ICS-5000 Modular, Metal-Free System Ideal for Bioanalyses

The ICS-5000 Ion Chromatography system is the world's first capillary ion chromatography (IC) system on the market and provides an impressive combination of increased productivity, expanded capabilities, and improved performance. Modular versatility, functional integration, and superior performance culminate in the most advanced ion chromatography system available.

The ICS-5000 Reagent-Free™ IC (RFIC™) system is the optimal liquid chromatography system for ion-exchange chromatography and biomolecule analysis. With a 100% metal-free system (autosampler, pump, flow path, and detector), the ICS-5000 system is able to overcome the limitations of traditional stainless steel or other metal chromatography systems affected by corrosive mobile phases. Metal flow paths can cause denaturation or absorption effects with some analytes, resulting in poor chromatography. Oxidized metals can poison columns, reducing separation performance and lifetime. The complete PEEK™ flow path of the ICS-5000 system, with its ability to resist pH values from 1–14, eliminates the possibility of system corrosion, and improves robustness. The all-PEEK pump can also perform quaternary gradients for demanding application needs (analytical scale).

The ICS-5000 is the first IC system to allow separation on the analytical (4 mm and 2 mm) and capillary (0.4 mm) scale. Add to this, the ability to inject small sample volumes (down to 0.4 µL) from a variety of sample formats, including well plates; the versatility of multiple detection schemes (electrochemical, conductivity, mass spectrometric [MS] and UV-vis) even in series; and the ease-of-use of Always on, Always Ready, makes the ICS-5000 the most versatile IC system for bioanalysis.

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Metabolomics

The ICS-5000 system is an excellent platform for metabolomics. Unlike HPLC, ion chromatography separates the components that normally only elute in the void volume (early, unretained peaks). Ions and other highly charged species are easily separated and detected using suppressed conductivity detection (Figures 1 and 2). Eluents are converted to water by the suppressor, making the effluent completely MS compatible. The ICS-5000 system offers the advantages of separating anions and cations in one dual system (saving bench space). It allows sample volumes as low as 0.4 µL for precious samples. Additional detectors, such as UV and electrochemical, can be added to determine sugars and un-derivatized amino acids, or to resolve coeluting metabolites (Figure 1).

Glycobiology

High-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) is widely used for determination of carbohydrates, including simple monosaccharides, oligosaccharides, sugar acids, such as sialic acids, sugar alcohols, sugar phosphates, and sugar nucleotides.

The development of recombinant-derived glycoproteins and monoclonal antibodies for therapeutic use has led to an increasing demand for methods to characterize their carbohydrate structures, especially asparagine-linked oligosaccharides that can impact the glycoprotein's function. The HPAE-PAD technique not only separates oligosaccharides according to charge, but can also resolve oligosaccharides with the same charge according to size, sugar composition, and linkage of monosaccharide units. Analysis of enzymatic digests of glycoproteins can reveal important information about their structure (Figure 3).

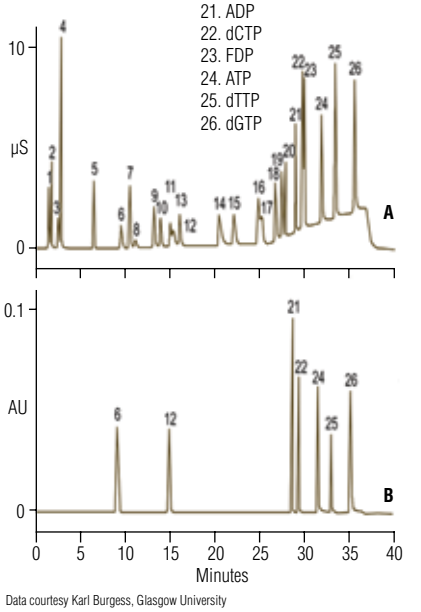
Pharmaceuticals

Manufacture of biological products, such as biofuels and pharmaceuticals, increasingly uses fermentation. The ICS-5000 system provides corrosion resistance for hydrolyzed samples, automation for matrix elimination or sample preparation, and a range of detection options, for example conductivity, amperometry, and UV-vis absorbance, singly or in series, to help detect the wide range of analytes and concentrations found in in-process samples and final products. Dionex systems and ion-exchange columns are suitable for many US and European Pharmacopeia analytical methods. For example, Dionex has solutions for determining galactosamine-containing impurities with amperometric detection (AN 233) and oversulfated chondroitin sulfate and dermatan sulfate with UV detection (AN 235) in Heparin Sodium.

