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Evaluation of Uncertainty in the Determination of Pesticide Residues in Tea using GC-ECD

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Abstract

Key indicator of quality in test results is the uncertainty value, which could be evaluated using several common approach. Uncertainty evaluation in α-endosulfan and bifenthrin in Oolong tea, and cypermethrin in green tea using bottom-up approach showed that uncertainty component arising from GC-ECD instrument calibration, method performance i.e. repeatability and recovery were the main contributors to total uncertainty. Uncertainty component arising from weighing, dilution factor, stock standard solution, calibration solution, and moisture correction had no significant effect to total uncertainty, hence they could be neglected. Relative standard uncertainty obtained for all of pesticides residue were 18.23, 10.44, dan 14.98% for α-endosulfan, bifenthrin, and cypermethrin, respectively. Comparison with 2/3 CV Horwitz of 17.33, 10.62, and 12.44% respectively for all pesticide residues indicated that the evaluation of uncertainties were realistic

INTRODUCTION

Tea is a popular plant originated from China and parts of India. It can be found in tropical and subtropical regions. Tea is now grown all over the world especially in China, India, Indonesia, Sri Lanka, and Japan. Tea is also cultivated in Africa, including Kenya, Malawi, Zimbabwe, and South Africa. It grows best at lower temperatures (5–25°C), high relative humidity (80–90%), and high annual rainfall (around 1500–2000 mm). Given such conditions, tea will grow at the altitude of up to 2100 m, and similar with wine, the aspects of soil, altitude, and climate will affect the flavour and characteristics of tea. Chemical composition of tea leaves consists of tanning substances, flavonols, alkaloids, proteins and amino-acids, enzymes, aroma-forming substances, vitamins, minerals, and trace elements [**1**].

Tea is known as one of the most popular beverage after water, and it is considered as a good source of many essential nutrition for human body, including essential trace elements [**2**]. A study about the content of trace elements in tea conducted by Street, R, et.al showed that the content was varied due to the type of tea (green tea or black tea), and likely influenced by many other factors like soil property, location, rainfall, altitude, genetical aspect, etc. Among essential minerals and trace elements, the content of Ca, Na, K, Mg are at g/kg, while Cr, Fe, Co, Ni, Cu, Zn are at mg/kg [**1**]. Another study has confirmed that the content of essential elements in tea drinkers are significantly higher than non tea drinkers [**2**].

In contrast to its nutritional function, tea has potential to harm human health as a consequence of the use of various chemicals in its cultivation process; one of the most important issue is pesticide. Because of its well known toxicicity, many countries have set up regulations regarding maximum level of pesticide residues in various comodities. Also international commission under United Nations like Codex Alimentarius Commission

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(CAC) has pay attention to this issue. The government of Indonesia through the Agriculture Minister regulation No: 88/Permentan/PP.340/12/2011 has also set up maximum level of pesticide residues in various commodities including tea. The forementioned regulations are then become a basic framework for assessment and monitoring activities of many agro commodities to manage negative impact of pesticide residues. In this context, laboratory testing of pesticide residues become routine to determine the effectiveness of the assessment and monitoring activities.

It is well known that the key indicator for quality of test results is the uncertainty value, in which all of the systematic and random error of the test method are accounted **[3,4]**. Thorough evaluation of uncertainty is a complex procedure that requires good knowledge of the testing methodology, performance of instruments, perfomance of personnel, influence of environmental condition, etc. This article described the process of uncertainty evaluation in the determination of α-endosulfan and bifenthrin in Oolong tea, and cypermethrin in green tea using Gas Chromatography - Electron Capture etector (GC-ECD). The evaluation was conducted based on bottom-up approach publicated by EURACHEM **[5]**.

2. EXPERIMENTAL SECTION

Certain amount of tea leaves (W_{Spl}) containing pesticide residues at certain level of concentration (C_S) was maserated using 50 mL acetone/dichloromethane 50/50 (V/V) overnight. This mixture was then centrifugated. The filtrate was vaporized at 40 ^oC to almost dry, then diluted quantitatively using n-hexane to certain weight (W_1). An aliquote of sample solution (W_2) was cleaned up using column containing 10 g of floricyl. The analyte then eluted with 150 mL mixture of n-hexane/diethyl ether 85/15 (V/V). The solution was vaporized once again at 40 ^oC to almost dry. The residue was diluted using n-hexane to certain weight (W_3), and injected in the amount of 2 uL into GC-ECD instrument.

