Asia Pacific Laboratory Accreditation Cooperation

APLAC T083 Preservatives in Soy Sauce Proficiency Testing Program

Final Report

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1. Introduction

This report summarizes the results of **APLAC T083 Preservatives in Soy Sauce** Proficiency Testing Program involving testing laboratories for the Asia Pacific Laboratory Accreditation Cooperation (APLAC).

2. Program Feature

(a) APLAC, EA and ILAC members were invited to participate in this proficiency testing program. There were total 22 laboratory participants nominated by 8 accreditation bodies (ABs) in this program. All samples were sent to the ABs and then transferred to the participating laboratories.

Table 1 Summary of Economies and Participants

Economy	No. of laboratories nominated	No. of laboratories returning result
Hong Kong, China (HKAS)	3	3
Malaysia(MS)	1	1
Singapore (SAC)	3	3
Australia (NATA)	2	2
Viet Nam (BoA)	3	3
Japan (JAB)	3	3
New Zealand (IANZ)	1	1
Taiwan (TAF)	6	6
Total	**22**	**22**

(b) Participating laboratories were supplied two bottles. Each bottle contains about 80 mL of soy sauce.

(c) Each participating laboratory was required to analyse the preservative concentration of Benzoic acid, Sorbic acid, Methyl p-hydroxy benzoate and Butyl p-hydroxy benzoate.

(d) Laboratories were requested to complete the test procedures for preservative in soy sauce according to their copy of the Instructions to Participants and to record their results on the accompanying Results Sheet, both of which were

distributed to participants with the samples. (The Instructions and the Results Sheet are shown in Appendix C)

(e) Each laboratory was randomly allocated a unique code number for the program to enable confidentiality of results. Its code number makes reference to each laboratory in this report.

3. Content of Appendices

APPENDIX A contains:

(a) The results and measurement uncertainties of test samples reported by the participants and the calculated z-score result for each test on each sample.

(b) A table of robust statistics for each test, such as number of results, median, robust average, standard deviation estimated from Horwitz equation, minimum, maximum and range.

(c) Z-scores and ordered z-score charts calculated for laboratories for each test.

(d) The information of test method reported for each laboratory.

APPENDIX B describes the sample preparation details and the homogeneity/ stability testing procedure of test samples.

APPENDIX C contains a copy of the *Instructions to Participants* and the *Results Sheet*, as supplied to participants.

APPENDIX D shows the details of statistical procedures and the calculations and formulae of statistic parameters.

4. Statistical design of the program

Two soy sauce samples were prepared. The two samples were labeled with Sample A and Sample B.

The robust statistical procedures were used to generate the z-score and a summary of statistics for the sample-number of results, original relative standard deviation, final relative standard deviation, robust average (x^*) and standard deviation estimated from Horwitz equation (s^*).

For each laboratory, z-score was calculated for each returned result. We can use the z-score of each laboratory to understand the degree how the result of that

laboratory deviates from the population. If the absolute z-score of the testing result is larger than three (| z-score | ≧3), it represents that the testing result of that laboratory is a unsatisfactory result and the laboratory will be denoted by "※". For the testing result of the laboratory with 2 < | z-score | < 3, one should make further assessments for the test procedure.

5.Assigned values and uncertainty

Assigned values were robust mean (x^*) calculated from participating laboratories results based on robust statistics in ISO 13528.

Sample A

	Benzoic acid	Sorbic acid No additives	Methyl p-hydroxy benzoate	Butyl p-hydroxy benzoate
Data submitted	22	20	14	10
Range(mg/kg)	0.452-863.993	ND-266	241-381	79.93-89.6
Median(mg/kg)	262	–	260.5	84.484
Robust mean(mg/kg)	263.5	–	259.8	84.40
Horwitz SD(mg/kg)	18.1	–	17.9	6.90
Robust standard deviation(mg/kg)	22.1	–	14.5	2.97
Uncertainty (mg/kg ,95 %)	5.6	–	4.8	1.2

Sample B

	Benzoic acid	Sorbic acid	Methyl p-hydroxy benzoate	Butyl p-hydroxy benzoate
Data submitted	22	20	14	10
Range(mg/kg)	0.23-1852	16-286	269-888.2	72.5-83.6
Median(mg/kg)	517.51	250.42	355.7	78.31
Robust mean(mg/kg)	515.5	250.4	359.8	78.82
Horwitz SD(mg/kg)	32.1	17.4	23.6	6.50
Robust standard deviation(mg/kg)	41.2	9.8	23.2	3.64
Uncertainty (mg/kg ,95 %)	11.0	2.7	7.8	1.4

6.Statistical Evaluation

(a)Performance of the participating laboratories is assessed using z-score which is calculated as:

$$z = \frac{(x_i - \bar{x})}{\sigma}$$

xi= reported result of individual participant

$\bar{x}$= robust mean

σ= standard deviation estimated from Horwitz equation

Horwitz equation $RSD=2^{(1-0.5\log C)}$

Where C=measured concentration of analyte expressed as a decimal

(b) The criteria for performance evaluation for z-scores as per ISO/IEC 17043 should be:

(i) $|z| \leq 2$: Satisfactory

(ii) $2 < |z| < 3$: Questionable

(iii) $|z| \geq 3$: Unsatisfactory

(c) Participants having $|z| \geq 3$ are regarded as giving unsatisfactory results and they should investigate the cause and take necessary follow up actions. Participants having z-score(s) in the range $2 < |z| < 3$ are encouraged to review their results.

(d) Uncertainty of assigned values was estimated from robust standard deviation(s^*) of robust statistics based on $1.25s^*/\sqrt{n}$, n was data submitted.

7. Satisfactory Results

A z-score tells how a single data point compares to normal data. A z-score says not only whether a point was above or below average, but how unusual the measurement is. The outliers for the feedback results of the participating laboratories are shown in Table 2 as below; and the detailed information of the test results, statistics and test procedures were referred to the summary of results in appendix A.

Table 2 List of satisfactory results

$\|z\| \leq 2$	Lab Code	The Proportion of Satisfactory (%)
Sample A		
Benzoic acid	1,2,3,4,6,7,11,12, 13,14,15,16,17,18, 19,20,21,22	81.8 %
Sorbic acid	---	---
Methyl p-hydroxy benzoate	2,3,4,11,12,15,16, 18,19,20,21,22	85.7 %
Buthyl p-hydroxy benzoate	2,3,4,,9,11,12,18, 19,21,22	100 %
Sample B		
Benzoic acid	1,2,3,4,6,7,9,11,12, 13,14,15,16,17,18, 19,20,21,22	86.4 %
Sorbic acid	1,2,3,4,6,7,11,12, 13,14,15,16,17,18, 19,20,21,22	90.0 %
Methyl p-hydroxy benzoate	2,3,4,9,12,15,16,18, 20,21,22	78.6 %
Buthyl p-hydroxy benzoate	2,3,4,9,11,12,18,,19, 21,22	100 %

8. Technical Comments

Total of 22 laboratories registered for this proficiency testing program on preservatives. Each participating laboratory received two test samples after registration. A maximum of four types of preservatives (benzoic acid, sorbic acid, methyl p-hydroxy benzoate, and butyl p-hydroxy benzoate) were added to each sample. The participating laboratories to conduct their analysis using their unique testing methods (such as the HPLC method and the GC method). The analysis

results and statistics for the two test samples are shown in Appendix A Tables 1.1 to 1.8. The z-score distribution and histogram are presented in Figs. 1.1 to 1.2.

The z-scores were used to assess the deviation of the each test results and the general average. As shown in Appendix A Table 1.1, the results of the 22 participating laboratories for Sample A-Benzoic acid, Lab Code 5, 8, and 10 displayed unsatisfactory test results, with | z-score | $\geq$ 3. Lab Code 8, in particular, had a comparatively smaller data, which may have been caused by erroneous data or units. The test result for Lab Code 9 was considered questionable with the z-score in the range of 2 < | z-score | < 3. Lab Code 9 should make further assessments for the test procedure.

Table 1.3 shows the test results of 14 participating laboratories for Sample A-Methyl p-hydroxy benzoate. Among them, Lab Code 5 had the z-score of | z-score | $\geq$ 3, indicating an unsatisfactory test result. The test result for Lab Code 9 was considered questionable with the z-score in the range of 2< | z-score | < 3. Lab Code 9 should make further assessments for the test procedure.

The test results of all 10 participating laboratories for Sample A-Butyl p-hydroxy benzoate, as shown in Table 1.4, were satisfactory at | z-score | $\leq$ 2.

As shown in Table 1.5, of the results of the 22 participating laboratories for Sample B-Benzoic acid, Lab Code 5, 8, and 10 displayed an unsatisfactory result, with | z-score | $\geq$ 3. Lab Code 8, in particular, had a comparatively smaller data, which may have been caused by erroneous data or units.

Among the results of the 20 participating laboratories for Sample B-Sorbic acid shown in Table 1.6, Lab Code 5 had the z-score of | z-score | $\geq$ 3, indicating an unsatisfactory test result. The test result for Lab Code 9 was considered questionable with the z-score in the range of 2< | z-score | < 3. Lab Code 9 should make further assessments for the test procedure.

Among the results of the 14 participating laboratories for Sample B-Methyl p-hydroxy benzoate shown in Table 1.7, Lab Code 5,11, and 19 had the z-score of | z-score | $\geq$ 3, indicating an unsatisfactory test result.

Finally, the test results for all 10 participating laboratories for Sample B-Butyl p-hydroxy benzoate, as shown in Table 1.8, had the z-score of | z-score | $\leq$ 2, indicating a satisfactory result.

In summary, excluding the two samples from Lab Code 8 that had smaller benzoic acid datas, which may have been caused by erroneous data or units, those with | z-score | $\geq$ 3 should review their results considering factors such as the primary operator, analytical methods, and procedures.

Additionally, based on previous sample preparation results in this proficiency testing program, if a sample contains two preservatives (sorbic acid and methyl p-hydroxy benzoate), sorbic acid, which has the same retention time as methyl p-hydroxy benzoate, can act as an interference that affects the quantitation of methyl p-hydroxy benzoate during testing for methyl p-hydroxy benzoate. Therefore, the mobile phase ratio for HPLC must be carefully adjusted. Only three types of preservatives (benzoic acid, methyl p-hydroxyl benzoate, and butyl p-hydroxy benzoate) were added to Sample A, whereas four types of preservatives (benzoic acid, sorbic acid, methyl p-hydroxyl benzoate, and butyl p-hydroxy benzoate) were added to Sample B. According to the results for Samples A and B (methyl p-hydroxy benzoate) presented in Tables 1.3 and 1.7, Lab Code 11 and 19 found no discrepancies in Sample A data; however, Sample B had a larger data, which contributed to the z-score of | z-score | $\geq$ 3. This was probably caused by the interference from sorbic acid in Sample B.

