ELSEVIER

Journal of Chromatography A, 920 (2001) 13–22

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Sub-ambient temperature effects on the separation of monosaccharides by high-performance anion-exchange chromatography with pulse amperometric detection Application to marine chemistry

Christos Panagiotopoulos[a], Richard Sempéré[a,*], Raymond Lafont[a], Philippe Kerhervé[b]

[a]*Laboratoire de Microbiologie Marine, CNRS-INSU UMR 6117, Centre d'Océanologie de Marseille, Université de la Méditerranée, Campus de Luminy-Case 907, 163 Av. de Luminy, 13288 Marseille Cedex 09, France*

[b]*Centre de Formation et de Recherche sur l'Environnement Marin (CEFREM), CNRS UMR 5110, Université de Perpignan, 52 Avenue de Villeneuve, 66860 Perpignan Cedex, France*

Abstract

The effects of column temperature in the range 10–45°C using high-performance anion-exchange chromatography (HPAEC) and pulse amperometric detection are described for the determination of monosaccharides. The influence of temperature was tested with an isocratic elution of NaOH at concentrations varying from 2.5 to 20 m*M* and with a post-column addition of 1 *M* NaOH. The results showed that small changes of temperature greatly affect retention times and resolution (R_s) of monosaccharides and particularly those of the both pairs xylose–mannose and rhamnose–arabinose which cannot be simultaneously detected at usual room temperature (~25°C). Our results suggest that a subambient temperature of 17°C and an eluent concentration of 19 m*M* are the more appropriate conditions for an acceptable separation ($R_{s\ rha/ara}$= 1.02, $R_{s\ man/xyl}$=0.70) in a short analytical run time (35 min). The results showed that within the range of temperatures studied, enthalpy and entropy are invariant of temperature indicating that changes in the retention processes are mainly due to temperature than other associated changes in the system. This study demonstrated the importance of controlling temperature during HPAEC of monosaccharides, both to accomplish highly reproducible retention times and to achieve optimal separation of sugars. This method gave acceptable results for detection of marine sugars.

Keywords: Temperature effects; Marine particles; Mucus; Monosaccharides

1. Introduction

Carbohydrates including simple sugars, some sugar derivatives and polysaccharides make up a large fraction of most organisms on the Earth and the largest identified fraction of organic matter in seawater [1,2]. Recent studies indicated that polysaccharides are the dominant component of high-molecular weight dissolved organic matter and colloidal material isolated from surface and deep water [3–5]. Additionally, carbohydrates contribute to transparent exopolymer particle formation and comprise a significant fraction of marine particles and sediments [6–8]. Carbohydrates are also important food sources for heterotrophic bacteria and rapid turn-over rates (hours–days) have been measured in various natural

*Corresponding author. Tel.: +33-4-9182-9050; fax: +33-4-9182-9051.

E-mail address: sempere@com.univ-mrs.fr (R. Sempéré).

0021-9673/01/$ – see front matter
PII: S0021-9673(01)00697-5

waters [9–11]. However, more informations are still necessary to generalize their molecular distribution and their degradation in marine water.

High-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC–PAD) is an interesting chromatographic tool for monosaccharides determination in marine samples [5–7,10–14]. This method, generally used under ambient temperature, permits detection of monosaccharides including fucose, rhamnose (deoxysugars), arabinose, xylose, ribose (aldopentoses), galactose, glucose, mannose (aldohexoses) and fructose (ketohexose) in an alkaline NaOH eluent. The separation is based on anion-exchange mechanisms and the order of elution of the aldoses depends mainly from their pK_a values. The detection is performed without derivatization by an electrochemical detector that applies a triple sequence of potentials to a gold electrode [15]. However, current HPAEC–PAD conditions do not allow a complete peak separation in one isocratic run since several authors reported coelution under ambient conditions of two couples of sugars, rhamnose/arabinose and xylose/mannose, which are significantly abundant in seawater [7,12–14].

In HPAEC–PAD, analysts are usually working under ambient temperature and column temperature has often been considered to often serve a minor role [6,7,10,13,16]. Nevertheless, column temperature is a very important factor because it modifies the selectivity and the retention time of the analyzed components. Rocklin and Pohl [17] noticed that temperature modified the selectivity of different types of sugars (disaccharides, sugars alcohol, oligosaccharides and monosaccharides) analyzed by anion-exchange chromatography (HPIC-AS6 column). Recent studies indicated that regulation of temperature is a valuable tool in achieving optimal separation of oligosaccharides by HPAEC–PAD or some monosaccharides found in fruit juices by using capillary zone electrophoresis (CZE) [18,19]. However, to our knowledge, there is no report specifically dealing with the impact of temperature for the separation of monosaccharides by HPAEC–PAD and especially xylose–mannose and rhamnose–arabinose.

Here, we discuss the role of the temperature for sugar determination by using the HPAEC separation followed by a gold–palladium PAD method. We also suggest the most appropriate temperature conditions which can improve separation and quantification of nine marine monosaccharides with one HPAEC–PAD run in an acceptable period of time. The validity of our method is also examined on natural monosaccharides extracted from hydrolysis of marine particulate organic material and from blue–green algae (mucus).