The GC-ECD instrument was calibrated against pure stock standard solution for each analyte using calibration curve. Purity of stock standards were 99.5 ± 0.5 %, 99.0 ± 0.5 %, and 99.0 ± 0.5 % for α-endosulfan, bifenthrin, and cypermethrine, respectively. Series of standards taken for calibration for α-endosulfan and cypermethrine were 1, 10, 20, dan 40 ng, while 100, 200, and 400 ng were taken for bifenthrin. To improve the accuracy, the dilution of stock standard solution into a series of calibration solutions was conducted gravimetrically. Concentration of analyte targeted in solution which injected to the GC-ECD was obtained from calibration curve (C_X). Concentration of analyte in original sample, Cs, was calculated using Equation 1, where DF was the dilution factor of the sample which equal to $(W_1.W_2)/(W_2.W_3)$. Please be noted that W_2 would be eliminated in the calculation of DF, however its uncertainty will still remain and should be accounted in the uncertainty evaluation.

$$C_S = \frac{C_X . DF}{W_{Spl}} \qquad (1)$$

Calculation of pesticide residues in tea samples was based on dry basis. Therefore, moisture correction was neccesary. To obtain the correction, experiments that determine the moisture content (M) of the samples were conducted gravimetrically and calculated based on weight loss which heated at 105 ^oC. Moisture correction which was needed to obtain dry mass concentration was calculated by multiplying the wet basis concentration (Cs) with M', which is equal to 1/(1-M).

$$C_S' = \frac{C_X . DF}{W_{Spl}} . M' . \frac{1}{\mathrm{Re}c} \qquad (2)$$

The performance of the method was evaluated by some experiments to assess its precision and trueness. Method precision was evaluated by calculating the standard deviation of the replicate sample from measurements under repeatable condition (Rep). Meanwhile, trueness was evaluated by recovery test using sample spiked with known amount of analyte

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(Rec), which was then used as a correction factor. Correction accounted for moisture content and recovery to obtain final concentration (C_S') was calculated using Equation 2.

3. RESULT AND DISCUSSION

Evaluation of uncertainty using bottom-up approach requires detail identification of all component that contribute to total uncertainty. Uncertainty of each component was estimated based on available information, called as quoted uncertainty (QU). There are several types of QUs, for example: there is a QU that account for only random error while the other account for both random and systematic error. Hence, all the QUs need to be converted into a value that is equal to one standar deviation (1s), hereafter called as standard uncertainty (SU). To do the conversion, those QUs were classified into type A and type B, and the convertion into SUs was conducted according to procedure suggested by EURACHEM guide. The next step was to combined SU of all components, which was calculated according to the guideline. Finally, the combined standard uncertainty value need to be expanded by multiplying it with a coverage factor (k) that correlated with the confidence level of the results.

3.1. Identification of all components contribute to uncertainty

Identification was conducted by careful inspection of the testing procedure and the equation used to obtain the final analyte concentration (C_S'). As results, weight of sample (W_{Spl}), dilution factor (DF), C_X concentration obtained from GC-ECD calibration curve, concentration of stock standard solution, and moisture correction (M') were several components that comes out from the identification step. In addition, the method performance, namely repeatability and recovery, were also identified as main contributors to total uncertainty. The component of W_{Spl} was obtained from the difference of two weighing steps, i.e. weighing of weighing bottle (m_0) and bottle containing sample (m_1). Each weighing step had uncertainty from calibration of the analytical balance (Cal). Thorough identification for the determination of α-endosulfan was presented in fishbone diagram which enable one to see the correlation of one component to another, and how all component will contribute to total uncertainty. Recovery and repeatability of the method were also put in the diagram. Having those in the diagram, then all random effects of the test method had accounted in the repeatability component and all systematic effects had accounted in the recovery component. Fishbone diagram for cypermethrine was similar to that for α-endosulfan because the testing and calibration procedure were exactly the same, in which series of calibration solution used were 1, 10, 20, dan 40 ng (Figure 1). Fishbone diagram for bifenthrin was slightly different in which different series of calibration solution used were, i.e. 100, 200, and 400 ng.

