



Analytical Methods

Multiresidue pesticide analysis in Korean ginseng by gas chromatography–triple quadrupole tandem mass spectrometry

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ABSTRACT

In this study, a new analytical method was developed based on gas chromatography–triple quadrupole tandem mass spectrometry (GC–MS/MS) and used to determine 32 multiclass pesticides in ginseng products. The analytical method was validated, yielding recovery rates in the range of 55.2–108.3%, with precision values expressed as relative standard deviation (RSD) lower or equal to 12% at the spiking levels of 30, 100, and 1000 µg/kg. Correlation coefficients and LOQs (limit of quantification) were in the range 0.9801–0.9989 and 0.15–70 g/kg, respectively. With these validation data and this method, multiresidue pesticides of ginseng samples (fresh ginseng ($n = 118$), red ginseng ($n = 24$), dried ginseng ($n = 10$)) were analysed. Among them, the most frequently detected pesticide was tolclofos-methyl. Tolclofos-methyl was detected in 86.4% of fresh ginseng (18.25–404.5 µg/kg), 91.7% of red ginseng (13.14–119.4 µg/kg), and 87.5% of dried ginseng (23.15–3673 µg/kg).

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1. Introduction

Ginseng (*Panax ginseng* C.A. Mayer) is a valuable herb that has been used extensively in Eastern Asian countries, such as Korea, China, and Japan, for more than 5000 years. Ginseng as a medicine has been studied thoroughly. Ginsenosides are major components having pharmacological and biological activities, including immune, cardiovascular, central nervous system, endocrine, anti-diabetic, anti-tumor, and antioxidant activities (Attele, Wu, & Yuan, 1999; Joint FAO/WHO, 1993). Ginseng is generally harvested after a 5- or 6-year cultivation period, or even a 10-year period. Such a long cultivation period combined with the widespread use of pesticides in ginseng production has led to the presence of pesticide residue in ginseng (KCPA, 2004; Kim & Lee, 2002; Roh, Kim, Ku, Jo, & Pyon, 2002).

Pesticide contamination of foods can negatively affect human health since many pesticides used in agriculture have toxic effects on living organisms. Governments and international organizations regulate the use of pesticides by setting maximum residue levels (MRLs) of pesticides in foods. In Korea, MRLs of 34 pesticides have been established in ginseng (KFDA, 2008). Thus, pesticide analysis in the cultivate region is an essential. Analysis of the large number of pesticides applied to crops generally requires the use of analytical tools such as gas chromatography (GC) or high-performance liquid chromatography (HPLC). Both techniques have been extensively utilised with class-selective detection methods,

especially GC with an electron-capture (ECD), flame photometric (FPD), or nitrogen-phosphorus (NPD) detector. In spite of their excellent selectivity, these detectors are often influenced by matrix components and do not provide unambiguous results. For this reason, mass spectrometry (MS) has become prevalent in pesticide residue analysis (Gamon, Lleo, Ten, & Mocholi, 2001). To quantify and confirm the results of MS, either its full scan or selected ion monitoring (SIM) spectra are used. However, it is not guaranteed that that matrix interference can be eliminated in all cases (Schachterle & Feigel, 1996; Garrido Frenich, Plaza-Bolanos, & Martinez Vidal, 2008).

Certainly, tandem mass spectrometry (MS/MS) provides a much higher degree of assurance in the identification of an analyte than any other single stage mass spectrometry technique (Walorczyk, 2007). One of the advantages of MS/MS is to increase S/N ratio and selectivity. Due to the power of MS/MS, the confirmation of target compounds can be achieved with a higher level of confidence. Among the various mass analysers that can perform tandem mass spectrometry, triple quadrupole mass spectrometers have recently been recommended for the analysis of pesticide residues in crops (Martinez Vidal, Arrebola, & Mateu-Sanchez, 2002; Walorczyk, 2007). Recently LC-MS/MS method for traditional Chinese medicine was published (Jia, Mao, Chen, Wang, & Ji, 2010). With LC-MS/MS the sample is not limited to thermostable or volatile properties.

The aim of this paper was to develop a robust multiresidue method for the analysis of 32 pesticides of different classes using GC/MS/MS. In addition, validation and uncertainty studies were carried out to provide clear verification of the proposed analytical

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method. Furthermore, the method was applied to analyse residue pesticides in ginseng (fresh ginseng, red ginseng, dried ginseng).

