In-house method validation and occurrence of alpha-, beta-endosulfan, endosulfan sulphate, lambda-cyhalothrin, procymidone and trifluralin residues in strawberry

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Abstract

A method for determination of organohalogen pesticides in strawberry by gas chromatography with electron capture detection was validated and applied in a monitoring program. Linearity, matrix effects, and day effect were evaluated for the analytes alpha-endosulfan, beta-endosulfan, endosulfan sulphate, lambda-cyhalothrin, procymidone, and trifluralin. The linear range varied according to the chromatographic response of the analyte. Significant matrix effects were observed. The mean recoveries ranged from 74.6 to 115.4%, with repeatability standard deviations between 1.6 and 21.0% and intermediate precision between 5.9 and 21.0%. Detection, quantification and decision limit, and detection capacity ranged from 0.003 to 0.007 mg/kg, 0.005 to 0.013 mg/kg; 0.003 to 3.128 mg/kg; and 0.005 to 3.266 mg/kg, respectively. The method was fit for the purpose of monitoring organohalogen residues in strawberries. Residues of these pesticides were detected in 124 of the 186 samples analyzed between 2009 and 2011 in the state of Minas Gerais. Nine of them did not comply with the current legislation requirements; among them, seven (3.8%) had residues of unauthorized pesticide for the culture of strawberry, one (0.5%) had residues above the maximum residue limit, and another one (0.5%) exhibited both non-conformities.

Keywords: gas chromatography with electron capture detection; organohalogen pesticides; monitoring program.

1 Introduction

The increase in world's population and in demand for food has motivated the use of large amounts of pesticides on crops to prevent or control pests in order to ensure higher productivity. Several chemical classes of pesticides have been used to improve the quality of agricultural production, which is strongly affected by undesirable forms of life such as insects and weeds (SANCHES et al., 2003). The role of pesticides in agriculture includes production and productivity increase, product quality improvement, and labor and energy cost reduction. Although these objectives have been achieved in recent decades, the indiscriminate use of these compounds causes serious health and environment problems (KRISTENSON et al., 2001; PINHO et al., 2009).

According to the Brazilian National Health Surveillance Agency – ANVISA (AGÊNCIA..., 2008), the use of pesticides in agricultural production and the consequent contamination of foods have been the subject of public health concern. Due to the toxicological effects caused by pesticides, it is necessary to monitor and quantify the residues of these chemicals in water (VIEIRA; NEVES; QUEIROZ, 2007), soil, food (AMARAL, 2007; GORENSTEIN, 2008; BERRADA et al., 2010) and in the atmosphere as a prerequisite for traceability and protection of the human health and the environment. Residues of pesticides have been found in foods often consumed raw by the population (UNITED..., 2009; SSEBUGERE et al., 2010).

In Brazil, there is the Program for the Analysis of Pesticides in Food (PARA) of ANVISA, which aims to continually evaluate the levels of pesticide residues in fresh food that are consumed *in natura* by the population, strengthening the government's ability to assure food security, thus avoiding possible health risks to the population (AGÊNCIA..., 2008). There is also the National Program for Control of Residues and Contaminants (PNCRC), of the Ministry of Agriculture, Livestock and Supply (MAPA), which aims to systematize the control of products of animal and vegetal origin with respect to their contamination by compounds that are used in agriculture, including pesticides (BRASIL, 2010a, b, c). In the state of Minas Gerais, this control in fruits and vegetables is carried out by the Minas Gerais Institute of Agriculture (IMA). IMA is a state company responsible for taking care of the sanitary and phytosanitary issues in Minas Gerais State. Its role includes the control of animal and plants transit, compulsory vaccination campaigns, pesticides and veterinary drug products, among others. In summary, IMA's mission is to establish procedures and control the status of animal and plant diseases in the state helping to provide food safety to the consumers.

The organohalogens are one of the most important groups of insecticides used in Brazil; therefore, the analysis of residues of these pesticides in food is essential. Considering the ANVISA program, 128 samples of strawberry were analyzed in 2009, and 50.8% had unsatisfactory results (unauthorized pesticide

or residues detected in levels above those permitted). In 2010, 112 strawberry samples were analyzed, among which 63% had unsatisfactory results (AGÊNCIA..., 2010, 2011). In the context of the PNCRC of the MAPA, for the 2009/2010 and 2010/2011 harvest, around 36.0% of non-conformities were reported for a total of 75 samples (BRASIL, 2010b, 2011a). Therefore, strawberry stands out as an important commodity to be monitored due to the high frequency of pesticide residues.

Analytical methods for the determination of residues of organohalogen pesticides are based on the use of chromatographic techniques, especially gas chromatography (GC). Although there are several detection systems available, the electron capture detector (ECD) is suitable for their quantitative analysis due to the presence of one or more halogens in the organohalogen molecules (CHEN; WANG, 1996; COLUMÉ et al., 2001; LENTZA-RIZOS; AVRAMIDES; VISI, 2001; VIEIRA; NEVES; QUEIROZ, 2007; ZAWIYAH et al., 2007; PINHO et al., 2009; MENEZES; SANTOS; PEREIRA, 2010).

The growing need for reliable and comparable analytical data is essential for the elimination of technical barriers between countries. Therefore, in order for the measurement to be accepted in other countries, accreditation and legal requirements must be observed. Accordingly, the international standards for quality management systems highlight the importance of a validation method to verify their fitness-for-purpose and ensure reliability of results (ASSOCIAÇÃO..., 2005). Thus, validation is a fundamental requirement of quality management (THOMPSON; ELLISON; WOOD, 2002), which ensures that an analytical method provides reliable results supporting decision making (RIBANI et al., 2004). Therefore, this study presents the validation of a method for detection of alpha-endosulfan, beta-endosulfan, endosulfan sulphate, lambda-cyhalothrin, procymidone, and trifluralin in strawberry by GC-ECD and its application in the IMA monitoring activities in the state of Minas Gerais between 2009 and 2011.

