

Development of Candidate Reference Materials of Endosulfan Sulfate and Bifenthrin in Black Tea

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ABSTRACT

The candidate reference materials of endosulfan sulfate and bifenthrin in black tea have been developed according to the requirements of ISO Guide 34 and 35. Preparation of candidate material includes grinding and sieving of the black tea leaves, spiking the black tea powder by both analytes, homogenization, and bottling. Homogeneity and short-term stability test were performed using a GC- μ ECD instrument. Meanwhile, the characterization was carried out by a collaborative study using both of GC- μ ECD and GC-MS instruments. The uncertainty budget was evaluated from sample inhomogeneity, short-term instability and variability in the characterization procedure. In a dry mass fraction, endosulfan sulfate was assigned to be $491 \mu\text{g kg}^{-1}$ with a relative expanded uncertainty of $\pm 33.2\%$, and bifenthrin was assigned to be $937 \mu\text{g kg}^{-1}$ with a relative expanded uncertainty of $\pm 18.5\%$. The candidate reference materials are aimed to support the need of matrix CRM especially for the measurement of pesticide residue for quality assurance work done by laboratories in Indonesia.

Keywords: bifenthrin; endosulfan sulfate; GC- μ ECD; pesticide residue; reference material

ABSTRAK

Kandidat bahan acuan endosulfan sulfat dan bifentrin dalam teh hitam telah dikembangkan berdasarkan ketentuan dalam ISO Guide 34 dan 35. Preparasi kandidat bahan meliputi penghancuran dan penyaringan daun teh hitam, penambahan kedua analit yang diinginkan pada bubuk teh hitam, homogenisasi, dan pengemasan ke dalam botol. Uji homogenitas dan stabilitas jangka pendek dilakukan dengan menggunakan instrumen GC- μ ECD. Sementara itu, karakterisasi dilakukan dengan pengujian bersama menggunakan instrumen GC- μ ECD dan GC-MS. Penentuan ketidakpastian dievaluasi dari ketidakhomogenan sampel, ketidakstabilan jangka pendek dan variasi dalam prosedur karakterisasi. Dalam berat kering, nilai endosulfan sulfat ditetapkan sebesar $491 \mu\text{g kg}^{-1}$ dengan ketidakpastian relatif yang diperluas sebesar $\pm 33,2\%$, dan nilai bifentrin ditetapkan sebesar $937 \mu\text{g kg}^{-1}$ dengan ketidakpastian relatif yang diperluas sebesar $\pm 18,5\%$. Kandidat bahan acuan ini ditujukan untuk mendukung kebutuhan CRM matriks terutama untuk pengukuran residu pestisida untuk menjamin kualitas yang dilakukan oleh laboratorium di Indonesia.

Kata Kunci: bahan acuan; bifentrin; endosulfan sulfat; GC- μ ECD; residu pestisida

INTRODUCTION

Indonesia is known as the sixth largest tea exporter in the world after China, India, Srilanka, Kenya and Vietnam. The values of exports of processed tea from Indonesia were amounted to \$162.8 million in 2008, \$174.4 million in 2009 and \$184.9 million in 2010 [1]. However, behind the increased of tea export value, actually, the production of tea in Indonesia has decreased. This can be happened because the tea plantation areas in Indonesia tend to be decline. Currently, the remaining tea plantation area in Indonesia are 126,251 ha, with the largest concentration in West Java (97,138 ha), Central Java (10,100 ha), and North Sumatra (5000 ha) [1].

The existence of pests also decreases the tea production by 30-50% thus the farmers have to use various pesticides to maintain the production. For example, tea plantations in West Java spend as much as 3.5 tons of insecticides and a total of 70 tons of fungicides, nematicides, and herbicides per year [2]. A large amount of pesticides used can cause the residue left on the bulk of tea and the processed tea. Therefore, an accurate and reliable measurement system for pesticide residue levels in tea become essential for quality control, environmental monitoring, public health protection, legislative enforcement as well as building stronger brand images [3].

Seeing the importance in developing capabilities for the analysis of pesticide, a meeting in Jakarta in

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2008 held by Developing Economies Committee (DEC) of the Asia Pacific Metrology Programme (APMP) submit proposals to organize proficiency testing (PT) program for pesticide residue levels in tea. The purpose of this PT program is to build a technical platform for evaluating the measurement capability of testing laboratories in the Asia Pacific region. The PT program conducted in 2009 was followed by 42 laboratories from 13 countries [3]. Ten laboratories in Indonesia took part in the PT. Meanwhile, in 2011, the PT program was followed by 20 laboratories. Both PT program were participated by Research Center for Chemistry-LIPI (RCChem-LIPI).

