Development of milk powder reference material and its use for evaluation of laboratory performance on analysis of mandatory nutrients for nutrition labeling

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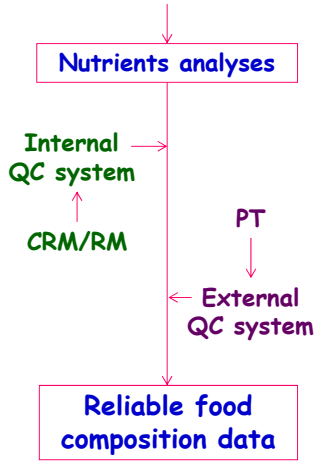
Outline

1. **Introduction:** RM, CRM, PT
2. **Objectives**
3. **Materials and methods:** homogeneity, stability, assigned value, z-score evaluation
4. **Results & Discussion**
5. **Conclusion**



Reference materials (RM):

material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process

Certified reference materials (CRM):

reference material, characterised by metrologically valid procedures for one or more specified properties, accompanied by a certificate that provides the values of the specified properties, associated uncertainty, and a statement of metrologically traceability.

References; ISO 17034 (2016), guide 35 (2017)

ISO guide 35 (2017)

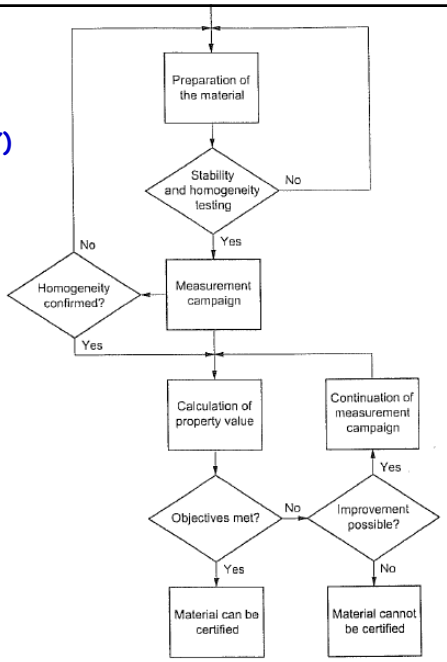
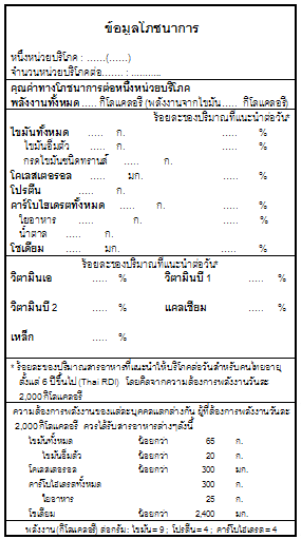


Figure 1. Layout of development of RM by collaborative study

Thai FDA regulation No. 182 BE 2541 (1998): Nutrition labelling (NL)

Mandatory nutrients:
Energy
Lipid
Saturated fat
Cholesterol
Protein
Carbohydrate
Dietary fibre
Sugars
Sodium
Vitamin A
Vitamin B1
Vitamin B2
Calcium
Iron


ข้อมูลโภชนาการ

หนึ่งหน่วยบริโภค : .....(.....)
จำนวนหน่วยบริโภคต่อ..... : .....

คุณค่าทางโภชนาการต่อหนึ่งหน่วยบริโภค
พลังงานทั้งหมด..... กิโลแคลอรี (พลังงานจากไขมัน..... กิโลแคลอรี)

		ร้อยละของปริมาณที่แนะนำต่อวัน*
ไขมันทั้งหมด	..... ก.	..... %
ไขมันอิ่มตัว	..... ก.	..... %
กรดไขมันชนิดทรานส์	..... ก.	
โคเลสเตอรอล	..... มก.	..... %
โปรตีน	..... ก.	
คาร์โบไฮเดรตทั้งหมด	..... ก.	..... %
ใยอาหาร	..... ก.	..... %
น้ำตาล	..... ก.	
โซเดียม	..... มก.	..... %

