https://dx.doi.org/10.21577/0103-5053.20200099

J. Braz. Chem. Soc., Vol. 31, No. 10, 1991-1999, 2020
Printed in Brazil - ©2020 Sociedade Brasileira de Química

Article

A Simple and Efficient Optimized Solid-Liquid Extraction with Low Temperature Partition Procedure for Dissipation and Translocation Study of Pesticide Residues in Rice Grains

Mariane B. R. de Ávila,[a] Lêda R. A. Faroni,[a] Fernanda F. Heleno,[a] Maria Eliana L. R. de Queiroz [b] and Rodrigo I. Almeida[a]*

[a]*Laboratório de Manejo Integrado de Pragas de Grãos Armazenados, Departamento de Engenharia Agrícola, Universidade Federal de Viçosa, 36570-900 Viçosa-MG, Brazil*

[b]*Laboratório de Química Analítica, Departamento de Química, Universidade Federal de Viçosa, 36570-900 Viçosa-MG, Brazil*

Pesticides are used globally to protect food against pest attack. This study evaluates the dissipation of bifenthrin and deltamethrin residues in unhulled rice and the translocation of these insecticide residues in husked rice stored at 25 °C in a biochemical oxygen demand (BOD) incubator for 35 days. For pesticide determination, the simple and efficient solid-liquid extraction methods with low temperature partition (SLE/LTP) followed by gas chromatography with electron capture detection (GC-ECD), for unhulled and husked rice were optimized and validated. These methods produced results of validated parameters consistent with Brazilian legislation, showing good efficiency (recovery rate above 95%) and limit of quantification (LOQ) < 0.090 mg kg^{-1} for bifenthrin and LOQ < 0.070 mg kg^{-1} for deltamethrin. There was a 40% dissipation of deltamethrin residues after 15 days of storage of the unhulled rice. For the insecticide bifenthrin, dissipation during the 35 days of storage was not verified. The remaining residues of dissipation in the rice grains were below the maximum residue level (MRL) prescribed by the law. The insecticide residues did not translocate in the husked rice grains destined for final consumption.

Keywords: stored grain, bifenthrin, deltamethrin, sample preparation, gas chromatography

Introduction

Protective insecticides are used in the production process and in the storage of rice.[1-3] These treatments are designed to ensure product quality by preventing the attack of insect pests.[3]

Organophosphates and pyrethroids constitute the insecticides groups authorized by Brazilian legislation, for use in rice grains protection during storage.[4] Pyrethroids are increasingly used on a large scale because they have a high level of efficacy in pest control.[5-7] However, because there is little variety in the active ingredients of pyrethroids, we have observed increased populations of resistant insects.[8-10] As a result, higher doses are used, and residues of these pesticides remain in food.[11-13] These residues can cause damage to the environment because the residues bioaccumulate and biomagnify affecting non-target organisms.[7,14-16] Moreover, the residues of these pesticides can pose a risk to the population due to the adverse effects that can cause long-term harm.[16,17]

*e-mail: meliana@ufv.br

Food safety is a common concern worldwide. Thus, there is a stimulus to investigate the quality of the food consumed by man.[2,16,18] Pyrethroids, such as bifenthrin and deltamethrin, are registered for treatment of stored rice grains.[4] The first effect of these insecticides on the nervous system is the induction of long-lasting repetitive activities such as tremors, excessive salivation, tearing, nasal hypersecretion, muscle cramps, and seizures.[7] For this reason, there is a need to monitor these residues in rice grains intended for human consumption.

Traditionally, for the determination of pesticide residues in food, the preferred analytical technique is gas chromatography. The methods used for the extraction of rice pesticide residue include solid phase extraction,[19] QuEChERS (quick, easy, cheap, effective, rugged, safe)[20] and matrix dispersion in the solid phase.[21] Another technique that has been used successfully for the extraction of pesticides in plant matrices is solid-liquid extraction with low temperature partition (SLE/LTP). This technique has some advantages over others such as practicality, extraction in one step, and use of small amounts of organic solvents, as well as being reliable, selective and not needing a cleanup step.[12,22,23]

The SLE/LTP is based on the partition of analytes between a solid phase and a liquid phase consisting of an organic solvent miscible with water. Upon subjecting the extraction solution to a temperature of −20 °C, the aqueous phase freezes into a solid matrix, and the analytes migrate into the organic phase, which is subsequently isolated and analyzed.[22,23] This method was efficient in the extraction and analysis of pesticides in grains, such as corn.[12] However, there are no reports of applying this technique to the extraction of rice grains. This study aimed to optimize and validate the SLE/LTP conditions in order to obtain a simple and efficient method that consumes little reagent, for the determination of bifenthrin and deltamethrin residues' dissipation in rice grains shell and the translocation of these residues from the shell to the husked rice.

Experimental

Reagents and solutions

Analytical standards of bifenthrin (98.8% m/m) and deltamethrin (99.7%, m/m) (Table S1, Supplementary Information (SI) section) were purchased from Sigma-Aldrich, Steinheim, Germany. A standard stock solution at a concentration of 1000.0 mg L^{-1} was prepared by dissolving both analytical standards in acetonitrile (99.5% v/v) (Sigma-Aldrich, Steinheim, Germany). The working solutions containing bifenthrin at a concentration of 50.0 mg L^{-1} and deltamethrin at a concentration of 10.0 mg L^{-1} were prepared by diluting the standard stock solution with the same solvent, acetonitrile. All solutions were stored in a freezer at −20 °C.

