HPAE-PAD for the Analysis of Carbohydrates

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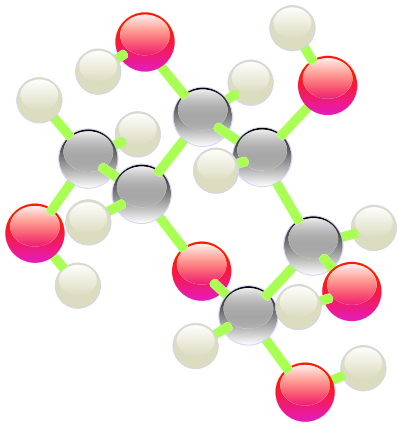
Executive Summary

High-performance anion exchange chromatography with derivatization-free, pulsed amperometric detection (HPAE-PAD) and mass spectrometry allows for a more complete analysis of carbohydrates. These compounds can be difficult to fully analyze given their polar nature, similar structural characteristics and lack of a suitable chromophore.



Keywords

Carbohydrates, High-Performance Anion Exchange Chromatography, Pulsed Amperometric Detection, Derivatization-Free, Weak Acids

How Are Carbohydrates Different from Other Analytes?

Carbohydrates play vital roles in a variety of biological functions, including cellular communication, gene expression, immunology, organism defense mechanisms, and growth and development. They are difficult to analyze as they are very polar compounds, exhibit similar structural characteristics, and do not have a suitable chromophore.

Methods for the liquid chromatographic analysis of carbohydrates have utilized silica-based amino-bonded or polymer-based, metal-loaded, cation-exchange columns, with refractive index (RI) or low-wavelength ultraviolet (UV) detection. The low sensitivity and selectivity of RI and low-wavelength UV detection methods limit their applications in trace carbohydrate analysis. Another popular HPLC technique exists called Hydrophilic Interaction Liquid Chromatography (HILIC) with fluorescence tag detection which requires derivatization. HILIC also requires mobiles phases of high organic content (50–80% acetonitrile) which could cause sample solubility problems at high concentrations.

Which Techniques Successfully Allow for the Separation and Quantification of Carbohydrates?

An improved chromatographic technique known as high-performance anion exchange (HPAE) takes advantage of the weakly acidic nature of carbohydrates to give highly selective separations at high pH using a strong anion-exchange stationary phase. Coupled with pulsed amperometric detection (PAD), it permits direct quantification of non-derivatized carbohydrates at high femtomolar concentration levels with minimal sample preparation and cleanup.

HPAE Chromatography — Superior Separations

HPAE chromatography can be used to separate analytes that can be ionized under high pH conditions. Carbohydrates typically have pKas in the range of 12–13. Once the pH rises above the pKa of the analyte, it becomes ionized in solution. This is accomplished using hydroxide-based eluents. And with the development of highly cross-linked, ethylvinyl benzene-divinyl benzene pellicular resins that have broad pH stability (0 to 14), separations at high pH conditions is

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feasible. The columns' nonporous resins have small anion-exchange microbeads carrying the anion-exchange functional groups which are permanently attached electrostatically to a larger cation-exchange resin particle. The nonporous nature of the resin minimizes band-broadening and imparts highly effective separation of a wide variety of carbohydrates, including branched oligosaccharides.

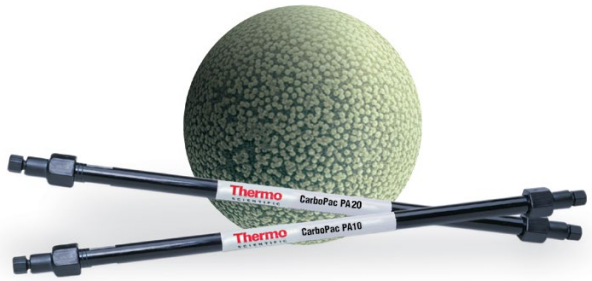
PAD — Sensitive, Selective Detection

The detection of underivatized analytes can be achieved using pulsed amperometric detection. The potential variations are known as a waveform. The variations result in oxidizing and reducing conditions on the electrode surface, which in turn causes the oxidation of analytes bound to the working electrode surface. Pulsed amperometry detects only those compounds that contain functional groups which become oxidized at the detection voltage employed. Detection is sensitive and highly selective for electroactive species, since many potentially interfering species cannot be oxidized or reduced, and are not detected. It is also important to note that neutral or cationic sample components in the matrix elute in, or close to, the void volume of the column. As a result, the carbohydrate components of interest are not impacted even if the neutral or cationic sample components are oxidized.

What Solutions Exist on the Market?

Thermo Scientific has coupled high-performance anion exchange chromatography with derivatization-free, pulsed amperometric detection and mass spectrometry for direct quantification and qualitative analyses of monosaccharides, sialic acids, glycols, etc. HPAE-PAD can be performed on the Thermo Scientific™ Dionex™ ICS-5000⁺ HPIC™ system with standard bore (4 mm i.d.) microbore (2 mm i.d.) or capillary (0.4 mm i.d.) column formats. HPAE-PAD can also be performed in a capillary scale on the Thermo Scientific Dionex ICS-4000 HPIC system. To identify unknown analytes, the Dionex ICS-5000⁺ and Dionex ICS-4000 can be configured for HPAE-PAD and then coupled with a mass spectrometer using a Thermo Scientific™ Dionex™ CMD™ Carbohydrate Membrane Desalter inline prior to the MS interface or to spot fractions onto MALDI targets for offline MALDI/MS analysis.

Additionally, the Thermo Scientific™ Dionex™ CarboPac™ column family offers a selection of columns, each for a different class of compounds. Combined with PAD, these columns support reliable techniques to provide high-resolution separations of glycoprotein oligosaccharides and complex carbohydrates from dietary fiber, including fructans, maltodextrins, and amylopectins. The Dionex CarboPac PA20 column line has been expanded to include a new 0.4 × 150 mm capillary column, which provides high-resolution separations of mono- and disaccharides with no need for derivatization.



References

1. Thermo Fisher Scientific, Analysis of Carbohydrates by High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD), Technical Note 20, TN70671_E. Sunnyvale, CA, 2004.
2. Thermo Fisher Scientific, Analysis of 2-aminobenzamide Labeled Dextran Ladder on a Solid Core HPLC Column, Application Note 20593, AN20593_E. Runcorn, Cheshire, UK, 2012.
3. http://www.dionex.com/en-us/markets/life-science/carbohydrate-sciences/lp-71623.html
4. http://www.dionex.com/en-us/products/columns/bio/carbohydrate/lp-73362.html
5. http://www.dionex.com/en-us/products/ion-chromatography/ic-rfic-solutions/hpae-pad/lp-111613.html

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