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Asia Pacific Laboratory Accreditation Cooperation (APLAC) Proficiency Testing Programme (APLAC PT T103)

Determination of Acesulfame Potassium and Sucralose in Cake Mix Flour

FINAL REPORT

A PT Programme Coordinated By:
Chin Poh Yin & Lee Jia Juan, Singapore Accreditation Council
Teo Tang Lin & Cheow Pui Sze, Health Sciences Authority, Singapore

Report Issued By:

Chin Poh Yin, Singapore Accreditation Council

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Summary

1. This proficiency testing (PT) programme was jointly organised by the Singapore Accreditation Council (SAC) and the Chemical Metrology Laboratory (CML) of the Health Sciences Authority (HSA), Singapore. The objective of the programme is to enable participating laboratories to demonstrate their competence in the determination of two commonly used artificial sweeteners in cake mix flour by various analytical techniques.

2. The analytes in the PT programme are acesulfame potassium and sucralose. The participating laboratories were allowed to register their participation in either one or both of the analytes. Each participating laboratory received one packet of the PT sample (about 50 g). In addition, certified reference materials (CRMs) of acesulfame potassium and sucralose (about 250 mg each) were provided to the participating laboratories as calibrants. The laboratories received either one or both CRMs, depending on the analytes that they registered for.

3. A total of 41 laboratories from 32 economies registered for the programme. 40 Participating laboratories registered for acesulfame potassium and 38 returned their results obtained using the provided CRM. 21 Participating laboratories registered for sucralose and 17 returned the results obtained using the provided CRM.

4. The participating laboratories' performance was primarily assessed using the z-scores calculated from results obtained using the provided CRMs. The values determined by HSA CML were used as the assigned values for the APLAC PT T103 programme. The robust standard deviation of the results reported by all participating laboratories was used as the standard deviation for proficiency assessment (σ_{PT}). The distribution of z-scores of the participating laboratories is summarised as follows:

z-Scores	Number of Participating Laboratories (Percentage in parenthesis)	
	Acesulfame Potassium	Sucralose
$\|z\| \leq 2.0$	30 (79.0%)	17 (100.0%)
$2.0 < \|z\| < 3.0$	4 (10.5%)	0 (0.0%)
$\|z\| \geq 3.0$	4 (10.5%)	0 (0.0%)
Total	38 (100.0%)	17 (100.0%)

1.0 Introduction

Artificial sweeteners are extensively used to substitute sugar as excessive consumption of sugar would lead to obesity and the possible development of other health conditions. However, animal studies had shown that artificial sweeteners can also cause headaches, respiratory difficulties, seizures, bladder cancer and other health hazards. Hence, regulatory controls on the maximum amount of some artificial sweeteners in food are put in place and their concentration levels are routinely analysed in food testing laboratories worldwide. Acesulfame potassium and sucralose are two such commonly used artificial sweeteners in confectionary products, e.g. cake mixes.

A proficiency testing (PT) programme (APLAC PT T103) on the determination of acesulfame potassium and sucralose in cake mix flour was coordinated by the Singapore Accreditation Council (SAC) and the Chemical Metrology Laboratory (CML) of the Health Sciences Authority (HSA) [1], Singapore, under the auspices of the Asia Pacific Laboratory Cooperation (APLAC). The programme was approved by the APLAC PT Committee in 2015.

In this PT programme, assigned values traceable to the International System of Units (SI) were used to evaluate the performance of the participating laboratories. These assigned values were determined by HSA CML using high accuracy isotope dilution mass spectrometry (IDMS). In addition, certified reference materials (CRMs) of acesulfame potassium and sucralose were also provided to the participating laboratories for use as calibrants. The PT programme thus enabled participating laboratories to compare their results against metrologically traceable assigned values. In this PT programme, each participating laboratory was randomly assigned with a confidential laboratory code which was used throughout the programme.

[1] HSA is a Designated Institute for chemical metrology in Singapore. One of its designated areas covers food safety. CML HSA is accredited by the SAC as a PT Provider since August 2013, in accordance with the requirements of ISO/IEC 17043:2010.

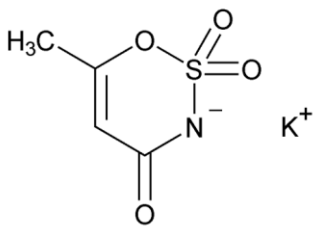
A total of 41 laboratories from 32 economies registered for the programme. 40 Participating laboratories registered for acesulfame potassium, in which 38 returned the results obtained using the provided CRM. 21 Of these laboratories also provided additional result obtained from their own source of standard. Due to an oversight, one laboratory only provided result obtained from its own sources of standard. 21 Participating laboratories registered for sucralose, in which 17 returned the results obtained using the provided CRM. Four of these laboratories also provided additional result obtained from their own sources of standard.

2.0 Objectives

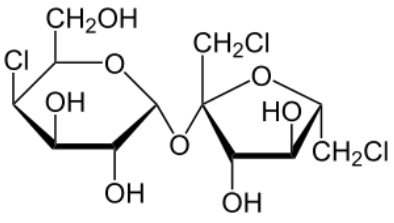
The PT programme aimed to evaluate the participating laboratories' competence in the determination of artificial sweeteners in cake mix flour by comparing their results against metrologically traceable assigned values. The programme also enabled the laboratories to identify problems and opportunities for further improvement.

3.0 The Analytes

The mass fractions (in mg/kg) of acesulfame potassium and sucralose in the cake mx flour were determined by HSA CML. The chemical structures and physical information of the analytes are as shown:



Acesulfame potassium
CAS No.: 55589-62-3
Chemical formula: $C_4H_4KNO_4S$
Molecular mass: 201.24 Da



Sucralose
CAS No.: 56038-13-2
Chemical formula: $C_{12}H_{19}Cl_3O_8$
Molecular mass: 397.64 Da

4.0 The PT Material

A suitable source of 'blank' cake mix flour was obtained from local supermarkets and screened for the presence of acesulfame potassium and sucralose using liquid chromatography coupled with mass spectrometry (LC-MS). The cake mix flour was fortified with the sweeteners to achieve the desired mass fraction values of the two analytes. The fortified cake mix flour was then adequately homogenised by mixing the flour in a drum mixer over a period of 14 days before being distributed into polyethylene pouches under an inert atmosphere and controlled conditions (temperature and humidity). About 140 packets of the PT samples were prepared and stored between 18 °C and 25 °C.

Homogeneity and stability studies for the PT material were carried out in accordance with the procedures described in APPENDICES I and II, respectively. The studies had found the material to be sufficiently homogeneous and stable for use as PT samples for the PT programme.

The participating laboratories were provided with one packet each of the PT sample (about 50 g) and CRM(s) of acesulfame potassium and/or sucralose (about 250 mg each) in a single parcel. The parcels were dispatched by courier on 19 January 2016 under ambient condition. All the recipients received the parcels intact and they were not exposed to temperatures above 40 °C (as indicated by the temperature strip pasted on each parcel).

5.0 Assigned Values and Associated Uncertainties

The assigned values of acesulfame potassium and sucralose in the PT samples were determined by HSA CML using liquid chromatography-isotope dilution mass spectrometric (LC-IDMS) method (APPENDIX III). The assigned values and associated expanded uncertainties at approximately 95% confidence with coverage factor, k = 2, are provided in the following Table:

Table 1

Analytes	Assigned Value ± Expanded Uncertainty[a] (Relative Expanded Uncertainty in parenthesis)
Acesulfame Potassium	1,092 ± 52 mg/kg (4.8%)
Sucralose	825 ± 38 mg/kg (4.6%)

[a] Coverage factor of k=2 at approximately 95% confidence level

The assigned values were traceable to the SI through the use of CRMs of acesulfame potassium and sucralose from HSA CML and reference weights calibrated by the National Metrology Center (NMC), Agency for Science, Technology and Research (A*STAR), Singapore. The associated uncertainties of the assigned values were estimated by including all possible uncertainties in the characterisation (u_{char}), homogeneity (u_{homo}) and stability (u_{stab}) of the PT samples.

6.0 Schedule

Table 2

Period	Events
10 Sep 2015	Call for nomination
2 Oct 2015	Deadline for submission of Nomination Form For Accreditation Body to SAC
15 Oct 2015	Distribution of Registration Form For Participating Laboratory to nominated laboratories
31 Oct 2015	Deadline for submission of Registration Form For Participating Laboratory to SAC
19 Jan 2016	Dispatch of PT packages to Accreditation Bodies (ABs)/participating laboratories
29 Apr 2016	Deadline for submission of Result Proforma
13 Jun 2016	Distribution of Interim Reports to ABs and participating laboratories, and collation of feedbacks/complaints/appeal
Sep/Oct 2016	Issue of Final Report for approval by APLAC Proficiency Testing Committee

7.0 Nominating Accreditation Bodies, Number of Nominated Laboratories and Economies

Table 3

No.	Nominating Accreditation Body	Economy	No. of Nominated Laboratories
1	National Association of Testing Authorities (NATA)	Australia	1
2	Standards Council of Canada (SCC)	Canada	1
3	China National Accreditation Service (CNAS)	China	1
4	Organismo Nacional de Acreditación de Colombia (ONAC)	Colombia	1
5	Czech Accreditation Institute (CAI)	Czech Republic	1
6	Organismo Salvadoreño de Acreditación	El Salvador	1
7	Estonian Accreditation centre (EAK)	Estonia	1
8	Comité français d'accréditation (COFRAC)	France	1
9	Deutsche Akkreditierungsstelle GmbH (DAkkS)	Germany	1
10	Hellenic Accreditation System (ESYD)	Greece	1
11	Hong Kong Accreditation Service (HKAS)	Hong Kong SAR, China	1
12	Komite Akreditasi Nasional (KAN)	Indonesia	2
13	L'Ente Italiano di Accreditamento (ACCREDIA)	Italy	1
14	Japan Accreditation Board (JAB)	Japan	1
15	Lithuanian National Accreditation Bureau (LA)	Lithuania	1
16	Institute for Accreditation of the Republic of Macedonia (IARM)	Macedonia	1
17	Philippine Accreditation Bureau (PAB)	Philippines	2
18	Polish Centre for Accreditation (PCA)	Poland	
19	Portuguese Accreditation Institute (IPAC)	Portugal	1
20	Federal Antimonopoly Service (FAS)	Russia	2
21	Accreditation Body of Serbia (ATS)	Serbia	1
22	Singapore Accreditation Council (SAC)	Singapore	4
23	Spanish Accreditation Body (ENAC)	Spain	1
24	Sudanese Standards & Metrology Organization (SSMO)	Sudan	1
25	Swiss Accreditation Body (SAS)	Switzerland	1
26	Taiwan Accreditation Foundation (TAF)	Taiwan	2
27	Turkish Accreditation Agency (TÜRKAK)	Turkey	1
28	Dubai Accreditation Centre (DAC)	United Arab Emirates	1
29	United Kingdom Accreditation Service (UKAS)	United Kingdom	1
30	ANSI-ASQ National Accreditation Board (ANAB)	United States	1
31	Bureau of Accreditation (BoA)	Vietnam	2
32	International Accreditation Service (IAS)	Saudi Arabia	2#
	Total	**32**	**41**

The laboratories nominated by IAS are based in Saudi Arabia.

8.0 Participating Laboratories' Data

8.1 Acesulfame Potassium

The Table below summarises the participating laboratories' results obtained using the acesulfame potassium CRM provided:

Table 4

Lab Code	Overall Result (mg/kg)	No. of Subsamples	u(x) (mg/kg)	Coverage Factor	Level of Confidence (%)	U(X) (mg/kg)
3	1053.85	7	-	-	35	-
4	1149.3	4	63.2	2	95	126.4
7	1055	8	72.8	2	95	146.65
9	1050	6	13	2	95	26
11	1129	-	14.9	2	95	29.8
12	1128	8	11.3	2	95	22.6
14	1085.32	6	25.02	2	95	50.04
16	887.8	2	-	-	-	-
18	1038	4	19.75	2	95	39
19	839.25	2	-	2	95	16.64
20	986.14	3	55	2	95	110
23	1103.99	3	21.00	2	95	42.00
25	1064	5	66.5	2	95	133
29	1075	3	8	2	95	16
31	1013	3	-	-	-	161.9
34	1038	3	53	2	95	31
37	1026.6	3	3.8	2	95	7.5
38	1133	3	67	2	95	130
41	1270.37	[a]	1.22	2	95	19
42	992.82	[a]	27.52	2	95	55.04
46	1071	3	75	2	95	150
50	1047	6	86	2	95	20
53	1060	3	24	2	95	49
57	1120	2	80	2	95	160

60	1061	3	44	2	95	88
63	955.8	3	26.5	2	95	53.0
65	1056.73	3	46.50	2	95	92.99
72	101295	3	3.0%	2	95	6.0
74	1064.5	2	-	-	-	-
77	1035	3	0.39	2	95	0.77
78	911	5	30	2	95	60
81	1093	6	25	2	95	50
85	884	4	29.8	2	95	59.5
88	1111	3	29	2	95	58
90	1091	2	-	-	-	-
94	1030	4	66	2	95	133
97	1008	3	125	2	95	250
98	951.92	[a]	61.87	2	95	123.75

[a] Sample ID was provided instead of the number of subsamples taken

The Table below summarises the participating laboratories' results obtained using their own sources of acesulfame potassium standard:

