Final Report

Asia Pacific Metrology Programme Supplementary Comparison (APMP.QM-S8)

Determination of Mass Fraction of Benzoic Acid, Methyl Paraben and n-Butyl Paraben in Soy Sauce

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1.0 INTRODUCTION

Preservatives such as benzoic acid and parabens are being tested on a regular basis by regulatory authorities and food testing laboratories as they are widely found in condiments and beverages such as fruit juices, sports drinks and soft drinks. The consumption of excessive amount of preservatives can lead to adverse health effects such as allergy and asthma. Also, the levels of these preservatives in food are strictly regulated and the maximum permitted concentrations in each type of food have been established in most countries.

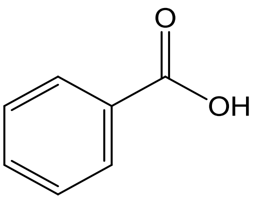
The study was first proposed by the Health Sciences Authority (HSA), Singapore as a key comparison and was presented at the APMP TCQM meeting in Taipei in 2013. It was subsequently presented at the CCQM OAWG meeting in April 2014. The meeting recognised the need for a key comparison to underpin new and existing CMCs, and it also noted that the topic involved a condiment (see Objectives) which is commonly used in the Asia Pacific region. Hence, the meeting recommended that the study should proceed as a subsequent comparison under the APMP. CCQM OAWG members from other RMOs would also be invited to participate. An approval was subsequently obtained from the APMP TCQM Chair in April 2014 to organise this supplementary comparison, along with a parallel pilot study.

2.0 OBJECTIVES

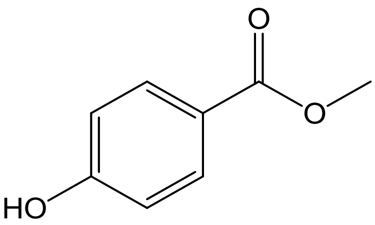
The comparison aimed to enable participating NMIs/DIs to demonstrate their competence in the determination of three common preservatives; namely: benzoic acid, methyl paraben and n-butyl paraben. The chosen matrix material was soy sauce, a traditional condiment commonly used in the preparation of Asian cuisines.

3.0 MEASURANDS

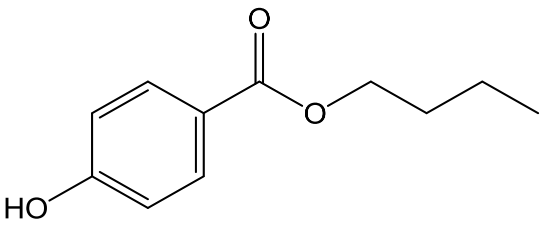
The mass fractions (in mg/kg) of benzoic acid, methyl paraben and n-butyl paraben in soy sauce, were determined by the participating NMIs/DIs. The chemical structures and physical information of the analytes are as shown:



Benzoic acid
CAS No.: 65-85-0
MW: 122.12
logK$_{OW}$: 1.87[1]



Methyl paraben
CAS No.: 99-76-3
MW: 152.15
logK$_{OW}$: 1.96[1]



n-Butyl paraben
CAS No.: 94-26-8
MW: 194.23
logK$_{OW}$: 3.57[1]

The mass fractions of benzoic acid, methyl paraben and n-butyl paraben in the study material were in the range of 50 to 200 mg/kg. The study material was intentionally prepared to contain a lower concentration of the analytes than the regulatory limits in most countries in order to provide a higher level of analytical challenge for the participating NMIs/DIs.

4.0 STUDY MATERIAL

4.1 PREPARATION

The study material was prepared by HSA. The soy sauce was sourced from local supermarkets and screened for the presence of preservatives using liquid chromatography-diode array detection (LC-DAD). A brand of soy sauce with no preservatives found was then used to prepare the study material. The soy sauce was poured into a clean, dry drum and spiked with benzoic acid (NIST, in-house qNMR purity value: 998 ± 4 mg/g), methyl paraben (Sigma Aldrich, in-house qNMR purity value: 994 ± 4 mg/g) and n-butyl paraben (Sigma Aldrich, in-house qNMR purity value: 1000 ± 5 mg/g) in methanolic solution. The mixture was then stirred under an atmosphere of nitrogen for 2 hours at room temperature (about 18 to 25 °C). Thereafter, the soy sauce was aliquoted into amber glass bottles. A total of 89 bottles of study material were prepared.

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[1] S.G. Machatha, S.H. Yalkowsky, International J. Pharmaceutics, 294 (2005), 185-192.

4.2 HOMOGENEITY STUDIES

The homogeneity of the study material was established by employing gas chromatography-isotope dilution mass spectrometry (GC-IDMS). Ten bottles were randomly and stratifically selected. Two subsamples were taken from each bottle. The sample size was about 1 g. Using one-way ANOVA at 95 % level of confidence, the material was found to be sufficiently homogeneous for the study. Summaries of the ANOVA for the homogeneity study are given in Tables 1-3.

Table 1: Summary of ANOVA for benzoic acid

Source of variance	*SS*	DF	MS	F	p-Value	$F_{critical}$
Between bottles	14.23	9	1.58	1.05	0.46	3.02
Within bottles	15.01	10	1.50			
Total	29.24					

Table 2: Summary of ANOVA for methyl paraben

Source of variance	*SS*	DF	MS	F	p-Value	$F_{critical}$
Between bottles	6.75	9	0.75	2.14	0.13	3.02
Within bottles	3.50	10	0.35			
Total	10.25					

Table 3: Summary of ANOVA for n-butyl paraben

Source of variance	*SS*	DF	MS	F	p-Value	$F_{critical}$
Between bottles	4.75	9	0.53	1.56	0.25	3.02
Within bottles	3.38	10	0.34			
Total	8.13					

The relative uncertainties of the between-bottle homogeneity, u_{bb}, for benzoic acid, methyl paraben and n-butyl paraben were found to be 0.37 %, 0.45 % and 0.32 %, respectively.

4.3 STABILITY STUDIES

The stability of the study material at about 40 °C and 25 °C was established by LC-DAD and GC-IDMS, respectively.

A short-term stability testing using isochronous design was carried out over a period of 28 days at a simulated transport temperature of about 40 °C. Two randomly selected bottles were stored at the reference temperature of about 4 °C. Two bottles were transferred from about 40 °C to the reference temperature each time, on two occasions over the study period. One subsample was taken from each bottle and analysed at least five times. The effect of exposure time on the stability of the analytes was determined by fitting linear regression lines to the data set. The slope (*b*) was tested for statistical significance using Student's *t* test at 95% confidence level, where the *t* value was calculating by dividing *b* by its standard deviation *s(b)* and compared against the critical *t* value. The statistical results given in Table 4 indicated that no significant trend at 95% confidence level was detected. Hence, no significant instability of the study material was observed at about 40 °C for a period of 28 days.

Table 4: Summary of stability study for benzoic acid, methyl paraben and n-butyl at about 40 °C for 28 days

Descriptions	Benzoic Acid	Methyl Paraben	n-Butyl Paraben
Slope of the regression line (*b*)	-0.149	0.056	0.048
Intercept of the regression line (b_0)	161.128	106.189	102.552
Variance of the points (s^2)	1.201	0.363	0.166
Standard deviation of the points (*s*)	1.096	0.602	0.408
Uncertainty of slope [*s(b)*]	0.0553	0.030	0.021
Calculated *t* ($\frac{\lvert b\rvert}{s(b)}$)	2.69	1.85	2.32
Critical *t* factor ($t_{0.95,n-2}$)	12.71	12.71	12.71

The same approach was used to determine the stability of the study material under the storage temperature of about 25 °C using a classical design. The study was carried out on six occasions over a period of 454 days. The statistical results for the significance test of the regression coefficient are given in Table 5. The results showed that the slopes of the line were not significantly different from zero, using Student's *t*-test at 95 % confidence level, i.e. no significant instability was observed when the study sample was stored at about 25 °C. The uncertainties of the stability of the analytes were estimated by calculating *s(b)*454*.

Table 5: Summary of stability study results for benzoic acid, methyl paraben and n-butyl paraben at about 25 °C for 454 days

Descriptions	**Benzoic Acid**	**Methyl Paraben**	**n-Butyl Paraben**
Slope of the regression line (*b*)	-0.0041	-0.0010	-0.0022
Intercept of the regression line (b_0)	156.606	100.768	98.386
Variance of the points (s^2)	2.301	0.985	0.376
Standard deviation of the points (*s*)	1.517	0.992	0.614
Uncertainty of slope [*s(b)*]	0.0038	0.0025	0.0016
Calculated *t* ($\frac{\lvert b \rvert}{s(b)}$)	1.06	0.41	1.40
Critical *t* factor ($t_{0.95,n-2}$)	2.78	2.78	2.78
Relative standard uncertainty of stability	1.12 %	1.14 %	0.72 %

5.0 SCHEDULE

Table 6: Schedule of the comparison

Period	Event
September 2014	Call for participation
January 2015	Distribution of study sample
May 2015	Deadline for submission of results

6.0 REGISTRATION, SAMPLE RECEIPT AND REPORT SUBMISSION

A total of ten NMIs/DIs participated in the comparison for benzoic acid, while six NMIs/DIs participated in the comparison for methyl paraben and n-butyl paraben.

One package comprising one bottle of study material and the Material Safety Data Sheet of soy sauce was sent to all participating NMIs/DIs on 26 Jan 2015. A temperature strip was pasted on the bottle to indicate any exposure of the study material to above 40 °C during transportation. Eight of the participating NMIs/DIs received the package within four days after dispatch. NIM, China received the package 16 days after dispatch due to customs issue. All the participating NMIs/DIs received the study sample intact and the study materials were not exposed to above 40 °C. The NMIs/DIs were informed to keep the study material at or below 25 °C, before analysis.

Due to the coincidence of the CCQM meeting and technical issues faced by a participating NMI/DI, the initial reporting deadline of 24 April 2015 was extended to 8 May 2015 and later re-extended to 22 May 2015, respectively, with the approval of the APMP TCQM Chair. All the participating NMIs/DIs submitted their results on or before the final deadline.

Information on participating NMIs/DIs, contacts, measurands registered, sample receipts and report submissions are summaried in Table 7.

Table 7: Information on participating NMIs/DIs, contacts, measurands registered, sample receipts and report submissions

No.	Participating Institutes, Department/Laboratory	Economy	Contact Person	Measurands Registered	Sample Receipt Date	Report Submission Date
1	EXHM/GCSL-EIM National Laboratory of Chemical Metrology/General Chemistry State Laboratories - Hellenic Institute of Metrology, Chemical Metrology Laboratory	Greece	Dr Elias Kakoulides	Benzoic acid	29 Jan 2015	21 May 2015
2	GLHK Government Laboratory, Hong Kong SAR, Additives, Contaminants and Composition Section	Hong Kong SAR, China	Dr Sin-Kam Hui	Benzoic acid	28 Jan 2015	19 May 2015
3	HSA Health Sciences Authority, Chemical Metrology Division, Chemical Metrology Laboratory	Singapore	Dr Teo Tang Lin	Benzoic acid, methyl paraben and n-butyl paraben	N.A.	6 May 2015
4	INMETRO Instituto Nacional de Metrologia, Qualidade e Tecnologia, Chemical Metrology Division (DQUIM), Organic Analysis Laboratory (LABOR)	Brazil	Eliane Rego	Benzoic acid	28 Jan 2015	12 May 2015
5	KRISS Korea Institute of Standards and Science, Center of Analytical Chemistry, Division of Metrology for Quality of Life, Center for Organic Analysis	Republic of Korea	Dr Seonghee Ahn	Benzoic acid, methyl paraben and n-butyl paraben	28 Jan 2015	21 May 2015
6	NIM National Institute of Metrology, Food Safety Laboratory	PR. China	Dr Xiuqin Li	Benzoic acid, methyl paraben and n-butyl paraben	11 Feb 2015	22 May 2015

7	RCChem-LIPI Research Center for Chemistry – Indonesian Institute of Sciences, Laboratory of Metrology in Chemistry	Indonesia	Dyah Styarini	Benzoic acid, methyl paraben and n-butyl paraben	27 Jan 2015	22 May 2015
8	STD-ITDI Industrial Technology Development Institute, Standards and Testing Division	Philippines	Dr Benilda S. Ebarvia	Benzoic acid	27 Jan 2015	21 May 2015
9	TISTR Thailand Institute of Scientific and Technological Research, Industrial Metrology and Testing Services Centre, Analytical Chemistry Laboratory	Thailand	Thippaya Junvee Fortune	Benzoic acid, methyl paraben and n-butyl paraben	27 Jan 2015	22 May 2015
10	UME TÜBİTAK Ulusal Metroloji Enstitüsü, Organic Chemistry Laboratory	Turkey	Dr Ahmet Ceyhan Gören	Benzoic acid, methyl paraben and n-butyl paraben	30 Jan 2015	22 May 2015

7.0 REPORTING OF RESULTS

A Report of Results Form was provided to the participating NMIs/DIs for completion. The participating NMIs/DIs were requested to report their results based on at least three subsamples, each with a recommended size of not less than 1 g. The results were reported in the unit of mg/kg, which included standard and expanded uncertainties (95 % level of confidence) for the mean of the replicate determinations. A complete description of the analytical procedure and the uncertainty estimation was also provided by the participating NMIs/DIs.

8.0 RESULTS SUBMITTED BY PARTICIPATING INSTITUTES

The reported results are summarised in Tables 8-10.

Table 8: Summary of results for benzoic acid

NMI/DI	Overall mean (mg/kg)	No. of subsamples	Combined standard uncertainty (mg/kg)	Coverage factor, k (95 % confidence level)	Expanded uncertainty to give 95 % confidence level (mg/kg)
INMETRO	148.6	9	1.6	2.10	3.3
TISTR	150.5	14	4.4	1.97	8.7
EXHM/GCSL-EIM	150.66	10	1.75	2.31	4.03
UME	152.38	6	3.46	2	6.92
NIM	154.5	6	1.6	2	3.2
GLHK	154.6	18	2.4	2	4.8
KRISS	155.03	5	1.90	2.262	4.29
HSA	155.5	9	2.4	2	4.8
STD-ITDI	155.6	4	1.9	2	3.8
RCChem-LIPI	157.5	13	3.9	2	7.8

Table 9: Summary of results for methyl paraben

NMI/DI	Overall mean (mg/kg)	No. of subsamples	Combined standard uncertainty (mg/kg)	Coverage factor, k (95 % confidence level)	Expanded uncertainty to give 95 % confidence level (mg/kg)
RCChem-LIPI	96.6	13	3.2	2	6.4
TISTR	98.9	15	3	1.99	5.9
HSA	100.9	9	1.0	2	1.9
NIM	101.0	6	1.1	2	2.2
UME	101.46	6	2.68	2	5.35
KRISS	101.49	5	1.10	2.228	2.44

Table 10: Summary of results for n-butyl paraben

NMI/DI	Overall mean (mg/kg)	No. of subsamples	Combined standard uncertainty (mg/kg)	Coverage factor, k (95 % confidence level)	Expanded uncertainty to give 95 % confidence level (mg/kg)
HSA	97.2	9	1.2	2	2.3
NIM	97.3	6	1.3	2	2.6
RCChem-LIPI	97.3	12	3.5	2	7.0
TISTR	100.8	15	3.7	2.01	7.5
KRISS	101.09	5	1.21	2.365	2.86
UME	103.58	6	2.77	2	5.54

8.1 ADDITIONAL INVESTIGATION

When the Draft A report was first submitted, the then APMP TCQM Chair, Dr Euijin Hwang, commented that while the results of benzoic acid and methyl paraben seemed very good and consistent, the results of n-butyl paraben would need more discussion. As there appeared to be two distinct groups of results for n-butyl paraben (one group from TISTR, KRISS and UME and the other from HSA, NIM and RCChem-LIPI), additional investigative work was carried out by HSA. In the investigation, a single analyst in HSA performed sample preparation and clean-up using the procedures of HSA (GC-IDMS), NIM (results similar to HSA's but using LC-IDMS/MS) and KRISS (results obtained using LC-IDMS/MS and with similar C18 columns as UME and TISTR; extensive sample preparation/clean-up). Three subsamples were taken and subjected to each sample preparation and clean-up procedure under repeatability conditions. All nine subsamples were analysed using LC columns used by NIM (BEH SHIELD RP18) and KRISS (BEH C18) on a LC-MS/MS. The measurements and associated standard deviation from the injections agreed within the reported results and associated combined standard uncertainty of the reported results made by HSA and KRISS.

Results of the investigation were presented at the 2015 CCQM OAWG meeting in October and 2015 APMP TCQM meeting in November. NIM confirmed their result after the APMP meeting. HSA updated the 2016 CCQM OAWG meeting in April that there was no technical reason for the appearance of the two groups of results.

8.2 REFERENCE MATERIALS USED

Summaries of the reference standards and internal standards used are given in Tables 12 to 14.

Benzoic acid (acidimetric) certified reference material (CRM) from NIST (SRM 350b) was used as calibrant by INMETRO, KRISS, STD-ITDI and UME. Additional purity assessment on the chromatographic purity, water content and non-volatile and volatile residues of the CRM was carried out by KRISS. INMETRO verified the calibrant using NMR and mass spectrometry, STD-ITDI verified the calibrant using mass spectrometry, while UME did not report verification of the calibrant. EXHM/GCSL-EIM used benzoic acid (acidimetric) CRM from BAM. Benzoic acid CRM from HSA was used as calibrant by GLHK, HSA, RCChem-LIPI, and TISTR. NIM used its benzoic acid solution CRM.