2 Materials and methods

2.1 Samples

For the validation experiments, strawberry samples were obtained from organic certified cultures. The samples were previously tested, and the absence of signals in the retention time of the studied analytes was confirmed. The samples destined for occurrence investigation were collected on rural properties by IMA fiscals in Alfredo Vasconcelos, Barbacena, Estiva, Pouso Alegre e Bom Repouso, the most important strawberry producers in Minas Gerais state between 2009 and 2011. Sampling was carried out according the Codex Alimentarius (1999) recommendations. At least 1 kg of strawberries was collected per sample, which were packaged into plastic bags, sealed, labelled, and immediately sent to the laboratory. Only those, for which the period between sampling and the arrival in the laboratory was not longer than 36 h, were selected for analysis.

The samples were homogenized and processed without the sepals, packaged into plastic bags, sealed, labelled and kept frozen between -25 °C and –15 °C until analyses.

2.2 Reagents and standards

The standards of the analytes allethrin, bifenthrin, vinclozolin and fipronil were supplied by Sigma-Aldrich (Seelze, Germany); cyfluthrin (I, II, III, IV), cypermethrin (cis and trans), aldrin, captan, cyproconazole, difenoconazole, dicofol, folpet, iprodione, bromopropilate, chlorotalonil, procloraz, and propiconazole were supplied by ChemService (West Chester, USA); deltamethrin, esfenvalerate, fenvalerate, lambda-cialothrin, cis-permethrin, trans-permethrin, dichlorodiphenyltrichloroethane (DDT) methabolites op'DDD, pp'DDD, op'DDT, pp'DDT, op'DDE, pp'DDE, dieldrin, dodecachloro, alpha-endosulfan, beta-endosulfan, endosulfan sulphate, endrin, hexachlorobenzene (HCB), alpha-hexachlorocyclohexane (HCH), beta-HCH, delta-HCH, lindane (gama-HCH), heptachlor, methoxyclor, procymidone, chlorfenapyr, tetradifon, and trifluralin were supplied by Dr. Ehrenstorfer GmbH (Augsburg, Germany).

Acetone, dichlorometane, n-hexane, and sodium sulphate used in the pesticide residues analysis (PRA grade) were purchased from Tedia (Ohio, USA). Acetone PRA was supplied by Vetec (Rio de Janeiro, Brazil), isooctano by PRA by Merck (Darmstadt, Germany), and the ultra-pure gases helium and nitrogen by IBG (São Paulo, Brazil).

2.3 Analytical procedure

The analytical procedure was adapted from Netherlands (1996). Aliquots of 15.00 ± 0.10 g of previously homogenized and processed strawberry samples and 15 ± 1 g of sodium sulphate were weighed into 400 mL flasks and mixed. Extraction was carried out with 40 mL of acetone for 30 s, followed by 60 mL of dichlorometane:n-hexane (1:1 v/v) for 30 s using an ultra turrax homogenizer (Marconi, MA102E). The mixture was filtrated, and the volume was completed with dichlorometane:n-hexane (1:1 v/v) to 100 mL. An aliquot of 5 mL of the extract was evaporated to dryness in a rotatory evaporator (Büchi, KRVRTD65/45 and W240N) and re-dissolved with 2 mL of n-hexane.

GC/ECD analyses were performed on a Varian CP3800 GC equipped with an autosampler CP8200 and a CP-SIL 19CB column (14% cyanopropylphenyl and 86% dimethylpolysiloxane, 30 m lengthx 0.25 mm internal diameter × 0.25 μm particle size; Varian, Santa Clara, USA). The optimized chromatographic conditions were: helium flow rate of 1.0 mL/min; injection mode split with a 1/20 rate; injector temperature of 250 °C; ECD temperature: 300 °C; the initial temperature of the column was 70 °C and was maintained for 1 minute, increased 30 °C/min until 180 °C and maintained for 2 minutes, increased 15 °C/min until to 250 °C and was maintained for 8 minutes, it was finally increased 15 °C/min until 280 °C and maintained for 17 minutes. The total run time was 35 minutes. Confirmation was carried out under the same chromatographic conditions with a CP-SIL 5CB column (100% dimethylpolysiloxane, 30 m × 0.25 mm i.d. × 0.25 μm particle size; Varian, Santa Clara, USA).

2.4 Validation

The performance characteristics of the method were established by in-house validation procedures employing assays

with standard solutions, sample blanks, and spiked samples. The analytes chosen for the validation studies were endosulfan (alpha, beta and sulphate), lambda-cyhalothrin, procymidone, and trifluralin because they were representative of organochlorines, pyrethroids, dicaboximides, and dinitroanilines herbicides, respectively (AGÊNCIA..., 2008, 2010, 2011).

