# Determination of Maltitol, Isomaltitol, and Lactitol by High-pH Anion-Exchange Chromatography with Pulsed Amperometric Detection

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Disaccharide alditols (DAs) such as maltitol, isomaltitol, and lactitol are increasingly being employed in food industry by virtue of their low hydroscopicity, high stability, and good bulking properties. Still, these compounds are reduced-calorie sweeteners, so they are successfully employed in many dietetic foods, like candies, chocolates, baked products, ice creams, and beverages. Here we describe the determination of maltitol, isomaltitol, and lactitol, along with other common carbohydrates, in some foodstuffs such as toffees, biscuits, creams, sponge cakes, chocolates, roasted malt, and chicory leaves. Separations were accomplished by high-pH anion-exchange chromatography (HPAEC) with pulsed amperometric detection using 40 mM NaOH + 1 mM Ba(CH$_3$COO)$_2$ as the mobile phase. The optimal detection potential ($E_{DET}$ = +0.10 V) was established in voltammetric experiments carried out in batch and flowing stream solutions. Under optimized conditions there was no need for both postcolumn addition of strong bases to the eluent and, even more important, column regeneration between runs. A pellicular column with a relatively low ion-exchange capacity was adopted, which allows a rapid separation of sorbitol, isomaltitol, lactitol, maltitol, glucose, fructose, sucrose, and lactose. The presence in the alkaline mobile phase of barium ions improved selectivity and reproducibility besides shorter analysis times as well. Limits of detection were on the order of 10−20 pmol injected. The contents of DAs and other free sugars in some dietetic foods were evaluated by calibration graphs.

**Keywords:** *Alditols; chromatography; amperometric detection; dietetic foodstuffs*

## INTRODUCTION

Traditional synthetic sweeteners such as saccharin and aspartame provide a very low amount of calories and, at the same time, have a sweetening power 200−300 times higher than that of sucrose. Despite these features, they possess only limited bulking properties and cannot be largely employed in the confectionery industries. Disaccharide alditols (DAs), also known as disaccharide sugar alcohols, are the reduced forms of the corresponding disaccharide compounds (Table 1); they are characterized by lower caloric values but similar sweetening power compared to that of sucrose. Maltitol, 4-*O*-α-D-glucopyranosyl-D-sorbitol, exhibits reduced laxative effects but similar high tolerance for humidity as D-mannitol, making it largely preferred in many dietetic products (Rapaille et al., 1995). Isomalt or palatinit is a white and crystalline mixture prepared from sucrose, which is composed of 6-*O*-α-D-glucopyranosyl-D-sorbitol, also known as isomaltitol (GPS), and 6-*O*-α-D-glucopyranosyl-D-mannitol (GPM). Isomalt has only 45−65% of sucrose's sweetening power and is then often used in combination with other sweeteners (Willibald-Ettle and Schiweck, 1996). The even milder sweetening power of lactitol (4-*O*-β-D-galactopyranosyl-D-sorbitol), which is equivalent to ca. 40%, makes it a very versatile ingredient in many low-calorie products (Grimble et al., 1988; Van Velthuijsen and Blankers, 1991).

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**Table 1. Structures of Disaccharide Alditols Studied**



Lactitol

Maltitol

Isomaltitol (GPS)

GPM

Isomalt (Palatinit)
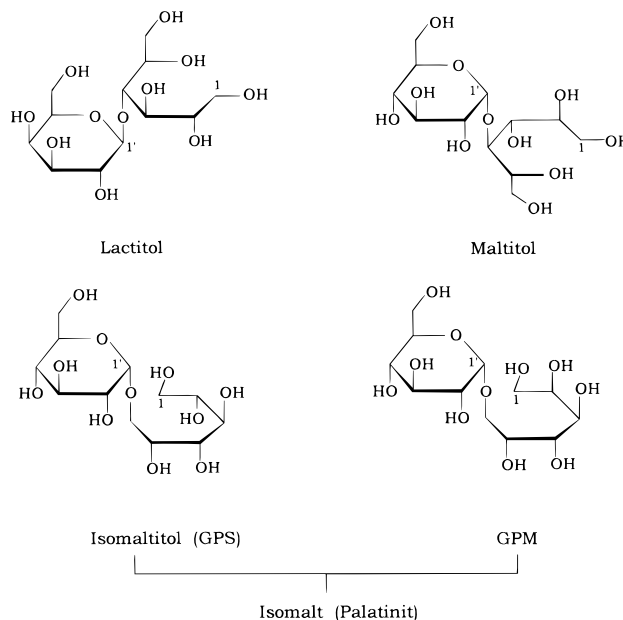
Together with traditional sweeteners or other alditols, it is especially employed for confectionery manufactures (Blankers, 1995; Armstrong, 1998). Conceivably, these compounds are provoking a growing interest in the food industry because they are characterized by low hydroscopicity, high stability, and good bulking properties (McNutt et al., 1996; Koutsou et al., 1996). Indeed, DAs

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are easily incorporated into processed foods and beverages and successfully used in many reduced-calorie products, such as candies, lollipops, chocolates, cough drops, baked products, and ice creams. Additionally, such compounds do not promote dental caries probably because they are not fermented significantly in the mouth (Grenby et al., 1989). The wide variety of foodstuffs in which DAs can be used is particularly helpful for diabetics, because alditols induce a reduced increase of insulin and glucose in the blood.

