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Measuring Lactose in Milk: A Validated Method

INTRODUCTION

Measurement of lactose, the major carbohydrate in milk, is important because it contributes to the sensory and functional properties of milk and has economic value since the price of milk is based on milk solids content. The physical characteristics of milk are highly complex because milk is composed of an intricate mixture of fat globules and protein (casein, whey) in an aqueous solution of lactose, minerals, and other minor constituents. In addition, milk's physical characteristics are affected by several factors including its processing. Measurement of milk's physical properties is used in processing to determine the concentration of milk components, and to evaluate the quality of milk products. The lactose content of cow's milk can vary from 3.8–5.3% (38,000–53,000 µg/mL). Modern 1% and 2% milk products have higher levels of lactose.

The Association of Analytical Communities (AOAC) Official Method 984.15 for determination of lactose in milk is both complex and time consuming. It involves the enzymatic hydrolysis of lactose to glucose and galactose at pH 6.6 by β-galactosidase. Subsequent oxidation of the β-galactose released to galactonic acid at pH 8.6 is catalyzed by β-galactose dehydrogenase, then followed by reduction of nicotinamide adenine dinucleotide (NAD^+). The amount of reduced NAD formed is measured at 340 nm and is proportional to the lactose content. The method requires seven different reagents, two of which must be prepared weekly.

This study describes a simple, rapid, and accurate method for the determination of lactose in milk using standard high-performance liquid chromatography (HPLC) with a Thermo Scientific Dionex Corona™ Charged Aerosol Detector (CAD™). The determination involves a simple 1:100 dilution of milk and high-performance liquid chromatography (HPLC) determination in 8 min. The method is validated with respect to specificity, linearity, accuracy, precision, and sensitivity.

EQUIPMENT

An HPLC system similar to the Thermo Scientific Dionex UltiMate™ 3000 HPLC system including a pump, autosampler, a Dionex Corona charged aerosol detector (P/N 70-9116), and a nitrogen generator (P/N 70-6003)

CONDITIONS

Column:	Prevail Carbohydrate ES, 4.6 × 250 mm, 5 µm
Mobile Phase:	65% (v/v) aqueous acetonitrile
Flow Rate:	1.0 mL/min
Inj. Volume:	10 µL
Column Temp.:	35 ºC
Detection:	Dionex Corona detector, 100 pA range, no filter

PREPARATION OF STANDARDS

Lactose standard was obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO). A stock standard was prepared at 1.0 mg/mL in 70% acetonitrile. Additional standard solutions at 50, 100, 200, and 500 µg/mL lactose were prepared in 70% acetonitrile and used to create the calibration curve. For recovery studies, a 10 mg/mL standard was prepared in water.

SAMPLE PREPARATION

Milk was first diluted from 0.50 mL to 5.0 mL with water, then 0.50 mL of this solution was diluted to 5.0 mL with 70% acetonitrile. After mixing, the sample was filtered using a syringe filter or microcentrifuge tube filter (Spin X; Corning Life Sciences, Lowell, MA) at 13,000 g.

RESULTS AND DISCUSSION

It was determined that a 1:100 (v/v) dilution of milk provided the optimum concentration range for analyte determination. A number of different sample preparation techniques were evaluated:

- Precipitation of protein and dilution with pure acetonitrile provided very poor recovery of lactose.
- Different concentrations of aqueous acetonitrile were compared. A 1:100 (v/v) dilution of milk with 50% or 70% aqueous acetonitrile showed good recovery.
- Dilution with other aqueous solvent mixtures (e.g., 1-propanol, 2-propanol, or methanol) provided poorer recovery.

Subtle changes in the sample preparation procedure affected the recovery of lactose. The best recovery achieved, determined empirically, was obtained by first diluting the milk 1:10 (v/v) with water followed by a 1:10 (v/v) dilution with 70% aqueous acetonitrile. Reversing this order resulted in poorer recovery. Dilution with 70% acetonitrile only produced lower recovery. Simultaneous dilution and precipitation of the milk with 1% perchloric acid, a common procedure used in the literature, also yielded poorer recovery (Table 1). As the first procedure was the fastest and least expensive and appears to give acceptable recovery, this method was chosen.

