Analysis of Lactose Free Dairy Products using the Agilent 1220 Infinity LC with Evaporative Light Scattering Detection

Application Note

Food Testing and Agriculture

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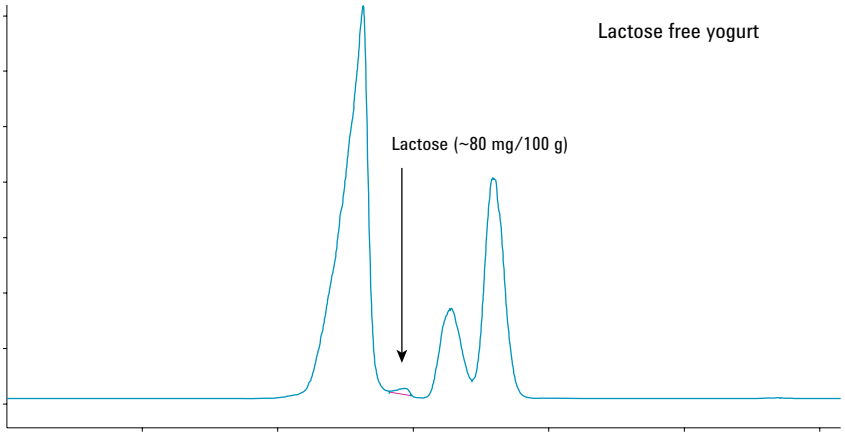
Abstract

This Application Note demonstrates the use of the Agilent 1220 Infinity LC in combination with the Agilent 1290 Infinity Evaporative Light Scattering Detector (ELSD) for the analysis of lactose in dairy products. A calibration for lactose including limit of detection (LOD), limit of quantification (LOQ) and statistical evaluation is shown. Finally, the developed method is applied to determine the lactose content in regular and lactose-free dairy products.



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Introduction

Dairy products are a group of high volume foods such as milk and yogurt products, which belong to staple diets in all industrialized countries. Regular dairy products can contain up to 5% of milk sugar (lactose). Unfortunately, the digestion of a medium percentage of the adult population (10–20% in Europe) is intolerant to lactose. The food industry judiciously attempts to produce lactose free dairy products to satisfy this portion of the population.

To control the content of lactose in dairy products, their quality is monitored by HPLC separation of the inherent compounds and their detection by refractive index detection (RID) or evaporative light scattering detection (ELSD) [1]. Sample preparation is typically done by the addition of *Carrez*-Reagent, which breaks up emulsions and makes lipids and proteins removable by filtration[2].

In principle, a dairy food product is allowed to be declared as lactose free, if the content of lactose is less than 100 mg/100 g product. This low amount is tolerated even by people who are very sensitive to lactose. That means, dairy products declared lactose free under these terms of condition, are safe for all lactose-intolerant people.

Experimental

Instrumentation

Description	Model number
Agilent 1220 Infinity LC	G4290B
Agilent 1290 Infinity ELSD	G4261B

Software

Agilent OpenLAB CDS ChemStation Edition for LC & LC/MS Systems, Rev. C.01.04

HPLC Method

Parameter	Condition
Column	Agilent Hi-Pex Ca, USP L19, 4.0 × 250 mm, (p/n PL1570-5810)
Solvent	Ultrapure water
Flow rate	0.25 mL/min
Elution conditions	Isocratic
Stop time	15 minutes
Injection volume	20 µL
Needle wash	In vial with water
Column temperature	80 °C

ELSD Method

Parameter	Condition
Nebulizer temperature	90 °C
Evaporation temperature	60 °C
Gas flow	1.2 SLM
Detector gain	1.2 PTM
Smoothing	2 seconds
Data rate	10 Hz

Chemicals

Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with an LC-Pak Polisher and a 0.22 µm membrane point-of-use cartridge (Millipak).

Lactose was purchased from Sigma-Aldrich, Germany. A 5 g/L amount was used as stock solution. The following dilutions were used for the calibration: 500, 250, 100, 50, 25, 10, 5, and 2.5 mg/L.

Samples and Sample Preparation

Dairy products (regular milk and yogurt as well as lactose-free milk and yogurt) were purchased in a local store.

A solution of 1 mL milk/1 g yogurt was diluted with 8 mL of water and vortexed. Then, 0.5 mL of *Carrez* Reagent 1 and 2 was added and vortexed for 1 minute. The mixture was allowed to settle for 15 minutes. The mixture was filtrated through a cellulose syringe filter (Agilent Captiva Premium Syringe Filter, Regenerated Cellulose, 0.45 µm, 25 mm, p/n 5190-5111). The resulting filtrate was diluted 1:10 for the regular dairy products and lactose free yogurt (final dilution: 1:100) and 1:5 for lactose free milk (final dilution: 1:50).

