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## Liquid chromatographic isolation of individual carbohydrates from environmental matrices for stable carbon analysis and radiocarbon dating

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1 **Liquid chromatographic isolation of individual carbohydrates from**  
2 **environmental matrices for stable carbon analysis and radiocarbon**  
3 **dating**

4  
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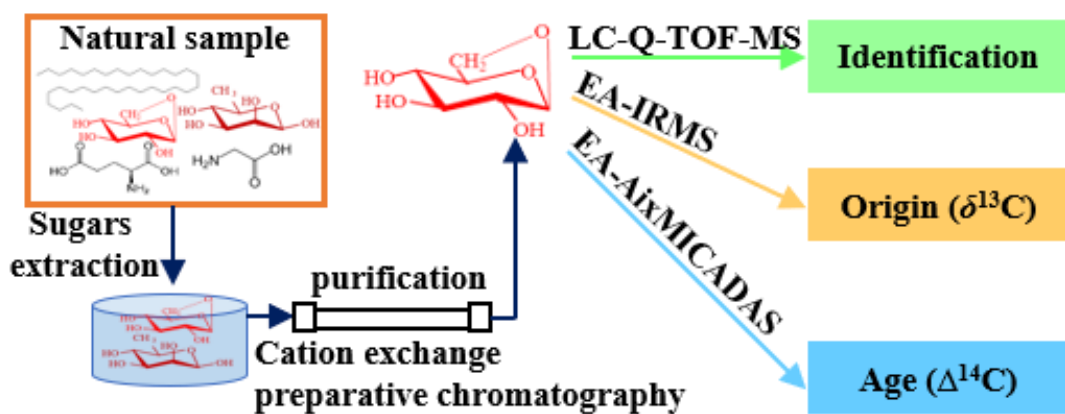
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23

## 24 Abstract

25 Carbohydrates are among the most abundant organic molecules in both aquatic and  
 26 terrestrial ecosystems; however, very few studies have addressed their isotopic signature  
 27 using compound-specific isotope analysis, which provides additional information on  
 28 their origin ( $\delta^{13}\text{C}$ ) and fate ( $\Delta^{14}\text{C}$ ). In this study, semi-preparative liquid  
 29 chromatography with refractive index detection (HPLC-RI) was employed to produce  
 30 pure carbohydrate targets for subsequent offline  $\delta^{13}\text{C}$  and  $\Delta^{14}\text{C}$  isotopic analysis.  $\delta^{13}\text{C}$   
 31 analysis was performed by elemental analyzer-isotope ratio mass spectrometer  
 32 (EA-IRMS) whereas  $\Delta^{14}\text{C}$  analysis was performed by an innovative measurement  
 33 procedure based on the direct combustion of the isolated fractions using an elemental  
 34 analyzer coupled to the gas source of a mini carbon dating system (AixMICADAS). In  
 35 general, four successive purifications with  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Pb}^{2+}$ , and  $\text{Ca}^{2+}$  cation-exchange  
 36 columns were sufficient to produce pure carbohydrates. These carbohydrates were  
 37 subsequently identified using mass spectrometry by comparing their mass spectra with  
 38 those of authentic standards.

39 The applicability of the proposed method was tested on two different environmental  
 40 samples comprising marine particulate organic matter (POM) and total suspended  
 41 atmospheric particles (TSP). The obtained results revealed that for the marine POM

42 sample, the  $\delta^{13}\text{C}$  values of the individual carbohydrates ranged from  $-18.5$  to  $-16.8\text{‰}$ ,  
43 except for levoglucosan and mannosan, which presented values of  $-27.2$  and  $-26.2\text{‰}$ ,  
44 respectively. For the TSP sample, the  $\delta^{13}\text{C}$  values ranged from  $-26.4$  to  $-25.0\text{‰}$ . The  
45 galactose and glucose  $\Delta^{14}\text{C}$  values were  $19$  and  $43\text{‰}$ , respectively, for the POM sample.  
46 On the other hand, the levoglucosan radiocarbon value was  $33\text{‰}$  for the TSP sample.  
47 These results suggest that these carbohydrates exhibit a modern age in both of these  
48 samples. Radiocarbon HPLC collection window blanks, measured after the addition of  
49 phthalic acid ( $^{14}\text{C}$  free blank), ranged from  $-988$  to  $-986\text{‰}$  for the abovementioned  
50 compounds, indicating a very small background isotopic influence from the whole  
51 purification procedure. Overall, the proposed method does not require derivatization  
52 steps, produces extremely low blanks, and may be applied to different types of  
53 environmental samples.

54

55

56 **Keywords:** Semi-preparative liquid chromatography; carbohydrates purification;  
57 carbohydrate-specific  $^{13}\text{C}$  and  $^{14}\text{C}$  analysis; EA-IRMS; EA-AixMICADAS

58

## 59 **1. Introduction**

60 Carbohydrates are among the most ubiquitous organic molecules and have been  
61 recorded in all geochemical systems, including terrestrial [1,2], marine [3,4], and  
62 atmospheric organic matter [5,6]. Although previous investigations have provided a  
63 wealth of information on their concentrations and distributions in all geochemical  
64 systems [7–11], very less is known about their sources and fate, which have not been  
65 thoroughly studied using carbon isotopes. Such information, obtained from carbon

66 isotope examination at the molecular level, may help trace the origin of the different  
67 components of organic matter and explain its reactivity during long-range transport  
68 [12].

69 Bulk carbon isotope analysis generally reflects the average of the isotopic  
70 composition of the whole panel of organic molecules inside the sample [13–18]. Further  
71 extraction of the sample with acids or organic solvents produces “purified” fractions  
72 (e.g. sugar- or lipid-like fractions) and aids in the determination of the isotopic  
73 composition of the hydrophilic and hydrophobic components of the sample [18–20]. For  
74 example, previous studies on dissolved organic matter (DOM) have reported  $\delta^{13}\text{C}$   
75 values for carbohydrate-like fractions in the ranges  $-29$  to  $-25\%$  in river estuaries [21]  
76 and  $-21.5$  to  $-20.3\%$  in the Atlantic and Pacific Oceans [22]. These  $\delta^{13}\text{C}$  values are  
77 typical for terrestrial and marine ecosystems, respectively. Moreover, the reported  $\Delta^{14}\text{C}$   
78 values for carbohydrate-like fractions in marine high molecular weight DOM showed a  
79 wide range of values spanning from  $7$  to  $-406\%$ , further implying that the age of  
80 carbohydrates spans from modern to very old (4180 yr BP) [22]. However, the bulk  
81 isotope analysis approach does not completely address the isotopic diversity of the  
82 individual molecules inside the sample.

83 In contrast with bulk isotope analysis, the compound-specific isotope analysis  
84 (CSIA) of the individual sugars offers valuable information on the origin ( $\delta^{13}\text{C}$ ) and  
85 age ( $\Delta^{14}\text{C}$ ) of the single molecules [23–27]. The CSIA technique is not a new approach;  
86 however, it requires high analytical skills for the purification and extraction of  
87 individual molecules from the sample. This step is crucial and might strongly affect the  
88 results. The two most commonly employed techniques for the stable carbon isotope  
89 analysis of carbohydrates are gas and liquid chromatography coupled with isotope ratio  
90 mass spectrometry (GC-IRMS and LC-IRMS, respectively). Since carbohydrates are

91 not volatile, derivatization steps (silylation or alditol acetate derivatization) are required  
92 for GC-IRMS analysis. This further necessitates carbon corrections on the carbohydrate  
93 isotopic signatures [23,28–30]. Another disadvantage of the GC-IRMS technique is that  
94 two different monosaccharides (e.g. glucose and fructose) can produce the same alditol  
95 (e.g. glucitol) during the reduction step of the derivatization procedure, thereby causing  
96 a loss of compositional information [31].

97 LC-IRMS is a good alternative to GC-IRMS because it does not require any  
98 derivatization steps [30,32–34]. However, this technique does not target all the sugar  
99 components of the sample; for example, neutral sugars, amino sugars, alditols, and  
100 anhydrosugars cannot be separated in a single run [35,36]. Moreover, both the GC-  
101 IRMS and LC-IRMS techniques cannot be used for  $\Delta^{14}\text{C}$  determination on single  
102 carbohydrates due to their low sensitivity toward the  $^{14}\text{C}$  isotope. To date, very few  
103 radiocarbon data exist on single carbohydrates comprising neutral sugars, alditols, and  
104 anhydrosugars. Alditols and anhydrosugars are important tracers of terrestrial  
105 vegetation [37,38] and burning biomass processes [39], respectively. Thus, their  
106 isotopic study may help to evaluate their long-range transport from land to sea and more  
107 importantly, to assess their reactivity in long time scales (fate) in the marine  
108 environment. Although a significant amount of work was done on the compound-  
109 specific radiocarbon analysis of individual lipids over the past 20 years [40–43] and  
110 more recently, on amino acids [44], a well-established technique for single carbohydrate  
111 purification from environmental samples for subsequent radiocarbon measurements has  
112 not been reported to date.

113 An interesting approach to produce pure carbohydrate targets for  $\delta^{13}\text{C}$  and  $\Delta^{14}\text{C}$   
114 analysis is the use of semi-preparative high-performance liquid chromatography with  
115 refractive index detection (HPLC-RI) on cation-exchange columns. Pure carbohydrates

116 can be analyzed by an elemental analyzer–isotope ratio mass spectrometer (EA-IRMS)  
117 for  $\delta^{13}\text{C}$  and/or an accelerated mass spectrometer for  $\Delta^{14}\text{C}$  analysis. HPLC-RI is a  
118 simple and well-established method for carbohydrate analysis; however, in  
119 environmental studies, it has been less explored [27,45] than traditional GC-MS or  
120 high-performance anion-exchange chromatography with pulsed amperometric detection  
121 (HPAEC-PAD) techniques [5,8,10,11,31,46,47].

122 This study employs HPLC-RI to demonstrate that four successive purifications on  
123  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Pb}^{2+}$ , and  $\text{Ca}^{2+}$  cation-exchange columns are sufficient to produce pure  
124 carbohydrates for compound-specific carbon isotope determination. In previous  
125 investigations, the isolated fractions were measured after combustion in evacuated  
126 quartz tubes and the produced  $\text{CO}_2$  was purified on vacuum lines. This was followed by  
127 graphitization for solid measurements or transfer in glass ampoules for gas  
128 measurements. On the other hand, this study employs an alternative procedure [48]  
129 based on the direct combustion of isolated fractions using an elemental analyzer  
130 coupled to the gas source of a mini carbon dating system (AixMICADAS) for  $\Delta^{14}\text{C}$   
131 analysis. Compared to the time-consuming vacuum line method, this procedure is  
132 relatively fast and can be adapted for sample masses  $\leq 10 \mu\text{g C}$  with moderate precision.  
133 The applicability of the method was tested on two different environmental samples,  
134 namely marine particulate organic matter (POM) and total suspended atmospheric  
135 particles (TSP).