When the Draft A report was first submitted, the then APMP TCQM Chair, Dr Euijin Hwang, suggested that the traceability issue related to the use of acidimetric CRMs for the determination of benzoic acid should be further discussed.

At the 2015 CCQM OAWG meeting in October, the results were presented and HSA sought the WG's view on results (INMETRO, STD-ITDI and UME), which were traceable to NIST SRM 350b. The WG acknowledged that in principle, the purity value of the SRM was assigned through the amount of H^+ and not benzoic acid. However, NIST vouched for the high purity of the SRM and also pointed out that the SRM had been widely used as the internal standard for qNMR measurements. KRISS had carried out purity assessment on the SRM using mass balance approach and also found the purity value to be high, except that its uncertainty is larger than that reported by NIST using a coulometric method. Despite the larger uncertainty, it would not change the overall uncertainty of the results due to its small contribution. In view of the high purity of the SRM, the WG considered this to be a special case.

Following the OAWG meeting, the results were presented at the APMP TCQM meeting in November 2015. The TCQM decided that it was necessary for INMETRO, STD-ITDI and UME to re-calculate the benzoic acid results with purity value (999.93±3.1mg/g, k=4.3 at approximately 95 % confidence level) from KRISS for traceability, even if it would not affect the result. EXHM/GCSL-EIM performed additional qNMR experiments on the benzoic acid (acidimetric) CRM from BAM using $DMSO_2$ as an internal standard (previously measured against 3,5-BTFMBA CRM 4601-a from NMIJ).

The re-calculated results of the four NMIs/DIs are given in Table 11. The results of STD-ITDI and INMETRO did not change, while those of EXHM/GCSL-EIM and UME did not deviate much from their original submitted results. Subsequently, the original submitted results of EXHM/GCSL-EIM and UME were used for evaluation.

Table 11: Original and re-calculated results for benzoic acid

Participating institute	Original result (mg/kg)		Re-calculated result (mg/kg)	
	Result	U (at 95 % CI)	Result	U (at 95 % CI)
EXHM/GCSL	150.66	4.03	150.65	4.04
INMETRO	148.6	3.4	148.6	3.4
STD-ITDI	155.6	3.8	155.6	3.8
UME	152.38	6.92	152.37	7.02

Labelled isotopes (D_5-benzoic acid or $^{13}C_6$-benzoic acid) were used as internal standard by all participating NMIs/DIs, except RCChem-LIPI which employed external calibration.

Methyl paraben CRM from HSA was used as calibrant by HSA, RCChem-LIPI and TISTR, while NIM used its methyl paraben CRM. Methyl paraben standards from commercial sources were used by KRISS and UME. The commercial standard was purity assessed in-house using qNMR by UME. Purity assessment on the chromatographic purity, water contents and non-volatile and volatile residues of the commercial standard was carried out by KRISS.

Labelled isotopes ($^{13}C_6$-, D_3- or D_4-methyl paraben) were used as internal standards by HSA, KRISS, NIM, TISTR and UME. RCChem-LIPI did not use any internal standard as external calibration was employed.

n-Butyl paraben CRM from HSA was used as calibrant by HSA, RCChem-LIPI and TISTR, while NIM used its n-butyl paraben CRM. n-Butyl paraben standards from commercial sources were used by KRISS and UME. Purity assessment on the chromatographic purity, water contents and non-volatile and volatile residues of the commercial standard was carried out by KRISS. The commercial standard was purity assessed in-house using qNMR by UME.

Labelled isotopes ($^{13}C_6$- or D_4-n-butyl paraben) were used as internal standards by HSA, KRISS, NIM, TISTR and UME. RCChem-LIPI did not use any internal standard as external calibration was employed.

Table 12: Reference standards and internal standards used for the determination of benzoic acid

NMI/DI	Calibrant						
	Source	Purity value	Expanded uncertainty of purity value	Traceability	Verification Technique	Purity assay	Internal Standard
EXHM/GCSL-EIM	BAM U1009	99.997%	0.066%	BAM			D_5-Benzoic acid
GLHK	HSA	999.9 mg/g	3.3 mg/g	HSA		Conducted by the producer of the calibrant (HSA)	$^{13}C_6$-Benzoic acid
HSA	HSA (HRM-1002A)	999.9 mg/g	3.3 mg/g	HSA			$^{13}C_6$-Benzoic acid
INMETRO	NIST SRM 350b	999.78 mg/g (99.9978 %)	0.044 mg/g (0.0044 %)	NIST	NMR chemical shifts and MS spectrum consistent with benzoic acid	No, a CRM was used instead	D_5-Benzoic acid
KRISS	NIST SRM	999.93 mg/g	3.1 mg/g	KRISS	LC/MS, LC-UV, K-F titration, TGA	Purity assayed by KRISS with chromatographic purity, water contents and non-volatile & volatile residues	$^{13}C_6$-Benzoic acid
NIM	NIM CRM GBW(E)100006	1.00 mg/mL	0.01 mg/mL （*U*=1%，*k*=2)	NIM			$^{13}C_6$-Benzoic acid
RCChem-LIPI	HSA, Singapore	999.9 mg/g	3.3 mg/g	HSA			

STD-ITDI	NIST (SRM 350b)	999.978 mg/g	0.044 mg/g	NIST	MS	D_5-Benzoic acid
TISTR	HSA (HRM-1002A)	999.9 mg/g	3.3 mg/g	HSA		$^{13}C_6$-Benzoic acid
UME	Benzoic acid SRM 350b	0.999978	0.000044	NIST		D_5-Benzoic acid

Table 13: Reference materials and internal standards used for the determination of methyl paraben

NMI/DI	Calibrant						
	Source	Purity value	Expanded uncertainty of purity value	Traceability	Verification Technique	Purity assay	Internal Standard
HSA	HSA (HRM-1003A)	999.5 mg/g	3.2 mg/g	HSA			$^{13}C_6$-Methyl paraben
KRISS	SIGMA	997.90 mg/g	0.44 mg/g	KRISS	LC/MS, LC-UV, K-F titration, TGA	Purity assayed by KRISS with chromatographic purity, water contents and non-volatile & volatile residues	$^{13}C_6$-Methyl paraben
NIM	NIM CRM GBW(E)100074	996 mg/g	1.992 mg/g (0.2% , *k*=2)	NIM			$^{13}C_6$-Methyl paraben
RCChem-LIPI	HSA, Singapore	999.5 mg/g	3.2 mg/g	HSA			
TISTR	HSA (HRM-1003A)	999.5 mg/g	3.2 mg/g	HSA			Methyl paraben-d_4
UME	Across Chemicals	0.99998	0.003	TÜBİTAK UME	NMR and Mass Spectrometry	QNMR	Methyl (CD_3) paraben

Table 14: Reference materials and internal standards used for the determination of n-butyl paraben

NMI/DI	Calibrant						
	Source	Purity value	Expanded uncertainty of purity value	Traceability	Verification Technique	Purity assay	Internal Standard
HSA	HSA (HRM-1004A)	999.2 mg/g	3.3 mg/g	HSA			$^{13}C_6$-n-Butyl paraben
KRISS	Dr. Ehrenstorfer	999.92 mg/g	0.7 mg/g	KRISS	LC/MS, LC-UV, K-F titration, TGA	Purity assayed by KRISS with chromatographic purity, water contents and non-volatile & volatile residues	$^{13}C_6$-n-Butyl paraben
NIM	NIM CRM GBW(E)100077	997 mg/g	1.994 mg/g (0.2% , *k*=2)	NIM			$^{13}C_6$-n-Butyl paraben
RCChem-LIPI	HSA, Singapore	999.2 mg/g	3.3 mg/g	HSA			
TISTR	HSA (HRM-1004A)	999.2 mg/g	3.3 mg/g	HSA			n-Butyl paraben-d_4
UME	Alfa Aesar	0.99990	0.00313	TÜBİTAK UME	NMR and Mass Spectrometry	QNMR	Butyl (C_4D_9) paraben

8.3 EXPERIMENTAL DETAILS

The participating NMIs/DIs used their own methods for the determination of benzoic acid, methyl paraben and n-butyl paraben. Details of the sample treatment, instrumental methods, preparation of calibration solutions and blends, and calibration methods are summarised in Tables 15 and 16. Generally, the participating NMIs/DIs performed simple dilution, liquid-liquid extraction or filtration before analyses.

For the determination of benzoic acid, gas chromatography coupled with mass spectrometry (GC-MS) analyses were carried out by INMETRO, GLHK and HSA; liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) analyses were carried out by EXHM/GCSL-EIM, GLHK, KRISS, NIM, STD-ITDI, TISTR and UME; and liquid chromatography coupled with diode array detection (LC-DAD) was carried out by RCChem-LIPI.

For the determination of methyl paraben, GC-MS analyses were carried out by HSA, LC-MS/MS analyses were carried out by KRISS, NIM, TISTR and UME; and LC-DAD was carried out by RCChem-LIPI.

For the determination of n-butyl paraben, GC-MS analyses were carried out by HSA and RCChem-LIPI; and LC-MS/MS analyses were carried out by KRISS, NIM, TISTR and UME.

Various GC columns (VF-1MS, DB-5MS and CP-Wax52 CB) have been used. Also, various LC columns (BEH Shield RP18, 100 SIELC PrimeSep 100, Poroshell HPH-C18, Hypersil 5µ C18 Phenomenex, AQUITY UPLC BEH C18, Agilent Zorbax Eclipse Plus C18, Porpshell 120, EC-C18 and Phenomenex Luna 5µ C18) were used.

Isotope dilution mass spectrometry with single-point, bracketing and multi-level calibration methods for quantification has been employed. EXHM/GCSL-EIM and TISTR used matrix-matched calibration blends for quantification. Most of the participating NMIs/DIs reported the use of qualifying ions/MRM transitions for verification of measurements.

Table 15: Participating NMIs/DIs' sample treatment and instrumental methods (BA denotes benzoic acid; MP denotes methyl paraben; and BP denotes n-butyl paraben)

NMI/DI	Sample size (g)	Extraction/Clean-up	Instrument used	Chromatographic column	Detection (Ionisation mode for MS)	Ions/MRM (Q: Quantifying; q: qualifying)	
						Analyte	Internal standard
EXHM/GCSL-EIM	1	Addition of internal standard, dilution with mobile phase, sonication then filtration.	LC-MS/MS	100 SIELC PrimeSep 100 (250mm x 2.1mm, 5µm)	MS [ESI (negative)]	BA: 121/77	BA: 126/82
GLHK	1	Acidification with 6N HCl, liquid-liquid extraction with diethyl ether, drying then reconstitution with $CHCl_3$ (for GC-MS) or mobile phase (for LC-MS/MS). Derivatisation with MSTFA (for GC-MS).	GC-MS and LC-MS/MS	GC: Agilent J&W DB-5MS (30m x 0.25mmID x 0.25µm) LC: Poroshell HPH-C18 2.1 x 50mm, 2.7µm	MS [EI for GC-MS, ESI (negative) for LC-MS/MS]	BA (GC-MS): 179 (Q),105 (q) BA (LC-MS/MS): 121/77 (Q), 121/121 (q)	BA (GC-MS): 185 (Q), 111 (q) BA (LC-MS/MS): 127/83 (Q)
HSA	1	Addition of internal standard, acidification with 5% HCl, dilution with CH_3CN, liquid-liquid extraction with NaCl, centrifugation, exchange with CH_3CN then filtration.	GC-MS	CP-Wax 52 CB (30m x 0.25mmID, 0.25µm)	MS (EI)	BA: 105.0 (Q), 77.0 (q), 122.0 (q) MP: 152.0 (Q), 93.0 (q), 121.0 (q) BP: 138.0 (Q), 194.0 (q), 121.0 (q)	BA: 111.0 (Q), 83.0 (q), 128.0 (q) MP: 158.0 (Q), 99.0 (q), 127.0 (q) BP: 144.0 (Q), 200.0 (q), 127.0 (q)

INMETRO	1	Addition of internal standard, liquid-liquid extraction with NaCl and ethyl ether, centrifugation, drying of organic extract under nitrogen and vacuum at 50 °C for 40 min, followed by derivatisation with MSTFA.	GC-MS	VF-1MS (30m x 0.25mmID, 0.25µm)	MS (EI, scan mode: m/z 50 to 300)	BA: 179 (Q), 194 (q)	BA: 184 (Q), 199 (q)
KRISS	1	Addition of internal standard, dilution with ammonium acetate, cleanup with SPE, drying, reconstitution with mobile phase then filtration.	LC-MS/MS	AQUITY UPLC BEH C18, 2.1 x 10 mm, 1.7 µm	MS [ESI (negative)]	BA: 121/77 MP: 151/92 BP: 193/92	BA: 127/83 MP: 157/98 BP: 199/98
NIM	1	Addition of internal standard, dilution with CH_3CN, ultrasonication, centrifugation, dilution of supernatant with water, then filtration.	LC-MS/MS	BEH Shield RP18 (1.7 µm, 2.1 mm×100 mm)	MS [ESI (negative)]	BA: 121.1/77.1 (Q), 121.1/103.1 (q) MP: 151.1/92.1 (Q), 151.1/136.1 (q) BP: 193.2/92.1 (Q), 193.2/136.2 (q)	BA: 127.1/83.1(Q), 127.1/109.1 (q) MP: 157.1/98.1 (Q), 157.1/142.1 (q) BP: 199.2/98.1 (Q), 199.2/142.1 (q)
RCChem-LIPI	2	Dilution, cleanup with SPE then filtration (for HPLC-DAD) and 5-fold dilution (for GC-MS).	BA and MP: HPLC-DAD BP: GC-MS	LC: Hypersil 5µ C18 Phenomenex 4,6 x 150 mm GC: DB-5MS (30m x 0.25mm x 0.25um)	DAD (BA: 230 nm; and MP: 254 nm) BP: MS (EI)	BP (underivatised): 121 (Q), 138 (q), 93 (q)	
STD-ITDI	0.2	Dilution with mobile phase	LC-MS	Agilent Zorbax Eclipse Plus C18, 4.6 x 100 mm, 3.5 µm	MS [ESI (negative)]	BA: 77.2	BA: 82.1

TISTR	1	Extract with CH_3OH, sonicate then filter	LC-MS/MS	Porpshell 120, EC-C18 2.7 µm,2.1x100mm	MS [ESI (negative)]	BA: 77 MP: 136 (Q), 92 (q) BP: 92 (Q), 136 (q)	BA: 77 MP: 140 (Q), 96 (q) BP: 96 (Q), 140 (q)
UME	1.2	Liquid-liquid extraction with methanol, filtration then dilution	LC-MS	Phenomenex Luna 5µ C18 column (150 x 2 mm i.d., 5µm particle size)	MS [ESI (negative)]	BA: 120.7/77.0 MP: 150.5/135.5 BP: 192.7/136.0	BA: 125.7/82.0 MP: 153.7/135.5 BP: 202.0/136.0

Table 16: Participating NMIs/DIs' preparation of calibration solutions and blends, and calibration methods (continued)

NMI/DI	Calibration solutions and blends		Calibration
	Preparation	Matrix/Solvent	
EXHM/GCSL-EIM	Dilution of multiple calibration solutions. Preparation of 10 different matrix-matched calibration blends.	Matrix-matched	IDMS (single-point, exact-matching)
GLHK	Gravimetric preparation of multiple calibration solutions. Preparation of multiple calibration blends.	Methanol	IDMS (bracketing)
HSA	Gravimetric preparation of multiple calibration solutions. Preparation of multiple calibration blends.	Methanol	IDMS (single-point, exact-matching)
INMETRO	Dilution of methanolic stock solutions of calibrants with water, followed by addition of NaCl and internal standard to prepare an eight-level calibration curve. Subjected solutions to same treatment as the samples.	Water	BA: IDMS (multi-level) MP: Internal calibration (multi-level)
KRISS	Gravimetric preparation of multiple calibration solutions. Preparation of multiple calibration blends.	5 mmol/L ammonium acetate buffer (pH4.1) / methanol (v/v, 50/50)	IDMS (single-point)
NIM	Dilution of multiple calibration solutions. Preparation of multiple calibration blends.	$CH_3CN:H_2O$ (1:1 v/v)	IDMS (single-point)
RCChem-LIPI	Dilution of multiple calibration solutions to prepare five-point calibration curves.	Methanol	External (both HPLC-DAD and GC-MS analyses
STD-ITDI	Preparation of two calibration blends each from four mixed standard solutions of natural BA, MP and BP and mixed solutions of their isotopically-labelled standards.	Mobile phase diluent (50:50, methanol:ammonium acetate buffer)	IDMS (single-point)

TISTR	Gravimetric preparation of multiple calibration blends.	Exact matrix-matching calibration.	IDMS (bracketing, exact matrix-matching)
UME	Preparation of multiple calibration solutions.	Matrix calibration solution	IDMS (single-point)

9.0 MEASUREMENT EQUATION AND ESTIMATION OF MEASUREMENT UNCERTAINTY

The measurement equations and contributors to the measurement uncertainty (MU) budgets are summarised in Table 17 below. The full uncertainty evaluation reported by the participating NMIs/DIs is given in Appendix I.

