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Purity Assessment of Organic Reference Materials with a Mass Balance Method: A Case Study of Endosulfan-II

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A mass balance method established in this laboratory was applied to determine the purity of an endosulfan-II pure substance. Gas chromatography-flame ionization detector (GC-FID) was used to measure organic impurities. Total of 10 structurally related organic impurities were detected by GC-FID in the material. Water content was determined to be 0.187% by Karl-Fischer (K-F) coulometry with an oven-drying method. Non-volatile residual impurities was not detected by Thermal gravimetric analysis (TGA) within the detection limit of 0.04% (0.7 μg in absolute amount). Residual solvents within the substance were determined to be 0.007% in the Endosulfan-II pure substance by running GC-FID after dissolving it with two solvents. The purity of the endosulfan-II was finally assigned to be (99.17 ± 0.14)%. Details of the mass balance method including interpretation and evaluating uncertainties of results from each individual methods and the finally assayed purity were also described.

Key Words : Purity assay, Oraganic substance, GC-FID, K-F coulometry, TGA

Introduction

Achievement of compatibility and reliability in chemical measurement has been a great concern in the chemical metrology communities as a chemical measurement usually consists of several complex operational steps and therefore is prone to be biased due to its inherent weakness in each step or involvement of improper operation in certain steps along the whole processes. It is now understood that the traceability of measurement results to International System (SI) of Units is the most important key for the achievement of compatibility and reliability in chemical measurements.[1,2] The traceability of chemical measurements in testing laboratories can be ensured by calibration or verification of their measurement results against proper Certified Reference Materials (CRMs) provided by authorized bodies, usually National Metrology Institutes (NMIs) or accredited CRM producers with linking traceability to NMIs' standards. Therefore, it is NMIs' role to make CRMs traceable to SI.[3,4] In theory, pure substances of target analytes are at the apex of the traceability as the amount of a target analyte in CRMs or samples should be calibrated against the amount of its pure substance used as a primary calibrator, or a calibration solution prepared with it. However, ideally 100% pure chemicals are not available, especially in the field of organic analysis due to the complexity of organic materials. Therefore, purity assessment of organic substances is one of the most critical step to link traceability of chemical measurements to SI units. For this reason, the Organic Analysis Working Group (OAWG) of the Consultative Committee for Amount of Substance-Metrology in Chemistry (CCQM) under International Committee for Weights and Measures (CIPM) regards the purity assessment for high-purity organic substances as one of the core competences of each individual NMI. The OAWG of the CCQM have regularly organized series of inter-laboratory comparisons among NMIs for the determination of the chemical purity of an organic substance to be use as a primary calibrator.[5] Its purpose is to investigate appropriateness and limitation of the various approaches and techniques currently practiced in NMIs to assign purities (mass fraction of principal component and impurities) to organic substances to be used as calibrators. The long-term goal of the inter-laboratory comparison series is to provide a harmonized best-practice approach under current technical limits and to further improve it.

An ideal approach for the purity determination may be employing a method which can measure the mass fraction of the principal component of each organic substance. Differential scanning calorimetry (DSC) has been one of strong candidates of a direct method for purity assay. Purity assay by DSC is based on the melting point depression due to impurities.[6] The technique works when all impurities dissolve in the melt and are insoluble in the crystal. This requirement severely limits the applicability of DSC to purity assay. Most of organic reference materials inherently have structurally related organic impurities, and some of them can co-crystallize with the principal component and cannot be detected by DSC as impurities. Impurities insoluble in the melt (water, inorganic impurities, and any foreign particles could be examples) are not detected by DSC.[7] Therefore, DSC cannot be a prior direct method for the purity assay of organic substances. A spectroscopic method with quantitative nuclear magnetic resonance (qNMR)

can measure the content of the principal component in comparison with a well-characterized reference material as an internal or external standard to calibrate ^{1}H signals.[8-10] In a few NMIs, qNMR is considered to be a relatively easy and economically sound method for purity assays of various organic substances. However, qNMR is still used as a confirmatory method to the mass-balance approach (*vide infra*) as it is considered to be further validated as a stand-alone purity assay method and also need to use an internal or external standard, which is well-characterized by the mass-balance approach.[11]

