Key development stages of reference material for lactose monohydrate composition

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Abstract: The article mirrors key stages of development of reference material (RM) for the composition of lactose monohydrate with certified values of mass fraction of water and basic material. The RM could be used in the analytical chemistry and food industry, as well as at identification and assessment of nutritive products safety, int. al., infant food. The RM under development will be intended for metrological assurance of mass fraction of water and basic substance measuring instruments based on usage of thermogravimetric, titrimetric and chromatographic methods, certification of measurement procedures and control of measurement results of lactose monohydrate mass fraction accuracy, used as the host material, and in compositions of organic substances and materials or in measurements of water mass fraction in solid substances and materials, as well as for other kinds of metrological control. Determination of certified value of lactose monohydrate mass fraction in the RM was performed by reference method of iodometric titration and affirmed by mass balance method (one hundred minus the number of impurities) and by calculating-experimental method. The certified value of mass fraction of water was assigned by the Fischer’s method of volumetric titration and confirmed by the thermogravimetric method. Considerable attention was focused to the study of the separation process of sorbed and crystallisation water in a high-purity lactose monohydrate reagent. The results of measurements of the quantitative content of the types of water were considered when assessing the purity of the target substance by various methods. Unlike existing RM of similar composition, the traceability of the certified values of the RM being developed will be ensured to units of values reproduced respectively by the state primary (GET 173-2017) and secondary (GVET 176-1-2010) standards.

Introduction

Lactose (milk sugar) is the most important disaccharide determining nutritive value of milk and dairy products, and widely used in analytical chemistry, food industry, and feed vitamins production. When the lactose enters the human organism, it relies on lactase enzyme. In case of lack of lactase, the lactose intolerance (hypolactasia), which can cause a variety of painful symptoms, is observed. The lactose monohydrate is the basic component in raw materials, as well as in composition of organic substances and materials or in measurements of water mass fraction in solid substances and materials, as well as for other kinds of metrological control. Determination of certified value of lactose monohydrate mass fraction in the RM was performed by reference method of iodometric titration and affirmed by mass balance method (one hundred minus the number of impurities) and by calculating-experimental method. The certified value of mass fraction of water was assigned by the Fischer’s method of volumetric titration and confirmed by the thermogravimetric method. Considerable attention was focused to the study of the separation process of sorbed and crystallisation water in a high-purity lactose monohydrate reagent. The results of measurements of the quantitative content of the types of water were considered when assessing the purity of the target substance by various methods. Unlike existing RM of similar composition, the traceability of the certified values of the RM being developed will be ensured to units of values reproduced respectively by the state primary (GET 173-2017) and secondary (GVET 176-1-2010) standards.
substances and materials. In connection with the above, the lactose content is monitored when assessing the safety of a number of food products, including infant food (TR CU 027/2012 [1], TR CU 033/2013 [2]). For most standardized methods for determining the lactose content (refractometry, polarimetry, infrared spectroscopy, enzymatic analysis, capillary electrophoresis, chromatography), it is necessary to build a calibration (graduation) dependence using a reference material (RM) with a certified value of the mass fraction of lactose monohydrate. In addition, this RM is necessary for monitoring the accuracy of measurement results, certification of methods, calibration, verification and testing of express measuring instruments of crystalline hydrate composition. The second area of application of RM is the improvement of metrological support of means and methods for measuring the mass fraction of water based on the use of the thermogravimetric method and the Karl Fischer titration method, since lactose monohydrate is a crystallohydrate with a constant value of the mass fraction of water.

**Analysis of existing legal framework regulating the permissible levels of lactose content in food products**

In accordance with TR CU 033/2013 [2], the labelling of concentrated or condensed dairy products and dry dairy products should contain information about the type of sugars (sucrose, fructose, glucose, lactose). The parameter of ice cream identification is the mass fraction of sucrose or total sugar (minus lactose). In a lactose-free milk processing product, the lactose content should not exceed 0.1 g per 1 litre of a ready-to-use product in which lactose is hydrolysed or removed. The requirements for the content of lactose in adapted or partially adapted initial or subsequent milk mixtures (including dry), dry fermented milk mixtures, milk drinks (including dry) for the nutrition of young children are established in TR CU 033/2013 [2] (Table 1).

