JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Application of Isotope Dilution Gas Chromatography−Mass Spectrometry in Analysis of Organochlorine Pesticide Residues in Ginseng Root

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A highly accurate and precise method based on isotope dilution gas chromatography−mass spectrometry was developed for the determination of five matrix-bound organochlorine pesticides, namely, hexachlorobenzene and hexachlorocyclohexanes (α-, β-, δ-, and γ- isomers), in a reference sample of *Panax gingseng*. Identification of the analytes was confirmed under selective ion monitoring mode by the presence of two dominant ion fragments within the specific time windows (±1% of the relative retention time with respect to the calibration standards) and matching of relative ion intensities of the concerned ions in samples and calibration standards (within ±5%). Quantification was based on the measurement of concentration ratios of the natural and isotopic analogues in the sample and calibration blends. To circumvent the tedious iterative process of exact isotope matching that is often used in isotope dilution mass spectrometry analysis, a single-point calibration procedure was adopted with the isotopic amount ratios in the sample and calibration blends close to unity (0.9−1.1). Under the described approach, intraday and interday repeatability of replicate analyses of organochlorine pesticides in the ginseng root sample were below 1.4%. The expanded relative uncertainty ranging from 4.0 to 6.5% at a coverage factor of 2 was significantly lower than those of conventional gas chromatographic methods using other calibration techniques (internal or external standards). A deviation of less than 2.0% from the certified values was achieved when applying the developed method to determine hexachlorobenzene, α-, and β-hexachlorocyclohexane in a certified reference material (CRM), BCR-CRM 115. Because of the unavailability of relevant CRMs of herbal origins, the concerned ginseng root sample, after verification of the "true values" of the concerned organochlorine pesticides by the valid primary method, is suitable for serving as an in-house reference material for quality assurance and method validation purposes.

KEYWORDS: Organochlorine pesticides; hexachlorobenzene; hexachlorocyclohexanes; isotope dilution; ginseng root; IDMS

INTRODUCTION

Persistent organochlorine pesticides (OCPs) such as hexachlorobenzene (HCB) and hexachlorocyclohexanes (HCHs of α-, β-, δ-, and γ- isomers) have been used extensively to control harmful pests and prevent vegetation infections in the past few decades. It was estimated that the total worldwide production exceeded 100000 tons and 10 million tons for HCB (*1*) and HCHs (*2*), respectively. HCB and HCHs were already banned in the 1970s in most countries because of their carcinogenicity and other adverse health effects. Nowadays, only γ-HCH (or lindane) is still permitted as a dresser for seed protection, but its use is strictly regulated at low levels. About 100 tons per year of lindane was consumed in the United Kingdom (*3*). Widespread consumption and high biodegradation resistance of OCPs have nevertheless led to the ubiquitous presence of these toxic chemicals in the global environment causing a significant disturbance to our ecosystems and intruding into the human food chain. As a consequence, the analysis of OCP residues in plant materials is of great importance to provide a clear understanding of contamination profiles, physiological absorption and transport pathways in plant species, average daily intake computation, and risk assessments.

There are large quantities of accessible scientific data in the literature concerning the quantification of OCP residues in various types of agricultural products. Recent studies showed that relatively high concentrations of HCB and HCHs were detected in leek (*4*), rye (*5*), olive (*6*), rice (*7*), and ginseng (*8*,

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10.1021/jf0637168 CCC: $37.00
Published on Web 04/04/2007

9). Analytical techniques such as gas chromatography−electron capture detection (GC-ECD) and gas chromatography−mass spectrometry (GC/MS) are often used for measurement of OCP residues in vegetation and other matrices. These techniques offer the advantages of good sensitivity and selectivity and are largely employed in fit-for-purpose methods for most trace-level measurements. However, the uncertainty is often not fit for the provision of a reference measurement. For instance, the spike recoveries of HCB and HCHs were found to be 65.6 and 74.3−89.2% (*6*), respectively, at the fortification level of 0.01 mg/kg and were 104 and 80.3−120% (*7*), respectively, at the level of 0.1 mg/kg. According to the recommendations stipulated in the Eurachem Guide (*10*), the uncertainty budgets for these trace-level organic measurements are expected to be high. Furthermore, unavailability of certified reference materials (CRMs) for OCPs in plant matrices hindered evaluation of the overall accuracy and the traceability of the measurements. Under these circumstances, primary measurement methods that are traceable to the International System of Units like isotope dilution mass spectrometry (IDMS) provide a viable alternative to estimate the "true values".