Table 5

Lab Code	Overall Result (mg/kg)	No. of Subsamples	u(x) (mg/kg)	Coverage Factor	Level of Confidence (%)	U(X) (mg/kg)
7	1023	6	70.6	2	95	142.20
9	1050	6	13	2	95	26
11	971	[a]	12.6	2	95	25.3
23	1088.14	3	22.60	2	95	45.2
25	1127	5	70.5	2	95	141
29	1077	3	10	2	95	19
31	1024	3	–	–	–	163.6
37	940.2	3	3.4	2	95	6.9
42	985.60	[a]	30.16	2	95	60.32
46	1064	3	75	2	95	150
50	1000	10	82	2	95	50
53	1026	3	24	2	95	47

54	1100	5	14	2.776	95	39
60	1042	3	42	2	95	85
65	1024.78	3	45.09	2	95	90.18
72	1107	3	3.0%	2	95	6.0%
77	1029	3	0.39	2	95	0.77
81	1099	6	25	2	95	50
85	882	4	24.2	2	95	48.4
88	1141	3	30	2	95	60
97	1014	3	125	2	95	250

[a] Sample ID was provided instead of the number of subsamples taken

The Table below summarises the information on the participating laboratories' own sources of acesulfame potassium standard:

Table 6

Lab Code	Source	Purity and Unit	U(X) and Unit	Verified Before Use?
7	Sigma-Aldrich, 0454	100%	0%	No
9	Fluka	≥ 99.0%	–	No
11	Sigma Aldrich	99.0%	–	Yes, Linearity R^2 = 0.9999
23	Sigma-Aldrich	100%	No	[a]
25	Supelco	99.9%	0.5%	[b]
29	Wako Pure Chemical Industries	995 mg/g	11.3 mg/g	No
31	Fluka ref 04054	≥ 99%	–	[c]
37	USP	100%	0	No
42	Supelco	99.9%	0.5%	No
46	Standard SUPELCO LC051146V	99.9%	0.5%	No
50	Supelco	99.9%	0.1%	No
53	Fluka	≥ 99%	–	No
54	Supelco Analytical	99.9%	N/A	No
60	LGC Dr Ehrenstorfer	99.0%	0.5%	No
65	Dr. Ehenstofer	99.5	–	–
72	Supelco	99.9%	0.5	[d]

77	TCI A1490	100.3	NA	No
81	Supelco	99.9%	0.5%	No
85	Sigma-Aldrich	≥ 99.0%	–	No
88	Sigma-Aldrich	≥ 99.0%	No information	No
97	Sigma-Aldrich	≥ 99.0%	–	No

[a] No calibration standard was verified, it was take into account the value reported by the certificate.
[b] When a new set of standards is prepared, we check DAD response of the quality control standard of 10mg/L.
[c] Check in using the certificate to open a lot and monitor factors of response and a reference material to each session
[d] Yes inject Multi points for 5 sts's

The Table below summarises the sample sizes and sample treatments carried out for the determination of acesulfame potassium:

Table 7

Lab Code	Sample Size (g)	Extraction Technique	Extraction Solvent/Reagents	Clean-Up
3	5	Liquid-liquid (shaking)	Water	No
4	5	Liquid-liquid (shaking) / Liquid-liquid (ultrasonic) / SPE	Buffer pH 4.5 (formic acid and trimethylamine in water)	Yes (SPE cleanup using Waters Oasis HLB cartridge)
7	5	Liquid-liquid (shaking)	Water	No
9	5.00	Liquid-liquid (ultrasonic)	Water	Purified with Carrez reagents
11	0.5	Liquid-liquid (ultrasonic)	Aquabidest; $K_4[Fe(CN)_6].3H_2O$; $ZnSO_4.7H_2O$	No
12	5.0000	Liquid-liquid (shaking)	Water	Yes; With syringe filter 0.45um PVDF
14	5	In liquid (shaking)/centrifuge/SPE	10mM KH_2PO_4 acidified weakly with H_3PO_4	Yes (over Accell Plus QMA cartridge 500mg elution to >0.2M KH_2PO_4 with 10% ACN)
16	1.00000	[a]	Buffer solution pH 4.5 (0.1% formic acid in water and adjusting to pH 4.5 with triethylamine)	No
18	1 and 2	Liquid-liquid (shaking)	Ultrapure water, Carrez I solution (Potassium Hexacyanoferrate), Carrez II solution (Zinc Sulfate)	No
19	10.1863	Ultrasonic, centrifugation (2000rpm)	MeOH / Water (1:1)	No
20	5	Liquid-liquid (shaking) / Liquid-liquid (ultrasonic)	ACN & deionized water	No
23	0.5	Solid-liquid (shaking + ultrasonic)	Water; ACN; Formic acid	No
25	5.00	Shaking with hot water at magnetic stirrer	Hot water at 60°C; Carrez I and Carrez II solutions, 3mL of each; To remove fat, proteins and starch matters	No
29	5	Liquid-liquid (ultrasonic)	Water	No
31	1	Liquid-liquid (shaking)	Tampon phosphate pH 3.5 + carrez	No

34	5	Liquid-liquid (shaking)	MeOH, water and clearing agents (potassium hexacyanoferrate and zinc acetate)	Yes; filtration
37	5.0	Liquid-liquid	Water	No
38	5	Liquid-liquid (shaking)	Water	No
41	10	Liquid-liquid (over-night)	Water	No
42	5	Liquid-liquid (shaking)	Aquabidest	No
46	5	Ultrasonic	Water	No
50	50	Liquid-liquid (ultrasonic)	Water	Yes; Kareza I, II
53	5	Solid-liquid (Ultrasonic, 40°C)	Water; Carrez I; Carrez II	No
54	1	Solid/Liquid	Deionized water	Yes; Filtered with 0.45μm PVDF filter
57	5	Liquid-liquid (shaking)	50% MeOH	Yes; Carrez 1 and Carrez 2
60	2.50-3.00	Liquid-liquid (shaking) / Liquid-liquid (ultrasonic)	Water	Yes; Clarification using Carrez I & II solutions, filtration using RC 0.45μm filters
63	0.5	Liquid-liquid (shaking)	Carrez solutions	No
65	5	Liquid-liquid (shaking)	Carez/Potassium hexacyanoferrate trihydrate 15g/100mL; Carez/Zinc sulphate heptahydrate 30g/100mL	Yes; Filtration with paper filter and Q-MaxRR syringe filters
72	2.5	[b]	Water	No
74	1.01	Ultrasonic	Water	No
77	10	Liquid-liquid (shaking) / Liquid-liquid (ultrasonic)	Ultrapure water, Carrez 1 and Carrez 2	Yes; Folded filter paper 597½ and Millex PVDF syringe filter
78	5	According to EN 12856:1999	According to EN 12856:1999	Yes; According to EN 12856:1999
81	[c]	Ultrasonic bath	Water	[d]
85	1-2	[b]	Distilled water	Yes; $K_3[Fe(CH)_6]$ + $Zn(CH_3COO)_2(ZnSO_4)$ to precipitate and centrifuged
88	5	Liquid-liquid (ultrasonic)	buffer pH 4.5; formic acid; triethylamine	Yes; SPE with MeOH

90	50	Solid-liquid extraction	Double distilled water	No
94	5	Liquid-liquid (ultrasonic)	50% MeOH in water (v/v)	No
97	5	Liquid-liquid (ultrasonic); 70°C	MeOH/water 7:3	No
98	5.0	Liquid-liquid (ultrasonic)	Water, Carrez I solution ($K_4[Fe(CN)_6].3H_2O$); Carrez II solution ($ZnSO_4.7H_2O$)	No

[a] Addition of 3mL buffer - vortex for 30s. Then, sonicate for 10 min. Centrifuge at 10000rpm for 10min (RT). Filter supernatant on 0.45um PTFE.
[b] No information was provided
[c] 5.2367; 5.4290; 5.1186; 5.1225; 5.1340; 5.5027
[d] 3 subsamples were treated with Carrez I and II and 3 subsamples were extracted only with water. No difference was observed between the two sample treatments.

The Table below summarises the analytical instruments and conditions used for the determination of acesulfame potassium:

Table 8

Lab Code	Instrument	Instrument Condition
3	HPLC-UV	–
4	HPLC-ELSD	C18 4.6 x 150 3.5u HPLC column; Flow rate 0.5mL/min; Column temp 30°C; Drift tube temp 70°C; Nebulizer temp 60°C; Inj vol 20uL
7	Capillary electrophoresis	Supporting electrolyte (20mmol/dm^3 sodium tetraborate and 40mmol/dm^3 sodium dodecyl sulfate); Wavelength 254nm; Voltage +25kV; Temp 20°C
9	HPLC-UV	Ion-pair reversed-phase HPLC, with UV detection; C18 Supelcosil 4.5um, 250 x 4.6mm; Gradient: 5% MeOH in water + TPrA-HSO_4 → 75% MeOH in water + TPrA-HSO_4
11	HPLC-UV	µ Bondapak C18 column; KH_2PO_4 0.0125M pH 3.5 : ACN (90:10); λ=220nm
12	HPLC-UV	Column LiChrospher 100 RP-18 250-4; Mobile phase 0.2g de sodium heptane Sulphonate in mixture of water and ACN (90:10)
14	Alliance Waters 2695 Separations module with PDA 2696	Nova-Pak C18 column; Mobile phase solution 20mM KH_2PO_4 pH ~4.2
16	HPLC-ELSD	Zorbax Eclipse XDB-C18 4.6x150mm 5um; Mobile phases A: 69/24/7(v/v/v) MeOH/Buffer/Acetone, B: 11/82/7(v/v/v) MeOH/Buffer/Acetone; Inj vol 10uL; Flow 0.5mL/min; Column temp RT; ELSD Neb 70°C Evap 50°C N_2 flow 1.6slm
18	HPLC-UV	Column Kinetex 2-6um C18 100A; Mobile phase 0.1% phosphoric in ACN & 0.1% phosphoric acid in water; Wavelength 210nm
19	HPLC-PDA	Column vp ODS (250x4.6mm 10); Mobile phase Buffer solution (potassium dihydrogen phosphate 0.0125M at pH 3.5) :acetronitrile (90:10); Column temp 30°C; Inj vol 20mL; Flow rate 1.000mL/min
20	HPLC-UV	Amendment column T.; Mobile phase ACN buffer
23	LC-MS	Shimpack VP-ODS 150x2.0mm ID-4.6um; MP 0.1% formic acid solution in water ACN (gradient); Ions (-)162.05; IM EST (-) SIIM
25	HPLC-UV	UHPLC Agilent 1290 Kinetex 1.7u XB C-18 100x3.0mm; Flow 0.7mL/min; Gradient elution ammonium acetate 5mM adjusted at pH 4.4 ACN (Channel B); DAD signal at 227nm
29	HPLC-UV	Develosil NH2-5 4.6x150mm; Mobile phase 1% phosphoric acid/Methanol (7:3)
31	HPLC-UV	Colonne chromatographique pour HPLC, en Phase inverse; Gradient tampon phosphate et acetonitrile
34	HPLC-UV-DAD	ACE3 C8; Mobile phase 25% MeOH, 75% 10mM tetra butyl ammonium hydrogen/40mM potassium dihydrogen

		orthophosphate adjusted to pH 3 with 4% ammonia
37	HPLC-PDA	Column C18; Mobile phase (15:85) ACN-0.01M Phosphate buffer adjusted to pH 4.0
38	HPLC-UV	Column Lichrospher 60 RP-Select B 5um 4.0x125mm; Mobile phase KH_2PO_4 (0.02M)/ACN; Ions NA; Ionisation mode NA
41	HPLC-UV	Column Phenyl 5um 4.6x150mm ; Mobile phase ACN:0.2% H_3PO_4=12:88
42	HPLC-PDA	Mobile phase acetic acid 6%; Column Eclipse Plus C18 4.6x250mm
46	HPLC-UV	Column C18 ; Mobile phase KH_2PO_4 2g/L (pH 4):CH_3CN (90:10)
50	HPLC-UV	Gemini 3u C18 110A column; mobile phase H_2O(KH2PO4 mol/cm^3, H_3PO_4 pH 3.5):CH_3CN=85:15
53	HPLC-PDA	Column RP 18e PurosphereStar 205x4.6mm 5um; Mobile phase 90% KH_2PO_4 0.02M pH 4.3 / 10% ACN; Oven 40°C; Flow rate 1mL/min; Vol inj 20uL
54	HPLC-UV	UV/vis detection 205nm; Column Supelcosil LC-18 4.6x250mm 5u; MP A: 12.2mM aq. KH_2PO_4; MP B :ACN; Flow rate 1.0mL/min
57	HPLC-DAD	Column C18; Mobile ACN and water with 0.1% formic acid; 256nm
60	HPLC-DAD	Column RP C18 4.6x250mm; Mobile phase Phosphate buffer (pH 3.5) / ACN 90/10
63	HPLC-UV	Column C18; Detector wavelength 227nm; Mobile phase Buffer KH_2PO_4 & ACN
65	HPLC-UV	ACE-5 C18 HL 250x4.6x5 column; Potassium dihydrophosphate solution with pH 3.5; ACN
72	HPLC-UV & UPLC-DAD	Column BEH-C18; MP: CAN:Acetonetrile 13:87
74	HPLC-UV	Agilent Eclipse Plus C18 5um 4.6x100mm; MeOH + 20mM ammonium acetate
77	HPLC-UV	Acquity UPLC BEH C18 1.7um 2.1x150mm; 0.0125M KH2PO4 buffer pH 3.5 - ACN
78	HPLC-UV	Column Atlantis dC18 3um 3.0x150mm; Mobile fase Metanol:Acetat puffer 35:65
81	HPLC-DAD	LiChrospher RP-Select B 4.0x125mm column; MF A(NaH_2PO_4, 100mM, pH 3.5) B(MeOH) A:B=75:25; Flow 1mL/min; λ=210nm
85	HPLC-PDA	Column Anion wake (4.6x150mm, 10um); MP: Na_2HPO_4 5mM pH 8.2: ACN
88	HPLC-ELSD	C18 (2) 100A 3x250mm 5um; Mobile phase A: MeOH-buffer pH 4.5-Acetone 69:24:7 (v:v:v); B: MeOH-buffer pH 4.5-Acetone 11:82:7 (v:v:v)
90	HPLC-UV	ACN / 0.2% phosphoric acid in the ratio of 12:88 (v/v)
94	HPLC-UV	Column C18 5um 4.6x250mm; Mobile phase A) 0.02M KH_2PO_4 (pH 4) in water; B) ACN; Program A:B (9:1) to A:B (8:2) in 10 minutes
97	HPLC-UV	Column RP-C8 ; Mobile phase: Acetate buffer/MeOH
98	HPLC-UV	Column Symmetry C18 5um; Mobile phase Phosphate buffer + MeOH