2. Material and methods

2.1. Chemicals

The 32 pesticides standards (ethylenbis -dithiocarbamate, cadusafos, α -hexachlorocyclohexane (α -HCH), β -hexachlorocyclohexane (β -HCH), quintozone, γ -hexachlorocyclohexane (γ -HCH), tefluthrin, chlorothalonil, tebuipirimfos, δ -hexachlorocyclohexane (δ -HCH), tolclofos-methyl, metalaxyl, diethofencarb, aldrin, cyprodinil, tolyfluanid, flutolanil, fludioxonil, *p,p*-dichlorodiphenyldichloroethylene (*p,p*-DDE), thifluzamid, kresoxim-methyl, dieldrin, endrin, *p,p*-dichlorodiphenyl-trichloroethane (*p,p*-DDT), *p,p*-dichlorodiphenyldichloroethane (*p,p*-DDD), *o,p*-dichlorodiphenyl-trichloroethane (*o,p*-DDT), trifloxystrobin, fenhexamid, carbosulfan, cypermethrin, difenoconazole, and azoxystrobin) were purchased from Ehrenstorfer GmbH Co. (Augsburg, Germany), Wako (Osaka, Japan) and Chem Service (West Chester, PA, USA). The purities of the standard pesticides ranged from 91.0% to 99.5%. Each stock solution was prepared in 1000 μ g/ml of acetonitrile solution and used for further dilutions. The stock and working solutions were stored in completely filled vials closed with parafilm at -20°C until analysis. Acetonitrile, acetone, and *n*-hexane (HPLC grade) were purchased from J.T. Baker (Phillipsburg, NJ, USA). Anhydrous sodium chloride for residue analysis was purchased from Fluka (Buchs, Switzerland).

2.2. Samples

Ginseng samples (fresh ginseng, red ginseng, dried ginseng) were obtained from local ginseng agricultural cooperative federations located in Seoul. All samples were produced in South Korea. Samples were analysed within 24 h and stored at 4°C until the moment of extraction. No degradation of pesticides was detected under the storage conditions.

2.3. Validation of method

2.3.1. Specificity

Ginseng matrix contains many compounds. In order to minimise matrix effects, ginseng was extracted in acetonitrile and cleaned by Florisil cartridge (Strata, Harbour City, CA, USA). The peaks for each pesticide in the chromatogram were compared with that of pesticide standard.

2.3.2. Calibration curve and linearity

Calibration standards for analysis of pesticides were prepared by injecting various concentrations of pesticide solutions (0.01–5.00 μ g/ml). Linearity was determined from the coefficients of determination (R^2).

2.3.3. Precision and accuracy

Intra-day precision (repeatability) was measured on a single day using five replicates of each spiked matrix under the same conditions (same analyst, apparatus, reagents, and short interval of time), whereas inter-day precision was calculated during five consecutive days also using five replicates of five standard injection concentrations (0.1, 0.5, 1, 5, 10 μ g/ml). Precision and accuracy are expressed as relative standard deviation (RSD, %) and recovery rate (%), respectively.

2.3.4. Limit of detection (LOD), limits of quantification (LOQ)

For determining LOD and LOQ, 0.01 μ g/ml of each pesticide standard was injected seven times, and standard deviations were

calculated. LOD and LOQ were calculated as $3 \times S_{y/x}/b$ and $10 \times S_{y/x}/b$, respectively. $S_{y/x}$ is the standard deviation of the estimate while b is the slope of the calibration curves.

2.3.5. Recovery rate

Ginseng without pesticides was used for the fortification experiments. Three sets of each pesticide standard solution (30, 100, 1000 μ g/kg) were spiked to contain 5 g of homogenised sample. Spiked samples were shaken for 2 h to allow pesticide absorption by the sample. Sample preparation was carried out and recovery rate calculated (Harris, 2001).

2.4. Sample preparation

The pesticides in ginseng were extracted and purified according to the method of Korea Food code (2008) with slight modifications. Briefly, 50 g of grinded ginseng was weighed into an Omni mixer (Omni International, Waterbury, CT, USA) bottle, after which the sample bottle was homogenised with 100 ml of acetonitrile for approximately 10 min. The sample was filtered through a 12 cm Shark skin filter paper (Whatman, Piscataway, NJ, USA). Then, 20 g of anhydrous sodium chloride was added to the extract and then vortexed immediately for 3 min. The extract was next centrifuged for 5 min at 4000 rpm at 4°C (Hanil Co., Korea). From the upper layer, a 10 ml aliquot was transferred into a 100 ml beaker. Then, evaporation of the solvent to dryness was carried out with nitrogen gas (35 – 40°C). The dried residue was re-dissolved with acetone: hexane (2:8; v/v). The mixture was passed through a Florisil Cartridge (Strata, Harbour City, CA, USA) preconditioned with 5 ml of acetone: hexane (2:8; v/v). By this way the analyte of interest pass the column while matrix constituents are retained. The solvent in the mixture was then evaporated slowly to dryness at 35 – 40°C under a gentle stream of nitrogen. The dried residue was re-dissolved with 2 ml of acetone for GC–MS/MS analysis.