ร้อยละของปริมาณที่แนะนำต่อวัน*

วิตามินเอ	..... %	วิตามินบี 1	..... %
วิตามินบี 2	..... %	แคลเซียม	..... %
เหล็ก	..... %		

* ร้อยละของปริมาณสารอาหารที่แนะนำให้บริโภคต่อวันสำหรับคนไทยอายุตั้งแต่ 6 ปีขึ้นไป (Thai RDI) โดยคิดจากความต้องการพลังงานวันละ 2,000 กิโลแคลอรี

ความต้องการพลังงานของแต่ละบุคคลแตกต่างกัน ผู้ที่ต้องการพลังงานวันละ 2,000 กิโลแคลอรี ควรได้รับสารอาหารต่างๆดังนี้

ไขมันทั้งหมด	น้อยกว่า	65	ก.
ไขมันอิ่มตัว	น้อยกว่า	20	ก.
โคเลสเตอรอล	น้อยกว่า	300	มก.
คาร์โบไฮเดรตทั้งหมด		300	ก.
ใยอาหาร		25	ก.
โซเดียม	น้อยกว่า	2,400	มก.

พลังงาน(กิโลแคลอรี) ต่อกรัม: ไขมัน = 9 ; โปรตีน = 4 ; คาร์โบไฮเดรต = 4

Proficiency testing (PT)

PT providers for nutrient analysis in Thailand

- Institute of Nutrition, Mahidol University (INMU) – first place
- Department of Science Service (DSS)
- National Food Institute (NFI)
- Department of Medical Sciences (DMSc)

During 1989 – 2013: **5 out of 10 PT rounds** were conducted on analyses of mandatory nutrients for nutrition labelling
(2 rounds at international level, 3 rounds at national level)

Previous PT studies by INMU

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Table 1. Summary of laboratory performance on mandatory nutrient analysis

Nutrient	Laboratory with good performance (%) |z-score| ≤ 2				
	PT-3	PT-4	PT-7	PT-9	PT-10
n	21	12	32	17	16
Lipid	33	85	58	76	69
Sat. Fat	-	40	45	73	80
Cholesterol	29	-	NE*	-	-
Protein	52	92	67	88	81
Dietary fibre	38	46	50	56	79
Sugars	27	46	40	40	NE*
Sodium	35	54	61	71	69
Vitamin A	57	67	NE*	-	-
Vitamin B1	71	36	56	73	57
Vitamin B2	67	80	74	NE*	NE*
Calcium	30	54	54	63	73
Iron	65	31	64	81	69
Moisture	59	67	82	82	88
Ash	68	69	70	29	75

Different test materials were used:

- PT-3 (1998) weaning food
- PT-4 (2000) instant noddle
- PT-7 (2003) milk powder
- PT-9 (2010) broad bean powder
- PT-10 (2011) cracker powder

Problematic nutrients:
- Nutrients: Sat. fat, cholesterol, dietary fibre, sugars, Vitamin A

- Amount of the nutrient in each matrix

of the nutrients

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Objectives of this study

- To assess laboratory performance on analyses of mandatory nutrients for nutrition labeling using milk powder as the test material
- To develop food reference materials with reference/consensus values of mandatory nutrients for nutrition labelling to be used in quality control system in laboratories

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Reference material production: ISO standard

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Candidate reference material

↓

Homogenise and sieve → sub-samples, pack in sealed containers

*Follow ISO Guide 34, 35, ISO 5725 & 13528

Testing for homogeneity and stability of representative nutrients

↓

Distribution of samples to expert/outstanding laboratories

PT participants

*Follow ISO/IEC 17043

Request for the analysis of required nutrients

Computation and statistical evaluation of results

- Robust statistics (ISO 13528)
- Uncertainty (ISO guide 35)

Values from good performance laboratories

Reference materials with reference values of nutrients
Mean ± SD or Mean ± U

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Materials and methods (cont.)

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Sample preparation: milk powder

Transfer sample to mixing machine & mix for 3 hrs.

Aliquot 1-2 kg

Packing (~50 g/bag)

Vacuum sealed

Test material

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Materials and methods (cont.)

