MULTI-RESIDUE ANALYSIS OF PESTICIDES AND POPS IN FRUITS, VEGETABLES AND DRIED GINSENG POWDERS

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Introduction

In recent years a globalized economy has greatly increased the number, type and origin of imported foods to many countries in the world. Pesticide and persistent organic pollutant (POPs) monitoring programs have focused their attention on imports where more frequent violations of tolerances can occur. Analytical methods used by regulatory agencies in the US have often not kept pace with these import changes. The methods are too frequently inefficient and not comprehensive for the array of chemicals that require screening.

Manual preparation procedures that are reported to be easy and more efficient have been proposed[1]. One procedure, called "QuEChERS" (quick, easy, cheap, effective, rugged and safe) has been successfully used with some fruits and vegetables[1]. One of the difficulties with the QuEChERS approach is that the extracts from some foods are not sufficiently purified for some GC based detection modes. A procedure was developed which combines the efficiency of dispersive solid-phase extraction of the "QuEChERS" method[1] and the effectiveness of the procedure developed by Fillion[3] *et al.*2000 to evaluate ~380 pesticides and metabolites in fresh fruits and vegetables.

Wong et al. 2007 demonstrated that this procedure could be used for ~100 organophosphorous pesticides in ginseng as well.[4] Co-extracted interferences produced difficulties with measuring the lowest pesticide fortifications with the commonly used gas chromatography low resolution quadrupole mass spectrometry using selective ion monitoring (GC/LRMS SIM). This prompted our search for alternative approaches for botanicals[4]. We report the development of a second method with dried ginseng for the measurement of ~380 target pesticides and metabolites. We also report the detection of persistent pesticides on ginseng, carrots and organic spinach. The goals of these studies are to develop efficient methods for comprehensive pesticide and POPs screening of all food types and evaluate various detection systems.

Methods

Food Collections

Organic spinach, carrots and oranges were purchased in the Washington DC area from organic grocers. Dried and powdered ginseng samples, *Panax quinquefolius* (American ginseng) and *P. ginseng* (Asian ginseng) were purchased in bulk packages from commercially available sources.

Homogenization

All fresh foods were cryogenically ground in a "robot coup" using dry ice until a frozen powder was obtained. Fresh food was kept at -40^{o}C until use in validation or incurred analysis. Ginsengs were obtained as dried powders or dried roots. Roots were ground in a geno-grinder for 5 min. then stored at -40^{o}C.

Fortification

Organic fruits and vegetables were measured first to determine if they were free of pesticide residues. In quadruplicate, six groups of mixed pesticides standards were used to fortify test portions. Three of the groups (168 pesticides) were intended for GC/MS screening while the other three groups (213 pesticides) were intended for ultra

high pressure liquid chromatography (UPLC)/MS/MS. Carrots, spinach and oranges were fortified in four batches of twelve. One batch was spiked with groups 1&2 for GC/MS, while a 2nd with 1&2 for LC/MS. The third batch was fortified with groups 1&3 for GC/MS and the fourth with groups 1&3 for LC/MS at 10 ppb (25ppb LC/MS), 100 and 1000 ppb (500 ppb LC/MS). Internal standards were added at extraction for modified QuEChERS. Tris trichloropropane phosphoric acid and tri-phenyl phosphate (TPP) were added for GC/MS. Bis (4-nitrophenyl) urea, 4-bromo-3,5-dimethylphenyl-N-methylcarbamate, fluconazole and carbanilide were added for LC/MS. Just prior to GC/MS, deuterated acenaphthalene, phenanthracene, chrysene (PAHs), were added and benzanilide just prior to LC/MS determinations. Ginseng test portions were fortified with all six pesticides groups in each test portion at 25ppb, 100, 500ppb. Internal standards were added just prior to GC(LC)/MS (PAHs and TPP for GC/MS and benzanilide for LC/MS).

Matrix matched standards

A set of 12 test portions of pesticide free produce or ginseng was extracted and purified in an identical way to the fortified test portions except the pesticide mixed standards were added at the end of the preparation to give matrix standards at 2.5, 5, 12.5, 25, 50, 100, 250, 500, 1250, 2500, 5000 and 10,000 ppb for each pesticide for GC/MS or 1.25, 2.5, 5, 12.5, 25, 50, 100, 250 and 500 ppb for UPLC/MS/MS. Each matrix standard contained the PAHs and TPP at 1 ppm for GC/MS and benzanilide at 0.5 ppm for LC/MS/MS.

