High-Performance Anion-Exchange Chromatography coupled with Pulsed Electrochemical Detection as a powerful tool to evaluate lactose content in lactose-free labeled products

Application Note

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Method	HPAEC-PAD
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Analytes	sucrose, galactose, glucose, fructose, lactose and maltose
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Abstract

An analytical method based on an Agilent Infinity LC 1260 coupled to a Decade Elite Electrochemical Detector has been developed to accurately determine lactose in lactose-free products with different matrices. In addition, the methodology presented herein opens the possibility to quantify with high resolution and accuracy mixtures of 6 common carbohydrates (sucrose, galactose, glucose, fructose and lactose) from different sugar-free matrices in less than 10 minutes.

Introduction

The analysis of lactose has become very important in the last decades due to the increasing population who suffers from lactose intolerance. Nowadays it has been established, following the recommendations of the European Food Safety Authority, that the lactose-free products must contain less than 0.01% (100 ppm) of lactose. This limit, though, may decrease in the upcoming years and therefore it is necessary to develop a robust and accurate method to determine very low lactose concentration in lactose-free products even below actual recommended limits.

Due to the high volume of carbohydrates that food industries process daily, a technique to analyze these compounds in the fastest way with minimum sample preparation is required. In addition, life sciences are also in need of methodologies to determine these compounds at very low concentration since carbohydrates take part of many important physiological processes.

In this application a methodology based on an Agilent 1260 Infinity Binary LC coupled with a Decade Elite Electrochemical Detector from Antec Scientific has been developed to determine accurately lactose with proper resolution in the 0.1-100 ppm range. This methodology has been extended to 6 out of the most common carbohydrates: sucrose, galactose, glucose, fructose, lactose and maltose in a concentration range between 50 and 5000 ppm in food samples with different matrices by only adapting the injection volume of sample. No additional hardware changes are required.

Experimental

Method parameters

The HPLC analysis have been performed using an Agilent Analytical HPLC system consisting of a 1260 Infinity Binary pump (G1312B), 1260 Infinity High Performance Degasser (G4225A), 1260 Infinity High Performance Autosampler(G1367E), an analytical column and the Decade Elite electrochemical detector in multistep pulse mode. The applied anion exchange column is stored in the detector. The Decade Elite is equipped with a cell containing a 2 mm Au working electrode and a HyRef reference electrode, which requires very little maintenance. The use of multistep pulses keeps the surface of the working electrode in optimum conditions and no additional polishing is required. Moreover, the HyRef reference electrode versus the commonly used Ag/AgCl has the advantage that is maintenance free.

MilliQ water, 18MΩ-cm resistance, filtered through a 0.2 µm filter has been used as a solvent for the mobile phase and the prepared standard solutions of lactose and the rest of carbohydrates, which contain a concentration range between 0.1-1000 ppm and 5-5000 ppm, respectively. The mobile phase consists of a solution of NaOH 100 mM prepared from a 50% w/w carbonate-free NaOH stock solution. The mobile phase is continuously sparged with N_2 to avoid further carbonation during the analysis. Alternatively, a CO2 filter can be used on top of the solvent bottle.

Prior to the analysis of any set of samples and/or standard solutions the system has to be flushed and equilibrated during 30 min at the initial conditions (2 mM NaOH).

The HPLC and detector parameters are summarized in Table 1 followed by a picture of the equipment (Figure 1).

Column	CarboPac SA10 (4 x 250 mm)
Mobile phase	Binary gradient H_2O –NaOH, from 2-35 mM
Flow rate	1.8 mL/min
Injection volume	0.5-10 µL
Temperature	40ºC (Column and flow cell)
Detection	Pulsed Amperometric Detection with multistep pulses
Cell	SenCell 2 mm Au HyRef
Detector range	10 µA; 10 % offset
Multistep pulses	E_{cell}: E1, E2, E3, E4: +0.1, -2.0, +0.6, -0.1 V ts, t1, t2, t3, t4: 0.2, 0.4, 0.02, 0.01, 0.07 s I_{cell}: >100 nA

Table 1. HPLC and PAD parameters

Figure 1. HPLC Agilent 1260 Infinity series coupled with a Decade Elite Electrochemical Detector used in this study.
The DECADE Elite can be used with any HPLC & (u)HPLC system from Agilent

Data acquisition uses an AD board input connected to the DECADE Elite 20-bit analog Output. CDS used: Agilent Openlab Chemstation

For instrument control the keyboard can be used or the free Dialogue software is available.

