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MÉTODO DE VALIDAÇÃO PARA DETERMINAÇÃO DE INSETICIDAS ORGANOCLORADOS EM GINSENG USANDO A CROMATOGRAFIA GASOSA ACOPLADA À ESPECTROMETRIA DE MASSA DE DILUIÇÃO ISOTÓPICA (ID-GC-MS)

METHOD VALIDATION FOR ORGANOCHLORINE INSECTICIDES DETERMINATION IN GINSENG BY USING ISOTOPE-DILUTION GAS CHROMATOGRAPHY-MASS SPECTROMETRY (ID-GC-MS)

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RESUMO

Os inseticidas organoclorados ainda são explorados entre os pesticidas mais importantes para fins de proteção de plantas. Conhecidos por serem perigosos para o ser humano e persistentes no meio ambiente, é necessário criar um método preciso para detectar inseticidas organoclorados em alimentos e substâncias ambientais. A espectrometria de massa por cromatografia em fase gasosa de diluição isotópica (ID-GC-MS) é um sistema de medição de acoplamento versátil e método de alta ordem que combina seletividade, sensibilidade e alta precisão. O presente trabalho tem como objetivo mostrar a metodologia da determinação de inseticidas organoclorados (alfa-HCH e gama-HCH) no ginseng usando ID-GC-MS. O método descrito abrangeu a preparação de amostras usando extração com solvente orgânico (hexano), seguido de limpeza com florisil. Após a reconstituição da base de solvente, a medição foi realizada usando ID-GC-MS no parâmetro ideal do instrumento. Usando as condições ideais determinadas, os parâmetros como sensibilidade, linearidade, precisão e exatidão foram estudados para validação do método ID-GC-MS. O limite de detecção e o limite de quantificação do instrumento foram de 0,5 ng/g e 2,0 ng/g para os dois analitos. O método mostrou linearidade com o coeficiente de correlação de 0,999 para alfa-HCH e gama-HCH na faixa de concentração de 1 - 300 ng/g. A precisão variou de 3,0 a 3,7% e 2,4 a 3,3% para alfa-HCH e gama-HCH, respectivamente. As recuperações médias para alfa-HCH e gama-HCH foram encontradas em 98,0 e 95,6%, respectivamente. Após a validação do método, a incerteza de medição da determinação de alfa-HCH e gama-HCH foi avaliada de acordo com o guia EURACHEM GUM com nível de confiança de 95% (k = 2). A incerteza expandida na medição de alfa-HCH e gama-HCH foi de 5,4% e 8,2%, respectivamente. Todos esses parâmetros demonstram alta sensibilidade do método oferecido e o sucesso do método descrito na determinação de alfa-HCH e gama-HCH na amostra de ginseng.

Palavras-chave: *química analítica, análise orgânica, pesticidas, padrão interno, cromatografia em fase gasosa*

ABSTRACT

Organochlorine insecticides are still exploited among the most prominent pesticides for plant protection purposes. Known for having hazardous to humans and persistent in the environment properties, it is necessary to build an accurate method for detecting organochlorine insecticides in food and environmental substances. Isotope-dilution gas chromatography-mass spectrometry (ID-GC-MS) is a versatile coupling measurement system and high order method that combines both selectivity, sensitivity and high accuracy. The present paper aims at showing the methodology of the organochlorine insecticides (alpha-HCH and gamma-HCH) determination in ginseng by using ID-GC-MS. The described method covered sample preparation using an organic solvent (hexane) extraction, followed by florisil cleaning-up. After the reconstitution of the solvent base, the measurement was conducted by using ID-GC-MS in the optimal instrument parameter. Using the determined optimal conditions, the parameters such as sensitivity, linearity, precision, and accuracy were studied for validation of the ID-GC–MS method. The limit of detection and the limit of quantitation of the instrument were 0.5 ng/g and 2.0 ng/g for both

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analytes. The method showed linearity with the correlation coefficient of 0.999 for both alpha-HCH and gamma-HCH over the concentration range of 1–300 ng/g. The precision ranged from 3.0 to 3.7% and 2.4 to 3.3% for alpha-HCH and gamma-HCH, respectively. The mean recoveries for alpha-HCH and gamma-HCH were found at 98.0 and 95.6%, respectively. Following method validation, the measurement uncertainty of the alpha-HCH and gamma-HCH determination was evaluated according to EURACHEM GUM guide at a 95 % confidence level (k = 2). The expanded uncertainty in the measurement of alpha-HCH and gamma-HCH was 5.4% and 8.2%, respectively. All these parameters demonstrate the high sensitivity of the offered method and the success of the described method in the determination of alpha-HCH and gamma-HCH in ginseng sample.

