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A rapid, environmentally friendly, and reliable method for pesticide analysis in high-fat samples

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ABSTRACT

This report describes the development and validation of a simple, rapid, and efficient method in which solid-phase extraction followed by analysis in a gas chromatograph equipped with an electron capture detector (SPE-GC-ECD) is used for the simultaneous determination of dicofol, dieldrin, endosulfan, and permethrin in rat adipose tissue. This study targeted pesticides for which controversies exist regarding the harm that they may cause to humans, such as endocrine disruption or cancer, and that have also been found in recent years in vegetables consumed by the Brazilian population. The analytical procedure was optimised for SPE extraction and for GC-ECD conditions. The optimised method includes the extraction of the samples with n-hexane followed by an SPE procedure in which deactivated neutral alumina cartridges are used as the sorbent and a mixture of n-hexane:dichloromethane is used for elution. Recovery studies with spiked samples were used to evaluate the method's efficiency. Mean recoveries ranged from 75% to 119% with relative standard deviations (RSD) $< 19\%$. Quantification limits (LOQs) were 0.05 mg kg^{-1} for dieldrin and endosulfan and 0.5 mg kg^{-1} for dicofol and permethrin. The matrix effect was pronounced for all of the pesticides studied and ranged from 26% to 49%. In comparison to other related methods, this method requires less time and solvent and allows for rapid isolation of the target analytes with high selectivity. This method therefore allows for the screening of numerous samples and can also be used for routine analyses.

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1. Introduction

Agricultural practices throughout the world are still highly dependent on pesticides; without their use, food production would be drastically affected. However, many questions have been raised about the need for pesticides in agriculture because of their potential risk to public health and the environment that arises from their improper use [1].

Pesticide residues, especially organochlorinates, are found in all environmental compartments. Although many pesticides have been banned for agricultural use, they are still studied because of their extended persistence in the environment. These highly lipophilic compounds easily bio-accumulate and are found in lipidic tissues, such as vegetables, fruits, and fish, mainly in the liver and adipose tissue [2–4].

To ensure public health safety, many countries have established monitoring programs for the analysis of pesticide residues in food and in the environment. In Brazil, the Programme of Analysis of Pesticide Residues in Foods (PARA–Programa de Análise de Resíduos de Agrotóxicos em Alimentos) was established by the Brazilian National Health Surveillance Agency (ANVISA–Agência Nacional de Vigilância Sanitária). Under this programme, between July 2001 and December 2004, ANVISA surveyed 4001 fresh-food samples (lettuce, bananas, potatoes, carrots, oranges, apples, papayas, strawberries, and tomatoes). In this study, pesticide residues were found in 3271 samples, of which 71.5% were within the maximum-residue limits (MRL). The remaining 28.5% either exceeded the MRL or contained residues of pesticides that are unauthorised for certain crops. In 2009, recent surveys revealed that of 3130 samples analysed by the ANVISA, 29.0% were considered unsatisfactory: 2.8% of the total results showed levels above the MRL, 23.8% were unauthorised pesticides, and 2.4% showed both problems. The food plants with the highest frequency of abnormalities were tomatoes, apples, strawberries, peppers, and cucumbers. Pesticides detected most frequently included six unauthorised pesticides (dicofol, dieldrin, endosulfan, monocrotophos, chlorpyrifos, and dichlorvos) and five pesticides that are allowed but showed residue levels above the

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MRL (methamidophos, permethrin, triazophos, dithiocarbamates, and cyhalothrin) [5].

The presence of pesticides in environmental and biological materials has been known for decades, but studies regarding their potential effects in low concentrations on carcinogenicity and endocrine disruption have only begun [2–8]. Therefore, several faculty members in chemistry, biology, and medicine at São Paulo State University (Universidade Estadual Paulista), Brazil initiated a study to expose rats to low doses of pesticide mixtures to evaluate their effects as carcinogens and endocrine disruptors. This study targeted pesticides for which controversies exist regarding the harm that they may cause to humans, such as endocrine disruption or cancer, and that have also been found in recent years by the ANVISA in vegetables consumed by the Brazilian population [5]. These pesticides were dicofol, dieldrin, endosulfan, dichlorvos, and permethrin.

The development of analytical methods for the determination of these compounds in biological matrices requires estimations of the amounts that have been bio-accumulated and of their relationship to the development of damage in various tissues. The extraction methods described in the literature for the determination of these pesticides in lipidic matrices, such as adipose tissue, are often lengthy procedures that involve several steps of extraction and purification with several sorbents [6,9,10]. Commonly used procedures include the soxhlet extraction process [4], pressurised liquid extraction (PLE) [11], microwave-assisted extraction (MAE) [12], accelerated solvent extraction (ASE) [13], supercritical fluid extraction (SFE) [14], and sonication extraction (SE) [15].

