Development of CRMs for Melamine Determination in Milk Powder and Food

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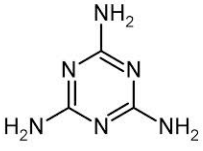
Introduction

- Melamine (1,3 ,5-triazine–2,4,6-triamine, $C_3H_6N_6$) was first synthesized in 1834 and had later been found to have extensive uses in the industry
- With high nitrogen content in the melamine molecule, the chemical could be unethically added to food products in order to increase the apparent protein content
- Melamine incidents that were due to tainted pet food in the United States in 2007 and tainted milk powder in China in 2008 have caused significant impact to the analytical chemistry world
- After the crises, the analysis of melamine becomes one of the routine measurements for food and laboratory testing. A maximum residue limit (MRL) of 1 mg/kg was set up for melamine in milk and 2.5 mg/kg for food



Chemical Formula: $C_3H_6N_6O_8$
Average Mass: 126.12 g/mol
CAS number: 108-78-1
IUPAC name: 1,3,5 -triazine triazine –2,4,6 –triamine

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Introduction (Cont'd)

Toxicity of melamine

Melamine is not metabolized in human body and is rapidly eliminated in the urine. Thus this compound has a low acute toxicity. However, long term of high dose exposure can cause the formation of bladder stones and crystals in the urine.

Thai Food and Drug Administration (Thai FDA) has set a regulation limits of melamine contamination in milk and related food as

- less than 1mg/kg for all kinds of milk powder
- less than 2.5 mg/kg for dairy products and related food

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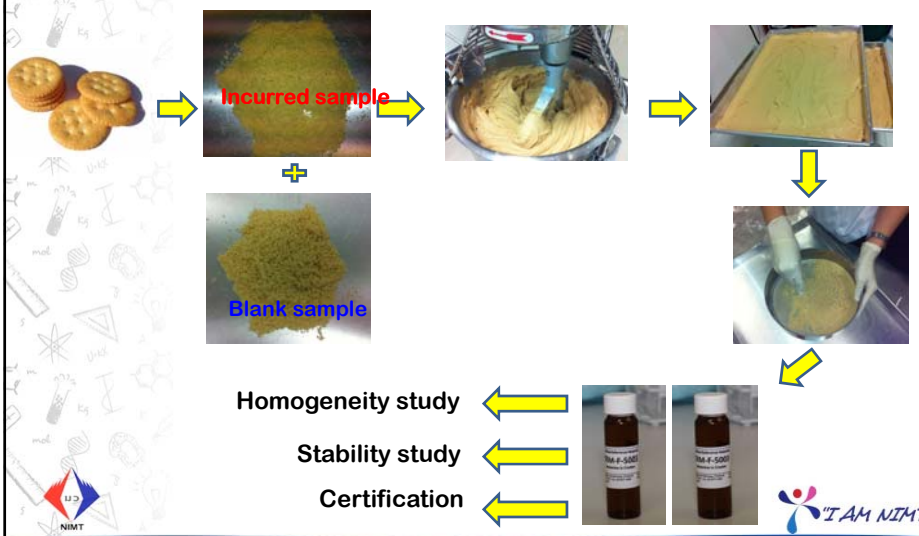
Preparation of Candidate CRM: Melamine in Cracker



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Preparation of Candidate CRM:
Melamine in Milk Powder

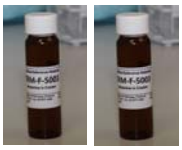
Blank sample

methanol

Homogeneity study

Stability study

Certification



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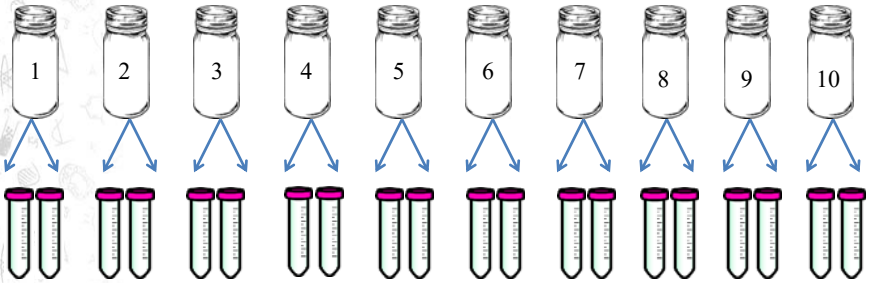
Homogeneity study