Appendix A

Summary of Results

Information of Test

Table 1.1 Laboratories' Test Results for **Benzoic acid** of Sample A

Sample A- Benzoic acid						
No	**Lab Code**	**Result (mg/kg)**	**Combined standard uncertainty**	**Coverage factor k (95% level of confidence)**	**Expanded Measurement Uncertainty**	**Z-score**
1	**1**	260	<5	–	–	-0.2
2	**2**	240	–	–	–	-1.3
3	**3**	273.87	8.69	2	17.39	0.6
4	**4**	264	–	–	–	0.0
5	**5**	474	9.15	2	18.31	**11.6※**
6	**6**	277.548	0.081	2	0.162	0.8
7	**7**	270	–	–	–	0.4
8	**8**	0.452	0.0147	2	0.0294	**-14.5※**
9	**9**	222	22	K=2	±44	**-2.3**
10	**10**	863.993	–	–	–	**33.1※**
11	**11**	259.167	9.246	2	18.492	-0.2
12	**12**	258.299	12.8584	2	25.7168	-0.3
13	**13**	255	–	–	±26	-0.5
14	**14**	300	–	–	–	2.0
15	**15**	239	8.5	2	17.0	-1.4
16	**16**	252	18	2	35	-0.6
17	**17**	240.340	1	2	2	-1.3
18	**18**	267.602	4.48	2	8.959	0.2
19	**19**	246	3.19%	2	6.38%	-1.0
20	**20**	299	–	2	17.0	2.0
21	**21**	284	8.5	2	±17	1.1
22	**22**	269	–	–	–	0.3

Note:"-" denotes results not provided.

Table 1.2 Laboratories' Test Results for **Sorbic acid** of Sample A

Sample A - Sorbic acid						
No	**Lab Code**	**Result (mg/kg)**	**Combined standard uncertainty**	**Coverage factor k (95% level of confidence)**	**Expanded Measurement Uncertainty**	**Z-score**
1	**20**	<1	–	2	16.8	–
2	**15**	<4	–	2	–	–
3	**16**	ND	–	–	–	–
4	**6**	not detected	0.069	2	0.138	–
5	**5**	266	5.16	2	10.33	–
6	**9**	N.D.	–	–	–	–
7	**3**	<0.10	0.002	2	0.004	–
9	**7**	<10	–		–	–
10	**21**	<5.0	–	–	–	–
11	**13**	0	–		–	–
13	**22**	ND(<10)	–	–	–	–
14	**2**	<10	–	–	–	–
15	**4**	ND(<5)	–	–	–	–
16	**14**	<40	–		–	–
17	**11**	N.D.	–	2	–	–
18	**17**	0	0	0	0	–
19	**19**	N.D.	4.07%	2	8.15%	–
20	**8**	0.230	0.0124	2	0.0248	–
21	**18**	N.D.	–	–	–	–
22	**12**	N.D.	N.D.	N.D.	N.D.	–

Table 1.3 Laboratories' Test Results for **Methyl p-hydroxy benzoate** of Sample A.

Sample A-Methyl p-hydroxy benzoate						
No	**Lab Code**	**Result (mg/kg)**	**Combined standard uncertainty**	**Coverage factor k (95% level of confidence)**	**Expanded Measurement Uncertainty**	**Z-score**
1	**2**	265	–	–	–	0.3
2	**3**	266.00	4.31	2	8.63	0.3
3	**4**	256	–	–	–	-0.2
4	**5**	381	5.60	2	11.19	**6.8※**
5	**9**	304	30	K=2	±60	**2.5**
6	**11**	249.714	2.497	2	2.994	-0.6
7	**12**	255.048	10.5871	2	21.1743	-0.3
8	**15**	250	9.2	2	18.4	-0.5
9	**16**	244	12	2	24	-0.9
10	**18**	268.274	7.08	2	14.167	0.5
11	**19**	267	3.90%	2	7.80%	0.4
12	**20**	247	–	2	15.5	-0.7
13	**21**	266	8.0	2	±16	0.3
14	**22**	241	–	–	–	-1.0

Note:"-" denotes results not provided.

Table 1.4 Laboratories' Test Results for **Butyl p-hydroxy benzoate** of Sample A

Sample A- Butyl p-hydroxy benzoate						
No	**Lab Code**	**Result (mg/kg)**	**Combined standard uncertainty**	**Coverage factor k (95% level of confidence)**	**Expanded Measurement Uncertainty**	**Z-score**
1	**2**	82.2	–	–	–	-0.3
2	**3**	79.93	2.46	2	4.92	-0.6
3	**4**	85.4	–	–	–	0.1
4	**9**	84.6	8.5	K=2	±17	0.0
5	**11**	84.468	0.929	2	1.858	0.0
6	**12**	82.343	3.8525	2	7.7051	-0.3
7	**18**	89.274	2.91	2	5.812	0.7
8	**19**	84.5	–	2	–	0.0
9	**21**	83.0	3.4	2	±6.8	-0.2
10	**22**	89.6	–	–	–	0.8

Note:"-" denotes results not provided.

Table 1.5 Laboratories' Test Results for **Benzoic acid** of Sample B

Sample B -Benzoic acid						
No	**Lab Code**	**Result (mg/kg)**	**Combined standard uncertainty**	**Coverage factor k (95% level of confidence)**	**Expanded Measurement Uncertainty**	**Z-score**
1	**1**	510	270	–	–	-0.2
2	**2**	480	–	–	–	-1.1
3	**3**	534.32	16.96	2	33.92	0.6
4	**4**	520	–	–	–	0.1
5	**5**	254	4.91	2	9.81	**-8.1※**
6	**6**	569.849	0.081	2	0.162	1.7
7	**7**	531				0.5
8	**8**	0.230	0.0147	2	0.0294	**-16.1※**
9	**9**	459	46	K=2	±92	-1.8
10	**10**	1852.033	–	–	–	**41.6※**
11	**11**	507.493	18.105	2	36.210	-0.2
12	**12**	516.023	20.3390	2	40.6780	0.0
13	**13**	524	–	–	±52	0.3
14	**14**	574	–	–	–	1.8
15	**15**	502	17.8	2	35.6	-0.4
16	**16**	484	34	2	68	-1.0
17	**17**	483.631	1	2	2	-1.0
18	**18**	522.163	8.74	2	17.482	0.2
19	**19**	498	3.19%	2	6.38%	-0.5
20	**20**	551	–	2	17.0	1.1
21	**21**	561	17	2	±34	1.4
22	**22**	519	–	–	–	0.1

Note:"-" denotes results not provided.

Table 1.6 Laboratories' Test Results for **Sorbic acid** of Sample B

Sample B - Sorbic acid						
No	**Lab Code**	**Result (mg/kg)**	**Combined standard uncertainty**	**Coverage factor k (95% level of confidence)**	**Expanded Measurement Uncertainty**	**Z-score**
1	**1**	270	–	–	–	1.1
2	**2**	242.0	–	–	–	-0.5
3	**3**	242.4	4.32	2	8.64	-0.5
4	**4**	254.0	–	–	–	0.2
5	**5**	16.0	0.25	2	0.50	**-13.5※**
6	**6**	248.1	0.069	2	0.138	-0.1
7	**7**	255.0	–	–	–	0.3
8	**9**	286.0	28	K=2	±56	**2.1**
9	**11**	250.6	5.964	2	11.927	0.0
10	**12**	250.3	8.0877	2	16.1754	0.0
11	**13**	253.0	–	–	±25	0.2
12	**14**	273.0	–	–	–	1.3
13	**15**	259	9.5	2	18.9	0.5
14	**16**	237	12	2	24	-0.8
15	**17**	241.140	1	2	2	-0.5
16	**18**	253.680	8.73	2	17.461	0.2
17	**19**	247.0	4.07%	2	8.15%	-0.2
18	**20**	248	–	2	16.8	-0.1
19	**21**	264.0	8.7	2	±17	0.8
20	**22**	246.0	–	–	–	-0.3

Note:"-" denotes results not provided.

Table 1.7 Laboratories' Test Results for **Methyl p-hydroxy benzoate** of Sample B

Sample B Methyl p-hydroxy benzoate						
No	**Lab Code**	**Result (mg/kg)**	**Combined standard uncertainty**	**Coverage factor k (95% level of confidence)**	**Expanded Measurement Uncertainty**	**Z-score**
1	**2**	345	–	–	–	-0.6
2	**3**	368.77	5.98	2	11.96	0.4
3	**4**	356	–	–	–	-0.2
4	**5**	269	3.95	2	7.90	**-3.8※**
5	**9**	396	39	K=2	±78	1.5
6	**11**	888.187	8.882	2	17.763	**22.3※**
7	**12**	360.989	13.2206	2	26.4412	0.0
8	**15**	352	13.0	2	26.0	-0.3
9	**16**	341	17	2	34	-0.8
10	**18**	355.402	9.38	2	18.768	-0.2
11	**19**	877	3.90%	2	7.80%	**21.9※**
12	**20**	350	–	2	15.5	-0.4
13	**21**	389	12	2	±24	1.2
14	**22**	337	–	–	–	-1.0

