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Simultaneous determination of neutral sugars, alditols and anhydrosugars using anion-exchange chromatography with pulsed amperometric detection: Application for marine and atmospheric samples

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Abstract

An improved high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) method is described for the simultaneous determination of neutral sugars (hexoses, pentoses and deoxysugars), alditols and anhydrosugars commonly found in atmospheric and marine samples. The method uses a CarboPac MA1 column, at a flow rate of 0.3 mL min^{-1}, and a NaOH gradient (250-700 mM). The proposed method applies a temperature gradient (from 25 to 28 °C) to the column for the first 30 min of the analysis, followed by a constant temperature at 28 °C until the end of the analysis. These analytical conditions allowed the separation of 15 out of 17 carbohydrates in 75 min with resolution factors better than 0.5 for the critical pairs levoglucosan/arabitol, galactosan/arabinose, arabinose/mannose and glucose/xylose.

The application of this method to field samples revealed that anhydrosugars represented 53% of total neutral carbohydrates (TCHO) in total suspended atmospheric particles (TSP), whereas they were detected for the first time in marine particulate organic matter (POM) and high-molecular-weight dissolved organic matter (HMWDOM) samples accounting 2% and 3% of TCHO, respectively. Levoglucosan and/or galactosan were the major anhydrosugars in all samples however, their concentrations are undoubtedly underestimated because hydrolysis was applied to the marine samples prior to the HPAEC-PAD analysis. Despite this underestimation, their presence in the marine samples clearly indicates possible terrestrial input most likely via atmospheric deposition as these compounds are considered terrestrial burring biomass tracers. Finally, deoxysugars were also detected for the first time in the TSP sample representing 1% of TCHO, while alditols accounted for 0.4% and 0.3% of TCHO in POM and HMWDOM, respectively.

1. Introduction

Carbohydrates are among the most abundant organic molecules on the earth because they are detected in all marine and terrestrial ecosystems (Giorio et al., 2018; He et al., 2010; Mopper, 1977; Mopper et al., 1980; Panagiotopoulos and Sempéré, 2005a; Repeta et al., 2015; Theodosi et al., 2018). The determination of carbohydrates at the molecular level has been often used to determine different biogeochemical signatures such as terrestrial inputs in coastal marine environments (Cowie and Hedges, 1984; da Cunha et al., 2002; Panagiotopoulos et al., 2014), organic matter sources (He et al., 2010; Panagiotopoulos et al., 2012; Wicks et al., 1991), and decomposition pathways (Amon et al., 2001; Giroldo et al., 2003; Hedges et al., 1994; Opsahl and Benner, 1999).

Previous environmental studies on atmospheric suspended particulate matter ($PM_{2.5–10}$) showed that carbohydrates account for 0.2–3% and 0.7–11% of organic carbon (OC) and water-soluble organic carbon (WSOC), respectively (Theodosi et al., 2018; Tsai et al., 2015; Yang et al., 2005). The dominant carbohydrates reported in aerosols are glucose and sucrose released by terrestrial plants (Medeiros et al., 2006; Samaké et al., 2019), levoglucosan, galactosan and mannosan from biomass burning processes (Bhattarai et al., 2019; Simoneit et al., 2004, 1999), as well as mannitol and arabitol from airborne fungal spores and/or various vascular plants (Bauer et al., 2008; Samaké et al., 2019). Other minor carbohydrates reported in aerosols include some neutral sugars (e.g., arabinose, fructose, galactose, and mannose) and alditols (e.g., xylitol and sorbitol; Barbaro et al. 2015; Theodosi et al. 2018). It is worth noting that fucose and rhamnose, two important deoxysugars consistently found in bacterial heteropolysaccharides (Giroldo et al., 2003), soil organic matter (Bock et al., 2007), freshwater ecosystems (Cheng and Kaplan, 2003) as well as vascular plants (Opsahl and Benner, 1999) have never been reported in atmospheric samples.

Monomeric constituents of carbohydrates after acid hydrolysis (e.g., fucose, rhamnose, arabinose, galactose, glucose, mannose and xylose) have also been detected in the marine environment. As revealed by chromatographic techniques, these monosaccharides account for 10–15%, <10% and <20% in particulate, dissolved and sedimentary organic carbon, respectively (Panagiotopoulos and Sempéré, 2005a and references therein). These monosaccharides have also been reported in marine high-molecular-weight dissolved organic matter (HMWDOM) at equimolar concentrations although ^{1}H nuclear magnetic resonance spectroscopy (NMR) showed the presence of much broader spectra of carbohydrates including unhydrolyzed polysaccharides and methylated sugars (Panagiotopoulos et al., 2007). Other minor carbohydrate classes also reported in marine samples include amino sugars (Benner and Kaiser, 2003; Kaiser and Benner, 2009) and uronic acids (Bergamaschi et al., 1999; Engel and Händel, 2011; Hung et al., 2001).

Surprisingly, alditols and anhydrosugars, two major carbohydrate categories consistently found in aerosols and ice cores (Gambaro et al., 2008; Giorio et al., 2018), have scarcely been reported in marine samples despite the growing evidence that dry or wet atmospheric deposition is an important process between the atmospheric and marine compartments (Pantelaki et al., 2018; Zheng et al., 2018). It is worth noting that only Panagiotopoulos et al. (2013) have identified anhydrosugars (levoglucosan, mannosan, and galactosan) in surface marine HMWDOM, in addition to the seven neutral sugars described above. This finding raises questions about the possible sources of anhydrosugars in marine water (terrestrial *vs* marine), which have been recently examined using compound-specific carbon isotopes analysis (Nouara et al., 2019). Nevertheless, to the best of our knowledge a well-established technique for the measurement of anhydrosugars and alditols does not exist for marine samples. Moreover, the lack of information about deoxysugars in atmospheric samples

hinders our understanding of their dynamics and clearly points to the need for a universal method to measure these carbohydrates in any environmental sample.

High-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) is the most employed chromatographic technique for the analysis of carbohydrates in environmental samples (Benner and Kaiser, 2003; Caseiro et al., 2007; Iinuma et al., 2009; Mopper et al., 1992; Panagiotopoulos and Sempéré, 2005a; Sempéré et al., 2008). However, simple carbohydrates (e.g., monosaccharides) are not ionized in the same manner in alkaline media used in the HPAEC mobile phase, and therefore different concentrations of NaOH must be applied to separate these monosaccharide families (e.g., alditols, neutral sugars, and uronic acids). Due to this "limitation", most environmental applications of the HPAEC-PAD technique have focused on one family of carbohydrates (e.g., neutral sugars, or amino sugars) and few attempts have been made to date to separate a much broader spectrum of carbohydrate categories in the same run (Engel and Händel, 2011).

The main objective of the present study is to establish a universal HPAEC-PAD method capable of simultaneously identifying several carbohydrate families including neutral sugars, alditols and anhydrosugars commonly found in terrestrial and marine ecosystems. This objective was achieved after the evaluation of the performance of two different anion-exchange columns and the optimization of the mobile phase concentration, column temperature and flow rate. The proposed method was validated in three different environmental matrices, namely, total suspended atmospheric particles (TSP), marine particulate organic matter (POM), and marine HMWDOM.

