Quantitative Analysis of Trace *pp'*-DDE in Corn Oil by Isotope Dilution Mass Spectrometry : Uncertainty Evaluations

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A current interest in chemistry concerns traceability of analytical measurements to the International System of Units (SI) and the proper estimation of their uncertainties in accordance with the internationally agreed guide provided by the International Organization for Standardization (ISO). Isotope dilution mass spectrometry (IDMS) is regarded as a primary method, which make the measurement results traceable to SI units without significant empirical correction factors. Our laboratory, as the national standards institute of Korea, participated in an intercomparison of environmental analysis, *pp'*-DDE in corn oil, which was organized by the CCQM under supervision of the CIPM to test feasibility of IDMS as a primary method for the trace analysis of organic compounds. In this report, we provide basic equations used for the calculation of the concentration of the analyte in a sample and a precise description of the processes for the evaluation of the uncertainties of the measurement results. Also, we report the experimental conditions adopted to improve the accuracy of the IDMS measurement. The principles contained in ''Guide to the Expression of Uncertainty in Measurement'' provided by ISO are followed for the uncertainty evaluation.

Introduction

In modern industrial era, measurements in chemistry are closely linked with commercial and public affairs such as trade, regulation, health and safety. Therefore, making measurement results of a specific laboratory reliable and comparable to other laboratories worldwide are highly demanded. Comparability and reliability of a measurement result can be achieved by making the property of the measurement result traceable to long-term stable references which are ultimately anchored to physical principles of nature, through an unbroken chain of comparisons all having stated uncertainties.[1,2] This can be best done by linking all measurements to the SI. Keeping a traceability chain to the SI unit makes all measurement results internationally comparable regardless of measurement entities and sample matrix.[1-5]

The realizations of the SI unit are usually kept in each country by the national metrology institute as the national standards of measurement. The national metrology institute provides the realizations of the SI unit by primary methods which stand alone and do not need any references of the same quantity. [1-5] International traceability and uniformity of measurement is then established on a world-wide scale through a mechanism of high-level comparisons between the national metrology institutes. The Comité Consultatif pour la Quantité de Matière (CCQM), established by the Comité International des Poids et Mesures (CIPM) in 1993, has taken the lead in the development of the metrology system in chemistry.

Since a typical chemical measurement consists of a number of measurement steps, it requires careful design of measurement procedure to keep the traceability chain to the SI unit.[4] To make a measurement result traceable to the SI unit, it is also necessary to evaluate the uncertainty of every step in the measurement procedure and combine them to meet the principles of the internationally agreed guide, [''Guide to the Expression of Uncertainty in Measurement'' provided by International Organization for Standardization (ISO) in 1993].[6] The guide establishes general rules for evaluating and expressing uncertainty in the broad spectrum of measurement and is accepted in all field of measurements. However, it is very difficult to estimate and combine uncertainties for every step involved in chemical measurements following the ISO guide due to the complexity of the procedures. Recently, Eurachem provided a document, [''Quantifying Uncertainty in Analytical Measurement'',[7] Eurachem, 1995], describing how the concepts in the ISO guide can be applied in chemical measurements. Though the document has been a useful guideline for analytical chemists, it is still important to provide a practical guidance on estimating uncertainty for each of widely used chemical measurement method to make the ISO guide properly applicable.

A few chemical measurement methods are recognized as primary methods[5,8] that make the measurement results traceable to the SI units directly without significant empirical correction factors. Isotope dilution mass spectrometry (IDMS) has been considered as a strong candidate of a primary method for the analysis of trace organic compounds in complex matrix.[9-14] As IDMS method overcomes difficulty of correcting recovery yield in sample preparation and separation, it has been widely accepted as a reliable analysis method for highly accurate determination in clinical chemistry,[12,5-17] toxicology,[12,18,19] food and drug analysis,[12] and environmental analysis.[12,20] To test feasibility of IDMS as a primary method for the analysis of trace organic compounds and the international uniformity of the measurement, the

CCQM under supervision of the CIPM carried out or is preparing intercomparison programs with several national metrology institutes. National metrology institutes having or building up robust chemical metrology system were involved into the intercomparison programs. Our laboratory has participated in most of the intercomparison programs as the national metrology institute representing Korea. Recently, we participated in the intercomparison of environmental analysis, (*p,p'*-dichlorodiphenyl)dichloroethylene (*pp'*-DDE) in corn oil.

