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QuEChERS-Based Method for Pesticides Analysis in Adipose Tissue Associated with Rat Ovaries

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Abstract

The concomitant exposure to low doses of various pesticides is one of the most relevant issues in human toxicology today. An experimental toxicology study was developed to evaluate the effects of this type of exposure on the reproductive capacity of females of three species of rats that were exposed to mixtures of dicofol, dieldrin, endosulfan and permethrin at low doses (LOAEL and NOAEL). In this context, we have developed a method for determining pesticides in adipose tissue (0.5 g, 49% lipid) associated with the ovaries, based on the QuEChERS (quick, easy, cheap, effective, rugged and safe) strategy. The method quantification limit (LOQ) was 0.5 mg/kg for dicofol and permethrin, 0.05 mg/kg for endosulfan and dieldrin and 0.2 mg/kg for diclorobenzophenone. Mean recoveries ranged from 75% to 93% with a relative standard deviation <13%. The unspecific selectivity (matrix effect) indicates the mandatory use of analytical curves constructed on the matrix extract. All the analyzed samples (53 adipose tissue associated to ovaries) showed residues of dichlorobenzophenone + dicofol, dieldrin and *cis*-permethrin while *trans*-permethrin were detected in 40% of the samples but were below the LOQ. The data indicated the bioaccumulation characteristics of these substances.

Introduction

Pesticides have been used since the 1940s and are still essential to ensure productive and secure food supplies. However, using pesticides irregularly entails severe environmental and public health concerns. In addition to poisoning, which is the most well-known problem, pesticides can be responsible for other diseases, such as allergies, cancer and neurological and reproductive disturbances (1). Many of these complications are directly linked to the ability of pesticides to interfere with the endocrine system in humans and animals; thus, some pesticides are considered endocrine disrupters (EDs) (2).

Humans can be exposed to EDs, among other ways, through contamination of drinking water and food (3). Fruits and vegetables are often contaminated with pesticides because many of these foods are lipophilic (4). Additionally, pesticides can bioaccumulate in the various strata of the food chain (5) and can be present in eggs, meat, milk, etc.

To strengthen the Brazilian's government ability to provide food safety and prevent possible damage to the population's health, the Brazilian National Health Surveillance Agency (ANVISA—Agência Nacional de Vigilância Sanitária) was established in 2001 the Programme of Analysis of Pesticide Residues in Foods (PARA—Programa de Análise

de Resíduos de Agrotóxicos em Alimentos) aims to continuously evaluate the levels of pesticide residues in fresh food for Brazilian consumers (6).

At the time of the last report, covering the year 2012, a total of 1,665 samples were analyzed, and 29% had unsatisfactory results. Within the irregularities found, active ingredients not allowed in the culture were found in 416 samples, corresponding to 25% of the total samples analyzed (6).

The population's exposure to pesticides occurs through food in low doses and involving a mixture of pesticides. A group of researchers that cover different areas of research have proposed a project to evaluate in experimental animal models (rats) the effects of ED when these animals were exposed in conditions similar to the conditions found in humans. For these experiments, the pesticides most commonly found in foods and most consumed when the project began were selected (dicofol, dieldrin, endosulfan and permethrin).

To determine the accumulation potential of these substances and aggregate the results from toxicological tests involving dysfunction in the reproductive system, adipose tissue associated with the ovaries of rats (animal testing of biological tissues) was chosen for analysis.

For matrices with high-lipid content, the main extraction methods are solid–liquid for solid matrices and liquid–liquid for liquid matrices. At this stage of the work, solvent choice is of extreme importance because the solvent is directly related to the recovery of analytes and the amount of impurities. The most commonly used solvents include hexane (7, 8), petroleum ether (4, 9), acetonitrile (10, 11) and some solvent mixtures (12, 13).

Because most pesticides are non-polar, the use of a non-polar solvent ensures high recovery, but increases the co-extraction of the matrix, and in this case adding a cleaning step (cleanup) of the extract is required. To remove interferents, the most widely used method is solid phase extraction (SPE) (4, 9, 13), and in some cases, this technique is used in conjunction with matrix solid phase dispersion (MSPD) (14, 15).