Selectivity, linearity, matrix and day effects, recovery, precision, detection limit (LD), quantification limit (LQ), decision limit (CCα), and detection capability (CCβ) were investigated as described by Souza (2007). This validation procedure was in accordance with the International Standards Organization (ISO), International Union of Pure and Applied Chemistry (IUPAC) and Association of Official Analytical Chemists (AOAC), and international harmonized protocol (THOMPSON; ELLISON; WOOD, 2002); they were referred in Brazilian guidelines related to method validation (INSTITUTO..., 2010; BRASIL, 2011b). Linearity was assessed by linear regression based on the procedure proposed by Souza and Junqueira (2005) in the range from 1.0 to 9.0 ng/mL for alpha-endosulfan, from 2.0 to 24.0 ng/mL for trifluralin and beta-endosulfan, and from 3.0 to 45.0 ng/mL for endosulfan sulphate, lambda-cyhalothrin, and procymidone. The evaluation of the day effect was based on the calibration curves generated from the linearity study and obtained in three different days. The slopes and interceptions were compared by the t test, applying two orthogonal contrasts (AMARAL, 2007). Matrix effects were assessed by the method of standard additions. Two calibration curves were prepared; one of them was prepared in n-hexane (usual curve) and the other one in extracts of the studied matrix (matrix-matched curve). The slope, intercept, and the respective variances of both curves were calculated by OLSM. All assumptions were tested, and linearity was also assessed for these curves. The slopes and interceptions obtained from the solvent and matrix-matched calibration curves were compared for significant differences by the Student's t test. For recovery and precision under repetitivity and intermediate precision conditions studies, blank and spiked samples were prepared at five levels in the linear range, with twelve independent replicates for each level, which were divided into four analytical batches to obtain the intermediate precision conditions. Lack of trueness was investigated through mean recovery obtained for twelve replicates of spiked samples at 0.005, 0.008 and 0.019 mg/kg for alpha-endosulfan; 0.005, 0.011 and 0.032 mg/kg for beta-endosulfan and trifluralin; and 0.013, 0.032 and 0.107 mg/kg for endosulfan sulphate, lambda- cyhalothrin and procymidone. The replicates were divided into four analytical batches, which were analyzed on different days by different analysts. Precision, under repeatability and intermediate precision conditions were estimated by ANOVA and expressed in terms of relative standard (RSD) deviation for each level. The criteria for the acceptability of mean recovery and the relative standard deviations obtained under repeatability (RSD_r) and intermediate precision (RSD_R) conditions were based on European Commission (2010). The LQ was stated as a concentration below which the method could not operate with an acceptable precision and trueness. The LD was the lowest concentration of the organohalogens in strawberry samples that was detectable, but not necessarily quantified and distinguished from zero (signal / noise ≥ 3).

These limits were established based on the mean recovery and relative standard deviation results obtained for the replicates of spiked samples. CCα and CCβ were estimated using the recovery results obtained in the intermediate precision experiments, and the maximum residues limits (MRL) established by the national legislation according the statistical procedure described by Van Loco and Beernaert (2003).

2.5 Occurrence

186 strawberries samples were analyzed, 94 in 2009, 75 in 2010, and 17 in 2011. 46 organohalogens were investigated by the validated method, including the pyrethroids allethrin, bifenthrin, cyfluthrin (I, II, III, IV), cypermethrin (cis and trans), deltamethrin, esfenvalerate, fenvalerate, lambda-cialothrin, cis-permethrin and trans-permethrin; the organochlorines aldrin, op'DDD, pp'DDD, op'DDT, pp'DDT, op'DDE, pp'DDE, dicofol, dieldrin, dodecachloro, alpha-endosulfan, beta-endosulfan, endosulfan sulphate, endrin, HCB, alpha-HCH, beta-HCH, delta-HCH, lindane, heptachlor, and methoxyclor; the fungicides triazole cyproconazole, difenoconazole, and propiconazole; the fungicides dicarboximides captan, folpet, iprodione, procymidone, and vinclozolin; and other classes such as bromopropilate, chlorfenapyr, chlorotalonil, fipronil, procloraz, tetradifon, and trifluralin.

3 Results and discussion

3.1 Validation

Selectivity

The mean retention times and peak resolution obtained for the organohalogens studied, considering 27 runs, are described in Table 1. The minimal value estimated for the peak resolution was 1.5, considering all of the analytes, which was satisfactory according to the Kealey and Haines (2002) criteria. The resolution indicated the ability of the method to determine all organohalogens tested without mutual interference between the analytes, even for the pairs of isomers of lambda-cyhalothrin. Typical chromatograms of blank samples, standard solution of the organohalogens prepared in solvent, standard solution of the organohalogens prepared in strawberry extracts and spiked samples are shown in Figure 1. In all cases, the peaks in the

Table 1. Retention times and peak resolution obtained for the studied organohalogens.

Analyte	Retention time (min)*	Resolution (R)*
Trifluralin	9.865 ± 0.009	54.0
Procymidone	13.918 ± 0.014	79.7
Alpha-endosulfan	14.490 ± 0.011	10.8
Beta-endosulfan	15.481 ± 0.013	17.8
Endosulfan sulphate	16.447 ± 0.013	15.9
Lambda-cyhalothrin I	20.079 ± 0.027	44.3
Lambda- cyhalothrin II	20.586 ± 0.030	4.9

*means of 27 runs, R = 2 Δt_r / (W_1+W_2), Δt_r is the variation of the retention time between two peaks and W the width of the peaks baseline.

chromatograms of the spiked samples were sharp and similar to those of the standard solution of comparable concentrations.

All blank samples analyzed showed non-detected results for SEM (signal / noise < 3). Cardoso et al. (2010) also evaluated

Figure 1. Typical chromatograms obtained for (a) blank sample, (b) standard solution of the organohalogens in hexane, (c) standard solution of the organohalogens prepared in strawberry extracts and (d) spiked sample. (a) Trifluralin, (b) Procymidone, (c) Alpha-endosulfan, (d) Beta-endosulfan, (e) Endosulfan sulphate, (f) Lambda-cyhalothrin I e (f´) Lambda-cyhalothrin II.

selectivity by the analysis of spiked samples with the pesticides gama-HCH, chlorotalonil, fenitrothion, chlorpyrifos, and procymidone in tomato.