In this report, we provide basic equations used for the quantitative analysis of the corn oil sample and a description of the procedures for the evaluation of the uncertainty of the result of the IDMS measurement. This report also describes details on experimental conditions used to increase the accuracy of the IDMS measurement.

Experimental Section

The IDMS measurement consists of spiking a known amount of isotope-enriched *pp'*-DDE-$^{13}C_{12}$ to a known amount of corn oil, clean-up of the sample to separate oil matrix, and GC/MS measurement of the extract. The measured isotope ratio of spiked corn oil sample was calibrated by bracket method using two calibration standard mixtures containing known amounts of *pp'*-DDE and *pp'*-DDE-$^{13}C_{12}$.

Materials. Two levels of analyte materials of 0.05 μg/g (solution 1) and 5 μg/g (solution 2) were provided by the Laboratory of the Government Chemist, U. K., which is the pilot laboratory of this intercomparison program. Sample of each level was provided in duplicate bottles. The pilot laboratory also provided a *pp'*-DDE calibration solution and a *pp'*-DDE-$^{13}C_{12}$ spike solution, both in 2,2,4-trimethylpentane. The concentration of the calibration solution is quoted by the laboratory to be 7.911 ± 0.005 μg/g on the nominal basis (uncorrected for a chemical purity of *pp'*-DDE raw material) and 7.879 ± 0.032 μg/g on an absolute basis (corrected for a chemical purity of 99.6 ± 0.4%). The concentration of the spiked solution is quoted to be 7.78 μg/g on the nominal basis. The numbers quoted after ± are the expanded uncertainties ($U = ku$) calculated using a coverage factor (k) of 2 which gives a level of confidence of 95%.

Sample Preparation and Clean-up. For solution 2, 2 g of sample was spiked with an appropriate amount of the *pp'*-DDE-$^{13}C_{12}$ spike solution. The amount of the spike solution to be added were determined to make the mass ratio of *pp'*-DDE/ *pp'*-DDE-$^{13}C_{12}$ in the spiked corn oil sample near 1 : 1. The spiked sample was diluted to 10 mL with ethylacetate/ cyclohexane (1+1 in volume). A 2 mL aliquot of this solution was then subjected to clean-up by gel permeation chromatography (GPC).[21, 22] The GPC column (25 mm I.D. × 500 mm height) was packed with Bio-Bead SX-3 with 200-400 mesh from Bio-Rad Laboratories. Ethylacetate/ cyclohexane (1+1) was used as a mobile phase. The column flow rate was set to 6 mL/min. The appropriate fraction (145 mL to 220 mL) containing the *pp'*-DDE and *pp'*-DDE-$^{13}C_{12}$ was collected. The extract was then concentrated to a volume of approximately 1 mL. The 1 mL extract was then added to the top of a preconditioned solid phase extraction cartridge (Silica, 500 mg from Waters) and eluted using 10 mL of ethyl acetate/cyclohexane (1+1). The extract was concentrated to a volume of approximately 1 mL using a suitable evaporator. The *pp'*-DDE content was then determined using GC/MS by injecting 1 μL of the final extract. Solution 1 was handled in similar method as solution 2, but a few modifications were done due to its low concentration. A portion of the original spike solution was diluted to ~0.7 μg/g by weight to reduce the uncertainty associated with the amount of *pp'*-DDE-$^{13}C_{12}$ spiked. 4 g of the sample was spiked with an appropriate amount of the diluted *pp'*-DDE-$^{13}C_{12}$ spike solution. Amount of corn oil sample loaded for GPC clean-up is twice of solution 2, and the final extract from the solid phase extraction is concentrated to approximately 50 μL. Thus the concentration of *pp'*-DDE and *pp'*-DDE-$^{13}C_{12}$ in the final extract is around a half of that of the extract of solution 2. 2 μL of the final extract is injected for GC/MS measurement. Thus, similar amount of *pp'*-DDE and *pp'*-DDE-$^{13}C_{12}$ were injected to GC/MS for both levels of samples.