2. Materials and methods

2.1. Chemicals

Sugar standards were purchased from Sigma (St. Louis, MO, USA) as the purest available grade and diluted in photo-oxidized Milli-Q plus water obtained from a Millipore unit. Standard sugar stock solutions at a concentration of 1 m*M* were prepared and stored at 4°C in the dark with addition of 0.02% NaN_3 in order to inhibit bacterial growth. For calibration, diluted standards were prepared daily. Ultrapur Normatom 30% HCl was used for hydrolysis of natural marine particles. Low-carbonate sodium hydroxide solutions (NaOH) (J.T. Baker, analytical concentrate) were used for HPAEC mobile phase.

2.2. Chromatographic system

The HPAEC–PAD system was constructed with the use of commercially available material from several manufacturers and it consists of three modules including pump, column and detector. The chromatographic instrument that we used consisted of a Waters 626 pump gradient controller and four reservoirs. For the reduction of solvent pump pulses, both pumps are equipped with pulse dampening units installed (silk system). Sample injection was performed with a manual Rheodyne injector and one sample loop of 20 μl. The separation of the monosaccharides was performed on a Dionex CarboPac PA-1 analytical column (250×4 mm I.D.) (anion-exchange capacity 100 μequiv.) and a Dionex CarboPac PA-1 guard column (50×4 mm I.D.). The influence of column temperature was controlled by using a Jones chromatography oven (model 7955) that operates within the temperature range 0–50°C

with a system based on Peletier effect. The gradient of the temperature inside the oven is about 1°C and 30 min are needed to reach equilibration inside the oven.

We used a Decade (Antec) electrochemical detector which operated in this study in the PAD mode. Table 1 summarizes the operating parameters and the details for the electrochemical detector including the settings given by the manufacturer (Antec). We noticed that the settings used for EG&G or Dionex detectors [7,10–13] were not well adapted to the Decade detector. The full-scale current (I) range used was related to the measured concentrations and ranged from 0.2 to 2 μA for standards analyzed (nano–micromolar ranges). A strong base (1 *M* NaOH) was added to the eluent stream by a post-column pump having a flow-rate of 0.2 ml min^{-1} to increase PAD sensitivity and to minimize the baseline drift [20]. The background noise was about 2–3 mV. Data acquisition and processing were performed with a Waters Millenium software (Waters Chromatography Division, Millipore). We established a calibration curve for each of the analyzed compound since the molar response of the solute measured with PAD is different for each compound.

2.3. HPAEC conditions

The analysis of monosaccharides were made by isocratic elution with the use of a mobile phase 2.5, 5, 15 and 20 m*M* NaOH at a flow-rate of 0.7 ml min^{-1}. As the NaOH has the tendency to absorb carbonates, the mobile phases (A: 20 m*M* NaOH; B: 1 *M* NaOH; C: Milli-Q) were degassed before use and constantly held under pure helium pressure at a flow-rate of 4 ml min^{-1}. Moreover, gases such as CO_2 or O_2 were removed as possible from the eluent with a degasser (Waters) located between the solvents and the pumps. Additionally a carbonate retarder column ATC-1 (Dionex Chrom Ion PAC) was installed between the Rheodyne injector and the pump.

To regenerate the column, a 1 *M* NaOH solution was pumped for 1 h at the end of each analysis day, whereas the same solution was systematically pumped (30 min) after each sample analysis. The minimum time of reequilibration of the column after each cleaning was 30 min. In order to discriminate nine monosaccharides (fucose, rhamnose, arabinose, galactose, glucose, xylose, mannose, fructose, ribose) and especially the critical coeluted couples rhamnose–arabinose, xylose–mannose, we tested different column temperatures ranging from 10 to 45°C for different NaOH concentrations in the mobile phase (2.5, 5, 15 and 20 m*M*). These experiments were performed by injection of 20 μl of standard solutions diluted in Milli-Q water at a final concentration of 2.5 μ*M* for each monosaccharide.

2.4. Collection of marine samples

Large particles were collected at 50 m depth in Southern Indian Ocean (63°E; 43°S) in January–February 1999 with an in situ pump equipped with precombusted (450°C, 6 h) 0.7-μm pore size-glass fiber filters (GF/F, 145 mm). Samples of mucus which was produced during the development of a blue–green algae bloom were collected in September 1999 from surface waters of Euvoikos Gulf in

Table 1
Conditions for the Decade (Antec) detector (PAD mode) for monosaccharide detection

Flowcell	VT-03 with 3.0 mm gold working electrode mounted with 50-μm spacer			
Reference electrode	Palladium			
Flow rate	0.9 ml min^{-1}			
Temperature	35 °C			
I-cell	ca. 120 nA			
Settings	s	1	2	3
t (ms)	20	480	120	130
E (mV)		0.15	0.75	−0.80

s, delay applied; E_1, oxidation potential; E_2 and E_3, cleaning potentials; and *t*, time corresponding to the potentials E_1–E_3.

Aegean Sea. Samples were stored in precombusted vials in the dark at −17°C.

2.5. Sample preparation

In order to examine the concentrations and composition of monosaccharides, samples were hydrolyzed with 1.8 *M* HCl for 3.5 h at 100°C [21]. After cooling, HCl was evaporated to dryness in a rotary evaporator and the residue washed with a small volume of water (1 ml) which was removed by evaporation. The residue was taken up in 10 ml of Milli-Q water. Duplicates of 20 μl were then injected into the HPAEC–PAD system. The monosaccharides values were not corrected from losses during the hydrolysis procedure.