The weak acidic properties of carbohydrates allow their chromatographic separation through ion-exchange mechanisms. Indeed, high-pH or high-performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC−PAD) is a very powerful tool for the determination of sugars and alditols (Johnson and LaCourse, 1990, 1995; Lee, 1996). Sensitivity, selectivity, and reliability of this technique are relevant aspects for the analysis of food products, often characterized by complex matrixes. Sugar alcohols owing to their even weaker acidity are generally well-separated with a macroporous anion-exchange column using highly basic mobile phases, that is, 0.4−0.6 M NaOH (Dionex, 1994a,b; Corradini et al., 1997; Cataldi et al., 1997a). Interestingly, in this work a pellicular anion-exchange column, characterized by a relatively lower ion-exchange capacity (Dionex, 1995), was successfully employed for the separation of some relevant alditols and sugars. Good resolution of commonly occurring carbohydrates in dietetic foodstuffs was obtained upon optimization of the alkaline eluent composition. Efficiency and reproducibility of the chromatographic results were significantly improved by addition of barium ions to the mobile phase as has been previously described (Cataldi et al., 1997b). We determined the linearity, sensitivity, reproducibility of response, and limits of detection. The applicability of the method has been demonstrated by the analysis of some typical dietetic food products, where maltitol, isomalt, and lactitol are used as sweeteners.

## MATERIALS AND METHODS

**Chemicals.** Sodium hydroxide, 50% solution in water (1.515 g/mL), Ba(CH$_3$COO)$_2$ (99%), α-D-lactose monohydrate (97%), D-sorbitol (99+%), D-xylose (99%), lactitol monohydrate (98%), and MgSO$_4$ (99%) were purchased from Aldrich Chemical Co. (St. Louis, MO), maltitol 98% was from Fluka Chemie (Buchs, CH), NaN$_3$, α-D-glucose (99.5%), sucrose (>99.5%), D-fructose (99%), maltose monohydrate (99%), and isomaltitol (98%) were from Sigma Chemical Co. (Steinheim, Germany). Other chemicals were purchased from Carlo Erba (Milan, Italy) and were used as received. Doubly distilled, deionized water was used throughout for preparing solutions. Sodium hydroxide solutions used as the eluents were prepared by diluting a carbonate-free 50% (w/w) NaOH solution in water, previously filtered with a 0.45-μm membrane and degassed with N$_2$ gas. The exact concentration of hydroxide ions in the mobile phase was determined by titration against a standard solution of hydrochloric acid. Stock solutions of sugars were prepared in pure water and were stabilized with 0.1% sodium azide to prevent microbial growth. Carbohydrate standard solutions to be injected were prepared fresh daily by dilution of the stock solutions. Sample solutions were filtered just prior to injection with nylon membranes, 0.2 μm, Nylaflo (Aldrich).

**Voltammetric Apparatus.** Voltammetric data were obtained at a rotating disk electrode (RDE) using a model EDI101 rotator from Radiometer Analytical (Copenhagen, Denmark) and a model 263A potentiostat (EG&G Princeton Applied Research, Princeton, NJ). Data acquisition and potentiostat control were accomplished with a computer running the M270 electrochemical research software (EG&G) version 4.11. Electrochemical experiments were made using a conventional single-compartment glass cell (PAR). A gold disk electrode (2.0-mm diameter; Radiometer Analytical) was used for all voltammetric investigations, and the gold surface was polished with 0.05 μm of alumina oxide powder on microcloth using water as the lubricant prior to each series of experiments. A Pt auxiliary electrode and a Ag|AgCl (saturated KCl) reference electrode were used. High-purity N$_2$ gas was bubbled through the cell to remove dissolved O$_2$ prior to scan sweeps, and an atmosphere of N$_2$ was maintained over the cell solutions during voltammetric experiments. The current−potential ($i$−$E$) curves reported correspond to the stable profiles obtained after four potential cycles. All experiments were carried out at room temperature, ∼20 °C.

