

Articles

The Assessment of Carbendazim, Cyazofamid, Diethofencarb and Pyrimethanil Residue Levels in *P. ginseng* (C. A. Meyer) by HPLC

Jeong-Heui Choi,^{†,a} A. M. Abd El-Aty,^{†,‡,a} Young-Seok Park,[§] Soon-Kil Cho,[§] and Jae-Han Shim^{†,*}

[†]Natural Products Chemistry Laboratory, Institute of Agricultural Science and Technology, Chonnam National University, Gwangju 500-757, Korea. *E-mail: jhshim@chonnam.ac.kr

[‡]Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, 12211-Giza, Egypt and Konkuk University, Seoul 143-701, Korea

[§]National Agricultural Products Quality Management Service, Products Safety Inspection Laboratory, Gwangju 506-824, Korea
Received November 21, 2006

A fast and simple high-performance liquid chromatography (HPLC) method for the simultaneous determination of four pesticides having fungicide properties has been proposed for *Panax ginseng*, C. A. Meyer grown for 4, 5, or 6 years. Analytical separation was performed on C₁₈ columns using ultraviolet detector under gradient conditions. Spiked blank samples were used as standards to counteract the matrix effect observed in the chromatographic determination. The HPLC response for all pesticides was linear, with determination coefficients > 0.9986. The average rate of recovery for pesticides spiked with 2 fortification levels was > 72% with relative standard deviations < 9%. The limits of quantification (LOQ) ranged from 0.03 to 0.16 ppm. These LOQs were lower than the respective maximum residue limits (MRL) established by the Korean Food and Drug Administration (KFDA), except for cyazofamid. The proposed method was used to determine pesticide residue levels in samples of ginseng obtained from Jeonnam Province (Republic of Korea). None of the pesticides were found in ginseng samples grown for 4, 5, or 6 years.

Key Words : *Panax ginseng*, Pesticides, Liquid chromatography

Introduction

Panax ginseng (Asian ginseng), *P. pseudo-ginseng* (Japanese ginseng) and *P. quinquefolius* (North American ginseng) represent primary sources of the herb that is commonly referred to as ginseng. The roots of *P. ginseng*, C.A. Meyer, which has been used in traditional herbal remedies and medicines in Eastern Asia for more than 2000 years, are often used to make a tonic to increase energy, reduce susceptibility to various disorders and for recovery from illness.^{1,2} Ginseng is composed of a mixture of glycosides, essential oils, and a variety of complex carbohydrates and phytosterols, as well as amino acids and trace minerals.³ The principle active ingredient in ginseng (responsible for the biological effects) is believed to be a complex mixture of more than 30 triterpenoid saponins (each with a different set of properties) that are commonly referred to as ginsenosides and are present in the leaf, stem, and berries of the plant, in addition to their traditional source the root.⁴ The pharmacological effects of ginseng include enhancement of carbohydrate and lipid intermediary metabolism,⁵ and anti-stress mechanisms.⁶ There are also reports of antioxidant^{7,8} and anti-tumor activity for ginseng.⁹

Ginseng is highly susceptible to fungal diseases such as anthracnose (20%-47%), damping-off (5%-50%), root rot

(1%-60%), and alternaria blight (10%-20%).¹⁰ This susceptibility is associated with its requirement for artificial shade and the fact that these plants have to grow for 4 to 6 years before they can be harvested. Pesticides are essential to control these diseases and increase harvest productivity; however, because of their potentially dangerous effects on human health, the control of pesticide residue in food is of great importance. The simultaneous presence of pesticides (organochlorine, carbamate, and pyrethroid) in *P. ginseng* has rarely been considered in multiresidue analyses.¹¹⁻¹³