Calibration Standards. Two sets, each containing three calibration standard mixtures, were prepared independently by combining weighted portions of the *pp'*-DDE calibration solution and the *pp'*-DDE-$^{13}C_{12}$ spike solution provided by the pilot laboratory. The mass ratio of *pp'*-DDE to *pp'*-DDE-$^{13}C_{12}$ for the first set was near 0.96 and that of the other set was near 1.09. They were tested with GC/MS to check the repeatability of the preparation processes.

GC/MS Conditions. The instrumentation consists of a gas chromatography (Hewllet Packard 6890) with an automatic liquid sample injector, a double focusing magnetic sector mass spectrometer (Jeol JMS 700), and its control and data acquisition system. The GC was equipped with a Rtx-5ms column (30 m long, 0.25 μm i.d., 0.25 μm film thickness). Helium was used as carrier gas at a flow rate of 1.0 mL/min. Its injection port was kept at 300 °C. The split ratio of the injection port was set to 5 : 1. The temperature of the GC oven started at 150 °C and maintained for 1 min and was ramped to 300 °C at the rate of 20 °C/min and held for 3 min. The interface to the mass spectrometry was maintained at 300 °C. The mass spectrometer was operated under electron impact ionization condition at 70 eV with a source temperature of 250 °C and an ionization current of 0.25 mA. Chromatograms of ions at m/z 318 and ions at m/z 330, which correspond to [M+2] ions of the unlabeled and labeled *pp'*-DDE, respectively, were monitored with the selected ion monitoring mode. Switching between the selected ions was accomplished by changing the acceleration voltage at every 50 ms with the magnet field fixed. Ion optics and slits were adjusted to give a near rectangular ion peak profile with a flat top in the acceleration voltage scan mode. It minimizes a gradual change on the relative response between the two ions which would be caused by a long-term drift of magnetic field.

Measurement Procedures. Sample solution in each

bottle (two bottles for each level) was analyzed in quadruplicate in four different batches. Thus, sample of each level was subject to 8 independent IDMS measurements. For each *single IDMS measurement*, we carried out sample weighing, spiking isotope analogue, clean-up, and GC/MS measurements of the final extract and two calibration mixtures. The two calibration standard mixtures were chosen: one from each set. For the GC/MS measurements, the two standard mixtures and one sample were subjected to 6 GC/MS runs for each in succession usually in the order of a standard solution with lower mass ratio, sample, a standard solution with higher mass ratio. In the end of the GC/MS measurements, the standard solution measured at first was measured again to check any instrumental drift. No drift was observed for a usual 6 hour measurement period.

Mathematical Expression

For the uncertainty evaluation of a measurement result following the ISO guide,[6] it is necessary to build a mathematical model that can express the relationship of the final measurement result with all sub-measurements and related parameters needed to reach it. If the isotope ratio of *pp'*-DDE to *pp'*-DDE-$^{13}C_{12}$ in the spiked corn oil sample, IR_x, is obtained from GC/MS measurement. Then, the concentration of *pp'*-DDE in the sample, C_x, can be expressed as following.[23]

$$C_x = \frac{M_{sp,x} C_{sp}}{M_x} IR_x \tag{1}$$

where:

C_x is the concentration of *pp'*-DDE in the corn oil sample;
IR_x is the isotope ratio of *pp'*-DDE / *pp'*-DDE-$^{13}C_{12}$ in mass in the spiked sample solution;
C_{sp} is the concentration of the isotopically labeled (*pp'*-DDE-$^{13}C_{12}$) spike solution;
M_x is the mass of the corn oil sample taken for analysis;
$M_{sp,x}$ is the mass of the *pp'*-DDE-$^{13}C_{12}$ spike solution added to the sample solution.

As the GC/MS measurement was calibrated by bracket method, IR_x can be replaced with 2-point calibration (by bracketing) equation as following.[7]

$$C_x = \frac{M_{sp,x} C_{sp}}{M_x}\left[\left(\frac{AR_x - AR_1}{AR_2 - AR_1}\right)(IR_2 - IR_1) + IR_1\right] \tag{2}$$

where:

AR_i is the observed area ratio of *pp'*-DDE /*pp'*-DDE-$^{13}C_{12}$ for calibration standard mixture *i* (=1, 2) from GC/MS measurement ;
AR_x is the observed area ratio of *pp'*-DDE /*pp'*-DDE-$^{13}C_{12}$ for the sample solution from GC/MS measurement ;
IR_i is the isotope ratio of *pp'*-DDE /*pp'*-DDE-$^{13}C_{12}$ for calibration standard mixture *i* (=1, 2).