3. Results and discussion

3.1. Resolution factor (R_s) as a function of NaOH concentration and temperature

Elution of critical pairs of compounds were studied through examination of R_s plotted as a function of temperature (Fig. 1a–d). With one only exception (5 m*M* NaOH), our results showed that at low temperatures (<15°C) acceptable separation was achieved for all monosaccharides. For NaOH concentrations of 2.5 and 5 m*M* we observed drastic changes of $R_{s\ rha/ara}$ which ranged from 0 to 0.91. The resolution plots ($R_{s\ rha/ara}$) for NaOH concentration of 15 and 20 m*M* were always superior or equal to 1 for all the temperatures tested, suggesting that this pair is adequately separated under these conditions. On the other hand, we noticed that for all studied concentrations of NaOH, $R_{s\ man/xyl}$ decreases as the temperature increases indicating that this pair is more sensitive to temperature variations in comparison with the other pair. A reverse elution order was observed for the pair mannose–xylose at 15 and 20 m*M* which is in agreement with the results obtained by Germain [22].

Best results for simultaneous separation pairs xylose–mannose, rhamnose–arabinose were obtained for 2.5 m*M* (45°C), 2.5 m*M* (10°C), 5 m*M* (40°C), 15 m*M* (10°C) and 20 m*M* (15°C) (Fig. 1). It should be noticed that for a NaOH concentration of 20 m*M*, we obtained better results at 15 than 10°C (as seen in Fig. 1d) since we observed a coelution between mannose and glucose at 10°C ($R_{s\ glc/man}$=0.67; 10°C vs. $R_{s\ glc/man}$=0.86; 15°C). At 45°C for a NaOH concentration of 2.5 m*M*, there was an acceptable separation ($R_{s\ rha/ara}$=0.67, $R_{s\ xyl/man}$=0.63) of the coeluted pairs, though fructose was not detected. Surprisingly, we observed that for temperatures higher than 40°C and whatever the NaOH concentration a decrease of the surface area of fructose which might be due to a degradation of this sugar at relatively high temperatures (~40°C). This trend was more pronounced for low concentrations of mobile phase (2.5 and 5 m*M*) and is consistent with the results obtained by Andersen and Sørensen [23]. In such a way, these results (i.e., 2.5 m*M* 45°C, 5 m*M* 40°C) were excluded from further investigations. Adequate separation of all the monosaccharides studied was observed for 2.5 m*M* at 10°C ($R_{s\ rha/ara}$= 0.91, $R_{s\ xyl/man}$=0.99) and approached for 20 m*M* at 15°C ($R_{s\ rha/ara}$=1.2, $R_{s\ xyl/man}$=0.81).

However, the run time was much shorter for a NaOH concentration of 20 m*M* at 15°C (35 min) than for 2.5 m*M* at 10°C (90 min). For 20 m*M* (15°C) we observed slight coelutions of glucose–xylose–mannose and an acceptable separation between rhamnose and arabinose and other sugars. Additional experiments indicated that the most appropriate conditions were actually found for 19 m*M* NaOH at 17°C (Figs. 1e and 2b). These results are by far better (good separation of both pairs rhamnose–arabinose and mannose-xylose) than those obtained at usual room temperature (20–25°C) (Fig. 2a,b) [6,7,10,13,16]. The role of temperature has been already discussed for the separation of oligosaccharides by using a Dionex PA-100 column [18]. Our results clearly indicated that small changes in temperature (±5°C) as those found in uncontrolled laboratory conditions play a significant role on the separation of monosaccharides by HPAEC–PAD. Moreover, such modifications driven by ambient temperature may induce poor reproducibility of monosaccharides determination as well as different results between laboratories.

As suggested by Pohl et al. [24], temperature changes may affect retention processes by alteration