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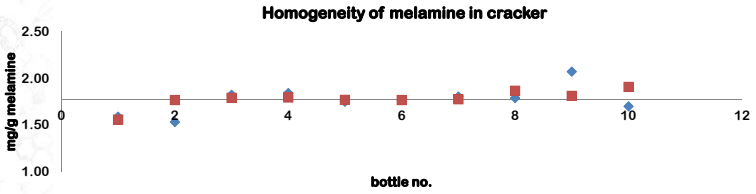
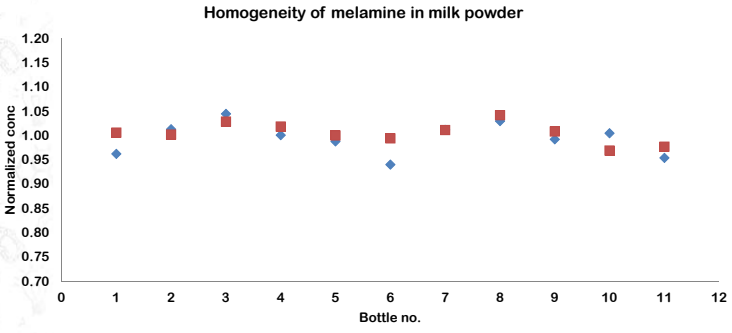
Homogeneity Study



ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.000927	1	0.000927	0.061545	0.806879	4.413873
Within Groups	0.271059	18	0.015059			
Total	0.271985	19				

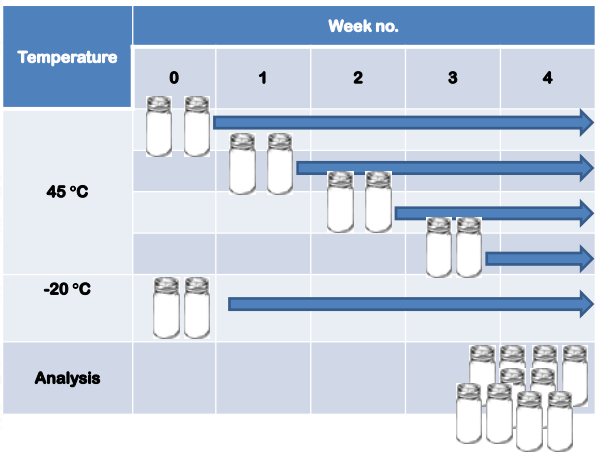
Homogeneity Study (Cont'd)



ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.00028	1	0.00028	0.352	0.560	4.414
Within Groups	0.01415	18	0.00079			
Total	0.01442	19				

Short-term stability-isochronous scheme



Analyze all samples under repeatability conditions

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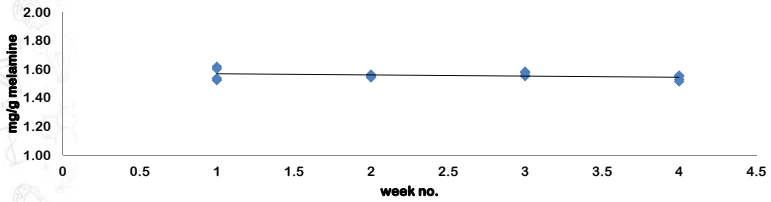
Short-term stability study

Stability of melamine in cracker at 45 oC



Statistical parameter	Value
Slope (b), µg/g	-14.7515
Standard error of slope (s_b), µg/g/week	10.42588
Degree of freedom	14
$t_{calc} = \frac{\lvert b \rvert}{s_b}$	1.4149
t_{crit} = t(0.05, 14)	2.145
Statistical significance at 95% CI	No