Results and Discussion

With the above method, a dilution series of lactose from 500 mg/L to 2.5 mg/L was measured for the construction of a calibration curve as well as the determination of the LOQ and LOD. The LOQ was determined to be at a signal-to-noise (S/N) ratio of 10 (S/N = 10) and the LOD at S/N = 3. A concentration of 2.5 mg/L was identified for the LOD and a concentration of 5 mg/L was identified for the LOQ (Figure 1). The calibration curve for the measured areas and their concentrations is shown in a double logarithmic scale with excellent linearity (Figure 2).

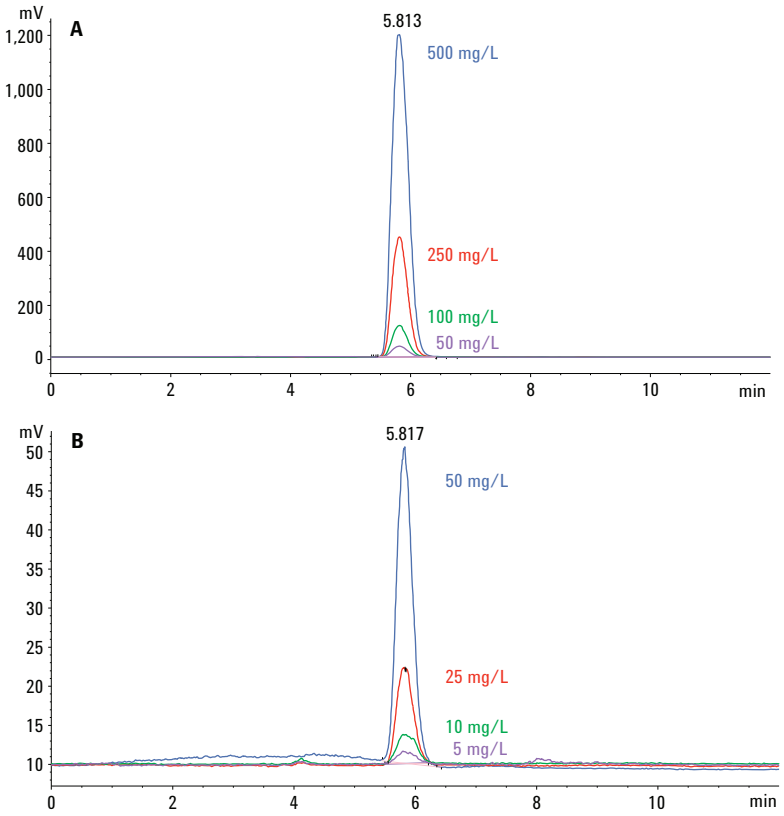


Figure 1. Injection of lactose for calibration from:
(A) 500–50 mg/L
(B) 50–5 mg/L, LOQ 5 mg/L at S/N > 10, LOD at 2.5 mg/L at S/N < 3

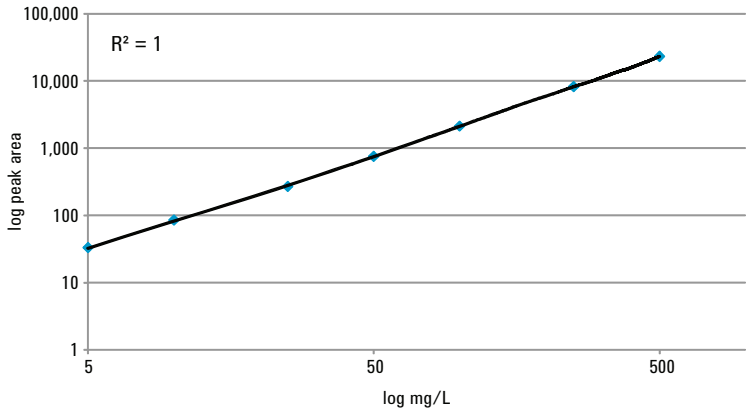
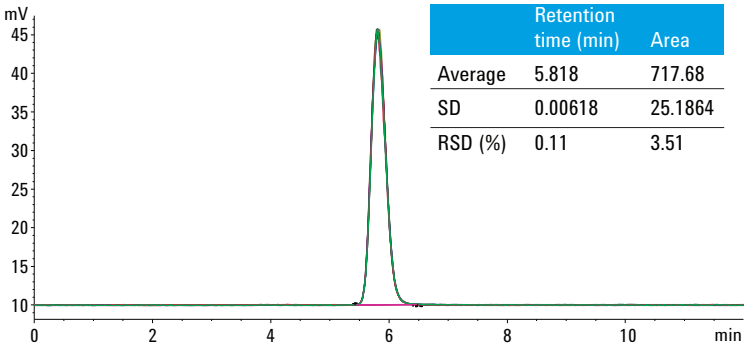


Figure 2. Calibration curve of lactose 500–5 mg/L by ESLD in double logarithmic scales.