The Table below summarises the calibration method, use of internal standard, method recovery and test methods used for the determination of acesulfame potassium:

Table 9

Lab Code	Calibration (Cal) Method	Internal Standard(s) used?	Method Recovery Investigated?	Correction for Method Recovery Made?	Test Method Employed
3	External Cal	No	No	No	Accredited
4	External Cal	No	Yes (81%)	Yes	Accredited; EN:15911 (2011)
7	External Cal	No	No	No	Accredited; M 04-59-2009 (2014)
9	External Cal	No	105%	No	Accredited; CEN/TS 15606:2009
11	External Cal	No	No	No	[a]
12	External Cal	No	Yes (99.0%)	No	Not accredited
14	External Cal	No	Yes (109%)	No	[b]
16	External Cal	No	Yes (82.2%; spiked sample)	No	Not accredited
18	Internal Cal	No	No	Yes	Accredited; Reference to BS EN 12856:1999
19	External Cal	No	Yes (84.41%)	No	Not accredited
20	External Cal	No	Yes (97%)	Yes	Accredited; EN12856
23	External Cal	No	No	No	Not accredited
25	External Cal	No	Yes (96.5%)	No	Accredited; Own method validated in house
29	External Cal	No	Yes (102.3%)	No	Not accredited
31	External Cal	No	No	Yes	Accredited; Norme NF EN 12856
34	External Cal	No	Yes (100.2%)	No	Accredited; Method code KSS M23
37	Conventional Interpolative Method	No	Yes (97.2%)	[c]	Not accredited
38	External Cal	No	Yes (98, 94, 97%)	Yes	Accredited; FSL MSOP ADD-A10
41	Standard Addition	No	No	No	Not accredited
42	External Cal	No	Yes (95.80%)	No	Not accredited
46	External Cal	No	No	[c]	Not accredited

50	External Cal	No	No	No	Accredited; TOCT P EH 12856-2010
53	External Cal	No	No	No	Accredited; EN 12856:1999
54	External Cal	No	No	No	Not accredited; J.AOAC 1993, 76 (2), 268-274
57	External Cal	No	Yes (88%)	Yes	Not accredited
60	External Cal	No	Yes (99.6%)	No	The method is fully validated and based on EN 12856
63	External Cal	No	Yes (80%)	No	Accredited; BS EN 12856:1999
65	Standard Addition	No	Yes (101.2%)	No	Accredited; LST EN ISO 12856:2001
72	[c]	No	Yes (101.2%)	No	Accredited
74	External Cal	No	Yes (96.4%)	No	Accredited; GB/T 5009.140-2003
77	External Cal	No	No	No	Accredited; LI-00.450-2
78	External Cal	No	No	No	Accredited; Modifaed EN 12856:1999
81	External Cal	No	No	No	Accredited; EN 12856
85	External Cal	No	Yes (96.1-98.6%)	No	Accredited; ISO 17025
88	External Cal	No	Yes (104%)	No	Not accredited
90	External Cal	No	Yes (80.5%)	Yes	[d]
94	External Cal	No	Yes (94%)	No	Accredited; In-house method
97	External Cal	No	Yes (116%)	No	Accredited; Z1028/1003
98	Internal Cal (the cal curve)	No	No	No	Accredited; PN-EN 12856:2002

[a] Accredited; MU/INST/15 (Prodolliet & Bruelhart. 1993. Determination of Acesulfam-K in Foods. Journal of AOAC International. Vol. 76. No 2, P.268-27A.)
[b] Accredited; Determination of sweeteners in food by HPLC/DAD method, SOP 1/1.5 CSN EN 12856
[c] No information was provided
[d] Accredited (FDA, Ministry of Health and Welfare, Taiwan. No. 1021950329) Method of test for Sweeteners in Foods - Test of Acesulfame Potassium, Saccharin, Dulcin and Cyclamate

8.2 Sucralose

The Table below summarises the participating laboratories' results obtained using the sucralose CRM provided:

Table 10

Lab Code	Overall Result (mg/kg)	No. of Subsamples	u(x) (mg/kg)	Coverage Factor	Level of Confidence (%)	U(X) (mg/kg)
3	811.33	13	–	–	35	–
4	836.2	4	20.9	2	95	41.8
12	878	3	76.6	2	95	153.2
16	868.7	2	–	–	–	–
19	753.05	2	–	2	95	18.59
20	873	3	33.5	2	95	67
23	775.72	3	17.33	2	95	34.67
29	878	3	13	2	95	25
34	845	3	203	2	95	117
38	732	3	30	2	95	60
57	830	2	37	2	95	74
60	900	6	35	2	95	70
69	762	3	–	1.96	95	21.0
78	885	5	34	2	95	68
88	893	3	13	2	95	26
94	821	4	68	2	95	136
97	808	3	120	2	95	240

The Table below summarises the information on the participating laboratories' own sources of sucralose standard:

Table 11

Lab Code	Overall Result (mg/kg)	No. of Subsamples	u(x) (mg/kg)	Coverage Factor	Level of Confidence (%)	U(X) (mg/kg)
23	791.25	3	18.95	2	95	37.90
29	870	3	13	2	95	27
88	908	3	13	2	95	26
97	798	3	120	2	95	240

The Table below summarises the information on the participating laboratories' own sources of sucralose standard:

Table 12

Lab Code	Source	Purity and Unit	U(X) and Unit	Verified Before Use?
23	Sigma-Aldrich	96.80%	No	No calibration standard was verified, it was take into account the value reported by the certificate.
29	Wako Pure Chemical Industries	993 mg/g	11.3 mg/g	No
88	Alfa Aesar	98.9%	No information	No
97	Sigma-Aldrich	≥ 98.0%	–	No

The Table below summarises the sample sizes and sample treatments carried out for the determination of sucralose:

Table 13

Lab Code	Sample Size (g)	Extraction Technique	Extraction Solvent/-Reagents	Clean-Up
3	5	Liquid-Liquid (shaking)	Water	No
4	5	Liquid-liquid (shaking) / Liquid-liquid (ultrasonic) / SPE	Buffer pH 4.5 (formic acid and trimethylamine in water)	Yes (SPE cleanup using Waters Oasis HLB cartridge)
12	5.0000	Liquid-Liquid (shaking)	Water	Yes; With syringe filter 0.45um PVDF
16	1.00000	[a]	Buffer solution pH 4.5 (0.1% formic acid in water and adjusting to pH 4.5 with triethylamine)	No
19	10.1863	Ultrasonic, centrifugation (2000rpm)	MeOH / Water (1:1)	No
20	5	SPE / Liquid-liquid (shaking) / Liquid-liquid (ultrasonic)	ACN & buffer	Yes
23	0.5	Solid-liquid (shaking + ultrasonic)	Water; ACN; Formic acid	No
29	5	Liquid-liquid (ultrasonic)	Water	Yes; Sep-Pak Plus C18 cartridge
34	5	Liquid-liquid (shaking) / SPE	MeOH, water and clearing agents (potassium hexacyanoferrate and zinc acetate)	Yes; filtration
38	5	Liquid-liquid (shaking)	Water	Yes; SPE
57	5	Liquid-liquid (shaking)	50% MeOH	Yes; Carrez 1 and Carrez 2
60	2.00-2.50	Liquid-liquid (shaking)/ Liquid-liquid (ultrasonic) and centrifugation	Water	No; Filtration using RC 0.45um filters
69	5.0	Solvent extraction	ACN; water	No
78	5	According to EN 12856:1999	According to EN 12856:1999	Yes; According to EN 12856:1999
88	5	Liquid-liquid (ultrasonic)	buffer pH 4.5; formic acid; triethylamine	Yes; SPE with MeOH
94	5	Liquid-liquid (ultrasonic)	50% MeOH in water (v/v)	No
97	5	Liquid-liquid (shaking)	MeOH/water 1:1	No

[a] Addition of 3mL buffer - vortex for 30s. Then, sonicate for 10 min. Centrifuge at 10000rpm for 10min (RT). Filter supernatant on 0.45um PTFE.

The Table below summarises the analytical instruments and conditions used for the determination of sucralose:

Table 14

Lab Code	Instrument	Instrument Condition
3	HPLC-UV	–
4	HPLC-ELSD	C18 4.6 x 150 3.5u HPLC column; Flow rate 0.5mL/min; Column temp 30°C; Drift tube temp 70°C; Nebulizer temp 60°C; Inj vol 20uL
12	HPLC-IR	Column LiChrospher 100 RP-18 250-4; Mobile phase Acetonitrilo-Water (3:17)
16	HPLC-ELSD	Zorbax Eclipse XDB-C18 4.6x150mm 5um; Mobile phases A: 69/24/7(v/v/v) MeOH/Buffer/Acetone, B: 11/82/7(v/v/v) MeOH/Buffer/Acetone; Inj vol 10uL; Flow 0.5mL/min; Column temp RT; ELSD Neb 70°C Evap 50°C N2 flow 1.6slm
19	HPLC-RID	Column vp ODS (250x4.6mm 10); Mobile phase MeOH/water (30:70); Column temp 30°C; Cell temp 30°C; Flow rate 1mL/min; Inj vol 50mL
20	HPLC-ELSD	Amendment coluum T.; Mobile phase MeOH & Acetone & buffer
23	LC-MS	Column Shimpack VP-ODS 150x2.0mm ID-4.6um; MP 0.1% formic acid solution in water ACN (gradient); Ions (-) 442.90; IM EST (-) SIIM
29	HPLC-RI	Shodex sugar SP0810 8x300mm; Mobile phase water
34	HPLC-RI	Column Spherisil ODS 2 with a MeOH:water mobile phase (25:75)
38	HPLC-ELSD	Inertsil ODS-3 3um 3.0x100mm column; Mobile phase 25% MeOH; Ions NA; Ionisation mode NA
57	LC-MS	Column C18; mobile ACN and water with 0.1% formic acid; 395 m/z; Negative
60	HPLC-RI	Column RP C18 4.6x250mm; Mobile phase MeOH/Water 30/70
69	HPLC-RI	C18 column; Mobile phase 15% ACN
78	HPLC-RI	Column Atlantis dC18 5um 4.6x250mm; Mobile fase ACN:Water 15:85
88	HPLC-ELSD	C18 (2) 100A 3x250mm 5um; Mobile phase A: MeOH-buffer pH 4.5-Acetone 69:24:7 (v:v:v); B: MeOH-buffer pH 4.5-Acetone 11:82:7 (v:v:v)
94	HPLC-RI	Column C18 5um 4.6x250mm; Mobile phase 30% MeOH : 70% water
97	HPTLC	Detection: Fluorescence

The Table below summarises the calibration methods, use of internal standard, method recovery and test methods used for the determination of sucralose:

Table 15

Lab Code	Calibration (Cal) Method	Internal Standard(s) used?	Method Recovery Investigated?	Correction for Method Recovery Made?	Test Method Employed
3	External Cal	No	No	No	Accredited
4	External Cal	No	Yes (100%)	No	Accredited; EN:15911 (2011)
12	External Cal	No	Yes (99.8%)	No	Not accredited
16	External Cal	No	Yes (76.1%; spiked sample)	No	Not accredited
19	External Cal	No	Yes (98.86%)	No	Not accredited
20	External Cal	No	NA	NA	Not accredited
23	External Cal	No	No	No	Not accredited
29	External Cal	No	Yes (100.5%)	No	Not accredited
34	External Cal	No	Yes (103%)	No	Accredited; Method code KSS M279
38	External Cal	No	Yes (96, 97, 96%)	Yes	Accredited; FSL MSOP ADD-S07
57	External Cal	No	Yes (95%)	Yes	Not accredited
60	External Cal	No	Yes (100.5%)	No	Not accredited
69	External Cal	No	No	No	Not accredited
78	External Cal	No	No	No	Not accredited
88	External Cal	No	Yes (102%)	No	Not accredited
94	External Cal	No	Yes (92%)	No	Accredited; In-house method
97	External Cal	No	No	No	Not accredited

9.0 Evaluation of Performance of Participating Laboratories

The performance evaluation was conducted in accordance with the statistical methods described in ISO 13528 Statistical methods for use in proficiency testing by interlaboratory comparisons.