However, the most prominent technique for multiresidue analysis of pesticides is dispersive SPE (d-SPE), and this method is known as QuEChERS (quick, easy, cheap, effective, rugged and safe), as proposed by Anastassiades *et al.* (16). At first, the method has been proposed for food matrices with low-fat content such as apples, oranges and lettuce. Several studies use this method, with some modifications, for different matrices (17, 18).

First, this treatment consists of sample extraction using acetonitrile in the presence of water, anhydrous magnesium sulfate and sodium chloride. The hydration of magnesium sulfate is an exothermic reaction, and the heat generated by this reaction helps to extract the analytes, while adding sodium chloride promotes the separation of the polar and organic phases (acetonitrile and water) (16). The next step is the cleanup (d-SPE) with a portion of the extract, where the main adsorbent is a primary secondary amine (PSA), which is modified silica with a short carbon chain and two amine groups. In this technique, the adsorbents are agitated with just the extract. For the lipid matrix, adsorbents, such as C18, that are capable of retaining non-polar interfering substances are more commonly used (19, 20).

The objective of this study was to validate a method of extraction based on the QuEChERS method for determination of organochlorine pesticides (dieldrin, dicofol and endosulfan) and permethrin (pyrethroid class) in adipose tissue associated with the ovaries of rats, and the subsequent application of this method to experimental animals for toxicological study.

Experimental

Chemicals and standards

Standards of the pesticides dieldrin (97.9%), endosulfan (99.9%, with 67% alpha and 33% beta isomers, w/w), permethrin (98.0%, with 40% *cis* and 60% *trans* isomers, w/w) and dicofol (97.6%) were obtained from the laboratory supplier Sigma-Aldrich GmbH Laborchemikalien (Seelze, Lower Saxony, Germany). The solvents used were isooctane and acetonitrile (HPLC grade, Mallinckrodt, USA). The chemical reagents used were PSA (47–60 µm, Varian; 45 µm, Agilent), C18 (45 µm, Sigma-Aldrich), anhydrous magnesium sulfate (Sigma-Aldrich) and sodium chloride (Sigma-Aldrich). Individual stock solutions (100 mg L^{-1}) of each pesticide were prepared by dissolving 10 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 remade every 30 days.

Apparatus and chromatographic conditions

The studies were performed on a Varian 450 (Agilent, Walnut Creek, USA) gas chromatograph equipped with an electron capture detector (GC-ECD) with a Varian (Agilent, Walnut Creek, USA) VF-5MS column fused-silica capillary (30 m, 0.25 mm internal diameter), a 0.25 µm-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 optimization of the analysis conditions by GC-ECD was based on Toledo Netto *et al.* (8). The column temperature program was as follows: an initial temperature of 200°C (4 min) was then increased to 280°C (6 min) at 5°C/min, total run time was 26 min; injector temperature: 280°C; 1 µL of volume injection, in a splitless mode (30 seconds) and split 1:50; nitrogen as carrier and makeup gas; carrier gas flow: 1 mL min^{-1}; makeup gas flow: 32 mL min^{-1}; detector temperature: 300°C.

For the tests with the standard of dicofol a Varian 3800 (Agilent, Walnut Creek, USA) gas chromatograph equipped with an ion trap mass spectrometer detector was used, model Varian Saturn 2000 operating in the MS and MSMS modes, with a Varian (Agilent, Walnut Creek, USA) VF-5MS column fused-silica capillary (30 m, 0.25 mm internal diameter), 0.25 µm-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). Column temperature program: initial temperature of 90°C (3 min), increased to 230°C at 20°C/min and then increased to 280°C (8 min) at 5°C/min, injector temperature: 270°C; 1 µL of volume injection, in a mode split 1:10. He carrier gas flow: 1 mL min^{-1}. Conditions of the detector: Transfer line: 200°C; manifold: 50°C; ion trap: 180°C; 70 eV electron ionization; Electron multiplier: 1800 V; 15 µA was the emission current; scan mode, range: 70–420 amu.

