ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Application of Solid phase Extraction Cleanup for the Determination of Pesticide Residues in Fresh Food Items Followed by GC-ECD

Abdulnaser Hajisamoh[1]*, Sarimoh Sosanae[2], Nawawi Lemeahmad[2] and Jamilah Abu[2]

[1]Faculty of Science Technology and Agriculture, Yala Rajabhat University, Yala, Thailand
[2]Science and Applied Science Center, Yala Rajabhat University, Yala, Thailand

ABSTRACT

A solid phase extraction (SPE) cleanup procedure was modified and successfully applied for the determination of organochlorine pesticide residues (OCPs) in fresh food items. The samples were extracted with a solvent mixture of dichloromethane and acetone. Sample cleanup was performed by C18 SPE cartridges. The pesticide residues were eluted with (1:1) hexane and petroleum and the eluate was preconcentrated by nitrogen gas before being analyzed by GC-ECD. The recoveries of all target OCP residues were in the range of 74.55 % to 102.4 %. The instrumental limit of detection (LOD) and limit of quantification (LOQ) for OCPs were ranging from 0.005 to 0.035 ng/mL and 0.015 to 0.106 ng/mL, respectively. The present study showed that commonly detected OCPs in analysed samples were HCHs, heptachlor and ensosulfan at wide range concentrations. We found that carrot samples were contaminated with higher levels of HCHs and endosulfan sulfate at the concentration range of 12.36-31.74 ng/mL and 8.20-12.55 ng/mL, respectively. This study provides important information on the current contamination status of fresh food items which widely consumed by local people and point to the action needed for controlling.

Keywords: SPE cleanup, GC-ECD, OCPs, Fresh food items

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Organochlorine pesticides (OCPs) have been used extensively in agricultural activities in the early 1950s. Due to their unique properties of persistence in natural environment, bioaccumulation through food chain, most of these pesticides are not allowed to be used in many countries. However, many developing countries still allow their use in agricultural and public health sector. Because of their highly persistence and lipophilic property, these chemical have led to contamination in the environment, especially food and vegetable that can pose powerful effects to human health and natural environment [1]. A number of toxicity studies reported that these pesticides had caused various ill-effects to human health [2, 3]. Several studies have reported the cleanup methods for the determination of OCP residues in various food items using SPE C18 cartridge with various eluting solvents [3-5].

The SPE cleanup procedure offers several advantages over conventional method of extraction and column cleanup such as efficient isolation, pre-concentration, shorter analysis time, easy automation and minimal volume of solvent use.

Our study was designed to determine the contamination levels of 18 OCPs in various fresh food items using C18 SPE cartridge cleanup in order to remove co-extracts from sample matrix. Some samples contained high sugar, chlorophyll, fats or waxes. Hence, the validation of SPE cleanup procedure for the determinative proposes is essential.

The comprehensive data on organochlorine residues contaminated in fresh food items of the southern area are very scarce, as these types of food items are routinely consumed. This research work would be the significant effort to determine current contamination status of OCPs in fresh food items in Yala province which is very important to the health of consumer.

MATERIALS AND METHODS

Reagents and Glassware

All glassware and chemicals were prepared and maintained properly as stated in the US-EPA method 1664 [6]. Organic solvents and chemicals were of analytical grade and used without further purification. The 1000 mg/L TLC standard mixture of 18 OCPs and C18 endcapped SPE cartridges (1 g sorbent in 6 mL tube) were purchased from Supelco. 200 ng/mL stock solutions were prepared and diluted into several concentration levels of working solutions for method validations and instrument calibrations.

Sample Preparation and Extraction

The selected samples for this study were carrot, cucumber, broccoli, mustard, long bean and auliflower. Extraction was carried out based on the procedure described by Steinwandter [7], with some modification to suit our laboratory. 10 g of sample was homogenised with 200 mL of acetone/dichloromethane (1:1 ratio) and 5 g of sodium chloride in a blender for 3 minutes. The mixture was allowed to separate into organic and aqueous phases in a beaker for 5 minutes. The organic layer was then transferred into a

flask containing 5 g of sodium sulphate to eliminate the remaining water before being cleaned up with C18 SPE cartridge.

