Lactose in Milk: A Validated Method

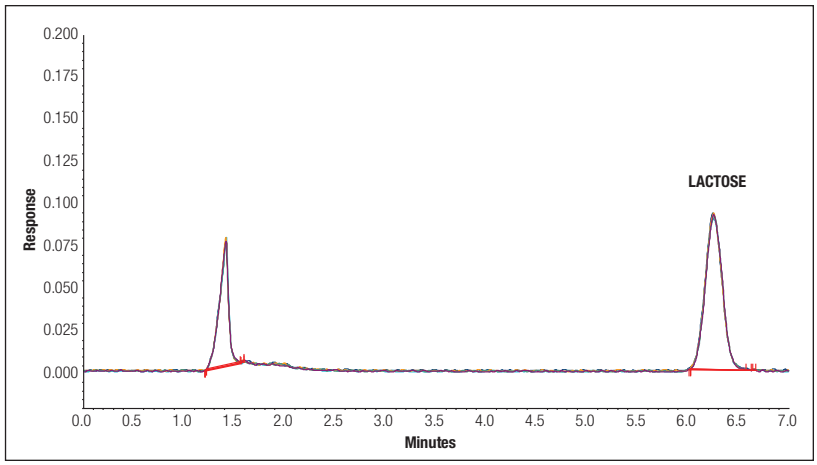

Figure 1. Analysis of lactose. Five replicate determinations of the 50 µg/mL standard.

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. Milk's physical characteristics are affected by several factors including the composition and processing of milk. Measurement of milk's physical properties is used in processing to determine the concentration of milk's components and to evaluate the quality of milk products. Lactose is the major carbohydrate in milk. Measurement of lactose in milk is important because it contributes to the sensory and functional properties of milk. It also has economic value since the price of milk is based on milk solids content. 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 have higher levels of lactose.

The AOAC Official Method (984.15) for lactose in milk is both complex and time consuming. It involves enzymatic hydrolysis of lactose to glucose and galactose at pH 6.6 by β-galacto-sidase. Subsequent oxidation of the β-galactose released to galactonic acid at pH 8.6, as catalyzed by β-galactose dehydrogenase, then occurs with concomitant 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 application note describes a simple, rapid and accurate method for the determination of lactose in milk using standard high-performance liquid chromatography (HPLC) with the Thermo Scientific Dionex Corona Charged Aerosol Detector. The determination involves a simple 1–100 dilution of milk and HPLC determination in eight minutes. The method was validated with respect to specificity, linearity, accuracy, precision, and sensitivity.

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Method Parameters

Column:	4.6 × 250 mm, 5 µm
Column Temperature:	35 °C
Mobile Phase:	65% (v/v) aqueous acetonitrile
Flow Rate:	1.10 µL
Corona™:	100 pA range, no filter

Standard Preparation

Lactose standard was obtained from Sigma-Aldrich (St. Louis, USA). 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 0.50 mL to 5.0 mL with water followed by a 0.50 mL to 5.0 mL dilution with 70% acetonitrile. After mixing, the sample was filtered using a syringe filter or micro-centrifuge tube filter 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 the Corona determination. A number of different sample preparation techniques were evaluated:

- Precipitation of protein and dilution with pure acetonitrile gave very poor recovery of lactose.
- Different concentrations of aqueous acetonitrile were then 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) gave poorer recovery.

Subtle changes in the sample preparation procedure affected the recovery of lactose. The best recovery 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 gave poorer recovery. Dilution with 70% acetonitrile only also gave lower recovery. Simultaneous dilution and precipitation of the milk with 1% perchloric acid, a common procedure used in the literature, also gave poorer recovery (Table 1).

Table 1. Effect of sample preparation on lactose recovery levels

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 µìg/mL
1:10 dilution with 70% acetonitrile 1:10 dilution with 70% acetonitrile	511 µg/mL	518 µg/mL

The calibration curve is presented in Figure 2, and showed an R^2 value of 1.000 when plotted with the concentration and response axes inversed (see Liu *et al.*, (2008), J. Pharmaceut. Biomed. Anal., **46**, 639-644). The experimental values generated for different calibrators using this curve is presented in Table 2.

