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An On-line Method for the Determination of Organochlorine Pesticide Residues in Soybean Derivative

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Um método "on-line" foi desenvolvido para determinação de resíduos de pesticidas organoclorados em amostras de bagaço de soja. A extração dos pesticidas e a purificação dos extratos é realizada em uma única etapa pela transferência direta das amostras para uma coluna empacotada com alumina e sílica gel. Os pesticidas são eluídos com n-hexano (1ª fração) e n-hexano:diclorometano 8:2 (2ª fração) e analisados por CG com detector por captura de elétrons. Os valores médios de recuperação obtidos para amostras fortificadas "in vitro" com treze pesticidas em níveis entre 1,5 e 600 ppb variaram de 73 a 104%. A comparação com outros métodos descritos na literatura aplicados a amostras ricas em lipídios, indica que o método proposto utiliza uma quantidade reduzida de reagentes e solventes e permite uma análise rápida e eficiente de organoclorados em amostras de bagaço de soja.

An on-line method for the determination of organochlorine pesticide residues in soybean bagasse is described. The extraction and cleanup steps are combined into one step by transferring the sample to a chromatographic column prepacked with alumina and silica gel. The pesticides are eluted with n-hexane (fraction 1) and n-hexane:dichloromethane 8:2 (fraction 2). The fractions are analyzed using gas liquid chromatography with electron capture detection. Mean recoveries of the 13 "in vitro" fortified pesticides at levels of 1.5-600 ppb ranged from 73 to 104%. Comparison of this method with other methods for fatty samples indicates that this is an improvement in accuracy, precision, for analysis time.

Keywords: *organochlorine pesticides, soybean bagasse*

Introduction

Organochlorine pesticides have become universal contaminants found throughout the environment in all segments of the food chain. Kaphalia *et al.*[1] mentioned that several studies on organochlorine contamination have demonstrated that 80-90% of total intake of those residues in non-occupationally exposed humans was accumulated via food. Because of the uncertain consequences for human beings the application of organochlorine pesticides has been prohibited or at least restricted in many countries. But they are still used in several developing nations in agriculture as well as vector control programs.

In Brazil, the use of organochlorine pesticides is restricted but the application of endosulfan to soybean, coffee, cotton and cocoa cultures is permitted. The recommended tolerance limit is 1 μg/g for total endosulfan in soybean seeds[2].

Soybeans are an important world crop grown in many countries, but mainly in the United States, Brazil, China and Argentina. They now dominate edible oil and vegetable protein production[3].

Although Brazil is the second major soybean producer, the acceptance of soybean by-products by the Brazilian population is problematic[4], with only margarine and oil being widely consumed.

When submitted to a special procedure the soybean grains provide two by-products: an extract, called soybean milk and a solid residue, called soybean bagasse.

The soybean bagasse is used for baking bread and crackers which are offered to primary school students in São Paulo state, Brazil.

In this study, the authors report the development of an analytical method that combines extraction and cleanup into a single step for the determination of selected organochlorine pesticides in soybean bagasse.

Experimental Details

Equipments

(a) Gas chromatograph - Intralab model 3300, equipped with a ^{63}Ni electron capture detector and a 200 cm 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. Operation conditions: injector t° 200-210 °C, column t° 190 °C, detector t° 300 °C, carrier gas nitrogen at 30 ml/min.

(b) Gas chromatograph - CG model 35370, equipped with a ^{63}Ni electron capture detector and a 183 cm x 2 mm i.d. glass column packed with 5% OV-210 on 100-120 mesh Chromosorb WHP. Operation conditions: injector t° 210-220 °C, column t° 190 °C, detector t° 254-268 °C, carrier gas nitrogen at 40 ml/min.

(c) Chromatographic column - Glass, 30 cm x 1 cm i.d., equipped with 20 ml reservoir and polytetrafluoroethylene (PTFE) stopcock.

Reagents

(a) Solvents - dichloromethane (pesticide grade); iso-octane (p.a.) and n-hexane (p.a.) purified as described by Polese *et al.*[5] were used.

(b) Neutral alumina (70-230 Mesh ASTM) was activated at 600 °C for 4 hours as described by Wells and Johnstone[6]; deactivated to 4.6% with deionized water; stored in a stoppered bottle.

(c) Silica gel 60 (70-230 Mesh ASTM) was washed with distilled water and n-hexane; dried at 130 °C for 24 hours; deactivated to 10% with deionized water; stored in a stoppered bottle.

(d) Pesticide standards - All standard solutions were prepared in iso-octane, using reference standards obtained from the U.S. Environmental Protection Agency.