As a form of dissemination of the PT program participation, as Designated Institute (DI) for the development of chemical metrology in Indonesia, we try to develop a reference material for the measurement of pesticides in tea considering availability of reference standards or reference materials to support traceability to the SI and accuracy of testing results for pesticide residues in food, especially in tea, is very limited in the local and global market [4].

In the previous project, Sin et al. [5] presented the preparation of a candidate reference material of cypermethrin in green tea GLHK-11-01a according to the requirements of ISO Guide 34 and 35. Certification of the material was performed using isotope dilution mass spectrometry (IDMS) approach, with gas chromatography-high resolution mass spectrometry (GC-HRMS) and gas chromatography-tandem mass spectrometry (GC-MS/MS).

In this project, we try to develop a candidate reference material of endosulfan sulfate and bifenthrin in black tea. Based on some research that has been conducted, endosulfan sulfate and bifenthrin are included in the type of pesticides commonly used in the tea plantation in Indonesia [6]. If used at the recommended dose of 350 g and 700 g of active ingredient per hectare, the endosulfan leaving a deposit residues under the required MRL at the beginning of the application (day 0) [6]. However, since 2012, endosulfan and its isomers are banned for use in more than 50 countries due to their acute toxicity and potential for endocrine disrupting effect. In the environment, endosulfan breaks down into endosulfan sulfate which has a structure similar to the parent compound and is also of toxicological concern [7]. This compound has been classified into the "extreme toxicity category" (i.e., highly acutely toxic) by the US EPA [8]. Meanwhile, bifenthrin which applied at dose 60-100 g of active ingredient per hectare leaving a deposit residues under the required MRL at day 10 after application [6]. Bifenthrin is one of pyrethroid pesticide that legally used in Indonesia [9]. Although previously pyrethroid pesticides were regarded to have low toxicity and safe to humans, but later reports have shown that chronic

exposure to these compounds can result in neurotoxic, genotoxic and immunotoxic effects [10].

The development of the candidate reference material is according to ISO Guide 34 [11] and 35 [12] includes homogeneity tests, stability tests and characterization process, using gas chromatography- μ -electron capture detector (GC- μ ECD). The assign values are derived through collaborative studies between RCChem-LIPI and National Quality Control Laboratory for Drug and Food (NQCLDF).

EXPERIMENTAL SECTION

Materials

The materials used are black tea leaves as raw materials (Research Center for Tea and Kina (PPTK), Gambung, West Java), reference standard endosulfan sulfate P-1372 Batch 96-19416 (purity $99.2 \pm 0.3\%$ at confidence level 95%, NMIA, Australia), bifenthrin with purity min 99% (Chem Service West Chester, PA USA), 2,4'-DDT as internal standard (in nonane, Cambridge Isotope Laboratories, Inc), Quechers extract pouches EN method (Agilent), Quechers dispersive SPE 15 mL for pigment sample EN with CH (Agilent), n-hexane p.a grade (Merck) and acetonitrile p.a grade (Merck).

Instrumentation

The instruments used are a stainless steel grinder (Grinder CX-750A), an automatic siever (Restch), a GC- μ ECD instrument (Agilent 6890), a rotary evaporator (Büchi R-124), a vortex mixer Caframo REAX 2000, and a drying oven (Heraeus T12P).

Procedure

Preparation of bulk sample

The black tea leaves were grounded for 90 sec using a stainless steel grinder (Grinder CX-750A) to make a fine powder which then sieved by an automatic siever (Restch) with the pore size of 500, 250, 150, and 50 μ m to obtain a homogeneous powder. Black tea powder with particle size less than 150 μ m has been selected.

Pre-analysis by using GC- μ ECD (Agilent 6890) confirmed that the tea samples do not contain endosulfan sulfate and bifenthrin. Therefore, these pesticides were spiked into the samples.