**HPAEC−PAD System.** Carbohydrates analyses were performed using a Dionex system (Dionex Corp., Sunnyvale, CA) composed of a metal-free isocratic pump model IP20 with on-line degas, a pulsed amperometric detector (model ED40), and a metal-free rotary injection valve equipped with an injection loop of 10 μL. A Dionex CarboPac PA100 analytical column (250-mm × 4-mm i.d.) preceded by a guard CarboPac PA100 column (50-mm × 4-mm i.d.) was adopted for the optimized separations. Such a column is composed by sulfonated ethylvinylbenzene−divinylbenzene particles agglomerated with 350 μm of Micro Bead quaternary amine-functionalized latex. Alternatively, a Dionex CarboPac MA1 column, 8.5-μm bead diameter (250-mm × 4-mm i.d.), coupled with a guard CarboPac MA1 column (50-mm × 4-mm i.d.) was used. The flow-through detection cell (Dionex) is made from a 1.0-mm diameter gold working electrode and a pH−Ag|AgCl combination reference electrode; the titanium body of the cell served as the counter electrode. Sodium hydroxide eluents were kept in plastic bottles, and a nitrogen headspace was maintained on the solutions with a Dionex eluent organizer (EO1). A personal computer equipped with the Kontron PC Integration Pack software (Kontron Instruments, Milan, Italy) allowed acquisition and processing of chromatograms. Unless stated otherwise, the pulsed amperometric detector settings were the following: $E_{DET}$ = +0.10 V ($t_{DET}$ = 440 ms, $t_{INT}$ = 240 ms), $E_{OX}$ = +0.80 V ($t_{OX}$ = 180 ms), $E_{RED}$ = −0.80 V ($t_{RED}$ = 360 ms); and $E_{DET}$ = +0.05 V ($t_{DET}$ = 450 ms, $t_{INT}$ = 300 ms), $E_{OX}$ = +0.65 V ($t_{OX}$ = 190 ms), $E_{RED}$ = −0.15 V ($t_{RED}$ = 340 ms) for separations carried out with a CarboPac PA100 and a CarboPac MA1 column, respectively. Flow-injection experiments were carried out by the same aforementioned equipment except for the column.

As detection mode, pulsed amperometric detection (PAD) was adopted. Current is measured and integrated with respect to time to give a net faradic charge ($q$) for the detection cycle, so the response is measured in coulombs (LaCourse, 1997). All experiments were carried out at room temperature in isocratic elution using a flow rate of 0.5 and 0.4 mL/min with CarboPac PA100 and MA1 columns, respectively. As described in previous work (Cataldi et al., 1997b), the addition of barium

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acetate to the alkaline solution was accomplished a few hours before using the eluent, so to allow the precipitation of barium carbonate in the eluent reservoir. In this way there is no need to filter out the modified eluent (Cataldi et al., 1998b). Compared to 0.5–0.6 M NaOH eluents, we recognize that better results are obtained when an inert gas headspace is kept on the eluent solution, especially with lower NaOH concentrations, i.e., $\leq$50 mM (Cataldi et al., 1998a).

**Samples.** Sponge cake and cream preparations from Giusto (Latina, Italy), dietetic toffees from Sella (Schio, Italy), Perfetti "Golia-Activ Plus-Forest Fruit" (Milan, Italy), dietetic biscuits from Dieterba (Latina, Italy), and dietetic chocolate from Gazzoni (Bologna, Italy) were purchased from a local retailer. Roasted malt was kindly provided by a local beer manufacturer. Carbohydrates were extracted following the procedures described in the next section. All samples were analyzed in triplicate.

**Sample Preparation.** *Roasted Malt.* A suspension of finely ground sample, 0.5 g in 20 mL of water, was sonicated for 20 min. Removal of proteins and fats was accomplished by treating a part of the extracted liquid with Carrez clearing reagents (Andrews, 1984); 10 mL of the sonicated solution was mixed with 1.75 mL of a 7.2% (w/w) $K_4Fe(CN)_6 \cdot 3H_2O$ water solution and 1.75 mL of a 14.4% (w/w) $ZnSO_4 \cdot 7H_2O$ water solution. Then, the mixture was stirred for 15 min. Afterward, 6.5 mL of a phosphate buffer (pH 7.5) solution containing 0.1% (w/w) $MgSO_4 \cdot 7H_2O$ and 0.3% (w/w) $NaN_3$ was added, and the mixture was stirred again for 15 min and finally filtered. The solution was diluted 1:5 with water, filtered, and injected in the column.

*Chicory.* Chicory crushed leaves (2 g) were extracted by sonication in 25 mL of water for 20 min. The suspension was then centrifuged and the supernatant filtered, diluted 1:2, and injected.

*Biscuits.* Some biscuits were broken into little pieces, and a sample was suspended in water (0.5 g/20 mL); then the mixture was sonicated for a few minutes; 10 mL was treated with the Carrez agents, and an aliquot of the final solution was finally diluted 1:300, filtered, and injected.

*Toffees.* To allow the dissolution of the toffee, 0.5 g of ground sample was sonicated in 20 mL of water for 15 min; a solution aliquot was then diluted 1:2000, filtered, and injected.

*Chocolate.* A suspension of crushed sample in pure water (i.e., 0.5 g in 20 mL) was sonicated for 20 min. The resulting mixture was then filtered, and 10 mL of suspension was treated with Carrez reagents to remove proteins and fats from the extract, following the procedure described above for roasted malt; the final solution was then diluted 1:200, filtered, and injected.

*Cream and Sponge Cake Preparations.* A sample powder was suspended in water (0.5 g in 20 mL) and sonicated for 20 min. The solutions were treated with Carrez agents, then diluted 1:1500 (cream) or 1:500 (sponge cake), filtered, and injected.

