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Analytical Methods

Development of a fast multiresidue method for the determination of pesticides in dry samples (wheat grains, flour and bran) using QuEChERS based method and GC–MS

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ABSTRACT

In this study a multiresidue method for the determination of 24 pesticides in wheat, white flour and bran using gas chromatography coupled to mass spectrometry with negative chemical ionisation and selected ion monitoring (GC–MS (NCI–SIM)) was developed and validated. The QuEChERS method was used for the extraction of different pesticides. The method was validated evaluating the following parameters: linearity, limit of detention, limit of quantification, matrix effect as well as precision and accuracy, evaluating the percentage of recovery at four different spike levels. The linear range used in the calibration curves was from 1.0 to 100 μg L^{-1} for wheat and 2.0 to 200 μg L^{-1} for flour and bran, both with values of $r^2 > 0.99$. The recoveries had been considered satisfactory presenting values between 70% and 120% with RSD < 20% for the majority of compounds.

1. Introduction

Pesticides have played a very important role in the development of the agriculture and were still irreplaceable until the present time. Although pesticides and veterinary drugs found their way into wide applications and have played a significant part in constantly boosting agricultural and animal production, the hazards they have brought along with them to food safety and human health have increasingly become the focus of world attention. Therefore, different countries have promulgated their own residue limits in the international trade. There are many methods for determination of pesticides multiresidue in agricultural products and animal derived foods, but the key technique is: firstly, how several dozens of varieties or even hundreds of pesticides residues can be thoroughly extracted from the complex matrixes; secondly, how a great deal of interfering matters co-extracted with the pesticides can be cleaned up; thirdly, what analytical modes should be adopted for the pesticides requiring determination (Pang et al., 2006).

Liquid extraction is the fundamental method utilised for the isolation of pesticide residues from various food matrices. Many aspects such as ability to cover pesticides of a wide polarity range, selectivity involved in extraction and clean-up step and compatibility with separation techniques have to be considered. The choice of the solvent is one of the most important decisions to take in a multiresidual method (Hercegová, Dömötörová, & Matisová, 2007). In the last few years acetone, acetone in combination with dichloromethane, ethyl acetate and acetonitrile are the extraction solvents most commonly used in extraction methods for the determination of pesticide residues in food (Maštovská & Lehotay, 2004). Anastassiades, Lehotay, Štajnbaher, and Schenk (2003) published a method that provided high quality results with a minimum number of steps and a low solvent and glassware consumption. The method was given an acronymic name QuEChERS that reflected its major advantages (quick, easy, cheap, effective, rugged and safe). Sample preparation is always the major in the complete analytical procedure for the determination of pesticide residues in food products (Lehotay, Maštovská & Lightfield, 2005). The QuEChERS multiresidue procedure omits or replaces many complicated analytical steps commonly employed in traditional methods by easier ones. The original procedure consists of extracting the homogenised sample by hand-shake or Vortex with the same amount of acetonitrile in order to have a final extract concentrated enough without the need of a solvent evaporation step. The technique has attracted the attention of pesticide analysis studies worldwide (Aysal, Ambrus, Lehotay, & Cannavan, 2007; Díez, Traag, Zommer, Marinero, & Atienza, 2006; Hercegová, Dömötörová, Kružlicova, & Matisová 2006; Lehotay, 2007; Lehotay, Kok, Hiemstra & Bodegraven, 2005; Lehotay, Maštovská & Yun, 2005; Lesueur, Knittl, Gartner, Mentler, & Fuerhacker, 2008; Walorczyk, 2007).