The mass-balance approach is more like a comprehensive method measuring all detectable impurities in an organic calibrator by applying several analytical techniques. Purity (mass fraction) of the principal component can be obtained by subtracting contents of all detected impurities from 100% (kg/kg).[12,13] For this approach, GC-FID or/and high performance liquid chromatography with UV/VIS detector (HPLC-UV) are generally used for the determination of structurally related organic impurities.[8] GC-FID is commonly used when the principal component is a volatile or semi-volatile and thermally stable organic compound. Its capability of high chromatographic separation is important to resolve and detect all organic impurities. FID detection is known to have a wide dynamic range (10^6) and to linearly response to the number of C-H moieties of molecules, approximately equi-mass response to organic molecules.[12,13] This FID character provides an assumption that peak areas of the principal component and all detected impurities in the material are proportional to their mass fraction in the material. In this respect, GC-FID is widely used as a universal method for the purity assessment of volatile and semi-volatile organic substances. HPLC-UV is used for thermally labile compounds for which GC-FID is not applicable, or as a confirmatory to the GC-FID analysis. Residual solvents in organic substances can be analyzed by a head space GC (HS-GC), or by comparing GC-FID chromatograms of the organic substance obtained after dissolving it into two organic solvents with quite different retention time. Water is not detected by those chromatographic methods, and it can be determined by Karl-Fischer (K-F) Coulometry.[14] A thermal gravimetric analysis (TGA) under inert gas is used for the determination of non-volatile (organic and inorganic) residues which are not eluted by GC.[15] TGA under air is used as an ash analysis for the determination of inorganic impurities which is not detected by HPLC-UV. Mass spectrometry combined with HPLC or GC is used for the identification of the principal component and organic impurities when it is necessary, especially when contents of certain organic impurities are significant and to be determined with calibration by the corresponding compounds. Many other analytical techniques can be employed depending on substances and impurities in them.

For a mass balance method, it is required to apply as many analytical methods as possible to determine all possible impurities, but some analytical methods are technically not applicable for specific target substances and applying all analytical methods is practically impossible due to economical reasons. Therefore, it is important to design a best-practice approach employing all analytical methods required to determine all impurities with significant mass fraction in the organic substance to be measured.[8] Two mass-balance approaches have been established and commonly used in most of NMIs including our laboratory. One approach is mainly based on the GC-FID analysis for thermally stable compounds. The other approach is based on the HPLC analysis for thermally labile. For the both approaches, K-F Coulometry, TGA, and residual solvent analysis were accompanying to determine impurities which are not detected by GC-FID or HPLC-UV. Also, employing confirmatory methods such as qNMR and DSC is beneficial to check whether any impurity with significant amount is not detected by the mass-balance approach.

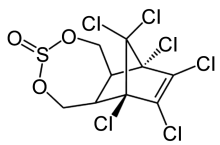

Figure 1. Structure of endosulfan-II ($C_9H_6Cl_6O_4S$, CAS # 1031-07-8, MW: 422.924).

In this article, we describe details of a mass-balance approach based-on the GC-FID analysis, which is established in this laboratory for the purity assessment of organic substances. We chose a pure substance of endosulfan-II as a representative material for this approach as the compound and organic impurities (mostly structurally related with endosulfan-II) are semi-volatile and applicable for GC-FID analysis. Endosulfan-II (shown in Figure 1) is an isomer in an endosulfan group of non-specific insecticide and has been commonly used to control various insect pests in agricultural and horticultural areas. Recently, many countries including European Union and the United States had regulated and/or banned the use of endosulfans with respect to the Stockholm Convention in April 2011, and Korea has implemented similar regulation.[16-18] Therefore, the analysis of endosulfan-II in food and environmental sample is an important subject and our laboratory developed several food CRMs for the analysis of pesticides including endosulfan-II to disseminate national standards to link traceability for the measurement of those pesticides by testing laboratories. For the purity assay of the endosulfan-II substance, the principle component, endosulfan-II, and organic impurities were measured by GC-FID with two different types of column (non-polar vs polar). Residual solvent in the material were measured with GC-FID after dissolving it in two different solvents. K-F coulometric titration was conducted for the determination of water content, and TGA was used for the determination of non-volatile organic residues and inorganic impurities. Those analytical methods were optimized and tested to determine targeted impurities with proper uncertainty levels. Furthermore, identification of the principle component and organic impurities detected by GC-FID were conducted with GC/MS. Finally, the purity of the endosulfan-II substance and its uncertainty were evaluated from those analytical results.

Experimentals

Reagents and Chemicals. An endosulfan-II substance was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany) and to be used as a primary calibrator for the certification of related CRMs. The purity provided by the manufacturer was (99.5 ± 0.50) %, which was based on only a few limited analytical techniques, GC-FID analysis with rapid temperature program and K-F Coulometry. *n*-Hexane (HPLC grade, Meet ACS specifications) and toluene (Batch No.: MKBF6324, Cat. No.: 650579) were purchased from B&J (Muskegon, MI, USA) and Sigma-Aldrich (Kyounggi-do, Korea), respectively.

