Journal of Environmental Sciences Vol.16, No.2, pp.268—271, 2004

ISSN 1001—0742
CN11—2629/X

Article ID: 1001-0742(2004)02-0268-04 **CLC number**: X132 **Document code**: A

Separation of chlorinated hydrocarbons and organophosphorus, pyrethroid pesticides by silicagel fractionation chromatography and their simultaneous determination by GC-MS

WANG Li-gang[1], JIANG Xin[1], WANG Fang[1], BIAN Yong-rong[1], Stephan Forster[2], Dieter Martens[2]

(1. Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China. E-mail: jiangxin@issas.ac.cn; 2. Institute of Ecological Chemistry, National Research Center for Environment and Health, Neuherberg 85764, Germany)

Abstract: A silicagel fractionation procedure for environmental sample extracts, which separates chlorinated hydrocarbons(CHCs) and organophosphorus, pyrethroid pesticides into two groups for subsequent instrumental analysis, was developed in this study. This method was achieved by optimizing the fraction cut-off volume of elution and different solvents. Using fully activated silica gel and cut-off CHCs collection after 10 ml 10% dichloromethane (DCM) in *n*-hexane passing through the column resulted in satisfactory separation of CHCs and organophosphorus, pyrethroid pesticides. This procedure had a higher reliability for CHCs than for organophosphorus, pyrethroid pesticides, because there is a relatively reliable recovery for CHCs. This approach is less expensive due to reducing sample pre-treatment time and solvent consumption.

Keywords: CHCs; organophosphorus pesticides; pyrethroid pesticides; silicagel fractionation

Introduction

Chlorinated hydrocarbons(CHCs) are persistent organic pollutants(POPs) in environment(Oxynos, 1989; Xu, 1992; 1994), they had been intensively and worldwidely used as effective insecticides such as DDT, hexachlorocyclohexane, and related compounds in the past decades. Improper use and disposal of these pesticides had resulted in the contamination of the environment including soil, water system etc.(Marth, 1999; O'Connor, 2002). Although the production of some CHCs has been drastically reduced or banned, they are still distributed all over the world due to their high resistance to chemical transformation, low biological degradation and high accumulation factors in fatty tissues (Kastanek, 1999). Organophosphorous pesticides(OPPs) enjoy wide use as an alternative to organochlorine pesticides for pest control owing to their relatively rapid decomposition and low accumulation in biological food chains (Fitzell, 1996), however, such chemicals are left in natural environment and are highly toxic to human beings. Pyrethroid pesticides have also been used in plant protection because of their desirable environment properties of short persistence and nontoxicity to mammals (Baker, 1982). The rapid increase in their application and the recent commercial introduction of some new pyrithroids require a method by which the residue of many pyrethroids can be determined. With the extensive utilization of OPPs and pyrethroide pesticides in agricultural practice, amount of these pesticides and their metabolites remain in soil, their intake by plant usually cause food pollution, or entry into water system even cause ecological and environmental question, especially in recent years, increasing public concern about possible health risks from pesticide resides has had emphasis on food quality and safety, therefore, special care must also be taken to the analysis of CHCs and OPPs, pyrethroids in environmental samples. The analytical methods for each kind of pesticides including CHCs, OPPs, pyrethroides have been stated in many documents, but little attention has been paid to their simultaneous determination, a modern trend in multiresidue analytical methodology is moving to the development of reliable procedures capable of determining as many pesticides as possible in the most rapid and accurate manner. Silicagel permeation chromatography is a widely used efficient technique for separation, purification and large molecule removal from sample extracts(Helreich, 1990). In analytical procedure for multiresidue, acetone and dichloromethane have shown high effectiveness (Holland, 1992). The main goal of this paper is to separate CHCs and OPPs, pyrethroid pesticides into two groups from their mixture through silicagel permeation chromatography eluted with 10% DCM in *n*-hexane and acetone, and develop a complete analytical methodology for simultaneous analysis of them with GC-MS in selected-ion monitoring(SIM) mode.

1 Materials and methods

1.1 Chemicals

The pesticides analyzed and their retention times and m/z selected for MS are listed in Table 1, all of them and pentachlorotoluene (PCT) were purchased from Dr. Ehrenstorfer GmbH, Germany. *n*-hexane, acetone, nonane and dichloromethane used are manufactured by Riedel-de-Haën; water used was distilled. Silicagel 60(0.063—0.200 mm particle size) and anhydrous sodium sulphate

Foundation item: The Chinese Ministry of Science and Technology(2002CB410805) and Chinese Academy of Sciences(KZCXZ-401) and Germany BMBF

manufactured by Merck were used.