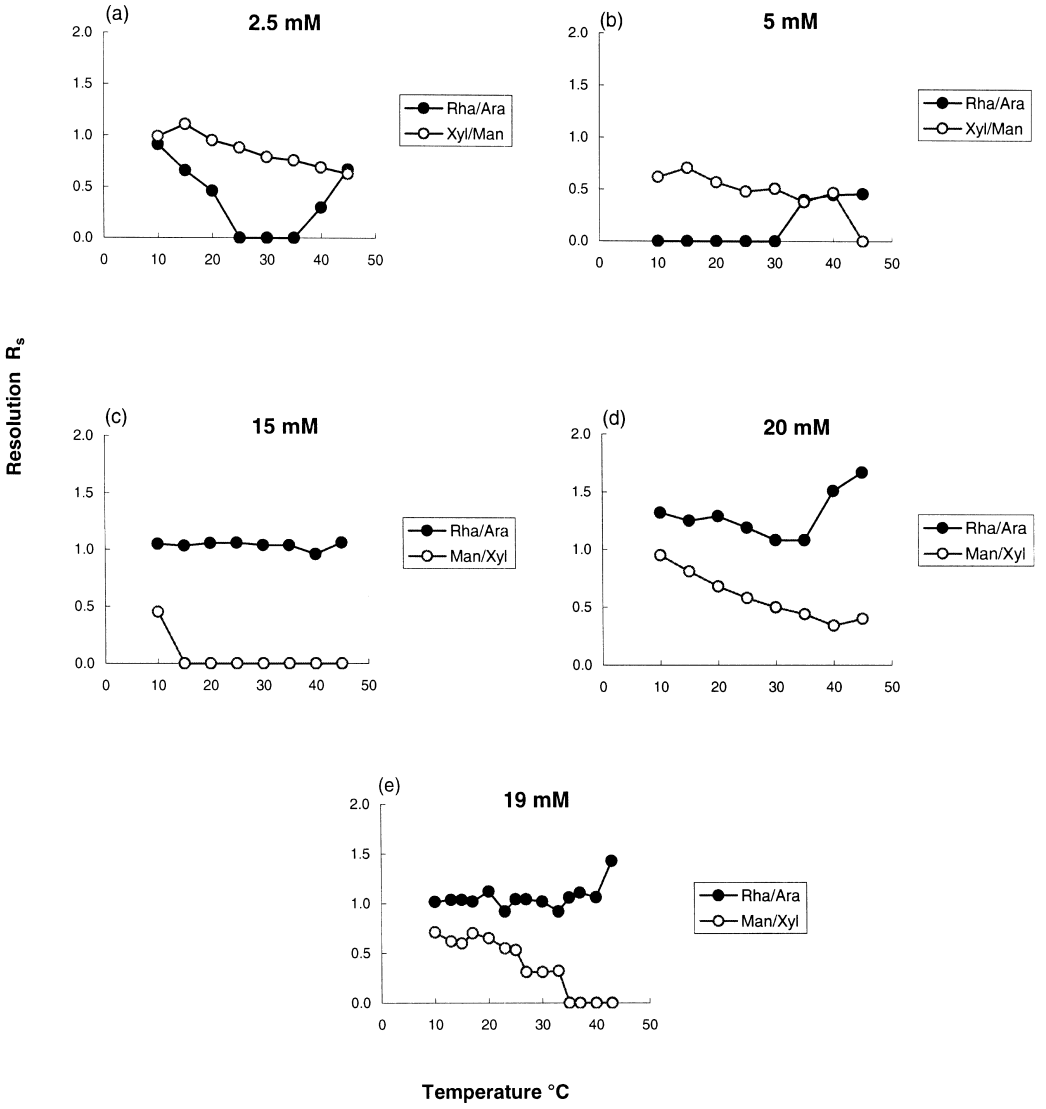

Fig. 1. Resolution factor (R_s) versus temperature for a monosaccharide mixture (individual concentrations 2.5 μ*M*; sample loop 20 μl) by HPAEC with a 1 *M* NAOH post-column addition (flow-rate 0.2 ml min^{-1}) with PAD in isocratic mode, the total flow-rate being 0.9 ml min^{-1}. Concentrations of the mobile phase (NaOH) were: 2.5 m*M* (a); 5 m*M* (b); 15 m*M* (c); 20 m*M* (d); and 19 m*M* (e). Note that the elution order for the pair xylose–mannose is reversed at 15, 19 and 20 m*M* NaOH. The resolution (R_s) between two peaks in a chromatogram is given by the formula $R_s=2[(t_r)_A-(t_r)_B]\times(W_A+W_B)^{-1}$ where $(t_r)_A$ and $(t_r)_B$ are the retention times of the peaks A and B; and W_A, W_B are the widths at the base of peaks A and B, respectively. In our study, we considered that complete resolution is on the order of $R_s=1.5$.

of the stationary mass transfer characteristics. Retention times usually increased at low temperatures because of the poor mass transport. Such process might explain the better separation of the pairs rhamnose/arabinose and mannose/xylose at low temperatures (2.5 m*M*, 10°C; 15 m*M*, 10°C; 20 m*M*, 15°C; and 19 m*M*, 17°C). According to the same authors certain solutes (such as monosaccharides) or functional groups may change their degree of ionization at elevated temperatures, leading to changes in