Linearity

The statistics obtained for the linearity assessment is shown in Table 2. The outliers detected by the Jacknife residuals were removed. The assumptions that the errors are normally distributed, homoscedastic, and independent were confirmed. The high significance (p < 0.001) of the regression, although the lack of fit was not significant (p > 0.05), indicated linearity in the range from 2.0 to 7.0 ng/mL (corresponding to 0.005 to 0.019 mg/kg of strawberry) for alpha-endosulfan, from 2.0 to 17.0 ng/mL (corresponding to 0.005 to 0.032 mg/kg of strawberry) for trifluralin and beta-endosulfan, and from 5.0 to 40.0 ng/mL (corresponding to 0.013 to 0.1079 mg/kg of strawberry) for endosulfan sulphate, lambda-cyhalothrin, and procymidone. The linearity assessment over three different days indicated that the regression parameters were stable only for beta-endosulfan and trifluralin. For the other analytes studied, the curves did not show the same chromatographic responses (Table 3). These results confirmed those obtained by Amaral (2007), in which the calibration response obtained for pesticides, even for solutions prepared in solvents, cannot be reused for the quantification in different days.

Matrix effects

When the slopes and interceptions from the solvent standard curves were compared with those from strawberry matrix-matched curves, significant differences (p < 0.05) were observed, demonstrating that the chromatographic response of the analytes was affected by the presence of the matrix (Figure 2).

For the organohalogens endosulfan sulphate and lambda-cyhalothrin, there were significant differences (p < 0.001) between the slopes, while differences between the interceptions were observed for alpha-endosulfan (p < 0.05). For the other studied analytes, there were no significant matrix effects (p > 0.05) in the respective concentration ranges. As for lamba-cyhalothrin, considering that the residual variances of the matrix matched and solvent calibration curves were heteroscedastic (p < 0.01), the comparison between slopes and interceptions was carried out by the t test (Table 4). Based on these results, it was possible to conclude that matrix matched curves need to be used to calculate organohalogen concentrations in strawberry samples.

According to González et al. (2002), effects on the slope cause proportional systematic error in the quantification, while constant systematic errors are observed when the interception is affected by the presence of the matrix. Ambrus (2000) states that the matrix effects vary from day to day during the regular use of the method, depending on the conditions of the GC system and the type of injection. This means that the preliminary assessment of the matrix effects for the method validation is not sufficient. This parameter should be continuously assessed when the option is the use of usual calibration curves in the absence of the matrix. As demonstrated in this paper, the
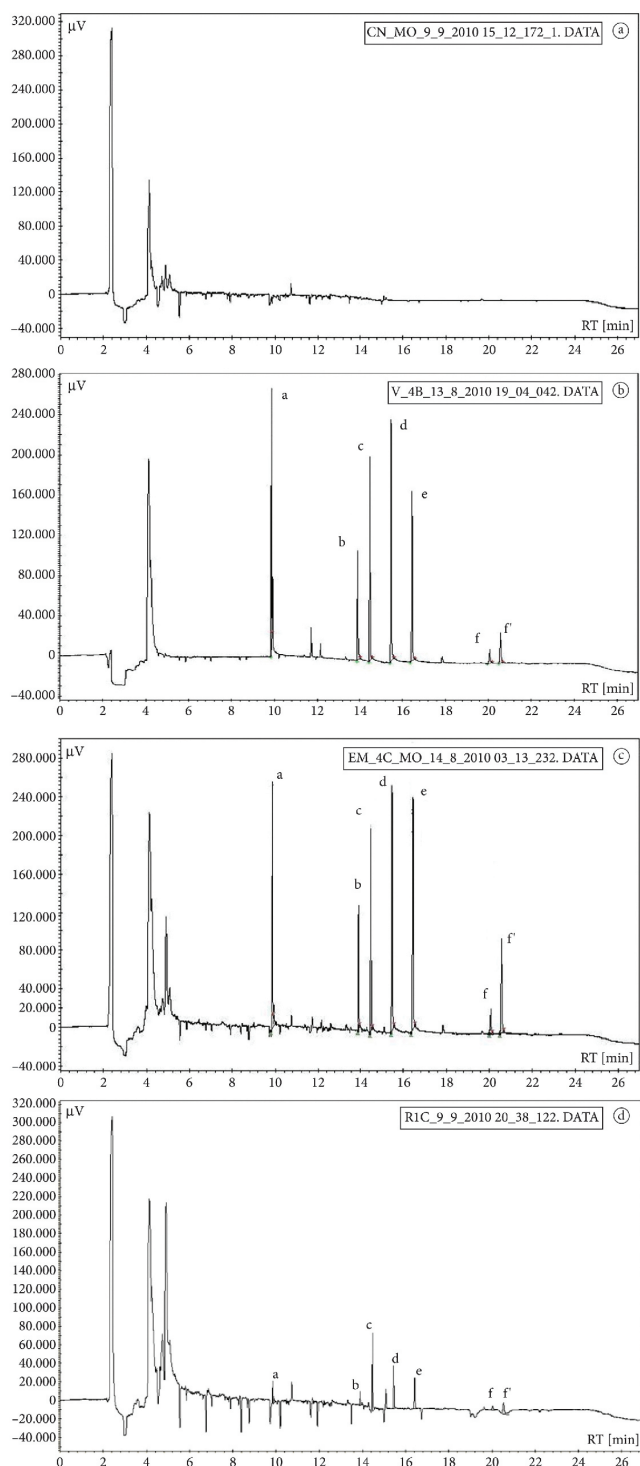
Table 2. Linearity assessment for standard solutions of the organohalogens prepared in hexane in three different days.