Table 17: Participating NMIs/DIs' measurement equations and contributors to measurement uncertainty budgets.

NMI/DI	Measurement Equation	Contributions to measurement uncertainty budget
EXHM/GCSL-EIM	$w_{B,S} = w_{B,C} \frac{m_{D,S}}{m_{B,S}} \times \frac{m_{B,C}}{m_{D,C}} \times \frac{R_S}{R_B}$ w_{BS} = mass fraction of benzoic acid in test material, (*mg/kg*); w_{BC} = mass fraction of benzoic acid in calibration solution, (*mg/kg*); $m_{D,S}$ = mass of benzoic acid-d5 solution added to sample blend, (*g*); $m_{B,S}$ = mass of test material in sample blend, (*g*); $m_{B,C}$ = mass of benzoic acid solution added to calibration blend, (*g*); $m_{D,C}$ = mass of benzoic acid-d5 solution added to calibration blend, (*g*); R_S = measured peak area ratio of the selected ions in the sample blend; R_C= measured peak area ratio of the selected ions in the calibration blend Equation used to estimate measurement uncertainty: $u(w_{BS}) = w_{BS}\sqrt{\left(\frac{SDr/\sqrt{n}}{w_{BS}}\right)^2 + \sum\left(C_j\frac{u(m_i)}{m_i}\right)^2 + \sum\left(C_j\frac{u(R_i)}{R_i}\right)^2 + \left(C_j\frac{u(w_{BC})}{w_{BC}}\right)^2}$ where SD_r is the standard deviation under reproducibility conditions, *n* the number of determinations and C_j the sensitivity coefficients associated with each uncertainty component. The uncertainty of the peak area ratios was considered to have been included in the estimation of method precision.	• Method precision • Mass fraction of benzoic acid in calibration solution • Mass fraction of benzoic acid-d5 solution added to sample blend • Mass of test sample in sample blend • Mass of benzoic acid solution added to calibration blend • Mass of benzoic acid-d5 solution added to calibration blend • Measured peak area ratio of the selected ions in the sample blend • Measured peak area ratio of the selected ions in the calibration blend

GLHK	$c_X = c_Z \cdot \frac{m_Y}{m_X} \cdot \frac{m_{Zc}}{m_{Yc}} \cdot \frac{R_B}{R_{Bc}}$ C_x: mass fraction of native analyte in sample; C_Z: mass fraction of the native standard used to prepare the calibration blend; m_Y: mass of labelled standard added to sample; m_X: mass of sample used; m_{Zc}: mass of the native standard added to the calibration blend; m_{Yc}: mass of the labelled standard added to the calibration blend; R_B: measured ion ratio (native/labelled) of the sample blend; R_{Bc}: measured ion ratio (native/labelled) of the calibration blend	• Precision • Method bias • Purity of calibrants
HSA	$C_X = C_Z \cdot \frac{m_Y \cdot m_{Zc}}{m_X \cdot m_{Yc}} \cdot \frac{R_Y - R_B}{R_B - R_X} \cdot \frac{R_{Bc} - R_Z}{R_Y - R_{Bc}}$ C_Z: mass fraction of benzoic acid/methyl paraben/n-butyl paraben in the calibration standard solution used to prepare the calibration blend; m_Y: mass of internal standards solution added to the sample blend; m_{Yc}: mass of internal standard solution added to the calibration blend; m_{Zc}: mass of standard solutions added to the calibration blend; m_X: mass of study material in the sample blend; R_X: observed isotope abundance ratio in the study material; R_Y: observed isotope abundance ratio in the internal standard; R_Z: observed isotope abundance ratio in the calibration standard; R_B: observed isotope abundance ratio in the sample blend; R_{Bc}: observed isotope abundance ratio in the calibration blend.	• Method precision • Bias in the result due to choice of instrument (GC-MS and LC-MS) • bias in the result due to choice of ion pair • Method recovery • Concentration of calibration standard solution (purity of calibration standard, weighing, different calibration blends) • Mass of study material, standard solution, internal standard solutions

INMETRO	$w_a = \left(\frac{A_a}{A_{IS}} - b0\right) \times \frac{m_{IS} \times m_{solIS} \times Pa}{b1 \times m_{sample} \times m_{totalsolIS}}$; $C_X = F_p.F_i.F_{ip}.F_r.C_Z \cdot \frac{m_Y \cdot m_{ZC}}{m_X \cdot m_{YC}} \cdot \frac{R_Y - R_B}{R_B - R_X} \cdot \frac{R_{BC} - R_Z}{R_Y - R_{BC}}$ w_a = Mass of anlyte; A_a = Area of analyte signal; A_{IS}= Area of internal standard signal; $b0$= Linear coefficient; m_{IS}= Mass of internal standard weighted in the stock solution; m_{solIS} = Mass of internal standard solution added in the sample; Pa= Purity of analyte; $b1$= Angular coefficient; m_{sample} = Mass of sample weighted; $m_{totalsolIS}$ = Total mass of internal standard stock solution. Subsequently, w_a was multiplied by 1000000 in order to express the mass fraction in mg/kg.	• Area ratio • Mass of sample • Mass of internal standard solution • Angular coefficient (b1) • Calibration Curve • Linear coefficient (b0) • Purity of calibrant
KRISS	$\mathrm{C_{sample}} = \frac{\mathrm{M_{is-sol,spiked}} \cdot \mathrm{AR_{sample}} \cdot \mathrm{M_{s-sol,std.mix.}} \cdot \mathrm{C_{s-sol}}}{\mathrm{M_{sample}} \cdot \mathrm{AR_{std.mix.}} \cdot \mathrm{M_{is-sol,std.mix.}}}$ $C_{\text{sample:}}$ is the concentration of analytes in the sample; $C_{\text{s-sol:}}$ is the concentration of the analytes standard solution; $M_{\text{sample:}}$ is the mass of the sample taken for analysis; $M_{\text{is-sol, spiked:}}$ is the mass of the isotope standard solution added to the sample aliquot; $M_{\text{is-sol, std. mix.:}}$ is the mass of the isotope standard solution added to the isotope ratio standard solution; $M_{\text{s-sol, std. mix.:}}$ is the mass of the standard solution added to the isotope ratio standard solution; AR_{sample}: is the area ratio of analyte/isotope for sample extract, observed by GC/MS; $AR_{\text{std. mix.:}}$ is the area ratio of analyte/isotope for the isotope ratio standard solution, observed by GC/MS. Combined standards uncertainties: $u_{total.} = \sqrt{u^2_{\text{systematic}} + \frac{s^2}{n}}$	Systematic ($u_{systematic}$) • Uncertainty of purity assay • Uncertainty of gravimetric preparation for standard solutions • Uncertainty of gravimetric mixing for calibration isotope standard mixtures • Uncertainty of observed area ratio of calibration isotope standard mixture by LC/MS ($AR_{\text{std. mix}}$) Random(u_{random}) • Uncertainty of observed area ratio of each subsample by LC/MS (AR_{sample}). The uncertainty is included into s. • Uncertainties of weighing sample (M_{sample}) and isotope standard solution spiked to the sample ($M_{\text{is-sol, spiked}}$). The uncertainties are included into s. Method precision • Standard deviations (s) of multiple measurement results from five subsamples (n=5). It includes u_{random}. Therefore, u_{random}. is not combined into u_{total}.

NIM	$X=\left(\frac{A_s}{A_i}\right)_s \times \left(\frac{A_i}{A_s}\right)_r \times \left(\frac{M_s}{M_i}\right)_r \times \frac{(M_i)_s}{W}$ X : Content of measurands in soy sample (mg/kg); $\left(\frac{A_s}{A_i}\right)_s$: Peak area ratio of measurand and internal standard in sample solution; $\left(\frac{A_i}{A_s}\right)_r$: Peak area ratio of internal standard and measurand in standard solution; $\left(\frac{M_s}{M_i}\right)_r$: Mass ratio of measurand and internal standard in standard solution; $(M_i)_s$: Mass of internal standard added in soy sample (µg); *w*: Mass of soy sample (g).	• Method precision (between batch precision for the method as a whole) • Standard solution (purity of standard solution, weight of standard solution, weight of internal standard solution, weight of solvent) • Sample pretreatment (weight of sample, weight of internal standard solution, weight of solvent)
RCChem-LIPI	HPLC: $X = C_{HPLC} * \frac{m_{centrifuge}}{m_{sample}} * \frac{m_{hplc}}{m_{spe}} * \frac{1}{\mathrm{Re}c}$ GC-MS: $X = C_{GCMS} * \frac{m_{centrifuge}}{m_{sample}} * \frac{m_{hplc}}{m_{spe}} * \frac{m_{final}}{m_{200ul}} * \frac{1}{\mathrm{Re}c}$ X: mass fraction of the analyte (mg/kg); C_{HPLC} : Concentration analyte from calibration curve in the HPLC system; C_{GCMS}: Concentration analyte from calibration curve in the GCMS system; $M_{centrifuge}$: mass of solution of sample in water; M_{sample} : mass of 2 ml of sample; M_{hplc} : mass of final methanol solution after celan up with SPE; M_{SPE} :mass of 1 ml aliquot of sample in water solution that is pass through in to the SPE cartridge; M_{final} : mass of the final solution for analysis with GCMS M_{200ul} : mass of the 200 uL of final methanol solution aliquot to be diluted Rec : recovery factor	HPLC: • Calibration curve • Mass of final methanol solution • Mass of 1 ml aliquot of sample in water solution that is pass through in to the SPE cartridge • Mass of sample • Mass of solution of sample in water • Recovery • Repeatability sample • Middle standard solution GC-MS • Mass of sample • Mass of solution of sample in water • Mass of 1 ml aliquot of sample in water solution that is pass through in to the SPE • Mass of final methanol solution after celan up with SPE • Mass of the 200 uL of final methanol solution to be diluted • Mass of the final solution for analysis with GCMS • Presision • Calibration curve • Recovery • Middle standard solution

STD-ITDI	$C_{Measurand} = \frac{m_{is-sp}\ x\ m_{std}\ x\ AR_{sample}\ x\ C_{stock,std}}{m_{sample}\ x\ AR_{std}\ x\ m_{is-sp,std}}$ $C_{Measurand}$:concentration of measurand; $m_{is-sp,sample}$: mass of isotope solution added to the sample solution; m_{std}: mass of standard stock solution in the standard-isotope calibration blend; AR_{sample}: observed response ratio of the standard/isotope in the sample; $c_{stock,std}$: concentration of the standard stock solution; m_{sample}: mass of the sample taken for analysis; AR_{std}: observed response ratio of standard/isotope in the standard-isotope calibration blend; $m_{is-sp,std}$: mass of isotope stock solution in the standard-isotope blend	• Concentration of standard stock solution purity and gravimetric preparation) • Calibration standard mixture (gravimetric mixing of blends) • Mass of sample for analysis • Mass of internal standard in the sample • Peak area ratio of analyte and internal standard in calibration blend • Peak area ratio of analyte and internal standard in the sample
TISTR	$C_X = C_Z \cdot \frac{M_Y \cdot M_{Zc}}{M_X \cdot M_{Yc}} \cdot \frac{R'_B}{R'_{Bc}}$ For uncertainty estimation: $w_x = F_P.F_E.w_{z,c} \cdot \frac{m_y \cdot m_{z,c}}{m_x \cdot m_{y,c}} \cdot \frac{R'_b}{R'_{b,c}}$ w_x = mass fraction of analyte in sample; $w_{Z,C}$ = mass fraction of analyte in the calibration solution used to prepare the calibration blend; m_Y = mass of spike solution added to sample blend; $m_{Y,C}$ = mass of spike solution added to calibration blend; m_x =mass of sample added to sample blend; $m_{Z,C}$ = mass of solution added to calibration blend; R'_B , $R'_{B,C}$ = observed isotope amount ration in the sample blend and the calibration blend, respectively; F_P = method precision factor; F_E = Extraction efficiency factor	Benzoic acid • Method precision • Weighing • Mass fraction of analyte in the calibration solution • R'b and R'bc • Extraction effects/matrix effects Methyl paraben and n-butyl paraben • Method precision • Weighing • Mass fraction of analyte in the calibration solution • R'b and R'bc • Matrix effects • Interference from two different ion pairs
UME	$C_{sample} = \frac{Area_{sample}}{Area_{IS}} \frac{C_{Std}}{C_{IS}} \frac{Area_{STD-IS}}{Area_{Sample}} C_{Sample-IS}$	• Weighing of sample • Weighing of internal standard • Native stock solution • Labelled stock solution • Intermediate precision • Recovery • Repeatability

10.0 SUPPLEMENTARY COMPARISON REFERENCE VALUES

Table 18 summarises the proposed supplementary comparison reference values (SCRVs) and their associated standard uncertainties for the three measurands. Two statistical approaches were undertaken, using all results submitted in the supplementary comparison.

The proposed SCRVs were presented at the 2016 CCQM OAWG meeting in April and 2016 APMP TCQM meeting in November. The meetings agreed to the use of the median approach for calculation of the SCRVs for all three measurands as there was no significant difference between the two approaches.

Table 18: Proposed SCRVs and associated uncertainties calculated by arithmetic mean and median approaches

	Measurands		
	Benzoic acid	Methyl paraben	n-Butyl paraben
No. of results (*N*)	10	6	6
Approach 1:			
Arithmetic mean	153.49	100.06	99.55
Standard deviation (SD)	2.81	1.94	2.68
Standard uncertainty (SD/√N)	0.89	0.79	1.09
Approach 2:			
Median	154.55	100.95	99.05
Median absolute deviation (MAD)	1.61	0.53	1.80
1.483×MAD (MAD_e)	2.39	0.78	2.67
Standard uncertainty (1.25×MAD_e/√N)	0.94	0.40	1.36

The participating NMIs/DIs' results with associated reported standard uncertainties, as well as the SCRV (median) and their associated standard uncertainty (1.25×MAD_e/√N) for benzoic acid, methyl paraben and n-butyl paraben are plotted in Figures 1, 2 and 3, respectively.

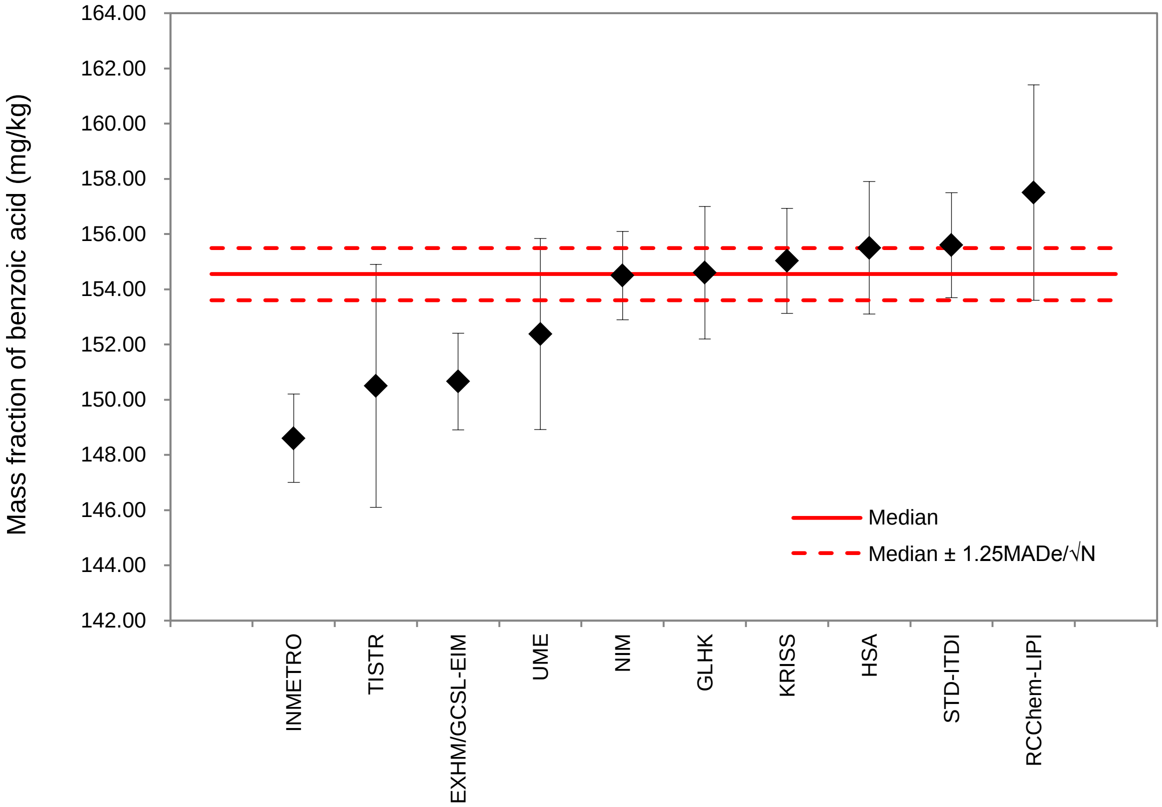


Figure 1: SCRV (solid red line) and its standard uncertainty (red dotted line) for benzoic acid, with participating NMIs/DIs' results and the associated reported standard uncertainties