Column: IonPac® AS11, 2 × 250 mm
Eluent: 2 to 12 mM NaOH in 15 min, to 20 mM in 25 min, to 70 mM in 35 min
Flow Rate: 2.0 mL/min
Detection: A. Suppressed conductivity B. UV absorbance at 260 nm

Peaks (2.5 nmol each):

1. Acetate
2. Pyruvate
3. Glyoxylate
4. Chloride
5. Nitrate
6. cAMP
7. Succinate
8. Unknown
9. Glucose-6-phosphate
10. Fructose-6-phosphate
11. Ribose-5-phosphate
12. AMP
13. Sedoheptulose-7-phosphate
14. Phosphate
15. 6-Phosphogluconate
16. 3-Phosphoglycerate
17. 2-Phosphoglycerate
18. Citrate
19. Isocitrate
20. Phosphoenolpyruvate
21. ADP
22. dCTP
23. FDP
24. ATP
25. dTTP
26. dGTP



Data courtesy Karl Burgess, Glasgow University

Figure 1. Separation of a series of metabolic intermediates.

Column: IonSwift™ MAX-100 Capillary, (0.25 × 250 mm) and guard (0.25 × 50 mm)
Eluent: 0.1 mM–2 mM KOH in 5 min, 2 mM–25 mM in 15 min, 25 mM–65 mM KOH in 10 min
Eluent Source: EGC KOH capillary
Flow Rate: 12 µL/min
Inj. Volume: 0.4 µL
Temperature: 35 °C
Detection: Suppressed conductivity, ACES™ 300 AutoSuppression recycle mode

Peaks:

1. Fluoride	0.6 mg/L	10. Carbonate	4
2. Acetate	2	11. Sulfate	2
3. Formate	2	12. Oxalate	2
4. Butyrate	2	13. Tungstate	4
5. Galacturonate	2	14. Phosphate	4
6. Chloride	1	15. Chromate	4
7. Nitrite	2	16. Citrate	4
8. Bromide	2	17. Isocitrate	4
9. Nitrate	2	18. *cis*-Aconitate	4
		19. *trans*-Aconitate	4

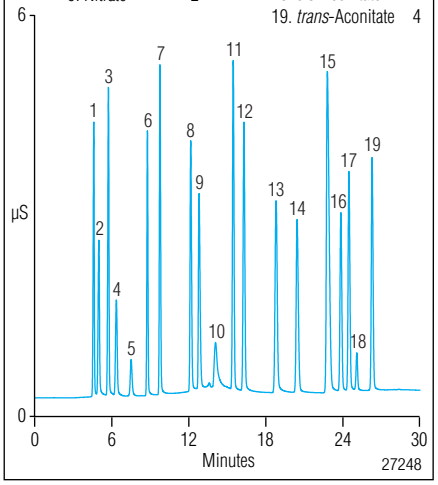


Figure 2. Separation of organic acids and inorganic anions using the IonSwift MAX-100 capillary column.

Column: CarboPac® PA200 (3 × 250 mm) and guard (3 × 50 mm)
Eluent: 0–5 min: 100 mM NaOH/5 mM NaOAc 60 min: 100 mM NaOH/180 mM NaOAc
Temperature: 30 °C
Flow Rate: 0.5 mL/min
Inj. Volume: 9 µL from 10 µL loop
Detection: PAD (Au) Waveform A (TN 21)
Samples: A) PNGase F digest of human IgG B) Sialic acids standard C) Neuraminidase digest of Sample A

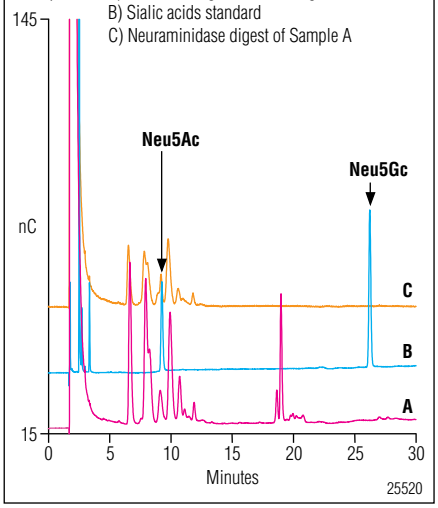


Figure 3. Monitoring release of sialic acids from human polyclonal lgG N*-linked oligosaccharides by HPAE-PAD.*

Fermentation and Feedstocks

Manufacture of biological products, such as biofuels and pharmaceuticals, increasingly utilizes fermentation. The ICS-5000 provides corrosion resistance for hydrolyzed samples, automation options for in-line matrix elimination and sample preparation, and electrochemical detection for determination of carbohydrates in feedstocks (Figure 4) and analysis of alcohols, glycols, and carbohydrates in fermentation broths for in-process monitoring. The suppressed effluent from ion chromatography is also ideal for injection into a mass spectrometer for analysis of feedstock components, such as low molecular mass organic acids.

