การพัฒนาวัสดุอ้างอิงสำหรับตัวอย่าง Water Soluble Chlorides (as NaCl) ในอาหารสัตว์
Development of a reference material for water soluble chlorides (as NaCl) in feeding stuffs

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บทคัดย่อ

งานรายงานนี้เป็นการนำเสนอแนวทางการให้ค่ากำหนดและค่าความไม่แน่นอนของวัสดุอ้างอิงในตัวอย่าง Water Soluble Chlorides (as NaCl) ในอาหารสัตว์ โดยวัสดุอ้างอิงนี้เป็นตัวอย่างที่ผลิตขึ้นจากกิจกรรมทดสอบความชำนาญห้องปฏิบัติการ (PTCH-FA03-1401) จัดโดยศูนย์บริหารจัดการทดสอบความชำนาญห้องปฏิบัติการ กรมวิทยาศาสตร์บริการ การผลิตวัสดุอ้างอิงจะดำเนินการเป็นไปตามมาตรฐานสากล ISO/IEC 17043, ISO Guide 34 และ ISO Guide 35. วัตถุประสงค์การใช้งานวัสดุอ้างอิงนี้เพื่อประโยชน์ในการตรวจสอบความสมเหตุสมผลของวิธีทดสอบ การทวนสอบวิธีทดสอบ และการควบคุมคุณภาพของห้องปฏิบัติการ ในช่วงการใช้งาน 2.00 – 2.50 g/100g และค่าความไม่แน่นอนเป้าหมายไม่เกิน 0.10 g/100g ผลการศึกษาแสดงให้เห็นว่า ค่ากำหนดของวัสดุอ้างอิงนี้คือ 2.292 g/100g และค่าความไม่แน่นอนคือ 0.064 g/100g ที่ระดับความเชื่อมั่น 95% (k=2)

Abstract

This paper presented how to assign reference material value and measurement uncertainty for Water Soluble Chlorides (as NaCl) in Feeding Stuffs. This reference material was produced and its value was assigned from the proficiency testing round (PTCH-FA03-1401) organized by Center for Laboratory Proficiency Testing, Department of Science Service in accordance with ISO/IEC 17043, ISO Guide 34 and ISO Guide 35. The reference material is intended to be used for method validation, method verification and laboratory quality control. Target range for the reference material is set at 2.00 – 2.50 g/100g. The target measurement uncertainty for the reference material should not be more than 0.10 g/100g. The result show that the assigned value for the reference material is 2.292 g/100g and the measurement uncertainty is 0.064 g/100g at the 95% confidence level (k=2).

คำสำคัญ: วัสดุอ้างอิง อาหารสัตว์ ค่ากำหนด ค่าความไม่แน่นอน
Keywords: Reference material, Feeding stuff, Assigned value, Measurement uncertainty

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1. Introduction

Reference Materials (RM)[1] are the materials, sufficiently homogeneous and stable with reference to specified properties, which have been established to be fit for its intended use in measurement or in examination of nominal properties. Certified reference materials (CRM)[1] are the reference materials, accompanied by documentation issued by an authoritative body and providing one or more specified property values with associated uncertainties and traceability, using valid procedures. Reference materials and Certified Reference Materials are important tool in realizing a number of aspects of measurement quality and are used for method validation, calibration, estimation of measurement uncertainty, training and for internal quality control and external quality assurance (proficiency testing) purposes.

The production of CRM requires a great deal of planning prior to undertaking any actual activity in the project. A substantial part of the planning deals with the amount of material needed, as well as with the design of the homogeneity, stability and characterization studies. The number of samples to be produced is a very important variable in the planning process. The number of samples and the amount of raw material depend on all these factors.

Modelling a characterization process for the evaluation of uncertainty is neither a routine task nor a strictly mathematic one. The establishment of a proper model for a property value of a specific candidate CRM is a complex task, which should be carried out with great care to account for all relevant details of the procedures followed to produce and certify the material. One of the basic requirements of the model is that all factors that could significantly contribute to the uncertainty associated with the property values of the CRM are included. Therefore, in order to be complete, the combined standard uncertainty on a reference material should acknowledge that homogeneity and both long- and short-term stability also play an important role in addition to the characterization of the batch.

