Simplifying Carbohydrate Testing to Meet Food Quality and Labeling Requirements Using Ion Chromatography and Pulsed Amperometric Detection

Webinar: April 2, 2015. Presented by Dr. Khalil Divan
Thermo Fisher Scientific, Hemel Hempstead, United Kingdom

Introduction

Carbohydrate analysis of food and beverages continues to be the center of attention when monitoring the quality or labeling of these products. Such analysis helps to remove doubts about a food's provenance; establishes its nutritional value and purity and ensures its conformity to regulation and quality standards.[1]

This webinar, delivered by Dr. Khalil Divan (Thermo Fisher Scientific), discusses an ideal solution suited to carbohydrate analysis.[2] High performance anion-exchange chromatography coupled to pulsed amperometric detection (HPAE-PAD) delivers versatility, sensitivity, and ease-of-use to the analyst. Unlike other analytical techniques, a range of different carbohydrates – from the smallest of monomers to more complex forms such as oligosaccharides and polysaccharides – are readily profiled in a wide range of samples. Very often food and beverage matrices are complex, containing not only the carbohydrates of interest but also many other components such as fats and proteins that can interfere with analysis.

Ion chromatography with pulsed amperometric detection offers the analyst the opportunity to separate and measure carbohydrates with high resolution and without derivatization. HPAE-PAD offers extremely low detection levels, with high efficiency and reproducibility that is unparalleled. Specific methods and applications are illustrated and reference papers supporting the examples are available in the webinar so the viewer can conduct a much more detailed investigation on this subject.

Webinar Summary

The viewer is presented with a review of the importance of carbohydrates and reasons for analysis. An overview of industrial and regulatory standards coupled to why food labeling requirements need to be met leads then to a basic summary on carbohydrate classification and chemistry. This introduction drives the viewer to appreciate the challenges brought by this complex and fascinating group of molecules.

Thermo Scientific™ Dionex™ ICS-5000+ ion chromatography system.

Various chromatographic techniques applied to carbohydrate separation are reviewed based on carbohydrate chemistry. The principals for chromatographic separation are explained as well as the reason why taking advantage of the weakly acidic nature of carbohydrates lends itself to anion-exchange separations. The high pH of the mobile phase used for separation converts the carbohydrate to an anionic species which makes them highly amenable to anion-exchange separation. The resolving power of HPAE means molecules with subtle differences in structure are resolved during anion-exchange separation without having to resort to complex work-up procedures beforehand or elaborate mobile phases.[3]

Columns for Carbohydrate Separation

Thermo Fisher Scientific manufactures a variety of ion chromatography columns under the Thermo Scientific™ Dionex™ CarboPac™ trade name. The column's packing material is discussed demonstrating why it has the mechanical strength, chemical stability, and resolving power to discriminate between simple carbohydrates and polysaccharides. The viewer is shown a diverse range of ion chromatography columns specific to the separation of various carbohydrate classes and tailored to an analyst's specific requirements.

Thermo SCIENTIFIC

Detection

Chromatographic separation is meaningless without a suitable detection method. A brief review highlights why electrochemical detection, among the range of techniques available, is ideal for molecules without a chromophore such as carbohydrates. A synopsis of the advantages of electrochemical techniques precedes a review of more specific amperometric detection methods. This is bolstered by a survey of amperometry and why PAD, in particular, is so useful compared to other methods.[3]

The principal of regenerating the working electrode on-line electrolytically is discussed as it lends itself to making the technique ultra-reproducible, robust, and user friendly. PAD eliminates complications associated with detection reproducibility and loss of sensitivity. If mass spectrometry (MS) is required rather than amperometric detection, a simple device can be employed that replaces metal cations from the mobile phase with hydronium ions so that MS is performed without compromising chromatographic separation. As alkaline eluents are required for oxidation of the sample to allow detection, the durability of the Dionex CarboPac columns is discussed.[3] The use of PAD and the manner by which electrode potentials are manipulated with pulsed waveforms are examined further.[4]

Case Studies

A number of different case studies and examples illustrate the power of HPAE-PAD. Carbohydrate detection and analysis have been crucial in resolving adulteration issues and defining products for labelling, quality, and regulatory purposes. The use of Dionex CarboPac columns for many of the HPAE-PAD applications described in official food methods such as those published by the Association of Analytical Communities (AOAC), reinforces Thermo Fisher Scientific's direct involvement in helping the analyst meet the challenge of food regulation compliance.