Preparation of Standard Solution. For GC-FID or GC/MS measurements, the endosulfan-II substance was dissolved in *n*-hexane at 10 mg/g level. For the measurement of residual solvents, a standard solution of 10 mg/g level in toluene was also prepared.

GC-FID Analysis for Purity/Impurities. An Agilent 6890N GC (Agilent Technologies, Ramsey, MN) equipped with a flame ionization detector (FID) and an Agilent 6873 series sample injector was used for the determination of mass fractions of endosulfan-II and organic impurities in the endosulfan-II substance. For the GC-FID analysis, two different types of columns were used. First, the endosufan-II solution in *n*-hexane was analyzed with a non-polar GC column (DB-5, 60 m × 0.53 mm i.d., 0.25-μm film thickness; J&W Scientific, Folsom, CA) by injecting through the on-column injection port. Injection volume was 1 μL. The carrier gas was helium at 6.0 mL/minute. The initial oven temperature was 50 °C and held for 3 minutes. The temperature was increased to 260 °C at 20 °C/minute, to 270 °C at 2 °C/minute, to 300 °C at 20 °C/minute, and held for 30 minutes at 300 °C. Second, the solution was analyzed with a polar GC column (Rtx1701, 30 m × 0.53 mm i.d., 0.25-μm film thickness; Restek, Bellefonte, PA). Injection volume and flow rate of the carrier gas were same as the above mentioned. The initial temperature was 50 °C and held for 3 minutes. The temperature was increased to 150 °C at 20 °C/minute and then to 260 °C at 5 °C/minute, and held for 10 minutes at 260 °C. Mass fractions of endosulfan-II and detected impurities were calculated as following:

$$P_{\mathrm{GC-FID}} = \frac{A_{\mathrm{endosulfan-II}}}{A_{\mathrm{endosulfan-II}} + \sum_i A_{\mathrm{impurity},i}} \quad (1)$$

$$P_{\mathrm{impurity},i} = \frac{A_{\mathrm{impurity},i}}{A_{\mathrm{endosulfan-II}} + \sum_i A_{\mathrm{impurity},i}} \quad (2)$$

where, $P_{\mathrm{GC\text{-}FID}}$ and $P_{\mathrm{impurity},i}$ are mass fractions of endosulfan-II and impurity i, respectively, $A_{\mathrm{endosulfan\text{-}II}}$ is the peak area of endosulfan-II and $A_{\mathrm{impurity},\,i}$ is the peak area of impurity i in the GC chromatogram. Note that contents of impurities undetected by the GC-FID analysis such as residual solvents, non-volatile residues, and water were not included in the equation. Therefore, those mass fractions represent those from total of organic components detectable by the GC-FID analysis.

GC/MS Analysis. An Agilent 6890N GC interfaced with a single quadrupole mass spectrometer (Agilent 5973 MSD, Ramsey, MN) was used to identify endosulfan-II and other impurities. The endosulfan-II solution in *n*-hexane was injected to a Rtx-5MS GC column (30 m × 0.25 mm i.d., 0.25-μm film thickness; Restek, Bellefonte, PA) using an Agilent 7683 series injector. The analytical conditions were as following: Injection volume was 1 μL with split injection mode (split ratio of 5:1), and the injector temperature was 280 °C. The carrier gas was helium at 1.0 mL/minute. The initial temperature was 100 °C and held for one minute. The temperature was increased to 200 °C at 10 °C/minute, increased to 280 °C at 5 °C/minute, increased to 300 °C at 20 °C/minute, and held for 5 minutes at 300 °C. The mass spectrometer was operated in a scan mode (scan range: *m/z* 50 to *m/z* 555, full scan time: 3.25/second) with electron impact ionization (EI) at 70 eV, and solvent delay was 5.0 minutes. The retention time of endosulfan-II was 18.46 minute. The unknown impurity peaks were searched and identified with the National Institute of Standards and Technology (NIST) MS library.

GC-FID Analysis for Residual Solvent. A standard solution (−10 g/kg) of the endosulfan-II substance was prepared with toluene. Toluene was chosen as its retention time in GC is much later then *n*-hexane, and therefore possible residual solvent, which could be overlapped and/or undistinguished from huge peaks of *n*-hexane and its impurities in the above GC-FID purity analysis, can be detected. The standard solution was injected to a non-polar GC column (DB5, 60 m × 0.53 mm i.d., 0.25-μm film thickness; J&W Scientific, Folsom, CA) through the on-column injection port. The analytical conditions were as following: the basic conditions including injection volume were same as the above mentioned (the section of "*GC/FID analysis for purity assay*"). The initial temperature was 50 °C and held for 3 minutes. The temperature was increased to 130 °C at 5 °C/minute, increased to 250 °C at 30 °C/minute, increased to 300 °C at 5 °C/minute, and held for 5 minutes at 300 °C. The mass fraction of the sum of residual solvents in the endosulfan-II substance, $P_{\mathrm{residual\ solvent}}$, was calculated as followings.