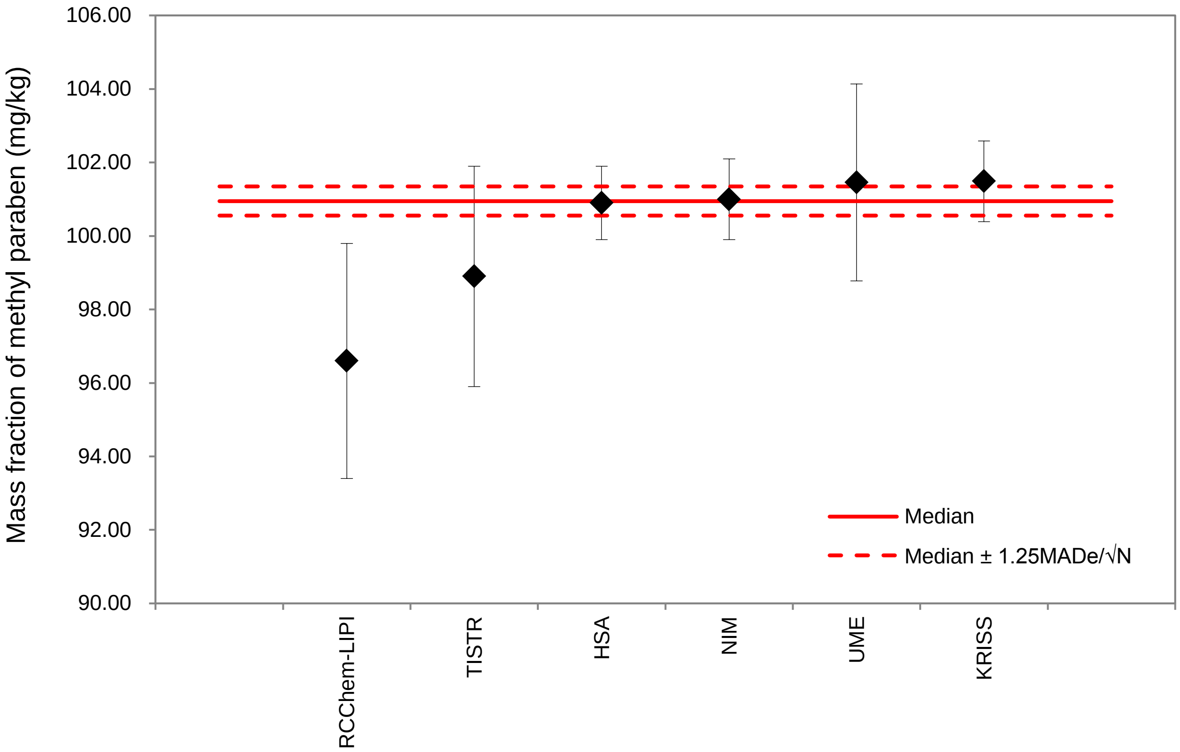


Figure 2: SCRV (solid red line) and its standard uncertainty (red dotted line) for methyl paraben, with participating NMIs/DIs' results and the associated reported standard uncertainties

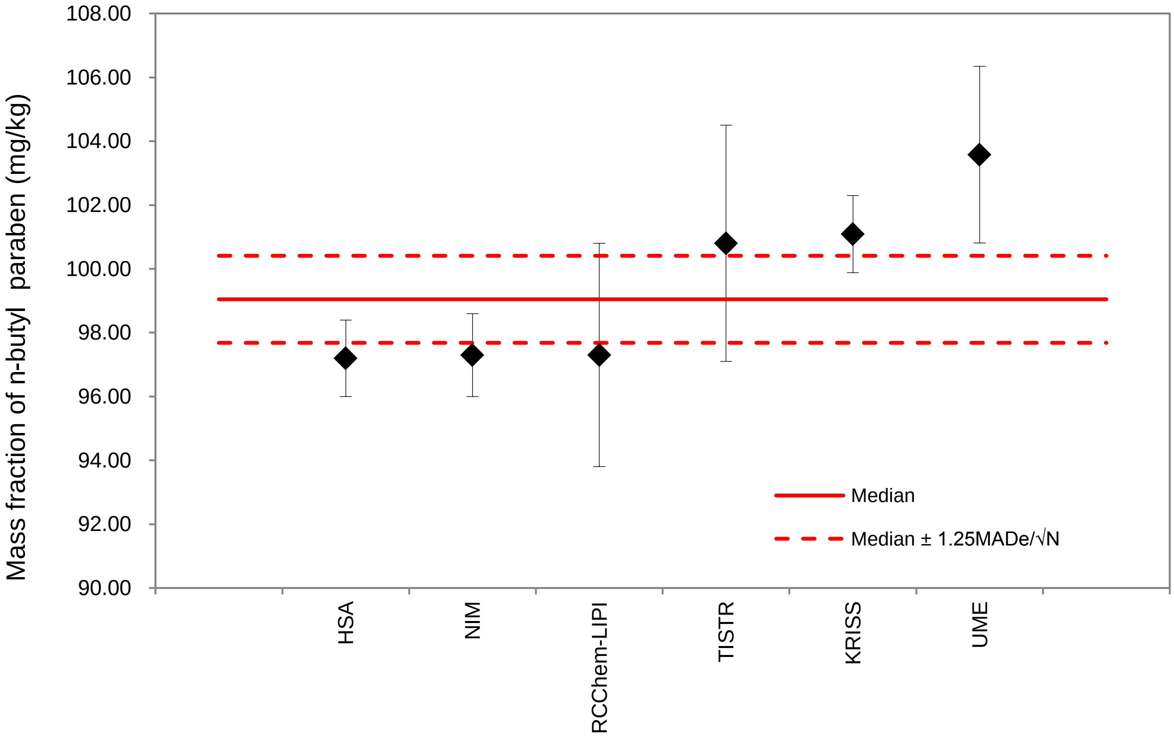


Figure 3: SCRV (solid red line) and its standard uncertainty (red dotted line) for n-butyl paraben, with participating NMIs/DIs' results and the associated reported standard uncertainties

11.0 DEGREES OF EQUIVALENCE (DOE) CALCULATION

The degree of equivalence (D_i) for each participating NMI/DI's result was calculated using the SCRV for each measurand. The D_i was calculated as follows:

$$D_i = X_i - X_{SCRV}$$

where X_i is the reported result of a participating NMI/DI; and X_{SCRV} is the SCRV.

The uncertainty associated with D_i for each participating NMI/DI was estimated as follows:

$$u(D_i) = \sqrt{u^2(X_i) + u^2(X_{SCRV})}$$

The expanded uncertainty of the D_i, i.e. $U(D_i)$ was calculated as:

$$U(D_i) = \sqrt{k_i^2 \times u^2(X_i) + k_{SCRV}^2 \times u^2(X_{SCRV})}$$

where k_i is the coverage factor reported by the participating NMI/DI; and k_{SCRV} is the coverage factor of the SCRV calculated from t(0.05; degrees of freedom) at 95% confidence level.

Tables 19 to 21 summarise the participating NMIs/DIs' D_i (mg/kg), $U(D_i)$ (mg/kg) and $D_i/U(D_i)$ for the three measurands. Figures 4 to 6 present the plots of D_i and their expanded uncertainties [$U(D_i)$] with the respective k_{SCRV} at 95% confidence level for the three measurands.

Table 19: Participating NMIs/DIs' D_i (mg/kg), U(D_i) (mg/kg) and D_i / U(D_i) for benzoic acid

NMI/DI	Benzoic acid		
	D_i	$U(D_i)$	$D_i/U(D_i)$
INMETRO	-5.95	3.98	-1.49
TISTR	-4.05	8.93	-0.45
EXHM/GCSL-EIM	-3.89	4.57	-0.85
UME	-2.17	7.24	-0.30
NIM	-0.05	3.85	-0.01
GLHK	0.05	5.25	0.01
KRISS	0.48	4.80	0.10
HSA	0.95	5.25	0.18
STD-ITDI	1.05	4.36	0.24
RCChem-LIPI	2.95	8.09	0.36

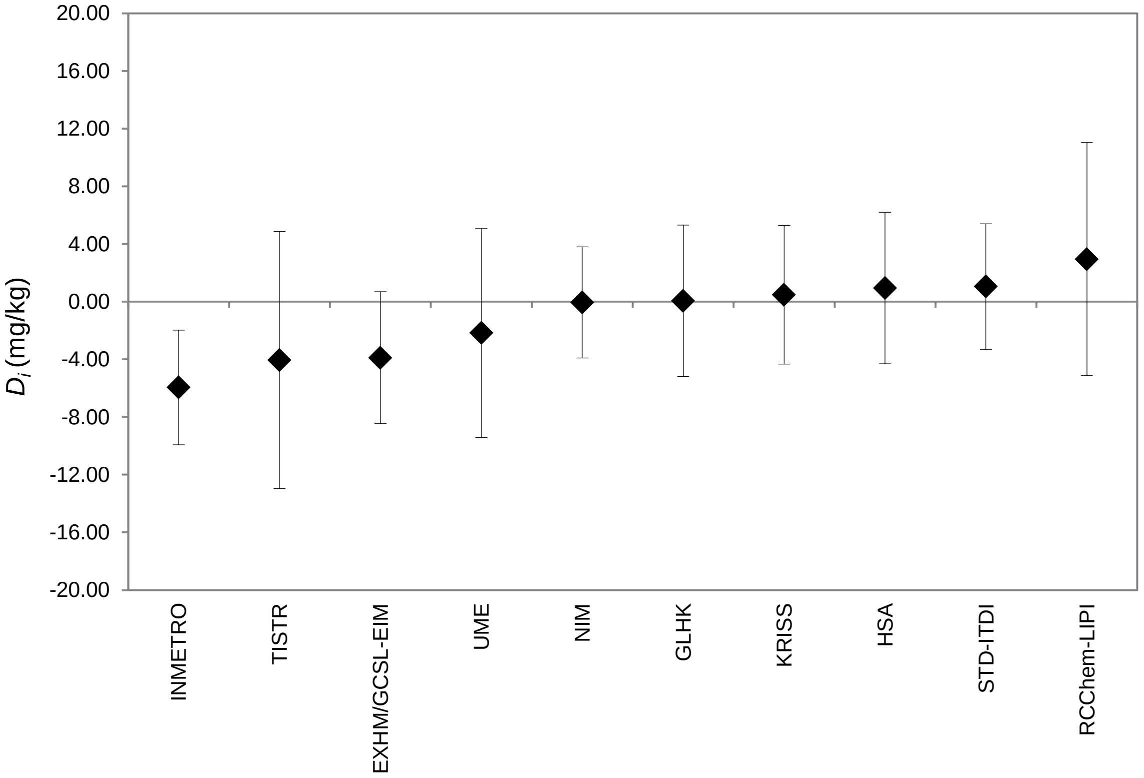


Figure 4: Plot of degrees of equivalence (D_i) and their expanded uncertainties [$U(D_i)$] with k=2.26 at 95% confidence level for benzoic acid.

Table 20: Participating NMIs/DIs' D_i (mg/kg), U(D_i) (mg/kg) and D_i / U(D_i) for methyl paraben

NMI/DI	Methyl paraben		
	D_i	$U(D_i)$	$D_i / U(D_i)$
RCChem-LIPI	-4.35	6.48	-0.67
TISTR	-2.05	6.06	-0.34
HSA	-0.05	2.25	-0.02
NIM	0.05	2.43	0.02
UME	0.51	5.46	0.09
KRISS	0.54	2.66	0.20

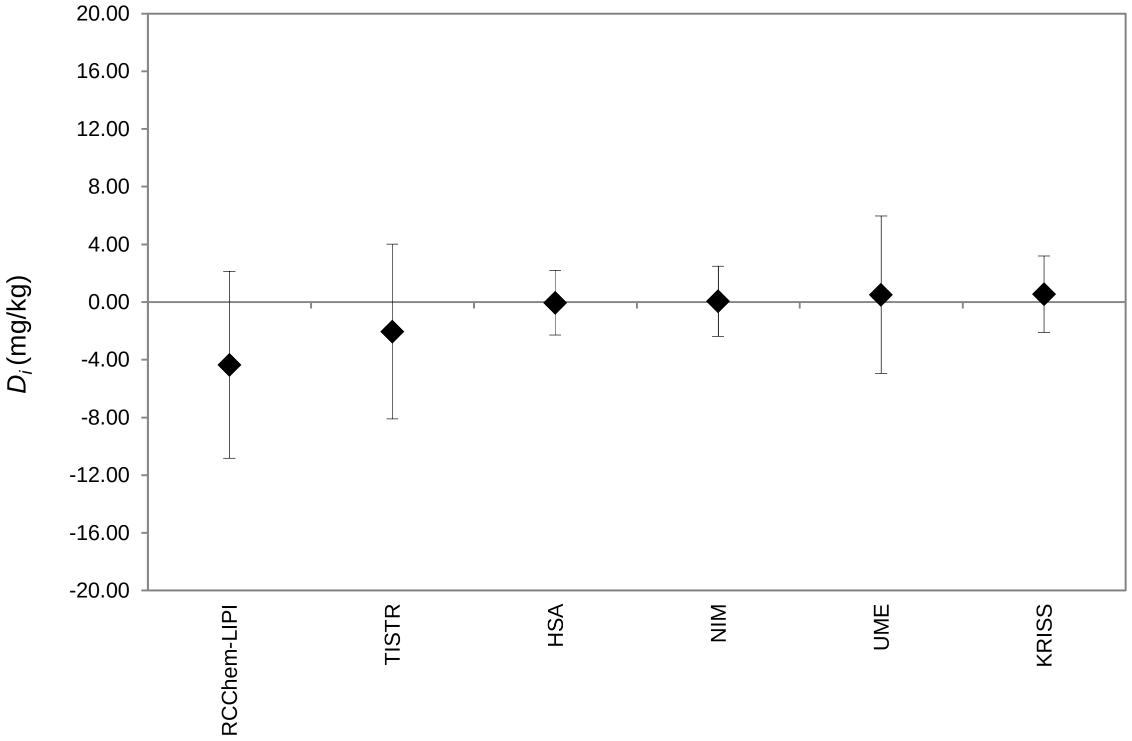


Figure 5: Plot of degrees of equivalence (D_i) and their expanded uncertainties [$U(D_i)$] with k=2.57 at 95% confidence level for methyl paraben.

Table 21: Participating NMIs/DIs' D_i (mg/kg), U(D_i) (mg/kg) and D_i / U(D_i) for n-butyl paraben

NMI/DI	n-Butyl paraben		
	D_i	$U(D_i)$	$D_i/ U(D_i)$
HSA	-1.85	4.25	-0.44
NIM	-1.75	4.36	-0.40
LIPI	-1.75	7.83	-0.22
TISTR	1.75	8.22	0.21
KRISS	2.04	4.52	0.45
UME	4.53	6.55	0.69

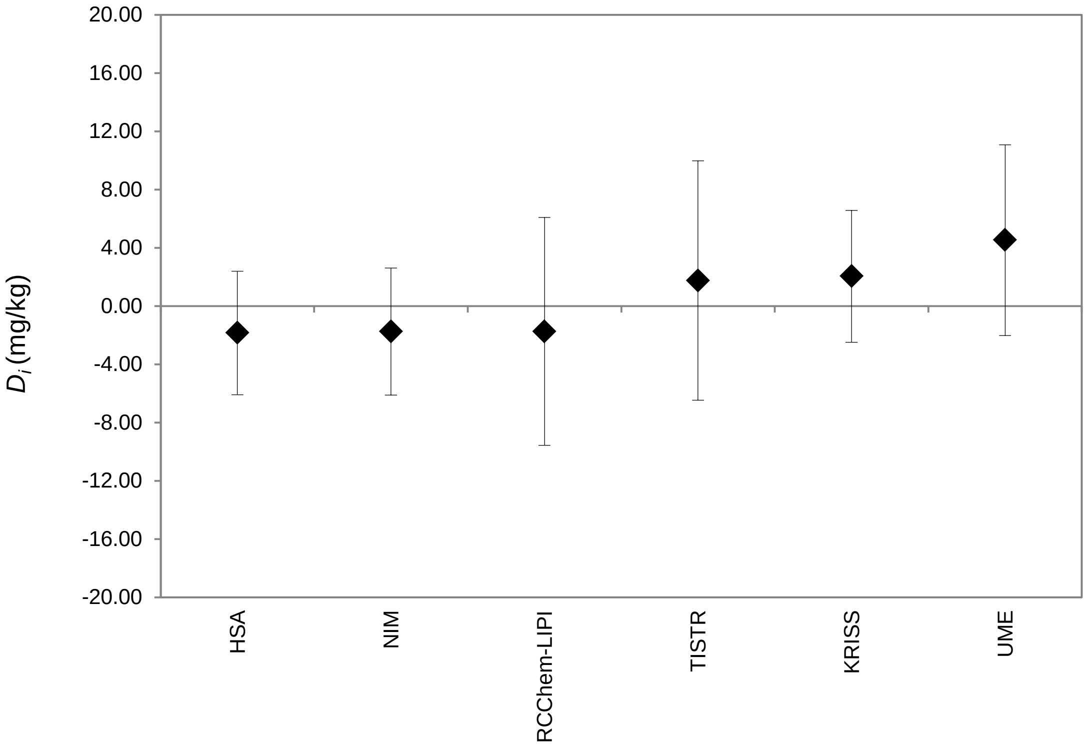


Figure 6: Plot of degrees of equivalence (D_i) and their expanded uncertainties [$U(D_i)$] with k=2.57 at 95% confidence level for n-butyl paraben.