The expected concentration of both pesticides in tea matrix were about 400 μ g kg^{-1} of endosulfan sulfate and 800 μ g kg^{-1} of bifenthrin. The spiking technique was done by adding a standard solution containing both pesticides into the black tea powder. For making

the standard solution, endosulfan sulfate with purity of 99.2% (NMIA, Australia) and bifenthrin with purity of 99% (Chem Service West Chester, PA USA) were dissolved with n-hexane. This standard solution than poured into 100 g of the black tea powder inside a rotary evaporator flask and allowed to stand for a while until all powder in the flask become wet. The solvent was then dried by a rotary evaporator (Büchi R-124). After dried, the spiked black tea powder then mixed with 100 g of unspiked black tea powder and homogenize for 1 h. The homogenization process continued by adding another unspiked tea step by step up to 2 kg of total sample weight and homogenize for 1 h every addition of unspike black tea powder.

A total of 100 clean amber bottles with a capacity of 50 g were prepared for the samples. The bottles were labeled with number 1 to 100 to determine the filling order. Aliquots of about 20 ± 1 g were packaged into the bottles one by one according to the filling order and then closed tightly. Finally, all bottled samples (100 bottles) were stored at a temperature less than -10 °C before further study and analysis.

Moisture content

Moisture content was determined by oven technique at 105 °C. Three sets of 1 g of test samples were dried overnight in the oven. The materials were weighed until a constant mass was obtained. The moisture content of the sample is calculated by using Equation 1.

$$\text{moisture content} = \frac{w_1 - w_2}{w_1} \times 100\% \quad (1)$$

where:

w_1 = initial sample weight

w_2 = weight of the dried sample

Quechers sample preparation method

Quechers sample preparation method was used for the quantification of the target analytes. Each 2 g of sample was weighted into a 60 mL centrifuge tube, then added by 8 mL of distilled water and vortexed for 1 min. 10 mL of acetonitrile being added and weighted to each and the mixture was shaken vigorously for 1 min. Then, Quechers En extraction salt packet was added and the tube being shaken immediately after addition of the salt. Then each sample was shaken vigorously for 30 sec., and centrifuged at 4000 rpm for 5 min. 6 mL of the supernatant was transferred into a dispersive SPE 15 mL tube containing PSA, graphitized carbon black and MgSO_4 . The tube was vortexed for 1 min. and centrifuged at 4000 rpm for 5 min. The extracts were filtered by Whatman 0.2 μm and transferred into a screw-capped vial.

For analysis, 2,4'-DDT (as internal standard (IS)) was added to the sample. 1 μL of the extracts were

injected into the GC- μECD instrument. A series of standard solution in pure solvent were prepared by dilution of the standard mixture solution in acetonitrile at the range of 25 $\mu\text{g kg}^{-1}$ to 250 $\mu\text{g kg}^{-1}$.

Method validation

Determination of working and linear range. Sets of a total of 2 g black tea powder were weighed into extraction tubes. Then each of the target analyte: endosulfan sulfate dan bifenthrin, were added at the concentration range of 100-1000 $\mu\text{g kg}^{-1}$. The measurements were performed using a GC- μECD instrument.

Determination of precision. To determine the precision of the method, the analysis performed on samples containing spiked endosulfan sulfate around 400 $\mu\text{g kg}^{-1}$ and bifenthrin around 800 $\mu\text{g kg}^{-1}$. Sets of spiked samples were analyzed by the analysts to see the repeatability and reproducibility of the method. From the measurements, the average value and standard deviation were obtained. Precision is expressed in Relative Standard Deviation (RSD), following Equation 2.

$$\%RSD = \frac{SD}{\text{mean}} \times 100\% \quad (2)$$

Homogeneity test

For the homogeneity test, 5 test samples (2 g portion) were selected from the bulk by random stratified order and were analyzed in duplicate at two different days in a randomized order by a validated GC- μECD method. A statistical approach using two-factor analysis of variance (ANOVA) without replication was applied to evaluate within-bottle and between-bottle inhomogeneity. Between-bottle standard deviation (s_{bb}) was determined in accordance with ISO Guide 35 [12] using Equation 3.

$$s_{bb} = \sqrt{\frac{MS_{\text{among}} - MS_{\text{within}}}{n}} \quad (3)$$

where,

s_{bb} = between-bottle (in)homogeneity standard deviation

MS_{among} = mean square between groups

MS_{within} = mean square within groups

n = number of replication

Stability test

For the stability test, short-term stability (sts) of the analytes of random samples (2 g portion) were determined for assessing the conditions during delivery. The short-term stability test was performed using an isochronous technique. Eight bottles were selected from the bulk and were stored in an oven at the temperature of 40 °C for 4 weeks. Every week

about two bottles were randomly picked and moved to the freezer with the temperature less than $-10\text{ }^{\circ}\text{C}$ (reference temperature). All bottles were analyzed at the same time with a repeatability conditions to assess the short-term stability.