$$P_{\mathrm{residual\ solvent}} = \frac{\sum_i A_{\mathrm{residual\ solvent},i}}{A_{\mathrm{endosulfan-II}}} \quad (3)$$

Where, $A_{\mathrm{endosulfan\text{-}II}}$ is a peak area of endosulfan-II in the GC chromatogram, $A_{\mathrm{residual\ solvent},i}$ is the peak area of residual solvent i in the GC chromatogram, and $P_{\mathrm{GC\text{-}FID}}$ is the mass fraction of endosulfan-II measured by GC-FID using Eq. (1).

Karl-Fischer Coulometry. Water content in the organic substance was measured with the Karl-Fischer (K-F) coulometric titrator C30 connected with an oven-drying sample changer (Mettler-Toledo, Switzerland). Nitrogen gas was used as a carrier gas which transferred water from the oven-drying sample changer to the measuring cell. Before sampling, all the vials were dried at 100 °C for 30 minutes then

cooled down in a desiccator with silica-gel in effort to minimize background moisture in the vials. Prior to a sample measurement, a drift of the K-F coulometric titrator was stabilized below 15 μg/minute level. Temperature of the oven sample changer was set at 100 °C, which was set-up by considering the melting point (MP) of endosulfan-II (usually 20 °C or lower than MPs of target organic substances).

20 mg of the endosulfan-II substance was weighed into a vial, and an oven-drying method was used for the measurement of water content in the sample. Dry nitrogen gas at 50 mL/minute was used as a carrier gas. Carrier gas from drying oven introduced into the reaction cell for 5 minutes, and then titration was conducted for 5 minutes. In our experience, the titration for 5 minute was adequate, if water content in organic substances was below 10%. At least six blank samples (pre-baked empty vials) and a single subsample were measured in the present study. The six blank runs were done to measure the system blank (sum of water introduced from carrier gas, residual water in the vial). Water content in sample was calculated as followings

$$P_{\text{water}} = \left[\left(\frac{\text{ICEQ}}{10.712} - \text{Time} \times \text{Drift} - \text{Blank}\right)/m\right] \times C \qquad (4)$$

where, P_{water} is the mass fraction (kg/kg) of water in endosulfan-II, ICEQ is a total consumed electric charge, which is converted to total detected mass of water (in μg). Time is a total K-F coulometric measurement time in minute (10 minutes in this case), drift (μg/minute) is a systematic water content measured by K-F coulometric titrator before the analysis, Blank is the system blank measured from the six empty vials, m is a sample weight (in g) used for a K-F coulometric analysis, and C is a constant, 1×10^6, to convert μg/g unit to kg/kg.

Thermo Gravimetric Analysis (TGA). Non-volatile residual impurities like inorganic materials or non-volatile/high-molecular weight organic materials cannot be detected by the GC-FID method (*vide supra*). Therefore, they were measured with a thermo gravimetric analysis (TGA/DSC1, Mettler Toledo, Switzerland). A few (2 to 5) mg of sample was load on an alumina pan, which was pre-baked for 5-7 times with the same temperature program used for sample analysis to eliminate some unknown impurities in alumina pan and properly aging the pan. Sample measurement began at 25 °C, and held for 10 minutes in order to equilibrate temperature between sample and TGA furnace. Furnace temperature in TGA was increased to 600 °C at 10 °C/minute, and then held for 1 h at 600 °C. Dry nitrogen was used as both a reactive gas at 5 mL/minute and a protective gas at 30 mL/minute. The internal micro-balance (UMX1, Mettler Toledo, Switzerland) in TGA recorded weight change of the sample pan following the temperature program, and provide a sample evaporation profile. However, the precision of weighing with the internal balance is a few tens of micrograms due to heating and gas (N_2) flow. When sample size is limited to a few milligrams, even a few percentages of residual non-volatile impurities cannot be determined with the weighing method. Thus, an external micro-balance (MX5, Mettler Toledo, Switzerland) was used for weighing the alumina pan before and after loading sample and after sample run by the TGA. In this way, the weight of sample (W_{sample}) loaded for measurement and the weight of total non-volatile impurities ($W_{\text{non-volatile residues}}$) remained on the pan were precisely determined by weighing-by-difference with the standard uncertainty of 0.7 μg. The content of total non-volatile impurities ($P_{\text{non-volatile residues}}$) can be estimated by following equation.