## RESULTS AND DISCUSSION

**Electrochemical Oxidation of Disaccharide Alditols in Barium-Containing Alkaline Solutions.** Electrochemical characterization was performed using lactitol as a model compound. Figure 1 shows the voltammetric response (*i*–*E*) of 0.25 mM lactitol at a gold rotating disk electrode (RDE) in deareated alkaline solutions, 40 mM NaOH (dashed line) and 40 mM
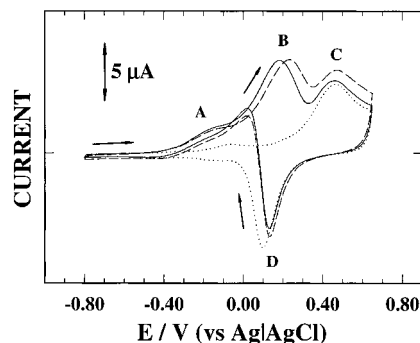


**Figure 1.** Cyclic voltammetric response for 0.25 mM lactitol at a Au RDE in deareated 40 mM NaOH + 1 mM Ba(CH₃-COO)₂ (solid curve) and 40 mM NaOH (dashed curve) solutions. The dotted line represents the voltammetric profile of the gold electrode in the Ba-containing alkaline electrolyte. Electrode rotation speed, 900 rpm (94.2 rad/s); scan rate, 50 mV/s.
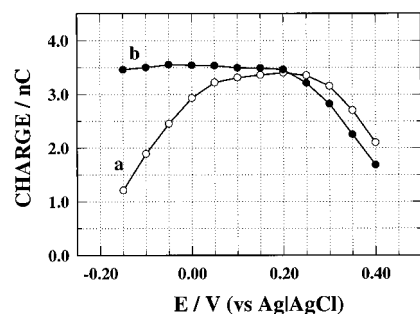


**Figure 2.** Effect of barium on the detection potential for 100 $\mu$M lactitol in flow-injection PAD using 40 mM NaOH (a) and 40 mM NaOH + 1 mM Ba(CH₃COO)₂ (b) as the carrier electrolyte. Flow rate, 0.5 mL/min; loop, 10 $\mu$L.

NaOH + 1 mM Ba(CH₃COO)₂ (solid line); the dotted line is the voltammetric profile of Au in the blank supporting electrolyte. As can be seen, the anodic response of lactitol during the forward scan exhibits two oxidation waves. The first one (A) starts at ca. $-0.30$ V vs Ag|AgCl, whereas the large anodic wave (B) displays a peak maximum at $+0.23$ V in 40 mM NaOH (see dashed line). The peak potential is influenced by the presence of barium ion in the supporting electrolyte, because the oxidation peak is shifted by ca. 50 mV toward a less positive one (solid line). The anodic wave (C) formed during the forward scan, which exhibits a peak potential at about $+0.47$ V, corresponds to gold oxide formation and continues until the potential scan is reversed at $+0.65$ V. Its subsequent reduction is identified in the cathodic peak (D) during the reverse scan. The oxidation of maltitol is similar to that of lactitol.

To verify the effect of Ba²⁺ on the current signal, the applied potential to the working electrode for the chromatographic determination of DAs was optimized in flow-injection experiments. Figure 2 shows the plots of the faradic anodic charge versus detection potential resulting from the flow-injection analysis of lactitol before (curve a) and after (curve b) addition of 1 mM Ba(CH₃COO)₂ to 40 mM NaOH as the carrier electrolyte. A typical pulsed amperometric waveform was applied to the working electrode as described in Materials and Methods; the detection potential ($E_{DET}$) was incremented by 50 mV steps, starting from $-0.20$ to $+0.40$ V, and the charge signal was recorded upon 10-$\mu$L injections of a 100 $\mu$M lactitol solution. In the absence

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**Table 2. Capacity Factors (*k*) of Some Alditols and Sugars in HPAEC**

| | column A[a] | | column B[b] | | |
|---|---|---|---|---|---|
| | 0.6 M NaOH | 0.5 M NaOH | 0.075 M NaOH | 0.060 M NaOH | 0.040 M NaOH |
| sorbitol | 1.77 | 1.97 | 0.49 | 0.57 | 0.66 |
| isomaltitol | 2.01 | 2.59 | 0.90 | | |
| lactitol | 2.33 | 2.80 | 1.05 | 1.20 | 1.54 |
| maltitol | 3.46 | 4.22 | 1.87 | 2.42 | 2.97 |
| glucose | 2.73 | 3.43 | 2.03 | 2.80 | 3.72 |
| fructose | 3.45 | 4.30 | 2.42 | 3.50 | 4.84 |
| sucrose | 5.76 | 7.33 | 3.97 | 5.21 | 7.02 |
| lactose | 3.32 | 4.28 | 4.15 | 6.02 | 8.30 |

[a] Dionex CarboPac MA1 plus guard column; flow rate, 0.4 mL/min; back pressure, 110 bar; $t_M$ = 5.6 min. [b] Dionex CarboPac PA100 plus guard column; flow rate, 0.5 mL/min; back pressure, 98 bar; $t_M$ = 2.54 min. Capacity factor evaluated as $k' = (t_R - t_M)/t_M$, where $t_R$ is the retention time and $t_M$ is the column dead time.