**Table 1 – Permissible levels of lactose content in products for the nutrition of little baby in accordance with TR CU 033/2013**

| Group of products                                                                 | Maximum percentage of lactose from the total amount of crystalline hydrate, % |
|-----------------------------------------------------------------------------------|-------------------------------------------------------------------------------|
| Adapted milk mixtures (dry, liquid, fresh, fermented milk) and products based on partially hydrolysed proteins for the nutrition of children aged 0 to 6 months | 65                                                                            |
| Subsequent adapted milk mixtures (dry, liquid, fresh and fermented milk) and products based on partially hydrolysed proteins for the nutrition of children over the age of 6 months | 50                                                                            |
| Adapted milk mixtures (dry, liquid, fresh, fermented milk) and products based on partially hydrolysed proteins for the nutrition of children from 0 to 12 months | 65                                                                            |
| Subsequent partially adapted milk mixtures (dry, liquid, fresh, fermented milk) for the nutrition of children over the age of 6 months | 50                                                                            |

TR CU 027/2012 [1] establishes acceptable levels of mass concentration of lactose in low-lactose and lactose-free products (Table 2).

**Table 2 – Permissible values of the mass concentration of lactose in accordance with TR CU 027/2012**

| Group of products                                           | Maximum mass concentration of lactose, g/l |
|-------------------------------------------------------------|------------------------------------------|
| Low-lactose products for infants of the first year of life  | 10                                       |
| Lactose-free products for infants of the first year of life | 0.1                                      |
| Low-lactose milk processing products for young children     | 16                                       |
Search for analogues
The analysis of currently existing RMs of similar composition, including foreign production, has been carried out. The following certified reference material (CRM) is registered in the State Register: Certified reference material for the mass fraction of water (AQUASTAR™ LACTOSE STANDARD 5 % MERCK) GSO 10980-2017 manufactured by Merck KGaA (Germany). The material of the RM is a solid organic substance of white colour (lactose monohydrate). RM substance is packaged in 10 g in hermetically sealed plastic bottles, which have labels pasted on them. The method of establishing the certified value is the usage of certified measurement procedures. The metrological characteristics of the RM are presented in Table 3.

| Certified characteristic | Unit designation | The range of permissible certified values of the RM | The limits of the permissible values of the absolute error of the certified value of the RM at \( P = 0.95 \) | The permissible value of the absolute extended uncertainty of the certified value of the RM (at \( k = 2 \)) |
|-------------------------|------------------|--------------------------------------------------|------------------------------------------------|--------------------------------------------------|
| Mass fraction of water  | %                | 5.00 – 5.50                                      | ±0.10                                             | 0.10                                             |

Among the RMs of foreign production, the CRM for lactose monohydrate (Cat. № PHR1024, Lot. № P500189) manufactured by Sigma Aldrich can be highlighted. The certified reference material is a white lactose monohydrate powder packaged in 1 g vials of dark glass. The method of establishing the certified value is the mass balance method. The metrological characteristics of the CRM are presented in Table 4. The disadvantage of existing CRMs of similar composition is the lack of requirements for metrological traceability.

| Certified characteristic | Certified value, % | Extended uncertainty of certified value (\( k = 4.1 \)), % |
|-------------------------|--------------------|----------------------------------------------------------|
| Mass fraction of lactose monohydrate | 99.7              | 1.5                                                       |

The objective of the work is to create a reference material for the lactose monohydrate composition which will be intended for metrological assurance of measuring instruments for the mass fraction of the water and host substance, based on the usage of thermogravimetric, titrimetric and chromatographic methods. The RM being developed will compare to current requirements for metrological traceability and the presence of uncertainty properties. The key feature of the developed RM, in contrast to existing analogues, will be the availability of analytical information on the mass fractions of sorbed and crystallisation water.

