Bull Environ Contam Toxicol (2012) 89:1057–1061
DOI 10.1007/s00128-012-0818-1

Multiresidue Determination of Organophorous Pesticides in Camellia Oil by Matrix Solid-Phase Dispersion Followed by GC-FPD

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Received: 15 June 2012 / Accepted: 31 August 2012 / Published online: 21 September 2012

Abstract A novel analytical approach has been developed and evaluated for the quantitative analysis of 15 organophorous pesticides residues in camellia oils. The proposed methodology is based on acetonitrile/water (3:1, V/V) extraction, followed by matrix solid-phase dispersion, using aminopropyl as dispersant material. Then gas chromatography-flame photometric detection was applied for the pesticide residue analysis. The optimal sorbent quantity was studied. The results demonstrated that the method achieved acceptable quantitative recoveries of 71.5 %–104.2 % with relative standard deviations <19 %, and the method limit of detection at or below the regulatory maximum residue limits for the pesticides were achieved.

Keywords Pesticide · Camellia oil · Residue · MSPD

Camellia oil is a concentrated, hydrophobic liquid containing volatile aromatic compounds extracted from *Camellia oleifera*. Camellia oil is popularly used in food processing and cooking. The camellia oil in China was about 270,000 tons per year, and the production is increased year after year. Studies have shown that camellia oil with high content of monounsaturated fatty acids and antioxidative substances is beneficial to human health (Zhang et al. 2011), and camellia oil rivals olive oil in nutrition (Li et al. 2011). Currently, it is conventional practice for *C. oleifera* growers to apply pesticides (mostly organophorous pesticides) to control diseases and pests. The presence of pesticide residues in camellia oil is one possible risk source for consumers, due to their possible long adverse health effects (Kojima et al. 2011; Moser 2011). Thus many countries, China being no exception, have established Maximum Residue Limits (MRLs) for camellia oil. So it is necessary to monitor and control residual levels in camellia oil in order to meet regulatory requirements and protect the consumer and the environment. However, vegetable oil, such as camellia and olive oil, is well known to be difficult to analyze because of the high amounts of fats (>95 % triglycerides) that co-extract with the analyte thus tends to adsorb in injection port and column, resulting in poor chromatographic performance (Sobhanzadeh et al. 2011). Therefore sample preparation is a key step in the analytical procedure since even small amount of lipids can harm columns and detectors or cause signal suppression.

The most commonly used methodology for fatty samples is based on gel permeation chromatography (GPC) (Cavaliere et al. 2008). GPC represents much analysis time and is typically bottleneck of the analytical procedure, and furthermore large amount of organic wastes are produced that require safe disposal. However, the practical needs for an appropriate pesticide control are mainly focused on simple and fast sample treatment methods that may be easily implemented in routine laboratories. Matrix solid-phase dispersion (MSPD) has been found, in many cases, to provide equivalent or superior results comparatively to older official methods conducted by more classical countercurrent extraction and/or SPE techniques (Perez-Parada et al. 2011; Mu et al. 2012; Zhang et al. 2012). Further, it has been rather consistently observed that MSPD requires approximately 95 % less solvent and can be performed in 90 % less time when compared to such classical methods. The use of smaller sample sizes, combined with lower solvent consumption, purchase and disposal, make MSPD

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competitive with such methods on several levels and should be considered as an alternative when developing new analytical protocols. Several papers have reported the pesticide residue determination method for olive oil (Benincasa et al. 2011), soybean oil (Dong et al. 2010), palm oil (Zainudin et al. 2009), etc. There has no any report about pesticide residue determination for camellia oil. The work described in this paper was focused on the development and evaluation of a simple and rapid multiresidue method based on MSPD as the cleanup technique, followed by capillary GC with flame photometric detection (FPD) for the determination of pesticide residues in camellia oil.

