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DEVELOPMENT OF REFERENCE MATERIAL FOR ORGANOCHLORINE PESTICIDES IN HERBAL SAMPLE

Yiu-chung Wong[1], Tat-ting Kam[2], Serena Chan[3]

[1] *Analytical & Advisory Services Division, Government Laboratory, Hong Kong, ycwong@govtlab.gov.hk*
[2] *Analytical & Advisory Services Division, Government Laboratory, Hong Kong, ttkam@govtlab.gov.hk*
[3] *Analytical Laboratories in Anaheim, Inc., Los Angeles, USA, serenac_2001@yahoo.com*

Abstract: Development of reference material for four organochlorine pesticides, namely hexachlorobenzene and three isomers (α-, β- and γ-) of hexachlorocyclohexane, in ginseng root sample (*Panax ginseng*) to ascertain the quality control and validation processes was presented. A total of more than 300 bottles each containing 25g of *Panax ginseng* samples was prepared and the reference values were characterised using a primary measurement method, isotope dilution gas chromatography-mass spectrometry. The concentrations (± expanded uncertainty) of hexachlorobenzene, α-, β- and γ-hexachlorocyclohexane in the reference material are 0.198 ± 0.015 mg/kg, 0.450 ± 0.025 mg/kg, 0.213 ± 0.014 mg/kg and 0.370 ± 0.031 mg/kg respectively. A portion of the samples was also used in a proficiency testing (PT) scheme for assessing the testing capabilities of field laboratories and the consensus mean values from the 70 participants in the programme were deviated from -2.7% to -14.1% as the assigned reference materials.

Keywords: reference material, organochlorine pesticides, isotope dilution mass spectrometry, proficiency testing programme

1. INTRODUCTION

Organochlorine pesticides (OCP) have been used extensively to control harmful pests and prevent vegetation infections during the last century. Owing to the persistent properties, large amount of these chemicals that released through all kinds of anthropogenic activities led to ubiquitous long-term contaminations in the environment and food chains. As a consequence, determinations of residual OCP in agricultural products and food are of great importance for comprehensive understandings of contamination patterns, physiological absorption, distribution, transport pathways as well as the estimation of average daily intake in risk assessments. Since the recognition of the adverse effects of OCP to our ecosystem, a huge quantity of accessible scientific data concerning the analysis of residual OCP in various types of environmental, agricultural and animal samples has been reported in the literature. In particular, with the signing of the Stockholm convention on persistent organic pollutants and frequent of global corporation programs [1], there is an ever-increasing trend for laboratories worldwide to determine OCP.

Analytical methods for OCP are widely available IN the literature. Among all the commonly used screening and quantification methodologies, GC-ECD and GC-MSD are the most preferred methods because the instrumentations offer good degree of sensitivity and selectivity for OCP in a variety of matrices. Despite the majority of OCP test methods that claimed having high recovery and reasonably good precision was mainly relied upon the results of spiked or fortified samples, it was found that the actual bias might always be underestimated. As reviewed by a recent survey on the measurements of residual OCP in food matrices [2], the expectable minimum of the combined relative standard uncertainty was significantly in the range of 33–49%. To ensure the reliability of analytical data and to fully comprehend the deficiencies of OCP analysis, quality assurance through the use of reference materials (RM) and the participation of proficiency testing schemes are regarded as the crucial prerequisites in ISO17025 [3]. The usefulness and applications of RM, either non-certified or certified RM (CRM), in quality controls of chemical analysis have been clearly explicated [4]. The certification processes for CRM are tedious and the production, in terms of quantity and variety, is often too small to satisfy laboratories' demands. Commercially available matrix CRM for OCP are even severely limited [5] and such CRM in herbal matrices is nowhere identified. The circumstances have recently been discussed by the Reference Materials Committee of ISO/REMCO [6] and the uses of non-certified RM for quality control work of analytical methods were recommended as the best alternative whenever suitable CRM is not present. As a universal rule for all good practice laboratories, analytical techniques that are used to monitor the levels and fate of contaminants in the samples must be calibrated using appropriate calibration materials, and the methods must be properly validated using fit-for-purpose matrix-matched RM, to ensure validity of data being produced.