2. Materials and procedures

2.1. Reagents

Carbohydrate standards were purchased from Sigma-Aldrich or Interchim, at their purest available grade (>98%). A mixture of standard solutions at concentrations ranging from 50

nM to 10 µM was prepared by dilution with ultrapure water of a standard stock solution (1 mM) of neutral sugars (fucose, rhamnose, arabinose, galactose, glucose, mannose, xylose, fructose and ribose), alditols (xylitol, arabitol, sorbitol and mannitol), anhydrosugars (levoglucosan, mannosan and galactosan), and one disaccharide (sucrose). All the prepared solutions including the stock solution were stored in the dark at –25 °C until use. Despite that no degradation was observed after melting and refrozen, the standard solutions (50 nM–10 µM) were renewed after 2 weeks. The HCl solution (37%, Sigma-Aldrich) diluted with ultrapure water to 1 M was used for sample hydrolysis, while the NaOH solution (50% *w/v* in H_2O, low carbonate; PanReac AppliChem ITW Reagents) diluted with ultrapure water was used for the mobile phase of the HPAEC-PAD chromatography. The ultrapure water used in this work was produced by a Millipore Milli-Q system (Molsheim, France).

2.2. Sampling and extraction of carbohydrates

2.2.1. TSP

The aerosol sample was collected on a precombusted (450 °C, 6 h) weighed Whatman quartz fiber filter (20.3 cm × 25.4 cm, nominal retention size, 0.7 µm) using an automatic sampler (Tisch Environmental USA; flow rate 85 m^3 h^{-1}). The sample was collected from March 10th to March 17th of 2016, from the rooftop of the Endoume Marine Station (Marseille; 43° 16' N - 5° 21' E). After collection, the sample was dried for 24 h in a desiccator, weighed, and then stored in a freezer at –25 °C in precombusted aluminum foil (450 °C, 6 h). A 1-cm^2 section of the filter was extracted with 6 mL ultrapure water in an ultrasonic bath for 1 h and filtered through a Pasteur pipette packed with quartz wool (both precombusted at 450 °C for 6 h) to remove any remaining particles, and then analyzed immediately (Theodosi et al., 2018). The recovery yields of the extraction procedure were estimated after the extraction of a precombusted (450 ° C, 6 h) Whatman quartz fiber filter

spiked with a standard mixture of neutral carbohydrates spanning a concentration range from 100 to 1000 nM (Theodosi et al., 2018). The obtained yields of neutral sugars for all the concentrations tested were between 101 and 119% (n = 5). The abovementioned analytical procedure allowed the extraction and identification only of the free neutral sugars present in the TSP sample and as such the composition of combined sugars was not determined in this study.

2.2.2. Marine POM

Sinking particles (marine POM) were collected over seven-day periods from January 6th to March 3rd, 2013, in the upwelling system located offshore of Lima (Peru) in the Pacific Ocean (12° 02’ S - 77° 40’ W), using sediment traps (PPS3, Technicap) deployed in the oxycline/upper oxygen minimum zone (OMZ) layer at a depth of 34 m (Bretagnon et al., 2018). To avoid the bio-degradation of POM, a solution of seawater with 5% formaldehyde was added to the bottom of the collection chamber. After trap recovery, the living and dead swimmers were carefully removed so that only detrital particles remained in the sample. These detrital particles (marine POM) were stored in the dark at 4 °C in the initial chambers used in the trap. On land, the samples were filtered through 25 mm precombusted (450 °C, 6 h) Whatman GF/F filters, freeze-dried, and subsequently stored in the dark at 4 °C until further analysis. Five portions (40–60 mg each) of each of the five samples obtained from the respective collection chambers of the sediment trap were pooled together, resulting in ~265 mg dry POM powder. To release neutral sugars, the POM powder was hydrolyzed with 1 M HCl at 100 °C for 20 h (Panagiotopoulos et al., 2014). The acid-soluble fraction recovered after centrifugation (2000 rpm) was then transferred into a precombusted (450 °C, 6 h) glass vial, and the acid was removed from the sample by three successive lyophilizations. The POM recovery (by weight) after acid hydrolysis was 84%. A part of the final dry powder was

weighed (25.61 mg), redissolved in 1 mL ultrapure water and filtered through a Pasteur pipette packed with quartz wool (both precombusted at 450 °C for 6 h). The filtrate was diluted 500 times with ultrapure water and filtered again to remove any remaining particles prior to injection into the chromatographic system.

2.2.3. *Marine HMWDOM*

The surface (15 m) seawater sample was collected from the Hawaii area (19° 43′ N - 156° 03′ W) in December 2003 and processed at Woods Hole Oceanographic Institution (USA) as described by Panagiotopoulos et al. (2013). Briefly, the sample (8640 L) was filtered through 0.8 µm and 0.2 µm duel cartridge filters, and then ultrafiltered using a cross-flow ultrafiltration system. The concentrated sample (40 L) that included the HMWDOM was further desalted by diafiltration with ultrapure water to a final volume of 2 L and freeze dried to obtain a fluffy white powder. The carbon composition of the HMWDOM collected was 35% by weight, with a carbon/nitrogen ratio of 16. A part of the resultant HMWDOM powder (104 mg) was hydrolyzed to release the neutral sugars with 1 M HCl at 100 °C for 20 h, then the hydrolysis was stopped by placing the sample in an ice bath. The HCl was removed from the sample by lyophilization (3 times; final pH = 7). Then, a portion of the sample (55.83 mg) was redissolved in 1 mL ultrapure water, filtered through a Pasteur pipette packed with quartz wool (both precombusted at 450 °C for 6 h) and stored frozen at –20 °C. Before analysis, the sample was diluted 3 times with ultrapure water.

2.3. Chromatographic system

Carbohydrate analysis was performed using a Thermo-Dionex ICS-3000 anion-exchange chromatograph (HPAEC) equipped with a 250-µL injection loop and a pulsed amperometric detector (PAD). The waveform used for pulsed amperometric detection was the standard

quadruple potential for carbohydrate analysis (Panagiotopoulos et al., 2012). The ultrapure water used to prepare the mobile phase was sparged with high purity N_2 for 30 min prior to use. The mobile phase consisted of ultrapure water (eluent A) and NaOH at 1 M (eluent B) prepared by dilution in degassed ultrapure water. The eluents were kept continuously under pure N_2 pressure to avoid exposure to atmospheric CO_2, which can cause a drastic decrease in column selectivity and loss of sugar resolution. In addition, an on-line degasser (RFIC eluent degasser, Thermo Fisher) was added before the analytical column to decrease interference from dissolved oxygen (Cheng and Kaplan, 2001).

In this study, two anion-exchange analytical columns were tested for sugar separation: a CarboPac MA1 (7.5 µm, 4 × 250 mm; Thermo Fisher) and a CarboPac PA1 (10 µm, 4 × 250 mm; Thermo Fisher), both fitted with their corresponding guard column (4 × 50 mm; Thermo Fisher). The Chromeleon software (Thermo Fisher) was used for processing and data acquisition. The performance of the chromatographic separation (resolution factor, R_s) between the two-carbohydrate species A and B is calculated according to the following formula:

$$R_s = 2\,[(t_R)_B - (t_R)_A] \times [W_A + W_B]^{-1}$$

Where $(t_R)_A$ and $(t_R)_B$ are the retention times of the two species A and B, respectively, and W_A and W_B are their corresponding peak widths.

