XIX IMEKO World Congress
Fundamental and Applied Metrology
September 6−11, 2009, Lisbon, Portugal

QUALITY CONTROL MATERIALS FOR ANALYSIS OF VITAMINS IN FOOD

Isabel Castanheira, Elsa Vasco , Ana Cristina Flores, Inês Coelho

[1] National Institute of Health Dr Ricardo Jorge, Lisbon Portugal, isabel.castanheira@ insa.min-saude.pt

Abstract – One of priorities in research undertaken to support Food Composition Analysis was the production of a range of reference materials relevant to determine nutrients in selected foods.

This paper describes the implementation and outcomes of activities on reference material production program and their contribution to international developments in the harmonization of Quality System of Food Composition Databanks

In order to enhance the quality assurance of analytical result. Infant formula as food matrix was evaluated to check for the suitability as quality control matrices for the analysis vitamin B2 and vitamin E.

Homogeneity and stability of samples was evaluated by means of official methods of analysis. Statistical analysis was carried out to check for significant differences between results. Results showed that both nutrients were homogeneous for all selected parameters. Regarding stability, the infant formula under study can be used in-house quality control materials at different temperatures and for different periods of time.

The results have demonstrated that quality control materials prepared under the frame of ISO 34 and ISO 17025 standards can fit the purpose of measurement process of nutrient values that enter in food composition database.

Keywords: Reference materials; Quality control materials; Vitamins;

1. INTRODUCTION

One of the key uses of National Food Composition Databanks (FCDBs) in Europe is to provide data, in accordance with international standards, to assist multi-centre studies investigating diet and health relationships and to provide data for nutritional labelling information.[1]

Currently available food composition databases contain compositional values of differing quality, reflecting the different ways in which they were obtained [2].

Data used worldwide must be of consistent quality so that they can be used for different purposes. Moreover, it is now recognised that for a food laboratory to produce sound reliable data, suitable for the purpose of table composition, it must implement a program of adequate quality assurance measures.[3]

Quality Control materials (RMs) have emerged with the aim of improving analytical methods performance, in what regards to their validation, of increasing the comparability of measurements between laboratories[4].

In order to enhance the quality assurance of analytical results, a commercially available formula of baby milk powder was evaluated to check for its suitability as quality control matrix for the analysis of vitamins B2 and Vitamin E.

.

2. METHODS

The analytical methods followed for the determination of vitamins B2 and E were based on EN 14152[5]and EN 14152 [6], respectively. Reference material BCR421 – Milk powder was used for vitamin B2 and for vitamin E. The analysis were carried out by laboratories hold ISO 17025 accreditation[7].

Methods of analysis

In brief the method of analysis used to perform the measurements was reverse-phase HPLC with fluorescence. High-resolution HPLC analyses were performed with a solvent delivery system and UV detector. Chromatograms were registered and stored. With an integrator, Vitamin standards, controls and spikes were injected automatically into the HPLC.

A three point standard calibration was performed with each HPLC run, with calibrates injected after every four-sample injections during each run. In all cases separations were performed at a constant temperature of 35 ºC on a reverse phase (C18 column) making a gradient with solvent A (water) and solvent B (acetonitrile).

Homogeneity

Homogeneity and stability studies were designed according to the directions of ISO Guide 34 (2000)[8]. So, for homogeneity assessment, the following scheme was laid out:

ISBN 978-963-88410-0-1

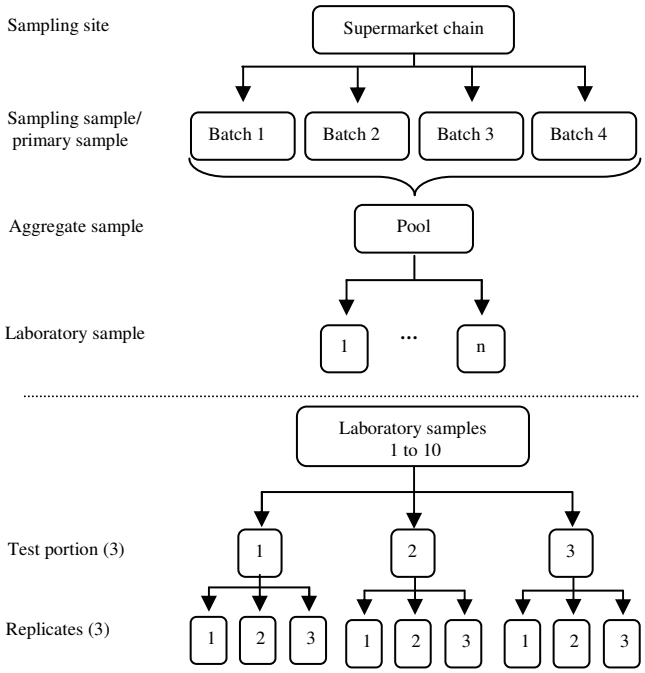

Fig. 1. Homogeneity study lay-out.

