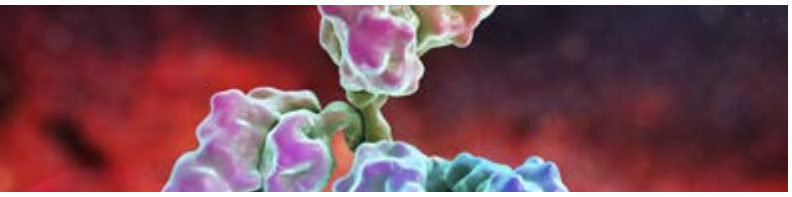
thermoscientific

Ion Chromatography for Pharma and Biopharma

Complete IC solutions for pharmaceuticals and biopharmaceuticals

ThermoFisher SCIENTIFIC

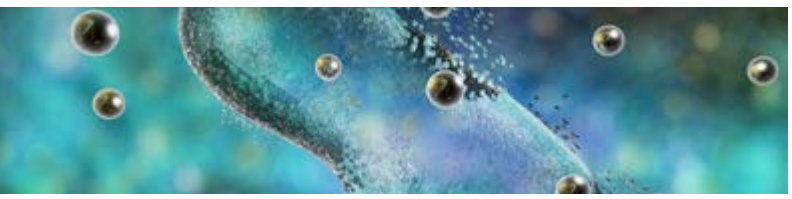
Ion Chromatography Pharma/Biopharma



Monosaccharides,
Sialic Acids & Glycans

Cell Culture Media
& Fermentation Broths

Counterion and
Impurity Analysis



Aminoglycosides

Solutions for Applied Markets

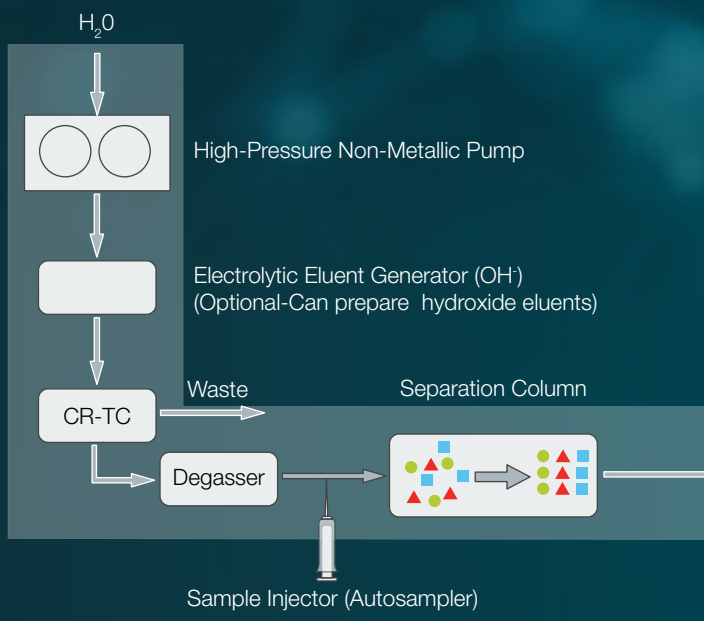
Ion Chromatography – Making It Easier To Get Results

Universal	No need for sample derivatization
Fast	Little to no sample preparation needed
Safe	Minimal handling of toxic reagents
Simple	Sample can simply be reconstituted in water and directly injected
Reliable	Maintains sample integrity & stability, no interferences due to labelling reagents
Green	No generation of hazardous chemical waste

Separation

High **P**ressure **I**on **C**hromatography (HPIC™)
– Our metal-free integrated and modular systems are capable of running up to 5,000 psi. Higher pressure tolerance enables the use of smaller particle size columns and allows faster flow rates

Reagent-**F**ree **I**on **C**hromatography (RFIC)
– No handling of acids or bases
– Just add water! RFIC feature eliminates eluent preparation and provides ease-of-use, reproducibility and precise gradients

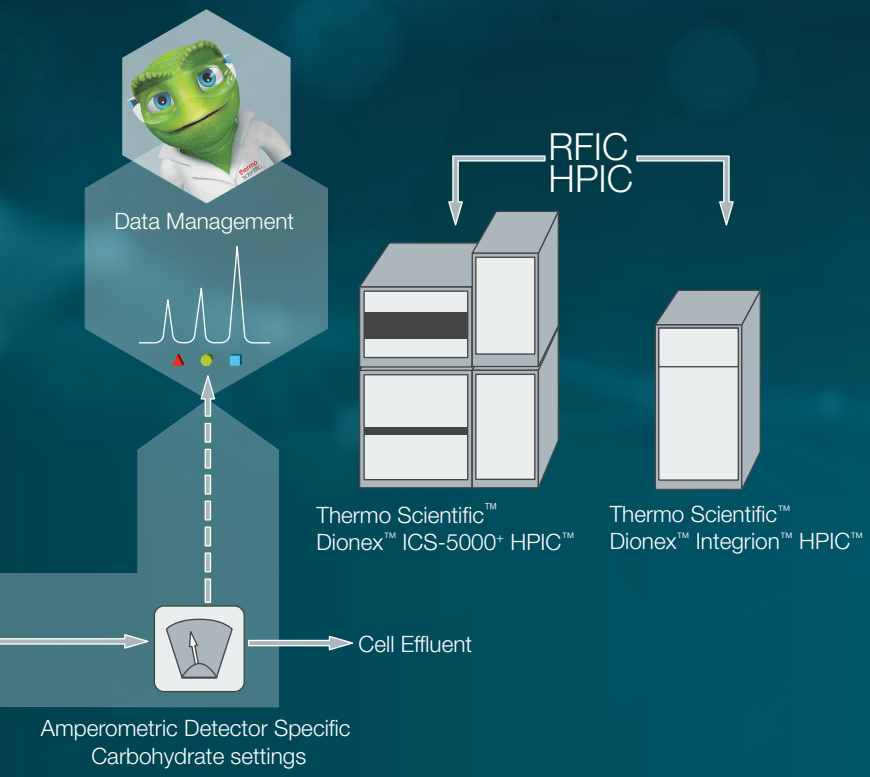


Basics of High Performance Anion Exchange (HPAE) Chromatography

- Carbohydrates are separated as oxyanions at high pH (≥12) using hydroxide-based eluents
- For High Mannose, Complex and Hybrid oligosaccharides, separations can be facilitated and further improved by using sodium acetate gradients in sodium hydroxide
- Separations of carbohydrates ranging from mono- to oligosaccharides are achieved using our diverse column portfolio
- For more information: www.thermofisher.com/ICColumns

Thermo Scientific™ Dionex™ CarboPac™ Columns

Detection



High-**P**erformance **A**nion-**E**xchange chromatography coupled with **P**ulsed **A**mperometric **D**etection (HPAE-PAD) – Technique where non-derivatized carbohydrates and amino acids (ranging from simple sugars to complex carbohydrates) are analyzed and detected

Disposable Gold (Au) Working Electrode: Greater reproducibility of PAD between electrode to electrode and user to user

Basics of Pulsed Amperometric Detection (PAD)

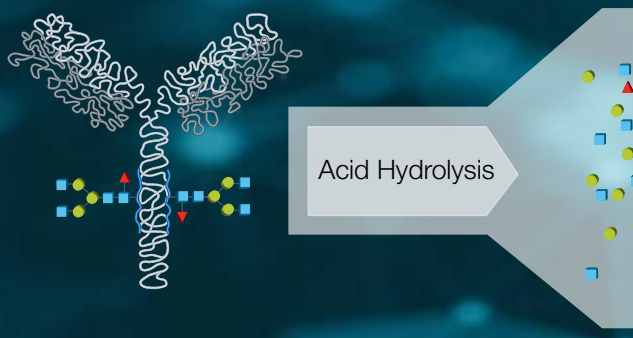
- Non-derivatized carbohydrates are detected on a gold working electrode at high pH by pulsed amperometric detection (PAD)
- PAD applies a series of potentials (a waveform) to a working electrode
- Pulsed amperometry detects analytes containing functional groups which are oxidized at the applied detection voltage
- For more information: www.thermofisher.com/Carbohydrates