3. Results and discussion

3.1. Method optimization

Although previous investigations described the simultaneous discrimination of some neutral sugars, alditols and anhydrosugars using CarboPac PA1 or CarboPac MA1 columns (Caseiro et al., 2007; Iinuma et al., 2009), the analytical HPAEC-PAD conditions used in previous studies were unable to fully resolve the 17-carbohydrate mixture assessed in the

present study. Application of the analytical protocol using the CarboPac MA1 column in Iinuma et al. (2009) revealed that the critical monosaccharide groups that required a further efficient separation were, by elution order: (a) levoglucosan, arabitol, fucose and rhamnose, (b) mannosan and sorbitol, (c) mannose, arabinose and galactosan, and (d) glucose and xylose. The optimization of the method therefore involved evaluating the effects of basic chromatographic parameters (flow rate, mobile phase composition and column temperature) to achieve optimal separation of the 17-carbohydrate mixture. Charged carbohydrates (e.g., uronic acids and phosphorylated sugars) were not considered in this study because they require different mobile phase conditions compared to neutral carbohydrates using CH_3COONa as the eluent (Engel and Händel, 2011; Hu et al., 2012).

3.1.1. Column selection

Although several types of anion-exchange columns are available for carbohydrate analysis (CarboPac PA10, 20, 100 and 200; Ronkart et al. 2007; Meyer et al. 2008; Raessler et al. 2010; Raessler 2011), the most recommended are: (1) the CarboPac PA1 column for analysis of neutral sugars (Cheng and Kaplan, 2001; Kerhervé et al., 2002; Panagiotopoulos et al., 2012; Panagiotopoulos and Sempéré, 2005a; Theodosi et al., 2018), and (2) the CarboPac MA1 column for analysis of alditols, anhydrosugars and neutral sugars (Iinuma et al., 2009; Jung et al., 2014; Zhang et al., 2013).

The CarboPac PA1 column is well-suited for the analysis of neutral monosaccharides. However, when alditols and anhydrosugars are included in the same run, a NaOH gradient is required because these compounds are eluted at lower NaOH concentrations (<1 mM) compared to neutral monosaccharides (>15 mM). However, the use of a NaOH gradient causes disequilibrium of the baseline signal, which further results in a positive (Sullivan et al.,

2011) or a negative peak (Caseiro et al., 2007; Theodosi et al., 2018) that may potentially mask sugar peaks that are eluted within this time window.

The CarboPac MA1 column was introduced much later than the CarboPac PA1 column and, to date, has never been applied to marine samples. Nevertheless, its macroporous stationary phase has a higher anion-exchange capacity over the CarboPac PA1 column (1450 μeq/column *vs* 100 μeq/column), which further allows the use of gradient conditions without affecting the baseline stability (Iinuma et al., 2009; Zhang et al., 2013). Moreover, because the CarboPac MA1 column operates at high NaOH elution conditions (>200 mM), the pH values of the mobile phase are >12.5 and as such all the hemiacetal groups of sugars are deprotonated. This further increases the retention of sugars on the column and thus results in a better resolution (Thermo Fisher, Technical Note 20). Finally, the higher baseline stability of the CarboPac MA1 column over the CarboPac PA1 column offers an additional advantage in terms of the retention time of chromatographic peaks, which does not significantly shift over successive injections (Gremm and Kaplan, 1997). Due to these reasons, this study was conducted using the CarboPac MA1 column and different chromatographic parameters (flow rate, etc.) were appropriately adjusted for separation of different classes or sugars.

3.1.2. Optimization of flow rate, mobile phase and column temperature

According to the manufacturer, the CarboPac MA1 column operates at a recommended range of flow rates of 0.2–0.5 mL min^{-1}, and the flow rate is generally fixed at 0.4 mL min^{-1} for environmental applications (Iinuma et al., 2009; Jung et al., 2014; Li et al., 2016). Nevertheless, the latter authors did not report co-elution problems because they did not consider fucose, rhamnose, arabinose, sorbitol and xylose in their standards. To overcome the co-elution problem, we first decreased the flow rate to 0.3 mL min^{-1}. This resulted in a slight

improvement in the separation of the above compounds, and therefore this flow rate was maintained for all further improvements.

Previous investigations employing the CarboPac MA1 column used NaOH concentrations ranging from 300 to 800 mM (Andersen and Sørensen, 2000) to elute alditols, anhydrosugars, and some neutral sugars (Iinuma et al., 2009). Nonetheless, such conditions were not optimal for the 17 carbohydrates considered in this study. Therefore, different NaOH gradient tests were performed at a fixed column temperature of 25 °C and a flow rate of 0.3 mL min^{-1}. The improved separation of the first seven eluted monosaccharides (xylitol, levoglucosan, arabitol, fucose, rhamnose, sorbitol and mannosan) was achieved by applying an NaOH gradient ranging from 250 mM to 350 mM in the first 30 min (Fig. 1 a, Table 1). Unfortunately, fucose and rhamnose were not resolved (R_s = 0) under these conditions despite additional adjustments of the NaOH gradient. The 250 to 350 mM gradient was then followed by a 15 min-NaOH gradient increasing from 350 to 450 mM that allowed an acceptable separation (with resolution factors close to or higher than 1) of mannitol, galactosan, arabinose and mannose.

Following the chromatographic separation after 45 min, the NaOH concentration was increased from 450 to 700 mM and held for 55 min to elute glucose, xylose, galactose and fructose. Under these elution conditions, the xylose/galactose and galactose/fructose pairs were fully resolved (R_s > 1.5), while the glucose/xylose pair was partially resolved (R_s = 0.3). Additional tests of decreasing or increasing the final NaOH concentration (700 mM) did not improve the glucose/xylose resolution. Finally, by prolonging the 700 mM NaOH concentration for an additional 20 min, the last two carbohydrates (ribose and sucrose) were eluted with excellent resolution (R_s = 8.1). After 75 min, the column was equilibrated for 20 min with the initial NaOH concentration (250 mM). Regeneration and clean-up of the column

were not necessary, because the high NaOH concentration (700 mM) reached at the end of the analysis was sufficient to restore column performance.

Because pairs of levoglucosan/arabitol, fucose/rhamnose, galactosan/arabinose, arabinose/mannose and glucose/xylose were completely or partially overlapped under the NaOH gradient conditions optimized above, we also explored the effect of temperature on the resolution of these monosaccharide pairs. Earlier investigations have shown that temperature plays an important role in monosaccharide separation using the CarboPac PA1 column and that sub-ambient temperatures strongly affect the resolution of closely eluting monosaccharide pairs, notably fucose/rhamnose and mannose/xylose (Panagiotopoulos et al., 2001). The results of this study indicated that glucose co-eluted with xylose at temperatures <20 °C, while arabinose overlapped with mannose at >30 °C. Moreover, fucose and rhamnose always co-eluted under both these temperature conditions. Overall, these results suggest that sub-ambient temperature conditions do not have a significant impact on the separation of the abovementioned carbohydrate pairs on the CarboPac MA1 column.

By narrowing the temperature range between 25 and 30 °C, we found that these monosaccharide pairs were better resolved (except the fucose/rhamnose pair which again co-eluted), as shown by their corresponding resolution factors (Fig. 2). The results showed that except for the arabinose/mannose ($R_s = 0.4$) and fucose/rhamnose pairs ($R_s = 0$), the resolution factors for the levoglucosan/arabitol ($R_s = 0.7$), galactosan/arabinose ($R_s = 1.3$), and glucose/xylose ($R_s = 0.6$) pairs were significantly improved at a temperature of 28 °C. Therefore, a temperature gradient was applied between 25 and 28 °C for the first 30 min of analysis. The latter temperature was maintained for the next 45 min to distinguish the galactosan/arabinose, arabinose/mannose and glucose/xylose pairs (Table 1, Table A 1; Fig. 1 a). After 75 min, the temperature was reset to 25 °C during column re-equilibration (20 min) until the next injection.

