Thailand Reference Material (TRM-F-2002) for Analysis of Trace and Essential Elements in Prawn

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Introduction

Thailand is the world's leading exporter of seafood products, especially prawns.

Obstacles: Competitions, Regulations, Policies, Trade barriers and Lack of confidence in the certificates of seafood products

Testing laboratories are not used certified reference materials (CRM) for validation and quality control of analytical methods.

The main reasons CRM does not bring to use are high cost, improper analyte composition and matrix.

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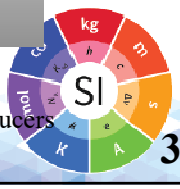
Thailand Reference Material (TRM)

TRM is a CRM issued by NIMT and its designated institute.
- ✓ **According to ISO 17034 ensuring their consistent quality**
- ✓ **Production with a well-defined traceability linkage to SI unit (kg).**

TRM-F-2002 Trace and Essential Elements in Prawn

Cadmium (Cd)	Copper (Cu)	Lead (Pb)	Zinc (Zn)



ISO 17034:2016 General requirements for the competence of reference material producers

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TRM-F-2002

Elements

Total malachite green

Raw materials:
Prawn from Thai local market

- **Preliminary studied (Cd, Cu, Pb, Zn contents)**
- **Ground and spiked standard (Cd, Cu, Pb, Zn) mixture**

Blended, Freeze-dried, Ground, Sieved , Homogenized
Pre-homogeneity testing result: RSD ≤ 3%

Placed into amber glass bottles

- **Homogeneity testing**
- **Stability testing**
- **Moisture content**
- **Assigned value + uncertainty**

Accuracy and validation:
TORT-2 Lobster hepatopancreas marine CRM for trace metals (NRC, Canada)

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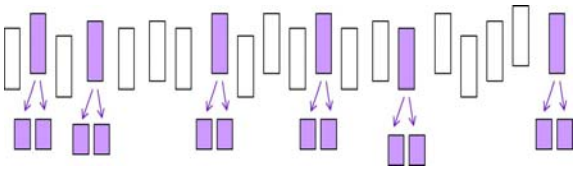
Homogeneity testing

- Random selection: 10 bottles
- 2 sub-samples for each bottle
- Sampling weight : 0.2 g
- Method: External calibration ICP-MS
- Criteria:



Within-sample variation	Between-sample variation
Cochran's maximum range test	ANOVA F-test

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Homogeneity testing

*at 95 % confidence level

Within-sample variation	Between-sample variation
Cochran's maximum range test*	ANOVA F-test*
Criteria: Ratio $C = \frac{D^2_{max}}{\Sigma D_i^2}$ $C_{max} < C_{crit}$	Criteria: $F_{statistics} < F_{critical}$
good homogeneity in each bottle	good homogeneity between bottles

Candidate TRM:

- ✓ Sufficiently homogeneous
- ✓ Minimum sampling weight is 0.2 g
  (to ensure no effect from sample inhomogeneity)

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D_{max} the maximum difference of the duplicate D_i difference of each pair of duplicates

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Stability testing

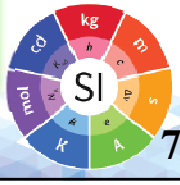
1. Short-term stability (the stability of the material during its transport)

- **APMP.QM-S5** 政府化驗所 Government Laboratory

  Essential and Toxic Elements in Seafood (Prawn)

- **Storage condition: 20, 40 and 50 ºC**
- **Sampling point: 1, 2 and 4 weeks**
- **Criteria: ISO Guide 35:2006**

No instability was observed at 20, 40 and 50 ºC during the testing period



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Stability testing

Elements

Total malachite green

2. Long-term stability (the behavior of the test material under storage in laboratory)

- **Storage condition: -20 and 25 ºC**
- **Analyze in 3 randomly bottles at specified periods**
- **Method: Exact matching double isotope dilution ICP-MS**
- **Criteria:**

Application Note 1
Comparison of a measurement result with the certified value
ERM European Reference Materials

$$|c_m - c_{CRM}| \leq 2\sqrt{u_m^2 + u_{CRM}^2}$$

c_m mean measured value
c_{CRM} certified value
u_m uncertainty of the measurement result
u_{CRM} uncertainty of the certified value

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Stability testing

$$|c_m - c_{CRM}| \leq 2\sqrt{u_m^2 + u_{CRM}^2}$$

At 95% confidence level, no significant difference between the measurement result (C_m) and the certified value (C_{CRM})

Long-term stability

Element	$\lvert c_m - c_{CRM} \rvert$		$2\sqrt{u_m^2 + u_{CRM}^2}$	Stability observed at 26 months
Cd	0.04	<	0.28	✓
Cu	1.29	<	2.86	✓
Pb	0.10	<	0.30	✓
Zn	0.65	<	7.10	✓

The candidate TRM was stable throughout the study period.

✓ Homogeneity ✓ Stability
The candidate TRM was considered fit for the purpose of TRM development.

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Moisture content

"to know its moisture content at the time of analysis to allow analyte mass fractions to be corrected to dry mass basis"

3 separate portions (0.5 g each) of the sample

placed over anhydrous calcium sulphate (DRIERITE®) in a desiccators at room temperature

minimum of 10 days until reaching a constant mass

The moisture content of the candidate TRM was approximately 4.9%.