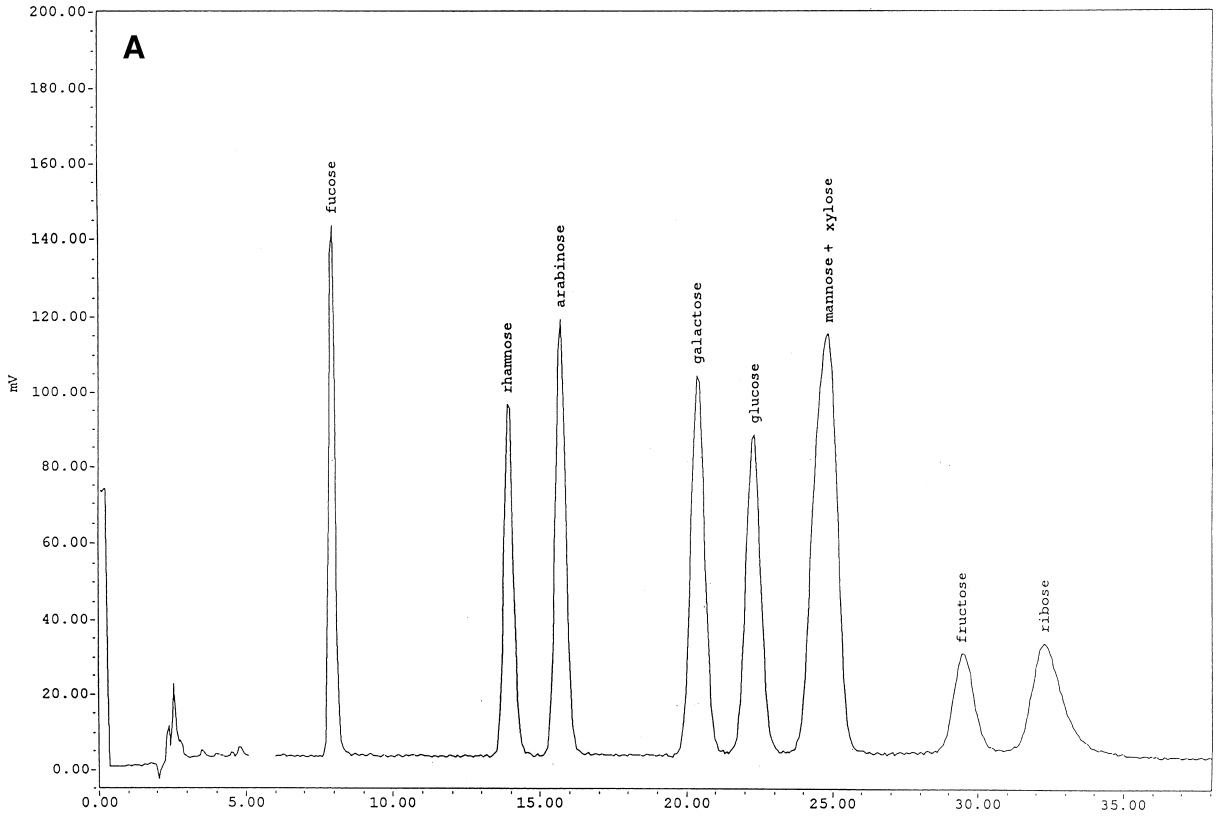

Fig. 2. Typical chromatograms of standards as well as hydrolyzed marine samples including marine particles and mucus of nine monosaccharides by HPAEC with integrated PAD using isocratic elution of NaOH: standard 15 m*M* 25°C (a), standard 19 m*M* 17°C (b), marine particles 19 m*M* 17°C (c), mucus 19 m*M* 17°C (d). Standards were diluted in Milli-Q water at a final concentration (each) of 2.5 μ*M*. Analytical conditions were 1 *M* NaOH post-column addition (flow-rate 0.2 ml min^{-1}) with PAD and a total flow-rate of 0.9 ml min^{-1}; sample loop 20 μl.

the solute retention and selectivity. This may explain why we observed an improvement in selectivity of both pairs rhamnose–arabinose and mannose–xylose at elevated temperatures (2.5 m*M*, 45°C; 5 m*M*, 40°C).

3.2. Van't Hoff plots

We also examined $\ln(k')$ plotted versus $1/T$ (Van't Hoff representation) for the five conditions of mobile phase tested (i.e., 2.5, 5, 15, 20, 19 m*M* NaOH) in order to explain our previous results in terms of enthalpy and entropy. The slopes of the curves in Van't Hoff plots, are related to the enthalpy of the retention process. Our results showed that the Van't Hoff plot generates a linear relationship indicating that enthalpy and entropy of the retention processes (for solute transfer from the mobile to the stationary phase) remain constant in the temperature range 10–45°C for each monosaccharide (Table 2). This was observed for all the NaOH concentrations tested (results not shown). Therefore, the effects of temperature on the retention time are mainly due to direct dependence of k' which is in agreement with Landberg et al. [18] study related to oligosaccharide retention on PA100 column. The lack of a transition, over the temperature interval investigated (10–45°C) may suggest that the bonded phase (quaternary amines) does not change abruptly with temperature [25]. This further indicates that changes in the

