

## Mixed Column Application in Pesticide Residues Analysis

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Uma técnica de "clean-up" para análise de resíduos de pesticidas organoclorados foi avaliada pelo estudo dos parâmetros envolvidos na recuperação dos pesticidas selecionados e na eficiente purificação dos extratos. O teor de desativação dos adsorventes, o tempo de repouso da sílica e a velocidade de eluição do sistema de solvente, representam fatores significativos para o desenvolvimento do procedimento. A otimização dos parâmetros experimentais empregando soluções padrões resultou em recuperação maior que 88% para todos os pesticidas estudados, exceto para HCB. A aplicação do procedimento às amostras gordurosas forneceu um valor médio de gordura residual de 0,04%. Os dados obtidos indicaram a viabilidade de se efetuar a extração dos pesticidas e a purificação dos extratos em uma única etapa. As análises foram realizadas em cerca de 40 min, empregando pequena quantidade de reagentes e de solventes.

A clean-up technique for organochlorine pesticide residues analysis was evaluated through the study of the parameters involved in recovery of selected pesticides and in a efficient purification of the extracts. The degree of water deactivation of adsorbents, the standing time of the silica and the flow rate elution were all significant factors for the development of the procedure. Optimization of the experimental conditions using standard solutions resulted in >88% recoveries for all studied pesticides, except for HCB. The application of the method to lipid samples provided an average residual fat value *ca.* 0,04%. The data demonstrated the viability of the procedure to promote the pesticide extraction and the purification of the extracts in a single step. The analysis were performed in about 40 min employing small quantities of reagents and solvents.

**Key words:** *organochlorine; pesticide residues; soybean bagasse; gas chromatography.*

### Introduction

The problems related to pesticide residue analysis of fat samples, have been discussed in literature<sup>1-5</sup>, in connection to the presence of lipids and co-eluting compounds which interfere in the determination by gas chromatography. On the other hand the time required to isolate fat and to purify extracts (which in the conventional methods may include partition and/or adsorption chromatography techniques) limits the number of samples that can be analyzed.

The development of methods in small scale using micro-columns<sup>6-10</sup> and of procedures on-line<sup>11-14</sup> represent modifications which allow a considerable reduction in the time and the amounts of reagents required for analysis.

By combining the principles suggested in these reports with the purpose of working with samples rich in lipids, we investigated the experimental parameters of a clean-up technique by adsorption chromatography, employing available reagents in the Brazilian market.

### Experimental

**Apparatus and Reagents.** The solvents and the reagents used were: iso-octane p.a., Fisher, n-hexane p.a., Grupo Química, n-hexane pesticide grade, acetone p.a., dichloromethane p.a., sodium hydroxide p.a., aluminium oxide 90 active, neutral (70-230 mesh ASTM) and silica gel 60 (70-230 mesh ASTM and silica gel 60 (70-230 mesh ASTM), Merck.

The n-hexane, p.a. was treated with solution of sodium hydroxide 50% in the ration of 7:2. The organic phase was washed with water, dried with anhydrous sodium sulphate and purified by a procedure developed in our laboratory<sup>15</sup>. The dichloromethane p.a. was refluxed with sodium carbonate (20 g/l) under magnetic stirring and distilled according to the procedure described by Albert<sup>16</sup>.

The standards of organochlorine pesticides were provided by the US Environmental Protection Agency (EPA), Research Triangle Park, N.C., U.S.A., and their solutions were prepared in iso-octane, p.a., Fisher.

Chromatographic columns 20 cm length x 1 cm i.d., with a teflon tap at the lower end and a reservoir at the top, were used. The chromatographic analysis were performed in: a) gas chromatograph Intralab model 3300, equipped with a <sup>63</sup>Ni electron capture detector and 200 cm length x 2 mm i.d. glass column packed with 1,5% OV-17 + 1,95% QF-1 on 80-100 mesh Chromosorb W-AW DMCS under the following operation conditions: injector 200-210°C, column at 190°C, detector at 250°C, carrier gas nitrogen at 30 ml/min. b) gas chromatograph CG model 35370, equipped with a <sup>63</sup>Ni electron capture detector and 183 cm length x 2mm i.d. glass column packed with 5% OV-210 on 100-120 mesh Chromosorb WHP under the following operation conditions: injector 213-218°C, column at 190°C, detector at 254-263°C, carrier gas nitrogen at 40 ml/min.