Keywords: *analytical chemistry, organic analysis, pesticides, internal standard, gas chromatography*

1. INTRODUCTION:

A pesticide is any substance (or in the form of mixtures) from chemical or biological ingredients which are used to control, repel or destroy any pest, or to regulate the growth of the plant (FAO, 2013). The pests are avoided because they can harm during, or otherwise interfering with, the production, processing, storage, or marketing of food, agricultural commodities, wood and wood products, or animal feedstuffs, or which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies (FAO, 2003).

The word pesticide is a parent term to cover all herbicides, fungicides, insecticides, wood preservatives, rodenticides, garden chemicals, and household disinfectants that may be used to destroy some pests (Zacharia & Tano, 2011; EPA, 2009). Insecticides are chemical compounds used in agriculture and are addressed to control and kill insects and related invertebrate pest species (Matsumura, 2009).

The organochlorine group is one class of insecticides that have been used around the world for centuries. Organochlorine pesticides include cyclodienes, DDT-type compounds, and hexachlorocyclohexane (HCH) isomers. Some organochlorine pesticides are members of persistent organic pollutants (POPs) and are classified as the chemicals targeted by the Stockholm Convention (UNEP, 2013). Initially, the use of the chlorinated hydrocarbons or organochlorines was as fire retardants and for dielectrics. As insecticides, the first use of these compounds occurred with the finding that the mixture of benzene and liquid chlorine killed insects in the field (Gupta and Milatovic, 2014).

HCH isomers, also called benzene hexachloride (BHC), are the most common halogenated organic insecticides that have been used for crop protection (Li, 1999; Vijgen, 2006; Simonich and Hites, 1995). The danger of HCHs is contributed by its lipophilic properties and persistence in the environment, causing possibly bioaccumulated and biomagnified up in the food chain (Berntssen *et al.*, 2012). The residue of HCHs was found at quite significant concentration in soil, air, fish, and mammals because of the usage of HCHs for agricultural purpose (Liu and Meng, 2011; Gong *et al.*, 2004; Concha-Grana *et al.*, 2006; Lammel *et al.*, 2007; Fang *et al.*, 2017). In terms of toxicity for mammals, gamma-HCH is the most toxic, followed by the alpha-, delta-, and beta-isomers when it is acute exposure. Beta-HCH shows the most significant toxicity at the chronic exposure, followed by the alpha-, gamma-, and delta-HCH isomers (Jackovitz and Hebert, 2015). All HCH isomers can cause liver hyperplasia and liver tumors. The International Agency for Research on Cancer (IARC) classifies HCHs as carcinogenic to humans in the Group 2B category (Berntssen *et al.*, 2012). Related to this property, HCH is legally prohibited in many countries (historically since 1981 in the European countries). The use of HCHs has decreased in recent years. However, others, particularly developing countries, still advantage this compound for economic reasons.

With the use of insecticides in the agriculture field and or the existence of pesticides in water and soil, the plant or food is wide open to be contaminated to the compounds. The overall assessment in 12 vegetables and eight fruits is that 73 % contain pesticide residues (Baker *et al.,* 2002). Ginseng, as one of the agriculture commodities, has the same potential to contaminated. This is because ginseng, or its root as the central part in utilization, generally needs 4-6 years (depending on the type of ginseng) to grow and ready for harvest, adequate time for chemical contaminants such as insecticides accumulated in ginseng plant (Yun, 2001). Several papers reported the presence of HCHs and or other organochlorine pesticides in ginseng (Khan *et al.*, 2001; Durgnat *et al.*, 2005; Leung *et al.*, 2005; Lee and Jo, 2012).

Ginseng has a full-range application which is available in many forms and preparations such as fresh root, extracts, capsules, teas, cigarettes,

both alone and in combination with other ingredients. It also appears in Japan, China, Germany, France, Austria, and the UK Pharmacopoeias (Thompson Coon and Ernst, 2002). Ginseng roots as one of the most popular and expensive raw drugs have been advantaged to boost the quality of life (Ellis and Reddy, 2002; Coleman *et al.*, 2003).