12.0 CORE COMPETENCY AND HOW FAR DOES THE LIGHT SHINE?

This comparison covered a subject which is of wide interest and importance. It enabled participating NMIs/DIs to demonstrate their measurement capabilities in the determination of common preservatives in soy sauce, using procedure(s) that required simple sample preparation and selective detection in the mass fraction range from 50 to 1,000 mg/kg. The APMP TCQM has earlier agreed that the study can be extended to include other polar food preservatives (e.g. sorbic acid, propionic acid and other alkyl benzoates) in water, aqueous-based beverages (e.g. fruit juices, tea extracts, sodas, sports drinks, etc) and aqueous-based condiments (e.g. vinegar, fish sauce, etc).

The Core Competency Tables of the participating NMIs/DIs for the measurands reported in APMP.QM-S8 are presented in APPENDIX II.

13.0 USE OF REPORT

This report is intended to be used as an internal reference for the participating NMIs/DIs and APMP TCQM. Its content shall not be disclosed to other parties or used for other purposes.

ACKNOWLEDGEMENT

The Coordinators of this comparison would like to express their sincere thanks to all the participating institutes for their contributions, as well as for the support of Dr Euijin Hwang & Prof Liandi Ma, Chairs, APMP, TCQM and Dr Lindsey Mackay, Chair, CCQM OAWG.

APPENDIX I

Full Uncertainty Evaluation Reported By the Participating NMIs/DIs

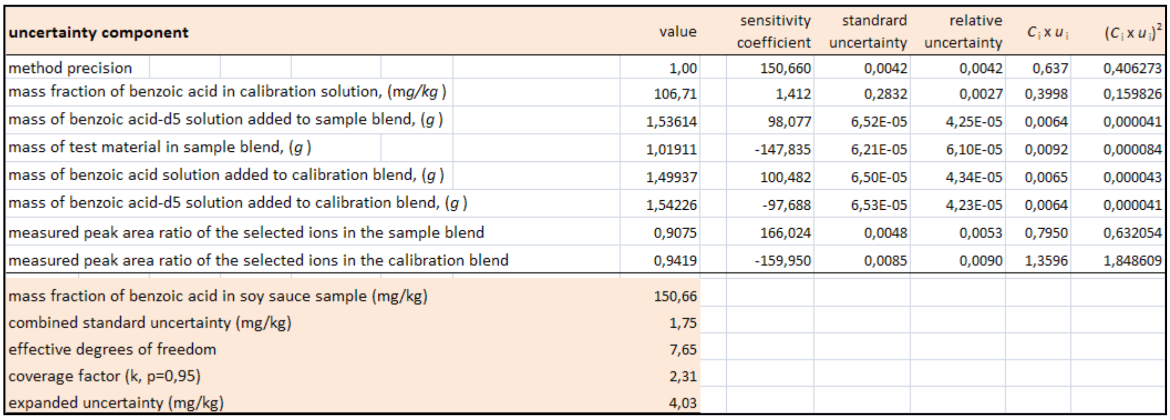
EXHM/GCSL-EIM

The equation used to estimate standard uncertainty is :

$$u(w_{BS}) = w_{BS}\sqrt{\left(\frac{SDr/\sqrt{n}}{w_{BS}}\right)^2 + \sum\left(C_j\frac{u(m_i)}{m_i}\right)^2 + \sum\left(C_j\frac{u(R_i)}{R_i}\right)^2 + \left(C_j\frac{u(w_{BC})}{w_{BC}}\right)^2}$$

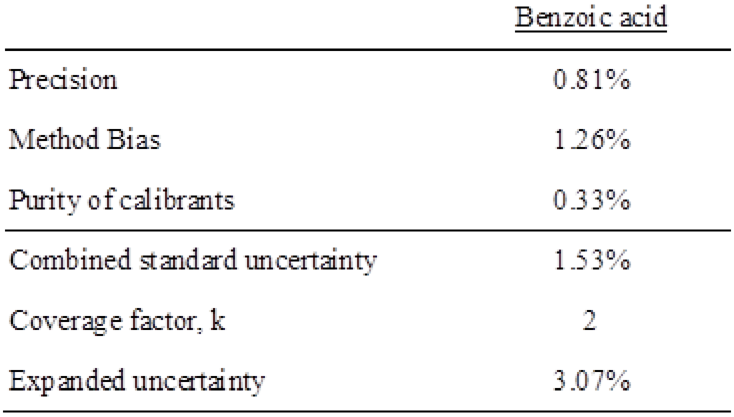
where SD_r is the standard deviation under reproducibility conditions, n the number of determinations and C_j the sensitivity coefficients associated with each uncertainty component. The uncertainty of the peak area ratios was considered to have been included in the estimation of method precision.

Uncertainty estimation was carried out according to JCGM 100: 2008. The standard uncertainties were combined as the sum of the squares of the product of the sensitivity coefficient (obtained by partial differentiation of the measurement equation) and standard uncertainty to give the square of the combined uncertainty. The square root of this value was multiplied by a coverage factor (95% confidence interval) from the t-distribution at the total effective degrees of freedom obtained from the Welch-Satterthwaite equation to give the expanded uncertainty.



uncertainty component	value	sensitivity coefficient	standrard uncertainty	relative uncertainty	$C_i \times u_i$	$(C_i \times u_i)^2$
method precision	1,00	150,660	0,0042	0,0042	0,637	0,406273
mass fraction of benzoic acid in calibration solution, (*mg/kg*)	106,71	1,412	0,2832	0,0027	0,3998	0,159826
mass of benzoic acid-d5 solution added to sample blend, (*g*)	1,53614	98,077	6,52E-05	4,25E-05	0,0064	0,000041
mass of test material in sample blend, (*g*)	1,01911	-147,835	6,21E-05	6,10E-05	0,0092	0,000084
mass of benzoic acid solution added to calibration blend, (*g*)	1,49937	100,482	6,50E-05	4,34E-05	0,0065	0,000043
mass of benzoic acid-d5 solution added to calibration blend, (*g*)	1,54226	-97,688	6,53E-05	4,23E-05	0,0064	0,000041
measured peak area ratio of the selected ions in the sample blend	0,9075	166,024	0,0048	0,0053	0,7950	0,632054
measured peak area ratio of the selected ions in the calibration blend	0,9419	-159,950	0,0085	0,0090	1,3596	1,848609
mass fraction of benzoic acid in soy sauce sample (mg/kg)	150,66					
combined standard uncertainty (mg/kg)	1,75					
effective degrees of freedom	7,65					
coverage factor (k, p=0,95)	2,31					
expanded uncertainty (mg/kg)	4,03					

GLHK



	Benzoic acid
Precision	0.81%
Method Bias	1.26%
Purity of calibrants	0.33%
Combined standard uncertainty	1.53%
Coverage factor, k	2
Expanded uncertainty	3.07%

HSA

$$C_X = F_p.F_i.F_{ip}.F_r.C_Z \cdot \frac{m_Y \cdot m_{ZC}}{m_X \cdot m_{YC}} \cdot \frac{R_Y - R_B}{R_B - R_X} \cdot \frac{R_{BC} - R_Z}{R_Y - R_{BC}}$$

where

additional factors (*F*) contributing to biases in the result value of benzoic acid or methyl paraben or n-butyl paraben were included by assigning a mean value of 1, with an associated uncertainty value to this mean value.

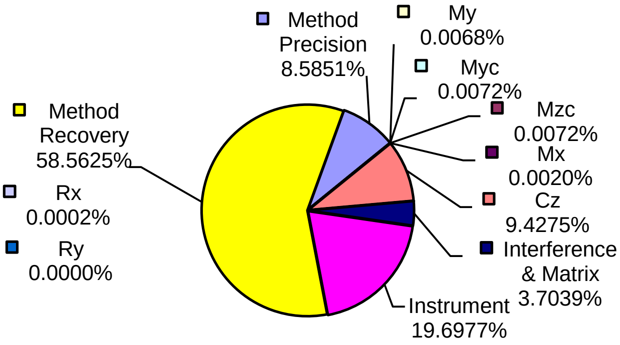
F_p = Factor representing method precision

F_i = Factor representing any bias in the result due to choice of instrument

F_{ip} = Factor representing any bias in the result due to choice of ion pair

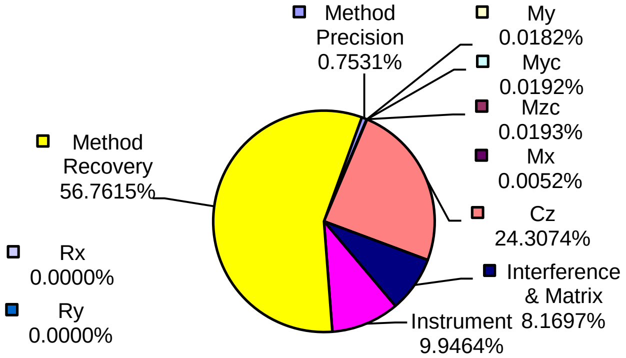
F_r = Factor representing method recovery

The full uncertainty budget for the determination of benzoic acid is given in the Table below:



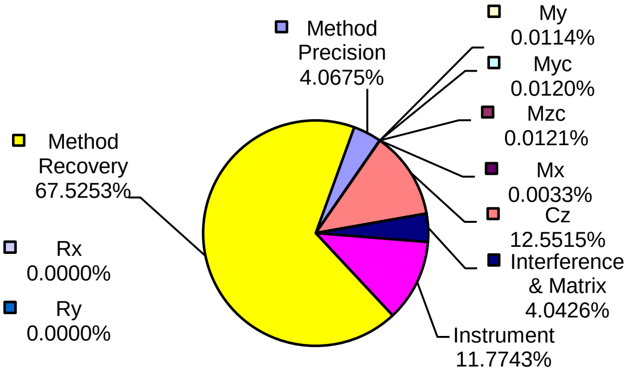
Parameter	x_i	u_{xi}	u_{xi}/x	Contribution	*Sources of uncertainty*
F_p	1	0.004540	0.4543 %	8.5851%	Standard deviation of the mean of 9 independent determinations on the study material
F_i	1	0.006882	0.6882 %	19.6977%	Comparison of results obtained using GC-MS and LC-MS
F_{ip}	1	0.002984	0.2984 %	3.7039%	Comparison of results obtained using different ion pairs
F_r	1	0.011866	1.1866	58.5625%	Uncertainty in method recovery
C_Z	100.9371	0.480540	0.4761 %	9.4275%	• Uncertainty in the purity value of benzoic acid certified reference material (HRM-1002A) • Uncertainty in weighing based on value from the in-house balance calibration certificate
m_Y	0.5517	0.000071	0.0128	0.0068%	Uncertainty based on value from the in-house balance calibration certificate
m_{Yc}	0.5371	0.000071	0.0132	0.0072%	
m_{Zc}	0.5366	0.000071	0.0132	0.0072%	
m_X	1.0304	0.000071	00069	0.0020%	
R_X, R_Y, R_Z	Uncertainty included in method precision				
R_B, R_{Bc}	Negligible				

The full uncertainty budget for the determination of methyl paraben is given in the Table below:



Parameter	x_i	u_{xi}	u_{xi}/x	Contribution	*Sources of uncertainty*
F_p	1	0.000824	0.0824 %	0.7531%	Standard deviation of the mean of 9 independent determinations on the study material
F_i	1	0.002993	0.2993 %	9.9464%	Comparison of results obtained using GC-MS and LC-MS
F_{ip}	1	0.002712	0.2712 %	8.1697%	Comparison of results obtained using different ion pairs
F_r	1	0.007150	0.7150	56.7615%	Uncertainty in method recovery
C_Z	63.6893	0.297979	0.4679 %	24.3074%	• Uncertainty in the purity value of methyl paraben certified reference material (HRM-1003A) • Uncertainty in weighing based on value from the in-house balance calibration certificate • Comparison of results obtained from different calibration blends bracketing the same sample blend
m_Y	0.5517	0.000071	0.0128	0.0182%	Uncertainty based on value from the in-house balance calibration certificate
m_{Yc}	0.5371	0.000071	0.0132	0.0192%	
m_{Zc}	0.5366	0.000071	0.0132	0.0193%	
m_X	1.0304	0.000071	0.0069	0.0052%	
R_X, R_Y, R_Z	Uncertainty included in method precision				
R_B, R_{Bc}	Negligible				

The full uncertainty budget for the determination of n-butyl paraben is given in the Table below:



Parameter	x_i	u_{xi}	u_{xi}/x	Contribution	*Sources of uncertainty*
F_p	1	0.002420	0.2420 %	4.0675%	Standard deviation of the mean of 9 independent determinations on the study material

F_i	1	0.004118	0.4118 %	11.7743%	Comparison of results obtained using GC-MS and LC-MS
F_{ip}	1	0.002413	0.2413 %	4.0426%	Comparison of results obtained using different ion pairs
F_r	1	0.009861	0.9861 %	67.5253%	Uncertainty in method recovery
C_Z	67.0825	0.285183	0.4251 %	12.5515%	• Uncertainty in the purity value of n-butyl paraben certified reference material (HRM-1004A) • Uncertainty in weighing based on value from the in-house balance calibration certificate • Comparison of results obtained from different calibration blends bracketing the same sample blend
m_Y	0.5517	0.000071	0.0128 %	0.0114%	Uncertainty based on value from the in-house balance calibration certificate
m_{Yc}	0.5371	0.000071	0.0132 %	0.0120%	
m_{Zc}	0.5366	0.000071	0.0132 %	0.0121%	
m_X	1.0304	0.000071	0.0069 %	0.0033%	
R_X, R_Y, R_Z	Uncertainty included in method precision				
R_B, R_{Bc}	Negligible				

INMETRO

Uncertainty was firstly estimated during the analysis of each subsample. Tables 1 and 2 show examples of the determination of benzoic acid and methyl paraben, respectively, for one of the subsamples. The same is shown in Figures 1 and 2 in order to highlight the major contributions. Combined standard uncertainty was then calculated by the square root of the sum of the squares of the standard uncertainties calculated for each subsample. This resulted in 1.6 mg/kg for benzoic acid and for methyl paraben, as reported in item 1. Coverage factors were calculated considering the effective degrees of freedom.

Table 1 – Uncertainty budget for benzoic acid

Sources of uncertainty	***Type***	***Distribution***	***Standard uncertainty***	***Sensitivity coefficient***	***Uncertainty component***
Area ratio	A	Normal	0.00562311	0.00011228	6.314E-07
Mass of sample	B	Normal	0.000015	-0.00014586	2.188E-09
Mass of IS solution	B	Normal	0.000015	0.00014895	2.234E-09
Angular coefficient (b1)	A	Normal	0.00459928	-0.00013970	-
Linear coefficient (b0)	A	Normal	0.00478592	-0.00011228	-
Calibration Curve	A	Normal	-	-	2.327E-07
Purity of calibrant	B	Normal	0.00072093	0.00014921	1.076E-07
Standard uncertainty					6.814E-07
Standard uncertainty (mg/kg)					0.6814

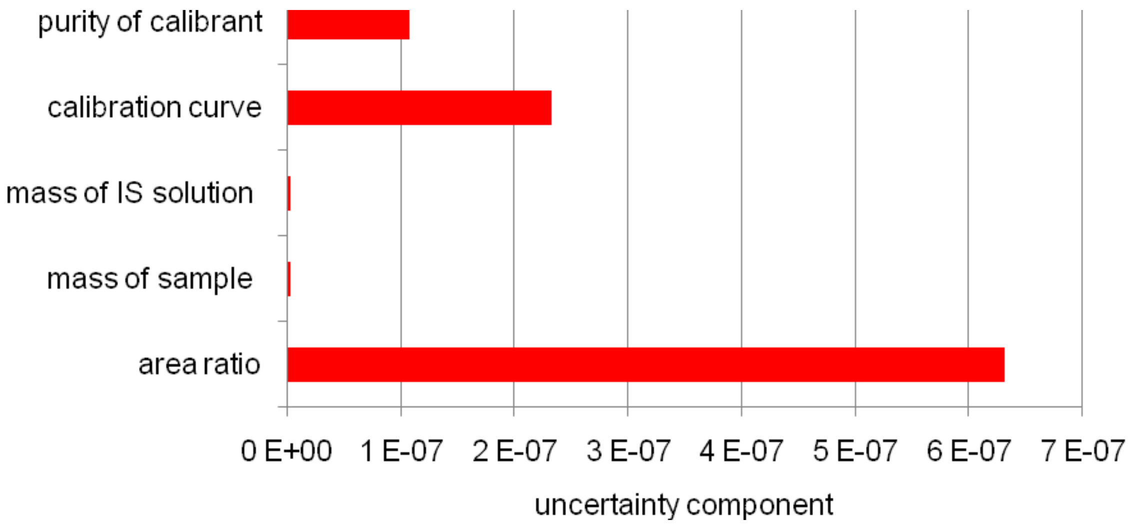


Figure 1 – Magnitude of contribution of uncertainty sources for benzoic acid determination

KRISS

Combined standards uncertainties were obtained by combining systematic uncertainties and random uncertainties, which can be experimentally estimated by the standard deviation (s) of measurement results of multiple subsamples, as shown below equation

$$u_{total.} = \sqrt{u_{\mathrm{systematic}}^2 + \frac{s^2}{n}}$$

Detailed for the full uncertainty budget is provided the below table.