Sugars and Sugar Alcohols

The applications begin with a separation of a variety of sugars and sugar alcohols followed by examples of carbohydrate separations in coffee, chewing gum, sugar cane molasses, apple juice, and maltodextrins in soda and sports drinks.[5] Each example is supported by a discussion on how resolution was achieved specific to the application. The Dionex CarboPac PA1[6] and Dionex CarboPac MA1 columns are brought to the forefront in these applications. Interest in the close links with AOAC methodology are examined further with the determination of carbohydrates in coffee.[7] Compliance with formulation is illustrated by a great example of the sweetener sucralose found in soft drinks.[8]

Adulteration

Adulteration is a common cause of mistrust for the consumer about food products. We are able to see illustrations of how cheap sugar alternatives are detected in orange juice. Nowadays more exotic commercially sensitive juices such as pomegranate and other high value juices have been adulterated, and the fact that illegal additions of sugar alternatives may be detected illustrates the power of HPAE-PAD as a forensic tool.

Fermentation Monitoring

Process monitoring of carbohydrate profiles during fermentation is described for beer but can equally be applied to cider, wine, and other alcoholic beverages. If we can follow fermentation by monitoring the changes in sugar content, producers can routinely produce beverages with consistency and a quality that removes uncertainty from the process.[9]

Lactose

Some individuals exhibit intolerance to the disaccharides lactose and lactulose in food products. Measurement of these analytes in milk, dairy, or medicinal foods was evaluated[10] using HPAE-PAD and a 4 µm resin particle with performance superior to previous column resin packing formats. Characterization of starch, such as its size and degree of branching, dictates its functional capabilities when used as a thickening agent for liquids or pastes. This is readily determined from various food sources using HPAE-PAD. Likewise, inulin is an important dietary fiber found in smoothies and health foods. A recently developed Dionex CarboPac PA200 column demonstrates improved chain-length resolution while an example that assesses how the degree of polymerization of chicory inulin can be monitored is also presented.

The technology of HPAE-PAD is simple and direct, offering unparalleled sensitivity of detection. Minimal sample preparation is needed and both versatility and flexibility is offered. All the examples using HPAE-PAD can currently be viewed on-line[11] and many of the methods relating to beverages are to be found in the monograph Beverages Applications Notebook.[12]

Questions and Answers

To conclude the presentation, a number of questions were posed which received the following answers.

Q. Ion chromatography columns are commonly prone to metal contamination. Do columns supplied by Thermo Fisher Scientific suffer from this issue as other chromatographic columns do when analyzing for carbohydrates?

A. Systems used for ion chromatography with electrochemical detection such as HPAE-PAD are made of PEEK and are completely inert. The challenge for analysts using conventional high pressure liquid chromatography (HPLC) systems made of stainless steel is that the eluents used for ion exchange separations of carbohydrates – such as strong hydroxide, NaOH and KOH for example – leach metals off the system and deposit them on the column. Thermo Fisher Scientific developed a completely inert separation and column system so strong alkali eluents would not cause leaching of any metals onto the packing material.

Q. How easy is it to prepare the mobile phase for carbohydrate separation with HPAE-PAD?

A. The typical mobile phase used for carbohydrate separations with ion exchange is aqueous sodium or potassium hydroxide. Analysts can prepare these mobile phases in a very straightforward manner. Eluents can be prepared manually by degassing the deionized water and then adding the appropriate amount of hydroxide. Thermo Fisher Scientific has made matters simpler by developing a Reagent-Free™ ion chromatography (RFIC™) where the mobile phase is prepared from deionized water electrolytically. Deionized water is pumped into a module called the Eluent Generator Module. The water is electrolyzed by applying a current to produce hydroxide ions. Potassium counter ions from the cartridge in the module team up with the hydroxide ion to produce ultra pure potassium hydroxide which is used for the separation. The concentration of the mobile phase required is controlled by software which the user simply selects the appropriate concentration and gradient.

Q. Food samples are complex. How rugged is the column chemistry and how would you clean the chromatography columns?

A. A food sample is not simple – it's not like injecting standards. Column packing material is prepared from highly cross-linked polymeric resins that are designed to be resistant to acids and bases, and have excellent solvent compatibility. Contaminating materials that stick to the column packing material can be easily removed by washing the column with strong alkali or acid and solvents too, which solubilize these contaminants. The column is rugged enough to withstand such conventionally harsh cleaning treatment and its lifetime is extended dramatically.

Q. Amperometric detection is very sensitive and certain food samples contain high concentrations of sugars. How do you overcome dilution errors which would affect results? What is your recommendation for a workhorse column for carbohydrate analysis?

A. The majority of methods, including the official Methods, use the Dionex CarboPac PA1 column routinely as the workhorse column. This is a highly robust column for separation that covers a range of sugar classes from monosaccharides and disaccharides to small oligosaccharides.