Preparation of standard solutions and samples

All the solutions have been prepared from a stock standard solution containing 1000 ppm of lactose, sucrose, galactose, glucose, fructose and maltose which were prepared dissolving 0.1 g of each carbohydrate in 100 mL of MilliQ water. The carbohydrates contained in food samples with different matrices were extracted using Carrez solutions where no further dilution of the sample was needed. After the extraction, the samples were filtered through a 0.2 µm filter and 10 µL were injected in the HPLC system.

Results

Determination of lactose in lactose-free products

The calibration curve has been determined as the average of three consecutive calibration curves in the range 0.1 to 100 ppm.

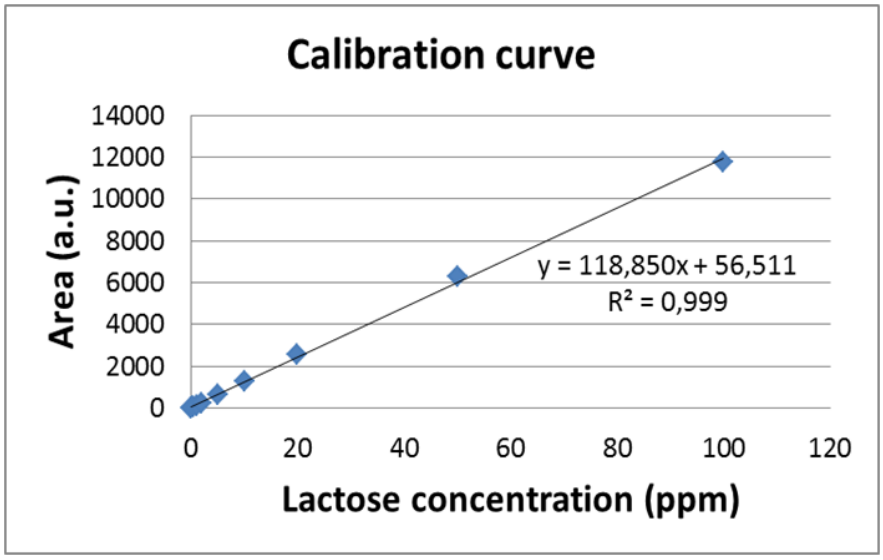


Figure 2. Calibration curve of the Lactose from 0.1 to 100 ppm

The reproducibility of the retention time (RSD) is 0.2% after the three calibration curves have been measured. The detection and quantification limits for this technique by using these standard solutions have been calculated:

Detection Limit: 0.003 ppm
Quantification limit : 0.030 ppm

This methodology has been applied in lactose-free milk samples and some of them have been doped with 10 ppm of lactose, respectively. The following figure (Figure 3) shows the chromatogram of the milk sample doped with 10 ppm of lactose. The lactose compound appears at **4.97** min under the given chromatographic conditions.

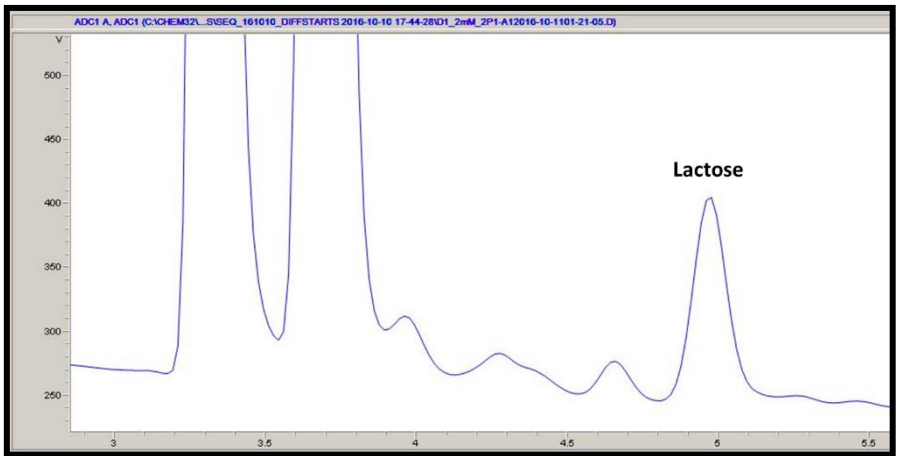


Figure 3. Chromatogram of Lactose-free milk doped with 10 ppm of lactose

Moreover, it has also been demonstrated that the reproducibility of the retention time after 24 h of continuous analysis is **0.5%**. A control calibration sample has been measured after 24 h of analysis and the difference in the retention time is **1.3%** and the difference in the area is **0.2%**.