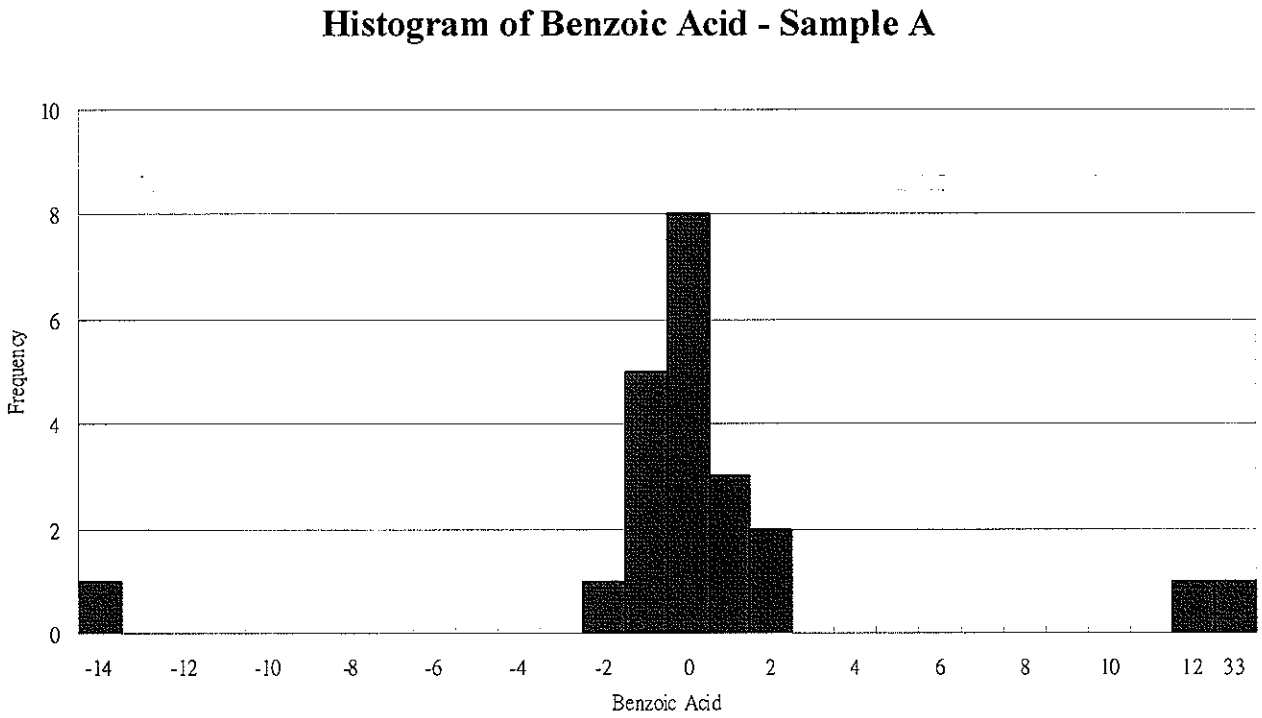
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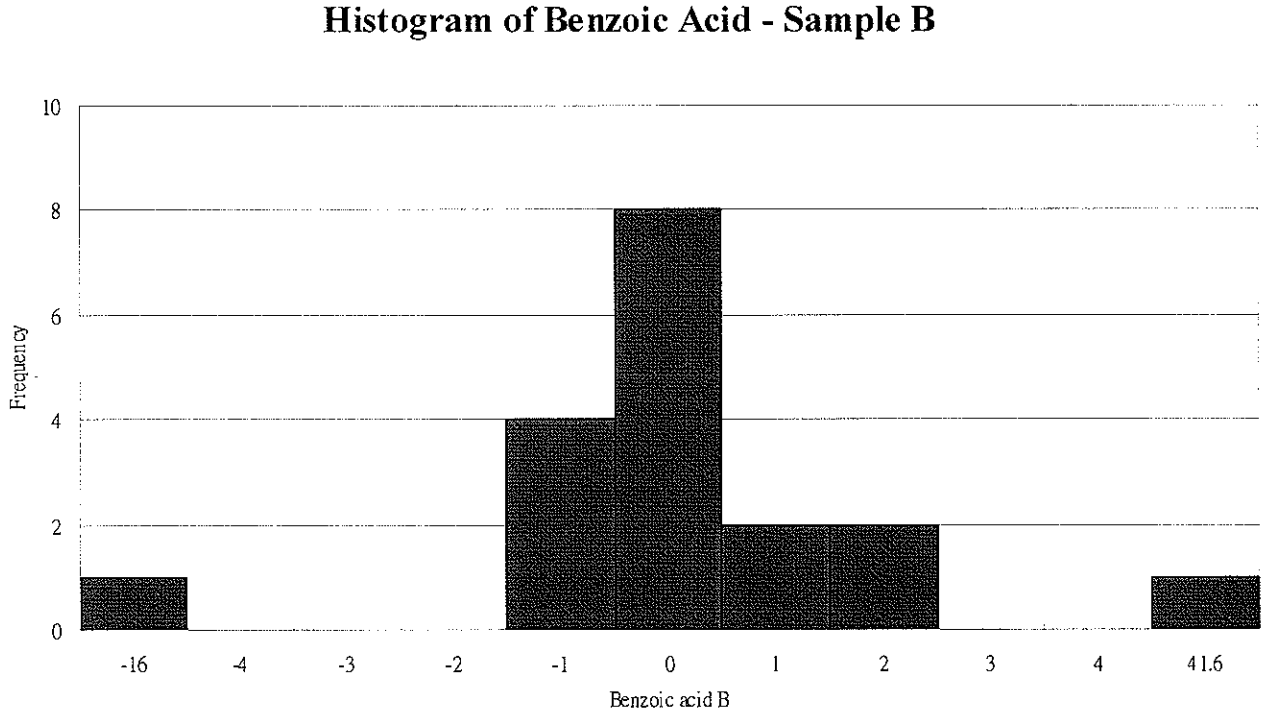
Table 1.8 Laboratories' Test Results for **Butyl p-hydroxy benzoate** of Sample B

Sample B -Butyl p-hydroxy benzoate						
No	Lab Code	Result (mg/kg)	Combined standard uncertainty	Coverage factor k (95% level of confidence)	Expanded Measurement Uncertainty	Z-score
1	**2**	72.5	–	–	–	-1.0
2	**3**	76.05	2.34	2	4.68	-0.4
3	**4**	80.8	–	–	–	0.3
4	**9**	77.2	7.5	K=2	±15	-0.2
5	**11**	78.552	0.864	2	1.728	0.0
6	**12**	78.072	3.6663	2	7.3325	-0.1
7	**18**	81.740	2.66	2	5.322	0.4
8	**19**	77.2	–	2	–	-0.2
9	**21**	83.6	3.4	2	±6.8	0.7
10	**22**	82.0	–	–	–	0.5

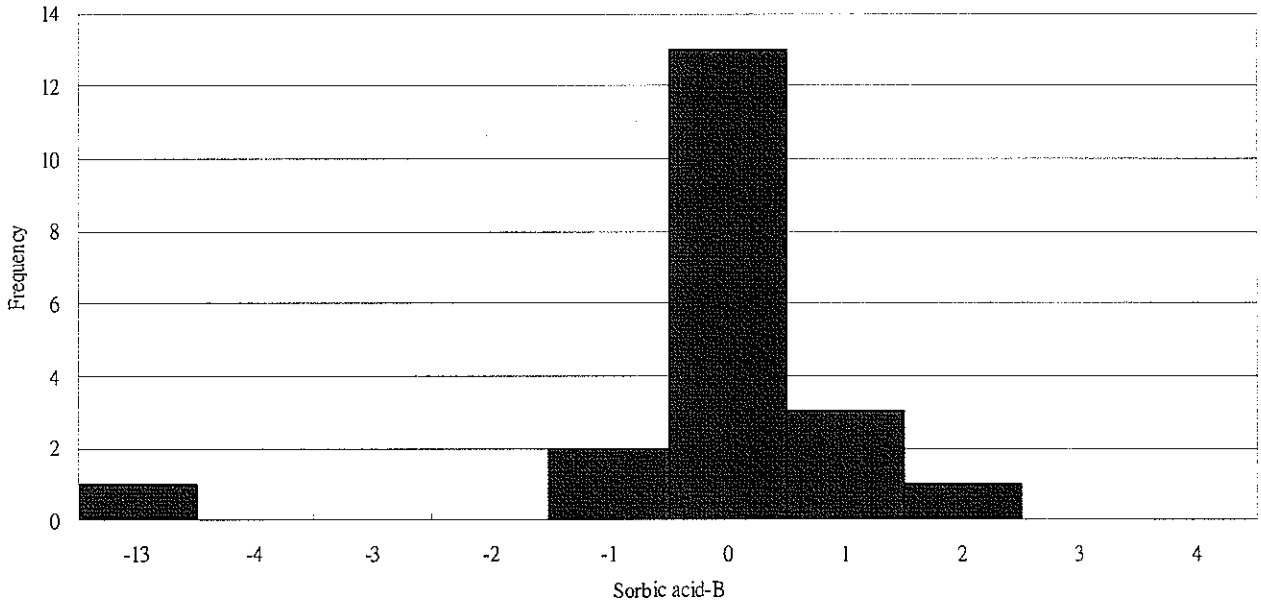
Note:"-" denotes results not provided.