Analyte (Range)	Day	Statistic					
		n	R	d	t_L	$F_{Regression}$	$F_{Lack-of-fit}$
Alpha-endosulfan (2.0 to 7.0 ng/mL)	1	16	0.9739	2.177	0.695	576.8	1.642
	2	16	0.9646	1.842	0.799	518.2	0.880
	3	17	0.9927	1.633	1.630	1688.7	1.42
Beta-endosulfan (2.0 to 17.0 ng/mL)	1	18	0.9744	2.367	0.425	813.6	1.252
	2	18	0.9564[#]	1.224	0.454	367.6	2.569
	3	16	0.9594	2.055	0.173	668.2	3.380
Endosulfan sulphate (5.0 to 40.0 ng/mL)	1	15	0.9390[#]	2.343	1.549	356.4	1.183
	2	14	0.9847	2.334	1.052	563.7	0.819
	3	16	0.9750	2.453	0.022	227.0	1.715
Lambda-cyhalothrin (5.0 to 40.0 ng/mL)	1	16	0.9633	1.900	1.951	265.1	1.901
	2	14	0.9762	2.535	1.700	380.0	0.321
	3	15	0.9816	1.602	0.415	429.8	2.465
Procymidone (5.0 to 40.0 ng/mL)	1	16	0.9866	1.539	0.350	2500.1	3.141
	2	14	0.9602	1.155	0.137	1119.3	3.468
	3	15	0.9874	1.654	2.109	533.5	1.842
Trifluralin (2.0 to 17.0 ng/mL)	1	18	0.9773	2.322	0.285	1543.4	0.411
	2	18	0.9581	1.997	0.558	1450.4	2.734
	3	15	0.9839	1.507	0.181	1336.1	3.361

n: number of observations after the outliers treatment by Jack-Knife residual test (p < 0.05), R: Ryan-Joiner correlation coefficient (p > 0.10 or [#]p > 0.05), d: Durbin-Watson statistic (p > 0.10), tL: Levene t statistic (p > 0.05), $F_{Regression}$: variance ratio (p < 0.001), $F_{Lack-of-fit}$: variance ratio (p > 0.05).

Table 3. Evaluation of the day effect based on the calibration curves of the organohalogens prepared in hexane in three different days.

Statistic	Analyte					
	Alpha-endosulfan	Beta-endosulfan	Endosulfan sulphate	Lambda-cyhalothrin	Procymidone	Trifluralin
	Slope					
$t_{(Y1b)}$	2.863**	1.754	2.831**	0.4925	0.7775	0.6505
$t_{(Y2b)}$	1.567	0.9772	0.1531	7.139***	2.351*	0.7036
	Interception					
$t_{(Y1a)}$	4.333***	1.256	0.2475	0.0867	0.1060	0.1060
$t_{(Y2a)}$	5.016***	0.6224	0.6430	1.046	0.9093	0.9093

*p < 0.05, **p < 0.01, ***p < 0.001, t: t statistic for the contrasts (Y) between slopes (β = b) and interceptions (β = a), $Y_{1\beta} = \beta_1 - \beta_2$, $Y_{2\beta} = \beta_1 + \beta_2 - 2\beta_3$.

Table 4. Slope and intercept comparisons between strawberry matrix matched and solvent (hexane) calibration curves.

Statistic	Analyte					
	Alpha-endosulfan	Beta-endosulfan	Endosulfan sulphate	Lambda- cyhalothrin	Procymidone	Trifluralin
	Homoscedasticity					
F	2.369	2.233	1.407	5.238**	2.021	1.286
	Slope					
$t_{(Yb)}$	1.670	0.1642	5.161***	9.939***	1.752	1.195
	Interception					
$t_{(Ya)}$	2.096*	0.5732	1.260	0.5957	0.2098	0.0851

*p < 0.05, **p < 0.01, ***p < 0.001, F: variances ratio, $t_{(Yb)}$: t statistic for the contrasts (Y) between slopes (b), $t_{(Ya)}$: t statistic for the contrasts (Y) between interceptions (a).

chromatographic response was affected by the day of analysis, even in the absence of the matrix (Table 3). The matrix effect has been attributed primarily to the presence of components in the sample that may be adsorbed in the active sites of the injector and change the behavior of the analytes in the vaporization chamber. Consequently, the amount of analyte reaching the column in extracts containing the matrix can be different from the amount injected in pure solvent. Therefore, the matrix

effects can generate analytical problems due to the possible overestimation or underestimation of the analyte concentration (ZROSTLÍKOVÁ et al., 2001; ANASTASSIADES et al., 2003; POOLE, 2007; PINHO et al., 2009).