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Gas chromatography coupled with quadrupole mass spectrometry detection is considered a powerful technique for the quantitative determination of lower levels of contaminants in complex matrices. In this sense, it has been used for the determination of pesticide residues in matrices such as vegetables, honey, beer, baby food and meat (Garrido-Frenich et al., 2006). The electron ionisation (EI) mode is the most widely used for identification and quantitation of unknown compounds in complex mixtures. However, quite often the lack of information due to the extensive fragmentations of the molecular ions demand the use of a softer ionisation mode, the chemical ionisation (CI) with positive (PCI) or negative (NCI) ion detection, which produces fewer fragment ions (Béguin, Jadas-Hécart, Tabet, & Communal, 2006). If the CI mode is used the chromatograms obtained are cleaner due to the minimisation of background interferences and because there is less chance for interferences from ions derived from the sample matrix than when using EI (Barreda et al., 2006). NCI is specially recognised for the improved selectivity and sensitivity for organochlorine and organophosphorus compounds. Usually, only a few ions of high abundance are observed in the NCI mass spectra, this fact enhances analyte detectability if the selected ion monitoring (SIM) mode is applied (Louter, Hogenboom, Slobodník, Vreuls, & Brinkman, 1997).

The main objective of this work was demonstrating the potential sample preparation of a miniaturized acetonitrile-based extraction method followed by a dispersive solid phase extraction (D-SPE) clean-up step. In addition evaluating the GC coupled with a mass spectrometry analyser operated in NCI–SIM mode for sensitive and reliable pesticide multiresidue determination for dry samples such as wheat grains, white flour and bran.

2. Materials and methods

2.1. Materials and reagents

Acetonitrile (MeCN) pesticide residue grade and acetic acid (HAc) analytical grade were obtained from Merck (Darmstadt, Germany). Toluene and isooctane were both pesticide residue grade (J.T. Baker, Phillipsburg, USA). Water was freshly purified using a Direct UV3® system (Millipore, Molsheim, France). Anhydrous magnesium sulphate ($MgSO_4$), anhydrous sodium acetate (NaAc), sodium citrate tribasic dehydrate, sodium chloride (NaCl) all reagent grade were purchased from Merck (Rio de Janeiro, Brazil). Adsorbent C_{18} (55 μm) was obtained from Phenomenex (Torrance, USA). Polypropylene centrifuge tubes (Sarstedt, Nümbrecht, Germany), 50 mL volume for initial extraction, and 15 mL volume for D-SPE step were used.

Pesticide standards (purity >94.0%) were obtained from Dr. Ehrenstorfer (Augsburg, Germany). Purity was considered in the calculation of actual concentration of each standard solution. Individual stock standard solutions (1000 mg L^{-1}) were prepared in toluene. Working standard mixture in toluene, containing 1 mg L^{-1} of each pesticide, were prepared for use as spiking solution.

2.2. Samples

Ground wheat grains, white flour and bran were obtained in a local green shop and were produced without pesticides applications. The samples were stored in freezer at −18 °C until the sample preparation step.

2.3. GC–MS system

The GC–MS system comprising of a CP-3800 gas chromatograph equipped with electronic flow control (EFC), a 1079 injector, a CP 8400 autosampler and a 1200 triple quadrupole MS (Varian, Walnut Creek, USA). Data acquisition and processing were performed using a Varian Star Workstation, version 6.6. A capillary fused silica column VF-5 MS (30 m × 0.25 mm i.d., 0.25 μm film thickness) from Varian (Middelburg, The Netherlands) was used. Analytical balance HR-120 (A & D, Tokyo, Japan), Ultraturrax blender (Kinematica, Lucerne, Switzerland), food processor K-650 Braun (Kronberg im Taunus, Germany) and a Jouan C 412 (Saint-Herblain, France) centrifuge were employed.

Aliquots of 2 μL of sample extract were injected into the gas chromatograph. The injector temperature was held at 80 °C for 0.1 min during injection, then programmed at 200 °C min^{-1} to 300 °C which was held for 13 min. The injector liner (3.4 mm i.d.) was filled with a Carbofrit plug (Restek, Bellefonte, USA). The GC oven temperature programme was 80 °C for 1.0 min, followed by a 25 °C min^{-1} ramp to 180 °C and a final ramp of 5 °C min^{-1} to 280 °C (held for 5 min). Carrier gas was helium (99.9999% purity; Air Products, Allentown, USA) at a constant flow-rate of 1.3 mL min^{-1}.