Historically, ginseng was first cultivated around 11 BC and had a medical history (as a wild herb) stretching back more than 5000 years (Kennedy and Scholey, 2003). Immune system modulation, anti-stress activity, anti-cancer, anti-aging, a medicine for cardiovascular diseases, improvement of cognitive and physical performance, and sexual function and anti-diabetic activities are the most notable features of ginseng in laboratory and clinical practices (Vogler *et al.*, 1999; Shibata, 2001; Kiefer and Pantuso, 2003; O'Hara *et al.*, 1998). Therefore, rapid, effective, and validated methods for the determination of organochlorine insecticides residues in ginseng that can decrease the product value of ginseng are crucial needed. Moreover, accurate and precise measurements to detect hazard organic contaminants are requisite for ensuring appropriate diagnosis and essential decisions in trade consideration.

Gas Chromatography-Mass Spectrometry instrument is most commonly used to confirm and quantitate the residues of organochlorines in dried fruits, fat-rich cereals, herbal medicine, celery, rape, scallion and spinach (Surma *et al.*, 2014; Rasche *et al.*, 2015; Mao *et al.*, 2012; Zhang *et al.*, 2012;). Mass spectrometry (MS) detector is a powerful analytical tool that has been used successfully for detecting and identifying various volatile organic compounds. One trend in recent years related to the analysis based on MS is the development of isotope dilution mass spectrometry (ID-MS) technique. ID-MS is the optimum analytical method to obtain the accuracy of analytical results through the advantage of spiked target analyte isotope in the sample. By the addition of a known amount of a spike, the amount of analyte in the ID-MS technique is computed based on the change of the isotope ratio, the abundance ratio of two isotopes (Henrion, 1994).

In the analysis of organic and biomolecule compounds, labeled analytes are available commercially in the form of ^{13}C or ^{2}H (Rodríguez-González and Alonso, 2018). The equilibrium condition of natural and isotopic analyte provides a stable ratio between them in every sub-sample of the mixture along with the sample preparation. The loss during extraction, clean-up, evaporation will not much affect the accuracy (Sargent *et al.*, 2002). Some successful works employing ID-MS in the determination of organic substances have been published in recent years both GC-based or LC-based (Goldschmidt and Wolf, 2010; Bi *et al.*, 2012; Huertas-Pérez *et al.*, 2019; Huertas-Pérez *et al.*, 2015; Bercaru *et al.*, 2006). However, the challenge in the analysis still presents due to the complex matrix and the shallow maximum residue limits (MRLs) in some regulations.

The goal of this study was to obtain a high order method by using ID-GC-MS for the determination of alpha-HCH and gamma-HCH residue in ginseng roots. The approach to evaluating the measurement uncertainty of the method is also described.

2. MATERIALS AND METHODS:

The organochlorine standard mixture (2000 mg/L), which was used in the validation study, was purchased from SUPELCO. Pure Certified Reference Material (CRM) of lindane was from NMIA, Australia (P1332) and the CRM for α-HCH was from NIST, USA (SRM 2275). The high purity isotope for both Lindane (CLM-1282-S) and α-HCH (CLM-2482-S) was obtained from Cambridge Isotopes Laboratory. The preparations of standard analytical solutions were done by using the gravimetric method.

MERCK supplied analytical grade acetonitrile (ACN), hexane, diethyl ether, florisil, and sodium chloride (NaCl). Sigma Aldrich provided magnesium sulphate ($MgSO_4$). Ultrapure water (18 MOhm) was produced by a Milli-Q Plus 185 (France).

2.1. Sample Preparation

All the sample preparation was conducted using gravimetric dilution. About 2 grams of ginseng sample was wetted by 10 mL of water for two hours. After two hours, 8 mL of acetonitrile was added to the mixture and the extraction using vortex was applied for 1 minute at room temperature. After the extraction, 4 grams of $MgSO_4$ and 1 gram of NaCl were added to the mixture and shake vigorously for 30 seconds before centrifuge for 5 minutes at 4000 rpm. A 1 mL of supernatant then filtered by using a 0.2 μm PTFE syringe filter before evaporation using nitrogen gas into dryness. The solid residue was reconstituted with 1 mL of n-hexane. The cleaning up process followed by using 1 gram of activated florisil with 10 mL of n-hexane/diethyl ether (85/15) mixture as eluent. The activated florisil was first

conditioned with n-hexane. The 10 mL of extract was then evaporated again by using nitrogen into dryness, followed by reconstitution with 1 mL of n-hexane. The sample was then ready to be injected into the GC/MS.

In the isotope-dilution experiment, the same treatment was applied, except the labeled standard (isotope solution) was spiked to the sample before the sample extraction step as in the conventional internal standard technique.