The underlying principle (*11*) of IDMS is rather straightforward with the initial addition of a known amount of an isotopically labeled analogue (e.g., ^{13}C, ^{2}H for organic analytes) to the sample (or called the "sample blend") and to the blank (the "calibration blend"). After equilibration, the ratio of the natural and labeled isotopes in both the sample and the calibration blends is quantified by mass spectrometry enabling concentration of the targeted analyte to be accurately determined. Because they are applicable to inorganic (*12*, *13*) and organic (*14*, *15*) analytes in a diversity of matrices, IDMS methods are often employed by national metrology institutes (NMIs) and designated metrology institutes for high-accuracy measurements such as key comparisons with other NMIs/designated institutes and certification of reference materials (*16*). Our laboratory has applied IDMS techniques in a number of studies/interlaboratory comparisons organized by the Consultative Committee for Amount of Substance—Metrology in Chemistry (CCQM) and the Asia-Pacific Metrology Programme (APMP) in the past few years, for example, CCQM-P31.b.1, PCB congeners in solution, and CCQM-P31.c.1, chlorinated pesticides in solution (detailed results can be assessed at the respective official websites of BIPM at www.bipm.org and APMP at www.apmpweb.org). The experience has reinforced our confidence toward the development of an in-house metrological infrastructure, among others, making use of primary ratio methods including IDMS methods for meeting the laboratory's various analytical commitments.

Considering a lack of CRMs for OCPs in plant and herbal materials and the safety concerns arising from the ubiquitous presence of HCB and HCHs in the environment, the objectives of the present work are (i) to develop a high-accuracy ID-GC/MS method for matrix-bound HCB and HCHs in ginseng matrix and (ii) to ascertain the concentrations of HCB and HCHs in the respective sample for future in-house method validation work.

MATERIALS AND METHODS

Chemicals. Neat chemicals of OCPs with claimed purity >98% were obtained from ChemService (West Chester, PA). The actual purity of neat chemicals was verified with CRMs SRM 2261 (NIST, Gaithersburg, MD) for HCB and γ-HCH, SRM2275 (NIST) for α-HCH and β-HCH, and EPA-1078 (Ultra Scientific, North Kingstown, RI) for δ-HCH. The isotopic $^{13}C_6$-HCB and $^{13}C_6$-HCH standards with concentrations ranging from 50 to 100 μg/mL in nonane and claimed purities of 99% were obtained from the Cambridge Isotope Laboratories (Andover, MA). BCR-CRM 115 (Institute for Reference Materials and Measurement, Geel, Belgium) and analytical-grade isooctane, dichloromethane, ethyl acetate, and petroleum ether (Labscan Asia, Bangkok, Thailand) were redistilled before use. Anhydrous sodium sulfate (Fluka, St. Gallen, Switzerland), high-purity activated copper powder RDH12806 (Sigma, St. Louis, MO), and concentrated sulfuric acid (BDH, Poole, England) were used as cleanup reagents. About 1 kg of raw ginseng root (*Panax ginseng*) was purchased from the local markets, briefly rinsed with distilled water, dried, and vortexed in a domestic shaker to remove dirt and foreign matters. The pretreated sample was ground in a blender (model ZM200, Retsch, Germany) and sieved through 100 μm sieves (Retsch). The fine powder was subjected to thorough mixing for 5 days and then stored in a nitrogen-purged container before use.

Standard and Sample Preparation. Calibration and spiking solutions were prepared by dissolution and gravimetric dilution of the neat standards using an analytical balance (CP224S, Sartorius AG, Germany). All weighing data were automatically processed and stored in an in-house-developed computer spreadsheet. Individual calibration solutions of each of the natural OCP at the concentrations of 100−1000 μg/g in isooctane were prepared. Approximately 0.5−1.0 g of the calibration solutions was accurately weighed into 10 mL volumetric flasks, and then, a quantitative amount of isooctane was added and made up to the marks. Appropriate dilution was carried out until the final concentrations of the solutions were fit for spiking into the ginseng sample. Similarly, dilution, if necessary, for the standard solutions of labeled OCPs was performed to obtain solutions of an appropriate concentration range for spiking purposes.