Determination of assigned value

The determination of the assigned value for endosulfan sulfate and bifenthrin in the black tea, conducted by carrying the analysis of the sample in collaborative studies between RCCChem-LIPI and NQCLDF that have competency in the pesticide compound testing. Both laboratories analyzed the sample by using the validated analytical method, and reported the result with its uncertainty value in a mass fraction in dry base as uncertainty from characterization process (u_{char}).

The combined u_{char} is calculated by the root of the sum of the squares of each u_{char} and standard deviation from concentration values reported by both laboratories (Equation 4).

$$\text{combined } u_{\text{char}} = \sqrt{(u_{\text{char lab1}})^2 + (u_{\text{char lab2}})^2 + sd^2} \quad (4)$$

In quantitative analysis, each laboratory using CRM of the pure standard derived from NMIA, Australia to make the calibration curve. The average of both value then determined as the assigned value.

The uncertainty budget was evaluated from sample inhomogeneity, stability, and combined characterization process. At a coverage factor of 2, expanded uncertainty (U) was calculated using Equation 5:

$$U = 2 \cdot \sqrt{u_{\text{bb}}^2 + u_{\text{sts}}^2 + \text{combined } u_{\text{char}}^2} \quad (5)$$

RESULT AND DISCUSSION

Preparation of Bulk Sample

Sample grinding and sieving are the initial steps in the preparation of solid reference material. These steps were conducted to obtain optimal particle size and distribution in the sample characterization process. Generally, a small particle size and a uniform particle distribution are needed to ensure the desirable level of homogeneity. In this experiment, about 2 kg of black tea with the particle size less than $150\text{ }\mu\text{m}$ were selected as the candidate material and spiked with the target analytes: endosulfan sulfate and bifenthrin.

The moisture content was used to convert the certified values obtained from the characterization methods to dry mass fraction. The average moisture content of the material ($n = 3$) was 8.24% (w/w) with a relative standard deviation of 1.67%.

In this experiment, the Quechers sample preparation method was used to quantify the target analytes. This method has been reported to be widely

used in the determination of many pesticides [13-16]. In the extraction tube, the mix of MgSO_4 , NaCl and citrate buffer works to yield larger recoveries of analytes and ensured the stability of pH-sensitive pesticides. Meanwhile, the dispersive tube contains [14]: (i) magnesium sulfate – to separate water from the organic solvent, (ii) primary secondary amine (PSA) – to remove various polar organic acids, polar pigments, some sugars and fatty acids, (iii) graphitised carbon black (GCB) – to remove sterols and pigments such as chlorophyll.

Fig. 1 show chromatograms of the black tea sample, the spiked black tea sample, the target analytes and internal standard.

The concentration of target pesticides was calculated by the following Equation 6.

$$C_x = \frac{C_{\text{GC}} \times V_{\text{final}}}{m} \times \frac{1}{\text{Rec}} \times \text{fp} \times \frac{1}{(1-M)} \quad (6)$$

where C_x is the concentration of analyte in the sample, C_{GC} is the concentration of analyte derived from GC instrument, V_{final} is the weight of final volume of the extract solution, m is the mass of tea sample, Rec is the recovery value, fp is the dilution factor, and M is the moisture content.

Method Validation

Determination of working and linear range

For method linearity, in the range of concentrations from $100\text{--}1000\text{ }\mu\text{g kg}^{-1}$, the r-values were 0.9615 and 0.9387 for endosulfan sulfate and bifenthrin, respectively. For working area, in the range of $25\text{ }\mu\text{g kg}^{-1}$ to $250\text{ }\mu\text{g kg}^{-1}$, the r-values were higher than 0.99 both for endosulfan sulfate and bifenthrin.

Precision

For repeatability study, 7 sets of blank samples were spiked with around of $400\text{ }\mu\text{g kg}^{-1}$ of endosulfan sulfate and $800\text{ }\mu\text{g kg}^{-1}$ of bifenthrin. The samples were then analyzed by one analyst on the same days. Table 1 summarizes the repeatability study. The recovery value for endosulfan sulfate were 105.54%, with the relative standard deviation was 5.56%. The recovery values for bifenthrin were 137.82%, with the relative standard deviation was 6.42%.