Organophosphorus pesticides have also been studied, because some of them are used extensively.¹² In our previous study, we used gas chromatography (GC) as a separation technique to analyze the pesticide residues in *P. ginseng*. Because of its high separation power and the availability of a variety of sensitive and selective detectors (*e.g.*, the electron capture detector and nitrogen-phosphorus detector), 18 pesticides (among 32 pesticides registered for ginseng in Republic of Korea) were monitored in ginseng matrices.¹³ The pesticide tolclofos-m has been detected in ginseng obtained from Jeonnam Province in the Republic of Korea.¹³ In the present contribution, we measured the residue levels for 4 pesticides (carbendazim, cyazofamid, diethofencarb and pyrimethanil) that had been used in *P. ginseng*, based on liquid partitioning with an organic solvent (acetonitrile), purification by solid-phase extraction, and high performance liquid chromatography. This method was applied to the determination of

^aThis paper is equally contributed by the two authors.

pesticides in ginseng samples obtained from the southern region of the Korean peninsula. It should be noted that the remaining compounds including metam-sodium, mancozeb, copper sulfate, polyoxin B D zinc salt, iminoctadine triacetate, metaldehyde, fludioxonil, chlorothalonil, and carbosulfan, could not be simultaneously detected by GC or HPLC.

Experimental Section

Materials. HPLC-grade acetonitrile, *n*-hexane, dichloromethane, methanol, and anhydrous sodium sulphate were supplied by Merck (Darmstadt, Germany). The pesticides carbendazim (96.5%), cyazofamid (97.0%), diethofencarb (98.5%), and pyrimethanil (98.5%) were obtained from Dr. Ehrenstorfer (Augsburg, Germany); the chemical structures of these compounds are illustrated in Table 1. Water was distilled and filtered through a Milli-Q apparatus (Millipore, Seoul, Korea) before use. Stock pesticide solutions (1000 ppm) were prepared by dissolving each pesticide standard in methanol and storing at 4 °C in a brown bottle with a Teflon-lined screw cup. Pesticide working-strength solutions were prepared by diluting the stock solutions in methanol. No degradation of the pesticides was detected under the storage conditions used at any time during the study.

Samples. Twelve samples (weight approx. 2 kg) of ginseng roots harvested 4, 5, or 6 years after planting were

Table 1. Chemical structures, uses, and the retention times for the tested pesticides

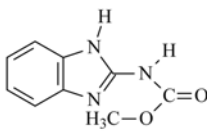
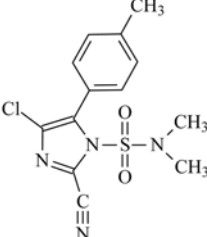
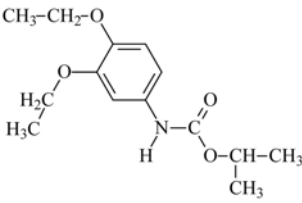
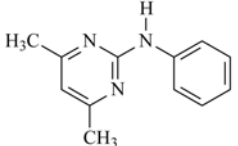
No	Compound	Structure	Action & uses	Retention Time
1	carbendazim		Fungicide against gray mold rot	8.57
2	cyazofamid		Fungicide against late blight	29.70
3	diethofencarb		Fungicide against gray mold rot	30.65
4	pyrimethanil		Fungicide against alternaria blight	39.63

Table 2. Conditions of HPLC analysis of pesticides in *Panax ginseng* (C. A. Meyer)

Instrument	HP-1100 Series (Agilent Technologies, Inc., Palo Alto, Calif, USA)
Column	Luna C ₁₈ (25 cm, particle size 5 μm, Phenomenex Products, USA)
Detector	UVD Wavelength: 254 nm
Injection volume	10 μL
Mobile phase	H ₂ O:CH ₃ CN=70:30, 1.0 mL/min (from 0 to 5.0 min) H ₂ O:CH ₃ CN=15:85, 1.0 mL/min (from 5.0 to 50.0 min)

collected between April and October 2004 from 4 agricultural areas, Haenam, Naju, Yeongam and Yeonggwang in Jeonnam Province in the southern region of the Korean peninsula. Prior to the analysis, each sample was blended and divided into 4 parts. A 20-g sample was selected randomly and tested in triplicate for pesticide residue levels using HPLC with a-UV detector.