Soxhlet extraction may require a long time period as well as the consumption of large quantities of organic solvents; therefore, it is being replaced by more rapid techniques, such as those previously cited (PLE, MAE, ASE, SFE, and SE). Other studies have evaluated liquid–liquid partition [16], gel permeation chromatography [17], preparative chromatographic columns [18], multiple cleanup [6,19], and treatment with acid or base [7]. However, these methods are also slow and consume large amounts of solvents and sorbents.

The objective of the present study was to optimise, validate, and implement a method for the analysis of residues of three organochlorine pesticides (dicofol, dieldrin, and endosulfan) and a pyrethroid (permethrin) in the adipose tissue of rats using solid-phase extraction and gas chromatography combined with an electron capture detector. We sought to develop a simple and efficient procedure that involves fewer experimental steps.

2. Experimental

2.1. Chemicals and standards

Standards of the pesticides dieldrin (97.9%), endosulfan (99.9%), and permethrin (98.0%) (Riedel de Haën) were obtained from Sigma-Aldrich GmbH Laborchemikalien (United Kingdom). Dicofol (96.5%) was obtained from Dr. Ehrenstorfer GmbH (Germany); this standard is constituted by the isomers o,p′-dicofol and p,p′-dicofol, which were designated dicofol I and II, respectively. The two peaks were used for quantification because the analytical curves refer to the sum of the areas of peaks I and II, as recommended by the ANVISA for dicofol monitoring in food [20].

The solvents used were n-hexane, acetone, isooctane, dichloromethane, and acetonitrile (HPLC grade, Mallinckrodt, USA). The chemical reagents used were neutral alumina (70–230 mesh ASTM, Sigma-Aldrich, Germany), Florisil (60–100 mesh ASTM, Fluka, Germany), C18 (200–400 mesh, Sigma-Aldrich, Germany), silica gel 60 (70–230 Mesh ASTM, Sigma-Aldrich, Germany), anhydrous sodium sulphate (Sigma-Aldrich, Germany), Celite (Sigma-Aldrich, Germany), and concentrated sulphuric acid (JT Baker, Mexico). Individual stock solutions (200 μg mL^{-1}) of each pesticide were prepared by dissolving 2 mg of each authentic standard in 10 mL of isooctane. Working standard solutions were obtained by diluting the individual stock solutions with isooctane. All solutions were stored at −20 °C and were renewed every 30 days.

Table 1
Chromatographic conditions used for pesticide analysis by a gas chromatograph equipped with an electron capture detector (GC-ECD).

Conditions	Varian GC-ECD 3800
Chromatography column	VF-5MS
Column temperature programme	Initial temperature 200 °C, held for 4 min, and then heated at 5 °C/min until 280 °C and held for 6 min. Run time = 26 min.
Injector temperature (°C)	280
Volume (μL)/injection mode	1/splitless
Carrier and makeup gas	Nitrogen
Carrier gas flow (mL min^{-1})	1.0
Makeup gas flow (mL min^{-1})	32.0
Detector temperature (°C)	300

2.2. Apparatus and chromatographic conditions

The studies were performed on a Varian 3800 (Agilent, Walnut Creek, USA) gas chromatograph equipped with an electron capture detector (GC-ECD) with a Varian (Agilent, Walnut Creek, USA) VF-5MS fused-silica capillary column (30 m, 0.25 mm internal diameter), 0.25 mm-thick film coated with 5% phenyl–95% methylpolysiloxane, and a split/splitless injector. The data-acquisition software used was Varian Galaxie™ (Agilent, Walnut Creek, USA). The chromatographic conditions were optimised based on previously published studies [8,9,21], and the parameters are detailed in Table 1.

2.3. Procedures

2.3.1. Obtaining samples of rat adipose tissue

This experiment was performed by the research group of Dr. J.L.V. Camargo, Faculty of Medicine (UNESP). All aspects involved in the animal experimentation were approved by the institutional ethics committee. The animals were evaluated weekly throughout the trial period, and all observed changes were recorded. After eight weeks, they were sacrificed by exsanguination under narcosis induced in a CO_2 chamber.

The rats were given feed spiked with the pesticides dicofol, endosulfan (α and β), dieldrin, and permethrin (*cis* and *trans*) in LOEL (lowest observed effect level) and NOEL (no observable effect level) doses according to Lu [22].