As calibration standard mixtures were prepared by mixing weighted portions of the *pp'*-DDE calibration solution and the *pp'*-DDE-$^{13}C_{12}$ spike solution provided by the pilot laboratory, IR_i can be expressed as following.

$$IR_i = \frac{M_{s,i} C_s}{M_{sp,i} C_{sp}} \tag{3}$$

where:

C_s is the concentration of the *pp'*-DDE calibration solution;
$M_{s,i}$ is the mass of the *pp'*-DDE calibration solution added to the calibration standard mixture *i* (=1, 2);
$M_{sp,i}$ is the mass of the *pp'*-DDE-$^{13}C_{12}$ spike solution added to the calibration standard mixture *i* (=1, 2).

By replacing IR_i of equation 2 with equation 3, the concentration of *pp'*-DDE in the corn oil sample can be expressed as following.

$$C_x = \frac{M_{sp,x} C_s}{M_x}\left[\left(\frac{AR_x - AR_1}{AR_2 - AR_1}\right)(MR_2 - MR_1) + MR_1\right] \tag{4}$$

where, MR_i (=$M_{s,i}/M_{sp,i,}$), i =1, 2, is the mass ratio of the *pp'*-DDE calibration solution to the spike solution added to calibration standard mixture *i*. In the equation, C_{sp} is cancelled out as the same spike solution was added to sample and standard mixtures. Therefore, its exact chemical purity, isotopic purity, its concentration, and the uncertainties associated with these parameters are unimportant for the calculation of C_x and the evaluation of its uncertainty.

For convenience, the equation is written as following.

$$C_x = \frac{M_{sp,x} C_s}{M_x} Q \tag{5}$$

where Q is as following

$$Q = \left[\left(\frac{AR_x - AR_1}{AR_2 - AR_1}\right)(MR_2 - MR_1) + MR_1\right] \tag{6}$$

As Q has little correlation with $M_{sp,x}$, C_s, and M_x, the uncertainty of Q can be evaluated separately using equation 6 and combined to C_x in equation 5. [See Appendix A for the description of the ISO guide for determining combined standard uncertainty and expanded uncertainty]

Results and Discussion

Instrumental Performance. *pp'*-DDE and *pp'*-DDE-$^{13}C_{12}$ coelute at 7.5 minute under the chromatographic conditions described above, which is required for the accuracy and repeatability of the isotope ratio measurement. The full peak width at 10% height was 2 second. Thus, more than 20 detection cycles are allowed within a GC peak with the acceleration voltage switching mode at the switching rate of 50 ms. Switching between selected ions could be accomplished by varying magnetic field. However, the fastest magnet field switching rate of the mass spectrometer[24] used in this experiment is 100 ms and it does not make enough number of detection points within a chromatographic peak. Thus, the acceleration voltage switching mode at the rate of 50 ms

was adopted in this work. Under the selected experimental conditions, the area ratio of the two ions (AR_x and AR_i in equation 6) from six repeated GC/MS runs shows about 0.1 % of relative standard uncertainty.

Examination of Materials. The *pp'*-DDE-$^{13}C_{12}$ spike solution and the *pp'*-DDE calibration solution were examined by GC/MS at the same condition described above to test their cross contamination, which can lead bias in the final results. The *pp'*-DDE-$^{13}C_{12}$ spike solution does not show *pp'*-DDE peak above noise level on the ion chromatogram of m/z 318. Based on the signal to noise ratio of *pp'*-DDE-$^{13}C_{12}$ peak on its ion chromatogram of m/z 330, the contamination level of the solution by the unlabeled *pp'*-DDE is estimated to be less than 0.005% of *pp'*-DDE-$^{13}C_{12}$. Also, GC/MS measurement of the *pp'*-DDE solution indicates that it is free from contamination by the labeled compound.

Isotopic Differentiation in Clean-up Processes. Any isotopic differentiation in the two stages of extensive clean-up processes could lead to bias in the final results. A portion of a calibration standard mixture was subjected to the clean-up, and its final extract and the original mixture were examined by GC/MS. The measured isotope ratios for the two solutions agree within our experimental precision, indicating that there is no noticeable isotopic differentiation in the clean-up stage.

