thermo scientific

APPLICATION NOTE 73009

An HPAE-PAD method for determination of saccharides in atmospheric aerosol samples

Authors

Sachin Patil and Jeffrey Rohrer

Thermo Fisher Scientific,
Sunnyvale, CA

Keywords

Anhydro sugars, CarboPac MA1 column, levoglucosan, mannosan, galactosan, wood burning

Goal

To describe a single column method using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) to determine anhydro sugars, simple sugars, and sugar alcohols present in atmospheric aerosol samples

Introduction

Anhydro sugars, sugar alcohols, monosaccharides, and disaccharides are among the major classes of water-soluble organic compounds (WSOC) in atmospheric aerosols.[1,2] Atmospheric saccharides originate from two main sources: biomass burning (natural or anthropogenic) and natural biogenic detritus.[1,3-6] Saccharides have been proposed as markers to evaluate the contribution of these sources to atmospheric aerosols.[2] Anhydro sugars levoglucosan, mannosan, and galactosan originate from the combustion of cellulose and hemicellulose due to wildfires or residential wood burning.[1,2]

High performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) has been used to determine saccharides from atmospheric aerosol samples.[7-12] This technique separates native carbohydrates (i.e., underivatized) at high pH (>12) and detects them by PAD on a gold working electrode. HPAE is capable of separating complex mixtures of carbohydrates, and PAD is a powerful detection technique with a broad linear range and very low detection limits. For complex samples, the high resolving power of HPAE and the specificity of PAD allow the determination of carbohydrates, glycols, sugar alcohols (alditols), and other alcohols such as ethanol and methanol (though with much less sensitivity), with little interference from matrix components.

ThermoFisher SCIENTIFIC

The HPAE-PAD methods for saccharide determination in atmospheric aerosol samples typically use a Thermo Scientific™ Dionex™ CarboPac™ MA1 Analytical & Guard Column[7] for separation of anhydro sugars and sugar alcohols. Some studies have used a Thermo Scientific™ Dionex™ CarboPac™ PA10 Analytical & Guard Column[9] or a Thermo Scientific™ Dionex™ CarboPac™ PA1 Analytical & Guard Column[10] to separate anhydro sugars and monosaccharides. Sometimes, both CarboPac MA1 columns and either CarboPac PA10 or PA1 columns are used together.[8] However, these methods have drawbacks. In some cases, the resolution between key saccharide pairs, for example arabitol and levoglucosan, is poor.[10] A single column method can result in smaller saccharide coverage,[9] whereas using two separate columns in combination makes saccharide analysis cumbersome.[8] To address these concerns and to design a good single column method, we first tested a Thermo Scientific™ Dionex™ CarboPac™ PA20 Column and a Thermo Scientific™ Dionex™ CarboPac™ SA10 Analytical and Guard Column. Both columns are excellent for the separation of simple sugars.[13,14] Unfortunately, both columns yielded poor separation of anhydro sugars from sugar alcohols (unpublished data). This makes either of these columns alone unsuitable for designing a single column method for atmospheric aerosol samples.

This application note describes a single column method using HPAE-PAD to determine anhydro sugars, simple sugars, and sugar alcohols present in atmospheric aerosol samples. Using a Dionex CarboPac MA1 column, 11 saccharides can be resolved in a single 55 min method. Additionally, three sugar alcohols can be resolved by modifying the initial eluent concentration, taking the total number of analytes that can be resolved to 14. The 14 saccharides separated here include major saccharides found in atmospheric aerosols from four classes—anhydro sugars, sugar alcohols, monosaccharides, and disaccharides, as described above. Hence, the method reported here offers significant advantages over other reported methods that use two different columns, one for separating anhydro sugars and another for separating mono and disaccharides.[8] The Dionex CarboPac MA1 column (4 mm) is optimized for weakly ionizable compounds such as sugar alcohols. The resin technology used in the Dionex CarboPac MA1 column makes it an efficient tool for the determination of mono- and disaccharide alditols. Because of its higher capacity, the macroporous Dionex CarboPac MA1 column resolves many carbohydrates that are poorly retained on pellicular anion-exchange columns, successfully separating alditols such as glycerol, arabitol, sorbitol, dulcitol, and mannitol found in food products, physiological fluids, tissues, and reduced glycoconjugate saccharides. Results for method precision, calibration, and accuracy are reported here.