Figure 1.1 Histogram of z-score

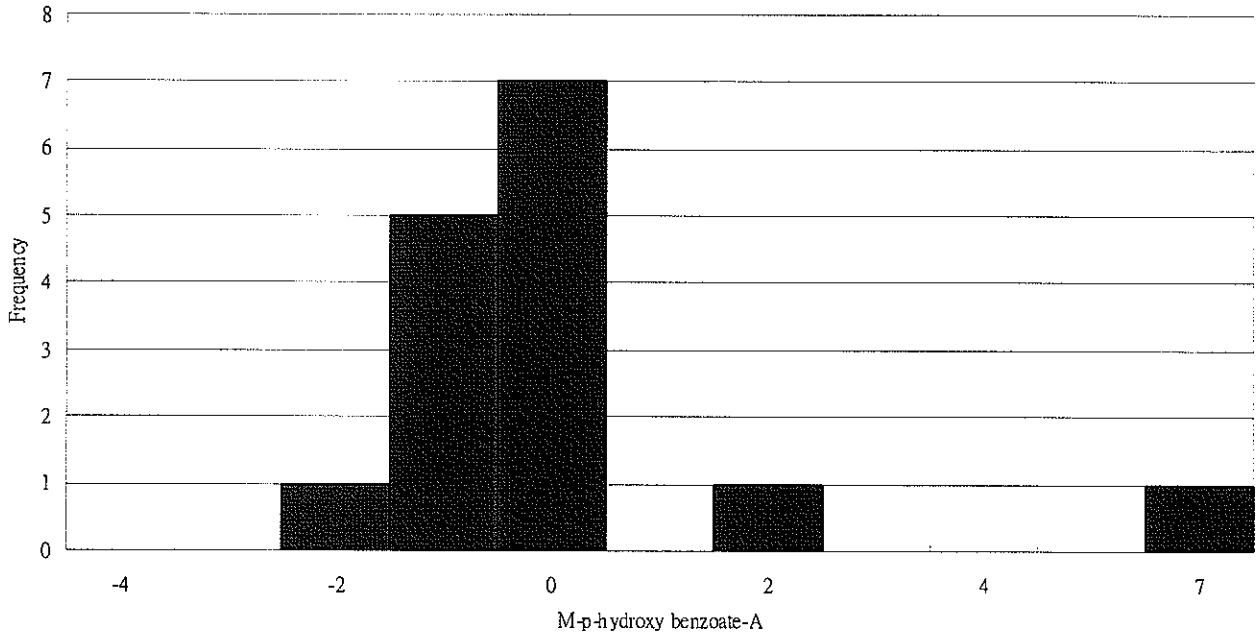




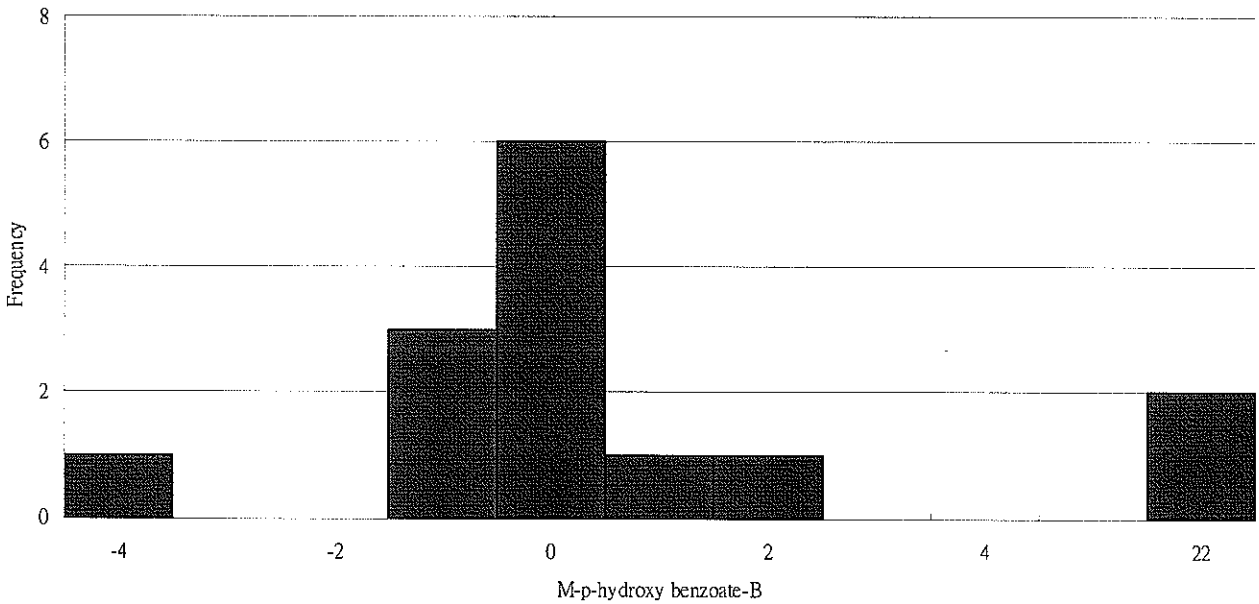
Histogram of Sorbic Acid - Sample B



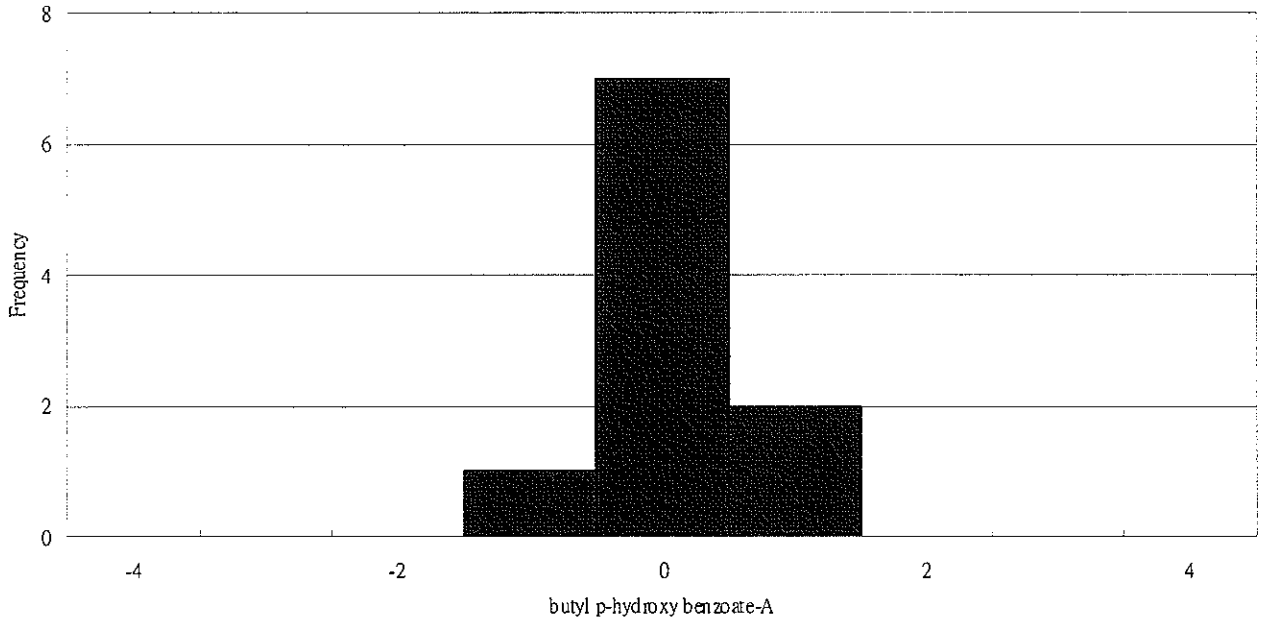
Histogram of Methyl-p-hydroxy Benzoate - Sample A