The performance of the participating laboratories was primarily assessed using the *z*-scores. The calculation of *z*-score was as follows:

$$z = \frac{x - X}{\sigma_{PT}}$$

where, x is participating laboratory's result, X is the assigned value determined by HSA CML and σ_{PT} is the standard deviation for proficiency assessment.

A *z*-score with absolute value of: $|z| \leq 2.0$ is satisfactory

$2.0 < |z| < 3.0$ is questionable

$|z| \geq 3.0$ is unsatisfactory

The σ_{PT} for both analytes (summarised in the following Table) was determined using the robust standard deviation of the results reported by all the participating laboratories, calculated using Algorithm A in ISO 13528.

Table 16

Analytes	**σ_{PT}**
Acesulfame Potassium	61 mg/kg
Sucralose	58 mg/kg

For laboratories which reported the uncertainties of their results, the ζ-scores were also calculated. The ζ-score provided an indication of whether a participating laboratory's estimate of uncertainty was consistent with the observed deviation from

the assigned value. It was complementary to the *z*-score in the assessment of laboratory performance. The calculation of ζ-score was as follows:

$$\zeta = \frac{x - X}{\sqrt{u_x^2 + u_X^2}}$$

where, u_x is the standard uncertainty of the participating laboratory's result x and u_X is the standard uncertainty of the assigned value X determined by HSA CML.

A ζ-score with absolute value of: $|\zeta| \leq 2.0$ is satisfactory

$2.0 < |\zeta| < 3.0$ is questionable

$|\zeta| \geq 3.0$ is unsatisfactory

The participating laboratories' ζ-scores are presented and discussed in the following Sections.

9.1 Acesulfame Potassium

The Table below summarises the z-scores and ζ-scores calculated from the results obtained using the CRM provided and the participating laboratories' own sources of standard:

Table 17

Lab Code	z-Score (X=1092 mg/kg, σ_{PT} = 61 mg/kg)		ζ-score (X=1092 mg/kg, u_X = 26 mg/kg)	
	Based on CRM provided	Based on laboratory's own source of standard	Based on CRM provided	Based on laboratory's own source of standard
3	-0.6	[a]	[b]	[a]
4	0.9	[a]	0.8	[a]
7	-0.6	-1.1	-0.5	-0.9
9	-0.7	-0.7	-1.4	-1.4
11	0.6	-2.0	1.2	-4.2
12	0.6	[a]	1.3	[a]
14	-0.1	[a]	-0.2	[a]
16	-3.4	[a]	[b]	[a]
18	-0.9	[a]	-1.7	[a]
19	-4.2	[a]	[b]	[a]
20	-1.7	[a]	-1.7	[a]
23	0.2	-0.1	0.4	-0.1
25	-0.5	0.6	-0.4	0.5

29	-0.3	-0.2	-0.6	-0.5
31	-1.3	-1.1	[b]	[b]
34	-0.9	[a]	-0.9	[a]
37	-1.1	-2.5	-2.5	-5.8
38	0.7	[a]	0.6	[a]
41	2.9	[a]	6.9	[a]
42	-1.6	-1.8	-2.6	-2.7
46	-0.3	-0.5	-0.3	-0.4
50	-0.7	-1.5	-0.5	-1.1
53	-0.5	-1.1	-0.9	-1.9
54	[c]	0.1	[c]	0.3
57	0.5	[a]	0.3	[a]
60	-0.5	-0.8	-0.6	-1.0
63	-2.2	[a]	-3.7	[a]
65	-0.6	-1.1	-0.7	-1.3
72	1651.2	0.2	33.0	0.4
74	-0.5	[a]	[b]	[a]
77	-0.9	-1.0	-2.2	-2.4
78	-2.98	[a]	-4.6	[a]
81	0.0	0.1	0.0	0.2
85	-3.4	-3.5	-5.3	-5.9
88	0.3	0.8	0.5	1.2
90	0.0	[a]	[b]	[a]
94	-1.0	[a]	-0.9	[a]
97	-1.4	-1.3	-0.7	-0.6
98	-2.3	[a]	-2.1	[a]

[a] The laboratory did not report any result with the use of its own source of standard
[b] Result was reported but standard uncertainty was not reported
[c] The laboratory did not report any result with the use of the CRM provided

The distribution of the above scores are summarised in the following Table:

Table 18

Score	**No. of Participating Laboratories (% in parenthesis)**			
	z-Score (Based on CRM provided)	**z-Score** (Based on laboratory's own source of standard)	**ζ-Score** (Based on CRM provided)	**ζ-Score** (Based on laboratory's own source of standard)
|score| ≤ 2.0	30 (79.0%)	19 (90.4%)	23 (71.9%)	15 (75.0%)
2.0 < |score| < 3.0	4 (10.5%)	1 (4.8%)	4 (12.5%)	2 (10.0%)
|score| ≥ 3.0	4 (10.5%)	1 (4.8%)	5 (15.6%)	3 (15.0%)
Total	38 (100.0%)	21 (100.0%)	32 (100.0%)	20 (100.0%)

9.2 Sucralose

The Table below summarises the z-scores and ζ-scores calculated from the results obtained using the CRM provided and the participating laboratories' own sources of standard:

Table 19

Lab Code	z-Score (X=825 mg/kg, σ_{PT} = 58 mg/kg)		ζ-score (X=825 mg/kg, u_X = 19 mg/kg)	
	Based on CRM provided	Based on laboratory's own source of standard	Based on CRM provided	Based on laboratory's own source of standard
3	-0.2	[a]	[b]	[a]
4	0.2	[a]	0.4	[a]
12	0.9	[a]	0.7	[a]
16	0.8	[a]	[b]	[a]
19	-1.2	[a]	[b]	[a]
20	0.8	[a]	1.3	[a]
23	-0.9	-0.6	-1.9	-1.2
29	0.9	0.8	2.3	2.0
34	0.4	[a]	0.1	[a]
38	-1.6	[a]	-2.6	[a]
57	0.1	[a]	0.1	[a]
60	1.3	[a]	1.9	[a]
69	-1.1	[a]	[b]	[a]
78	1.0	[a]	1.5	[a]
88	1.2	1.4	2.96	3.6
94	-0.1	[a]	-0.1	[a]
97	-0.3	-0.5	-0.1	-0.2

[a] The laboratory did not report any result with the use of its own source of standard
[b] Result was reported but standard uncertainty was not reported

The distribution of the above scores are summarised in the following Table:

Table 20

Score	No. of Participating Laboratories (% in parenthesis)			
	z-Score (Based on provided CRM)	**z-Score** (Based on laboratory's own source of standard)	**ζ-Score** (Based on provided CRM)	**ζ-Score** (Based on laboratory's own source of standard)
|score| ≤ 2.0	17 (100.0%)	4 (100.0%)	10 (76.9%)	3 (75.0%)
2.0 < |score| < 3.0	0 (0.0%)	0 (0.0%)	3 (23.1%)	0 (0.0%)
|score| ≥ 3.0	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (25.0%)
Total	17 (100.0%)	4 (100.0%)	13 (100.0%)	4 (100.0%)

10.0 Graphical Presentation of Results

10.1 Acesulfame Potassium

The z-scores, ζ-scores and participating laboratories' results obtained using the CRM provided are presented in the following Figures:

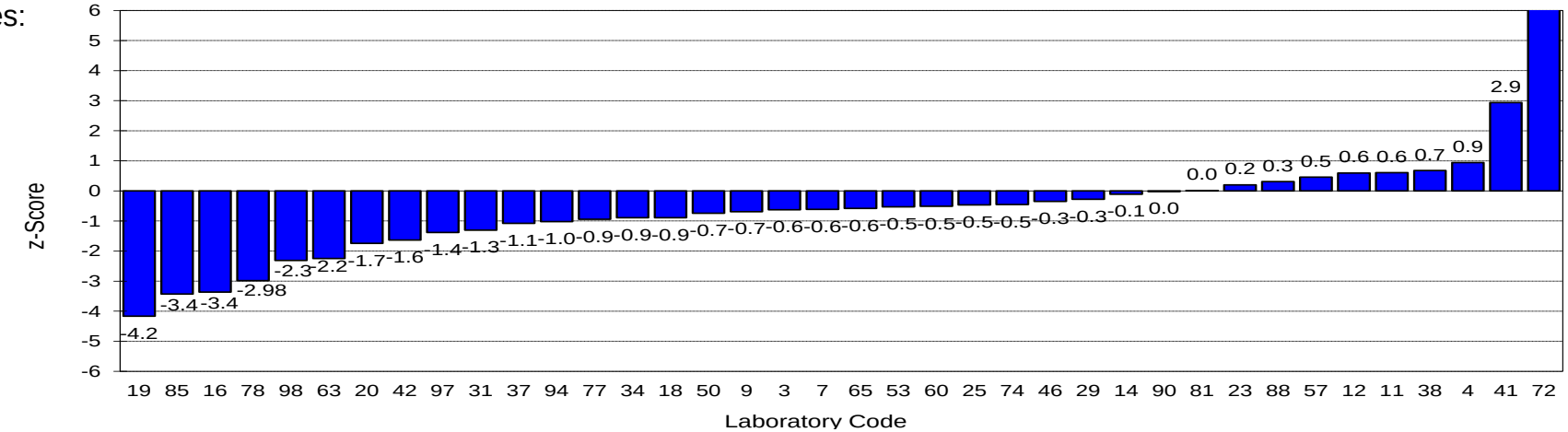


Figure 1: z-Scores for acesulfame potassium. Note: The z-score of Lab 72 lies outside the range of the plot.

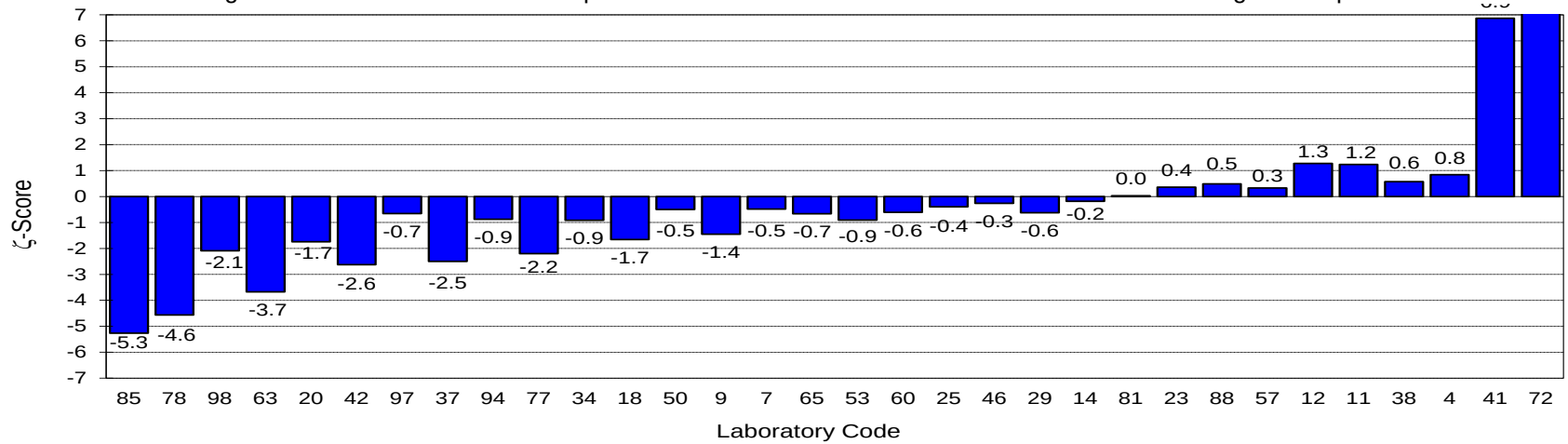


Figure 2: ζ-Scores for acesulfame potassium. Note: The ζ-score of Lab 72 lies outside the range of the plot. Six laboratories did not report the uncertainty of their results.