Uncertainties in Weighing. A balance(Mettler Toledo AT201) used in this work is readable down to 0.01 mg, however the precision of the mass obtained from weighing by difference is 0.1 mg in the working range of this experiment.[25] The uncertainty of the balance zero calibration falls to zero. Therefore, the uncertainty of the mass of the sample solution (M_x) taken for analysis is 0.0001 g for both solution 1 and 2. The uncertainty of the mass of the spike solution ($M_{sp,x}$) added to the sample is 0.0001 g for solution 2. However, it is 0.00001 g for solution 1 as the spiked solution is diluted before spiking. The buoyancy correction factors of the masses of the *pp'*-DDE calibration solution and the spike solution are canceled out in the mass ratio of the two solutions in a standard mixture (MR_i in Equation 4, 5, and 6) as the two solutions have same density. The buoyancy correction factor for $M_{sp,x}/M_x$ in equation 5 is 1.0001, which contributes only 0.01% correction on the final C_x. Thus, the uncertainty associated with the buoyancy correction factor for $M_{sp,x}/M_x$ contributes very little to the uncertainty of the final result.

Standards Cross-Check. The accuracy of results is limited by the accuracy of the calibration standard mixtures used for the calibration. To test the consistency of the whole processes of preparing calibration standard mixtures, independently prepared calibration standard mixtures were tested with GC/MS using the measurement conditions described above. The measurement results are shown in Table 1. The relative response of each calibration standard mixture is obtained from dividing the measured area ratio of *pp'*-DDE to *pp'*-DDE-$^{13}C_{12}$ by the weight-in ratio. The variation of the relative response between calibration standard

Table 1. Test of Calibration Standard Mixtures (*pp'*-DDE and *pp'*-DDE-$^{13}C_{12}$)

Standard	Ratio(unlabeled/labeled)		Relative Response
	Weight-in[a]	Area Ratio[b]	(Area Ratio/Weight-in Mass Ratio)
1-1	0.9622	1.0100±0.0006	1.0497±0.0006
1-2	0.9627	1.0090±0.0008	1.0482±0.0008
1-3	0.9633	1.0090±0.0010	1.0474±0.0010
		mean of the relative response	1.0484
		standard uncertainty of the mean	0.0006
2-1	1.0878	1.1435±0.0006	1.0512±0.0006
2-2	1.0983	1.1535±0.0011	1.0503±0.0012
2-3	1.0961	1.1501±0.0009	1.0493±0.0009
		mean of the relative response	1.0503
		standard uncertainty of the mean	0.0006

[a]Ratio of *pp'*-DDE to *pp'*-DDE-$^{13}C_{12}$ in mass as provided by mixing weighted portions of the *pp'*-DDE calibration solution and the *pp'*-DDE-$^{13}C_{12}$ spike solution. Calculated using Equation 3 in main text. The purity-corrected concentration of the calibration solution is used. [b]Area ratio measured by GC/MS. The number after "±" is the standard uncertainty (u) from 4 repeated measurements. $u^2 = s^2/n$, where s is the standard deviation of the area ratios and n is the number of measurements.

Table 2. Determination of *pp'*-DDE in Corn Oil Samples

Sample	Vial No.	Measure-ment[a]	Concentration (μg/g)	
			Nominal[b]	Absolute[c]
Solution 1	3-13	1	0.0713	0.0711
		2	0.0714	0.0711
		3	0.0711	0.0708
		4	0.0713	0.0710
	3-14	1	0.0719	0.0716
		2	0.0719	0.0716
		3	0.0718	0.0715
		4	0.0720	0.0718
	Mean of Measurements		0.0716	0.0713
	Standard Uncertainty[d]		0.00013	0.00013
Solution 2	7-37	1	4.751	4.732
		2	4.756	4.737
		3	4.754	4.735
		4	4.745	4.725
	7-38	1	4.748	4.729
		2	4.749	4.730
		3	4.749	4.730
		4	4.749	4.730
	Mean of Measurement		4.750	4.731
	Standard Uncertainty[d]		0.0013	0.0013

[a]Each single measurement consists of clean-up of an independently spiked portion of sample and 6 GC/MS runs for each of the sample and two calibration mixtures. [b]The concentration is calculated based on the nominal (uncorrected for the purity of *pp'*-DDE raw material). [c]The concentration is calculated based on the absolute (corrected for the purity of *pp'*-DDE raw material). [d]$u_{method} = s_{method}/\sqrt{n}$, where s_{method} is the standard deviation of 8 (= 4+4) measurement results and n = 8. It represents the reproducibility of the whole analysis method.

mixtures in a set is within the measurement uncertainty of a single solution, indicating that the processes of preparing calibration standard mixtures are well established.