$$P_{\text{non-volatile residues}} = \frac{W_{\text{non-volatile residues}}}{W_{\text{sample}}} \qquad (5)$$

Results and Discussion

GC-FID Determination of the Principal Component and Organic Impurities. In general, a purity assessment of organic substances by a chromatographic method like GC or HPLC is based on assumptions as followings: high mass fraction of a principle component (> 95%), complete separation of all impurities from a principle component, same detection responses of all impurities compared with the principle component.[19] GC-FID, especially, is a common method for the purity assessment of volatile or semi-volatile

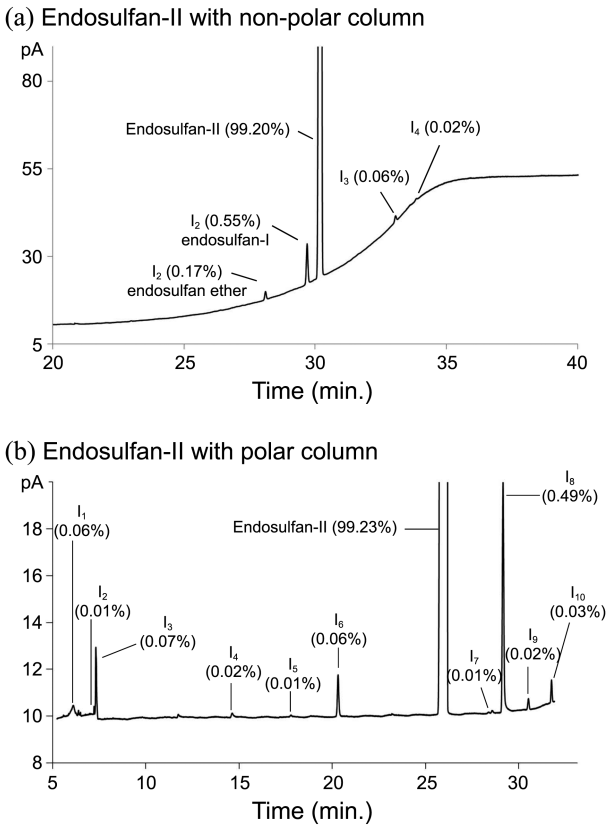

Figure 2. Representative chromatograms of endosulfan-II by GC-FID (a) with a non-polar column (DB-5) and (b) with a polar-column (Rtx1701). Impurity peaks marked with identified chemical constituents were based on a GC/MS analysis.

Table 1. Summary of measurement results from individual analytical methods for purity assay of endosulfan-II

Assay type	Measurement results, % (kg/kg)	Standard uncertainty, % (kg/kg)[b]
Purity assay by GC-FID, $P_{\text{GC-FID}}$[a]	99.20	0.003 (v = 9)
Residual solvent by GC-FID, $P_{\text{residual solvent}}$	0.007	0.0005 (v = 4)
Water content by KFC, P_{water}	0.18	0.05 (v = 5)
Non-volatile Residues by TGA, $P_{\text{non-volatile residues}}$	0	0.04 (v = ∞)
Putiry, $P_{\text{endosulfan-II}}$	99.17	0.06 (v = 11)
Expanded uncertainty of $P_{\text{endosulfan-II}}$ (95% level of confidence)	-	0.14

[a] For $P_{\text{GC-FID}}$, results with the nonpolar column were used for the final purity assessment as discussed in the text. [b] v is the effective degree of freedom for the corresponding uncertainty.

organic substances due to its high resolution and approximately linear response to the amount (mass) of organic compounds in most of cases.[8] In this study, GC-FID analysis was carried out with a non-polar column and a polar column. In this way, any possible significant impurity overlapped with the large peak of the principal component with a column can be detected with the other.

Figure 2(a) showed a representative chromatogram of the endosulfan-II substance by GC-FID with the non-polar column. Four impurity peaks were observed in addition to a large dominant peak for the principal component. Following the integration of peak areas, the mass fraction of the principal component ($P_{\text{GC-FID}}$) was estimated, according to Eq. (1), to be 99.20% (kg/kg) with its standard uncertainty ($u_{P_{\text{GC-FID}}}$) of 0.003% (kg/kg) (Table 1). Mass fractions of four detected impurities were estimated and marked in Figure 2(a). Mass fraction for the total of the four organic impurities was 0.80% (kg/kg). Using the polar column (Figure 2(b)), the mass fraction of endosulfan-II was 99.23% (kg/kg) with its standard uncertainty ($u_{P_{\text{GC-FID}}}$) of 0.04% (kg/kg). The standard uncertainty ($u_{P_{\text{GC-FID}}}$) during the purity assay by GC-FID was evaluated with the standard deviation ($s_{P_{\text{GC-FID}}}$) from multiple measurements (n = 10) (Table 2). $P_{\text{GC-FID}}$ values from the two columns agree to each other within their uncertainties. More impurities (10 impurity peaks) were detected with the polar column as shown in Figure 2(b). In comparing details of two chromatograms, we note that I_2 impurity in Figure 2(a) separated mainly to I_8 impurity and other much smaller impurity peaks in Figure 2(b). Also, I_1 impurity in Figure 2(a) separated to much smaller impurity peaks. Comparing the two chromatograms, there is very little chance that any significant impurity is overlapped with the principal component and is not detected with the both columns. Further correlating peaks observed in the two chromatograms are difficult and not necessary for purity assay of the substance. In the case of the endosulfan-II substance, the mass fraction obtained with the non-polar column was used as it provides more amounts of impurities than the other.