The calibration solution at a concentration of 50 mg/L was injected 10 times and used for the statistic evaluation of relative standard deviation (RSD) of retention time and of the peak area (Figure 3). For the average retention time at 5.818 minutes, an RSD value of 0.11% was calculated and for the peak area RSD, a value of 3.5%. The carryover of the method was determined by an injection of the highest point in the calibration curve (500 mg/L) followed by a blank injection. No carryover could be identified in this case (Figure 4).



	Retention time (min)	Area
Average	5.818	717.68
SD	0.00618	25.1864
RSD (%)	0.11	3.51

Figure 3. Overlay of 10 runs at 50 mg/L and calculation of RSD for retention times and peak areas.

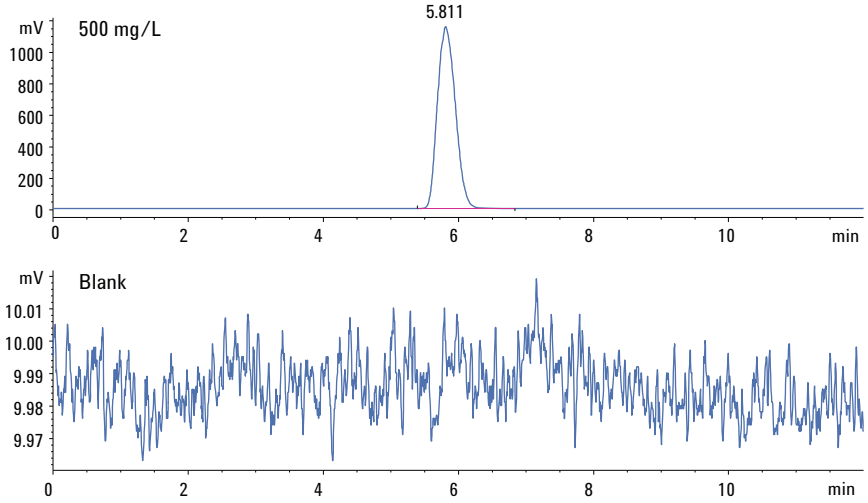


Figure 4. Determination of carryover in first blank injection after injection of 500 mg/L sample. Carryover was not detectable for the used method.

To prove the method, samples of regular milk and regular yogurt were prepared as described above. After injection, the measured areas for lactose could be found in the higher area of the calibration range. Taking the dilution factor of 1:100 into account, the concentration of lactose in the regular dairy products could be calculated. For regular milk a concentration of 5 g/100 mL and for regular yogurt a concentration of 4.3 g/100 mL was determined (Figure 5). This content is in accordance to the product descriptions on the food packages. The same experiment was done for samples of lactose free milk and yogurt products for the determination of residual lactose. Lactose free milk in a dilution of 1:50 showed a tiny lactose signal at the LOQ (Figure 6A). The content can be calculation to be < 25 mg/100 mL. For the lactose free yogurt, a signal above the LOQ was determined for the dilution at 1:100. The calculated residual content of lactose in this product was approximately 80 mg/100 g. The content of lactose in both product descriptions was less than 100 mg/100 g (mL).