2.5. GC–MS/MS analytical method

Pesticide analysis was performed using a Varian 3800 gas chromatograph equipped with an electronic flow control (EFC) and fitted with a triple quadrupole mass spectrometer (GC–MS/MS) (Varian Instrument, Sunnyvale, CA, USA). Chromatographic separation was performed on Varian VF-5 ms Columns (30 m \times 0.32 mm, 25 μ m film, Walnut Creek, CA, USA). Injection temperature was held at 250°C , and injection volume was 1 μ l. The oven temperature was held for 2 min at 70°C , ramped to 200°C at $20^{\circ}\text{C}/\text{min}$, increased to 220°C at $2^{\circ}\text{C}/\text{min}$, and then held for 3 min and finally increased to 300°C (hold 3 min) at $10^{\circ}\text{C}/\text{min}$ (total run time equalled 35 min) with a flow of 1.0 ml/min. Helium (99.999%) was used as the carrier gas and the linear velocity was 30 cm/s. Triple quadrupole system was operated in electron impact ionisation mode (EI, 70 eV). Argon (99.999%) was used as the collision gas. The dwell time was 0.5 s. The trap, manifold, and transfer line temperatures were set at 200°C , 40°C , and 280°C , respectively. Split ratio and split vent valve state were programmed as follows: initial (open, 5:1); 0 min (closed, off); 1.5 min (open, 100:1); 3.0 min (open, 30:1). The MS/MS system entails two fundamental steps between the formation and detection of ions. In first step, the precursor ion or an entire cluster of parent ions is isolated in the trap, and in the second step, the dissociation of the precursor ion or ions is performed by collision with an inert gas (Arrebola, Martinez Vidal, Mateu-Sanchez, & Alvarez-Castellon, 2003). The precursor ion of each analyte and the corresponding product ions are also reported in Table 1. The multiplier voltage of 1300 V and collision energy were optimised for each compound as shown in Table 1.

Table 1

Retention time and MS/MS fragmentation ions in mass spectrum.

Pesticides	RT	M. W.	Q 1 ^a	Q 3-1 ^b	Q 3-2 ^c	V ^d	Ions
EBDC	8.938	–	102	102	74	20	235, 165, 199, 176, 212
Cadusafos	9.856	270	159	131	97	15	159, 88, 127, 213
α -HCH	10.142	288	181	145	109	25	181, 219, 109, 145
β -HCH	10.595	288	181	109	145	30	109, 181, 219, 145
Quintozene	10.667	293	237	142	119	25	237, 142, 214, 249, 295
γ -HCH	10.776	288	181	145	109	10	181, 109, 219, 145
Tefluthrin	10.978	419	177	127	157	10	177, 197, 141, 127, 161
Chlorothalonil	11.095	264	266	168	231	25	266, 109, 124, 229
Tebupirimfos	11.276	318	261	233	153	15	152, 234, 137, 261, 318
δ -HCH	11.359	288	181	109	145	10	181, 219, 109, 145
Tolclofos-methyl	12.179	300	265	250	93	20	265, 125, 79, 93, 250
Metalaxyl	12.307	279	206	132	162	10	206, 170, 155, 96, 198
Diethofencarb	13.326	267	225	196	168	5	124, 225, 168, 196, 267
Aldrin	13.528	362	263	193	228	30	263, 66, 79, 91, 293
Cyprodinil	14.464	225	224	224	208	25	224, 77, 210
Tolyfluanid	14.754	346	238	137	91	35	238, 137, 181, 92, 104
Flutolanil	16.595	323	173	145	95	20	173, 145, 281, 323
Fludioxonil	16.847	248	248	154	182	10	248, 127, 154, 182, 77
<i>p,p'</i> -DDE	17.219	316	246	176	211	25	246, 318, 176, 105, 210
Thifluzamide	17.220	528	194	166	125	20	194, 166, 125, 449
Kresoxim-methyl	17.490	313	131	116	89	20	116, 131, 206, 89, 142
Dieldrin	17.582	378	263	193	209	30	263, 79, 173, 215
Endrin	18.485	378	263	228	193	20	345, 81, 263, 147, 345
<i>p,p'</i> -DDT	19.047	352	235	199	165	20	235, 165, 199, 176, 212
<i>p,p'</i> -DDD	19.318	318	235	165	200	30	235, 165, 199, 212
<i>o,p'</i> -DDT	19.416	352	235	199	165	20	235, 165, 199, 176, 212
Trifloxystrobin	21.131	408	131	89	116	25	116, 135, 222, 59
Fenhexamid	21.139	301	177	113	78	20	177, 97, 55, 179
Carbosulfan	23.524	380	118	76	62	20	160, 118, 135, 91, 149
Cypermethrin	28.449	415	181	152	127	30	181, 163, 91, 127, 209
Difenoconazole	30.252	406	323	265	202	30	323, 265, 202, 130, 173
Azoxystrobin	30.841	403	344	329	183	20	344, 388, 345, 75

^a precursor ion.^b quantification ion.^c identification ion.^d collision energy (eV).