Under the chromatographic tested conditions, it has not been necessary any column conditioning step as it is not observed any retention time shifting.

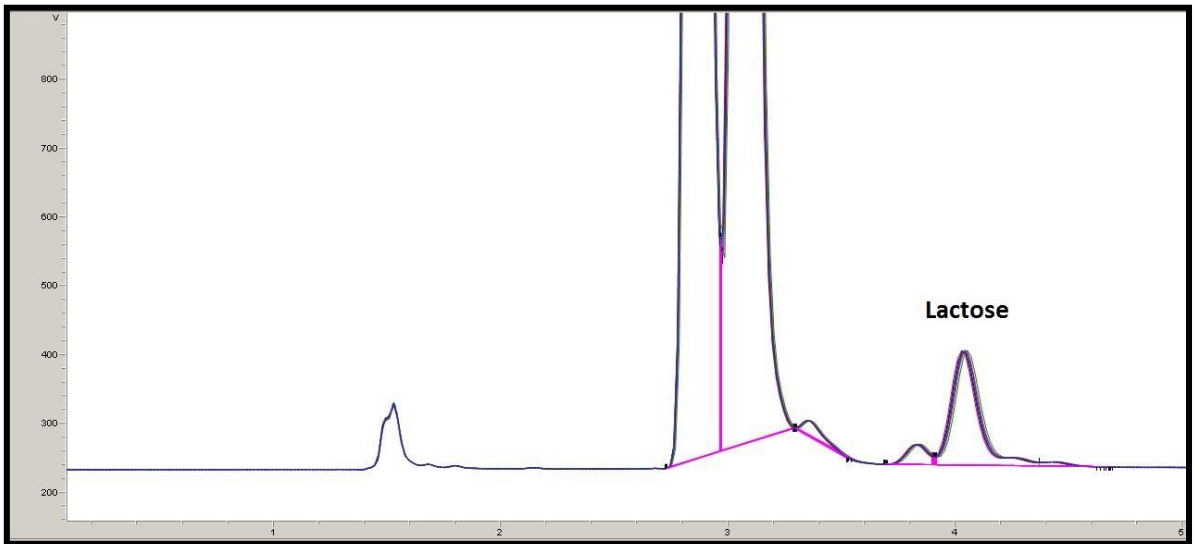


Figure 4. Repeatability (n10) of a Lactose-free milk sample doped with 10 ppm lactose

Determination of carbohydrates in food samples

The methodology developed for the analysis of lactose in lactose-free labeled products was extended to other carbohydrates within the same concentration range (up to 100 ppm). Figure 5 shows the chromatogram of a sample containing 100 ppm of each carbohydrate and their retention times, which have also been summarized in table 2.