of Ba$^{2+}$, the signal maximum lies at about +0.25 V, whereas with the modified carrier electrolyte a signal plateau from +0.20 to −0.20 V was observed, thus suggesting the possibility of using a lower detection potential. Note that −0.20 V represents the most negative $E_{DET}$ investigated because of the large background noise. A similar behavior was observed for the other DAs of interest, in agreement with previously reported data on D-sorbitol, D-glucose, and sucrose (Cataldi et al., 1997c). Based on the recommended values reported by LaCourse and Johnson (1993) when amperometric experiments are performed in 40 mM sodium hydroxide, the detection potential that should be applied for the highest signal-to-noise ratio is about +0.23 V vs Ag|AgCl. Interestingly, in the presence of Ba$^{2+}$ and according to the results obtained by voltammetric studies, it is possible to apply a lower $E_{DET}$ (i.e., <+0.20 V) to the working electrode. A detection potential equal to +0.10 V vs Ag|AgCl was therefore chosen as the optimal value, since massive reduction of dissolved oxygen hinders the application of a less positive potential. An advantage to appling such a potential is to increase selectivity of detection, thus preventing interference from other species likely occurring in real samples.

**Optimization of the Chromatographic Conditions.** As monosaccharide alditols and DAs are weaker acids than sugars, they are successfully separated employing a macroporous anion-exchange column, characterized by a relatively high ion-exchange capacity (column A in Table 2), using strongly alkaline eluents. Despite the general applicability of such a choice, as has been confirmed in some recent publications (Cataldi et al., 1997b, 1998a; Corradini et al., 1997), we noted that even upon changing the concentration of the basic eluent the chromatographic selectivity is not suitable for certain compounds (see Table 2). For instance, an accurate quantification of maltitol, fructose, and lactose is hindered as they elute at approximately the same retention time. A different column was thus tested for the separation of DAs and other commonly occurring sugars in reduced-calorie products. Column B in Table 2, which is characterized by a relatively lower ion-exchange capacity (90 $\mu$equiv/column) is tailored for oligosaccharide analysis. By employing 40 mM NaOH as the eluent, such a column allowed excellent separation of the sample molecules of interest; more concentrated alkaline solutions provide a less effective resolution of some sugar molecules, as reported in Table 2.

It is recognized that the reproducibility of the chromatographic data in HPAEC is strongly affected by the interference of carbonate. Such a divalent ion is always present despite the precautions used during the alkaline eluent preparation. It seems that carbonate ions tend to progressively occupy the active sites of a column, thereby progressively decreasing the retention of sugar molecules (Cataldi et al., 1998b). The problem is particularly striking when relatively low concentrated alkaline mobile phases are employed (i.e., ≤50 mM NaOH), since OH$^-$ does not effectively remove carbonate ions from the ion-exchanging sites. Recently, we have demonstrated that the addition of millimolar concentrations of Ba$^{2+}$, Ca$^{2+}$, or Sr$^{2+}$ in the alkaline eluents causes an effective minimization of carbonate (Cataldi et al., 1997b). In the case described here, the modification of the mobile phase with Ba(CH$_3$COO)$_2$ has allowed an excellent within-day working reproducibility of the chromatographic data without recurring to regeneration steps after each run. To compare the inherent advantage of using barium ion as an eluent additive, in Table 3 are reported the retention times of sugars and alditols evaluated over about 7 h during which the sample mixture was repetitively injected. Upon column regeneration the retention times obtained using 40 mM NaOH + 1 mM Ba(CH$_3$COO)$_2$ as the eluent exhibited excellent reproducibility; the relative standard deviation for all investigated compounds was lower than that evaluated for sucrose, i.e., 1.3%. On the contrary, the employment of a conventional eluent (e.g., 40 mM NaOH) imparted a gradual reduction of the retention for all compounds; after about 6 h of operation the retention time of lactose decreased up to 45% of the initial value.

Figure 3 shows a typical chromatographic separation of a sample mixture of alditols and carbohydrates using HPAEC in conjunction with PAD accomplished under isocratic conditions using a mobile phase composed of 40 mM NaOH + 1 mM barium acetate. Note that the elution of all compounds was completed in about 22 min of chromatographic run. This duration is considerably shorter than that required with a column, especially designed for the separation of alditols using stronger alkaline eluents (see data for column A in Table 2). It is worthy of note also that in the presence of Ba$^{2+}$, the column efficiency was noticeably increased, especially for sugar alcohols, which exhibited more tailed peaks using unmodified eluents. The effect of barium has been explained by invoking specific interactions between Ba$^{2+}$ and the sorbitol or mannitol moiety of DAs. It is recognized that the complexes between metal cations and carbohydrates possessing triol sequences such as sorbitol are relatively strong (Angyal, 1989).