The calibration curve presented in Figure 1 shows an R^2 value of 1.000 when plotted with the concentration and response axes inversed.[1] The response in charged aerosol detection is curved.

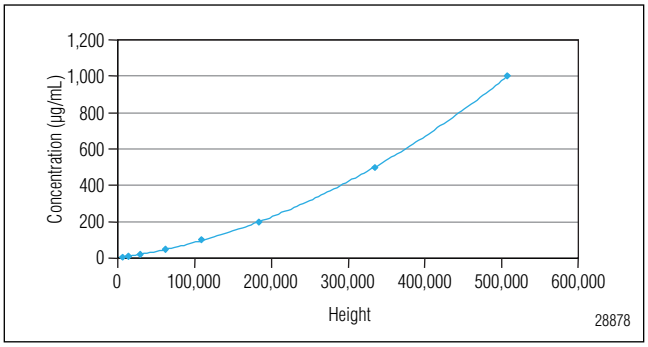

Figure 1. Lactose calibration curve response on the Dionex Corona detector.

Table 1. Effect of Sample Preparation on Lactose Recovery

Sample Preparation	Lactose 1% Milk	Lactose 2% Milk
1-10 dilution with water 1-10 dilution with 70% acetonitrile	533 µg/mL	529 µg/mL
1-10 dilution with 1% perchloric acid 1-10 dilution with 70% acetonitrile	522 µg/mL	515 µg/mL
1-10 dilution with 70% acetonitrile 1-10 dilution with 70% acetonitrile	511 µg/mL	518 µg/mL

Table 2. Experimental Calibrator Values From Figure 1

Calibrator Value (µg/mL)	Experimental Value (µg/mL)
5.0	5.8
10.0	10.8
20.0	20.9
50.0	48.7
100	97.6
200	200
500	502
1000	999

The data shows a high degree of correlation when a quadratic fit is used. Due to idiosyncrasies in the implementation of the equations it has been found that reversing the axis produces the best correlation. The experimental values generated for different calibrators using this curve are presented in Table 2.

A 1% milk product was spiked with lactose at 1% (1 g/100 mL), 2%, and 4% concentrations. The determination was performed on three different nonconsecutive days over a period of one week.

Table 3. Recovery				
	Unspiked Milk	**1% Spike**	**2% Spike**	**4% Spike**
	n = 5	n = 5	n = 5	n = 5
Day 1 (g/100 g)	4.84 ± 0.10%	5.79 ± 0.10%	6.81 ± 0.07%	8.78 ± 0.17%
Recovery (%)	n/a	99.2	99.6	99.3
Day 2 (g/100 g)	4.75 ± 0.04%	5.77 ± 0.08%	6.72 ± 0.09%	8.97 ± 0.08%
Recovery (%)	n/a	100.3	99.6	102.5
Day 3 (g/100 g)	4.93 ± 0.09%	5.86 ± 0.03%	6.78 ± 0.06%	8.96 ± 0.09%
Recovery (%)	n/a	98.8	97.9	100.3
Average (g/100 g)	4.84 ± 0.11 %	5.81 ± 0.08%	6.77 ± 0.08%	8.90 ± 0.14%
Ave % Recovery		**99.4**	**99.0**	**100**

Table 4. Intraday Precision*				
	Unspiked Milk	**1% Spike**	**2% Spike**	**4% Spike**
	n = 5	n = 5	n = 5	n = 5
Day 1	2.0% RSD	1.8% RSD	1.0% RSD	1.9% RSD
Day 2	0.8%	1.4%	1.3%	0.9%
Day 3	1.9%	0.5%	0.9%	1.0%
Ave % RSD	**1.6%**	**1.2 %**	**1.1%**	**1.3%**

*Based on amount

Table 5. Interday Precision*			
Unspiked Milk	**1% Spike**	**2% Spike**	**4% Spike**
n = 15	n = 15	n = 15	n = 15
2.3% RSD	1.4% RSD	1.2% RSD	1.6% RSD

*Based on amount

These recovery data are presented in Table 3. Intraday and interday precision data are presented in Tables 4 and 5, respectively. The estimated limits of detection (LOD) (3 × standard deviation [SD]) and limits of quantitation (LOQ) (10 × SD), based on 10 replicate determinations of lactose-reduced low fat milk samples, were 0.006% (6 ng) and 0.02% (20 ng), respectively.