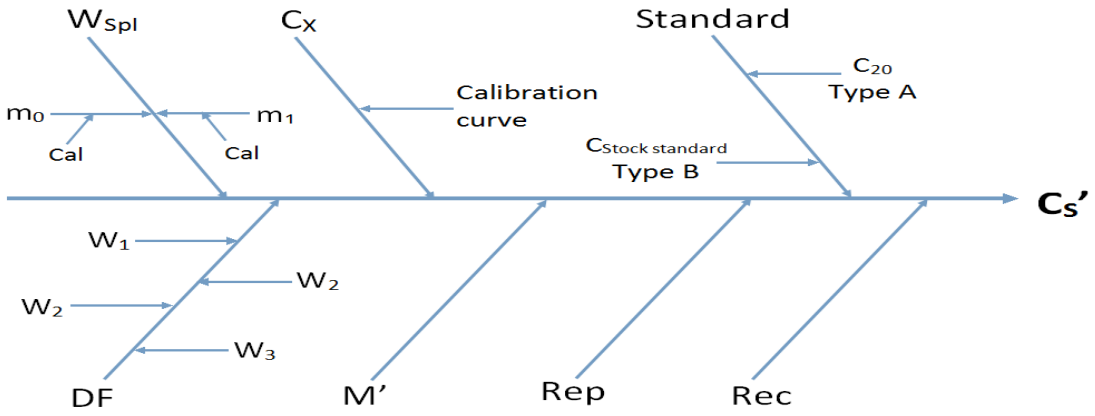

Fig 1. Fishbone diagram of the determination of α-endosulfan using GC-ECD

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3.2. Estimation of uncertainty of each component

Fishbone diagram provided systematic mapping of all components which contribute to total uncertainty. The next step was estimating the size of uncertainty of each component. Available QU information could be a good starting point for this purpose. One could examine the size of QU whether it is equal to 1s or not; if yes then the QU could be assumed to be equal to SU, otherwise convertion would be required. Conversion from QU into SU was conducted according to publish guideline from EURACHEM [**5,6]**. Recapitulation of SU from all components in the determination of α-endosulfan, bifenthrin, and cypermethrine are presented in Table 1.

The SU value of C_X which represents the analyte concentration in the measured solution, was estimated from uncertainty from calibration curve. Calibration curve itself has intrinsic uncertainty arising from instrument effects, acurracy of calibration solutions, and another random and systematic effects. As consequence, concentration of C_X which was calculated based on calibration curve would also has certain size of uncertainty.

Main factors that contributed to the uncertainty of C_X were number of calibration points, coefficient of correlation of the curve, number of replication of sample measurements, sensititify of instrument (indicate by slope of the curve), and the difference of sample absorbance to the mean absorbance of standard solutions (the bigger the difference, the bigger it would contribute to the uncertainty). Uncertainty component arising from C_X gave significant contribution to total uncertainty, simply because there were so many significant contributing factors as described above.

The SU value of $C_{stock\ standard}$ and series of calibration solutions were not accounted yet in the estimation uncertainty from Cx, though it was believe that they will give some contribution to total uncertainty. Hence, they were put in the fishbone as sources of uncertainties. Uncertainty of $C_{stock\ standard}$ was estimated by accounting effects arising from purity of standard, it's weight, and dissolution process. To estimate the uncertainty of series of calibration solutions, one calibration solution was selected to represent the series. In case of α-endosulfan and cypermethrine, C_{20} was selected (Figure 1), while C_{200} was selected in the case of bifenthrin.

The SU value of dilution factor (DF) was estimated from serial gravimetric dilution, hence accuracy of balance was the main contributor. It was true also for SU of W_{Spl}, where the weghing was conducted using a balance. The SU value of M' was estimated from replication of moisture determination of samples and the obtained standard deviation which then used as a basis for estimating the SU. In general compared to other components, all of these three components only gave slight contribution to the total uncertainty. Hence, the effects were not significant.

