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DEVELOPMENT OF A CERTIFIED REFERENCE MATERIAL FOR NICOTINAMIDE IN INFANT FORMULA

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Abstract - Nicotinamide, one kind of Vitamin B, plays an important role for humans. To ensure quality control of nicotinamide in infant formula, a Certified Reference Material for nicotinamide in infant formula is being prepared in China for the first time. Studying on the procedure of sample preparation and HPLC analysis with different detectors like UV and MS, HPLC-UV and HPLC-IDMS are selected as the value assignment methods. And HPLC-IDMS method was validated in the international comparison CCQM-P78 and CCQM-K62. Homogeneity and stability are discussed in this paper as well.

Keywords: Nicotinamide, Infant Formula, Certified Reference Material

1. INTRODUCTION

Because of a recognized lack of analytical methods and reference materials for food regulations, the National Institutes of Metrology (NIM) was mandated by General Administration of Quality Supervision, Inspection, and Quarantine of the People's Republic of China (AQSIQ). NIM has developed reference materials to support the needs of the food safety. A Certified Reference Material for nicotinamide in Infant Formula was developed.

Nicotinamide, one kind of Vitamin B, plays an important role for humans. The fortification of food products with vitamins is generally achieved with premixes that contain high concentration of vitamins, thus, there is a need to have analytical methods and CRMs for their quality control during production and at the end of shelf life.

There are other institutes (e.g. NIST) that have worked extensively on certifying Vitamins in foods including infant formula. They have certified of Whole Egg Powder (NIST RM 8415), Whole Milk Powder (NIST RM 8435) and Infant Formula (milk-based) (NIST SRM 1846) Reference Materials [1].

This paper describes the preparation and value assignment of certified reference material for nicotinamide in Infant Formula. This material is intended for use as a primary control material when assigning values to in-house control materials and for validation of analytical methods for measurement nicotinamide.

2. PREPARATION

The CRM is a milk-based infant formula powder. It was purchases from market in Beijing. Test of physical and chemical properties and determination of purity, if the results meet the requirements, packing the infant formula with brown glass bottles filled with nitrogen, each containing approximately 30 g of material and packing these glass bottles with opaque sealed bags in vacuum.

3. VALUE ASSIGNMENT

3.1 Analytical approach for determination of nicotinamide

Assignment of the concentration of the nicotinamide was measured by using combinations of two HPLC methods with absorbance or MS detection. And the HPLC-IDMS method was validated in the international inter-comparison CCQM-P78 and CCQM-K62.

3.2 Uncertainty of the certified value

The uncertainty of the certified value included the combined effects of method imprecision, possible bias effects among methods, standard solution, measurement process, homogeneity and stability.

3.3 Homogeneity Assessment

The homogeneity of nicotinamide in Infant Formula was assessed using the HPLC–UV method. 15 bottles of candidate CRM sample were randomly selected for analysis and 7 sub-sample of one bottle were subjected to independent measurement. RSD(%) were 1.66% (n=15) and 1.35%(n=7), respectively. The F-test and T-test method were used and no statistically significant differences among bottles were observed.

The minimum sample amount is 500 milligram.

3.4 Stability Testing

The stability is regularly monitored using the HPLC–UV method for more than one year by NIM. Random sampling at regular intervals was made to test.

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4. ANALYSIS

NIM analysis of nicotinamide in Infant Formula was measured by using combinations of two HPLC methods with absorbance or MS detection. Calibrants were prepared gravimetrically, at levels intended to approximate the levels of the nicotinamide in the Infant Formula.

4.1 Sample preparation

For each method, two 2.0g test portions from each of bottles were diluted in hot ultra pure water (60 ◦C). After cooling, the solution was sonicated for 10 min，and the pH was adjusted to 1.7±0.1 by addition of aqueous HCl (5 mol/L)，waiting for 2 min，the pH was adjusted to 4.5±0.1 by addition of aqueous NaOH (5 mol/L), then the solution was transferred to a 50mL amber volumetric flask and pure water was added to the mark. The solution was filtered through a 0.45 μm syringe filter before the LC-analysis.

Samples were diluted with a water solution of D4-nicotinamide (internal standard) for HPLC–IDMS analysis. Two levels were used.

Low level：nicotinamide: D4-nicotinamide is about 0.9:1(2.4 μg/g:2.6 μg/g);

High level：nicotinamide: D4-nicotinamide is about 1.1:1(2.8 μg/g:2.6 μg/g).

4.2 HPLC with absorbance detection, method 1

An isocratic HPLC method with 1:2:7（V/V） water containing 91g/L 1-Octanesulfonic acid sodium salt monohydrate（pH=2.6）:iso-propanol: methanol as mobile phase and a Inertsil ODS-SP C18 column (5μm particle size, 4.6×250 i.d.mm; DIKMA) was used for HPLC–UV determination of nicotinamide at room temperature;. The flow rate was 1.0 mL min−1, The separation was monitored at λ=261nm. A typical separation is shown in Fig. 1.