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Sample preparation

Particle size distribution ← Random Sampling → **Storage sample** at -20^0 C

10 packages (5A, 5B)

Homogeneity testing

Total fat, saturated fat, protein, cholesterol, total sugars, dietary fibre, vitamin A, vitamin B_1, vitamin B_2, sodium, calcium, iron, ash, moisture

6 packages (3A, 3B)

Stability testing

Vitamin A, B_1, B_2, moisture at 0, 0.5, 3, 6, 12 mo.

2 packages, to each participating laboratory

Analysis of mandatory nutrients

Homogeneity testing: statistical analysis

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1). Checking for within sample variation (precision of the analyst): using Cochran' s maximum range test ⟶ Pass

2). Checking for between sample variation (check homogeneity of the test materials) using

- ISO 13528:2005 $s_s \leq 0.3\sigma_{pt}$
- IUPAC (Thompson et al., 2006) $s_{sam}^2 < c, \quad c = F_1\sigma_{all}^2 + F_2 s_{an}^2$
- %RSD, HORRAT approach $RSD_r/pRSD_R$

1) ISO 13528: 2005 Statistical methods for use in proficiency testing of interlaboratory comparisons.

2) Thompson M, Ellison SLR, Wood R. The International harmonised protocol for the proficiency testing of analytical chemistry laboratories (IUPAC Technical Report). Pure Appl Chem 78: 145-196, 2006.

3) ISO 5725-1994: Accuracy (trueness and precision) of measurement methods and results.

Table 2. Homogeneity testing: Between sample variation

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Parameter (per sample)	Mean	Standard deviation (SD)	RSD_r (%CV)	Horwitz predicted Value (RSD_p)	HORRAT (RSD_r/ RSD_p)	Acceptable HORRAT** (0.3-1.3)
Total fat (g)	18.38	0.22	1.2	2.6	0.46	**pass**
Saturated fat (g)	7.11	0.07	1.0	3.0	0.32	**pass**
Cholesterol (mg)	22	1	3.3	7.1	0.47	**pass**
Protein (g)	18.13	0.16	0.9	2.6	0.34	**pass**
Dietary fibre (g)	1.87	0.01	0.7	3.6	0.18	**pass**
Total sugar (g)	41.70	0.87	2.1	2.3	0.91	**pass**
Sodium; Na (mg)	248	13	5.3	4.9	1.08	**pass**
Vitamin A (µg)	795	61	7.7	11.7	0.65	**pass**
Vitamin B1 (mg)	0.79	0.03	3.4	11.7	0.29	**pass**
Vitamin B2 (mg)	0.87	0.02	2.3	11.5	0.20	**pass**
Calcium; Ca (mg)	650	18	2.8	4.3	0.65	**pass**
Iron; Fe (mg)	7.3	0.09	1.3	8.4	0.15	**pass**
Moisture (g)	2.15	0.06	2.7	3.6	0.74	**pass**
Ash (g)	4.35	0.02	0.4	3.2	0.12	**pass**

Stability testing: statistical analysis

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1). ISO 13528 approach: $\left|\bar{x}_{hom} - \bar{y}_{sta}\right| < 0.3\hat{\sigma}$

where X_{hom} = mean value from homogeneity of test material

Y_{sta} = mean value from stability of each storage time

= Horwitz's predicted standard deviation

2). ISO guide 35: $\dfrac{|b_1|}{s(b_1)} < t_{0.95,n-2}$ ⇒

- ✓ the slope is insignificant
- ✓ no instability was observed

where b_1 = slope of stability data

$s(b_1)$ = standard deviation of slope

$t_{0.95}$ = critical t at 95% confidence limit for n-2 degree of freedom

n = number of stability data

1) ISO 13528: 2005 Statistical methods for use in proficiency testing of interlaboratory comparisons.

2) ISO guide 35: Reference materials - General and statistical principles for certification, Geneva, Switzerland 2006.