3. Results and discussion

3.1. Validation for the analysis of pesticides

The GC and MS/MS analytical conditions in this study allowed us to correctly resolve all target compounds. All 32 pesticides could be analysed by a single chromatographic run of 35 min. All pesticides in the chromatogram were satisfactorily separated with high sensitivity and selectivity. GC–MS/MS calibration curves were prepared after injection of the standard mixtures based on the peak area. Table 2 summarizes the slopes, intercepts, and correlation coefficient values of the validation study. Reasonable linearity was found in the concentration range studied, with correlation coefficients between 0.9801 and 0.9989.

The precision (repeatability) of the overall method was evaluated at five concentration levels and expressed as relative standard deviation (RSD). Table 3 shows the results with RSD values of 0.06–12.0% for all pesticides. Accuracy was also evaluated at five concentration levels. As seen in Table 3, the recovery values were in the range of 80.0–108.3% for all pesticides, except for ethylenebis-dithiocarbamate. All results were within the acceptable range and indicate that the method was accurate and precise. In a report by Wong et al. the recovery rates of organophosphorus pesticides in dried ginseng using GC-FPD (flame photometric detector) were below 90% (Wong et al., 2007). Lower (<60%) recoveries most likely resulted from the physical state of pesticides, including volatility and matrix effect.

The LOD of most pesticides ranged from 0.04 to 3.9 $\mu\text{g/kg}$, and the LOQ ranged from 0.15 to 13 $\mu\text{g/kg}$, with the exception of ethylenebis-dithiocarbamate, flutolanil, and fenhexamide. With GC-FPD, the LODs were reported as lower than 50 $\mu\text{g/kg}$ (Wong et al.,

Table 2

Calibration parameters for 32 pesticides.

Pesticides	Standard calibration equation ($y = ax + b$)		R^2
	Slope (a)	Intercept (b)	
EBDC	3.6904e + 7	–1.9131e + 7	0.9934
Cadusafos	5.6410e + 8	–7.7260e + 7	0.9896
α -HCH	1.2192e + 8	–5.7815e + 6	0.9984
β -HCH	6.8986e + 7	–4.7560e + 6	0.9983
Quintozene	3.0729e + 7	–2.3328e + 6	0.9984
γ -HCH	7.0687e + 7	–4.4321e + 6	0.9973
Tefluthrin	2.3368e + 8	–3.2027e + 7	0.9873
Chlorothalonil	9.4145e + 7	–3.3678e + 7	0.9916
Tebupirimfos	8.4015e + 7	–1.4330e + 7	0.9892
δ -HCH	5.5139e + 7	–3.0525e + 6	0.9986
Tolclofos-methyl	2.5150e + 8	–2.4624e + 7	0.9969
Metalaxyl	4.3842e + 7	–6.4853e + 6	0.9872
Diethofencarb	2.8580e + 7	–8.3080e + 6	0.9831
Aldrin	4.4717e + 7	–2.7870e + 6	0.9989
Cyprodinil	2.3139e + 8	–3.2713e + 7	0.9919
Tolyfluanid	3.0239e + 7	3.3886e + 6	0.9937
Flutolanil	6.5200e + 7	–1.1927e + 7	0.9917
Fludioxonil	1.6050e + 8	–4.8987e + 7	0.9907
<i>p,p'</i> -DDE	2.3687e + 8	–1.7841e + 7	0.9979
Thifluzamid	2.9998e + 8	–6.1156e + 7	0.9892
Kresoxim-methyl	1.58899e + 7	–9.2323e + 5	0.9988
Dieldrin	9.8953e + 7	–1.4109e + 7	0.9919
Endrin	3.6420e + 7	–4.3692e + 6	0.9937
<i>p,p'</i> -DDT	4.2160e + 6	–5.9265e + 5	0.9949
<i>p,p'</i> -DDD	8.0117e + 8	–7.4720e + 7	0.9975
<i>o,p'</i> -DDT	8.4408e + 8	–1.0444e + 8	0.9950
Trifloxystrobin	1.8855e + 8	–3.1433e + 7	0.9886
Carbosulfan	7.9862e + 6	–2.3463e + 6	0.9916
Fenhexamid	1.9086e + 8	–8.2268e + 7	0.9801
Cypermethrin	7.6538e + 7	–1.1150e + 7	0.9913
Difenoconazole	1.6342e + 8	–2.7328e + 7	0.9898
Azoxystrobin	2.3645e + 8	–3.3513e + 7	0.9920

Table 3

The recovery rates of pesticides analysed by GC/MS/MS.

