Validation of method for analysis of glucose in food additives

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Abstract. The dangers of consuming various food and drink products containing substances with a potential risk to human health are the subject of increased interest. There is a drive among manufacturers and the scientific community to ensure safety in the use of various foods and beverages with proven and/or suspected links to one or another disease state. Fast and reliable methods of analysis are increasingly sought for the purpose of establishing the type and concentrations of the contained substances and elements, which methods support the implementation of strict control of the quantitatively regulated levels of various indicators, including main components and impurities. The current work presents a validation procedure of an iodometric method for determining the content of glucose as the main component of three types of nutritional supplements. To evaluate the method, results for the analytical characteristics specificity, repeatability, accuracy and linearity are presented and analyzed.

1 Introduction

Glucose is the main sugar found and freely circulating in the body's bloodstream [1]. It serves as food for muscle cells, through which they receive "quick" energy, and is the main source of energy for red blood cells. However, from the point of view of science of nutrition, glucose contains no vital nutrients. Together with fructose, it is contained in equal amounts in sugar, which is used in various food products and is considered the main culprit for the increased incidence of type 2 diabetes [1]. For example, Tankova [2] reports that in eight studies with 310,819 participants, it was found that the intake of sugar-sweetened beverages is associated with the development of type 2 diabetes. Krastev [3] indicates that in the presence of certain risk factors, among which immobility, abuse of refined sugars and obesity, the incidence of the disease diabetes mellitus is increasing significantly.

Food supplements are concentrated sources of certain substances with a nutritional or physiological effect and are intended to supplement the normal diet. These substances, alone or in combination, are intended to be taken in small measured amounts [4] and are usually dosed in the form of capsules, dropper bottles, tablets, pills, and more. In order to protect human health and due to the fact that these products are consumed directly by humans, certain regulatory restrictions are placed on them, such as: absence of toxic levels of certain elements, a certain expiration date, a requirement for constant quality control, and others. When assessing their safety, a number of factors should be monitored: the quantitative presence of a given substance; the oxidation state of the element in the product; application method; and others. However, for this purpose, reliable methods of analysis should be applied to ensure their safety.

The reliability of the analytical methods, for its part, should be established through a validation process, by means of which it is confirmed that the applied analytical procedure is suitable for performing the relevant analysis [5, 6], as well as that the validated method is fully characterized, and the obtained results correspond to the purposes for which this method is applied [7]. Adherence to the validation protocol is therefore critical, as all aspects of the analytical procedures, including the instrumental technique and sample preparation process, must be validated and declared acceptable. The validation process should be considered effective only if appropriate analytical characteristics and evaluation criteria are selected [8, 9].

The method proposed and described in this article is a classic method – iodometry, which aims to determine glucose (d-glucose/dextrose) in food supplements. It is widely known in practice and has been described by a different of authors [10 - 12] in the direct determination of glucose content. In this case, the basis for its adaptation to the studied products is given by their composition, namely, d-glucose is added as the main ingredient to all three types of food supplements studied. This method differs from that recommended in the Ph. Eur. and BP for similar type of products, namely determination of the optical rotation of a solution of the test product of a certain concentration and its polarimetric comparison with that of a standard dextrose solution in the sample matrix [13, 14].

The aim of this work is to present the results of the performed validation procedure of a method for the iodometric determination of glucose in food supplements.

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2 Materials and methods

In the experimental work, a nutritional supplement (three types) was used, with the main composition glucose, which is a commercial product falling under trade secret protection. An average sample is taken from each of the three products, which is homogenized and further used for the analysis.

The following reagents were used to perform the experimental work: 0.1 mol/l I_2 (pure for analysis, Ferak), 0.1 mol/l NaOH (pure for analysis, Valerus), 1 mol/l H_2SO_4 (pure for analysis, Valerus), 0.1 mol/l $Na_2S_2O_3.5H_2O$ (pure for analysis, Reachim), 1.5% starch (pure for analysis, Valerus) and distilled water.