Procedures

Obtaining samples of rat adipose tissue

The research group of Dr J.L.V. Camargo, Faculty of Medicine (UNESP), performed this experiment. All aspects involved in the animal experimentation were approved by the institutional ethics committee. Female rats from the Wistar, Sprague-Dawley and Lewis species were randomized into three groups: one group received a semipurified diet (non-treated); two groups received a semipurified diet containing low dose mixtures (dieldrin 0.025 mg/kg, endosulfan

0.7 mg/kg, dicofol 0.5 mg/kg, dichlorvos 0.23 mg/kg and permethrin 5 mg/kg) or an effective dose mixture (dieldrin 0.05 mg/kg, endosulfan 3.8 mg/kg, dicofol 2.1 mg/kg, dichlorvos 2.3 mg/kg and permethrin 25.0 mg/kg). Euthanasia was performed between the 10th and the 12th experimental week.

Adipose tissue associated with the ovaries from each animal was collected, wrapped in foil, properly identified and immediately frozen at −18°C. The individual samples were taken from the freezer, opened on Petri dishes and homogenized by fragmentation using a scalpel, wrapped in foil again and returned to the freezer at −18°C. This procedure was performed quickly to prevent the tissue from melting.

Determination of lipid content

Lipids were determined using a gravimetric method based on the procedure from Phillips *et al.* (21). Of the note, 10 mL of hexane was added to 0.5 g of fat and this mixture was ultrasonicated for 5 min. An aliquot of 0.1 mL of the extract 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.

Sample treatment

In this work, the method was based on Cunha *et al.* (19), which used a QuEChERS method to analyze multiclass pesticides from olive oil.

The final optimized method involved (i) extraction and partitioning of adipose tissue (0.5 g) with acetonitrile (10 mL), water (7 mL), NaCl (1 g) and $MgSO_4$ (4 g); shake with a vortex (1 min) followed by centrifugation (1 min); (ii) d-SPE with Acetonitrile (ACN) extract (1 mL), PSA (50 mg), C18 (50 mg) and $MgSO_4$ (150 mg); shake with a vortex (20 s) followed by centrifugation (1 min); (iii) 200 µL extract is transferred to a vial, dried with N_2, dissolved in isooctane (200 µL) and analysed (1 µL) by GC-ECD.

Method validation

To assess the reliability of the proposed extraction method, the following parameters were evaluated: selectivity, matrix effect, accuracy, precision and limits of detection (LODs) and quantification (LOQs). For all the studies, diclorobenzophenone was evaluated separately from the dicofol standard.

To evaluate the selectivity, the proposed method was tested in a control sample (without pesticides) to evaluate the possibility of substances interfering with the analytes retention times.

The matrix effect is the increase or decrease of the chromatographic response that occurs due to the presence of the matrix components after extraction, which are evaluated by comparing the calibration curves for the solvent and matrix extract. The extract curve was prepared with control samples of fat tissue. After the whole extraction procedure for adipose tissue without the pesticides (control sample), the extracts were suspended in 200 µL solutions containing a mixture of pesticides at known concentrations.

Two models were proposed to estimate the effect of the matrix: the first model was Student's *t*-test for comparison of the means, using as parameters the linear and angular coefficients of the equations of analytical curves prepared in the matrix extract and in the solvent. Additionally, Thompson *et al.* (22) suggested to first assess if the residual variances of the two curves are significantly different by the *F* test and then apply the most appropriate Student's *t*-test: two samples assuming equal or different variances. To perform this calculation, the software Microsoft® Excel® 2010 was used. The matrix effect was observed when the calculated *t* value was greater than the tabulated *t* value at a given significance level. The second model was based on Toledo Netto *et al.* (8) that assessed the matrix effect by the comparison of the slopes of the analytical curves for the analytes prepared in the matrix (adipose tissue from non-exposed rats) with the slopes of those substances prepared in isooctane, without a statistical test.