Rat adipose tissue was collected, wrapped in aluminium foil, labelled, and then immediately frozen at −18 °C. The mass of the collected adipose tissue ranged from 0.5 g to 2.5 g (perirenal, subcutaneous, and peritoneal), depending on the animal weight; 0.5 g sub-samples were used to perform the extraction procedure described in the next section. Samples were removed from the freezer, opened over Petri dishes, and homogenised by fragmentation using a spatula and a stainless-steel cutting blade. This procedure was performed quickly to prevent the tissue from melting. After homogenisation, the sample was again wrapped in aluminium foil and returned to the freezer at −18 °C.

2.3.2. Extraction.

The main difficulty in this extraction was to establish an experimental procedure that allowed for the removal of lipids without the loss of pesticides. Therefore, several experimental conditions were evaluated for the elimination, including solid-phase extraction involving various combinations of adsorbents and extraction of the sample by freezing.

To determine the best extraction conditions for the analysis of the pesticides in adipose tissue, two extraction methods were tested and optimised prior to implementation of the final method. In method I, which was based on that of Hong et al. [21], the pesticides were extracted by ultrasonication in an acetone: n-hexane mixture. Most of the lipids in the extract were eliminated by freezing-lipid filtration with acetonitrile prior to solid-phase extraction (SPE) cleanup using Florisil as an adsorbent. Method II was based on those of Nardelli et al. [8] and Criado et al. [9]: the adipose tissue was extracted with n-hexane, and this mixture was then mechanically shaken using a vortex agitator. Next, Florisil was added and the mixture was shaken, followed by the addition of acidified silica (44%) and sodium sulphate and subsequent centrifugation. Celite and 4.6% deactivated alumina were also evaluated separately in place of Florisil.

The final, optimised method was as follows: (1) the rat adipose tissue sample (0.5 g) in 10 mL of n-hexane was ultrasonicated with constant stirring for 5 min; (2) 1 mL of the resulting extract was submitted to solid-phase extraction using 4.6% deactivated neutral alumina and was subsequently eluted with n-hexane:dichloromethane (15 mL, 7:3, v-v); and (3) the eluate was dried and dissolved in 250 μL isooctane, and 1 μL was analysed by GC-ECD.

2.3.3. Determination of lipid content

Lipids were determined using a gravimetric method based on that of Phillips et al. [23]. An aliquot of 0.1 mL of the extract, which was obtained as described in the proposed method, was transferred to a small, preweighed flask. After total evaporation of the solvent, the flask was reweighed, and the difference between the initial and final weights was used to calculate the percentage of lipids.

2.3.4. Method validation

The accuracy of the method was estimated using Student's t test according to the equation described by Van der Voet et al. [24]. For this study, samples free of pesticides (obtained from no-exposed isogenic rats from same specie of exposed ones) were spiked with standard solutions of compounds, prepared in isooctane, which resulted in fortified samples in three different concentrations (0.05 mg kg^{-1}, 0.1 mg kg^{-1}, and 0.5 mg kg^{-1} for dieldrin and endosulfan; 0.5 mg kg^{-1}, 1.0 mg kg^{-1}, and 5.0 mg kg^{-1} for permethrin and dicofol). The samples were prepared in triplicate for each fortification level.

The interaction time between the matrix and the solutions of the pesticides was 3 h, which is in agreement with the results reported in similar studies [9,18,25–27]. These parameters were expressed in terms of the percentage recovery (accuracy) and the percentage relative standard deviation (RSD) (precision).

The limits of quantification (LOQ) and detection (LOD) of this method were calculated according to the method of Thier and Zeumer [28]. This procedure is considered the most suitable to estimate the method detection limit because it corrects for variations in the pesticide-free samples and in the lowest level of fortification, such as impurities in solvents and reagents, instrument noise, and co-extracted compounds.

2.3.5. Evaluation of matrix effect

The matrix effect can cause an increase or decrease of the chromatographic response of an analyte present in the extract of the sample compared to the same analyte in a pure solvent. This phenomenon was investigated for all pesticides studied. The matrix effect was assessed by comparison of the slopes of the analytical curves for analytes prepared in the matrix (adipose tissue from non-exposed rats) with the slopes of those prepared in isooctane. The calculation to evaluate this difference was performed using the equation given by Thompson et al. [29].

This procedure evaluates whether the matrix causes a positive (increased) or negative (decreased) effect on the sensitivity. When the result is greater than 10%, a matrix effect is considered to exist, which may influence the quantitative analysis [29].

One means of correcting for this effect is to prepare the analytical curves using the extract of the matrix. Each extract of the pesticide-free samples obtained using the proposed extraction method was evaporated under a gentle flow of N_2; after it was completely dry, it was dissolved in 250 μL of the mixture of pesticides prepared in isooctane at concentrations that corresponded to different points on the analytical curves.