Gas chromatography with electron capture detector (GC-ECD) conditions

The chromatographic analyses of the extracts were performed using a gas chromatograph (GC), model GC-2014 (Shimadzu, Kyoto, Japan), equipped with an electron capture detector (ECD) and an AOC-20i auto injector. The chromatographic separation of the analytes was achieved using the HP-5 capillary column (Agilent Technologies, Palo Alto, CA, USA) with a stationary phase consisting of 5% diphenyl and 95% dimethylpolysiloxane (30 m × 0.25 mm, film thickness of 0.1 μm). Nitrogen was used as the carrier gas (1.2 cm^3 min^{-1}). The extract/standard solution (1 μL) was injected into the chromatograph, 1:5 split ratio, under the following chromatographic conditions: injector temperature of 280 °C and the column started at 200 °C and heated at a rate of 30 °C min^{-1} to 290 °C. The column was maintained at this temperature for 5 min, and the temperature of the detector was 300 °C. The total analysis lasted 8 min. The compounds were identified by comparing the retention time of the peaks present in the extracts of the samples with the standard retention times. The insecticides were quantified by the matrix-matched method.[24]

SLE/LTP sample preparation and insecticide extraction

It was used unhulled rice grains, with no pesticides application, from the 2012/2013 harvest. The grains were provisioned by the Agricultural Research Company of Minas Gerais (EPAMIG) and stored at 10 °C until the analysis was performed and data collection. For the analysis, grains of husked rice and unhulled rice were ground in a Wiley mill (Fritsch Pulverisette 14, Oberstein, Germany). For sample spiking, 2.0000 g of milled rice were conditioned in a 22 mL flask and spiked with 28 and 200 μL of bifenthrin and deltamethrin working solutions, respectively. Thus, the samples of milled husked rice and milled unhulled rice were spiked with a concentration of 0.7 and 1 mg kg^{-1} of bifenthrin and deltamethrin and vortexed (Marconi, MA 162, Piracicaba, Brazil) for 3 s to ensure insecticide distribution in all sample. Then, 4.0 mL of distilled water and 4.0 mL of acetonitrile were added to the milled husked rice sample. This mixture was vortexed for 1.0 min, and kept in a freezer (Consul Slim 160, Joinville, Brazil) (−20 °C) for approximately 6 h. Then, a 1.0 mL aliquot of the supernatant of the extract obtained was collected and transferred into a 1.8 mL vial for GC-ECD analysis.

The insecticide extraction from the milled unhulled rice samples was made by adding 2.0 mL of distilled water and 4.0 mL of acetonitrile to the sample. This mixture was vortexed (Marconi, MA 162, Piracicaba, Brazil) for 1.0 min and kept in a freezer (Consul Slim 160, Joinville, Brazil) (−20 °C) for approximately 6 h. Then, a 1.0 mL aliquot of the supernatant extract obtained was collected and transferred into a 1.8 mL vial for GC-ECD analysis.

Optimization of SLE/LTP of bifenthrin and deltamethrin in rice

The SLE/LTP extraction methods were optimized by performing an experimental assay to evaluate the best chromatographic responses for the insecticides, using different levels of the tested variables. For this experiment, a multivariate optimization was performed using a full factorial design 2^4, with two replications. The model was generated by statistical software (Statistica, version 8.0),[25] which describes the influence of the combination of the four variables tested on the chromatographic responses.

The four variables were the volume of extracting solvent, the volume of distilled water, the time of vortexing and the cooling time in freezer. For a total of 16 treatments, 32 assays were performed for the optimization based on the experimental design outlined in Table 1. Experimental results were evaluated according to the chromatographic responses (areas) obtained in each test.

Validation of extraction methods and calibration curves

The analytical methods were validated for the assessment of selectivity, limit of detection (LOD), limit of quantification (LOQ), linearity, matrix effect, precision (repeatability and intermediate precision) and accuracy.

The selectivity of the method was evaluated by comparing chromatograms of the extracts of the samples free from rice insecticides (blank) with the chromatograms of the extracts of the rice samples spiked with bifenthrin and deltamethrin at concentrations of 1.4 and 2.0 mg kg^{-1}, respectively.

LOD and LOQ were determined with a calculation based on 3.3 and 10 times the ratio between the standard deviation of the intercept and the slope estimated from the calibration curve of the analytes.[24] The concentration of calibration curves were 0.04; 0.06; 0.08; 0.1; 0.3 and 0.5 mg kg^{-1}.

To evaluate the linearity, analytical curves were performed based on matrix matching. The blank matrix extract was fortified to obtain nine concentrations (n = 9), equally spaced, ranging between LOQ up to twice the maximum residue limit (MRL). The values of the MRL are 0.7 and 1.0 mg kg^{-1} for bifenthrin and deltamethrin, respectively.[26] Standard solutions were analyzed in triplicate by GC-ECD, and the chromatographic peak areas for each replica of standard solutions were observed to obtain the calibration curves.