Electrochemical Detector Cell

Monosaccharides and Sialic Acids

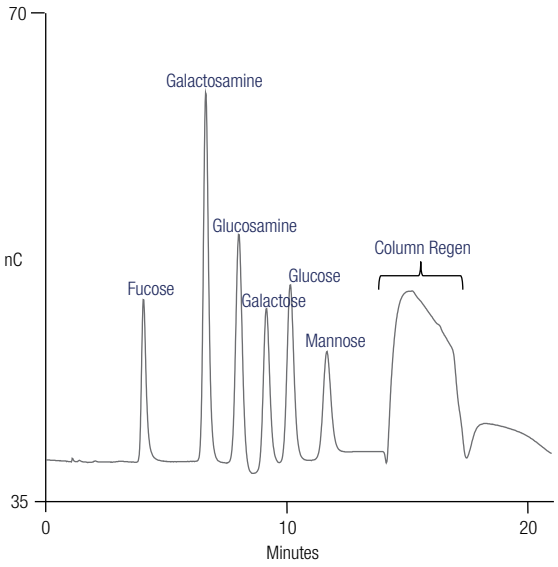
Benefits of Ion Chromatography

- High-resolution separations of carbohydrates using our diverse CarboPac columns
- Sensitive detection with no derivatization
- Same system can analyze a variety of carbohydrates and amino acids
- Allows measurement of total sugars and amounts of specific monosaccharides and sialic acids

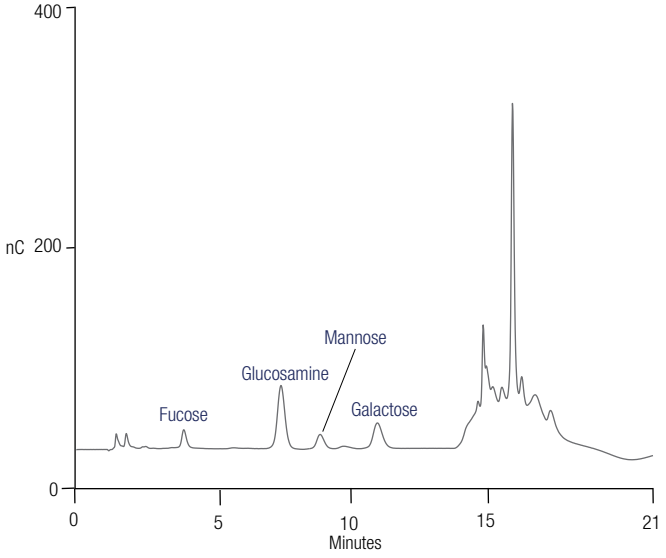


Glycoprotein

Glycoprotein Monosaccharide Analysis



Robust method for monosaccharide composition analysis with high precision for screening changes in glycosylation



Fast and reproducible monosaccharide composition analysis in a digested glycoprotein sample

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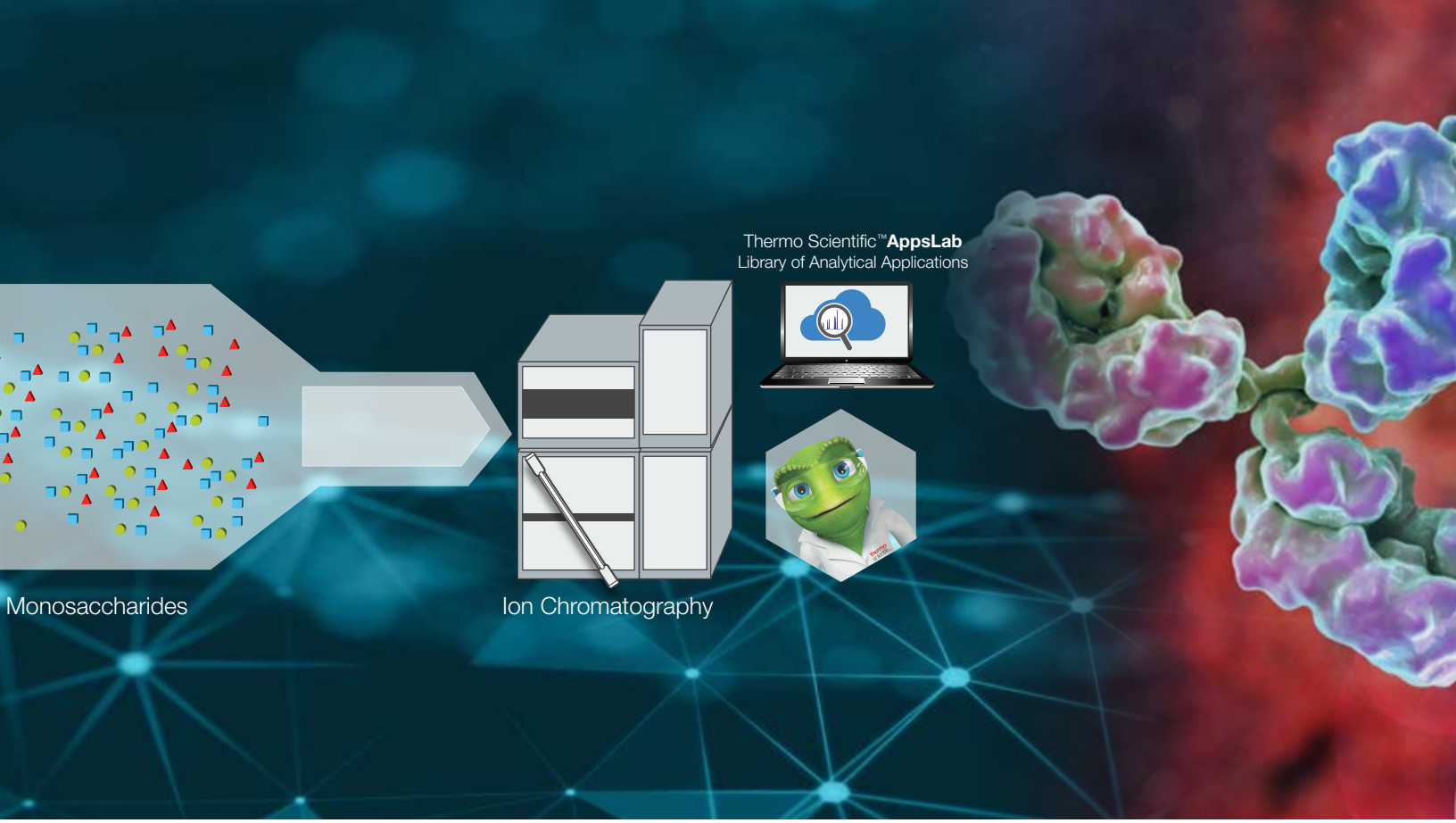
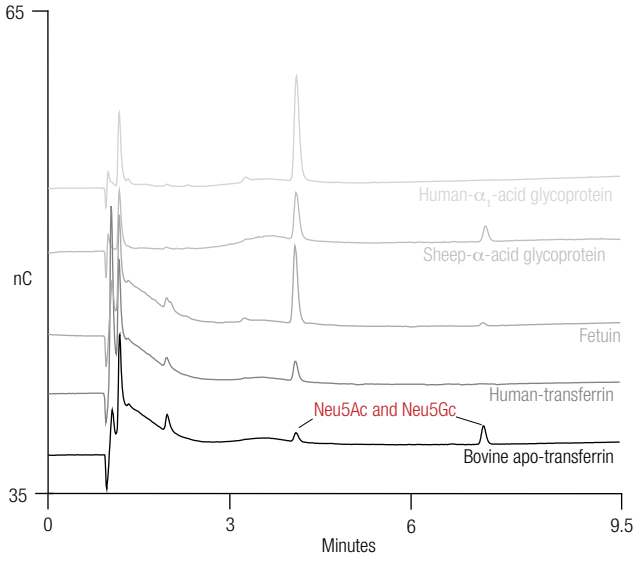
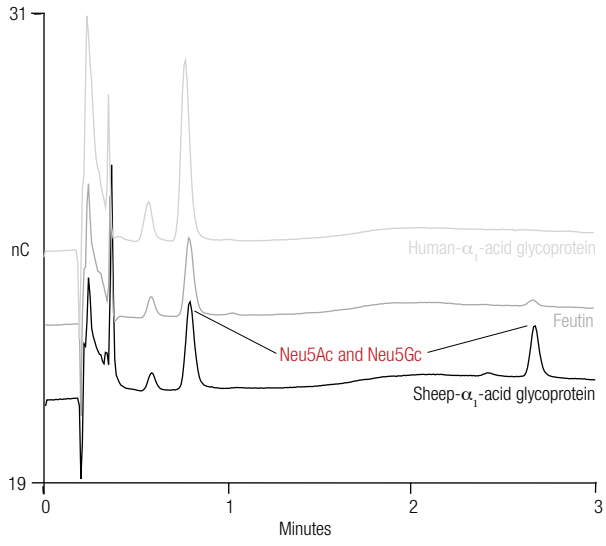


