

Production and Certification of Certified Reference Materials for Veterinary Drugs in Food and Feed

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Outline:

- Introduction
- Malachite green in prawn-CRM
- Clenbuterol and salbutamol in feed-CRM
- Other activities

Veterinary drug

- Any substance applied or administered to any food-producing animal, such as meat or milk producing animals, poultry, fish or bees, whether used for therapeutic, prophylactic, or diagnostic purposes, or for modification of physiological functions or behavior. **(Definitions adopted by the Codex Alimentarius Commission - Codex Procedural Manual).**
- Vet drugs include antibiotics, antimicrobial agents, antihistamine, hormone, anti-inflammatory, steroid etc.
- These drugs improve the rate of weight gain, feed efficiency, prevent and treat diseases in food producing animals.
- Drugs residues might be due to improper drug usage and failure to apply the withdrawal period.

Veterinary drug residues

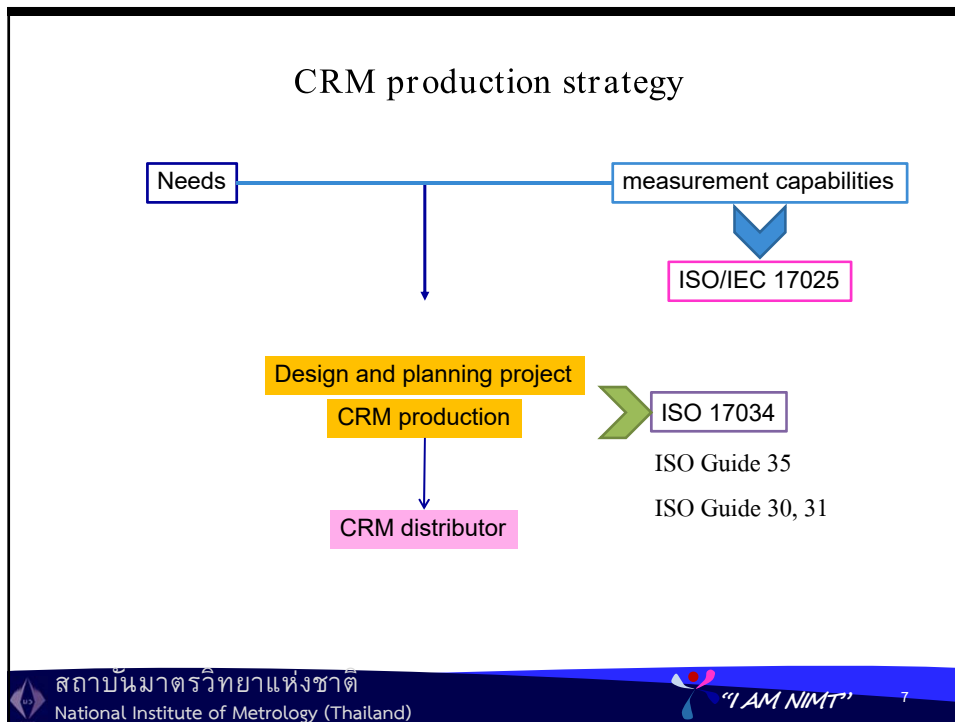


Veterinary drug- risk of adverse effects

- Development of antimicrobial drug resistance
- Hypersensitivity reaction in consumer
- Carcinogenicity, mutagenicity, teratogenicity
- Disruption of intestinal normal flora
- Some vet drugs are banned from use in food producing animals
- Accurate measurement results of the drug residues are needed

Matrix CRMs

- Method validation, quality control purposes
- Not many matrix certified reference materials of vet drugs are available
- Need matrix certified reference materials for accurate measurement results