Przybylski and Hommet (2008) compared the slope of calibration curves obtained from standard solutions prepared with 12 pesticides in solvent and in extracts of carrot baby
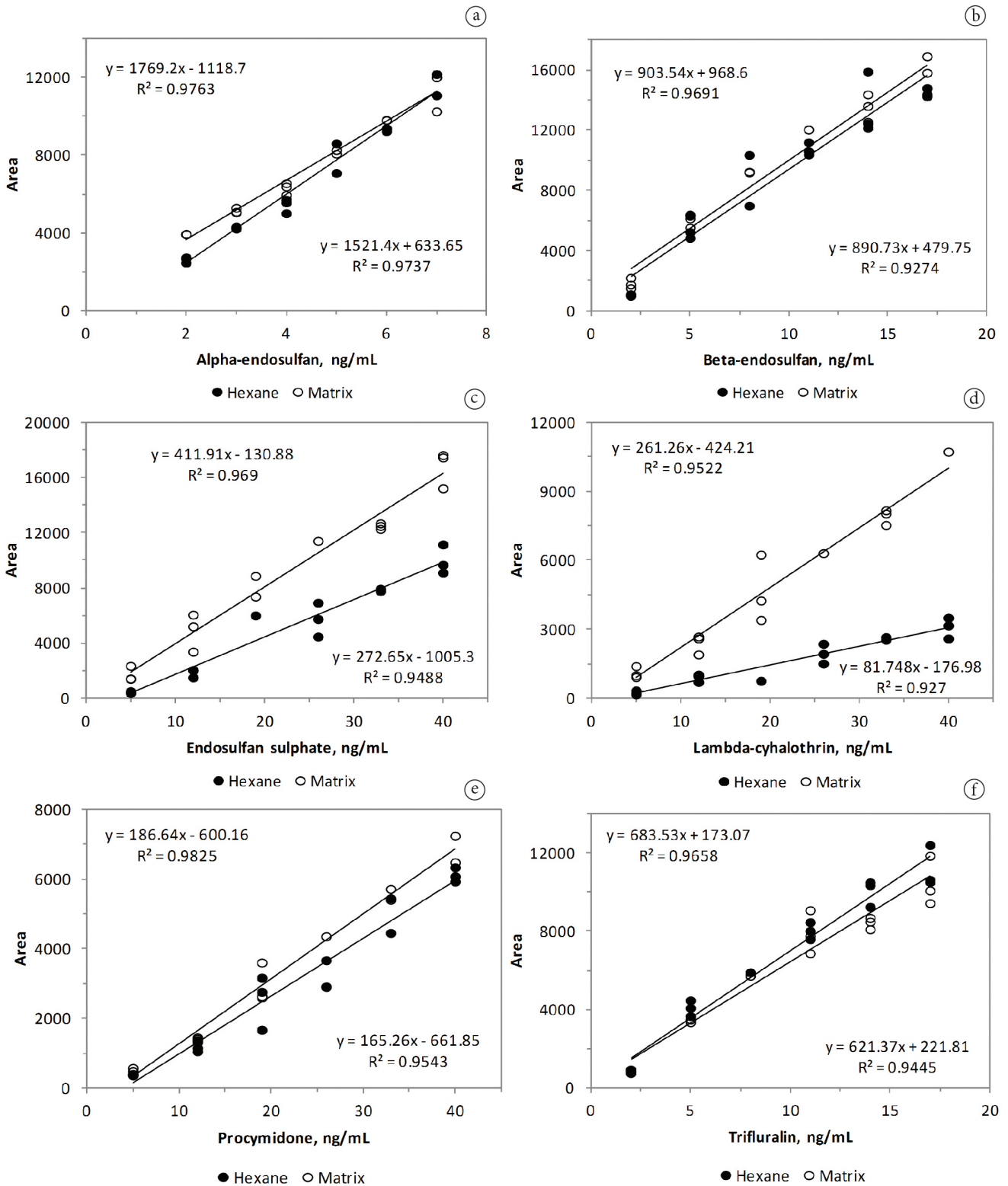
Figure 2. Comparison between solvent (hexane) and strawberry matrix matched calibration curves for the studied organohalogens.

food, beans, potatoes, and peas. The results indicated that the matrix effects may be positive or negative, depending on the characteristics of the pesticide and the complexity of the matrix being analyzed by GC. Cardoso et al. (2010) tested the matrix effects for five pesticides and confirmed these effects for chlorpyrifos and fenitrothion, while for the gamma HCH, procymidone, and chlorothalonil no significant effects of the matrix were observed, confirming the results obtained in the present study, in which no matrix effects were detected for procymidone. In general, hydrophobic compounds, such as

organochlorine pesticides, do not create pronounced matrix effects due to the fact that they are less susceptible to adsorption by active sites of the injector (PINHO et al., 2009). Although the pyrethroids are less polar than organochlorines, they have significant matrix effects due to their high molecular weight (above 400 g/mol), which hinders volatilization and consequently produces smaller signals. In the present study, the matrix effect of lambda-cyhalothrin, a pyrethroid compound was confirmed, in accordance with the results observed by Pinho et al. (2009). However, among the three organochlorines studied, two showed matrix effects, contradicting the trend indicated by these authors. Matrix effects are complex and are difficult to predict, but pesticides containing functional groups P=O (organophosphorus),-O-CO-NH-(carbamates),-OH (hydroxy), -N= (imidazoles and benzimidazoles),-NH-CO-NH-(urea derivatives) are more susceptible to these effects (SCHENCK; LEHOTAY, 2000).

Recovery and precision

The recovery results were analyzed for the presence of outliers. Acceptable mean recoveries were achieved for all analytes (**Table 5**). However, estimates of RSD_r and RSD_R above 20% were observed for alpha-endosulfan at the level of 0.005 mg/kg. These results showed no lack of trueness and precision of the validated method in the range of 0.005 mg/kg to 0.032 mg/kg for beta-endosulfan and trifluralin, 0.013 mg/kg to 0.107 mg/kg for endosulfan sulphate, procymidone, and lambda-cyhalothrin; and 0.008 mg/kg to 0.019 mg/kg for alpha-endosulfan considering the established criteria. The average recovery ranged from 74.59% to 115.41%, and RSD_r ranged between 1.63% and 20.98% and RSD_R between 5.86%

and 20.98%. Disregarding the value of 20.98%, that exceeded the limits established by the European Commission (2010); the largest acceptable value of RSD_r and RSD_R was 19.91%. These results were similar to those reported in the literature; although, most of them addressed concentration levels higher than those investigated here and carried out precision experiments under repeatability conditions (Table 6).

Limits of detection, quantification, decision, and detection capability

The limits established in this study, as shown in Table 7, were suitable for monitoring the activities, considering the Brazilian legislation: the maximum MRL of 3 mg/kg for procymidone and 0.5 mg/kg for lambda-cyhalothrin and the non-authorized inseticides alpha-, beta-endosulfan and endosulfan sulfate. These estimated LD were similar to those found in the literature. Zawiyah et al. (2007) determined the LD for six organochlorines and three pyrethroids in the range of 0.003 mg/kg to 0.015 mg/kg for grape samples, considering signal / noise ≥ 3. Adopting the same criterion, Cardoso et al. (2010) established LD for tomato samples: 0.006 mg/kg for chlorothalonil and procymidone; 0.005 mg/kg for HCH and fenitrothion; and 0.004 mg/kg for chlorpyrifos. Menezes, Santos and Pereira (2010) determined LD for pesticide residues in sleeve samples in the range from 0.001 mg/kg to 0.033 mg/kg, but they did not describe how the limits were calculated.