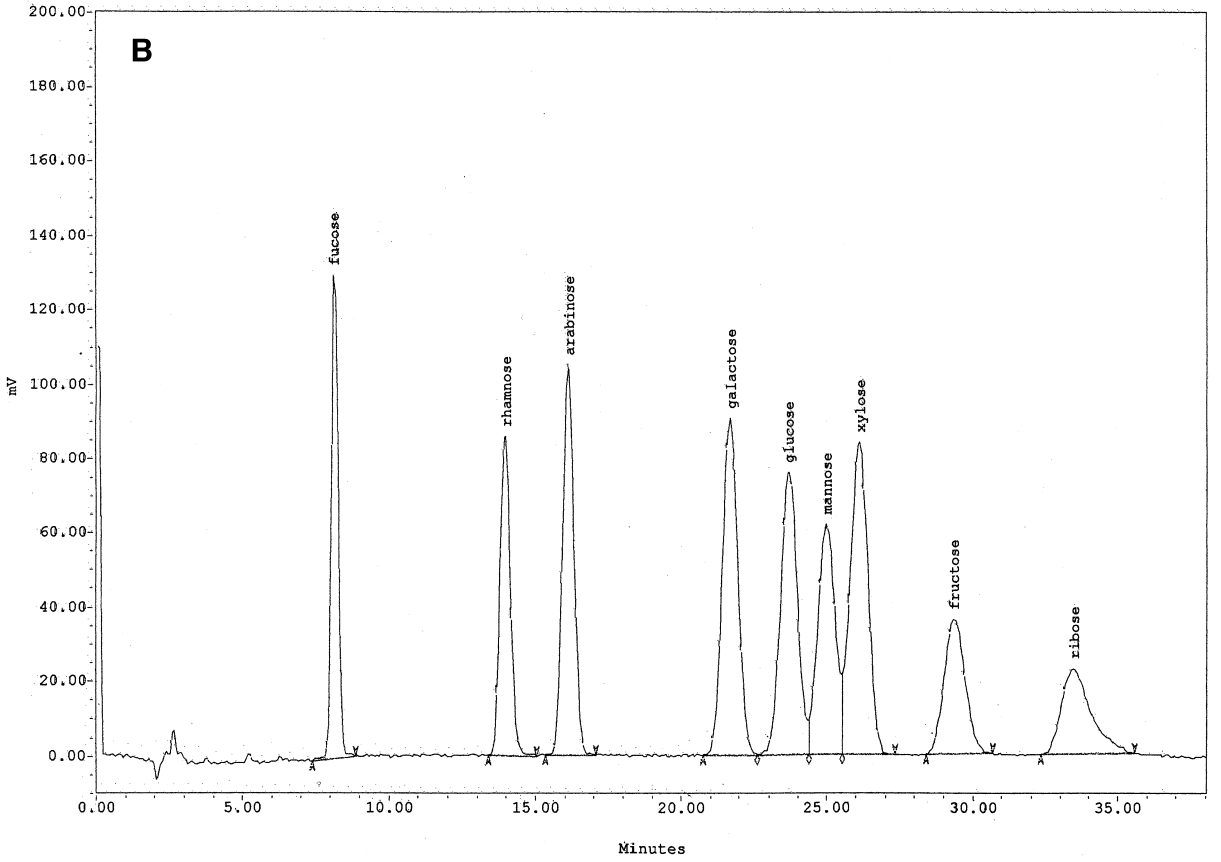

Fig. 2. (*continued*)

retention processes are mainly due to temperature than other associated changes in the system.

3.3. Calibration, precision and detection limit

The calibration range we used in this study varied from 250 to 2500 n*M* (loop 20 μl), corresponding to the same order of concentrations found in marine hydrolyzate samples. The response was linear for all the nine sugars under the applied conditions of 19 m*M* NaOH at 17°C. The results clearly showed the high correlation coefficients ($r>0.99$) that makes feasible the quantitative determination of these monosaccharides within this concentration range. Precision was tested by performing a series of six identical runs (19 m*M* NaOH at 17°C) with a nine-component sugar standard solution (2.5 μ*M* per sugar). The relative standard deviation (RSD) obtained for the retention time as well as for peak area were 0.5 and 5%, respectively. The precision for the surface area for the ribose (17%) was found to be higher in comparison with the other monosaccharides as it has been previously observed [7]. The detection limit of the method using our HPAEC–PAD system under isocratic elution of 19 m*M* NaOH at 17°C was found to be about 10 n*M* ($S/N=3$, loop 200 μl) for all the monosaccharides. This is in agreement with previous results obtained with other detectors and other analytical conditions including eluent concentrations and column temperature [10,12,13].

3.4. Application to marine samples

Our method was tested on two different kinds of

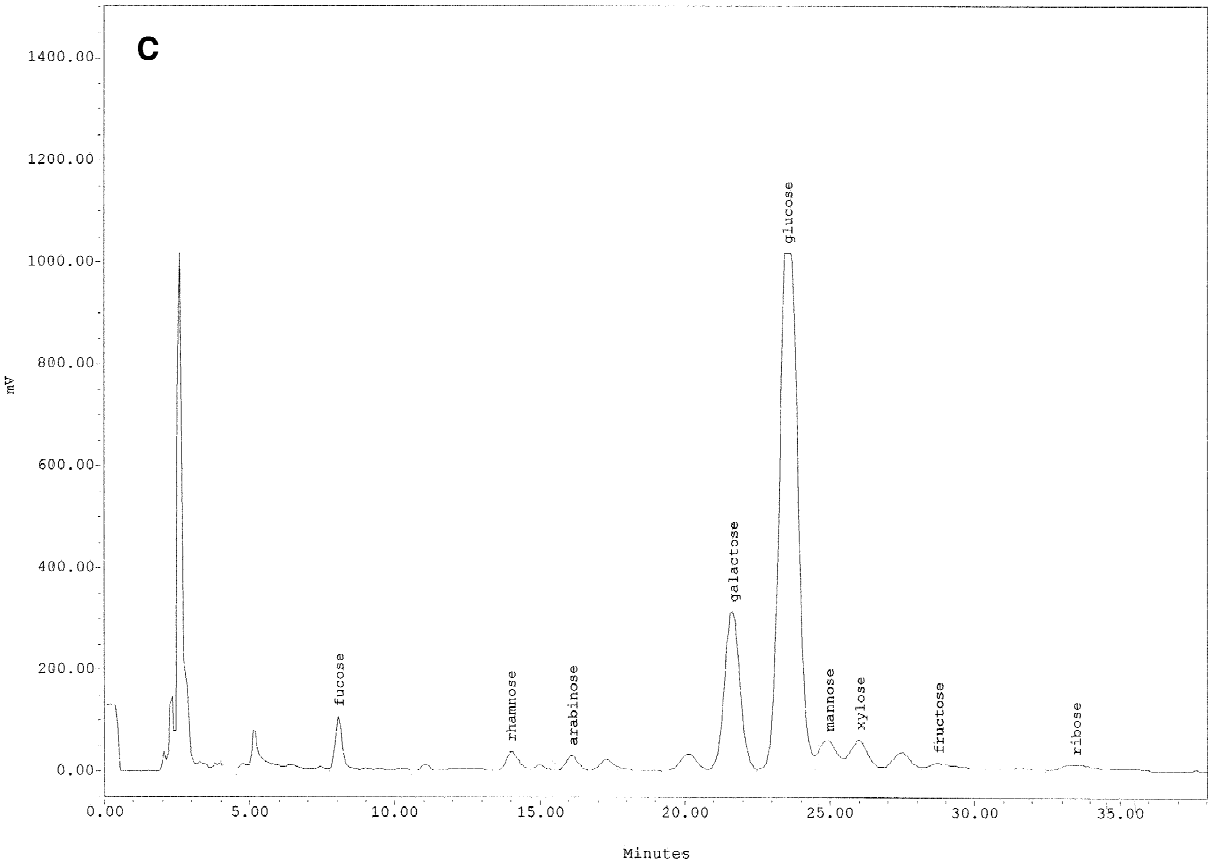

Fig. 2. (*continued*)