Histogram of Methyl-p-hydroxy benzoate - Sample B



Hustogram of Butyl p-hydroxy benzoate - Sample A



Histogram of Butyl-p-hydroxy benzoate - Sample B

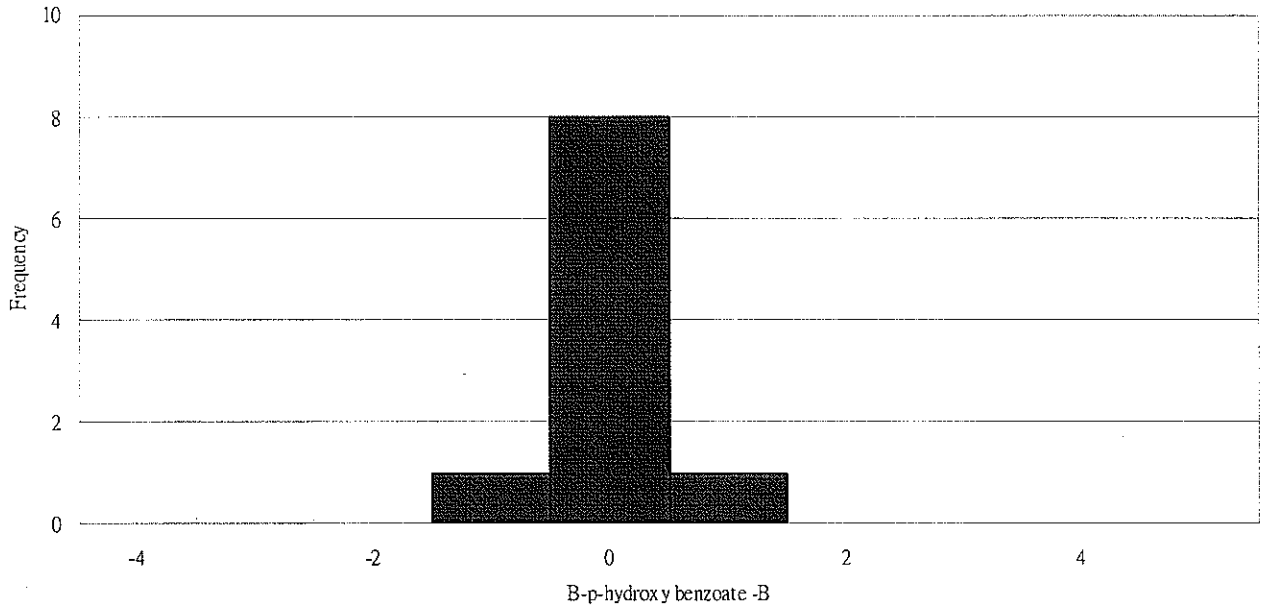
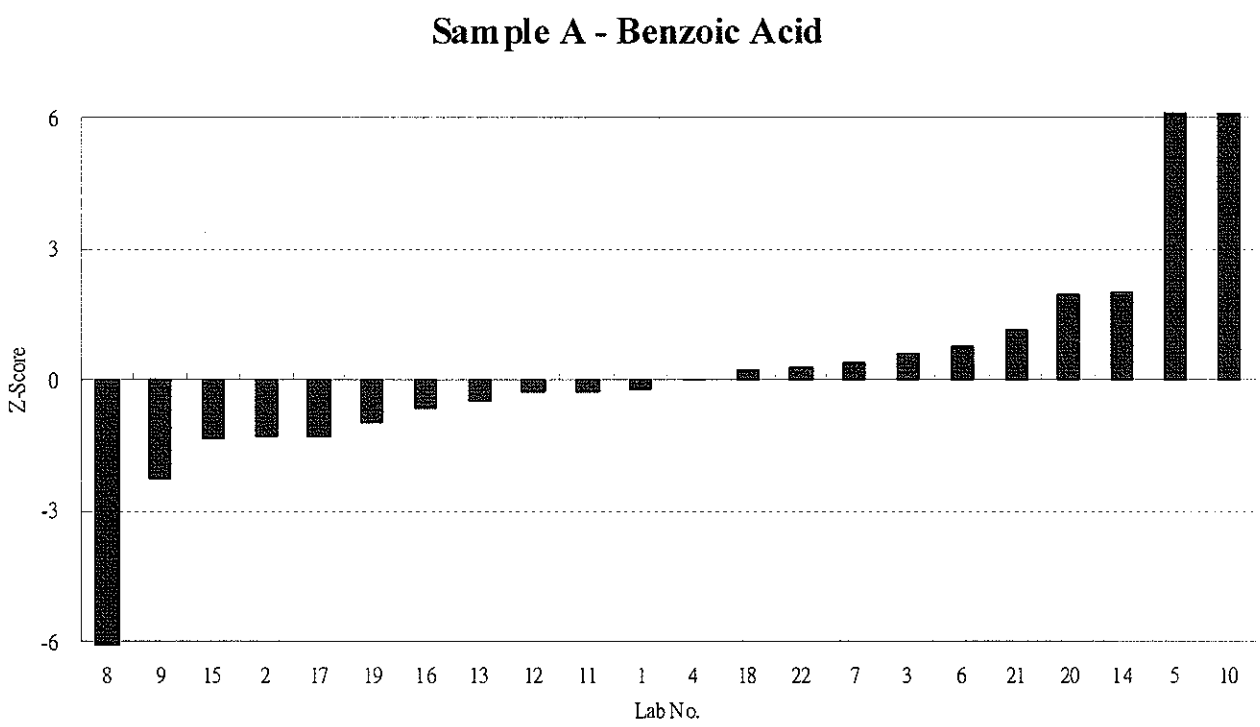
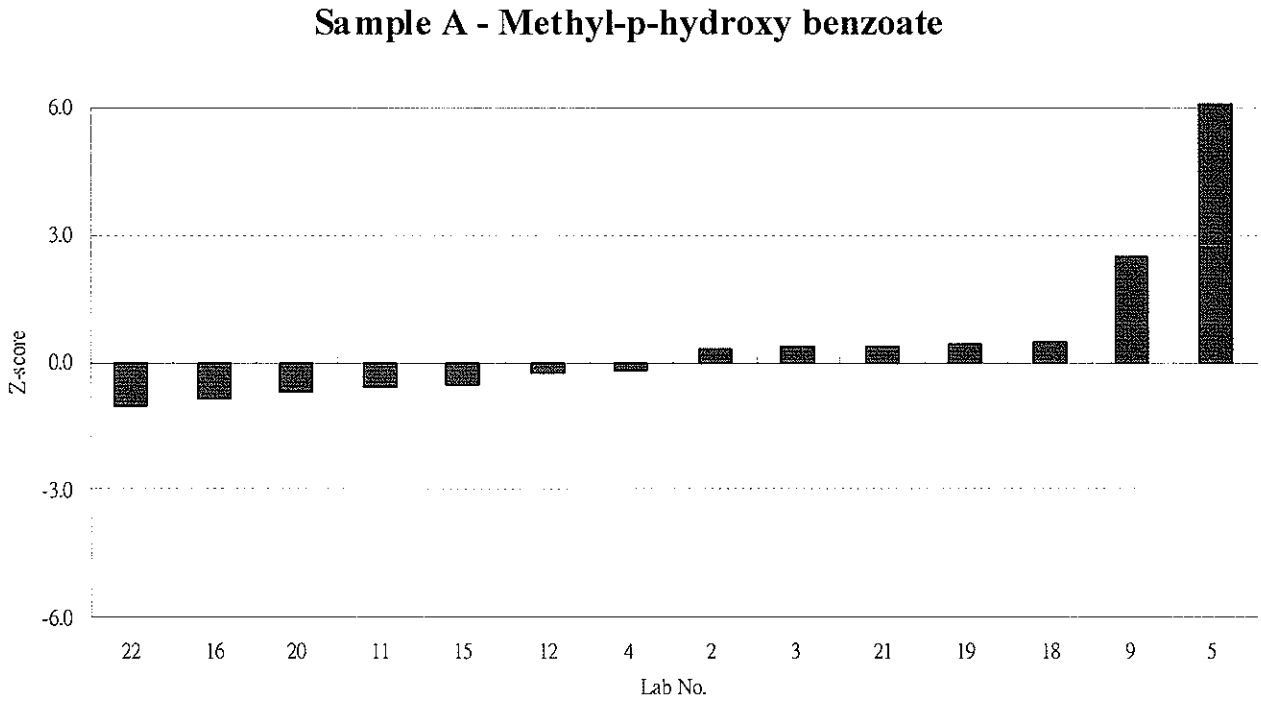
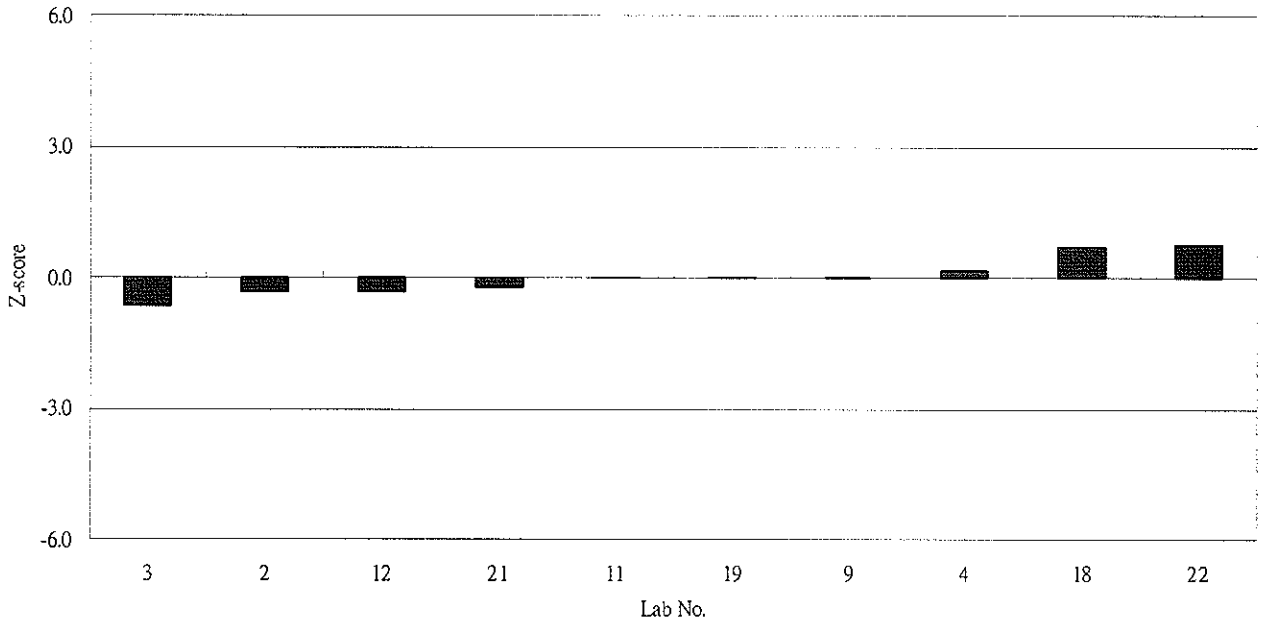