2.2. Conditions of GC-MS

A Gas Chromatography-Mass Spectrometry method was developed for their separation and detection. GC-MS was performed using GC Agilent 7890 tandem with MSD 5977A (United States). The chromatographic separation was carried out using an HP-5 MS UI (30 m x 0.250 mm x 0.25 µm). The temperature program was as follows: 70ºC as initial temperature and held for 2 min; increased to 150ºC at rate of 25ºC/min without holding; ramped to 200ºC at rate of 3ºC/min without holding; and ramped to 280ºC at rate of 8ºC/min, hold for 10 min. The front inlet pressure was set at a constant flow of 1 mL/min, and 2 µL of sample solution was injected into the GC system. The total run time was 41.9 min. The MS transfer line temperature was held at 280ºC.

Mass spectrometric parameters were configured as follows: electron impact ionization with 70 eV energy; ion source temperature, 230ºC; MS quadrupole temperature, 150ºC, and solvent delay 4 min. The MS system was continuously set in selective ion monitoring (SIM) mode, and each compound was quantified based on peak area using one target and two qualifier ion(s). Retention times of the analytes and complete SIM profile are shown in Table 1. Agilent Masshunter software was used for data processing.

2.3. Validation Method

Initially, the analytical method was validated by using external calibration technique. Linearity for all of the compounds in pure solvent was obtained by plotting the peak area from MS response against the concentration of the corresponding calibration standards between 1 and over 300 ng/g.

Limit of detection (LoD) and limit of quantification (LoQ) were estimated by performing serial dilution method of standard mix solution from the lowest calibration standard with signal to noise (S/N) of 3 and 10, respectively, and observed in 7 times experiment.

Precision was evaluated at two-level concentrations, 20 and 100 ng/g. The relative standard deviation (RSD), and also from the precision of the method, was calculated and compared to the Horwitz equation as the acceptance criteria.

The recovery study was conducted to assess the performance of an analytical procedure or sample preparation. The study was evaluated by spiking analysis, adding the known value to the sample, and measure the substantial value with the usual calibration curve. The authors reviewed the recovery in three different levels of spiking at 50, 300, and 800 ng/g.

After validation of the external calibration, the authors tested the performance of the ID-GC-MS method by checking the linearity firstly. In the ID-MS technique, the calibration curve will cover the plot between the ratio of the concentration of the analyte to concentration of internal standard and the ratio of the area of analyte to internal standard, where the isotope form of HCHs is the internal standard in this case. The HCHs concentration varied in the same range in the external calibration linearity while the isotope was guarded at a level of around 150 ng/g.

The recovery for the ID-GC-MS technique was also evaluated by analyzing CRM from KRISS (ginseng powder), compared the laboratorium result with the CRM certificate value and expressed as the percent value.

The authors applied the ID-GC-MS technique to determine the HCHs concentration in the ginseng roots sample, below equation 1. The definition of each parameter in the equation is described in Table 2.

$$C_x = C_z * \frac{m_y * m_{zc}}{m_x * m_{yc}} * \frac{R_B}{R_{Bc}} * \frac{1}{f_d} \qquad \text{(Eq. 1)}$$

The technique is familiarly known as exact-matching ID-MS. This time-consuming method is capable of reaching a high accuracy result (Sargent *et al.*, 2002).

The evaluation of the measurement uncertainty was studied based on the Guide to the Expression of Uncertainty in Measurement (JCGM, 2008) to consider all parameters in equation 1 that significantly contribute to the account. Other possible sources of uncertainty such as precision (F_p) and different calibration blend (F_{CB}), are accounted for in the final uncertainty budget. The measurement uncertainty (U), which is the expanded uncertainty, was obtained by multiplying the combined standard

possibility of all parameters by a coverage factor, k = 2, which gives a confidence level of approximately 95%.

3. RESULTS AND DISCUSSION:

The GC part in this method previously was the developed method for pesticides in black tea analysis (Aryana *et al.*, 2016). Comparing the black tea method, there are minor modifications in this ginseng method due to the different compounds of pesticides and retention time area observation. The change includes the detector the authors employed in the study, which is a mass spectrometry detector, while in the prior research, the authors used μECD sensor. The method described in Section 2 gave a satisfying result as the standard mixture of organochlorine has good separation, as shown in Figure 1. The identity of the alpha-HCH and gamma-HCH was confirmed through selective ion monitoring (SIM) mode by the presence of two dominant ion fragments from their particular MS fragmentation within specific time windows. The relative ion intensities of the ions targeted in samples and calibration standards were matched as a confirmation.