Figure 1: Stability study (ISO guide 35)

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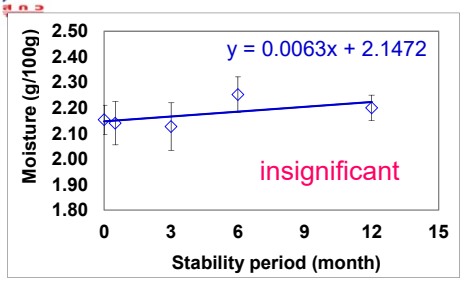
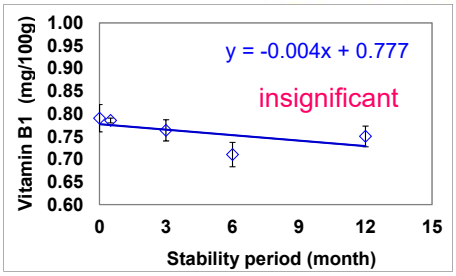
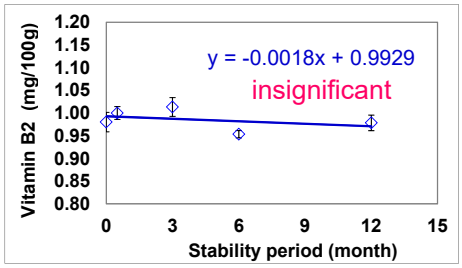


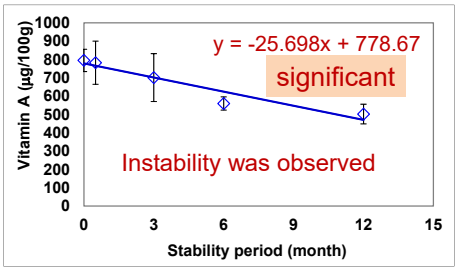
(a) Moisture



(b) Vitamin B1



(c) Vitamin B2



(d) Vitamin A

Establishing assigned values:

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Consensus from participants in a round of PT scheme

(Algorithm A in Annex C of ISO 13528)

Assigned value: Robust mean (x^*) ± robust SD (s^*),

Robust mean (x^*) ± uncertainty (u_x)

Standard uncertainty $u_x = \frac{1.25 \times s^*}{\sqrt{p}}$

s^* = the robust standard deviation of the results, calculated using Algorithm A in Annex C.' p = number of participants' reported data

Table 3: Assigned values of nutrients in milk powder estimated by ISO 13528

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Measurand	Unit per 100 g	Robust mean	Robust SD	%RSD_R	Standard uncertainty
Total fat	g	19.25	0.59	3.1	0.16
Saturated Fat	g	7.29	0.59	8.2	0.19
Cholesterol	mg	20.00	2.90	14.4	0.90
Protein (N x 6.38)	g	18.00	0.23	1.3	0.07
Dietary fibre	g	2.15	1.17	54.5*	0.39
Total sugar	g	42.89	2.42	5.6	0.69
Sodium	mg	223	26	11.6	8
Vitamin A*	µg	720	282	39.2*	88
Calcium	mg	809	76	9.4	23
Iron	mg	8.56	0.81	9.4	0.24
Vitamin B1	mg	0.66	0.11	15.9	0.04
Vitamin B2	mg	0.79	0.10	12.0	0.03
Moisture	g	2.37	0.20	8.2	0.05
Ash	g	4.50	0.06	1.2	0.02

* Not evaluate due to high variation

Laboratory performance by estimation of robust z-score: Between laboratory variation

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$$\text{Robust z-score}_{\text{between}} = \frac{x_i - \overline{X}}{SD}$$

where x = average value of reported data from each participant

$\overline{X}$ = robust mean (x^*) obtained by ISO 13528

SD = robust standard deviation (s^*) obtained by ISO 13528 or predicted SD of Horwitz (SD_p)