Table 1 Pesticides analyzed and their retention time and m/z selected

Substances	RT, min	m/z	Substances	RT, min	m/z
Chlorinated hydrocarbons			Pyrethroid and organophosphorus		
Pentachlorobenzene	10.114	250/252	β-cyfluthrin 1	28.96	163/206
Hexachlorobenzene	12.12	284/286	β-cyfluthrin 2	29.03	163/206
Octachlorostyrene	14.92	343/345	Cypermethrin 1	29.21	163/181
Pentachloroanisole	12.10	265/267	Cypermethrin 2	29.40	163/181
α-hexachlorocyclohexane	11.98	217/219	Cypermethrin 3	29.50	163/181
β-hexachlorocyclohexane	13.25	217/219	Cypermethrin 4	29.57	163/181
γ-hexachlorocyclohexane	12.65	217/219	Fenpropathrin	26.45	181/209
δ-hexachlorocyclohexane	13.77	217/219	Fenvalerat 1	31.11	167/209
Dieldrin	16.50	263/265	Fenvalerat 2	31.49	167/209
Heptachlor	13.79	337/339	Diazinon	16.76	277/305
2,4′-DDT	16.97	235/237	Disulfoton	17.22	153/274
2,4′-DDE	15.60	246/248	Isofenphos	20.55	213/255
2,4′-DDD	16.43	235/237	Malathion	19.27	125/127
4,4′-DDT	17.80	235/237	Parathion-ethyl	19.99	291/292
4,4′-DDE	16.26	246/248	Parathion-methyl	18.81	263/265
4,4′-DDD	17.20	235/237	Phentoat	20.80	246/274
2,4,4′-PCB	13.70	256/258	Sulfotep	15.12	266/322
2,2′,5,5′-PCB	14.19	290/292	Bifenthrin	25.27	166/181
2,2′,4,5,5′-PCB	15.76	326/328	λ-cyhalothrin	26.71	181/197
2,2′,3,4,4′,5′-PCB	17.82	360/362	Phenothrin 1	26.13	123/183
2,2′,4,4′,5,5′-PCB	17.26	360/362	Phenothrin 2	26.04	123/183
2,2′,3,4,4′,5,5′-PCB	19.31	394/396	Tetramethrin 1	25.64	123/164
Oxy-chlordan	15.08	387/389	Tetramethrin 2	25.71	123/164
Cis-heptachloroepoxide	15.31	353/355	Azinphos-methyl	27.28	132/160
Trans-heptachloroepoxide	15.42	353/355	Resmethrin 1	24.52	123/171
Trans-chlordan	16.01	373/375	Resmethrin 2	24.65	123/171
Mirex	20.72	272/274	Chlorfenvinphos	20.82	323/325
Cis-chlordan	15.86	373/375	Fonophos	16.95	246/247
α-endosulfan	16.02	339/341	Phosalon	26.93	182/184
			Pyrazophos	27.27	221/373
			Triazophos	19.94	285/313
			Cis-permethrin	27.82	163/183
			Tr-permethrin	28.02	163/183

Notes: Preparation for measuring standard: 10 μl pest 1(10 ng/μl), 10 μl PCT(10 ng/μl) and 80 μl nonane were added in a microvial, both CHCs and organophosphorous, pyrethroid pesticides were measured simultaneously in measuring standard

1.2 Instrumental condition

Fisons 8000 GC with autosampler AS800 and quadrupole-MS MD800 was used for qualitative and quantitative analysis. XCB column (30 m length, 0.25 mm internal diameter, 0.25 μm film thickness) was used for gas chromatographic separation. Carrier gas was Helium, column head pressure was 50 kPa. Temperatures programmed for CHCs was as follows: initial temperature was 60℃ for 1 min, then up to 140℃ at 20 ℃/min, finally to 280℃ at 12℃/min, and held for 5 min. This program was named method Ⅰ. Temperatures programmed for organophosphorus and pyrethroid pesticides was as follows: initial temperature was 60℃ for 1 min, then up to 140℃ at 12 ℃/min, finally to 300℃ at 8 ℃/min, and held for 5 min. This program was named method Ⅱ. The injector and detector temperatures were 250℃ and 280℃, respectively. 1 μl sample was injected. The correct peak in chromatogram was identified by relative retention time with PCT as reference peak.