	Sources
Systematic ($u_{systematic}$)	Uncertainty of purity assay
	Uncertainty of gravimetric preparation for standard solutions
	Uncertainty of gravimetric mixing for calibration isotope standard mixtures
	Uncertainty of observed area ratio of calibration isotope standard mixture by LC/MS ($AR_{\mathrm{std.\ mix}}$)
Random(u_{random})	Uncertainty of observed area ratio of each subsample by LC/MS (AR_{sample}). The uncertainty is included into s.
	Uncertainties of weighing sample (M_{sample}) and isotope standard solution spiked to the sample ($M_{\mathrm{is\text{-}sol,\ spiked}}$). The uncertainties are included into s.
Method precision	Standard deviations (*s*) of multiple measurement results from five subsamples (*n*=5). It includes u_{random}. Therefore, u_{random}. is not combined into u_{total}.

NIM

Uncertainty budget of benzoic acid				
Parameter	Source of uncertainty	Source of data	x_i	$u_{rel}(x_i)$
Method precision	Between batch precision for the method as a whole	Replicate analysis of samples among six batches	154.5 mg/kg	0.82%
Standard solution	Purity of standard solution	RM specification	1.00 mg/mL	0.5%
	Weight of standard solution	Balance calibration certificate	1 g	0.019%
	Weight of internal standard solution		0.23g	0.083%
	Weight of solvent		10 g	0.0019%
Sample pretreatment	Weight of sample		1 g	0.019%
	Weight of internal standard solution		0.1 g	0.19%
	Weight of solvent		8 g	0.0024%
Combined standard uncertainty		0.98 %		
Relative expanded uncertainty (k=2)		2.0 %		
Uncertainty Analysis Results				
Cx=		154.5 mg/kg		
u(x) =		1.6 mg/kg		
k=		2		
U(x) =		3.2 mg/kg		

Uncertainty budget of methyl paraben				
Parameter	Source of uncertainty	Source of data	x_i	$u_{rel}(x_i)$
Method precision	Between batch precision for the method as a whole	Replicate analysis of samples among six batches	101.0 mg/kg	0.95%
Standard solution	Purity of standard	RM specification	996 mg/g	0.1%
	Weight of standard		0.01 g	0.19%
	Weight of standard solution	Balance calibration certificate	1 g	0.019%
	Weight of internal standard solution		0.23g	0.083%
	Weight of solvent		10 g	0.0019%
Sample pretreatment	Weight of sample		1 g	0.019%
	Weight of internal standard solution		0.1 g	0.19%
	Weight of solvent		8 g	0.0024%
Combined standard uncertainty		1.0%		
Relative expanded uncertainty (k=2)		2.0%		
Uncertainty Analysis Results				
Cx=		101.0 mg/kg		
u(x) =		1.1mg/kg		
k=		2		
U(x) =		2.2 mg/kg		

Uncertainty budget of n-Butyl paraben				
Parameter	Source of uncertainty	Source of data	x_i	$u_{rel}(x_i)$
Method precision	Between batch precision for the method as a whole	Replicate analysis of samples among six batches	97.3 mg/kg	1.21%
Standard solution	Purity of standard	RM specification	997 mg/g	0.1%
	Weight of standard		0.01 g	0.19%
	Weight of standard solution	Balance calibration certificate	1 g	0.019%
	Weight of internal standard solution		0.23g	0.083%
	Weight of solvent		10 g	0.0019%
Sample pretreatment	Weight of sample		1 g	0.019%
	Weight of internal standard solution		0.1 g	0.19%
	Weight of solvent		8 g	0.0024%
Combined standard uncertainty		1.3%		
Relative expanded uncertainty (*k*=2)		2.6%		
Uncertainty Analysis Results				
Cx=		97.3 mg/kg		
u(x) =		1.3mg/kg		
k=		2		
U(x) =		2.6 mg/kg		

RCChem-LIPI

HPLC-DAD

$$C = \frac{C_{hplc} \times M_{hplc} x M_{centrifuge}}{M_{SPE} x M_{sample}} x \frac{1}{\mathrm{Re}\,c}$$

$$u_{combine} = C \sqrt{\left(\frac{u_{Chplc}}{C_{hplc}}\right)^2 + \left(\frac{u_{Mhplc}}{M_{hplc}}\right)^2 + \left(\frac{u_{MSPE}}{M_{SPE}}\right)^2 + \left(\frac{u_{Mcentrifuge}}{M_{centrifuge}}\right)^2 + \left(\frac{u_{Msample}}{M_{sample}}\right)^2 + \left(\frac{u_{\mathrm{Re}\,c}}{\mathrm{Re}\,c}\right)^2 + \left(\frac{u_{rep}}{1}\right)^2 + \left(\frac{u_{Cs\tan dard}}{C_{s\tan dard}}\right)^2}$$

Benzoic Acid

Mass fraction (mg/kg)	**157.527**
Replication	13
sd	4.942
%RSD	3.137

The uncertainty budget is summarized in the following table

No	Source of uncertainty	Symbol	Value	u	Unit	Relative standard uncertainty (%)
1	Calibration curve	Co_{hplc}	11.1374	0.193886065	mg/kg	1.741
2	mass of final methanol solution	M_{hplc}	2.9817	2.12132E-05	g	0.000711
3	mass of 1 ml aliquot of sample in water solution that is pass through in to the SPE cartridge	M_{SPE}	1.0200	2.12132E-05	g	0.00208
4	mass of sample	M_{sample}	2.1023	0.000141421	g	0.00673
5	mass of solution of sample in water	$M_{sentrifuge}$	10.1237	0.000141421	g	0.00140
6	Recovery	Recovery	103.9089	1.580259091	%	1.521
7	Repeatability sample	Repeatability	1	0.008701578	-	0.870
8	Middle standard solution		10.5472	0.030070407	mg/kg	0.285
	X (C)	**157.5271755**		**mg/kg**		
	$u_{c(X)}$	**3.91668857**		**mg/kg**	**2.5**	
Coverage factor at confidence level of 95%		**2**				
Expanded uncertainty		**7.83337714**		**mg/kg**		
Result		**157.5 ± 7.8**		**mg/kg**		

Methyl Paraben

Mass fraction (mg/kg)	96.579
Replication	13
sd	2.410
%RSD	2.495

The uncertainty budget is summarized in the following table

No	**Source of uncertainty**	**Symbol**	**Value**	**u**	**Unit**	**Relative Standard Uncertainty (%)**
1	Calibration curve	Co_{hplc}	6.9015	0.095503547	mg/kg	1.384
2	mass of final methanol solution	M_{hplc}	2.9817	2.12132E-05	g	0.000711
3	mass of 1 ml aliquot of sample in water solution that is pass through in to the SPE cartridge	M_{SPE}	1.0200	2.12132E-05	g	0.00208
4	mass of sample	M_{sample}	2.1023	0.000141421	g	0.00677
5	mass of solution of sample in water	$M_{centrifuge}$	10.1237	0.00014142	g	0.00140
6	Recovery	Recovery	103.2608	3.04839692	%	2.952
7	Repeatability sample	Repeatability	1	0.00692142	-	0.692
8	Middle standard solution		10.4444	0.027436596	mg/kg	0.263
	X (C)	**96.57882833**	**mg/kg**			
	$u_{C(X)}$	**3.228987879**	**mg/kg**			**3.3**
Coverage factor at confidence level of 95%		**2**				
Expanded uncertainty		**6.457975**	**mg/kg**			
Result		**96.6 ± 6.5**	**mg/kg**			

GCMS

Without derivatization technique

$$X = C_{GCMS} * \frac{m_{centrifuge}}{m_{sample}} * \frac{m_{hplc}}{m_{spe}} * \frac{m_{final}}{m_{200ul}} * \frac{1}{\mathrm{Re}\,c}$$

$$u_{combine} = C\sqrt{\left(\frac{u_{CGC}}{C_{GC}}\right)^2 + \left(\frac{u_{Mhplc}}{M_{hplc}}\right)^2 + \left(\frac{u_{MSPE}}{M_{SPE}}\right)^2 + \left(\frac{u_{Mcentrifuge}}{M_{centrifuge}}\right)^2 + \left(\frac{u_{Msample}}{M_{sample}}\right)^2 + \left(\frac{u_{\mathrm{Re}\,c}}{\mathrm{Re}\,c}\right)^2 + \left(\frac{u_{rep}}{1}\right)^2 + \left(\frac{u_{Cs\tan dard}}{C_{s\tan dard}}\right)^2 + \left(\frac{u_{Mfinal}}{M_{final}}\right)^2 + \left(\frac{u_{200ul}}{M_{200ul}}\right)^2}$$

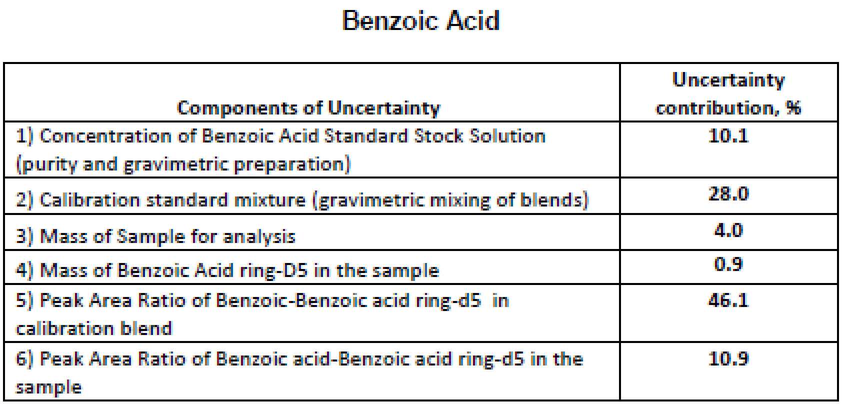
Butyl paraben

Mass fraction (mg/kg)	97.298
Replication	12
sd	4.801
%RSD	4.934

The uncertainty budget is summarized in the following table

No	Source of uncertainty	Symbol	Value	u	Unit	Relative Standard Uncertainty (%)
1	mass of sample	M_{sample}	2.361723	0.000141421	g	0.0059791
2	mass of solution of sample in water	$M_{centrifuge}$	10.37422	0.000141421	g	0.001363196
3	mass of 1 ml aliquot of sample in water solution that is pass through in to the SPE cartridge	M_{spe}	1.0403	2.12132E-05	g	0.00203914
4	mass of final methanol solution after celan up with SPE	M_{hplc}	2.5990	2.12132E-05	g	0.000816206
5	mass of the 200 uL of final methanol solution to be diluted	M_{200ul}	0.1589	2.12132E-05	g	0.013350
6	mass of the final solution for analysis with GCMS	M_{final}	0.8008	2.12132E-05	g	0.002649
7	Presision	Repeatability	1	0.013685199		1.3685199
8	Calibration curve	C_{GC}	1.8120	0.0113	mg/kg	0.62362
9	Recovery	Recovery	1.0169	0.032558472		3.2017378
10	Middle standard solution	Standard solution	3.1895	0.011289444	mg/kg	0.353956
	X (C)	**97.29764**	**mg/kg**			
	$u_{C(X)}$	**3,458882568**	**mg/kg**			**3.6**

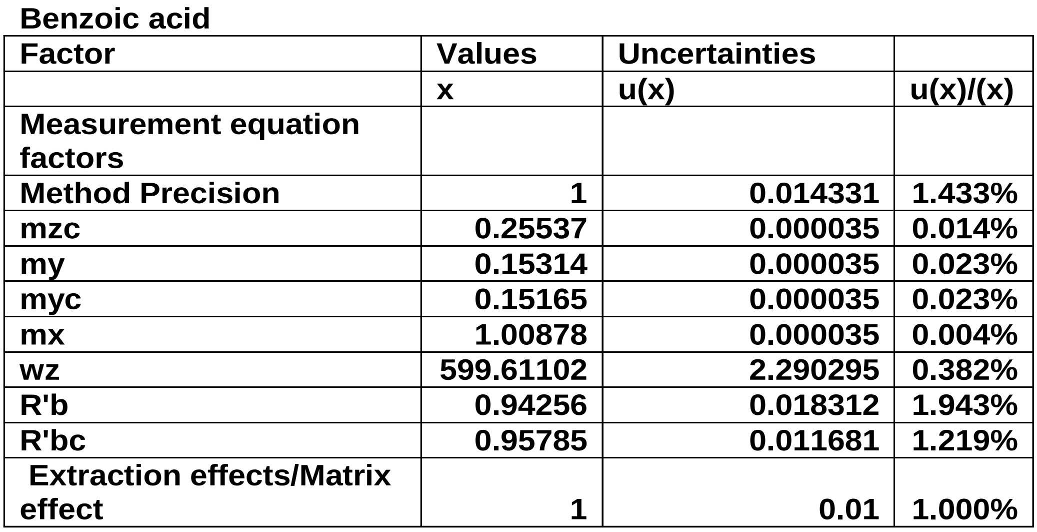
STD-ITDI



Benzoic Acid

Components of Uncertainty	Uncertainty contribution, %
1) Concentration of Benzoic Acid Standard Stock Solution (purity and gravimetric preparation)	10.1
2) Calibration standard mixture (gravimetric mixing of blends)	28.0
3) Mass of Sample for analysis	4.0
4) Mass of Benzoic Acid ring-D5 in the sample	0.9
5) Peak Area Ratio of Benzoic-Benzoic acid ring-d5 in calibration blend	46.1
6) Peak Area Ratio of Benzoic acid-Benzoic acid ring-d5 in the sample	10.9

TISTR



Benzoic acid

Factor	Values	Uncertainties	
	x	u(x)	u(x)/(x)
Measurement equation factors			
Method Precision	1	0.014331	1.433%
mzc	0.25537	0.000035	0.014%
my	0.15314	0.000035	0.023%
myc	0.15165	0.000035	0.023%
mx	1.00878	0.000035	0.004%
wz	599.61102	2.290295	0.382%
R'b	0.94256	0.018312	1.943%
R'bc	0.95785	0.011681	1.219%
Extraction effects/Matrix effect	1	0.01	1.000%

n-butyl paraben			
Factor	**Values**	**Uncertainties**	
	x	**u(x)**	**u(x)/(x)**
Measurement equation factors			
Method Precision	1.0000	0.02598	2.598%
m_{zc}	0.18838	0.000035	0.0188%
m_{y}	0.10174	0.000035	0.0348%
m_{yc}	0.10618	0.000035	0.0333%
m_{x}	0.99996	0.000035	0.0035%
w_{z}	533.5706	3.466894	0.6498%
R'b	1.0847	0.015236	1.4045%
R'bc	1.1295	0.019938	1.7651%
Additional Factors		Enter u(x) = 0 and veff = 1 for unused factors.	
matrix effects	1.000	0.0100	1.000%
Interference from two different ion pairs	1.000	0.0053	0.534%
Methyl paraben			
Factor	**Values**	**Uncertainties**	
	x	**u(x)**	**u(x)/(x)**
Measurement equation factors			
Method Precision	1.0000	0.01897	1.897%
m_{zc}	0.18838	0.000035	0.0188%
m_{y}	0.10174	0.000035	0.0348%
m_{yc}	0.10618	0.000035	0.0333%
m_{x}	0.99996	0.000035	0.0035%
w_{z}	532.9885	2.348845	0.4407%
R'b	1.1473	0.015370	1.3397%
R'bc	1.1462	0.016863	1.4712%
Additional Factors		Enter u(x) = 0 and veff = 1 for unused factors.	
matrix effects	1.000	0.0100	1.000%
Interference from two different ion pairs	1.000	0.0043	0.431%

UME

Uncertainty budget of Benzoic Acid				
		Value	u(x)	u(x)/x
Weighing of sample (mg)		1250	2.56E-04	2.05E-07
Weighing of IS (mg)		140	2.02E-04	1.44E-06
Native stock solution (mg/kg)		1200	6.60E-03	5.50E-06
Labelled stock solution (mg/kg)		1200	1.10	9.18E-04
Intermediate precision		100	8.68E-01	8.68E-03
Recovery		1	1.32E-02	1.32E-02
Repeatability		100	1.62	1.62E-02
				2.27E-02
Result (mg/kg)	152.38			
Combined uncertainty		3.46		
Expanded uncertainty		6.92		
% Relative uncertainty		4.54		
% Relative standard uncertainty		2.27		

Uncertainty budget of methyl paraben				
		Value	u(x)	u(x)/x
Weighing of sample (mg)		1250	2.02E-04	1.62E-07
Weighing of IS (mg)		140	2.56E-04	1.83E-06
Native stock solution (mg/kg)		800	1.50E-02	1.88E-05
Labelled stock solution (mg/kg)		800	2.94E-02	3.68E-05
Intermediate precision		100	6.36E-01	6.36E-03
Recovery		1	2.40E-02	2.40E-02
Repeatability		100	8.78E-01	8.78E-03
				2.64E-02
Result (mg/kg)	101.46			
Combined uncertainty		2.68		
Expanded uncertainty		5.35		
% Relative uncertainty		5.28		
% Relative standard uncertainty		2.64		