For the recovery study, control samples obtained from animals that received none treatment (semipurified diet without pesticides) were spiked at three different levels with standard solutions of compounds (0.5, 3.0 and 7.0 mg/kg for dicofol and permethrin; 0.05, 0.3 and 0.7 mg/kg for dieldrin and endosulfan; 0.2, 3.0 and 7.0 mg/kg for DBD) in triplicate. The spiking process was carried out by weighing 0.5 g of homogenized sample in a glass tube. The spiked sample remained in a freezer for 4 hours and was held for 10 min at ambient temperature before extraction.

The LOD and the LOQ of this method were calculated according to the method of Thier and Zeumer (23). The LOQ is defined as the lowest concentration of the substance under study fortified in that matrix that has recovery values between 70 and 120%, with coefficients of variation (CVs) <20%. The LOD of this method was estimated using the results for the lowest level of recovery and extraction performed with the control sample.

Results and Discussion

Estimation of lipids extracted from rat adipose tissue

The lipid content found in the rat adipose tissue was 49 ± 6%, and the tissues were high fat in comparison to food, which is classified as a high-fat food when the lipid content is greater than 20% (20). Therefore, we can say that the matrix of this study has a high-lipid content, which demonstrates the complexity of the matrix under study and justifies the need for an effective extraction method to remove the maximum amount of lipids and other matrix constituents without the loss of pesticides.

Dicofol study

During this study, dicofol presents instability in the peak numbers and the intensities of the peaks. Beyond the isomers, dicofol can be degraded by light to 4,4′-dichlorobenzophenone that is also one of the principal metabolites found in rats (24). To elucidate the identity of these peaks, this sample was analyzed in a solution of dicofol by GC–MS with electron ionization. The chromatogram and the mass spectrum obtained are presented in Figure 1.

The GC–MS chromatogram showed two peaks with very similar mass spectra. An analysis of the spectrum indicated the presence of the three characteristic ions of dicofol (*m/z* 111, 139 and 251), and the three characteristic ions of 4,4′-diclorobenzophenone (*m/z* 111, 139 and 250). A difference of only one unit of mass was observed (*m/z* 250 and 251). This difference, although not as significant, is used to identify the analytes dicofol (*m/z* 251) and diclorobenzophenone (*m/z* 250) in GC–MS analysis (25).

In addition to the GC–MS analysis, a standard of diclorobenzophenone was used to check the retention time (t_R) in GC-ECD using the optimized conditions. Dicofol standard had two peaks, the first at t_R = 8.35 min, indicating the presence of degradation product of dicofol (DBP), and the second at t_R = 15.87 min, related to dicofol. The cause of the observed degradation is difficult to explain, and could have occurred in the preparation of the standard solutions, during the extraction or in the chromatographic system (25–28). Due to this degradation and diclorobenzophenone being indicated

