STUDY ON REMOVING COLOR IN THE EXTRACT SOLUTION FROM VEGETABLES FOR ANALYZING ORGANOCHLORIDE PESTICIDES RESIDUE IN HANOI MARKETS

Vu Van Tu, Nguyen Thanh Thao, Nguyen Thi Phuong Thao, Phan Quang Thang, Nguyen Thi Hue

Institute of Environmental Technology, Vietnamese Academy of Science and Technology,

18 Hoang Quoc Viet, Caugiay distr., Hanoi, Vietnam

Abstract

The vegetables and fruit extract solutions was always accompanied by the color in organochlorine pesticides analysis.The samples should be decolozated and reduced impurities before injecting to GC-ECD. Using active silcagel and/or florisil material was applied to the extract solution clean-up process in this paper. Eluted solution, the weigh ratio of silicagel/florisil and the percentage of sulfuric acid were selected. 20 organochlorine pesticides is used for a standard solution. The results showed that the recovery was of 85-110% for 20 organochlorine pesticides when using a mixed silicagel with 10% active carbon (w/w). The color can be fastly reduced by adding 1 or 3 % H2O in Chinese pea and cucumber samples. The highest concentration of pesticides were found in cucumber samples from 13.4 to 32.1 µg/kg and in Chinese pea samples from 21.1 to 36.8 µg/kg at Long Bien market. DDT is detected in the cucumber sample with low level.

Key words: 20 organochloride pesticides, activated carbon, GC-ECD, H_2SO_4, silicagel, florisil, vegetable.

1. Introduction

Vietnam is located in the tropical region. Twenty organochloride (OC) pesticides had been banned from 1990 to 1998 [1]. But these compounds residue is still affected to soil, water and vegetable. Vegetables can contaminated from water and soil using in plant process or other cause from farmer, who used pesticides and pesticides relation in the plant protection. Agricultural chemicals including organochlorine (OC) pesticides such as HCHs and DDTs, are among the most widely known groups of contaminants because of their ubiquitousness, high accumulation potential and harmful biological effects. However, OC pesticides residue in spinach, cabbage, Chinese pea and cucumber have not mentioned recently. Determination of contaminants at trace levels often requires extensive cleanup procedure to remove color and organic coumppounds interference contributed by this study. Low level analysis of OC pesticides in vegetable can suffer from interferences when ECD or MS are used for detection.

Chromatography column separation can involve adsorption, partition, gel permeation, ion exchange and etc. Clean-up methods in the chromatography column include acid partition, dichloromethane - hexane partition. Silicagel was soaked into sulfuric acid with the different percentage ratios, florisil impregnated water are selected for cleaning-up process.

Therefore, the determination of residue of OC pesticides in vegetable using decolozating techniques in the extracted solution is necessary.This research concentrated to clean up techniques for vegetable extract solution and increasing the recovery of OC pesticides in real samples. The purposes of this study are therefore to focus on the clean up techniques for OC pesticides analysis in vegetable samples in Hanoi markets by GC-ECD and to investigate the recovery of clean up processes.

2. Experiments

2.1. Chemical

A mixture solution of 20 compounds of OC Pesticide, having the concentration of 40ppm in n-hexane is used. The list of compound is described in table 1 and chromatograph in Figure1. The solvents as N-Hexane(n-Hex), acetone (Ace), dichloromethane (DCM) were purchased from Merck company. An activated carbon, NaCl, Na_2SO_4 anhydrous, H_2SO_4 were also purchased from Merck Company for pure analysis.

Table 1. List of 20 OC pesticide using in the study

No.	*Compound*	*No.*	*Compound*	*No.*	*Compound*
1	Alpha-BHC	8	Gama-chlodane	15	Endosulfan II
2	Gama-BHC	9	4,4 DDE	16	Endrin aldehyde
3	Beta-BHC	10	Endosulfan	17	Endrin Sulfate
4	Delta-BHC	11	Alpha Chlodane	18	4,4 DDT
5	Heptachlor	12	Dieldrin	19	Endrin Ketone
6	Aldrin	13	Endrin	20	Methoxychlor
7	Heptachlor eproxide	14	4,4 DDD		

2.2. Measurements

OC pesticides are analyzed by Gas Chromatography with electron capture detector (GC-ECD, Shimadzu 2010, Japan). The column was carried out using an Equity-5 (30m x1.8um x 0.25mm). For concentration, using an Evapoter system Buchi R – 200 (Germany) with V-800 control to concentrate the extract or column chromatography eluent. Rotary evaporation is chosen depends on the boiling point of the target compounds. The silmulation sample by homogenize equipment (IKA, Korea). The sample was extracted by ultrasonic (Germany) equipment and separated by centrifuge (9000 rpm, Henttich, Germany). OC pesticides concentrations were calculated from the peak area of the sample to the corresponding external standard. A blank sample procedure was implemented for every set of 5 samples to check for the contamination of OC pesticides and correction of samples.

