceutical ingredient (API) with ionizable functional groups, salt formation can be used to improve the physicochemical properties including aqueous solubility, hygroscopicity, solution pH, melting point, dissolution rate, chemical stability, crystal form, and mechanical properties.

The ability to measure the API, its counter-ion(s), and trace contaminants is a necessity. Their simultaneous measurement can be achieved by using the Acclaim Trinity P1 column and the Corona Veo charged aerosol detector.

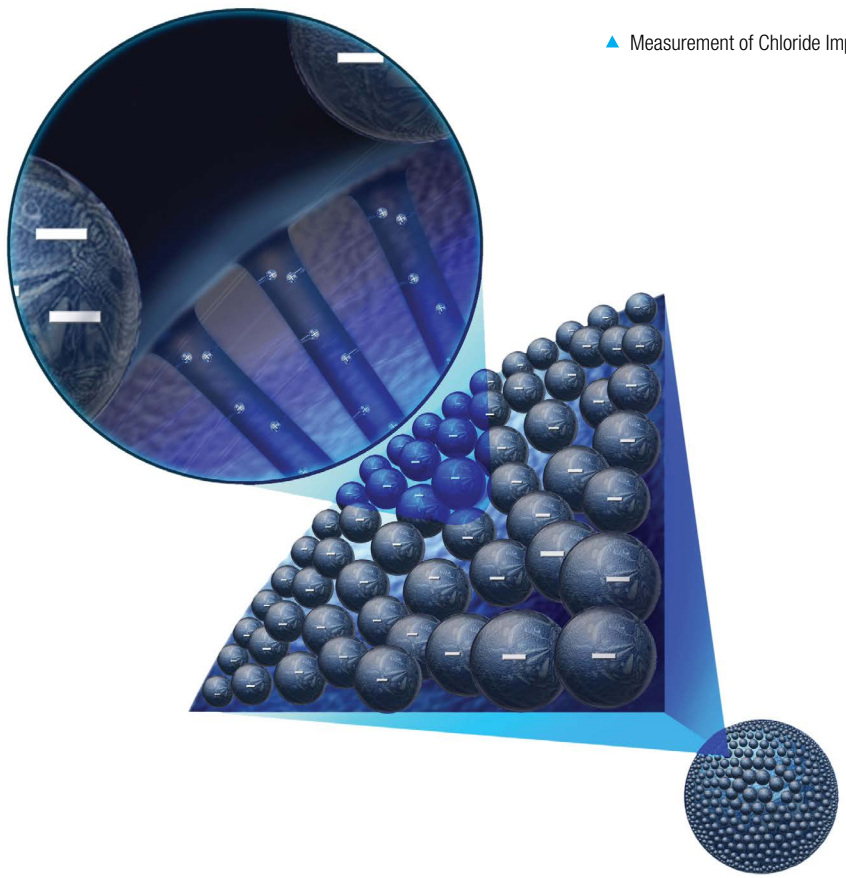
Column:	Acclaim Trinity P1 3 µm, 3.0 × 50 mm
Mobile phase:	75% Acetonitrile:25% 200mM Ammonium acetate, pH 4
Flow rate:	0.8 mL/min
Inj. volume:	5 µL
Column temperature:	30 °C
Evaporation temperature:	60 °C



▲ Measurement of Diclofenac-Sodium Salt (1mg/mL)