3. Results and discussion

3.1. Chromatographic optimisation

The optimisation of the analysis conditions by GC/ECD was based on literature sources [8,9,21]. Solutions of dicofol, dieldrin, endosulfan, and permethrin were analysed by GC-ECD. The analysis run time was 26 min, and the resolution between the peaks was greater than 2.3.

3.2. Estimation of lipids extracted from rat adipose tissue

The lipid content found in the rat adipose tissue was $90 \pm 5\%$. This value is higher than that of the contents found by other investigators: 21% (chicken eggs), 23% (fish feed), and 85% (abdominal beef fat), found by Valsamaki et al. [30], Nardelli et al. [8], and Criado et al. [9], respectively. This high percentage of lipids indicates the complexity of the matrix and also indicates the need for an efficient extraction method that allows for good selectivity and chromatographic performance.

3.3. Development and optimisation of the extraction method

With the use of pure solvents or mixtures such as acetone and n-hexane in solid–liquid extraction, large amounts of lipids may be extracted and adsorbed in the capillary column and/or injection system. These lipids may contaminate the injector liner and the detector and interfere with the chromatographic analysis. Therefore, several cleanup procedures were used to remove the lipid material; these procedures involve numerous experimental steps and can require excessive amounts of time.

In many cases, the lipids can be effectively removed by saponification. However, many organochlorine pesticides are degraded in basic or acid hydrolysis. Tunistra et al. [31] have reported that 40% of endosulfan molecules lost their sulphate group under acidic conditions. Dieldrin is also significantly degraded; acid hydrolysis produces dechlorinated compounds, whereas other organochlorines are slowly hydrolysed under basic conditions. Accordingly, steps that involve treatment with an acid or base should be avoided in general organochlorine analyses. The results of the two extraction

methods evaluated prior to implementation of the final method are presented below:

3.3.1. Method I

This procedure resulted in adequate (approximately 47%) removal of lipids. However, the chromatogram showed a high degree of interference, and this method was therefore deemed inappropriate for the analysis using GC-ECD. In contrast, the chromatographic profile was improved with the use of deactivated alumina (4.6%); however, the recovery was less than 53% for all of the pesticides.

3.3.2. Method II

Lipids were removed using Florisil, and the extract showed no turbidity after being frozen at −20 °C for 4 h. However, the recovery percentages, at less than 5% for all of the pesticides, were unsatisfactory. Other modifications of this method, including the replacement of Florisil by alumina or Celite and the replacement of the extraction solvent with n-hexane:dichloromethane (9:1, v/v or 7:1, v/v), resulted in a slight increase in the recovery of some pesticides.

3.3.3. Final method

The proposed method described in Section 2.3.2 involved extraction with 10 mL of n-hexane; however, only a 1 mL aliquot of the extract was used for cleanup. This dilution step improved the amount of lipid removed, although most previous studies have not included this step.

The chromatogram of the control sample (adipose tissue from non-exposed animals) (Fig. 1) shows that the extract was clean and interference-free, which indicates the selectivity of the method.

3.4. Analytical curves

The analytical curves from standards prepared in isooctane and in the matrix (extract from control samples) were constructed for

Table 3
Evaluation of the method performance using spiked samples. Accuracy (% recovery, R), precision (coefficient of variation, CV), limit of detection (LOD), and limit of quantification (LOQ).

Pesticides	Spiking level(ng g^{-1})	R[a] (%)	CV[b] (%)	LOD[c] (ng g^{-1})	LOQ[d] (ng g^{-1})
Dicofol	500	87	18	352	500
	1000	76	10		
	5000	88	3		
α-endosulfan	50	92	12	25	50
	100	113	11		
	500	111	6		
Dieldrin	50	94	10	23	50
	100	96	7		
	500	116	4		
β-endosulfan	50	75	7	50	50
	100	94	14		
	500	106	7		
cis-permethrin	500	86	8	374	500
	1000	89	10		
	5000	119	14		
trans-permethrin	500	82	19	354	500
	1000	94	18		
	5000	101	12		

[a] Recovery.
[b] Coefficient of variation.
[c] Detection limit of the method, calculated as described in Section 2.3.4.
[d] Quantification limit of the method, estimated as described in Section 2.3.4.