Sialic Acid Analysis



Direct and accurate quantification of sialic acids – a convenient method for screening sialic acids in glycoprotein hydrolysates



Faster separations - the two most commonly analyzed forms of sialic acid can be determined under 3 minutes

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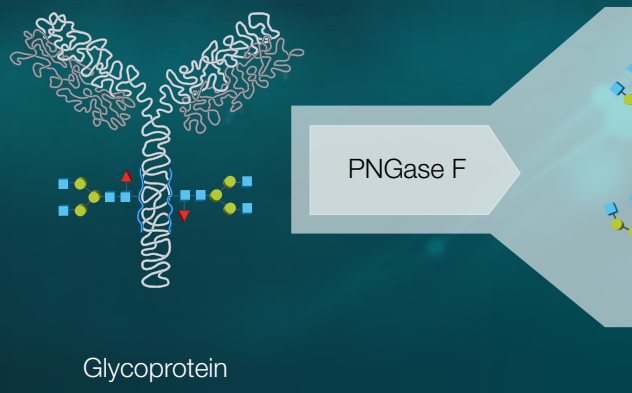
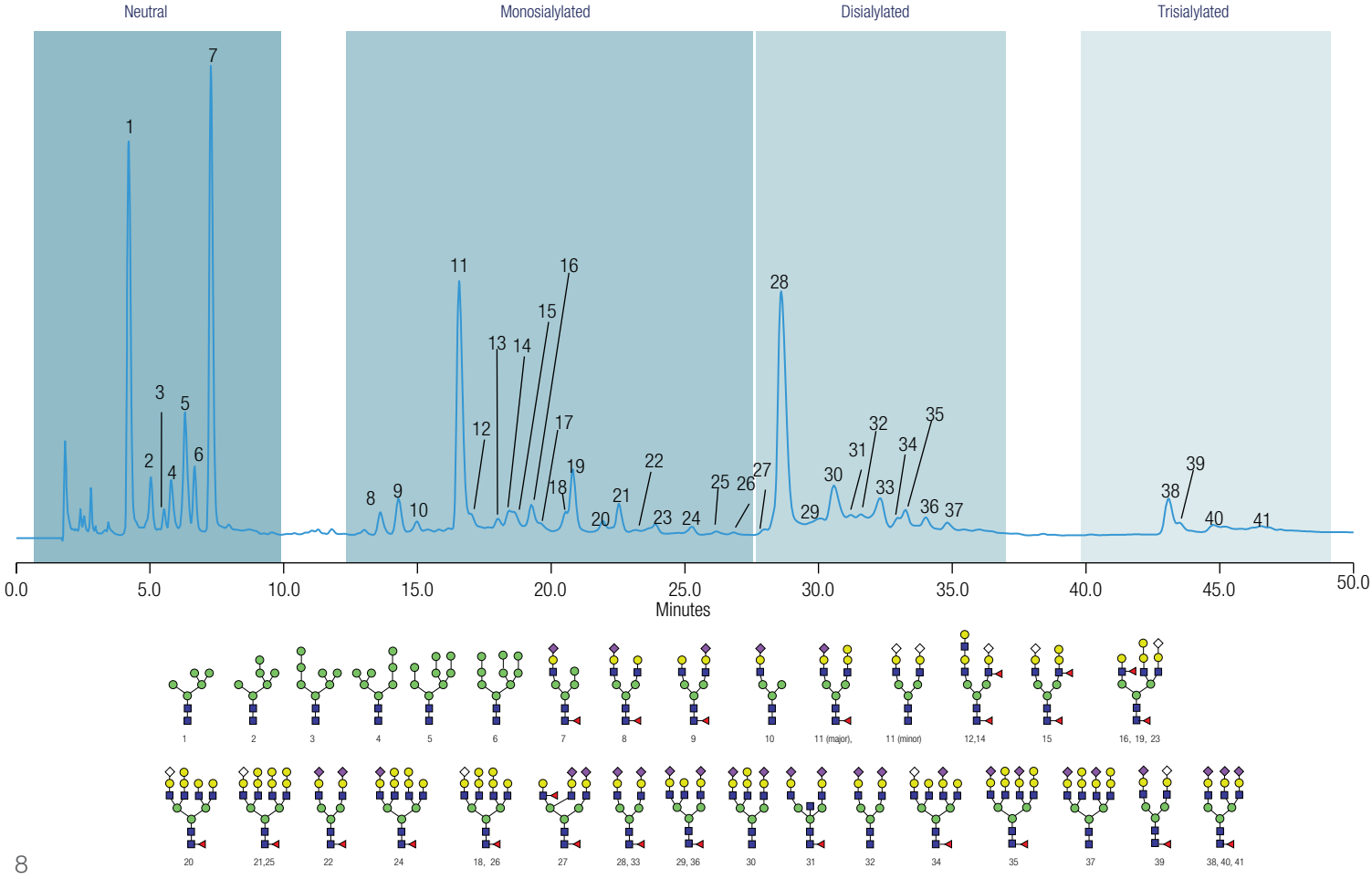
Non-derivatized *N*-linked Glycans