Some samples contain sugar levels that are so high in concentration they have to be measured in percentage terms. The intention is to avoid using large dilution factors as much as possible. First, the sensitivity of electrochemical detection can be lowered by simply increasing the cell volume. Thermo Fisher Scientific supplies a gasket which can be placed into the cell to increase the volume of the electrochemical detector, and the corresponding electrochemical sensitivity can be lowered.

Second, applying small injection volumes using an injection valve with a small volume internal loop or a smaller sample loop helps in turn to reduce sample sensitivity so large dilutions are not required. In the past, the analyst had to dilute 100- or even 1000-fold when dealing with samples containing high levels of sugars. Now, only a 1 in 10 dilution may be needed, which is effective enough for maintaining the required resolution.

Q. Carbohydrate analysis covers routine measurement of simple monosaccharides to more complex oligosaccharides. How simple is it to do both routine and complex analysis simultaneously on the same system?

A. The Dionex CarboPac PA100 column was designed specifically to address this issue. Simple to complex carbohydrates are resolved on one column which avoids the need for the analyst operating one or two separate methods. It is feasible to cover a range of sugar sizes using a NaOH gradient and the electrochemical detector is compatible with all gradient capabilities. A weak hydroxide elution is applied initially to release small saccharides and sugar alcohols before ramping up the NaOH concentration elution gradient. This permits separation in even the most complex food matrices.

Q. Is this solution easy to use and if so why?

A. The solution is straightforward. It is a direct detection mechanism, so there is no need for a derivatization step for analysts to perform when doing carbohydrate analysis. The development of column chemistries has led to increased resolving power on these highly durable ion exchangers. A simple isocratic separation is all that is needed for most sugar analysis. There are also predefined waveforms for carbohydrates so no waveform development or tuning is required. Advanced column technology coupled with the newly introduced reagent-free chromatography capabilities only requires water to be added to the system making routine analysis very easy to conduct.

Q. How easy is it to couple mass spectrometry to ion chromatography given NaOH elution is used?

A. The typical perception is if NaOH or KOH is used as the mobile phase then it is never coupled to an MS system. However, Thermo Fisher Scientific has developed a hydroxide desalter that replaces the cation of the eluent with a hydronium ion so that highly alkaline eluents can now be converted to water before entering the MS system. The desalter device is placed between the chromatographic column and the MS. The highly ionic eluent is converted in the device to deionized water which has no further impact on the operation of MS. To help with solvation and evaporation of the eluent and achieve better sensitivity within the mass spectrometer, post-column solvent can be added such as acetonitrile or methanol, or an adduct-forming mobile phase such as lithium chloride.

References

1. Cui, S.W. *Food Carbohydrates: Chemistry, Physical Properties, and Applications*. Taylor & Francis CRC; Boca Raton, Florida, USA, 2005. [Online] https://ttngmai.files.wordpress.com/2012/09/foodcarbohydrates.pdf (accessed Sept 29, 2015).
2. Divan, Khalil. *Simplifying Carbohydrate Testing In Food and Beverages to Meet Food Quality and Labelling Requirements Using Ion Chromatography and Pulsed Amperometric Detection;* Webinar; Thermo Fisher Scientific, April 2, 2015. [Online] https://event.on24.com/eventRegistration/EventLobbyServlet?target=registration.jsp&eventid=947701&sessionid=1&key=7B8AED093AE569B4FB545321CBD77D24&partnerref=UBMACI&sourcepage=register (accessed Sept 29, 2015).
3. Rohrer, J. *Analysis of Carbohydrates by High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection* (HPAE-PAD); Technical Note 20; Thermo Fisher Scientific; Sunnyvale, CA, USA; 2013. [Online] http://www.thermoscientific.com/content/dam/tfs/ATG/CMD/CMD%20Documents/Application%20&%20Technical%20Notes/Chromatography/Ion%20Chromatography/IC%20and%20RFIC%20Columns/TN-20-Analysis-Carbohydrates-HPAE-PAD-TN70671-EN.pdf (accessed Sept 29, 2015).