All glassware apparatus was rinsed with acetone followed by water. It was then immersed in 10% aqueous solution of alkaline Extran, kept in ultrasound by 30 minutes, rinsed with water and dried in an oven.

The cotton used as a support to the adsorbent was washed with diluted solution of hydrogen peroxide 130 v(1:1), rinsed with distilled water, dried in an oven at 60°C for 4 h; the silica gel was washed with water and with methanol and activated at 130°C for 24 h. The deactivation of the adsorbents was achieved by adding the correct amount of deionized water. The stoppered flask was shaken until all lumps disappeared. The adsorbents were kept in a desiccator for 24 h before use.

The adsorption columns were freshly prepared for each sample. Each empty column was rinsed with n-hexane. The columns were packed with a slurry of 3.0g of alumina in n-hexane and 0.5 g of silica and were settled by tapping the column side. A 1.0 ml volume of a standard solution was applied on top of the column and allowed to soak into the packing. Next, the elution was processed under a definite flow rate. The eluate was received in a modified flask<sup>17</sup>, and concentrated to 1 ml in a rotary evaporator. The final drying was achieved by passing a gentle stream of nitrogen. The residue was taken up in 1.0 ml of iso-octane and 5 µl were analyzed by GC.

Two solvent systems were studied: n-hexane-dichloromethane (80:20 - v/v) and n-hexane both under elution rates of 40 or 60 drops/minute. The water content of adsorbents tested were: alumina 4.6%, activated silica (0%) and deactivated silica 1.5% and 10%.

Procedure blanks, consisting of all reagents and glassware used during the analyses were carried out to check contamination. Recoveries were calculated from the chromatograms of the standard solution before and after use of the adsorption column.

## Results and Discussion

Since the elution behaviour of pesticides depends on many parameters<sup>6</sup> and that minor experimental variations lead to different results, the recovery studies developed with standard solutions, were performed by detailed testing the following parameters:

- elution rate to each solvent system;
- influence of the water content on silica to each solvent system;
- comparison between two solvent systems for the same column packing.

In the first part of this study it was evaluated the effect of

elution rate in the recovery of organochlorine pesticides. It was used deactivated alumina with 4.6% H<sub>2</sub>O and activated silica (0% H<sub>2</sub>O). The elution was performed with n-hexane (L) and n-hexane-dichloromethane (80:20-v/v)(M). The results are shown in Table 1 and indicate that the elution rates of 40 and 60 drops/minute respectively, were adequate to M and L'. In these conditions a better reproductibility of the recovery values and number of pesticides with recovery higher than 70% were obtained. Also it could be verified that the Endrin recovery values are very low under the conditions specified as M'. The average recoveries under conditions L' and M confirmed those results and also showed that the proce-

**Table 1.** Influence of elution rate on organochlorine pesticides recovery

Pesticide (µg/l)	Recovery (%)									
	L		L'		L'*		M		M'	
HCB (4.0 x 10 <sup>-3</sup> )	19	38	37	68	35	34	36	22	46	38
α-HCH (6.0 x 10 <sup>-3</sup> )	46	33	63	68	45	44	51	32	52	53
γ-HCH (6.0 x 10 <sup>-3</sup> )	67	40	74	67	55	59	67	46	58	66
Heptachlor (8.0 x 10 <sup>-3</sup> )	56	45	71	75	52	58	60	47	54	61
Aldrin (4.0 x 10 <sup>-3</sup> )	64	44	77	79	57	86	66	85	113	70
p,p'-DDE (9.6 x 10 <sup>-3</sup> )	107	69	101	101	106	101	74	79	80	90
Dieldrin (9.6 x 10 <sup>-3</sup> )	-	-	-	-	-	97	69	78	86	95
Endrin (12 x 10 <sup>-3</sup> )	-	-	-	-	-	97	80	18	25	95
p,p'-DDD (20 x 10 <sup>-3</sup> )	123	75	106	102	96	103	82	106	113	89
p,p'-DDT (40 x 10 <sup>-3</sup> )	124	98	98	100	97	102	100	98	85	99

\* Values indicate mean of 6 analysis.

