Food Chemistry 153 (2014) 378–386

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Establishment of the purity values of carbohydrate certified reference materials using quantitative nuclear magnetic resonance and mass balance approach

Can Quan *

Division of Chemistry, National Institute of Metrology, Beijing 100013, China

ARTICLE INFO

Article history:
Received 8 December 2012
Received in revised form 29 October 2013
Accepted 19 December 2013
Available online 3 January 2014

Keywords:
Carbohydrates
Certified Reference Material (CRM)
Quantitative Nuclear Magnetic Resonance (QNMR)
Mass balance approach
Uncertainty

ABSTRACT

This work described the assignment of purity values to six carbohydrate certified reference materials, including glucose, fructose, galactose, lactose, xylose and sucrose, according to the ISO Guides 34 and 35. The CRMs' purity values were assigned based on the weighted average of quantitative nuclear magnetic resonance method and mass balance approach with high resolution liquid chromatography – evaporative light scattering detection. All the six CRMs with following value amount fractions: glucose (GBW10062) at a certified purity $P \pm U$ ($k = 2$) of (0.99 ± 0.005)%; fructose (GBW10063) at (0.99 ± 0.005)%; galactose (GBW10064) at (0.99 ± 0.007)%; lactose (GBW10065) at (0.99 ± 0.008)%; xylose (GBW10066) at (0.99 ± 0.007)% and sucrose (GBW10067) at (0.99 ± 0.008)%, respectively were certified. The homogeneity of the CRMs was determined by an in-house validated liquid chromatographic method. Potential degradation during storage was also investigated and a shelf-life based on this value was established.

1. Introduction

Carbohydrates, quantitatively the largest organic compound group on earth (Bowman, 1999), analysis of mono and disaccharides (simple sugars) in foods is required when their presence is greater than 1%, in accordance to the Food and Drug Administration' new policy (Weaver and Finke, 2003). Furthermore, carbohydrate content must be included on food labels (Kreuter, Brennan, Scharff, & Lukwago, 1997).

A certified reference material (CRM) is a material or substance that its one or more property values are sufficiently homogeneous, stable, and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials (Gallorini, 1995; Emons, Linsinger, & Gawlik, 2004).

A common characteristic of carbohydrate is that most of the molecular ultraviolet absorption occurs towards the end of the ultraviolet region. Not only do these compounds lack the characteristic ultraviolet absorption zone utilized in chromatographic analysis, the ultraviolet absorption coefficient of each impurity is different (Dubois, Gilles, Hamilton, Rebers & Smith, 1956; Bhandari, Kumar, Singh, & Kaul, 2008). The evaluation and control of carbohydrate levels are routinely performed by the international scientific community, in collaboration with food control organizations and different analytical laboratories. While it is desirable for all laboratories to achieve comparable and accurate results, this is not an easy task especially during the analysis of complex matrices. Therefore, various authorities have emphasized the need for CRMs to ensure metrological traceability of the results. However, CRM of carbohydrates are currently unavailable. Therefore, the China National Institute of Metrology (NIM) had undertaken the task to develop GBW10062–GBW10067, carbohydrate CRMs including glucose, fructose, galactose, lactose, xylose and sucrose, following the principles of ISO Guides 34 and 35 (ISO, 2000; ISO, 2006). NIM employed two methods to certify the carbohydrates namely the quantitative nuclear magnetic resonance (QNMR) and the mass balance approaches. The QNMR method does not require consideration of impurities or a reference material of known content, and only uses a common chemical substance of known content to determine the absolute content of the target compound. This method is rapid, convenient, accurate, and highly specific, and does not require the determination of absorption coefficient (Saito et al., 2009; Wells, Cheung, & Hook, 2004).

In the mass balance approach all the impurities (as well as moisture and ash) are quantified and subtracted from 100%. Although the mass balance approach serves as a basic guideline for establishment of chemical reference materials and has long been recommended by the World Health Organization (WHO, 1999a), the European Pharmacopoeia and the International Pharmacopoeia (DQMCE, 2007; WHO, 2003), reports documenting the

* Tel./fax: +86 10 6452 4785.
E-mail address: superfluidcan@hotmail.com

0308-8146/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2013.12.086

use of the mass balance approach for the establishment of carbohydrate purity reference materials had not be found.

This paper detailed the production of six carbohydrates CRMs, i.e., glucose (GBW10062), fructose (GBW10063), galactose (GBW10064), lactose (GBW10065), xylose (GBW10066) and sucrose (GBW10067) including evaluating the homogeneity and stability among its units and within a same unit, and the strategy to assign reference values in the materials. The procedures included qualitative analysis, quantitative analysis, homogeneity study, stability study and uncertainty evaluation.

2. Experimental

2.1. Instrumentation

^{1}H NMR measurement was made on a Bruker AV600 spectrometer operating at 600 MHz. The HPLC system consisted of an Agilent 1100 chromatograph equipped with a binary pump, an automated sample injector, a TCC thermostated column compartment and a SEDEX 7 evaporative light scattering detector. Agilent 6410 triple quad system and Thermo Nicolet iS10 spectrophotometers were used for qualitative analysis. A Mettler Tolerdo DL39 Karl Fischer titrator was used for water measurement of all the samples. The Agilent 7500CE ICP-MS was used for analysis of trace metal residues.