Method performance was also contributed to total uncertainty, which was estimated from its two main components, i.e. repeatability and recovery. Careful consideration should be done by putting these two components in the fishbone since repeatability represents for random error while recovery represents for systematic error; double counting of uncertainty components must be avoided. Hence, all random error i.e. weighing precision, volumetric precision, instrument precision, personnel precision, and other random effects were already took into account in the estimation of uncertainty of repeatability. Similarly, all systematic error that could be arise from inefficient analyte extraction from samples, clean-up step, measurement bias, and other systematic effects were took into account in the estimation of uncertainty of recovery. The SU value arising from method performance undoubtedly gave significant contribution to total uncertainty. The result was similar previous studies regarding uncertainty arising from method performance [**7,8]**.

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Table 1. Recapitulation of SU values of all uncertainty components in determination of α-endosulfan, bifenthrin, and cypermethrine

Uncertainty Component	□-Endosulfan		Bifenthrin		Cypermethrine		Unit	Source of QU	Type
	Typical value	SU	Typical value	SU	Typical value	SU			
C_X	7.7182	1.125	234.41	6.245	31.977	0.647	ng	calibration curve	B
$C_{Stock\ Standard}$ (type B)	23.455	0.0886	215.98	0.82	19.744	0.075	mg/mL	purity of standard	B
C_{20} (type A)	23.455	0.0375	-	-	19.744	0.0318	ng	gravimetric dilution	A
C_{200} (type A)	-	-	215.98	0.46	-	-	ng	gravimetric dilution	A
DF	8.005	0.00095	7.843	0.0009	7.489	0.00086	-	gravimetric dilution	B
W_{Spl}	2.05215	0.00014	2.0457	0.00014	2.0482	0.00014	g	balance	B
M'	1.036	0.0002	1.036	0.0002	1.049	0.00019	-	replicate moisture determination	A
Rec	0.7858	0.069	0.9019	0.079	0.3437	0.03	-	spiked sample	B
Rep	1	0.065	1	0.05	1	0.12	-	replicate sample measurements	A

3.3. Expanded uncertainty

Each uncertainty component could be compared one to another to see their relative contribution to total uncertainty. This could be done easily by calculating relative SU for each component and visualized it in a graphical presentation that enable quick assesment of the relative contribution of each component (Figure 2).

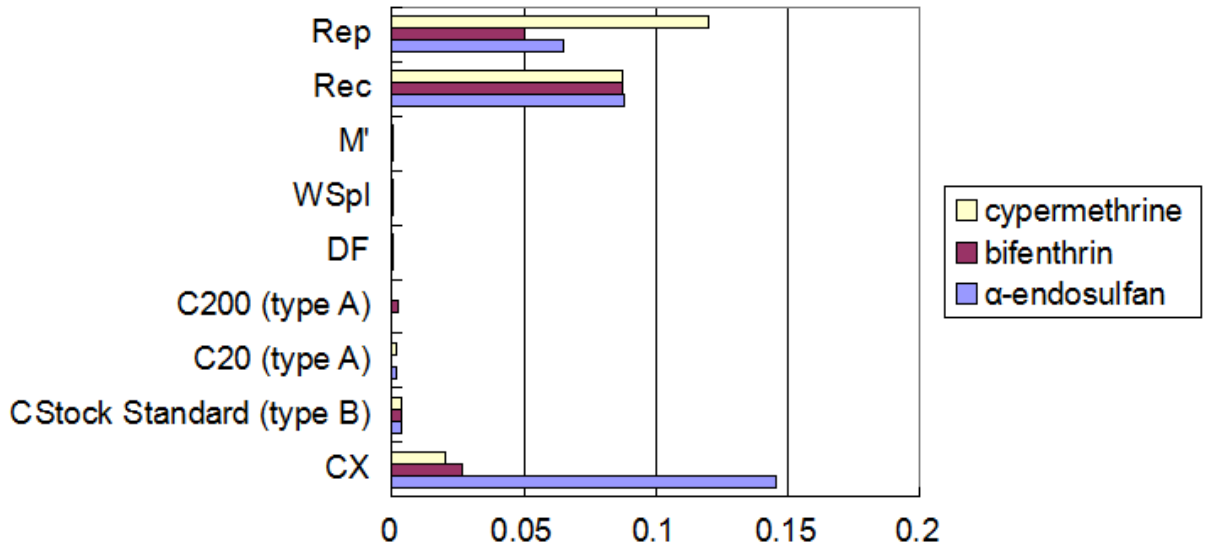

Fig. 2. Histogram of relative SU of all components in the determination of α-endosulfan, bifenthrin, dan cypermethrine