According to ISO Guide 35, the data evaluation for characterization can be evaluated from either

1. Single method in a single laboratory
2. Multiple methods in a single laboratory
3. Method-defined parameters
4. A network of methods and/or laboratories.

In this paper, we used a network of laboratories with similar method from the proficiency testing program to evaluate the reference value and their measurement uncertainty detailed in section 2.

2. Experimental

2.1 Setting target measurement uncertainty for reference material

The candidate reference materials are concentrated feed which the target range for water soluble chlorides (as NaCl) is 2.00 – 2.50 g/100g. The target measurement uncertainty for the reference material should not be more than 0.10 g/100g (%CV = 5%) that is suitable for method validation, method verification and laboratory quality control for feed testing laboratories.

2.2 Homogeneity test

The candidate reference material was checked for homogeneity. Randomly ten packs of samples were measured for water soluble chlorides (as NaCl). The results, together with their statistical evaluations are given the between samples standard deviation (s_{bb}) from a one-way analysis of variance approach. The s_{bb} can be calculated by

$$s_{bb}^2 = \frac{MS_{among} - MS_{within}}{n_0} \quad \text{.....(1)}$$

where n_0 is the number of replicate for within sample

MS_{among} is the variance of the between samples

MS_{within} is the variance of the within samples

In this case, the between samples standard deviation s_{bb} is identical to u_{bb}

2.3 Study short term stability

There are two types of (in)stability to be considered in the certification of reference materials which are the short term stability and long term stability (shelf life).

The short term stability for the sample was calculated

by the deviation between the general average of the measurements obtained in the homogeneity testing and the general average of the results obtained in the stability testing. The standard uncertainty for short term stability u_{sts} is calculated by

$$u_{sts} = \frac{|A-B|}{\sqrt{3}} \quad \text{.....(2}$$

where A is the general average of the measurements obtained in the homogeneity testing.

B is the general average of the results obtained in the stability testing

2.4 Providing proficiency testing and assigned the value from the group of participants

Proficiency testing program (PTFF-FA03-1401) was organized by the Center for Laboratory Proficiency Testing, Department of Science Service (CLPT, DSS). The results of the proficiency testing program on Water Soluble Chlorides (as NaCl) were summarized from 43 laboratories registered in this program (3 governmental laboratories and 40 private laboratories).

2.4.1 The assigned value (x_{pt})

The assigned value for test samples used in the proficiency testing scheme is the robust average (x^*) that is the consensus value from all participants. This assigned value was calculated by using Algorithm A (ISO 13528: 2015)

2.4.2 The standard deviation (σ_{pt}) is the target standard deviation from the standard method ISO 6495: 1999 which is 15% of the assigned value.

2.5 Standard uncertainty of characterization (u_{char})

The standard uncertainty (u_(char)) of the assigned value is estimated from standard uncertainty of the proficiency testing value (u(x_pt)) (ISO 13528: 2015) as:

$$u_{char} = u(x_{pt}) = \frac{1.25 \times s^*}{\sqrt{n}} \quad \text{.....(3)}$$

where $u(x_{pt})$ is standard uncertainty of the proficiency testing value.

s^* is standard deviation of the consensus value calculated by using Algorithm A.

n is the number of results.

2.6 Study long term stability

In this paper, only classical stability is studied for 1 month, 12 months and 24 months. The standard uncertainty for 24 months long term stability (u_{lts}) as

Time (months) x_i	Water soluble chloride (as NaCl) (g/100g) y_i
x_1	y_1
x_2	y_2
...	...
x_n	y_n

$$u_{lts} = s(b_1) \times t \quad \text{.....(4)}$$

$$s(b_1) = \frac{s}{\sqrt{\sum_{i=1}^{n}(x_i-\bar{x})^2}} \quad \text{.....(5)}$$

$$s^2 = \frac{\sum_{i=1}^{n}(y_i-b_0-b_1x_i)^2}{n-2} \quad \text{.....(6)}$$

$$b_0 = \bar{y} - b_1\bar{x} \quad \text{.....(7)}$$

$$b_1 = \frac{\sum_{i=1}^{n}(x_i-\bar{x})(y_i-\bar{y})}{\sum_{i=1}^{n}(x_i-\bar{x})^2} \quad \text{.....(8)}$$

where $s(b_1)$ is standard uncertainty associated with the slope.

t is the number of months.

