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Original Article

Determination of glucosamine and lactose in milk-based formulae by high-performance liquid chromatography

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Abstract

A simple method for the determination of glucosamine and lactose in milk-based formulae by high-performance liquid chromatography (HPLC) with refractive index detector was developed and validated. Samples were cleaned up just by protein precipitation before HPLC analysis. Separation was achieved with a Shodex Asahipak NH2P-50 column. The method showed good linearity, sensitivity, precision and recovery, and proved very simple and rapid for routine analysis since separation was completely achieved at 10 min.

Keywords: Milk-based formulae; Glucosamine; Lactose; HPLC

1. Introduction

The dairy industry has made several attempts to improve the quality and the nutrient contents of milk-based formulae. In order to develop special products for specific people, some ingredients have been added to the milk powder. For example, calcium is added to prevent osteoporosis that frequently affects old people, while some vitamins and minerals are used in the formulae for pregnant women. And recently glucosamine (GLcN) is started to be added into the milk powder. GLcN is a natural component of the glycoproteins present in connective tissue and gastrointestinal mucosal membrane and acts as a building block of glycosaminoglycans (Setnikar and Rovati, 2001; Bassleer et al., 1998; Barclay et al., 1998; Hawker, 1997). In vitro studies have shown that GLcN stimulates the production of proteoglycans via chondrocytes activation and increases the absorption of sulfate uptake by articular cartilage, thus helping to rebuild cartilage, slow down or even stop the progression of osteoarthritis (OA) (Houpt et al., 1999). OA is a type of arthritis that is caused by the breakdown and eventual loss of the cartilage of one or more joints. Among the over 100 different types of arthritis conditions, OA is the most common, and occurs more frequently with ageing. Its prevalence after the age of 65 years is about 60% in men and 70% in women (Sarzi-Puttini et al., 2005).

Lactose is the main carbohydrate in dairy products. This disaccharide is composed of glucose and galactose and is the only saccharide synthesized by mammals. Lactose plays an important role in the formation of the neural system and the growth of skin (texture), bone skeleton and cartilage in infants. It also prevents rickets and saprodontia (Emmett and Rogers, 1997).

GLcN and lactose are both saccharides. The classic method for the determination of saccharides is chemical analysis as well as the enzyme-catalyzed method and spectrophotometry (Brereton et al., 2003; Gonzáles et al., 2003; Salvador et al., 2002). The major disadvantage of these three methods consists in the difficulty of evaluating different saccharides simultaneously. One of the methods commonly used in saccharide analysis is gas chromatography (GC), which has been used in the study of milk

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(Troyano et al., 1994a, b; Olano et al., 1989), dried skim milk (Troyano et al.,1994a, b) and pasteurized milk (Ruas-Madiedo et al., 2000). Although GC is a sensitive method for saccharide analysis, sample preparation is laborious because saccharides must be derivatized before GC analysis. The procedure is too tedious to be used routinely. Lately, in many studies, high-performance liquid chromatography (HPLC) has been used to separate and determinate the saccharides. HPLC with refractive index (RI) detection is a powerful technique for quantifying various types of saccharides (Chávez-Servín et al., 2006; Knutsen et al., 2001; Yuan and Chen, 1999).

Our main object in this study was to establish and validate a sensitive, reliable and selective analytical method for the determination of GLcN in milk samples. Since lactose is a main saccharide in milk samples, it is best to determine it simultaneously in the method established. To meet these requirements, we developed an HPLC method with RI detection. The sensitivity, precision and accuracy of this method should be sufficiently acceptable to permit quantification of the GLcN and lactose in milk-based formulae.

2. Materials and methods

2.1. Equipment

The chromatographic analysis was carried out in a Waters high-performance liquid chromatograph equipped with a 515 Series pump, a Waters column heater module, a 2410 refractive index detector and a Rheodyne 7725 manual injector (Cotati, CA, USA) with a 20 μL sample loop. The chromatographic separation was achieved with a Shodex Asahipak NH2P-50 column (4.6 × 250 mm, 5 μm) from Showa Denko (Japan).