Experimental

Equipment

- A Thermo Scientific™ Dionex™ ICS-5000 Reagent-Free™ Ion Chromatography (RFIC™) System is an integrated ion chromatograph that includes:
  - Thermo Scientific™ Dionex™ ICS-5000 SP Single Pump or Thermo Scientific™ Dionex™ ICS-5000 DP Dual Pumps with degas option
  - Thermo Scientific™ Dionex™ ICS-5000 DC Detector/ Chromatography Compartment with single temperature zone

    Note: This method can also be run on a Thermo Scientific™ Dionex™ ICS-5000⁺ System or Thermo Scientific™ Dionex™ ICS-6000 High Pressure Ion Chromatography (HPIC™) System.

  - Thermo Scientific™ Dionex™ Electrochemical Detector (P/N 072042) and Thermo Scientific™ Dionex™ Electrochemical Detector Cell (P/N 072044)
  - Thermo Scientific™ Dionex™ Electrochemical Detector Ag/AgCl pH Reference Electrode (P/N 061879)
  - Thermo Scientific™ Dionex™ Electrochemical Detector Gold on PTFE Disposable Electrode, pack of six (two 2.0 mil gaskets included) (P/N 066480)
  - 25 µL sample loop
- Thermo Scientific™ Dionex™ AS-AP Autosampler (P/N 074926) with cooling tray option (recommended)
- Thermo Scientific™ Dionex™ AS-AP Autosampler Vial Kit, polypropylene, 0.3 mL, with caps (P/N 055428)
- Thermo Scientific™ Nalgene™ Rapid-Flow™ Sterile Disposable Filter Units with Nylon Membrane, 0.2 µm filter units, 1000 mL, 90 mm diameter (Fisher Scientific, P/N 164-0020)
- Whatman™ Grade GF/A Glass Fiber Filter Paper without

Conditions

Column:	Dionex CarboPac MA1 column 4 × 250 mm (P/N 044046) Dionex CarboPac MA1 guard column 4 × 50 mm (P/N 044067)
Column temp.:	30 °C
Compartment temp.:	25 °C
Flow rate:	0.4 mL/min
Eluents:	A) DI water B) 1M sodium hydroxide
Elution conditions:	Table 1

Table 1. Gradient program for elution

Time (min)	A%	B%	Comment
0	80	20	Initial 200 mM NaOH
5	80	20	Maintain 200 mM NaOH for 5 min
34	30	70	Increase NaOH concentration from 200 to 700 mM NaOH
34.1	20	80	Increase NaOH concentration from 700 to 800 mM NaOH
40	20	80	Maintain NaOH concentration at 800 mM NaOH for 5.9 min
40.1	80	20	Reduce NaOH concentration to 200 mM and maintain for 14.9 min
55	80	20	Stop

Detection:	Pulsed amperometric, gold on PTFE disposable working electrode (P/N 066480) using a 2 mil gasket (P/N 060141)
Typical backpressure:	~1400 psi (Note: The maximum recommended operating pressure for the Dionex CarboPac MA1 column is 2000 psi or 13.79 MPa).
Sampler tray temp.:	4 °C
Inj. volume:	25 µL (full loop mode)

Binder (Whatman, P/N 1820-037)