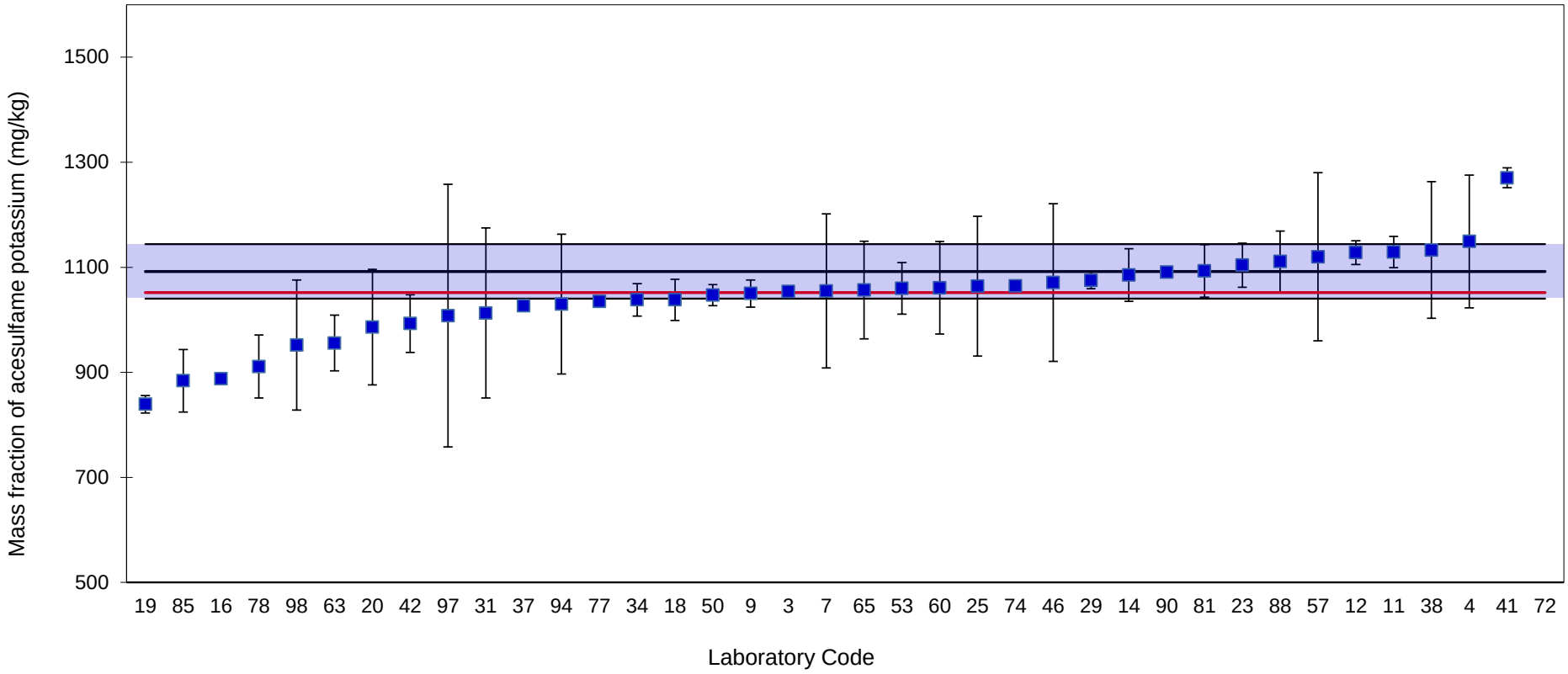


Figure 1: Participating laboratories' results, assigned values and associated expanded uncertainties (mg/kg) for acesulfame potassium.
Note: The result reported by Lab 72 lies outside the range of the plot.

10.2 Sucralose

The z-scores, ζ-scores and participating laboratories' results obtained using the CRM provided are presented in the following Figures:

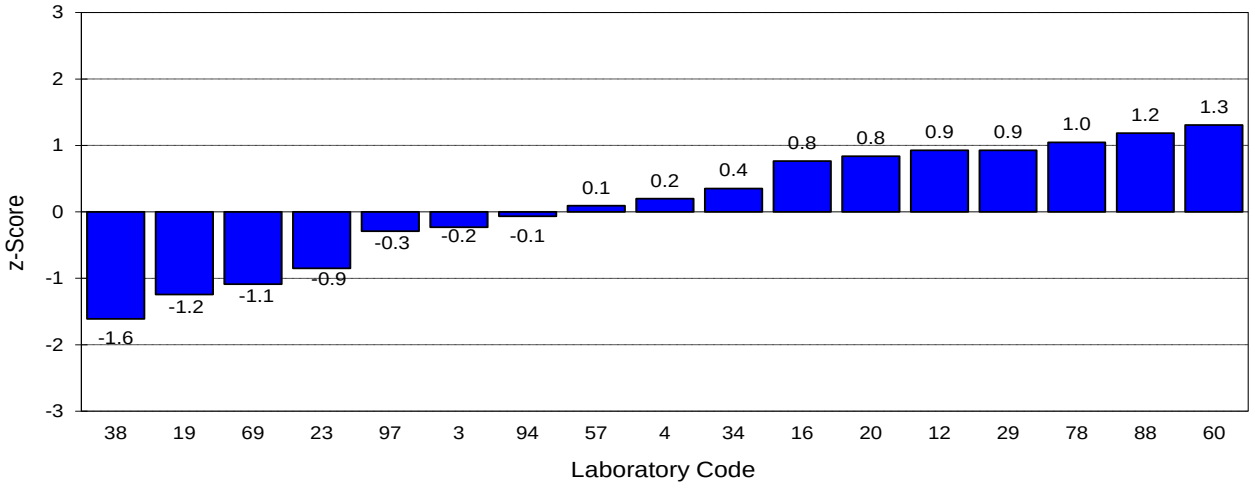


Figure 2: z-Scores for sucralose

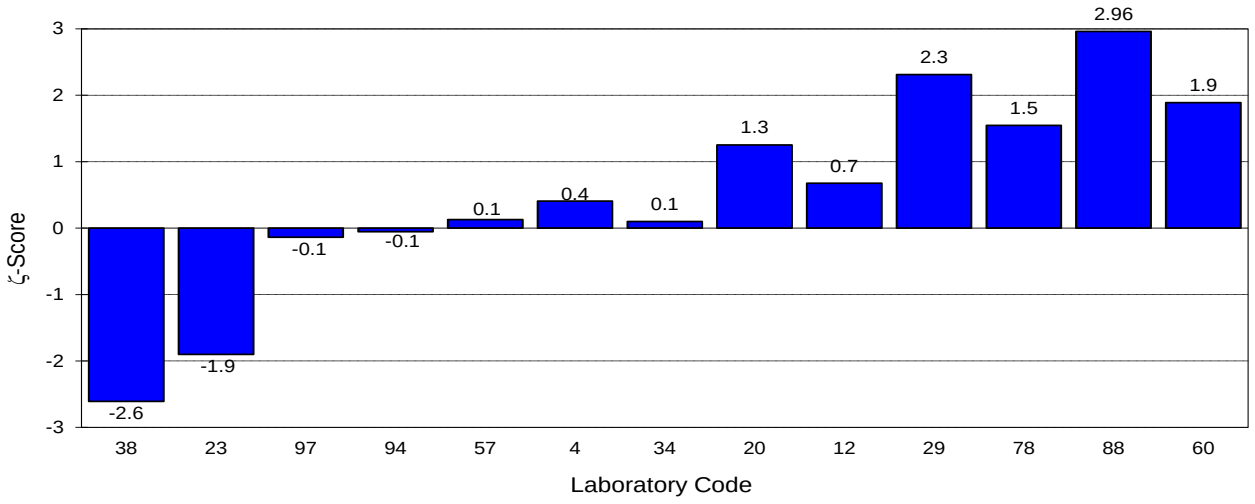


Figure 3: ζ-Scores for sucralose. Note: Four laboratories did not report the uncertainty of their results.

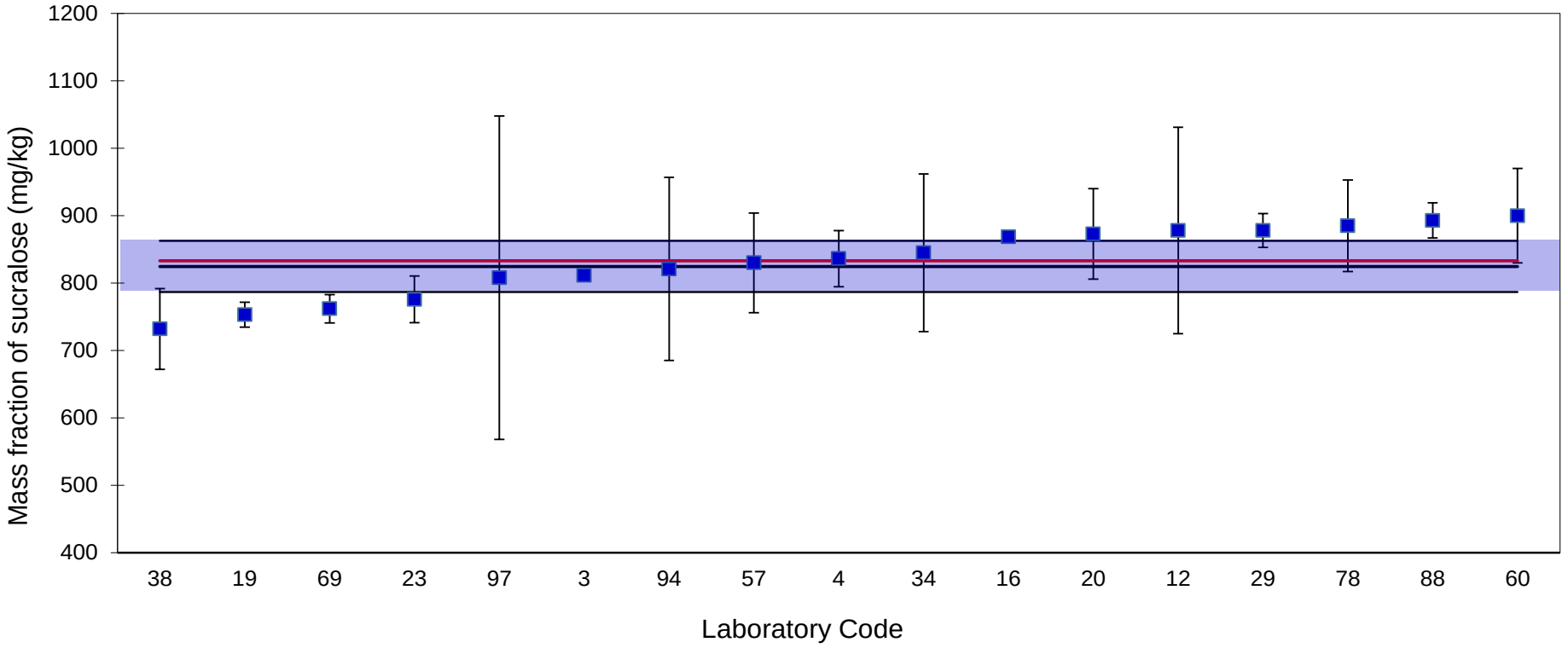


Figure 4: Participating laboratories' results, assigned values and associated expanded uncertainties (mg/kg) for sucralose

11.0 Results and Discussions

11.1 Acesulfame potassium

The performance of the participating laboratories was primarily assessed by z-scores calculated from the results obtained using the CRM provided by HSA only. A total of 38 participating laboratories returned their result obtained using the CRM provided. An overview of the participating laboratories' results is summarised in the following Table:

Table 21

No. of Results Received	38
Assigned Value	1,092 mg/kg
Robust Mean of Laboratories' Results (Relative Difference from Assigned Value in parenthesis)	1,052 mg/kg (-3.7%)
Mean of Laboratories' Results (Relative Difference from Assigned Value in parenthesis)	1,045 mg/kg (-4.3%)
Median of Laboratories' Results (Relative Difference from Assigned Value in parenthesis)	1,055 mg/kg (-3.4%)

The consensus values (robust mean, mean and median) calculated from the participating laboratories' results were observed to be generally lower than the assigned value.

A total of 21 participating laboratories provided additional result obtained using their own sources of acesulfame potassium standard as calibrant. One of the participating laboratories (Laboratory 54) only returned the result for acesulfame potassium determined using its own source of standard for calibration[2].

While the ζ-score is not used as the primary tool for performance evaluation, it is a useful tool recommended in ISO/IEC 17043 and ISO 13528 in evaluating the closeness of a participating laboratory's result to the assigned value, within its

[2] Reason provided by participating laboratory in Section D of Result Proforma: "Due to internal miscommunication we neglected to run the samples with the provided CRM."

claimed uncertainty. The information is useful as measurement uncertainty is a requirement under ISO/IEC 17025. Of the 38 participating laboratories which reported results using the CRM provided, 34 laboratories reported the expanded uncertainties. 3 Of the remaining 4 laboratories indicated that their methods were accredited but did not report their expanded uncertainties. The relative expanded uncertainties of the results obtained using the CRM provided were between 0.1% and 24.8%.

Laboratory 37 attained satisfactory z-score ($|z| \leq 2.0$) with the use of the CRM provided as calibrant but attained questionable z-score ($2.0 < |z| < 3.0$) with the use of its own source of standard as calibrant. As the result from the latter was lower and the purity value of the standard was reported to be 100%, the reason for the lower result could not be attributed to the purity value of the standard. Laboratory 85 attained unsatisfactory z-score ($|z| \geq 3.0$) with both the CRM provided and its own source of standard. Laboratory 72 made an obvious transcription error in its Result Proforma. However, as re-submission of the Result Proforma due to modification of any information could not be accepted, the Coordinators were unable to ask the laboratory to re-submit its data.

Based on the results obtained using the CRM provided, Laboratories 37, 42 and 77 attained satisfactory z-scores ($|z| \leq 2$) but questionable ζ-scores ($2 < |\zeta| < 3$), while Laboratories 41, 63 and 78 attained questionable z-scores ($2 < |z| < 3$) but unsatisfactory ζ-scores ($|\zeta| \geq 3$). The discrepancy in z-score and ζ-score could be attributed to the underestimation of the measurement uncertainty. For example, the relative standard uncertainties reported by Laboratories 37 and 77 were 0.4% and 0.04%, respectively, were much smaller than u_{char} of 1.2% for the assigned values.

Of the 39 participating laboratories (including Laboratory 54), 26 (66.7%) employed accredited methods, 12 (30.7%) employed non-accredited methods, while 1 (2.6%) did not indicate clearly if it had employed accredited method for the PT programme.

