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Determination of lactose in milk and dairy products by HPLC-RID method

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Abstract

Fast, simple high-performance liquid chromatography (HPLC) method with refractive index detection (RID) was optimized and validated for the quantification of lactose in milk and dairy products. The method showed good linearity with determination coefficient 0.9975. The limit of detection was 0.04 mg/mL, and the limit of quantification 0.12 mg/mL. The relative standard deviations for repeatability were 4.00 and for reproducibility 4.98 in reference material, while in different tested matrix the relative standard deviations for repeatability were in range 2.58–6.51. Average efficiency value was 97%. The applicability of the method was confirmed by its application in different samples. The presence of this sugar was not detected (lower than 0.1%) in the samples of milk and yogurt labelled as lactose-free, as well as in parmesan, cheddar, gouda and trappist cheese samples. In various dairy products the measured lactose content had values from 0.493±0.026% in home-made rolled cheese to 4.42±0.180% in cooking sour cream.

Keywords: lactose, determination, milk, dairy products, HPLC-RID

1. Introduction

Lactose is a disaccharide, a sugar naturally found in milk and dairy products. A significant percentage of the human population is lactose intolerant, and people with this problem tolerate a certain amount of lactose differently [1]. Therefore, it is necessary to determine the concentration of lactose in each product. Besides, the nutrition label must include the information about sugar content [2,3].

Currently, there is no single lactose concentration below which dairy products are defined as lactose-free. In some European countries, the lactose-free threshold is <0.1% (w/w), while in others the threshold is <0.01% (w/w), and in China products with <0.5% (w/w) lactose are labelled as lactose-free [4]. Due to the importance of achieving the lowest possible limit of detection in the determination of lactose, but also due to the complicated and different types of samples, methods of determining lactose are the subject of scientific interest and continuous improvement. Lactose in milk and products can be determined by using a range of various techniques. Traditional methods are based on mid-infrared detection [5], polarimetry [6] or gravimetry [7]. These methods are often simple, fast, or inexpensive to operate, but because of the tendency to develop methods that are more sensitive and/or specific enough to quantify lactose at low levels, the development of modern techniques is topical today. Nowadays, the methods of determining lactose include enzymatic methods [8-10], chromatographic methods [11,12] such as ultra-high performance chromatography coupled to mass spectrometry (UHPLC-MS) [13] or tandem mass spectrometry (UHPLC-MS/MS) [14,15], gas chromatography [16], high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) [17], and other innovative methods.

Since there is always a tendency for the simplest as possible method with cheap sample preparation and fast detection, in this paper we applied the high performance liquid chromatography (HPLC) method with refractive index detection (RID). The method was optimized and evaluated for its application for different and complex matrix of

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dairy product samples. Furthermore, the applicability of this method to different samples was confirmed in this research.

2. Materials and Method

For the purposes of optimization and validation of the analytical method, two milk powder samples with familiar lactose content were used (Table 1, Fapas 25189/2020, and 25205/2021). For repeatability testing, there were used dairy product samples with different characteristics were used, in order to verify methods on the different matrices with different lactose content (Table 2). Additionally, to validate method for low lactose content, two lactose-free milk samples, yoghurt, and four cheeses (parmesan, cheddar, gouda and trappist) were used for examination.

After method validation, cheese samples which are most commonly used for human consumption were selected for the analysis (Table 3). The samples were obtained from a supermarket (samples No 1 – 10), and the rest of the samples (the "home-made" samples No 11 – 15) were obtained from a market in Novi Sad, Vojvodina province, Serbia. All samples were made from cow's milk, and kept in the refrigerator at 4 ºC.

Standard substance d-lactose monohydrate (Sigma Aldrich 61339, ≥99.5%, HPLC), was used to prepare calibration standard solutions, and to construct a calibration curve at concentrations of 0.5 to 10 mg/mL in deionized water.