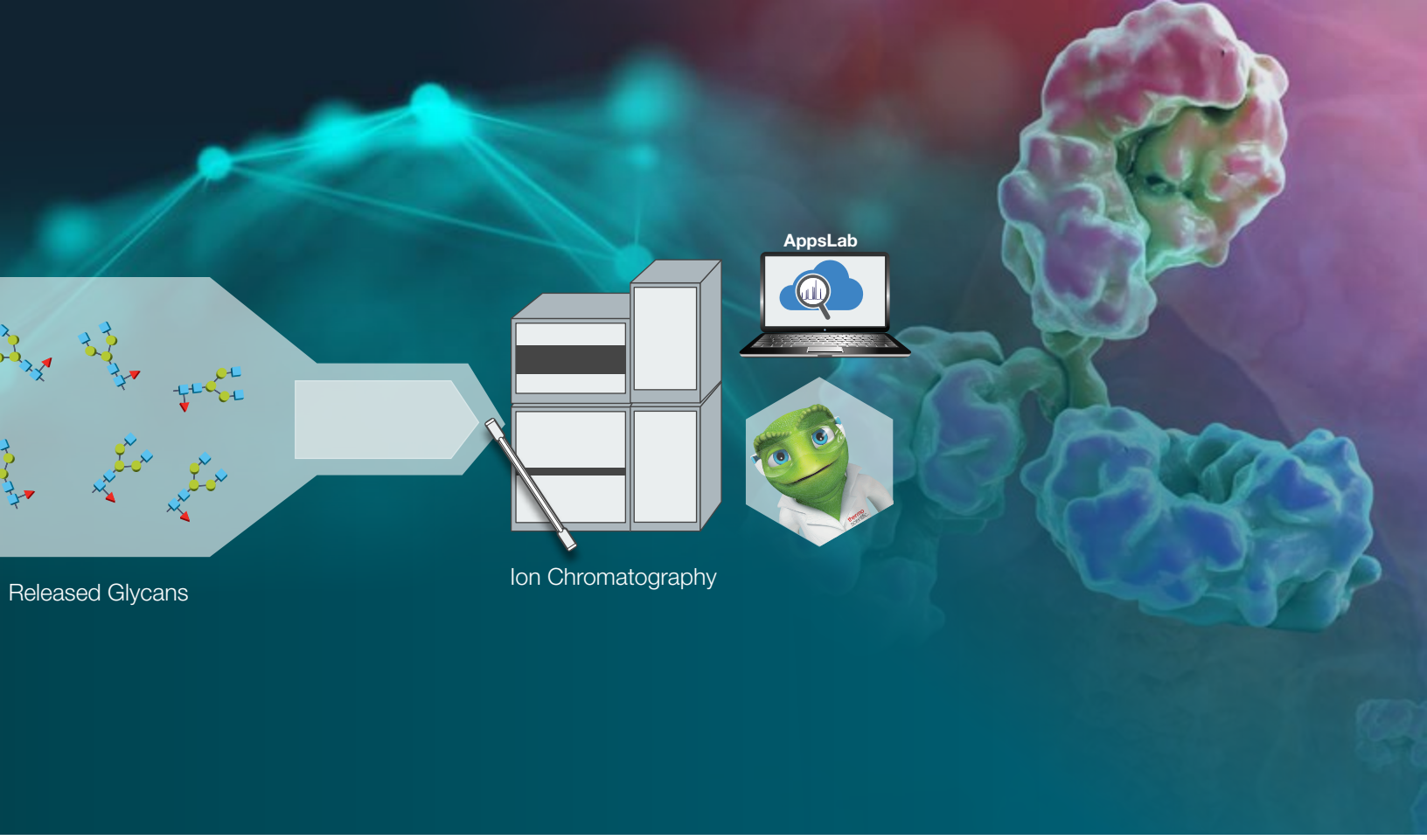
Benefits of Ion Chromatography

- Direct detection for profiling native glycans
- Sensitive separations based on charge, linkage, positional isomerism, and fucosylation
- Rapid workflow to release glycans with excellent reproducibility