For reproducibility study, 13 sets of blank samples were spiked with around of $400\text{ }\mu\text{g kg}^{-1}$ of endosulfan sulfate and $800\text{ }\mu\text{g kg}^{-1}$ of bifenthrin. The samples were then analyzed by three analysts on three different days. Table 1 summarized the reproducibility study. The recovery values for endosulfan sulfate ranged from 81.80% to 128.11%, with the relative standard deviation of 12.48%. The recovery values for bifenthrin ranged from 118.75% to 151.71%, with the relative standard deviation was 8.25%. The mean of recovery

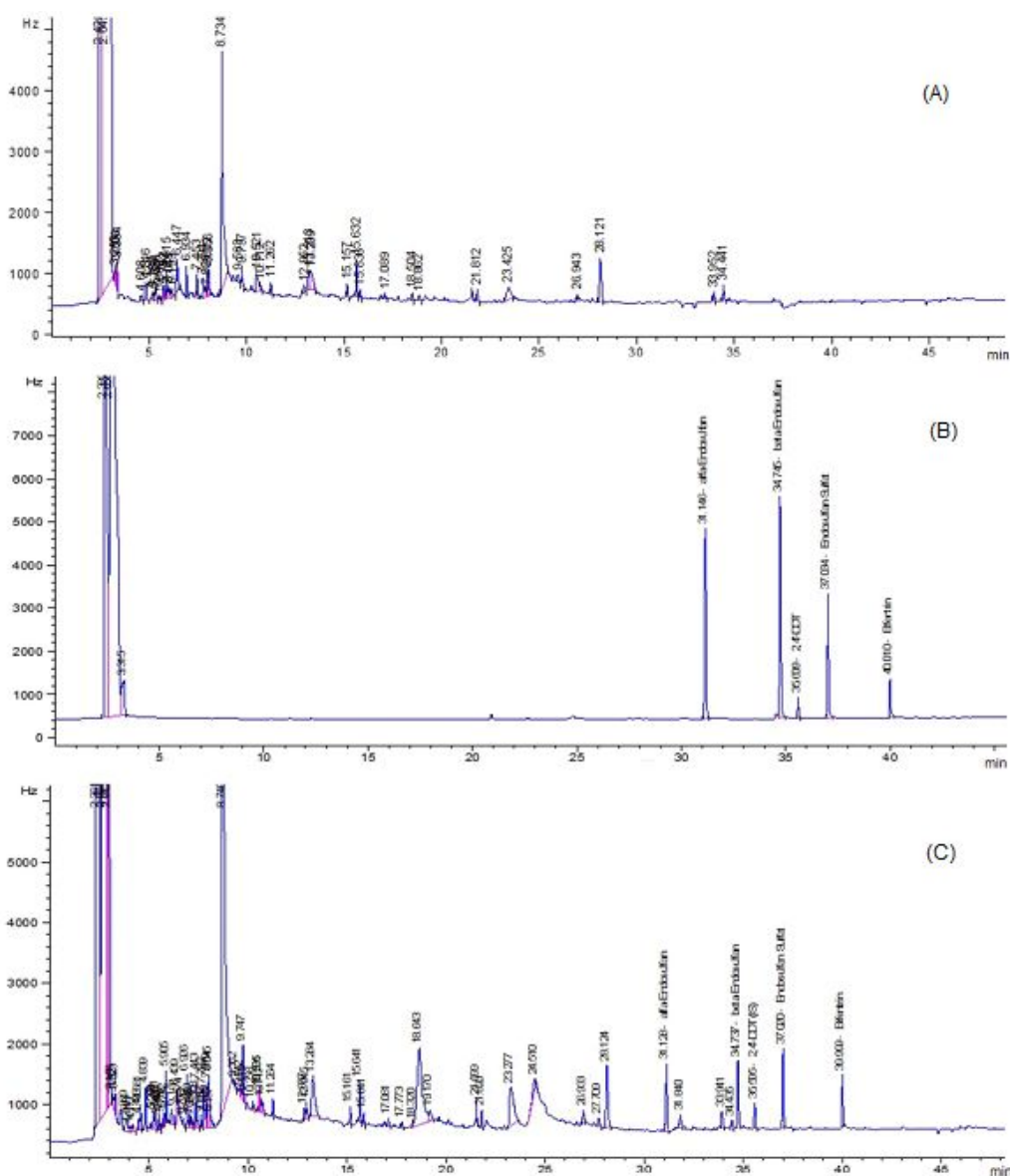


Fig 1. Chromatograms of (a) the black tea sample; (b) the target analytes and internal standard; and (c) the spiked black tea sample. (Operating condition of the GC- μ ECD instrument: type of instrument Agilent 6890; DB-5 capillary column, dimension 250 mm \times 250 μ m \times 30 m; carrier gas Helium with flow rate 1 mL/min; make up gas Nitrogen with flow rate 60 mL/min, oven temperature: 70 $^{\circ}$ C hold for 1 min, increase the temperature by 20 $^{\circ}$ C/min until reach 150 $^{\circ}$ C, increase the temperature by 2 $^{\circ}$ C/min until reach 200 $^{\circ}$ C, increase the temperature by 5 $^{\circ}$ C/min until reach 280 $^{\circ}$ C and hold for 10 min; injector temperature 250 $^{\circ}$ C; detector temperature 300 $^{\circ}$ C; splitless injection mode; injection volume 1 μ L)

value for endosulfan sulfate and bifenthrin were 106.86% and 136.42%, respectively. Both of the relative standard deviation are below the Horwitz value, thus meet the official requirements on precision for trace analysis.

Assessment of Homogeneity

For endosulfan sulfate, the mean value of the homogeneity test was 493 μ g kg^{-1} and the relative

standard deviation was 10.14%. The between-bottle variance and within-bottle variance were determined by two-factor ANOVA without replication. The statistical results of 5 sub-samples showed that the sample was homogenous, where calculated F value ($F_{\text{calc}} = 5.17$) was less than critical F value ($F_{\text{crit}} = 6.39$) at 95% confidence level. This indicates the homogeneity status of endosulfan sulfate in the material prepared was satisfactory. s_{bb} for endosulfan sulfate was 43.04 μ g kg^{-1} and the relative standard uncertainty (u_{bb}) due to sample