Preparation of the CRM material
A high-purity \( D \)-lactose monohydrate reagent for biochemistry manufactured by Merck (Germany) was selected as the source material of the developed RM, the main properties of which are presented in Table 5. The material of the RM was thoroughly mixed and packaged weighing from 5 to 10 g in glass vials with an airtight lid, pre-UV-treated for 30 minutes. The packing of the RM material was carried out in the box of the abacterial air media BAVnp-01- “Laminar-C”-1.2 (14.120-02). Each
specimen was provided with a label designed under the requirements of GOST R 8.691-2010 [3], placed in a plastic bag, which was sealed with a contact welding appliance. The prepared RM specimens were stored in a thermostat at ambient temperature (7 ± 3) °C.

Tab. 5 – Description and main properties of the high-purity $D$-lactose monohydrate reagent selected as the source material

| Name of the substance | Lactose monohydrate |
|-----------------------|----------------------|
| Synonyms              | $\beta$-$D$-Galactopyranosyl-(1$\rightarrow$4)$-\alpha$-$D$-glucopyranose monohydrate, $D$-lactose monohydrate, milk sugar |
| Form                  | White or almost white crystalline powder or white crystals |
| Molecular formula     | C$_{12}$H$_{22}$O$_{11}$ $\cdot$ H$_2$O |
| CAS Number            | 64044-51-5 |
| EC Number             | 200-559-2 |
| Lot. Number           | FN1432060 918 |
| Molecular mass        | 360,31 g/mol |
| Solvability           | The material is easily soluble in water. Very slightly soluble or practically insoluble in 96% alcohol, practically insoluble in chloroform |
| Specific rotary power | From $+54.4$ up to $+55.9$ calculated with reference to the anhydrous substance |
| Water                 | Not less than 4.5 % and not more than 5.5 % |

The validity of the RM material was confirmed using an infrared Fourier spectrometer Nicolet iS5 manufactured by Thermo Fisher Scientific (USA) with an auxiliary device in diffuse reflection mode. Radiation source: high-temperature ceramic; detector: deuterated L-alanine doped triglycine sulphate (DLaTGS); spectrum registration area – 4000 - 400 cm$^{-1}$, resolution – 4 cm$^{-1}$, number of scans – 16.

It was stated that the absorption bands of the analysed substance by position and intensity correspond by 94.5% to the substance «lactose powder (hydrous)» from the library “Georgia State Crime Lab Sample Library, 120” of the database of the software of the spectrometer “OMNIC” (Figure 1).

Fig. 1 – IR spectrum of the source material for RM (top) and library spectrum of lactose monohydrate comparison (bottom)
Description of performed measurement methods
Verification of the vapour-gas mixture extracted by heating the material for the presence/absence of volatile components other than water was performed using a standard installation based on thermogravimetric analysis with mass spectrometric detection (TGA/MC) from the composition of GET 173-2017 [4].

Quantitative determination of the mass fraction of water in the RM material was performed on standard installations from the composition of GET 173-2017: air-thermal drying; based on volumetric titration by the Karl Fischer method.

Quantitative determination of the host substance (lactose monohydrate) mass fraction in the RM material was carried out by three methods:
1. Method of iodometric titration by State Secondary Standard of mass fraction and mass (molar) concentration units of components in solid and liquid substances and materials, based on volumetric titrimetric analysis method GVET 176-1-2010 (direct method);
2. Mass balance method (one hundred minus amount of impurities) with usage of State Primary Standard of mass (molar, atomic) fraction and mass (molar) concentration units of components in solid and liquid substances and materials on the base of coulometry GET 176-2019; State Primary Standard of mass fraction and mass (molar) concentration units of water in solid and liquid substances and materials GET 173-2017, and State Secondary Standard of mass fraction and mass (molar) concentration units of organic components in liquid and solid substances and materials on the base of gas and liquid chromatography GVET 208-1-2016 (indirect method);
3. Calculating-experimental method (stoichiometry).

Determination of RM metrological characteristics
The research of the metrological characteristics of the RM was carried out in accordance with the procedure established in GOST ISO Guide 35 [6]. To assess the homogeneity, 6 samples of the RM were used, randomly selected from prepared batches. In each of the 6 RM samples, 5 parallel determinations of the mass fraction of the main substance were carried out by iodometric titration using GVET 176-1-2010 and 8 parallel determinations of the mass fraction of water by volumetric titration by the Karl Fischer method using GET 173-2017. The processing of the measurement results obtained for 6 RM specimens was carried out according to the scheme of single-factor analysis of variance. The measurement results obtained during the study of the uniformity of the RM sample material were used to calculate the certified values.