## 136 **2. Materials and Methods**

### 137 *2.1. Chemicals and reagents*

138 All the carbohydrate standards used in this study were purchased from  
139 Sigma-Aldrich or Interchim at the purest available grade (>98%). The standard stock

140 solutions of the individual (1 mM) and a mixture of 14 monosaccharides (glucose,  
141 galactose, mannose, fucose, rhamnose, arabinose, xylose, fructose, xylitol, sorbitol,  
142 mannitol, levoglucosan, mannosan, and galactosan, 1 mM and 50  $\mu$ M each) were  
143 prepared by dilution with ultrapure water. The prepared solutions were stored in the  
144 dark at  $-15\text{ }^{\circ}\text{C}$  until use. HCl (37%, Sigma-Aldrich), diluted with ultrapure water (final  
145 concentration of 1 M), was used for sample hydrolysis. The ultrapure water used in this  
146 work was produced by a Millipore Milli-Q system (Molsheim, France).

## 147 *2.2. Sampling and carbohydrate extraction*

### 148 *2.2.1. Marine POM*

149 Sinking particles (marine POM) were collected over seven-day periods from January  
150 6th to March 3th, 2013, in the upwelling system located offshore Lima (Peru) in the  
151 Pacific Ocean ( $12^{\circ} 02' \text{ S} - 77^{\circ} 40' \text{ W}$ ), using sediment traps (PPS3, Technicap)  
152 deployed in the oxycline/upper oxygen minimum zone (OMZ) layer at a depth of 34 m  
153 [49]. To avoid POM bio-degradation, a solution of seawater with 5% formaldehyde was  
154 added to the bottom of the collection chamber. After trap recovery, the living and dead  
155 swimmers were carefully removed so that only detrital particles remained in the sample.  
156 These detrital particles (marine POM) were stored in the dark at  $4\text{ }^{\circ}\text{C}$  in the initial  
157 chambers used in the PPS3. On land, the samples were filtered through 25 mm  
158 pre-combusted ( $450\text{ }^{\circ}\text{C}$ , 6 h) Whatman GF/F filters (nominal retention size,  $0.7\text{ }\mu\text{m}$ ),  
159 freeze-dried, and subsequently stored in the dark at  $4\text{ }^{\circ}\text{C}$  until further analysis. The  
160 organic carbon (OC) content of the particles was in the range 20–29%. To obtain  
161 sufficient material for isotopic analysis, five portions (40–60 mg each) of each of the  
162 five samples obtained from the respective collection chambers of the sediment trap were  
163 pooled together. This resulted in  $\sim 265$  mg dry POM powder which was hydrolyzed with  
164 1 M HCl at  $100\text{ }^{\circ}\text{C}$  for 20 h [50]. The acid-soluble fraction recovered after



165 centrifugation (2000 rpm) was then transferred into a pre-combusted (450 °C, 6 h) glass  
166 vial and the acid was removed from the sample by three successive lyophilizations. The  
167 afforded dry powder was weighed (25.61 mg), redissolved in 1 mL ultrapure water and  
168 filtered through a Pasteur pipette packed with quartz wool (both pre-combusted at 450  
169 °C for 6 h) to remove any remaining particles prior to chromatographic injection.

### 170 2.2.2. TSP

171 The aerosol sample was collected on a pre-combusted (450 °C, 6 h) weighed  
172 Whatman quartz fiber filter (20.3 cm × 25.4 cm) using an automatic sampler (Tisch  
173 Environmental USA; flow rate 85 m<sup>3</sup> h<sup>-1</sup>). The sample was collected from the 10th to  
174 17th March, 2016, from the rooftop of the Endoume marine station (Marseille; 43° 16'  
175 N - 5° 21' E). After collection, the sample was dried for 24 h in a desiccator, weighed,  
176 and then stored in a freezer at -25 °C in pre-combusted aluminum foil (450 °C, 6 h).  
177 Three portions (17.34 cm<sup>2</sup> each) of the filter were extracted with 18 mL ultrapure water  
178 in an ultrasonic bath for 1 h and then filtered through a Pasteur pipette packed with  
179 quartz wool (both pre-combusted at 450 °C for 6 h) to remove any remaining particles  
180 [5]. Finally, the sample was freeze-dried and stored in a freezer at -35 °C until  
181 chromatographic injection.

### 182 2.3. Chromatography

183 The carbohydrates were analyzed using an HPLC system (Thermo Scientific  
184 UltiMate 3000) equipped with a vacuum degasser and a 100-μL loop auto-injector. The  
185 carbohydrates were detected with a refractive index detector (Shodex RI-101) and  
186 eluted in isocratic mode with ultrapure water, which was previously degassed with high  
187 purity N<sub>2</sub> for 30 min.

188 Three cation-exchange analytical columns packed with a polymeric resin (sulfonated  
189 polystyrene-divinyl benzene) were used to purify the carbohydrates (Table S.1). The

190 first column ( $\text{Na}^+$ : 4% cross-linked  $\text{Na}^+$ ;  $200 \times 10$  mm,  $12 \mu\text{m}$ ; REZEX™  
191 RNO-Oligosaccharide; Phenomenex) was used to separate the oligosaccharides from  
192 the monosaccharides after acid hydrolysis. The column temperature was set at  $85 \text{ }^\circ\text{C}$   
193 and the carbohydrates were eluted with ultrapure water at a flow rate of  $0.3 \text{ mL min}^{-1}$ .  
194 The second column ( $\text{Ca}^{2+}$ : 8% cross-linked  $\text{Ca}^{2+}$ ;  $300 \times 7.8$  mm,  $9 \mu\text{m}$ ; REZEX™  
195 RCM-Monosaccharide; Phenomenex) was used to separate the neutral sugars from  
196 sugar alcohols and anhydrosugars. The column temperature was maintained at  $85 \text{ }^\circ\text{C}$   
197 throughout the analysis at a flow rate of  $0.6 \text{ mL min}^{-1}$ . The third column ( $\text{Pb}^{2+}$ : 8%  
198 cross-linked resin  $\text{Pb}^{2+}$ ;  $300 \times 7.8$  mm,  $8 \mu\text{m}$ ; REZEX™ RPM-Monosaccharide;  
199 Phenomenex) was used to further separate the monosaccharides at  $75 \text{ }^\circ\text{C}$  and a flow rate  
200 of  $0.6 \text{ mL min}^{-1}$ . The injected samples never exceeded  $10 \text{ mg/injection}$  and the columns  
201 were cleaned at the end of each day by washing overnight with ultrapure water at a flow  
202 rate of  $0.1 \text{ mL min}^{-1}$ . The carbohydrate fractions and/or individual monosaccharides  
203 were collected by an automatic Foxy R1 fraction collector (Teledyne ISCO, USA)  
204 placed after the RI detector. The system was controlled via Chromeleon  
205 chromatography software (ThermoFisher).

206 The detection limit of the HPLC-RI system was  $\sim 1 \mu\text{M}$  at a signal-to-noise ratio  
207 (S/N) of three for all the carbohydrates on the three tested columns; this was in  
208 agreement with previous results reported in the literature [51]. The precision of the  
209 method was evaluated by calculating the relative standard deviation (RSD%) for six  
210 replicate HPLC-RI injections of the standard mixture of the 14 carbohydrates at the  $5$   
211  $\mu\text{M}$  level. The RSD was  $<10\%$  for the peak area and  $<1\%$  for the retention time for all  
212 the columns tested. Additional details on the system optimization are included in the  
213 supplementary information (section S1 and Fig. S1).

#### 214 *2.4. Isotopic measurements*

#### 215 2.4.1. EA-IRMS

216 Prior to EA-IRMS processing, the samples were acidified with HCl (final  
217 concentration, 0.01 M) to avoid any errors related to the isotopic signature of inorganic  
218 carbon mainly from atmospheric CO<sub>2</sub> absorbed in the sample [52]. The samples were  
219 placed in a tin capsule (5 mm × 9 mm; light; Santis) and dried under a N<sub>2</sub> stream. The  
220 stable carbon isotope composition and the carbon content of the purified carbohydrates  
221 were measured using an elemental analyzer (Flash EA 1500; Thermo Finnigan,  
222 Germany) coupled with an isotope ratio mass spectrometer (IRMS Delta<sup>plus</sup>, Thermo  
223 Finnigan, Germany). Briefly, this technique measures the <sup>13</sup>C/<sup>12</sup>C ratio of total carbon of  
224 the dried sample as follows: in a continuous helium flow, the sample is oxidized at 1000  
225 °C in the presence of O<sub>2</sub> and catalysts; the resulting CO<sub>2</sub> is separated from the other  
226 combustion products and transferred by the helium flow to a gas source, magnetic  
227 sector, triple collector mass spectrometer. The latter determines the <sup>13</sup>C/<sup>12</sup>C ratio of  
228 CO<sub>2</sub>-carbon. The stable carbon isotope composition is conventionally expressed as δ<sup>13</sup>C  
229 values according to the formula:

$$\delta^{13}\text{C} \text{ ‰} = \left[ \frac{(^{13}\text{C}/^{12}\text{C})_{\text{Sample}}}{(^{13}\text{C}/^{12}\text{C})_{\text{VPDB}}} - 1 \right] \times 1000$$

230 where VPDB is the Vienna Pee Dee Belemnite standard.

231 International Atomic Energy Agency (IAEA)-CH-7 polyethylene (δ<sup>13</sup>C = -32.2‰)  
232 and IAEA-CH-6 sucrose were used as the calibration standard and control, respectively  
233 [53]. The latter yielded a mean value of -10.5‰. The δ<sup>13</sup>C and carbon content of the  
234 samples were corrected for the contribution of carbon in the tin capsules. The δ<sup>13</sup>C  
235 standard deviations were determined from replicated measurements of the IAEA-CH-6  
236 standard and were ± 0.2‰, ± 0.2‰, ± 0.4‰, and ± 0.7‰ for samples containing 20, 10,

237 5, and 2  $\mu\text{g C}$ , respectively. The precision on the carbon content (precision on the  
238 absolute amount of carbon analyzed) was  $\pm 0.1 \mu\text{g C}$ .

#### 239 2.4.2. EA-AixMICADAS

240 Direct radiocarbon measurement of the  $\text{CO}_2$  gas was carried out, after combustion,  
241 using an elemental analyzer (EA) coupled to the gas interface system (GIS) of the  
242 AixMICADAS system [48]. Briefly, the main characteristic of the EA is that it works  
243 with a combustion tube filled with tungsten oxide heated to  $1050 \text{ }^\circ\text{C}$  to allow the  
244 introduction of silver boats containing the sample material. The purified extracts were  
245 recovered in ultrapure water without acidification, transferred into silver capsules (4  
246  $\text{mm} \times 8 \text{ mm}$ , Elemental Microanalysis Ltd), and dried at  $80 \text{ }^\circ\text{C}$  on a hotplate under a  $\text{N}_2$   
247 stream. The silver capsules were baked at  $800 \text{ }^\circ\text{C}$  for 3 h prior to use to eliminate any  
248 organic contamination [54,55]. The  $\text{CO}_2$  produced by the EA is captured in the zeolite  
249 trap inside the GIS. The  $\text{CO}_2$  is then released by heating the zeolite trap to  $450 \text{ }^\circ\text{C}$  and is  
250 mixed inside the syringe with a helium flow in order to obtain 5%  $\text{CO}_2$  in the gas  
251 mixture, which is sputtered into the ion source. The  $\text{CO}_2$  is injected from the GIS into  
252 the ion source through a small fused silica capillary continuously fed by the syringe,  
253 which is driven by a stepping motor controlled by the GIS software. A carbon flow of  
254  $2.80 \mu\text{g C min}^{-1}$  keeps the total pressure constant inside the syringe (filled to 1300  
255 mbar), allowing the ion source to produce stable currents. The tuning procedure and  
256 main operation parameters used in the gas configurations are described in the literature  
257 [48]. The measurements were normalized with the oxalic acid 2 standard ( $\sim 100 \mu\text{g C}$ ;  
258 OxA2 SRM 4990 C, National Institute of Standards and Technology) and corrected for  
259 blanks using phthalic anhydride acid ( $F^{14}\text{C} = 0.0027$ ;  $n = 3$ ) prepared from the same  
260 protocol as that used for OxA2 (i.e. silver capsules measured by EA-GIS).