Figure 1.2 Bar Chart of z-score

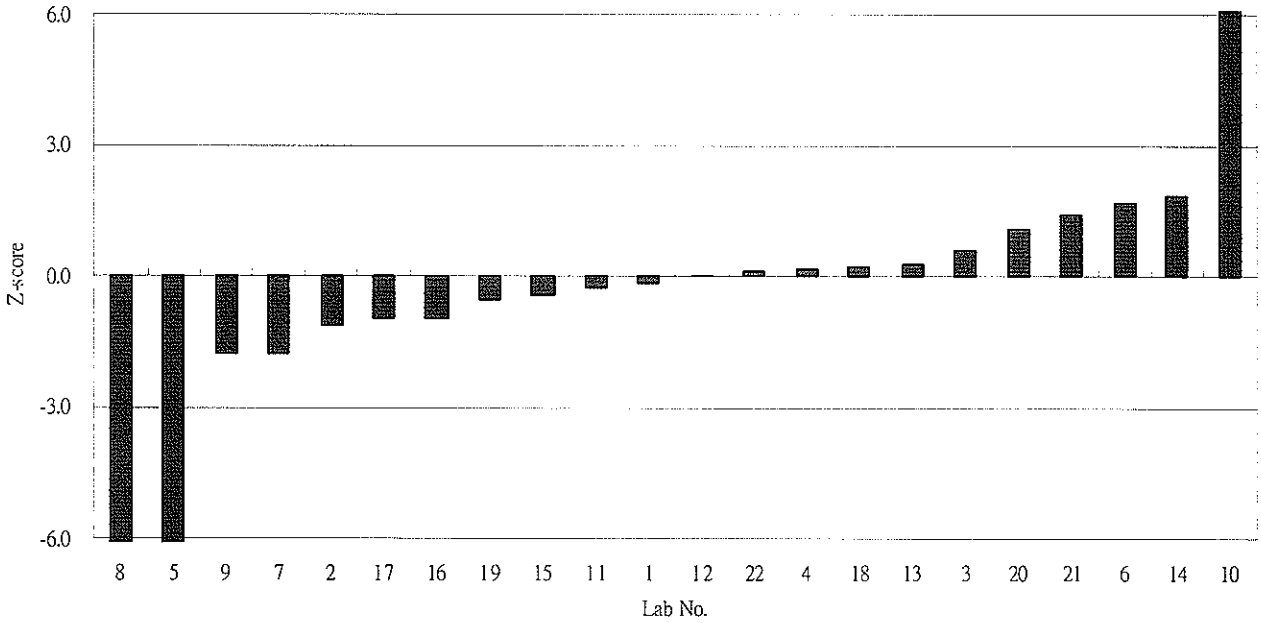




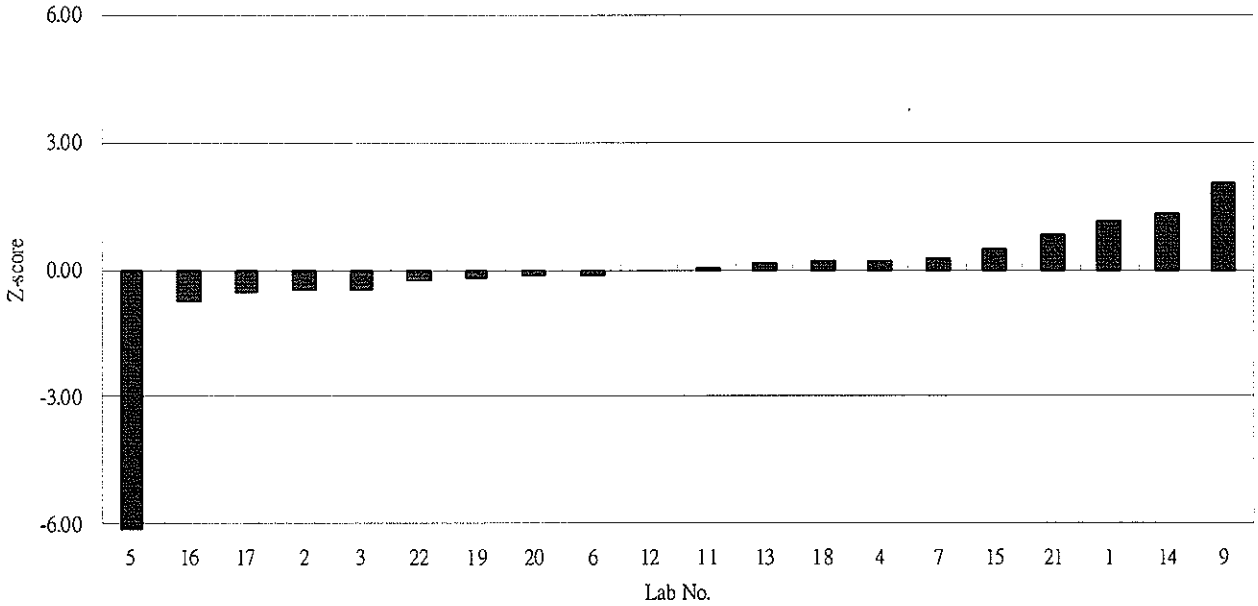
Sample A - Butyl-p-hydroxy benzoate



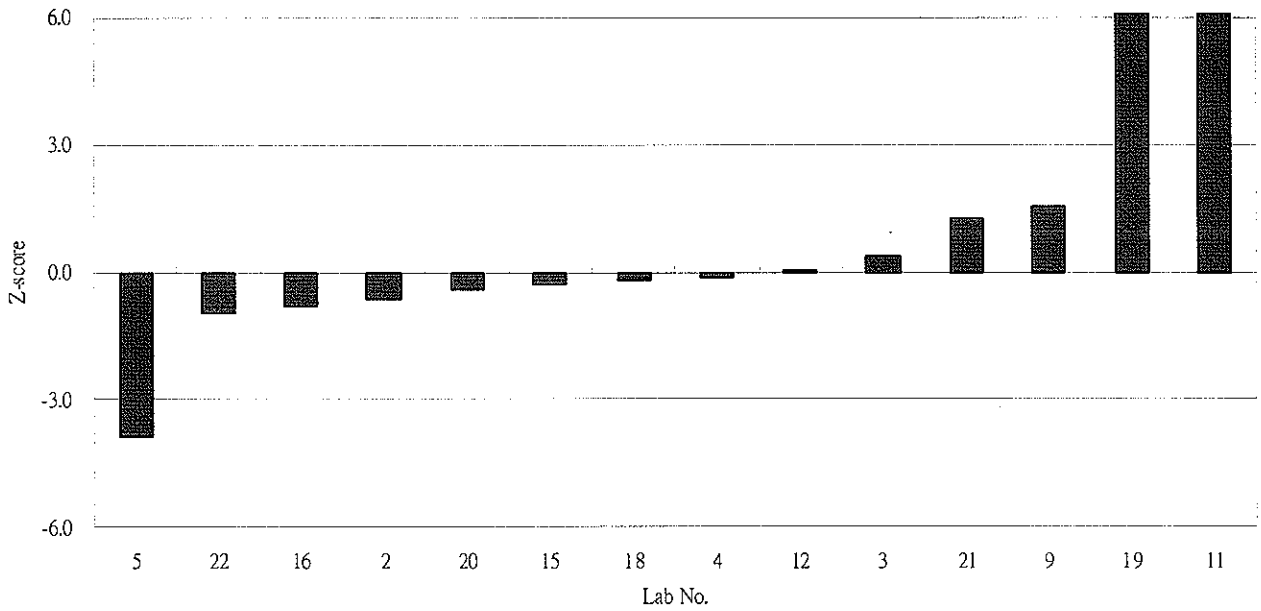
Sample B - Benzoic Acid



Sample B - Sorbic Acid



Sample B - Methyl p-hydroxy benzoate



Sample B - Butyl-p-hydroxy benzoate

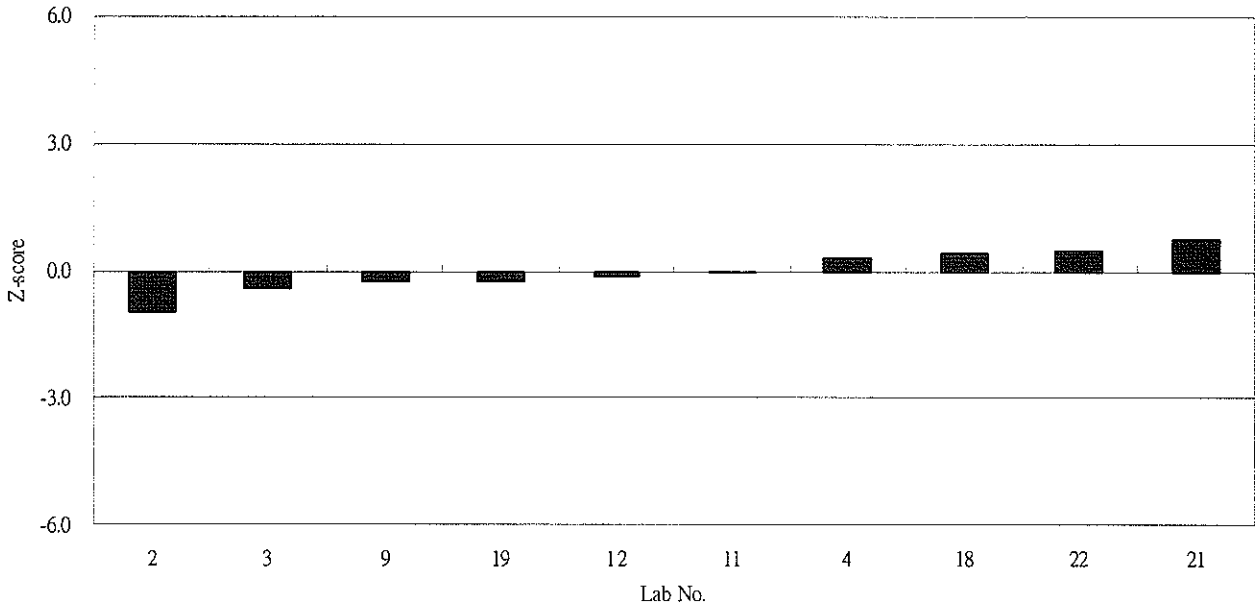


Table 1.9 Information of test methods in participating laboratories

Lab Code	Instrument	Accredited Status	Other additional information
1	HPLC/UV	Yes	
2	HPLC/UV	Yes	
3	HPLC/PDA	Yes	
4	HPLC/UV	No	
5	HPLC/UV	Yes	
6	HPLC/PDA	Yes	
7	HPLC/PDA	Yes	
8	GC/FID	Yes	According to CNS 10949 Method of test for preservatives in foods, Yangmei QC section laboratory only identified benzoic acid and sorbic acid, the idenfication of methy p-hydroxy benzoate and butyl p-hydroxy benzoate are not included in the method.
9	HPLC/UV	Yes	N.D = Not Detected
10	Other: AOAC 963.19	Yes	
11	HPLC/UV	Yes	
12	HPLC/PDA	Yes	
13	HPLC/UV	Yes	
14	HPLC/PDA	Yes	
15	HPLC/PDA	Yes	
16	HPLC/UV	Yes	
17	HPLC/PDA	Yes	
18	HPLC/PDA	Yes	
19	HPLC/UV	Yes	
20	HPLC/PDA	Yes	
21	HPLC/UV	No	
22	HPLC/UV	Yes	

Appendix B

Preparation of the samples

Homogeneity and stability test

1. Preparation of the samples

The soy sauce samples were prepared by Food Industry Research and Development Institute, Taiwan. The following details the sample preparation procedure.

1.1 Preparation of pre mix preservatives

(1) Calculate total batch weight of soy sauce for sample A and B.

(2) Approximate concentration ranges of the four preservatives(benzoic acid, sorbic acid, methyl p-hydroxy benzoate and butyl p-hydroxy benzoate) are as follows:

Preservatives	Approximate Conc. Range (mg/kg)
Benzoic acid	125 to 1500
Sorbic acid	125 to 1500
Methyl p-hydroxy benzoate	125 to 1500
Butyl p-hydroxy benzoate	125 to 1500

(3) Weight the three standeards (benzoic acid, methyl p-hydroxy benzoate and butyl p-hydroxy benzoate) into a volumetric flask and dissolve completely with acetonitrile for pre mix preservative A. Another pre mix preservative B contains four standeards (benzoic acid, sorbic acid, methyl p-hydroxy benzoate and butyl p-hydroxy benzoate).

1.1 Preparation the soy sauce samples

(1) Weight the required amount of soy sauce to two containers for sample A and B.

(2) Separately add the pre mix preservative A and B to two containers.

(3) Separately mix the two soy sauce samples for 1 hour.

(4) Fill each bottle contains about 80 mL of soy sauce and label "APLAC Sample A" and "APLAC Sample B".

(5) The samples will be placed at room temperature (about 25 °C) for homogeneity and stability analysis.

2. Homogeneity and stability test

2.1 Homogeneity testing

In homogeneity testing, not less than 10 samples were taken randomly from the final package of samples and analyzed in duplicate for determining the sample inhomogeneity in accordance with the recommendation stipulated in ISO 13528:2005.

The collected data of homogeneous test were analyzed by using ISO 13528:2005 Annex B. Homogeneous test for each sample tested 2 replicates under the same conditions. Calculate the general average, the standard deviation of sample averages s_x, the within-samples standard deviation s_w, the between-samples standard deviation s_s. The between-samples standard deviation s_s was compared with performance standard deviation σ_R (estimated using Horwitz equation). The samples may be considered adequately homogeneous if $s_s \leqq 0.3\,\sigma_R$. The results are listed in Table 2.1~2.7 and showed that the samples were sufficiently homogeneous.

The data from a homogeneity check are represented by:

$$x_{t,k}$$

where

t represents the sample (t = 1 , 2 ..., g)

k represents the test portion (k = 1, 2)

Define the sample averages as:

$$x_{t,\cdot} = (x_{t,1} + x_{t,2}) / 2$$

and the between-test-portion ranges as:

$$w_t = \left| x_{t,1} - x_{t,2} \right|$$

Calculate the general average:

$$\bar{x}_{\cdot\cdot} = \sum \bar{x}_{t,\cdot} / g$$

the standard deviation of sample averages:

$$s_x = \sqrt{\sum \left(x_{t,\cdot} - \bar{x}_{\cdot\cdot} \right)^2 / (g - 1)}$$

and the within-samples standard deviation:

$$s_w = \sqrt{\sum w_t^2 / (2g)}$$

where the summations are over samples (t = 1, 2, ..., g).