Extraction. Approximately 20 g of *P. ginseng* that had been chopped into small pieces was weighed and placed in a 250-mL glass bottle containing acetonitrile (100 mL), and then sodium chloride (18 g) was added to the mixture. The mixture was homogenized at 3000 rpm for 5 min in a high-speed homogenizer (SMT High-Flex, HG-92, Japan). After the extraction procedure, the solution was transferred to a separation funnel and vigorously shaken for 3 min and the extract was centrifuged for 5 min at 3000 rpm. The organic layer was collected in a round-bottomed flask, and concentrated to dryness in a rotary evaporator (Büchi Rotavapor R-114, Germany) at 40 °C in a Büchi Waterbath B-480.

Purification. The NH₂ cartridge (Phenomenex, Torrance, Calif, USA) was washed with 6 mL dichloromethane (for activation), and the dried residue was dissolved in 2 mL of *n*-hexane. The mixture was then pre-loaded in the cartridge and eluted with 15 mL of dichloromethane containing 5% methanol. The eluent was filtered through sodium sulphate, evaporated at 40 °C, and dissolved in 2 mL of methanol: water (1:1, v/v). The extract was then transferred to a 5 mL vial for chromatographic analysis. The details of the chromatographic conditions are shown in Table 2.

Analyte recovery rate. The extraction efficiency (recovery rate) of these pesticides was determined in triplicate at 2 fortification levels in spiked samples of blank *P. ginseng* (*i.e.*, containing none of the pesticides of interest). The samples were processed according to the extraction procedure just described. The recovery rate was calculated by comparing peak areas for spiked ginseng (with known amounts of pesticides in the range of calibration curves concentrations) with standard solutions dissolved in organic solvent and injected directly into the analytical column.

Results and Discussion

Method Validation

Table 3. Determination coefficient, rate of recovery, RSD, LOD, LOQ and MRL for the pesticides tested*

No	Compound	r^2	Fortification levels (ppm)	Recovery rate (%)	RSD (%)	LOD (ppm)	LOQ (ppm)	MRL (ppm)
1	Carbendazim	0.9986	12.5	93.2 ± 3.57	3.8	0.039	0.12	0.125
			1.6	118.8 ± 10.50	8.8			
2	Cyazofamid	0.9995	17.3	80.6 ± 0.66	0.8	0.054	0.16	0.075
			2.2	80.2 ± 5.13	6.4			
3	Diethofencarb	0.9999	4.7	97.4 ± 1.31	1.4	0.015	0.05	0.125
			0.6	81.2 ± 0.00	0.0			
4	Pyrimethanil	0.9999	3.1	72.7 ± 0.67	0.9	0.010	0.03	0.075
			0.4	72.4 ± 3.92	5.4			

*Pesticide components were analyzed by HPLC. r^2 , determination coefficient; RSD, relative standard deviation; LOD, limit of determination; LOQ, limit of quantitation; MRL, maximum residue limit.