The range of linearity for analytes was evaluated based on the correlation coefficient (r) for each analyte by ordinary last squares, on the residual plots and on the homoscedasticity of the data obtained, as described by Barbosa *et al.*[27] For this analysis, it was used the software OriginPro.[28]

The matrix effect was evaluated by comparing the calibration curve from standard addition to the extract with the standard curve in acetonitrile at concentrations ranging from 0.06 to 2.80 mg L^{-1} for bifenthrin, and from 0.14 to 4.00 mg L^{-1} for deltamethrin, for the extraction method in husked rice. In the case of the extraction method in unhulled rice, the concentrations of the calibration curve ranged from 0.18 to 2.8 mg L^{-1} for bifenthrin, and from 0.12 to 4.00 mg L^{-1} for deltamethrin. The enhancement or reduction of chromatographic response was used to determine the

Table 1. Experimental design used for optimization of SLE/LTP methods for husked and unhulled rice

Trial	Independent variable							
	Water volume / mL		Acetonitrile volume / mL		Vortexing time / min		Cooling time / h	
	Actual	Coded	Actual	Coded	Actual	Coded	Actual	Coded
1 and 17	2.00	–	4.00	–	1.00	–	3.00	–
2 and 18	4.00	+	4.00	–	1.00	–	3.00	–
3 and 19	2.00	–	8.00	+	1.00	–	3.00	–
4 and 20	4.00	+	8.00	+	1.00	–	3.00	–
5 and 21	2.00	–	4.00	–	3.00	+	3.00	–
6 and 22	4.00	+	4.00	–	3.00	+	3.00	–
7 and 23	2.00	–	8.00	+	3.00	+	3.00	–
8 and 24	4.00	+	8.00	+	3.00	+	3.00	–
9 and 25	2.00	–	4.00	–	1.00	–	6.00	+
10 and 26	4.00	+	4.00	–	1.00	–	6.00	+
11 and 27	2.00	–	8.00	+	1.00	–	6.00	+
12 and 28	4.00	+	8.00	+	1.00	–	6.00	+
13 and 29	2.00	–	4.00	–	3.00	+	6.00	+
14 and 30	4.00	+	4.00	–	3.00	+	6.00	+
15 and 31	2.00	–	8.00	+	3.00	+	6.00	+
16 and 32	4.00	+	8.00	+	3.00	+	6.00	+

The signs "+" and "–" represent the maximum and minimum levels of each variable, respectively.

matrix effect. This was calculated as: ((matrix-matched slope/solvent calibration slope) – 1) × 100.[29] In addition, the matrix effect was evaluated comparing the slope of matrix-matched curve with slope of standard curve in acetonitrile by Student's *t*-test, at 5% probability.

The precision of the method was evaluated by the relative standard deviation (RSD) of repeated measurements, either on the same day (n = 7, repeatability) or on different days (n = 21, intermediate precision) at three concentration levels (0.5 × MRL, 1.0 × MRL and 1.5 × MRL). Accuracy was determined from spiked sample recovery rates in seven replicates and at three different concentrations (0.5 × MRL, 1.0 × MRL and 1.5 × MRL). The results were expressed as percent recovery.

Method application

Unhulled rice grains, with a water content of 14%, were sprayed with a solution containing bifenthrin at a concentration of 0.4 mg kg^{-1}, and with a solution containing deltamethrin at a concentration of 0.5 mg kg^{-1}. These solutions were prepared by diluting commercial products ProStore 25 CE® (bifenthrin, 16 mL t^{-1}) and K-25 Obiol CE® (deltamethrin, 20 mL t^{-1}) at the recommended doses by the manufacturer in 2.0 L of water. It was sprayed 20 mL of the insecticide solution in 10 kg of rice. The spray solution was applied to the grains over a plastic canvas using a hand sprayer. After spraying, the grains were homogenized with the aid of a squeegee and remained at rest for approximately 12 h. Then, the grains were stored in biochemical oxygen demand (BOD) chambers (Marconi MA 415, Piracicaba, Brazil) at 25 °C for 35 days.

The dissipation and translocation of pesticide residues were evaluated according to the security interval established by Brazilian National Health Surveillance Agency (ANVISA).[26] Periodically, 0, 7, 14, 21, 28 and 35 days, portions of 100 g of the pulverized rice with bifenthrin and periodically (0, 7, 14 and 21 days) portions of 100 g of the pulverized rice with deltamethrin were removed. Of these samples, 50 g of pulverized rice with each insecticide were ground and subjected to the extraction method for unhulled rice, and 50 g of pulverized rice with each insecticide were peeled, ground and submitted to the extraction method for husked rice for GC-ECD analysis. The extraction was performed in triplicate with samples of 2.0000 g of the milled rice.

Results and Discussion

Optimization of the SLE/LTP for rice

In the proposed experimental design, the variables evaluated were the volume of the organic solvent (acetonitrile), the volume of distilled water, the time for vortexing and the cooling time. Figure 1 presents the Pareto graphics for the effects of these factors on the extraction of pesticides from husked rice. For the analysis of the effects of the variables on the chromatographic response of bifenthrin and deltamethrin, the minimum volume of the solvent extractor (4.0 mL) was found to have a significant effect ($p < 0.05$) for both insecticides. The maximum water volume (4.0 mL) and the maximum cooling time were significant only for deltamethrin, improving the chromatographic response of this insecticide. There was a notable interaction between the volume of water and volume of acetonitrile factors for bifenthrin. At the same significance level, the vortexing time was negligible for both insecticides. According to the results, we opted for the use of 4.0 mL of acetonitrile and 4.0 mL