3.2. Linearity, detection limit, precision and blanks

The optimized method showed a linear response for all monosaccharides (R^2 >0.999) over a concentration range of 50 nM to 10 µM, which is typical for environmental monosaccharide concentrations. The detection limits calculated at a signal-to-noise ratio (S/N) of three were 4−51 nM for neutral sugars, 2−15 nM for alditols and 8−32 nM for anhydrosugars (Table A 1), in agreement with previous studies using different elution conditions and anion-exchange columns for monosaccharide determination (Caseiro et al., 2007; Iinuma et al., 2009; Panagiotopoulos et al., 2001; Theodosi et al., 2018). The high detection limit obtained for fructose (51 nM), also observed in a previous study (Iinuma et al., 2009), is probably due to the low detector response toward this compound. The precision of the method was calculated by performing six identical runs with the standard mixture of the 17 carbohydrates (50 nM each). The relative standard deviation (RSD%) ranged from 1.8 to 16.6% for the peak area and from 0.2 to 1.7% for the retention time (Table A 1). The procedural blank for the marine POM consisted of a 25-mm Whatman GF/F filter (precombusted at 450 °C for 6 h), hydrolyzed in the same manner as the samples. Similarly, the procedural blank for the TSP consisted of a Whatman quartz fiber filter (precombusted at 450 °C for 6 h) extracted with the ultrapure water. The results showed no detectable sugars in blanks (Fig. S 1).

3.3. Comparison with the CarboPac PA1 column

The final optimized MA1-HPAEC-PAD method (Table 1, Fig. 1 a) was compared with a different method employing the CarboPac PA1 column (Caseiro et al., 2007; Theodosi et al., 2018). The chromatogram obtained with the latter column (Fig. 1 b) showed that, at low NaOH concentrations (1 mM; 0−15 min), xylitol, sorbitol, mannitol and mannosan peaks were partially resolved, while the arabitol/levoglucosan and galactosan/fucose pairs completely overlapped. Similar observations were made at high NaOH concentrations (19

mM; 15−53 min) for rhamnose/arabinose, mannose/xylose and ribose/sucrose pairs. Based on the above co-elutions, these results clearly suggest that loss of molecular level information may occur when a sample containing all of the abovementioned monosaccharides is analyzed with the CarboPac PA1 column. Moreover, these co-elutions may further induce erroneous quantification of these monosaccharides. Overall, these results indicate that the CarboPac MA1 column is more appropriate than the CarboPac PA1 column for environmental applications in terms of monosaccharide resolution and baseline stability.

3.4. Application to environmental samples

The method established in this study was tested on three different environmental matrices including TSP, marine POM and HMWDOM. The chromatograms of these matrices are presented in Fig. 3 and indicated the presence of large quantities of monosaccharides (Table 2).

3.4.1. TSP

The major carbohydrate classes identified in the TSP sample included anhydrosugars and disaccharides, followed by neutral sugars and alditols. The sum of their concentration was 563 ng m^{-3}, representing the total neutral carbohydrate (TCHO) content recorded in the TSP sample. The results of this study showed that levoglucosan (261 ng m^{-3}) and sucrose (178 ng m^{-3}) were among the major neutral carbohydrates (Table 2; Fig. 3 a), which is in agreement with other studies of European urban atmospheres (Yttri et al., 2007). Anhydrosugars represented 53% of TCHO in the TSP sample and were dominated by levoglucosan and mannosan, which accounted for 87% and 10% of the anhydrosugar pool, respectively (Fig. 4 a). Galactosan was also detected in the TSP sample but in lower concentrations (Table 2).

Neutral sugars accounted for 11% of the TCHO pool (Fig. 4 a) with fructose and glucose exhibiting the highest concentrations (Fig. 4 a, Table 2). The presence of these neutral sugars in the TSP sample strongly supports the influence of biogenic emission, which is typical of plants growth during spring (Fu et al., 2012; Medeiros et al., 2006). Other minor neutral sugars found in the TSP sample included the arabinose, mannose and xylose (Table 2). Finally, alditols represented the least abundant carbohydrate class, accounting for 4% of TCHO in the TSP sample (Fig. 4 a). The alditols decreased in abundance in the order of xylitol, mannitol, arabitol and sorbitol (Table 2), and their presence in the sample indicates inputs from fungal spores and/or microbial activities (Bauer et al., 2008; Dahlman et al., 2003; Loos et al., 1994).

Although fucose and rhamnose co-eluted under the current HPAEC-PAD conditions, they were also identified and quantified in the aerosol sample for the first time and accounted for 1% of TCHO and 8% of neutral sugars. Fucose and rhamnose may have multiple sources in atmospheric particles including soil organic matter (Gunina and Kuzyakov, 2015; Ogner, 1980; Simoneit et al., 2004), vascular plants (Bianchi, 2007; Popper et al., 2004; Schädel et al., 2010), microorganisms and bacteria (Cowie and Hedges, 1984; Fox et al., 1993; Petit et al., 2013), while recent studies indicated that marine sources via water/air exchange may also be an important contributor (Rastelli et al., 2017).

Nevertheless, field measurements using gas chromatography (Alves et al., 2011) or nuclear magnetic resonance spectroscopy (Chalbot et al., 2013) identified only fucose or rhamnose but never simultaneously reported both sugars in atmospheric samples. On the other hand, studies using HPAEC-PAD have never reported fucose and rhamnose in atmospheric particles, probably because of the co-elution of these deoxysugars with other monosaccharides (fucose with galactosan and rhamnose with arabinose after analysis with the

CarboPac PA1 column; Fig. 1 b). Therefore, the presence of fucose and rhamnose in the sample has never been thoroughly assessed to date.

The lack of data on deoxysugars in atmospheric samples may also be related to their original structure. As deoxysugars are mostly found in bacterial lipopolysaccharides (Perry et al., 1996; Weckesser et al., 1970; Zdorovenko et al., 2007) and/or plant structural polysaccharides (Colombini et al., 2002; Grössl et al., 2005; Popper et al., 2004; Schädel et al., 2010), they are either not efficiently recovered after water extraction (case of lipopolysaccharides) or are recovered in the water phase but cannot be released from carbohydrate polymer (case of structural polysaccharides) as no hydrolysis is applied.

3.4.2. Marine POM

The major carbohydrate classes identified in the marine POM sample included the neutral sugars followed by anhydrosugars and alditols. The sum of their concentration was 16.2 mg g^{-1}. Neutral sugars accounted for 98% of the TCHO pool (Fig. 4 b) and were dominated by galactose (5.7 mg g^{-1}) and glucose (3.8 mg g^{-1}) (Table 2; Fig. 3 b). These monosaccharides were followed by fructose (2.0 mg g^{-1}) and fucose/rhamnose (co-eluted; 1.8 mg g^{-1}) while xylose, mannose and arabinose exhibited concentrations <1 mg g^{-1}. These results are in good agreement with previously reported monosaccharide patterns for marine POM in several oceanic regimes (Hernes et al., 1996; Panagiotopoulos and Sempéré, 2005b; Skoog and Benner, 1997). Fructose and ribose, occasionally reported in marine POM samples due to their low recovery after acid hydrolysis (Borch and Kirchman, 1997; Panagiotopoulos and Sempéré, 2005a), were also identified in this sample (Table 2). This finding most likely indicates the presence of storage polysaccharides (fructans) and nonstructural labile compounds such as RNA or nucleotides originating from marine organisms (Cowie and Hedges, 1984; Haug and Mykelstad, 1976; Hicks et al., 1994; Panagiotopoulos and Sempéré, 2005b). Alternatively, fructose may originate from sucrose. As sucrose was not detected in

the marine POM sample (the same holds for the HMWDOM sample), this most likely implies its complete hydrolysis under the acid conditions used for its extraction, which results in two monosaccharides (fructose and glucose).