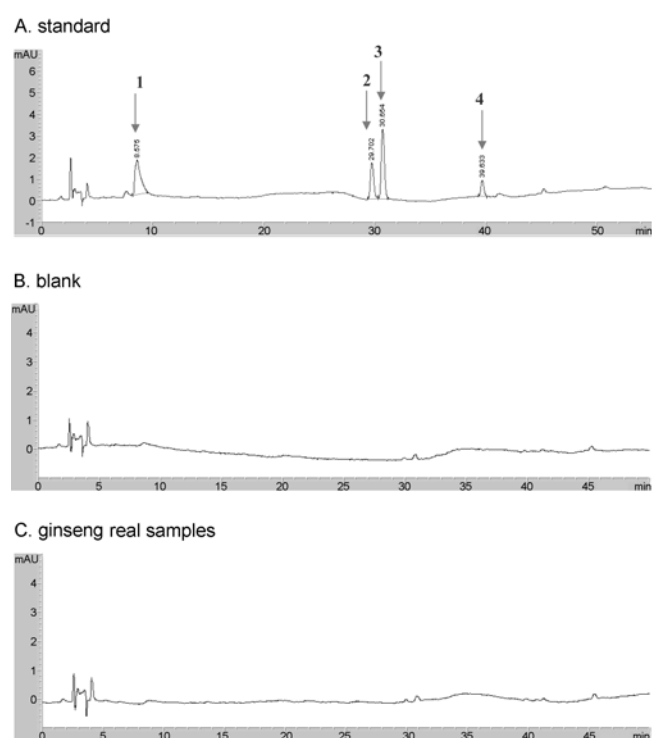


Figure 1. Typical HPLC-UV-D chromatograms of carbendazim (1.6 ppm), cyazofamid (2.2 ppm), diethofencarb (4.7 ppm) and pyrimethanil (3.1 ppm) standards dissolved in organic solvent (A), blank (B), and ginseng (real) samples (C). See Table 1 for peak identification.

Linearity. The linearity of all the pesticides was determined using blank samples. Good determination coefficients were obtained for all of the compounds and ranged from 0.9986 to 0.9999 (Table 3).

Repeatability. The repeatability of our chromatographic findings was determined by analyzing a sample spiked at 50 ppm. The sample was injected 10 times with an automatic injector, and the relative standard deviation (RSD) values for retention times ranged from 0.02% to 0.04%; whereas for peak areas the values ranged from 1.1% to 4.2%. Therefore, the repeatability of the results of the HPLC analysis achieved by automatic injection was very good.

Stability. Stock standard solutions and working solutions were found to be stable for at least 3 months and 1 week,

respectively, when stored at 4 °C. Moreover, when the stability of a fortified blank sample kept in the autosampler for 24 h was assayed, differences of < 3% were obtained.

Specificity. The specificity of the proposed procedure was assessed by analyzing blank samples. The absence of background peaks above a signal-to-noise ratio of 3 at the retention time for each pesticide indicated that no interference occurred (Figure 1).

Recovery. The rate of recovery for all pesticides ranged from 72.4% to 118.8%, with RSDs lower than 9% (Table 3). These results show that the rates of recovery for pesticides were good. These values are similar to those reported elsewhere for other pesticide detected in ginseng.¹¹⁻¹³

Detection and quantification limits. Both limits of detection (LOD) and limits of quantitation (LOQ) were calculated based on the minimum standard concentration detected by the analytical instrument as a peak. To determine this concentration, extracts of nonfortified ginseng (referred to as blanks in this study) were analyzed. Around each retention time of interest, signals below a certain response were considered noise, and the average noise, in terms of Hz or counts, was manually calculated. To qualify as a peak, a signal's response had to be either equal to (or higher than), three times the average noise in case of LOD or 10 times the average noise for LOQ.¹⁴ Table 3 summarizes the LOD and LOQ for each pesticide. The LODs varied from 0.010 to 0.054 ppm, and the LOQs were lower than the maximum residue limits (MRLs) authorized by the Korean Food and Drug Administration,¹⁵ except for cyazofamid.

Analysis of ginseng samples. The analytical method was applied to the analysis of ginseng samples. No pesticide residue was found in any of the ginseng samples analyzed in this study (Figure 1).

Conclusions

We have described a method for monitoring the levels of certain pesticides in *P. ginseng* (C. A. Meyer) using HPLC. This method was simple, easy, and inexpensive and provided linear and repeatable data. For all the pesticides, the sensitivity of this method was good enough to ensure a reliable determination at pesticide levels that were much

lower than the respective MRLs established by the Korean FDA. With slight modifications, it might also be applicable to other matrices.

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