261 The samples were blank corrected with procedural blanks of the same size (section  
262 3.3) and a conservative uncertainty of 30% of the blank value was propagated in the  
263 final error calculation. The samples were in the size range 50–150  $\mu\text{g C}$ , which  
264 translates to a precision of  $\sim 1\%$  for a modern sample. However, samples down to 10  $\mu\text{g}$   
265 C could be measured with less precision. The accuracy of  $^{14}\text{C}$  measurements of small  
266 samples ( $< 100 \mu\text{g C}$ ) with the gas ion source of AixMICADAS has been tested with  
267 numerous measurements on various standards (NIST 4990C, IAEA-C1, IAEA-C2, in-  
268 house carbonate standards) [54,55]. For example, the analysis of oxalic acid NIST  
269 4990C was replicated 132 times over 2.5 years, giving an average  $F^{14}\text{C}$  of 1.3403 and a  
270 standard deviation (SD) of 0.0078 (i.e. 6%). This arithmetic mean and its associated  
271 error (std error = 0.0007) are compatible with the weighted mean (1.3405) and the  
272 weighted error (0.0008), and closely agree with the NIST reference value of  $1.3407 \pm$   
273 0.0005.

274 The  $^{14}\text{C}$  analyses were reported as  $\Delta^{14}\text{C}$  corrected for decay [56,57] according to the  
275 formula:

$$\Delta^{14}\text{C} \text{ ‰ (corrected for decay)} = \left( \frac{A_{SN} e^{\lambda_C (1950 - x)}}{A_{ON}} - 1 \right) \times 1000$$

276 where  $A_{SN}$  is the normalized sample activity,  $A_{ON}$  is the normalized standard activity,  
277  $x$  is the year of formation or growth, and  $\lambda_C = (1/8267) \text{ yr}^{-1}$ .

### 278 2.5. Identification of purified monosaccharides

279 The identification of the isolated purified monosaccharides was checked with Liquid  
280 Chromatography coupled with Time-of-Flight Mass Spectrometry (LC-Q-TOF-MS)  
281 Agilent 6500 system. The chromatographic separation was performed with a Luna  
282 HILIC column (100 mm  $\times$  2.00 mm I.D., 3  $\mu\text{m}$  particle size; Phenomenex). The mobile  
283 phase was ultrapure water with 13 mM  $\text{CH}_3\text{COONH}_4$  (A) and acetonitrile (LC-MS

284 grade) with 13 mM CH<sub>3</sub>COONH<sub>4</sub> (B) and sugars were eluted isocratically (20% A and  
285 80% B). The flow rate and the column temperature were set at 200 μL min<sup>-1</sup> and 25 °C,  
286 respectively for the whole run. Monosaccharides were injected without prior  
287 derivatization and ionized in the ESI positive mode. The main ion source parameters  
288 were optimized as follows: source temperature 350 °C, sheath gas temperature 350 °C,  
289 gas flow 11 L min<sup>-1</sup>, and drying gas at 8 L min<sup>-1</sup>. The MS scan was 50 – 1700 m/z, and  
290 the scan rate was 1 spectra s<sup>-1</sup>. The capillary voltage and the nozzle voltage were set at  
291 4000 V and 500 V, respectively. Samples and standards were diluted in acetonitrile  
292 before injection.

### 293 **3. Results and discussion**

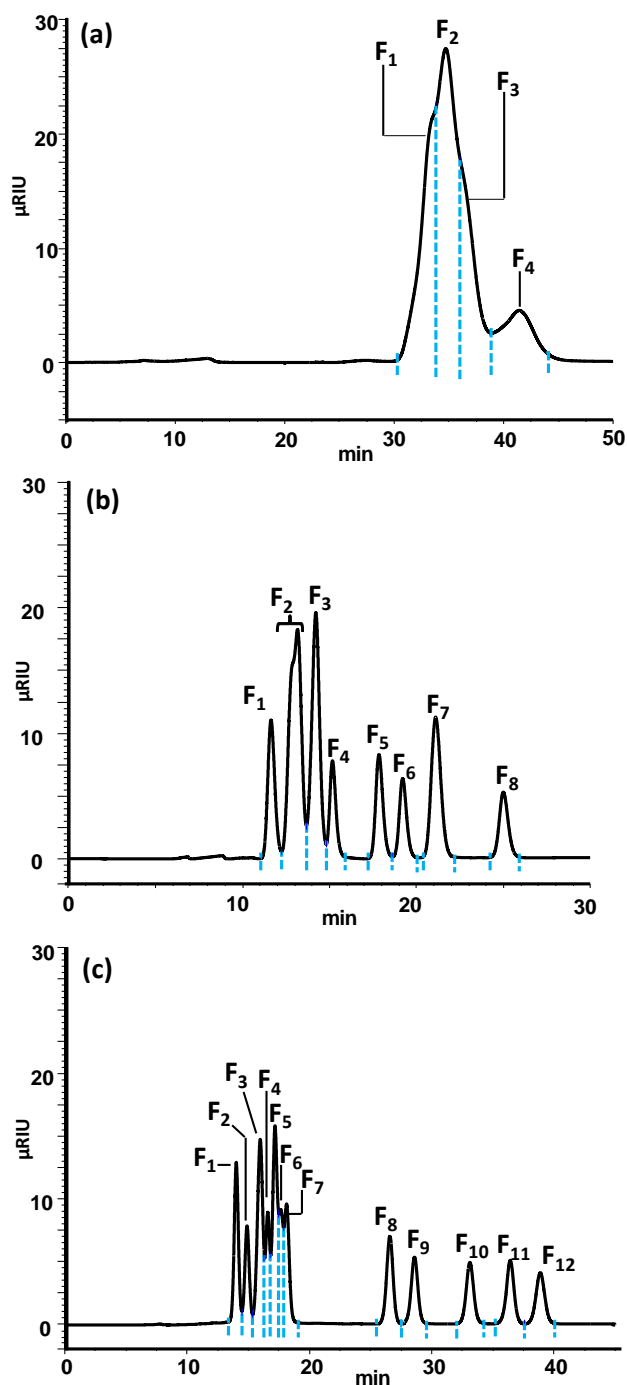
#### 294 *3.1. Cation-exchange column selection and purification procedure*

295 Cation-exchange chromatography allows the separation of poly-, oligo-, and  
296 monosaccharides based on their degree of polymerization [45,58]. The chromatographic  
297 resolution and selectivity can be modulated by changing the cation-exchange column.  
298 The separation mechanism is based on the strength of the complex formed between the  
299 hydroxyl groups of the carbohydrate and the metal (e.g. K<sup>+</sup>, Na<sup>+</sup>, Pb<sup>2+</sup>, Ca<sup>2+</sup>, Cd<sup>2+</sup>, and  
300 Cu<sup>2+</sup>).

301 In this study, Na<sup>+</sup>, Ca<sup>2+</sup>, and Pb<sup>2+</sup> columns were selected for optimum purification of  
302 the set of studied carbohydrates. The Na<sup>+</sup> column is ideal to separate polysaccharides  
303 from oligosaccharides and monosaccharides, whereas the Ca<sup>2+</sup> and Pb<sup>2+</sup> columns  
304 provide further and complementary separation among the different monosaccharides  
305 (e.g. neutral monosaccharides, alditols, anhydrosugars; Fig. 1 a-c). Compared to NH<sub>2</sub><sup>-</sup>  
306 columns (also employed for sugar preparative chromatography), Na<sup>+</sup>, Ca<sup>2+</sup>, and Pb<sup>2+</sup>  
307 columns have the advantage of running with ultrapure water as eluent instead of  
308 acetonitrile. This considerably reduces the organic carbon contamination delivered by

309 the eluent, while ultrapure water is easily removed by evaporation with a minimum  
310 organic carbon background left behind.

311 Because carbohydrates in environmental samples can also be polymers  
312 (polysaccharides and oligosaccharides) and acid hydrolysis is not always 100%  
313 effective [59], an initial purification on a  $\text{Na}^+$  column was necessary to separate the  
314 polymers from the monosaccharides [60]. The next step was the sequential purification  
315 of the monosaccharides on the  $\text{Ca}^{2+}$ ,  $\text{Pb}^{2+}$  and again, when necessary, on the  $\text{Ca}^{2+}$   
316 columns. This provided further and complementary separation among the different  
317 monosaccharides (e.g. neutral monosaccharides, alditols, anhydrosugars; Fig. 1 b-c)  
318 [61]. In summary the purification procedure flowchart proposed in this study is:  $\text{Na}^+ \rightarrow$   
319  $\text{Ca}^{2+} \rightarrow \text{Pb}^{2+} \rightarrow \text{Ca}^{2+}$ .



320

321 **Fig. 1.** Chromatogram of a standard monosaccharide mixture (1 mM each): (a)  $\text{Na}^+$   
 322 column:  $F_1$  (glucose + rhamnose + mannitol)/ $F_2$  (xylitol + sorbitol + fructose + mannose  
 323 + galactose + xylose)/ $F_3$  (fucose + arabinose + galactosan)/ $F_4$  (levoglucosan +  
 324 mannosan); (b)  $\text{Ca}^{2+}$  column:  $F_1$  (glucose)/ $F_2$  (xylose + galactose + mannose +  
 325 rhamnose)/ $F_3$  (fucose + fructose + arabinose)/ $F_4$  (galactosan)/ $F_5$  (mannitol)/ $F_6$   
 326 (levoglucosan)/ $F_7$  (sorbitol + xylitol)/ $F_8$  (mannosan); and (c)  $\text{Pb}^{2+}$  column:  $F_1$   
 327 (glucose)/ $F_2$  (xylose)/ $F_3$  (galactose + rhamnose)/ $F_4$  (galactosan)/ $F_5$  (arabinose +



328 fucose)/F<sub>6</sub> (mannose)/F<sub>7</sub> (fructose)/F<sub>8</sub> (mannitol)/F<sub>9</sub> (levoglucosan)/F<sub>10</sub> (xylitol)/F<sub>11</sub>  
329 (sorbitol)/F<sub>12</sub> (mannosan). Dashed vertical lines correspond to the duration of the eluted  
330 compound(s) and were used as the starting and ending points of peak collection by the  
331 fraction collector.