The applied method is based on the titration of the glucose present in the product, which has reducing properties and can be oxidized by an elementary molecule of iodine to gluconic acid. The reaction takes place in an alkaline medium, whereby the gluconic acid is neutralized to the corresponding salt. A peculiarity is that the oxidation proceeds at a very low rate, therefore the titration cannot be carried out directly, but the residual method is used, in which a certain volume of standard iodine solution is added in excess to a volume of the sample weighed for analysis, and the remaining excess of iodine is determined by titration with a standard solution of sodium thiosulphate, with starch as an indicator. The specific procedure is as follows: 0.2 g (± 0.0001 g) of the homogenized sample mass is placed in a 300 cm^3 iodine number flask and 30 cm^3 of distilled water is added to them. The flask is shaken until the product is completely dissolved, after which 25 cm^3 of 0.1 M iodine solution and 30 cm^3 of 0.1 M sodium base solution are successively added to it. The flask is closed and placed in the dark for 10 - 15 min, after which it is acidified with 15 cm^3 of 1 M sulfuric acid solution and the excess iodine is titrated with sodium thiosulfate.

Validation of the method was performed by calculating and monitoring the following analytical characteristics: specificity; repeatability; accuracy; and linearity. For the assessment of these characteristics, specific values of the parameters were used: average value; standard deviation (RSD); confidence interval and coefficient of variation (CV). Analyses were performed under normal conditions and reflect normal variations in method parameters and environment during ongoing routine operation in a testing laboratory.

3 Results and discussion

The specificity of the method for all three types of analyzed products was tested by analysing "placebo" samples (all components, without d-glucose content, prepared and provided by the manufacturer) differing in the contents of the individual components according to the manufacturing formulations: the expected concentration of glucose, according to the manufacturer's data, is respectively: in type I – 1513 mg/2500 mg mass; in the II type – 1435 mg/2500 mg mass; and in type III – 1430 mg/2500 mass. The results of the performed analyses show that when proceeding to the analysis in a concentration of the titrant suitable for the titration, the amount required for the occurrence of the equivalence point is equivalent to the input amount of standard iodine solution, which is a sure indication that no reaction leading to the production of gluconic acid, and subsequent reaction of residual iodine in the solution. This also confirms the specificity of the method with regard to the analyzed component for all three types of supplements investigated.

The repeatability of the method was tested in the analysis of seven independent single samples of the product, with an acceptance criterion – a coefficient of variation of no more than 3.0%. The results are shown in Table 1.

Table 1. Method repeatability validation results

Product	Type I		Type II		Type III	
Sample	Spent cm^3 of titrant for the analysis	Glucose content, g	Spent cm^3 of titrant for the analysis	Glucose content, g	Spent cm^3 of titrant for the analysis	Glucose content, g
0 (placebo)	25.00	-	24.95	-	24.95	-
1	18.05	1.485	18.35	1.385	18.80	1.355
2	18.30	1.501	18.60	1.402	18.45	1.387
3	17.90	1.472	18.45	1.364	18.70	1.422
4	18.20	1.469	18.75	1.351	18.65	1.384
5	18.05	1.478	18.90	1.386	18.50	1.369
6	18.10	1.481	18.60	1.414	18.45	1.395
7	18.25	1.490	18.75	1.347	18.70	1.371
Average value		1.482		1.378		1.383
RSD		0.011		0.025		0.022
Confidence interval (t, P=0.95)		0.010		0.024		0.020
CV, %		0.74		1.84		1.56

The obtained values of the coefficient of variation below 2.0% correspond to the previously set acceptance criterion. However, relatively higher values for the II type of product indicate a certain inhomogeneity of the samples.

Table 2. Results of verification of the accuracy of the method for the 1st type of product

Added glucose, %	Added glucose, mg	Determinate glucose content, mg	Analytical yield of glucose, %
50	756.5	747.7	98.84
		742.8	98.19
		758.6	100.28
100	1513.1	1491.6	98.59
		1481.0	97.89
		1510.3	99.82
150	2269.5	2229.7	98.25
		2258.9	99.53
		2233.8	98.43
Average value			98.87
RSD			0.82
Confidence interval (t, p = 0.95)			0.83
CV			0.28

According to USP Chapter 232 guidelines [8], accuracy can be estimated by incremental yield. For this

reason, this parameter was verified by analysing samples containing 50, 100 and 150 % of the recipe content of the analyzed element, in three independent tests. As an acceptance criterion, the requirement that the coefficient of variation should be within the metrological limits of expectations – below 3.0%, and the analytical yield should be 97 ± 3% – should be respected. The results regarding the accuracy parameters are presented in Tables 2 - 4.