Uncertainty budget of butyl paraben				
		Value	u(x)	u(x)/x
Weighing of sample (mg)		1250	1.01E-05	8.11E-09
Weighing of IS (mg)		140	1.48E-04	1.06E-06
Native stock solution (mg/kg)		800	1.57E-02	1.96E-05
Labelled stock solution (mg/kg)		800	1.51E-02	1.88E-05
Intermediate precision		100	4.60E-01	4.60E-03
Recovery		1	2.58E-02	2.58E-02
Repeatability		100	5.47E-01	5.47E-03
				2.68E-02
Result (mg/kg)	103.58			
Combined uncertainty		2.77		
Expanded uncertainty		5.54		
% Relative uncertainty		5.35		
% Relative standard uncertainty		2.68		

APPENDIX II

Core Competency Tables for Measurands Reported in APMP.QM-S8

APMP.QM-S8	***EXHM/ GCSL-EIM***	**Determination of Mass Fraction of Benzoic Acid, Methyl Paraben and n-Butyl Paraben in Soy Sauce**
Scope of Measurement: The supplementary comparison enables participating NMIs/DIs to demonstrate their measurement capabilities in the determination of common preservatives in soy sauce, using procedure(s) that required simple sample preparation and selective detection in the mass fraction range from 50 to 1000 mg/kg. The study can be extended to include other polar food preservatives (e.g. sorbic acid, propionic acid and other alkyl benzoates) in water, aqueous-based beverages (e.g. fruit juices, tea extracts, sodas, sports drinks, etc) and aqueous-based condiments (e.g. vinegar, fish sauce, etc).		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	Pure material Benzoic acid: BAM, U1009
Identity verification of analyte(s) in calibration material.#	N/A	
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).#	✓	Performed additional qNMR experiment on the benzoic acid (acidimetric) CRM using $DMSO_2$ as internal standard (previously measured against 3,5-BTFMBA CRM 4601-a from NMIJ). The re-calculated result (150.65 mg/kg ± 4.04 mg/kg) did not deviate much from the original submitted result (150.66 mg/kg ± 4.03 mg/kg).
For calibrants which are a calibration solution: Value-assignment method(s).#	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Retention time, MRM mode with one ion pair, MS ion ratios
Extraction of analyte(s) of interest from matrix	✓	Liquid-liquid extraction, sonication
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	Filtration
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	✓	LC-MS/MS
Calibration approach for value-assignment of analyte(s) in matrix	✓	IDMS Single-point calibration, matrix-matched, exact-matching

Verification method(s) for value-assignment of analyte(s) in sample (if used)	✓	Standard additions
Other	N/A	

APMP.QM-S8	***GLHK***	**Determination of Mass Fraction of Benzoic Acid, Methyl Paraben and n-Butyl Paraben in Soy Sauce**
Scope of Measurement: The supplementary comparison enables participating NMIs/DIs to demonstrate their measurement capabilities in the determination of common preservatives in soy sauce, using procedure(s) that required simple sample preparation and selective detection in the mass fraction range from 50 to 1000 mg/kg. The study can be extended to include other polar food preservatives (e.g. sorbic acid, propionic acid and other alkyl benzoates) in water, aqueous-based beverages (e.g. fruit juices, tea extracts, sodas, sports drinks, etc) and aqueous-based condiments (e.g. vinegar, fish sauce, etc).		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	Benzoic acid: HSA (HRM-1002A) (999.9 ± 3.3 mg/g) Methyl paraben: HSA (HRM-1003A) (999.5 ± 3.2 mg/g)
Identity verification of analyte(s) in calibration material.#	N/A	
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).#	N/A	
For calibrants which are a calibration solution: Value-assignment method(s).#	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	GCMS: Retention time and the ion ratio LCMSMS: Retention time and MRM transition ratio
Extraction of analyte(s) of interest from matrix	✓	Added 6N HCl to 1g of sample
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	Liquid-liquid extraction by diethyl ether. Dried and reconstituted in chloroform (for GC-MS) or in mobile phase : MeOH (95:5) (for LC-MS/MS)
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	✓	For ID-GC-MS, the extraction solution was derivatized by N-Methyl-N-trimethylsilyl-trifluoracetamide (MSTFA) for detection.
Analytical system	✓	ID-GC-MS

		ID-LC-MS/MS
Calibration approach for value-assignment of analyte(s) in matrix	✓	a) IDMS b) Bracketing (The sample was calibrated by isotope dilution of $^{13}C_6$-Benzoic acid (for benzoic acid) and $^{13}C_6$-Methyl paraben (for methyl paraben). The calibration blends were prepared by gravimetric method. The value-assignment of analytes in matrix was calculated by bracketing method.)
Verification method(s) for value-assignment of analyte(s) in sample (if used)	✓	Matrix CRM from HSA (HRM – 1005A) (Benzoic Acid, Methyl Paraben and n-Butyl Paraben in Soy Sauce) was used to verify the method for value-assignments.
Other	N/A	

APMP.QM-S8	***HSA***	**Determination of Mass Fraction of Benzoic Acid, Methyl Paraben and n-Butyl Paraben in Soy Sauce**
Scope of Measurement: The supplementary comparison enables participating NMIs/DIs to demonstrate their measurement capabilities in the determination of common preservatives in soy sauce, using procedure(s) that required simple sample preparation and selective detection in the mass fraction range from 50 to 1000 mg/kg. The study can be extended to include other polar food preservatives (e.g. sorbic acid, propionic acid and other alkyl benzoates) in water, aqueous-based beverages (e.g. fruit juices, tea extracts, sodas, sports drinks, etc) and aqueous-based condiments (e.g. vinegar, fish sauce, etc).		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	Pure materials Benzoic acid: HSA, HRM-1002A Methyl paraben: HSA, HRM-1003A n-Butyl paraben: HSA, HRM-1004A
Identity verification of analyte(s) in calibration material.[#]	N/A	
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).[#]	N/A	
For calibrants which are a calibration solution: Value-assignment method(s).[#]	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Retention times, SIM mode with three ions on GC-MS, MS ion ratios
Extraction of analyte(s) of interest from matrix	✓	Liquid-liquid extraction, shaking, centrifugation
Cleanup - separation of analyte(s) of	✓	Filtration

interest from other interfering matrix components (if used)		
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	✓	GC-MS
Calibration approach for value-assignment of analyte(s) in matrix	✓	IDMS Single-point calibration, exact-matching
Verification method(s) for value-assignment of analyte(s) in sample (if used)	✓	Two qualifying ions per measurand
Other	N/A	

APMP.QM-S8	***INMETRO***	**Determination of Mass Fraction of Benzoic Acid, Methyl Paraben and n-Butyl Paraben in Soy Sauce**
Scope of Measurement: The supplementary comparison enables participating NMIs/DIs to demonstrate their measurement capabilities in the determination of common preservatives in soy sauce, using procedure(s) that required simple sample preparation and selective detection in the mass fraction range from 50 to 1000 mg/kg. The study can be extended to include other polar food preservatives (e.g. sorbic acid, propionic acid and other alkyl benzoates) in water, aqueous-based beverages (e.g. fruit juices, tea extracts, sodas, sports drinks, etc) and aqueous-based condiments (e.g. vinegar, fish sauce, etc).		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	NIST SRM 350b (highly-pure benzoic acid). 999.93 mg/g ± 3.1 mg/g, k=4.3, 95% (balance mass approach)
Identity verification of analyte(s) in calibration material.[#]	✓	GC-MS and NMR (NMR chemical shifts and MS spectrum consistent with benzoic acid)
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).[#]	N/A	
For calibrants which are a calibration solution: Value-assignment method(s).[#]	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	GC-MS
Extraction of analyte(s) of interest from matrix	✓	Liquid-liquid extraction with ethyl ether
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	N/A	
Transformation - conversion of	✓	Derivatisation with MSTFA, resulting in

analyte(s) of interest to detectable/measurable form (if used)		Benzoic acid trimethylsilyl ester
Analytical system	✓	GC-MS
Calibration approach for value-assignment of analyte(s) in matrix	✓	GC-IDMS using calibration curve approach with isotope labeled internal standard
Verification method(s) for value-assignment of analyte(s) in sample (if used)	✓	Recovery studies of fortified blank matrix
Other	✓	The purity value (999.93 mg/g ± 3.1 mg/g, k=4.3, 95% CI) of the benzoic acid CRM determined by KRISS (based on mass balance approach) was used in place of NIST's certified value (based on coulometric assay). Using this purity value, INMETRO's re-calculated result did not change. The uncertainty associated with the result for benzoic acid would be increased by 4.0% to cross the DoE line (Di = -5.95 mg/kg for reported result of 148.6 mg/kg) for the purpose of CMC claim in future.

DoE result for benzoic acid does not cross zero. The reported value was not consistent with the KCRV. No specific competency in the Table above was identified as the reason. However, INMETRO would increase the uncertainty of their measurement in future.

APMP.QM-S8	***KRISS***	**Determination of Mass Fraction of Benzoic Acid, Methyl Paraben and n-Butyl Paraben in Soy Sauce**
Scope of Measurement: The supplementary comparison enables participating NMIs/DIs to demonstrate their measurement capabilities in the determination of common preservatives in soy sauce, using procedure(s) that required simple sample preparation and selective detection in the mass fraction range from 50 to 1000 mg/kg. The study can be extended to include other polar food preservatives (e.g. sorbic acid, propionic acid and other alkyl benzoates) in water, aqueous-based beverages (e.g. fruit juices, tea extracts, sodas, sports drinks, etc) and aqueous-based condiments (e.g. vinegar, fish sauce, etc).		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	Benzoic acid was purchased from NIST, methyl paraben from SIGMA, and n-butyl from Dr. Ehrenstorfer. Purities of three compounds were assayed by KRISS
Identity verification of analyte(s) in calibration material.[#]	✓	LC/MS/MS
For calibrants which are a highly-	✓	Mass balance: LC/UV (related organic

pure substance: Value-Assignment / Purity Assessment method(s).#		impurities), TGA (non-volatile residue) Headspace GC/MSD (residual solvent) and Karl-Fisher titmetry (water contents)
For calibrants which are a calibration solution: Value-assignment method(s).#	✓	Gravimetrically prepared four solutions were analysed and cross checked by ID-LC/MS/MS.
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	LC/MS/MS
Extraction of analyte(s) of interest from matrix	N/A	No extraction
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	C18 SPE
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	No transformation
Analytical system	✓	ID-LC/MS/MS
Calibration approach for value-assignment of analyte(s) in matrix	✓	One-point exact-matching calibration with an isotope ratio standard solution.
Verification method(s) for value-assignment of analyte(s) in sample (if used)	✓	KRISS CRM 108-06-001
Other	N/A	

APMP.QM-S8	***NIM***	**Determination of Mass Fraction of Benzoic Acid, Methyl Paraben and n-Butyl Paraben in Soy Sauce**
Scope of Measurement: The supplementary comparison enables participating NMIs/DIs to demonstrate their measurement capabilities in the determination of common preservatives in soy sauce, using procedure(s) that required simple sample preparation and selective detection in the mass fraction range from 50 to 1000 mg/kg. The study can be extended to include other polar food preservatives (e.g. sorbic acid, propionic acid and other alkyl benzoates) in water, aqueous-based beverages (e.g. fruit juices, tea extracts, sodas, sports drinks, etc) and aqueous-based condiments (e.g. vinegar, fish sauce, etc).		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	Calibration solution: NIM-CRM GBW(E)100006 Benzoic acid in water 1.00 mg/mL (*U*=1%, *k*=2) Highly-pure substance: NIM-CRM GBW(E)100074 Methyl Paraben 996 mg/g (*U*=0.2%, *k*=2) NIM-CRM GBW(E)100077 n-Butyl Paraben 997 mg/g (*U*=0.2%, *k*=2)

Identity verification of analyte(s) in calibration material.#	N/A	/
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).#	N/A	/
For calibrants which are a calibration solution: Value-assignment method(s).#	N/A	/
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	LC-MS/MS quantifying ions, qualifying ions and retention time
Extraction of analyte(s) of interest from matrix	✓	Liquid –liquid extraction and ultrasonic extraction
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	N/A	/
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	/
Analytical system	✓	LC-MS/MS (ACQUITY UPLC™ Xevo TQ-S)
Calibration approach for value-assignment of analyte(s) in matrix	✓	Single-point calibration (IDMS)
Verification method(s) for value-assignment of analyte(s) in sample (if used)	✓	HPLC-DAD external calibration
Other	N/A	/

APMP.QM-S8	***RCChem-LIPI***	**Determination of Mass Fraction of Benzoic Acid, Methyl Paraben and n-Butyl Paraben in Soy Sauce**
Scope of Measurement: The supplementary comparison enables participating NMIs/DIs to demonstrate their measurement capabilities in the determination of common preservatives in soy sauce, using procedure(s) that required simple sample preparation and selective detection in the mass fraction range from 50 to 1000 mg/kg. The study can be extended to include other polar food preservatives (e.g. sorbic acid, propionic acid and other alkyl benzoates) in water, aqueous-based beverages (e.g. fruit juices, tea extracts, sodas, sports drinks, etc) and aqueous-based condiments (e.g. vinegar, fish sauce, etc).		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	We used "highly-pure substance" from Health Science Authority(HSA), Singapore • HSA HRM-1002A (Benzoic acid, Purity 999.9±3.3 mg/g) • HSA HRM-1003A(Methyl paraben,

		Purity 999.5±3.2 mg/g) • HSA HRM-1004A(n-Butyl paraben, Purity 999.2±3.3 mg/g)
Identity verification of analyte(s) in calibration material.[#]	N/A	
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).[#]	N/A	
For calibrants which are a calibration solution: Value-assignment method(s).[#]	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Identification of analyte(s) in sample was done by using GC-MS.
Extraction of analyte(s) of interest from matrix	✓	Extraction and Clean up was done by employing conditioned C18 Solid Phase Extraction cartridge. The cartridge containing sample was then washed with 4 ml of 10% methanol in 1% phosphoric acid and then the analytes were eluted with 3 ml of methanol.
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	✓	HPLC-DAD and GC-MS were used for quantitative analysis of analyte(s) in matrix.
Calibration approach for value-assignment of analyte(s) in matrix	✓	External calibration approach was used for value-assignment of analyte(s) in matrix.
Verification method(s) for value-assignment of analyte(s) in sample (if used)	✓	The validated analytical method was used for value-assignment of analyte(s) in sample.
Other	✓	CRM matrix (HSA HRM-1005A) was used as quality control material for evaluating the performance of the analytical method.

APMP.QM-S8	***STD-ITDI****	**Determination of Mass Fraction of Benzoic Acid, Methyl Paraben and n-Butyl Paraben in Soy Sauce**
Scope of Measurement: The supplementary comparison enables participating NMIs/DIs to demonstrate their measurement capabilities in the determination of common preservatives in soy sauce, using procedure(s) that required simple sample preparation and selective detection in the mass		

fraction range from 50 to 1000 mg/kg. The study can be extended to include other polar food preservatives (e.g. sorbic acid, propionic acid and other alkyl benzoates) in water, aqueous-based beverages (e.g. fruit juices, tea extracts, sodas, sports drinks, etc) and aqueous-based condiments (e.g. vinegar, fish sauce, etc).		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	The benzoic acid standard (NIST SRM-350b) was purchased from NIST.
Identity verification of analyte(s) in calibration material.#	✓	The identity of benzoic acid was confirmed by LCMSMS
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).#	N/A	
For calibrants which are a calibration solution: Value-assignment method(s).#	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	The identity of benzoic acid was confirmed by LCMSMS
Extraction of analyte(s) of interest from matrix	✓	Extraction with mobile phase (methanol:ammonium acetate buffer 50:50)
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	N/A	
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	✓	LCMSMS
Calibration approach for value-assignment of analyte(s) in matrix	✓	Exact matching IDMS
Verification method(s) for value-assignment of analyte(s) in sample (if used)	✓	HPLC-PDA (gravimetric). Use of CRM from HSA (HRM-1001A) as quality control material.
Other	✓	The purity value (999.93 mg/g ± 3.1 mg/g, k=4.3, 95% CI) of the benzoic acid CRM determined by KRISS using mass balance approach was used in place of NIST's certified value (based on coulometric assay). Using this purity value, STD-ITDI's re-calculated result did not change.

***Important Note: the metrology in chemistry activities under STD-ITDI will eventually be part of the National Metrology Laboratory of ITDI. However, there will be no major**

changes to the manpower and infrastructure supporting the capability demonstrated in this supplementary comparison following the change in organisational structure.