The mass spectrometer (MS) was operated in negative chemical ionisation (NCI) mode using methane (99.999% purity; AGA, São Paulo, Brazil) as the reagent gas. A collect delay time of 7.0 min was set to prevent instrument damage. The MS was calibrated with perfluorotributylamine (PFTBA). The MS system temperatures of the detector interface was set at 250 °C, the source of ionisation at 235 °C and the manifold at 40 °C. The MS system was set in selective ion monitoring (SIM) mode and each compound was quantified based on peak area using three qualifier ions (Table 1). The identification and confirmation of the pesticides were performed as recommended by the European SANCO Guidelines (SANCO, 2006).

2.4. Sample homogenisation, extraction and clean-up

The QuEChERS extraction method was designed for samples with more than 75% moisture, and it had to be adapted to dry samples like wheat grains, white flour and bran. For products with a water content lower than 25% like cereals, dried fruits and spices, the sample amount may have to be reduced and water has to be added to make sample pores more accessible to the extraction solvent (Díez et al., 2006; Pizzutti et al., 2007; Walorczyk, 2007, 2008). Wheat grains were previously processed in a food processor. A sub-sample of 500 g of wheat, white flour and bran were blended in Ultraturrax at high speed with 1500 mL of purified water to give an homogeneous slurry (paste) from which aliquots are taken for analysis.

A 10 g of each slurry previously homogenised were weighed in a 50 mL centrifuge tube. Ten millilitres of acetonitrile, containing 1% (v/v) of acetic acid, were then added to the sample, and the mixture was hand-shaken for 1 min. Afterwards, 3 g of $MgSO_4$ were added and the tube was hand-shaken immediately for 20 s. Later, 1.7 g of sodium acetate and 1 g of sodium citrate were added and the tube was hand-shaken for another 1 min to provide well-defined phase separation after 8 min of centrifugation at 4000 rpm. During the clean-up step, 4 mL aliquot of the upper layer was transferred to a centrifuge tube (15 mL) containing 0.6 g $MgSO_4$ and 0.5 g C18. The tube was hand-shaken for 1 min and centrifuged at 4000 rpm for 8 min. A aliquot of the supernatant was transferred into a autosampler vial to its injection into the GC–MS system. Fig. 1 shows a scheme of the QuEChERS method used in this work.

2.5. Method performance and validation

For the validation of the QuEChERS method we selected 24 multiclass (organochlorine, organophophorus, pyretroids and others) pesticides (Table 1) for the GC–MS (NCI–SIM) analysis, based on

Table 1
Chromatography and mass spectrometry parameters for the pesticides.

Nr.	Pesticides	t_R (min)	Monitored ions	Segment	*Time window* (min)
1	Dimetoate	8.1	157 + 159 + 158	2	
2	Lindane	8.5	71 + 73 + 255 + 253	2	7.0–9.1
3	Chlorothalonil	8.9	266 + 264 + 268	2	
4	Chlorpyrifos-methyl	9.8	141 + 214 + 212	3	
5	Pirimifos-methyl	10.5	141 + 304 + 290	3	9.1–10.9
6	Fenitrothion	10.6	168 + 141 + 169	3	
7	Malathion	10.8	157 + 159 + 172	3	
8	Chloropyrifos-ethyl	11.0	169 + 214 + 212	4	
9	Aldrin	11.2	237 + 235 + 239	4	10.9–12.0
10	Parathion-ethyl	11.2	154 + 169 + 155	4	
11	Dicofol	11.5	250 + 252 + 251	4	
12	Endosulfan-alpha	13.3	242 + 240 + 244	5	
13	Dieldrin	14.2	237 + 239 + 235	5	12.0–15.0
14	Endrin	14.8	237 + 239 + 240	5	
15	Endosulfan-beta	15.2	99 + 242 + 240	6	
16	Endosulfan-sulphate	16.4	97 + 386 + 80 + 99	6	15.0–17.0
17	Bifenthrin	18.1	205 + 241 + 206	7	17.0–19.0
18	Tetradifon	19.2	320 + 318 + 245	8	
19	Cyhalothrin-lambda	20.0	205 + 241 + 243	8	19.0–23.0
20	Permethrin-cis	21.5	207 + 209 + 171	8	
21	Permethrin-trans	21.8	207 + 209 + 171	8	
22	Cypermethrin	23.4	207 + 209 + 171	9	
23	Deltamethrin	26.4	79 + 81 + 137 + 139	9	23.0–30.0
24	Azoxystrobin	26.8	371 + 356 + 301	9	