The calibration blend was prepared in duplicate by spiking an appropriate quantity of natural and $^{13}C_6$-OCPs into 5 g portions of the analyte-free ginseng powder in an extraction thimble. To achieve a higher degree of accuracy, the calibration and sample blends were prepared in such a way that their concentration ratios of the natural to labeled isotopes were close to unity (0.9−1.1). The concentrations of the natural OCPs present in the sample were predetermined by a reliable method, in this case a validated modified GC-ECD method (method 8081B, USEPA, 2000), before the ID-GC/MS experiments. The sample blend was prepared by spiking an appropriate quantity of $^{13}C_6$-labeled isotope solutions into 5 g portions of ginseng sample. The analytical sequence was arranged by interspersing a sample blend with two calibration blends. The mean of the respective isotope ratio in the bracketing calibrations blends was used to calculate the concentrations of OCPs in the sample bracketed in between.

About 10 g of anhydrous sodium sulfate was added to each of the calibration and sample blend. The extraction thimble of a given calibration/sample blend was covered with glass wool, placed into a Soxhlet extraction apparatus, and then extracted with ethyl acetate: petroleum ether (7:3; v/v) for 4 h with not less than four cycles per hour. The extract was transferred to a 250 mL flat-bottom flask, and the solvent mixture was removed by rotary evaporation. The residue was reconstituted in 10 mL of dichloromethane, and the flat-bottom flask was further rinsed with another 5 mL of dichloromethane. The rinsing was combined and put into a 15 mL capped glass tube and was then dried under a gentle stream of nitrogen. The extract residue was reconstituted in 2 mL of isooctane, and 3 mL of concentrated sulfuric acid was added to digest insoluble matters and other interfering organic substances. The mixture was thoroughly shaken in a vortex mixer for about 5 min and was centrifuged at 4000 rpm for 10 min. About a 1 mL portion of the upper organic layer was transferred into a GC vial and 200 mg of acid washed copper powder was added to remove sulfur-containing interference prior to GC/MS analysis.

Chromatography and Mass Spectrometry. An Agilent GC/MS (HP6890 GC and a quadrupole MS HP5973, Palo Alto, CA) with a 30 m × 0.25 mm, 0.25 μm film HP-1707MS column (J & W Scientific, Folsom, CA) was used. Helium carrier gas was set at a flow rate of 1.0 mL/min, and separation of the five OCPs was carried out under a temperature program 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 ionization energy was under electron ionization mode at 70 eV. Aliquots of 1 μL were injected into the GC/MS system under splitless mode, and the

Table 1. Abundance Ratios of the Qualifier Ions (Q1 and Q2) to Target Ions (Mean ± SD) of OCPs in the Standard Solutions and the Ginseng Root Sample ($n = 6$)

	retention time (min)	target (*m/z*)	qualifier (*m/z*)		standard		sample	
			Q1	Q2	Q1/target	Q2/target	Q1/target	Q2/target
HCB	6.2 ± 0.05	284	282	286	0.49 ± 0.01	0.84 ± 0.03	0.49 ± 0.01	0.85 ± 0.01
α-HCH	7.2 ± 0.05	181	183	217	0.92 ± 0.01	0.56 ± 0.01	0.90 ± 0.02	0.55 ± 0.01
β-HCH	10.6 ± 0.1	181	183	217	1.01 ± 0.01	0.76 ± 0.01	0.99 ± 0.03	0.75 ± 0.03
γ-HCH	8.4 ± 0.05	183	217	221	0.62 ± 0.02	0.54 ± 0.01	0.61 ± 0.02	0.52 ± 0.01
δ-HCH	11.1 ± 0.1	181	183	217	0.80 ± 0.01	0.23 ± 0.01	0.79 ± 0.01	0.23 ± 0.01

Table 2. Validation of the Purity of Neat ^{12}C-OCP Standards against CRMs (Section A) and Estimation of Uncertainty Budget of Each^{12}C-OCP Calibration Standard (Section B)

