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A Validated HPLC-RID Method for Quantification and Optimization of Total Sugars: Fructose, Glucose, Sucrose, and Lactose in Eggless Mayonnaise

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Abstract: Mayonnaise is an oil-in-water emulsion containing 70–80% finely dispersed droplets of oil in a continuous phase of water. Since mayonnaise has a sour and acidic taste, its sugar profile is barely noticed and thus often disregarded. However, today, there are various variants of mayonnaise available on the market; hence, it is crucial to understand their mono- and disaccharide profile, in order to determine the precise total sugar composition. The traditional methods of sugar analysis available, such as titration, can only quantify sucrose and are unable to differentiate between mono- and disaccharides. The aim of this study was to develop and validate a method for the quantification of total sugars, including fructose, glucose, sucrose, and lactose, in eggless mayonnaise, using a high performance liquid chromatography refractive index detector (HPLC-RID). Sugars were separated on an amino column with an oven temperature of 35 °C, using an isocratic solvent system consisting of a 75:25 *v/v* mixture of acetonitrile and HPLC water, at a 0.9 mL/min flow rate with RID. Method validation was performed for the linearity, specificity, precision, accuracy, LOD, LOQ, and robustness. A linearity for total sugars, with a regression coefficient of 0.9998, was obtained within the range of 0.05024 to 10.048 mg/mL. The relative standard deviation was less than 2.0% for the intra-day and inter-day precision. The accuracy was found to be 96.78–108.88% using a three-level recovery method. The LOD and LOQ were also found to be suitable. The samples used in this study contained 0.24–10.32% total sugars. The sucrose value obtained matched the label claim of the products and no significant differences were observed between results in a paired sample *t*-test. This showed the applicability of the proposed method for analyzing the sugar profile in a finished product. Routine analysis of total sugars in eggless mayonnaise and similar finished products can thus be performed using this technique, which was found to be simple, rapid, and reproducible.

Keywords: total sugars; monosaccharide; disaccharide; eggless mayonnaise; HPLC-RID method



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1. Introduction

One of the most common ingredients in the diet is carbohydrate [1–6]. Monosaccharides, disaccharides, oligosaccharides, polysaccharides, and nucleotides make up the five primary classes of carbohydrates [7]. Monosaccharides such as fructose, galactose, and glucose, and disaccharides such as sucrose, lactose, and maltose have a characteristic sweet taste [8]. According to certain definitions, the term “sugars” mostly refers to mono- and disaccharides [9,10]. The Food and Drug Administration’s (FDA) new policy states that when the sugar content in foods is greater than 1%, an analysis is necessary [11,12].

With the increasing prevalence of public health issues such as obesity and diabetes, it is important to increase consumer awareness about sugar consumption and monitoring its intake from processed foods. Various regulatory authorities such as the European Union (EU), Food and Drug Administration (FDA), Food Safety and Standards Authority of India (FSSAI) etc. have made it mandatory to declare the sugar content on product labels [13,14]. Mono- and disaccharide determination is one of the most commonly requested tests in

the food analysis laboratory [15]. Sugar analysis is useful for monitoring sugar-labelling claims in calorie-reduced foods; determining food energy content; testing fruit juice quality or adulteration [16]; determining the amount of lactose in milk; measuring the amount of lactose in low-lactose or lactose-free foods [17]; and monitoring sugar content in sugar beet, cane molasses, and regular and high-fructose corn syrups [18]. Glucose, fructose, sucrose, lactose, and maltose must all be analyzed, in order to determine the total sugar content in food.