Finally, calculate the between-samples standard deviation as:

$$s_s = \sqrt{s_x^2 - \left(s_w^2 / 2 \right)}$$

Table 2.1 Results of homogeneity test of sample A-Benzoic acid

Sample A-Benzoic acid			
Analysis method：HPLC/DAD			
Results (mg/kg)			
Test number / Sample number	X_{t1}	X_{t2}	Average
1	260	254	257
2	249	258	253
3	254	254	254
4	256	254	255
5	256	255	256
6	258	254	256
7	255	256	256
8	264	267	266
9	256	257	256
10	259	267	263
The general average, $\bar{\bar{x}}$			257
The standard deviation of sample averages, s_x			4.01
The within-samples standard deviation, s_w			3.29
The between-samples standard deviation, s_s			3.26
$0.3\,\sigma_R$			5.35

Table 2.2 Results of homogeneity test of sample A-Methyl p-hydroxy benzoate

Sample A-Methyl p-hydroxy benzoate			
Analysis method：HPLC/DAD			
Results (mg/kg)			
Test number / Sample number	X_{t1}	X_{t2}	Average
1	259	259	259
2	262	260	261
3	261	258	259
4	263	260	262
5	261	261	261
6	258	260	259
7	260	261	261
8	263	262	263
9	258	261	260
10	262	263	263
The general average, $\bar{\bar{x}}$			261
The standard deviation of sample averages, s_x			1.40
The within-samples standard deviation, s_w			1.23
The between-samples standard deviation, s_s			1.10
$0.3\,\sigma_R$			5.42

Table 2.3 Results of homogeneity test of sample A-Butyl p-hydroxy benzoate

Sample A-Butyl p-hydroxy benzoate			
Analysis method：HPLC/DAD			
Results (mg/kg)			
Test number / Sample number	X_{t1}	X_{t2}	Average
1	87.0	86.9	87.0
2	86.6	88.0	87.3
3	85.9	86.6	86.2
4	87.8	85.8	86.8
5	87.2	81.6	84.4
6	86.1	84.4	85.3
7	84.5	84.7	84.6
8	82.4	84.5	83.5
9	81.7	85.8	83.8
10	84.7	83.0	83.8
The general average, $\bar{\bar{x}}$			85.3
The standard deviation of sample averages, s_x			1.46
The within-samples standard deviation, s_w			1.79
The between-samples standard deviation, s_s			0.72
$0.3\,\sigma_R$			2.10

Table 2.4 Results of homogeneity test of sample B-Benzoic acid

Sample B-Benzoic acid			
Analysis method：HPLC/DAD			
Results (mg/kg)			
Test number / Sample number	X_{t1}	X_{t2}	Average
1	521	508	514
2	539	528	533
3	531	522	526
4	511	513	512
5	506	516	511
6	512	512	512
7	504	510	507
8	502	509	505
9	510	504	507
10	528	517	523
The general average, $\bar{x}$			515
The standard deviation of sample averages, s_x			9.34
The within-samples standard deviation, s_w			5.83
The between-samples standard deviation, s_s			8.38
$0.3\sigma_R$			9.66

Table 2.5 Results of homogeneity test of sample B-Sorbic acid

Sample B-Sorbic acid			
Analysis method：HPLC/DAD			
Results (mg/kg)			
Test number / Sample number	X_{t1}	X_{t2}	Average
1	257	250	253
2	266	260	263
3	261	255	258
4	250	247	249
5	243	251	247
6	249	249	249
7	246	252	249
8	247	251	249
9	251	247	249
10	257	252	254
The general average, $\bar{\bar{x}}$			252
The standard deviation of sample averages, s_x			5.14
The within-samples standard deviation, s_w			3.78
The between-samples standard deviation, s_s			4.38
$0.3\,\sigma_R$			5.26

Table 2.6 Results of homogeneity test of sample B-Methyl p-hydroxy benzoate

Sample B-Methyl p-hydroxy benzoate			
Analysis method：HPLC/DAD			
Results (mg/kg)			
Test number / Sample number	X_{t1}	X_{t2}	Average
1	363	358	360
2	363	362	363
3	362	365	363
4	363	364	364
5	365	366	365
6	363	360	361
7	363	364	364
8	362	363	363
9	362	362	362
10	361	365	363
The general average, $\bar{\bar{x}}$			363
The standard deviation of sample averages, s_x			1.42
The within-samples standard deviation, s_w			1.80
The between-samples standard deviation, s_s			0.64
$0.3\,\sigma_R$			7.17

Table 2.7 Results of homogeneity test of sample B-Butyl p-hydroxy benzoate

Sample B-Butyl p-hydroxy benzoate			
Analysis method：HPLC/DAD			
Results (mg/kg)			
Test number / Sample number	X_{t1}	X_{t2}	Average
1	80.4	82.7	81.5
2	83.0	81.0	82.0
3	83.9	80.8	82.3
4	83.0	84.2	83.6
5	81.9	83.8	82.8
6	80.9	80.5	80.7
7	85.1	82.5	83.8
8	82.2	83.8	83.0
9	82.9	84.0	83.5
10	82.8	83.6	83.2
The general average, $\bar{\bar{x}}$			82.7
The standard deviation of sample averages, s_x			0.99
The within-samples standard deviation, s_w			1.34
The between-samples standard deviation, s_s			0.31
$0.3\,\sigma_R$			2.04

2.2 Stability testing

For the stability test, at least 3 samples prepared in their final package will be selected in random, and measurement results for each of tests will be obtained under repeatability conditions. The samples will be placed at room temperature (about 25 °C) for monitoring the stability of analytes between sample dispatch and after submission of results. Samples will be tested on the contents of preservatives every 10~15 days. Stability of the samples will be evaluated in accordance with the recommendation stipulated in ISO13528:2005.

The general average of the measurements obtained in the homogeneity check was compared with the general average of the results obtained in the stability check. The samples may be considered to be adequately stable if $|\bar{x} - \bar{y}| \leqq 0.3\sigma_R$. The results are listed in Table 2.8~2.14 and showed that the samples were sufficiently stable.

Table 2.8 Results of Stability testing of sample A- Benzoic acid

Sample A - Benzoic acid							
Analysis method：HPLC/DAD							
Results (mg/kg)							
Day	y_{t1}	y_{t2}	Average	The general average $\bar{y}$	The general average $\bar{x}$	$\lvert \bar{x}-\bar{y} \rvert$	0.3σR
10	253	249	251	253	257	3.66	5.35
	249	258	253				
	260	251	256				
20	259	249	254	256		1.13	
	258	257	258				
	252	262	257				
60	257	262	259	258		0.86	
	259	259	259				
	259	253	256				
90	253	258	255	253		3.86	
	256	260	258				
	248	244	246				

Table 2.9 Results of Stability testing of sample A- Methyl p-hydroxy benzoate

Sample A - Methyl p-hydroxy benzoate							
Analysis method：HPLC/DAD							
Results (mg/kg)							
Day	y_{t1}	y_{t2}	Average	The general average $\bar{y}$	The general average $\bar{x}$	$\lvert \bar{x}-\bar{y} \rvert$	0.3σR
10	268	265	266	261	261	0.77	5.42
	265	254	260				
	256	260	258				
20	262	268	265	262		1.12	
	253	256	254				
	268	265	266				
60	266	250	258	258		2.33	
	264	253	259				
	262	254	258				
90	250	257	253	256		4.99	
	258	257	257				
	257	256	256				

Table 2.10 Results of Stability testing of sample A- Butyl p-hydroxy benzoate

Sample A - Butyl p-hydroxy benzoate							
Analysis method：HPLC/DAD							
Results (mg/kg)							
Day	y_{t1}	y_{t2}	Average	The general average $\bar{y}$	The general average $\bar{x}$	$\lvert \bar{x}-\bar{y} \rvert$	0.3σR
10	86.5	85.4	86.0	85.0	85.3	0.24	2.10
	85.8	82.3	84.0				
	86.3	83.8	85.0				
20	81.5	83.2	82.3	83.8		1.45	
	85.3	85.2	85.3				
	85.5	82.2	83.9				
60	82.2	83.3	82.7	83.8		1.51	
	84.3	83.8	84.1				
	84.6	84.4	84.5				
90	86.4	83.2	84.8	83.2		2.02	
	85.1	79.6	82.4				
	83.3	81.9	82.6				

Table 2.11 Results of Stability testing of sample B- Benzoic acid

Sample B - Benzoic acid							
Analysis method：HPLC/DAD							
Results (mg/kg)							
Day	y_{t1}	y_{t2}	Average	The general average $\bar{y}$	The general average $\bar{x}$	$\lvert \bar{x}-\bar{y} \rvert$	0.3σR
10	498	509	504	510	515	5.61	9.66
	509	512	510				
	516	513	515				
20	505	521	513	514		1.16	
	519	510	514				
	512	517	514				
60	509	519	514	508		7.55	
	505	516	511				
	502	495	498				
90	507	503	505	505		9.65	
	507	504	505				
	508	505	506				

Table 2.12 Results of Stability testing of sample B- Sorbic acid

Sample B - Sorbic acid							
Analysis method：HPLC/DAD							
Results (mg/kg)							
Day	y_{t1}	y_{t2}	Average	The general average $\bar{y}$	The general average $\bar{x}$	$\lvert \bar{x}-\bar{y} \rvert$	0.3σR
10	252	257	255	254	252	1.95	5.26
	257	248	253				
	256	253	254				
20	253	261	257	254		2.54	
	259	254	257				
	255	245	250				
60	258	243	250	256		3.89	
	262	256	259				
	261	255	258				
90	252	247	249	247		5.11	
	249	241	245				
	247	245	246				

Table 2.13 Results of Stability testing of sample B- Methyl p-hydroxy benzoate

Sample B - Methyl p-hydroxy benzoate							
Analysis method：HPLC/DAD							
Results (mg/kg)							
Day	y_{t1}	y_{t2}	Average	The general average $\bar{y}$	The general average $\bar{\bar{x}}$	$\lvert \bar{\bar{x}}-\bar{y} \rvert$	0.3σR
10	371	363	367	368	363	5.50	7.17
	364	378	371				
	363	370	367				
20	372	372	372	368		4.85	
	359	365	362				
	370	367	369				
60	365	368	366	366		2.34	
	368	360	364				
	368	364	366				
90	354	355	355	356		7.06	
	358	355	356				
	358	354	356				

Table 2.14 Results of Stability testing of sample B- Butyl p-hydroxy benzoate.