marine samples. The chromatograms are presented in Fig. 2c,d and indicated the presence of large amounts of monosaccharides. Seven monosaccharides, including fucose, rhamnose, arabinose, galactose, glucose, mannose and xylose were adequately separated and quantified by using 19 m*M* NaOH as eluent concentration at 17°C. Fructose and ribose were probably degraded during hydrolyses and found as minor peaks, which is consistent with the studies of Borch and Kirchman [12]. Glucose was by far the most abundant monosaccharide found in marine particles followed by galactose and xylose, whereas glucose, xylose and fucose were the dominant peaks in the mucus sample (Fig. 2c,d). Similar molecular distribution for the particulate organic matter sample (high relative abundance of glucose, galactose and xylose) have already been reported for dissolved monosaccharides in surface waters [3,12]. The concentrations of monosaccharides ranged from 30 n*M* (arabinose) to 1.24 μ*M* (glucose) and from 2.96 μg g^{-1} (xylose) to 0.32 μg g^{-1} (rhamnose) for the particles and the mucus sample, respectively.

The results obtained from the calibration curve with standard monosaccharides were verified by spiked experiments on the diluted marine sample. Table 3 gives the results of the spiked experiment of the most six common monosaccharides abundant in the marine particles. The concentrations linearly increased with the amounts of monosaccharides spiked and all the coefficients of correlation were comprised within the range 0.998–0.999. The slopes of the lines ranged from 0.77 to 0.91 among the

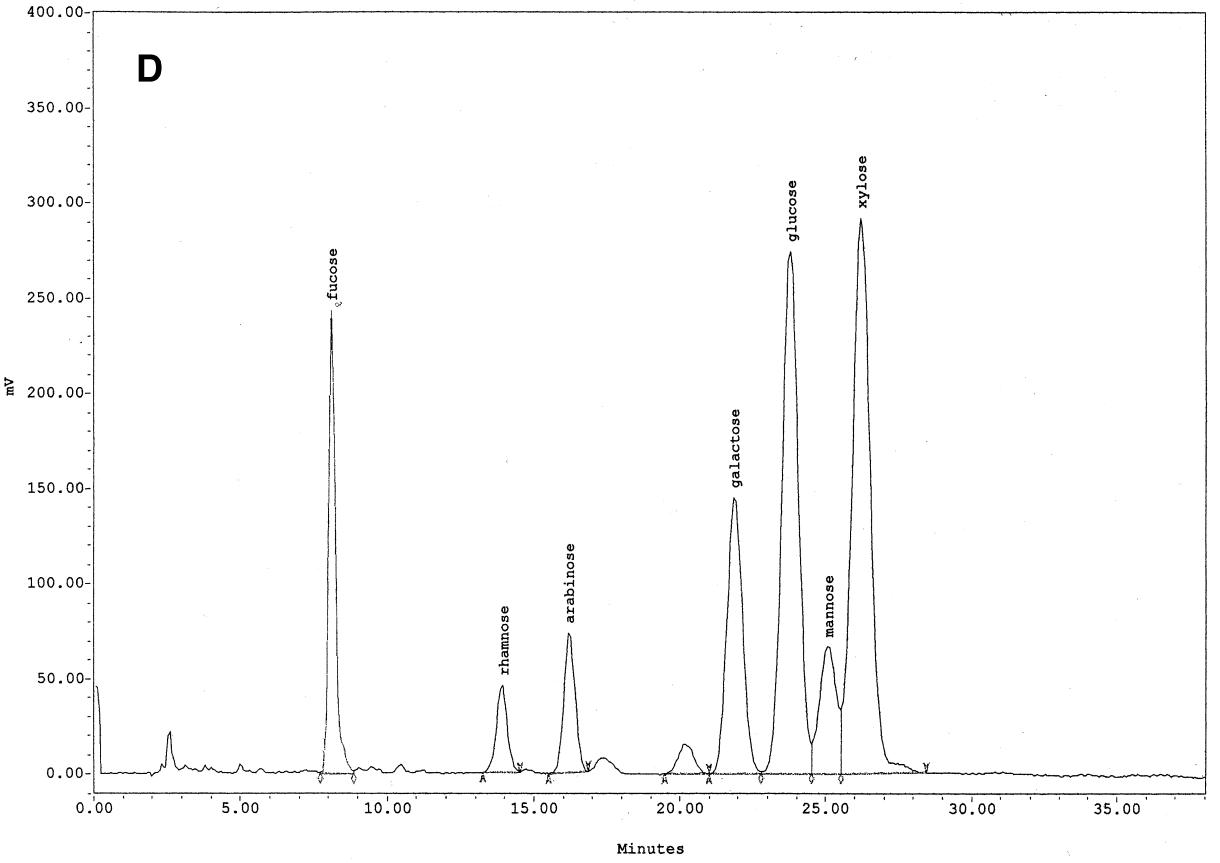

Fig. 2. (*continued*)

Table 2
Plots of $\ln k'$ as a function of temperature (Van't Hoff plots)