2.2. Materials

Six carbohydrate reference material candidates in this study including glucose, fructose, galactose, lactose, xylose and sucrose (with purity greater than 99%) purchased from Aldrich (Steinheim, Germany). They were stored under controlled conditions of temperature and related humid. Dimethyl Sulfoxide-d_6 (>99.9%, 0.03%, *v/v*, TMS) was bought from Cambridge Isotope Laboratories Inc., UK. Methanol and ethyl acetate (used as solvent or mobile phase) with HPLC grade were obtained from Kingchemtune Co. Ltd. (Shandong Province, China). The NIST SRM Benzoic acid (SRM 350a) was selected as the internal standard after preliminary QNMR optimization.

2.3. Methods

2.3.1. Preparation of candidate CRM

Preparation of candidate CRM was conducted according to the guideline of the National Institute of Scientific Investigation Ethics Committee (NIM, 2009; AQSIQ, 1994). All samples were stored in an airtight container with desiccant after pretreatment. The procedure for the preparation and characterization of the reference material and the homogeneity study had been carefully designed and optimized step by step (WHO, 2003; Quan et al., 2011).

The use of glass boats for weighing and transferring high-purity materials was intended for good repeatability of operation and good visibility of materials which may contribute to the reduction of preparation variation. An analytical balance with maximum weight capacity of 22 g (Model XP26, Mettler Toledo, resolution of 0.001 mg) was used to weigh high-purity materials, since the precision and accuracy of weighing directly affected the certified values (NIM, 2009). Brown glass vial was used as container with package amount of about 2 g and then stored in desiccant at 4 °C for following characterization.

2.3.2. Characterization of candidate CRM

2.3.2.1. Liquid chromatography–mass spectrometry. LC/MS analysis. All the carbohydrate reference material solutions, diluted to concentration of 20 μg mL^{-1} in acetonitrile, were quantified at flow injection analysis mode with an Agilent 6410 triple quad system with electro-spray ionization source (ESI). The target molecule was qualified by comparing the mass spectrum peak with the available literature mass spectrum (NIMC, 2011; NIST, 2011) or analysis the mass spectrum peak molecular weight at the positive ion polarity mode at specified MS scan range of 100–400.

The liquid chromatography conditions optimized were as following: flow rate of 0.3 mL min^{-1}, isocratic, 70% acetonitrile/30% 0.1% ammonium acetate solution and column temperature of 35 °C. The mass spectrometer conditions, optimized with flow injection analysis in an ESI positive ion mode; fragmentor voltage was set 40 V; drying gas temperature was 300 °C; drying gas flow was 10 L min^{-1}; capillary voltage was 3500 V; and nebulizer pressure was 30 psi. Scanning mode was used to obtain the spectra shown in the results. The ions monitored by SIM were the $[M + NH_4]^+$ ion that correspond to $m/z = 198$ for glucose, fructose and galactose, $m/z = 360$ for lactose and sucrose, and $m/z = 168$ for xylose, respectively. Duplicate injections were made for each sample.

2.3.2.2. Fourier transform infrared spectroscopy. Infrared spectra were collected with FT-IR spectrometer Thermo Nicolet IS10 spectrophotometers via KBr pellets technique. Specimens were ground with dried spectroscopic grade KBr powder and the mixture was compressed to pellets for FT-IR measurements. The sample to KBr mass ratio was optimized as 1:100. All the spectra were collected in the 4000–400 cm^{-1} range at 8 cm^{-1} resolution.

2.3.3. Quantitative nuclear magnetic resonance spectroscopy (QNMR)

The QNMR method is based on the directly proportional relationship between the signal response (integrated signal area, I_x) and the number of nuclei generating the corresponding resonance line, which is represented by Eq. (1) as follows (Jancke, 1998; Wells, Hook, Al-Deen, & Brynn, 2002).

$$P_x = \frac{m_x}{m_s} = \frac{I_x}{I_{std}} \cdot \frac{N_{std}}{N_x} \cdot \frac{M_x}{M_{std}} \cdot \frac{m_{std}}{m_s} \cdot P_{std} \quad (1)$$

Where P_x is purity of analyte; I_x and I_{std} are the integrated signal areas of analyte and internal standard, respectively; N_x and N_{std} are the spin numbers of the analyte and internal standard, respectively; M_x and M_{std} are molar mass of analyte and internal standard, respectively; m and m_{std} are the mass of analyte and internal standard, respectively; P_{std} is the purity of the internal standard.

The QNMR measurements were performed with a Bruker AV600 spectrometer (Bruker, Billerica, MA). Spectra were run with the following optimized parameters: probe size, 5 mm; probe temperature, was 23.0 °C; excitation pulse angle, 45°; 32 K time domain points; 16 K spectral data points; pulse delay, 4.15 μs; relaxation delay, 32 s and number of scans, 32. A sample solution containing about 10 mg benzoic acid, 70 mg of carbohydrate samples diluted in 0.5 mL DMSO was analyzed by QNMR. The total mass of analyte in the sample solution was determined using Eq. (1).

2.3.4. Mass balance approach

The mass balance approach involves quantifying all of the detectable impurities (including water and trace metal residues) with available techniques and subtracting the sum of these impurities from 100% (DQMCE, 2007; WHO, 2003), according to which the content of the analyte can be calculated as following by Eq. (2):

$$P(\%) = \left(1 - \sum_{i=1} P_{im}\right)(1 - P_w - P_v - P_a) * 100\% \quad (2)$$

Where P_{im}, P_w, P_v and P_a were the content of HPLC impurities, water, volatile material and ash, respectively. Impurity analysis of the reference material candidates was carried out by HPLC-ELSD