Analysis of Corn Oil. The results of the IDMS measurements are shown in Table 2. The concentration of *pp'*-DDE was obtained from the analysis of corn oil sample from each bottle in quadruplicate in four different batches. Thus, 8 independent IDMS measurements were performed for the sample of each level. The mean of the 8 measurements are taken as the concentration of *pp'*-DDE in the sample solution. The standard deviation (s_{method}) of the 8 measurements is divided by $\sqrt{8}$ to obtain the standard uncertainty of the mean (u_{method}), which represents only the reproducibility of the whole experimental method. The mean for solution 2 is 4.731 μg/g on the absolute basis and its u_{method} is 0.0013 μg/g, which is only 0.03% of the mean. The mean for solution 1 is 0.0713 μg/g on the absolute basis and its u_{method} is 0.0001 μg/g, which is 0.14% of the mean. For both levels of samples, all measurement processes were similar and the same standard mixtures were used. Also, the level of concentration and the GC injection volume of the final extract of solution 1 were determined to have similar signal to noise ratio with solution 2. Therefore, the higher relative uncertainty for solution 1 is attributed to the uncertainty related with spiking small amount of the *pp'*-DDE-$^{13}C_{12}$ solution.

Uncertainty Analysis. The uncertainty of the final result can be obtained by combining the uncertainty associated with the variation of the values from 8 independent IDMS measurements, u_{method}, and the standard uncertainty associated with C_x of each single IDMS measurement, $u_{s,m}$, by using the equation $[u_{s,m}^2 + u_{method}^2]^{1/2}$.

Here, $u_{s,m}$ can be estimated by combining all the uncertainty sources of a IDMS measurement procedure. In the preceding sections, we already discussed uncertainty components of the IDMS measurement. For convenience, we first evaluated the uncertainty of factor Q in equation 5, based on the equation 6. The value of Q is near 1. The uncertainty components of Q are listed in Table 3. As discussed above, the uncertainty of 0.06% associated with MR_i is obtained from the intercomparison of three calibration standard mixtures prepared independently, and considered as Type B. The standard uncertainties of AR_x and AR_i is 0.1%

Table 3. Uncertainty of factor Q in equation 5

Parameter (x_i)	Source of Uncertainty	x_i	$u(x_i)$	$c_i(=\partial Q/\partial x_i)$	Degrees of freedom	Type	Source of data
MR_1	Between batch precision for preparing calibration standard mixtures	0.9749	0.00059	0.655	large	B	Intercomparison of 3 calibration standards mixtures prepared independently (by GC/MS analysis)
MR_2	"Same as MR_1"	1.1100	0.00067	0.344	large	B	"Same as above"
AR_1	Measurement of *pp'*-DDE / *pp'*-DDE-$^{13}C_{12}$ for calibration standard mixture 1	1.00095	0.001	-0.593	5	A	Repeated GC/MS analysis
AR_2	Measurement of *pp'*-DDE / *pp'*-DDE-$^{13}C_{12}$ for calibration standard mixture 2	1.1502	0.001	-0.312	5	A	Repeated GC/MS analysis
AR_x	Measurement of *pp'*-DDE / *pp'*-DDE-$^{13}C_{12}$ for the sample	1.0524	0.001	0.905	5	A	Repeated GC/MS analysis
Q (=0.9900)	$u(Q)=[\Sigma(c_i\,u(x_i))^2]^{1/2}$ = 0.00121, ν_{eff} = 12 (using Welch-Satterthwaite equation)						

Table 4. Uncertainty of C_x of Solution 1 (using equation 5)