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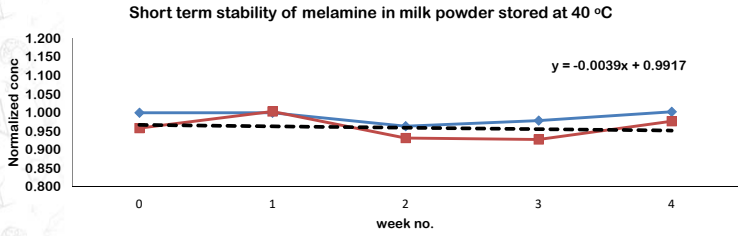
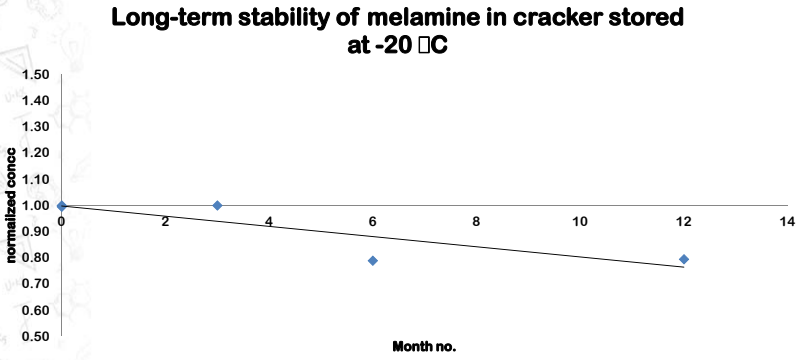
Short-term stability study (Cont'd)

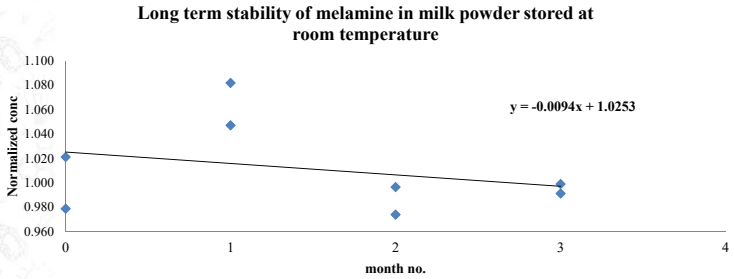


Statistical parameter	Value
Slope (b), µg/g	-0.0039
Standard error of slope (s_b), µg/g/week	0.0067
Degree of freedom	8
t_{calc} =	0.58
t_{crit} = t(0.05, 8)	2.306
Statistical significance at 95% CI	No

Long-term stability study



Long-term stability study (Cont'd)



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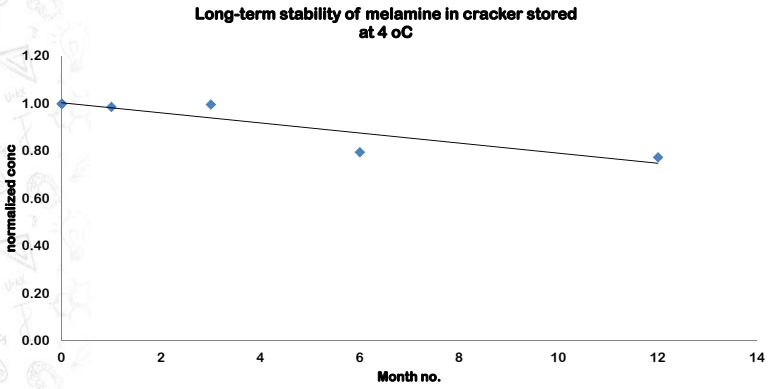
Long-term stability study



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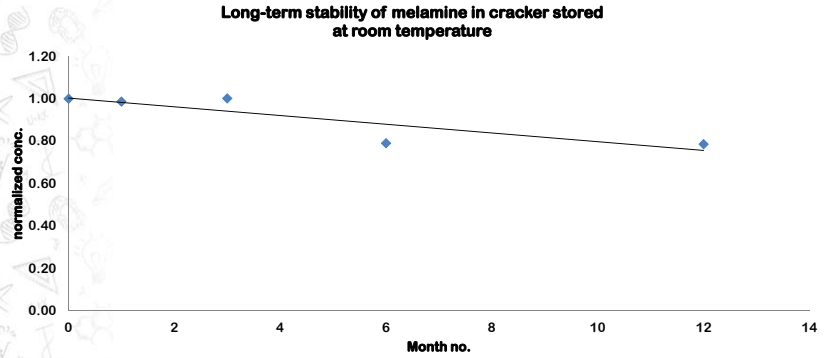
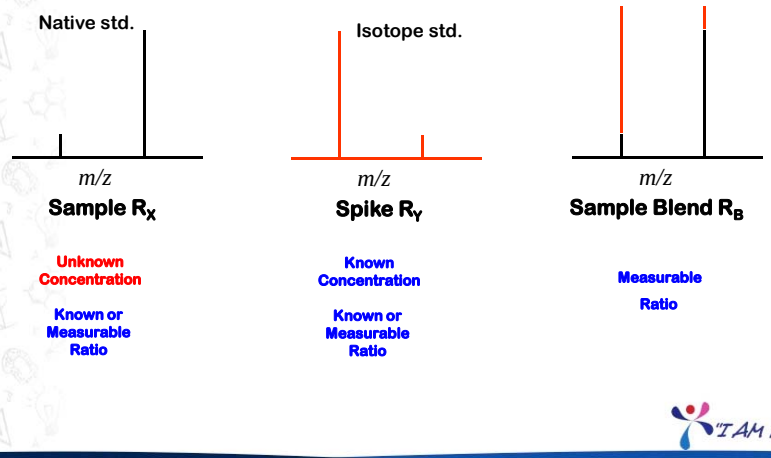
Long-term stability study