2.2. Chemicals

The chemicals such as sodium oxalate, sodium hydrogen phosphate and lead acetate used for sample preparations were of analytical reagent grade. Acetonitrile was of HPLC grade and purchased from B&J (Muskegon, USA). The standard saccharides (glucosamine hydrochloride, lactose), obtained from Sigma (St. Louis, MO, USA), were ⩾99.0% pure.

2.3. Sample

The milk-based formulae powder supplemented with glucosamine was obtained from a firm in Qingdao, China. According to the label, the composition of the formulae was as follows: carbohydrate 45%, protein 34%, fat 15%, Vitamin A 1300 IU/100 g, Vitamin B1 500 mg/100 g and calcium 650 mg/100 g. The samples were maintained at room temperature.

2.4. Sample preparation

Powdered sample of 0.5 g was accurately weighed and transferred to a 25 mL volumetric flask with 5 mL of distilled water added and homogenized for 2 min. Then 1.5 mL lead acetate solution (20%, w/v), 1.5 mL mixed solution of sodium oxalate (1.5%, w/v) and sodium hydrogen phosphate (3.5%, w/v) were added and re-homogenized for an additional minute. The solution was made up to 25 mL with distilled water. The sample was then left to stand for 30 min and filtered by dry filter paper. Finally the filtrate was passed through a 0.22 μm nylon filter from Yadong Resin Ltd. (Shanghai, China) before liquid chromatographic analysis.

2.5. Chromatographic conditions and quantification

Separation was performed at 35 °C using water–acetonitrile (30/70, v/v) as the mobile phase and at a flow rate of 1 mL/min. The injection volume was 20 μL (filling the loop completely). The total run time was 10 min between each routine injection. Peaks were identified by comparing retention time with sugar standards. The respective peak areas were used for the quantitative analysis. Calibration curves for each sugar were prepared at six levels, from 0.2 to 10 mg/mL for glucosamine and 1–20 mg/mL for lactose, all dissolved in water.

3. Results and discussion

3.1. Optimization of chromatographic conditions

We first tested the mobile phase of water–acetonitrile (35/65, v/v). Since complete separation of the peaks was not possible, we orderly lowered the quantity of water to 30% and 25%, and encountered the best resolution and effective separation of the chromatographic peaks with the mobile phase of water–acetonitrile (30/70, v/v). Fig. 1 shows the typical chromatograms of saccharide standards and saccharides in milk-based formulae.

3.2. Sample preparations tested

Since the protein content in the milk-based formulae was high, precipitation was needed to prevent the protein from emulsification. We tested several precipitators, such as acetic acid (Brendon and Harvey, 2007), zinc acetate and potassium ferrocyanide (Li and Han, 2002), lead acetate, sodium oxalate and sodium hydrogen phosphate (Liu and Chen, 1995). The last kind showed better results than the other precipitators, for its higher recoveries and less solvent consumption. It had the highest recovery of 96.5%, while zinc acetate and potassium ferrocyanide 92.5% and acetic acid 93.2%. The precipitators used completely precipitated all the substances which might interfere with sugar analysis and got stable baseline.

3.3. Validation of the proposed method

3.3.1. Linearity

Under the chromatographic conditions described above, a linear relationship between the concentrations of saccharides and peak areas was found with standard solutions at different concentrations. For glucosamine and lactose, the r^2 values were both >0.99 (Table 1).