Pesticides	Spiked grade	Recovery (%)			Recovery mean (%)	RSD (%)
		1	2	3		
EBDC	Low ^a	50.00	63.33	60.00	57.78	12.00
	Middle ^b	56.00	57.00	64.00	59.00	7.39
	High ^c	52.40	60.40	52.80	55.20	8.17
Cadusafos	Low	90.00	86.87	80.00	85.62	5.97
	Middle	82.00	97.00	86.00	88.33	8.79
	High	90.60	91.50	86.60	89.57	2.91
α -HCH	Low	96.67	93.33	90.00	93.33	3.57
	Middle	99.00	97.00	98.00	98.00	1.02
	High	98.90	99.90	101.00	99.93	1.05
β -HCH	Low	93.33	93.33	90.00	92.22	2.09
	Middle	98.00	94.00	98.00	96.67	2.39
	High	96.80	92.50	95.20	94.83	2.29
Quintozene	Low	83.33	80.00	86.67	83.33	4.00
	Middle	89.00	87.00	85.00	87.00	2.30
	High	86.20	85.30	80.80	84.10	3.44
γ -HCH	Low	96.67	96.67	93.33	95.56	2.01
	Middle	99.00	98.00	98.00	98.33	0.59
	High	96.90	97.80	97.80	97.50	0.53
Tefluthrin	Low	96.67	93.33	93.33	94.44	2.04
	Middle	86.00	91.00	90.00	89.00	2.97
	High	96.30	93.50	95.30	95.03	1.49
Chlorothalonil	Low	83.33	80.00	83.33	82.22	2.34
	Middle	89.00	90.00	87.00	88.67	1.72
	High	87.90	85.70	84.70	86.10	1.90
Tebupirimfos	Low	83.33	80.00	86.67	83.33	4.00
	Middle	84.00	87.00	90.00	87.00	3.45
	High	98.70	91.70	90.60	93.67	4.69
δ -HCH	Low	96.67	93.33	96.67	95.56	2.01
	Middle	98.00	97.00	98.00	97.67	0.59
	High	90.60	93.80	93.60	92.67	1.93
Tolclofos-methyl	Low	96.67	93.33	90.00	93.33	3.57
	Middle	98.00	97.00	95.00	96.67	1.58
	High	98.90	95.90	97.20	97.33	1.55
Metalaxyl	Low	83.33	80.00	80.00	81.11	2.37
	Middle	85.00	87.00	85.00	85.67	1.35
	High	103.00	99.90	105.00	102.60	2.50
Diethofencarb	Low	93.33	96.67	96.67	95.56	2.01
	Middle	91.00	90.00	90.00	90.33	0.64
	High	99.90	98.70	99.90	99.50	0.70
Aldrin	Low	96.67	93.33	96.67	95.56	2.01
	Middle	101.00	101.00	99.00	100.33	1.15
	High	91.20	94.50	93.20	92.97	1.79
Cyprodinil	Low	93.33	90.00	93.33	92.22	2.09
	Middle	108.00	101.00	99.00	102.70	4.60
	High	107.50	101.10	101.20	103.30	3.55
Tolyfluanid	Low	93.33	90.00	86.67	90.00	3.70
	Middle	94.00	98.00	98.00	96.67	2.39
	High	105.40	99.90	100.10	101.80	3.06
Flutolanil	Low	93.33	96.67	90.00	93.33	3.57
	Middle	97.00	95.00	96.00	96.00	1.04
	High	99.80	99.70	99.80	99.77	0.06
Fludioxonil	Low	90.00	86.67	90.00	88.89	2.17
	Middle	89.00	90.00	90.00	89.67	0.64
	High	98.90	97.90	97.70	98.17	0.65
<i>p,p</i> -DDE	Low	96.67	93.33	96.67	95.56	2.01
	Middle	93.00	98.00	97.00	96.00	2.76
	High	94.20	99.40	106.00	99.87	5.92
Thifluzamid	Low	90.00	93.33	83.33	88.89	5.73
	Middle	101.00	101.00	104.00	102.00	1.70
	High	108.20	101.00	109.00	106.10	4.15
Kresoxim-methyl	Low	93.33	90.00	93.33	92.22	2.09
	Middle	80.00	82.00	79.00	80.33	1.90
	High	93.10	94.10	92.70	93.30	0.77
Dieldrin	Low	90.00	90.00	93.33	91.11	2.11
	Middle	97.00	102.00	106.00	101.70	4.44
	High	101.30	99.90	99.50	100.20	0.94
Endrin	Low	96.67	90.00	93.33	93.33	3.57
	Middle	94.00	93.00	89.00	92.00	2.88
	High	108.80	90.80	94.70	98.10	9.65
<i>p,p</i> -DDT	Low	96.67	90.00	96.67	94.44	4.08
	Middle	94.00	99.00	107.00	100.00	6.56
	High	95.40	103.90	106.70	102.00	5.77
<i>p,p</i> -DDD	Low	96.67	93.33	90.00	93.33	3.57