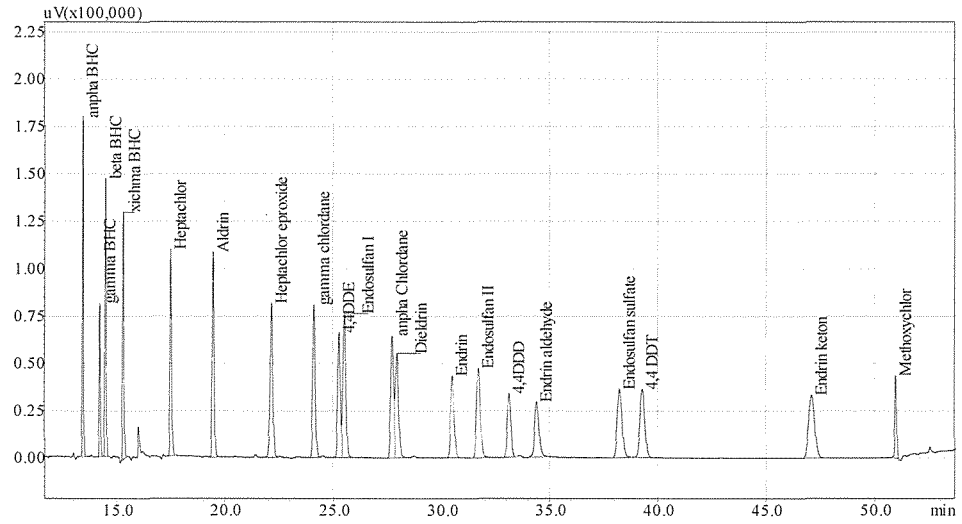

Fig.1. Chromatograph of 20 organochlorine pesticides

2.3. Analytical condition

GC-ECD injection temperature is 230°C while the temperature of detector is 300°C. The temperature program is described as follows: start at 80°C hold on 1 minute and then increase with the rate of 15°C/minute. Hold on for 40 minutes at 210°C and then growing up to 280°C with the rate of 25°C/minute and keeping for 3 minutes. Total time is 55.5 minutes for analyzing a sample.

3. Sample preparation method

3.1. Extraction / separation methods

Solvents such as hexane and DCM are usually used for the extraction of OC compounds from vegetable. Hexane is suitable for extraction of non-polar compounds. DCM has high extraction efficiency for a wide rage of non-polar to polar compounds. Because of DCM 's boiling point is low and easy to reconcentrate after extraction. Therefore, DCM - n-Hex was selected as rinsing solvent to avoid too much color and impurities.

Giving the 10 grams of vegetable sample, which is cut a small pieces and put into a glass cup. The sample were homogenized and extracted with 20 ml of acetonitril by ultrasonic vibration (3 times). A 3g of sodium chloride (corresponding to 5% of the sample volume) was added into the extracted solution. The extracts are concentrated to 1.0mL and cleaned up by using clean up material (silicagel, florisil, activated carbon). A mix of OC pesticide standard was added to sample before extracting to check recovery of extract and clean up process. The eluted are concentrated and summitted for GC-ECD analysis. Vacum rotary evaporation was carried out at 40°C with speed of 30rsp/min. The evaporation pressure is varied from 600mbar to 250 mbar for 3 min upto 15 min.

3.2. Clean-up process

The extracts are cleaned up by using florisil and /or silicagel chromatograph column if the extracts are not colored and contaminated. The florisil chromatograph column is conditioned by eluting with n-hexane.

Silicagel clean-up

Sonication method was used to extracted pesticides from vegetable [2], DCM-n-Hex was selected as rinsing solvent to avoid very much color and impurities.