Stability Studies

Stability studies were planned according to the isochronous method. However control samples were analysed every 3 months. Three temperatures were under study, -20, 4ºC and 20ºC. The reference temperature was -70ºC

Short Term stability studies was carried out at 40ºC since this temperature represents transport under extreme conditions.

Long-term stability was evaluated by two methods: classic method and isochronous method. In the classic method, samples are stored at different temperatures and analysed at selected storage times. In the isochronous method, samples are stored at the reference temperature (-70ºC) and then they are taken after 1, 8 and 15 days, 3 weeks and 1 month and put at different temperatures.

For results analysis a one way ANOVA was applied to homogeneity results and linear regression was used to assess the existence of trends in stability studies.

3. RESULTS

Food Matrices

In the present paper, the suitability of using commercial samples as in-house reference materials was evaluated for the determination of Vitamin B2 and Vitamin E. In this work, matrices suitability as Quality Control Materials is carried out by comparison with those achieved in our tests the nutrient content in select food matrix and value included in food databases. The results are in accordance with data published in literature [9].

Sampling

After matrices selection, special attention was given to sampling because this is a critical element of data quality. The sampling plan was crucial to obtained concentration that fit the purpose. This is one of key steps, quality control reference materials should have the nutrients contents in the working range. Therefore several batches were used for selected the best approach.

Meanwhile handling of laboratory sample was carried out with precautions. For the reason special sampling protocols were made to select the appropriate infant formula, according to EuroFir project[10]. The scheme is presented in figure 2.

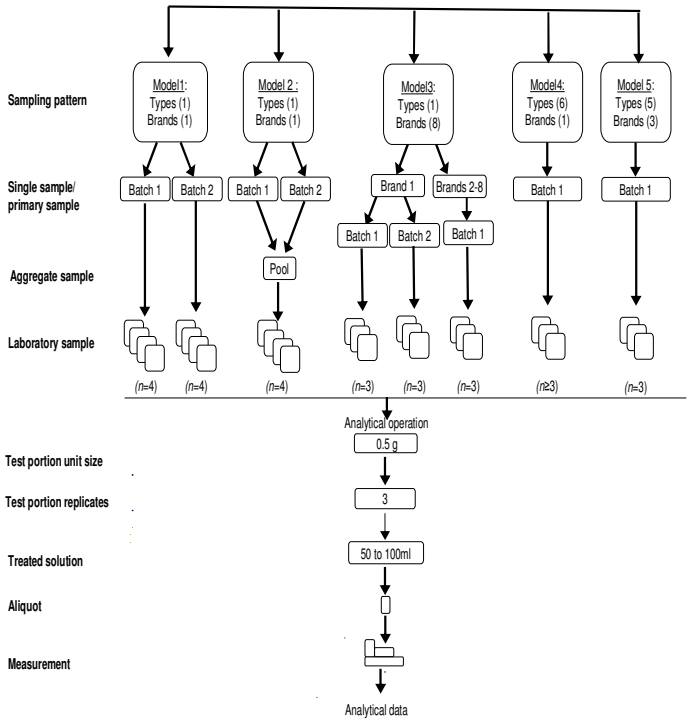

Figure 2 Sampling pattern adopted in the work

Homogeneity

The results of homogeneity studies obtained for Vitamin B2 are presented in the Figure 3. The results from ANOVA at the 95% confidence level for the homogeneity study show that there is no significance differences for any of the parameters/nutrients studies. Therefore, infant formula can be considered suitable as quality control materials for the analysis of these parameters.

The uncertainty given in the same Figure 3 shows that the variation within sachet is less than the variation between sachets. This indicates that the Vitamin B2 presents a good homogeneity within sachet.

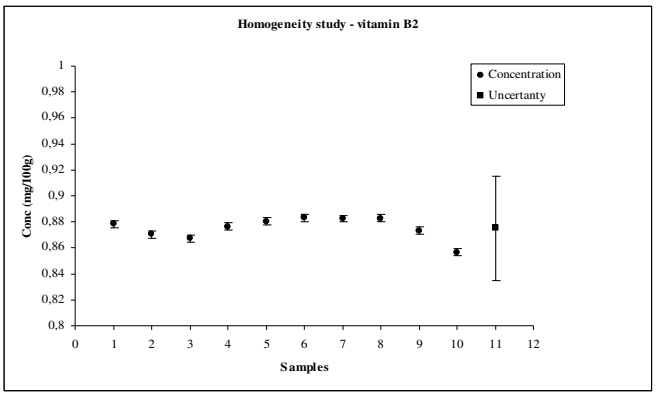

Figure 3 - Results of Homogeneity study for Vitamin B2

The value and associated measurement between sachets are good and the low value was 0.83 mg/100 g of milk and highest 0.89 mg/100 g. The vitamin B2 presents an appropriate homogeneity for the parameters studied F= 0,7534261 when F critical is 2,39281661. Only one sachet presents outlier values and this is consider as a failure in this particularly packaged