SPE Cleanup

In order to obtain good recoveries for OCPs cleanup, several parameters affecting SPE conditions such as solvent polarity, the pressure of SPE manifold and flow rate of eluent were optimised. In this study, we used C18 SPE cartridge for sample cleanup before being analysed by GC. The cartridge was conditioned with 6 mL (1:1, v/v) hexane and petroleum ether. Steps in the SPE process are shown in figure 1

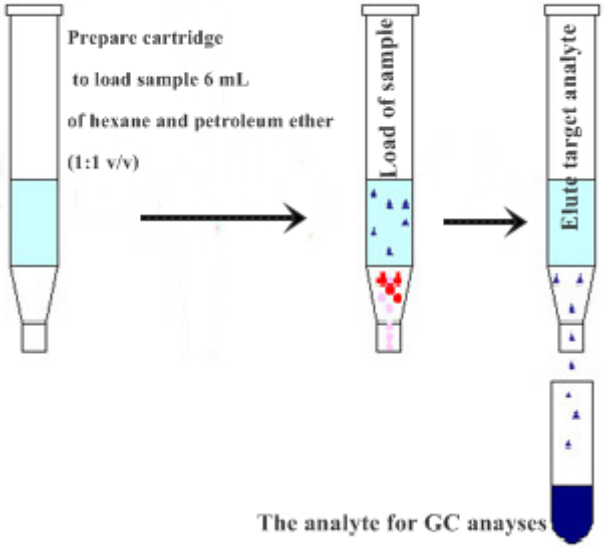

Figure 1: Steps in SPE cleanup for this study

Then, 2 mL of concentrated extract was transferred to SPE cartridge, followed by 6 mL (1:1, v/v) hexane and petroleum ether. The final eluate was then concentrated by purging with nitrogen gas and adjusted the volume to exactly 1 mL before being analysed by GC-ECD.

GC Analysis

A Varian 3600 Cx gas chromatograph equipped with electron captured detector (ECD) was used for the analysis of 18 OCPs. The analytes were separated on SPB-5 capillary column (30 m x 0.25 mm i.d. x 0.25 µm film thickness). The column oven was programmed from 120 ^{o}C (maintained for 1 min) increased to 195 ^{o}C at 15.0 ^{o}C/min (maintained for 0.5 min), then increase to 240 ^{o}C at 4.7 ^{o}C/min and held at final temperature for 4.82 minutes. The temperature of injection port and detector were set at 250 ^{o}C and 300 ^{o}C, respectively.

All data were processed and quantified by external standard of five point calibrations with correlation coefficients of 18 OCPs calibration curves were greater than 0.99. The GC instrumental limit of detection (LOD) for OCPs was ranging from 0.014 to 0.035 ng/mL. In most cases, the recoveries of validated SPE cleanup procedure for 18 OCPs standards mixture were in the range of 74.55 % to 102.4 %, which fall within the acceptable ranges of the US-EPA protocol [8].

ISSN: 0975-8585

Method blank was analyzed with each set of samples to verify the absence of interferences from either sorbent material or eluting solvent used. The standard mixtures of 18 OCPs used for calibration were routinely checked for area counts in order to maintain a proper value of concentration during sample quantifications.

RESULTS AND DISCUSSION

In this study, we found that a mixture of non-polar solvent such as hexane and petroleum ether in 1: 1 ratio (v/v) resulted in good recoveries for all pesticides selected. However, increasing the solvent polarity did not result in good recoveries for all pesticides. This is due to the nature of non-polar OCPs selected in this study. The critical factor to be observed was that SPE cartridge should not be left dry after loading samples since low recoveries may result.

Table 1: The concentration ranges of OCPs (ng/g wet wieght) detected in various samples of fresh food items

OCPs	Broccoli	Cauliflower	Mustard	Cucumber	Long bean	Carrot
ΣHCHs	2.22-9.27 (6.55)	0.95-5.50 (3.00)	1.12-3.44 (2.10)	1.20-5.58 (2.43)	0.12-6.89 (3.10)	12.36-31.74 (18.4)
ΣHeptachlor	0.19-0.39 (0.27)	0.12-0.21 (0.23)	0.53-1.24 (1.02)	1.01-2.22 (1.82)	0.41-1.38 (0.72)	0.12-1.43 (1.23)
ΣAldrin	1.23-1.88 (1.55)	0.98-2.76 (1.75)	ND	ND	1.20-3.29 (1.52)	ND
ΣEndrin	0.30-0.70 (0.32)	0.28-0.36 (0.32)	ND	ND	ND	ND
ΣEndosulfan	0.45*	2.20-9.73 (6.01)	1.87*	1.92-4.42 (2.14)	ND	8.20-12.55 (9.47)
ΣDDT	5.18-16.56 (12.10)	10.81-18.06 (15.53)	ND	1.12-2.18 (1.42)	ND	ND
ΣOCPs	**9.12-28.80 (20.80)**	**15.34-36.62 (26.81)**	**1.65-4.68 (3.12)**	**5.25-14.40 (7.81)**	**1.73-11.56 (5.34)**	**20.68-45.72 (29.38)**