The proposed analytical method allowed the identification and quantification of alditols (0.4% of TCHO) and anhydrosugars (2% of TCHO), the two monosaccharide families that have been largely overlooked to date in marine environmental studies (Fig. 4 b). The results showed that xylitol was the major alditol type detected in the POM sample, followed by sorbitol, while arabitol and mannitol were not detected in the sample (Table 2). Alditols are generally reported as terrestrial tracers derived from fungal spores and/or microbial activities (Dahlman et al., 2003; Vandeska et al., 1995), therefore, their presence in the marine POM sample may suggest possible terrestrial input (van Pinxteren et al., 2012). Alternatively, alditols may have marine origin. As carbohydrates are an important fraction of the organic matter produced by marine primary production or released into the environment via degradation processes, it is highly possible that alditols are also part of the carbohydrate pool. Nevertheless, additional measurements using compound-specific ^{13}C analysis are warranted to test this hypothesis and constrain the sources of alditols in the marine waters.

Compositionally, anhydrosugars were dominated by galactosan followed by levoglucosan and mannosan, which represented for 63%, 25% and 13% of total anhydrosugars, respectively (Fig. 4 b). The dominance of galactosan over levoglucosan has also been reported for sea surface microlayer samples in Baltic sea (van Pinxteren et al., 2012) during different seasons of the year however, more data are warranted in the same time from both atmospheric and marine compartments to validate/confirm these findings.

However, the anhydrosugar concentrations found in this sample (and in the HMWDOM sample) are most likely underestimated because these compounds are converted to their respective hexoses after acid hydrolysis (Blanco et al., 2018). Indeed, in an additional set of

experiments to investigate whether the hydrolysis conditions affect the stability of monosaccharides, we performed acid hydrolysis (1 M HCl, 100 °C, 20 h) on a standard monosaccharide mixture containing neutral sugars, alditols and anhydrosugars. The results showed that levoglucosan, mannosan and galactosan were completely converted to glucose, mannose and galactose, respectively, while minimal loss was observed for neutral sugars and alditols (Fig. S 2), which is consistent with previous studies (Skoog and Benner, 1997; Wang et al., 2016).

Overall, these results revealed that the concentration of anhydrosugars is biased in environmental samples if these sugars are acid-extracted (POM and HMWDOM sample). Therefore, an alternative extraction method should be considered (e.g., extraction with water or MeOH). Despite the possible underestimation of the amount of the anhydrosugars found in the marine POM, their presence in this sample clearly indicates an external input, most likely via atmospheric deposition, as shown by the isotopic signature ($\delta^{13}C$) of levoglucosan and mannosan pointing to a terrestrial origin (Nouara et al., 2019).

3.4.3. Marine HMWDOM

The major carbohydrate classes identified in the HMWDOM sample included the neutral sugars followed by anhydrosugars and alditols. The sum of their concentration was 766 µg g^{-1}. Neutral sugars accounted for 97% of the TCHO pool in the HMWDOM (Fig. 4 c). The results of this study revealed that fucose & rhamnose (300 µg g^{-1}), glucose (122 µg g^{-1}), mannose (103 µg g^{-1}), galactose (83 µg g^{-1}), xylose (74 µg g^{-1}), and arabinose (34 µg g^{-1}) were among the major monosaccharides, which agrees with the neutral sugar pattern reported in previous studies (Aluwihare et al., 1997; Repeta et al., 2002) (Table 2; Fig. 3c). Fructose and ribose were also quantified in the HMWDOM sample, and similar to POM, they most likely originate from storage polysaccharide or RNA.

Anhydrosugars accounted for 3% of TCHO and were dominated by levoglucosan, galactosan and mannosan, which represented 61%, 22% and 17% of total anhydrosugars, respectively (Fig. 4 c). The lower concentration of anhydrosugars in the HMWDOM sample (the same holds for the POM sample) compared to the TSP sample maybe due to the high dilution occurring at sea and also to photodegradation processes by OH^* radicals which take place in the atmosphere for these compounds (Hoffmann et al., 2010; Lai et al., 2014). In addition, as suggested for marine POM, the concentration of anhydrosugars in HMWDOM is most likely biased due to the acid extraction procedure. The presence of the anhydrosugars in the sample may indicate possible input of terrestrial organic matter (burning biomass tracers), although additional studies using $\delta^{13}C$ are warranted to confirm this hypothesis. Finally, the least abundant carbohydrate class was the alditols, which accounted 0.3% of TCHO and represented by mannitol and xylitol (Fig. 4 c).

4. Conclusions

The results of this study indicated that under the optimized analytical conditions, neutral sugars, alditols, and anhydrosugars commonly found in most environmental samples can be analyzed with a single run in 75 min with satisfactory resolution except for the fucose/rhamnose pair that completely co-eluted. These deoxysugars were identified for the first time in the TSP sample by this improved HPAEC-PAD technique, which will facilitate future investigations by shedding light on bacterial and/or microorganism activities on atmospheric samples. However, additional deconvolution of these deoxysugars signals using the traditional HPAEC-PAD method for neutral sugar determination (Panagiotopoulos et al., 2001) might also be useful.

The proposed improved HPAEC-PAD method allowed, for the first time, the identification and quantification of anhydrosugars and alditols in marine samples (marine POM and

HMWDOM). These two carbohydrate families have largely been overlooked in the dissolved free monosaccharide pool (DFMS), and, to the best of our knowledge, they have never been reported in marine DOM. Indeed, anhydrosugars and alditols can easily be extracted from seawater using cation and anion-exchange resins (Kirchman et al., 2001; Mopper et al., 1992; Sempéré et al., 2008; Skoog and Benner, 1997; van Pinxteren et al., 2012) in the same manner as neutral sugars. However, because the subsequent PA1-HPAEC-PAD analysis has always been performed at >15 mM NaOH to elute the neutral sugars; anhydrosugars and alditols always co-elute at time window of 2–5 min, therefore, they have never been identified in the DFMS pool.

On the other hand, dissolved combined monosaccharides (DCHO) or polysaccharides in marine samples are generally measured as their monomeric constituents that are released after acid hydrolysis. Thus, if anhydrosugars are present in the sample, they will be counted in the hexose pool as the sample is hydrolyzed. Therefore, we recommend that DCHO analysis always be preceded or accompanied by the analysis of DFMS to evaluate the contribution of anhydrosugars – if present – in the sample. This strategy is particularly critical for samples from coastal areas that receive significant terrestrial inputs, particularly in wintertime, and are characterized by strong influence from wood burning.

The proposed HPAEC method provides an acceptable resolution for most of the carbohydrates examined, and therefore its coupling with mass spectrometry may further help with the structural elucidation and confirmation of the analyzed carbohydrates. Moreover, its high potential may further be explored in other environmental matrices, including sediments, seawater, and marine biota, which will provide a more complete profile of carbohydrates in the environment. This complete profile, in turn, may be helpful to improve our understanding on carbohydrate dynamics in the marine and terrestrial ecosystems and potentially better evaluate the impact of biomass burning processes on marine waters.

