

Lactose Content in 'Lactose-Free' Labelled Dairy Products (Lactose Intolerance)

J.-P. Chervet¹, H.-J. Brouwer¹, M. Eysberg¹, D. Friscione²

¹Antec Scientific, Zoeterwoude, The Netherlands; ²Alfatech SpA, Genova, Italy;



Introduction

Lactose-intolerance is a widespread condition, which prevents a large number of people (ca. 65% of the global population) of consuming dairy products as a part of their daily diet [1]. The global market for 'lactose-free' dairy products is rapidly growing and the criteria for 'lactose-free' labelled products are becoming stricter. In Europe for instance, the threshold limit for lactose has been lowered to 10 mg per 100 gr product in a number of EU member states in the last years [2]. A 'green' HPAEC-PAD method is presented for the fast and sensitive analysis of lactose, and lactose isomers using Antec's ALEXYS Carbohydrate Analyzer.



Figure 1: Left: ALEXYS Carbohydrate Analyzer with DECADE Elite electrochemical detector. Right: SenCell with Au working electrode and Pd/H₂ (HyREF) reference electrode.

Method

Table 1. Conditions

LC system	Quaternary HPLC pump (Antec Scientific)
Detector	DECADE Elite electrochemical detector (Antec Scientific)
Columns	CarboPac PA210G-4µm column, 150 x 2.0 mm, CarboPac PA210G-4µm column, 30 x 2.0 mm ID, BorateTrap inline trap column, 50 x 4.0 mm ID (Thermo-Scientific)
Mobile phase (MP)	A: deionized (DI) water (resistivity > 18 MOhm.cm and TOC<10ppb) B: 200 mM KOH Eluents blanketed with Helium 5.0 to keep CO ₂ free
Flow rate	0.2 mL/min
Step gradient	0-10 min 20 mM KOH (elution & detection) 10-15 min 100 mM KOH (column clean-up regeneration) 15-30 min 20 mM KOH (equilibration at starting conditions)
Injection	2.5 µL
Temperature	30°C for separation, 35°C for detection
Flow cell	SenCell with Au WE, stainless steel AE and HyREF, AST 2
Potential waveform (4-step)	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	about 0.2–0.4 µA
ADF	0.5 Hz
Range	500 nA/V or 5 µA/V
Sample preparation	Carrez clarification procedure described in reference [3]

Results

An example chromatogram of a standard mixture of 10 sugars of interest is shown in figure 2. Lactose and its isomers allolactose, lactulose and epilactose are eluting within 8 minutes. The Linearity in the concentration range of 34 µg/L - 34 mg/L for lactose and its isomers was excellent, with correlation coefficients (peak area) > 0.999 for all sugars. Detection limits around 10 nmol/L are attainable for most of the sugars. The calculated LOQ (10x S/N) for lactose is approximately 0.010 mg/L, which is a factor 100 below the upper limit of the lactose concentration expected in samples of 'lactose-free' labelled products.

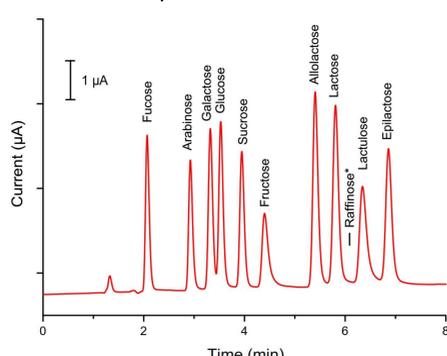


Figure 2: Chromatogram of a 2.5 µL injection 10 µM sugar standard mix. (1) Fucose, (2) Arabinose, (3) Galactose, (4) Glucose, (5) Sucrose, (6) Fructose, (7) Allolactose, (8) Lactose, (9) Raffinose (*not shown) (10) Lactulose and (11) Epilactose.

Analysis of dairy products

6 different types of 'lactose-free' dairy samples were analyzed. Table 3 shows the calculated lactose and allolactose contents and recoveries based on standard addition. The lactose contents in all samples were within the specified limit of < 10 mg/100 g (dairy products) or < 100 mg/100 g (Chocolate paste, Chocolate-covered rice cake).