Preparation of Samples

Soybean bagasse was obtained from the city of Américo Brasiliense, in the state of São Paulo. The samples were dried at 75 °C for 12 hours, and ground and screened through a 1 mm sieve. The particle size suitable for our purposes was a mesh range of 8-18 (ASTM). All samples were stored frozen.

Sample extraction and cleanup

To a chromatographic column containing a glass-wool plug was added an n-hexane slurry of 3.0 g of alumina and an n-hexane slurry of 0.5 g of silica. The column was tapped to ensure proper setting of the granules. After, 0.5 g of the sample was transferred to the top of the column and the elution was processed with 40 ml n-hexane (fraction 1) and 20 ml n-hexane:dichloromethane 8:2 (fraction 2) at 3 ml/min. Both fractions were collected in modified flasks[7], the eluate was evaporated to *ca.* 1 ml in a rotary evaporator and a gentle stream of nitrogen was applied to the concentrate up to dryness. The residue was reconstituted to 1.0 ml with iso-octane.

Determination of the extract lipid content

The lipid content of the extracts was determined by collecting the column eluate in a tared vessel. The solvent was evaporated up to dryness under a stream of cool air, and the lipid eluted from the column was quantified by weight.

Recovery procedure

The study of pesticide recovery for the proposed method was determined using samples fortified at three concentration levels.

Half a milliliter of the pesticide standard solution was added to 0.5 g of soybean bagasse. After 1 hour at ambient conditions (25 °C), the sample was homogenized and submitted to the extraction procedure.

Gas chromatographic analysis

A 5 µl aliquot of each fraction, and of the standard solution, was injected into a gas chromatograph operated as described before. Blank analyses were performed in order to check the presence of interfering compounds in the reagents and solvents. The results (as percentages) were calculated by comparing the peak size (height or area) of each pesticide in the extract with those of the standard solutions of pesticides.

Results and Discussion

The study of the experimental parameters of the proposed method, described in a previous paper[8], was performed with standard solutions containing HCB, α- and γ-HCH, heptachlor, aldrin, p,p'DDE, dieldrin, endrin, p,p'-DDD and p,p'-DDT. The average recoveries for six analyses ranged from 88 to 102% for all compounds, except for HCB (60%).

In the present study fortified and un-fortified samples were submitted to the one step procedure under these conditions.

GC chromatograms obtained for blank and un-fortified sample analyses were free of interfering peaks and confirmed the improved cleanup of the proposed method.

As a first step, the cleanup efficiency was tested by means of the determination of the lipid content of the extracts. After cleanup only 0.7% of extractable fat contained in 0.5 g of sample was eluted from the column.

The usage of columns prepacked with two adsorbents for analysis of fat samples was discussed by other authors. Venant and Borrel[9] described a method which employs 9.0 g of silica gel and 2.0 g of Florisil and although interfering impurities have not been completely removed 0.5 g of fat could be analyzed. Another procedure[10] is based on the use of a column made of 4.0 g of silica, 2.0 g of alumina and 1 g of sodium sulphate, which allows application of about 60 mg of lipids of animal origin.

To determine the usefulness and the sensitivity of the described procedure, recovery data were obtained for fortified samples with the selected pesticides at three different levels. As shown in Table 1, the recoveries ranged from 84 to 104% for α- and γ-HCH, heptachlor, aldrin, p,p'-DDE,

endosulfan I, dieldrin, endrin, p,p'-DDD, p,p'-DDT, endosulfan II and endosulfan sulphate at these studied levels.. The recovery of HCB ranged from 73 to 83%, and it was better than that reported in the recovery study performed with standard solutions[8]. This result demonstrates that the presence of the sample affects the HCB recovery.

Table 1. Recovery and precision of the on-line method.

Pesticides	Added (ng/g)	Recovery[a] (%)	Standard Deviation
HCB	1.5	73	9.1
	3.0	83	6.2
	6.0	80	7.1
α-HCH	1.5	96	8.3
	3.0	90	8.3
	6.0	84	10.6
γ-HCH	1.5	95	8.3
	3.0	100	4.3
	6.0	92	8.0
Heptachlor	2.0	100	8.4
	4.0	97	9.3
	8.0	91	6.9
Aldrin	2.0	94	7.2
	4.0	95	5.7
	8.0	86	6.8
p,p'-DDE	2.5	101	1.6
	5.0	99	4.5
	10.0	94	6.8
Endosulfan I	8.0	93	6.4
	16.0	101	6.4
	200	95	5.2
Dieldrin	2.5	103	3.4
	5.0	100	3.0
	10.0	96	4.1
Endrin	3.8	98	8.4
	7.5	95	8.0
	15.0	97	3.9
p,p'-DDD	6.0	101	4.4
	12.0	98	3.4
	24.0	97	3.4
p,p'-DDT	6.0	102	2.5
	12.0	102	3.6
	24.0	96	9.2
Endosulfan II	8.0	91	6.0
	16.0	99	4.7
	200	97	2.5
Endosulfan Sulphate	24.0	95	6.5
	48.0	104	2.0
	600	95	4.2