Amino Acid Analysis

Cell cultures and fermentation broths are complex mixtures of nutrients, waste products, cells and cell debris, and desired products, such as protein biotherapeutics (cytokines, monoclonal antibodies, etc.). Recently, interest has been growing for the characterization of the amino acids and their metabolic by-products in fermentation broths because these components affect the yield of the desired products. Integrated pulsed amperometric detection (IPAD) is a powerful detection technique with a broad linear range and very low detection limits. The AminoPac® PA10 column can separate numerous amino acids and carbohydrates in one run (Figure 5), and the small sample size requirement and inert fluidic paths of the ICS-5000 system are ideal for monitoring fermentation broths.

3D Amperometry

The 3D Amperometry technique is an extension of conventional amperometry, enabling the continuous acquisition of current throughout the entire waveform period. The complete data set enables, among other things, postchromatographic current integration of the amperometric data. Because different chemical compounds oxidize differently at given applied voltages, subtle differences in the amount of current generated through a waveform can provide additional information about the identity and purity of the substances being analyzed. In addition, a waveform integration range selective to carbohydrates or specific amino acids can reduce or eliminate peak interferences and improve their peak integrations.

For example, resolution may be improved between asparagine and glucose through reduction of the peak area for asparagine relative to glucose. The resolution factor equation relates the differences in retention times of the two peaks to the sum of both peak widths (U.S. Pharmacopeia definition), or to the peak widths at 50% peak height (European Pharmacopeia definition). Although the retention time difference between two peaks remains unchanged by integration range modifications, the relative peak widths can be reduced for the two peaks using this technique, thus increasing the resolution factor (Figure 6).

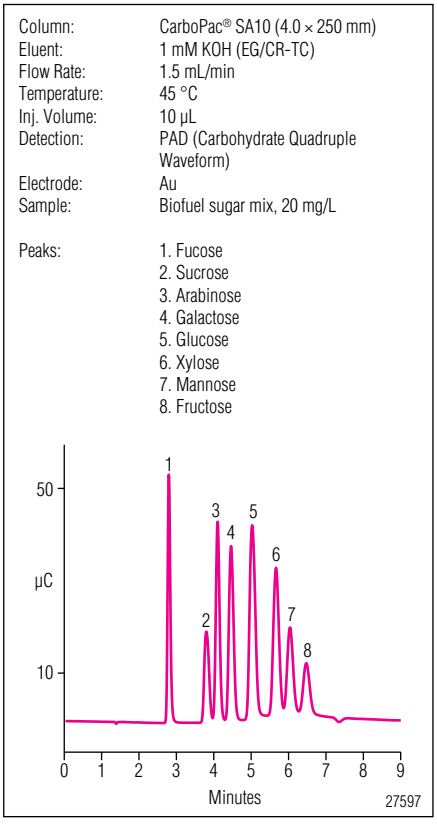


Figure 4. Fast 7 min separation of biofuel sugars on the CarboPac SA10.

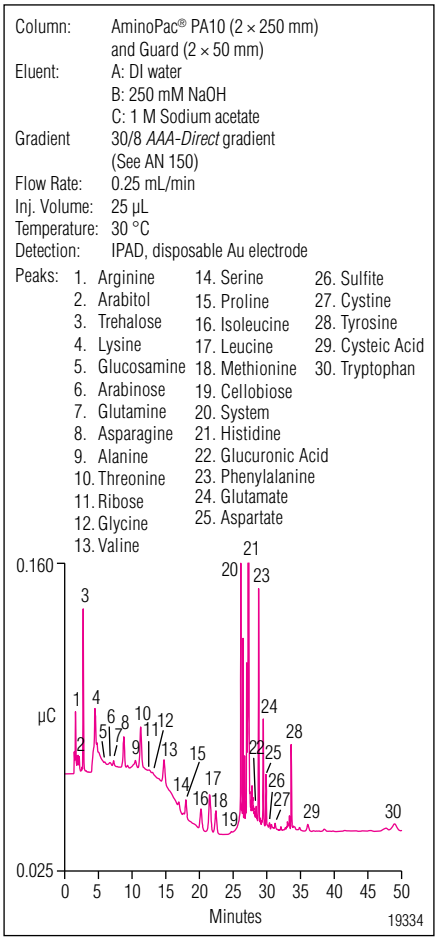


Figure 5. Determination of amino acids and carbohydrates in LB Broth supernatant (100-fold dilution).