The principle of the method is based on the extraction of lactose with 7% perchloric acid, neutralization of the extract with sodium hydroxide, and determination by liquid chromatography with refractive index detection (HPLC-RID) [18]. Five grams of milk and crushed and homogenized cheese sample or 1 g of milk powder was used for extraction by shaking in 50 mL of a 7% perchloric acid. The sample was centrifuged, and 20 mL aliquot after neutralization to pH 7 was transferred to a volumetric flask, and filled up to 50 mL with deionized water. The prepared sample was injected into the HPLC system after filtration with a 0.22 µm PTFE syringe filter. Liquid chromatography equipment in our study included HPLC Dionex UltiMate 3000 Series system equipped with a refractive index detector RefractoMax521 (Thermo Scientific, Germany) at 35ºC and the HPLC column was Hypersil GOLD Amino 150x3 mm (particle size 3 µm), fitted with a guard column Hypersil GOLD Amino 10x3 mm (particle size 3 µm). The mobile phase was acetonitrile:water (85:15, v/v) filtered through 0.22 µm membrane filter, at a flow rate of 1 mL/min, injected volume of 5 µL. All measurements were conducted at room temperature. The system was controlled by Chromeleon® 7 software (Thermo Scientific, Germany).

The external calibration curves produced by standard solutions were used to quantify the amount of lactose in the samples. Lactose was identified and quantified by comparing retention time and peak area with that of standard sugar solution. The results were expressed as gram sugar per 100 g of milk and product (%). All samples were analyzed in duplicate.

3. Results and Discussion

The calibration curve for lactose determination was constructed based on the peak area from chromatograms on Figure 1. Linearity is expressed through a coefficient of determination of > 0.99. The standards for calibration are runs in parallel with the samples in each series of tests.

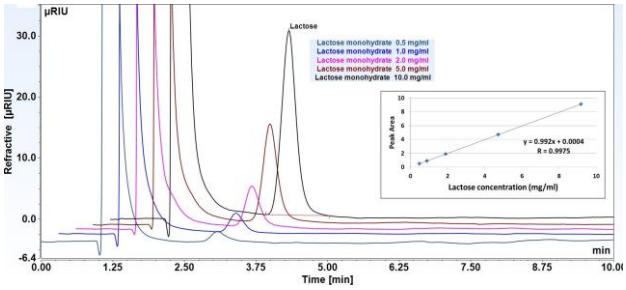

Figure 1. Chromatograms of lactose standard solutions used for calibration and calibration results

The limits of detection (LOD, signal-to noise ratio of 3:1) and quantification (LOQ, signal-to-noise ratio of 10:1) of the HPLC–RID method were calculated from the chromatograms of standard solutions. LOD was 0.04 mg/mL, while LOQ was 0.12 mg/mL. When it comes to dilution according to sample preparation, the LOD in the sample is 0.1% (0.5% for milk powder) and the LOQ is 0.3% (1.5 for milk powder) of lactose content. EU Regulation 609/2013 [2] defines the conditions about labelling the absence of lactose or it low content. The term "lactose free" can be used in milk and dairy products with content lower than 0.1 g per 100 g or mL. On the other hand, the term "low lactose content" can be quoted only if the content of

disaccharide is lower than 0.5 g per 100 g or mL. The limit of detection of the validated method is at the level of regulated values and can be used as a qualitative screening method for "lactose free" samples, and as a confirmatory method for samples "low lactose content".

For the evaluation of the repeatability of the method, six replicate determinations of lactose content in two reference milk powder materials were carried out on the same day. For reproducibility, six determinations of the same reference samples on different days were done. The standard deviations (SD), and relative standard deviations (RSD) show good precision (Table 1) within the limits of acceptable variability in methods of analysis. The efficiency or accuracy of the method was calculated based on the results of determining the same reference samples. Recovery value expressed as the mean for both reference samples was 97%.

The accuracy and precision of the method satisfies the requirements of AOAC for Single Laboratory Validation methods [19], in the range of quantification 0.1–10.0 mg/mL.

In order to validate the method for different matrices of samples, the lactose content was determined in the series of samples shown in Table 2. It can be seen that the repeatability is satisfactory, and obtained RSD is in accordance with the different samples homogeneity. Namely, it is lower for liquid samples that are more homogeneous than cheese. The obtained results are comparable with the results of other authors who applied the same method of detection, but with different preparation of the milk sample [20,21].