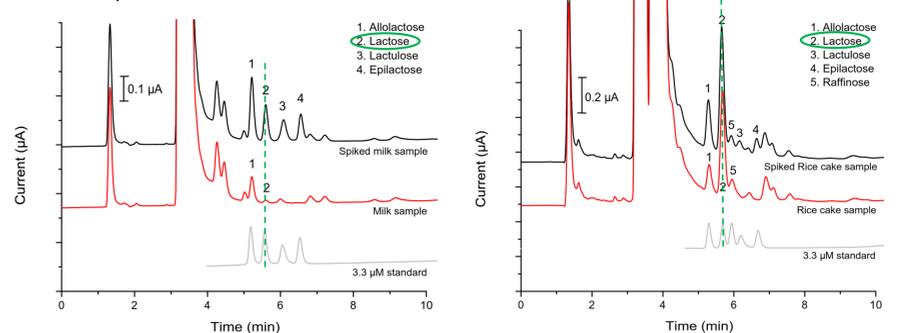


Figure 3: Left: Chromatogram of a milk sample (red), spiked milk sample (black) and 3.3 µM standard of lactose and isomers (grey) Right: Chromatogram of a chocolate-covered rice cake sample (red), spiked rice cake sample (black) and 3.3 µM standard (grey).

Table 2. Lactose & allolactose, contents and sample recovery

Product	Lactose		Allolactose	
	mg/100 g product [#]	Recovery (%)	mg/100 g product	Recovery (%)
Semi-skimmed milk UHT	0.7	95.6	7.5	96.8
Cream cheese	6.7	90.5	17.6	94.4
Low-fat yoghurt	3.3	91.7	12.2	95.9
Matured cheese	-	94.6	-	-*
Chocolate paste	0.6	85.9	-	98.0
Chocolate-covered rice cake	60.5	87.1	13.9	95.2

*) recovery could not be calculated due to coeluting interference.

#) The concentrations shown in table were calculated using the standard addition method (single point calibration).

Analysis of glucose, galactose and other sugars

The method is also suitable to assess the contents of other sugars present in dairy products. See example in figure 4. The total amount of sugar found (3.2 g/100 g) was in agreement with the product label (average sugar contents of 3.0 g/100 g).

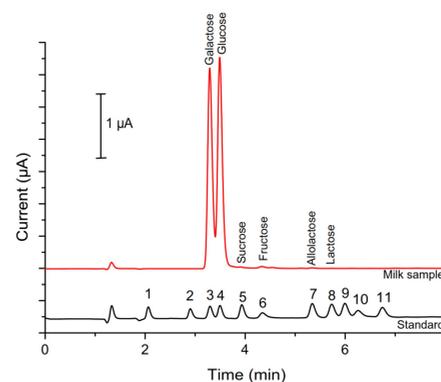


Table 3. Contents of sugars in milk sample

Sugar	Contents (mg/100 g)
Galactose	1451
Glucose	1685
Sucrose	4.6
Fructose	22.9
Allolactose	7.5
Lactose	0.7
Total	3172

Figure 4: Chromatogram of a 2.5 µL injection of a 10 x dilution of the worked-up milk sample (red) and a standard sugar mix (black). See fig 2 for the legend of the sugars in the standard. Right: table with calculated amount of sugars in the milk sample.

Conclusion

The ALEXYS Carbohydrate Analyzer, equipped with a narrow-bore 'fast-4µm' HPAE column, offers a fast and sensitive solution for the quantification of lactose and isomers in commercial labelled "Lactose-free" products. The presented HPAEC-PAD method allows fast separation (within 8 min) of Lactose and isomers. Besides the quantification of low concentrations of lactose (100 times below the upper limit for lactose free labelling) also the major sugars in dairy products (Galactose, Glucose, Sucrose and Fructose) can be analyzed using the presented method. A four-fold reduction in mobile phase consumption was achieved by using a 2 mm ID column instead of a standard bore version (4 mm ID).

References

- [1] T.M Bayless, et al., Lactase Non-persistence and Lactose Intolerance, Curr Gastroenterol Rep, 19 (2017), 23
- [2] EFSA panel, Scientific Opinion on lactose thresholds in lactose intolerance and galactosaemia, EFSA Journal, 8(9) (2010), 1777, <https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2010.1777>
- [3] Antec Scientific, Application note 220_009 Analysis of Lactose and isomers in 'Lactose-free' labelled products