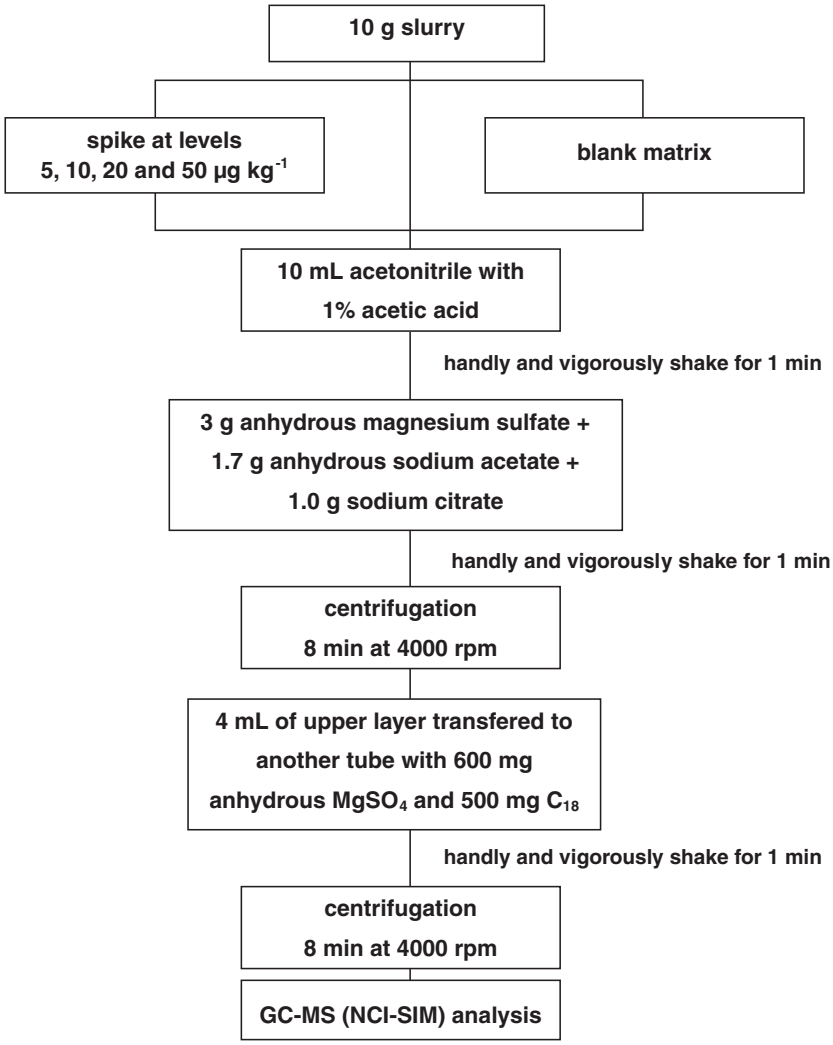

Fig. 1. Scheme of the QuEChERS extraction method.

their relevance in wheat cultivation and storing conditions. Based on the pesticides retention time, the GC–MS acquisition method was divided into as many time-windows as possible in order to maximise signal for pesticides that gave low response (Walorczyk, 2008). This method consisted of nine retention time-windows (segments).