A total of 30 (76.9%) participating laboratories used the minimum number of 3 subsamples for analysis, as indicated in the Instructions for Participating Laboratories. 5 (12.8%) Laboratories used less than 3 subsamples for analysis while another 4 (10.3%) laboratories did not provide the relevant information. 28 (71.8%) Laboratories used the minimum sample size of 5 g for analysis, as indicated in the Instructions for Participating Laboratories. 11 (28.2%) Laboratories used less than the stated minimum sample size (0.5 g to 3.0 g) for analysis.

20 Participating laboratories reported results obtained using both the CRM provided and their own sources of standard for calibration. One laboratory reported result using only its own source of standard for calibration. Majority of the laboratories employed external calibration method.

24 (61.5%) Of the participating laboratories investigated the method recovery (80.0% to 116.0%) and 5 of these laboratories also carried out correction for method recovery. The method recoveries reported by the 5 laboratories ranged from 80.5% to 98.0%.

The purity values of the acesulfame potassium standards used by 21 participating laboratories ranged from 99.0% to 100.3%. Only 8 (38.1%) of the purity values were reported with an associated measurement uncertainty. 4 (19.0%) Laboratories indicated that they have verified the standards before use.

Liquid-liquid extraction with shaking was employed by 17 (43.6%) of the participating laboratories. Liquid-liquid extraction with sonication was employed by 14 (35.9%) of the laboratories. Liquid-liquid extraction with both shaking and sonication was employed by 5 (12.8%) of the laboratories. 3 (7.7%) Laboratories did not indicate clearly the extraction method used. Water was used as the main extraction solvent by 17 (43.6%) of the participating laboratories.

No further clean-up was applied by 24 (61.5%) of the participating laboratories before instrumental analysis. LC coupled with absorption spectroscopic detection methods (UV/DAD/PDA) was used by 34 (87.2%) of the laboratories. Other instruments included LC-ELSD (3, 7.7%), CE (1, 2.6%) and LC-MS (1, 2.6%).

The z-scores of the participating laboratories which employed the most commonly used sample preparation method (liquid-liquid extraction) and instruments (LC-UV, DAD or PDA) were further analysed. The z-scores of 19 laboratories which did not apply further sample clean-up before subjecting the extracts to LC analyses were extracted. Of these laboratories, 10 and 9 employed liquid-liquid extraction with shaking and liquid-liquid extraction with sonication, respectively. As shown in Figure 7, neither extraction methods were found to be favourable over the other. The number of laboratories which employed both shaking and sonication methods was too small to be included in the data analysis.

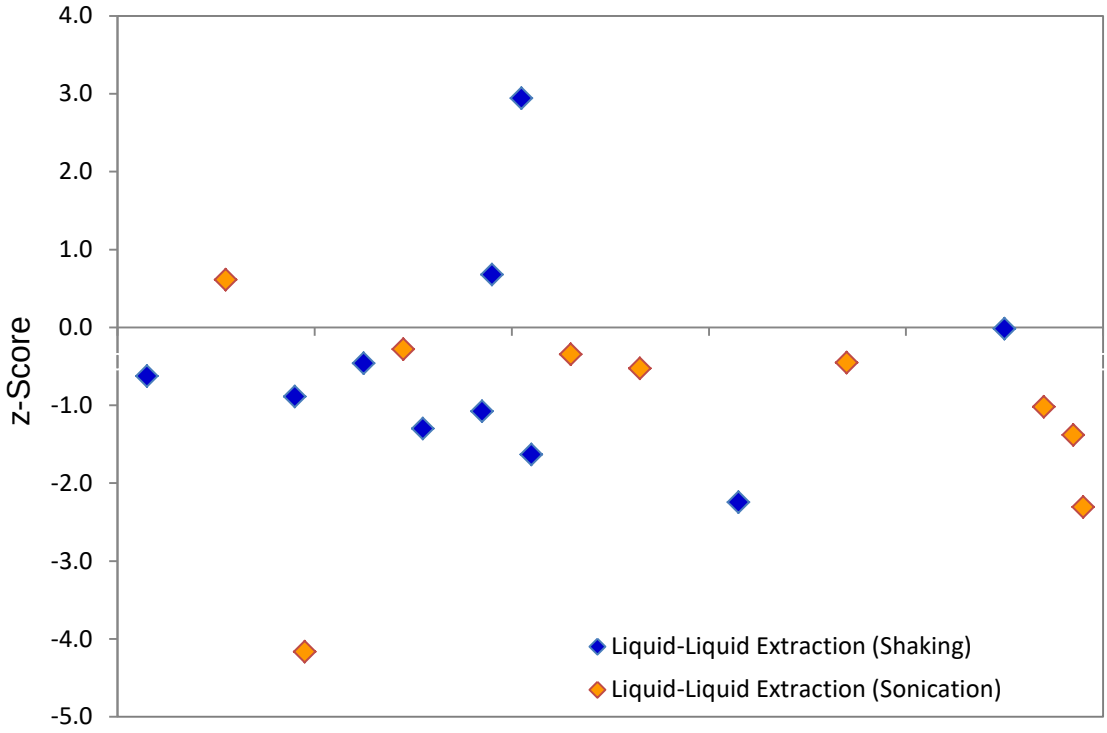


Figure 5: Distribution of z-scores of participating laboratories which employed liquid-liquid extraction with shaking or sonication

Similarly, the z-scores of 15 laboratories which employed liquid-liquid extraction (shaking) and LC coupled with absorption spectroscopic analysis were extracted. Of these laboratories, 5 applied further sample clean-up while 10 did not. As shown in Figure 8, the additional step did not appear to affect their results. Laboratory 81 (z-score: 0.0) also indicated that they had treated three subsamples with Carrez I and II solutions and another three were extracted with water only, and found no difference between the results of these two sets of experiments.

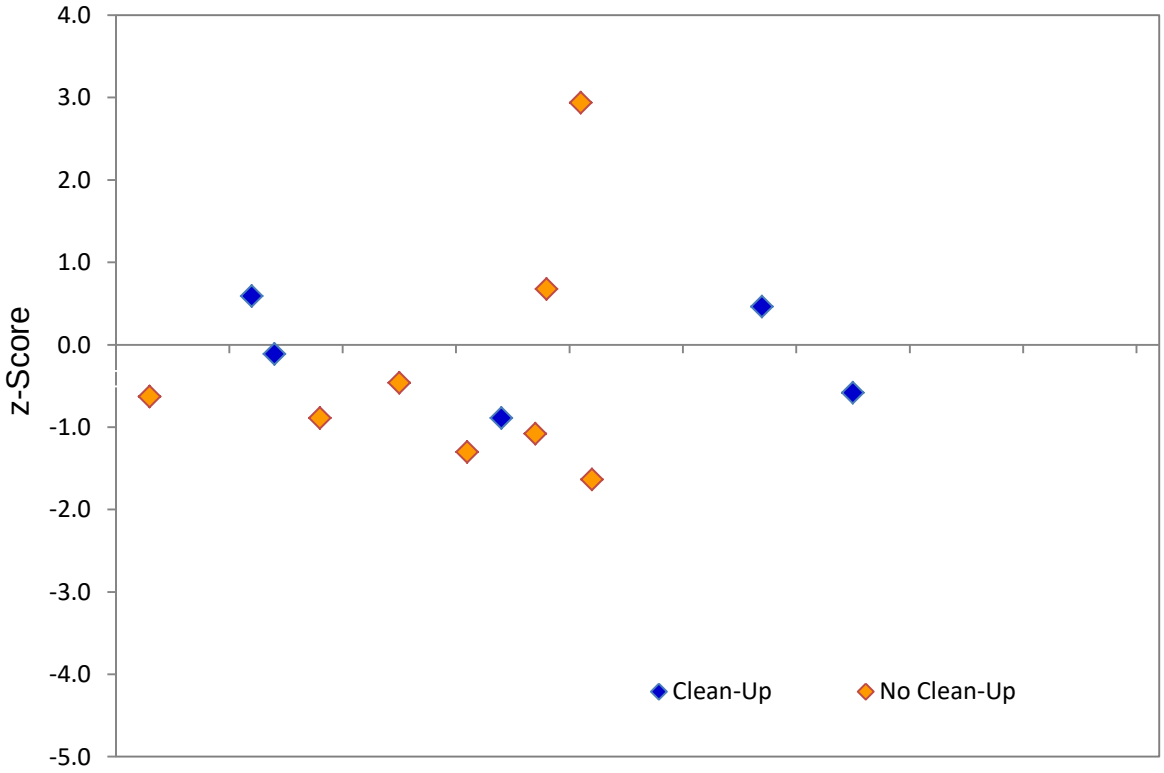


Figure 6: Distribution of z-scores of participating laboratories which carried out or did not carry out additional clean-up before instrumental analysis

11.2 Sucralose

The performance of the participating laboratories was primarily assessed by z-scores calculated from the results obtained using the CRM provided by HSA only. A total of 17 participating laboratories returned their result obtained using the CRM provided. An overview of the participating laboratories' results is summarised in the following Table:

Table 22

No. of Results Received	17
Assigned Value	825 mg/kg
Robust Mean of Laboratories' Results (Relative Difference from Assigned Value in parenthesis)	833 mg/kg (+1.0%)
Mean of Laboratories' Results (Relative Difference from Assigned Value in parenthesis)	832 mg/kg (+0.8%)
Median of Laboratories' Results (Relative Difference from Assigned Value in parenthesis)	836 mg/kg (+1.3%)

The consensus values (robust mean, mean and median) calculated from the participating laboratories' results were observed to be slightly higher than the assigned value. The differences were much smaller than those observed in acesulfame potassium.

A total of 4 participating laboratories provided additional result obtained using their own sources of sucralose standard as calibrant. All the laboratories attained satisfactory z-scores ($|z| \leq 2$) for results obtained using the CRM provided and their own sources of standard for calibration.

Of the 17 participating laboratories which reported results using the CRM provided, 15 laboratories reported the expanded uncertainties. 1 Of the remaining 2 laboratories indicated that their methods were accredited but did not report their expanded uncertainties. The relative expanded uncertainties of the results obtained using the CRM provided were between 2.5% and 29.7%.

Based on the results obtained using the CRM provided, Laboratories 29, 38 and 88 attained satisfactory z-scores ($|z| \leq 2$) but questionable ζ-scores ($2 < |\zeta| < 3$), which could be attributed to the underestimation of the measurement uncertainty.

Of the 13 participating laboratories which reported the standard uncertainty of their results using the CRM provided, 2 laboratories (Laboratories 29 and 88) reported relative combined standard uncertainties smaller than u_{char} (in %) associated with the assigned value. Since the CML had used high accuracy and precision LC-IDMS method, u_{char} would generally be expected to be smaller instead. Hence, it is recommended that the laboratories review their measurement uncertainty estimations.

A total of 5 (29.4%) participating laboratories employed accredited methods while 12 (70.6%) employed non-accredited methods for the PT programme.

Out of the 17 participating laboratories, 14 (82.4%) used the minimum number of 3 subsamples for analysis, as indicated in the Instructions for Participating Laboratories. The remaining 3 (17.6%) laboratories used less than 3 subsamples for analysis. 14 (82.4%) Laboratories used the minimum sample size of 5 g for analysis, as indicated in the Instructions for Participating Laboratories. The remaining 3 (17.6%) laboratories used less than the stated minimum sample size (0.5 g to 2.5 g) for analysis.

All the participating laboratories employed external calibration method. 11 (64.7%) Of the participating laboratories investigated the method recovery (76.1% to 103.0%) and two of these laboratories carried out method recovery correction. The method recoveries reported by the two laboratories ranged from 95.0% to 97.0%.

The purity values of the sucralose standards used by the laboratories ranged from 96.8% to 99.3%. Only 1 (25.0%) of the purity values was reported with an associated measurement uncertainty. All the laboratories indicated that the standards were not verified before use.

Majority of the participating laboratories employed liquid-liquid extraction (shaking) followed by LC (installed with C18 column) coupled with RI/ELSD.

Liquid-liquid extraction with shaking was employed by 7 (41.2%) of the participating laboratories. Liquid-liquid extraction with sonication was employed by 5 (29.4%) of the laboratories. Liquid-liquid extraction with both shaking and sonication was employed by 4 (23.5%) laboratories, while 1 did not clearly indicate the extraction method used. Acetonitrile/Methanol-water mixtures were used as the extraction solvent by most of the participating laboratories.

LC-RID and LC-ELSD were used by 7 (41.1%) and 5 (29.4%) of the laboratories. Other instruments used included LC-MS (2, 11.8%), LC-UV (1, 5.9%), HPTLC (1, 5.9%) and HPLC-IR (1, 5.9%).

12.0 References

[1] ISO/IEC 17043:2010 Conformity Assessment – General Requirements for Proficiency Testing.

[2] ISO 13528:2015 Statistical Methods for Use in Proficiency Testing by Interlaboratory Comparison.

[3] ISO/IEC Guide 98-3:2008 Uncertainty of Measurement – Part 3: Guide to the Expression of Uncertainty in Measurement (GUM:1995).

[4] S L R Ellison and A Williams (Eds). Eurachem/CITAC guide: Quantifying Uncertainty in Analytical Measurement, Third edition, (2012) ISBN 978-0-948926-30-3. Available from www.eurachem.org.

[5] ISO Guide 35:2006(E) Reference materials – General and statistical principles for certification.

