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On-Line DDT Determination in Blood Serum: Experimental Parameters

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DDT is largely used in Brazil for the malaria vector control. Due to this application, problems related to contamination risks of exposed professionals are often detected. Analysis of this pesticide and its analogs in blood can be used to estimate their concentrations in human body. Studies of the determination of organochlorine pesticide residues in human serum have been accomplished in Brazil. Almeida (1972), Fernicola and Azevedo (1982) as well as Lara et al. (1987) have determined chlorine levels in human serum in groups with different degrees of exposure to HCH. More recently, Carvalho et al. (1989) have reported data concerning the occupational exposure of HCH workers in cacao fields in the State of Bahia.

All these analyses have been accomplished by using the methodology described by Dale et al. (1970). In spite of the great use of this method, several analytical procedures (Henderson et al. 1971; Guilford and Hickman 1977; Gupta et al. 1978; Smrek et al. 1981; Burse et al. 1983; Sabbah et al. 1987) and monitoring studies (Siddiqui et al. 1981; Saxena et al. 1987) have been described recently, for chlorine residue determination in serum and whole blood. From these, three deal with the use of adsorbents in the extract purification. Smrek et al. (1981) had reported a method in which methanol was used to deproteinate the serum; the extraction is promoted with n-hexane and the clean-up step is performed on silica gel column chromatography. Based on the above summarized method, Burse et al. (1983) and Sabbah et al. (1987) have determined organochlorine residues in human serum. The main differences between these three procedures seem to be related to the extraction solvent system and deproteination agent. For the first author, methanol was used in this step whereas Sabbah et al. (1987) had employed 97% formic

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acid. Elutions from silica gel or florisil columns were performed with pentane and benzene or ethyl acetate--benzene and hexane (1:19:80).

In the present paper it is shown that extraction and purification of extracts containing DDT and its metabolites can be accomplished in a single step. The method employs chromatography by alumina adsorption. The serum sample is homogenized with silica gel in order to permit its direct passage to the column without previous extraction of pesticides. The study of experimental parameters related to the adsorbent selection and to the choice of elution systems for DDT and its metabolites is discussed in this manuscript.

MATERIALS AND METHODS

The organochlorine pesticide standards were given by EPA (United States Environmental Protection Agency) and their solutions were prepared in redistilled isooctane (Merck, p.a.). Acetone and n-hexane (Merck, pesticide grade) and redistilled methanol (Grupo Química, p.a.). Silica gel 60 (Merck, 70-230 Mesh ASTM) have been successively washed with distilled water and methanol, activated at 130°C for 24 hours and also stored in sealed erlenmeyer being protected from light. Once silica had been prepared in this way, it is possible to remain adequate for using for seven days. Neutral alumina (Merck, 70-230 Mesh ASTM), was activated at 600°C for 4 hours, then it was desactivated at 4.6% with deionized water and stored in sealed erlenmeyer being protected from light. The adsorbent remains good to use for 4 days.

Gas chromatograph CG 35370 equiped with electron capture detector (^{63}Ni). Glass column with 180 cmx2mm i.d. packed with 1.5% OV-17 and 1.95% QF-1 on Chromossorb WHP, 80-100 Mesh. Operating conditions: injector t° 215°C, column t° 200°C, detector t° 250-265°C; carrier gas nitrogen at 40 ml/min.

Chromatographic column (1.0 cm i.d. x 20 cm height), was packed with a small plug of glass wool, 2.0 g desactivated alumina at 4.6% as slurry.

Serum samples corresponding to 1.0 ml were homogenized in a mortar with 1.6 g silica gel until the mixture was free from lumps. The powder obtained was directly transferred to the column and the elution was processed with 20 ml n-hexane:acetone (9:1) at 24 drops/min. The eluate was collected in a 25 ml Kontes tube, attached to a Snyder micro-column, concentrated to 1 ml in a 75°C water bath and evaporated to dryness by passing a gentle stream of nitrogen. The residue was taken up in 1.0 ml of n-hexane and analysed by G.C.. The