332

### 333 *3.2. Monosaccharide standards purification and recovery yields*

334 The analytical procedure flowchart established in the previous section was tested on  
335 monosaccharide standards (neutral monosaccharides, alditols, and anhydrosugars; initial  
336 concentration of 50 μM) to evaluate the separation and peak isolation of the single  
337 monosaccharides. The monosaccharide standards were sequentially purified on Ca<sup>2+</sup> and  
338 Pb<sup>2+</sup> columns and if necessary, again on a Ca<sup>2+</sup> column. Note that for the purpose of this  
339 exercise the Na<sup>+</sup> column was not included because the polysaccharides and  
340 oligosaccharides were not considered in the standard (section 3.1).

341 The first separation was achieved on a Ca<sup>2+</sup> column (Fig. S2 a) and resulted in eight  
342 peaks corresponding to fractions F<sub>1</sub>–F<sub>8</sub>, which were collected into 6 mL pre-combusted  
343 (450 °C, 6 h) glass tubes. The obtained results indicated that at this stage of the  
344 purification process only the F<sub>8</sub> fraction (e.g. mannosan) was pure because it was eluted  
345 significantly after the other monosaccharides (Fig. S2 a). Therefore, this  
346 monosaccharide was not purified further. The collected fractions F<sub>1</sub>–F<sub>7</sub> were freeze  
347 dried, redissolved into 300 μL ultrapure water, and injected individually into the Pb<sup>2+</sup>  
348 column (Fig. S2 b). It is worth noting that fractions in the time periods between the two  
349 adjacent peaks were also collected and their purity was checked after injection into the  
350 Pb<sup>2+</sup> column. The monosaccharide(s), if any, obtained in such a way was (were) further  
351 pooled with fraction(s) that contained the same monosaccharide to increase its recovery.  
352 Following the second purification, 10 peaks were obtained corresponding to fractions

353 F<sub>1</sub>–F<sub>10</sub>. The last purification was performed on a Ca<sup>2+</sup> column to ensure the collection of  
354 ultrapure monosaccharide targets (Fig. S2 c).

355 The obtained results revealed that the recovery yields after three successive  
356 purifications (Ca<sup>2+</sup> → Pb<sup>2+</sup> → Ca<sup>2+</sup>) of the standard mixture ranged from 12.38 ± 0.01%  
357 to 36.32 ± 0.02% (n = 3), with the highest values observed for glucose, mannosan,  
358 levoglucosan, and mannitol and the lowest for arabinose (Table S2). Notably, for  
359 environmental sample analysis (section 3.5), the monosaccharides xylose, fucose, and  
360 rhamnose were also included in the standard (in total 14 monosaccharides) despite the  
361 fact that their recovery yield was not estimated via this exercise.

### 362 3.3. Chromatographic system blanks and background isotopic signature

363 The blanks were run on the HPLC-RI system with ultrapure water and the amount of  
364 carbon released from the chromatographic columns (column bleeding) and isotopic  
365 signature were evaluated (Table 1). Column blanks were recorded during the whole run  
366 for each column: 50 min for the Na<sup>+</sup> column, 30 min for the Ca<sup>2+</sup> column, and 45 min  
367 for the Pb<sup>2+</sup> column. Subsequently, the amount of carbon released from these three  
368 columns was measured via EA-IRMS. The obtained values were significantly close for  
369 the Ca<sup>2+</sup> and Pb<sup>2+</sup> columns (4.84 ± 0.25 and 2.80 ± 0.21 µg, respectively), while those of  
370 the Na<sup>+</sup> column were much higher (28.64 ± 1.57 µg). Despite these differences, all the  
371 blanks resulted in a similar δ<sup>13</sup>C signature ranging from -28.4 ± 0.4 to -27.8 ± 0.4‰  
372 (Table 1).

373 The next step was to estimate the total carbon release after sequential purification on  
374 the Na<sup>+</sup> → Ca<sup>2+</sup> → Pb<sup>2+</sup> → Ca<sup>2+</sup> columns. The obtained results indicated that the  
375 purification procedure produced a blank that contained 19.76 ± 1.80 µg carbon (Table  
376 1) with a δ<sup>13</sup>C value of -27.9 ± 1.1‰; these results were similar to those observed for  
377 each column. Blanks corresponding to the collection time window of the three

378 monosaccharides (glucose, galactose, and levoglucosan) after  $\text{Na}^+ \rightarrow \text{Ca}^{2+} \rightarrow \text{Pb}^{2+} \rightarrow$   
379  $\text{Ca}^{2+}$  purification were also run. The respective carbon inputs and the  $\delta^{13}\text{C}$  signatures  
380 after  $\text{Na}^+ \rightarrow \text{Ca}^{2+} \rightarrow \text{Pb}^{2+} \rightarrow \text{Ca}^{2+}$  purification were  $0.58 \pm 0.004 \mu\text{g}$  and  $-24.4 \pm 2.9 \text{‰}$   
381 for glucose and  $0.54 \pm 0.15 \mu\text{g}$  and  $-26.7 \pm 1.5\text{‰}$  for levoglucosan (Table 1).

382 Because of the elevated cost of radiocarbon analysis, radiocarbon blanks were only  
383 run for the collection time trap of the three monosaccharides (glucose, galactose and  
384 levoglucosan) measured in this study. Moreover, as the amount of carbon delivered for  
385 the whole purification procedure ( $\text{Na}^+ \rightarrow \text{Ca}^{2+} \rightarrow \text{Pb}^{2+} \rightarrow \text{Ca}^{2+}$  columns) for each of the  
386 these monosaccharides was too small for a reliable  $\Delta^{14}\text{C}$  measurement, phthalic acid  
387 ( $^{14}\text{C}$  free blank sample) was added to the final collected blank and the sample was  
388 processed as the monosaccharide sample (i.e. transferred into Ag capsules with  
389 ultrapure water and combusted by the EA coupled to the gas interface of  
390 AixMICADAS). The amount of phthalic acid was adjusted according to the size of the  
391 sample to correct for constant contamination offsets. The results revealed that the  
392 radiocarbon blanks exhibited values ranging from  $-988$  to  $-986\text{‰}$  (Table 1).

393 These  $\Delta^{14}\text{C}$  values are slightly higher than those observed for the phthalic acid  
394 samples ( $\approx -997\text{‰}$ ) measured directly after addition into the silver capsules (i.e. without  
395 any transfer from the collection tube to the silver cups). This suggests that little  
396 exogenous carbon was added to the sample from HPLC purification (column bleeding  
397 and organic residues), glassware contamination (collection vials, Pasteur pipets) and  
398 airborne particle deposition during sample collection and transfer. Moreover, the  $\Delta^{14}\text{C}$   
399 values ( $-989.7 \pm 3.5\text{‰}$ ) and carbon amounts ( $0.52 \pm 0.34 \mu\text{g C}$ ) of the ultrapure water  
400 samples (10 mL corresponding to  $\sim 10$  times the volume of the collection window of a  
401 pure monosaccharide) indicated that the addition of exogenous carbon from the eluent  
402 was also negligible.

403 **Table 1.** Carbon content ( $\mu\text{g}$ ),  $\delta^{13}\text{C}$  (‰) and  $\Delta^{14}\text{C}$ (‰) values of the procedural blanks. Phthalic  
 404 acid was added to the collected time window of the three monosaccharide samples and the  
 405 ultrapure water sample to make the measurement feasible. The mass of phthalic acid was  
 406 adjusted according to the sample mass of the respective time window of the examined  
 407 environmental samples.

408

Blank	Carbon $\pm$ SD	$\delta^{13}\text{C}$ $\pm$ SD	$\Delta^{14}\text{C}$ $\pm$ SD
$\text{Na}^+$ column ( $n = 3$ ) *	$28.64 \pm 1.57$	$-28.3 \pm 0.8$	ND
$\text{Ca}^{2+}$ column ( $n = 3$ ) *	$4.84 \pm 0.25$	$-28.4 \pm 0.4$	ND
$\text{Pb}^{2+}$ column ( $n = 3$ ) *	$2.80 \pm 0.21$	$-27.8 \pm 0.4$	ND
$\text{Na}^+ \rightarrow \text{Ca}^{2+} \rightarrow \text{Pb}^{2+} \rightarrow \text{Ca}^{2+}$ columns ( $n = 3$ )	$19.76 \pm 1.80$	$-27.9 \pm 1.1$	ND
Retention time window of glucose ( $n = 3$ ) **	$0.58 \pm 0.00$	$-24.4 \pm 2.9$	$-986.2 \pm 5.8^{\S}$
Retention time window of galactose ( $n = 2$ ) **	$< 2 \pm 2.0^{\dagger}$	ND	$-988.2 \pm 3.1$
Retention time window of levoglucosan ( $n = 3$ ) **	$0.54 \pm 0.15$	$-26.7 \pm 1.5$	$-988.2 \pm 0.9^{\ddagger}$
Ultrapure water ( $n = 3$ )	$0.52 \pm 0.34$	$-21.9 \pm 5.9$	$-989.7 \pm 3.5$

409

410

411

412

413

414

415

416

ND: Not determined

\* Measured for the whole HPLC -RI run time for each column (Table S1)

\*\* Measured for the whole purification procedure ( $\text{Na}^+ \rightarrow \text{Ca}^{2+} \rightarrow \text{Pb}^{2+} \rightarrow \text{Ca}^{2+}$ )

$\S$   $n = 4$

$\dagger$  Measured with EA-AixMICADAS

$\ddagger$   $n = 2$

417 Despite the significantly low amount of contaminants ( $< 1\%$  for both the glucose and  
 418 levoglucosan samples from the estimated carbon content of the retention time window)  
 419 and the low  $\Delta^{14}\text{C}$  procedural blanks, the results presented in this study were blank  
 420 corrected. Finally, it is worth noting that the direct combustion of the small silver cups  
 421 via an elemental analyzer allows a low background (equivalent age of 46 000 yr BP)  
 422 and is therefore a good alternative to the time-consuming conventional method based on  
 423 purification with a vacuum line.

424 Overall, the above results clearly suggest that the proposed purification procedure  
 425 does not induce any significant contamination in the samples or affect their isotopic  
 426 signature.

427 *3.4. Hydrolysis effects*

428 The marine sample was submitted to hydrolysis (1 M HCl) prior to its  
429 chromatographic purification (Section 2) to release the monosaccharides from the  
430 biopolymer macrostructure. Moreover, additional experiments were performed on the  
431 standard mono- and polysaccharides to investigate whether the hydrolysis conditions  
432 affect the isotopic composition of the released monosaccharides. The three levoglucosan  
433 standard solutions (90 µg C each) presented similar  $\delta^{13}\text{C}_{\text{levo}}$  values, namely  $-11.2 \pm$   
434  $0.2\text{‰}$  ( $n = 3$ ) and  $-11.2 \pm 0.1\text{‰}$  ( $n = 3$ ) before and after hydrolysis, respectively.  
435 Similar results were obtained by Wang et al. [19] for the glucose standard ( $\delta^{13}\text{C}_{\text{glc}} =$   
436  $-9.8\text{‰}$  and  $\delta^{13}\text{C}_{\text{glc}} = -9.9\text{‰}$  before and after processing, respectively); however their  
437 study included additional purification steps comprising anion and cation-exchange  
438 resins.