Materials and Methods

Pesticide analytical standards were purchased from National information center for Certified Reference Materials (Beijing, China), certified quality. Individual pesticide stock solution (100 mg/L) were prepared in methanol or acetonitrile and stored at −20°C. Then, a working solution containing the mixture of standards was prepared (10 mg/L) in methanol and also freezed. HPLC-grade acetonitrile and methanol were obtained from Merck (Darmstadt, Germany). A Milli-Q-Plus ultrapure water system from Millipore (Milford, MA, USA) was used throughout the study to obtain the HPLC-grade water used during the analyses. Other solvents were from Shanghai Sanying Chemical Reagents (Shanghai, China), pesticide residue analysis quality. Aminopropyl (PSA) was from Agela (Agela, USA).

5 g oil sample was weighed in a 50 mL plastic centrifuge tube and 20 mL of acetonitrile/water (3:1) were added. The sample was homogenized by a high-speed blender for 1 min. After addition of 3 g of NaCl and 5 g of $MgSO_4$, the mixture was shaken by hands for 2 min to separate aqueous and organic phase. Then 12 mL of the organic phase was collected and evaporated to near 0.5 mL. The extract was transferred to a glass mortar, where it was gently blended and homogenized together with 2 g of PSA until obtaining a fine powder. This mixture was then transferred to a commercially available minicolumn. The column was washed with 5 mL of acetonitrile. The final elution was evaporated until near dryness, being then dissolved in 0.5 mL of acetone. Prior to analysis, the obtained extract was filtered through a 0.45 μm PTFE filter (Milford, MA, USA).

Organophorous pesticides were determined by FPD in the phosphorus mode. Pesticide residue analysis was conducted with a Agilent 6890 N gas chromatograph equipped with a programmed temperature vaporization injector, a Agilent AOC-20i autosampler, and Agilent flame photometric detector. The injector and detector temperature was 250°C. Nitrogen was used as the carrier gas at a constant flow rate of 3.0 mL/min. The flow rates of air and hydrogen were 450 and 40 mL/min, respectively. The makeup flow was 45 mL/min. Injection volume was 1.0 μL. OP pesticides were determined with a ZB-17 silica capillary column (30 m × 0.32 mm id, 0.25 μm film thickness). The temperature of the column oven was programmed from an initial value of 100–150°C at 5°C/min (3 min hold), and then to 200°C at 5°C/min (10 min hold), finally to 250°C at 10°C/min (5 min hold).

For recovery studies, the samples were spiked with the studied pesticides before the corresponding extraction procedure. A representative 50 g portion of camellia oil was weighted and fortified homogeneously with appropriate volume of working standard solution. The mixture was then gently blended in the mortar for 1 h, to assess the homogeneity of the sample. Then the sample was incubated at room temperature for 24 h, to make sure the solvent was completely evaporated. Then the samples were dealed with the method described above.

Results and Discussion

Figure 1 shows typical gas chromatograms of standards for the 15 pesticides. Standard calibration curves were obtained by plotting analyte concentrations against the peak area. Linearity of the GC-FPD method was tested with standard mixtures at five concentration levels (n = 3) in the range 10–1,000 ng/mL for each compound. Correlation coefficients above 0.995 were obtained for all of the compounds. Relative standard deviations (RSD%) for n = 5 consecutive injections of a standard, containing all species at the 100 ng/mL level (precision within days), ranged from 1.3 to 4.5 were obtained.

According to Rodrigues et al. (2010), the ratio between the sample and the sorbent is extremely important for the success of the method. The best ratio guarantees that the sample is totally homogenized and dispersed in the sorbent, making it easier to transfer to the column of MSPD and decrease sample loss in this process. In this study, ratios of 1:1, 1:2, 1:3, 1:4 and 1:6 (sample:PSA) were evaluated, using 0.5 mL of the extracted solvent. The results are shown in Fig. 2. Ratios 1:3 and 1:4 presented better recovery (R%) than other ratios, with relative standard deviation (RSD) lower than 20 % for all compounds. However, the R values from ratio 1:3 for paraoxon and methidathion were beyond 110 %, which might result in overestimation effect for pesticide residue determination. Therefore, 2.0 g PSA was chosen as the optimal sorbent quantity.