[6] Zygler, A., Wasik, A., Namiesnik, J.; Analytical methodologies for the determination of artificial sweeteners in food. *Trends in Analytical Chemistry*, 2009, 28(9), 1082-1102.

[7] Chattopadhyay, S., Raychaudhuri, U., Chakraborty, R.; Artificial sweeteners – a review. *Journal of Food Science and Technology*, 2014, 51(4), 611-621.

[8] Whitehouse, C.R., Boullata, J., McCauley, L.A.; The potential toxicity of artificial sweeteners. *Journal of the American Association of Occupational Health Nurses*, 2008, 56(6), 251-259.

13.0 Use of Report

This report is intended to be used as an internal reference for all the participating accreditation bodies and laboratories of APLAC PT T103, and the APLAC. Its content shall not be disclosed to other parties or used for other purposes.

Acknowledgements

The Coordinators of APLAC PT T103 would like to express their sincere thanks to all the participating accreditation bodies and laboratories for their contributions, as well as for the support of Dr Koichi Nara, Chair of APLAC PT TC and APLAC PT TC Members.

APPENDIX I: Homogeneity Study

1. The homogeneity of the PT samples was established using LC-MS, following the guidelines in ISO 13528:2015. Homogeneity testing was performed on 11 packets, with two subsamples taken from each packet. The packets were selected using stratified random sampling approach. Each subsample with a minimum sampling size of 5 g was taken and analysed under repeatability conditions.

2. HSA CRMs [acesulfame potassium (HRM-1012A) and sucralose (HRM-1015A)] were used as the calibration standards and saccharin (from Tokyo Chemical Industry) was used as the internal standard for the homogeneity testing using calibration curve method.

3. The calibration curve method was derived from five calibration blends gravimetrically prepared with the mass ratios of acesulfame potassium/saccharin and sucralose/saccharin ranging from 0.7 to 1.7. Both calibration blends and sample blends were extracted using water. Each sample blend and calibration blend were subjected to the same extraction procedure, i.e., vortexing, shaking, sonication, shaking then centrifugation. The extract was filtered through 0.45 µm PVDF syringe filter, then analysed using Waters 2767 sample manager and 2545 binary solvent manager coupled to a 3100 Single Quadrupole Mass Detector with the following parameters:

   Column: Kinetex Phenyl-Hexyl (5 µm, 4.6 x 250 mm)
   Mobile phase A: 0.1% formic acid in water
   Mobile phase B: 0.1% formic acid in acetonitrile
   Flow rate: 1 mL/min
   Gradient: 0 min, 88% A; 14.0 min, 88% A; 14.1 min, 10% A; 15.0 min, 10% A; 15.1 min, 88% A; 21.0 min, 88% A

The characteristic ions (m/z) monitored in the LC-MS were:

Analytes	Monitored Ion (m/z)
Acesulfame potassium	161.9
Sucralose	395.0
Saccharin	182.0

4. The between-packet standard deviation was compared against 0.3*standard deviation estimated from the Horwitz Equation. The samples were found to be sufficiently homogeneous in accordance with the recommendations of ISO 13528:2015.

5. The uncertainty due to between-packet inhomogeneity for acesulfame potassium and sucralose was 1.5 % and 1.1 %, respectively.

APPENDIX II: Stability Studies

The stability of the PT samples under storage and transportation conditions were investigated. The measurements were carried out following the sample preparation and instrumental conditions described in APPENDIX I.

a) Stability Test under Storage Condition

1. The stability of the PT samples stored between 18 °C and 25 °C was established using LC-MS following the guidelines in ISO 13528:2015. The stability of the PT samples was monitored for three test occasions: (i) storage period of about 3 months after packaging, (ii) before dispatch of samples, and (iii) after dispatch of samples. This period spanned from sample dispatch to result submission.

2. Using a classical design, three randomly selected packets, with two subsamples (5 g each) taken from each packet, were analysed for each test occasion.

3. The difference in results determined for each test occasion and homogeneity data (APPENDIX I) was compared against 0.3*standard deviation estimated from the Horwitz Equation. The study showed that the samples were sufficiently stable throughout the period of this PT programme.

4. The uncertainty due to instability of acesulfame potassium and sucralose was 1.4 % and 1.3 %, respectively.

b) Stability Test under Transportation Condition

1. The stability of the PT samples under the maximum allowable transportation condition of 40 °C for a period of 1 month was established using LC-MS.

2. Three packets were randomly selected and stored in a refrigerator maintained at 4 ºC. Another six packets were also randomly selected and stored into an oven maintained at 40 ºC. After 10 days and 30 days, three packets were removed from the oven on each day and transferred into the refrigerator. Following an isochronous design, all the nine packets were analysed on the same day. Two subsamples (5 g each) were taken from each packet, i.e. a total of 18 subsamples were analysed.

3. The difference in results between samples not exposed to 40 °C and those exposed to 40 °C for up to 30 days, was compared against 0.3* standard deviation estimated from the Horwitz Equation. The study showed that the samples were sufficiently stable at 40 ^{o}C during the period.

APPENDIX III: Method for Value Assignment

Exact-matching IDMS method using LC-MS (Waters 2767 sample manager and 2545 binary solvent manager coupled to a 3100 Single Quadrupole Mass Detector) was employed for the determination of both analytes in the PT material.

HSA CRMs [Acesulfame potassium (HRM-1012A) and Sucralose (HRM-1015A)] were used as calibrants for the IDMS measurements. D_4-Acesulfame potassium and D_6-Sucralose (from Toronto Research Chemicals) were used as the internal standards.

The calibration blends (CBs) were prepared gravimetrically by spiking appropriate amounts of calibration standard and internal standard solutions. The study sample was diluted prior to preparation of sample blends (SBs), by accurately weighing 5 g of the sample and 12 g of water to form slurry. The SBs were then prepared by spiking appropriate amounts of internal standard solutions into 5 g of the slurry. The CBs and SBs were prepared such that the ratio of native/labelled analytes in both blends was closely matched. Quality controls (QCs) were prepared by spiking appropriate amounts of internal standard solutions into 5 g of slurry prepared from fortified flour and water. These QCs were then analysed together with the SBs. Appropriate amount of deionised water was added to the blends to achieve a total volume of 30 mL. They were then subjected to the same extraction procedure, i.e. vortexing, shaking, sonication, shaking then centrifugation. The supernatant was then transferred into a 50 mL centrifuge tube. Approximately 10 mL of water was added into the residues and the extraction procedure was repeated another time.

The combined supernatant was filtered through 0.45 um PVDF syringe filter, then analysed using Waters 2767 sample manager and 2545 binary solvent manager coupled to a 3100 Single Quadrupole Mass Detector following the parameters described in APPENDIX I. The characteristic ions monitored were:

Analytes	Monitored Ion (m/z)	
	Native	Labelled
Acesulfame potassium	161.9	165.9
Sucralose	395.0	401.0

APPENDIX IV: Nomination Form for Accreditation Body

Please provide details of your nominated laboratory/laboratories and return this form to the Coordinator by **02 October 2015**:

Attn: Ms Lee Jia Juan, Singapore Accreditation Council
Email: lee_jia_juan@spring.gov.sg

Section A: Information on Accreditation Body

Name of Accreditation Body	:	
Salutation	:	Dr / Mdm / Mr / Mrs / Ms / Professor *
Name of Contact Person	:	
Designation	:	
Email Address	:	
Telephone Number	:	
Fax Number	:	
Postal Address (for PT Package)	:	
Postal Code	:	
City	:	
Country	:	

* Please delete where appropriate

Do you require a special custom permit for the Proficiency Testing (PT) package(s) to be sent to your institute/company? [Please tick (✓) If yes, please attach details in a separate sheet of paper.]

( ) Yes ( ) No

Please note that any import taxes or charges imposed on the PT package(s) during transportation shall be borne by the Accreditation Body.

Section B: Information on Nominated Laboratory/Laboratories

Gentle reminder: An APLAC member may nominate a maximum of two laboratories to participate in this PT programme. A non-APLAC member may nominate a maximum of one laboratory to participate in the PT programme.

Nominated Laboratory 1

Name of Institute	:	
Name of Laboratory/Department	:	
Salutation	:	Dr / Mdm / Mr / Mrs / Ms / Professor *
Name of Contact Person	:	
Designation	:	
Email Address	:	
Telephone Number	:	
Fax Number	:	
Postal Address	:	
Postal Code	:	
City	:	
Country	:	
Is the nominated laboratory accredited?	( )	Yes (In accordance with ISO/IEC 17025 / ISO 9001 / Others: _____)*
	( )	No

* Please delete where appropriate

For use by Coordinator only:

Nominated Laboratory 2

Name of Institute	:	
Name of Laboratory/Department	:	
Salutation	:	Dr / Mdm / Mr / Mrs / Ms / Professor *
Name of Contact Person	:	
Designation	:	
Email Address	:	
Telephone Number	:	
Fax Number	:	
Postal Address	:	
Postal Code	:	
City	:	
Country	:	

Is the nominated laboratory accredited?	( ) Yes (In accordance with ISO 9001 / ISO/IEC 17025 / Others: _____)*

* Please delete where appropriate

For use by Coordinator only:

The Accreditation Body and the laboratories will be informed on the acceptance of their participation in the PT programme after the deadline for nomination. A Registration Form For Participating Laboratory will be sent to the laboratories for completion.

Completed by : _____

Signature : _____

Date : _____

APPENDIX V: Registration Form for Participating Laboratory

Please complete this form and return it to the Coordinator by **16 November 2015**:

Attn: Ms Lee Jia Juan, Singapore Accreditation Council
Email: lee_jia_juan@spring.gov.sg

Section A: Contact details of participating laboratory and personnel to whom the PT package will be dispatched.

Name of Laboratory/Department	:	
Salutation	:	Dr / Mdm / Mr / Mrs / Ms / Professor *
Name of Contact Person	:	
Designation	:	
Email Address	:	
Telephone Number	:	
Fax Number	:	
Postal Address (for PT Package)	:	
Postal Code	:	
City	:	
Country	:	
Name of Accreditation Body For the Laboratory	:	

* Please delete where appropriate

Section B: Confirmation of Participation

I, on behalf of my laboratory, agree to participate in the above APLAC Proficiency Testing programme.

Name : _____
Designation : _____
Name of Laboratory/Department : _____
Name of Institute : _____
Email Address : _____

Our Laboratory would like to register for participation in the following analyte(s) [please tick (✓)]:

Signature : _____

Date : _____

APPENDIX VI: Instructions for Accreditation Bodies

1. Nomination of Participating Laboratories

Each Accreditation Body (AB) who is an APLAC member may nominate a maximum of two laboratories to participate in the programme. An AB who is a non-APLAC member can nominate a maximum of one laboratory to participate in the programme. Due to the limited number of Proficiency Testing (PT) samples available, the total number of participating laboratories for this PT programme will be restricted to about 80. The Coordinator will, as far as possible, allow at least one laboratory from each AB to participate in the PT programme. Priority will be given to accredited laboratories. The participating laboratories will then be selected on a first-come, first-served basis.

The Coordinator will notify the ABs and the laboratories on the acceptance of their participation in the PT programme after the deadline for nomination. A Registration Form For Participating Laboratory will be sent to the laboratories for completion and must be returned to the Coordinator before the deadline for registration. The participating laboratories are allowed to register for one or both of the analytes, depending on their services.

2. Sample Receipt and Distribution

The PT packages (each comprising one packet of PT sample, one bottle of acesulfame potassium certified reference material and one bottle of sucralose certified reference material) will be delivered to the ABs. The ABs will be responsible for distributing the PT packages to their respective participating laboratories.

The PT packages will be delivered to the ABs under non-controlled temperature conditions. A temperature strip will be pasted on each package to enable the recipient to check if it has been exposed to a high temperature. The AB should then promptly acknowledge receipt of the PT packages to the Coordinator by returning the Sample Receipt Form For Accreditation Body. The AB should not break the seal to remove any items from the PT package.

The PT samples and CRMs should be stored between 18 ^{o}C to 25 ^{o}C if they are not immediately distributed to the participating laboratories.

3. Contact Details of Coordinator

Ms Lee Jia Juan
Senior Manager
Singapore Accreditation Council
SPRING Singapore
2 Fusionopolis Way
#15-01, Innovis
Singapore 138634

Telephone: +65 6279 3672

Fax:+ 65 6659 0640
Email: lee_jia_juan@spring.gov.sg

APPENDIX VII: Instructions for Participating Laboratories

1. About the Proficiency Testing Sample

The Proficiency Testing (PT) material was prepared by the Chemical Metrology Laboratory (CML), Health Sciences Authority (HSA), Singapore by fortifying cake mix flour (purchased from a local supermarket) with acesulfame potassium and sucralose. The fortified cake mix flour was then adequately homogenised by mixing the flour in a drum mixer over 14 days before being distributed into polyethylene pouches under an inert atmosphere and controlled conditions (temperature and humidity). About 140 packets of the PT samples were prepared and stored between 18 °C and 25 °C. The mass fraction ranges of acesulfame potassium and sucralose are expected to be 500 – 1400 mg/kg and 500 – 1200 mg/kg, respectively.

The PT samples were evaluated for homogeneity and stability (at storage temperature of 18 °C to 25 °C for 3 months and maximum allowable transportation of 40 °C for 1 month) with reference to ISO 13528:2005. Statistical evaluations showed that the PT samples were sufficiently homogeneous and stable for the programme. The stability of the PT samples under the storage condition will be evaluated again before dispatch.