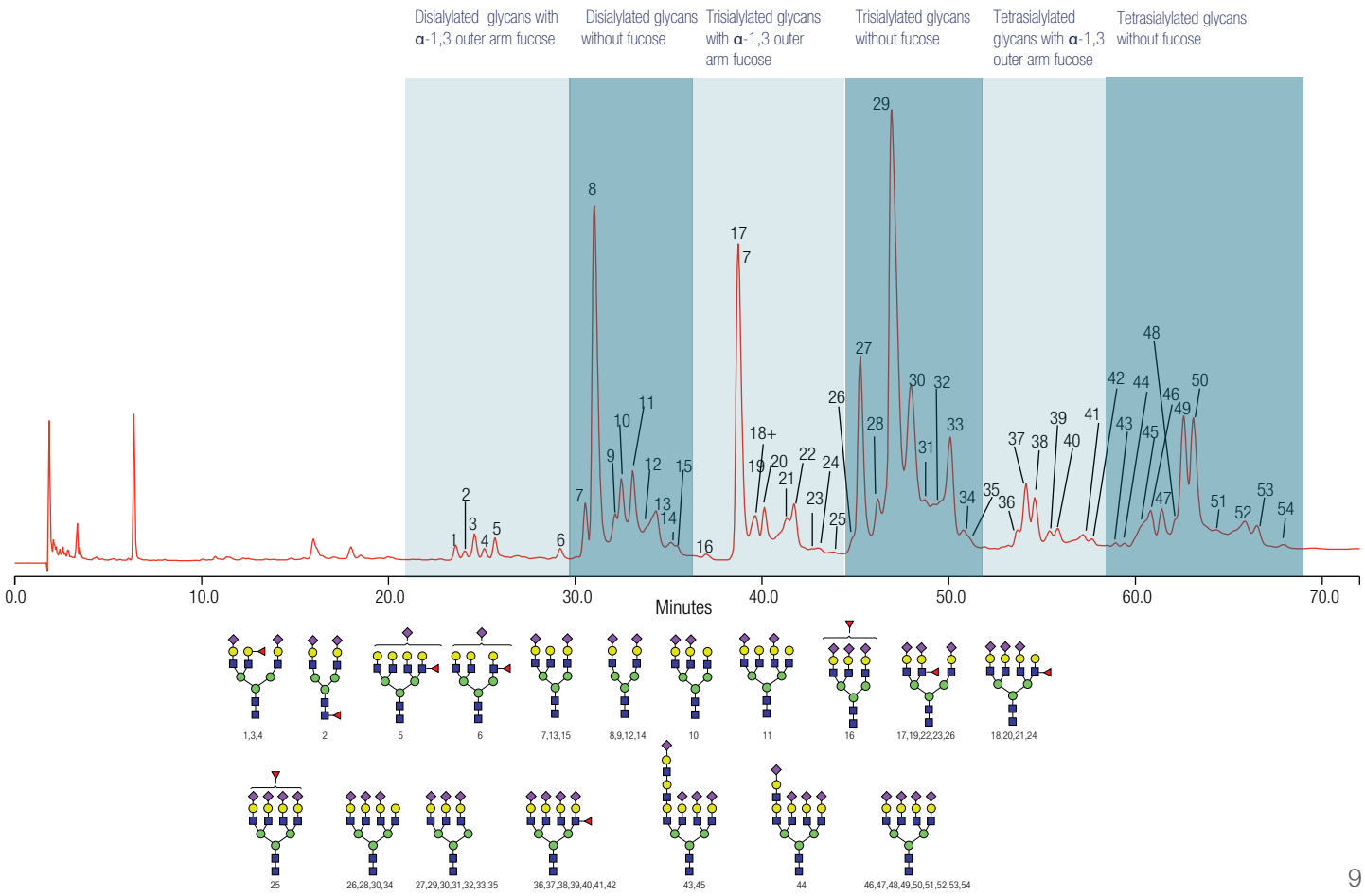


Released Glycan Analysis from Bovine Thyroglobulin





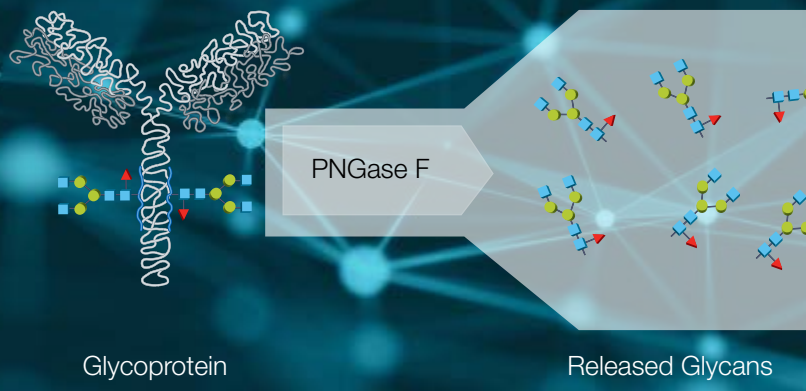
Released Glycan Analysis from hAGP



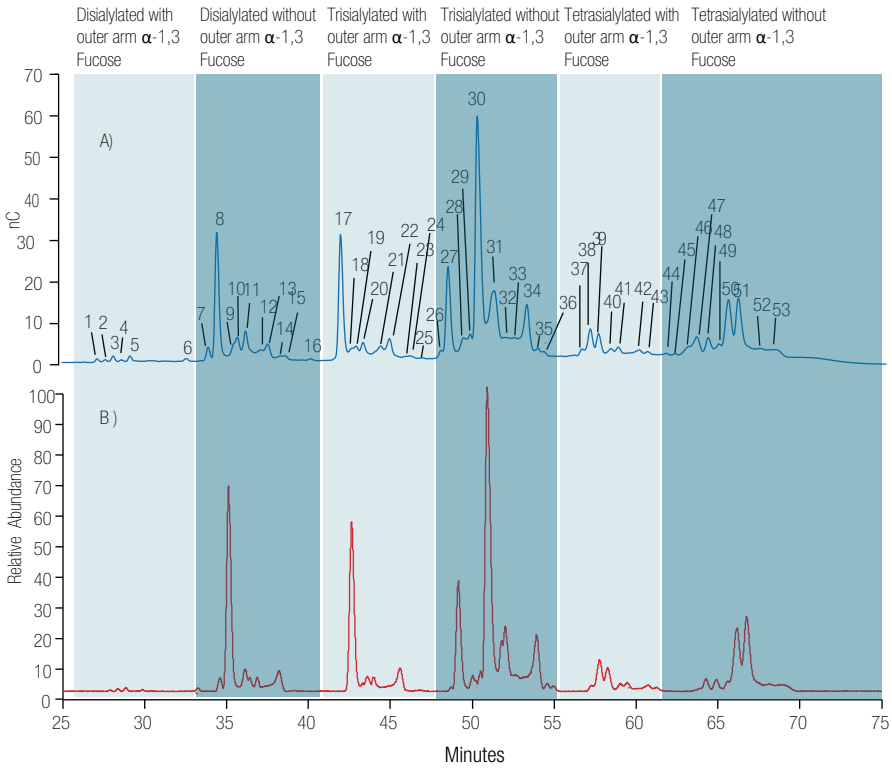
Non-derivatized *N*-linked Glycans

Benefits of Ion Chromatography

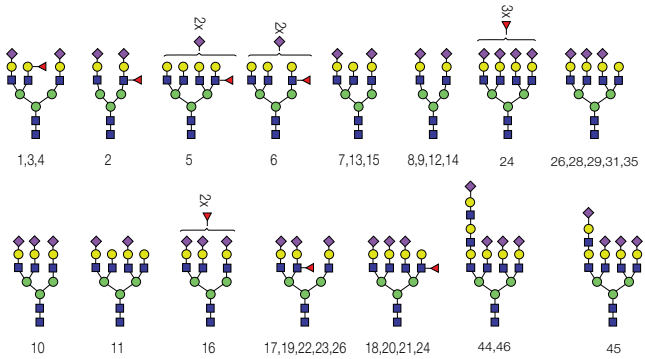
- Simultaneous separation and detection of neutral and sialylated (charged) glycans
- Easily interfaced with mass spectrometry (MS)
- In-depth characterization of all released glycans including low abundant glycans
- Excellent reproducibility of peak area distribution



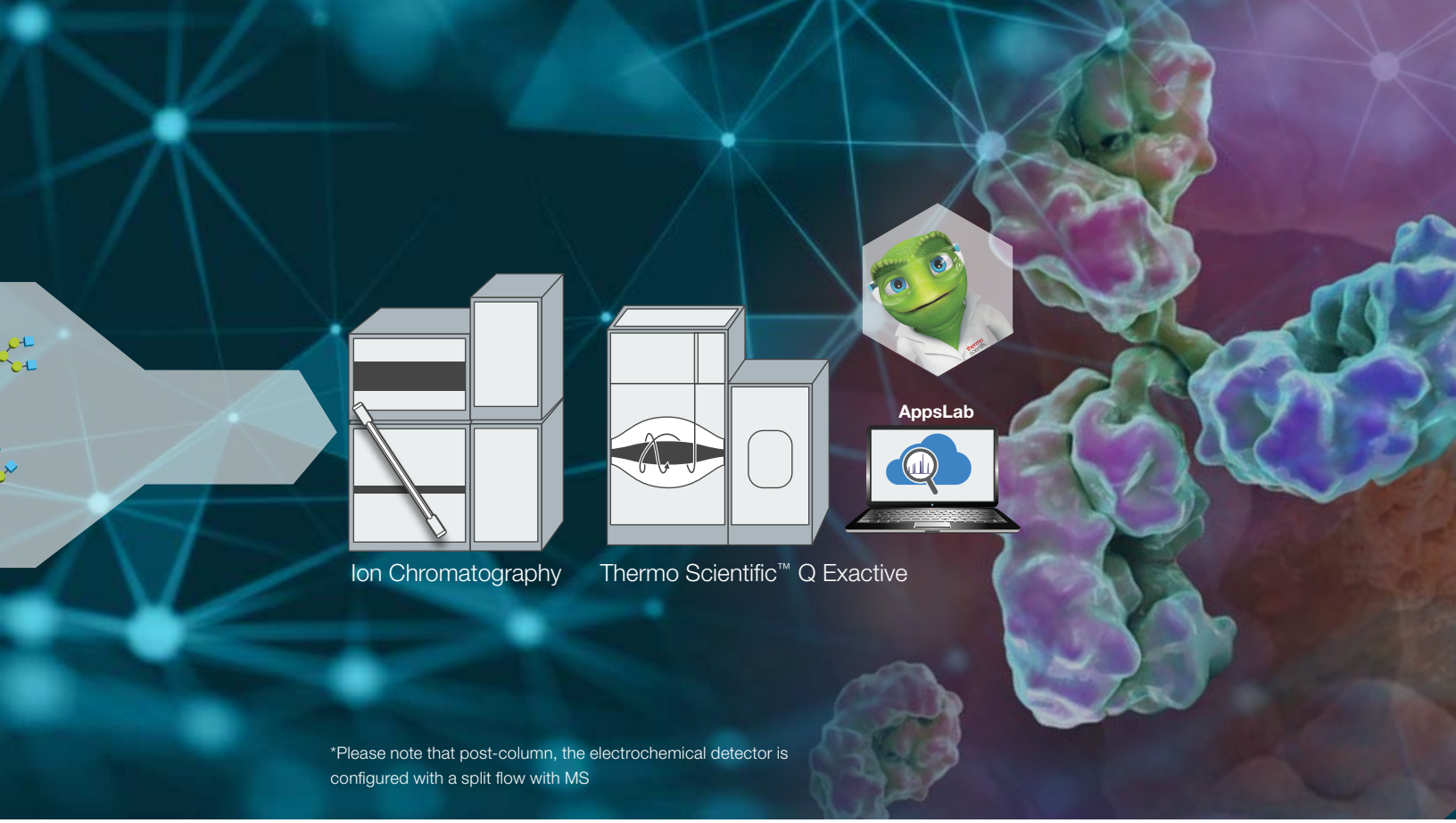
PAD (A) and MS (B) Chromatograms of hAGP Glycans



Annotated glycans on hAGP

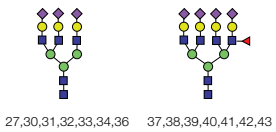


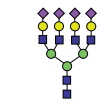
- Bias-free in-depth analysis of native *N*-linked glycans
- High resolution separation of glycans based on charge, linkage, position, and fucosylation
- MS2 spectra with diagnostic fragments allows highly reliable annotation of glycan species



Glycans Identified from hAGP

(X denotes unknown linkages of sialic acid)