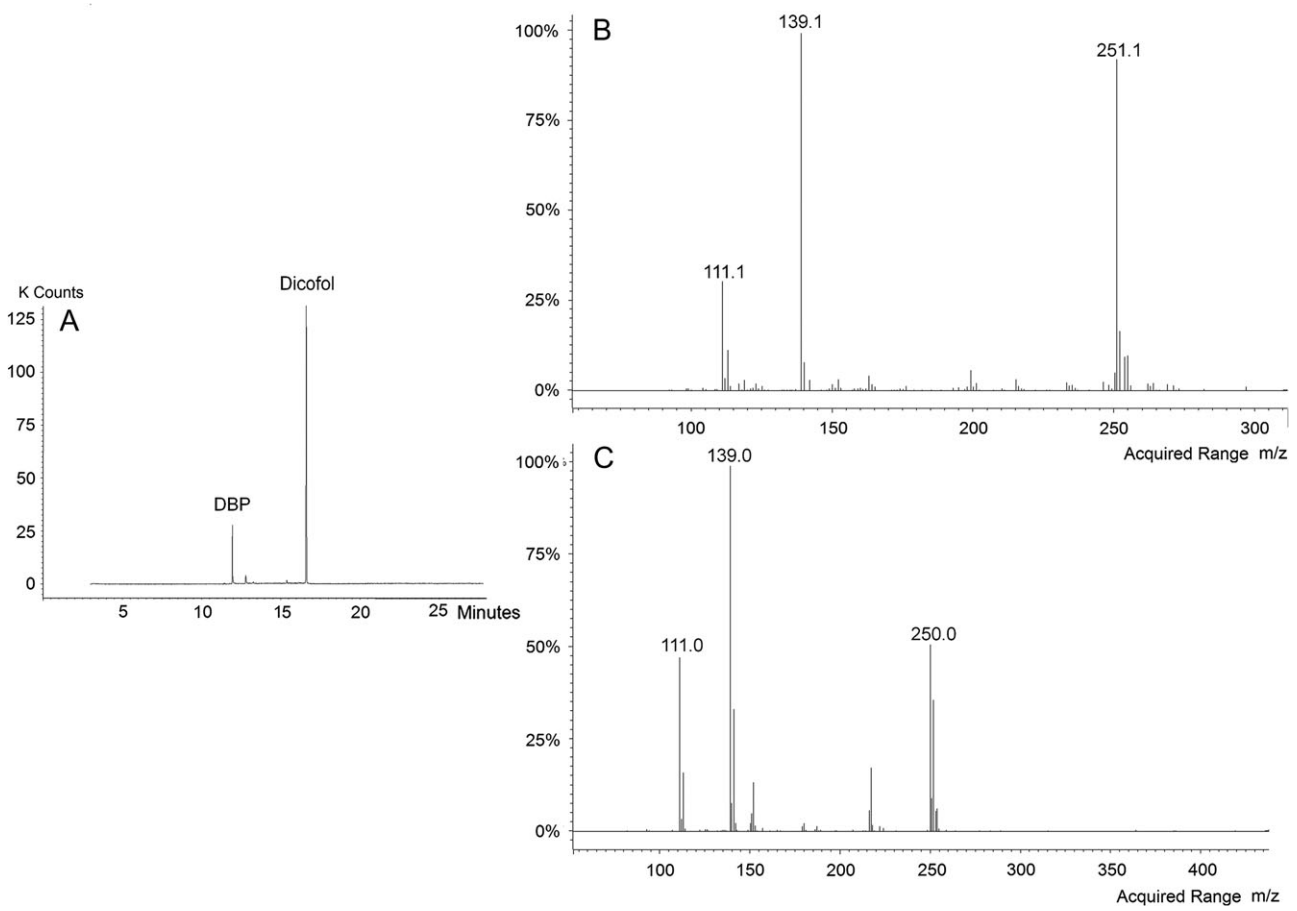

Figure 1. GC–MS chromatogram of dicofol (100 mg L^{-1}). (A) Extracted ion chromatogram of the selected mass, (B) mass spectrum obtained for dicofol and (C) mass spectrum obtained for DBP.

as a metabolite of dicofol in animals (24), we chose to evaluate the presence of both the analytes: dicofol (t_R = 15.87) and its degradation product, DBP (t_R = 8.35).

Extraction method validation

Selectivity

The QuEChERS extraction method was initially tested to verify the possible presence of interference from the matrix (specific selectivity). Figure 2 shows the chromatogram obtained from carrying out the extraction process with a control sample (without pesticides) also shown with a chromatogram of the pesticides mixture standard solution, which indicates that the extraction method is selective because no interfering substances were present at the same retention times of the analytes.

Analytical curves and matrix effects

Table I shows the equations of the analytical curves, the correlation coefficients were above 0.99, and the matrix effect for each pesticide.

Some approaches can be used to evaluate the presence of a matrix effect (8, 22, 29) by comparing the means (by Student's *t*-test) or the variance homogeneity (by *F* test), and these approaches can be applied when the matrix without the analytes (control sample) is available. In this approach, first, the *F* test is applied to verify that the variances of the samples (control sample and control sample spiked) can be considered equal in the following equation:

$$F = \frac{s_1^2}{s_2^2} \quad (1)$$

where s_1^2 and s_2^2 are the variances of each sample with the highest variance in the numerator. At the same time, the $F_{tabulated}$ value is calculated with (n_1-1) degrees of freedom in the numerator and (n_2-1) degrees of freedom in the denominator; adopting a confidence level of 95%. There are two cases:

(I) If the *F* test is not significant, then the calculated *F* is smaller than the tabulated *F*, and the matrix does not have a major effect on the accuracy of the method in the concentration range studied. In this case, the standard deviations of the test groups can be pooled and the significance of the mean differences of the two sets of samples may be tested with Student's *t*-distribution using the following equation:

$$t = \frac{\left|\bar{x}_1 - \bar{x}_2\right|}{\sqrt{s^2\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}} \quad (2)$$

where $\bar{x}_1$ and $\bar{x}_2$ are the averages of the slope coefficients of the equations of standard curves prepared in the solvent and

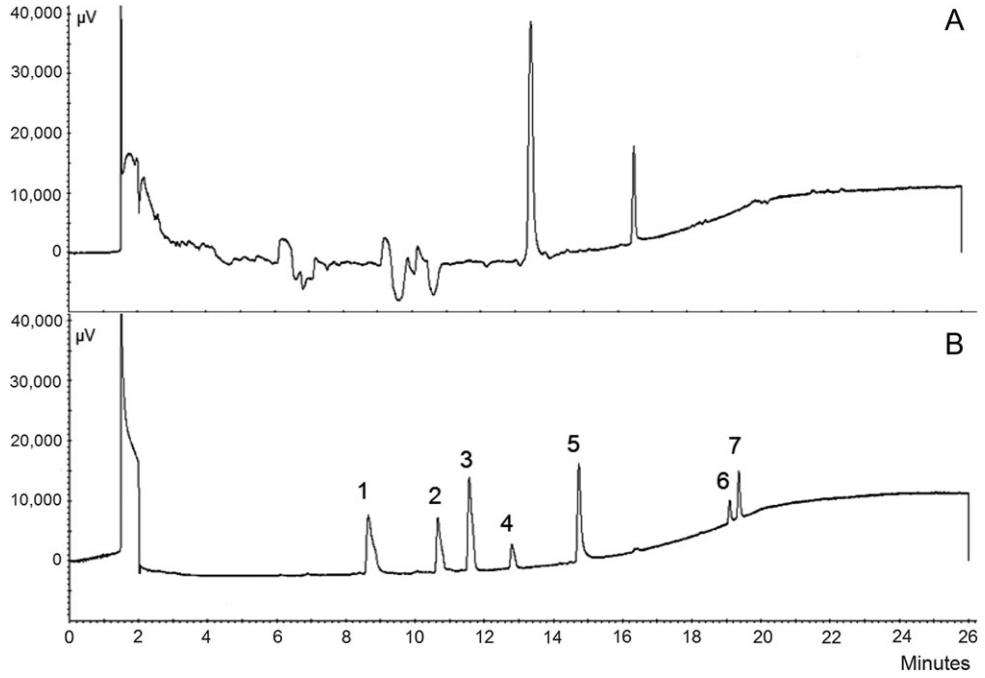

Figure 2. Superimposed GC-ECD chromatograms of the control sample (without pesticides—A) versus pesticides (B) by (1) diclorobenzophenone (within dicofol comercial standard), (2) 6.7 μg/L of α-endosulfan, (3) 10 μg/L of dieldrin, (4) 3.3 μg/L of β-endosulfan, (5) 100 μg/L of dicofol, (6) 40 μg/L of *cis*-permethrin and (7) 60 μg/L of *trans*-permethrin.

matrix, $\bar{n}_1$ and $\bar{n}_2$ are the sample sizes and s^2 is the estimate of the pooled variance. The $t_{tabulated}$ value was obtained from the table for the Student's distribution for $(n_1 + n_2 - 2)$ degrees of freedom and with 95% confidence.

(II) If the *F* test is significant, then the array has an important effect on the accuracy of the method in the concentration range studied and the variances can be considered unequal and $t_{calculated}$ is obtained by the following equation:

$$t = \frac{\left|\bar{x}_1 - \bar{x}_2\right|}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}} \quad (3)$$

where $\bar{x}_1$ and $\bar{x}_2$ are the averages of the slope coefficients of the equations of standard curves prepared in the solvent and matrix, $\bar{n}_1$ and $\bar{n}_2$ are the sample sizes and s_1^2 and s_2^2 are the estimates of the variances within each group. In this case, the $t_{tabulated}$ value was obtained as described by the guide from the National Institute of Metrology, Quality and Technology (INMETRO—Instituto Nacional de Metrologia, Qualidade e Tecnologia) (30).