439 In another set of experiments a polysaccharide standard (laminarin) containing  
440 glucose units was submitted to acid hydrolysis and the isotopic signature of the released  
441 glucose was compared to that of the original laminarin. The results indicated that  
442 glucose and laminarin exhibited similar isotopic signatures in terms of  $\delta^{13}\text{C}$  ( $\delta^{13}\text{C}_{\text{glc}} =$   
443  $-10.4\text{‰}$  and  $\delta^{13}\text{C}_{\text{lam.}} = -13.0\text{‰}$ ;  $n = 1$ ) and  $\Delta^{14}\text{C}$  ( $\Delta^{14}\text{C}_{\text{glc}} = -85.4\text{‰}$  and  $\Delta^{14}\text{C}_{\text{lam.}} =$   
444  $-84.90\text{‰}$ ;  $n = 1$ ) indicating few differences between the isotopic composition of the  
445 original polysaccharide and its monomeric constituent (Repeta; unpublished results).

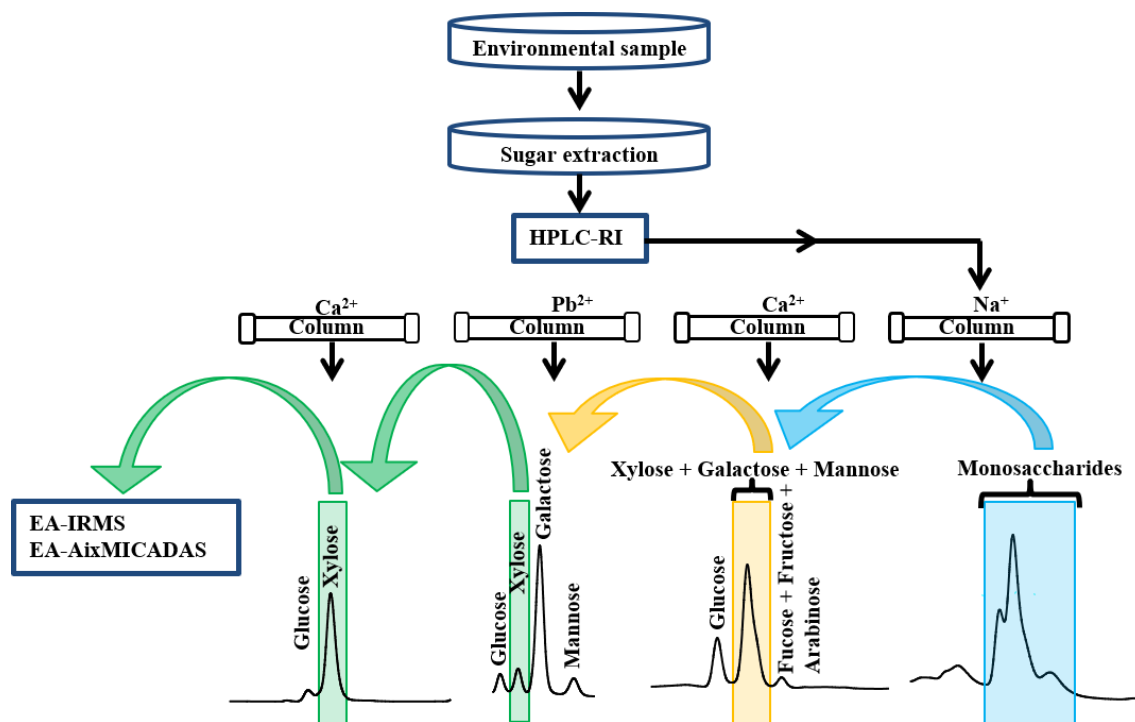
446

447 *3.5. Application to environmental samples*

448 The purification procedure, including sample preparation, employed in this study is  
449 briefly summarized in Fig. 2. Two distinct environmental samples, namely, a marine  
450 POM and a TSP sample were considered. The choice of samples was made with respect

451 to the opportunities and the logistics set to obtain them but more importantly of their  
452 high carbohydrate content.

453



454

455 **Fig. 2.** Procedural flowchart of this study with a simplified example of the purification  
456 of xylose isolated from the marine particulate organic matter (POM) sample. The last  
457 purification of xylose on the  $\text{Ca}^{2+}$  was obtained after pooling the xylose fraction  
458 collected from the  $\text{Pb}^{2+}$  column and the xylose purified from the adjacent fractions.

459

### 460 3.5.1. Marine POM

461 The hydrolyzed marine sample was processed on a  $\text{Na}^+$  column and the collected  
462 fractions ( $\text{F}_3$ – $\text{F}_6$ ), corresponding to the monosaccharides, were further purified on  
463  $\text{Ca}^{2+} \rightarrow \text{Pb}^{2+} \rightarrow \text{Ca}^{2+}$  columns (Fig. 3). The results indicated that after four sequential  
464 purifications the major monosaccharides obtained were: galactose (368  $\mu\text{g}$ ), glucose  
465 (273  $\mu\text{g}$ ), mannose (65  $\mu\text{g}$ ), xylose (38  $\mu\text{g}$ ), and fucose/arabinose (16  $\mu\text{g}$ ). It is worth  
466 noting that levoglucosan (1.8  $\mu\text{g}$ ) and mannosan (1.5  $\mu\text{g}$ ) were also collected for the

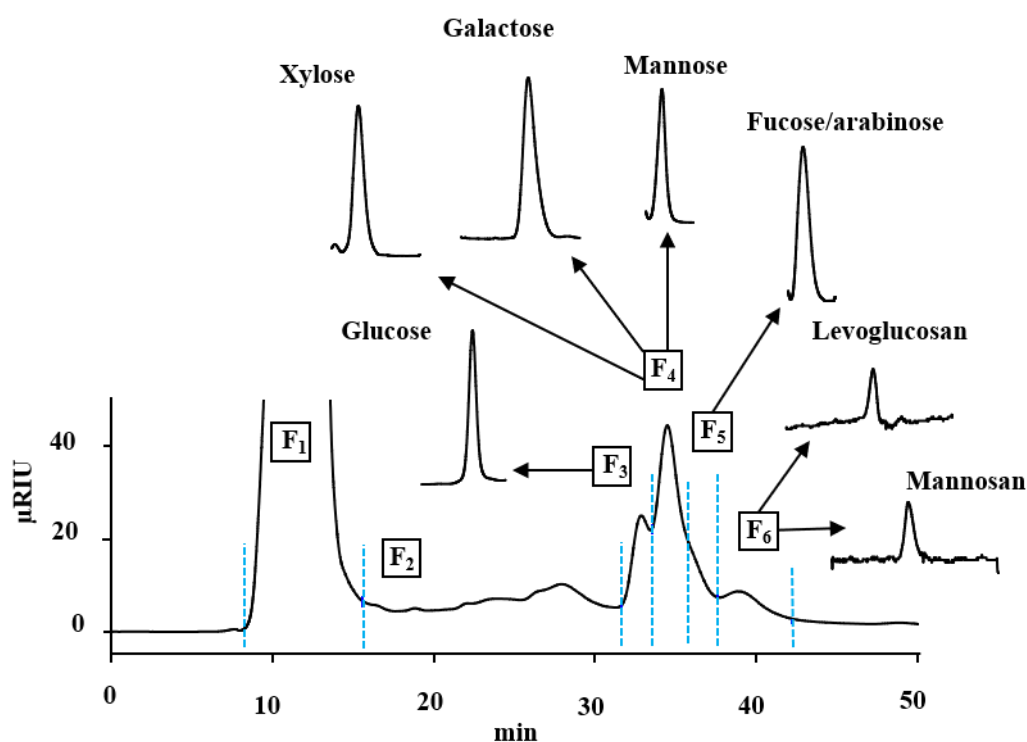
467 first time from marine POM; however, in very low amounts. The presence of the  
468 abovementioned anhydrosugars in the hydrolyzed marine sample was also confirmed by  
469 high-performance anion-exchange chromatography (Nouara et al., *submitted*). Finally,  
470 although rhamnose and galactosan were detected in the marine POM sample they  
471 yielded very small recovered amounts ( $< 0.5 \mu\text{g}$ ), which were insufficient for isotopic  
472 measurement.

473 The results revealed that the marine POM sample exhibited a  $\delta^{13}\text{C}$  value of  $-19.6 \pm$   
474  $0.6\text{‰}$  which is typical of marine origin and consistent with the  $\delta^{13}\text{C}$  values reported for  
475 the surface coastal sinking POM (range:  $-23$  to  $-20 \text{‰}$ ) [18,62]. The  $\delta^{13}\text{C}$  signature of  
476 the POM hydrophilic extract (fraction comprising most of the hydrophilic components  
477 of the sample including monosaccharides) was slightly enriched ( $-17.2 \pm 0.5\text{‰}$ )  
478 compared to that of the bulk POM and agreed well with the  $\delta^{13}\text{C}$  values recorded for  
479 glucose, galactose, mannose, xylose, and fucose/rhamnose ( $-18.5$  to  $-16.8\text{‰}$ ; Table 2).

480 The  $\delta^{13}\text{C}$  signature recorded for these individual monosaccharides agrees well with  
481 the isotopic values measured for the individual monosaccharides in marine high-  
482 molecular-weight dissolved organic matter (HMWDOM) [27], and thereby pointing  
483 toward a marine origin. The slight depletion of the  $\delta^{13}\text{C}$  values of the bulk POM relative  
484 to its individual carbohydrate component may be due to the presence of other organic  
485 compounds (e.g. amino acids and lipids) in the sample, which may have a lighter stable  
486 carbon isotope signature than that observed for carbohydrates [20,22]. Regardless,  
487 further molecular level isotopic analysis on individual amino acids and/or lipids is  
488 warranted to test this hypothesis.

489 On the other hand, the levoglucosan ( $-27.2\text{‰}$ ) and mannosan ( $-26.2\text{‰}$ ) isotopic  
490 signatures exhibited depleted  $\delta^{13}\text{C}$  values when compared to those of the other  
491 monosaccharides, indicating the different origin of these two monosaccharides (Table

492 2). Indeed, these sugars are well known tracers of terrestrial biomass burning processes  
 493 [39], and thus their presence in marine POM indicates an external terrestrial input  
 494 probably via atmospheric deposition [5,10] from C3 land plant tissue ( $\delta^{13}\text{C}$ :  $-32$  to  
 495  $-20\%$ ; mean:  $-27\%$  [63]). The presence of levoglucosan and mannosan in the POM  
 496 sample is not surprising since the sampling site was located a few kilometers offshore  
 497 from the Lima area (section 2.2).



498

499 **Fig. 3.** Chromatogram of a marine particulate organic matter (POM) sample on a  $\text{Na}^+$   
 500 column (F<sub>1</sub>: polysaccharides; F<sub>2</sub>: oligosaccharides; F<sub>3</sub>: glucose, rhamnose; F<sub>4</sub>: xylose,  
 501 galactose, and mannose; F<sub>5</sub>: fucose + arabinose, and galactosan; F<sub>6</sub>: mannosan and  
 502 levoglucosan). The final purified compounds (after  $\text{Na}^+ \rightarrow \text{Ca}^{2+} \rightarrow \text{Pb}^{2+} \rightarrow \text{Ca}^{2+}$   
 503 purification) are also indicated with arrows.