Reagents

1. Levoglucosan (Toronto Research Chemicals, P/N A168400)
2. Mannosan (Toronto Research Chemicals, P/N A652500)
3. Galactosan (Toronto Research Chemicals, P/N A641000)
4. Arabitol (Sigma-Aldrich, P/N A3381)
5. Erythritol (Pfanstiehl, P/N E100)
6. Xylitol (Sigma-Aldrich, P/N X3375)
7. Sorbitol (Sigma-Aldrich, P/N S1876)
8. Galactitol (Pfanstiehl, P/N RGG100)
9. Mannitol (Sigma-Aldrich, PN M9546)
10. Galactose (Sigma-Aldrich, P/N G0625)
11. Glucose (Fisher, P/N 1910-01)
12. Mannose (Sigma-Aldrich, P/N M6020)
13. Fructose (Sigma-Aldrich P/N F2543)
14. Sucrose (Sigma-Aldrich P/N 84097)
15. 50% w/w sodium hydroxide solution (Fisher P/N SS254-500)

Preparation of solutions and reagents

1. Atmospheric aerosol matrix sample:

Normally, atmospheric aerosol samples are prepared using high volume air samplers. But here, an atmospheric aerosol matrix sample was prepared by using passive diffusion on a 3.7 cm Whatman GF/A filter paper. The filter paper was attached to a vehicle that moved for ~2 h in San Francisco Bay Area traffic. The diffused saccharides were extracted in 10 mL DI water using sonication for 10 min. An untreated control was prepared in the same manner using untreated filter paper.

2. Simulated atmospheric aerosol sample:

Simulated atmospheric aerosol sample was prepared by spiking saccharides into the atmospheric aerosol matrix sample (Table 2).

Table 2. Composition of the simulated atmospheric aerosol sample

Saccharide	Concentration (mg/L)
Erythritol	0.02
Levoglucosan	3.4
Arabitol	0.04
Mannosan	0.6
Mannitol	0.7
Galactosan	0.3
Mannose	0.4
Glucose	0.1
Galactose	0.01

3. Calibration standard solutions

Stock solutions containing 1000 mg/L of each analyte of interest in DI water were prepared in 125 mL polypropylene bottles. The standard stock solutions or secondary standards containing 5 mg/L, or 0.05 mg/L were used directly for making calibration standards used in this study. The calibration standards used here were 0.024, 0.049, 0.098, 0.195, 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25.0, and 50.0 mg/L.

4. 1 M Sodium hydroxide

It is essential to use high-quality water of high resistivity (18 MΩ-cm) as free of dissolved carbon dioxide as possible. It is extremely important to minimize contamination by carbonate, a divalent anion at high pH that binds strongly to the columns, causing a loss of chromatographic resolution and efficiency. Commercially available sodium hydroxide pellets are covered with a thin layer of sodium carbonate and should not be used. A 50% (w/w) sodium hydroxide solution is much lower in carbonate and is the preferred source for sodium hydroxide. Dilute 52.3 mL of 50% (w/w) sodium hydroxide solution into ~850 mL of DI water, and adjust the volume to 1 L to yield 1 M sodium hydroxide. Keep the eluents blanketed under 5 psi of nitrogen at all times. Do not shake the 50% (w/w) NaOH bottle or pipette the required aliquot from the top or bottom of the solution where sodium carbonate may have formed or has settled.[15]

Results and discussion

Separation

Based on literature review,[7,8,10] 14 saccharides, including some that were noted as difficult to resolve, were designated as minimal list for the current study. Our initial studies (not shown) demonstrated that some saccharides are difficult to separate under a single set of conditions. Therefore, a 55 min method (Method A) that uses the gradient program shown in Table 1 was designed. This method enables separation of 11 saccharides commonly observed in atmospheric aerosol samples.[7,8,10] Figure 1 shows a well-resolved separation of an injection containing 12.5 mg/L of each of the 11 saccharides. All the peaks are well resolved as shown by all resolution values above 1.6 in Table 3. This is significant improvement over separations reported in the literature.[8,10] Sucrose is the last saccharide to elute and does so during the wash step. This distorts its peak shape. Sucrose originating from plants and soil biota is sometimes present,[10] and the ability to resolve it makes the method suitable for such samples. If a better sucrose peak shape is required for such samples, the wash step can be delayed.