The instrumental limits of detection (LOD) for the studied pesticides were determined as the lowest concentration giving responses of 3 times the average of the baseline noise, and ranged from 13 to 61 ng/g. The limits of quantification

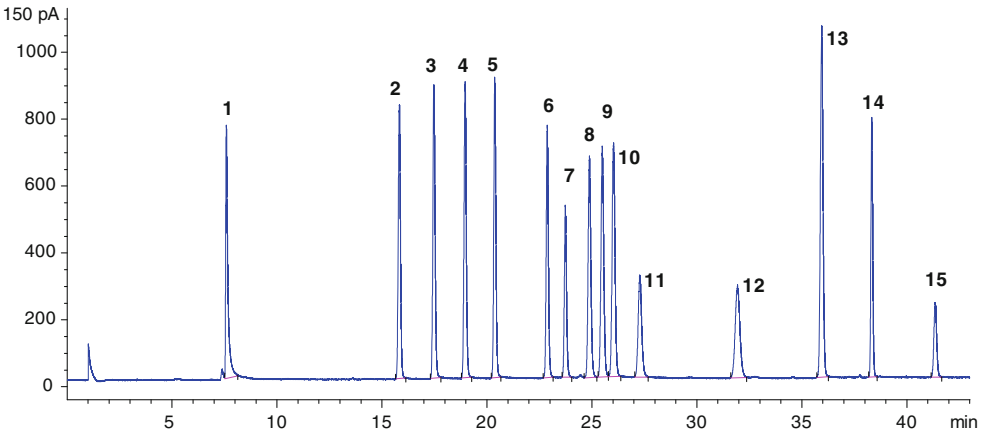

Fig. 1 Gas chromatogram of the 15 OPs standards. *1* Methamidophos, *2* Ethoprophos, *3* Phorate, *4* Terbufos, *5* Disulfoton, *6* Fenchlorphos, *7* Paraoxon, *8* Parathion, *9* Ethyl pirimiphos, *10* Fenthion, *11* Isocarbophos, *12* Methidathion, *13* Ethion, *14* Triazophos, *15* Phosmet

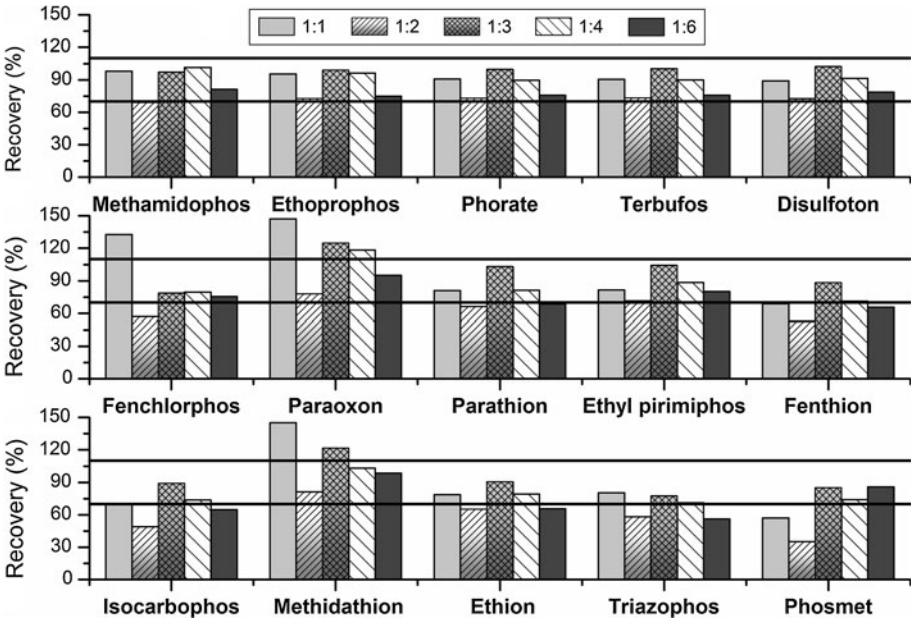

Fig. 2 Mean recovery (R%) and RSD (%) of the pesticide using different sorbents. The *two horizontal lines* represented the R values were 70 % and 110 %, respectively