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Table and Figure captions

Table 1: Optimal analytical conditions for the simultaneous analysis of neutral sugars, alditols, anhydrosugars and sucrose

Table 2. Carbohydrate composition in atmospheric TSP, marine POM and marine HMWDOM samples. The carbohydrate concentrations presented in this table are not corrected for losses during the hydrolysis or the extraction procedure (see materials and methods), and therefore they should be considered as minimum concentrations found in the samples.

Fig. 1. HPAEC-PAD chromatograms of a standard mixture of 17 monosaccharides (1 µM each) obtained with the (a) CarboPac MA1 column after optimization according to Table 1 (Peak identification: 1 = xylitol, 2 = levoglucosan, 3 = arabitol, 4 = fucose/ rhamnose, 5 = sorbitol, 6 = mannosan, 7 = mannitol, 8 = galactosan, 9 = arabinose, 10 = mannose, 11 = glucose, 12 = xylose, 13 = galactose, 14 = fructose, 15 = ribose, 16 = sucrose) and (b) CarboPac PA1 column (Peak identification: 1 = xylitol, 2 = arabitol/levoglucosan, 3 = sorbitol, 4 = mannitol, 5 = mannosan, 6 = galactosan, 7 = fucose, 8 = rhamnose/arabinose, 9 = galactose, 10 = glucose, 11 = mannose/xylose, 12 = fructose, 13 = ribose/sucrose). The dotted line and secondary axis indicate the NaOH gradient. The analytical HPAEC-PAD conditions for the CarboPac PA1 column are as follows: flow rate 0.7 mL min^{-1}, 0−15 min : 1 mM NaOH; 15−38 min : 19 mM NaOH (curve 5), column temperature 17 °C and detector temperature 20 °C (Theodosi et al., 2018).

Fig. 2. The resolution of levoglucosan/arabitol (♦), fucose/rhamnose (×), galactosan/arabinose (■); arabinose/mannose (▲), and glucose/xylose (●) pairs with fixed temperatures (25, 28 and 30 °C) applied during the whole HPAEC-PAD run under the optimized mobile phase and flow rate conditions.

Fig. 3. HPAEC-PAD chromatograms according to Table 1 of (a) total suspended atmospheric particles (TSP), (b) marine particulate organic matter (POM) and (c) marine high-molecular-weight dissolved organic matter (HMWDOM). Peak identification: 1 = xylitol, 2 = levoglucosan, 3 = arabitol, 4 = fucose/rhamnose, 5 = sorbitol, 6 = mannosan, 7 = mannitol, 8 = galactosan, 9 = arabinose, 10 = mannose, 11 = glucose, 12 = xylose, 13 = galactose, 14 = fructose, 15 = ribose, 16 = sucrose.

Fig. 4. Relative abundances (%) of neutral sugars, alditols, anhydrosugars and disaccharides within the TCHO pool for (a) total suspended atmospheric particles (TSP), (b) marine particulate organic matter (POM) and (c) marine high-molecular-weight dissolved organic matter (HMWDOM). The relative abundances (%) of levoglucosan, galactosan and mannosan within the anhydrosugar pool are given as well for the abovementioned environmental samples.

Table 1

Optimal analytical conditions for the simultaneous analysis of neutral sugars, alditols, anhydrosugars and sucrose.

Parameters	Conditions	
Column	CarboPac MA1 (250 × 4 mm I.D.), Thermo Fisher	
Eluent flow rate (mL min^{-1})	0.3	
Detector temperature (°C)	25	
Sample loop size (µL)	250	
Injection volume (µL)	230	
Column oven temperature (°C)	25–28	(0–30 min); curve 5
	28	(30–75 min)
	25	(75–95 min)
NaOH gradient (mM)	250–350	(0–30 min) ; curve 5
	350–450	(30–45 min) ; curve 5
	450–700	(45–55 min) ; curve 5
	700	(55–75 min)
	250	(75–95 min)

Table 2

Carbohydrate composition in atmospheric TSP, marine POM and marine HMWDOM samples. The carbohydrate concentrations presented in this table are not corrected for losses during the hydrolysis or the extraction procedure (see materials and methods), and therefore they should be considered as minimum concentrations found in the samples.

Carbohydrate	TSP (ng m^{-3})	POM (mg g^{-1})	HMWDOM (µg g^{-1})
Xylitol	11	0.05	0.13*
Levoglucosan	261	0.08	12
Arabitol	3.4	ND	ND
Fucose & Rhamnose	5.0	1.8	300
Sorbitol	1.9	0.02	ND
Mannosan	30	0.04	3.4
Mannitol	7.8	ND	2.3
Galactosan	9.4	0.2	4.4
Arabinose	2.1	0.1	34
Mannose	1.2	0.8	103
Glucose	15	3.8	122
Xylose	1.2	0.9	74
Galactose	ND	5.7	83
Fructose	36	2.0	11
Ribose	ND	0.7	19
Sucrose	178	ND	ND

ND = Not detected

* Xylitol concentration was outside the calibration range (50-1000 nM) but ~6 times higher than its detection limit (Table A 1), thus it was quantified with an uncertainty of ~24%.

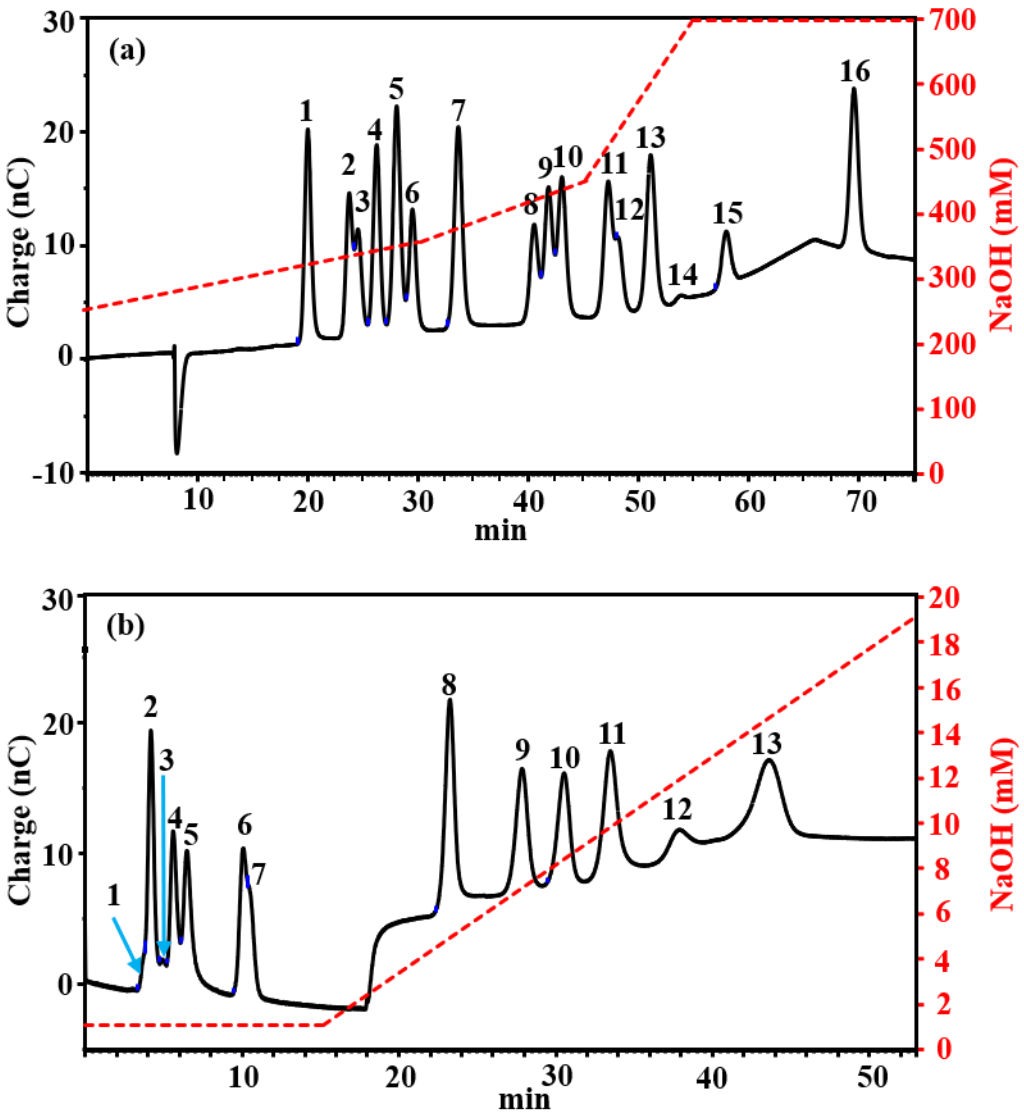
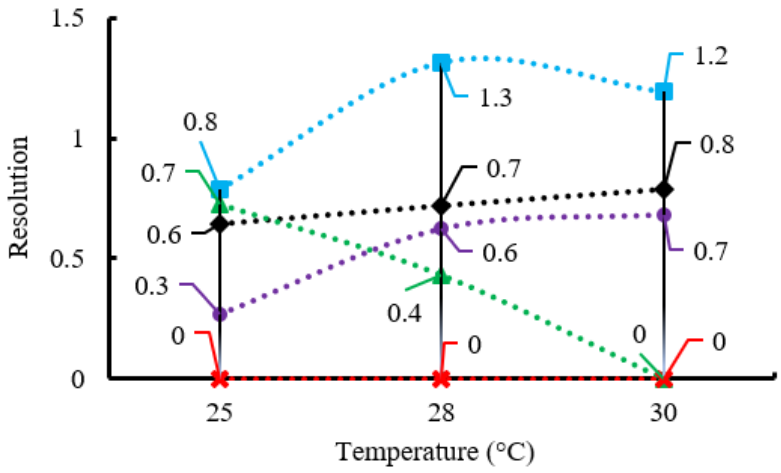