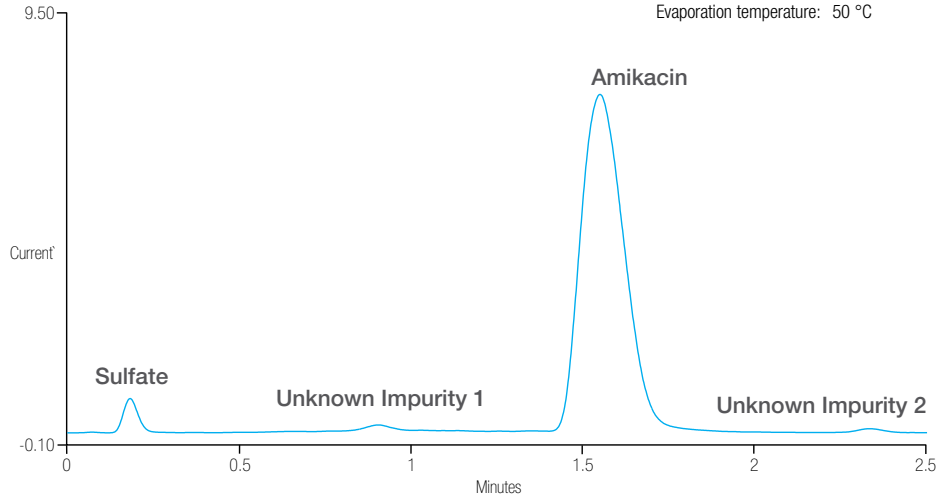
▲ Measurement of Chloride Impurity



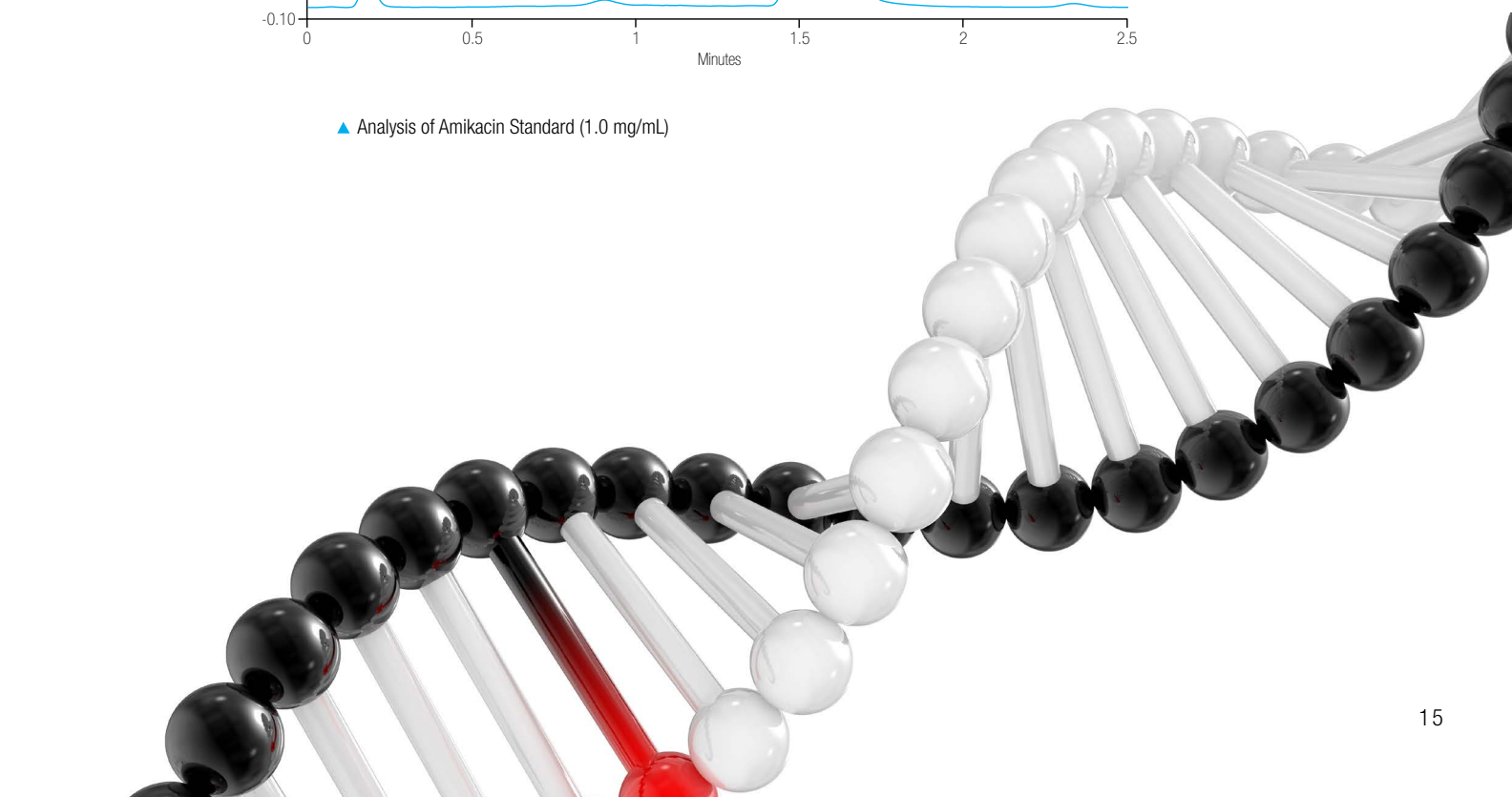
Impurity testing

Impurities occur in essentially all small molecule drug substances and drug products and often include starting materials and drug intermediates. Impurities provide no health benefits to patients and can even be (geno)toxic. As it is not feasible to completely avoid the presence of impurities, they have to be monitored and kept below a level that is sufficiently safe when administered to humans. As external standards are not always available, the use of charged aerosol detection with analyte response being independent of chemical structure, is proving to be extremely useful in estimating impurity levels.

Column:	C18 3 µm, 1.0 × 35 mm		
Mobile phase A:	0.2% Pentafluoropropionic acid in water		
Mobile phase B:	Methanol		
Gradient:	Time (min)	0% Mobile phase B	Flow Rate (µl/min)
	-3.00	10	50
	0.00	10	50
	0.30	10	50
	4.50	60	50
Flow rate:	50 µL/min		
Inj. volume:	0.5 µL		
Column temperature:	55 °C		
Evaporation temperature:	50 °C		



▲ Analysis of Amikacin Standard (1.0 mg/mL)



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