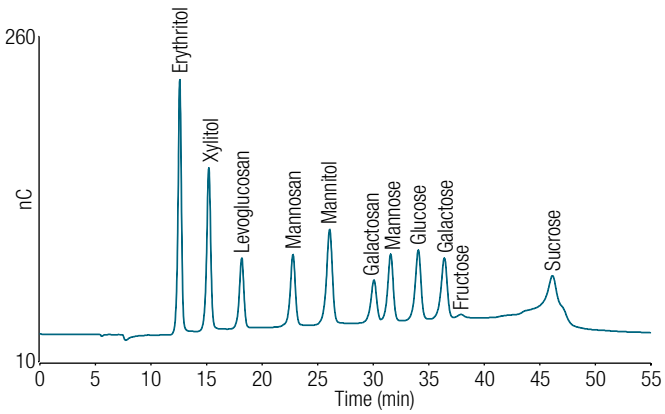

Figure 1. Separation of an 11-saccharide standard using method A

Table 3. Retention time and resolution of 11 analytes

Peak Number	Peak Name	Ret. Time (min)	Resolution
1	Erythritol	12.58	4.42
2	Xylitol	15.18	4.36
3	Levoglucosan	18.15	6.04
4	Mannosan	22.76	3.78
5	Mannitol	26.06	4.27
6	Galactosan	30.04	1.68
7	Mannose	31.56	2.76
8	Glucose	34.04	2.46
9	Galactose	36.38	1.62
10	Fructose	37.89	7.66
11	Sucrose	46.08	n.a.

Additional experiments were performed to demonstrate separation of three other important but poorly resolved saccharides by Method A that are generally present in atmospheric aerosol samples. These are arabitol, galactitol, and sorbitol. Figure 2 shows separation using Method A of a six-saccharide standard that contains these three plus mannosan, levoglucosan, and mannitol. The three alcohol sugars, mannitol, sorbitol, and galactitol, are not resolved using Method A elution conditions. As shown in Figure 3, sorbitol is resolved from mannosan using a method that uses 60 mM initial hydroxide concentration (Method B) as opposed to 200 mM initial hydroxide concentration used in Method A. To create Method B replace the 0, 5, and 55 min time points with A% 94 and B% 6.

