PESTICIDES MORNITORING IN THE HAIR OF VIETNAMESE PEOPLE BY MEANS OF SUPERCRITICAL FLUID EXTRACTION COUPLED WITH CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS

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Introduction

The speedy development of chemical industry is tightly connected with a very strong negative impact of human activities on our environment[1,2]. The most wide-scale and influential of all is the chemical pollution of the environment with xenogenic artificial chemical substances. Among them, are: toxic chemical elements, organic pesticides, and other substances of industrial and household genesis[3,4]. These polluting substances stay in the ecological nutritional chains for quite a long time, which leads to apparition of different acute and chronic diseases (cancer, Alzheimer…), as well as to changes in the ecosystem parameters (population density, dominant structure, species diversity...)[5].

Due to this, the problem of eco-monitoring of organic pollutants in the environment and in human organisms is very acute. Studies of such ecological problems are quite new to the Vietnamese science. That is why this work has a great theoretical and practical meaning. From the theoretical point of view, it is one of the complex researches of ecological problems and situations in typical wet-waste tropical conditions of Vietnam, with its actively developing chemical industry. From the practical point of view, its importance is connected with the fact that it reflects all of the most influential human impacts on the environment and gives us the possibility to choose correctly the priorities and directions of activity in improving the ecological situation around us.
Human hair show cumulative properties and their structure reflect the general condition of human health[6,7]. Due to this, hair is a widely spread bio-material used for ecological and epidemiological research. They also represent a convenient diagnostic substrate for screening surveys of large groups of people.

Materials and methods

The chemicals used as analyte standards were all reagent-grade or better. Hexachlorobenzene, p,p'DDT, p,p'DDE, butapon, 2,4-D, lindane, chlorofos, dichlorvos were purchased from «Metrologia+ Scientific and production company» Ltd (Russia). All solvents (methanol, *n*-hexane, acetone, dichloromethane) were supplied by Chimreaktiv (Moscow, Russia). During the process of supercritical fluid extraction, the edible carbon dioxide (99.8%), passed through a filter-drier where dehydrated silica gel, was used. Ultra pure water obtained from a Simplicity-185 purifier system (Millipore Corp., France). All glassware and polyethylene bottles were thoroughly washed and then soaked in nitric acid, and rinsed with ultra pure water before use.

The process of supercritical fluid CO2-extraction from the hair samples was carried out with the help of the supercritical fluid extraction instrument of circulatory type with fluid pumps by Thar Technologies Inc. company (USA).

The chromato-mass-spectrometric analysis was performed on the DFS Thermo Electron Corporation (USA). The ion source was operated in the electron ionization mode (EI; 70 eV, 280^{o}C). All mass spectra were obtained with the open-tubular column DB-5MS "Agilent" (length 30 m, column diameter 0,254 mm, phase width 0,25 μm). Helium, at a flow-rate of 1.0 ml/min, was used as carrier gas. Processing of mass-spectrometric

data was controlled by a computer running Xcalibur software. The injector port temperature was kept at 280°C. The oven temperature program was as follows: 120°C held for 1 min, ramp to final temperature 280 °C at 10°C/min, held for 30 min. Sample injection was done with flow split 1:20, using an injection volume of 1µl.

The hair samples were collected from 65 Vietnamese who have been living in Danang and Ho Chi Minh cities, Vietnam. The hair samples were cut close to the scalp with stainless steel scissors and were placed into identified envelopes.

Before the digestion procedure for the determination of organic pesticides, 20 mg of the hair sample was comminuted with stainless steel scissors to homogenize. The spike sample was placed in the stainless steel extraction vessel with a volume of 1.2 ml and extraction vessel put in the thermostat. Further on, as part of preparation for the main experiment, a brief CO_2-extraction process was set, for the purpose of additional sample purification and elimination of endogenous substances which may have caused distortions. For this, in the SFE instrument which you can see on Fig. 1, the static (lasting for 5 minutes) and dynamic (lasting equally, with discharge of 0.4 g/min) processing modes were performed in turns, with t=35°C and P=100 bar.

The carbon dioxide taken from reservoir *1*, passed through drier filter *3* which was filled with dehydrated silica gel. Afterwards, CO_2 was cooled up to 268 K and was drawn up by the pump *6*. The co-solvent used was passed from reservoir *12* with the help of pump *7*. After carbon dioxide and co-solvent flows got mixed, the modified extractant moved to the extraction vessel *9*, filled with hair. Further on, during the barbotage process of the extractant flow and the extract flowed through the collection vessel (organic solvent in the V-like collection vessel *11*), extract solution in the correspondent organic solvent was formed. The releasing carbon dioxide escaped outside.