**Retention, Calibration Data, and Limits of Detection.** Excellent chromatographic separation was obtained using the optimized chromatographic conditions, which imply the presence of barium in the alkaline eluent. The results including linear range, capacity factors, detection limits, and data of the peak area calibration plots are summarized in Table 4. The linearity extends over 2 or 3 orders of magnitude above the limit of detection (LOD), with correlation coefficients (*r*) greater than 0.999. Each data point (*n* = 6−7 concentrations in the linear range) was generated from at least three separate injections. Note that although the dynamic range of each compound extended to 3 orders of magnitude above the LOD, alditols and especially DAs were featured by a restricted linear range, which is typical of PAD for strongly adsorbed

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**Table 3. Retention Times of Sugars and Alditols Evaluated by HPAEC–PAD during Repetitive Injections of a Sample Mixture Using 40 mM NaOH and 1 mM Ba(CH₃COO)₂ as the Eluent[a]**

| | | retention time, $t_R$ (min) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| run | time (h) | sorbitol | isomaltitol | lactitol | maltitol | glucose | xylose | fructose | sucrose | lactose |
| 1 | first inj | 3.94 | 5.03 | 5.66 | 8.55 | 10.73 | 11.55 | 12.89 | 17.14 | 19.48 |
| 2 | 0.9 | 3.95 | 5.10 | 5.65 | 8.50 | 10.72 | 11.55 | 12.90 | 16.95 | 19.40 |
| 3 | 1.5 | 3.94 | 5.09 | 5.62 | 8.50 | 10.71 | 11.55 | 12.90 | 16.88 | 19.38 |
| 4 | 3.5 | 3.99 | 5.08 | 5.70 | 8.62 | 10.91 | 11.75 | 13.12 | 17.50 | 19.94 |
| 5 | 4.5 | 3.95 | 5.09 | 5.66 | 8.53 | 10.76 | 11.58 | 12.93 | 17.12 | 19.53 |
| 6 | 6.8 | 3.95 | 5.13 | 5.66 | 8.52 | 10.74 | 11.56 | 12.90 | 17.07 | 19.46 |
| average $t_R$ | | 3.95 | 5.11 | 5.66 | 8.53 | 10.76 | 11.59 | 12.94 | 17.12 | 19.53 |
| RDS% | | 0.48 | 0.14 | 0.44 | 0.60 | 0.70 | 0.68 | 0.70 | 1.3 | 1.1 |

[a] Column, CarboPac PA100 with guard column; flow rate, 0.5 mL/min. Other experimental conditions are reported in Figure 3.

**Table 4. Quantitative Parameters of Some Carbohydrates Determined by HPAEC with Integrated PAD Using 40 mM NaOH and 1 mM Ba(CH₃COO)₂ as the Eluent[a]**

| | | linear portion of the calibration graph:[b] $y = a + bC$[c] | | | | |
|---|---|---|---|---|---|---|
| compound | $K$ | $a \pm t_{95}s_b$ (nC min) | $b \pm t_{95}s_a$ (nC min/$\mu$M) | $r$ | linear range ($\mu$M) | LOD[d] (pmol) |
| sorbitol | 0.54 | $0.2 \pm 0.5$ | $0.45 \pm 0.01$ | 0.9999 | 1–100 | 10 |
| isomaltitol | 1.01 | $0.4 \pm 0.7$ | $0.44 \pm 0.02$ | 0.9997 | 2–100 | 20 |
| lactitol | 1.23 | $0.8 \pm 0.7$ | $0.67 \pm 0.08$ | 0.9997 | 1–50 | 10 |
| maltitol | 2.37 | $0.7 \pm 0.7$ | $0.66 \pm 0.07$ | 0.9995 | 1–50 | 10 |
| glucose | 3.22 | $0.5 \pm 0.8$ | $0.45 \pm 0.01$ | 0.9998 | 2–250 | 20 |
| xylose | 3.53 | $0.6 \pm 0.9$ | $0.35 \pm 0.01$ | 0.9998 | 3–250 | 30 |
| fructose | 4.05 | $-0.7 \pm 0.6$ | $0.35 \pm 0.01$ | 0.9996 | 2–250 | 20 |
| sucrose | 5.74 | $0.5 \pm 0.7$ | $0.44 \pm 0.03$ | 0.9994 | 2–50 | 20 |
| lactose | 6.67 | $0.2 \pm 0.8$ | $0.61 \pm 0.03$ | 0.9995 | 1–50 | 10 |

[a] Column, CarboPac PA100 with guard column; flow rate, 0.5 mL/min; sample loop, 10 $\mu$L; other data are reported in Figure 3. [b] Peak area signal corresponding to nC min. [c] $C$ represents the concentration in $\mu$M; slope, $a$, and intercept, $b$, were evaluated at the 95% confidence level. [d] Limit of detection evaluated as $c_L = 2\kappa[s_B^2 + s_a^2 + (a/b)^2 s_b^2]^{1/2}/b$, where $\kappa = 1.64$ at 95% confidence level and the peak-to-peak noise value $s_B^2$ is comparably lower than $s_a^2$ and $(s_b a/b)^2$. Noise measurements over 2–5-min periods of flat baseline in different chromatograms gave a mean value of 10 pC.
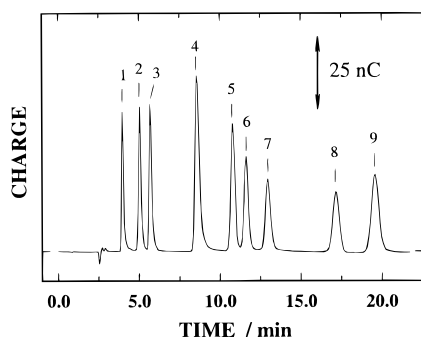