2.5.1. Linearity study and detection and quantification limits

The calibration curves was evaluated with a matrix-matched standard calibration in blank extracts of wheat grains, white flour and bran in the concentrations 1.0; 2.0; 4.0; 8.0; 20.0; 50.0; 100.0 and 200.0 $\mu g\ L^{-1}$, where this sequence was injected six times ($n = 6$). Calculations were performed considering the average peak areas, relative standard deviations (RSD), the determination coefficients (r^2) and also linear ranges were determined for each pesticide analysed.

From the calibration curves data and the repeatability (RSD%) at the lowest concentration levels of each pesticides, the method limit of detection (LODm) were estimated. The real method limit of quantification (LOQm) was based on the recovery results and was defined as the lowest validated spike level meeting the requirements of recovery within the range of 70–120% and RSD $\leqslant$ 20% (Walorczyk, 2007).

2.5.2. Recovery study (accuracy and precision)

During the recovery experiments, the main objective was to determine the method accuracy, comparing the real concentration of each pesticide measured by performing the complete procedure

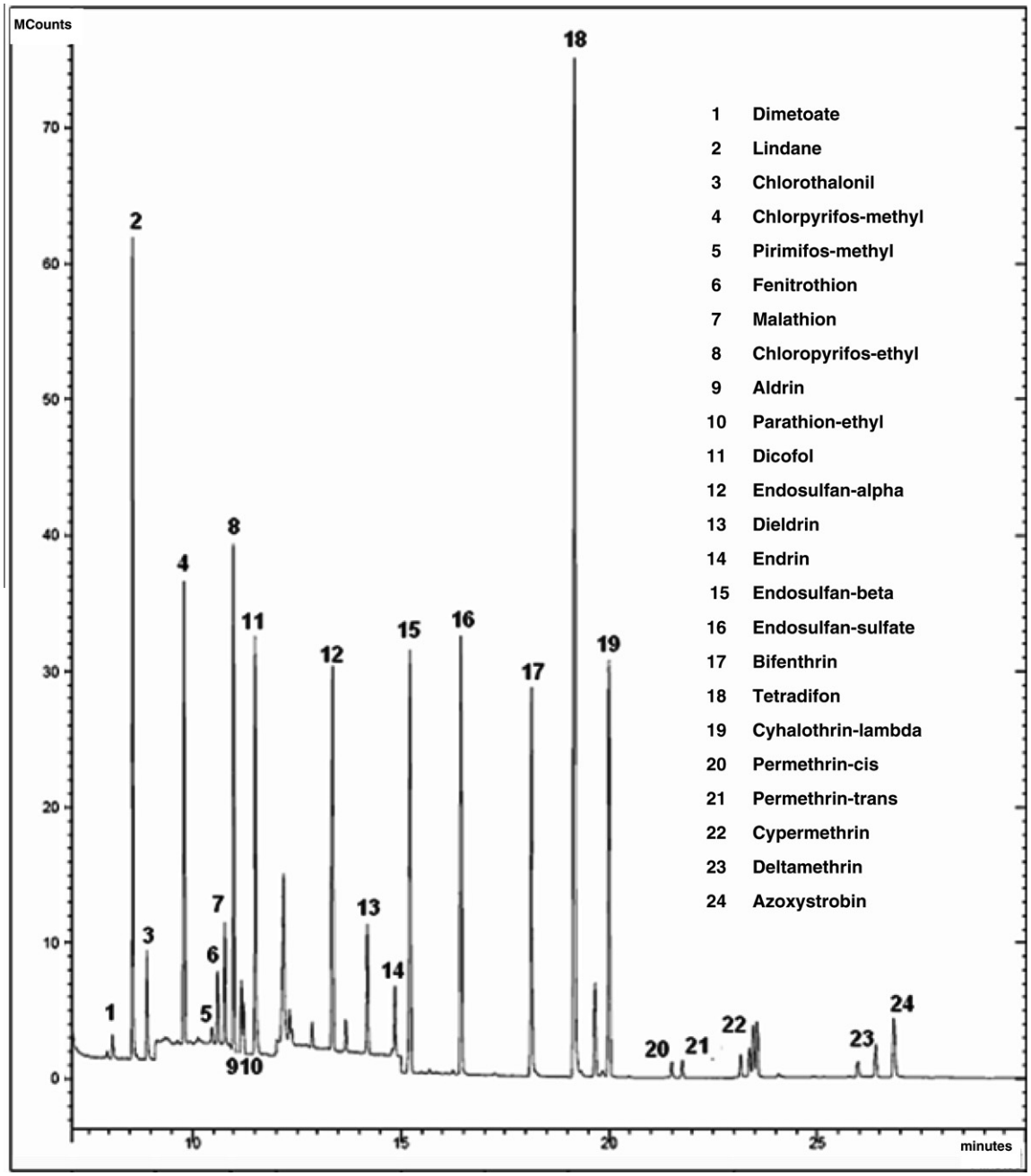

Fig. 2. GC-MS (NCI–SIM) chromatogram of wheat blank spiked with all 24 pesticides at 0.1 mg kg^{-1}.

with the known pesticide concentration initially added to the blank matrix at four levels 5, 10, 20 and 50 μg kg^{-1}. The 10 μg kg^{-1} level was chosen based on European Union legislation (EU, 2003), which is the most restrictive about pesticide residues, and the 50 μg kg^{-1} level was chosen because it is the most currently pesticide maximum residue level. The method precision was expressed as the repeatability (RSD%) of the recovery determinations at the four different spiking levels.

3. Results and discussion

3.1. Chromatographic determination by GC–MS (NCI–SIM)

A good resolution of all pesticides studied was achieved with the proposed chromatographic programme (Fig. 2).

All pesticides showed determination coefficient (r^2) $\geqslant$0.99 and linear range from 1.0 to 100 μg L^{-1} for wheat and from 2.0 to 200 μg L^{-1} for white flour and bran (Table 2). For all the analytes the LODm and LOQm ranged from 2.5 to 5 μg kg^{-1} and from 5 to 10 μg kg^{-1}, respectively (Table 2).