Parameter (x_i)	Source of Uncertainty	x_i	$u(x_i)$	$c_i(=\partial C_x/\partial x_i)$	Degrees of freedom(ν)	Type	Source of data
Pmethod	Between batch precision for the method as a whole	0.0713 μg/g (0.0716)	0.00013 μg/g (0.00013)	1	7	A	Replicate analysis of sample across 8 batches
Q	See above	0.9900	0.00121	0.0720 (0.0723)	12	A	
M_x	Balance Precision	4.00059 g	0.0001 g	-0.0178 (-0179)	large	B	Balance calibration certificate
M_{sp}	Balance Precision	0.03657 g	0.00001 g	1.950 (1.958)	large	B	Balance calibration certificate
C_s	Concentration of the standard solution	7.879 μg/g (7.911)	0.016 μg/g (0.0025)	0.00950 (0.00905)	large	B	Suppliers specification

For Single measurement (except Pmethod)	C_x(purity corrected):	$u_{s,m}$ = 0.00017 μg/g,	ν_{eff}=174,	k(95% CI) = 2,	U = 0.00034
	C_x': (purity uncorrected):	$u_{s,m}$ = 0.00009 μg/g,	ν_{eff}= 15,	k(95% CI) = 2.13,	U = 0.00018
Total (including Pmethod)	C_x(purity corrected):	u_c = 0.00021 μg/g,	ν_{eff}=46,	k(95% CI) = 2,	U = 0.00042
	C_x: (purity uncorrected):	u_c = 0.00016 μg/g,	ν_{eff}=14,	k(95% CI) = 2.145,	U = 0.00034

*Values inside parenthesis are on the nominal basis (purity uncorrected); Values outside parenthesis are on the absolute basis (purity corrected).
*Equation used for $u_{s,m}$ is as following.

$$u_{s,m}=\left[\left[\frac{\partial C_x}{\partial M_{sp,x}}u(M_{sp,x})\right]^2+\left[\frac{\partial C_x}{\partial C_s}u(C_s)\right]^2+\left[\frac{\partial C_x}{\partial M_x}u(M_x)\right]^2+\left[\frac{\partial C_x}{\partial Q}u(Q)\right]^2\right]^{1/2}$$

*$u_c = [u_{s,m}^2 + u_{method}^2]^{1/2}$, where u_{method} from Pmethod. *$u(C_s)$ was obtained by dividing the expanded uncertainty of C_s quoted by the pilot laboratory with the stated coverage factor (2).

Table 5. Uncertainty of C_x of Solution 2 (using equation 5)

Parameter	Source of Uncertainty	x_i	$u(x_i)$	$c_i(=\partial C_x/\partial x_i)$	Degrees of freedom(ν)	Type	Source of data
Pmethod	Between batch precision for the method as a whole	4.731 μg/g (4.750)	0.0013 μg/g (0.0013)	1	7	A	Replicate analysis of sample across 8 batches
Q	See above	0.9900	0.00121	4.779 (4.798)	12	A	
M_x	Balance Precision	2.00404 g	0.0001 g	-2.361 (-2.370)	large	B	Balance calibration certificate
M_{sp}	Balance Precision	1.21548 g	0.0001 g	3.893 (3.908)	large	B	Balance calibration certificate
C_s	Concentration of the standard solution	7.879 μg/g (7.911)	0.016μg/g (0.0025)	0.601 (0.600)	large	B	Suppliers specification

For Single measurement (except Pmethod)	C_x(purity corrected):	$u_{s,m}$ = 0.0113 μg/g,	ν_{eff}=148,	k(95% CI) = 2,	U = 0.022
	C_x: (purity uncorrected):	$u_{s,m}$ = 0.0060 μg/g,	ν_{eff}= 14,	k(95% CI) = 2.145,	U = 0.013
Total (including Pmethod)	C_x(purity corrected):	u_c = 0.0114 μg/g,	ν_{eff}=151,	k(95% CI) = 2,	U = 0.023
	C_x: (purity uncorrected):	u_c = 0.0062 μg/g,	ν_{eff}=15,	k(95% CI) = 2.13,	U = 0.013

*Values inside parenthesis are on the nominal basis (purity uncorrected); Values outside parenthesis are on the absolute basis(purity corrected).

of their values. These uncertainties are combined following the ISO guides [see Appendix A for the brief description of the guide] to obtain the uncertainty of *Q*. The sensitivity coefficient of each uncertainty component, $c_i = \partial Q/\partial x_i$, is also listed in Table 3. The combined uncertainty of *Q* is 0.00119, which is about 0.1% of *Q* value. The effective degrees of freedom for the uncertainty is calculated using the Welch-Satterthwaite equation (equation A2 in appendix A). The uncertainty of *Q* is combined in equation 5 with other uncertainty components to obtain $u_{s,m}$. Those uncertainty components and their sensitivity coefficients are listed in Table 4 for solution 1 and in Table 5 for solution 2. For both high and low level samples, the standard uncertainty of a single measurement result is mostly attributed to the uncertainty of the concentration of the *pp'*-DDE calibration standard solution.