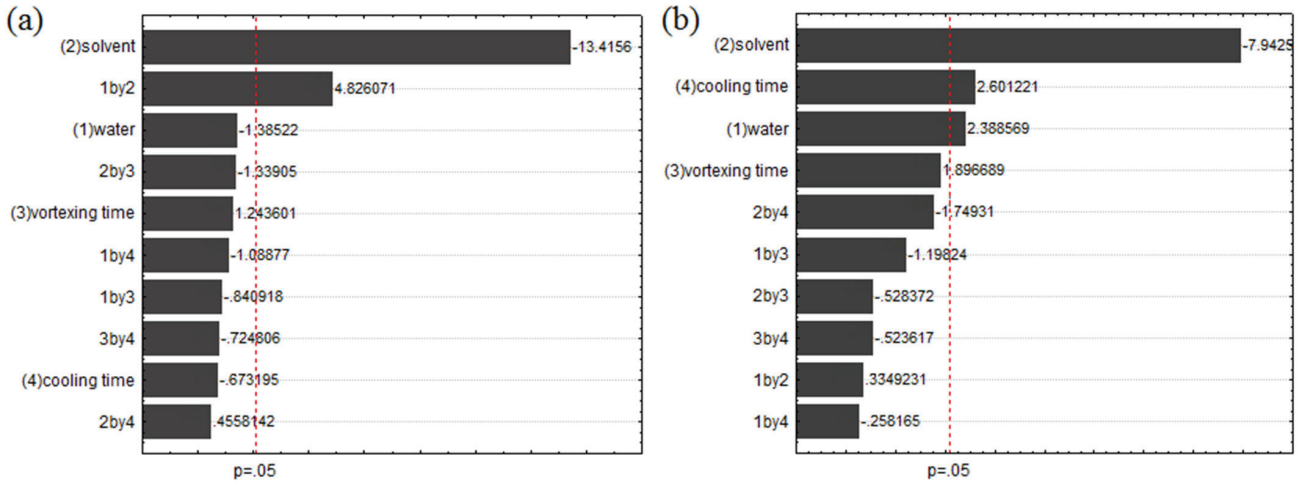

Figure 1. Pareto diagrams of the effects of varying volumes of acetonitrile, water volume, vortexing and cooling times on the extraction of (a) bifenthrin and (b) deltamethrin from husked rice samples.

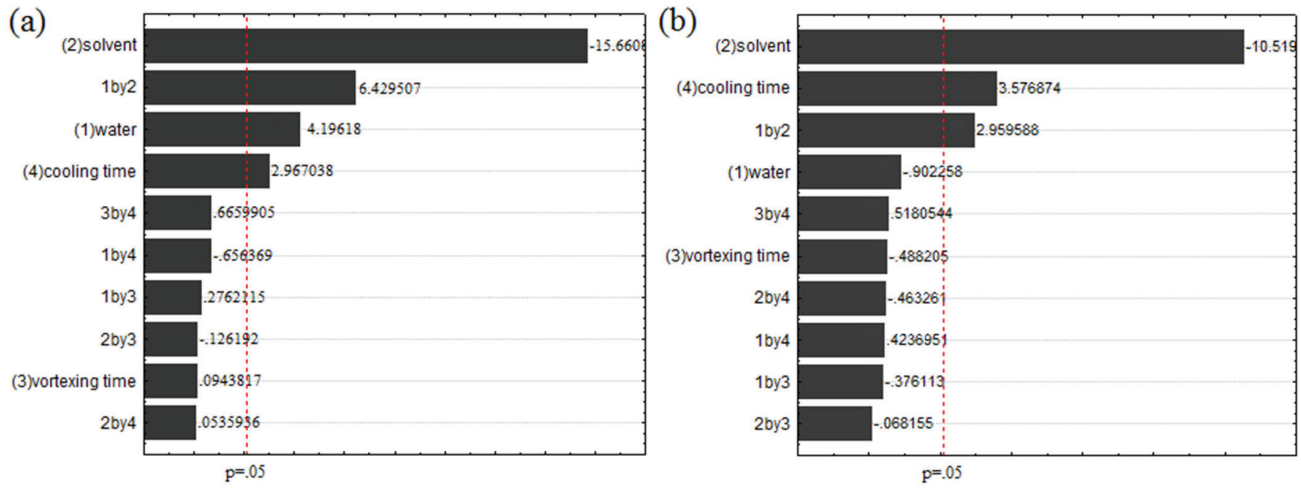

Figure 2. Pareto diagrams of the effects of varying acetonitrile volume, water volume, vortexing and cooling times on the extraction of (a) bifenthrin and (b) deltamethrin from unhulled rice samples.

of water as extraction solvents, 1 min of vortexing and 6 h of cooling at −20 °C.

Figure 2 shows the Pareto graphic with the chromatographic response of bifenthrin and deltamethrin in unhulled rice. The lower limit of the variable volume of acetonitrile (4.0 mL) and the maximum cooling time limit (6 h) were found to show a significant effect at 95% significance ($p < 0.05$) for both insecticides. The minimum water volume (2.0 mL) was significant only for bifenthrin, improving the response of the chromatographic analysis of the insecticide. There was also a significant interaction between the water volume and acetonitrile volume factors for both insecticides. At the same significance level, the vortexing time was not significant for either insecticide. In this way, we opted to use 4.0 mL of acetonitrile and 2.0 mL of water as extraction solvents, 1 min of vortexing and 6 h of cooling at −20 °C.

The optimized SLE/LTP methods showed a low consumption of sample and extracting solvent. It is not necessary to implement evaporation and solvent exchange steps, which reduces the risk of contamination and loss of sample. Little variation was observed between the methods for husked and unhulled rice, possibly due to the difference between the matrices because the rice shell is more complex and has in its composition various elements, such as silicon, the main element. However, the rice grain consists mainly of starch.[30,31] Extraction results using the same technique were reported by Freitas *et al.*,[12] who optimized and validated a method for the extraction of bifenthrin and pirimiphos-methyl in maize. In the optimization method, the authors found that the best recovery percentage was obtained using a volume of 4.0 mL water and 8.0 mL of acetonitrile as the extracting solution, 1 min of vortexing time and a cooling time of 3 h in a freezer (−20 °C).