The objectives of the stability study of the RM material were checking the suitability of the packaging of the RM material; establishing the shelf life and storage conditions (long-term stability); choosing transportation conditions (short-term stability). To determine the shelf life of the RM and assess the long-term stability of the RM material, the classical method was used. The duration of the study of long-term stability, equal to half of the estimated shelf life, was 6 months.

In terms of microbiology, the stability of the storage of CO material may be affected by the presence of bacteria (microorganisms) in it. This is due to several factors. First of all, a chemical reagent has been selected as the RM material, so its contamination by bacteria during production and packaging is not excluded. Secondly, lactose monohydrate, as well as other mono- and disaccharides, when stored in conditions of high humidity can turn into an environment favourable for nutrition and reproduction of bacteria. Thirdly, even at low humidity, with prolonged exposure to elevated storage temperatures, this can lead to partial destruction of the crystallohydrate and the release of sorbed and crystallization water, which will also lead to the proliferation of bacteria, and as a consequence to a decrease in the lactose content in the reference material. Fourthly, for the most part, the violation of storage conditions, albeit short-term, occurs precisely during transportation. Since temperatures below zero do not affect the growth of bacteria, studies of the short-term stability of the reference material were carried out at temperatures from +2 °C to +30 °C and humidity from 20 % to 60 %, created under thermostating conditions, for 14 days.
Results and discussion
1. Determining of sorbed and crystallisation water mass fraction

The literature data [7] presents the results of studies to determine the mass fractions of sorbed and crystallised water in lactose monohydrate by air-heat drying and azeotropic distillation with benzene and toluene. Thus, the values of the mass fraction of total water were obtained and conclusions were formulated that it is impossible to draw a clear boundary between the quantitative determination of sorbed and crystallisation water for lactose monohydrate. It is connected with the beginning of the destruction of the material at a temperature of about 70 °C, as well as with a low hydration temperature. However, these studies were conducted back in 1980. At present, thanks to the modern equipment, it is possible to separate the sorbed water from the crystallisation water, while considering the value of the mass fraction of sorbed water for assessing the purity of the RM material and the formation of uncertainty by the mass balance method according to 2.2.

To check the vapor-gas mixture extracted from the material during heating for the presence/absence of volatile components other than water, as well as to select the temperature of air-thermal drying, measurements were performed on a standard installation implementing the TGA/MC method from the composition of GET 173-2017 [4]. When the test material is heated, crystallization water is released with characteristic mass numbers m/z = 17 a.e.m. and m/z = 18 a.e.m. at temperatures from 120 °C to 180 °C, other compounds are not detected (Figure 2). For measuring the mass fraction of sorbed and crystallisation water by air-thermal drying, the recommended temperature range is 130 °C.

1.1 Air-heat drying

The mass fraction of sorbed and crystallisation water $W_{H_2O}$, %, was calculated by formula (1):

$$W_{H_2O} = \frac{m_1 - m_0}{m - m_0} \cdot 100,$$  \hspace{1cm} (1)

where,

$m_0$ – the mass of weighing cup with sand and glass rod, g;
$m$ – the mass of weighing cup with sand, a glass rod and a sample weight of lactose monohydrate before drying, g;
$m_1$ – the mass of weighing cup with sand, a glass rod and a sample weight of lactose monohydrate after drying, g.

1.2. Volumetric titration by Karl Fischer method

The mass fraction of sorbed and crystallisation water $W_{H_2O}$, %, was calculated by formula (2):

$$W_{H_2O} = \frac{T \cdot V \cdot 100}{m_1 - m_2},$$  \hspace{1cm} (2)

where,

$T$ – the titre of titrant solution, mg/cm$^3$;
$V$ – the volume of titrant solution used for titration, cm$^3$;
$m_1$ – the mass of sample weight in container, mg;
$m_2$ – mass of container, mg.