Section A

	certified value (ng/mL)			neat standard value (ng/mL)		
	NIST2261	NIST2275	EPA-1078	nominal	determined[a]	purity (%)
HCB	3005 ± 14			2996	3018 ± 40.7	100
α-HCH		3000 ± 15		3039	3026 ± 31.8	99.6
β-HCH		2980 ± 12		3021	2986 ± 21.2	98.8
γ-HCH	3012 ± 15			3022	3057 ± 12.8	100
δ-HCH			5015[b]	4965	4962 ± 56.6	99.9

Section B

	CRM (RSD_1)[c]	neat standard (RSD_2)[c]	gravimetric dilution (RSD_3)[c]	combined relative uncertainty (%)
HCB	0.466	1.35	0.064	1.43
α-HCH	0.500	1.05	0.032	1.16
β-HCH	0.403	0.71	0.036	0.81
γ-HCH	0.498	0.42	0.025	0.65
δ-HCH	0.499	1.14	0.083	1.25

[a] Mean (±SD) values of seven replicates. [b] The value was obtained after gravimetric dilution of in EPA-1078 (certified value of δ-HCH, 1003 ± 5 μg/mL) by 200-folds. [c] RSD1, stated uncertainty of CRM; RSD2, uncertainty determined from seven replicates in Section A; and RSD3, uncertainty arising from serial gravimetric dilution of the neat standards.

analytes were respectively monitored under the single ion monitoring mode at multiple mass channels at *m*/*z* 181, 183, and 284 for ^{12}C-OCPs and 187, 189, and 290 for $^{13}C_6$-OCPs.

Quality Control. Identification of OCP residues was confirmed by matching the relative ion abundance ratios of two major pairs of mass fragments in the ginseng sample to those obtained from the calibration solutions at the specified retention time windows. The quality assurance criteria were set as follows: The target isotope ratios of ^{12}C to $^{13}C_6$ in the calibration and sample blends should be within 1.0 ± 0.1, and the relative isotope ratios of the calibration blend to that of the sample blend should be within 1.0 ± 0.05. The relative percentage deviation (RPD) of the bracketing calibration blends should be less than 1.5%. Method detection limits (MDL) and method quantification limits (MQL) were, respectively, calculated as three and 10 times the signal-to-noise of OCP residues in blank samples. MDL and MQL were found to be 0.5 (ranging from 0.2 to 0.5 μg/kg) and 2.0 μg/kg (ranging from 0.7 to 2.0 μg/kg), respectively, for HCB and HCHs.

RESULTS AND DISCUSSION

To ensure proper implementation of an ID-GC/MS analysis of high accuracy, some critical factors that caused systematic errors such as the extraction efficiency, completeness of isotopic equilibration, matrix interferences, and purity of isotopes used to prepare standards were cautiously evaluated. First, the performance of Soxhlet extraction for OCPs was checked. Soxhlet is a traditional technique for extracting organic analytes, and it is still widely used nowadays for extraction of multiresidual pesticides in solid matrices (*17*). The technique was also reported to give satisfactory and comparable extraction efficiency for OCPs when compared to the efficient fluidized-bed extraction (*18*). In this study, the amounts of OCP residues present in the second round Soxhlet extract were confirmed to be less than the respective MDL. The extraction duration of 4 h was found to be sufficient as there was an insignificant difference ($p \leq 0.01$, $n = 3$) using analysis of variance, in terms of extractable quantity, between a 4 h extraction to that of an overnight 16 h extraction. Second, on the basis of the reported isotopic equilibration time of 20 min (*19*) to a minimum of 1 h (*20*) at room temperature for some matrix-bound organic compounds, complete equilibration of OCPs in the ginseng root sample was expected to be achieved during the 4 h extraction process. Third, the abundances of two pairs of major ions in each of the OCPs under study were found to have good agreement ($p \leq 0.01$) between the calibration solutions and the sample extracts within the specified retention time windows (**Table 1**). These indicated that simple cleanup using sulfuric acid and copper powder was effective for the removal of matrix interference for residual OCPs in the ginseng root sample. Finally, the purity of neat chemicals that were used for preparing standards was verified using three independent CRMs. Because the concentration of δ-HCH in EPA-1078 was high and direct injection into the GC/MS system was impracticable, the standard was gravimetrically diluted to two hundredths of its original concentration. The purity of each OCP was determined from the nominal value of the neat standard solution and the mean ($n = 7$) obtained by calibration against the respective CRM. As shown in **Table 2**, Section A, all neat standards have a purity of more than 98.5% and matched with the manufacturer's claims, and the combined relative uncertainty of neat chemicals (**Table 2**, Section B) could be estimated from the certified uncertainty of CRM, the relative standard deviation (RSD) of seven replicate analyses, and the gravimetric serial dilutions of the neat standards.