Interpretation

$|z| \leq 2$ Satisfactory (acceptable) result

$2 < |z| < 3$ Questionable (warning) result

$|z| \geq 3$ Unsatisfactory (unacceptable) result

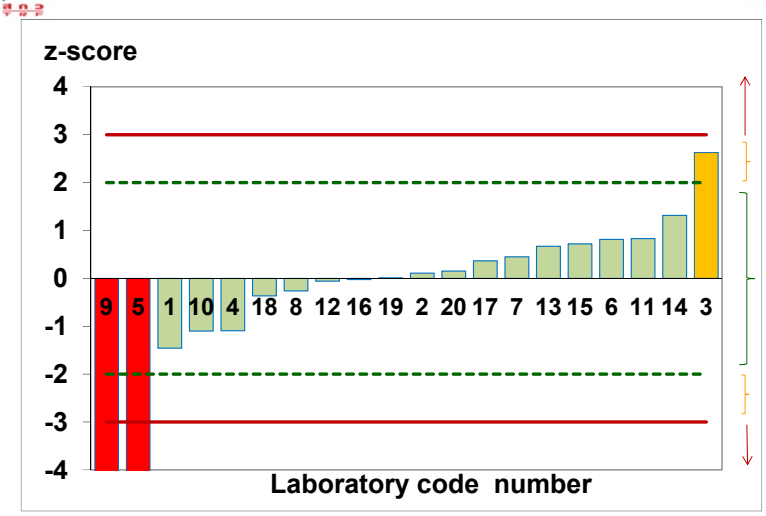
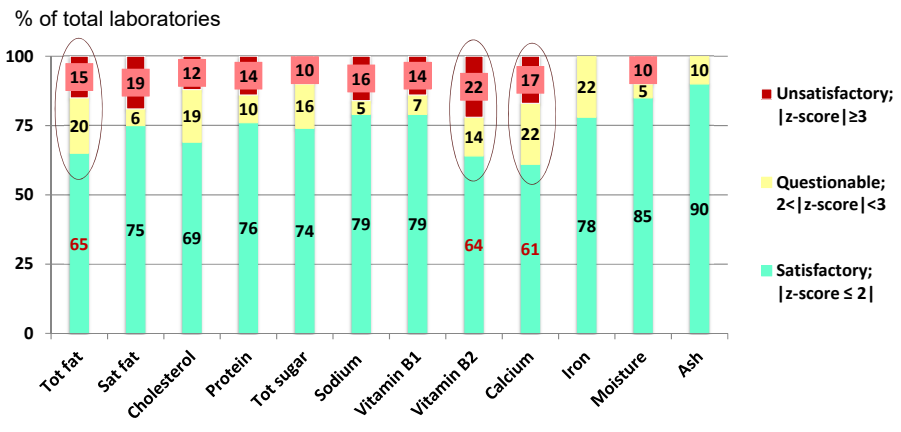


Figure 2. Bar-chart of z-scores for total fat in milk powder

Figure 3. Summary of z-score evaluation according to each parameter



Problematic nutrients: total fat, vitamin B2, calcium

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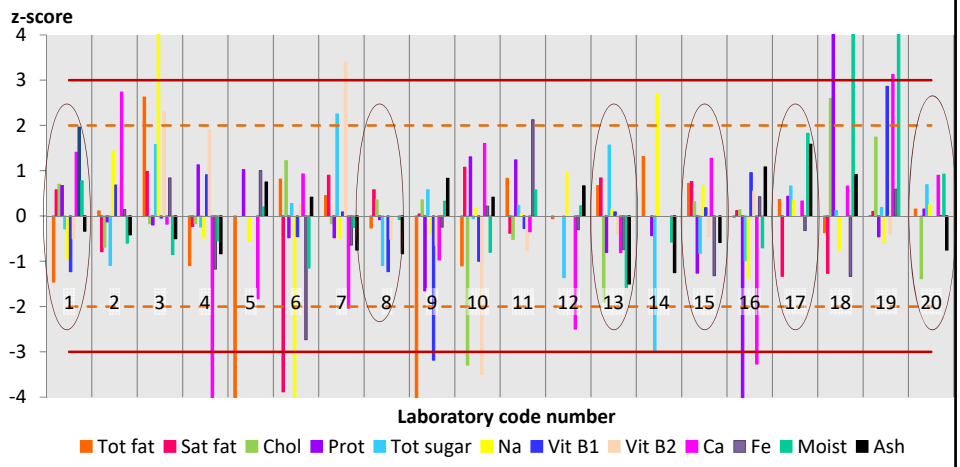
Figure 4. Summary of z-score evaluation according to laboratory number