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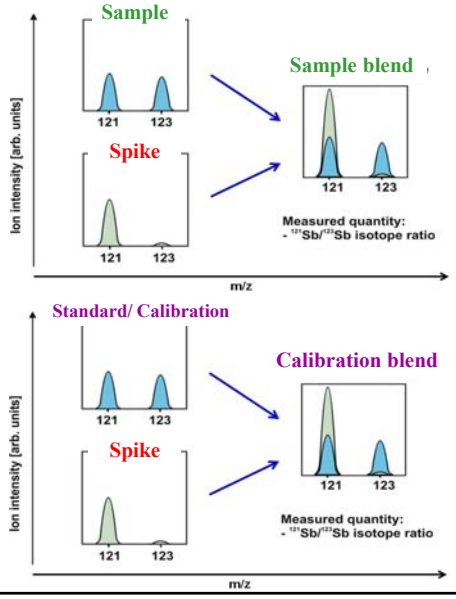
Assigned value: Exact matching double isotope dilution mass spectrometry (IDMS)



a known amount of isotope (or 'spike') of the element of interest is added to a known amount of sample and standard. The isotope ratio of the sample blend and calibration blend are measured by mass spectrometry, and the concentration of the element of interest is calculated.

Spike: ☑ **Chemically identical to the analyte**

☑ **Physically distinguished by mass**

Sample blend vs Calibration blend

- ✓ **match isotope ratio**
- ✓ **match ion intensities**

Figure is adapted from https://www.researchgate.net/ publication/ 225942041 "Isotope Dilution_Mass_Spectrometry_A_Primary_Method_of_Measurement_and_Its_Role_for_RM_Certification

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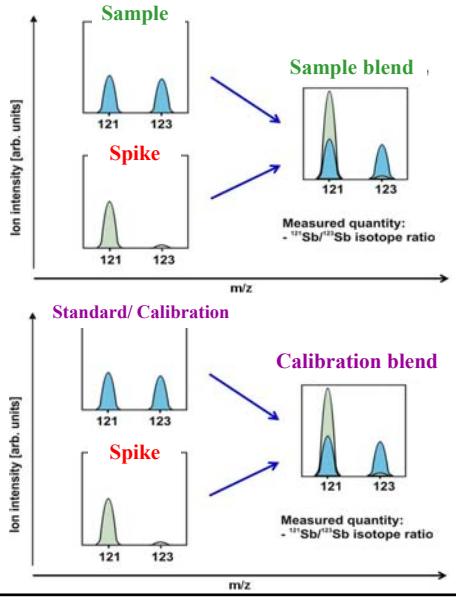
Assigned value: Exact matching double isotope dilution mass spectrometry (IDMS)



$$W_X = W_{Zc}\frac{M_Y M_{Zc}}{M_X M_{Yc}}\frac{R'_B}{R'_{Bc}}$$

(W_X : mass fraction of element in the sample)

- ✓ **High accuracy method**
  (SI unit: kg)
- ✓ **Minimize the effect of systematic error**
  - ➢ **Sample preparation**
  - ➢ **Matrix effects**
  - ➢ **Instrument drift**
- × **High cost (time, money, resources)**

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Uncertainty evaluation

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

$$U_{CRM} = 2 \times u_{CRM}$$

(ISO Guide 35)

- u_{CRM} standard uncertainty of the CRM
- u_{char} standard uncertainty due to characterization
  Analyzed 6 bottles and used Exact matching double IDMS
- u_{homo} standard uncertainty due to homogeneity
- u_{lts} standard uncertainty due to long-term stability
- U_{CRM} expanded uncertainty of the CRM

The expanded uncertainties were estimated using coverage factor k=2, corresponding to an estimated confidence interval of approximately 95 %.

ISO Guide 35 Reference materials-Guidance for characterization and assessment of homogeneity and stability

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Uncertainty budget

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

$$U_{CRM} = 2 \times u_{CRM}$$

(ISO Guide 35)

Element	u_{char}	u_{homo}	u_{lts}	u_{CRM}	U_{CRM} (95%CI)
Cd	0.03	0.03	0.03	0.05	0.11
Cu	0.53	0.41	0.24	0.72	2
Pb	0.02	0.04	0.02	0.05	0.10
Zn	0.87	1.38	0.92	1.87	4

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Certified values

TRM-F-2002 Trace and Essential Elements in Prawn		
Parameter	Mass fraction (mg/kg)	Expanded uncertainty (mg/kg)
Cadmium (Cd)	2.05	0.11
Copper (Cu)	49	2
Lead (Pb)	1.80	0.10
Zinc (Zn)	81	4

The expanded uncertainties were estimated using coverage factor k=2, Corresponding to an estimated confidence interval of approximately 95 %.

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Conclusions

TRM-F-2002 Trace and Essential Elements in Prawn

- **TRM-F-2002 is now disseminated together with its certification from NIMT. (www.nimt.or.th/etrm/en/)**
- **TRM was certified in compliance with ISO 17034 by an ISO/IEC 17025:2005 accredited laboratory.**
- **TRM will be of interest to testing laboratories requiring development, validation and quality assurance of measurements for elemental analysis in prawn tissue, marine biota and similar sample type.**

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NIMT

Chemical Metrology and Biometry Department (NIMT)

Inorganic analysis group	Organic analysis group
Trace and essential elements	**Total malachite green**
Pranee P.	Kittiya S.
Nattikarn O.	Nittaya S.
Sutthinun T.	Pornhathai K.
Usana T.	Preeyaporn P.