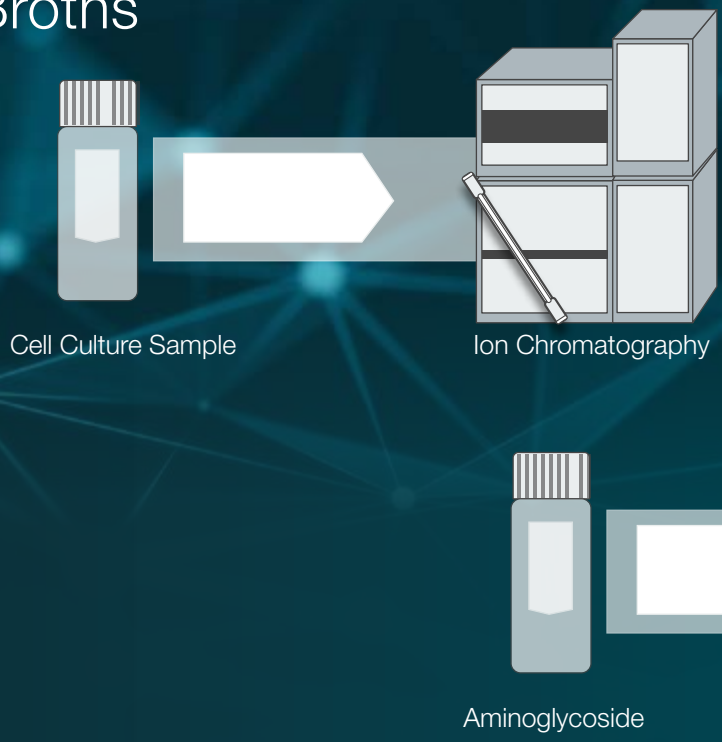


	Peak No.	Mass accuracy (ppm)	Glycan	Characteristic Fragments
Trisialylated without outer arm fucose	26	0.2314	A4F(3)1G(4)4S(X)3	B5Z3 (D ion), 2,4A5/Z4β, C4/Y4β, 0,4X6/Z4β
	26	0.15414	A4G(4)4S(X)3	C5/Z3 (D ion), 2,4X4α/Y3, 0,4X6β/B4, 2,4A6/Y3α
	27	0.62524	A3G(4)3S(X)3	B5/Z3 (D ion), 3,5A5, 1,3X6α/Y3, C5/Y3α
	28	0.2466	A4G(4)4S(X)3	C5/Z3 (D ion), 2,4X4α/Y3, 0,4X6β/B4, 2,4A6/Y3α
	29	0.7294	A3G(4)3S(X)3	B5/Z3 (D ion), 3,5A5, 1,3X6α/Y3, C5/Y3α
	30	0.06167	A4G(4)4S(X)3	C5/Z3 (D ion), 2,4X4α/Y3, 0,4X6β/B4, 2,4A6/Y3α
	30	0.7294	A3G(4)3S(X)3	B5/Z3 (D ion), 3,5A5, 1,3X6α/Y3, C5/Y3α
	31	0.3126	A3G(4)3S(X)3	B5/Z3 (D ion), 3,5A5, 1,3X6α/Y3, C5/Y3α
	32	0.6252	A3G(4)3S(X)3	B5/Z3 (D ion), 3,5A5, 1,3X6α/Y3, C5/Y3α
	33	0.7294	A3G(4)3S(X)3	B5/Z3 (D ion), 3,5A5, 1,3X6α/Y3, C5/Y3α
	34	0.0616	A4G(4)4S(X)3	C5/Z3 (D ion), 2,4X4α/Y3, 0,4X6β/B4, 2,4A6/Y3α
	35	0.5210	A3G(4)3S(X)3	B5/Z3 (D ion), 3,5A5, 1,3X6α/Y3, C5/Y3α
Tetrasialylated, outer arm fucose	36	-0.3260	A4F(3)1G(4)4S(X)4	2,4A6/Y5γ, 1,5X5/B4, 0,2X6α/B4α, 0,2X4β/Y3α
	37	-0.5705	A4F(3)1G(4)4S(X)4	2,4A6/Y5γ, 1,5X5/B4, 0,2X6α/B4α, 0,2X4β/Y3α
	38	-0.5705	A4F(3)1G(4)4S(X)4	2,4A6/Y5γ, 1,5X5/B4, 0,2X6α/B4α, 0,2X4β/Y3α
	39	-0.65195	A4F(3)1G(4)4S(X)4	2,4A6/Y5γ, 1,5X5/B4, 0,2X6α/B4α, 0,2X4β/Y3α
	40	-0.89645	A4F(3)1G(4)4S(X)4	2,4A6/Y5γ, 1,5X5/B4, 0,2X6α/B4α, 0,2X4β/Y3α
	41	-0.5705	A4F(3)1G(4)4S(X)4	2,4A6/Y5γ, 1,5X5/B4, 0,2X6α/B4α, 0,2X4β/Y3α
	42	-0.5705	A4F(3)1G(4)4S(X)4	2,4A6/Y5γ, 1,5X5/B4, 0,2X6α/B4α, 0,2X4β/Y3α

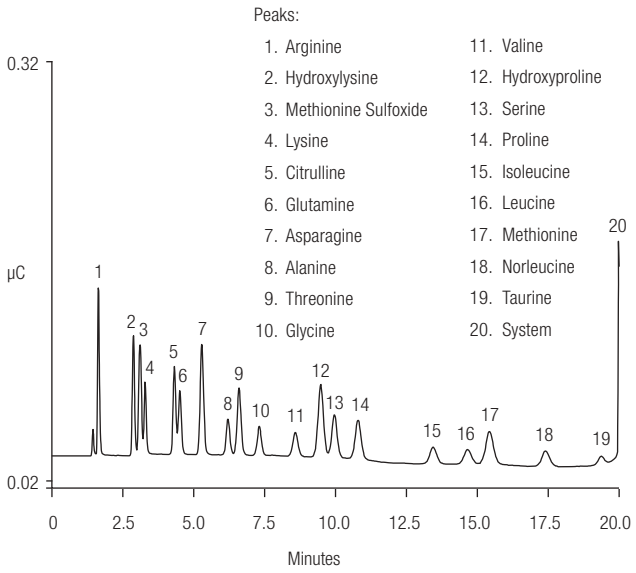
Cell Culture and Fermentation Broths

Benefits of Ion Chromatography

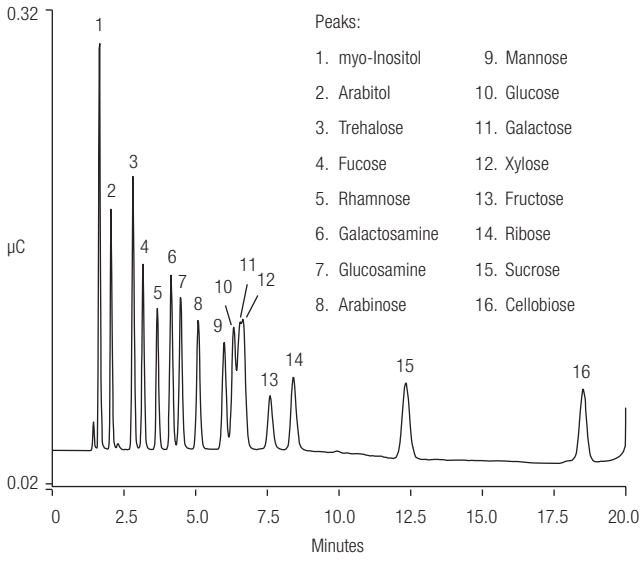
- Simultaneous detection of carbohydrates and amino acids in cell cultures
- Mid-femtomole to low-picomole detection limits
- Compatible with all commonly used hydrolysis procedures
- Compatible with on-line monitoring and analysis



Amino Acids in Cell Culture Media



Carbohydrates in Fermentation Broths



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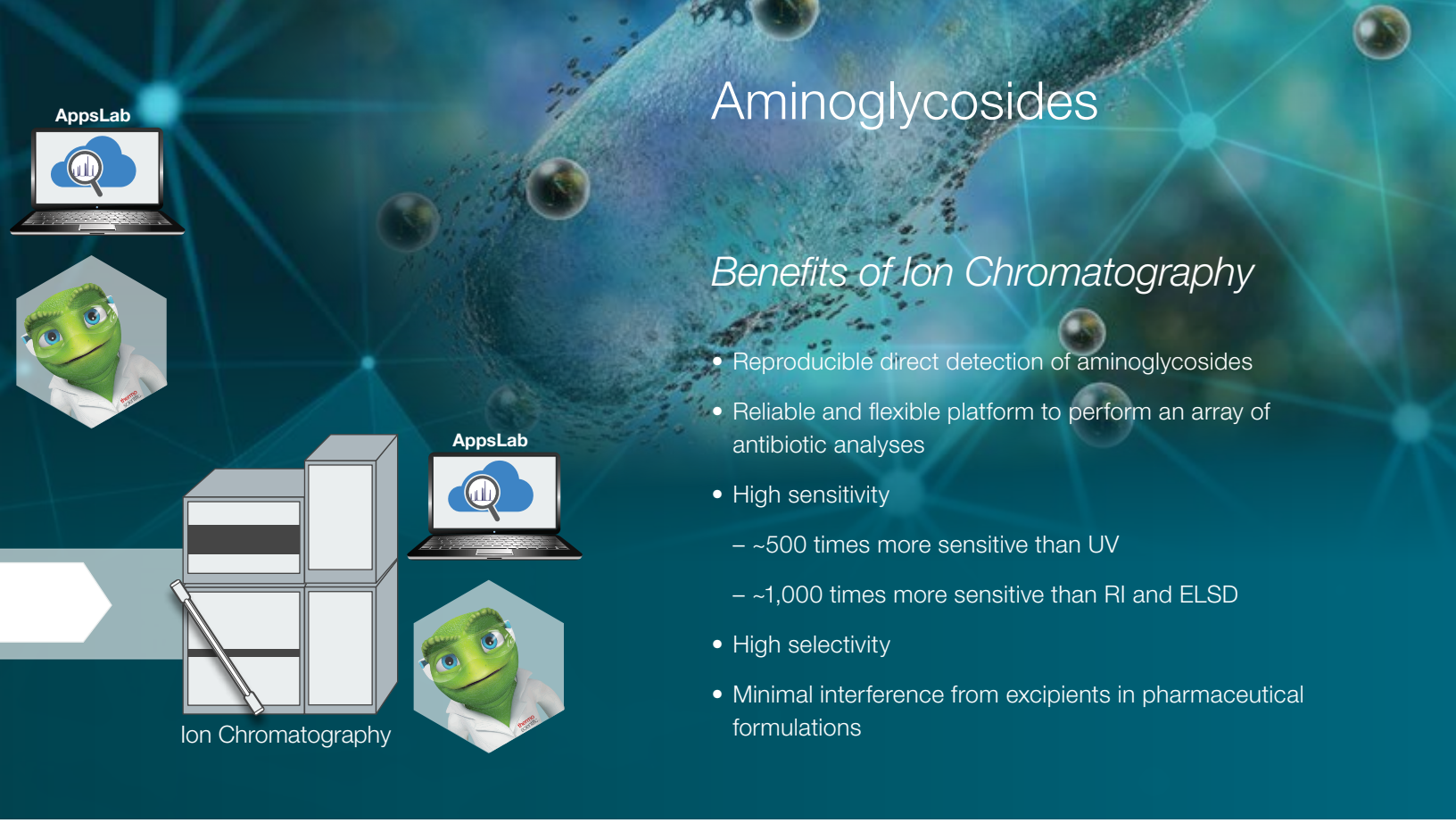
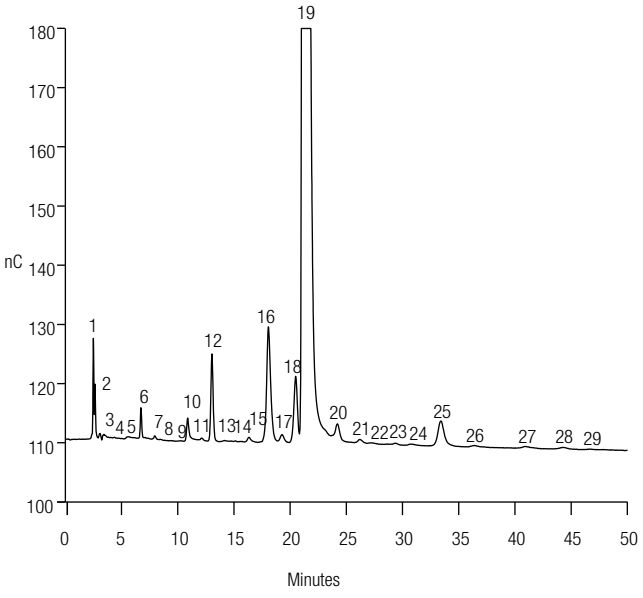