The slope should be checked as a consequence (no instability) by

$$|b_1 1| < t_{0.95,n-2}.s(b_1)$$

where $t_{0.95,n-2}$ is the student's t-factor for n-2 degree of freedom and p=0.95 (95% level of confidence)

2.7 Calculate measurement uncertainty for reference material

The uncertainty associated with a properly value of a CRM can be expressed as

$$u_{CRM} = \sqrt{u_{char}^2 + u_{bb}^2 + u_{sts}^2 + u_{lts}^2} \quad \text{.....(9)}$$

Where u_{char} is the standard uncertainty of the certified value analyzed by the robust average from the group of laboratories participated the proficiency testing scheme.

u_{bb} is the standard uncertainty transferred to a single package (homogeneity)

u_{sts} is the standard uncertainty as dispatched to the customer (short-term stability)

u_{lts} is the standard uncertainty at the time of kept (long-term stability)

3. Results and Discussion

3.1 Assigned value for the reference material

This reference material is part of samples from the proficiency testing program on the PTCH-FA03-1401: Water –soluble chlorides (as NaCl) in Feeding stuffs organized by the Center for Laboratory Proficiency Testing, Department of Science Service (CLPT, DSS). The total of 43 laboratories were participated. The assigned value is the robust average (x^*) calculated from 43 participants in the PT program by using Algorithm A (ISO 13528:2015). Standard deviation of the consensus value (s^*) calculated by using Algorithm A (ISO 13528:2015). The Summary statistics for water-soluble chlorides (as NaCl) in Feeding stuffs is shown in Table 1.

Table 1 Summary statistics for water-soluble chlorides (as NaCl) in feeding stuffs

Summary statistics	Value
No. of results	43
Assigned value (x_{char}) (g/100g)	2.292
Standard deviation of the consensus value (s^*) (g/100g)	0.130
Standard uncertainty of assigned value (u_{char}) (g/100g)	0.025

3.2 Homogeneity study

Ten randomly selected samples were analyzed in duplicate under repeatability conditions. The results together with their statistical evaluations are given in Table 2-4.

The results, together with their statistical evaluations are given the between samples standard deviation (s_{bb}) from a one-way analysis of variance approach.

Table 2 Homogeneity study for water-soluble chlorides (as NaCl) in feeding stuffs

Sample No.	Water soluble chloride (as NaCl) (g/100g)	
	replicate 1	replicate 2
001	2.335	2.339
002	2.339	2.340
003	2.340	2.336
004	2.347	2.340
005	2.341	23.41
006	2.353	2.341
007	2.342	2.344
008	2.343	2.349
009	2.341	2.341
010	2.341	2.343

Table 3 ANOVA single factor

ANOVA						
Source of Variance	SS	df	MS	F	P-value	F crit
Between Groups	0.0001862	9	0.0000207	1.53251	0.257359	3.02
Within Groups	0.000135	10	0.0000135			
Total						

Table 4 Calculation uncertainty for homogeneity study

Average (A)	2.342
MS between	0.0000207
MS within	0.0000135
S_{bb}	0.0000036
u_{bb}	0.0000036

3.3 Short term stability and long term stability

The short term stability for the sample was calculated by deviation between the general average of the measurements obtained in the homogeneity testing (Table 4) and the general average of the results obtained in the stability testing (Table 5).

Table 5 One month stability study

Sample No.	Water soluble chloride (as NaCl) (g/100g)	
	replicate 1	**replicate 2**
011	2.341	2.343
012	2.338	2.339
013	2.337	2.339
014	2.340	2.343
015	2.345	2.339
Average (B)	2.342	

The standard uncertainty for short term stability u_sts is calculated by

$$u_{sts} = \frac{|A-B|}{\sqrt{3}} = 0 \qquad \text{.....(10)}$$

For long term study, we studies 12 months and 24 months to support 2 years self-life. The information for 0 month, 1 month, 12 months and 24 months are shown in Table 6.