We had recently developed a highly precise isotope dilution gas chromatography mass spectrometry (ID-GCMS) method for analysing OCP in ginseng root [7] and reported it as a method of choice for the production of RM. In view of rapid growth of herbal medicine trade and its subsequent testing volume and lacking relevant CRM in laboratory testing for OCP in herbal matrices, we present the development RM for HCB, α-, β- and γ-HCH in ginseng root sample for method validation and quality control. The assigned reference values of the RM were determined by an accurate GC-IDMS method and the associated expanded uncertainties were estimated from the homogeneity and stability testing, and the precision of the GC-IDMS method.

2. EXPERIMENTAL

2.1. Preparation of bulk sample

About 20 kg of raw radix ginseng (*Panax ginseng*) that contained certain levels of targeted residual OCP were purchased from local market. The samples were rinsed with distilled water to remove dirt and other foreign matters, then were freeze-dried at about -50 °C and < 70 mT for 7 days. The dried ginseng root was ground to coarse powder by a domestic blender, then to fine powder by a high-speed centrifugal mill (ZM200, Retsch, Germany). The powder obtained was subject to passing through 100 μm calibrated sieves. About 8 kg of fine powder were collected and transferred to a 40-litre commercial blender for thorough mixing within a humidity- and temperature-controlled environment (relative humidity and temperature were respectively maintained at 50% and 20ºC). Homogeneity of the bulk was checked regularly by taking portions of sample during mixing and analysing the concentrations of the OCP using a validated GC-ECD method. Satisfactory and complete mixing was confirmed at day seventh after operation. Aliquots of about 25 g were packaged into clean and nitrogen-purge amber glass bottles in a clean room (Class 1000) and 300 bottles were finally prepared. All bottled samples were disinfected by γ-ray (Gammacell-1000 Elite, MDS Nordion, Canada) at a dose of about 0.8 kGy to prevent microbial growth, then vacuum-sealed in polypropylene bags.

2.2. Homogeneity Testing

A total of 16 randomly selected samples were analysed in duplicate by a validated GC-ECD method and the sequence of analysis (32 injections) was arranged in a randomised order. The analysis was performed using the same instrument and completed within the shortest time as possible for minimizing errors arising from instrumental bias. Statistical approaches were applied to evaluate: A one-way ANOVA was applied to evaluate within-bottle and between-bottle difference, hence the inhomogeneity status of the samples. Samples were considered homogeneous if the F-test values were smaller than those of the critical values.

2.3. Stability Testing

Stability testing aiming at assessing the stability of residual OCPs of randomly selected samples was performed at 25ºC for twelve months (Jan 2006 to Jan 2007). Samples were considered statistically stable if the mean in each triplicate analysis was ≤ 0.3 σ_p, where σ_p was the Horwitz standard deviation of the mean value of the OCP obtained from homogeneity testing.

2.4. Moisture Content

The bottles were kept in electronic desiccators at 25°C and 50% RH. The moisture content of the sub-samples (n = 10) was estimated by taking five bottles randomly and found to be 5.1% (RSD = 0.85%) at the time of bottling.

2.5. Preparation of Calibration Blend and Sample Blend

Calibration blend (by spiking appropriate quantity of $^{12}C_6$-OCP and $^{13}C_6$-OCPs) and sample blend samples (by spiking appropriate quantity of $^{13}C_6$) were respectively prepared in accordance with the reported procedure [7]. The concentration ratios of the natural to labelled isotopes in both blends should be close to unity (0.9 – 1.1) in order to achieve a high degree of accuracy in the ID-GCMS determinations.