1.3 Procedure

Preparation of pest 1: pest 1 was a mixture and contained every substance in Table 1 which concentration was 10 ng/μl, came from stock solution of every substance.

Preparation of silicagel cartridge: Silica gel was activated at 130℃ for 24 h, after cooled down in a desiccator, deionized water was added according to 1.5% content of silica gel weight. Empty cartrtidge (polypropyline, 6 ml) with 3 frits was used. First, one frit was placed at the bottom of empty cartridge, 1 g silicagel (containing 1.5% water) was weighted and put into the cartridge, then the second frit was set on the top of silicagel layer in the cartridge; after these, 1 g Na_2SO_4 was weighted and put into the cartridge, finally, the third frit are set on the top of the Na_2SO_4 layer. When the procedures above were being done, the compact ad neat silicagel layer and Na_2SO_4 layer were needed for separation before the cartridge being used. The cartridge was washed two times with 4 ml *n*-hexane. The solvent has to be pressed through the column under pressure,

to remove the air in the silicagel column.

10 μl pest 1, 1 ml methanol/H_2O (1:1) and 1.5 ml *n*-hexane were added into a 5 ml test tube with cap, shook it vigorously, then let it still and phase isolated. Finally 1 ml *n*-hexane phase was separated, and was drawn on top of the column. The cartridge was eluted with 10 ml 10% DCM in *n*-hexane first, and the eluate was collected in a 10 ml test tube, named fraction 1. Then the cartridge was eluted with 10 ml acetone, and the eluate was collected in another 10 ml test tube, named fraction 2. 100 μl nonane was added in each test tube, then evaporate each fraction under nitrogen stream to nearly 100 μl, 10 μl PCT(10 ng/μl) was added, which was recovery standard, finally fraction 1, fraction 2 were transferred to different microvial for measurement respectively. Both CHCs and organophosphorous, pyrethroid pesticides were measured simultaneously in each fraction.

Recovery of every substance was calculated by the following formula:

$$Rec_A = \frac{H_A \cdot V_R c_R}{H_R \cdot V_A c_A} \cdot \frac{H_{R,St} \cdot c_{A,St}}{H_{A,St} \cdot c_{R,St}} \cdot 100.$$

Where, R_{ecA} = recovery of analyte (%); H_A = peak high of analyte; H_R = peak high of recovery standard; V_A = volume of added analyte (μl); C_A = concentration of added analyte (ng/μl); V_R = volume of added recovery standard (μl); c_R = concentration of added recovery standard (ng/μl); $H_{A,St}$ = peak high of analyte in the measuring standard; $H_{R,St}$ = peak high of recovery standard in the measuring standard; $C_{A,St}$ = concentration of analyte in the measuring standard (ng/μl); $c_{R,St}$ = concentration of recovery standard in the measuring standard (ng/μl).

Percentage of every substance in each fraction was calculated by the following formula:

$$Y_{A1} = X_1/S_A,\ Y_{A2} = X_2/S_A;\ S_A = X_1 + X_2;$$
$$X_1 = H_{af1}/H_{PCTf1},\ X_2 = H_{af2}/H_{PCTf2}.$$

Where, Y_{A1}: percentage of analyte in fraction 1; Y_{A2}: percentage of analyte in fraction 2; H_{af1}: peak height of analyte in fraction 1; H_{PCTf1}: peak height of PCT in fraction 1; H_{af2}: peak height of analyte in fraction 2; H_{PCTf2}: peak height of PCT in fraction 2.

2 Results and discussion

2.1 Separation of organophosphorus and pyrethriod pesticides and their recovery

The results in Table 2 indicates that a considerable proportion of the organophosphorus and pyrethroid pesticides tested were eluted into fraction 2, their percentage in fraction 2 account for more than 90% except bifenthrin, resmethrin 1, disulfoton; the percentage of diazinon, parathion-ethyl, phosalon, sulfotep and triazophos in fraction 2 account for 100%, while that of bifenthrin in fraction 2 is the lowest, only account for 57.5%; there are differences among recovery of each substance. The recovery of bifenthrin is also the lowest, only account for 42.1%, while these of malathion, parathion-ethyl, parathion-methyl, phosalon, pyrazophos, fenvalerat 1, β-cyfluthrin 1 are more than 90%, the deviation may come from the different characteristics and response to GC-MS and silicagel of each pesticide compared with PCT. Therefore, further work which choose proper recovery standard for certain group of pesticides or even a pesticide respectively is essential, although it is not easy to do. However, the contented separation of organophosphorus and pyrethroid pesticides has been shown in this experiment. Therefore, the measurement for OPPs and pyrethroids can be done only in fraction 2.