6 laboratories have good performance on analyses of mandatory nutrients

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Table 4. Summary of consensus/reference values for milk power reference material

Measurand	Unit per 100 g	N(1)	Robust mean	Robust SD	RSD_R (%CV)	Expanded Uncer. (k=2)	% Relative Uncertainty
Total fat	g	13	19.25	0.40	2.1	0.28	1.4
Saturated Fat	g	12	7.39	0.43	5.9	0.31	4.2
Cholesterol	mg	12	19.4	2.4	12.4	1.7	9.0
Protein (N x 6.38)	g	15	18.01	0.19	1.1	0.13	0.7
Total sugar	g	14	42.99	2.26	5.3	1.51	3.5
Sodium	mg	11	221	20	8.8	15	6.6
Calcium	mg	11	814	35	4.4	27	3.3
Iron	mg	14	8.44	0.75	8.8	0.50	5.9
Vitamin B1	mg	11	0.64	0.08	12.4	0.06	9.4
Vitamin B2	mg	8	0.78	0.08	10.0	0.07	8.8
Moisture	g	17	2.34	0.17	7.3	0.10	4.4
Ash	g	18	4.49	0.05	1.2	0.03	0.7

(1) N = Number of laboratories, identified as "satisfactory performance"

ISO guide 35: certified value

Mean ± U (expanded uncertainty)

$$u_{CRM} = \sqrt{u_{char}^2 + u_{bb}^2 + u_{lts}^2 + u_{sts}^2}$$

u_{CRM} = Standard uncertainty of the CRM
u_{char} = standard uncertainty due to characterization
u_{bb} = standard uncertainty due to between-bottle homogeneity
u_{lts} = standard uncertainty due to long-term stability
u_{sta} = standard uncertainty due to short-term stability

Uncertainty of characterization: Mean ± uncertainty

Calculation of uncertainty……. 5 approaches

1. Unc. from mean of the means $u = \frac{SD}{\sqrt{p}}$
2. Unc. from weighted mean (based on uncertainty) $u^2 = \sum w_i^2 u_i^2$
3. Unc. from weighted mean (based on SD) $u^2 = \sum w_i^2 SD_i^2$
4. Unc. from one way ANOVA $u = \sqrt{\frac{MS_{withinLab}^2}{n_{Lab} \cdot n_0} + \frac{MS_{betweenLab}^2}{n_{Lab}}}$
5. Unc. - NIST approach: used law of propagation $u = \sqrt{\left(\frac{1}{n}\right)^2 u_{\bar{x}_1}^2 + \left(\frac{1}{n}\right)^2 u_{\bar{x}_2}^2 \ldots\ldots \left(\frac{1}{n}\right)^2 u_{\bar{x}_n}^2}$

Example
Determination of property values: by ANOVA

- Property value by grand mean

$$\bar{\bar{x}} = \frac{1}{\sum_{i=1}^{p} n_i} \sum_{i=1}^{p} \sum_{j=1}^{n_i} x_{ij}$$

- Standard uncertainty associated with the grand mean

$$u(\bar{\bar{x}}) = \sqrt{\frac{s_L^2}{p} + \frac{s_r^2}{n.p}}$$

n = number of observations
p = number of laboratories in a collaborative study
s_L = standard deviation from laboratories = $\sqrt{\frac{MS_{among} - MS_{within}}{n_0}}$ from ANOVA
s_r = repeatability standard deviation = $\sqrt{MS_{within}}$ from ANOVA

Uncertainty of homogeneity

$$s_{bb}^2 = s_A^2 = \frac{MS_{among} - MS_{within}}{n_0}$$

$$s_{bb} = u_{bb} = \sqrt{s_A^2}$$

Note: if $MS_{within} > MS_{between}$ then $s_{bb}^2 = 0$ Limited/insufficient repeatability

s_{bb} = between-bottle (in)homogeneity standard deviation

s_A^2 = between-bottle variance

u_{bb} = standard uncertainty due to between-bottle (in)homogeneity

MS_{among} = Mean square between (ANOVA)