3.2. Evaluation of the QuEChERS method

Sample preparation was carried out following a QuEChERS procedure since it provided high throughput with adequate validation parameters and low cost per sample.

The recoveries of the studied pesticides at four different spike concentration levels 5, 10, 20 and 50 μg kg^{-1} were checked using the calibration curves prepared in the blank of the respective matrix (Table 3). Mean results shown recoveries between 70% and 120% with RSD $\leqslant$ 20%. Recoveries of aldrin were bellow 70% at levels 10, 20 and 50 μg kg^{-1}, indicating that this pesticide may only be partly recovered. At the lowest (5 μg kg^{-1}) and highest levels (50 μg kg^{-1}) 33% of the pesticides like chlorotalonil, fenithrotion, azoxystrobin, chlorpyrifos-methyl, dimetoate, malathion, parathion-methyl and pyrimiphos-methyl not satisfied the recovery criteria for validation method, and showed recoveries between 123% and 139%. These recoveries values are in the accordance with Koesukwiwat, Lehotay, Miao, and Leepipatpiboon (2010), which concluded that QuEChERS buffered version (acetonitrile/acetic acid and sodium acetate) has led to recoveries >120% in some commodities with large starch amount, because buffering caused a greater degree of co-extractives (fatty acids).

3.3. Method application

The QuEChERS method was also applied to the determination of pesticides in five commercial samples of wheat, white flour and bran in order to verify the effectiveness of the proposed approach. All the samples presented residues of more than one pesticide (Table 4). Pirimiphos-methyl was present in all the samples in concentrations between 0.01 and 3.57 mg kg^{-1}, reflecting that this compound is a common pesticide used in cereals.

Table 4 shows also the different Maximum Residue Limits (MRLs) established by the Brazilian and European Union Community legislation to safeguard consumer health and to promote Good Agricultural Practice (GAP) in the use of pesticides. These MRL values vary from country to country depending on the pesticides available, the crops being treated and the way the pesticides are used. All the found pesticide residues were below the LMR values established by both legislations.