L and L' - n-hexane, 40 and 60 drops/minute, respectively,

M and M' - n-hexane:dichloromethane (80:20 v/v), 40 and 60 drops/minute, respectively,

Solvent volume = 40 ml.

cedure could be applied to determination of DDT and its metabolites. Furthermore, Dieldrin and Endrin can be analyzed when the elution is processed with n-hexane: dicloromethane.

The choice of deactivation degree of alumina was established based on previous experiences developed in our laboratory with samples of edible oils<sup>18</sup>. To silica, this selection was based on the water content described in the literature for organochlorine pesticide residues analysis<sup>4,6,11,19</sup>.

The effect of deactivation of adsorbents on the pesticides recovery was studied maintaining the deactivated alumina 4.6% and using deactivated silica 1.5% and 10%. The results given in Table 2 show that the better experimental conditions,

**Table 2.** Effect of water deactivation of silica on organochlorine pesticides recovery.

Pesticide	Recovery (%)							
	1.5%				10%			
	L*	$\sigma$	M <sup>Δ</sup>	$\sigma$	L*	$\sigma$	M <sup>Δ</sup>	$\sigma$
HCB	64	8.4	69	5.0	69	8.1	48	3.5
$\alpha$ - HCH	82	5.4	87	5.9	80	8.6	57	10.6
$\gamma$ - HCH	77	4.2	84	5.1	84	8.7	72	12.9
Heptachlor	73	5.9	80	6.2	81	8.3	63	10.5
Aldrin	81	4.8	86	7.1	80	5.9	69	12.5
p,p'-DDE	102	6.8	104	8.5	98	4.6	92	19.2
Dieldrin	-	-	102	3.4	98	2.9	85	22.3
Endrin	70	7.2	99	8.1	90	4.9	90	23.4
p,p'-DDD	96	4.4	96	10.7	98	6.3	93	19.3
p,p'-DDT	96	4.9	95	3.2	99	4.5	96	25.0

\* Values indicate mean of 6 analysis

Δ values indicate mean of 5 analysis

evaluated by recovery percentage values and their reproducibilities ( $\sigma$ ), are obtained when using silica 10%/n-hexane (L\*) or silica 1.5%/n-hexane-dichloromethane (M).

In view of inexpressive differences in the recovery results under the two above conditions, it was chosen to employ the parameters codified as L. In this case, the procedure is simplified and the analysis time is reduced.

During the course of this work it was verified that the recovery values are affected by the time that silica remains on standing. The results given in Table 3, show that 24 h is the adequate time to apply this procedure. In these conditions the recoveries are within the 88 - 102% range with an excellent reproducibility, as shown by the standard deviation values (1.4 - 9.4), except to HCB.

Table 3 also includes a comparison of our results with those published by Voogt *et alii*<sup>1</sup>. As should be noticed the

**Table 3.** Effect of rest time of deactivated silica on organochlorine pesticides recovery.

Pesticide	Recovery %						Method of Voogt <i>et alii</i> <sup>1</sup>
	3 Hours		5 Hours		24 Hours		
	L*	$\sigma$	L*	$\sigma$	L <sup>Δ</sup>	$\sigma$	
HCB	46	17.3	53	32.3	60	12.1	123
$\alpha$ - HCH	68	10.5	75	31.5	91	6.8	68
$\gamma$ - HCH	80	10.4	79	13.1	96	4.8	69
Heptachlor	72	6.4	69	22.0	88	9.4	110
Aldrin	72	5.8	69	18.7	94	5.7	81
p,p'-DDE	93	6.8	91	9.9	99	2.4	109
Dieldrin	93	9.2	93	7.9	101	2.4	88
Endrin	67	43.9	82	20.2	99	3.6	88
p,p'-DDD	96	6.1	95	7.1	102	1.4	83
p,p'-DDT	94	7.9	95	8.7	101	2.2	84

\* Values indicate mean of 0 analysis,

Δ values indicate mean of 6 analysis.

values here described, despite the clean-up differences, are in good agreement with the results of Voogt.

The methodology above described was applied to soybean bagasse. Samples with uniform granulometry (8-18 mesh) were directly transferred to the column. The extracts were examined and found to be efficiently purified. The average residual fat value (0.04%) obtained from 0.5 g of sample allows to realize the pesticide extraction and the purification of the extracts in a single step, in about forty minutes employing small quantities of reagents and solvents.

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