Silicagel is the most popular material for clean up. The research was implemented with 2.5, 5 grams of silicagel and rinsing solvent DCM-n-Hex with ratio of 1:4 and 1:3, respectively.

Florisil clean-up

Florisil is a material which is very useful for dirty sample because of higher attractive with organic mater. Active of florisil is depend to water contain. Vietnam ambiemt has high humidity so every material is easy absord water from air. Florisil free water can keep OC pesticides tightly, event rinsing solvent is polar solvent such as acetone. After activated at 130°C in 12 hours, florisil was added water to reduce activate with pesticides. Using 5 grams of florisil, which is soaked 0%, 1%, 3% water (w/w) to clean up extract solution of spinach, chinese pea, cabbage and cucumber. A 70ml of a mixed solution DCM-n-Hex with a volume ratios of 1:4 was applied to rinsing solvent.

Silicagel impregnated sulfuric acid clean-up

Silicagel impregnated with sulfuric acid make a new material for clean up for removed color. Combine parts concentrated sulfuric acid with parts silica gel in a screw capped bottle and mix until a uniform mixture is obtained [3]. The combination of silicagel and sulfuric acid solved color problem but some pesticides were disappear or low recovery when using sulfuric acid to clean up and how much sulfuric acid is suitable for high color level sample such as spinach. The research has tried 2 levels of sulfuric acid (22 and 44 %) with silicagel.

Silicagel impregnated activated carbon clean-up

Activated carbon has great surface area so attractive capacity is higher for organic mater. However, If only activated carbon is very difficult to rinse pesticides out. Silicagel impregnated activated carbon will support together. The ratio of activated carbon has studied for clean up extract solution of spinach, cabbage, Chinese pea, cucumber.

4. Result and discussion

In the case clean-up by Silicagel, the results showed that using a mixed solution of DCM-n-Hex with ratio of 1:4 is suitable for rinsing, with ratio 1:3 color is easy pass through clean up column. Silicagel can not use for clean up Cabbage and Spinach. Chinese pea and Cucumber can be used 5 gram of silicagel for cleaning-up. However, eluted solution is still yellow color. Recovery of 20 organochloride pesticides is low such as 4,4 DDE recovery was of 35% and 4,4 DDD was of 68%.

For florisil clean up, the results indicated that eluted solution has dark green color when using florisil with any water contain for Spinach and Cabbage. With 5 gram of Florisil (1, 3 % H2O) can remove green color for Chinese pea and Cucumber. However, eluted solution has yellow color but the recovery of 20 organochloride pesticides is accepted. Figure 2 showed that, the recovery was from 0-41% for using florisil which contains 0% of water and from 75 to 99 % for florisil contains 1% and 3% of water.

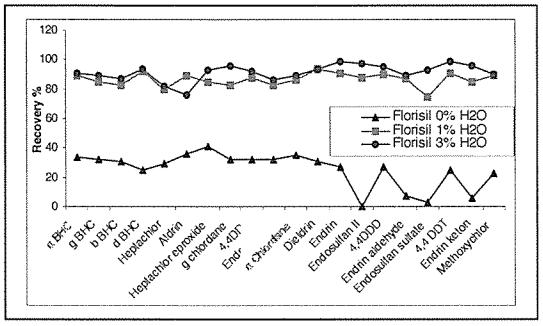

Fig. 2. Effect of water contain to activity of Florisil

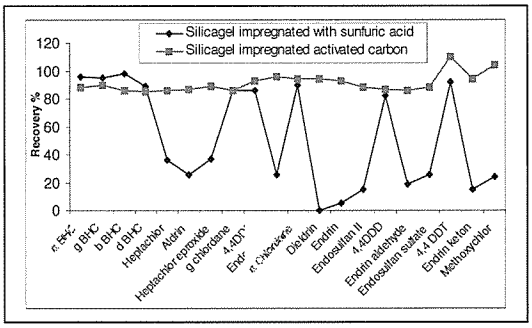

Fig.3. Recovery of 20 pesticides with different clean up material

For silicagel impregnated with sulfuric acid clean up, the result in Figure 3 showed that silicagel with 44% H_2SO_4, the 9/20 OC pesticides were a good recovery from 85% to 96 %. Observating the extract solution after eluting is colorless. The experiments demonstated that a mixed material is very useful for decoloration in vegetable. Some compounds in OC pesticides are aldrin, dieldrin, endosunfan, heptachlor, and methoxychlor can not apply this method.