1% milk product was spiked with lactose at the 1% (1 g/100 mL), 2%, and 4% concentrations. The determination was performed on three different, non-consecutive days over a period of one week. These recovery data are presented in Table 3. Intra-day and inter-day precision data are presented in Tables 4 and 5, respectively. The estimated limit of detection (LOD) (3 × SD) and limit of quantification (LOQ) (10 × SD), based on ten 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 minutes (Figure 1).

Representative chromatograms for 1%, 2%, fat-free, and lactose-reduced milk samples are presented in Figure 3.

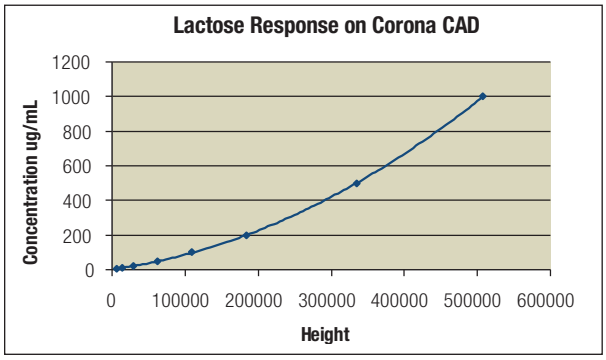

Figure 2. Lactose calibration curve.

Table 2. Experimental calibrator values from Figure 2

Calibrator Value (µg/mL)	Experimental Value (µg/mL)
5	5.8
10	10.8
20	20.9
50	48.7
100	97.6
200	200
500	502
1000	999

Table 3. Recovery

	Unspiked Milk	1% Spike	2% Spike	4% Spike
	(n = 5)	(n = 5)	(n = 5)	(n = 5)
Day 1	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	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	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	4.84 ± 0.11%	5.81 ± 0.08%	6.77 ± 0.08%	8.90 ± 0.14%
Average % Recovery		**99.4**	**99**	**100**

Table 4. Intra-day 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% 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**

Table 5. Inter-day 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

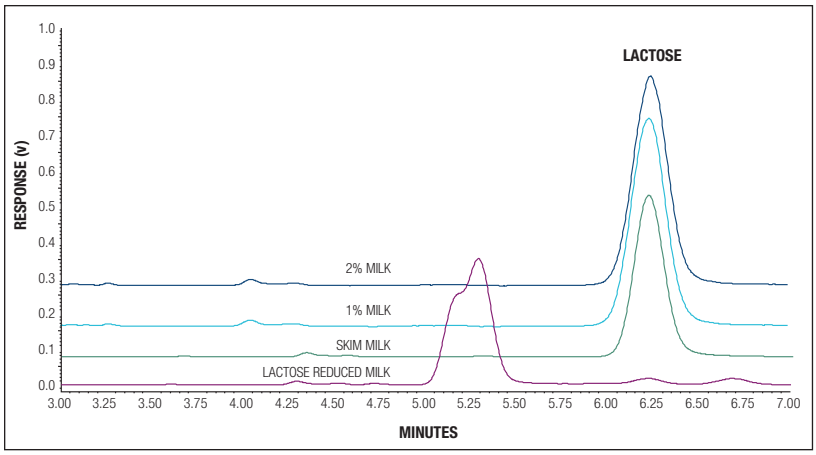

Figure 3. Overlay of different milk samples.

Conclusion

HPLC with Corona charged aerosol detector provides a simple and rapid procedure for the determination of lactose in milk. The greater sensitivity of the Corona charged aerosol detector allows lactose determination in milk at a 1:100 (v/v) dilution of the milk sample. The high sensitivity also enabled an 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 were performed to evaluate 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.

Australia +61 3 9757 4486
Austria +43 1 616 51 25
Benelux +31 20 683 9768
+32 3 353 42 94
Brazil +55 11 3731 5140
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