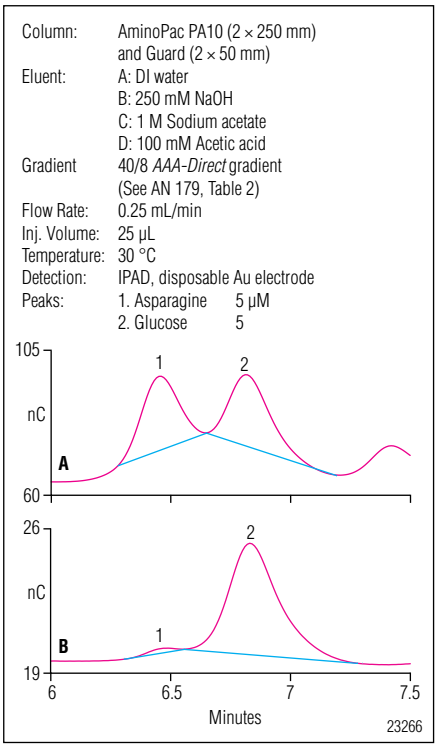


Figure 6. Post-run reintegration allows selective removal of asparagine, allowing better quantification of glucose.

Related Dionex Applications Literature

Dionex has an extensive library of methods and techniques for determining a wide variety of analytes important to the biotechnology and pharmaceutical industries. Below is a selected list of applications covered in this brochure. For more information, please visit www.dionex.com and click on the Documents tab, then on Application Notes and Updates, or contact your local Dionex representative.

IC SOLUTIONS FOR BIOANALYSIS		
Analyte	Matrix	Resource
Alcohols, glycols	Fermentation broths	AN 188: Determination of Alcohols and Glycols in Fermentation Broths Using Ion-Exclusion Chromatography and Pulsed Amperometric Detection
Amino acids	Cell cultures, fermentation broths	AN 150: Determination of Amino Acids in Cell Cultures and Fermentation Broths
Amino acids, carbohydrates	Cell cultures	AN 179: Carbohydrate and Amino Acid Analysis Using 3-D Amperometry
Biogenic amines	Beer, wine	AN 182: Determination of Biogenic Amines in Alcoholic Beverages by Ion Chromatography with Suppressed Conductivity and Integrated Pulsed Amperometric Detection
Biogenic amines	Meats, cheeses	AN 183: Determination of Biogenic Amines in Fermented and Non-Fermented Foods Using Ion Chromatography with Suppressed Conductivity and Integrated Pulsed Amperometric Detection
Biogenic amines	Fruits, vegetables, chocolate	AU 162: Determination of Biogenic Amines in Fruit, Vegetables, and Chocolate Using Ion Chromatography with Suppressed Conductivity and Integrated Pulsed Amperometric Detection
Carbachol	Opthalmic solutions	AN 194: Determination of Charbachol in Opthalmic Solutions Using a Reagent-Free Ion Chromatography System
Carbohydrates	Corn stover hydrolysate	AN 225: Rapid Method for the Estimation of Total Free Monosaccharide Content of Corn Stover Hydrolysate Using HPAE-PAD
Citrate, phosphate	Pharmaceutical formulations	AN 164: Assay for Citrate and Phosphate in Pharmaceutical Formulations Using Ion Chromatography
Galactosamine	Heparin	AN 233: Determination of Galactosamine Containing Organic Impurities in Heparin by HPAE-PAD Using the CarboPac PA20 Column
Inorganic anions	Water-insoluble pharmaceuticals	AN 220: Determination of Inorganic Anion Impurities in a Water Insoluble Pharmaceutical by Ion Chromatography with Suppressed Conductivity Detection
Mannose-6-phosphate	Biological fluids	AN 202: High Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD) Analysis of Mannose-6-Phosphate
N-Methylpyrrolidine	Cefepime	AN 199: Determination of *N*-Methylpyrrolidine in Cefepime Using a Reagent-Free Ion Chromatography System
Nucleotides	Amplification cocktails	AN 162: Determination of Nucleotides by Ion Chromatography with UV Absorbance Detection
Oligosaccharides	Polyclonal IgG	AN 215: Separation of Asparagine-Linked (N-Linked) Oligosaccharides from Human Polyclonal IgG Using the CarboPac PA200 Column
Oversulfated chondroitin	Heparin	AN 235: Determination of Oversulfated Chondroitin Sulfate and Dermatan Sulfate in Heparin Sodium Using Anion-Exchange Chromatography with UV Detection
Paramomycin	Pharmaceutical formulations	AN 186: Analysis of Paramomycin by HPAE-PAD
Phosphate content	Phosphorylated proteins	AN 210: Determination of the Protein Content of Phosphorylated Proteins
Protein concentration	Biological fluids	AN 163: Determination of Protein Concentrations Using *AAA-Direct*
Streptomycin, impurities	Fermentation broths, pharmaceutical formulations	AN 181: Determination of Streptomycin and Impurities Using HPAE-PAD
Sulfate	Aminoglycoside	AN 190: Determination of Sulfate Counter Ion and Anionic Impurities in Aminoglycoside Drug Substances by Ion Chromatography with Suppressed Conductivity Detection
Trifluoroacetate (TFA)	Protein buffers, peptides	AN 160: Determination of Residual Trifluoroacetate in Protein Purification Buffers and Peptide Preparations by Reagent-Free Ion Chromatography

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