The advent of more complex food matrixes and product innovations have made it essential to analyze the sugar content of food products, in a wide variety of foods, such as cereal products, dairy products, sweets, beverages, sauces, etc. [15]. Mayonnaise has been produced since its origins in France [19] and is commonly consumed globally. It is an oil-in-water emulsion, despite containing 70–80% fat. This oil in water emulsion consists of finely dispersed droplets of oil in a continuous phase of water or a dilute aqueous solution [19]. Despite the fact that the value of mayonnaise and similar sauces in the global market is constantly rising, there are still growing health concerns regarding the nutritional profile of conventional mayonnaise, due to its high caloric content, consumption of cholesterol content from eggs, and quick auto-oxidation of unsaturated fatty acids in the lipid fraction. These problems conflict with the growing demand for natural, wholesome, and more nutrient-dense food items, which is necessary to satisfy both business and health needs. For these reasons, eggless, low-calorie mayonnaise is the preferred consumer choice and is in increasing demand [20,21]. It is consumed often and is relished for its distinctive flavor and smooth mouthfeel [22]. Due to the acidic nature of mayonnaise, it has a longer shelf life [23]. Owing to its slight sour taste, the sugar profile of mayonnaise is usually disregarded. However, labelling norms mandate the declaration of sugars on the label; thus, the estimation of its sugars is crucial. Due to the presence of acid in mayonnaise, over time, heat may hydrolyze the sugars such as sucrose into fructose and glucose. Estimating the fructose and glucose content in the product can help in determining its stability, product quality, and label compliance.

Physical, chemical, and biological approaches are still being used in the analysis of carbohydrates, even though chromatographic methods are currently preferred [15]. A number of chemical methods are used to determine the total concentration of monosaccharides and oligosaccharides as total sugars, based on reducing properties, which can react with other additives to yield precipitates or colored complexes, which can be quantified. However, there are limitations to quantifying the actual concentration of individual non-reducing and reducing sugars using these hydrolyzation techniques.

There are many traditional methods available for quantifying carbohydrates. These methods can generally be classified into three categories: titration, gravimetric, and colorimetric.

- (1) **Titration Method:** The Lane–Eynon technique is an example of this category that can quantify the concentration of reducing sugars in a sample. Using a burette, a sample solution is added to a flask containing a known amount of boiling copper sulfate solution and a methylene blue indicator. The reducing sugar available in the sample reacts with copper sulfate. As soon as the entire copper sulfate in solution has reacted, any further addition of reducing sugars causes the indicator to change its color from blue to white. The volume of sample solution required to attain this end point is recorded. Since this reaction is non-stoichiometric, it is important to prepare a calibration curve using standard solutions with a known carbohydrate concentration. The disadvantages of this method is that the results are dependent on the reaction time, temperature, and amount of reagent used, thus these factors should be precisely considered. This method also cannot differentiate between different types of reducing sugars nor determine the concentration of non-reducing sugars. It is also time consuming, tedious, and susceptible to interference from different molecules that act as reducing agents [24].
- (2) **Gravimetric method:** The Munson and Walker method is the common method in this division. This method is used to measure the concentration of reducing sugars in a

sample. Carbohydrates are oxidized in the presence of heat and an excess of copper sulfate and alkaline tartrate under controlled conditions. This results in the formation of a copper oxide precipitate. The quantity of precipitate formed is directly related to the concentration of reducing sugars in the sample, which can be determined gravimetrically (by way of filtration, drying, and weighing), or titrimetrically (by way of re-dissolving the precipitate and titrating with a suitable indicator). This technique has the same disadvantages as the Lane–Eynon technique; nevertheless, it is more reproducible and accurate.

- (3) **Colorimetric method:** The anthrone method is an example of a calorimetric method, in which the concentration of sugars in the sample can be estimated using the principle of colored complex formation. In this method, the sample is mixed with sulfuric acid and anthrone reagent, which is further boiled until the reaction is completed. Sugars react with the anthrone reagent under acidic conditions to yield a blue-green color. The absorbance at 620 nm is measured after the solution is cooled down using a spectrophotometer. Similarly, the phenol–sulfuric acid technique is another colorimetric method that is widely used to estimate the total concentration of carbohydrates present in food. A clear aqueous solution of the sample to be analyzed is placed in a test tube, after which phenol and sulfuric acid are introduced gradually. The interaction of carbohydrates with phenol turns the color of the solution yellow–orange. Sulfuric acid causes all non-reducing sugars to be converted to reducing sugars, this helps in the estimation of total sugars. The absorbance is measured at 420 nm. In both these methods, there is a linear relationship between the absorbance and amount of sugar present in the sample, which helps the quantification. They have the same limitations as observed with the previous techniques [24].