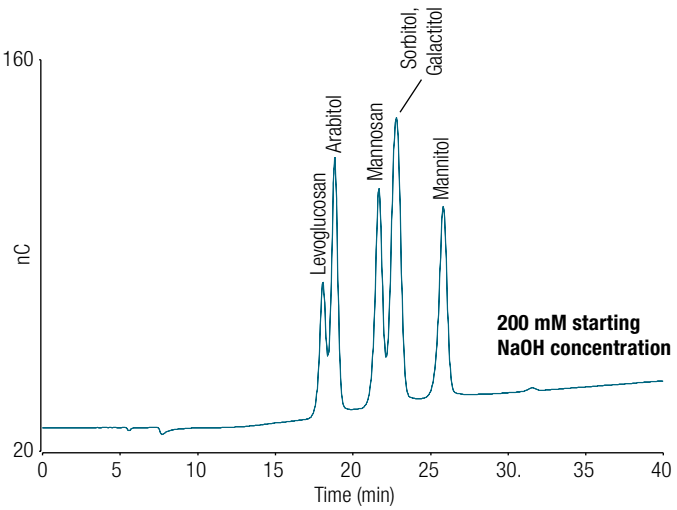

Figure 2. Separation of a 6-saccharide standard using Method A

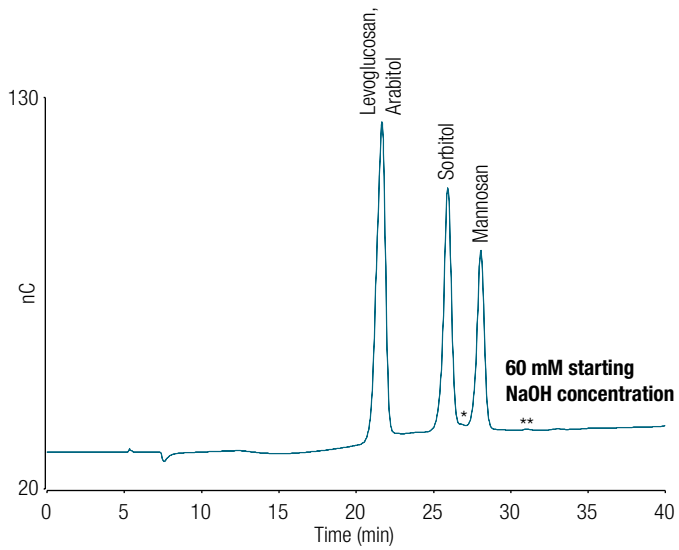

Figure 3. Improvement in separation between sorbitol and mannosan using Method B. (Note: Galactitol and mannitol retention times are indicated by * and **, respectively)

Arabitol is poorly resolved from levoglucosan using the method A elution conditions. Another sugar alcohol, galactitol, is poorly resolved from the sorbitol and mannosan pair, but can be separated using a higher starting hydroxide concentration of 400 mM (Method C, Figure 4). Under these conditions sorbitol and mannosan co-elute.

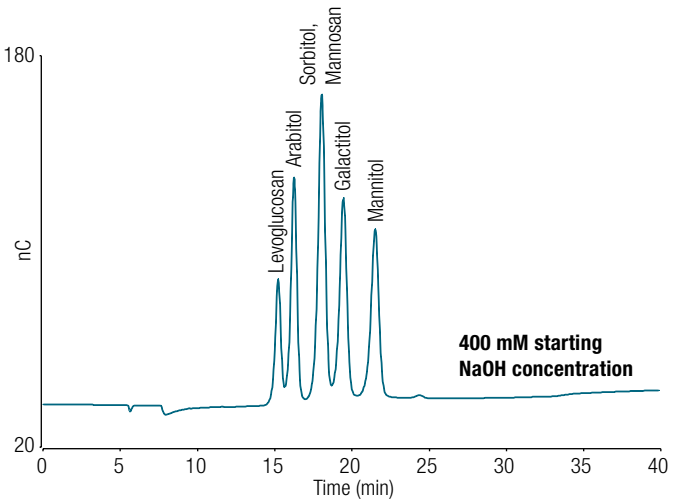

Figure 4. Improvement in separation of arabitol and galactitol using Method C

Finally, a simulated atmospheric aerosol sample was prepared by spiking blank sample matrix prepared previously using passive diffusion. The analytes and their spiked concentrations are as follows: erythritol, 0.02 mg/L; levoglucosan, 3.4 mg/L; arabitol, 0.04 mg/L; mannosan, 0.6 mg/L; mannitol, 0.7 mg/L; mannose, 0.2 mg/L; galactosan, 0.3 mg/L; glucose, 0.1 mg/L; and galactose 0.01 mg/L. Figure 5 shows a chromatogram of the simulated atmospheric aerosol sample.

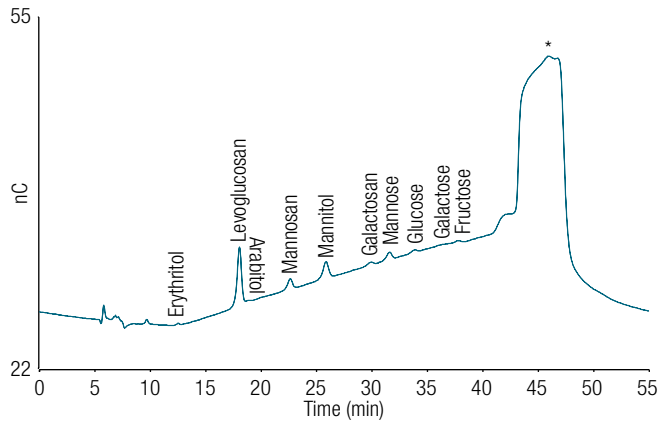

Figure 5. Simulated atmospheric aerosol sample analyzed using Method A described in the text (*sucrose present in the sample matrix)

Precision and linearity

Method validation studies were performed for Method A, starting with method precision. Table 4 contains RT and peak area RSD data for a 6.25 mg/L injection. Both sets of RSDs indicate good method precision.