Table 3 (continued)

Pesticides	Spiked grade	Recovery (%)			Recovery mean (%)	RSD (%)
		1	2	3		
<i>o,p</i> -DDT	Middle	98.00	100.00	103.00	100.30	2.51
	High	103.50	107.70	113.70	108.30	4.73
	Low	93.33	90.00	96.67	93.33	3.57
Trifloxystrobin	Middle	96.00	95.00	91.00	94.00	2.81
	High	100.00	100.70	100.40	100.40	0.35
	Low	90.00	93.30	86.67	90.00	3.70
Fenhexamid	Middle	104.00	108.00	115.00	109.00	5.11
	High	108.50	109.00	103.30	106.90	2.95
	Low	80.00	76.67	83.33	80.00	4.17
Carbosulfan	Middle	88.00	89.00	85.00	87.33	2.38
	High	91.10	87.90	92.10	90.37	2.43
	Low	90.00	93.33	90.00	91.11	2.11
Cypermethrin	Middle	101.00	109.00	108.00	106.00	4.11
	High	100.50	98.90	98.10	99.17	1.23
	Low	93.33	96.67	90.00	93.33	3.57
Difenoconazole	Middle	101.50	104.00	103.30	102.90	1.25
	High	104.50	105.10	105.30	104.90	0.40
	Low	90.00	90.00	93.33	91.11	2.11
Azoxystrobin	Middle	101.00	105.00	100.80	102.30	2.32
	High	102.30	104.20	103.00	103.20	0.93
	Low	83.33	80.00	80.00	81.11	2.37
	Middle	89.00	91.00	91.00	90.33	1.28
	High	97.50	97.50	96.40	97.13	0.65

^a Spike level 30 µg/kg.^b Spike level 100 µg/kg.^c Spike level 1000 µg/kg.

Table 4

LOD (limit of detection), LOQ (limit of quantification) and MRLs (multi residue limits) of pesticides in ginseng.

Pesticides	LOD (µg/kg)	LOQ (µg/kg)	MRLs (µg/kg)		
			Ginseng	Red-gin	Dried-gin
EBDC	21.0	70.0	300	–	–
Cadusafos	0.16	0.53	50	–	200
α-HCH	0.61	2.04	10	50	50
β-HCH	0.88	2.92	10	50	50
Quintozene	2.37	7.90	100	500	500
γ-HCH	1.23	4.08	10	50	50
Tefluthrin	0.29	0.97	100	–	–
Chlorothalonil	0.87	2.92	100	–	–
Tebupirimfos	0.40	1.32	10	–	–
δ-HCH	0.64	2.13	10	50	50
Tolclofos-methyl	0.12	0.40	300	500	500
Metalaxyl	1.17	3.92	500	500	500
Diethofencarb	2.83	9.45	300	300	300
Aldrin	0.84	2.79	10	50	50
Cyprodinil	0.16	0.55	2000	2000	2000
Tolyfluanid	0.73	2.45	200	10	200
Flutolanil	9.93	33.1	1000	–	–
Fludioxonil	0.43	1.45	500	1000	1000
<i>p,p</i> -DDE	0.16	0.52	10	50	50
Thifluzamid	0.18	0.61	1000	2000	2000
Kresoxim-methyl	1.53	5.11	200	100	1000
Dieldrin	0.57	1.89	10	50	50
Endrin	0.77	2.57	10	50	50
<i>p,p</i> -DDT	0.15	0.52	10	50	50
<i>p,p</i> -DDD	0.04	0.15	10	50	50
<i>o,p</i> -DDT	0.34	1.15	10	50	50
Trifloxystrobin	0.30	0.99	100	200	200
Fenhexamid	1.07	33.6	300	300	300
Carbosulfan	0.41	1.37	100	200	500
Cypermethrin	3.91	13.0	100	100	100
Difenoconazole	1.73	5.78	200	200	200
Azoxystrobin	0.67	2.23	500	500	500

2007). Therefore, the LOD of this study was quite lower than those of previous ones. The LOD and LOQ of ethylenebis-dithiocarbamate were higher than those of other pesticides. For most of the compounds, the LOQs were lower than their MRLs, as shown in Table 4.

3.2. Pesticides levels in ginseng

The validated method was applied to the routine pesticide analysis of ginseng samples. Fresh ginseng ($n = 118$), red ginseng

Table 5
Pesticides levels detected in fresh ginseng ($n = 118$).