Method validation

The sample chromatograms of the extracts obtained from the SLE/LTP rice spiked with bifenthrin and deltamethrin with and without shell and the chromatograms of the extracts obtained from the SLE/LTP rice with and without shell free from insecticides (blank) are presented in Figure 3. The methods are selective because the blank of the extracts shows no interfering peaks at the same retention times as the compounds analyzed in both methods. The presence of two peaks is attributed to deltamethrin isomers in the conversion during the injection of the sample into the gas chromatograph.[32] The quantification of deltamethrin was made considering the sum of the peak areas of the isomers in the chromatograms of the samples and standards.

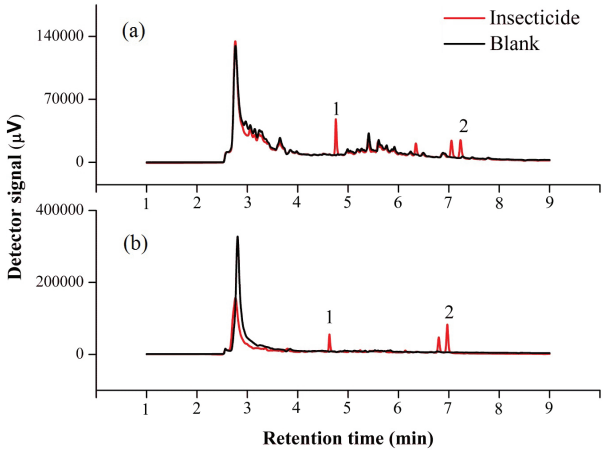

Figure 3. GC-ECD chromatographic analyses of spiked samples with 1.4 and 2.0 mg kg^{-1} of bifenthrin and deltamethrin, respectively, and the pesticide-free samples (blank). The peak numbers refer to the compounds: 1: bifenthrin, 2: deltamethrin. Chromatograms for the method for (a) unhulled rice and (b) husked rice.

There is a difference between the matrixes husked and unhulled rice. The difference is observed in the chromatogram: with the rice husks, a greater number of coextracted compounds are observed because its composition is more complex than the husked rice.[30,31]

The LOD and LOQ of the methods were estimated from the parameters of the calibration curve (Table 2), and they are far below the MRLs established by the ANVISA,[26] 0.7 mg kg^{-1} for bifenthrin and 1.0 mg kg^{-1} for deltamethrin. These values correspond to lower levels of the studied concentrations of insecticides that can be detected and quantified by the gas chromatograph used for analysis.[24] Similar LOD (0.09 mg kg^{-1}) were obtained in GC-ECD, for analysis of bifenthrin in corn.[12] In rice, Nguyen *et al.*[33] obtained the LOQ of 0.002-0.05 mg kg^{-1}, in a GC-MS-SIM (gas chromatography-mass spectrometry in selective ion monitoring mode) for determination of 203 pesticides. Liu *et al.*[34] obtained the LOD of 0.26-87 μg kg^{-1}, in a GC-MS, for determination of 40 pesticides, and Hou *et al.*[35] obtained the LOD of 0.1-7.0 μg kg^{-1} in the GC-MS/MS for analyses of 124 pesticides. Recently, Lee *et al.*[36] reported LOQ ranging from 1 to 10 ng g^{-1} using UHPLC-MS/MS in brown rice, orange, and spinach. The results of this study, corroborated by literature, make the SLE/LTP methods suitable to perform the analysis of the dissipation of residues of pesticides in unhulled rice grains and the translocation of these insecticide residues to husked rice grains in view of the national legislation.

The linearity was studied using the simple and weighted least square linear regression models in the concentration range between 0.03 and 1.40 mg kg^{-1} for bifenthrin and from 0.07 to 2.00 mg kg^{-1} for deltamethrin in the husked rice. For the unhulled rice matrix, the analytical curve was obtained in the range between 0.09 and 1.40 mg kg^{-1} for bifenthrin and from 0.06 to 2.00 mg kg^{-1} for deltamethrin.

The data of analytical curves for bifenthrin and deltamethrin in husked rice fit the calibration by simple least squares in the concentration range studied, as indicated by standard deviation analysis (Figure S1, SI section). Bifenthrin in unhulled rice data, obtained by simple regression ($\hat{y}$ = 5242.74 + 25979.77x, coefficient of determination (r^2) = 0.9849) was submitted to the adjustment procedure of exclusion of calibration points, with the criteria of keeping the minimum of five calibration levels. After exclusion of calibration points, the new regression parameters were obtained (Table 2), and its standard deviation plot is presented in Figure S1 (SI section).

The standard deviation plots indicate that only data of deltamethrin in unhulled rice was heteroscedastic (Figure S1, SI section). For this condition, the equation for the analytical curve obtained via simple least squared linear regression was not suitable because of the variance inconstancy throughout the concentration. Thus, the regression parameters were estimated via weighted least squared linear regression (Figure S2, SI section). The simple and weighted regression parameters and r^2 obtained after statistical analysis of linear regression (Table 2) indicate a directly proportional response in relation to the concentration of the analytes of interest, and are in accordance with SANTE[29] and Ribani *et al.*[24]

The repeatability of the methods was evaluated at three levels of concentration of bifenthrin (0.35, 0.70 and 1.05 mg kg^{-1}) and deltamethrin (0.50, 1.00 and 1.50 mg kg^{-1}). The repeatability was expressed in terms of coefficient of variation (CV) (Table 3). The intermediate precision was also expressed in terms of CV for the analysis of data obtained on three consecutive days (Table 3), under the same working conditions.

The percentages of the recovery methods (Table 3) are consistent with the data the analytical procedure must be able to recover at each fortification level, from 70 to 120% on average, with precision expressed in terms of CV < 20%.[24] Because the values obtained are within this range, one can infer that the recoveries and the coefficient of variation for bifenthrin and deltamethrin are appropriate.