4. Conclusions

In this study we optimised operation parameters and evaluated performance characteristics of GC–MS with negative chemical ionisation for the analysis of multiple pesticides in wheat grains, white flour and bran. The QuEChERS extraction method used in this study minimised the time, labour and cost of the sample preparation. The method proposed allowed the determination at low detection limits with good precision and accuracy. The combination of quick extraction and simultaneous determination for a 24 pesticides enables rapid and efficient monitoring. It was confirmed that the proposed method is suitable for routine residue monitoring in wheat, white flour and bran.

Table 2
Determination coefficient (r^2), LODm (μg kg^{-1}) and LOQm (μg kg^{-1}) for the matrix matched curves of the studied pesticides.

Pesticides	Calibration range (μg L^{-1})			LODm (μg kg^{-1})			LOQm (μg kg^{-1})		
	1–100 Wheat	2–200 White flour	2–200 Bran	Wheat	White flour	Bran	Wheat	White flour	Bran
Aldrin	0.9964	0.9965	0.9983	2.5	2.5	2.5	5.0	5.0	10.0
Azoxystrobin	1.0000	1.0000	0.9997	2.5	2.5	2.5	10.0	5.0	10.0
Bifenthrin	0.9992	0.9991	0.9896	2.5	2.5	2.5	5.0	5.0	5.0
Chlorpyrifos-ethyl	0.9993	0.9993	0.9900	2.5	2.5	2.5	5.0	5.0	5.0
Chlorpyrifos-methyl	0.9990	0.9999	0.9970	2.5	2.5	2.5	10.0	5.0	5.0
Chlorthalonil	0.9996	0.9999	0.9985	2.5	2.5	2.5	10.0	5.0	5.0
Cyhalothrin-lambda	0.9999	1.0000	0.9993	2.5	2.5	2.5	5.0	5.0	5.0
Cypermethrin-beta	0.9998	1.0000	0.9993	2.5	2.5	2.5	5.0	5.0	5.0
Deltamethrin	0.9998	1.0000	0.9998	2.5	2.5	2.5	5.0	5.0	5.0
Dicofol	0.9996	1.0000	0.9999	2.5	2.5	2.5	5.0	5.0	5.0
Dieldrin	0.9992	0.9999	0.9979	2.5	2.5	2.5	5.0	5.0	5.0
Dimetoate	0.9994	1.0000	0.9999	2.5	2.5	2.5	10.0	5.0	10.0
Endosulfan-alpha	0.9992	0.9998	0.9957	2.5	2.5	2.5	5.0	5.0	5.0
Endosulfan-beta	0.9998	1.0000	0.9993	2.5	2.5	2.5	5.0	5.0	5.0
Endosulfan-sulphate	0.9999	0.9999	0.9999	2.5	2.5	2.5	5.0	5.0	5.0
Endrin	0.9991	0.9998	0.9943	2.5	2.5	2.5	5.0	5.0	5.0
Fenitrothion	0.9999	0.9998	0.9987	2.5	2.5	2.5	10.0	5.0	5.0
Lindane	0.9957	0.9999	0.9981	2.5	2.5	2.5	5.0	5.0	5.0
Malathion	0.9997	0.9999	0.9959	2.5	2.5	2.5	10.0	5.0	5.0
Parathion-ethyl	1.0000	0.9999	0.9969	2.5	2.5	2.5	10.0	5.0	10.0
Permethrin-cis	0.9983	0.9999	0.9971	2.5	2.5	2.5	5.0	5.0	5.0
Permethrin-trans	0.9999	1.0000	0.9990	2.5	2.5	2.5	5.0	5.0	5.0
Pirimifos-methyl	1.0000	1.0000	0.9993	2.5	2.5	2.5	10.0	5.0	10.0
Tetradifon	0.9997	0.9999	0.9982	2.5	2.5	2.5	5.0	5.0	5.0

Table 3
Recovery (%) and repeatability (RSD%) from samples spiked at four levels, where measurements were performed six times ($n = 6$).

