The Analysis of Milk Sugars with a Modern Refractive Index Detector and a Carbohydrate Column

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Varian Application Note

Number 4

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Introduction

Sugar analysis is a common industrial analytical requirement. It is particularly important in the analysis of food products such as milk. Though lactose is the main carbohydrate in milk (1) and was typically the only sugar of concern, recently interest has expanded in analyzing milk products with reduced lactose. The lactose in milk can be reduced by hydrolyzing lactose into two other sugars, glucose and galactose, with an enzyme. The result is a product with reduced lactose that can more easily be consumed by large percentage of the world's population that are lactose intolerant (2).

There are two major HPLC approaches to the analysis of milk sugars. One method is to use a specific resin based carbohydrate column with a sensitive, modern, refractive index detector. This is an excellent approach as these columns have the ability to separate sugars within a group (i.e., C5 and C6 sugars), the corresponding sugar alcohols, and some larger sugars. The standard mobile phase for such a column is 100% water. A second approach uses an amine modified silica column with either a refractive index detector or a UV detector at a low wavelength. These amine columns have been used extensively. Currently, there are distinct advantages to using resin type carbohydrate columns for sugar analysis. Resin columns have proven to be more stable since amine columns can be modified by certain samples and therefore demonstrate less stability (3). Silica based amine columns require acetonitrile/water mobile phases at flow rates of 1.5 - 2.0 mL/min producing substantial amounts of hazardous waste. Additionally, these factors allow a cost analysis to be made:

	Carbohydrate Column	**Amine Column**
Solvent	Water: $90.00/case	ACN: $140.00/case
	—	Water: $90.00/case
Disposal	no	yes
Flow Rate	0.50 mL/min	1.5 mL/min
	240 mL/day	720 mL/day
Cost	$1.35/day	$6.47/day

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Experimental

This application note describes the analysis of milk sugars in whole milk and lactose reduced milk using a carbohydrate column and the RI-4 refractive index detector. This requires the separation of three sugars: lactose, glucose, and galactose. The glucose and galactose are produced as a result of the hydrolysis of lactose (2). The samples were prepared by precipitating the milk proteins with isopropanol and water and centrifuging the samples. The supernatant was filtered through a 0.45 μm filter, and the samples were diluted with water. This separation with commercially available lactose-reduced milk is shown in Figure 1.

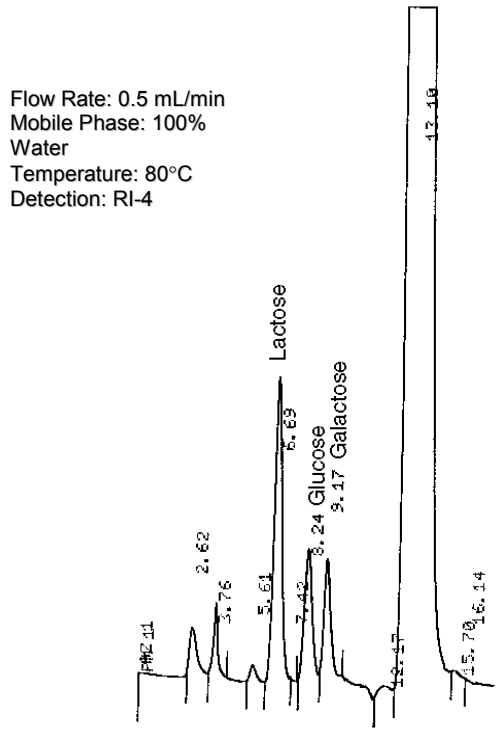

Figure 1. Separation of Lactose Reduced Milk (1:10 dilution)

Whole milk can be analyzed using the same method with lactose as the only sugar of concern (Figure 2).

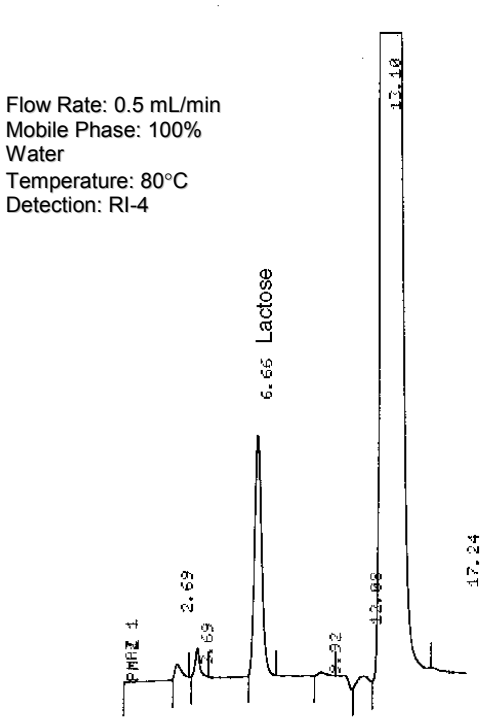

Figure 2. Separation of Whole Milk (1:25 dilution)

The chromatographic system consisted of a Varian Star 9010 pump, SPH99 column oven, a MicroPak carbohydrate column, RI-4 detector, and 4400 Integrator. The column was heated to 80°C and the detector to 50°C.

Results

The average recovery of the lactose was 104% (n=6). The quantitation was done using an external standard method (range 0.014 mg/mL to 0.68 mg/mL) with a linear calibration curve (R=0.99998) (Figure 3). The baseline stability and retention time reproducibility (Figure 4) was excellent for both samples (6.68 min ±0.42%, n=20) and standards (6.68 min ±0.18%, n=21). The acidophilus (lactose reduced) milk samples showed a reduction in lactose of over 50%, as expected.

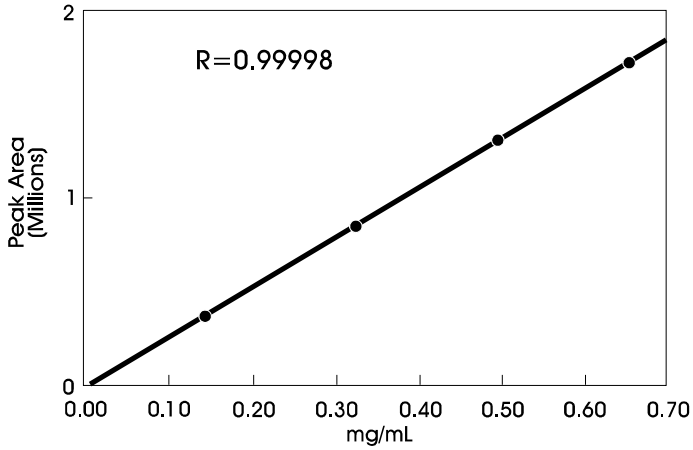

Figure 3. Calibration Curve for Lactose

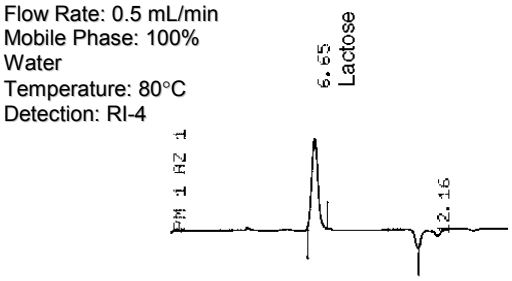

Figure 4. Lactose Standard @ 0.014 mg/mL

Conclusions

Sugar analysis on an ion exchange carbohydrate column with an aqueous mobile phase is a simple, sensitive method. Additionally the cost of mixing and disposal of solvents is eliminated, making this a cost effective method of analyzing sugars.

References

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