2.6. Instrumental conditions for ID-GCMS

A GC-MS (HP6890 GC and a quadrupole MS HP5973, Palo Alto, USA) with a 30 m x 0.25 mm, 0.25 μm film HP-1707MS column (J & W Scientific, Folsom, USA) was used. Helium carrier gas was set at a flow-rate of 1.0 mL/min, and separation of the four OCP was carried out under a temperature programme as follows: injector temperature at 200°C, column temperature at 90°C for 1 min, ramped to 200°C at 50°C/min and held for 6 min, then to 280°C at 20°C/min and held for 5 min. The transfer line and the ion source were set at 280°C, and the ionisation energy under electron ionisation mode at 70 eV. Aliquots of 1 μL were injected into the GC-MS system under splitless mode, and the analytes were respectively monitored under the single ion monitoring mode at multiple mass channels at m/z 181, 183, 284 for ^{12}C-OCP and 187, 189, 290 for $^{13}C_6$-OCP.

3. RESULTS and DISCUSSIONS

3.1. Homogeneity and Stability Testing

Within variance (CV_w) and between variance (CV_b) of samples were derived from the duplicate analysis of the 16 samples. At the 95% confidence level, the critical value is 2.352 for n = 16. Since $F_{test} < F_{critical}$, statistical significant difference between samples was not detected. The uncertainty of the sample inhomogeneity (U_{bb}) was estimated as:

$$U_{bb} = \sqrt{(CV_b - CV_w)/2} \tag{1}$$

When CV_w was equal to or greater than CV_b, $U_{bb'}$ was estimated as the upper detection limit by the following equation, where n is the number of replicate and df is the degree of freedom:

$$U_{bb'} = \sqrt{\frac{CV_w}{n}} \cdot \sqrt[4]{\frac{2}{df}} \tag{2}$$

As shown in Table 1, the uncertainty arising from sample inhomogeneity was ranging from 0.46% to 2.8%.

Table 1. Inhomogeneity of 16 randomly selected samples

OCP	F_{test}	CV_w	CV_b	U_{bb}
HCB	2.252	3.8	2.5	2.8
α-HCH	0.800	1.6	1.8	1.1
β-HCH	1.042	3.1	3.0	0.46
γ-HCH	0.492	2.1	3.0	1.8

Stability testing indicated that all the four OCP were stable at both 25°C and 37ºC over the 12-month study period. Deviations of OCP from the mean values obtained by homogeneity testing at were ranging from 0.1 to 7.5% at 25°C and from 0.1 to 13.5% at 37°C respectively. The RSDs (U_{stab}) of OCP at 25°C were the uncertainty component of stability testing for the reference material.

Table 2. Stability of OCP at 25°C and 37°C

	HCB	α-HCH	β-HCH	γ-HCH
At 25°C:				
% Deviation	0.10 – 7.0	0.86 – 5.4	1.4 – 7.5	0.58 – 4.2
RSD (%)	4.7	3.3	4.1	2.4

3.2. Results of ID-GCMS

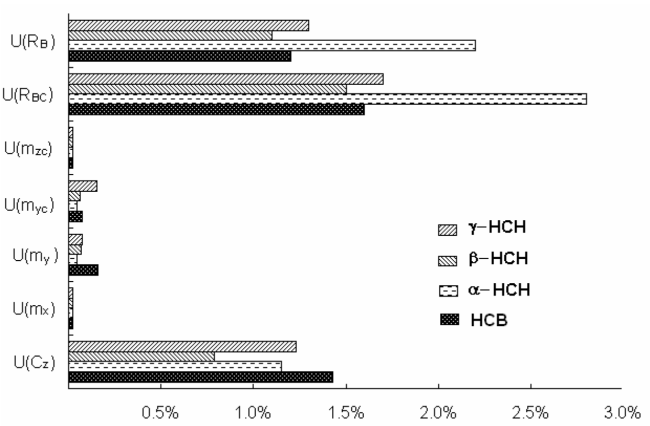
IDMS is recognised as a primary method for chemical analysis and hence an ideal method used for assigning reference values by RM producers [8]. Apart from being a high accurate technique, IDMS also offers a provision of well definable uncertainty values, in which the overall uncertainty could be conveniently derived. As shown in equation 1, the uncertainty of the reference values (C_x) was contributed by weighing of sample (m_x), standard solutions of the natural (m_{zc}) and labelled isotopes (m_y, m_{yc}), purity of standards (C_z) and the bias of isotope ratios in the sample blend (R_B) and calibration blend (R_{BC}):

$$C_x = C_z \times \frac{m_{zc}}{m_{yc}} \times \frac{m_y}{m_x} \times \frac{R_B}{R_{BC}} \qquad (3)$$