Satisfactory results were obtained by testing lactose-free products by the developed method. Namely, the presence of this sugar was not detected (lower than 0.1%) in two samples of milk, and yogurt labelled as lactose-free, as well as in certain types of cheeses known for their low lactose content due to production technology: parmesan, cheddar, gouda and trappist cheese samples. The sensitivity and specificity of the method is illustrated in the Figure 2, where the chromatograms of the sample with and without lactose are shown. It can be concluded that there are no interfering peaks, the separation is good and the retention times coincide with the standard lactose solution.

The results of the determination of lactose in samples with different lactose content are presented in Table 3, without correction for recovery. As can be seen from the obtained results, the tested cheeses that are traditionally used in the diet in Serbia contain between 1 and 3% lactose. Also, it can be seen that soured milk (Sample No. 2) and cooking cream (Sample No. 10) contain more lactose than cheese, while home-made dairy products have the lowest percentage of lactose (Samples No. 13-15).

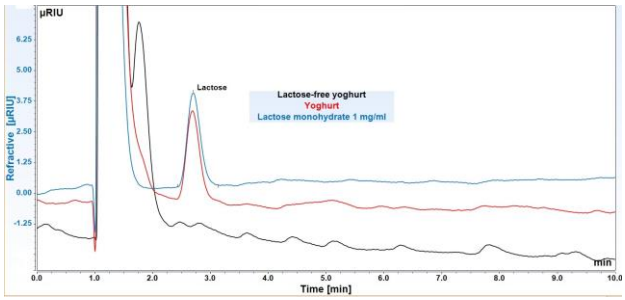

Figure 2. Chromatograms of lactose-free and common yoghurt sample in comparison with lactose standard solution chromatogram

Dairy products, cheese in particular, are a significant part of the traditional diet in Serbia. Also, Serbia is known for a large number of different characteristic dairy products. Although experiential data on the range to which lactose and sugar content can be found in some dairy products are available in the literature [22,23], there are no more specific data on the measured values. The measured values in our research fit into the literature data, but at the same time provide more precise knowledge about the lactose content in certain types of dairy products.

Table 1. Precision of the lactose determination method, based on lactose determination in internal reference materials

	Repeatability (n=6)			**Reproducibility (n=6)**		
Reference value±SD (%)	Average (%)	SD (%)	RSD (%)	Average (%)	SD (%)	RSD (%)
34.2±2.4	32.06	1.28	4.00	32.71	1.38	4.22
44.0±2.6	43.38	1.59	3.67	44.57	2.22	4.98

Table 2. Repeatability of method for different matrices of dairy samples (n=3)

Sample type	Mean (%)	SD (%)	RSD (%)
Milk	4.35	0.112	2.58
Rolled cheese	0.493	0.026	5.29
Soft cheese	2.69	0.175	6.51
Cooking sour cream	4.42	0.180	4.06

Table 3. Content of lactose in different dairy products

No of sample	Sample type	Lactose content±SD (%)
	Original dairy products from dairies (Supermarket)	
1	Yogurt	2.14±0.081
2	Soured milk	3.41±0.180
3	Young cheese	2.97±0.118
4	White cheese	1.19±0.086
5	Feta cheese	1.13±0.076
6	Sour cream	2.17±0.100
7	Soft cheese	2.69±0.175
8	Processed cheese	2.10±0.080
9	Cream cheese	2.01±0.102
10	Cooking sour cream	4.42±0.180
	Home-made dairy products from the market	
11	Soft cheese No1	2.66±0.163
12	Soft cheese No2	2.10±0.151
13	"Kajmak"	1.57±0.080
14	White cheese	0.850±0.060
15	Rolled cheese	0.493±0.026

4. Conclusions

In this research we described performances of a simple and fast HPLC–RID method for detection and determination of lactose. This method is suitable for routine analysis of this important disaccharide in milk and milk-based products, in order to monitor the safety of lactose free products, as well as for determination of milk sugar in order to obtain a reliable nutritional declaration. The method provides acceptable precision, recovery and sensitivity. The measured values of lactose in traditional Serbian dairy products, give more precise insight into the content of this health-important disaccharide in certain types of dairy products.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards. Authors declare that they present their own literature survey and results/discussion/conclusion in the article.

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