The authors obtained LoD and LoQ by injecting HCHs standard solutions (in hexane) at low-level concentration. LoD and LoQ were defined based on the signal-to-noise ratio, higher than 3:1 and 10:1, respectively (Uhrovčík, 2014; NATA, 2006; EMEA, 2006). LoD was determined to be 0.5 ng/g, while LoQ was 2 ng/g for both analytes.

Linearity study was established by the least-squares linear regression analysis of the standard solution set from 1 to 300 ng/g and evaluated by assessing the coefficient of determination (r^2) using ANOVA data analysis ($P < 0.05$). For alpha-HCH and gamma-HCH, the r^2-values were 0.9999 and 0.9997 respectively and the deviations of all data points in the calibration lines were lower than 10%, described in Figure 2.

Accuracy and precision of the present method were evaluated by recovery and repeatability experiments. Precision was checked by calculating the relative standard deviation (RSD) of the analytical results using standard mixture of 20 and 100 ng/g for each analyte. The experiments were carried out on intra-day observation. The standard mixture solutions at each concentration level was analyzed for five times. The results are listed in Table 3. The peak area of each compound was measured to determine the average values and the RSD (%).

As one of the parameters in the analytical method, recovery is an essential consideration in choosing the appropriate techniques for the calibration laboratory. The term recovery in this article means the amount of substance obtained in the last quantification step (after extraction) to the amount of substance added to the material before extraction and is expressed as a percentage. The authors tested the recoveries of the analytes by using the spiking technique (adding the known amount of the analytes) to the blank matrix. The recovery of the analytes was in the range of 85.5-107.1% with an average value at 98.0 and 95.6% for alpha-HCH and gamma-HCH, respectively. The RSDs of the set of measurements was found at less than 7% for both analytes (Table 3). This recovery criteria meet the satisfactory acceptance as the requirement of the AOAC Guidelines for Single Laboratory Validation of Chemical Methods were set as 80-110% at 100 ng/g. RSDs is also meet the satisfactory criteria which shows value below 22.6%, based on the Horwitz equation in 100 ng/g concentration as the acceptance (Hanley, 2016; Thompson and Lowthian, 1997).

In the application of the ID-GC-MS technique, the authors also checked the linearity profile to show the excellent relationship between the concentration ratio of natural and labeled analyte and the response ratio of natural and labeled analyte. This ratio concept is general in the analytical process by using internal standard calibration (Kościelniak and Wieczorek, 2016). The excellent linearity was described in Figure 3 where r^2-values for alpha-HCH and gamma-HCH was 0.9995 and 0.9990, respectively. For the other criteria, such as limit of detection, limit of quantitation, and intra-day precision would be in the same range in the previous study (external calibration) since the ID-MS only differs in the calibration technique. To cover the repeatability performance, the deviation of different days observation was taken into account in the uncertainty budget as a method precision parameter.

CRM matrix Ginseng Powder KRISS CRM 108-10-013 was used as quality control material for evaluating the performance of the analytical method for gamma-HCH only, as present in the CRM material. The CRM was analyzed by using the exact matching IDMS technique. The result, as shown in Table 3, shows good performance where the recovery value and the repeatability are in the level of acceptance.

The optimized ID-GC-MS technique was applied to measure alpha-HCH and gamma-HCH in the ginseng roots sample. Figure 4 showed the

chromatogram of the analysis result. The material was found to contain 448.9 ± 24.3 ng/g alpha-HCH and 98.4 ± 8.0 ng/g gamma-HCH. The uncertainties associated with these numbers are expanded (coverage factor k=2) to give a 95% confidence interval. The measurement uncertainty for HCHs determination in ginseng roots was evaluated entirely according to the guide to the expression of uncertainty in measurement (GUM) and the ID-MS equation used. Contributors of the overall uncertainty, including the weighing process, both standards and sample, the concentration of HCH standards, repeatability of measurements, dry mass factor and different calibration blend, were taken into consideration.

The uncertainty associated with the method precision, the calibration blend, and the repeatability of the measurement were the three main factors of the uncertainty budget for alpha-HCH and gamma-HCH. In the gamma-HCH's budget, the recovery of the method was also another significant source. This is caused by the parameter from CRM analysis (recovery study), which the authors take into account. Results of ginseng analysis for alpha-HCH and gamma-HCH and its uncertainty in measurement were summarized in Tables 4 and 5. The relative uncertainty was observed at 5.4% and 8.2% for alpha-HCH and gamma-HCH, respectively, representing the unknown true value is located in these interval around the measured result with a confidence level of 95% for each analyte.