Long-term stability study

Melamine Storage temp	Student's t (tcrit)	Parameters from regression analysis Slope, abs (b1)	 Error of slope s(b1)	tcalc	Interpretation
-20 °C	2.3646	0.0379	0.1288	0.294	stability was observed
4 °C	2.2622	0.0426	0.0999	0.426	stability was observed
Room Temp	2.2622	0.0404	0.1066	0.379	stability was observed

Isotope Dilution Mass Spectrometry (IDMS)



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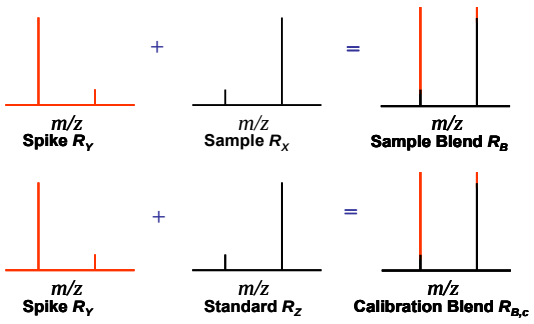
Assigning values
Isotope Dilution Mass Spectrometry (IDMS)



$$w_X = w_Z \cdot \frac{m_Y . m_{Z,c}}{m_X . m_{Y,c}} \cdot \frac{R_Y - R'_B}{R'_B - R_X} \cdot \frac{R'_{B,c} - R_Z}{R_Y - R'_{B,c}}$$

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Assigning values

- Modified AOAC method, 990.33, 49.2.17 (1996) with IDMS technique were applied to characterize property values of the CRM

$$w_X = \frac{w_Z}{} \cdot \frac{m_Y \cdot m_{Z,c}}{m_X \cdot m_{Y,c}} \cdot \frac{R'_B}{R'_{B,c}}$$

w_X, w_Z = mass fraction of analyte in the sample and the calibration standard
m_Y = mass of labelled solution added in the sample blend
$m_{Y,c}$ = mass of labelled standard solution added in the calibration blend
m_X = mass of sample in the sample blend
$m_{Z,c}$ = mass of standard added in the calibration blend
R'_B, R'_{BC} = peak area ratio in the sample and calibration blend

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Measurement uncertainty

Uncertainty budget for melamine in cracker

Factor	Value	Uncertainty	Relative Uncertainty
	x	u(x)	u(x)/(x)
Measurement equation factor			
Method Precision	1	0.02321	2.32%
m_{zc}	0.14526	0.000071	0.05%
m_y	0.15513	0.000071	0.05%
m_{yc}	0.15457	0.000071	0.05%
m_x	1.00513	0.000071	0.01%
w_z	10.1968	0.110669	1.09%
Additional Factor			
Homogeneity	1	0.007	0.70%
Long term stability	1	0.03	3.00%
Dry mass	1	0.0004	0.0004
Extraction effects	1	0.01	1.00%
Interference from two different ion pairs	1	0.0054	0.54%

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Measurement uncertainty

Uncertainty Analysis Results		
Wx =	1.563	mg/kg
u(x) =	0.065	mg/kg
u(x)/x =	4.17%	
Veff(total) =	129.809	
k =	1.98	(@ 95% level)
U(x) =	0.129	
%U(x) =	8.24%	

Certified value for melamine in cracker = 1.56 ± 0.13 mg/kg

Conclusions

1. Two matrix CRMs were prepared from incurred and spiked samples in accordance with ISO 17034.
2. Certified values are based on exact matching isotope Dilution mass Spectrometry (IDMS) technique.
3. Certified values for melamine in milk powder and melamine in cracker are 1.03 ± 0.16 mg/kg and 1.56 ± 0.13 mg/kg, respectively.
4. These CRMs can be used for method validation or as a quality control sample


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