MS_{within} = Mean square within (ANOVA)

n_0 = (effective) number of (sub)group members (ANOVA)

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Accepted criteria for homogeneity testing

$S_r < S_{bb}$ use S_{bb} as u_{bb}

$S_r > S_{bb}$ limited/insufficient repeatability

calculated upper limit for the between bottle variation

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Uncertainty of stability

Trend analysis calculated as slope/SD slope

$$\frac{|b_1|}{s(b_1)} < t_{0.95,n-2}$$

- ✓ the slope is insignificant
- ✓ no instability was observed

where b_1 = slope of stability data

$s(b_1)$ = standard deviation of slope

$t_{0.95}$ = critical t at 95% confidence limit for n-2 degree of freedom

n = number of stability data

$$u_{lts} = s_b \cdot t$$

where u_{lts} = standard uncertainty due to long-term stability

s_b = standard deviation of slope

t = shelf life

Short-term stability

- u_{sts} reflects the uncertainty about changes during transport
- Tested by exposing the material to severe conditions, e.g., high temperatures etc.
- Estimation of uncertainty similar to u_{lts} via regression over the short-time stability study

⇓

Double counting this effect

⇓

u_{sts} can be assumed negligible

Table 5. Reference value of food reference material

Source of uncertainty	Description	Moisture	Vitamin B1	Vitamin B2
u_{char} [%]	Relative standard uncertainty of characterisation exercise	3.4	3.7	3.2
u_{bb} [%]	Relative standard uncertainty due to between-bottle heterogeneity	4.3	2.3	2.0
u_{lts} [%]	Relative standard uncertainty of long-term stability	2.7	4.7	3.0
u_{sts} [%]	Relative standard uncertainty of short-term stability	-	-	-
u_{CRM}, relative [%]	Combined relative standard uncertainty of certified value	6.1	6.4	4.8

Parameters	Moisture (g/100g)	Vitamin B1 (mg/100g)	Vitamin B2 (mg/100g)
Certified value	**2.34**	**0.64**	**0.78**
Combined standard uncertainty	0.14	0.04	0.04
Expanded uncertainty (k=2)	**0.28**	**0.08**	**0.08**
Relative expanded uncertainty	12.2	12.7	9.7

Certify value ± U = 2.23 ± 0.28 | 0.64 ± 0.08 | 0.78 ± 0.08

Choice of a coverage factor

Conventional is for 95% coverage

- k = 2 is acceptable for normal distribution
- when the (effective) number of degree of freedom is considered to be low, Student's t-distribution may be used
- If distribution is not symmetric, a confidence interval should be stated rather than the expanded uncertainty and a coverage factor.

Conclusion & Take home message

- Laboratory performance on analyses of mandatory nutrients for nutrition labeling & development of food reference materials of some mandatory nutrients were successful achieved.
- Test material with consensus values of nutrients becomes reference material with assigned values and can be used for internal and external quality control system.
- Test material used in each PT, by nature, may not suitable for analysis of all mandatory nutrients → some nutrients cannot be evaluated due to low concentration.
- Laboratories with unsatisfactory results must conduct corrective action.
- To produce CRM, isochronous stability approach should be used → reduce uncertainty due to long term stability.
- Regular participation in PT programme is required for ISO 17025 accreditation and strongly recommended for producing reliable FCD.

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PRACTITIONER'S REPORT

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Abstract This study investigated the laboratory performance on the analyses of mandatory nutrients for nutrition labelling in Thailand and aimed to upgrade the proficiency test (PT) material into a reference material (RM). This milk powder with sufficient homogeneity and multiple nutrient compositions provided an acceptable PT material. Twenty ISO 17025 accredited laboratories participated in this study, and analytical performance was investigated using ISO 17043 and ISO 13528 standards. Satisfactory performance ($|z| \leq 2$, for both within- and between-laboratory developed RM can be used as quality control sample or as PT material with reference nutrient values among the laboratories in Thailand.

Keywords Laboratory performance · Mandatory nutrient · Reference material · Milk powder

Introduction

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Thank you for your attention
Sa-was-dee-krab