Table 1. Summary of the repeatability and reproducibility study

		Repeatability	Reproducibility
Endosulfan sulfate	n	7	13
	Mean of % Recovery	105.54	106.86
	%RSD	5.56	12.48
Bifenthrin	n	7	13
	Mean of % Recovery	137.82	136.42
	%RSD	6.42	8.25

Table 2. Summary of uncertainty budget for the analytical results of endosulfan sulfate by GC- μ ECD

Parameter	Value, x_i	Unit	Uncertainty ($u(x_i)$)
Mass of sample (m)	2	g	0.00063
Ratio concentration of analyte	2.6		0.15
C_{IS}	48	ng g ⁻¹	0.32
Mass of final volume	7.77	g	0.00063
Recovery	1.07		0.054
Concentration of stock solution (C_{stock})	1430.24	μ g g ⁻¹	28.23
Ratio concentration of standard calibration solution ($C_{std/CIS}$) at middle point	1.57		0.019
Precision	1		0.10
M'	1.09		0.00036

Table 3. Summary of uncertainty budget for the analytical results of bifenthrin by GC- μ ECD

Parameter	Value, x_i	Unit	Uncertainty ($u(x_i)$)
Mass of sample (m)	2	g	0.00063
Ratio concentration of analyte	5.7		0.29
C_{IS}	50	ng g ⁻¹	0.32
Mass of final volume	7.77	g	0.00063
Recovery	1.36		0.060
Concentration of stock solution (C_{stock})	1260.77	μ g g ⁻¹	28.08
Ratio concentration of standard calibration solution ($C_{std/CIS}$) at middle point	3.5		0.045
Precision	1		0.05
M'	1.09		0.00036

inhomogeneity was 8.72%.

For bifenthrin, the mean value of the homogeneity test was 886 μ g kg⁻¹ and the relative standard deviation was 5.16%. The between-bottle variance and within-bottle variance were determined by two-factor ANOVA without replication. The statistical results of 5 sub-samples showed that the sample was homogenous, where calculated F value ($F_{calc} = 1.09$) was less than critical F value ($F_{crit} = 6.39$) at 95% confidence level. This indicates the homogeneity status of bifenthrin in the material prepared was satisfactory. s_{bb} for bifenthrin was 26.96 μ g kg⁻¹ and the relative standard uncertainty (u_{bb}) due to sample inhomogeneity was 3.04%.