Interlaboratory comparison on Veterinary drugs

Description	Range	References
Mass fraction of chloramphenicol as residue in milk	0.2-1.0 µg/kg	CCQM-P90 (2007)
Malachite green in fish tissue	MG 0.5-5 µg/kg LMG 3-10 µg/kg	CCQM-P88 (2007)
Chloramphenicol in pig muscle	0.1 – 1 ng/g	CCQM-K81 (2009)
Malachite green in fish tissue	MG 0.5 – 5 µg/kg, LMG 3 – 10 µg/kg	CCQM-K85 (2011)
Clenbuterol in porcine meat	1-50 µg/kg	APMP.QM-S6 (2013)
Enrofloxacin and sulfadiazine in bovine tissue	EFX 20-200µg/kg SDZ 500-5000 µg/kg	CCQM-K141 (2016)

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64 CMCs in Chemistry

BIPM
Bureau International des Poids & Mesures

Home | Key and supplementary comparisons | Calibration and Measurement Capabilities - CMCs

KCDB home > Free search results

The BIPM key comparison database

Refine your search: **CMC AREA** (64) | **CMCs Chemistry** (64)

Result of the search: Your query 'NIMT' produced 64 results

Thailand, NIMT (National Institute of Metrology (Thailand))
Complete CMCs in Chemistry for Fuels for Thailand (.PDF file)

Matrix or material	Analyte or component	Dissemination range of measurement capability	
		Mass fraction in mg/kg	Relative expanded uncertainty (k = 2, 95%) in %
alcohol and gasohol	copper	0.05 to 1.0	3.0 to 4.0

Mechanism(s) for measurement service delivery: Provision of reference values for both in-house and external PT/RM samples
Uncertainty convention 1.
Approved on 13 June 2013.
Internal NIMT service identifier: NIMT/12021-60504

Thailand, NIMT (National Institute of Metrology (Thailand))
Complete CMCs in Chemistry for Fuels for Thailand (.PDF file)

Matrix or material	Analyte or component	Dissemination range of measurement capability	
		Mass fraction in mg/kg	Relative expanded uncertainty (k = 2, 95%) in %
biodiesel fuel	magnesium	0.5 to 10	3.5 to 4

Mechanism(s) for measurement service delivery: Provision of reference values for both PT and RM samples
Uncertainty convention 1.

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6 CMCs for organic analysis

Calibration and Measurement Capabilities

Amount of substance, Food, Thailand, NIMT (National Institute of Metrology (Thailand))

In the case where an uncertainty range is given, the expanded uncertainty range spans from the smallest numerical value of the uncertainty to the largest numerical value of the uncertainty found within the quantity range.

NMI Service Identifier	Measurement Service Sub-Category	Matrix	Measurand		Dissemination Range of Measurement Capability			Range of Expanded Uncertainties as Disseminated					Mechanism(s) for Measurement Service Delivery	Comments	
			Analyte or Component	Quantity	From	To	Unit	From	To	Unit	Coverage Factor	Level of Confidence			Is the expanded uncertainty a relative one?
12021-60901	Nutritional constituents	seafood	iron	Mass fraction	50	500	mg/kg	4	5	%	2	95%	Yes	Provision of reference values for both in-house and external PT/RM samples	Approved on 19 December 2013
12021-60211	Nutritional constituents	soybean and soybean powder	magnesium	Mass fraction	10	3000	mg/kg	3.0	3.5	%	2	95%	Yes	Provision of reference values for PT/RM samples	Approved on 19 June 2014
12020-60304	Nutritional constituents	cereal and cereal products	calcium	Mass fraction	1000	30000	mg/kg	3.0	3.5	%	2	95%	Yes	Provision of reference values for both PT and RM samples	Approved on 19 June 2014
12020-60501	Nutritional constituents	cereal and cereal products	copper	Mass fraction	5	100	mg/kg	2.0	2.5	%	2	95%	Yes	Provision of reference values for both PT and RM samples	Approved on 19 June 2014
12010-60107	Contaminants	aquatic farm animal tissue or meat	malachite green	Mass fraction	0.5	20	µg/kg	9	11	%	2	95%	Yes	Provision of reference values for both in-house and external PT/RM samples	Approved on 20 January 2016
12010-60108	Contaminants	aquatic farm animal tissue or meat	leuco-malachite green	Mass fraction	0.5	20	µg/kg	8	10	%	2	95%	Yes	Provision of reference values for both in-house and external PT/RM samples	Approved on 20 January 2016