The method's linear calibration range was determined using twelve concentrations from 0.024 mg/L to 50 mg/L. Table 5 shows coefficient of determinations for all 11 analytes. In most cases, the coefficient of determination value is 0.998 or better, indicating good linearity at the concentrations studied.

Accuracy

A spike recovery experiment using spike levels of 5 and 0.5 mg/L of all 11 sugars in the atmospheric aerosol matrix sample was performed to assess method accuracy. The results in Table 6 show excellent recoveries indicating good method accuracy.

Conclusions

This application note reports a method that can resolve 11 saccharides, which include anhydro sugars, sugar alcohols, and mono- and disaccharides. Unlike methods reported in the literature, the method reported here uses a single column, greatly simplifying determination of these analytes. The method shows good precision, linearity, and accuracy. By modifying elution conditions, three additional sugar alcohols can be determined taking the total analytes that can be determined to 14. This method can be used for routine analysis of atmospheric aerosol samples.

Table 4. Retention time and peak area RSDs for 11 analytes using 6.25 mg/L standard (n=3)

RSD										
Erythritol	Xylitol	Levoglucosan	Mannosan	Mannitol	Galactosan	Mannose	Glucose	Galactose	Fructose	Sucrose
Retention Time										
0.04	0.03	0.04	0.06	0.06	0.06	0.04	0.05	0.07	0.05	0.04
Peak Area										
0.33	0.97	1.83	1.54	0.60	0.80	0.87	0.89	3.61	4.30	0.33

Table 5. Calibration data

Peak Number	Peak Name	Concentration Level (mg/L)	Coefficient of Determination (r^2)
1	Erythritol	0.024–50	1.000
2	Xylitol	0.024–50	1.000
3	Levoglucosan	0.024–50	1.000
4	Mannosan	0.024–50	0.999
5	Mannitol	0.024–50	1.000
6	Galactosan	0.024–50	0.999
7	Mannose	0.024–50	1.000
8	Glucose	0.024–50	1.000
9	Galactose	0.024–50	1.000
10	Fructose	0.098–50	0.999
11	Sucrose	0.024–50	0.998

Table 6. Spike Recovery studies (n=3)

Recovery (%)										
Erythritol	Xylitol	Levoglucosan	Mannosan	Mannitol	Galactosan	Mannose	Glucose	Galactose	Fructose	Sucrose
Spike 0.5 mg/L										
89.8	93.7	89.9	86.1	89.5	82.3	86.9	99.4	92.1	116	122
Spike 5 mg/L										
98.5	99.7	100.5	98.3	99.7	98.0	99.4	100	99.8	103	108