a - 8 replicates at each level, except for endosulfan sulphate (6 replicates at each level).

Table 2. Comparison of the recovery values for the literature procedures and the proposed method.

Pesticides	Venant[a] & Borrel(7)	Voogt[b] (8)	Ahmad[c] & Marolt(9)	Proposed[d] Method
HCB	90	123		73
α-HCH	89	68		96
γ-HCH	100	69		95
Heptachlor		110		100
Aldrin		81		94
p,p'-DDE	93	109	58,5	101
Endosulfan I		48		93
Dieldrin	89	88		103
Endrin		88		98
p,p'-DDD	95	83	89,2	101
p,p'-DDT	91	84	88,1	102
Endosulfan II		0		91
Endosulfan Sulphate				95

a - added: 5-10 ng/g
b - added: not indicated
c - added: 100 ng/g
d - added: 1.5 - 24 ng/g

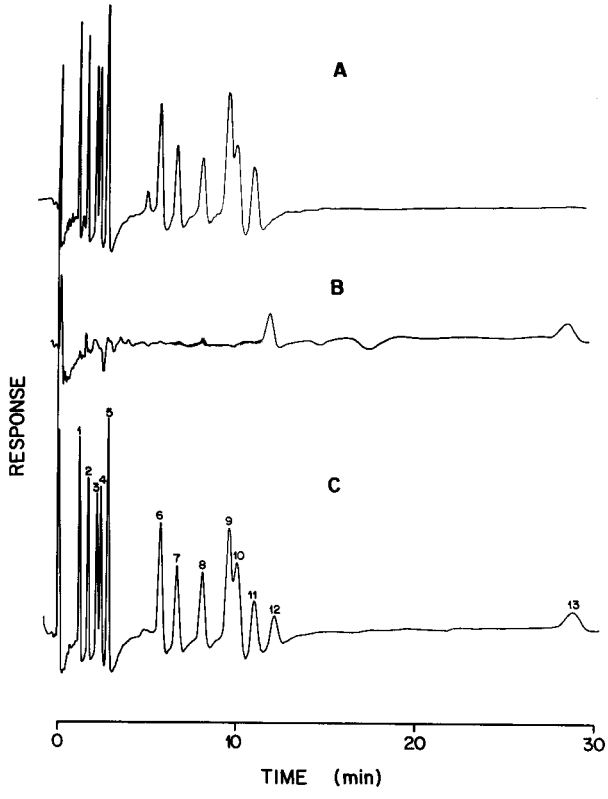

Figure 1. Chromatograms of fraction 1 (A); fraction 2 (B) and standard solution (C): 15 pg HCB (1); 15 pg α-HCH (2); 15 pg γ-HCH (3); 20 pg heptachlor (4); 20 pg aldrin (5); 20 pg endosulfan I (6); 25 pg p,p'-DDE (7); 25 pg dieldrin (8); 50 pg endrin (9); 60 pg p,p'-DDD (10); 60 pg p,p'-DDT (11); 20 pg endosulfan II (12) and 60 pg endosulfan sulphate (13). Conditions described in Equipments (b).

Two solvents were necessary for the elution of the pesticides, the first being n-hexane (40 ml) and the second n-hexane:dichloromethane 8:2 (20 ml). When the first 40 ml of n-hexane was followed by a further 20 ml of the same solvent, none of the pesticides of the second fraction were eluted. Gas chromatograms illustrative of fraction 1 and fraction 2 of the fortified sample and the standard solution are shown in Figure 1. The pesticides endosulfan II and endosulfan sulphate, eluted in fraction 2, showed recoveries ranging from 91 to 104%.

Table 2 shows the percentage of recovery for an on-line procedure[11], two methods which employ two adsorbents in the cleanup step[9-10], and the proposed method. By comparing the data with these methods we can see the efficiency of our method.

The method presented here is simple, reliable and offers advantages in terms of speed of analysis and cost of reagents.

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