Table 6 Stability data for Water soluble chloride (as NaCl) (g/100g)

	Month (x)	**Water soluble chloride (as NaCl) (g/100g)**	**n**
	0	2.342	2
	1	2.342	2
	12	2.340	2
	24	2.390	2
average	9.25	2.354	10

Table 7 Calculation from stability data

Month (x)	NaCl (y)	$(x_i-\bar{x})$	$(y_i-\bar{y})$	$(x_i-\bar{x})(y_i-\bar{y})$	$(x_i-\bar{x})^2$	$(y_i-(b_0-b_1 x_i)$	$(y_i-(b_0-b_1 x_i)^2$
0	2.342	-9.25	-0.012	0.111	85.5625	0.0065	0.000042
1	2.342	-8.25	-0.012	0.099	68.0625	0.0045	0.000020
12	2.340	2.75	-0.014	-0.039	7.5625	-0.0195	0.000380
24	2.390	14.75	0.036	0.531	217.5625	0.0065	0.000042

$$b_1 = \frac{\sum_{i=1}^{n}(x_i - \bar{x})(y_i - \bar{y})}{\sum_{i=1}^{n}(x_i - \bar{x})^2} = 0.002$$

$$b_0 = \bar{y} - b_1\bar{x} = 2.354 - (0.002\times 9.25) = 2.3355$$

$$s^2 = \frac{\sum_{i=1}^{n}(y_i - b_0 - b_1 x_i)^2}{n-2} = 0.0002425$$

$$s(b_1) = \frac{s}{\sqrt{\sum_{i=1}^{n}(x_i - \bar{x})^2}} = 0.00080$$

$$u_{lts} = s(b_1)\times t = 0.00080\times 24 = 0.020$$

$$t_{0.95,n-2}.\, s(b_1) = t_{0.95,8}\times 0.00080 = 2.31\times 0.00080 = 0.0018$$

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

So the slope is insignificant. As a consequence, no instability was observed.

3.4 Expanded uncertainty for the CRM: water soluble chloride (as NaCl) (g/100g)

The expanded uncertainty of the CRM is estimated by combining the contributions of characterizations, homogeneity and stability to the overall uncertainty associated with the property values:

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

$$U_{CRM} = 2 * \sqrt{0.025^2 + 0.0000036^2 + 0^2 + 0.020^2} = 0.064 \text{ g/100g}$$

The certificate of reference material is shown below

RM Report No. XXXX/2016

Center for Laboratory Proficiency Testing
Department of Science Service

Certification of Reference Material

Water - soluble chlorides (as NaCl) in Feeding stuffs

Expired Date : December 2016

Contents : Water - soluble chlorides (as NaCl) in Feeding stuffs (fish feed).

Stability : The original unopened container can be used until December 2016.

Storage : The reference material should be kept in the original packaging and stored at room temperature until testing commences.

This reference material was produced from the proficiency testing round (PTFF - FA03 - 1401) which are complied with ISO/IEC 17043, ISO Guide 34 and ISO Guide 35.

The reference material is intended to be used for method validation and quality control.

ASSIGNED VALUE AND CORRESPONDING STATISTICAL DATA

RMFF - FA03 - 1401

Test items	No. of results	Assigned Value	Measurement uncertainty	Standard deviation
Water - soluble chlorides (as NaCl) (g/100g)	43	2.292	0.064	0.130

Notes : 1. The assigned value is the robust average from the data of participation laboratories in PT scheme : Water - soluble chlorides (as NaCl) in Feeding stuffs (PTFF - FA03 - 1401).

2. The standard deviation is the target standard deviation from the standard method ISO 6495 : 1999.

3. Measurement uncertainty is the expanded uncertainty at confidence level 95% (k=2) and derived based on ISO Guide 35 : 2006.

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Approved by

(Mrs. Rachada Hemapattawee)
Director of Center for Laboratory Proficiency Testing
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4. Conclusion

The reference material is intended to be used for method validation, method verification and laboratory quality control. Target range for the reference material is set at 2.00 – 2.50 g/100g. The target measurement uncertainty for the reference material should not be more than 0.10 g/100g. The result show that the assigned value for the reference material is 2.292 g/100g and the measurement uncertainty is 0.064 g/100g at 95% confidence level (k=2) which are fit for the target setting.

5. Acknowledgement

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6. Reference

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