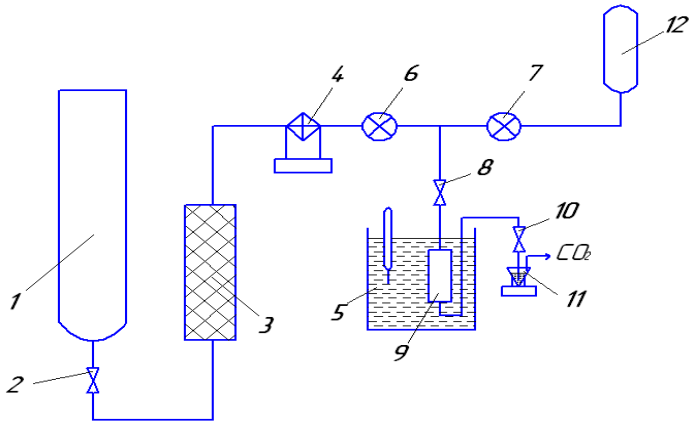

Fig. 1. Schematic view of supercritical fluid extraction instrument:
1 – reservoir with CO_2; 2,8,10 – gates; 3 – drier filter; 4 – cooling unit; 5 – thermostat; 6,7 – pumps; 9 – extraction vessel; 11 – collection vessel; 12 – reservoir with co-solvent

Results and discussion

Standard curves were made by computing regression lines of peak area of dichlorvos, chlorofos, hexachlorobenzene, lindane, butapon, p,p'DDE, p,p'DDT and 2,4-D after addition of 10 to 200 ng/g standards. Correlation coefficients were greater than 0,9825; detection limits were defined by a minimum signal-to-noise ratio of 3 (see Table 1).

Table 1. Linearity and detection limits of OCPs and OPPs

Pesticides	Concentration (ng/g)	Calibration curve	γ	MDL (ng/g)
Dichlorvos	10-200	y = 0,33x + 2,4	0,9946	1,3
Hexachlorobenzene	10-200	y = 0,122x + 0,8	0,9896	0,7
Lindane	10-200	y = 0,318x - 2,4	0,9863	1,2
Butapon	10-200	y = 0,544x + 2,2	0,9908	1,2
p,p'DDE	10-200	y = 0,332x - 3	0,9909	0,9
p,p'DDT	10-200	y = 0,19x + 0,6	0,9926	0,9
2,4-D	10-200	y = 0,112x - 0,8	0,9825	1,1
Chlorofos	10-200	y = 0,214x + 0,4	0,9966	1

The chromatograms of standards and extract from the hair samples are shown in Fig. 2. Residual OCPs and OPPs in human hair after a dose with organic solvent and put overnight in Petri plate were investigated. Six pesticides (dichlorvos, hexachlorobenzene, lindane, butapon, p,p'DDE, p,p'DDT) were indentified in Vietnamese's hair.

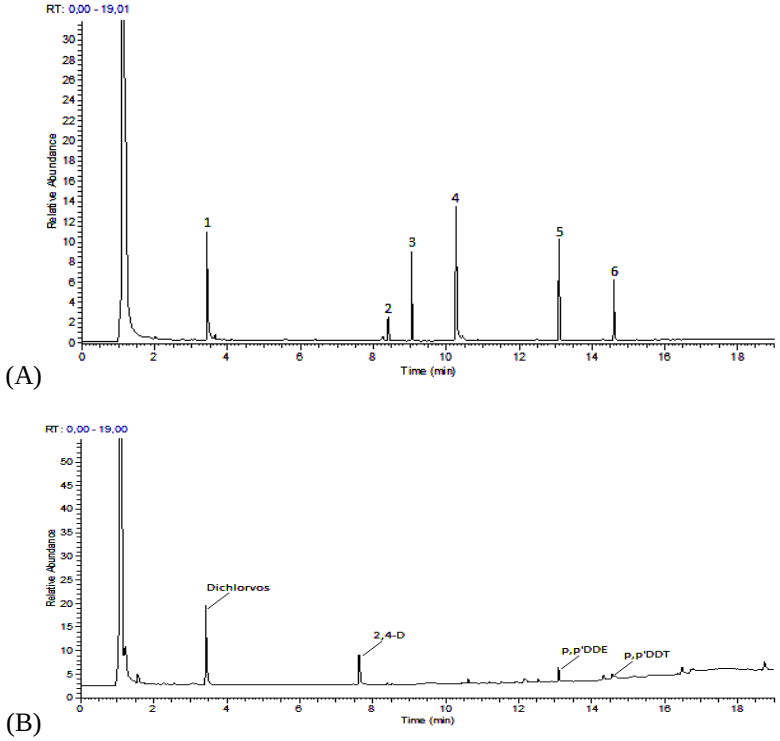

Fig. 2. Chromatograms of standard mixture (A) and blank human hair (B). (1: dichlorvos, 2: hexachlorobenzene, 3: lindane, 4: butapon, 5: DDE, 6: DDT)

The results of determining the concentration of pesticides in the Vietnamese's hair by the SFE-GC/MS method under the abovementioned optimal regimen conditions (P = 350 bar, t=45^{o}C, τ = 60 min) showed that people who have been living in Danang city have a high concentration of OCPs in their hair.

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