The BIPM key comparison database, January 2016

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Calibration and Measurement Capabilities

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NMI Service Identifier	Measurement Service Sub-Category	Matrix	Measurand		Dissemination Range of Measurement Capability			Range of Expanded Uncertainties as Disseminated					Mechanism(s) for Measurement Service Delivery	Comments	
			Analyte or Component	Quantity	From	To	Unit	From	To	Unit	Coverage Factor	Level of Confidence			Is the expanded uncertainty a relative one?
12010-60101	Contaminants	aquatic farm animal tissue or meat	total malachite green	Mass fraction	1	40	µg/kg	6.2	8	%	2	95%	Yes	Provision of reference values for both in-house and external PT/RM samples	Approved on 20 January 2016
12010-60103	Contaminants	aquatic farm animal tissue or meat	chloramphenicol	Mass fraction	0.1	10	µg/kg	7	9	%	2	95%	Yes	Provision of reference values for both in-house and external PT/RM samples	Approved on 20 January 2016
12010-60102	Contaminants	milk powder and dairy products	melamine	Mass fraction	0.1	5	mg/kg	5.5	7.5	%	2	95%	Yes	Provision of reference values for both in-house and external PT/RM samples	Approved on 20 January 2016
12010-60106	Contaminants	porcine meat, beef, animal feed	clenbuterol	Mass fraction	1	50	µg/kg	5.0	7.0	%	2	95%	Yes	Provision of reference values for both in-house and external PT/RM samples	Approved on 20 January 2016
12021-60211	Contaminants	cereal and cereal products	cadmium	Mass fraction	0.1	0.5	mg/kg	4.6	5.0	%	2	95%	Yes	Provision of reference values for both in-house and external PT/RM samples	Approved on 20 January 2016
12021-60105	Contaminants	cereal and cereal products	arsenic	Mass fraction	0.05	0.5	mg/kg	7.0	7.5	%	2	95%	Yes	Provision of reference values for both in-house and external PT/RM samples	Approved on 20 January 2016

The BIPM key comparison database, January 2016

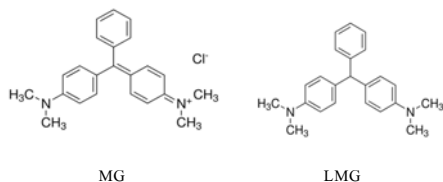
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Our Network



- Worked with Chachoengsao Coastal Fisheries Research and Development Center, under ministry of agriculture and cooperatives
- Prepared incurred samples of antibiotic drugs in prawn at the field
- 4 nitrofurantoin parent drugs, tetracycline, quinolone, chloramphenicol, malachite green) in prawn were produced
- Matrix CRM for total malachite green in prawn was prepared

Total MG in prawn CRM



- Incurred CRM
- Freeze dried form
- 5 g

Our Network on veterinary drugs



- Bureau of Quality Control of livestock products (BQCLP), Department of livestock Development (DLD), Thailand
- ASEAN Food Reference Laboratory for veterinary drug residues (AFRLs) since 2004

- Technical training and support
- Provide reference value for the PT

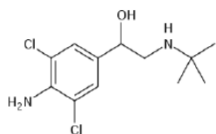
Production of a CRM for determination of Clenbuterol and salbutamol in feed

- In 2013 data survey by the Feed Quality Control Laboratory Division (FQCL), Department of Livestock Development found 128 out of 314 samples (40%) contaminated with beta-agonist in feed at 174.81-39092.74 ppb
- For the sampling samples (beef, chicken, pork) found positive 15 out of 38 samples (45.4%)

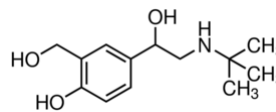


Production of a CRM for determination of Clenbuterol and salbutamol in feed (2015-2016)

- Clenbuterol and salbutamol were selected.