Monosaccharides	Slope	*y* Intercept	Correlation coefficient (*r*)
Fucose	0.545	−0.857	0.992
Rhamnose	0.670	−0.627	0.994
Arabinose	0.634	−0.345	0.991
Galactose	0.852	−0.774	0.994
Glucose	0.967	−1.072	0.995
Mannose	0.787	−0.388	0.993
Xylose	0.941	−0.876	0.995
Fructose	0.648	+0.261	0.992
Ribose	0.866	−0.348	0.991

Analytical conditions: mobile phase 19 m*M* NaOH, monosaccharides 2.5 μ*M* each, sample loop 20 μl, post-column addition 1 *M* NaOH (flow-rate 0.2 ml min^{-1}), total flow-rate 0.9 ml min^{-1}. Ln k' has been shown to be related to the temperature through the following equation: $\ln k' = -\Delta H^\circ/RT + \Delta S^\circ/R + \ln \Phi$, where k' is the capacity factor, ΔH° and ΔS° are, respectively, enthalpy and entropy of transfer (mobile to stationary phase), R is the ideal gas constant, T is the absolute temperature (K), and Φ is the phase ratio (volume stationary phase/volume mobile phase).

different sugars indicating that the response of the individual compounds measured with PAD is somewhat different for each compound which is consistent with previous expertise [26].

Table 3
A spiked experiment for marine particles

Monosaccharides	Slope	*y* Intercept	Correlation coefficient (*r*)
Fucose	0.819	0.139	0.999
Rhamnose	0.795	0.068	0.999
Arabinose	0.770	0.060	0.999
Galactose	0.891	0.969	0.999
Glucose	0.906	4.346	0.999
Mannose	0.909	0.232	0.998
Xylose	0.810	0.236	0.999

Monosaccharides standards were spiked into 10 ml of marine sample and injected (20 μl) at 17 °C and for a NaOH concentration of 19 m*M*.

Acknowledgements

We are grateful to Professor A. Siouffi and Drs. B. Charrière, D. Delmas, G. Pepe, and D. Repeta for comments on the MS. and discussions. The authors also thank Dr. A. Metaxatou and Dr. L. Ignatiades, who kindly provided mucus samples, as well as Dr. M. Denis chief scientist of the Antares 4 oceanographic cruise. Financial support for C.P. came from ''Conseil Général des Bouches du Rhône'' France and the Alexander S. Onassis Public Benefit Foundation Greece. This work was partly supported by France-JGOFS Antares-4 project.

References

[1] S.M. Henrichs, P.M. Williams, Mar. Chem. 17 (1985) 141.
[2] J.D. Pakulski, R. Benner, Mar. Chem. 40 (1992) 143.
[3] M.D. McCarthy, J. I Hedges, R. Benner, Chem. Geol. 107 (1993) 503.
[4] L.I. Aluwihare, D.J. Repeta, R.F. Chen, Nature 387 (1997) 166.
[5] A. Skoog, R. Benner, Limnol. Oceanogr. 42 (1997) 1803.
[6] J. Zhou, K. Mopper, U. Passow, Limnol. Oceanogr. 43 (1998) 1860.
[7] P. Kerhervé, B. Charrière, F. Gadel, J. Chromatogr. A 718 (1995) 283.
[8] K. Mopper, Mar. Chem. 5 (1977) 585.
[9] C.M. Burney, Limnol. Oceanogr. 31 (1986) 427.
[10] R.J. Wicks, M.A. Moran, L.J. Pittman, R.E. Hodson, Appl. Environ. Microbiol. 57 (1991) 3135.
[11] J.H. Rich, H.W. Ducklow, D.L. Kirchman, Limnol. Oceanogr. 41 (1996) 595.
[12] N.H. Borch, D.L. Kirchman, Mar. Chem. 57 (1997) 85.
[13] K. Mopper, C. Schultz, L. Chevolot, C. Germain, R. Revuelta, R. Dawson, Environ. Sci. Technol. 26 (1992) 133.
[14] N.O.G. Jørgensen, R.E. Jensen, FEMS Microbiol. Ecol. 14 (1994) 79.
[15] D.C. Johnson, W.R. LaCourse, Anal. Chem. 62 (1990) 589.
[16] K. Mopper, J. Zhou, K.S. Ramana, U. Passow, H.G. Dam, D.T. Drapeau, Deep-Sea Res. II 42 (1995) 47.
[17] R.D. Rocklin, C.A. Pohl, J. Liq. Chromatogr. 6 (1983) 1577.
[18] E. Landberg, A. Lundblad, P. Påhlsson, J. Chromatogr. A 814 (1998) 97.
[19] A. Klockow, A. Paulus, V. Figueiredo, R. Amadò, H.M. Widmer, J. Chromatogr. A 680 (1994) 187.
[20] Dionex Corporation Sunnyvale, Dionex Technical Note 21 TN21, 1989.
[21] V. Ittekkot, E.T. Degens, Limnol. Oceanogr. 27 (1982) 770.
[22] C. Germain, Ph.D. Thesis, Perpignan University, December 1989, p. 230.
[23] R. Andersen, A. Sørensen, J. Chromatogr. A 897 (2000) 195.
[24] C.A. Pohl, J.R. Stillian, P.E. Jackson, J. Chromatogr. A 789 (1997) 29.
[25] L.C. Sander, S.A. Wise, Anal. Chem. 61 (1989) 1749.
[26] Y.C. Lee, J. Chromatogr. A 720 (1996) 137.