Confirmation of Endosulfan-II and Identification of its Impurities by GC/MS. Endosulfan-II and its impurities were identified with GC/MS. The mass spectrum of the principal component was well matched with that of endosulfan-II in the NIST library. Major components of I_1 and I_2 impurities in Figure 2(a) were identified to be endosulfan ether and endosulfan-I, respectively, which are structurally-related with the principal component. Other two impurities (I_3 and I_4) were not clearly identified due to low quality of their MS spectra. In the present study and in most of purity assay studies, the GC/MS analysis is optimized for only

Table 2. Evaluation of measurement uncertainty of each individual analytical techniques

Analytical techniques	Standard uncertainty (u)	
	Multiple measurements ($n \geq 3$)[a]	Single measurement (n = 1)
Purity assay withGC-FID	$u_{\text{GC-FID}} = \frac{s_{\text{GC-FID}}}{\sqrt{n}}$	-[b]
Determination of residual solvent with GC-FID	$u_{\text{residual solvent}} = \frac{s_{\text{residual solvent}}}{\sqrt{n}}$	-[b]
Determination of water content with KFC	$u_{\text{water}} = \frac{s_{\text{water}}}{\sqrt{n}}$	$u_{\text{water}} = \frac{s_{\text{KFC system blank}}}{W_{\text{sample}}}$ [c]
Determination of non-volatile impurity with TGA	$u_{\text{non-volatile residue}} = \frac{s_{\text{non-volatile residue}}}{\sqrt{n}}$	$u_{\text{TGA}} = \frac{u_{\text{weighting-by-difference}}}{W_{\text{sample}}}$ [d]

[a] u_x is the standard uncertainty, which is estimated from the standard deviation (s_x) of the corresponding measurement value P_x from multiple measurements, $u_x = s_x/\sqrt{n}$. See Table 1 for the notation of each specific P_x. [b] Multiple measurements are usually carried out by these techniques. [c] $s_{\text{KFC system blank}}$ is the standard deviation of K-F coulometer's system blank, obtained by multiple measurement of empty vials, and is assumed to be a major source of variation in multiple measurement of a homogenous sample. [d] $u_{\text{weighing-by-difference}}$ is the standard uncertainty of weighing-by-difference of the sample pan before sample loading and after TGA run, and can be estimated from the certificate of the balance.

qualitative analysis, not for quantitative analysis, and therefore, quantitative evaluation of observed peaks was not carried out.

Determination of Residual Solvent by GC-FID. A trace level of residual solvents can be present in organic substances, because various organic solvents like benzene, toluene, methanol, or propanol are typically used during the manufacturing processes including synthesis, isolation, extraction and clean-up steps.[8] In GC-FID analysis for purity assay, organic substances are dissolved into a volatile solvent (*n*-hexane in this study) and the solution is injected into GC. The front part of the chromatogram is largely dominated by huge peaks due to the solvent and its impurities. Volatile residual solvents in organic substances usually co-elute in the solvent front zone and may not be distinguished (any residual solvent which is not overlapped with the solvent front zone must be already detected as an impurity in the above GC-FID analysis). Those undistinguished residual solvent can be determined by GC-FID analysis of the same substance after dissolving it into a different solvent whose own solvent front zone is not overlapped with that of the first solvent. In this study, after testing several organic solvents like methanol, dimethylsulfoxide, acetonitrile, and toluene, we chose toluene as an organic solvent. As shown in Figure 3(a), its solvent front zone is well behind and separated from that of *n*-hexane (retention time difference ≥ 5 minutes) and endosulfan-II has good solubility in toluene. Figure 3(b) shows a typical GC-FID chromatogram of the endosulfan-II substance in toluene. Two unknown residual solvent peaks, which were buried with the solvent front zone when *n*-hexane was used as a solvent, were distinguished. A residual solvent notated as RS-2 in the figure was overlapped with a peak due to a trace impurity in toluene. The amount of RS-2 from the endosulfan-II substance was obtained by subtracting blank toluene chromatogram. By using Equation 3, the total amount of the two residual solvents ($P_{\text{residual solvent}}$) was estimated to be 0.007% (in kg/kg) of that of endosulfan-II with its standard uncertainty ($u_{\text{residual solvent}}$) of 0.0005% (in kg/kg). The sum of their mass fraction is too small and negligible as the value is much less than uncertainties of other sources in the whole purity assay works. Therefore, further accurate quantitation of their mass fractions with external calibration was not carried out.