504

505 The radiocarbon results of this study indicated that relatively to its hydrophilic  
 506 fraction ( $\Delta^{14}\text{C} = 124 \pm 5\%$ ), the marine POM sample was depleted ( $\Delta^{14}\text{C} = 28 \pm 8\%$ ).



507 This agrees very well with previous investigations, which have reported similar results  
 508 for a wide variety of environmental samples comprising sediments, sinking POM,  
 509 planktons [19,64] including riverine, and marine HMWDOM [22,65]. Intermediate  
 510 radiocarbon values were recorded for glucose and galactose ( $\Delta^{14}\text{C}_{\text{glc}} = 43\text{‰}$  and  $\Delta^{14}\text{C}_{\text{gal}}$   
 511  $= 19\text{‰}$ ; Table 2) and are consistent with the radiocarbon monosaccharide signature  
 512 reported for surface marine HMWDOM [27].

513

514 **Table 2.**  $\delta^{13}\text{C}$  (‰)  $\pm$  SD and  $\Delta^{14}\text{C}$  (‰)  $\pm$  SD values of the examined environmental samples.  
 515 The  $\Delta^{14}\text{C}$  values of galactose, glucose and levoglucosan are blank-corrected.

Sample	Bulk OM <sup>§</sup>	Hydrophilic OM <sup>§</sup> extract	Pure monosaccharides		
	$\delta^{13}\text{C} / \Delta^{14}\text{C}$ ( <i>n</i> = 3)	$\delta^{13}\text{C} / \Delta^{14}\text{C}$ ( <i>n</i> = 3)	$\delta^{13}\text{C}$ ( <i>n</i> = 3)	$\Delta^{14}\text{C} \pm 1 \sigma$	
POM			Mannose	$-17.6 \pm 0.9$	–
			Xylose	$-16.8 \pm 0.2$	–
			Fuc./Ara. <sup>§§</sup>	$-18.5 \pm 0.5$	–
			Galactose	$-16.9 \pm 0.1$	$18.9 \pm 16.3$ ( <i>n</i> = 3)**
			Glucose	$-17.6 \pm 0.1$	$43.3 \pm 9.9$ ( <i>n</i> = 1)
			Levoglucosan	$-27.2^*$	–
			Mannosan	$-26.2^*$	–
TSP			Glucose	$-25.1 \pm 0.1$	–
			Fructose	$-25.3 \pm 0.3$	–
			Levoglucosan	$-25.0 \pm 0.4$	$33.0 \pm 9.7$ ( <i>n</i> = 1)
			Mannosan	$-25.7 \pm 0.5$	–
			Mannitol	$-26.4^*$	–

516 <sup>§</sup> Organic matter

517 <sup>§§</sup> Fucose / Arabinose

518 <sup>\*</sup> *n* = 1

519 <sup>\*\*</sup>  $\Delta^{14}\text{C} \pm \text{SD}$

520

521 Overall, the above radiocarbon data indicate a modern age for the purified  
 522 monosaccharides that further support the hypothesis that these monosaccharides are  
 523 incorporated into a common family of polysaccharides (acylpolysaccharides) present in  
 524 all terrestrial and aquatic ecosystems [66,67]. Nevertheless, more  $\Delta^{14}\text{C}$  data on the

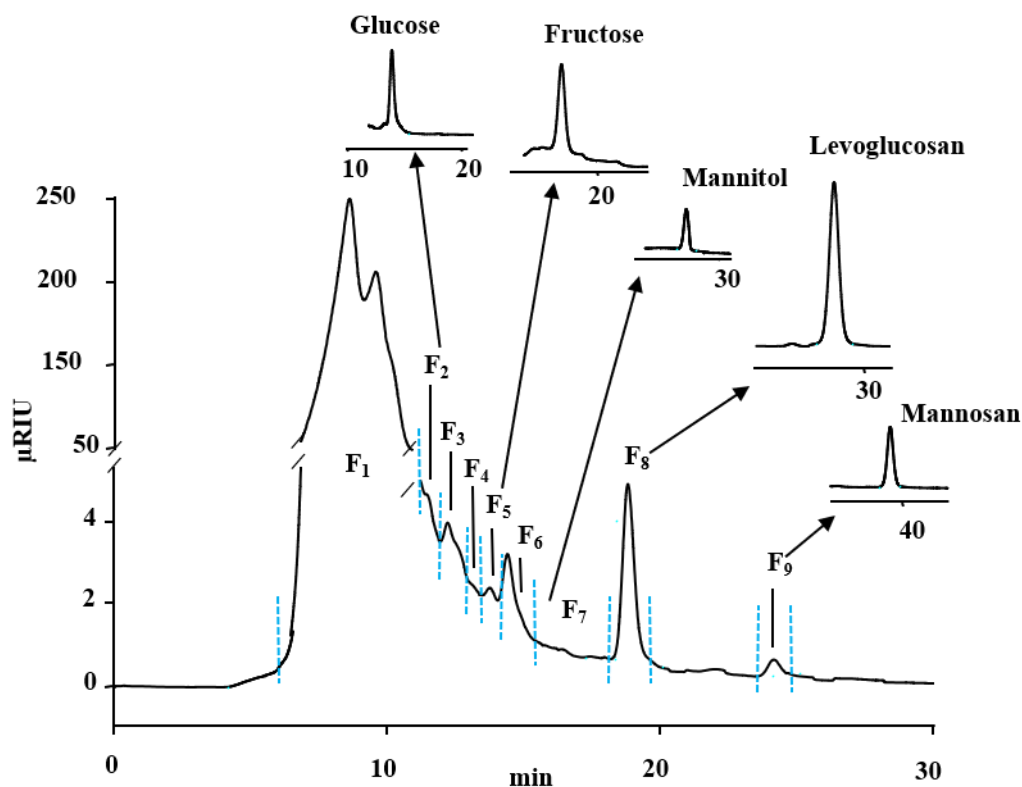
525 individual monosaccharides including other environmental matrices (e.g. sediments, and  
526 riverine POM and DOM) are warranted before any generalizations can be made.

527

### 528 3.5.2. TSP

529 The purification of the atmospheric sample was performed, without prior processing,  
530 on the Na<sup>+</sup> column. This was due to the low complexity of the sample and to avoid  
531 further possible losses and contamination that may occur during additional  
532 manipulation. The purification was conducted in a Ca<sup>2+</sup> column, followed by a Pb<sup>2+</sup>  
533 column and again by a Ca<sup>2+</sup> column (Fig. 4). The obtained results indicated that after  
534 three purifications the major monosaccharides obtained were: levoglucosan (134 µg),  
535 fructose (40 µg), mannosan (38 µg), glucose (36 µg), and mannitol (4 µg). The  
536 abundance of these monosaccharides is in agreement with the results generally reported  
537 for PM<sub>10</sub> particles [5,10]. Similar to the abovementioned results galactose, arabinose  
538 and galactosan were detected in the TSP sample. However, these were present in very  
539 low amounts (< 0.5 µg) and no isotopic measurements were performed.

540 The δ<sup>13</sup>C value recorded for the TSP sample (−25.9 ± 0.0‰) was similar to that of its  
541 hydrophilic extract (−24.8 ± 0.1‰) and within the range of that of the isolated  
542 monosaccharides (−26.4 to −25.0‰; Table 2). These results reflect a dominant  
543 terrestrial origin from C3 vegetation and/or microorganisms, including fungal spores  
544 [6,37]. The δ<sup>13</sup>C values of levoglucosan and mannosan were in agreement with the  
545 values reported by Sang et al. [25] for hardwood and stalk plant combustion. The δ<sup>13</sup>C  
546 values reported in this study are also in good agreement with those measured for *n*-  
547 alkanes (C<sub>23</sub>–C<sub>33</sub>: −26.7 to −28.5‰), *n*-alkanols (C<sub>22</sub>–C<sub>32</sub>: −23.9 to −30.4‰), and long-  
548 chain *n*-alkanoic acids (C<sub>22</sub>–C<sub>32</sub>: −22.6 to −27.4‰) in the north western African dust  
549 over the Atlantic Ocean [41].



550

551 **Fig. 4.** Chromatogram of the total suspended atmospheric particles (TSP) on a  $\text{Ca}^{2+}$   
 552 column (F<sub>1</sub>: polymer; F<sub>2</sub>: glucose; F<sub>3</sub>: unknown; F<sub>4</sub>: galactose; F<sub>5</sub>: arabinose and  
 553 fructose; F<sub>6</sub>: unknown and galactosan; F<sub>7</sub>: mannitol; F<sub>8</sub>: levoglucosan; F<sub>9</sub>: mannosan).  
 554 The final purified compounds (after  $\text{Ca}^{2+} \rightarrow \text{Pb}^{2+} \rightarrow \text{Ca}^{2+}$  purification) are also indicated  
 555 with arrows.

556

557 The radiocarbon signature of the TSP sample (Table 2) indicated that the bulk  
 558 sample was highly depleted ( $-175 \pm 5\%$ ; 1545 yr) relative to its hydrophilic component  
 559 ( $-64 \pm 13\%$ ; 530 yr). This is consistent with the characteristics already observed for the  
 560 POM sample (section 3.5.1). The purified levoglucosan exhibited a modern radiocarbon  
 561 age (33‰; Table 2), implying a very recent synthesis of this monosaccharide, which  
 562 agrees with the radiocarbon signature of contemporary biosphere carbon ( $\Delta^{14}\text{C} = 0$  to  
 563 200‰) and concurs with the radiocarbon monosaccharide data of the marine POM  
 564 sample. Although this result is a single and unique up-to-date measure of the  $\Delta^{14}\text{C}$  of

565 levoglucosan, it can also explain, in part, the lack of stability of levoglucosan in the  
566 atmosphere over time since this compound has a modern age [68,69]. The radiocarbon  
567 signature of the TSP sample (1545 yr) relative to those of the purified water extract (530  
568 yr) and levoglucosan (modern), may be due to the contribution of aged organic material  
569 such as black carbon (>50,000 yr). This material is known to be ubiquitous in the  
570 atmosphere as a result of fossil fuel emissions. This observation is further supported by  
571 the location of the sampling site (Marseille, France), which is characterized by the high  
572 influence from anthropogenic emissions and is in agreement with previous radiocarbon  
573 data performed on fossil fuels, soot aerosols, and PM<sub>2.5</sub> particles in other areas [70–72].  
574 However, more TSP radiocarbon data in conjunction with compound specific  
575 radiocarbon analyses (i.e. monosaccharides, lipids, polyaromatic hydrocarbons) are  
576 required to confirm this hypothesis.

577

### 578 *3.6. Identification of purified monosaccharides: comparison with authentic* 579 *standards*

580 Isolated monosaccharides recovered after the whole extraction and purification  
581 procedure were compared with authentic standards to check their purity. Depending on  
582 the amount of carbon recovered and the number of subsequently performed replicate  
583 EA-IRMS analysis, only three monosaccharides were further explored: levoglucosan,  
584 glucose, and galactose. The results from this study indicated that the mass spectra of the  
585 isolated monosaccharides well matched those of authentic standards (Table S3), as  
586 revealed by liquid chromatography coupled with quadrupole time-of-flight mass  
587 spectrometry (Q-TOF-LC/MS; Fig. S3). These results indicate that this proposed  
588 approach is specific, valid and reliable.