Table 3. Relative Responses of OCPs in the Sample Blend (R_B) and the Calibration Blend (R_{BC})

	HCB		α-HCH		β-HCH		γ-HCH		δ-HCH	
run	R_B (R_{BC})	R_B/R_{BC}	R_B (R_{BC})	R_B/R_{BC}	R_B (R_{BC})	R_B/R_{BC}	R_B (R_{BC})	R_B/R_{BC}	R_B (R_{BC})	R_B/R_{BC}
#1	0.9393 (0.9299)	1.010	0.9606 (0.9618)	0.9988	1.046 (1.044)	1.002	1.034 (1.033)	1.001	0.9431 (0.9430)	1.000
#2	0.9255 (0.9167)	1.010	0.9884 (0.9811)	1.007	1.039 (1.052)	0.9880	0.9959 (0.9980)	0.9979	0.9731 (0.9626)	1.011
#3	0.9467 (0.9440)	1.003	0.9616 (0.9654)	0.9961	1.039 (1.036)	1.003	0.9921 (0.9869)	1.005	0.9303 (0.9328)	0.9974
#4	0.9253 (0.9282)	0.9968	0.9559 (0.9730)	0.9824	1.013 (1.013)	0.9999	0.9759 (0.9816)	0.9942	0.9494 (0.9502)	0.9991
#5	0.9247 (0.9227)	1.002	0.9641 (0.9596)	1.005	1.041 (1.041)	0.9991	0.9826 (0.9840)	0.9986	0.9481 (0.9431)	1.005
#6	0.9335 (0.9412)	0.9918	0.9479 (0.9755)	0.9717	1.024 (1.034)	0.9902	0.9819 (0.9997)	0.9822	0.9578 (0.9343)	1.009
mean	0.9325 (0.9305)	1.0022	0.9631 (0.9694)	0.9935	1.034 (1.037)	0.9970	0.9937 (0.9972)	0.9965	0.9503 (0.9468)	1.004
SD	0.009 (0.011)	0.007	0.014 (0.008)	0.014	0.013 (0.010)	0.006	0.021 (0.019)	0.008	0.014 (0.010)	0.006
%RSD		0.71		1.4		0.63		0.79		0.56

Exact matching IDMS protocol involves iterative adjustment of the amount of spike solutions added to the calibration and sample blends leading to exact matching of both blends each containing the "exact" concentrations of the natural and labeled isotopes under study. Such a technique has been shown to provide the highest degree of accuracy and precision for mass spectrometric quantification as the instrumental concentration-dependent bias is eliminated. In our experience, exact matching might require a minimum of 3−5 iterations and is a time-consuming process. To circumvent the tedious process, we adopted a single-point approach in the present study, which has been verified to be a good alternative (*21*) for achieving satisfactory results on the condition that the concentrations of the spiked standards should be as close as possible to that of the sample. In this study, the approximate concentrations of OCP residues in the sample were preliminarily determined using an in-house-validated GC/MS method. On the basis of the replicate analyses ($n = 6$), the mean concentration (%RSD) was 124 μg/kg (4.5%) for HCB, 443 μg/kg (4.4%) for α-HCH, 144 μg/kg (4.3%) for β-HCH, 259 μg/kg (3.6%) for γ-HCH, and 1320 μg/kg (4.1%) for δ-HCH, respectively. Then, three different sets of calibration and sample blends were prepared by spiking an appropriate amount of isotopes into the blank and the sample. The relative responses of individual ^{12}C- and $^{13}C_6$-OCPs in the blends were analyzed by ID-GC/MS, and the one that gave the response ratios nearest to unity was assigned as the reference concentration for the subsequent single-point ID-GC/MS determination. Analysis was arranged by interspersing a calibration blend between each sample blend. As shown in **Table 3**, the isotopic response ratios of $^{12}C/^{13}C_6$-OCPs for the six sample blends (R_B, the peak area ratio of the natural/labeled isotopes in the sample blend) and calibration blends (R_{BC}, the peak area ratio of the natural/labeled isotopes in the calibration blend) ranged from 0.92 to 1.05, and their respective ratios, that is, R_B/R_{BC}, were very close to unity (ranging from 0.99 to 1.01) and had a good match of ±1% with a RSD of ≤1.5%. Furthermore, a recent comparative study (*22*) of the effect of mass bias for ID-GC/MS indicated that the degree of ratio matching was more sensitive in inorganic analysis than in organic analysis. A scenario of a 5% difference in R_B/R_{BC} from unity led to a bias of more than 0.2% for tin analysis, but there was a virtually insignificant (0.01%) effect to that of cholesterol analysis. Still, we cautiously limited the isotopic ratios of 1.0 ± 0.05 to minimize any possible propagated errors (*23*) that might occur in the IDMS method.