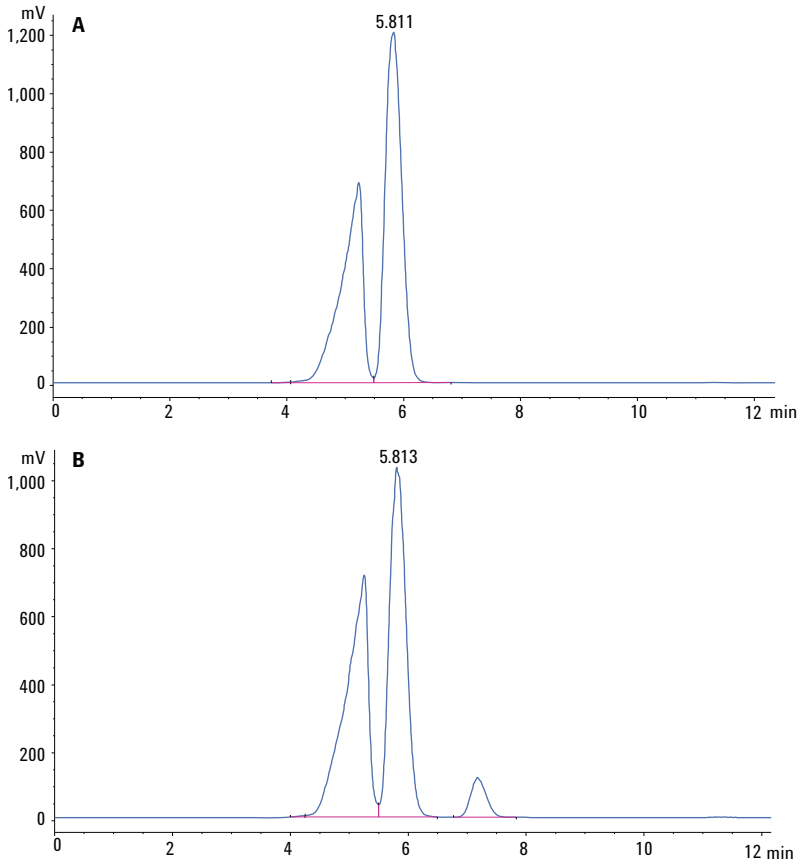


Figure 5.
A) Regular milk, approximately 5 g lactose/100 mL
B) Regular yogurt, approximately 4.3 g lactose/100 g

Conclusion

This Application Note describes the Agilent 1220 Infinity Gradient LC in combination with the Agilent 1290 Infinity ESLD to be used for quantitative measurement of lactose in dairy products. It shows that the system can be calibrated with excellent linearity and repeatability. RSDs of retention time and peak area were in a good range. Finally, it shows that the system has the capability to quantify lactose in regular dairy products as well as residual lactose in lactose-free products.

References

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2. H. E. Indyk, M. J. Edwards, D. C. Woollard, "High performance liquid chromatography analysis of lactose hydrolyzed milk", *Food Chemistry*, Vol. 57, No. 4, pp. 575-580, **1996**.

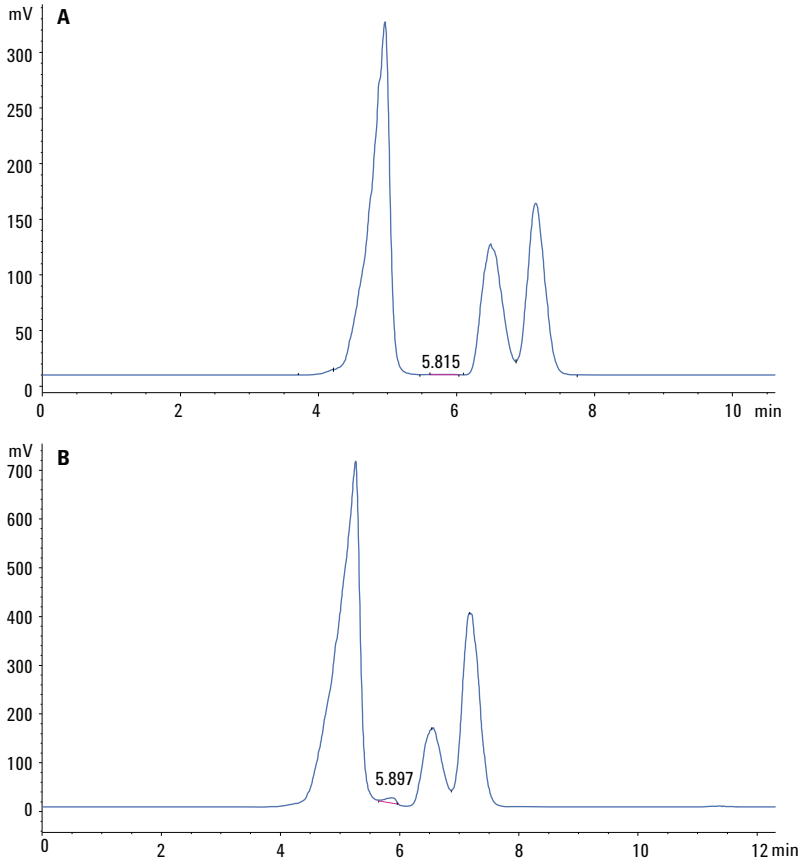

Figure 6.
A) Lactose-free milk, approximately < 25 mg/100 mL
B) Lactose-free yogurt, approximately 80 mg/100 g

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