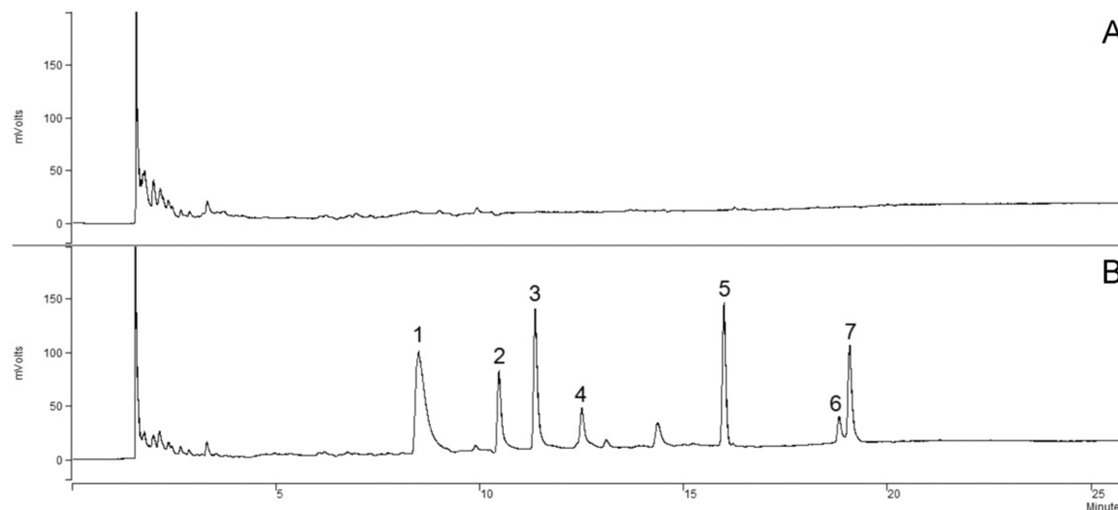

Fig. 1. Superimposed chromatograms of the control sample (without pesticides-A) versus pesticides (B) by GC-ECD (chromatography conditions as in Table 1): (1) 120 pg μL^{-1} of dicofol I (o, p′-dicofol), (2) 60 pg μL^{-1} of α-endosulfan, (3) 60 pg μL^{-1} of dieldrin, (4) 60 pg μL^{-1} of β-endosulfan, (5) 120 pg μL^{-1} of dicofol II (p, p′-dicofol), (6) 120 pg μL^{-1} of *cis*-permethrin, and (7) 120 pg μL^{-1} of *trans*-permethrin.

Table 2
Analytical curve parameters and matrix effect.

Pesticides	Equation in isooctane	Correlation coefficient	Equation in rat adipose tissue	Correlation coefficient	Matrix effect* (%)
Dicofol	$y=4471x-90312$	0.993	$y=2852x-13898$	0.997	−36
α-endosulfan	$y=9585x+13400$	0.998	$y=5638x-12466$	0.997	−41
Dieldrin	$y=14691x+5066$	0.995	$y=8563x+7422$	0.998	−42
β-endosulfan	$y=3423x+5212$	0.991	$y=2519x-1312$	0.998	−26
cis-permethrin	$y=210.6x+3051$	0.996	$y=106.9x+6402$	0.991	−49
trans-permethrin	$y=813x+22516$	0.993	$y=541.2x+18039$	0.990	−33

* Difference in slopes (%) of solvent and matrix curves.

all of the pesticides in the linear range of 80–1200 pg μL^{-1} for permethrin and dicofol and 5-120 pg μL^{-1} for dieldrin and endosulfan. Table 2 shows the equations for the curves and the correlation coefficients. Good correlation coefficients were obtained for all of the pesticides (> 0.99). Table 2 also shows the matrix effect for each pesticide, calculated as the difference between the angular coefficients for the two curves (solvent and matrix), as described in Section 2.3.5. The matrix effect was pronounced for all of the pesticides (greater than 26%).

The matrix effect can be explained by the complexity of rat adipose tissue, which consists primarily of lipids (90%). Even with the purification steps, lipids may have been extracted together