**Figure 3.** Separation in HPAEC with integrated PAD of a mixed standard solution containing: (1) D-sorbitol, 10 $\mu$M; (2) isomaltitol, 10 $\mu$M; (3) lactitol, 10 $\mu$M; (4) maltitol, 20 $\mu$M; (5) D-glucose, 20 $\mu$M; (6) D-xylose, 20 $\mu$M; (7) D-fructose, 20 $\mu$M; (8) sucrose, 20 $\mu$M; and (9) lactose, 20 $\mu$M. Eluent, 40 mM NaOH + 1 mM Ba(CH₃COO)₂ at a flow rate of 0.5 mL/min. The eluent was prepared by carbonate-free 50 wt % NaOH using degassed water continuously sparged with purified N₂. Column, Dionex CarboPac PA100. Detection potential at the gold working electrode, $E_{DET} = +0.10$ V vs Ag|AgCl.

compounds (Johnson and LaCourse, 1990). The standard deviation (*s*) of slope and intercept was estimated at the 95% confidence level. Using the linear regression method (Miller and Miller, 1993) the LODs were calculated to be below 30 pmol injected for all compounds investigated. Good repeatability was obtained as well; using three replicate injections at a concentration level 10 times higher, the LOD peak area RSDs were usually better than 2%. The above results suggest that predominantly alkaline eluents spiked with barium ions should be used to retain the advantages discussed in this study in terms of selectivity of detection and long-term reproducibility of retention and peak area measurements.

**Selected Applications.** As anticipated above, maltitol, isomalt, and lactitol are relatively new sweeteners, which are increasingly being employed in low-calorie, sugar-free foods and beverages. The determination of mono- and disaccharide alditols is currently of interest not only in nutrition and biotechnology but also for understanding their possible role in the biological processes of fruits and vegetables (Loescher et al., 1992). In this section, some selected applications of commercially available dietary products along with vegetal food samples after Carrez clarification are reported. A summary of the quantitation results in HPAEC–PAD using the above-described procedure is presented in Table 5. For all the samples reproducible separations with no appearance of unidentified peaks were obtained.

*Dietetic Chocolate and Biscuits.* Maltitol is characterized by a low hydroscopicity and is therefore ideal for exterior coating of good quality sugar-free chocolates; moreover, the flavor and body allow its use as a bulk sweetener, replacing all the sugar from the chocolate composition. In particular, only the employment of maltitol has allowed the production of dietetic chocolates with a calorie reduction of up to 15% (Rapaille et al., 1995). A typical chromatogram of a commercially available milk-chocolate sweetened with maltitol is shown in Figure 4. Note the presence of lactose at a retention time of 18.92 min (see inset); such a peak is not resolved from that of maltitol using the macroporous column A (see Table 2). As mentioned previously, the bulking properties along with the high-temperature stability of DAs allow their large employment in the industry of baked products. As an example of this category of foods, in Table 5 is also reported the sugar content of biscuits sweetened with maltitol.

**Table 5. Quantitative Results of the Free Sugar Content (g/100 g) in Some Food Samples Determined by HPAEC−PAD[a]**

|  | roasted malt | chicory leaves | toffee I | toffee II | biscuit | cream | sponge cake | chocolate |
|---|---|---|---|---|---|---|---|---|
| sorbitol |  |  | 0.8 | 2.4 |  | 7 |  | 0.2 |
| isomaltitol |  | 43 |  |  |  |  |  |  |
| lactitol |  |  |  | 62 |  | 37 | 39 |  |
| maltitol | 0.05 | 0.003 | 26 | 30 | 22 |  |  | 34 |
| glucose | >2.00 | 0.057 |  |  |  |  |  |  |
| fructose | 0.30 | >0.17 |  |  |  |  |  |  |
| sucrose | 1.70 | 0.023 |  |  |  | 0.9 | 0.15 | 1.3 |
| lactose |  |  |  |  | 0.6 | 12 | 0.6 | 10 |
| maltose | 5.0 |  |  |  |  |  |  |  |

[a] See experimental conditions described in Figure 3.
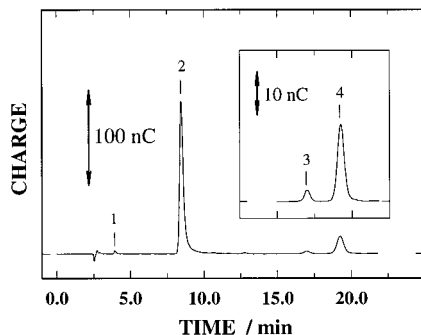


**Figure 4.** Anion-exchange chromatogram with integrated PAD of a reduced-calorie chocolate sample. Peak identification: (1) D-sorbitol, (2) maltitol, (3) sucrose, and (4) lactose (see inset). Experimental conditions were as in Figure 3.
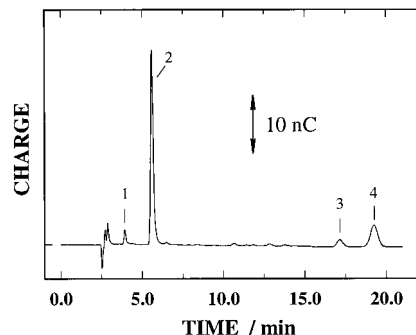