Under the described chromatographic conditions, residual ^{12}C-OCPs and matrix interference in the blank samples were not detected. Typical chromatograms of the OCPs in the ginseng sample are shown in **Figure 1**. HCB, α-HCH, β-HCH, γ-HCH, and δ-HCH were consistently eluted with good separation and peak shape at 6.2 ± 0.05, 7.2 ± 0.05, 10.6 ± 0.1, 8.4 ± 0.05, and 11.1 ± 0.1 min, respectively. Similar chromatographic peaks with exact retention times were also obtained for $^{13}C_6$-OCPs. This was one of the major advantages of using $^{13}C_6$ over deuterated isotopes because no separation difference between ^{12}C and $^{13}C_6$ isotopes ensured a simultaneous mass spectrometric measurement and minimized errors from short-term instrument instability (*24*), resulting in higher accuracy. The mass fraction in μg/kg (C_x) of OCP residues in the sample was calculated according to the following equation:

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

where C_z is the mass fraction (μg/kg) of ^{12}C-OCPs calibration solution, m_{zc} is the mass of ^{12}C-OCPs solution in the calibration blend, m_{yc} is the mass of $^{13}C_6$-OCPs solution in the calibration blend, m_y is the mass of $^{13}C_6$-OCPs solution spiked into the sample, and m_x is the mass of sample used. **Table 4** shows the intraday and interday repeatability of the OCP concentrations in the sample that were determined, respectively, from four independent determinations each comprised of six replicates within the 6 month study period. The RSD was less than 1.5%, suggesting that the ID-GC/MS method was highly precise. The respective OCP concentrations in the ginseng sample were 122.7 ± 1.37 μg/kg for HCB, 437.8 ± 4.85 μg/kg for α-HCH, 143.8 ± 1.97 μg/kg for β-HCH, 255.5 ± 3.23 μg/kg for γ-HCH, and 1314 ± 9.71 μg/kg for δ-HCH. The accuracy obtained was similar to and even slightly better than that of a previous ID-GC/MS analysis (*25*) of *p,p′*-DDE in bran. In addition to high accuracy and precision, another characteristic merit of a primary measurement method like IDMS is the clear provision of definable uncertainty values (*11*). As given in the above equation, the uncertainty associated with ID-GC/MS analysis was contributed by weighing of sample, standard solutions of the natural and labeled isotopes, purity of standards, and the bias of R_B and R_{BC}. The histogram shown in **Figure 2** illustrates the contribution of each identified uncertainty component. All components were below 2.5% where R_B and R_{BC} were among the major contributions. The expanded relative uncertainties at a coverage factor of 2 were calculated as 4.4% for HCB, 4.2% for α-HCH, 4.4% for β-HCH, 6.5% for γ-HCH, and 4.0% for δ-HCH, respectively.