Modern Methods: Chromatographic techniques are advanced and effective analytical techniques used to analyze both the quantity and type of sugars present in food. Thin layer chromatography (TLC), gas liquid chromatography (GLC), and high performance liquid chromatography (HPLC) are generally used to separate and identify carbohydrates. The separation is based on the differential adsorption characteristics of individual sugars. Partition coefficient, polarity, and size and type of column used are the main factors affecting separation [24]. HPLC and GLC are both favored methods that over the past 20 years have been actively employed, due to their high specificity and capacity to simultaneously determine many sugars [25,26]. A prerequisite for GLC is that the sample should be either volatile or in derivatized state, whereas HPLC samples can be analyzed as such, with a simple sample preparation with no derivatization, which saves time.

HPLC and GLC are commonly used along with nuclear magnetic resonance (NMR) or mass spectrometry, so that the chemical structure of the molecules that make up the peaks can also be identified. [24]. Due to its simplicity and relative sensitivity, ultraviolet (UV)-based detection is frequently utilized in HPLC. However, a refractive index (RI) detector is frequently employed, as a general-purpose detector to identify substances such as carbohydrates that lack UV chromophores. While there are some commonalities between these detection techniques, using various detectors results in a varied sensitivity and stability for a given analyte. Since UV chromophores are absent in sugars, RI detection may be preferable for sugar analysis. There are methods used for sugar analysis with UV at a wavelength below 200 nm; however, this is subject to interference from the solvent, which results in lower resolution of peaks, hence RI is used [27].

RID is a universal detector. Its detection principle involves measuring the change in refractive index of an effluent flowing through the flow-cell relative to the mobile phase. The signal increases with the difference in refractive indices between the mobile phase and effluent. It is impossible to detect an eluted component if it shares the same refractive index as the mobile phase. RID is highly sensitive to temperature changes, so it is crucial to maintain a stable temperature throughout the analysis. This is the detector of choice for sugar analysis [28].

The sugar determination techniques that are (association of official agricultural chemists) AOAC approved include indirect physical, enzymatic, or semi-empirical chemical techniques [10,29,30]. Out of the methods mentioned by the AOAC, HPLC is the ideal method for determining the amount of simple sugars in a variety of food products [18]. There have been no HPLC studies reported for the analysis of the mono and disaccharides in eggless mayonnaise. Therefore, it is crucial to establish a validated method for the rapid sugar analysis in this complex food matrix, and which can be used for day-to-day analysis. A summary of the different methods of sugar estimation is shown in Table 1.

Table 1. Summary of the different methods of sugar estimation.

Sr. No.	Method	Advantages	Disadvantages
01	Traditional methods: Lane–Eynon technique (Titration), Munson and Walker method (Gravimetric Method), Anthrone method (Colorimetric method) Phenol–Sulfuric Acid technique (Colorimetric method).	<ul style="list-style-type: none"> - No instrumentation to very less instrumentation required 	<ul style="list-style-type: none"> - Time consuming and tedious - Low precision in titration - Safety concern in handling heating apparatus - One test at a time - Cannot differentiate different types of reducing sugars - Not able to directly determine the concentration of non-reducing sugars - Susceptible to interference from different molecules that act as reducing agents - Measures total sugar as sucrose only, not able to differentiate between mono and disaccharides - Chances of errors are more
02	HPLC-RID method	<ul style="list-style-type: none"> - Simple, accurate, and robust method - Rapid and low cost when compared to hyphenated techniques and easy to control - Able to differentiate between mono and disaccharide - Sample preparation is simple, less effort required, and is environmentally friendly 	<ul style="list-style-type: none"> - Sensitivity of RID detector is less compared to UV detector but is useful with absence of chromophore in the analyte of interest

2. Materials and Method

2.1. Eggless Mayonnaise Samples and Chemicals Used

Three different brands of eggless mayonnaise were purchased from the local market and kept in a refrigerator until use. Standards of fructose from Tate & Lyle, glucose from Maize products, sucrose from Merck group, and lactose from DFE Pharma were used. All standards were of high purity ($\geq 99.0\%$). HPLC-grade acetonitrile from Qualigens Pharma Pvt. Ltd. (Khopoli, Maharashtra, India) was acquired. Class A Borosilicate volumetric glassware were used for the analyses. HPLC-grade water was obtained from an in-house Milli-Q apparatus (Millipore, Bedford, MA, USA).