Fresh food method

A set of twelve 15 g test portions were weighed into 50 mL disposable polypropylene centrifuge tubes. Internal standards were added followed by pesticide standard groups 1, 2 or 3 in acetonitrile (ACN). Fifteen mL ACN was added and the mixture shaken for 10 sec. A mixture of 6 g $MgSO_4$ and 1.5 g NaCl was added and the mixture shaken for 2 min. The tubes were centrifuged for 5 min. at 4500 rpm. The supernatant was transferred to 12 centrifuge tubes containing 0.5 g C_{18} silica gel and 1.2 g $MgSO_4$, shaken for 2 min. and centrifuged (not done for LC/MS extractions). The supernatant was transferred to 12 tubes containing 0.4 g PSA, 0.2 g carbon and 1.2 g $MgSO_4$ (both GC and LC/MS). The mixture was vortexed for 5 sec. then sufficient toluene was added to make the extract 25%. The mixture was shaken for 2 min. then centrifuged. One mL of the extract was evaporated to dryness for UPLC/MS/MS analysis while 8 mL was evaporated for GC/MS analysis. The LC/MS portion was reconstituted in 1 mL 50%ACN/10mM $NH_4C_2H_3O_2$ containing 0.5 ppm benzanilide. The GC/MS portion was reconstituted in 1 mL toluene containing 1 ppm PAH internal standards.

Ginseng method

A set of twelve 2 g test portions of ginseng were weighed into 50 mL centrifuge tubes. Each tube was fortified with mixed pesticide standards (groups 1-6). Twenty-five mL of ethyl acetate was added along with two stainless steel ball bearings. The tubes were placed into a geno-grinder and shaken for 5 min. at 1100 strokes per min. The tubes are centrifuged and decanted and the extraction was repeated. After pooling, the extracts were evaporated on a Labconco rapid-vap to ~1 mL and then reconstituted to 5 mL in 70/30 ethyl acetate/cyclohexane and filtered through 0.2 µm PTFE filter. The extracts were loaded on a J2 Scientific Accuprep GPC with a 70/30 ethyl acetate/cyclohexane mobile phase and a 24g S-X3 biobead express column. The first 42.5 mL was discarded and the next 50 mL was collected for evaporation on the rapid-vap to 2 mL. The extracts were applied to pre-washed PSA/carbon SPE columns with 3 mL ethyl acetate/cyclohexane. The columns were eluted with 13 mL of 75% ethyl acetate/toluene. The column effluent was split with 12 mL going to GC/MS and 4 mL for LC/MS determinations.

GC/MS analysis

An Agilent 6890N gas chromatograph was equipped with an Agilent 5975 mass selective detector (MSD, Agilent Technologies, Little Falls, DE) and fitted with a deactivated guard column (5 m x 0.25 mm I.D) and HP-5MS column (30 m x 0.25 mm I.D. x 0.25 µm film thickness, Agilent Technologies) conditions are as described by Wong et al[4]. The MSD system was programmed in SIM mode using one target and two or three qualifier ions. The second

GCMS system used was a Waters Corporations GCT Premier interfaced with an Agilent Technologies 6890N gas chromatograph and fitted with a deactivated guard column (5 m x 0.25 mm I.D., Restek Corporation) and HP-5MS column (30 m x 0.25 mm I.D. x 0.25 μm film thickness, Agilent Technologies). Test samples, standards and blanks were injected (1 μL) into the GC in splitless mode at 280 °C using an Agilent 7683 series autoinjector. The GCT operated in EI mode with source temperature 200 °C and was scanned at a rate of 4 scans/sec with dynamic range enhancement(DRE) turned on in lock mass mode with m/z 265.9964 lock mass from tris 2,4,6-trifluromethyl 1,3,5 triazine. Waters Corporations Targetlynx method processed the raw data files using three accurate mass ions (one target and two qualifiers).

Table 1. Recoveries for carrots at 0.01 ppm (0.025 ppm LC/MS) (GC/MS used split extracts) using modified QuEChERS (na = not analyzed). Table values are %recovery ± standard deviation (Quan ion)