4. CONCLUSIONS:

The organic laboratory of sub-directorate of chemical metrology in Indonesia has developed the analytical method for alpha-HCH and gamma-HCH measurement in the range of 1-300 ng/g by using isotope-dilution gas chromatography-mass spectrometry (ID-GC-MS). From the validation study, the proposed method is selective, specific, sensitive, precise, and accurate under the observed level of concentration. The limit of detection and the limit of quantitation values are 0.5 and 2 ng/g, respectively. It can be said the method is entirely reasonable to be advantaged in the trace analysis. The measurement uncertainty was evaluated in this study to obtain the full profile of the analytical process where the relative uncertainty value is 5.4% and 8.2% for alpha-HCH and gamma-HCH, respectively. Future work could focus on participation in the inter-laboratory study to assess and demonstrate the performance of the described method. As a high order method, it would be valuable if the technique can be implied in the accurate and precise determinations such as assigning a value to a certified reference material.

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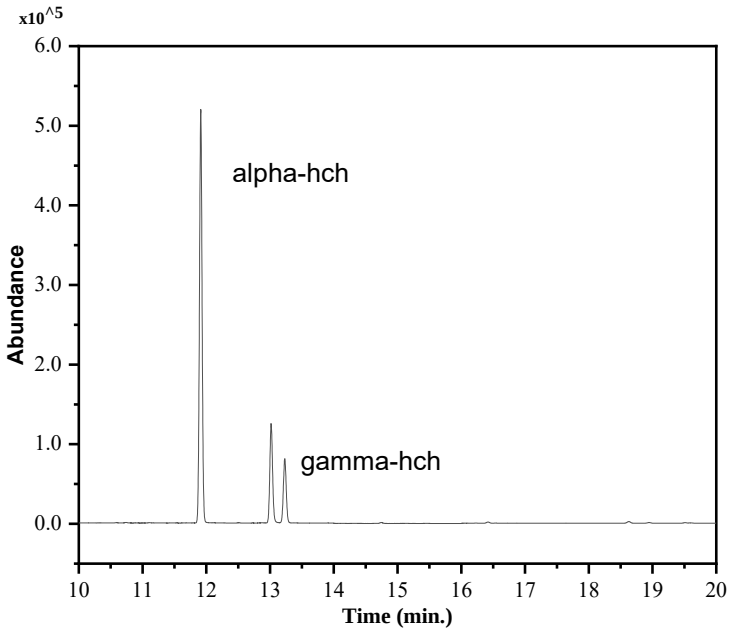

Figure 1. *Total ion chromatogram (TIC) of standard mixture of alpha-HCH and gamma-HCH*