Chromatography conditions were optimized to provide complete separation of lactose from other milk components within 7 min (Figure 2). Representative chromatograms for 1%, 2%, fat free, and lactose-reduced milk samples are presented in Figure 3.

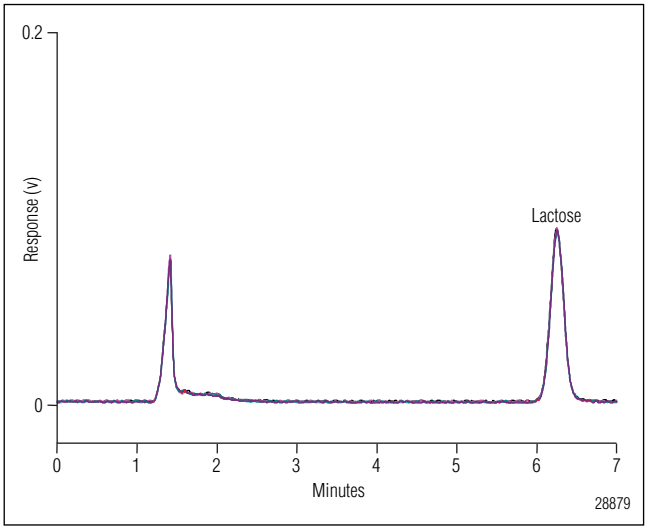

Figure 2. Five replicate injections of the 50 µg/mL lactose standard.

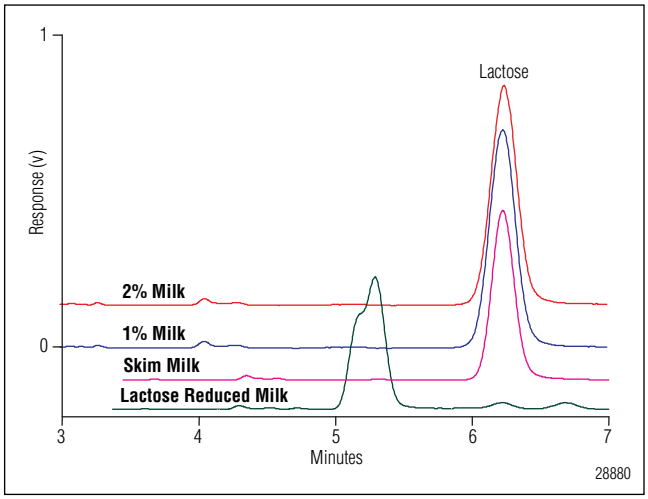

Figure 3. Overlay chromatograms of different milk samples.

CONCLUSION

High-performance liquid chromatography using the Dionex Corona Charged Aerosol Detector provides a simple and rapid procedure for the determination of lactose in milk. The greater sensitivity of the Dionex Corona CAD allows determination of lactose in milk at a 1:100 (v/v) dilution of the milk sample. The high sensitivity also enables evaluation of the effects of different methods of sample preparation. The highest recovery was observed when the milk sample was processed with an initial dilution of water followed by a dilution with 70% acetonitrile. Validation studies demonstrate the specificity, linearity, accuracy, precision, and sensitivity of the assay. The method demonstrates good accuracy and precision, and is suitable for the determination of lactose in different types of milk.

REFERENCE

1. Liu, X. K.; Fang, J. B.; Cauchon, N.; Zhou, P. Direct Stability-Indicating Method Development and Validation for Analysis of Etidronated Disodium Using a Mixed-Mode Column and Charged Aerosol Detector. *J. Pharm. Biomed. Anal.,* **2008,** *46,* 639–644.

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