Clenbuterol
Chemical Formula: $C_{12}H_{18}Cl_2N_2O$
Average Mass: 227.19 g/mol
CAS number: 37148-27-9
IUPAC name: 4-amino- α -[(tert-butylamino) methyl]-3,5-dichlorobenzyl alcohol



Salbutamol
Chemical Formula: $C_{13}H_{21}NO_3$
Average Mass: 239.31 g/mol
CAS number: 18559-94-9
IUPAC name: α -[(tert-Butylamino)methyl]-4-hydroxy-m-xylene- α,α' -diol, Albuterol

Production of a CRM for determination of Clenbuterol and salbutamol in feed

Blank feed after blended and sieved at 100 μm



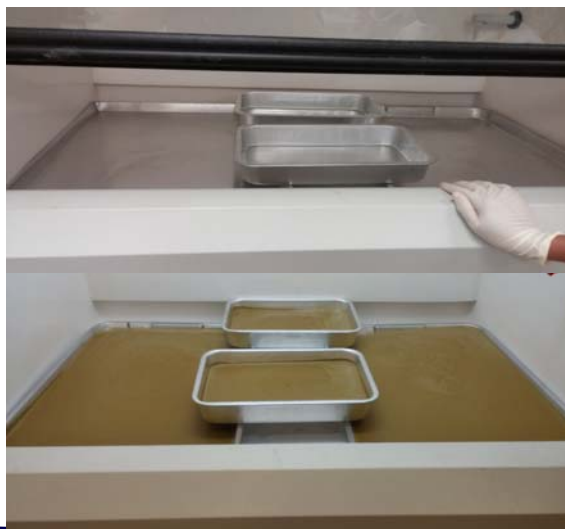
Production of a CRM for determination of Clenbuterol and salbutamol in feed

Prepared spiked material



Production of a CRM for determination of Clenbuterol and salbutamol in feed

- Leave to dry at the fume hood overnight in the dark
- Crush by hand and sieve



Production of a CRM for determination of Clenbuterol and salbutamol in feed



300 bottles
50 g each
Stored at -20 °C

Purity assessment of the pure standards using Mass Balance Method

Purity of salbutamol (%) = 100 % – % impurities



Karl Fischer Titration



HPLC-UV



TGA-MS

- Clenbuterol standard purity = 99.51 % ± 0.17 %
- Salbutamol standard purity = 99.32 % ± 0.13 %
(at the 95 % confidence level, $k = 2$)

Homogeneity testing-results

Analysis of Variance of homogeneity testing results of clenbuterol and salbutamol, at 95% confidence interval

ANOVA: Clenbuterol						
Source of variation	SS	df	MS	F	P-value	F critical
Between groups	12.1303	10	1.213	2.268	0.09767	2.854
Within groups	5.883	11	0.5349			

relative standard uncertainty of u_{bb} , 0.8%

ANOVA: Salbutamol						
Source of variation	SS	df	MS	F	P-value	F critical
Between groups	370.141	10	37.014	1.448	0.2757	2.854
Within groups	281.106	11	25.555			

relative standard uncertainty of u_{bb} , 2.9%

20/1 s_b

Stability testing-results

- Short-term stability testing : transport condition at 40 °C

Statistical parameters	Clenbuterol	Salbutamol
Slope (<i>b</i>), ng/g/month	-0.1348	-2.9089
Standard error of slope (<i>s_b</i>), ng/g/month	0.2646	0.7363
<i>df</i>	3	3
<i>t-cal</i> = <i>b</i> / <i>s_b</i>	0.5097	3.9505
<i>t-crit</i> = <i>t</i> _(0.05, df)	3.182	3.182
Statistical significance at 95% CI	No	Yes

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Assign value of candidate CRMs

Exact-matching double IDMS reference method

$$w_x = F_p \cdot F_E \cdot F_I \cdot w_z \cdot \frac{m_y \cdot m_{zc}}{m_x \cdot m_{yc}} \cdot \frac{R'_b}{R'_{bc}}$$

Where;