## 589 **4. Conclusions and Outlook**

590 The approach presented herein proved to be a powerful and robust tool for  $\delta^{13}\text{C}$  and  
591  $\Delta^{14}\text{C}$  determination of individual carbohydrates in the environmental matrices. Briefly,  
592 after four successive purifications on cation-exchange columns, the pure carbohydrates  
593 were produced and further assessed by EA-IRMS and/or EA-AixMICADAS. Particular  
594 attention was given to the procedural blanks, which were found to be extremely low  
595 during the whole cleanup procedure (0.5  $\mu\text{g C}$  per carbohydrate collected).

596 The applicability of the proposed procedure was tested on two different  
597 environmental samples comprising marine POM and TSP and the results revealed that  
598 the isotopic compositions of the individual carbohydrates, in terms of  $\delta^{13}\text{C}$ , were in  
599 good agreement with the data reported in the literature. Unfortunately, the  $\Delta^{14}\text{C}$  values  
600 for individual carbohydrates are scarce in literature and to the best of our knowledge,  
601 only one study, on marine HMWDOM, has been reported to date. Therefore, we could  
602 not directly compare the obtained results to  $\Delta^{14}\text{C}$  values of similar samples.  
603 Nevertheless, the obtained data suggested that the monosaccharides exhibit a modern  
604 age, further implying the recent synthesis of these compounds and their rapid cycling.  
605 Applying this method to a wide variety of environmental samples comprising marine  
606 sediments, riverine organic matter, deep water HMWDOM, soils, and rain may  
607 substantially help to improve the understanding of the carbohydrate dynamics and  
608 organic matter cycling in all geochemical systems.

609 Finally, the overall approach highlights the high potential of preparative liquid  
610 chromatography for application to the purification of specific compounds after selection  
611 of the adequate column(s) for subsequent molecular-level isotopic measurements.  
612 Moreover, future research employing two-dimensional cation-exchange  
613 chromatography may prove to be a very useful tool to speed up the whole purification  
614 procedure.

615

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623

## 624 **Appendix A. Supplementary data**

625 Supplementary data related to this article can be found at <https://doi.org/10.1016/j.aca>

## 626 **Table and Figure captions**

627 **Table 1.** Carbon content ( $\mu\text{g}$ ),  $\delta^{13}\text{C}$  (‰) and  $\Delta^{14}\text{C}$ (‰) values of the procedural blanks.  
628 Phthalic acid was added to the collected time window of the three monosaccharide  
629 samples and the ultrapure water sample to make the measurement feasible. The mass of  
630 phthalic acid was adjusted according to the sample mass of the respective time window  
631 of the examined environmental samples.

632

633 **Table 2.**  $\delta^{13}\text{C}$  (‰)  $\pm$  SD and  $\Delta^{14}\text{C}$  (‰)  $\pm$  SD values of the examined environmental  
634 samples. The  $\Delta^{14}\text{C}$  values of galactose, glucose and levoglucosan are blank-corrected.

635

636 **Fig. 1.** Chromatogram of a standard monosaccharide mixture (1 mM each): (a) Na<sup>+</sup>  
637 column: F<sub>1</sub> (glucose + rhamnose + mannitol)/F<sub>2</sub> (xylitol + sorbitol + fructose + mannose  
638 + galactose + xylose)/F<sub>3</sub> (fucose + arabinose + galactosan)/F<sub>4</sub> (levoglucosan +  
639 mannosan); (b) Ca<sup>2+</sup> column: F<sub>1</sub> (glucose)/F<sub>2</sub> (xylose + galactose + mannose +  
640 rhamnose)/F<sub>3</sub> (fucose + fructose + arabinose)/F<sub>4</sub> (galactosan)/F<sub>5</sub> (mannitol)/F<sub>6</sub>  
641 (levoglucosan)/F<sub>7</sub> (sorbitol + xylitol)/F<sub>8</sub> (mannosan); and (c) Pb<sup>2+</sup> column: F<sub>1</sub>  
642 (glucose)/F<sub>2</sub> (xylose)/F<sub>3</sub> (galactose + rhamnose)/F<sub>4</sub> (galactosan)/F<sub>5</sub> (arabinose +  
643 fucose)/F<sub>6</sub> (mannose)/F<sub>7</sub> (fructose)/F<sub>8</sub> (mannitol)/F<sub>9</sub> (levoglucosan)/F<sub>10</sub> (xylitol)/F<sub>11</sub>  
644 (sorbitol)/F<sub>12</sub> (mannosan). Dashed vertical lines correspond to the duration of the eluted  
645 compound(s) and were used as the starting and ending points of peak collection by the  
646 fraction collector.

647 **Fig. 2.** Procedural flowchart of this study with a simplified example of the purification  
648 of xylose isolated from the marine particulate organic matter (POM) sample. The last  
649 purification of xylose on the Ca<sup>2+</sup> was obtained after pooling the xylose fraction  
650 collected from the Pb<sup>2+</sup> column and the xylose purified from the adjacent fractions.

651

652 **Fig. 3.** Chromatogram of a marine particulate organic matter (POM) sample on a Na<sup>+</sup>  
653 column (F<sub>1</sub>: polysaccharides; F<sub>2</sub>: oligosaccharides; F<sub>3</sub>: glucose, rhamnose; F<sub>4</sub>: xylose,  
654 galactose, and mannose; F<sub>5</sub>: fucose + arabinose, and galactosan; F<sub>6</sub>: mannosan and  
655 levoglucosan). The final purified compounds (after Na<sup>+</sup>→Ca<sup>2+</sup>→Pb<sup>2+</sup>→Ca<sup>2+</sup>  
656 purification) are also indicated with arrows.

657

658 **Fig. 4.** Chromatogram of the total suspended atmospheric particles (TSP) on a Ca<sup>2+</sup>  
659 column (F<sub>1</sub>: polymer; F<sub>2</sub>: glucose; F<sub>3</sub>: unknown; F<sub>4</sub>: galactose; F<sub>5</sub>: arabinose and  
660 fructose; F<sub>6</sub>: unknown and galactosan; F<sub>7</sub>: mannitol; F<sub>8</sub>: levoglucosan; F<sub>9</sub>: mannosan).

661 The final purified compounds (after  $\text{Ca}^{2+} \rightarrow \text{Pb}^{2+} \rightarrow \text{Ca}^{2+}$  purification) are also indicated  
662 with arrows.

663

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## 936 **Appendix A**

### 937 **Supporting data and information for**

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### 939 **Liquid Chromatographic isolation of individual carbohydrates from** 940 **environmental matrices for stable carbon analysis and radiocarbon dating**

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960 February 27<sup>th</sup>, 2019

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969 (galactosan)/ $\text{F}_5$  (mannitol)/ $\text{F}_6$  (levoglucosan)/ $\text{F}_7$  (sorbitol + xylitol)/ $\text{F}_8$  (mannosan),  
970 followed by purification on (b)  $\text{Pb}^{2+}$  column of  $\text{F}_1$  and  $\text{F}_2$  fractions:  $\text{F}_1 + \text{F}_{2-1}$   
971 (glucose)/ $\text{F}_{2-2}$  (galactose)/ $\text{F}_{2-3}$  (mannose) and (c)  $\text{Ca}^{2+}$  column of  $\text{F}_{2-1}$  fraction :  $\text{F}_{2-1-1}$   
972 (unknown)/ $\text{F}_{2-1-2}$  (glucose). Colored boxes correspond to peak(s) collection. 46

973 **Fig. S3.** Extracted ion chromatograms corresponding to the analysis of underivatized  
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975 respective authentic standard ( $\sim 5\text{ppm}$  final concentration) and red line to the sample. In  
976 all spectra, the molecular ion was detected as a  $\text{Na}^+$  and  $\text{NH}_4^+$  additive. More details are  
977 given in Table S3. 47

978 Table S1: Columns characteristics and analysis conditions used for the HPLC-RI  
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980 **Table S2.** Monosaccharide purification yields after three sequential purifications  
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982 49

983 **Table S3.** Theoretical and measured masses (LC-Q-TOF-MS analysis) recorded as  $\text{Na}^+$   
984 and  $\text{NH}_4^+$  additives for levoglucosan, glucose and galactose. 49

985 **References** 50

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990 **S 1. Optimization of the HPLC-RI system**

991 During the optimization step, a systematic large negative peak was always observed in  
992 the chromatograms at 18-20 min (Fig. S1). Previous studies indicated that this peak is  
993 probably due to the presence of dissolved oxygen in the sample and can mask the eluted  
994 compounds at this time window or cause an increase in the baseline noise [1]. The peak  
995 intensity somehow decreased after sample degassing (with pure He) but was still  
996 significant at low sample concentrations. We overcame this problem by installing a  
997 degassing device (RFIC eluent degasser, Thermo Fisher) before the chromatographic  
998 column. We found that this device increases neither the back-pressure of the system nor  
999 the retention time of the carbohydrates. To shorten the analysis time of the  
1000 carbohydrates, no guard columns were used in this study. However, as a precaution, an  
1001 online polyether ether ketone (PEEK) filter equipped with a stainless-steel frit (pore size  
1002 0.5  $\mu\text{m}$ ) was placed just before the online degasser. The filter frit was cleaned regularly  
1003 by sonication in a methanol bath, followed by sonication in ultrapure water, once per  
1004 month and between each sample purification.

1005 Moreover, to maximize the collection efficiency of carbohydrates, the tubing between  
1006 the RI detector and the fraction collector was minimized. The delay time between the  
1007 detector and the fraction collector was calculated using 1 mM of vitamin B<sub>12</sub> without an  
1008 analytical column. Further adjustments of the working flow rates (0.3 mL min<sup>-1</sup> and 0.6  
1009 mL min<sup>-1</sup> for Na<sup>+</sup> and Ca<sup>2+</sup>/Pb<sup>2+</sup> columns, respectively) were made with a glucose  
1010 standard (50  $\mu\text{M}$ ). We found that the highest recovery (~80%) of the glucose was  
1011 obtained with a delay time of 110 s for the Na<sup>+</sup> column, and 60 s for the Ca<sup>2+</sup> and Pb<sup>2+</sup>  
1012 columns. These delay times were applied for all peak and fraction collections during the  
1013 purification procedure either of standards or samples.