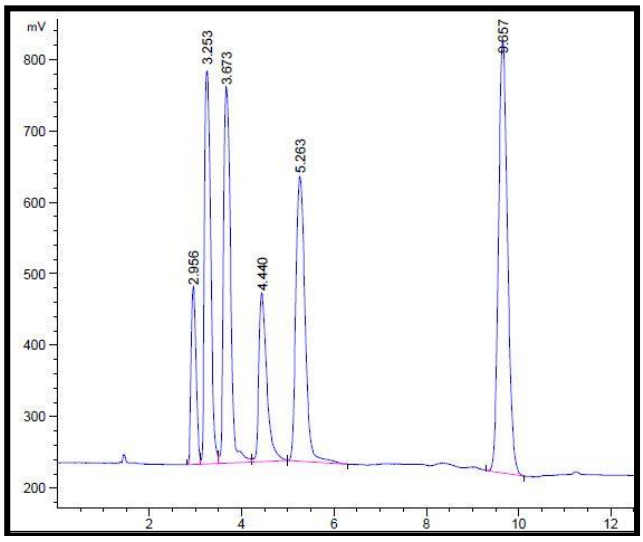
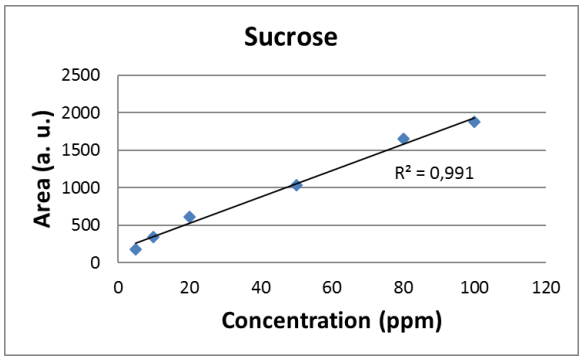


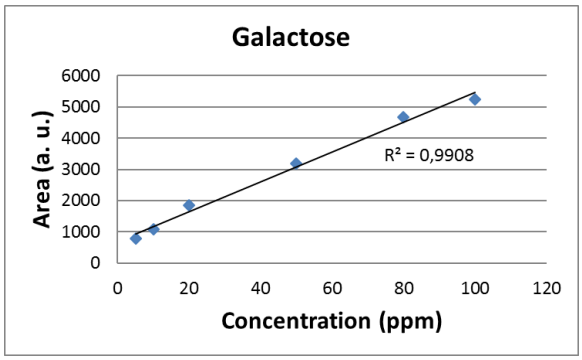
Figure 5. 6 Carbohydrates mixture sample (100 ppm)

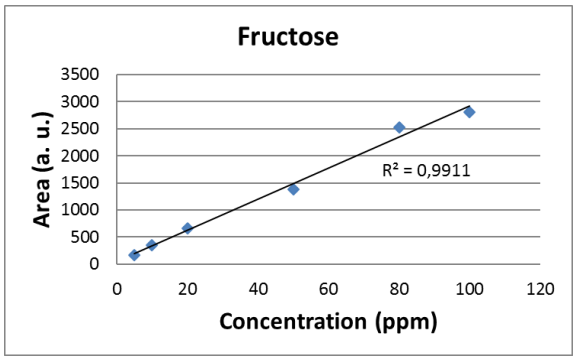
Table 2. Retention time of each carbohydrate

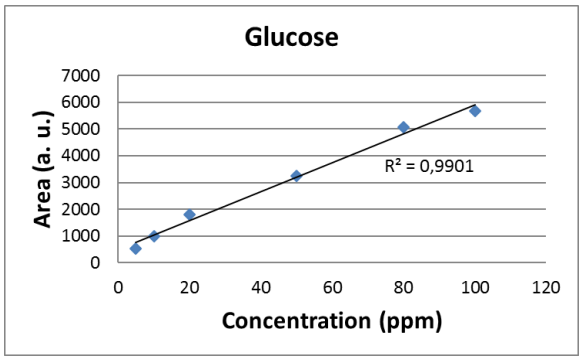
Compound	Ret. time (min)
Sucrose	2.96
Galactose	3.25
Glucose	3.67
Fructose	4.40
Lactose	5.26
Maltose	9.66

The calibration curves for each carbohydrate have been determined using the parameters listed in Table 1 and correlation factors higher than 0.99 have been obtained (Figure 6).