The uncertainty of the final result C_x, mean of the 8 measurement results, is then obtained by the equation $[u_{s,m}^2 + u_{method}^2]^{1/2}$. The calculated uncertainties, their effective degrees of freedom, and the coverage factors for 95% confidence level are listed in Table 4 and 5. For solution 2, u_{method} of 0.0013 μg/g is negligible compared to $u_{s,m}$ of 0.011 μg/g on the absolute basis. It indicates that the IDMS techniques can be used for high accuracy analysis in this level of concentration and that more accurate results could be achieved by improving the uncertainty associated with the concentration of the calibration standard. For low level sample, u_{method} of 0.00013 μg/g is compatible with $u_{s,m}$ of 0.00017 μg/g on the absolute basis. Thus, it indicates that this concentration is close to a limit, where the variation of the measurement values from repeated independent measurements becomes the major source of the uncertainty in the final result.

Summary of Results. The concentration of *pp'*-DDE in solution 1 is 0.0713 ± 0.00042 μg/g on the absolute basis (corrected for the chemical purity of *pp'*-DDE) and 0.0716 ± 0.00034 μg/g on the nominal basis (uncorrected for the purity). The concentration of *pp'*-DDE in solution 2 is 4.731 ± 0.023 μg/g on the absolute basis and 4.750 ± 0.013 μg/g on the nominal basis. The numbers following are the expanded uncertainties corresponding to the measurement results with the levels of confidence of approximately 95%. The coverage factors used for the calculations are listed in Table 4 and 5.

Conclusion

The IDMS method was applied to the analysis of *pp'*-DDE in corn oil. The principles contained in Guide to the Expression of Uncertainty in Measurement provided by the International Organization for Standardization (ISO) was successfully applied to the evaluation of the uncertainty of the result of IDMS measurement with a bracket method. Uncertainty sources that contribute to the uncertainties of the final result were investigated. For the sample of higher concentration, the uncertainty of the final result is mostly attributed to the uncertainty associated with the concentration of the calibration standard solution, and the uncertainty associated with the IDMS measurement processes is negligible. For the sample of lower concentration, the variation of the measurement values from repeated independent measurements becomes the major source of the uncertainty in the final result.

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Appendix A. Brief Description of the ISO Guides for the Determination of Expanded Uncertainty:

Here is a brief description of the ISO guide for the evaluation of the uncertainty of a measurement result. The combined standards uncertainty, $u_c(y)$, associated with the measurand y (= $f(x_i)$) is given by:

$$u_c(y) = \sqrt{\sum_{i=1}^{N} \left(\frac{\partial f}{\partial x_i}\right)^2 u^2(x_i)} \quad \text{(A1)}$$

where x_i (i = 1 to N) is an independent parameter with standard uncertainty $u(x_i)$. $u(x_i)$ is obtained from Type A or Type B evaluation. Type A evaluation of $u(x_i)$ could be obtained by using the equation, $u^2(x_i) = s^2(x_i)/n$, where $s(x_i)$ is the standard deviation of x_i from n repeated measurements. Type B evaluation is used for means other than the statistical analysis of series of observations such as previous measurement data, manufacturers specifications, or data provided in calibration and other certificate. In this case, the degrees of freedom for the uncertainty is assumed to be large.

The expanded uncertainty, U, is given by $U = ku_c(y)$ where k is an appropriate coverage factor. The value of the coverage factor is chosen based on the level of confidence of the interval y U to y + U and the effective degrees of freedom (ν_{eff}) for the combined standard uncertainty $u_c(y)$. The effective degrees of freedom can be calculated using the Welch-Satterthwaite equation

$$\nu_{eff} = \frac{u^4(y)}{\sum_{i=1}^{N} \frac{c_i^4 u^4(x_i)}{\nu_i}} \quad \text{(A2)}$$

where $c_i = \partial f/\partial x_i$ is the sensitivity coefficient for the parameter x_i , and ν_i is the degrees of freedom of $u(x_i)$.