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As predicted previously, the uncertainty component of Rep, Rec, and C_X were main contributors to total uncertainty. This phenomena was observed in all analytes, i.e. α-endosulfan, bifenthrin, and cypermethrine. The contribution of recovery was quite similar for all analytes. However, contribution of repeatability was relatively bigger in determination of cypermethrine, which caused by big standard deviation of the replication of sample measurements. Matrix effects on sample measurements and stability of analyte could be the main reason for this. The contribution of C_X were also not equal for the three analytes, where C_X in determination of α-endosulfan was relatively bigger than the other two (Figure 2). Some factors considered to be responsible were: a) the low level of the analyte (ng level) which made measurement become more difficult and caused the coefficient correlation of calibration curve using GC-ECD become worse than in higher concentration , b) the big difference of absorbance of sample to the mean of absorbance of calibration solutions. However for all three analytes, C_X was undoubtedly a major contributor to total uncertainty. This was also similar with previous study regarding the estimation of uncertainty in pesticide residues [**9**].

The SU value of $C_{stock\ standard}$, C_{200}, C_{20}, DF, W_{Spl}, dan M' individually did not give significant effect to total uncertainty, as could be seen in Figure 2. Hence, in routine uncertainty estimation in the determination of these residues, those components could be ignored in the estimation of total uncertainty.

3.4. Expanded uncertainty and report

Combined standard uncertainties of all components could be calculated with respect of how each component is related one to another. This was conducted easily by implementing the combination rules in the guideline published by EURACHEM [**5,6**]. Expanded uncertainty was obtained by multiplying the combined standard uncertainties with a coverage factor (k) which related to confidence level. In this case, k=2 had taken to give confidence level of 95%. The concentration of analyte and its expanded uncertainty for α-endosulfan in Oolong tea, bifenthrin Oolong tea, and cypermethrin in green tea were 39.8±14.5 (ng/g), 1029.0±214.9 (ng/g), and 359.0±107.6 (ng/g), subsequentially. All results were in dry basis. All of the uncertainty estimation results was compared to CV Horwitz prediction to see whether the estimation were reasonable or not.

Table 2. Comparison of CV experiment with CV Horwitz

Analit	CV_{Exp} (%)	$CV_{Horwitz}$ (%)	$2/3CV_{Horwitz}$ (%)
□-endosulfan	18.23	26.00	17.33
bifentrhin	10.44	15.93	10.62
cypermethrin	14.98	18.67	12.44

CV Horwitz is a CV that is derived from reproducibility data obtained from different laboratories. Therefore, in order for this to be able to be used for the comparison of repeatability data (obtained by only one laboratory under repeatable condition), a convertion factor of 2/3 is necessary. Comparison of CV of experiment (CV_{Exp}) with CV Horwitz is provided in Table 2. As could be seen in the table, CV of experiments were comparable with 2/3 CV Horwitz. Therefore, it could be concluded that the estimation were quite realistic.

4. CONCLUSION

Bottom-up approach in the evaluation of uncertainty in the determination of pesticide residues provides a systematic and holistic approach to the problem. This approach requires personnel which has a good understanding regarding the overall testing procedure, performance of main measuring instruments, level of quality of reagents and standards, effects of environmental condition, effects of personnel, and also performance of the testing method. Improving the quality of test results could be done simply by decreasing its uncertainty. This could be effectively conducted by foccusing the efforts to some components that give major contribution to total uncertainty. For routine uncertainty evaluation of these three pesticide residues

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determination, a spreadsheet application that allow to standardized the calculation format would be useful.

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