	GC/HR-TOFMS	GC/LRMS SIM	UPLC/MS/MS
mevinphos	98±13 (127)	99±3 (192)	95±10 (225→193)
methamidophos	na	na	76±10 (141 → 94)
β-BHC	109±3 (181)	99±5 (217)	na
γ-BHC	102±3 (181)	105±7 (219)	na
bendiocarb	na	na	90±13 (224 →109)
Fenuron	na	na	96±3 (165→72)
Pentachloroaniline	76±1 (265)	77±2 (265)	na
Quintozene	95±10 (265)	95±5 (295)	-
Thiabendazole	na	na	74±19 (202 →175)
Endrin	98±6 (317)	94±7 (345)	na
Hexachlorobenzene	42±3 (284)	63±3 (284)	na
Heptachlor	102±4 (272)	101±4 (272)	na
Diazinon	109±2 (304)	107±3 (304)	na
Aldrin	64±4 (263)	93±2 (263)	na
Dieldrin	118±9 (263)	99±6 (380)	na
p,p'-DDD	98±6 (235)	97±2 (235)	na
p,p'-DDE	112±6 (246)	89±3 (246)	na
p,p'-DDT	109±4 (235)	105±4 (235)	na
Transchlordane	72±4 (373)	77±3 (373)	na
Mirex	75±4 (272)	78±5 (272)	na
Azoxystrobin	na	na	99±4 (403 → 388)
Avermectin B_{1a}	na	na	97±16 (891 →568)

UPLC/MS/MS

A Waters Corporation Quattro Micro Premier operated in electrospray positive or negative ion mode was interfaced with a Waters Corporation UPLC fitted with an Acquity UPLC BEH C_{18} column (2.1 mm id x 100 mm with 1.7 μm particle size). A gradient was programmed from 90% 10mM $NH_4C_2H_3O_2$ /ACN to 90% ACN in 15 min. Test samples were injected (3μL)using a Waters Corporation sample injector. Each test sample was measured separately for either pesticide groups 1, 2 or 3 by monitoring two transitions from the pseudo molecular ions. Cone voltage and collision energy were optimized for each pesticide.

Results and Discussion:

Recoveries for pesticides were in the range of 60-130% for ~90% of the pesticides at all three fortification levels analyzed by GC/LRMS SIM, gas chromatography high resolution time-of-flight mass spectrometry (GC/HR-TOFMS) or UPLC/MS/MS. The recoveries for selected pesticides in carrots fortified at 10 ppb by the two GC/MS

instruments and UPLC/MS/MS are shown in table 1. Carbon always reduced hexachlorobenzene recoveries somewhat (Table 1), but was usually acceptable at ~65% (e.g. 71 & 82% for HCB in ginseng 25 ppb LRMS and TOFMS respectively). The standard deviations for repeat determinations were below 15% (Tables 1&2). Both fresh food and ginseng extracts were clean enough for determination by all three detection systems without interference. Limits of determination (LODs) for most pesticides were below the 10 ppb fortifying level. The dynamic ranges for both UPLC/MS/MS (1.25- ~500ppb) and GC/HR-TOFMS (2.5- ~500 ppb) were often reduced at the high concentration compared to GC/LRMS SIM, but extended to lower concentrations for many pesticides (Table 2). GC/HR-TOFMS provided full scan spectra which permitted circumventing interferences found in some foods for some pesticides by GC/LRMS SIM. LODs for GC/HR-TOFMS were comparable to the standard GC/LRMS SIM method. Ginsengs were found to have several pesticides including the POPs aldrin, hexachlorobenzene, pp´ DDT, as well as pp´ DDE, azoxystrobin, BHCs, diazinon, quintozene and its transformation products (Table 2). A sample of organic spinach and a conventional carrot were found to contain p,p'-DDE and p,p'-DDT (Table 2).

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Table 2. Residues found in ginseng, carrots and organic spinach (µg/kg)(n = 3;na = not analyzed; nd = not detected)

Ginseng sample #1	GC/HR-TOFMS	GC/LRMS SIM	UPLC/MS/MS
Azoxystrobin	-	-	7±0.3
Diazinon	18±1	19±1	na
Ginseng Sample #2			
p,p'-DDE	450±20	460±11	na
p,p'-DDT	86±3	96±4	na
p,p'-DDD	13±0.5	16±0.3	na
Ginseng Sample #3			
Pentachloroaniline	310±7	290± 29	na
Pentachlorothioanisole	73±1	68±3	
Quintozene	420±20	430± 10	na
γ-BHC	130±3	na	na
Hexachlorobenzene	59±0.9	55±1	na
Aldrin	1.9±0.4	nd	na
β-BHC	49±4	na	na
δ-BHC	309±3	na	na
Organic spinach			
p,p'-DDE	18±3	12±2	na
p,p'-DDT	2.6±1	nd	na
Carrots			
p,p'-DDE	34±2	45±3	na
p,p'-DDT	28±2	53±2	na

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