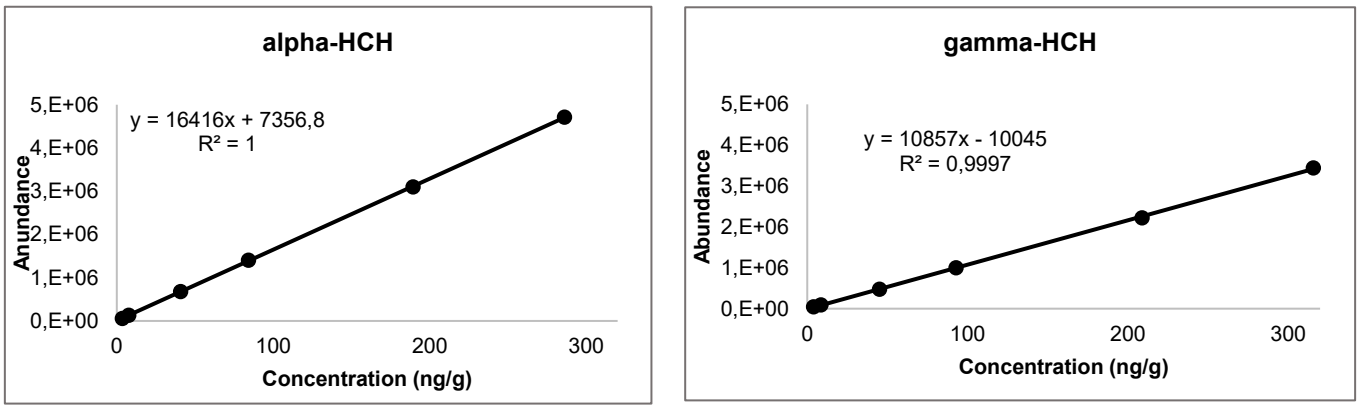

Figure 2. Linearity study for external calibration

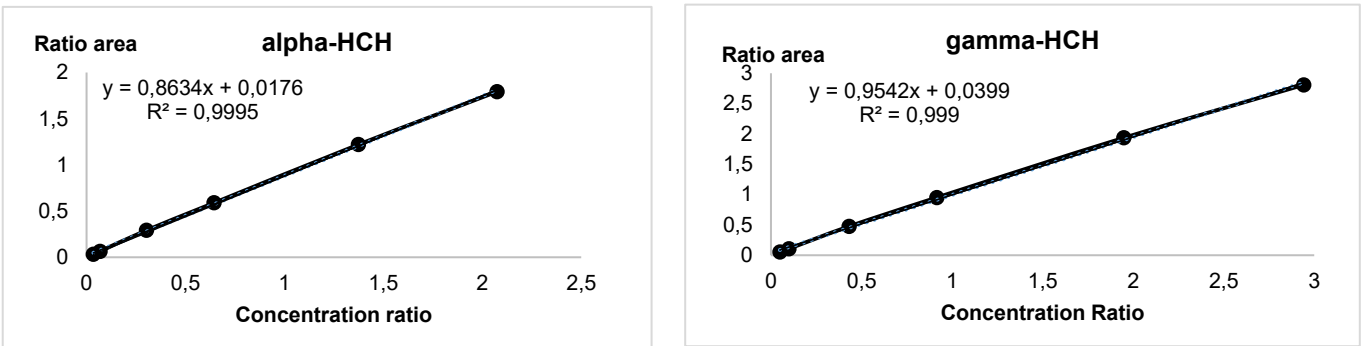

Figure 3. *Linearity study for isotope dilution calibration*

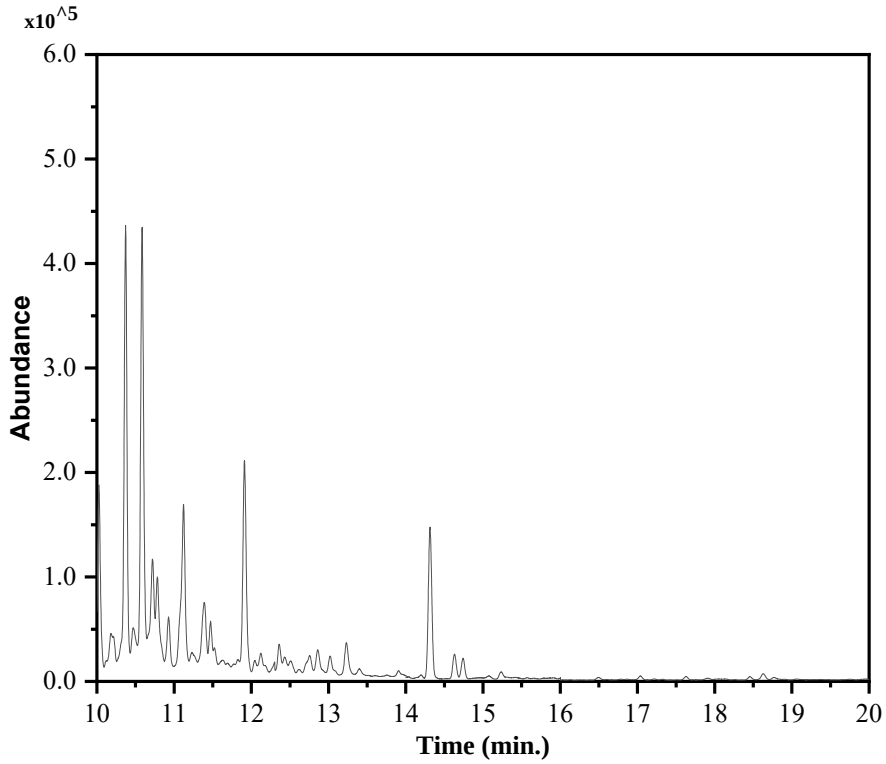

Figure 4. *Total ion chromatogram (TIC) of ginseng roots sample in exact-matching ID-GC-MS analysis*