Pesticides	Mean($\mu\text{g/kg}$)	Frequency (%)	Detection range($\mu\text{g/kg}$)
EBDC	N.D.	N.D.	N.D.
Cadusafos	N.D.	N.D.	N.D.
α -HCH	N.D.	N.D.	N.D.
β -HCH	N.D.	N.D.	N.D.
Quintozene	21.15	28.8	20.15–23.25
γ -HCH	N.D.	N.D.	N.D.
Tefluthrin	7.34	0.9	7.34
Chlorothalonil	132.1	0.9	132.1
Tebupirimfos	N.D.	N.D.	N.D.
δ -HCH	N.D.	N.D.	N.D.
Tolclofos-methyl	34.62	86.4	18.25–404.5
Metalaxyl	17.62	40.7	10.25–36.62
Diethofencarb	27.62	4.0	26.25–28.62
Aldrin	N.D.	N.D.	N.D.
Cyprodinil	16.26	24.6	15.62–24.25
Tolyfluanid	N.D.	N.D.	N.D.
Flutolanil	38.63	35.6	26.92–65.62
Fludioxonil	56.62	49.2	7.69–56.29
<i>p,p</i> -DDE	N.D.	N.D.	N.D.
Thifluzamid	20.63	6.8	7.20–47.29
Kresoxim-methyl	N.D.	N.D.	N.D.
Dieldrin	N.D.	N.D.	N.D.
Endrin	N.D.	N.D.	N.D.
<i>p,p</i> -DDT	N.D.	N.D.	N.D.
<i>p,p</i> -DDD	N.D.	N.D.	N.D.
<i>o,p</i> -DDT	N.D.	N.D.	N.D.
Trifloxystrobin	28.26	1.7	20.29–36.29
Carbosulfan	104.6	18.6	25.62–213.4
Fenhexamid	21.22	8.5	15.14–31.38
Cypermethrin	122.3	4.2	90.93–147.3
Difenoconazole	32.73	31.4	25.25–82.29
Azoxystrobin	18.36	3.4	4.29–34.17

Table 6
Pesticides levels detected in red ginseng ($n = 24$).

Pesticides	Mean($\mu\text{g/kg}$)	Frequency (%)	Detection range ($\mu\text{g/kg}$)
EBDC	N.D.	N.D.	N.D.
Cadusafos	N.D.	N.D.	N.D.
α -HCH	N.D.	N.D.	N.D.
β -HCH	N.D.	N.D.	N.D.
Quintozene	36.23	42.1	25.14–114.3
γ -HCH	N.D.	N.D.	N.D.
Tefluthrin	N.D.	N.D.	N.D.
Chlorothalonil	N.D.	N.D.	N.D.
Tebupirimfos	N.D.	N.D.	N.D.
δ -HCH	N.D.	N.D.	N.D.
Tolclofos-methyl	51.14	91.7	13.14–119.4
Metalaxyl	16.15	47.4	13.15–23.35
Diethofencarb	34.17	15.8	32.15–37.98
Aldrin	N.D.	N.D.	N.D.
Cyprodinil	29.25	79.0	19.38–115.2
Tolyfluanid	N.D.	N.D.	N.D.
Flutolanil	44.14	26.3	32.14–71.38
Fludioxonil	56.25	57.9	71.18–79.46
<i>p,p</i> -DDE	N.D.	N.D.	N.D.
Thifluzamid	20.25	6.8	7.16–47.62
Kresoxim-methyl	N.D.	N.D.	N.D.
Dieldrin	N.D.	N.D.	N.D.
Endrin	N.D.	N.D.	N.D.
<i>p,p</i> -DDT	N.D.	N.D.	N.D.
<i>p,p</i> -DDD	N.D.	N.D.	N.D.
<i>o,p</i> -DDT	N.D.	N.D.	N.D.
Trifloxystrobin	N.D.	N.D.	N.D.
Carbosulfan	113.11	4.2	113.11
Fenhexamid	109.35	4.2	109.35
Cypermethrin	N.D.	N.D.	N.D.
Difenoconazole	54.64	57.9	33.62–234.1
Azoxystrobin	26.24	4.2	26.24

($n = 24$), and dried ginseng ($n = 10$) were analysed following the sample preparation method described above. Detailed data on the

Table 7
Pesticides levels detected in dried ginseng ($n = 10$).