(LOQ) were determined as the lowest concentration giving a response of 10 times the average of the baseline noise. The LOQ values for these pesticides ranged from 43 to 202 ng/g. These data are summarized in Table 1.

Recovery studies were performed by spiking oil samples with composite working standard solution at two concentration levels. Three replicates were carried out at each spiking level to determine the mean recovery and RSD. Most of the observed RSDs of the analyzed samples were in general lower than 10 % that could be attributed to the experimental error. Obtained results from mean recoveries and RSD for all pesticides at two concentration levels are shown in Table 1. For all compounds in all samples, mean recoveries lie within an acceptable range, from 71.5 % to 104.2 % with RSD values from 4.0 % to 18.6 %. The method LOD for 11 OPs was 10 ng/g. This value for other pesticides, paraoxon, isocarbophos, methidathion and phosmet was 25 ng/g. The method LOD levels are considerably low since they are far below the maximum residue level regulations established for selected pesticides in this study. These results demonstrate the high sensibility of the proposed method based on MSPD and GC-FPD for the detection and quantification of the selected pesticides in camellia oil.

In conclusion, a method based on MSPD-GC-FPD for the trace analysis of 15 OPs in camellia oil has been developed. This method showed satisfactory validation parameters, such as accuracy, precision and lower limits of detection. For all of the pesticides, the sensitivity of the method was good enough to ensure reliable This method showed satisfactory validation parameters, such as accuracy, precision and lower limits of detection. For all of the pesticides, the sensitivity of the method was good enough to ensure reliable determination at levels lower than the respective MRLs. In addition, the procedure was simple and rapid and required only small samples and volumes of solvent. Less solvent waste supports in general efforts to decrease environmental pollution.

Table 1 Recoveries and precision (RSD), LOD and LOQ values of 15 OPs in spiked camellia oil samples

Pesticide	Spiked (ng/g)	Measured (ng/g)	RSD (%)	Recovery (%)	LOD (ng/g)	LOQ (ng/g)	MRL (ng/g)
Methamidophos	10	9.8	18.6	97.7	18	60	10
	100	85.8	7.4	85.8			
Ethoprophos	10	8.0	7.1	79.7	17	55	20[a]
	100	75.3	11.9	75.3			
Phorate	10	9.6	12.5	96.0	15	52	50[a]
	100	96.1	12.7	96.1			
Terbufos	10	10.4	7.9	103.5	15	51	50[a]
	100	97.9	11.2	97.9			
Disulfoton	10	8.8	4.0	88.3	15	50	–[b]
	100	94.2	4.2	94.2			
Fenchlorphos	10	7.7	7.4	76.7	18	60	–[b]
	100	83.8	12.5	83.8			
Paraoxon	25	23.2	14.5	92.8	26	88	–[b]
	100	104.2	9.9	104.2			
Parathion	10	8.5	5.5	85.3	20	68	10
	100	89.7	8.5	89.7			
Ethyl pirimiphos	10	10.0	11.0	99.7	20	66	5,000[c]
	100	93.0	4.1	93.0			
Fenthion	10	7.2	9.1	72.0	19	65	10
	100	71.5	9.6	71.5			
Isocarbophos	25	23.8	6.5	95.3	45	148	20[c]
	100	89.6	4.2	89.6			
Methidathion	25	20.7	7.6	82.9	50	165	2,000[c]
	100	85.7	7.2	85.7			
Ethion	10	7.7	12.5	76.7	13	43	500[b]
	100	80.9	10.8	80.9			
Triazophos	10	7.5	8.8	75.4	17	58	100[b]
	100	87.0	7.5	87.0			
Phosmet	25	18.4	6.5	73.7	61	202	50[b]
	100	81.1	6.1	81.1			