The mass fraction of sorbed and crystallisation water determined by the method of air-thermal drying was $(5.25 \pm 0.04)$ %, volumetric titration by the Karl Fischer method – $(5.35 \pm 0.13)$ %.
2. Determination of the host substance mass fraction

2.1. Iodometric titration method

The method of iodometric titration is based on the ability of iodine in an alkaline media to oxidize aldosaccharides into the corresponding uronic acids. 0.1 M sodium thiosulfate solution was used as a titrant, standardized according to the Certified Reference Material for the composition of potassium bicarbonate (potassium bichromate) of the 1st category GSO 2215-81.

The mass fraction of host substance $W_{\text{HM}}$, %, in the test sample was calculated by formula (3):

$$W_{\text{HM}} = \frac{C_{\text{Na}_2\text{S}_2\text{O}_3} \cdot \left(V_{\text{Na}_2\text{S}_2\text{O}_3, \text{before inversion}} - V_{\text{Na}_2\text{S}_2\text{O}_3, \text{before inversion}}\right) \cdot M_{\text{C}_12\text{H}_2\text{O}_1\text{H}_2\text{O}} \cdot V_{\text{m.f.}} \cdot 100}{1000 \cdot m_{\text{sample}} \cdot 2},$$  

where,

- $C_{\text{Na}_2\text{S}_2\text{O}_3}$ – molar concentration of sodium thiosulfate solution consumed for titration, mol/dm$^3$;
- $V_{\text{Na}_2\text{S}_2\text{O}_3, \text{before inversion}}$ – the volume of sodium thiosulfate consumed for the titration of iodine leftover after the reaction with a blank sample, cm$^3$;
- $V_{\text{Na}_2\text{S}_2\text{O}_3, \text{before inversion}}$ – the volume of sodium thiosulfate consumed for titration of iodine leftover after reaction with lactose monohydrate before inversion of the sample, cm$^3$;
- $M_{\text{C}_12\text{H}_2\text{O}_1\text{H}_2\text{O}}$ – the molar mass of lactose monohydrate, g/mol;
- $V_{\text{m.f.}}$ – the volume of the measuring flask in which the sample is dissolved (250 cm$^3$), cm$^3$;
- $V_{\text{al}}$ – aliquot volume taken for titration, cm$^3$;
- $m_{\text{sample}}$ – the weight of the sample taken for analysis, g;
- 2 – a number that takes into account stoichiometric coefficients in the reaction equation;
- 1000 – conversion of dm$^3$ into cm$^3$;
- 100 – conversion of fractions of units to %.
2.2 Method of mass balance

Confirmatory measurements of the mass fraction of the host substance were carried out by the mass balance method (“one hundred percent minus the number of impurities”) in accordance with MI 3561-2016 [5], which involves the measurement of probable groups of impurities in pure organic matter (related impurities, residual organic solvents, inorganic impurities, sorbed water).

To determine the mass fraction of sorbed water, the vacuum drying method is eminently suitable, since a small amount of thermal energy is supplied to the substance, which is sufficient to destroy only the physical-chemical bonds formed at the level of intermolecular interaction of water with the substance (according to P.A. Rebinder's classification [7, 8]). Physical-chemical bonds are the weakest compared to the chemical bonds characteristic of crystallization water, the destruction of which requires the supply of more thermal energy.

The mass fraction of host material $W_{HM}$, % was calculated by formula (4):

$$W_{HM} = 100 - (W_{\text{related}} + W_{\text{res. org. solvents}} + W_{\text{inorg. imp.}} + W_{\text{sorbed water}}),$$

(4)

where,

- $W_{\text{related}}$ – the mass fraction of related impurities obtained by high-performance liquid chromatography (HPLC) using a modular system for HPLC “Maestro-14” with subsequent registration of sample components by a refractometric detector using a Hi-Plex Ca (Duo) column (300 x 6.5 mm) from the composition of GVET 208-1-2016;
- $W_{\text{res. org. solvents}}$ – the mass fraction of residual organic solvents obtained by gas chromatography in accordance with the OFS.1.1.0008.15 [9] on GC/MS Triple Quad 7890A/7000 chromatography mass spectrometry from GVET 208-1-2016, %;
- $W_{\text{inorg. imp.}}$ – the mass fraction of inorganic impurities (metals, its oxides and salts) obtained by inductively coupled plasma mass spectrometry using a NexION-200 ICP mass spectrometer from the GET 176-2019 composition, %;
- $W_{\text{sorbed water}}$ – the mass fraction of sorbed water obtained by vacuum drying using the vacuum drying chamber VD 53 “WTB Vinder” from the composition of GET 173-2017, %.