The matrix effect was calculated according to the slope of calibration curve in the extract and in acetonitrile with the pesticide standard. The concentrations range corresponds to the respective LOQ and MRL of each compound. The calibration curves parameters are presented in Table 4 and Figure S3 (SI section).

Table 2. Limit of detection (LOD), limit of quantification (LOQ) and linearity from methods for husked and unhulled and rice

Pesticide	Regression equation	r^2	LOD / (mg kg^{-1})	LOQ / (mg kg^{-1})
Bifenthrin[a]	$\hat{y}$ = 9102 + 60882x	0.9930	0.010	0.030
Deltamethrin[a]	$\hat{y}$ = 3286 + 154226x	0.9991	0.023	0.070
Bifenthrin[b]	$\hat{y}$ = 5271.5 + 25996x	0.9901	0.030	0.090
Deltamethrin[b]	$\hat{y}$ = 1243 + 31862.87x	0.9966	0.018	0.060

[a]Measured by the method for husked rice; [b]measured by the method for unhulled rice. r^2: coefficient of determination; $\hat{y}$: chromatographic response; x: insecticide concentration.

Table 3. Repeatability and intermediate precision values expressed as CV, and recovery percentage of SLE/LTP methods for determination of bifenthrin and deltamethrin in husked and unhulled rice by GC-ECD

Pesticide	Concentration / (mg kg^{-1})		
	0.35[a] and 0.5[b]	0.7[a] and 1.0[b]	1.05[a] and 1.5[b]
	Repeatability (CV) / %		
Bifenthrin[c]	3.84	2.32	3.91
Deltamethrin[c]	3.16	3.03	1.97
Bifenthrin[d]	3.03	1.97	1.45
Deltamethrin[d]	3.62	1.78	2.09
	Intermediate precision (CV) / %		
Bifenthrin[c]	10.04	3.14	3.61
Deltamethrin[c]	5.90	6.32	7.21
Bifenthrin[d]	6.89	7.20	2.79
Deltamethrin[d]	9.50	8.36	7.35
	Recovery (mean ± RSD) / %		
Bifenthrin[c]	100.72 ± 11.07	101.22 ± 3.87	99.67 ± 2.48
Deltamethrin[c]	98.24 ± 2.27	98.26 ± 3.04	98.68 ± 7.14
Bifenthrin[d]	95.15 ± 4.7	105.41 ± 4.5	98.04 ± 1.5
Deltamethrin[d]	102.09 ± 4.4	102.77 ± 2.1	98.64 ± 2.8

[a]Bifenthrin concentration; [b]deltamethrin concentration; [c]measured by the method for husked rice; [d]measured by the method for unhulled rice; CV: coefficient of variation; RSD: relative standard deviation.

Table 4. Matrix effect and linearity parameters in solvent and rice matrices

Pesticide	Solvent curve		Matrix match curve		Matrix effect / %
	Slope	r^2	Slope	r^2	
Bifenthrin[a]	238756.71	0.9939	225308.11	0.9994	−5.63
Deltamethrin[a]	144221.51	0.9843	279343.94[c]	0.9850	93.7
Bifenthrin[b]	238645.83	0.9924	198409.43	0.9998	−16.86
Deltamethrin[b]	150681.27	0.9957	169804.31	0.9682	12.69

[a]Measured by the method for husked rice; [b]measured by the method for unhulled rice; [c]significant by the Student's *t*-test at 5% probability. r^2: coefficient of determination.

It can be observed that only deltamethrin in husked rice presented matrix effect, > 20%[29] and significant slope difference by Student's *t*-test at 5% probability. This result indicates that there was also an increase in the chromatographic response of deltamethrin. The matrix effect can be influenced by many factors, such as, pesticide physical-chemical properties, the matrix composition, injection process, chromatographic column and detector characteristics.[37,38] These results show that deltamethrin is more sensitive to a matrix effect than bifenthrin in rice matrix. The rice matrix components can interfere by competing for the active sites in the insert, allowing a greater amount of deltamethrin to be available into the chromatographic system, and consequently, detected.[38] Moreover, co-extracts that become deposited at the insert during repeated analyzes can increase the detector responses.[37,38] Therefore, we used the matrix-matched method for the quantification curves of insecticides with and without rice shell, so that this effect was reversed.

Dissipation and translocation of pesticide residues

Pyrethroid insecticide residues were found in unhulled rice at concentrations below MRL: 0.7 and 1.0 mg kg^{-1} for bifenthrin and deltamethrin, respectively. The concentration of bifenthrin in the grains did not vary significantly during storage for 35 days. Regarding deltamethrin, there was a significant difference after the second week in storage at 25 °C (Figure 4). There was a 40% dissipation of deltamethrin residues after 15 days of storage.