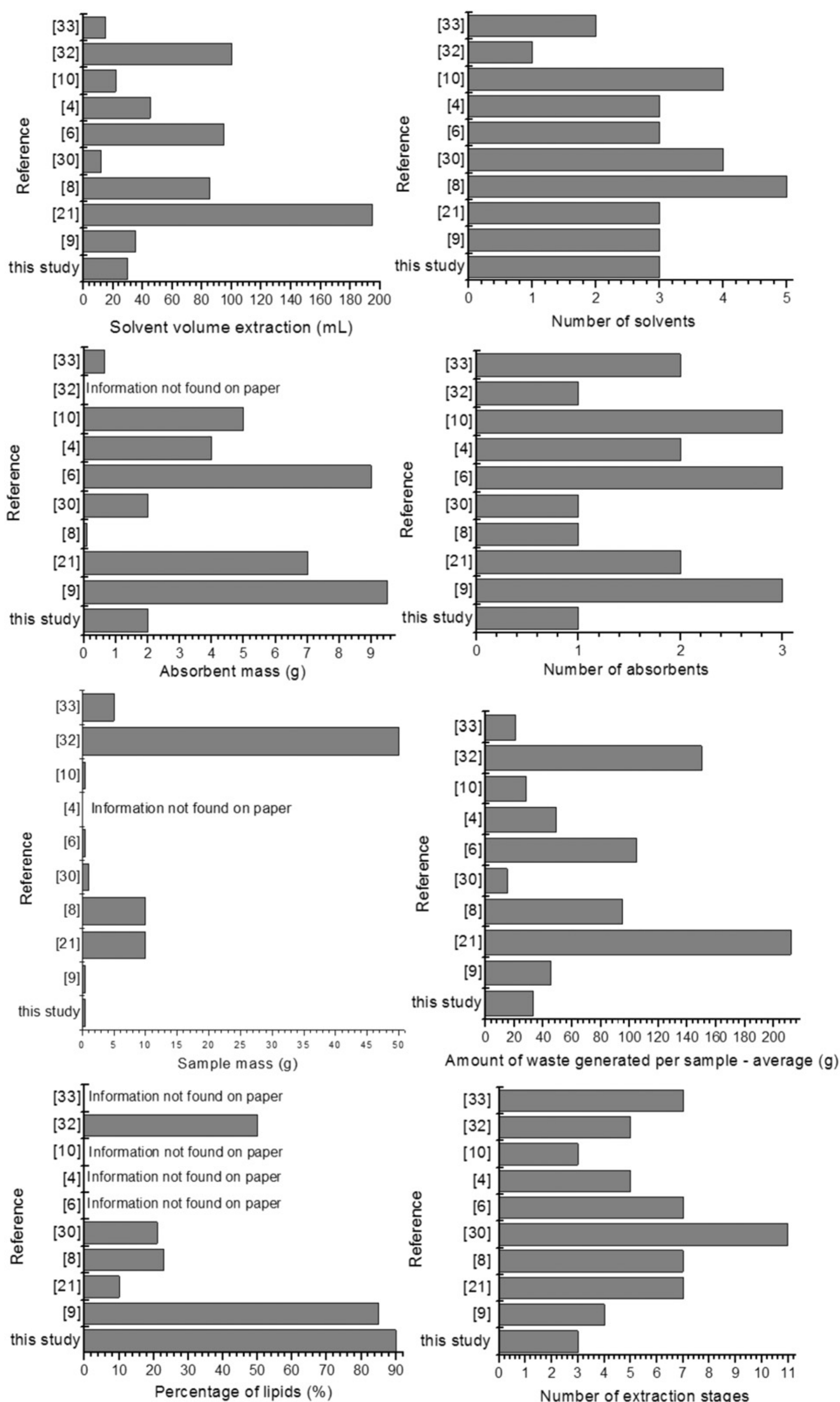

Fig. 2. Comparisons between the method proposed in this work and the literature.

Table 4
Recovery and coefficient of variation reported in different studies.

Reference	Concentration in spiked samples (ng g^{-1})	Matrix/compounds	Recovery range (%)	CV[a] (%)	Lipids (%)	LOD[b] (ng g^{-1})	LOQ[c] (ng g^{-1})
This study	50–5000	Animal fat (rat)/pyrethroid and organochlorinated pesticides	75–119	3–19	90	23–374	50–500
[9]	5–50	Animal fat (beef and chicken)/polychlorinated biphenyls	74–111	2–13	85	NP[d]	0.4–1.5
[21]	100	Fish/organochlorinated pesticides	78–115	1–14	10	0.5–20	NP[d]
[8]	100	Fish/organochlorinated pesticides	61–134	2–24	23	3	NP[d]
[30]	50	Chicken eggs/organochlorinated pesticides and polychlorinated biphenyls	82–110	2–8	21	< 0.7	< 0.7
[6]	NP[a]	Human adipose tissue/organochlorine	≥ 80	NP[d]	NP[d]	2–5	NP[d]
[4]	NP[a]	liver, muscle and fat tissue of rats/endosulfan	NP[d]	NP[d]	NP[d]	NP[d]	NP[d]
[10]	NP[a]	Chicken eggs and cucumbers/pesticides (several classes)	NP[d]	1–18	NP[d]	NP[d]	NP[d]
[32]	50–500	Fatty foods/organochlorine and organophosphate pesticides	76–104	1–14	50	0.9–12.5	2.7–37.8
[33]	40–160	Soybean oil/pesticides (several classes)	80–114	2–14	NP[d]	NP[d]	< 10^3

[a] Coefficient of variation.
[b] Limit of detection.
[c] Limit of quantification.
[d] Not provided.

with the pesticides, which may have interfered with the capture of β particles emitted by the electron capture detector during the gas chromatography analysis.