4. Rohrer, J. *Optimal Settings for Pulsed Amperometric Detection of Carbohydrates using the Dionex Electrochemical Detector;* Technical Note 21; Thermo Fisher Scientific; Sunnyvale, CA, USA; 2013. [Online] http://www.thermoscientific.com/content/dam/tfs/ATG/CMD/CMD%20Documents/Application%20&%20Technical%20Notes/Chromatography/Ion%20Chromatography/IC%20and%20RFIC%20Columns/TN-21-Optimal-Settings-Pulsed-Amperometric-Detection-Carbohydrates-ED40-TN70670-EN.pdf (accessed Sept 29, 2015).
5. Thermo Fisher Scientific. *Determination of Sugar Alcohols in Confections and Fruit Juices by High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection;* Application Note 87; Sunnyvale, CA, USA; 2013. [Online] http://www.thermoscientific.com/content/dam/tfs/ATG/CMD/cmd-documents/sci-res/app/chrom/ic/col/AN-87-IC-HPAE-PAD-Sugar-Alcohol-Confections-Fruit-Juice-AN71407-EN.pdf (accessed Sept 29, 2015).
6. Thermo Fisher Scientific, *Thermo Scientific Dionex CarboPac PA1 and PA10 Columns for Mono-and Disaccharide Analysis;* Product Specifications Sheet; Sunnyvale, CA, USA; 2013.
7. Basumallick, L. and Rohrer, J. *Carbohydrate in Coffee: AOAC Method 995.13 vs a New Fast Ion Chromatography Method;* Application Note 280; Thermo Fisher Scientific; Sunnyvale, CA, USA; 2014. [Online] http://www.thermoscientific.com/content/dam/tfs/ATG/CMD/CMD%20Documents/Application%20&%20Technical%20Notes/Chromatography/Liquid%20Chromatography/Liquid%20Chromatography%20Accessories/AN-280-IC-Carbohydrates-Coffee-HPAE-PAD-AN70231-EN.pdf (accessed Sept 29, 2015).
8. *Determination of Sucralose Using HPAE-PAD;* Application Note 159; Thermo Fisher Scientific; Sunnyvale, CA, USA; 2004. [Online] http://www.thermoscientific.com/content/dam/tfs/ATG/CMD/CMD%20Documents/Application%20&%20Technical%20Notes/Chromatography/Ion%20Chromatography/IC%20and%20RFIC%20Accessories/7121-AN159_LPN1574.pdf (accessed Sept 29, 2015).
9. Thermo Fisher Scientific. *Ion Chromatography: A Versatile Technique for the Analysis of Beer;* Application Note 46; Sunnyvale, CA, USA; 2014. [Online] http://www.thermoscientific.com/content/dam/tfs/ATG/CMD/cmd-documents/sci-res/app/chrom/ic/sys/AN-46-IC-Beer-Analysis-AN71410-EN.pdf (accessed Sept 29, 2015).
10. Fisher, C., Christison, T., Yang, H., Verma, M., Lopez, L. *Fast Determination of Lactose and Lactulose in Dairy Products Using a 4 µm Particle Column and High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection;* Poster Note 71016; Thermo Fisher Scientific; Sunnyvale, CA, USA; 2014. [Online] http://www.thermoscientific.com/content/dam/tfs/ATG/CMD/CMD%20Documents/PN-71016-IC-Lactose-Lactulose-Dairy-Products-HPAE-PAD-PN71016-EN.pdf (accessed Sept 29, 2015).
11. Hvizd, M.G. (2015) *Fast, Accurate and Direct Carbohydrate Analysis Using HPAE-PAD.* Presentation at Pittcon Conference and Expo, New Orleans, LA, USA, Mar 8-12, 2015; Thermo Fisher Scientific. [Online] http://www.thermoscientific.com/content/dam/tfs/ATG/CMD/cmd-documents/sci-res/pres/chrom/ic/sys/OT-90051-HPAE-PAD-Carbohydrates-Pittcon2015-OT90051-EN.pdf (accessed Jun 28, 2015).
12. *Beverages Applications Notebook. Sugars in Beverages;* LPN 3089; Thermo Fisher Scientific; Sunnyvale, CA, USA; 2012. [Online] http://www.thermoscientific.com/content/dam/tfs/ATG/CMD/CMD%20DocumentsAN-LPN-3089-Beverages-Applications-Notebooks-Sugars-LPN3089.pdf (accessed Sept 29, 2015).

Thermo Fisher Scientific, San Jose, CA USA is ISO 13485 Certified.

Africa +43 1 333 50 34 0
Australia +61 3 9757 4300
Austria +43 810 282 206
Belgium +32 53 73 42 41
Canada +1 800 530 8447
China 800 810 5118 (free call domestic) 400 650 5118
Denmark +45 70 23 62 60
Europe-Other +43 1 333 50 34 0
Finland +358 10 3292 200
France +33 1 60 92 48 00
Germany +49 6103 408 1014
India +91 22 6742 9494
Italy +39 02 950 591
Japan +81 45 453 9100
Korea +82 2 3420 8600
Latin America +1 561 688 8700
Middle East +43 1 333 50 34 0
Netherlands +31 76 579 55 55
New Zealand +64 9 980 6700
Norway +46 8 556 468 00
Russia/CIS +43 1 333 50 34 0
Singapore +65 6289 1190
Spain +34 914 845 965
Sweden +46 8 556 468 00
Switzerland +41 61 716 77 00
UK +44 1442 233555
USA +1 800 532 4752

WB71806-EN 1115S

Thermo SCIENTIFIC
A Thermo Fisher Scientific Brand