The matrix effect evaluated by the statistical approach indicated the presence of matrix effects only for DBP, α-endosulfan and dicofol, and this result was also observed by the strategy proposed by Toledo Netto *et al.* (8). However, the statistical test provides more reliability within a certain confidence level because the second approach does not have a maximum value to assume the absence of a matrix effect.

Even for pesticides (dieldrin, β-endosulfan, *cis*- and *trans*-permethrin) showing no matrix effect, the recovery study and the samples quantification were performed using the analytical curve prepared in the matrix extract for all the analytes.

Recovery, dicofol studies, LOD and LOQ of the method

In Table II, the recoveries and CVs found for all the pesticides at three levels of the concentration, the values for the LOD and the LOQ of the method are shown.

The values for DBP, α-endosulfan, dieldrin, β-endosulfan and *cis*- and *trans*-permethrin were determined within the range of recovery (70–120%) and CV (<20%) established by Thier and Zeumer (23) for pesticide analysis, indicating a good precision and accuracy of the developed method.

The dicofol recovery study was conducted separately, as previously described, the values for the recovery of BPD present in the dicofol standard, the value found for dicofol and the sum of the two peaks (BPD + dicofol) are shown.

Dicofol recovery values found for the second and third level of fortification were 30% and 27%, respectively, and are lower than the values indicated in the literature (70–120%) (23). Another negative factor was the CV was above 20%. Analyzing the same fortification levels (second and third) of DBP, degradation of dicofol was evident, causing the formation of DBP and decreasing the recovery of dicofol.

This degradation of dicofol was already addressed in another study with the QuEChERS method, and according to Lehotay *et al.* (25) this problem is hardly controlled due to various factors that may affect the problem, such as pH, solvent type, intensity of light, matrix components and their concentrations, temperature, water quantity and the analite concentration.

Regarding the work that addresses the issue of degradation, Meghesan-Breja *et al.* (26) pointed that dicofol is rapidly degraded, being sensitive to high temperatures used in GC analysis (at the injector or even during elution, in the column) and high pH, during sample preparation. In study developed by Engel *et al.* (27) appeared an unexpected chromatographic peak, which was later identified

Table I. Analytical curves and statistic tests for evaluation of the matrix effect

Pesticides	Equation in isooctane	Equation in rat adipose tissue	Statistics	Is there a matrix effect?	Matrix effect (%)
DBP	$y = 797x + 3{,}422$	$y = 1{,}008x + 19{,}201$	Test F; $F_{calc} < F_{tab}$ Test t; $t_{calc} > t_{tab}$	YES	26
α-Endosulfan	$y = 4{,}051x + 3{,}774$	$y = 3{,}212x–705$	Test F; $F_{calc} < F_{tab}$ Test t; $t_{calc} > t_{tab}$	YES	−21
Dieldrin	$y = 4{,}200x + 4{,}455$	$y = 3{,}951x + 7{,}746$	Test F; $F_{calc} < F_{tab}$ Test t; $t_{calc} < t_{tab}$	NO	−6
β-Endosulfan	$y = 3{,}351x + 1{,}144$	$y = 3{,}371x + 2{,}377$	Test F; $F_{calc} < F_{tab}$ Test t; $t_{calc} < t_{tab}$	NO	−5
Dicofol	$y = 1{,}245x - 17{,}431$	$y = 2{,}242x - 18{,}233$	Test F; $F_{calc} > F_{tab}$ Test t; $t_{calc} > t_{tab}$	YES	78
cis-Permethrin	$y = 245x - 272$	$y = 252x + 1{,}453$	Test F; $F_{calc} < F_{tab}$ Test t; $t_{calc} < t_{tab}$	NO	3
trans-Permethrin	$y = 230x - 535$	$y = 263x + 3{,}052$	Test F; $F_{calc} < F_{tab}$ Test t; $t_{calc} < t_{tab}$	NO	14