FIGURE 1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0.5
0
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0.7
0.6
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0
1.3
0.7
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1.2
0.8
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0
0
25
28
30
Temperature (°C)

FIGURE 2

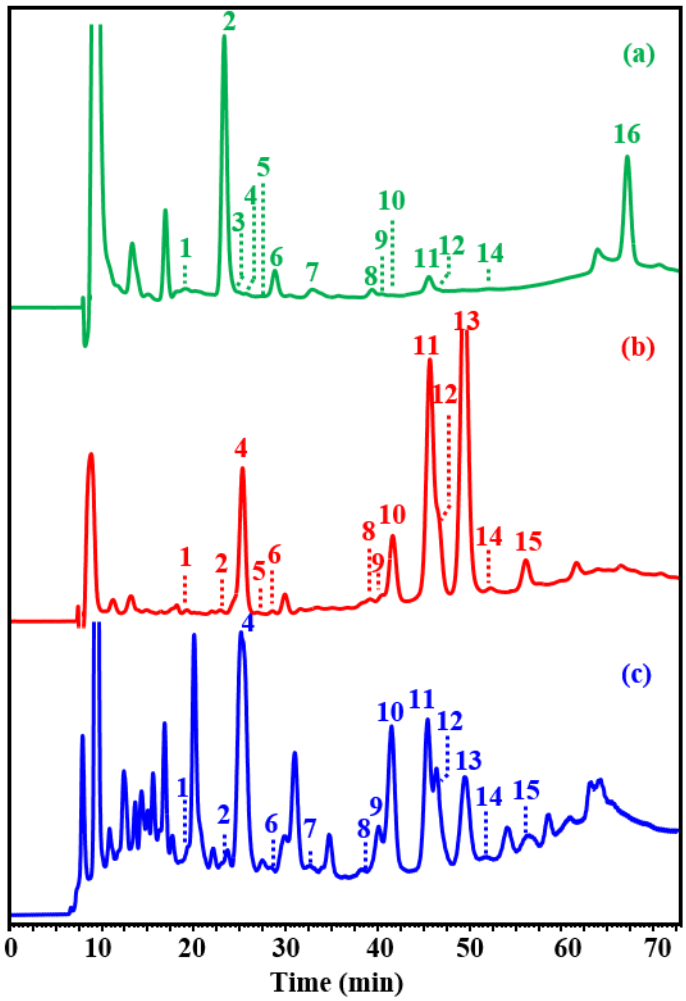

FIGURE 3

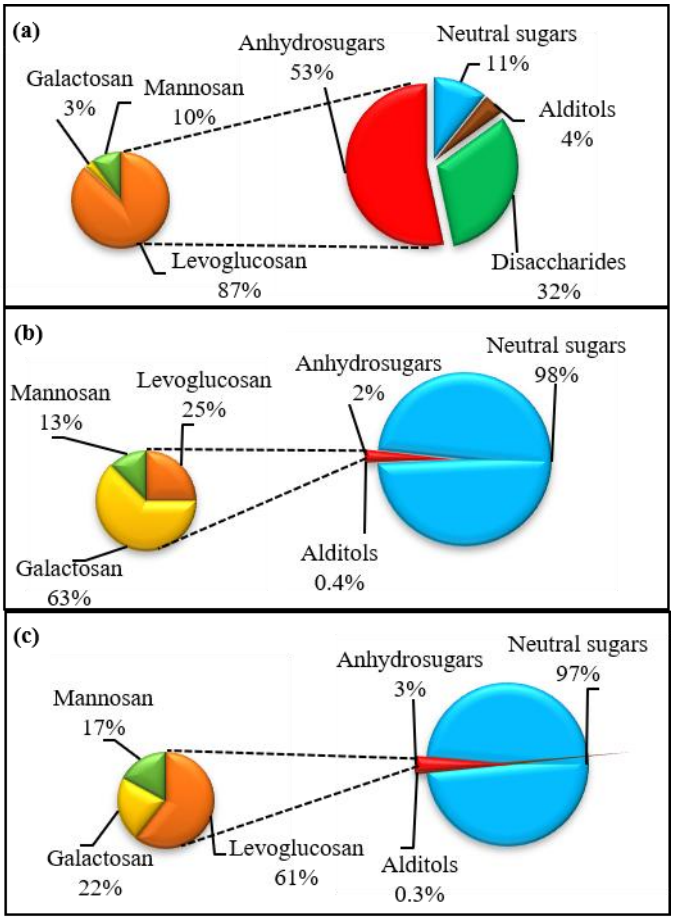
(a)
Anhydrosugars
53%
Neutral sugars
11%
Galactosan
3%
Mannosan
10%
Alditols
4%
Levoglucosan
87%
Disaccharides
32%
(b)
Neutral sugars
98%
Anhydrosugars
2%
Mannosan
13%
Levoglucosan
25%
Galactosan
63%
Alditols
0.4%
(c)
Anhydrosugars
3%
Neutral sugars
97%
Mannosan
17%
Galactosan
22%
Levoglucosan
61%
Alditols
0.3%

FIGURE 4

Supplemental Information for

Simultaneous determination of neutral sugars, alditols, and anhydrosugars using anion-exchange chromatography with pulsed amperometric detection: Application for marine and atmospheric samples