**Figure 6.** Anion-exchange chromatogram with integrated PAD of a sponge cream sample. Peak identification: (1) D-sorbitol, (2) lactitol, (3) sucrose, and (4) lactose. Experimental conditions were as in Figure 3.
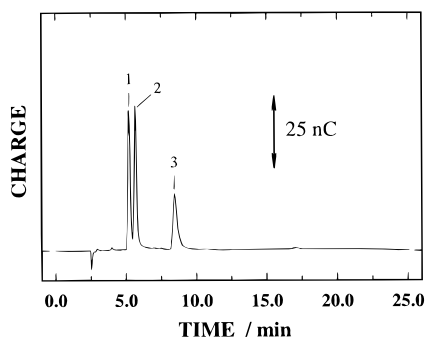


**Figure 5.** Anion-exchange chromatogram with integrated PAD of a toffee I sample. Peak identification: (1) isomaltitol, (2) GPM, and (3) maltitol. Experimental conditions were as in Figure 3.



**Figure 7.** Typical free carbohydrate profile of a roasted malt extract obtained by HPAEC−PAD. Peak identification: (1) D-sorbitol, (2) maltitol, (3) D-glucose, (4) D-fructose, (5) sucrose, and (6) maltose. Unlabeled peaks are unknown compounds. Experimental conditions were as in Figure 3.

*Candies.* The increasing diffusion of isomalt- and lactitol-based toffees is mainly due to the fact that these sweeteners do not influence taste. Indeed, they do not produce the cooling effect characteristic of monosaccharide alditols, like xylitol. Moreover, as already mentioned for maltitol, isomalt possesses a quite low hydroscopicity and is therefore used for external coating of candies. The presence of both isomalt components, together with maltitol, in a typical reduced-calorie toffee is illustrated in the chromatogram of Figure 5. In Table 5 are collected the results obtained for this sample and for a fruit-taste candy sweetened with lactitol and maltitol.

*Cream and Sponge Cake.* Lactitol, owing to its solubility and taste very similar to that of sucrose, is generally employed as a sweetener in the confectionery industry (Blankers, 1995). Figure 6 shows a chromatogram of an extract of cream preparation; four main compounds were identified and quantified: (1) sorbitol, (2) lactitol, (3) sucrose, and (4) lactose. A sponge cake preparation was also analyzed (not shown); a very prominent peak of lactitol was noted, and its content is reported in Table 5. Currently, industry uses reduction
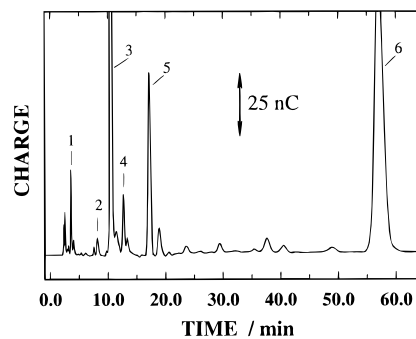
of lactose by catalytic hydrogenation to produce large quantities of food-grade lactitol (Hu et al., 1996). We suggest the usefulness and applicability of the present chromatographic method to follow lactose conversion.

*Natural Products.* Apparently, maltitol is widely diffused in nature, and for instance, we have found it in chicory and roasted malt samples. The high selectivity of the chromatographic conditions employed has allowed the determination of carbohydrate contents even in such relatively complex samples. A representative chromatogram of a roasted malt extract is shown in Figure 7. The major assigned peaks are (1) sorbitol, (2) maltitol, (3) glucose, (4) fructose, (5) sucrose, and (6) maltose, with a level of maltose equal to ca. 50 mg/100 g of dry weight. Of course, as a definite identification of naturally occurring carbohydrates based on the retention time is not conclusive, further support was given both by spiking authentic compounds to the sample extracts and by accomplishing chromatographic separations with different alkaline eluents (not shown). Note that when $E_{RED}$ was set equal to −0.28 V, which is the recommended value by LaCourse and Johnson

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(1993), using 40 mM NaOH as an eluent, some prominent peaks in the chromatograms exhibited a following negative peak. By a suitable choice of $E_{\text{RED}}$ equal to −0.80 V, this relevant problem has been solved, probably because such a potential is more effective at removing adsorbed oxidation products (Jensen and Johnson, 1997). The same experimental conditions were also applied to the separation of an extract of chicory sample; the amount of some identified compounds is reported in Table 5.

## CONCLUSIONS

An optimized HPAEC−PAD method for the determination of some disaccharide alditols and other free sugars in real samples has been developed, which is simple, sensitive, and reproducible. The use of a pellicular anion-exchange column allows rapid and effective separations of alditols and sugars present in many commercially available dietetic foodstuffs. For each sample the chromatogram is interference-free with a few peaks clearly recognizable. These results demonstrate that the eluent modification with $Ba^{2+}$ enhances both the amperometric response of the sugar molecules and the chromatographic data reliability (i.e., retention and peak area) without column regeneration and postcolumn addition of strong bases. The application of a lower detection potential to the Au electrode was also possible in the presence of barium ion.

## ACKNOWLEDGMENT

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