2.2. Method

HPLC: In this study, a Shimadzu (Kyoto, Japan) HPLC system (Model: LC-2030C 3D) equipped with thermostat column, vacuum degasser, quaternary pump, and refractive detector (RID) was used. The mobile phase was selected as per AOAC 977.20 with isocratic solvent system consisting (75:25 *v/v*) of acetonitrile and HPLC water at 0.9 mL/min flow rate. The RID was operated with polarity +, cell temperature 35 °C, and response 1.0 s. The column oven temperature was set at 35 °C. The chromatographic data were acquired, monitored, and processed using Shimadzu lab solutions software from Shimadzu corporation.

2.3. Selection of Chromatography Column

The key component of HPLC method is the selection of an appropriate chromatographic separation column, as this directly affects the component resolution and analytical outcomes. Understanding the column chemistry helps in choosing the right column

for separating the analyte of interest. In our study, a stainless-steel silica-based phenomenex luna amino (4.6 mm × 250 mm, 5 µm) column was found to be best suited to the desired separation.

2.4. Optimization of Mobile Phase

Strong polar solvents (such as water, methanol, and acetonitrile) should be used as the mobile phase, because sugar molecules contain polar groups. According to the AOAC (16), the amount of water in the mobile phase has a significant impact on the ability of the carbohydrates to be retained. An increase in the amount of water in the mobile phase caused the carbohydrates to elute more rapidly. Acetonitrile and water were selected as the mobile phase in the current study, at two different ratios 83:17 and 75:25 (*v/v*). With an optimized acetonitrile concentration, it was seen that the resolution (distance between two adjacent peaks in the chromatogram) improved. The best separation and shortest retention time were seen with an acetonitrile to water ratio of 75:25 (*v/v*). The resolution of fructose and glucose was not optimal at the ratio 83:17(*v/v*), when compared to 75:25 (*v/v*). Hence, the acetonitrile-to-water ratio of 75:25 (*v/v*) was used for subsequent studies.

2.5. Sample Preparation

Mixed Standard solution preparation: Fructose, glucose, and lactose at 5 mg each, and sucrose at 500 mg were accurately weighed and transferred to a 100 mL volumetric flask, 50 mL of diluent (HPLC Grade Water) was added; the solution mixed well and sonicated for 2 min. After sonication, the volume was made up with diluent (HPLC Grade Water) and subjected to HPLC analysis. The injection volume was 10 µL. Further standard solutions were prepared fresh each day, for intraday and interday analyses. Individual sugar standards solution: fructose, glucose, lactose, and sucrose were initially injected, to identify the respective peaks.

Test sample preparation: First, 1000 mg of eggless mayonnaise sample was accurately weighed and transferred to a 100 mL volumetric flask, 50 mL of diluent was added (HPLC Grade Water), it was mixed well, with for sonication for 2 min. After sonication, the volume was made up with diluent (HPLC Grade Water). Then, the solution was filtered through a 0.45 µ Polyvinyl Difluoride (PVDF) syringe filter and subjected to HPLC analysis. The injection volume was 10 µL. The concentrations of fructose, glucose, sucrose, and lactose were calculated based on the calibration curve equation.

3. Validation of the Method

The International Conference for Harmonization's guidelines were followed when validating the analytical procedure (ICH, 1996). The method's linearity, range, specificity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), and robustness were all validated.

Linearity: Linearity was determined using a mixed standard solution of sugars: fructose, glucose, sucrose, lactose. First, 0.05024 to 10.048 mg/mL of the standard solution was prepared. The peak area and concentration were used to plot the calibration graphs. Linearity was evaluated at seven points. Sample linearity was evaluated in the range 2.58–15.32 mg/mL.

Precision: The precision was determined by analyzing 10 mg/mL of eggless mayonnaise sample on the same day for intraday precision and on different day for inter-day precision, using the proposed method. Relative standard deviation (RSD) was used as key parameter for determining the precision of the method.

Accuracy: The accuracy of the method was tested by performing recovery studies at three different levels in the eggless mayonnaise sample, by adding a reference sugar standard. Standard fructose, glucose, sucrose, and lactose were added to the eggless mayonnaise sample at 50–150% level and further analyzed using the proposed HPLC RID method.