Table 3. Results of verification of the accuracy of the method for the 2nd type of product

Added glucose, %	Added glucose, mg	Determinate glucose content, mg	Analytical yield of glucose, %
50	717.5	721.2	100.51
		710.5	99.01
		698.7	97.37
100	1435	1427.6	99.49
		1432.2	99.80
		1399.7	97.54
150	2152	2116.2	98.34
		2146.6	99.75
		2108.1	97.96
Average value			98.86
RSD			1.11
Confidence interval (t, p = 0.95)			1.12
CV			0.37

Table 4. Results of verification of the accuracy of the method for the 3rd type of product

Added glucose, %	Added glucose, mg	Determinate glucose content, mg	Analytical yield of glucose, %
50	715	710.10	99.32
		722.03	100.98
		695.82	97.32
100	1430	1427.25	99.81
		1406.51	98.36
		1412.15	98.75
150	2145	2142.94	99.90
		2116.16	98.38
		2138.13	99.68
Average value			99.17
RSD			1.09
Confidence interval (t, p = 0.95)			1.09
CV			0.36

The obtained repeatability results for all three types of products show that the requirements set for the method, in terms of accuracy validation, are met - for all three types of products, the average analytical yield in the entire linearity interval is in the range of 97 - 101%, and the coefficient of variation is below 3%.

The linearity of the method was established by conducting an analysis of six independent tests of samples of the three types of products with an analytic content of 50, 75, 90, 100, 110, 125, and 150% of the prescription content, respectively.

An acceptance criterion, which is set in terms of linearity, is to have a linearity interval with a range of 50 - 150% in which the correlation coefficient is greater than 0.99. In the linearity interval, the accuracy expressed by the analytical yield and/or the coefficient of variation must be acceptable – respectively: 97 - 103% and/or ≤ 3.0%. The obtained numerical values are presented in the Tables 5 - 8, and graphical dependencies are presented in Figs. 1 - 3.

Table 5. Results of checking the linearity of the method for the determination of glucose in the I type of product

Added glucose, %	Added glucose, mg	Determine glucose content, mg	Spent cm³ titrant for analysis	Analytical yield of glucose, %
50	756.5	749.7	21.31	99.84
75	1134.8	1116.0	20.20	98.35
90	1361.7	1340.5	19.40	98.44
100	1513	1494.3	18.72	98.76
110	1664.5	1648.1	18.15	99.03
125	1891.3	1880.9	17.45	99.45
150	2269.5	2240.8	16.58	98.74

DATA=

905.2	21.45	21.4	21.15	21.25	21.3	21.3	21.31
1362.8	20.35	20.1	20.15	20.15	20.2	20.25	20.20
1638.5	19.35	19.4	19.55	19.5	19.25	19.35	19.40
1813.8	18.8	18.75	18.7	18.65	18.6	18.8	18.72
2000.1	18.15	18.1	18.15	18.2	18.15	18.15	18.15
2269.9	17.45	17.3	17.55	17.35	17.45	17.6	17.45
2722.3	16.5	16.55	16.6	16.6	16.6	16.6	16.58

Linear regression (Y = a + b*X) gives the following data:

Form of equation	Y = a + bX
Slope (b)	23.756
Cut (a)	-0.0033
Correlation coefficient	0.991

For this type of product, it was found that the analytic-signal relationship is linear with the opposite sign in the studied interval, with the obtained value for the correlation coefficient being above 0.99, and the analytical yield being in the interval 98 - 100 %. The linear relationship is valid in the interval for the expected content 50 - 150 %, which confirms that the requirements of the method are achieved.

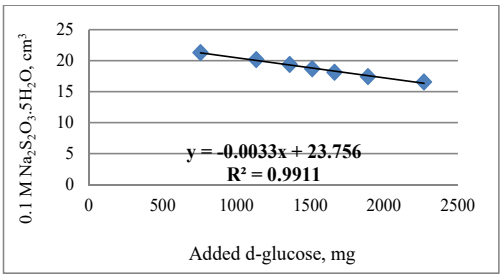

Fig. 1. Dependence between the amount of added glucose and the amount of spent titrant

Table 6. Results of checking the linearity of the method for the determination of glucose in the II type of product