(Mean), **ND**: not detected (below instrument LOD)
ΣHCHs: α-HCH+β-HCH+γ-HCH
ΣHeptachlor: heptachlor+heptachlor epoxide
ΣAldrin: aldrin+dieldrin
ΣEndrin: endrin+endrin aldehyde+endrin ketone
ΣEndosulfan: endosulfanI+endosulfanII+endosulfan sulfate
ΣDDT: p,p'-DDT+p,p'-DDD+p,p'-DDE
(*): detected in one sample only

Our findings showed that the optimal pressure of SPE manifold was -15 inch Hg. The 6 mL eluting solvent (hexane and petroleum ether in 1: 1 ratio by volume) was sufficient to condition the SPE cartridge and elution of target analytes. The optimal flow rate of eluting solvent for SPE cleanup at the optimal pressure was found to be 10 mL/min.

It was found that C18 SPE cartridge removed a substantial amount of matrix co-extractives from vegetable tested as colour intensity of extract were lighter after cleanup. No interfering peaks were observed in the chromatograms obtained from GC analyses. Table 1 summarizes the concentration ranges of individuals and total OCPs detected in fresh food items analysed in the present study

On the average, the analytical results showed that carrot samples were contaminated with total OCPs at the highest concentration level, especially HCHs and endosulfan were detected at the concentration ranges of 12.36-31.74 and 8.20-12.55 ng/g wet weight, respectively. In fact, endosulfan compound is routinely used for vegetable cultivation for long time. Other samples such as broccoli and cauliflower were also contaminated with total OCP residues at higher concentration ranges of 9.12-28.80 and 15.34-36.62 ng/g wet weight, respectively. On the other hand, mustard cabbage and long bean samples were contaminated with total OCPs at the lower concentration ranges.

Among the major OCP residues found in all samples of the present study were HCHs and heptachlor. Furthermore, all carrot samples analysed were contaminated with higher concentration levels of α-HCH residues. γ-HCH residue (lindane), the major isomer of HCHs categorized as the most toxic to human, was detected in one sample of broccoli at the concentration of 0.14 ng/g wet weight. DDTs was widely used for agricultural activities in the past and have been banned from use for long time, but still detected in several samples of cucumber, broccoli and cauliflower at the concentration ranges of 1.12-2.18, 5.18-16.56 and 10.18-18.06 ng/g wet weight, respectively. The p,p'-DDE isomer, the major degrade products of DDTs that categorised as more toxic than parent compound, was detected in cucumber samples at the concentration ranging from 1.68 to 2.08 ng/g wet weight. Based on the analytical results of this study, health risk through dietary intake for long-term hazards posed by these toxic contaminants are estimated to be low [9].

CONCLUSION

The results of the present study showed that C18 SPE cartridge has the potential to be used as cleanup for the determination of OCP residues in fresh food items. The recoveries for the multicomponents mixture of 18 OCPs obtained from SPE cleanup procedure were within the acceptable range of 70 % -130 % as recommended by the US-EPA protocol.

These findings revealed that carrot, cauliflower and broccoli samples were contaminated with higher levels of total OCPs as compared to those detected in long bean, mustard cabbage and cucumber samples. This work also provides an important information in the current contamination of OCPs in fresh food items which widely consumed in this area.

ACKNOWLEDGEMENTS

The author would like to acknowledge the Faculty of Science Technology and Agriculture (FSTA), Yala Rajabhat University (YRU) for funding this research project through short-term grant. The technical assistance rendered by the staff of the Chemistry Program is greatly acknowledged.

REFERENCES

[1] UNEP/GEF. Regionally Based Assessment of Persistent Toxic Substances: South East Asia and South Pacific Regional Report. United Nations, Geneva, Switzerland, 2002.

[2] Crouch MD and Barker SA. J Chromatogr A 1997; 774:287-309.
[3] Ahmad FE. Trend Anal Chem 2003;22:170-185.
[4] Lian Kuet AC and Seng L. Malaysian J Chem 2004;(6)1:039-047.
[5] Fillion J, Sauve F and Slewyn J. J AOAC Inst 2000; 83:698-713.
[6] US-EPA. Method 1664, Engineering and Analysis Division, 1995; pp. 1-24.
[7] Steinwandter H, Fresenius Z. Anal Chem 1985;322:752-754.
[8] US-EPA. Method number 3540A, SW-846; 1996.
[9] http://www.epa.gov/iris. (Retrieved October 8, 2011).