Considering the withdrawal periods established by ANVISA[26] (four weeks for bifenthrin and two for

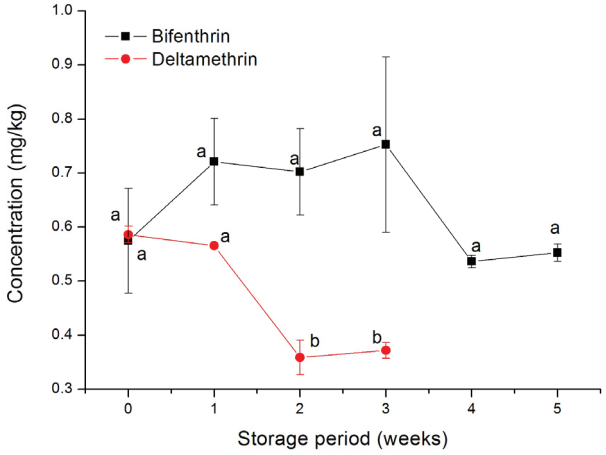

Figure 4. Change in concentrations of bifenthrin and deltamethrin insecticides in rice stored at 25 °C.

deltamethrin), residues of pesticides were found in stored rice grains, however, in concentrations below the maximum residue limit established by ANVISA.[26] The degradation rate of a pyrethroid depends on its molecular structure,[5,16] the storage temperature and the chemical composition of the grains to which it is applied because the pyrethroids are not very volatile compounds and have lipophilic characteristics.[39,40] The degradation rate also depends on the dose and frequency of application.[12,18]

Using the doses recommended by the manufacturer, Yu *et al.*[18] reported that the amount of pirimiphos-methyl and deltamethrin residues that can be quantified is below the maximum residue limit established by law. Afridi *et al.*[41] reported that permethrin residues (68.4 and 73.6%) persisted in wheat grain with a water content of 10 and 13%, respectively, when stored at 25 °C for 13 weeks. Residues levels reduced to 3.03 and 1.36% when the wheat grain at 10 and 13% moisture, respectively, was stored at 40 °C.

In the evaluation of bifenthrin and deltamethrin residue translocation for husked rice grains, chromatographic responses from the rice grains were below the LOQ of the method for this chromatographic analysis. The residues of bifenthrin and deltamethrin insecticides did not migrate from the hull to the rice grain during storage times of 35 and 21 days, respectively. In this regard, Dórea and Lima Sobrinho,[42] and Ma *et al.*[43] reported finding no pesticide residues in the rice trade.

In rice hulls, it is possible to have a total oil yield of 8.3%.[44] This amount of oil in the hull can be the reason for pesticides not to reach the grain that is intended for human consumption because pyrethroid compounds are lipophilic.[5,16,40] This fact is an advantage to human health because it ensures the quality of the product that will reach the consumer's table; however, it can enable the development of some insect pests such as *Sitophilus oryzae*. This insect attacks both in the field and in storage. The female makes a hole in the grain, lays the egg inside and seals the hole with a gelatinous layer. Thus, the offspring is protected because the development of the larvae will take place inside the grain until the adult stage is reached.[45,46]

Conclusions

The SLE/LTP optimized methods were simple, efficient and with low cost of analysis in GC-ECD. They were applied for the determination of pesticides in rice, showing good efficiency (recovery rate above 95%) and with LOQ < 0.090 mg kg^{-1} for bifenthrin and LOQ < 0.070 mg kg^{-1} for deltamethrin. The deltamethrin residues in rice grains had dissipated after 35 days of storage. The remaining bifenthrin residues in the grains, after 35 days of storage, were below the MRL established by law. The residues of bifenthrin and deltamethrin insecticides did not translocate to the husked rice grains after 35 days of storage.

Supplementary Information

Supplementary information (insecticides physical-chemical properties, statistical data from linearity analyses and matrix effect plots) is available free of charge at http://jbcs.sbq.org.br as PDF file.

Acknowledgments

We thank the Brazilian Agencies: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) finance code 001 and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for their financial support.

References

1. Kells, S. A.; Mason, L. J.; Maier, D. E.; Woloshuk, C. P.; *J. Stored Prod. Res.* **2001**, *37*, 371.
2. Carvalho, F. P.; *Food Energy Secur.* **2017**, *6*, 48.
3. Daglish, G. J.; Nayak, M. K.; Arthur, F. H.; Athanassiou, C. G. In *Recent Advances in Stored Product Protection*; Athanassiou, C. G.; Arthur, F. H., eds.; Springer: Berlin, Heidelberg, 2018, p. 45-63.
4. http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons, accessed in May 2020.
5. Hirata, R.; *Quim. Nova* **1995**, *18*, 368.
6. El-Sayed, Y. S.; Saad, T. T.; El-Bahr, S. M.; *Environ. Toxicol. Pharmacol.* **2007**, *24*, 212.
7. Gajendiran, A.; Abraham, J.; *Front. Biol. (Beijing)* **2018**, *13*, 79.