Added glucose, %	Added glucose, mg	Determine glucose content, mg	Spent cm³ titrant for analysis	Analytical yield of glucose, %
50	717.5	710.1	22.25	98.97
75	1076.4	1065.6	20.12	99.00
90	1291.5	1285.1	20.30	99.50
100	1435.0	1419.8	19.50	98.94
110	1578.5	1579.6	18.76	100.07
125	1795.0	1795.2	18.15	100.06
150	2152.0	2123.7	17.08	98.68

DATA=

717.5	22.35	22.15	22.25	22.3	22.25	22.2	22.25
1076.4	21.05	21.2	21.15	21.15	21.1	21.05	21.12
1291.5	20.25	20.4	20.2	20.25	20.4	20.3	20.30
1435	19.45	19.5	19.4	19.55	19.6	19.5	19.50
1578.5	18.75	18.8	18.75	18.8	18.75	18.7	18.76
1794	18.2	18.25	18.1	18.1	18.15	18.1	18.15
2152	17.05	17	17.05	17.1	17.15	17.15	17.08

Linear regression (Y = a + b*X) gives the following data:

Form of equation	Y = a + bX
Slope (b)	24.909
Cut (a)	-0.0037
Correlation coefficient	0.991

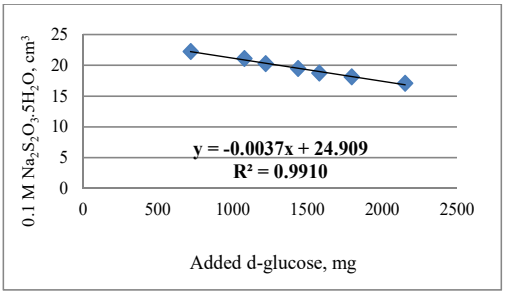

Fig. 2. Dependence between the amount of added glucose and the amount of spent titrant

And with this type of product, the analytic-signal relationship in the studied interval is linear with the opposite sign, and the value for the correlation coefficient is again above 0.99. The analytical yield is from 98 to 101 %.

Here, too, it was found that the requirements for the method were met – the analytic-signal correlation was linear in the studied interval (50 - 150% of the expected content), and the correlation coefficient was 0.99. The analytical yield for this type of product is from 98 to 100%.

Table 7. Results of checking the linearity of the method for the determination of glucose in the III types of products

Added glucose, %	Added glucose, mg	Determine glucose content, mg	Spent cm³ titrant for analysis	Analytical yield of glucose, %
50	715.0	709.3	23.18	99.21
75	1072.5	1052.7	21.58	98.15
90	1287.0	1278.6	20.35	99.35
100	1430.0	1415.3	19.47	98.97
110	1573.0	1543.2	18.75	98.11
125	1787.5	1771.9	17.90	99.13
150	2145.0	2130.4	16.45	99.32

DATA =

715	23.2	23.3	23.05	23.15	23.25	23.1	23.18
1072.5	21.6	21.65	21.8	21.4	21.5	21.5	21.58
1278	20.2	20.45	20.3	20.35	20.35	20.45	20.35
1430	19.45	19.35	19.55	19.5	19.5	19.45	19.47
1573	18.8	18.75	18.8	18.7	18.75	18.7	18.75
1787.5	17.85	17.95	17.9	17.95	17.85	17.9	17.90
2145	16.55	16.45	16.5	16.4	16.45	16.35	16.45

Linear regression (Y = a + b*X) gives the following data:

Form of equation	Y = a + bX
Slope (b)	26.560
Cut (a)	-0.0048
Correlation coefficient	0.994

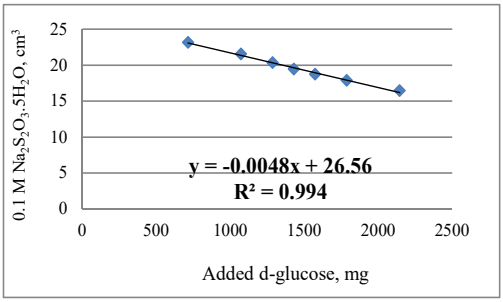

Fig. 3. Dependence between the amount of added glucose and the amount of spent titrant

4 Conclusion

The results of the performed analyses show that all the set criteria regarding the analytical characteristics: specificity, repeatability, accuracy and linearity are covered within the previously established limits. For this reason, the method can be considered applicable with a view to determining the glucose content in nutritional supplements with a similar composition to those studied.

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