

DEVELOPMENT OF A CERTIFIED REFERENCE MATERIAL FOR THE ANALYSIS OF VITAMINS IN INFANT FORMULA

Byungjoo Kim and Joonhee Lee

Division of Metrology for Quality of Life, Korea Research Institute of Standards and Science
Yuseong, Daejeon 305-600, Korea

Abstract: An infant formula certified reference material (CRM) for the analysis of vitamins was developed. Vitamin A, folic acid, niacin, were certified and their stability has been monitored by using isotope dilution-liquid chromatography/mass spectrometric (ID-LC/MS) methods developed in our laboratories as primary methods for the corresponding analytes. Certification of α -, δ -, and γ -tocopherols in the CRM is undergoing at the time of submitting this proceeding.

Keywords: Infant formula, CRM, vitamin A, folic acid, niacin, tocopherols

1. INTRODUCTION

Infant formula is usually the only nutrient source for non-breast-fed infant in its early ages. Therefore, producers have to ensure that their products contain all necessary nutrients within a specific range or above a specific minimum. Controlling the quality of the products depends on reliable analytical measurements. Well-characterized CRM will be used to validate or periodically control the quality of analytical measurements. Our laboratory, the National Metrology Institute of Korea, developed an infant formula CRM (KRISS CRM #108-02-001) for the analysis of several representative vitamins (vitamin A, folic acid, niacin, α -, δ -, and γ -tocopherols). KRISS traditionally uses isotope dilution mass spectrometry (IDMS) as a primary method for the certification of a matrix CRM. ID-LC/MS methods developed in this laboratory were used for the certification of vitamin A [1], folic acid [2], niacin [3]. Recently, we adopted a standard addition method in combination with isotope dilution techniques for the analysis of tocopherols to reduce bias in measurement results due to the difference in degradation of tocopherols and their dehydrated forms [4]. Tocopherols in the CRM are currently under certification by using this method. This article describes the certification results and the stability monitoring results of this CRM.

2. EXPERIMENTAL

Preparation of CRM. KRISS CRM #108-02-001 (batch number 090220) “Infant Formula (Milk Powder) for the Analysis of Organic Nutrients” was prepared based on the procedures maintained in our laboratory. About 60 kg of

infant formula was obtained from a local producer. The whole material was collected in the middle point of producing a single batch by the producer to obtain homogeneous material. The raw material was pulverized by using a laboratory mill (Pulverisette 14; Fritsch, Idar-Oberstein, Germany) with a 500 μm sieve ring, and sieved to select particle size of 50 – 250 μm by using vibrating sifter(V/SIFTER-141, Daega Co., Korea). The material was then homogenized for 10 hours of V-mixing. Around 54 kg of powder was finally obtained. The material was bottled into 120 mL wide-bore amber bottles in 30 g per unit. The bottles were purged with argon gas and tightly sealed with Teflon lined caps. A total of 700 units were prepared and stored at a -70 °C deep-freezer.

Value Assignment and Homogeneity Test. ID-LC/MS methods used for vitamin A [1], folic acid [2], niacin [3] were or will be published in separated articles. A standard addition method in combination with isotope dilution techniques [4] is used for the certification of tocopherols. For each analyte, at least 10 bottles with even intervals of bottling sequence were selected for the simultaneous value assignment and homogeneity test. One subsample from each bottle was analysed. The amount of subsample from a bottle was 1 g for vitamin A, folic acid, and tocopherols, and 2 g for niacin. The mean of all bottles was assigned as the certified value of each analyte. Between-bottle homogeneity of each analyte was assessed by the standard deviation among bottles (SD_{bb}).

Uncertainty Evaluation. Details for evaluating uncertainty of the certified value with this type of certification scheme were described in other paper [5, 6] and brief description is given here.

$$u(C_{cert}) = \sqrt{u_{char,sys}^2 + SD_{bb}^2}$$

Where $u_{char,sys}$ is the combined uncertainty of all uncertainty sources that give same systematic effects to the measurement value of all bottles. $u_{char,random}$, combined uncertainty of all uncertainty sources that cause random variation of multiple measurement results is included in SD_{bb} .

Stability Monitoring. As CRMs for the analysis of vitamins in infant formula has been known to have long-term stability from studies done by many other NMIs, this CRM was certified based on the initial value assignment study without stability test. Instead, we have been carrying

out periodic (at least one year interval) stability monitoring for the material stored at -70 °C. The same analytical methods used for the initial certification have been used for the stability monitoring.

3. RESULTS AND DISCUSSION

Certification of vitamin A, folic acid, and niacin was completed including stability monitoring as scheduled in the CRM production plan. Certification of α -, δ -, and γ -tocopherols in the CRM is undergoing at the time of submitting this proceeding and results will be included in the presentation at the conference.

Following figures show measurement results of 10 bottles chosen for the initial value assignment and homogeneity test of vitamin A (equivalent to retinol), folic acid, and niacin (equivalent to nicotinic acid) in the CRM. Table 1 listed the final certified values.