The analytical curve prepared in the matrix corrects for the observed effects and incorporates a correction into the recovery results, which thus show a value close to reality. Therefore, the quantification steps for the evaluation of the method recovery and for application to samples of rat adipose tissue were performed using the analytical curves prepared in the matrix extract.

3.5. Recovery percentage, accuracy, and limits of detection (LOD) and quantification (LOQ).

As shown in Table 3, the method is considered accurate because the recovery (75–119%) is in accordance with values reported in the literature [24,28] for pesticide residue analysis. The method is also precise because the coefficients of variation obtained (3–19%) were less than 20%. The limits of detection and quantification of the method, which were estimated using the statistical model described by Their and Zeumer [28], were appropriate for this study.

3.6. Comparison with other studies

Fig. 2 presents a comparison of our method with those of other studies that have compared the sample consumption and adsorbent mass, the types of adsorbents and solvents, the solvent consumption, the stages of the extraction procedure, the preparation of complex samples with high fat content, and the waste generated per sample.

As illustrated in Fig. 2, the method described in this study uses a relatively small amount of solvent (30 mL), unlike most of the cited studies (greater than 40 mL) [4,6,8,9,21,32]. The solvent types most often mentioned were n-hexane, isooctane, dichloromethane, acetone, acetonitrile, methanol, and ethyl acetate. However, a few investigators used cyclohexane, petroleum ether, or toluene [8,9,30].

The adsorbent mass used in this study was 2 g, which is a significantly smaller amount compared to that used by Munoz-de-Toro et al. [6], Criado et al. [9], and Frenich et al. [10] (an exception is Nardelli et al. [8], who used only 0.1 g). However, as previously mentioned, Nardelli et al. [8] used a large amount (85 mL) of five different types of solvent. Most studies [8,9,30,32,33] involved the use of two or more adsorbents, most often alumina, Florisil, and/or silica gel, whereas the method described here requires only one.

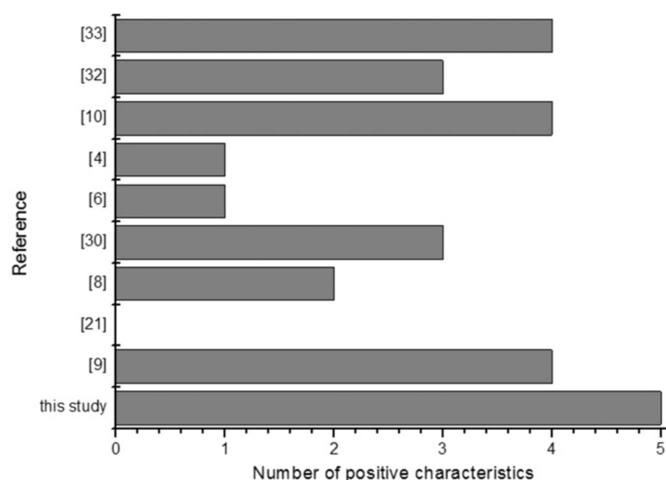

Fig. 3. Comparison of positive characteristics of the proposed method with others described previously.

The sample mass used in this study was only 0.5 g, which is similar to the amounts used in three previous studies [6,9,10]. Most of the other studies used approximately 10 g to 50 g (Kuvarega et al. [4] did not provide this information).

One of the requirements of "green" chemistry is the generation of only small amounts of waste. The waste amount generated was estimated by analysis in each study based on the extraction procedures. Many of the described methods generated approximately 40–210 g of waste per sample, which is undesirable because studies that involve large numbers of samples can produce large amounts of waste. The present study generated only 25 g of waste per sample, which is similar to amounts generated in the studies of Frenich et al. [10], Valsamaki et al. [30], and Nguyen et al. [33].

The percentage of lipids is an important factor in chromatographic analysis, and this information should be provided; however, some of the studies cited here did not mention the lipid content of their samples [4,6,10]. This characteristic posed the greatest challenge in the present study: removing these compounds without affecting the chromatographic analysis while maintaining the accuracy and precision of the method. This study and that of Criado et al. [9] had the highest amounts of lipids present in the matrix extract (90% and 85%, respectively), and both achieved good parameters of validation.