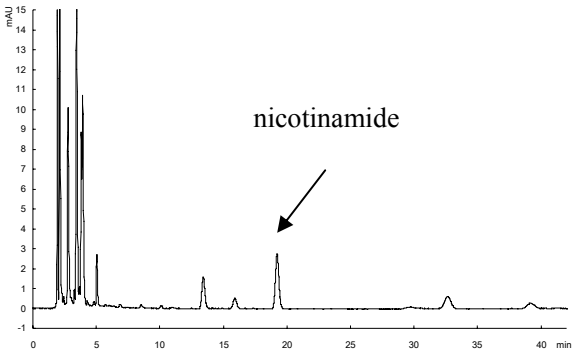

Fig. 1. HPLC-UV chromatograms of nicotinamide in Infant Formula sample

HPLC-IDMS, method 2

An isocratic LC method with 90:10(V/V)water containing 5 mmol/L ammonium acetate(pH=5.5): methanol as mobile phase and a C18 column (Atlantis 2.1×150 i.d.mm, 5μm particle size, Waters) was used for determination of nicotinamide and with electrospray ionization operated in positive ion mode. Sample injection volume was 10 μL. MS detector settings were: capillary potential -3500 V; vaporizer temperature 340 °C; drying gas flow rate 10 L/min; and nebulizer pressure 35 psi.

Two ions were monitored: m/z 123 for nicotinamide and m/z 127 for D4-nicotinamide. A typical separation is shown in Fig.2.

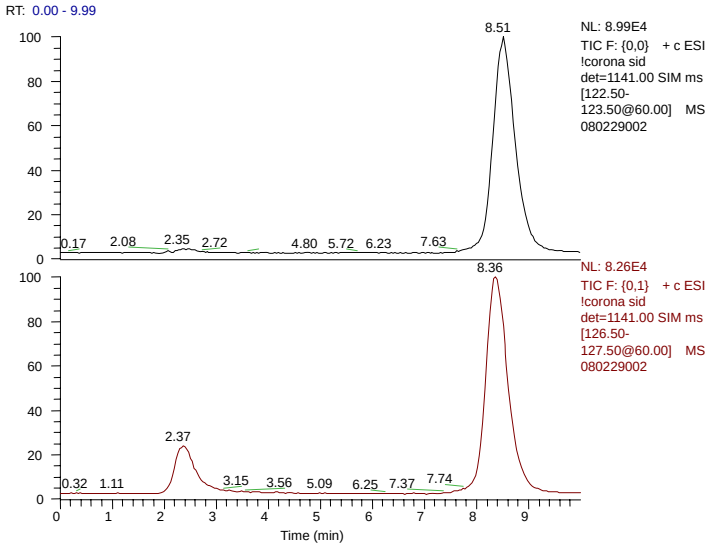

Fig. 2. HPLC-MS chromatograms of nicotinamide in Infant Formula samples

Above: *m/z*=123（nicotinamide），below: *m/z*=127（D_4-nicotinamide）

5. RESULTS

Determination of nicotinamide in Infant Formula by HPLC-UV and HPLC-IDMS methods were performed for the certification, as shown in table 1.

HPLC-IDMS method was applied in CCQM-P78 and CCQM-K62, and the results of comparisons showed that it is a highly precise method.

Table 2 showed the stability of nicotinamide.

Table 1 Analytical results obtained by two methods

	HPLC-IDMS（mg/kg）	HPLC（mg/kg）
values	65.64	64.29
	65.07	64.94
	64.74	63.58
	65.32	65.65
	65.31	64.14
	65.21	65.51
	66.11	66.21
	65.22	66.55
	65.60	65.80
$\overline{X}$	65.36	65.18
s	0.39	1.01
average	65.27	

Table2 the stability of nicotinamide in Infant Formula

time(month)	1	3	6	12	RSD
results	64.70	65.10	65.56	65.58	0.64%

The uncertainty of the certified value consisted of uncertainties related to the method variance (u_{M1}, u_{M2}), homogeneity (u_{H1}, u_{H2}) and stability (u_T). The combined standard uncertainty was calculated using the following equation:

$$u_v = \sqrt{u_{M1}^2 + u_{M2}^2} = 1.73\%$$

$$u_c = \sqrt{u_v^2 + u_{H1}^2 + u_{H2}^2 + u_T^2}$$

$$= \sqrt{1.73\%^2 + 1.66\%^2 + 1.35\%^2 + 0.64^2} = 2.8\%$$

The expanded uncertainty (u) of the certified value is:

u＝k×uc＝2.8%×2≈5.6%　(k=2)

Where uc is the combined standard uncertainty and k is a coverage factor. When k is set to 2, u corresponds to a 95% confidence interval. Therefore, the expanded uncertainty was calculated as 5.6 mg/kg (k=2).

As a result, the certified value and uncertainty for nicotinamide in infant formula is 65.3 mg/kg± 5.6 mg/kg (k=2).

6. CONCLUSION

A certified reference material of nicotinamide in infant formula was developed. The CRM is important for the study and monitoring of content in infant milk formula. It can be widely used in analytical work for nicotinamide measurement and control products in food inspection and scientific research in related areas.

REFERENCE

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