Table 2 Percentage in each fraction and recovery(R) in fraction 2 of organophosphorus and pyrethroid pesticides measured with method Ⅱ

	Fraction 1	Fraction 2	R in f 2		Fraction 1	Fraction 2	R in f 2
Azinphos-methyl	2.00	98.00	65.5	Resmethrin 1	19.00	80.50	81.2
Chlorfenvinphos	1.50	98.50	68.4	Resmethrin 2	0.00	99.50	78.0
Diazinon	0.00	100.00	56.0	Bifenthrin	42.50	57.50	42.1
Disulfoton	11.00	89.00	54.1	Tetramethrin 1	2.50	97.50	88.1
Fonophos	1.50	98.50	58.0	Tetramethrin 2	5.00	94.00	77.8
Isofenphos	0.00	99.50	71.2	Fenpropathrin	1.00	99.00	79.9
Malathion	1.50	98.50	90.6	Phenothrin 1	1.00	99.00	67.5
Parathion-ethyl	0.00	100.00	90.0	Phenothrin 2	0.50	99.00	67.7
Parathion-methyl	0.50	99.50	96.0	Cyhalothrin	1.00	99.00	75.7
Phentoat	0.50	99.50	70.1	*Cis*-permethrin	3.00	97.00	68.8
Phosalon	0.00	100.00	97.4	Tr-permethrin	1.00	98.50	72.5
Pyrazophos	5.00	95.00	98.6	β-cyfluthrin 1	3.50	96.50	97.9
Sulfotep	0.00	100.00	56	β-cyfluthrin 2	1.50	98.50	88.5
Triazophos	0.00	100.00	67.1	Cypermethrin 1	1.50	98.50	80.5
Fenvalerat 1	2.00	98.00	97	Cypermethrin 2	2.00	98.00	86.5
Fenvalerat 2	1.00	99.00	88.5	Cypermethrin 3	1.50	98.50	75.1
				Cypermethin 4	1.50	98.50	83.6

2.2 Separation of chlorinated hydrocarbons and their recovery

Table 3 shows that most of CHCs have better separation in fraction 1 except pentachlorobenzene(PeCB), δ-hexachlorocyclohexane, *cis*-chlordan, trans-chlordan and α-endosulfan. The percentage of hexachlorobenzene, heptachlor, oxy-chlordan, *cis*-heptachloroepoxide, *trans*-heptachloroepoxide, 4,4′-DDD, 2,2′, 3,4,4′,5,5′-PCB , mirex in fraction 1 account for 100%, while δ-hexachlorocyclohexane has the lowest percentage in fraction 1. The recoveries of pentachloroanisole, hexachlorobenzene, oxy-chlordan, *cis*-heptachloroepoxide, 2, 4′-DDE, 2, 4′-DDD, 4,4′-DDE, 2,4′-DDT, 2,2′,4,5,5′-PCB, 4,4′-DDD, 2,2′,4,4′,5,5′-PCB, 4,4′-DDT, 2,2′;3,4,4′,5′-PCB, 2,2′,3,4,4′,5,5′-PCB, mirex, trans-chlordan and α-endosulfan are more than 90%, while cis-chlordan has the lowest recovery. The recovery of each substance is basically consistent with their proportion in fraction 1, results of their recoveries have less variation, and are more reliable than these of organophosphorus and pyrethroid pesticides, that also mean PCT is a nicer recovery standard for CHCs than for organophosphorus and pyrethroid pesticides. Therefore, according to the result of Table 3, the conclusion can be drawn that all the CHCs have entered into fraction 1 considerably, and their recoveries are better, the measurement for CHCs can be done only in fraction 1.