The accuracy of the developed ID-GC/MS protocol was further reaffirmed with the aid of a CRM in this study. As discussed in a review (*26*) issued in 2001, commercially available OCP CRMs are very limited and none of the available CRMs is of plant origin. In this circumstance, a CRM of a relevant matrix could only be used. It was BCR-CRM 115, an animal feed that contained three of the concerned OCPs. To avoid having extra steps, gel permeation chromatography and Florisil cleanup were not applied prior to the ID-GC/MS

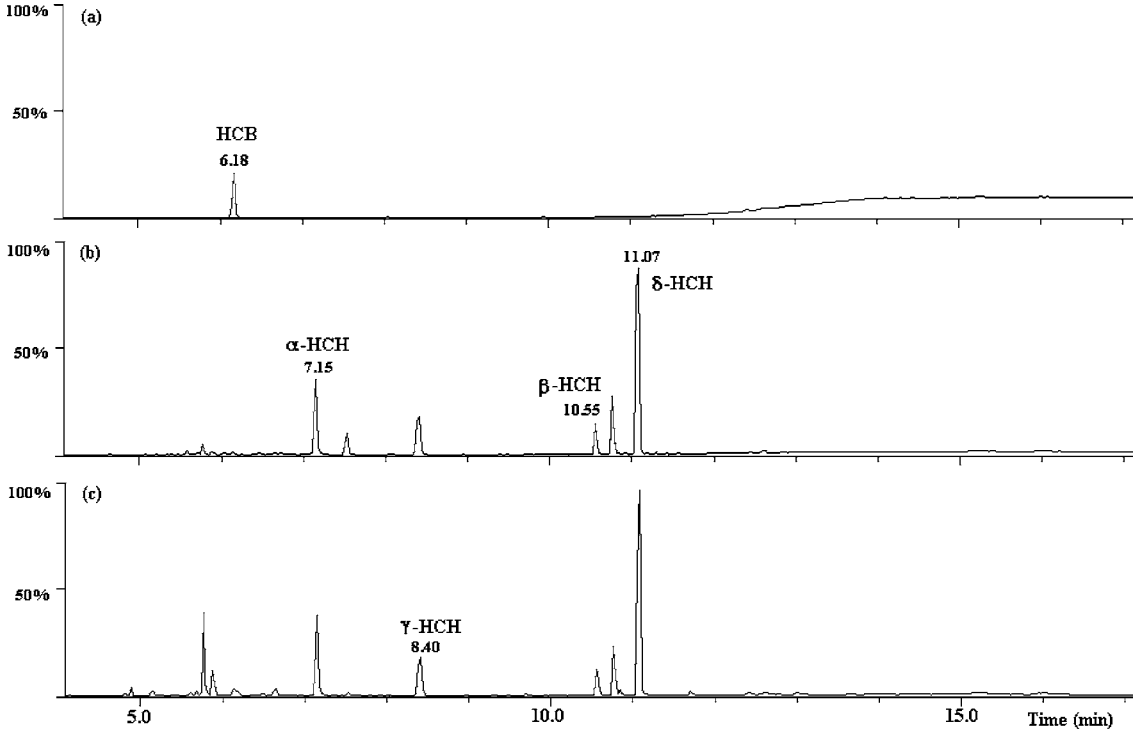

Figure 1. Chromatograms of matrix-bound OCPs in ginseng root sample: (**a**) HCB at *m*/*z* 284; (**b**) α-HCH, *β*-HCH, and *δ*-HCH at *m*/*z* 181; and (**c**) *γ*-HCH at *m*/*z* 183.

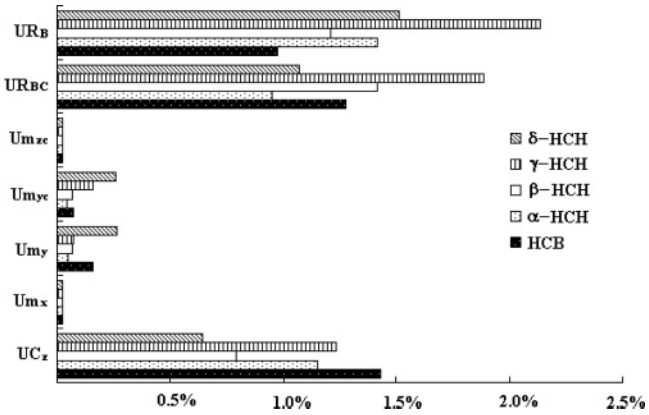

Figure 2. Distribution of uncertainty components (UR_B, UR_{BC}, Um_{zc}, Um_{yc}, Um_y, Um_x, and UC_x) for measurement of OCPs by the ID-GC/MS method.