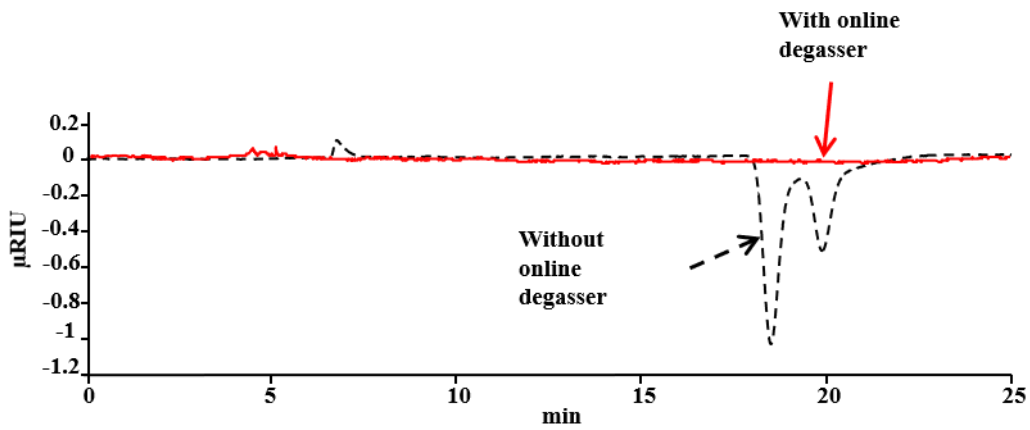
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1017 **S. 2. Supplementary figures and table legends**

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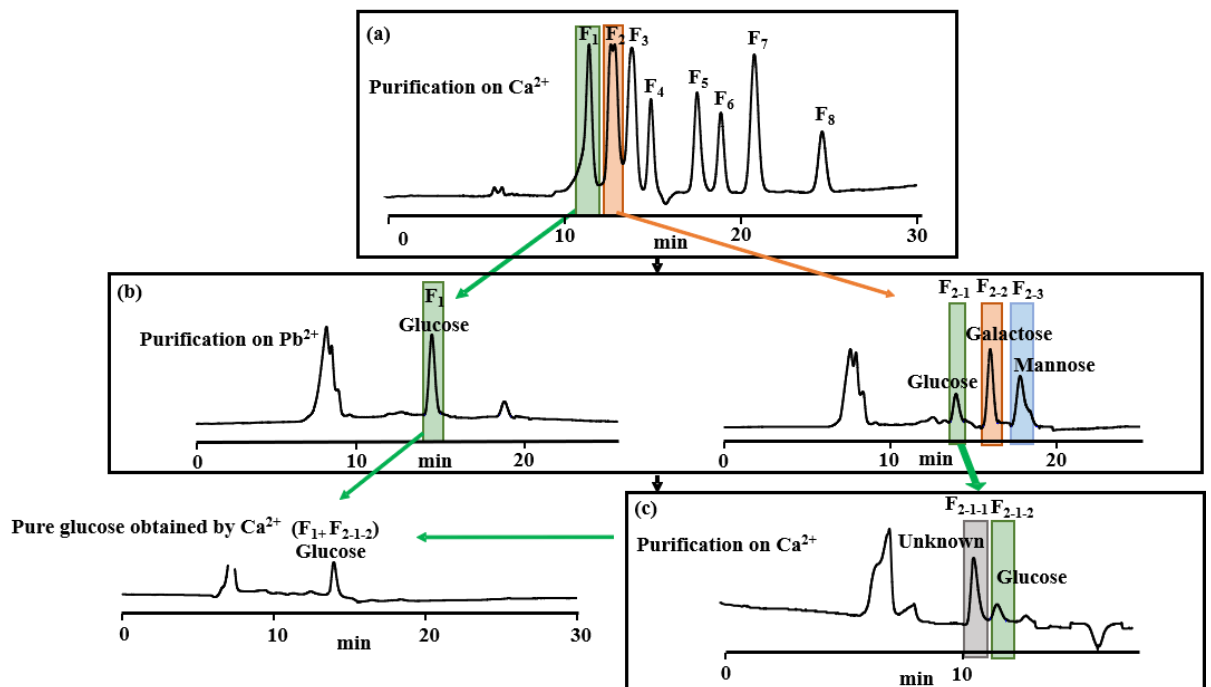


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1020 **Fig. S1.** Comparison of chromatograms with and without online degasser

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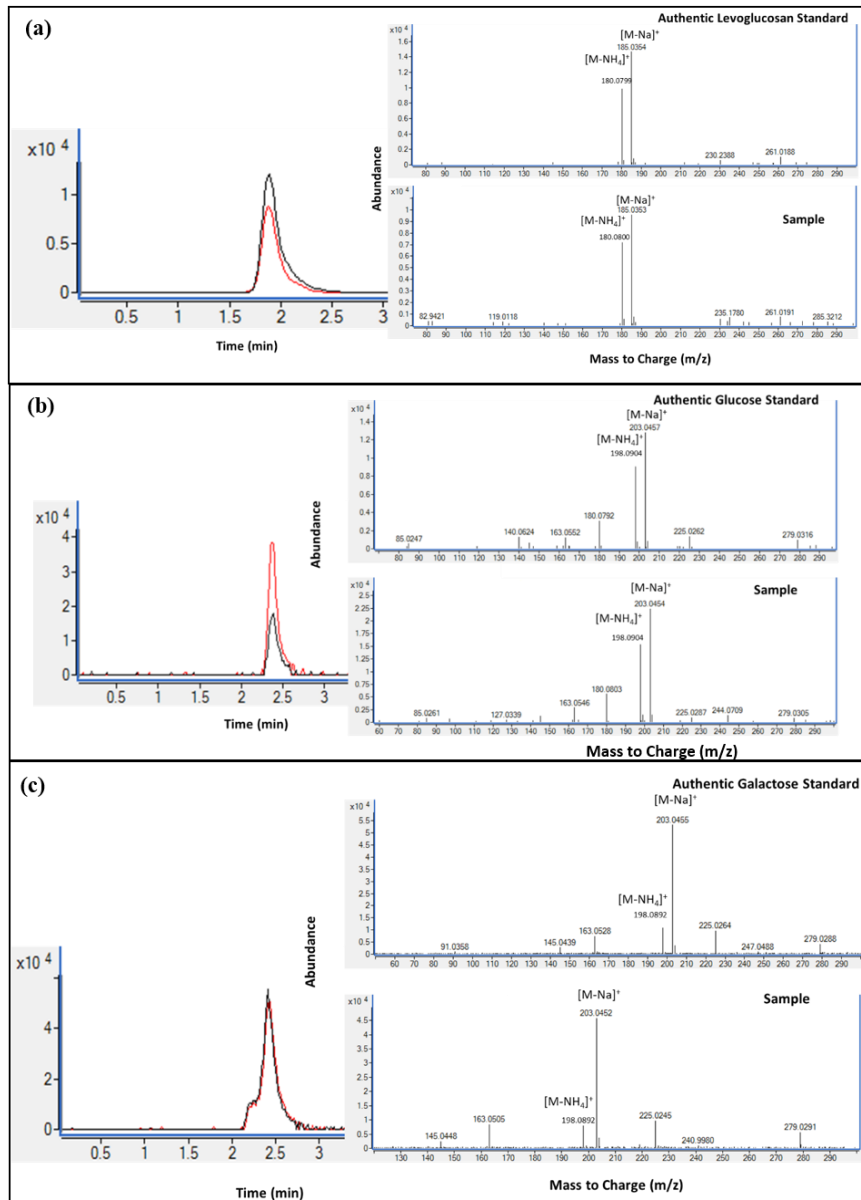
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1024 **Fig. S2:** Example of a standard monosaccharide mixture (50  $\mu\text{M}$  each) purification on

1025 (a)  $\text{Ca}^{2+}$  column:  $F_1$  (glucose)/ $F_2$  (galactose + mannose)/ $F_3$  (fructose + arabinose)/ $F_4$

1026 (galactosan) $F_5$  (mannitol)/ $F_6$  (levoglucosan)/ $F_7$  (sorbitol + xylitol)/ $F_8$  (mannosan),

1027 followed by purification on (b)  $\text{Pb}^{2+}$  column of  $F_1$  and  $F_2$  fractions:  $F_1 + F_{2-1}$   
 1028 (glucose)/ $F_{2-2}$  (galactose)/ $F_{2-3}$  (mannose) and (c)  $\text{Ca}^{2+}$  column of  $F_{2-1}$  fraction :  $F_{2-1-1}$   
 1029 (unknown)/ $F_{2-1-2}$  (glucose). Colored boxes correspond to peak(s) collection.  
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1033 **Fig. S3.** Extracted ion chromatograms corresponding to the analysis of underivatized  
 1034 levoglucosan (a), glucose (b) and galactose (c). The black line corresponds to the  
 1035 respective authentic standard ( $\sim 5$  ppm final concentration) and red line to the sample. In



1036 all spectra, the molecular ion was detected as a Na<sup>+</sup> and NH<sub>4</sub><sup>+</sup> additive. More details are  
1037 given in Table S3.

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1042 **Table S1:** Columns characteristics and analysis conditions used for the HPLC-RI  
1043 system.

<b>Column</b>	<b>RNO-Oligosaccharide</b>	<b>RCM-Monosaccharide</b>	<b>RPM-Monosaccharide</b>
<b>The ionic form</b>	Na <sup>+</sup>	Ca <sup>2+</sup>	Pb <sup>2+</sup>
<b>Column size</b>	200 × 10 mm	300 × 7.8 mm	300 × 7.8 mm
<b>Stationary phase</b>	Sulfonated styrene-divinylbenzene		
<b>Cross linking</b>	4%	8%	8%
<b>Particles size</b>	12 μm	9 μm	8 μm
<b>Temperature</b>	85 °C	85 °C	75 °C
<b>Mobile phase</b>	Degassed ultra-pure water		
<b>Flow rate</b>	0.3 mL min <sup>-1</sup>	0.6 mL min <sup>-1</sup>	0.6 mL min <sup>-1</sup>
<b>Run time</b>	50 min	30 min	45 min

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1046 **Table S2.** Monosaccharide purification yields after three sequential purifications  
 1047 ( $\text{Ca}^{2+} \rightarrow \text{Pb}^{2+} \rightarrow \text{Ca}^{2+}$ ) of a standard monosaccharide mixture (see text) at 50  $\mu\text{M}$  ( $n = 3$ ).

Standard	Average yield
	Mean $\pm$ Stdev
Glucose	36.32 $\pm$ 0.02
Galactose	29.28 $\pm$ 0.03
Mannose	25.17 $\pm$ 0.01
Fructose	20.44 $\pm$ 0.01
Arabinose	12.38 $\pm$ 0.01
Galactosan	15.22 $\pm$ 0.00
Mannitol	31.01 $\pm$ 0.01
Levoglucosan	31.49 $\pm$ 0.01
Xylitol	25.84 $\pm$ 0.01
Sorbitol	30.09 $\pm$ 0.03
Mannosan	32.21 $\pm$ 0.00

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1051 **Table S3.** Theoretical and measured masses (LC-Q-TOF-MS analysis) recorded as  $\text{Na}^+$   
 1052 and  $\text{NH}_4^+$  additives for levoglucosan, glucose and galactose.

Theoretical mass		Measured authentic standard mass		Measured purified compound mass	
$\text{Na}^+$	$\text{NH}_4^+$	$\text{Na}^+$	$\text{NH}_4^+$	$\text{Na}^+$	$\text{NH}_4^+$

	additive	additive	additive	additive	additive	additive
<b>Levoglucosan (TSP)</b>	185.0431	180.0877	185.0354	180.0799	185.0363	180.0800
<b>Glucose (POM)</b>	203.0537	198.0978	203.0457	198.0904	203.0454	198.0904
<b>Galactose (POM)</b>	203.0537	199.0978	203.0455	198.0892	203.0452	198.0892

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