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09th May 2019

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Table S 1. HPAEC-PAD performance after optimization (Table 1). The average retention time (t_R), and resolution factor (R_s) of the carbohydrate standards is given at 1 µM level (*n* = 3). The relative standard deviations (RSD) of peak area and retention time of the same standards is calculated at 50 nM level (*n* = 6), except for fructose (1 µM level; *n* = 6). The detection limit (DL) was calculated at signal to noise ratio of 3.....45

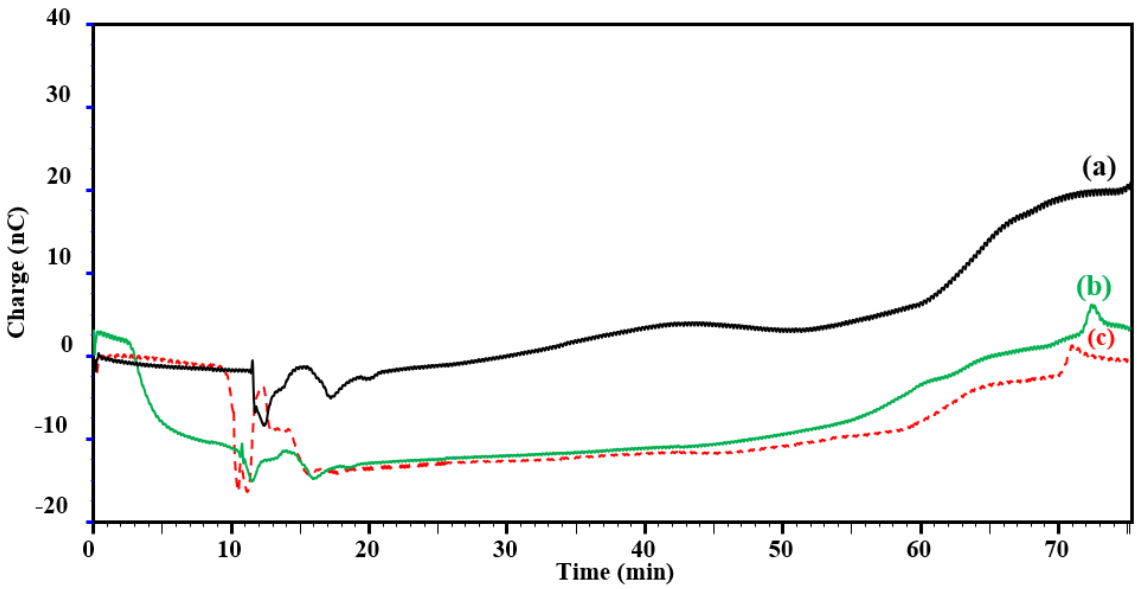
Fig S 1. Example of HPAEC-PAD chromatograms of procedural blanks (a) ultrapure water (b) TSP blank and (c) POM blank. Blanks were prepared in triplicate for each sample (TSP and marine POM) and run according to the method established in Table 1……..46

Fig S 2. High-performance liquid chromatography with refractive index detection (HPLC-RI) chromatograms of a monosaccharide mixture (1 mM each) before hydrolysis (black solid line) and after hydrolysis (red dotted line) with 1 M HCl under 100 °C for 20 h. Peak identification : 1 = glucose, 2 = xylose, 3 = galactose & rhamnose, 4 = galactosan, 5 = arabinose & fucose, 6 = mannose, 7 = fructose, 8 = mannitol, 9 = levoglucosan, 10 = xylitol, 11 = sorbitol, 12 = mannosan. The analytical HPLC-RI conditions are given in Nouara et al. (2019) and are as follows: cation exchange column in Pb^{2+} form, isocratic elution with ultrapure water at 0.6 mL min^{-1}, column temperature 75 °C. ……..47

Table S 1.

HPAEC-PAD performance after optimization (Table 1). The average retention time (t_R), and resolution factor (R_s) of the carbohydrate standards is given at 1 µM level (*n* = 3). The relative standard deviations (RSD) of peak area and retention time of the same standards is calculated at 50 nM level (*n* = 6), except for fructose (1 µM level; *n* = 6). The detection limit (DL) was calculated at signal to noise ratio of 3.

Carbohydrate	**t_R (min)**	**R_s**	**Area RSD (%)**	**t_R RSD (%)**	**DL (nM)**
Xylitol	19.9	3.1	5.6	0.2	2
Levoglucosan	23.8	0.6	4.0	0.5	8
Arabitol	24.4	1.3	6.7	0.4	13
Fucose & Rhamnose	26.0	1.3	6.5	0.3	17
Sorbitol	27.8	1.2	5.0	0.3	15
Mannosan	29.3	3.2	1.9	0.3	18
Mannitol	33.3	5.1	2.7	0.4	7
Galactosan	40.2	0.8	9.3	0.4	32
Arabinose	41.4	0.7	4.9	0.4	7
Mannose	42.6	2.4	1.8	0.3	6
Glucose	46.7	0.5	4.4	0.3	9
Xylose	47.7	1.6	6.7	0.3	24
Galactose	50.5	1.7	5.2	0.6	4
Fructose	53.1	3.0	16.6	1.7	51
Ribose	57.2	8.1	6.0	0.4	13
Sucrose	68.9	-	5.8	0.7	13



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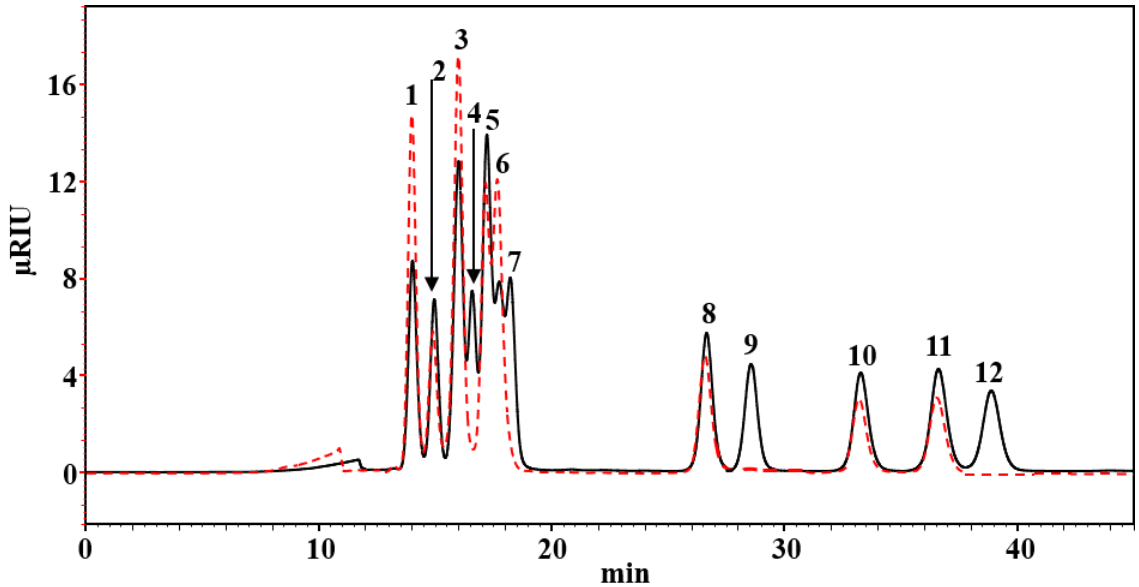

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