Assessment of Stability

By using regression analysis, the short-term stability (sts) for both endosulfan sulfate and bifenthrin were observed. It is found that p-value for both analytes

(p-value endosulfan sulfate = 0.58, p-value bifenthrin = 0.42) are higher than 0.05. This indicates that gradient is not significantly different from 0 which means there is no evidence of a significant or real trend of instability. Standard deviation of points (s) in sts for endosulfan sulfate and bifenthrin were 27.90 μ g kg⁻¹ and 25.31 μ g kg⁻¹ with the relative standard uncertainties were 6.05% and 4.00%, respectively.

Results of Characterization

The analytical results for the characterization are the mean of analytical results obtained by the GC- μ ECD method. Uncertainty budget for the analytical results of endosulfan sulfate and bifenthrin are summarized in Table 2 and 3. The uncertainty contributed to characterization (u_{char}) for endosulfan sulfate and bifenthrin were calculated to be 63 μ g kg⁻¹ and 79 μ g kg⁻¹, respectively.

Table 4. Summary of concentration of endosulfan sulfate and bifenthrin measured by RCChem-LIPI and NQCLDF

	Endosulfan sulfate ($\mu\text{g kg}^{-1}$)		Bifenthrin ($\mu\text{g kg}^{-1}$)	
	RCChem-LIPI	NQCLDF	RCChem-LIPI	NQCLDF
Concentration	493	489	886	988
U_{char}	63	7	78	6

Table 5. Summary of uncertainty components contributed to the certified value

	Endosulfan sulfate	Bifenthrin
Homogeneity		
\bar{X}_h	493 $\mu\text{g kg}^{-1}$	886 $\mu\text{g kg}^{-1}$
S_{bb}	43.04 $\mu\text{g kg}^{-1}$	26.96 $\mu\text{g kg}^{-1}$
U_{bb}	8.72 %	3.04 %
Stability		
\bar{X}_{sts}	461 $\mu\text{g kg}^{-1}$	632 $\mu\text{g kg}^{-1}$
S_{ts}	27.90 $\mu\text{g kg}^{-1}$	25.31 $\mu\text{g kg}^{-1}$
U_{sts}	6.05 %	4.00 %
Characterization		
Combined u_{char}	63.45 $\mu\text{g kg}^{-1}$	78.23 $\mu\text{g kg}^{-1}$
Combined u	81.59 $\mu\text{g kg}^{-1}$	86.53 $\mu\text{g kg}^{-1}$
U	163.18 $\mu\text{g kg}^{-1}$ (33.2%)	173.06 $\mu\text{g kg}^{-1}$ (18.5%)

Certified Values and Measurement of Uncertainty for the Reference Material

The concentration of endosulfan sulfate and bifenthrin measured by RCChem-LIPI and NQCLDF is summarized in Table 4. The uncertainty components due to sample inhomogeneity (u_{bb}), short-term instability (u_{sts}) and combined characterization process (combined u_{char}) are summarized in Table 5. The long-term stability tests are still in progress thus not included in the estimation of uncertainty.

The certified values (dry mass fraction) for the reference material were then calculated as the mean of the measurement results from RCChem-LIPI and NQCLDF. The certified values for endosulfan sulfate and bifenthrin were 491 $\mu\text{g kg}^{-1}$ and 937 $\mu\text{g kg}^{-1}$ respectively.

Compared to the previous project by Sin et al, the development of candidate reference material in this project results in bigger uncertainty though the sample inhomogeneity and stability are still confirmed satisfactory. For future development, the use of isotopes and more sensitive instrument such as GC-MS should be performed to make a better analysis.

CONCLUSION

The candidate reference materials, endosulfan sulfate and bifenthrin in black tea were developed. The candidate materials were characterized using GC- μECD and GC-MS. In a dry mass fraction, endosulfan sulfate was assigned to be 491 $\mu\text{g kg}^{-1}$ with a relative expanded uncertainty of $\pm 33.2\%$, and bifenthrin was assigned to

be 937 $\mu\text{g kg}^{-1}$ with a relative expanded uncertainty of $\pm 18.5\%$. These reference materials will be useful for the validation of the analytical methods and for quality assurance/quality control of endosulfan sulfate and bifenthrin analysis in tea matrices.

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