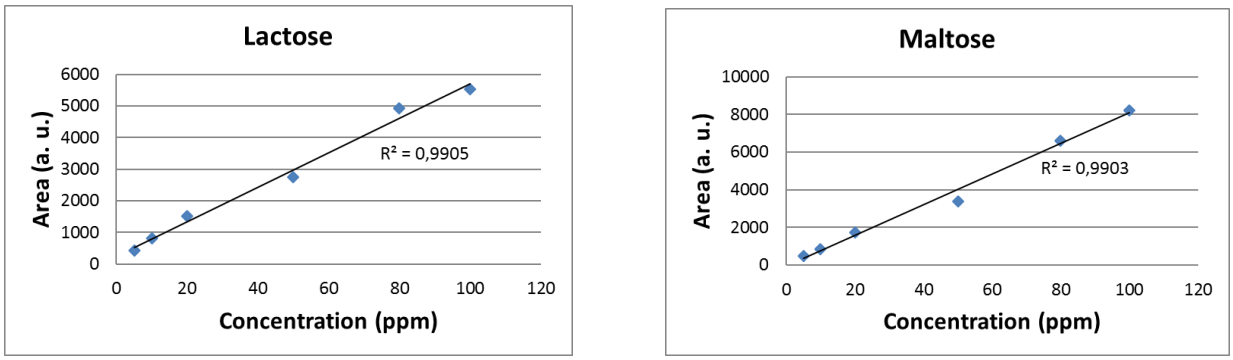
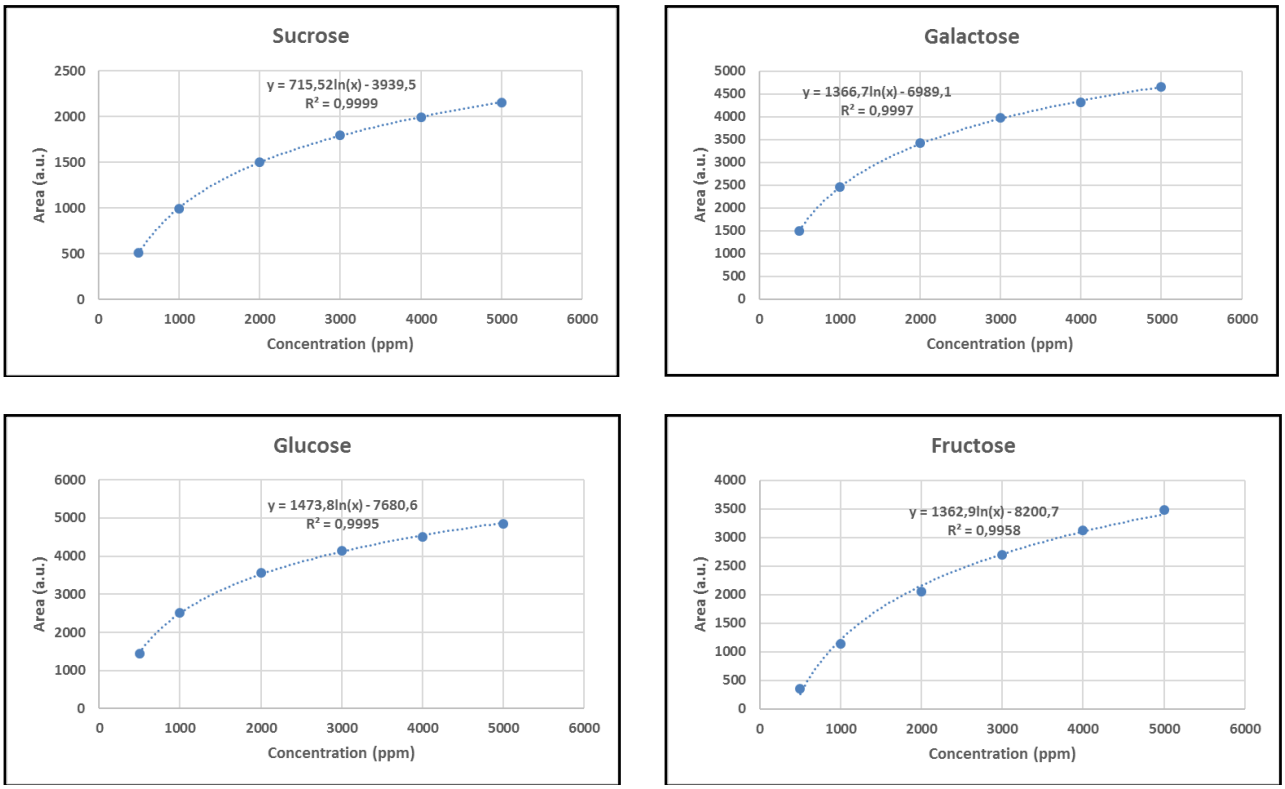


Figure 6. Calibration curves of the different carbohydrates from 0.1 to 100 ppm.