Each participating laboratory will be provided with one packet of the PT sample (about 50 g). In addition to the PT sample, pure substance certified reference materials (CRMs) of acesulfame potassium and sucralose (about 250 mg each) from HSA will also be provided. The CRMs should be used as calibrants for the determination of the analytes. The certified mass fraction values and associated expanded uncertainties of the pure substance CRMs are shown in Table 1.

Table 1: Information on pure substance CRMs of acesulfame potassium and sucralose

Pure substance CRM	CRM Code	Certified mass fraction value (mg/g)*	Expanded uncertainty (mg/g) at approx. 95% confidence level with coverage factor, k = 2)
Acesulfame potassium	HRM-1012A	999.2	5.0
Sucralose	HRM-1015A	985.4	4.8

*A certified mass fraction value of 1,000 mg/g is equivalent to a purity value of 100 %.

2. Storage of Proficiency Testing Sample and Certified Reference Materials

The PT packages will be delivered to the participating laboratories by their Accreditation Body (AB) under non-controlled temperature conditions. A temperature strip will be pasted on each package to enable the laboratories to check if it has been exposed to a high temperature. In addition, the laboratory should inspect the packages for any physical damage, then promptly acknowledge receipt of the PT packages or report any damages to the Coordinator by returning the Sample Receipt Form For Participating Laboratory.

The PT samples and CRMs should be stored between 18 ^{o}C to 25 ^{o}C. After opening the PT sample and CRMs, the polyethylene pouch and bottles should be resealed and recapped tightly.

3. Instruction for Use and Analysis

The participating laboratories are expected to apply the methods/procedures which they would normally use for routine analysis to determine the analytes in the PT sample. The CRMs provided shall be used as calibrants.

The participating laboratories should take a minimum of three subsamples from a minimum sample size of 5 g each for analysis.

It is essential for the participation laboratories to use the CRMs provided as calibrants to ensure that their results are included for performance evaluation. The laboratories may re-analyse the PT sample using their own standards as calibrants. However, the results will only be used for comparison purposes and not for performance evaluation.

4. Reporting and Submission of Results

The participating laboratories are expected to report the mass fraction of acesulfame potassium and sucralose using a Result Proforma. All results should be reported in mg/kg.

The participating laboratories shall report results obtained from the CRMs provided. The participating laboratories may report results obtained from their own standards but these results must be reported separately in the Result Proforma.

The uncertainty of measurement should be reported with a confidence level of approximately 95 %. The expanded uncertainty is to be reported to 2 significant figures and the results rounded off to the same number of decimal place(s) as the expanded uncertainty.

The participating laboratories are required to provide technical details of their methodologies in the Result Proformas. The completed Result Proformas must be returned to the Coordinator before the given deadline.

5. Safety and Handling

The PT sample and CRMs are intended for chemical analyses only. Disposal of the PT sample and CRMs should be carried out in accordance with existing regulations on waste management in the participating laboratories' country.

6. Evaluation of Performance of Participating Laboratories

The performance of the participating laboratories will be primarily assessed using the *z*-score. For laboratories which report the uncertainties of their results, the ζ-scores will also be calculated. The calculations of *z*-score and ζ-score are as follows:

(a) *z*-score: $z = \frac{x - X}{\sigma_{PT}}$

where, x is participating laboratory's result, X is the assigned value determined by HSA CML and σ_{PT} is the standard deviation for proficiency assessment.

A *z*-score with absolute value of: $|z| \leq 2.0$ is satisfactory
$2.0 < |z| < 3.0$ is questionable
$|z| \geq 3.0$ is unsatisfactory

(b) ζ-score: $\zeta = \frac{x - X}{\sqrt{u_x^2 + u_X^2}}$

where, u_x is the standard uncertainty of the participating laboratory's result x and u_X is the standard uncertainty of the assigned value X determined by HSA CML.

A ζ-score with absolute value of: $|\zeta| \leq 2.0$ is satisfactory
$2.0 < |\zeta| < 3.0$ is questionable
$|\zeta| \geq 3.0$ is unsatisfactory

The ζ-score provides an indication of whether a participating laboratory's estimate of uncertainty is consistent with the observed deviation from the assigned value. It is complementary to the *z*-score in the assessment of laboratory performance.

The σ_{PT} for both analytes will be determined using the robust standard deviation of the results reported by all the participating laboratories, calculated using Algorithm A in ISO 13528:2005.

Both z-score and ζ-score for the reported result(s) obtained using the CRMs provided and the participating laboratories' own standards will be calculated. However, only the results obtained from the use of the CRMs provided will be used to evaluate the performance of the participating laboratories.

7. Results Confidentiality

Each participating laboratory will be assigned a unique and confidential laboratory code. Both the identity and the code will only be known to staff of the Singapore Accreditation Council involved in the PT programme and the AB of the participating laboratory.

8. Collusion and Falsification of Results

It is the responsibility of the participating laboratory to avoid collusion of results. Collusion between participating laboratories will lead to the exclusion of the laboratories' results from the reports. In addition, a laboratory found to be falsifying results will have its results excluded from the reports.

9. Late Submission of Result Proforma

Participating laboratories should return their completed Result Proforma by the specified deadline in order to ensure that their results are included in the data analysis and report. Performance evaluation will not be carried out on results received after the reporting deadline.

10. Re-submission of Results

Re-submission of the Result Proforma due to modification of any information will not be accepted. Participating laboratories should ensure that all information in the Result Proforma are thoroughly checked before submission and submitted information are considered final.

11. Fees Payable

There is no participation fee for this PT programme for the participating laboratories nominated by their ABs.

12. References

ISO 13528:2005 Statistical methods for use in proficiency testing by interlaboratory comparisons, International Standards Organisation, Switzerland.

13. Contact Details of Coordinator

Ms Lee Jia Juan
Senior Manager
Singapore Accreditation Council
SPRING Singapore
2 Fusionopolis Way
#15-01, Innovis
Singapore 138634

Telephone: +65 6279 3672
Fax:+ 65 6659 0640
Email: lee_jia_juan@spring.gov.sg

APPENDIX VIII: Sample Receipt Form for Accreditation Body

Upon receipt of the Proficiency Testing (PT) package(s), please check to confirm that the seal is intact and that it was not exposed to a high temperature. Do not break the seal to remove any items from the PT package(s).

Please complete this form and promptly return it to the Coordinator:

Attn: Ms Lee Jia Juan, Singapore Accreditation Council
Email: lee_jia_juan@spring.gov.sg

Section A: Information on Recipient

Name of Accreditation Body	:	
Salutation	:	Dr / Mdm / Mr / Mrs / Ms / Professor *
Name	:	
Designation	:	
Email Address	:	
Date of Receipt	:	

* Please delete where appropriate

Section B: Condition of Items in PT Package(s)

Please tick (✓) where appropriate.

Name of laboratory on PT package : _____

S/N	Items	Conditions
1	Condition of seal on the box	( ) Intact ( ) Broken ( ) Missing
2	Indication of transportation temperature on temperature strip	( ) Did not exceed 40 °C ( ) Exceeded 40 °C

Name of laboratory on PT package : _____

S/N	**Items**	**Conditions**
1	Condition of seal on the box	( ) Intact ( ) Broken ( ) Missing
2	Indication of transportation temperature on temperature strip	( ) Did not exceed 40 °C ( ) Exceeded 40 °C

Received by : _____

Signature : _____

Date : _____

APPENDIX IX: Sample Receipt Form for Participating Laboratory

Upon receipt of the Proficiency Testing (PT) package, please check to confirm that the seal and items are intact and that it was not exposed to a high temperature.

Please complete this form and promptly return it to the Coordinator:

Attn: Ms Lee Jia Juan, Singapore Accreditation Council
Email: lee_jia_juan@spring.gov.sg

Section A: Information on Recipient

Name of Institute	:	
Laboratory Code	:	
Name of Laboratory/Department	:	
Salutation	:	Dr / Mdm / Mr / Mrs / Ms / Professor *
Name	:	
Designation	:	
Email Address	:	
Date of Receipt	:	

* Please delete where appropriate

Section B: Condition of Items in PT Package

Please fill in the blanks (_ _ _) and tick (✓) where appropriate.

S/N	Items on PT package	Condition
1	Seal on the box	( ) Intact ( ) Broken ( ) Missing
2	Indication of transportation temperature on temperature strip	( ) Did not exceed 40 °C ( ) Exceeded 40 °C

	Items in PT package		
	PT sample	Acesulfame potassium CRM (HRM-1012A)	Sucralose CRM (HRM-1015A)
	STY-0053-001-(_ _ _)	STY-0055-001-(_ _ _)	STY-0054-001-(_ _ _)

Conditions	( ) Intact ( ) Broken ( ) Missing	( ) Intact ( ) Broken ( ) Missing	( ) Intact ( ) Broken ( ) Missing

Received by : _____

Signature : _____

Date : _____

APPENDIX X: Result Proforma

NOTE: Please check all information provided in this form thoroughly before submitting. The information given is considered final once submitted and no further changes will be allowed.

Please complete and submit this form (in WORD format) to the Coordinator by **29 April 2016**:

Attn: Ms Lee Jia Juan, Singapore Accreditation Council
Email: lee_jia_juan@spring.gov.sg

Results received after the above deadline will not be evaluated.

Section A: Information on Participating Laboratory

Name of Institute	:	
Name of Laboratory/Department	:	
Submitted By	:	
Designation	:	
Email Address	:	

* Please delete where appropriate

Signature : _____

Date : _____

For use by Coordinator only:

Please complete Section B1. If your laboratory also re-analysed the PT sample using your own source of calibration standards, please complete Section B2.

(Please note that only the result from Section B1 will be used for performance evaluation. Results from Section B2 are used only for comparison purposes).

Section B1: Summary of Results Obtained Using CRMs Provided as Calibrants

	Acesulfame Potassium	**Sucralose**
Overall mean of results (mg/kg)		
No. of subsamples		
Combined standard uncertainty (mg/kg)		
Coverage factor, k		
Level of confidence (%)		
Expanded uncertainty (mg/kg)		

Note: All results should be reported in mg/kg. The uncertainty of measurement should be reported with a minimum confidence level of 95 %.The expanded uncertainty is to be reported to 2 significant figures and the result rounded off to the same number of decimal place(s) as the expanded uncertainty.

Section B2: Summary of Results Obtained Using Laboratory's Own Source of Calibration Standards

	Acesulfame Potassium	**Sucralose**
Overall mean of results (mg/kg)		
No. of subsamples		
Combined standard uncertainty (mg/kg)		
Coverage factor, k		
Level of confidence (%)		
Expanded uncertainty (mg/kg)		

Note: All results should be reported in mg/kg. The uncertainty of measurement should be reported with a minimum confidence level of 95 %.The expanded uncertainty is to be

reported to 2 significant figures and the result rounded off to the same number of decimal place(s) as the expanded uncertainty.

Please provide information on your laboratory's own source of calibration standards.

	Acesulfame Potassium	**Sucralose**
Source of calibration standard		
Purity value and its units		
Expanded uncertainty of purity value and its units		
Did you verify the calibration standard before use? If yes, please		

Note: Both z-score and ζ-score for the reported result(s) obtained using the CRMs provided and the participating laboratories' own calibration standards will be calculated. However, only the results obtained from the use of the CRMs provided will be used to evaluate the performance of the participating laboratories.

Section C: Analytical Methods

Please delete where appropriate and provide additional information in the lines below

	Acesulfame potassium		Sucralose
(1) Sample size used (g)	.....		.....
(2) Sample treatment			
(i) Extraction technique	Liquid-liquid (shaking) / Liquid-liquid (ultrasonic) / Solid phase extraction / Pressurised fluid extraction / Others (please specify):		Liquid-liquid (shaking) / Liquid-liquid (ultrasonic) / Solid phase extraction / Pressurised fluid extraction / Others (please specify):
(ii) Extraction solvent(s) and reagent(s) used	....		....
(iii) Clean-up	Yes / No (if Yes, please describe)		Yes / No (if Yes, please describe) ....

(3) Analytical instrument(s)	HPLC-UV / HPLC-ELSD / LC-MS/ LC-MS/MS / HPIC-ELCD / HPIC-UV / MEKC-UV / Others (please specify):	HPLC-UV / HPLC-ELSD / LC-MS/ LC-MS/MS / HPIC-ELCD / HPIC-UV / MEKC-UV / Others (please specify):
(i) Instrument condition (e.g. column and mobile phase used in LC, ions monitored in MS, ionisation mode in MS)		
(4) Calibration method	External calibration / Internal Calibration / Isotope dilution mass spectrometry / Standard addition / Others (please specify):	External calibration / Internal Calibration / Isotope dilution mass spectrometry / Standard addition / Others (please specify):
(5) Internal standard(s) used (if any)	Yes / No (if Yes, please specify)	Yes / No (if Yes, please specify)
(6) Investigation of method recovery	Yes / No (if Yes, please state recovery in %)	Yes / No (if Yes, please state recovery in %)
(i) Was correction for method recovery made	Yes / No	Yes / No

(7) Test method employed	Accredited / Not accredited (If the test method is accredited, please state standard method used)		Accredited / Not accredited (If the test method is accredited, please state standard method used) .....

Section D: Any Other Information

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