Spiking was carried out to ascertain the purity of the peaks. The spiked concentration in the sample was set as a % of actual amounts of individual sugars found in sample. A total of 50.91 mg, 101.82 mg, and 152.73 mg of sucrose; 2.07 mg, 4.14 mg, and 6.21 mg of lactose; and 1.87 mg, 3.74 mg, and 5.61 mg of fructose and glucose were added per 100 g of sample. The recovery and average recovery were calculated. Each concentration level was determined in triplicate.

Limit of Detection (LOD) and Limit of Quantitation (LOQ): Based on the ICH guidelines for the Registration of Pharmaceuticals for Human Use, a signal-to-noise ratio approach was used for determining the limits of detection and quantitation.

Robustness: Chromatographic parameters, such as the mobile phase composition and flow rate, were changed to determine their impact on the quantitative analysis and in order to assess the method's robustness.

4. Results and Discussion

Chromatography: Under the mentioned conditions, the fructose, glucose, sucrose, and lactose were eluted within 20 min. The peaks in the HPLC chromatogram of eggless mayonnaise sample were identified by comparing the retention time of sugars in the samples with the standards. Under the chromatographic conditions, a good separation was achieved among individual sugars (see Figure 1). The retention times of the fructose, glucose, sucrose, and lactose in the mixed standard solutions were 5.858, 6.467, 7.475, and 8.250 min, respectively. The retention time of individual sugars of the test samples are shown in Figure 2. Figure 1 shows the chromatogram of standards, Figure 2 illustrates the chromatogram of sugars in the eggless mayonnaise samples, whereas Figure 3 displays overlay chromatogram of both standards and sugars in the eggless mayonnaise samples.

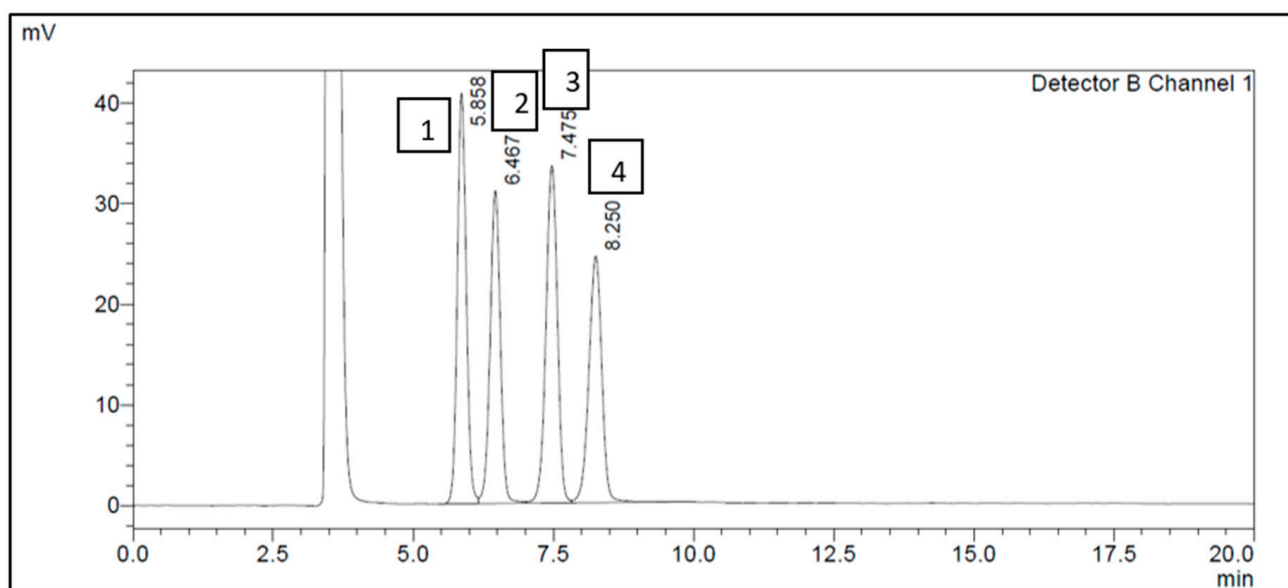


Figure 1. Chromatogram of Standard Sugars: (1) Fructose, (2) Glucose, (3) Sucrose, (4) Lactose.