Pesticides	Mean ($\mu\text{g/kg}$)	Frequency (%)	Detection range ($\mu\text{g/kg}$)
EBDC	N.D.	N.D.	N.D.
Cadusafos	N.D.	N.D.	N.D.
α -HCH	N.D.	N.D.	N.D.
β -HCH	N.D.	N.D.	N.D.
Quintozene	25.14	50.0	25.14
γ -HCH	N.D.	N.D.	N.D.
Tefluthrin	N.D.	N.D.	N.D.
Chlorothalonil	N.D.	N.D.	N.D.
Tebupirimfos	N.D.	N.D.	N.D.
δ -HCH	N.D.	N.D.	N.D.
Tolclofos-methyl	523.1	87.5	23.15–3673
Metalaxyl	N.D.	N.D.	N.D.
Diethofencarb	33.14	10.0	33.14
Aldrin	N.D.	N.D.	N.D.
Cyprodinil	23.35	75.0	20.14–34.35
Tolyfluanid	N.D.	N.D.	N.D.
Flutolanil	33.35	10.0	33.35
Fludioxonil	71.41	37.5	71.13–71.73
<i>p,p</i> -DDE	N.D.	N.D.	N.D.
Thifluzamid	N.D.	N.D.	N.D.
Kresoxim-methyl	N.D.	N.D.	N.D.
Dieldrin	N.D.	N.D.	N.D.
Endrin	N.D.	N.D.	N.D.
<i>p,p</i> -DDT	N.D.	N.D.	N.D.
<i>p,p</i> -DDD	N.D.	N.D.	N.D.
<i>o,p</i> -DDT	N.D.	N.D.	N.D.
Trifloxystrobin	N.D.	N.D.	N.D.
Carbosulfan	N.D.	N.D.	N.D.
Fenhexamid	N.D.	N.D.	N.D.
Cypermethrin	N.D.	N.D.	N.D.
Difenoconazole	37.32	50.0	35.26–39.52
Azoxystrobin	5.92	10.0	5.92

pesticide levels measured in fresh ginseng are shown in Table 5. We found 16 different pesticides in fresh ginseng samples. Especially, tolclofos-methyl was detected in 102 samples (ranging from 18.25 to 404.5 $\mu\text{g/kg}$). The detection level of fludioxonil was 56.62 $\mu\text{g/kg}$ and detection frequency was 49.2%. The residue level of chlorothalonil was higher than its MRLs, but detection frequency was not very high at 0.9%.

Detailed data of the residue pesticide detected in red ginseng are shown in Table 6. The results show that the most frequently detected pesticide was tolclofos-methyl, which was detected in 22 samples at concentrations ranging from 13.14 to 119.4 $\mu\text{g/kg}$. The detection frequencies of cyprodinil, fludioxonil, and difenoconazole were 79.0%, 57.9% and 57.9%, respectively, which were lower than their MRLs.

Table 7 shows analytical figures of residue levels detected in dried ginseng. The results indicate the existence of quintozone, tolclofos-methyl, cyprodinil, and difenoconazole. The detection frequencies of these pesticides were relatively higher than those of others. The residue level of tolclofos-methyl was higher than its MRL. Khan et al. reported that the concentration of pesticides in ginseng products was below 100 ppb (Khan et al., 2001). Considering that ginseng products are usually dried, our results are similar with the report.

As a summary, the present multiresidue method is a simple GC–MS/MS analytical technique that allows for the simultaneous determination of pesticides commonly found in ginseng. As a result of its high sensitivity and selectivity, GC–MS/MS has shown to have potential in the field of pesticide residue analysis. On the basis of the data, our results suggest a necessity for regular monitoring of ginseng for pesticide residues over long time periods. In addition, an important aspect of this study is its information on pesticide residue contamination of ginseng, which shows the need to perform further monitoring studies to improve food safety.

4. Conclusion

The data obtained in this study indicates the utility of triple quadrupole GC–MS/MS equipment for the detection and quantification of 32 different pesticides. The results show satisfactory validation parameters, such as linearity, precision, accuracy, and LODs. Most pesticides showed high sensitivity and/or good linearity of the calibration curves within the investigated calibration range (0.01–5.00 $\mu\text{g mL}^{-1}$ with $r^2 \geq 0.98$). For the instrument, the LOD values varied from 0.04 to 3.91 $\mu\text{g mg}^{-1}$. The recovery rates were between 80.0–108.3% with very good precision ($\text{RSD} \leq 9.7\%$), except for EBDC (ethylenebis-dithiocarbamate). The proposed method can be recommended for routine application in monitoring studies since it is simple, sensitive, and very useful. The applicability of the optimised method was proven by monitoring of pesticide residues in ginseng samples (fresh ginseng, red ginseng, dried ginseng) collected from local ginseng agricultural cooperative federations. The results show that the concentrations of pesticide residues in most ginseng samples were lower than their respective maximum residue limits (MRLs), except for tolclofos-methyl (dried ginseng). Tolclofos-methyl was detected in all three kinds of ginseng samples. The residue level of this pesticide was higher in comparison with the others.

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