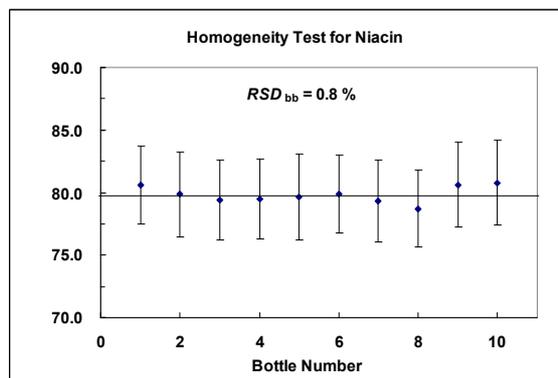


Figure 1. Homogeneity test results of vitamin A, folic acid, and niacin in infant formula CRM (KRISS CRM #108-02-001). RSD_{bb} is the relative standard deviation of measurement values among bottles. Error bars are expanded measurement uncertainties of the corresponding values with 95 % confidence levels

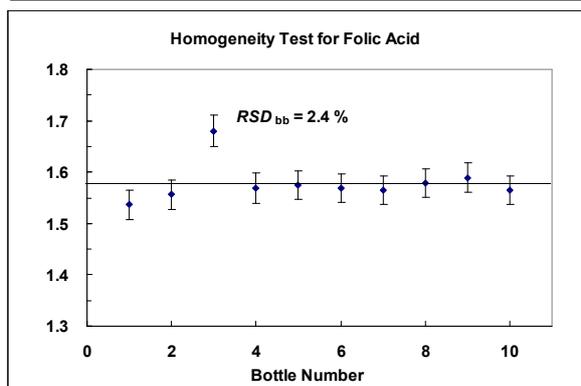
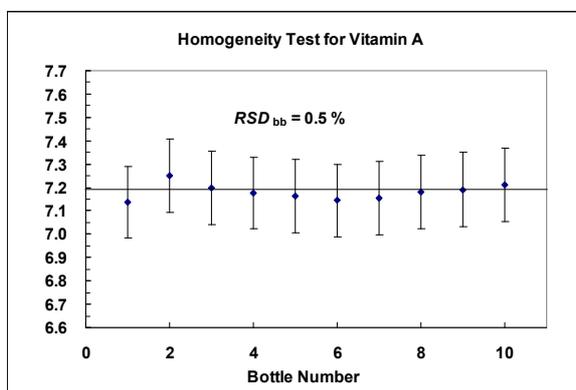


Table 1. Certified values of infant formula CRM (KRISS CRM #108-02-001)

Compound	Certified value (mg/kg)
Vitamin A (Equivalent to retinol)	7.18 ± 0.16
Folic acid	1.578 ± 0.090
Niacin (Equivalent to nicotinic acid)	80.5 ± 3.2
Tocopherols	Certification in process

Between-bottle homogeneities for vitamin A, folic acid, and niacin are 0.5 %, 2.4 %, and 0.8 %, respectively, expressed as among-bottle relative standard deviations. The CRM has very good homogeneity.

As this type of CRMs (for the analysis of vitamins in infant formula) has been known to have long-term stability from studies done by many other NMIs, this CRM was certified based on the initial value assignment study without stability test. After the certification of the CRM, periodic (at least one year interval) stability monitoring for the CRM have been carried out. The same analytical methods used for the initial certification was used for the stability monitoring. Figure 2 shows the stability monitoring results of the CRM up to the time of submitting this proceeding.

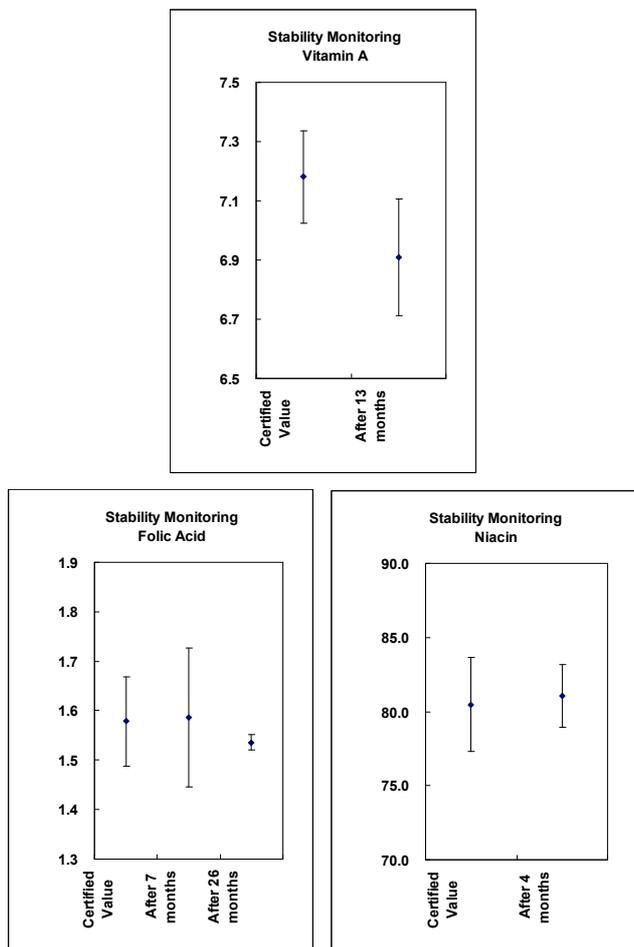


Figure 2. Stability monitoring results of vitamin A, folic acid, and niacin in the infant formula CRM (KRISS CRM #108-02-001) stored at $-70\text{ }^{\circ}\text{C}$

Figure 2 shows that results from stability monitoring agree with the certified value of the corresponding analytes. Therefore, the stability of the CRM at the storage condition was confirmed within the measurement uncertainty.

4. CONCLUSION AND FURTHER WORK

We developed an infant formula CRM for the analysis organic nutrients. Certification of vitamin A, folic acid, and niacin was completed and monitoring of their stability in the CRM has been and will be carried out. We are currently undergoing certification of α -, δ -, and γ -tocopherols. We plan to certify other vitamins as related analytical methods is developed and established in our laboratories.

5. REFERENCES

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