In addition to these concentration levels, it has also been possible to accurately quantify mixtures of **carbohydrates at concentration levels up to 5000 ppm without any hardware or configuration changes**. It has been only necessary to decrease the injection volume to 0.5 µL, while the other parameters remain the same. The obtained calibration curves for each carbohydrate are illustrated hereafter.



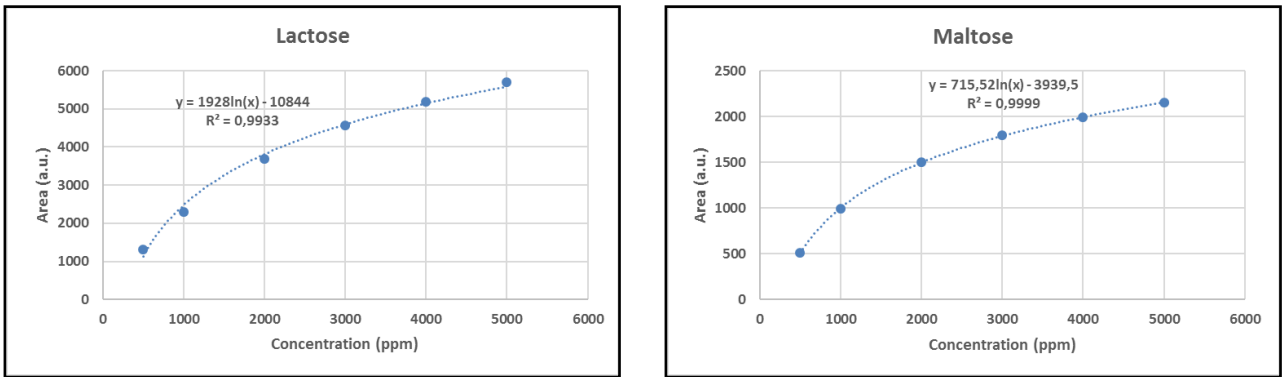


Figure 7. Calibration curves of the different carbohydrates up to **5000 ppm**.

As an example, a chromatogram of a 5000 ppm mixture of the different carbohydrates is depicted in Figure 8. The retention times have been summarized in Table 3. No shifting of the retention times has been observed after repeated analysis.

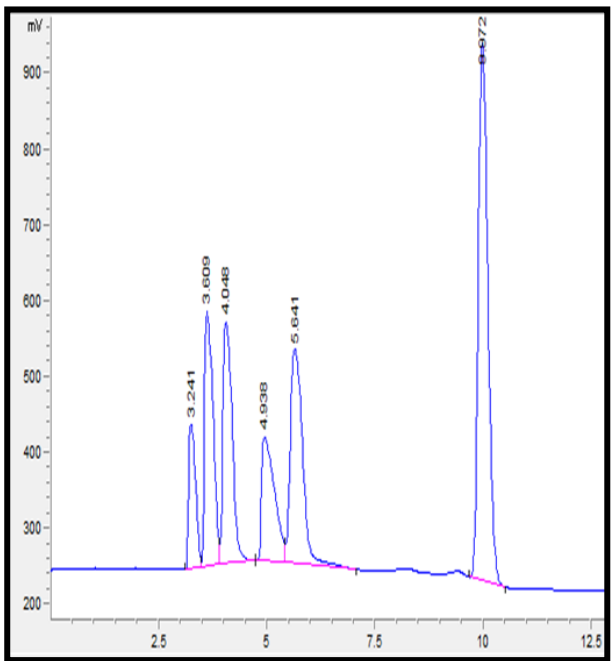


Figure 8. Carbohydrate mixture sample (5000 ppm)

Table 3. Retention time of each carbohydrate

Compound	Ret. time (min)
Sucrose	3.24
Galactose	3.61
Glucose	4.05
Fructose	4.94
Lactose	6.04
Maltose	9.97

Conclusions

The Analysis of lactose in lactose-free products using an Agilent 1260 Infinity HPLC coupled with a Decade Elite Electrochemical Detector is now possible without the need of a bioinert HPLC configuration. The same methodology and instrument configuration can be extended to the analysis of 6 out of the most common carbohydrates in food samples within a wide range of concentrations (up to 5000 ppm) by only adapting the injection volume without any additional hardware changes.

References

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