thermo scientific

References

1. Simoneit, B.R.T., Elias, V.O., Kobayashi, M., Kawamura, K., Rushdi, A.I., Medeiros, P.M., Rogge, W.F., Didyk, B.M. Sugars-- dominant water-soluble organic compounds in soils and characterization as tracers in atmospheric particulate matter. *Environ. Sci. Technol.* (38), **2004,** 5939–5949.
2. Yttri, K.E., Dye, C., Kiss, G., Ambient aerosol concentrations of sugars and sugar-alcohols at four different sites in Norway. *Atmos. Chem. Phys.* (7), **2007,** 4267–4279.
3. Medeiros, P.M., Conte, M.H., Weber, J.C., Simoneit, B.R.T., Sugars as source indicators of biogenic organic carbon in aerosols collected above the Howland Experimental Forest, Maine. *Atmos. Environ.* (40), **2006,** 1694–1705.
4. Elbert, W., Taylor, P.E., Andreae, M.O., Poeschl, U. Contribution of fungi to primary biogenic aerosols in the atmosphere: wet and dry discharged spores, carbohydrates, and inorganic ions. *Atmos. Chem. Phys.* 7, 4569–4588.
5. Tominaga, S., Matsumoto, K., Kaneyasu, N., Shigihara, A., Katono, K., Igawa, M. Measurements of particulate sugars at urban and forested suburban sites. *Atmos. Environ.* (45), **2011,** 2335–2339.
6. Fu, P.Q., Kawamura, K., Chen, J., Charriere, B., Sempere, R. Organic molecular composition of marine aerosols over the Arctic Ocean in summer: contributions of primary emission and secondary aerosol formation. *Biogeosciences* (10), **2013,** 653–667.
7. Linuma, Y., Engling, G., Puxbaum, H., Herrmann. H. A highly resolved anion-exchange chromatographic method for determination of saccharidic tracers for biomass combustion and primary bio-particles in atmospheric aerosol. *Atm. Environ.,* **2009,** (43), 1367–1371
8. Barbaro, E., Kirchgeorg, T., Zangrando, R., Vecchiato, M., Piazza, R., Barbante, C., Andrea Gambaro, A. Sugars in Antarctic aerosol, Atm. Environ., (118) 2015, 135-144
9. Engling, G., Carrico, C., Kreidenweis, S., Collett, J., Day, D., Malm, W., Lincoln, E., Hao, W., Iinuma, Y., Herrmann, H. Determination of levoglucosan in biomass combustion aerosol by high-performance anion-exchange chromatography with pulsed amperometric detection, *Atm. Enviro.*, (40), Supplement 2, **2006,** 299–311.
10. Caseiro, A., Marr, I., Claeys, M., Anne Kasper-Giebl, Hans Puxbaum, Casimiro A. Pio. Determination of saccharides in atmospheric aerosol using anion-exchange high-performance liquid chromatography and pulsed-amperometric detection, *J. Chromat. A,* (1171), 1–2, **2007,** 37–45.
11. Zhang, Z., Gao, J., Engling, G.,Tao, J., Chai, F., Zhang, L., Zhang, R., Sang, X., Chan, C., Lin, Z., Cao, J. Characteristics and applications of size-segregated biomass burning tracers in China's Pearl River Delta region. *Atmospheric Environment,* (102), **2015,** 290–301.
12. Yttri, K.E., Schnelle-Kreiss, J., Maenhaut, W., Alves, C., Bossi, R., Bjerke, A., Claeys, M., Dye, C. Evtyugina, M. Gacio, D., Gülcin, A., Hillamo, R., Hoffer, A., Hyder, M., Iinuma, Y., Jaffrezo, J.L. Kasper-Giebl, A. Kiss, G. López-Mahía, P., C. Pio, C. Piot12,16, C. Ramirez-Santa-Cruz, Sciare, J., Teinilä, K., Vermeylen, R., Vicente, A., Zimmermann, R. An intercomparison study of analytical methods used for quantification of levoglucosan in ambient aerosol filter samples. *Atmospheric Measurement Techniques Discussions,* (7), **2014,** 125–147.
13. Thermo Scientific Dionex Application Note 72580. (AN72580) [online] https://assets.thermofisher.com/TFS-Assets/CMD/Application-Notes/an-72580-hpae-pad-glycoprotein-monosaccharide-an72580-en.pdf (Accessed April 26h, 2019).
14. Thermo Scientific Dionex Application Note 72210 (AN72210). Fast determination of biofuel sugars by HPAE-PAD [Online] http://tools.thermofisher.com/content/sfs/brochures/AN-72210-IC-HPAE-PAD-Biofuel-Sugars-AN72210-EN.pdf (Accessed April 26h, 2019).
15. Thermo Scientific Dionex Technical Note 71 (TN71). Eluent Preparation for High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection. [Online] http://tools.thermofisher.com/content/sfs/brochures/TN-71-Eluent-Preparation-for-High-Performance-Anion-Exchange-Chromatogarphy-with%20APD-TN-70669.pdf (Accessed April 25th, 2019).

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