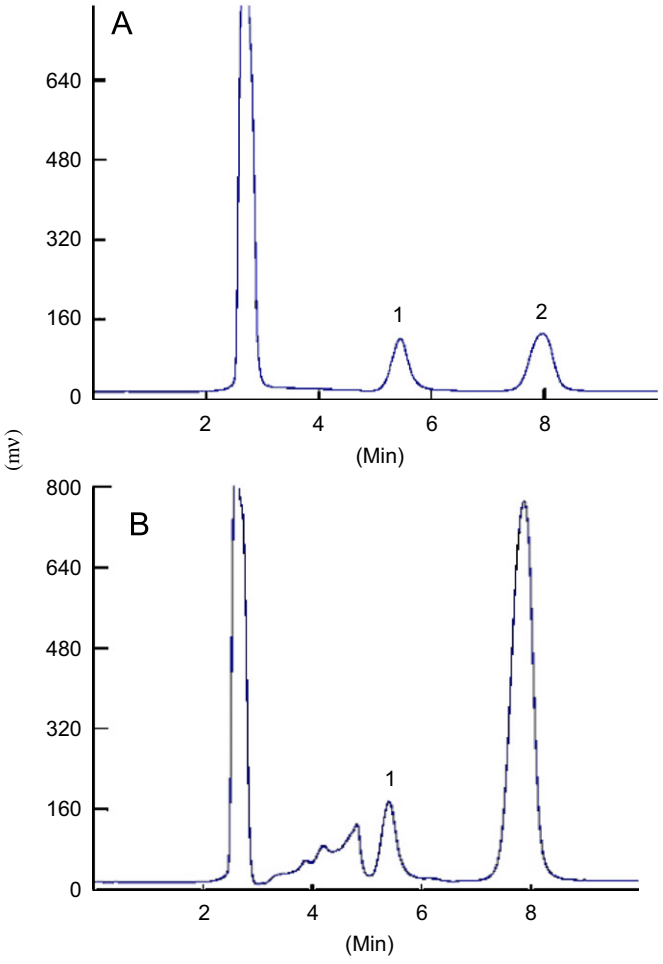

Fig. 1. Typical chromatograms of saccharide analysis by the HPLC method. Saccharide peaks: 1, glucosamine; 2, lactose. (A) Saccharide standards and (B) milk-based formulae sample.

3.3.2. Sensitivity

To check the sensitivity of the method under the working conditions proposed, the detection limit (DL) and the quantification limit (QL) were studied. DL and QL were determined by signal-to-noise ratio (S/N). The DL was defined as the lowest concentration level when the S/N was 3:1, while the S/N was 10:1 for the QL. The sensitivity results showed that the DLs for glucosamine and lactose were 0.11 and 0.13 mg/mL, while the QLs were 0.21 and 0.24 mg/mL. Results obtained showed good sensitivity (Table 1).

3.3.3. Precision

To evaluate the repeatability of the method, six replicate determinations of glucosamine and lactose in a milk-based formulae sample were performed using the same reagents and apparatus on the same day. The RSD value obtained was satisfactory (Table 2).

3.3.4. Recovery

For recovery, standards of glucosamine and lactose were added in a known mass at two levels in a previously analyzed formulae sample. Six determinations were carried out for each addition level. Results were reported in Table 3 and showed excellent recoveries. Recovery was

Table 2
Precision of the method

Component	Repeatability ($n = 6$)		
	Mean (g/100 g)	SD	RSD (%)
Glucosamine	4.85	0.06	1.26
Lactose	33.53	0.36	1.06

Table 3
Recoveries of the method

Component	Initial (g/100 g)	Added amount (g/100 g)	Found amount (g/100 g)	Recovery (%)	RSD (%)
Glucosamine	4.85	2.00	1.93	96.5	2.28
		6.00	5.89	98.2	2.74
Lactose	33.53	3.00	2.91	97.1	2.03
		10.00	9.79	98.0	2.45

Table 1
Regression equation and detection limit

Component	Regression equation[a] C (mg/mL)	r^{2b}	Linear range (mg/mL)	Detection limit (mg/mL)	Quantification limit (mg/mL)
Glucosamine	$Y = 14.613X-2.7433$	0.9987	0.2–10	0.11	0.21
Lactose	$Y = 16.79X-0.6534$	0.9991	1–20	0.13	0.24

[a] X: concentration (mg/ml); Y: peak area.
[b] Determination coefficient.

determined from

$$\%\text{Recovery} = \frac{\text{Increased amount}}{\text{Added amount}}.$$

4. Conclusion

The chromatographic method proposed allowed rapid and complete resolution of glucosamine and lactose in a chromatographic run. The results of saccharides analysis in milk-based formulae sample were given in Table 2, containing 4.85 ± 0.06 g/100 g of glucosamine and 33.53 ± 0.36 g/100 g of lactose. According to the results of the reliability study, the method proposed was precise and accurate, and showed appropriate sensitivity for application to the determination of glucosamine and lactose simultaneously in milk-based formulae.

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References

Barclay, T.S., Tsourounus, C., McCart, G.M., 1998. Comment: glucosamine: selecting appropriate study exclusion criteria. The Annals of Pharmacotherapy 32, 1371–1372.