The quantification data are shown in Table 2. Eggless mayonnaises of different brands were analyzed for their sugar profile and the results were found to be satisfactory. A paired sample *t*-test was performed for all three samples' sucrose value, the *p* value was 0.057, which is greater than 0.05 and shows no significant difference was observed between the sucrose values.

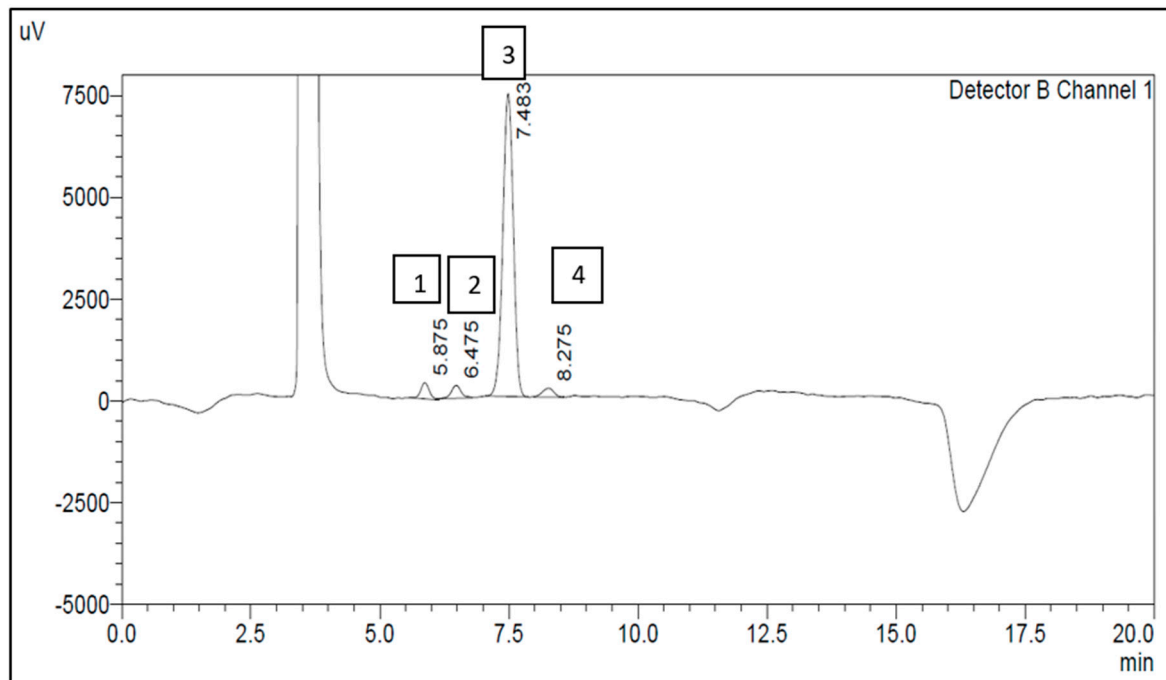


Figure 2. Chromatogram of Sugars in the Eggless Mayonnaise Sample (1) Fructose, (2) Glucose, (3) Sucrose, (4) Lactose.