8. Pimentel, M. A.; Faroni, L. R.; Guedes, R. N.; Sousa, A. H.; Tótola, M. R.; *J. Stored Prod. Res.* **2009**, *45*, 71.
9. Sousa, A. H.; Faroni, L. R. D.; Pimentel, M. A. G.; Guedes, R. N. C.; *J. Stored Prod. Res.* **2009**, *45*, 241.
10. Haddi, K.; Valbon, W. R.; Jumbo, L. O. V.; de Oliveira, L. O.; Guedes, R. N. C.; Oliveira, E. E.; *Sci. Rep.* **2018**, *8*, 16361.
11. Pinho, G. P.; Neves, A. A.; Queiroz, M. E. L. R.; Silverio, F. O.; *Food Chem.* **2010**, *121*, 251.
12. de Freitas, R. S.; de Queiroz, M. E. L. R.; Faroni, L. R. D.; Heleno, F. F.; de Moura, V. V.; *Quim. Nova* **2014**, *37*, 238.
13. de Freitas, R. S.; Faroni, L. R. D. A.; de Queiroz, M. E. L. R.; Heleno, F. F.; Prates, L. H. F.; *J. Stored Prod. Res.* **2017**, *74*, 1.
14. Corcellas, C.; Eljarrat, E.; Barceló, D.; *Environ. Int.* **2015**, *75*, 110.
15. Goulson, D.; Nicholls, E.; Botías, C.; Rotheray, E. L.; *Science* **2015**, *347*, 1435.
16. Chen, S.; Zhan, H. In *Microbial Metabolism of Xenobiotic Compounds*; Arora, P. K., ed.; Springer Nature: Singapore, 2019, p. 229-244.
17. Kunno, J.; Ong-Artborirak, P.; Taneepanichskul, N.; Robson, M. G.; Siriwong, W.; *Hum. Ecol. Risk Assess.* **2019**, DOI 10.1080/10807039.2019.1689098.
18. Yu, C.; Li, Y.; Zhang, Q.; Zou, N.; Gu, K.; Li, X.; Pan, C.; *Int. J. Environ. Res. Public Health* **2014**, *11*, 5372.
19. Khalilian, F.; Rezaee, M.; *Food Anal. Methods* **2017**, *10*, 885.
20. Melo, M. G.; Carqueijo, A.; Freitas, A.; Barbosa, J.; Silva, A. S.; *Foods* **2019**, *9*, 18.
21. Makkar, A.; Kaur, P.; Kaur, P.; Kaur, K.; *J. Liq. Chromatogr. Relat. Technol.* **2016**, *39*, 718.
22. Heleno, F. F.; Rodrigues, A. A. Z.; Queiroz, M. E. L. R.; Neves, A. A.; Oliveira, A. F.; Libardi, V. M.; *Microchem. J.* **2019**, *148*, 79.
23. Ramírez, A. C. R.; Teixeira, M. F. F.; Neves, A. A.; Queiroz, M. E. L. R.; da Silva, A. A.; Furtado, I. F.; de Oliveira, A. F.; *J. Exp. Agric. Int.* **2018**, *21*, DOI 10.9734/JEAI/2018/39554.
24. Ribani, M.; Bottoli, C. B. G.; Collins, C. H.; Jardim, I. C. S. F.; Melo, L. F. C.; *Quim. Nova* **2004**, *27*, 771.
25. *Statistica*, version 8.0; StatSoft Inc., Tulsa, OK, USA, 2008.
26. http://portal.anvisa.gov.br/documents/111215/117782/B26+Bifentrina/77c89cd6-4c54-40d0-b968-af0907faaa12, accessed in May 2020; http://portal.anvisa.gov.br/documents/111215/117782/D06+%E2%80%93+Deltametrina/f3f40b14-63aa-43d1-bf05-3245e8437e74, accessed in May 2020.
27. Barbosa, P. G. A.; Martins, F. I. C. C.; Lima, L. K.; Milhome, M. A. L.; Cavalcante, R. M.; do Nascimento, R. F.; *Food Anal. Methods* **2018**, *11*, 466.
28. *OriginPro*, version 9.6.0.172; OriginLab Corporation, Northampton, MA, USA, 2019.
29. SANTE/11813/2017: *Guidance Document on Analytical Quality Control and Method Validation Procedures for Pesticide Residues and Analysis in Food and Feed*; European Commission, 2017. Available at https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_2017-11813.pdf, accessed in May 2020.
30. Della, V.; Kuhn, I.; Hotza, D.; *Mater. Lett.* **2002**, *57*, 818.
31. Kik, M. C.; *J. Agric. Food Chem.* **1956**, *4*, 170.
32. Anastassiades, M.; Maštovská, K.; Lehotay, S. J.; *J. Chromatogr. A* **2003**, *1015*, 163.
33. Nguyen, T. D.; Han, E. M.; Seo, M. S.; Kim, S. R.; Yun, M. Y.; Lee, D. M.; Lee, G. H.; *Anal. Chim. Acta* **2008**, *619*, 67.
34. Liu, P.; Liu, Q.; Ma, Y.; Liu, J.; Jia, X.; *Chin. J. Chromatogr.* **2006**, *24*, 228.
35. Hou, X.; Han, M.; Dai, X.; Yang, X.; Yi, S.; *Food Chem.* **2013**, *138*, 1198.
36. Lee, J.; Shin, Y.; Lee, J.; Lee, J.; Kim, B. J.; Kim, J. H.; *Chemosphere* **2018**, *207*, 519.
37. Pinho, G. P.; Neves, A. A.; Queiroz, M. E. L. R.; Silvério, F. O.; *Quim. Nova* **2009**, *32*, 987.
38. Agatemor, C.; Beauchemin, D.; *Anal. Chim. Acta* **2011**, *706*, 66.
39. Holland, P. T.; Hamilton, D.; Ohlin, B.; Skidmore, M. W.; *Pure Appl. Chem.* **1994**, *66*, 335.
40. Liu, X.; Wang, P.; Liu, C.; Liang, Y.; Zhou, Z.; Liu, D.; *J. Agric. Food Chem.* **2017**, *65*, 7647.
41. Afridi, I. A. K.; Parveen, Z.; Masud, S. Z.; *J. Stored Prod. Res.* **2001**, *37*, 199.
42. Dórea, H. S.; Lima Sobrinho, L.; *J. Braz. Chem. Soc.* **2004**, *15*, 690.
43. Ma, Y.; Zhan, L.; Yang, H.; Qin, M.; Chai, S.; Cao, Z.; Mou, R.; Chen, M.; *J. Sci. Food Agric.* **2019**, *99*, 4602.
44. Karagöz, S.; Bhaskar, T.; Muto, A.; Sakata, Y.; *Fuel* **2005**, *84*, 875.
45. Lathrop, F. H.; *Ohio Nat.* **1914**, *14*, 321.
46. Richards, O. W.; *J. Zool.* **1947**, *117*, 2.

Submitted: December 18, 2019
Published online: May 26, 2020