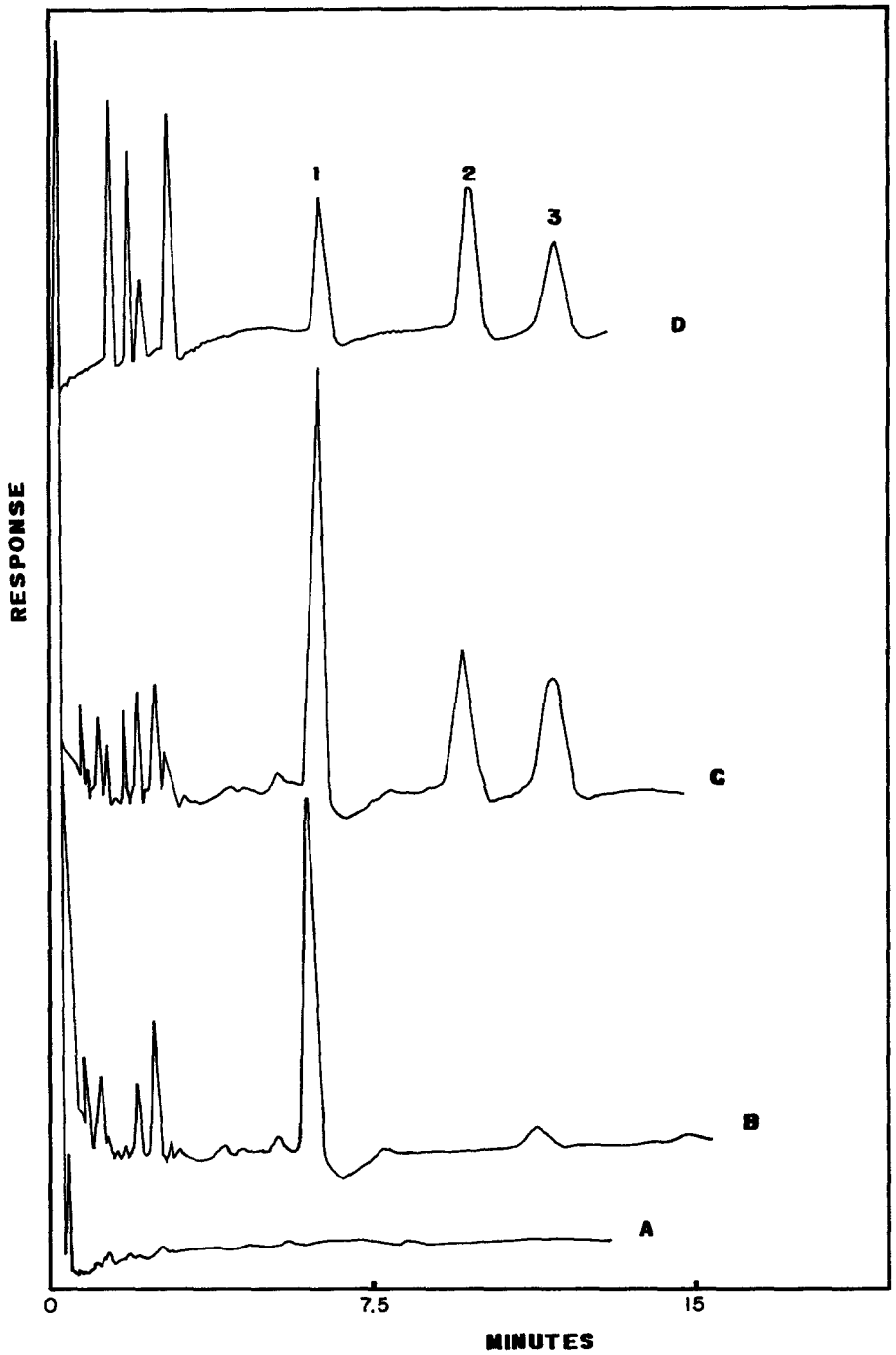

Figure 1. Gas Chromatograms of (A) Blank Analysis; (B) Serum Sample; (C) Fortified Sample; (D) Standard Solution: (1) p,p'-DDE (2) p,p'-DDD (3)p,p'-DDT.

recuperation analyses were performed by transferring 1.0 ml standard to an erlenmeyer, evaporating the solvent by passing a gentle stream of nitrogen and adding 10.0 ml of serum. The erlenmeyer was kept at the ambient temperature for 30 min.. Aliquots of 1.0 ml were submitted to the procedure. Blank analyses were performed in order to check to presence of interfering compounds (adsorbents, solvents, glassware). The chromatograms presented in Figure 1 are examples of these analyses.