APMP.QM-S8	***TISTR***	**Determination of Mass Fraction of Benzoic Acid, Methyl Paraben and n-Butyl Paraben in Soy Sauce**
Scope of Measurement: The supplementary comparison enables participating NMIs/DIs to demonstrate their measurement capabilities in the determination of common preservatives in soy sauce, using procedure(s) that required simple sample preparation and selective detection in the mass fraction range from 50 to 1000 mg/kg. The study can be extended to include other polar food preservatives (e.g. sorbic acid, propionic acid and other alkyl benzoates) in water, aqueous-based beverages (e.g. fruit juices, tea extracts, sodas, sports drinks, etc) and aqueous-based condiments (e.g. vinegar, fish sauce, etc).		
Competency	**Tick, cross, or “N/A”**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a “highly-pure substance” or calibration solution?	✓	Highly-pure substance purchased from HSA HSA (HRM-1002A) Benzoic acid HSA (HRM-1003A) Methyl paraben calibrant HSA (HRM-1004A) n-Butyl paraben calibrant
Identity verification of analyte(s) in calibration material.#	✓	Identity verification of analytes were done by LC-MS/MS, qualifying ions and retention time
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).#	N/A	
For calibrants which are a calibration solution: Value-assignment method(s).#	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Identity verification of analytes were done by LC-MS/MS, qualifying ions and retention time
Extraction of analyte(s) of interest from matrix	✓	Extraction with methanol followed by sonication
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	Filtration
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	✓	LC-MS/MS (Agilent 6400 Series Triple Quad LC/MS)

Calibration approach for value-assignment of analyte(s) in matrix	✓	Bracketing, exact-matching double IDMS. Matrix-matched calibration standards.
Verification method(s) for value-assignment of analyte(s) in sample (if used)	N/A	Recovery studies by spiking in blank matrix
Other	N/A	

APMP.QM-S8	***UME***	**Determination of Mass Fraction of Benzoic Acid, Methyl Paraben and n-Butyl Paraben in Soy Sauce**
Scope of Measurement: The supplementary comparison enables participating NMIs/DIs to demonstrate their measurement capabilities in the determination of common preservatives in soy sauce, using procedure(s) that required simple sample preparation and selective detection in the mass fraction range from 50 to 1000 mg/kg. The study can be extended to include other polar food preservatives (e.g. sorbic acid, propionic acid and other alkyl benzoates) in water, aqueous-based beverages (e.g. fruit juices, tea extracts, sodas, sports drinks, etc) and aqueous-based condiments (e.g. vinegar, fish sauce, etc).		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	-Benzoic Acid SRM350b (NIST) (KRISS and UME (qNMR) re-evaluated the value) -Methyl paraben calibrant(Across Chemicals) 0.99998 ±0.003 mg/g* -n-Butyl paraben calibrant (AlfaAesar) 0.9999±0.00313* *q NMR was used for value assignment
Identity verification of analyte(s) in calibration material.#	✓	LC-MS and qNMR
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).#	✓	qNMR
For calibrants which are a calibration solution: Value-assignment method(s).#	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	LC-MS
Extraction of analyte(s) of interest from matrix	✓	Liquid-liquid extraction with methanol
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	Filtration Sample Dilution
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	

Analytical system	✓	LC-MS/MS
Calibration approach for value-assignment of analyte(s) in matrix	✓	IDMS
Verification method(s) for value-assignment of analyte(s) in sample (if used)	✓	IDMS
Other	N/A	

APPENDIX III

OTHER INFORMATION PROVIDED IN REPORT OF RESULTS

UME

Synthesis of Calibrants

Methyl (CD_3) 4-hydroxybenzoate

4-hydroxybenzoic acid (1g, 7.24 mmol) was dissolved in methanol (CD_3OD, 6.66 mL, 0.164 mol). After adding concentrated sulphuric acid (1 mL, 10 M) the mixture was refluxed for 5 hours. Saturated sodium hydroxide (≈15 mL) was added to the cooled solution for neutralization. The resulting mixture was allowed to stand for 15 min, before being poured into a cool beaker which was made up to 500 mL with water. White crystalline solid (1.1 g, 98.2 %) was obtained after filtering and drying the precipitate.

^{1}H NMR, δ (600MHz, $CDCl_3$) 7.96 (2H, d, J 8.8 Hz, ArH); 6.89 (2H, d, J 8.8 Hz, ArH) ^{13}C NMR, δ (150 MHz, $CDCl_3$) 167.43; 160.18; 131.95; 122.32; 115.26. [M^-] *m/z* 153.7 .

Butyl (C_4D_9) 4-hydroxybenzoate

4-hydroxybenzoic acid (0.5 g, 3.62 mmol) was refluxed overnight in n-butanol(d10) (1.32 mL, 14.48 mmol) and toluene (0.765 mL, 7.24 mmol) using sulphuric acid as a catalyst (0.15 mL) . The solvent was distilled off and the residual oily liquid was poured into a cool beaker with ice-water. After filtration and drying the precipitates, the white solid of butyl paraben was obtained (88 %).

^{1}H NMR, δ (600MHz, $CDCl_3$) 7.96 (2H, d, J 8.8 Hz, ArH); 6.88 (2H, d, J 8.8 Hz, ArH) ^{13}C NMR, δ (150 MHz, $CDCl_3$) 166.87; 159.93; 131.87; 122.82; 115.17. [M^-] *m/z* 202.0.

Instrumunets and chromatographic conditions

Experiments were performed by a Zivak® HPLC and Zivak® Tandem Gold Triple quadrupole Mass Spectrometry equipped with a Phenomenex Luna 5u C18 column (150 x 2 mm i.d., 5μm particle size). The mobile phase was composed of (A, acetonitrile) in water (B, 5 mM ammonium acetate, pH:4.2), the gradient programme of which was 0-1.00 minute 20 % A and

80 % B, 1.01-8.00 minutes 80 % A and 20 % B, 8.01-8.06 minutes 20 % A and 80 % B and finally 08.06-12.00 20 % A and 80 % B. The flow rate of the mobile phase was 0.30 mL/min, and the column temperature was set to 30 °C. The injection volume was 10 μL (table 1, figure 1).

Table 1. LC-MS/MS parameters of selected compounds

	Compounds	**Parent ion**	**Daughter ion**	**Collision energy (V)**
1	Benzoic acid	120.70	77.00	10
2	Benzoic acid-IS	125.70	82.00	10
3	Methyl paraben	150.50	135.50	15
4	Methyl paraben-IS	153.70	135.50	15
5	Butyl paraben	192.70	136.00	15
6	Butyl paraben-IS	202.00	136.00	15

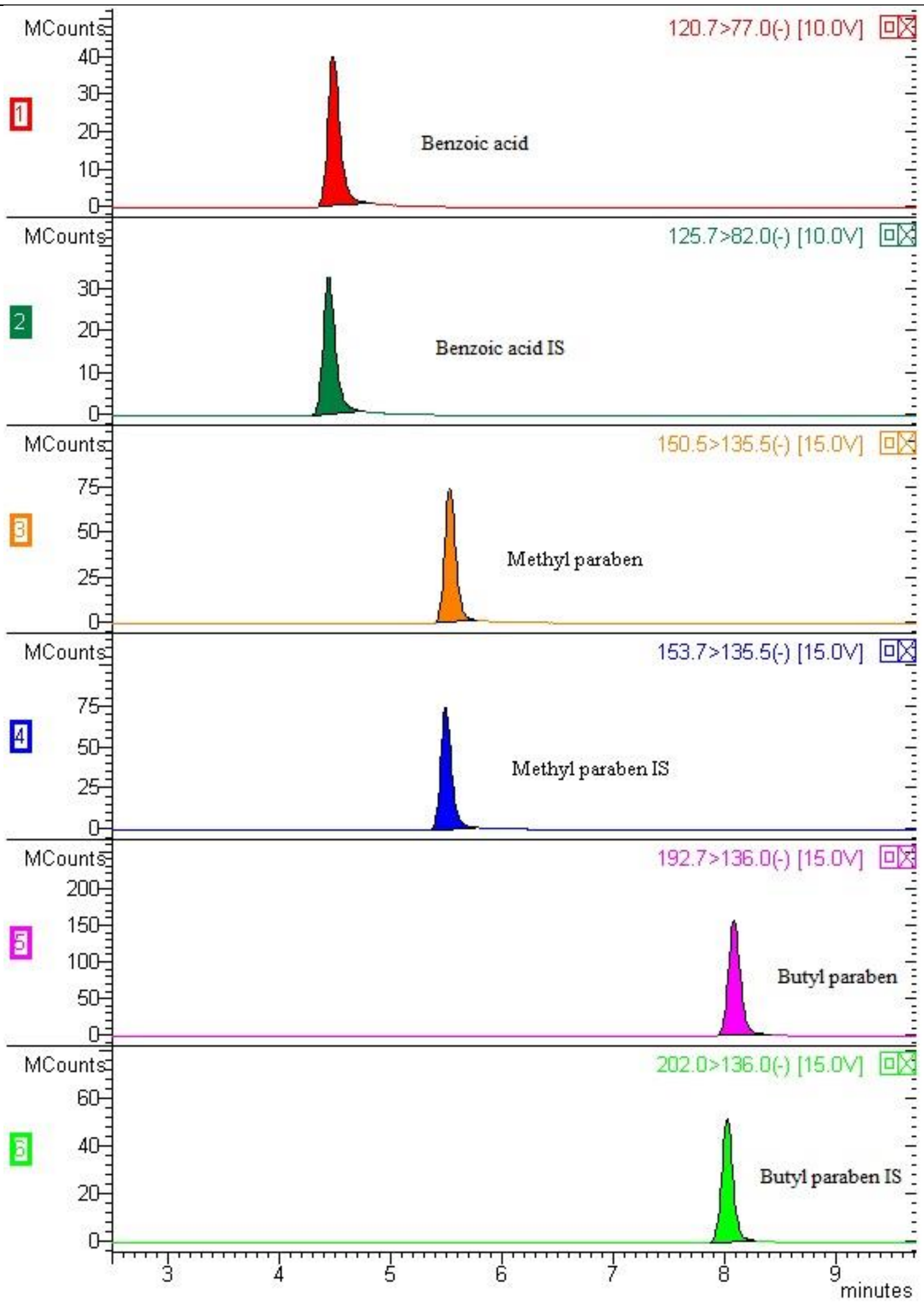


Figure 1. LC-IDMS chromatogram of methyl paraben, butyl paraben and benzoic acid.

Traceability of calibrants

The purity assessment of native and synthesized labeled parabens was done by quantitative nuclear magnetic resonance (qNMR). 1,3,5-trimethoxybenzene was used as internal standard (IS) and methanol-D_4 (Merck, 99.8%) was used as solvent. 1,3,5-trimethoxybenzene purity (99.798 % ± 0.144) was determined by Nist 350b benzoic acid CRM (NIST, Gaithersburg, US). Three different samples were prepared from each paraben and each sample was analyzed with three repetitions.

The sample solution of parabens was prepared by following steps: Paraben (20-30 mg) and 1,3,5-Trimethoxybenzene (20-30 mg) were accurately weighed, dissolved in CD_3OD (2.1 mL), stirred with vortex for 30 seconds and 0.7 mL solution transferred to an NMR tube. All NMR measurements were carried out on a Varian 600 spectrometer operating at 599.90 MHz. The probe used was a Varian's One NMR. The following parameters were employed for acquisition of spectra: spectral width, 16 ppm; acquisition time, 3.4 s; relaxation delay, 40 s; 90° pulse width, 7.2 µs; time domain, 64K data points; 32 scans; temperature, 298.15 K.

All NMR spectra were processed with the software Mestrenova 8.1.0. An exponential line broadening window function of 0.3 Hz was used in the data processing. After Fourier transformation of the free induction decays, the spectra were baseline corrected, phased, and integrated in the appropriate region. The peaks for the analyte and the internal standard were integrated inside, that is, excluding, the 13C satellites.

The calculation equation of qNMR for the purity is as follows:

$$P_x = \frac{I_x}{I_{Std}} \frac{N_{Std}}{N_x} \frac{M_x}{M_{Std}} \frac{m_{Std}}{m_x} P_{Std}$$

I_{Std}, N_{Std}, M_{Std}, m_{Std} and P_{Std} are the peak area, number of proton, molecular weight, mass and purity of the internal standard, respectively. Ix, Nx, Mx, mx and Px are the peak area, number of proton, molecular weight, mass and purity of the sample, respectively.

The calculation equation of the relative standard uncertainty is as follows:

$$\frac{u(Px)}{Px} = \sqrt{\left(\frac{u(Ix/Istd)}{Ix/Istd}\right)^2 + \left(\frac{u(Mx)}{Mx}\right)^2 + \left(\frac{u(Mstd)}{Mstd}\right)^2 + \left(\frac{u(mx)}{mx}\right)^2 + \left(\frac{u(ms)}{ms}\right)^2 + \left(\frac{u(Ps)}{Ps}\right)}$$

The uncertainty from balance is the most important component for the total uncertainty value so all of the samples were weighed with an advanced balance. The uncertainty from molecular weight is often pretty small than integration repeatability. All of the free induction decays of samples were processed by the same phase and baseline correction algorithms and used the same integral regions for the good repeatability (figure 2-4).

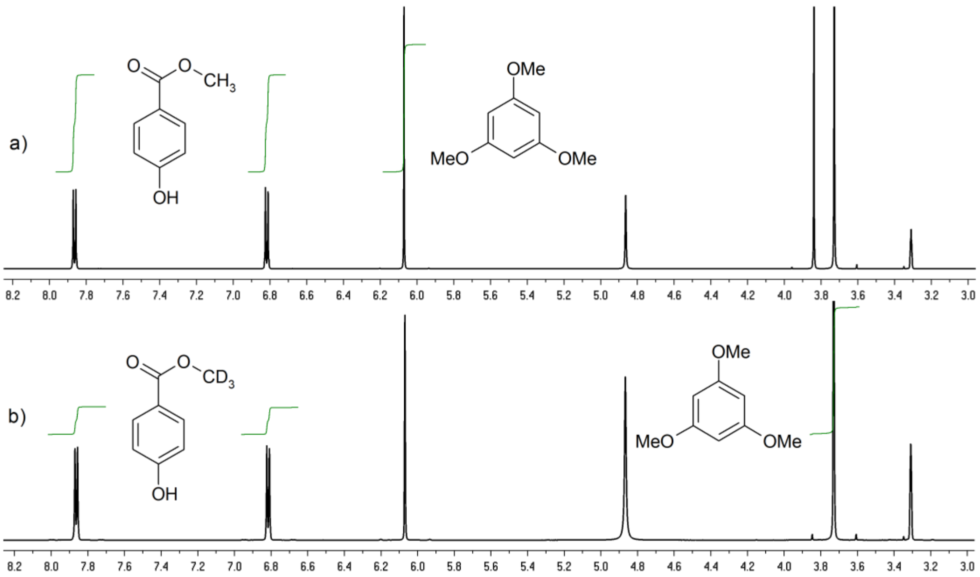


Figure 2. ^{1}H-NMR spectra of methyl paraben and methyl (CD_3) paraben with 1,3,5-trimethoxybenzene.

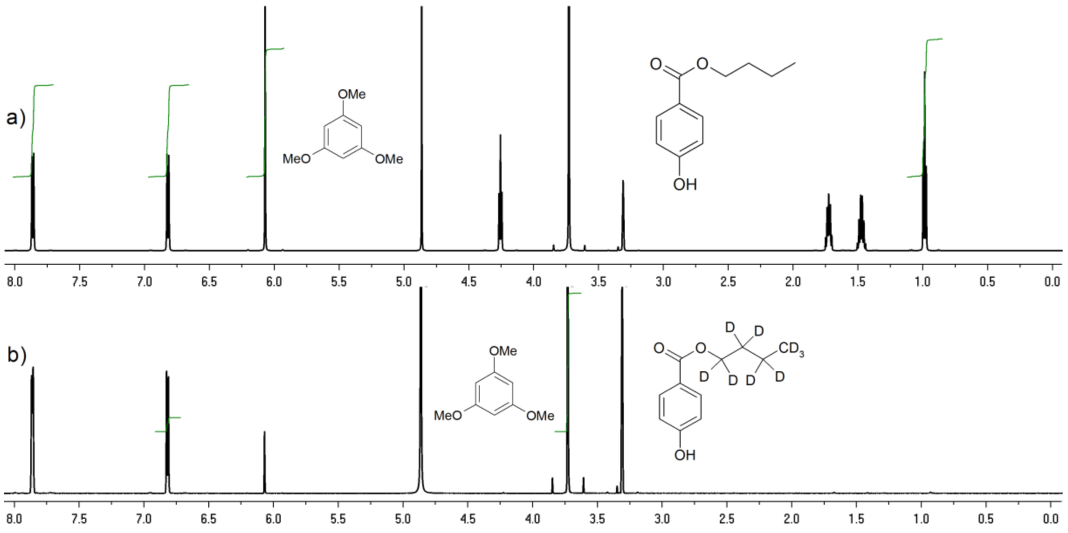


Figure 3. ^{1}H-NMR spectra of butyl paraben and methyl (C_4D_9) paraben with 1,3,5-trimethoxybenzene.

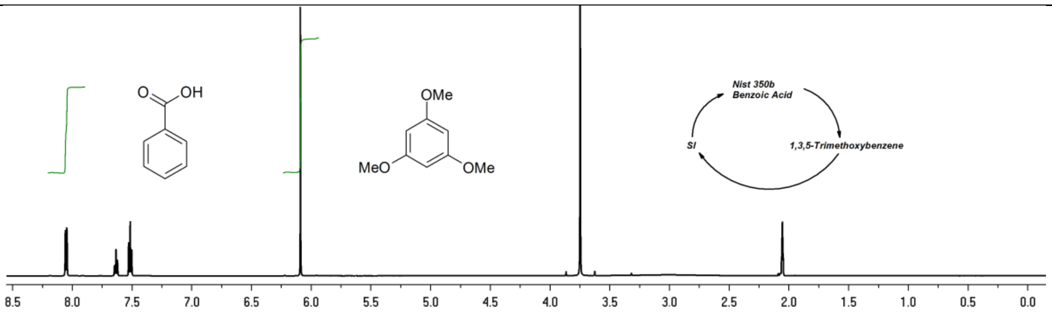


Figure 4. Traceability of 1,3,5-trimethoxybenzene via SRM 350b-Benzoic acid.