The established LQ, which corresponded to the lowest levels that indicated acceptable precision and accuracy, were smaller than those mentioned above; although the method has been validated for a closer range of linearity. Imoto, Fontoura and Freitas (2004) found LQ of 0.02 mg/kg, adopting the criterion

Table 5. Mean recovery and relative standard deviation under repeatability and intermediate precision conditions at the different concentration levels.

Analyte	Concentration (mg/kg)	MR (%)	RSD_r (%)	RSD_R (%)
Alpha-endosulfan	0.005	78.06	20.98	20.98
	0.008	88.04	4.90	13.42
	0.019	82.93	2.65	8.41
Beta-endosulfan	0.005	88.34	9.40	17.07
	0.011	92.20	6.60	12.57
	0.032	81.85	1.63	6.43
Endosulfan sulphate	0.013	83.94	19.91	19.91
	0.032	89.86	14.18	17.68
	0.107	76.70	4.66	10.10
Lambda- cyhalothrin	0.013	115.41	4.04	13.09
	0.032	81.79	6.19	13.61
	0.107	74.59	12.78	12.85
Procymidone	0.013	101.52	4.73	13.94
	0.032	82.98	6.42	7.35
	0.107	85.14	3.41	10.22
Trifluralin	0.005	108.93	5.86	5.86
	0.011	99.89	7.33	9.23
	0.032	91.65	4.15	6.08

MR: mean recovery of 12 replicates, RSD_r: repeatability relative standard deviation, RSD_R: intermediate precision relative standard deviation, Criteria: 70% ≤ MR ≤ 120 %, RSD_r ≤ 20,0% and RSD_R ≤ 20,0% (EUROPEAN..., 2010).

Table 6. Mean recovery and relative standard deviation reported in the literature for similar scopes.

Analyte	Matrix	Technique	Concentration (mg/kg)	MR (%)	RSD (%)	N	Reference
17 synthetic pyrethroids, including lambda-cyhalothrin	14 vegetables and fruits, including strawberry	gas chromatography/ electron capture detection	0.02 and 0.10	66-102	3-6	6	Columé et al. (2001)
Alpha- and beta-endosulfan, endosulfan sulphate, lambda-cyhalothrin and other 4 pyrethroids	olive oil	gas chromatography/ electron capture detection	0.20	71-91	6-17	5	Lentza-Rizos, Avramides and Visi (2001)
8 organohalogen, including alpha endosulfan, beta-endosulfan, endosulfan sulphate and trifluralin	apple	gas chromatography/ electron capture detection	0.02 and 0.10	74-111	5-14	5	Imoto, Fontouraand Freitas (2004)
Multi-residues for 43 pesticides and 9 pesticide metabolites	tomato, lemon, avocado and raisins	liquid chromatography / mass spectrometry	0.01 and 0.10	70-110	< 15	5	Hernández et al. (2006)
Procymidone and other 4 halogenated compounds	tomato	gas chromatography/ electron capture detection	0.020 to 2.5	72-110	2-15	6	Cardoso et al. (2010)
14 organochlorines	apricot, nectarine, cherry, plum, apple, pear and quince	gas chromatography/ mass spectrometry	0.008	71-172	< 17	2	Cieslik et al. (2011)

Table 7. Limits of detection, quantification, decision, and detection capability estimated for the studied organohalogens in strawberry.

Analyte	LD	LQ	CCα	CCβ
	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
Alpha-endosulfan	0.003	0.008	0.003	0.005
Beta-endosulfan	0.003	0.005	0.004	0.006
Endosulfan sulphate	0.007	0.013	0.025	0.042
Lambda- cyhalothrin	0.007	0.013	0.538	0.581
Procymidone	0.007	0.013	3.128	3.266
Trifluralin	0.003	0.005	0.004	0.006

LD: limit of detection, LQ: limit of quantification, CCα: decision limit, CCβ: detection capability.

signal / noise ≥ 10. Hernández et al. (2006) determined the LQ as 0.01 mg/kg for the multi-residue method, considering that at this level acceptable recovery and precision were obtained for all of the tested matrices. The LQ assessed by Zawiyah et al. (2007) in grape samples, considering six organochlorines and three pyrethroids, ranged from 0.01 mg/kg to 0.05 mg/kg.

The values estimated for CCα and CCβ were lower than those obtained by Garcia-Chao et al. (2010). These authors performed the validation of systemic insecticides in honey and calculated CCα and CCβ based on calibration curves. Values between 0.41 mg/kg and 2.33 mg kg for CCα and from 0.83 mg/kg to 4.83 mg/kg for CCβ were obtained. CCα is the limit at and above which it can conclude that a sample is non-compliant with a probability of error α corresponding to the rate of false noncompliant. CCβ is the smallest content of the analyte that can be detected, identified, or quantified in a sample with an error probability of β, corresponding to the false compliant (EUROPEAN..., 2002).

3.2 Occurrence

The occurrence of the 46 organohalogen residues in strawberry samples between 2009 and 2011 is shown in Table 8. Considering the 186 samples analyzed in the period, 62 (33.3%) had no organohalogens detected, and 114 (61.3%) contained residues of these pesticides below the MRL. Nine (4.8%) samples were classified as unsatisfactory, 7 (3.8%) of them had residues of unauthorized pesticides for the strawberry culture, 1 (0.5%) had residues above the MRL and 1 (0.5%) exhibited both non-conformities.

Of the 94 samples analyzed in 2009, 67 had one or more residues of pesticides, which accounts for 71.3% of the total.

Table 8. Occurrence of organohalogens residues in strawberry samples produced in the state of Minas Gerais between 2009 and 2011.