Determination of Water Content by Karl-Fischer (K-F) Coulometric Titration. As pure organic substances are usually precious and materials available for purity assay are limited like the case of this study, it is tricky to decide minimum sample amount required for K-F coulometric titration to measure water content in sample with a reasonable uncertainty.[8,20] System blank was determined by measuring pre-baked empty vials with the same method used for sample measurements. In the experimental conditions in this study, the system blank was around 390 μg and the standard deviation ($s_{\text{KFC system blank}}$) of multiple measurement results was 10 μg, which corresponds to 1.0, 0.2, 0.1, and 0.05% (kg/kg) of water in 1, 5, 10, 20 mg of sample, respectively. The standard deviation of multiple sample measurement is

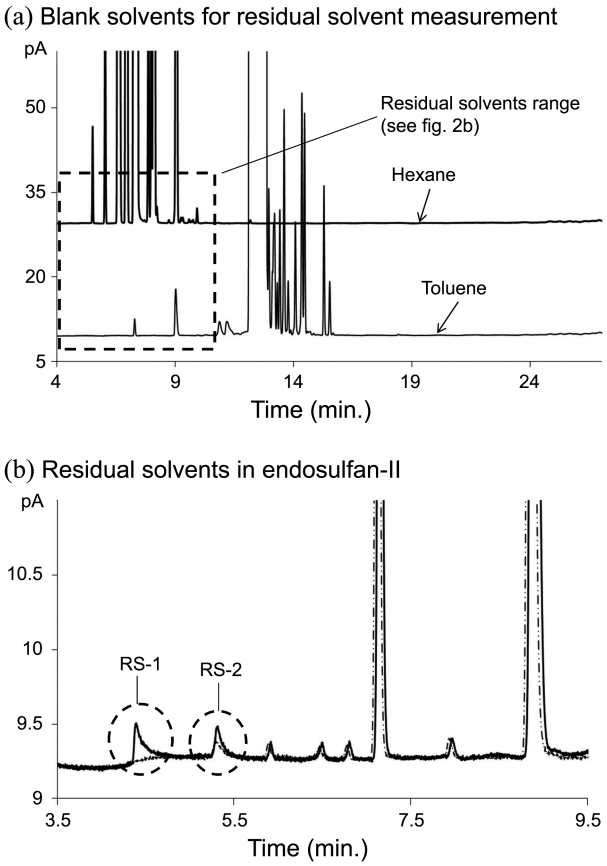

Figure 3. (a) Chromatograms of hexane and toluene and (b) chromatogram of the endosulfan-II organic substance dissolved in toluene for the measurement of residual solvents by GC-FID. Overlaid dotted chromatogram is from blank toluene.

expected to be similar to that of system blank as uncertainties from other operational step are usually negligible compared to the system blank. Therefore, the standard deviation of the system blank can be assigned as the standard uncertainty of water content measured by a single titration. In considering overall uncertainty of purity assay, we decided that more than 0.05% of water in the organic substance should be measured or that the uncertainty of water measurement should be less than 0.05%, which requires minimum 20 mg of sample. Therefore, we used 20 mg of sample for a single K-F titration in this study. Water content measured for 20 mg of the endosulfan-II substance was 37 μg, which corresponds to 0.18% (kg/kg) of water (P_{water}) in the sample with its standard uncertainty (u_{water}) of 0.05% (kg/kg), which is estimated from 10 μg of standard deviation in measurement of the system blank. If multiple measurement (n) is possible, u_{water} can be calculated by $s_{\text{water}}/\sqrt{n}$, where s_{water} is the standard deviation of P_{water} from multiple measurements. After this study, the K-F coulometric system is installed in a glove box, and is under test to reduce system blank and eventually to reduce minimum sample size required for water content measurement.