For silicagel impregnated activated carbon clean up, the result shown that only 1 gram of silicagel impregnated 10% activated carbon can apply for 4 kinds of vegetables above. The recovery of 20 organochloride pesticides belongs to range from 85 to 110 % (Figure 2).

Overall, silicagel impregnated 10% activated carbon is the most effective but in the case αBHC, γ BHC, β BHC, σ BHC, γ chlordane; 4,4 DDE, Chlordane, 4,4 DDD, 4,4 DDT needed analyzed so silicagel impregnated with sulfuric acid was selected. Florisil can not almost decolorazed. The remain color makes chromatography column corrupted.

Result of analysis vegetable samples in Hanoi market

4 kinds of vegetables as Spinach, cabbage, Chinese pea, cucumber have studied. The 40 samples were collect from Long Bien, Phia Nam, Xuan Dinh Markets on 16th Nov 2006. Sonication method is used to extract pesticides from samples. Clean up procedure has applied the silicagel impregnated

10% activated carbon. The OC pesticides concentration in cabbage samples is ranged between 1.1 and 19.5 μg/kg. The highest concentration found at Long Bien market (19.5 μg/kg); the OC pesticides concentration in cucumber samples is from 13.4 to 32.1 μg/kg; the OC pesticides in Chinese pea samples were from 21.1 to 36.8 μg/kg. DDT in 3/8 cabbage samples, 5/8 Chinese pea samples, 5/ 8 cucumber samples was detected but residue is too low in all samples. (in Fig.4).

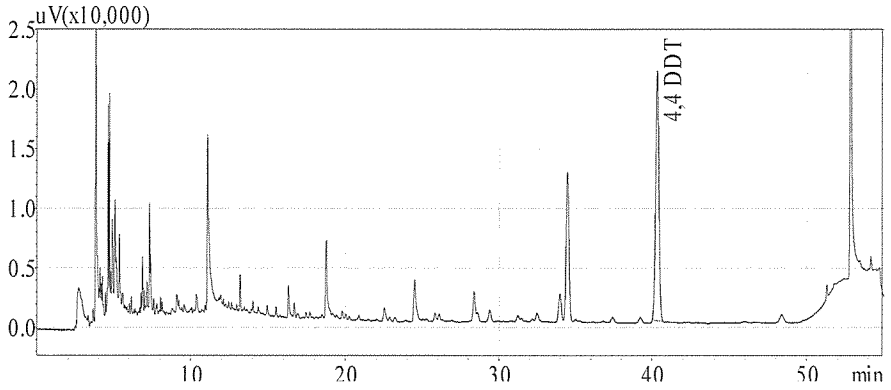

Fig.4. Chromatograph of 4,4- DDT in OC pesticides for cucumber sample

5. Conclusion

The paper has studied some techniques for clean up in vegetables and applied for analyzing 20 organochloride pesticides in Hanoi market. Silicagel impregnated 10% activated carbon was the best material for cleaning up vegetables extract solution with recovery from 85 and 110 %. Silicagel impregnated sulfuric acid is also good material for clean up process but 9/20 OC pesticides (αBHC, γ BHC, β BHC, σ BHC, γ chlordane; 4, 4 DDE, Chlordane, 4, 4 DDD, 4, 4 DDT) had recovery in ranged from 82 and 96 %. The other OC pesticides were lower than 40% for the recovery. Florisil activated at 130°C in 12 hours and reducing activity when adding 1% or 3 % H_2O can be used for Chinese pea and Cucumber samples but the eluted solution obtained yellow color. Silicagel impregnated 10% activated carbon should be used in analyzing OC pesticides for vegetable samples with an excellent decolozed ability and cheap. DDT still remained in vegetable in Hanoi market with a trace level. Other OC pesticides can not detected.

Acknowledgement

The work is supported from the core project of VAST.

References

1. Dang Quang Hung, Wolfram Thiemann. Contamination by selected chlorinated pesticides in surface waters in Hanoi, Vietnam. Chemosphere 47 (2002) p357–367
2. John R. Dean. Extraction Methods for Environmental Analysis. Wiley (1999)
3. Method 428 Determination of Polychlorinated Dibenzo-p-Dioxin (PCDD) and Polychlorinated Biphenyl emissions from stationary sources. California Environmental Protection Agency. (1990)