Tab. 6 – Results of mass fraction of host substance measurement by mass balance method

| Name of measurand* | Mass fraction, % | Absolute extended uncertainty $U$ ($k = 2$), % |
|---------------------|-----------------|------------------------------------------|
| Related impurities  | 0.44            | 0.05                                     |
| Residual organic solvents | 0.000 | 0.020                                   |
| Inorganic impurities (metals, oxides and salts thereof) | 0.020 | 0.012                                   |
| Sorbed water        | 0.37            | 0.08                                     |
| Host material (lactose monohydrate) considering all identified groups of impurities | 99.18 | 0.10                                     |

*with accordance to [5].
2.3. Calculating-experimental method

The method implies an assessment of the purity of the RM material by the values of the mass fraction of crystallisation water.

The calculating-experimental value of the crystallisation water mass fraction was evaluated by formula (5):

\[
W_{\text{crystal, water}} = W_{H_2O} - W_{\text{sorbed, water}},
\]

(5)

where,

\(W_{\text{sorbed, water}}\) – the mass fraction of sorbed water obtained by vacuum drying, in accordance with Table 6, %;

\(W_{H_2O}\) – the mass fraction of sorbed and crystallisation water obtained by the Karl Fischer method of volumetric titration, according to the formula (2), %.

Calculating-experimental value of the crystallisation water mass fraction in lactose monohydrate \(W_{\text{crystal, water}}\) was \((4.98 \pm 0.15)\) %.

The stoichiometric ratio was used to determine the theoretical value of the mass fraction of crystallisation water in lactose monohydrate \(W'_{\text{crystal, water}}\), considering the IUPAC nomenclature recommended for use \([10]\) – 5.00 %.

The purity of the RM material was evaluated by making a proportional relationship between the calculated experimental and theoretical values of crystallisation water according to the formula (6):

\[
W_{\text{HM}} = \frac{W_{\text{crystal, water}} \times 100}{W'_{\text{crystal, water}}},
\]

(6)

where,

\(W_{\text{crystal, water}}\) – calculating-experimental value of the crystallisation water mass fraction according to the formula (5), %;

\(W'_{\text{crystal, water}}\) – value of the crystallisation water mass fraction, evaluated by stoichiometry in reliance on IUPAC nomenclature for use, %.

The host substance (lactose monohydrate) mass fraction determined by iodometric titration was \((99.70 \pm 0.61)\) %; by mass balance method – \((99.18 \pm 0.10)\) % (considering each identified group of impurities according to Table 6); by calculating-experimental method – \((99.74 \pm 0.15)\) %.

The comparison of the methods was carried out to comply with the CCQM guidelines \([11]\) by the classical method of estimating the arithmetic mean (Figure 3). The results of measurements of the mass fraction of the host substance for the three methods are consistent within the stated uncertainties.

The metrological characteristics values of the RM are demonstrated in Table 7. The expected shelf life of a specimen of the RM is 1 year.
Tab. 7 – Metrological characteristics of the RM for composition of lactose monohydrate

| Certified characteristic | Certified value, % | Absolute uncertainty of certified value range (at $P = 0.95$), % | Absolute extended uncertainty of certified value (at $P = 0.95$, $k = 2$), % |
|-------------------------|------------------|---------------------------------------------------------------|---------------------------------------------------------------|
| Mass fraction of host material | 99.7 ± 0.7 | 0.7 |
| Mass fraction of water | 5.35 ± 0.20 | 0.20 |

Fig. 3 – Comparison of host material mass fraction determination methods

3. Homogeneity and stability assessment
The study of the homogeneity of the RM material was carried out, taking into account the provisions of GOST ISO Guide 35 [6] according to the scheme of one-factor analysis of variance. 6 RM specimens were randomly selected from the material. In each RM specimen, 5 parallel determinations of the mass fraction of the basic substance were carried out according to the method described in 2.1, using GVET 176-1-2010 and 8 parallel determinations of the mass fraction of water according to the method described in 1.2, using GET 173-2017. The standard uncertainty (error characteristic) from the inhomogeneity of the RM material, $u_h (\sigma_h)$, % was, respectively: 0.04% - when measuring the mass fraction of the base substance; 0.06% - when measuring the mass fraction of water.