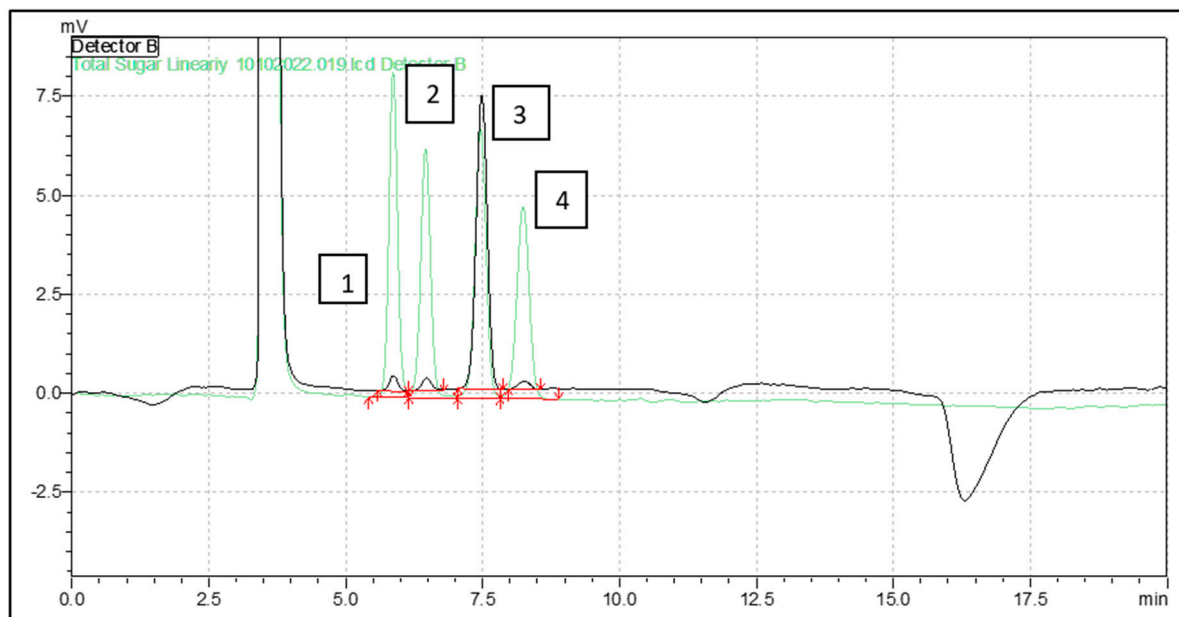


Figure 3. Overlay Chromatogram of Sugars in Eggless Mayonnaise Sample: (1) Fructose, (2) Glucose, (3) Sucrose, (4) Lactose, and standard sugars (green color chromatogram—standard sugars; Black color chromatogram—sugars in eggless mayonnaise sample).

Table 2. Sugar profile of the eggless mayonnaise samples.

Eggless Mayonnaise	Fructose (%)	Glucose (%)	Sucrose (%)	Lactose (%)
Sample 1 (Brand A) *	0.39 ± 0.0073	0.45 ± 0.0076	10.16 ± 0.1379	0.45 ± 0.0073
Sample 2 (Brand B)	0.37	0.37	10.08	0.41
Sample 3 (Brand C)	0.24	0.24	10.32	0.48

Mean % ±SD, n = 12. * Sample 1 (Brand A) was used for the validation studies. The sugar profile of Brand B and C was tested to understand the applicability of the validated method. Paired sample *t*-test: *p* value is 0.057 > 0.05 so there was no significant difference observed between the sucrose values.

The sucrose value obtained matched the label claim of the product. This demonstrated the applicability of proposed method for analyzing the sugar profile of finished products. The linearity, range, specificity, precision, accuracy, LOD, LOQ, and robustness were all validated. The calibration graphs for sugar standards: fructose, glucose, sucrose, and lactose were within the concentration range of 0.05024 to 10.048 mg/mL, with a correlation coefficient (r^2) of 0.9998 (Table 3). Sample linearity was evaluated in the range 2.584–15.324 mg/mL, with a correlation coefficient (r^2) of 0.99 (Table 4).

Table 3. Results of the validation parameters for sugar standards.

Parameters	Fructose	Glucose	Sucrose	Lactose
Range (mg/mL)	(0.050275–10.055)	(0.05024–10.048)	(0.05029–10.058)	(0.050365–10.073)
Linearity				
Correlation coefficient (r^2)	0.9998	0.9998	0.9998	0.9998
LOD (ppm)	15.8	18.26	20.56	22.97
LOQ (ppm)	47.89	55.34	62.32	69.59

The interday and intraday precisions of the individual standard sugars are provided in Table 3 and test samples are provided in Table 4. The results showed the acceptable precision of the method, with RSD values much lower than 2.0%. The recovery at three different levels of sugar (50%, 100%, and 150%) was found to be in the range of 90–110% (Table 4), which indicates the accuracy of the method. The LOD and LOQ are provided in Table 3.

These values show the high sensitivity of the method, which were calculated using the signal-to-noise (S/N) ratio. For LOD, the S/N ratio was 3:1, and for LOQ, it was 10:1.