In this experiment, three independent determinations each comprising of duplicate analysis of five samples were performed from February to May 2007. Using bracketing technique, analyses were arranged by interspersing a calibration blend between each sample blend and the respective ratios, ie. R_B/R_{BC}, were found to be close to unity (ranging from 0.96 to 1.05). Mean concentration (± SD) of 15 samples for HCB was 0.198 ± 0.004 mg/kg; α-HCH was 0.450 ± 0.008 mg/kg; β-HCH was 0.213 ± 0.004 mg/kg and γ-HCH was 0.370 ± 0.012 mg/kg respectively. The inter-day variations of the OCP were ranging from 1.7 to 3.2% indicating that the characterisation using the described ID/MS method was highly precise.

The relative expanded uncertainties at a coverage factor (k) of 2 were estimated as the square root of the sum of square of all error components that stated in equation (3) and found to be 5.0% for HCB, 4.4% for α-HCH, 4.9% for β-HCH and 7.8% for γ-HCH respectively. The estimations are in good agreements to our previous study. The measurement uncertainty of OCP obtained by IDMS were significantly better than those by conventional GC-ECD and GC-MS methods which could be as high as 50% in the concentration range of 0.01 – 10 mg/kg. As shown in Fig. 1, all the error components were below 3%; and similar to other IDMS studies, R_B and R_{BC} were amongst the major contribution. Furthermore, the overall bias of the method was assessed with an animal feed reference material (CRM BCR 115) with less than 2% deviation from the certified values for HCB, β-HCH and γ-HCH.

Figure 1. Distribution of error components in the IDMS method



3.3. Performance of PT

A portion of the samples was used in operating a PT programme for assessing the competence of routine OCP testing methods amongst participating laboratories [9]. The consensus means obtained from the 70 participants using robust statistics were found to be 0.170 ± 0.095 mg/kg for HCB; 0.393 ± 0.188 mg/kg for α-HCH; 0.219 ± 0.103 mg/kg β-HCH and 0.339 ± 0.149 mg/kg for γ-HCH respectively. The consensus values were in the same order but deviated from -2.7% to -14.1% when compared with the IDMS values. Generally, the performance of most pesticide PT programmes was found to be not impressive [10] and it also happened in the present programme. The wide distribution of the OCP results, with RSD ranging from 44% to 56%, indicated the difficulties and significant uncertainty in the determination of incurred OCP in herbal matrices from participants. Hence, the RM prepared could serve as an essential source for identifying the bias and improving the quality control of OCP determinations. Due to the unsatisfactory results from testing laboratories in the PT programme, the results were considered not suitable to be included in the calculation of reference values.

3.4. Uncertainty of Assigned Reference

Three main contributors, ie. homogeneity and stability of the materials and the measurements using ID-GCMS technique, to the uncertainty of the reference values were combined to estimate the relative expanded uncertainty (U) at a coverage factor (k) of 2 as follows:

$$U = \sqrt{U_{bb}{}^{2} + U_{stab}{}^{2} + U_{char}{}^{2}} \qquad (4)$$

Accordingly, the relative expanded uncertainty of the four OCP in the reference material was ranging from 5.6 % to 8.4% as summarized in Table 3.

Table 3. Expanded Uncertainty of the Reference Material

	HCB	α-HCH	β-HCH	γ-HCH
U (%)	7.4	5.6	6.4	8.4
U (mg/kg)	0.015	0.025	0.014	0.031

4. CONCLUSION

An RM for four OCP in ginseng root sample was developed. The characterisation for the reference values was determined by an accurate ID-GCMS technique while the homogeneity and stability of the samples were confirmed satisfactory. The relative expanded uncertainty of the reference values was estimated from all three components and found to be within 9%. The product was considered as a good alternative to use for method validation processes for residual OCP analysis in herbal matrices in the absence of any available CRM today.

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