Table 3 Percentage in each fraction and recovery(R) in fraction 1 of CHCs measured with method I

	Fraction 1	Fraction 2	R in f 1		Fraction 1	Fraction 2	R in f 1
Pentachlorobenzene	70	30	71	2,4′-DDD	97	3	114
α-hexachlorocyclohexane	97	3	89	4,4′-DDE	97	3	111
Pentachloroanisole	97	3	98	Dieldrin	92	8	82
Hexachlorobenzene	100	0	96	2,4′-DDT	97	3	113
γ-hexachlorocyclohexane	96	4	85	2,2′,4,5,5′-PCB	97	3	98
β-hexachlorocyclohexane	95	5	85	4,4′-DDD	100	0	106
2,4,4′-PCB	97	3	72	2,2′,4,4′,5,5′-PCB	97	3	96
δ-hexachlorocyclohexane	69	31	68	4,4′-DDT	97	3	111
Heptachlor	100	0	85	2,2′,3,4,4′,5′-PCB	100	0	99
2,2′,5,5′-PCB	98	2	86	2,2′,3,4,4′,5,5′-PCB	98	2	107
Octachlorostyrene	98	2	86	Mirex	100	0	115
Oxy-chlordan	100	0	120	*Trans*-chlordan	74	26	91
Cis-heptachloroepoxide	100	0	121	α-endosulfan	73	27	110
Trans-heptachloroepoxide	100	0	87	*Cis*-chlordan	75	25	61
2,4′-DDE	97	3	107				

3 Conclusions

This research conducted the fractionation of mixture using silicagel column for subsequent instrumental analysis of CHCs and organophosphorus, pyrethroid pesticides eluted with 10 ml 10% DCM in *n*-hexane and 10 ml acetone, CHCs and organophosphorus, pyrethroid pesticides were satisfactorily separated into two groups. The recovery of most substance are more than 80%. The main achievement of this research is that it puts forth a method which can be used for separating chlorinated hydrocarbon pesticides and organophosphorus, pyrethriod pesticides into two groups successfully, while these pesticides are widely distributed in environment, this method is very useful for their separation and exact analysis. The same research and results have not been found so far. Further improvement to the procedure presented in this paper can be achieved in several approaches. As mentioned in this research, PCT is not best recovery standard for organophosphorus and pyrethroid pesticides because of their obvious variation in different substance and fractionation. In addition, strict control on the moisture content of the silicagel, and consistency in the cut-off volume, are the most influencing parameters for the reproducibility of the fractionation.

References:

Baker P G, Bottomley P, 1982. Determination of residues of synthetic pyrethroids in fruit and vegetables by gas-liquid and high-performance liquid chromatography[J]. Analyst, 107: 206—212.

Baldi M, Previati M P, 1991. Purification of vegetable and lipidic samples by gel permeation chromatography technique and further determination by dual-channe l GLC (in Italian)[R]. GRIFA application. Note. 11. National Academy of Agriculture, Bologna .

Connor O, Thomas P, 2002. National distribution of chemical concentration in mussels and oysters in the USA[J]. Mar Environ Res, 53: 117—143.

Fitzell B L, Seiber W D, Parter J N *et al*., 1996. Ambient air concentration of pesticides in California [J]. Environ Sci Technol, 30:1365—1368.

Helrich K, 1990. Official methods of analysis of the Association of Official Analytical Chemists[M]. 15th ed. Arlington, VA: AOAC. 984

Holland P T, Malcolm C P, 1992. Emerging strategies for pesticide analysis[M] (Th. Cairns and J. Sherma ed.). Boca Raton, FL: CRC Press.71—84.

Holstege D M, Scharberg D L, Richardson E R *et al*., 1981. Improved multiredidue gas chromatographic determination of organophosphorus, organonitrogen and organohalogen pesticides in produce, using flame photometric and electrolytic conductivity detectors [J]. J Assoc off Anal Chem, 64: 1187—1195.

Kastanek F, Demnerova K, Pazlarova J *et al*., 1999. Biodegradation of polychlorinated biphenyls and volatile chlorinated hydrocarbons in contaminated soils and groundwater in field condition[J]. Int Biodeterioration Biodegradation, 44: 39—47.

Marth P, Schramm K-W, Martens D *et al*., 1999. Distribution of chlorinated hydrocarbons in different ecosystems in Germany[J]. Int J Environ Anal Chem, 75: 229—249.

Oxynos K, Schmitzer J, Kettrup A, 1989. Guidelines for environmental specimen banking in the Federal Republic of Germany[R]. Federal Environmental Agency, Berlin.

Xu Y, Wu W, Zhang Y *et al*., 1994. Mobility and transfer of residual hexachloro-cyclohexane(HCH) in aquatic environment[J]. Fresenius Environ Bull, 3: 557—562.

Xu Y, Zhang Y, Staudacher H *et al*., 1992. Annual report of FEBL GSF-Forschungszentrum [R]. Neuherberg, Germany. 87.

(Received for review January 2, 2003. Accepted February 24, 2003)