- w_x = mass fraction of clenbuterol/salbutamol in feed
- w_z = mass fraction of analyte in the calibration solution used to prepare the calibration blend
- m_y = mass of spike solution added to sample blend
- m_{yc} = mass of spike solution added to calibration blend
- m_{zc} = mass of standard solution added to calibration blend
- m_x = mass of sample added to sample blend
- F_E = extraction efficiency factor
- F_I = interference effect
- F_p = method precision factor
- R'_b and R'_{bc} = observed isotope amount ratios in the sample blend and the calibration blend, respectively

Factor *F* (for F_E , F_p and F_I) are additional factors added in the measurement equation and taken to have a value of unity when calculating the mass fraction.

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Estimation of measurement uncertainty

$$u_{CRM} = \sqrt{u_{charac}^2 + u_{homo}^2 + u_{stb}^2}$$

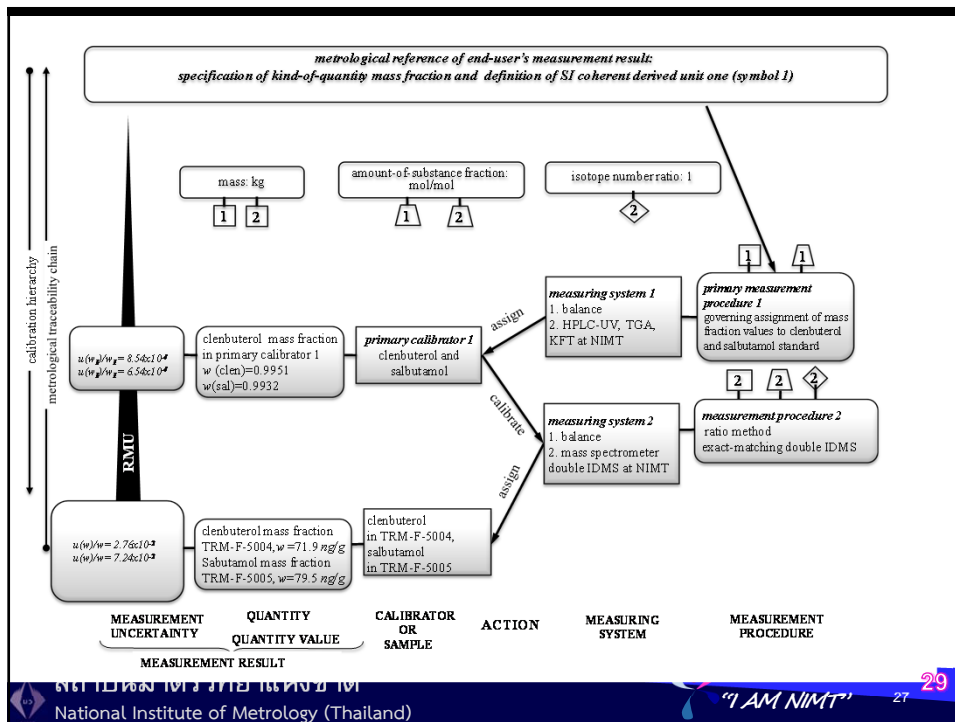
u_{homo} = homogeneity uncertainty

u_{charac} = characterization uncertainty

u_{stb} = stability uncertainty

Metrological Traceability

- The purities of the pure standards were assessed using Mass balance method.
- All standard and sample preparation were gravimetric using calibrated balances.
- The primary method of exact matching double IDMS was employed and it's measurement uncertainty rigorously assessed.



Other activities on veterinary drug

- Provide assign value for proficiency testing schemes
- Malachite green in prawn
- Chloramphenicol in prawn
- Fluoroquinolone group & Quinolone group in fish
- Sulfonamide group in prawn



บริษัท ห้องปฏิบัติการกลาง (ประเทศไทย) จำกัด
CENTRAL LAB THAI

Thailand Reference Material

- Registered Trademark for Thailand Reference Material (TRM) in 2015



Direct sale and distributors



Thank you!