Table 1. *Ions profile and retention time of analyte*

Analyte	Ion (m/z)	Retention time (min)
alpha-HCH	181	11.9
	183	11.9
	219	11.9
$^{13}C_6$ alpha-HCH	187	11.9
	189	11.9
	225	11.9
gamma-HCH	181	13.2
	183	13.2
	254	13.2
$^{13}C_6$ gamma-HCH	187	13.2
	189	13.2
	260	13.2

Table 2. *Parameters definition on ID-MS equation*

Parameter	Definition
C_x	mass fraction of HCH analyte in the ginseng sample (dry basis)
C_z	mass fraction of the HCH analyte in the standard solution used for preparing calibration blend
m_y	mass of internal standard solution added to the sample blend
m_{zc}	mass of native HCH calibration standard solution added to calibration blend
m_x	mass of study sample in the sample blend
m_{yc}	mass of internal standard solution added to the calibration blend
R_B	observed native and isotope ion abundance ratio in the sample blend
R_{Bc}	observed native and isotope ion abundance ratio in the calibration blend
f_d	dry mass factor

Table 3. *Analytical parameter for the determination of alpha-HCH and gamma-HCH by using ID-GC-MS*

Parameter	Analytes	
	alpha-HCH	gamma-HCH
Limit of detection (ppb)	0.5	0.5
Limit of quantitation (ppb)	2.0	2.0
Intraday precision		
%RSD (20 ppb)	3.7	3.3
%RSD (100 ppb)	3.0	2.4
Mean recovery CRM[a] (%)	-	97.8
%RSD recovery CRM	-	2.8
Mean recovery spiked[b] (%)	98.0	95.6
%RSD recovery spiked	7.0	6.0

a) Recovery study obtained using ID-GC-MS technique from CRM analysis
b) Recovery study obtained using external calibration technique from spiking analysis

Table 4. *Uncertainty budget for alpha-HCH determination in ginseng roots by exact matching ID-GC-MS*

Factor (Unit)	Values	Uncertainty		Sensitivity Coefficients		
	x	u(x)	u(x)/x	dCx/dx	$c^2.u(x)^2$	#CTV
Method precision	1.00000	0.00966	0.00966	448.94401	18.80711	12.75870%
m_{zc}	0.24985	0.00002	0.00008	1796.83619	0.00145	0.00099%
m_y	0.16508	0.00002	0.00013	2719.59542	0.00333	0.00226%
m_{yc}	0.16934	0.00002	0.00013	-2651.21808	0.00316	0.00215%
m_x	1.73616	0.00002	0.00001	-258.58459	0.00003	0.00002%
C_z	3.00	0.07500	0.02500	149.64800	125.96920	85.45719%
R_B	0.84358	Uncertainties captured in method precision				
R_{BC}	0.87126	Uncertainties captured in method precision				
F_d	0.90770	0.00010	0.00011	-494.59515	0.00255	0.00173%
Calibration blend	1.00000	0.00361	0.00361	448.94401	2.61938	1.77698%
C_x	448.9					
Combined Uncertainty	12.1					
Expanded Uncertainty (k=2)	24.3					
Total				2159.56982	147.40622	100%

Table 5. *Uncertainty budget for gamma-HCH determination in ginseng roots by exact matching ID-GC-MS*

Factor (Unit)	Values	Uncertainty		Sensitivity Coefficients		
	x	u(x)	u(x)/x	dCx/dx	$c^2.u(x)^2$	#CTV
Method precision	1.00000	0.01267	0.01267	98.39557	1.55391	9.84514%
m_{zc}	0.13944	0.00002	0.00015	705.64806	0.00022	0.00142%
m_y	0.14478	0.00002	0.00015	679.62908	0.00021	0.00132%
m_{yc}	0.15024	0.00002	0.00014	-654.94436	0.00019	0.00122%
m_x	1.73616	0.00002	0.00001	-56.67428	0.00000	0.00001%
C_z	1.41	0.01084	0.00769	69.82956	0.57262	3.62795%
R_B	0.787	Uncertainties captured in method precision				
R_{BC}	0.961	Uncertainties captured in method precision				
F_d	0.90770	0.000102	0.00011	-108.40098	0.00012	0.00077%
Calibration blend	1.00000	0.03337	0.03337	98.39557	10.78010	68.29970%
Method Recovery	0.983121597	0.016944838	0.017235775	100.0848382	2.87615	18.22247%
C_x	98.4					
Combined Uncertainty	4.0					
Expanded Uncertainty (k=2)	8.0					
Total				931.96306	15.78353	100%