Bassleer, C., Rovati, L., Franchimont, P., 1998. Stimulation of proteoglycan production by glucosamine sulfate in chondrocytes isolated from human osteoarthritis articular cartilage in vitro. Osteoarthritis Cartilage 6, 427–434.

Brendon, G., Harvey, I., 2007. Development and application of a liquid chromatographic method for analysis of nucleotides and nucleosides in milk and infant formulas. International Dairy Journal 17, 596–605.

Brereton, P., Hasnip, S., Bertrand, A., Wittkowski, R., Guillou, C., 2003. Analytical methods for the determination of spirit drinks. Trends in Analytical Chemistry 2, 19–25.

Chávez-Servín, J.L., Romeu-Nadal, M., Castellote, A.I., López-Sabater, M.C., 2006. Evolution of free mono- and di-saccharide content of milk-based formula powder during storage. Food Chemistry 9, 103–108.

Emmett, P.M., Rogers, I.S., 1997. Properties of human milk and their relationship with maternal nutrition. Early Human Development 49, 7–28.

Gonzáles, A.S.P., Naranjo, G.B., Malec, L.S., Vigo, M.S., 2003. Available lysine, protein digestibility and lactulose in commercial infant formulas. International Dairy Journal 13, 95–99.

Hawker, G., 1997. Update on epidemiology of the rheumatic diseased. Current Opinon in Rheumatology 9, 90–94.

Houpt, J.B., McMillan, R., Wein, C., Paget-Dellio, S.D., 1999. Effect of glucosamine hydrochloride in the treatment of pain of osteoarthritis of the knee. Journal of Rheumatol 26, 2423–2430.

Knutsen, S.H., Sletmoen, M., Kristensen, T., Barbeyron, T., Kloareg, B., Potin, P., 2001. A rapid method for the separation and analysis of carrageenan oligosaccharides released by iota- and kappa-carrageenase. Carbohydrate Research 331, 101–106.

Li, C., Han, H., 2002. Determination of oligolevulose in milk powder and powdered sugar by HPLC. Chinese Journal of Health Laboratory Technology 12, 307–308.

Liu, Z., Chen, H., 1995. Determination of lactose in milk powder by lodometric method and Lane and Eynor's method. China Dairy Industry 23, 286–288.

Olano, A., Calvo, M.M., Corzo, N., 1989. Changes in the carbohydrate fraction of milk during heating processes. Food Chemistry 31, 259–265.

Ruas-Madiedo, P., de los Reyes-Gavilan, C.G., Olano, A., Villamiel, M., 2000. Influence of refrigeration and carbon dioxide addition to raw milk on microbial levels free monosaccharides and myo-inositol content of raw and pasteurized milk. European Food Research and Technology 212, 44–47.

Salvador, L.D., Suganuma, T., Kitahara, K., Fukushige, Y., Tanoue, H., 2002. Degradation of cell wall materials from sweetpotato, cassava, and potato by a bacterial protopectinase and terminal sugar analysis of the resulting solubilized products. Journal of Bioscience and Bioengineering 93, 64–72.

Sarzi-Puttini, P., Cimmino, M.A., Scarpa, R., Caporali, R., Parazzini, F., Zaninelli, A., Atzeni, F., Canesi, B., 2005. Osteoarthritis: an overview of the disease and its treatment strategies. Seminars in Arthritis and Rheumatism 35, 1–10.

Setnikar, I., Rovati, L.C., 2001. Absorption, distribution, metabolism and excretion of glucosamine sulfate. A Review. Arzneimittel-Forschung 51, 699–725.

Troyano, E., Olano, A., Fernández-Díaz, M., Sanz, J., 1994a. Gas chromatographic analysis of free monosaccharides in milk. Chromatographia 32, 379–382.

Troyano, E., Olano, A., Martínez -Castro, I., 1994b. Changes in free monosaccharides during storage of dried milk. Journal of Agricultural and Food Chemistry 42, 1543–1545.

Yuan, J.P., Chen, F., 1999. Simultaneous separation and determination of sugars, ascorbic acid and Furanic compounds by HPLC—dual detection. Food Chemistry 64, 423–427.