RESULTS AND DISCUSSION

The establishment of experimental conditions was based on a methodology described by Ahmad and Marolt (1986) for DDT analysis in fish. The sample was macerated with sodium sulfate, transferred to an alumina and silicic acid column and eluated with n-hexane. In order to apply this operation sequence characteristic of one on-line procedure, it was important to submitt the serum samples to a treatment, so that they could be directly transferred to an alumina column. It should be mentioned that the use of sodium sulfate as described in that paper did not seem appropriate for the purposes of a small scale procedure: an excessive amount (~10g) of sodium sulfate was required. Thus, two adsorbents were also investigated: alumina and silica. The results had shown that 4.0 g of alumina and 1.6 g of silica were good enough to obtain a free flowing powder.

The adequate volume of pesticide elution for each solvent system (n-hexane, n-hexane:acetone 9:1 and 8:2) was investigated. The analyses performed with 4.0 g activated alumina and 40 ml n-hexane gave recovery values of 70% for p,p'-DDD and lower values for p,p'-DDE and p,p'-DDT. The elution with 20 ml n-hexane: acetone (9:1) did not promote apropriate purification of the samples for GC analysis.

Table 1. Percentage results of <u>in vitro</u> recovery from blood serum

Standard	Fortification Level (ng/g)	Recovery(%)*
p,p'-DDE	8	93
p,p'-DDD	16	102
p,p'-DDT	20	102

* average of 6 analyses

Elutions processed with 10 ml of this solvent system led to yellow extracts yielding mean recovery values

Table 2. Recovery of different methods for DDT analysis in blood serum

Method	p,p' - DDE		p,p' - DDD		p,p' - DDT	
	Fortification Level (ng/g)	Recovery (%)	Fortification Level (ng/g)	Recovery (%)	Fortification Level (ng/g)	Recovery (%)
Gupta (1987)	2.5×10^3	68.7	-	-	2.5×10^3	68.3
Burse (1983)	29.1	63.0	-	-	8.3	84.2
Smrek (1981)	31.2	90.5	10.5	90.5	20.7	90.8
Henderson (1971)	60.0	95.0	-	-	105.0	105.0
	300.0	93.0	-	-	750.0	96.0
Fernicola (1982)	-	86.0	-	105.0	-	52.0
Proposed	8.0	93.0	16.0	102.0	20.0	102.0

of 75, 104 and 121% for p,p'-DDE, p,p'-DDD and p,p'-DDT respectively.

When the analyses were performed with 1.6 g activated silica gel and 40 ml n-hexane, extremely low recuperation data (0-10%) were obtained. The replacement of the n-hexane for 10 ml of n-hexane:acetone 8:2 resulted in extracts inadequated for GC analysis. However when the elution with 20 ml n-hexane:acetone 9:1 was processed, excellent and reproductive recovery values were obtained (Table 1).

Recovery analyses in every condition above described performed with fortified samples with α-, γ- and β-HCH gave recuperation ranges from 29 to 39% (α-HCH), 51 to 59% (γ-HCH) and 47 to 74% (β-HCH), showing that these experimental conditions were not adequate to the determination of these pesticides in blood serum.

By comparing the percentage recovery data to four reported procedures, we may see the efficiency of the proposed method (Table 2). Gupta et al. (1978) had obtained some results which refer to serum treatment with formic acid and extraction with n-hexane:acetone (9:1). Henderson et al. (1971) had submitted whole blood samples to the action of sulphuric acid 60% and accomplished the extraction with n-hexane:acetone (9:1). Dale et al. (1970) procedure was applied by Fernicola and Azevedo.

As can be clearly deduced from analyses of Table 2, the most interesting comparisons are those which involve the data of Smrek et al. (1981), Burse et al. (1983) and Sabbah et al. (1987), as they also use a clean-up step by adsorption chromatography. The comparative analysis results have shown the method viability for DDT determination in blood serum. The procedure simplification has proved to be of great help for the data raising concerning its rapidity and significant saving of reagents.

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