Year	N of analyzed samples	Detection below the MRL		Detection of not permitted residues for the culture		Detection over the MRL		Detection of not permitted residues for the culture an over the MRL	
		N	%	N	%	N	%	N	%
2009	94	63	67.0	4	4.3	0	0.0	0	0.0
2010	75	47	62.7	1	1.3	1	1.3	0	0.0
2011	17	4	23.5	2	11.8	0	0.0	1	5.9
Total	186	115	61.8	7	3.8	1	0.5	1	0.5

N: number of samples, MRL: maximum residue limits.

Four (4.3%) samples were not in accordance with the current legislation. Among the 75 analyzed samples analyzed in 2010, 49 (65.3%) had residues of organohalogens; however, only two (2.6%) of them were unsatisfactory. In 2011, 17 samples were analyzed and seven (41.2%) had organohalogens residues. Three of them (17.6%) were not in accordance with the Brazilian legislation. The samples were considered unsatisfactory when they had residues above the MRL and residues of unauthorized pesticides for strawberry culture.

It should be noted that the presence of unauthorized residues was, in most cases, more common than that in the cases with residues above the MRL, confirming the tendency obtained by the national monitoring programs.

In the same period of time, despite the lower number of strawberry samples, the PNCRC of the MAPA found unsatisfactory results for 13.3% of the 30 samples analyzed for 170 pesticides in the 2009/2010 harvest and 51.1% of the 45 samples analyzed for 240 analytes in the 2010/2011 harvest. The unauthorized organohalogens found included cypermethrin, deltamethrin, and procloraz (BRASIL, 2010b, 2011a).

In 2009, 234 analytes were investigated in 128 strawberry samples by PARA, 49 (38.3%) had unauthorized pesticides, 11 (8.6%) had residues above the MLR, and 5 (3.9%) exhibited both non-conformities (AGÊNCIA..., 2010). Considering the 112 strawberry samples and the 119 pesticides analyzed by the ANVISA Program in 2010, 58 (51.8%) had residues of unauthorized residues, 3 (2.7%) had residues detected at levels above the permissible limits, and 10 (8.9%) exhibited both non-conformities (AGÊNCIA..., 2011).

Figure 3 shows the organohalogen compounds found in the 186 samples evaluated, highlighting the presence of procymidone, which was detected in 100 samples. Other relevant analytes detected were iprodione and lambda-cyhalothrin. It is noteworthy that 65.6% of the strawberry samples in 2009 and 67.9% in 2010 investigated by PARA showed residues of procymidone, confirming the profile obtained in this study.

In 2009 and 2010, the residues that were detected in levels below the MRL included iprodione, procymidone, lambda-cialothrin, and difenoconazole. In 2011, procymidone was detected, but it was within the limit established by Brazilian legislation. The unauthorized residues for strawberries cultures detected were deltamethrin, procloraz, chlorfenapyr, and chlorotalonil in 2009; chlorfenapyr in 2010 and chlorfenapyr, chlorotalonil, and endosulfan (alpha, beta and sulphate) in 2011. Difenoconazole was detected in levels above the MRL
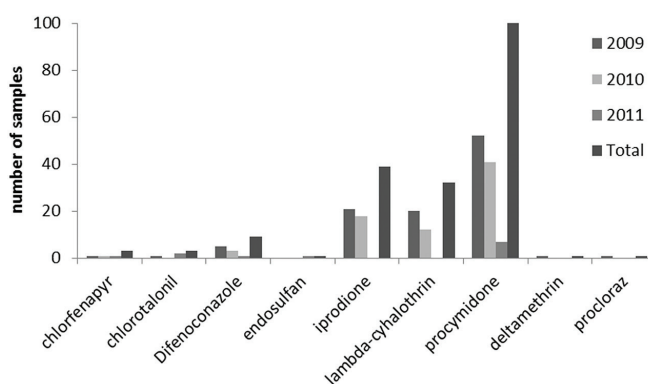

Figure 3. Organohalogens detected in strawberry samples in the years 2009, 2010 and 2011.

in the years of 2010 and 2011. Some samples contained more than one residue of pesticide, and in some cases four different organohalogen pesticides were detected.

Considering the USDA Pesticide Data Program from 1993 to 2003, more than 100,000 samples were analyzed, and 65% of them were of fresh fruit and vegetables. Strawberry is among the fresh fruits and vegetables commodities that have been sampled for two or more years and had detectable residues in 90% or more of the samples (PUNZI et al., 2005).

The 2010 final report of the United Kingdom Pesticide Residues Committee (PRC) oversaw a monitoring program to check traces of pesticides residues in food and drink indicated that for the 96 analyzed samples, 85 (88.5%) contained residues of these pesticides below the MRL and 76% of them contained more than one residue. In the present study, unauthorized residues and those above the MRL were not detected (PESTICIDE..., 2011).

In 2010, in Belgium, 1854 surveillance samples of fruit and vegetables were analyzed in the framework of the control program, and 31.2% were shown to be without residues of pesticides, 64.0% with residues at or below the MRL, and 4.8% with residues above the MRL. Like in previous years, imported products showed proportionally more MRL violations than the Belgium and European Union products. Main MRL violations in fruits were observed in strawberries imported from third world countries. However, the pesticide residues were different than those detected in this study (ethion, methomyl, chlorfenapyr, methomyl, and propargite) (BELGIUM, 2011).

4 Conclusions

The method validated was fit for the purpose. The results of the strawberry samples highlighted the importance of monitoring and evaluating actions by the states, which are complementary tools for national programs. Therefore, the results presented in this paper contribute to food safety, indicating the importance of appropriate validated methods, the need for the promotion of good agricultural practices and that strawberry is a critical culture in terms of pesticide residues in Brazil.

Acknowledgements

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