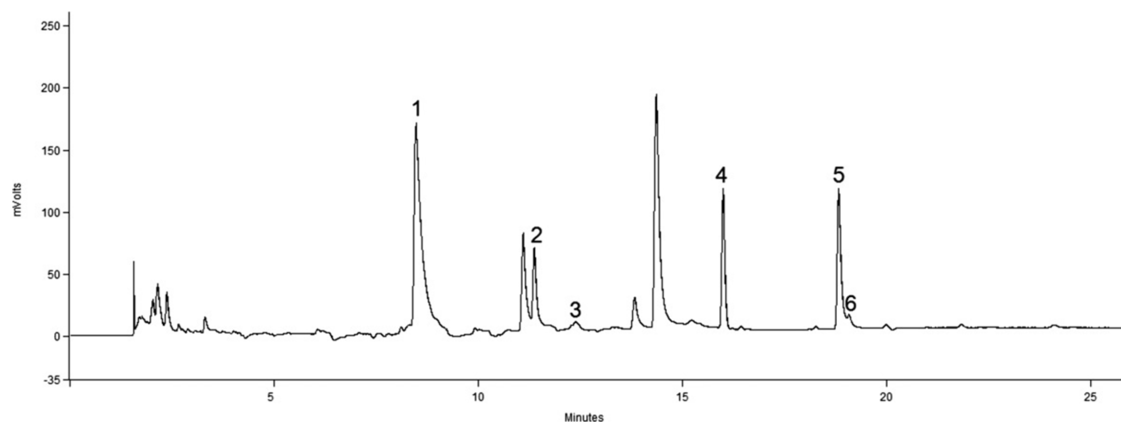

Fig. 4. GC-ECD chromatogram obtained from peritoneal rat adipose tissue extracted with the proposed procedure, containing: 4270 ng g^{-1} of dicofol total (sum of isomers o, p′-dicofol and p, p′-dicofol, peaks 1 and 4 respectively); 178 ng g^{-1} of dieldrin (peak 2); 63 ng g^{-1} of β-endosulfan (peak 3); 4278 ng g^{-1} of *cis*-permethrin (peak 5) and *trans*-permethrin (detected only—peak 6).

An important feature of an analysis method is the use of the fewest possible extraction steps, i.e., maximisation of its rapidity and ease. Therefore, we estimated the number of steps involved in each extraction method by considering the processes involved, such as sonication, cleanup, and concentration of the extract. The method presented here and that used by Frenich et al. [10] used the fewest experimental stages, i.e., they were simpler and faster than the other methods. However, Frenich et al. [10] did not mention the lipid percentage of the matrix studied, which made a comparison of their study with the present work difficult.

With respect to the validation parameters, our method showed good accuracy and precision, as recommended by the literature, as did the other studies. The detection and quantification limits are of the same order of magnitude as most of the studies cited here [6,8,9,21,30,32,33]. However, other studies did not provide these values (Table 4) [4,10].

These data (Fig. 2 and Table 4) indicate which studies showed more positive characteristics and allow a comparison with the results of the present study. The graph obtained is shown in Fig. 3.

In general, this study brought together positive characteristics (even with a highly lipid matrix), showed good validation parameters (percentage of recovery, coefficient of variation, LOD, and LOQ), used small amounts of solvents and adsorbents, generated relatively little waste per sample, and could be performed with few experimental steps.

3.7. Application to rat adipose tissue in a toxicological study

The validated and optimised procedure was applied to the determination of dicofol, endosulfan (α and β), dieldrin, and pemethrin (*cis* and *trans*) in 125 samples of rat adipose tissue, including peritoneal, subcutaneous, and perirenal tissues. Fig. 4 shows a sample chromatogram for a peritoneal adipose tissue.

In 54.4% of the samples of subcutaneous, peritoneal, and perirenal adipose tissues, at least one of the pesticides was detected.

4. Conclusion

This study has shown that for the extraction of the selected pesticides dicofol, endosulfan (α and β), dieldrin, and permethrin (*cis* and *trans*), the preferred method is SPE with 4.6% deactivated alumina, followed by a dilution step of the n-hexane extract to purify the extracts. The proposed method is simple and rapid, uses only one type of adsorbent, consumes a relatively small amount of solvent, and uses more accessible extraction and quantification techniques (SPE and GC-ECD). Based on the validation parameters appropriate for this type of analysis, recovery at three different levels of sample fortification gave proper accuracy (75–119%) and precision (3–19%).

The optimised SPE-GC-ECD method was applied successfully to the analysis of 125 samples of rat adipose tissue. Pesticide residues were detected in 54.4% of the samples, which shows that, even at low doses (NOEL and LOEL), these pesticides accumulate in tissues (especially *cis*-permethrin, which has not been reported in similar studies).

Finally, the method is also environmentally friendly (according to the principles of green chemistry) and thus represents an alternative to conventional methods. In addition, it provides for rapid isolation of the target analytes with high selectivity, which allows for the screening of numerous samples and the application of the method to routine analyses.

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