The robustness of the method was also assessed with minor modifications of the mobile phase composition (water: acetonitrile in the ratio of 73:27 *v/v* and 77:23 *v/v*) and the mobile phase flow rate (i.e., 0.7 mL/min and 1.1 mL/min) (Table 4). With minor variations in the chromatographic parameters, the method showed robustness.

Table 4. Results of the validation parameters for sugars in eggless mayonnaise samples.

Parameters		Fructose	Glucose	Sucrose	Lactose	Acceptance Criteria
Linearity Range (mg/mL)		2.584–15.324	2.584–15.324	2.584–15.324	2.584–15.324	(r ²) 0.99
Regression equation		y = 387.51x + 182.47	y = 408.16x + 5.5315	y = 9878.8x + 16.667	y = 502.32x – 325.3	
Correlation coefficient (r ²)		0.9927	0.9978	0.9999	0.9914	
In linearity; x is concentration of sugars (fructose, glucose, sucrose, and lactose) in mg/mL; y is the peak area						
Precision						RSD ≤ 2.0
a.	Intra-day % RSD (Relative standard deviation)	0.554	1.326	1.067	1.656	RSD—(Relative standard deviation)
b.	Inter-day % RSD (Relative standard deviation)	1.86	1.7	1.36	1.63	
Robustness						
1.	Change in Mobile phase ratio					RSD ≤ 2.0
(a)	73:27 v/v % RSD (Relative standard deviation)	0.921	0.123	0.409	1.913	RSD—(Relative standard deviation) RSD ≤ 2.0
(b)	77:23 v/v % RSD (Relative standard deviation)	1.102	1.512	1.536	0.618	
2.	Change in Flow rate					
(a)	0.7 mL/min RSD (Relative standard deviation)	1.553	0.588	0.89	0.053	
(b)	1.1 mL/min RSD (Relative standard deviation)	0.431	1.044	0.293	0.018	
Accuracy						
50% spiked level (% recovery)		104.65 ± 1.95	105.01 ± 0.69	100.53 ± 1.50	99.91 ± 0.17	90–110%
100% Spiked level (% recovery)		97.39 ± 0.36	99.29 ± 1.34	99.75 ± 1.70	96.78 ± 1.43	
150% Spiked level (% recovery)		108.88 ± 0.42	100.96 ± 1.93	100.93 ± 1.51	98.27 ± 1.07	

5. Conclusions

This paper highlights the superiority of modern analytical techniques, i.e., HPLC–RID, over traditional titration, gravimetric, and colorimetric methods for sugar analysis. The results of the traditional methods rely on an exact reaction time, temperature, and reagent concentration being used; they are unable to determine the concentration of non-reducing sugars directly and are vulnerable to interference from other types of molecules that function as reducing agents. The HPLC-RID technique is cutting-edge, efficient, and the method of choice for analyzing the amount and type of sugars present in food. The technique is less time- and solvent-consuming than the conventional methods, while being simple, accurate, reliable, quick, and environmentally friendly. This method can easily quantify the different types of mono- and disaccharides, i.e., fructose, glucose, sucrose, and lactose. Sample preparation is very simple and requires less effort. With integrated HPLC software, it is easy to produce accurate and reliable results. In this research, we developed and validated a HPLC-RID method for simultaneous assay, identification, and quantitation of sugars: fructose, glucose, sucrose, and lactose in eggless mayonnaise. The proposed method was proven to be robust using an experimental design with good resolution. This method could be successfully adopted in quality control labs for the routine analysis of sugars in eggless mayonnaise, because of the robust results observed with the tested samples. Additionally, this HPLC technique was found to be linear, sensitive, accurate, and robust during a successful validation utilizing the ICH Q2 (R1) guidelines. This method has a short run time (20 min); hence, a high throughput can be expected for the quality control and quick screening of prototype batches in product development laboratories. It also has the advantage of clear quantification of individual sugars compared to the traditional titration methods, where only total sugars, such as sucrose, are estimated. This method is affordable and environmentally friendly, because it uses minimal amounts of solvent for both the mobile phase and sample preparation. Furthermore, this highly efficient separation-based analytical method could be useful in the food industry to estimate and state the precise sugar content on food labels, and to maintain a “food integrity assurance”.

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