Table 4. Intraday and Interday Repeatability of the ID-GC/MS Measurement of OCPs in the Ginseng Root Sample

	HCB	α-HCH	*β*-HCH	*γ*-HCH	*δ*-HCH
		intraday ($n = 6$)			
minimum (*μ*g/kg)	121.6	432.6	141.1	254.6	1303
maximum (*μ*g/kg)	124.7	441.4	147.1	258.7	1333
mean (*μ*g/kg)	122.7	437.6	143.6	256.2	1315
SD	1.14	3.76	2.09	1.38	10.8
%RSD	0.93	0.86	1.5	0.54	0.82
		interday ($n = 4$)			
minimum (*μ*g/kg)	120.2	426.2	140.1	244.0	1300
maximum (*μ*g/kg)	125.7	444.2	147.7	260.2	1333
mean (*μ*g/kg)	122.7	437.8	143.8	255.5	1314
SD	1.37	4.85	1.97	3.23	9.71
%RSD	1.1	1.1	1.4	1.3	0.74

analysis, and only duplicate analyses were performed so as to minimize irreversible damages caused by the lipids and endogenous matrix substances in the feed to the analytical GC columns. The results (**Table 5**) indicated that mean values obtained by the developed method were in good agreement with the certified values.

Table 5. Bias Assessment of the Developed ID-GC/MS Method Using BCR-CRM 115

	certified value (mg/kg)	first run (mg/kg)	second run (mg/kg)	mean (mg/kg)	% deviation
HCB	0.0194 ± 0.0014	0.0195	0.0197	0.0196	1.03
β-HCH	0.023 ± 0.003	0.0215	0.0213	0.0214	−6.96
γ-HCH	0.0218 ± 0.0020	0.0233	0.0233	0.0233	6.88

With an estimation (*27*) of combined relative uncertainty at 17−25% for measurements in the concentration range of 0.01−10 mg/kg, the measurement uncertainty for residual levels of OCPs by conventional GC-ECD and GC/MS methods was significantly higher than that of ID-GC/MS. Similarly, some recent IAEA interlaboratory comparisons for OCPs have reported the coefficient of variation ranging from 12 to 130% in seaweed (*28*) and from 3 to 115% in fish homogenate (*29*) samples, respectively, among laboratories worldwide. The variable results substantiated the importance of the availability of CRMs for OCPs in plant matrices, particularly for matrix-bound analytes or "difficult matrices" (*30*) for validating the precision and accuracy of concerned testing methods. Being a designated metrology institute since 2005, the Government Laboratory of the HKSAR has started a metrology program, inter alia, to develop primary measurement methods for characterization of some suitable materials as in-house reference materials for internal quality assurance and method validation work wherever suitable CRMs are not available in the market for meeting its diversified service needs. In this connection, the high-accuracy ID-GC/MS method under study was applied to verify the concentrations of OCP residues in the concerned ginseng sample.

In conclusion, a primary measurement method based on ID-GC/MS was developed for the analysis of matrix-bound HCB and various isomers of HCHs in a control ginseng sample. The

method was validated and found to be accurate with less than 2% deviation from the certified values for HCB, β-HCH, and γ-HCH in CRM BCR 115. The 6 month intraday and interday repeatability was less than 1.5%. The achievable relative expanded uncertainty ranging from 4.0 to 6.5% was significantly lower than those of the GC-ECD and GC/MS methods using conventional calibration techniques other than ID. Although IDMS is generally expensive to be applied routinely, its accuracy and precision make it one of the reliable analytical tools for achieving results of higher analytical quality required for various analytical purposes such as high-level interlaboratory comparisons and certification of reference materials. The unavailability of suitable CRMs in some fields of chemical and physiochemical measurements further magnifies the importance of developing primary measurement techniques traceable to the internationally recognized and globally accepted units of measurement for establishing the traceability of measurements.

ACKNOWLEDGMENT

We express sincere thanks to Dr. T. L. Ting, Government Chemist of the Government Laboratory of HKSAR, for his support and encouragement during the course of study.

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Received for review December 21, 2006. Revised manuscript received February 28, 2007. Accepted March 06, 2007. The contents of this paper do not necessarily reflect the views of the Government of the HKSAR nor does mention of trade names or commercial products constitute endorsement or recommendations of use.

JF0637168