Aminoglycosides

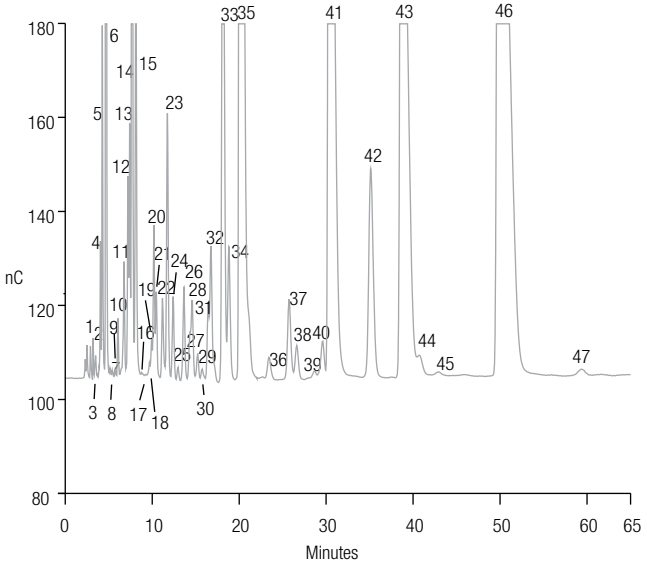
Benefits of Ion Chromatography

- Reproducible direct detection of aminoglycosides
- Reliable and flexible platform to perform an array of antibiotic analyses
- High sensitivity
  - ~500 times more sensitive than UV
  - ~1,000 times more sensitive than RI and ELSD
- High selectivity
- Minimal interference from excipients in pharmaceutical formulations

Detection of Impurities in Etimicin



Screening for Gentamicin Analogs and Impurities

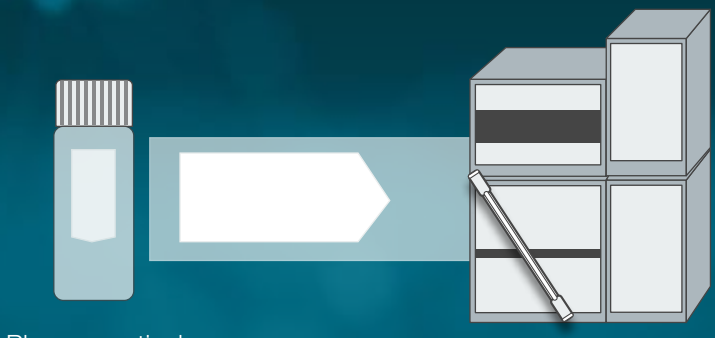


IC offers robust separations and sensitive detections for drug purity characterizations and screenings

Counterion and Impurity Analysis

Uses of Ion Chromatography

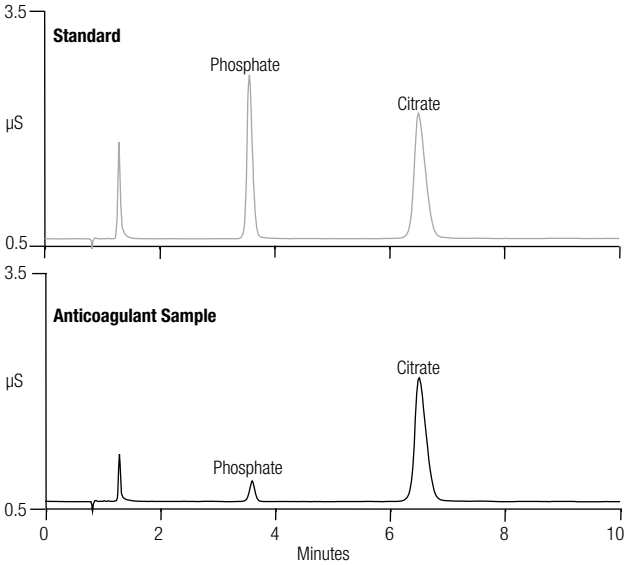
- Counterion determinations
- Impurity analysis in drug substances
- Assay of the drug substance
- Measuring excipients in drug products



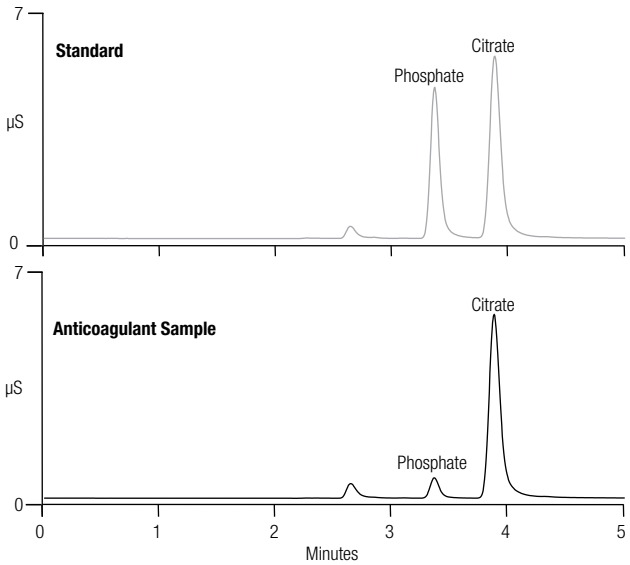
Pharmaceutical Formulation

Ion Chromatography

Assays



Determination of citric acid/citrate & phosphate in pharmaceutical formulations; USP General Chapter <345>



Modernized IC assay for citric acid/citrate and phosphate in pharmaceutical formulations