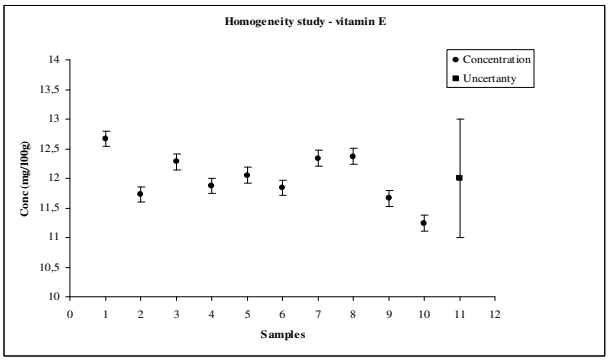

Figure 4 – Results of homogeneity study for Vitamin E

In what regards to Vitamin E , results are presented in Figure 4 and they correspond to the mean of three replicates.

Vitamin E presents a short inhomogeneity between and within sachets. ANOVA results indicate F= 1,52221871 for F critical 2,39281661.This reveals that vitamin E in infant formula is suitable as quality control material.

.

Short term stability studies

Short-term storage stability for vitamin B2 and Vitamin E at different temperatures (23 °C, 4 °C, –20 °C and -70 °C) under study (up to 10 days) provide good results. Uncertainty of the first day (homogeneity study) was used to compare with the CV of stability in semi-continuous studies. All of the results were normalized to reference temperature. Results of standard deviations that are equal or below the CV found in first day suggest that uncertainty due to stability is negligible.

The isochronous method and semi-continuous studies are adequate and can be applied to materials with large variability matrix effects, enabling comparative analysis under reproducible conditions.

Long term stability studies

Long-term stability was evaluated by two methods: classic method and isochronous method[11]. In the classic method, samples are stored at different temperatures and analysed at selected storage times.

Table 1 : Stability studies for Vitamin B 2

Time/Temperature	-20ºC	4ºC	20ºC
0	1,0222	1,0683	1,0382
90	0,9859	1,0581	1,0395
180	1,0031	1,0313	1,0366
365	1,0046	1,0348	1,0205

The results obtained in the stability study for vitamin B2 in infant formula, stored at different temperatures (-20, 4, and 20ºC) are presented in Table 3. All the results were normalized for the initial month (0 month). The relative a relative uncertainty was found to be better than 5% for all results. The stability test shows that all sachets analyzed remain stable for all tested temperatures. No trends were detected at any temperature.

Table 2; Stability studies for Vitamin E

Time/Temperature	4ºC
0	13,1458
90	13,2138
180	13,4248
365	13,2594

Measurement Uncertainty

Figures 5 shows the relevant individual contributions of combined standard uncertainty. These contributions were selected and estimated according to appropriate documents (Eurachem), [12] and other scientific papers [13, 14]. The input quantities were identified on the basis of the analytical process.In each step, uncertainty of type A was evaluated as standard deviations under repeatability conditions and that of type B was calculated on the basis of calibration certificate issues by Metrological Laboratories and IUPAC tables[15]. The applied model was previously described by van der Veen and Castanheira [16].

For the vitamins under study, homogeneity is the greatest contributor to uncertainty amongst the steps of the feasibility study, which is probably related to the variability of the vitamin content

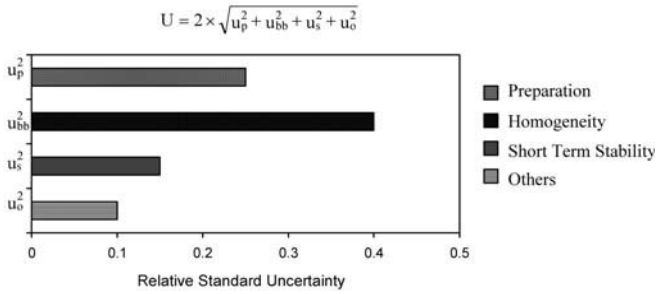

Figure 5 Uncertainty contributions

Decreasing the homogeneity contribution in the measurement uncertainty is a challenge for future studies.