The MRL values were set by Chinese Ministry of Agriculture

[a] The MRLs from other oils or theirs nuts, for example, peanut oil or peanut

[b] That there is no MRL for the corresponding pesticide

[c] The MRLs from other vegetables or fruits

The present study provides significant data on method development for pesticide residue determination in high fatty samples, especially oil samples.

Acknowledgments This work was supported by the Applied Research Project in the Public Interest of Zhejiang Province (2012C22090) and Special Fund for Forestry Scientific Research in the Public Interest (201204414).

References

Benincasa C, Perri E, Iannotta N, Scalercio S (2011) LC/ESI-MS/MS method for the identification and quantification of spinosad residues in olive oils. Food Chem 125:1116–1120

Cavaliere B, Macchione B, Sindona G, Tagarelli A (2008) Tandem mass spectrometry in food safety assessment: the determination of phthalates in olive oil. J Chromatogr A 1205:137–143

Dong NT, Hee LM, Ho LG (2010) Rapid determination of 95 pesticides in soybean oil using liquid–liquid extraction followed by centrifugation, freezing and dispersive solid phase extraction as cleanup steps and gas chromatography with mass spectrometric detection. Microchem J 95:113–119

Kojima H, Sata F, Takeuchi S, Sueyoshi T, Nagai T (2011) Comparative study of human and mouse pregnane X receptor agonistic activity in 200 pesticides using in vitro reporter gene assays. Toxicology 280:77–87

Li H, Zhou GY, Zhang HY, Liu JA (2011) Research progress on the health function of tea oil. J Med Plant Res 5:485–489

Moser VC (2011) Age-related differences in acute neurotoxicity produced by mevinphos, monocrotophos, dicrotophos, and phosphamidon. Neurotoxicol Teratol 33:451–457

Mu GF, Liu HT, Xu LN, Tian LH, Luan F (2012) Matrix solid-phase dispersion extraction and capillary electrophoresis determination of tetracycline residues in milk. Food Anal Meth 5:148–153

Perez-Parada A, Colazzo M, Besil N, Geis-Asteggiante L, Rey F, Heinzen H (2011) Determination of coumaphos, chlorpyrifos and ethion residues in propolis tinctures by matrix solid-phase dispersion and gas chromatography coupled to flame photometric and mass spectrometric detection. J Chromatogr A 1218: 5852–5857

Rodrigues SA, Caldas SS, Primel EG (2010) A simple; efficient and environmentally friendly method for the extraction of pesticides from onion by matrix solid-phase dispersion with liquid chromatography-tandem mass spectrometric detection. Anal Chim Acta 678:82–89

Sobhanzadeh E, Abubakar NK, Binabas MR, Nemati K (2011) An efficient extraction and clean-up procedure for pesticide determination in olive oil. Eur J Lipid Sci Technol 113:862–869

Zainudin BH, Abubakar NK, Muhamad H (2009) Determination of cypermethrin in palm oil matrices. Eur J Lipid Sci Technol 111: 1014–1019

Zhang Y, Chen QP, Luo XW, Dai TT, Lu BQ, Shen JF (2011) Mutagenicity and safety evaluation of the water extract of *Camellia oleifera* Abel. J Food Sci 76:T84–T89

Zhang YP, Xu X, Qi X, Gao WQ, Sun S, Li XT, Jiang CF, Yu AM, Zhang HQ, Yu Y (2012) Determination of sulfonamides in livers using matrix solid-phase dispersion extraction high-performance liquid chromatography. J Sep Sci 35:45–52