Sample B - Butyl p-hydroxy benzoate							
Analysis method：HPLC/DAD							
Results (mg/kg)							
Day	y_{t1}	y_{t2}	Average	The general average $\bar{y}$	The general average $\bar{\bar{x}}$	$\lvert \bar{\bar{x}}-\bar{\bar{y}} \rvert$	0.3σR
10	79.3	80.0	79.7	80.9	82.7	1.75	2.04
	80.5	81.8	81.1				
	81.0	82.8	81.9				
20	81.9	87.7	84.8	84.4		1.72	
	86.3	83.9	85.1				
	86.9	79.5	83.2				
60	78.5	83.2	80.8	80.9		1.71	
	80.2	81.9	81.0				
	80.1	81.9	81.0				
90	79.5	82.2	80.9	80.6		2.03	
	78.2	82.6	80.4				
	81.8	79.3	80.6				

Appendix C

Instructions to Accreditation Body

Instructions to Participants

Results Sheet

APLAC T083 Preservatives in Soy Sauce Proficiency Testing Program

Instructions to Accreditation Bodies

1. ORGNAIZATION

The Proficiency Testing Program on "Determination of Preservatives in Soy sauce" is organized by Taiwan Accreditation Foundation (TAF) under the auspices of Asia Pacific Laboratory Accreditation Cooperation (APLAC). The main purpose of this program is to evaluate the competence of participating laboratories on the concerned tests.

SAMPLES

Accreditation bodies (AB) will receive PT samples and instruction information (hard copy and soft copy) from the organizers. Each laboratory is supplied with two bottles that each contains about 80 mL of Soy sauce. Upon receipt, AB should carefully check the samples (number of bottles, physical conditions, etc.) and promptly acknowledge the TAF coordinator by returning the Receipt Form (for Accreditation Bodies) through E-mail and facsimile to

Taiwan Accreditation Foundation (TAF)
Ms. Cherry Cheng
FAX: +886-3-5726308
E-mail:cherry@taftw.org.tw

2. SAMPLES DISTRIBUTION

AB is recommended to promptly distribute the samples, together with the Instructions to Participating Laboratories and Results Sheet (hard and soft copy) to their respective participating laboratories. The samples shall be stored at room temperature before dispatch.

AB shall remind their participating laboratories to carefully check the samples upon receipt and promptly acknowledge the TAF coordinator by returning the Receipt Form (for Participating Laboratories) through E-mail and facsimile as soon as possible. New samples will be re-sent for any damaged samples.

In response to each country's State Customs import requests of liquid food, please AB

inform TAF that your country's State Customs request the documents of Customs declaration for international air or sea must be accompanied (Ex, The import request of US FDA... etc) while AB provide nomination for TAF. AB is responsible for Customs clearance formalities.

3. TESTS TO BE CARRIED OUT

Please refer to the Instructions to Participating Laboratories enclosed, which outline the tests to be performed and the instructions for test method and reporting results.

The Instructions to Participating Laboratories documents are master copies. The AB should make copies for distribution to the participating laboratories. Instructions could be e-mailed on request. If necessary, these documents should be translated into the appropriate language by the accreditation body.

4. SAMPLE DISTRIBUTION

Each accreditation body shall be responsible for the samples dispatching to the respective participating laboratories within one week of receipt date and the delivery costs in their own economy.

5. DOCUMENTS TO BE SUBMITTED

Within one week of the completion of the testing, participating laboratories are required to both facsimile and e-mail to their accreditation body the Results Sheet requested in the Instructions to Participating Laboratories. No other documentation is required. The accreditation body should ensure the information has been supplied by the participating laboratories and is complete. No later than **27th October 2011**, AB is required to forward these complete results forms to the TAF coordinator cherry@taftw.org.tw by **E-mail/Facsimile.**

6. GENERAL INFORMATION

For general queries, please contact:

Taiwan Accreditation Foundation (TAF)
Ms. Cherry Cheng
FAX: +886-3-5726308
E-mail:cherry@taftw.org.tw

APLAC

Asia Pacific Laboratory Accreditation Cooperation

APLAC T083 Preservatives in Soy Sauce Proficiency Testing Program

Receipt Form (for Accreditation Body)

In order to monitor the progress of the Proficiency Testing Program, we kindly ask each accreditation body, on receipt of the samples, to fill in this **Receipt Form** and E-mail/facsimile it to:

Taiwan Accreditation Foundation (TAF) Ms. Cherry Cheng FAX: +886-3-5726308 E-mail:cherry@taftw.org.tw

Thank you in advance for your cooperation.

1. _____* APLAC T083 samples were received on: _____ (date).
2. After inspection, are the contents of the package damaged:

   ☐ No ☐ Yes If yes, is it still suitable for use? ☐ Yes ☐ No
3. Is there a Declaration to Customs Officials and Shipping Agents enclosed in the plastic envelope and attached to the outside of the case? ☐ Yes ☐ No

* number of samples

Remarks: _____

_____

_____

Name of Accreditation Body: _____

Contact Person: _____

Fax: _____

E-mail: _____

APLAC T083 Preservatives in Soy Sauce Proficiency Testing Program

Instructions to Participating Laboratories

1. ORGNAIZATION

The Proficiency Testing Program on "Determination of Preservatives in Soy sauce" is organized by Taiwan Accreditation Foundation (TAF) under the auspices of Asia Pacific Laboratory Accreditation Cooperation (APLAC). The main purpose of this proficiency testing program is to evaluate the competence of participating laboratories on the concerned tests.

2. SAMPLES

Participating laboratories will be given two differences in concentrations sample bottles labeled APLAC Sample A and APLAC Sample B from their respective accreditation bodies (ABs). Each bottle contains about 80 mL of Soy sauce. Upon receipt of the samples, participating laboratories should carefully inspect the sample for any physical damages and defects. Participating laboratories shall promptly acknowledge receipt of the sample to AB and TAF. New samples will be replaced for any damaged claims. Intact sample is recommended to be stored in a secure environment at room temperature before use.

3. TESTS TO BE PERFORMED

Participating laboratories are required to determine the concentration (in mg/kg) of preservatives in the sample. HPLC (UV or PDA) and GC (FID) methods are most currently employed, such as AOAC 983.16 (Benzoic acid, and Sorbic acid in Food); however, participating laboratories could perform the test by the daily used method and specify the method and instrument used on the Results Sheet. Where possible, uncertainties should be calculated using the method in the ISO/IEC Guide 98-3:2008, *Uncertainty of measurement -- Part 3: Guide to the expression of uncertainty in measurement.*

4. HOMOGENEITY & STABILITY TESTS

In homogeneity checking, 10 samples will be randomly selected and tested. Each sample will be divided into two test portion, all test portions will be tested in random order in the repeatability condition. The samples will be placed at room temperature (about 25°C) for monitoring the stability of analytes between sample dispatch and after submission of results. Samples will be tested on the contents of preservatives every fifteen days. Homogeneity and stability of the samples will be determined in accordance with the recommendation stipulated in ISO13528:2005.

5. PERFORMANCE ASSESSMENT

Performance of the participating laboratories is assessed using z-score which is calculated as:

$$z = \frac{(x_i - \bar{x})}{\sigma}$$

x_i= reported result of individual participant

$\bar{x}$ = robust average

σ= standard deviation estimated from Horwitz equation

6. RESULTS AND DOCUMENTS TO BE SUBMITTED

Report all the data in Results Sheet, where possible, estimate and report the expanded uncertainty for individual analyte. Please identify the methods used in the Method column of the Results Sheet (e.g. HPLC, or GC, etc). Submit the results to TAF should be before 20th October 2011. Unless with special reasons, results submitted after the deadline might not be accepted.

Laboratories should make a copy of all documents and worksheets for their own records and keep these on file for an adequate period of time (at least until a final report has been issued).

7. CONFIDENTIALITY

Each laboratory will be assigned with a unique identification code. This unique code will be used throughout the programme. All information on the identities and results of participating laboratories will be kept confidential and not be disclosed.

8. GENERAL INFORMATION

For general queries, please contact with your accreditation body, additional information may be obtained from:

Taiwan Accreditation Foundation (TAF)

1. Ms. Cherry Cheng

TEL：+886-3-5714848 Ext 239

FAX：+886-3-5726308

E-mail:cherry@taftw.org.tw

2. Ms. Jean Yang

TEL：+886-3-5714848 Ext 224

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E-mail: jeanyang@taftw.org.tw

Asia Pacific Laboratory Accreditation Cooperation

APLAC T083 Preservatives in Soy Sauce Proficiency Testing Program

Results Sheet

Lab Code: ☐

Name of Accreditation Body: _____

Name of Participating Laboratory: _____

Contact person/E-mail: _____

Test Date: _____ (day/month/year)

Sample	Test Item	Result (mg/kg)	Combined standard uncertainty	Coverage factor k (95% level of confidence)	Expanded Measurement Uncertainty	Instrument
Sample A No:_____	Benzoic acid					
	Sorbic acid					
	Methyl p-hydroxy benzoate					☐GC/FID
	Butyl p-hydroxy benzoate					☐HPLC/UV
Sample B No:_____	Benzoic acid					☐HPLC/PDA
	Sorbic acid					☐Other:
	Methyl p-hydroxy benzoate					
	Butyl p-hydroxy benzoate					

Note:

1. Report all tests to 3 significant figures or 3 decimal places, whichever is appropriate
2. Reporting the expanded uncertainty at a coverage level of k = 2 and a confidence level about 95%.
3. Other additional information:

_____

- **It is the responsibility of the participants to avoid collusion and falsification of result so as to ensure a reliable assessment to be made in this proficiency testing program.**
- **Please submit this "Results sheet" before November 2nd , 2011. E-mail and facsimile to TAF.**

Signature: _____ Date: _____

Appendix D

Robust analysis

Robust analysis

The data are sorted into increasing order, and then calculate the initial mean (x*) and standard deviation (s*) of these data as below.

(x*)=median of the data

(s*)=1.483 multiply with median of the difference between the data and median

The δ value is the standard deviation (s*) multiply with 1.5.

$$\delta = 1.5s^*$$

For each data, the new value is calculated as the following formula:

$$x_i^* = \begin{Bmatrix} x^* - \delta & if\ x_i < x^* - \delta \\ x^* + \delta & if\ x_i > x^* + \delta \\ x_i & otherwise \end{Bmatrix}$$

The new mean (x*) and standard deviation (s*) are calculated as the following formula:

$$x^* = \frac{\sum x_i^*}{p}$$

$$s^* = 1.134\sqrt{\sum (x_i^* - x^*)^2 / (p-1)}$$

The new values of robust mean and standard deviation may be derived by an iterative calculation until the process converges. Convergence may be assumed when there is no change from one iteration to the next in the third significant figure of the robust range. This algorithm yields robust values of the mean.