Determination of Non-Volatile Residual Impurity by Thermal Gravimetric Analysis (TGA). In general, many organic substances could contain unknown impurities like

inorganic materials, elements, or/and non-volatile high-molecular weight organic impurities from the manufacturing processes. In the present study, the total amount of non-volatile residual impurities in the endosulfan-II substance was measured with a TGA under inert dry nitrogen gas so that non-volatile organic residues which was not detected by the GC-FID analysis [*vide supra*] can be measured in addition to those inorganic impurities. The evaporation profile of the organic substance was obtained by the TGA with the internal balance. Gradual evaporation processes was observed following the temperature program up to 250 °C and the weight of the sample pan did not show further change after that. By using the external balance, weights of the pan before and after loading 2 mg of sample and after the TGA analysis were measured. The weight of the pan after the TGA analysis was the same with that of the initial empty pan within the weighing uncertainty, indicating that the total amount of non-volatile impurities is less than the weighing uncertainty. Therefore, the mass fraction of non-volatile impurities ($P_{\text{non-volatile residue}}$) in the sample was assigned to be zero %(kg/kg) with the standard uncertainty ($u_{\text{non-volatile residue}}$) of 0.04% (kg/kg), which is estimated from the weighing uncertainty ($u_{\text{weighing-by-difference}}$) of 0.7 μg in 2 mg of sample (W_{sample}). When sample available for purity assay is limited like the case in this study, only a single TGA analysis can be carried out and the uncertainty can be estimated from that of weighing-by-difference of the pan before sample loading and after TGA run, $u_{\text{weighing-by-difference}}$. If multiple measurement (n) is possible, $u_{\text{non-volatile residue}}$ can be calculated by $s_{\text{non-volatile residue}}/\sqrt{n}$, where $s_{\text{non-volatile residue}}$ is the standard deviation of $P_{\text{non-volatile residue}}$ from multiple measurements. For the endosulfan-II substance, TGA analysis under air is not required as the total amount of non-volatile impurities (organic and inorganic), which cannot be detected by GC-FID, was determined by TGA analysis under inert gas.

Determination of the Final Purity and Evaluation of its Uncertainty. Table 1 summarizes measurement results from several analytical techniques, which have to be used for the calculation of the purity of the endosulfan-II substance, mass fraction of the principal component. The purity is calculated using the following equation from $P_{\text{GC-FID}}$, $P_{\text{residual solvent}}$, P_{water}, and $P_{\text{non-volatile residue}}$ [See Table 1 and text above for notations].

$$P_{\text{endosulfan-II}} = (1 - P_{\text{residual solvent}} - P_{\text{water}} - P_{\text{non-volatile residue}}) \times P_{\text{GC-FID}} \quad (6)$$

The first part of the equation, $(1 - P_{\text{residual solvent}} - P_{\text{water}} - P_{\text{non-volatile residue}})$, is the subtraction of mass fractions of impurities not detected by GC-FID purity analysis from 100%, and represents total amount of organic compounds to be detected by the GC-FID analysis. $P_{\text{GC-FID}}$ represents the mass fraction of the principal component out of those organic components detected by GC-FID, and can be estimated by Eq. (1). Measurement results from individual analytical techniques are summarized in Table 1. We already discussed how the uncertainty of the value from each individual analytical techniques can be evaluated in the corresponding sections above, and detailed equations used are listed in Table 2, and uncertainties estimated by those equations are listed in Table 1 in comparison with the corresponding measurement values. The final purity of the endosulfan-II substance was calculated to be 99.17% (kg/kg) by using Eq. (6). The standard uncertainty of the final purity was obtained by combining the uncertainties of values from each individual analytical techniques based on Eq. (6) by following the "Guide to the Expression of Uncertainty in Measurement".[21] The combined standard uncertainty was calculated to be 0.06% (kg/kg) with effective degree of freedom of 11, and its expanded uncertainty ($U_{\text{exp, endosulfan-II}}$) with the level of confidence of 95% was 0.14% ($k = 2.20$). The purity provided by the manufacturer agrees with our results. We note that the purity provided by the manufacturer was based on only a few limited analytical techniques, but we comprehensively examined and evaluated the purity of the endosulfan-II substance by a mass-balance method with applying several available analytical techniques to detect and quantify most of possible major impurities. Therefore, the assigned purity by this best-practice approach is more reliable and scientifically reasonable under current technical limits and economical burdens.

Conclusions

A mass-balance method for purity assessment of semi-volatile compounds was demonstrated in this study. The purity of an Endosulfan-II subsatnce was measured and its uncertainty was evaluated with the mass balance method as a representative model for semi-volatile organic substances. In this study, K-F coulometric method and TGA methods were optimized for trace level analyses of water and non-volatile residual impurities in small size samples. Furthermore, the approach for the estimation of uncertainty in measurement results from each individual analytical method was also developed and validated in this study. Therefore, the purity assay protocol used in this study can be applied in general to volatile and semi-volatile organic substances.

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