Table II. Evaluation of the method performance using spiked samples

	Spiking level						LOD (ng/g)	LOQ (ng/g)
	First		Second		Third			
	R (%)	CV (%)	R (%)	CV (%)	R(%)	CV (%)		
DBP	87	13	76	12	76	5	124	200
α-Endosulfan	85	5	85	7	80	5	3	34
Dieldrin	79	6	85	10	81	5	27	50
β-Endosulfan	81	7	83	7	86	5	3	17
cis-Permethrin	93	10	91	15	84	6	61	200
trans-Permetrhin	90	8	76	8	75	5	110	300
Dicofol								
DBP (within dicofol commercial standard)	73	10	133	19	170	14		
Dicofol	86	3	30	38	27	34		
Considering both peaks (DBP + dicofol)	82	5	58	10	53	12		

(by GC-HRMS) as DBP, a degradation product of dicofol. Considering that this degradation cannot be avoided in a GC analysis, DBP quantification was included in the pesticide mix to be determined. These authors cite a document from the European Food Safety Authority (EFSA) (28), which recommends that in the dicofol determination in food, the quantification of its metabolite due to be considered.

EFSA (2011) document (28) presents three possible phases of the analytical procedure where dicofol degradation may occur: the first is in sample storage where light can induce degradation, the second is during extraction of samples from water-rich matrices where can occur the hydrolysis, and the last one is in GC analysis, where thermal decomposition at the inlet of the injector may be possible.

With the sum of the two peaks present in the dicofol standard appropriate values were obtained for recovery and CV, indicating that even when degradation occurs, the compounds were successfully extracted considering the complexity of the matrix.

Application in rat adipose tissue associated to ovaries

After validation of the analytical method developed in the present study, the samples could be analyzed in a toxicological study. Analyses were performed in duplicate for all the samples and quantification were performed using the analytical curve prepared in the matrix extract. For minor variations in the results among the replicates, the peak areas of dicofol and DBP were summed because degradation was not systematic.

All the adipose tissue associated to the ovary samples showed residues of dicofol and dichlorobenzophenone (<LOQ to 16.22 mg/kg), dieldrin (<LOQ to 1.01 mg/kg) and *cis*-permethrin (<LOQ to 12.08 mg/kg). Among the quantified samples, the concentration values found in adipose tissue from the animals that ingested No Observed Adverse Effect Level (NOAEL) dose did not differ statistically from the animals that ingested Lowest Observed Adverse Effect Level (LOAEL) dose. It is interesting to note that although NOAEL doses correspond to 2–10 times the LOAEL doses, the accumulation of the studied pesticides in adipose tissue associated with the ovaries did not differ significantly between the two treatments.

The results show greater accumulation of DBP, dieldrin and *cis*-permethrin, as evident from the presence of these compounds in all the samples analyzed ($n = 53$). The pesticide dicofol was detected and quantified in most samples, whereas *trans*-permethrin was detected in several samples, but cannot be quantified because the concentration was below the limit of quantification of the method. Endosulfan (α and β isomers) was not detected in any sample.

Conclusion

This work presents the development of a new method to determine the levels of DBP, dieldrin, dicofol, α and β endosulfan and *cis*- and

trans-permethrin. This method is a modification of the QuEChERS method and has the advantages of being simple and fast because this method consists of only two steps, ACN extraction and subsequent cleanup with a d-SPE, making it much simpler than conventional methods (SPE and MSPD).

The method was validated according to the following parameters: selectivity, accuracy, precision, matrix effect, LOD and LOQ. The results show that this technique is adequate for the intended study.

Fifty-three rat adipose tissue samples associated with the ovaries were analyzed, and all of the samples contained residues of dicorobenzophenone, dieldrin and *cis*-permethrin. Furthermore, 75% of the samples had dicofol, which shows the accumulation characteristic of these substances. Another pesticide detected in 40% of the samples was *trans*-permethrin, but quantification was not performed because the concentrations of this pesticide were below the method LOQ.

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