The classical method was used to assess the long-term stability of the RM material. The duration of the long-term stability study was 6 months. 6 RM specimens were randomly selected from the RM material, labeled in order and stored at a temperature of $(20 \pm 5)$ °C and humidity from 30% to 60%, away from direct sunlight and daylight lamps. Once a month, one RM specimen was seized and measurements of the certified characteristics were carried out using the methods described above.

The short-term stability of RM may manifest itself during transportation. The greatest changes in the certified characteristic are likely in the extreme values of temperature and relative humidity. Possible conditions of RM transportation: ambient air temperature from plus 2 °C to plus 30 °C, relative humidity of the ambient air from 20% to 60%. For experimental confirmation of this fact, 4 samples were randomly selected from the RM material, and studies were conducted on the influence of changes in external conditions on the certified values. The study period of short-term stability is
equal to the maximum period of transportation and was 14 days. Modeling of extreme values of ambient air conditions was performed in the KBF-115 “Binder” climate chamber. After holding the RM specimens under the specified conditions, the values of the certified characteristics were measured using the methods described above.

The processing of the measurement results obtained during the stability assessment was carried out, taking into account the provisions of the GOST ISO Guide 35 [6]. The measurement results obtained during the study of the stability of the RM material are presented in Table 8.

Table 8 – Measurement results obtained in the study of the stability of the RM material

| Certified characteristic | Type of stability | Arithmetic mean, % | Absolute standard uncertainty from stability, % |
|--------------------------|-------------------|--------------------|-----------------------------------------------|
| Mass fraction of host material | short-term | 99.67 | 0.14 |
|                          | long-term  | 99.65 | 0.13 |
|                          | short-term | 5.29  | 0.06 |
|                          | long-term  | 5.32  | 0.04 |

The RM will be intended for calibration, establishment and control of the stability of the graduation (calibration) curve of measuring instruments, certification of measurement procedures and control of the accuracy of measurement results of the mass fraction of lactose monohydrate as the host component in raw materials, as well as in the composition of organic substances and materials; verification, calibration, establishment and control of the stability of the graduation (calibration) curve of measuring instruments based on the usage of the thermogravimetric method and Karl Fischer titration, certification of measurement procedures and control of the accuracy of measurement results of the mass fraction of water in solid substances and materials; control of metrological characteristics of measuring instruments during their tests, including for certification of the RMs; other types of metrological control in compliance with metrological characteristics with the requirements of metrological control procedures.

Conclusion
The new CRM for composition of lactose monohydrate was developed as the result of carried out works. The metrological traceability of certified values of mass fractions of the host material and water of the CRM is assured to the units realised by State Primary Measurement Standard of mass fraction and mass (molar) concentration units of water in solid and liquid substances and materials GET 173-2017 and State Secondary Measurement Standard of mass fraction and mass (molar) concentration units of water in solid and liquid substances and materials based on the volumetric titrimetric analysis method GVET 176-1-2010.

The quantitative determination of the mass fraction of sorbed and crystallisation water was performed for lactose monohydrate as for the host material for the first time; the first type of water was considered in assessing the purity of the CRM material by the verifying method of mass balance, the second - in assessing the purity of the CRM material by the calculating-experimental method.

The developed CRM was used in the construction of calibration graphs for measuring the mass fraction of sugars in milk powder by refractometry and HPLC methods with refractometric detection. The results of measurements of the mass fractions of sugars obtained by these methods amounted to 4.46 % and 4.40 %, respectively, which is in better agreement with the results of the titrimetric method – 4.43 %. Thus, the applicability of the developed CO for various methods of analysis is confirmed.

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