4. CONCLUSIONS

The establishment of traceability and evaluation of feasibility studies for the production of reference materials for analysis of vitamin B2 and Vitamin E were investigated by appropriate standards. The present methodology fulfils the requirements of ISO 17025 and ISO guide 34 for the production of reference materials.

The results of method optimization were presented and were accompanied by ANOVA evaluation. A procedure for obtaining appropriate homogeneity was established. The methods were checked against routine conditions in laboratory and have demonstrated that the results are appropriate for the purpose. The stability is nutrient dependent. The results have demonstrated that quality control materials prepared under the frame of ISO 34 and ISO 17025 can fill the gap in the traceability chain of measurement process of nutrient values that enter in food composition database.

Vitamins contents were within the range of concentration appears in food composition therefore these food matrices can be considered suitable as quality control materials, in other words, they are an economic contribution for the quality data of food databases the results show that quality control materials prepared under rigorous procedures are valuable tools in the food composition analysis.

ACKNOWLEDGMENTS

This work was completed on behalf of the EuroFIR consortium (FOOD-CT-2005-513944) and funded under the EU 6th FRAMEWORK FOOD QUALITY AND SAFETY

REFERENCES

[1] G. Deharveng, U.R.Charrondiere, N. Slimani, D.A.T Southgate, E. Riboli,(2009) "Comparison of nutrients in the food composition tables available in the nine European countries participating in EPIC". *European Journal of Clinical Nutrition,* 53,60-79.

[2] Slimani, N., Deharveng, G., Unwin, I., Vignat, J., Skeie, G., Salvini, S., Moller, A., Ireland, J., Becker, W., & Southgate, D.A.T. (2007). Standardisation of an European end-user nutrient database for nutritional epidemiology: what can we learn from EPIC Nutrient Database (ENDB) Project? *Trends in Food Science & Technology*, 18, 407-419.

[3] Castanheira, I.,· Andre, C., · Oseredczuk, M., · Ireland,. J., · Owen, L., · Robb, P., · Earnshaw, A., &· Calhau, M.A. (2007). Improving data quality in food composition databanks: a EuroFIR contribution. *Accreditation Quality Assurance*, 12(3-4), 117–125.

[4] Castanheira I. Abrantes C., Batista M., Coelho, I. Sanches-Silva A.Quality control materials in food composition databanks (2009).*Food Chemistry,*119,771-725.

[5] Castanheira. I., Ramos, S., Robb, P., Owen, L. , den Boer, H., Schmit, J. , Ent H.,& Calhau M.A.(2007). A proposal to demonstrate a harmonised quality approach to analytical data production by EuroFIR. *Journal of Food Composition Analysis*, 20(8), 725-732.

[6] EN 12822:2000 Foodstuffs - Determination of vitamin E by high performance liquid chromatography - Measurement of alpha-, beta-, gamma-, and delta-tocopherols.

[18] EN 14152:2003- Foodstuffs – Determination of Vitamin B2 by HPLC.

[8] ISO/IEC International Standard (2005). General requirements for the competence of calibration and testing laboratories. ISO/IEC 17025:2005.

[9] ISO International Standard (2000). General requirements for the competence of reference material producer. ISO GUIDE 34:2000

[9] Kuhnlein H.V.,_, Barthet, V. Farren, A. Falahi E., Leggee, D. Receveur, O. Berti P. (2006) Vitamins A, D, and E in Canadian Arctic traditional food and adult diets. *Journal of Food Composition and Analysis*, 19, 495–506.

[10]EuroFIR .European Food Information Resource Network. http://www.eurofir.net. (2008).

[11] van der Veen A, Linsinger T, Pauwels J (2001) Uncertainty calculations in the certification of reference materials. Stability study *Accreditation Quality Assurance* 6: 6:257–263.

[12] Eurachem/CITAC http://www.measurementuncertainty.org/.

[13] van der Veen A, Linsinger T, Pauwels J (2001) Homogeneity and stability of reference materials, *Accreditation Quality Assurance*, 6, 20-25

[14]Anglov, T., Petersen, I. M., & Kristiansen, J. (1999). Uncertainty of nitrogen determination by the Kjeldahl method. *Accreditation Quality Assurance*, 7(4), 504–510.

[15]BIPM, IEC, IFCC, ISO, IUPAC, IUPAP, OIML, (1993). International Vocabulary of Basic and General Terms in Metrology, 2nd edn. ISO, Geneva, Switzerland

[16] Batista, E., Sabrosa, A., Ferreira, M.C., Castanheira, I., Van der Veen, A.M.H.: Uncertainty Calculation in the Calibration of Volumetric Laboratory Glassware, *IX Congresso Internacional de Metrologia*, Brasil (2000)