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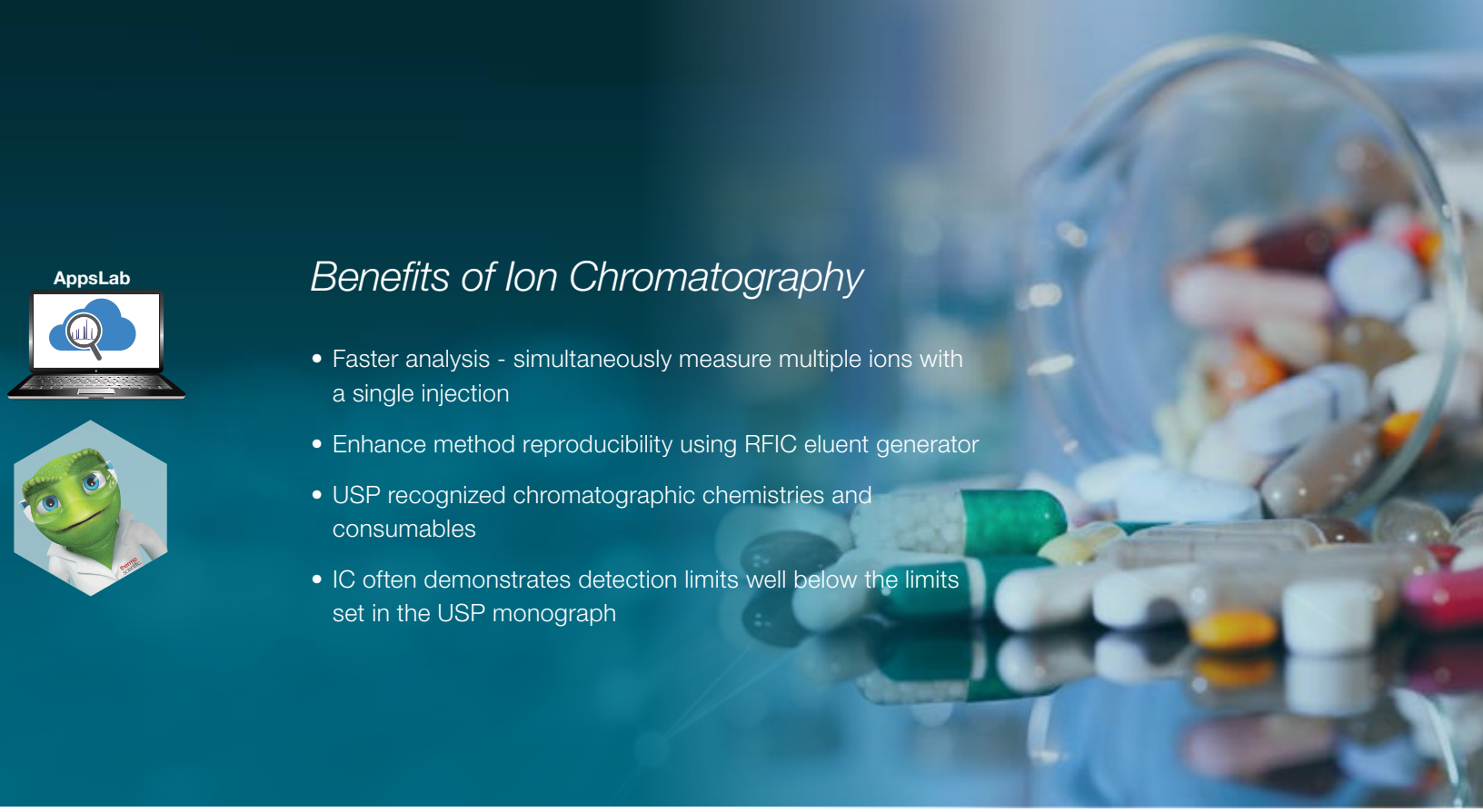
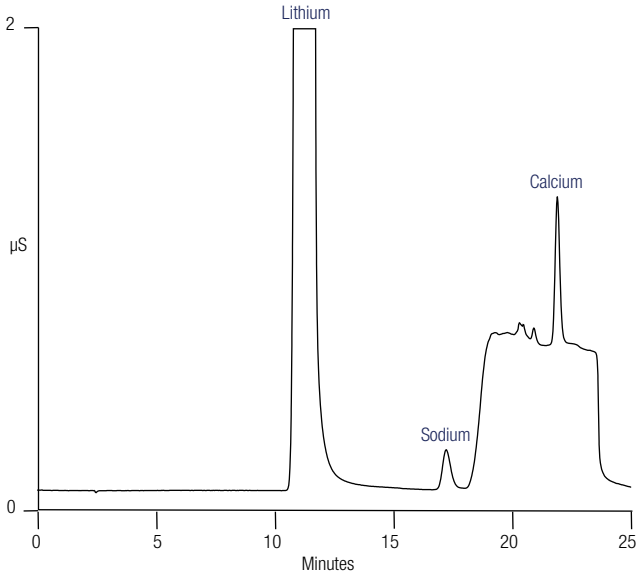


Benefits of Ion Chromatography

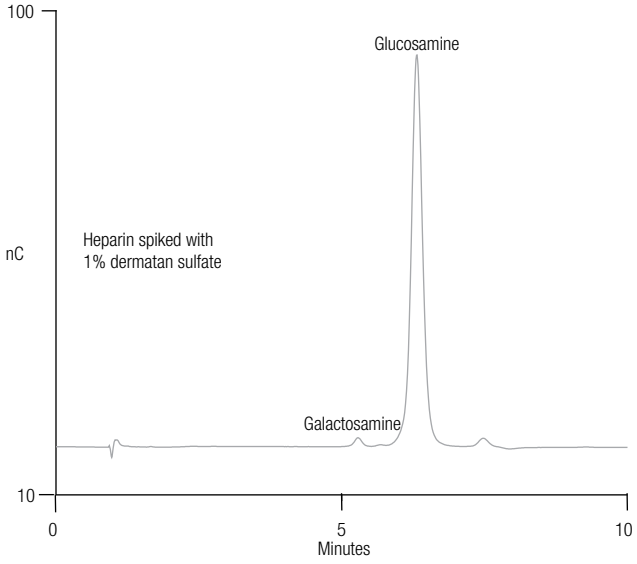
- Faster analysis - simultaneously measure multiple ions with a single injection
- Enhance method reproducibility using RFIC eluent generator
- USP recognized chromatographic chemistries and consumables
- IC often demonstrates detection limits well below the limits set in the USP monograph

Lithium Assay



Assay of lithium in lithium carbonate

Organic Impurity Analysis



Determination of galactosamine as an impurity in acid-hydrolyzed heparin samples

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Recommended Thermo Scientific™ Dionex™ column portfolio for carbohydrate and amino acid analysis

Column Options	Recommended application use
Thermo Scientific™ Dionex™ CarboPac™ PA200 Column Thermo Scientific™ Dionex™ CarboPac™ PA100 Column	Fast, pH-stable and high-resolution mapping and analysis of charged and neutral oligosaccharides; separations based on size, charge, degree of branching, and linkage isomerism.
Thermo Scientific™ Dionex™ CarboPac™ PA20 Column	High-resolution separations of mono- and disaccharides, including optimized resolution of glucosamine/galactose and glucose/mannose peak pairs.
Thermo Scientific™ Dionex™ CarboPac™ PA20 Fast Sialic Acid Column	Fast analysis of sialic acids (e.g., N-acetyl- and N-glycolylneuraminic acids) in glycoprotein acid hydrolysates.
Thermo Scientific™ Dionex™ CarboPac™ MA1 Column	For sensitive, rugged, and reliable separations of reduced sugars. Achieve baseline resolution of fucose, N-acetyl-(D)-glucosamine, N-acetyl-galactosamine, mannose, glucose, galactose, and neutral oligosaccharides in the same separation.
Thermo Scientific™ Dionex™ CarboPac™ PA10 Column	Separation of mono- and disaccharides in drugs and mammalian glycoproteins; separation of sialic acids when sodium acetate is added to the eluent.
Thermo Scientific™ Dionex™ CarboPac™ PA1 Column	Separation of mono- and disaccharides including sialic acids, as well as specific oligosaccharides using an isocratic eluent.
Thermo Scientific™ Dionex™ AminoPac™ PA10 Column	High-resolution separation of free amino acids using the Thermo Scientific AAA-Direct Amino Acid Analyzer.
Thermo Scientific™ Dionex™ IonPac™ AmG RP Column	High performance RP (C18) PEEK column for robust ion-pair separation of aminoglycosides.

www.thermofisher.com/ICColumns

Find out more at **thermofisher.com/PharmaIC**

ThermoFisher SCIENTIFIC

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