Pesticides	Average recovery % (RSD%)											
	5 μg kg^{-1}			10 μg kg^{-1}			20 μg kg^{-1}			50 μg kg^{-1}		
	Wheat	White flour	Bran	Wheat	White flour	Bran	Wheat	White flour	Bran	Wheat	White flour	Bran
Aldrin	87.2 (19.1)	81.6 (13.1)	115.9 (8.2)	67.6 (0.9)	78.4 (4.9)	67.8 (10.1)	67.1 (1.2)	73.0 (5.4)	69.1 (2.4)	66.6 (1.0)	73.0 (0.9)	69.4 (4.2)
Azoxystrobin	125.6 (0.1)	106.4 (5.5)	123.6 (2.9)	96.4 (3.1)	99.3 (1.1)	88.7 (2.8)	93.6 (2.2)	100.7 (0.9)	105.1 (5.4)	92.0 (0.8)	98.1 (2.7)	120.9 (9.4)
Bifenthrin	94.0 (0.7)	82.4 (2.6)	89.2 (1.2)	75.5 (1.7)	73.6 (10.4)	75.3 (0.7)	73.9 (0.8)	80.3 (2.1)	74.8 (2.4)	76.6 (1.4)	79.3 (2.1)	82.2 (5.0)
Chlorpyrifos-ethyl	115.4 (1.8)	90.6 (2.8)	102.6 (1.3)	90.4 (1.5)	91.8 (0.9)	87.2 (1.4)	87.9 (1.3)	95.7 (1.7)	92.1 (2.5)	82.4 (1.6)	92.1 (2.5)	103.5 (6.2)
Chlorpyrifos-methyl	127.0 (1.3)	104.0 (3.3)	113.3 (1.8)	96.2 (3.5)	96.7 (1.5)	90.7 (1.0)	92.3 (1.7)	101.3 (4.2)	103.3 (2.3)	90.7 (1.8)	99.0 (2.6)	126.5 (2.2)
Chlorthalonil	130.6 (3.1)	120.0 (8.5)	111.6 (2.9)	94.1 (3.8)	100.0 (4.1)	82.2 (1.7)	95.7 (1.7)	100.2 (5.0)	117.7 (4.2)	102.0 (1.9)	101.8 (3.0)	178.6 (2.3)
Cyhalothrin-lambda	116.8 (1.2)	100.6 (2.2)	113.3 (0.2)	91.1 (2.1)	93.4 (0.8)	89.5 (1.8)	90.5 (0.6)	96.6 (2.0)	92.0 (2.5)	89.8 (0.6)	94.5 (1.3)	103.5 (5.7)
Cypermethrin-beta	111.4 (5.5)	98.2 (2.3)	96.3 (1.7)	90.8 (1.7)	90.8 (4.5)	79.7 (0.8)	87.2 (1.4)	97.4 (1.8)	111.2 (3.1)	85.3 (2.0)	93.1 (1.6)	126.2 (1.4)
Deltamethrin	114.4 (1.3)	101.2 (2.8)	118.7 (8.4)	90.4 (4.2)	89.4 (0.9)	78.5 (7.1)	88.8 (0.2)	92.3 (1.1)	88.5 (1.4)	85.1 (1.1)	92.2 (1.6)	99.6 (5.6)
Dicofol	101.4 (7.1)	93.4 (2.5)	106.0 (0.4)	83.9 (0.4)	84.6 (0.3)	83.7 (1.6)	82.9 (1.2)	90.9 (1.9)	89.5 (2.1)	85.1 (1.8)	91.2 (3.0)	98.7 (0.3)
Dieldrin	105.8 (2.9)	100 (14.2)	115.5 (15.5)	79.8 (4.0)	85.4 (3.2)	78.4 (3.1)	81.7 (0.4)	91.9 (0.5)	85.5 (1.8)	83.2 (0.8)	87.1 (1.6)	88.3 (4.5)
Dimetoate	131.0 (5.6)	117.6 (7.8)	135.4 (1.6)	93.8 (4.1)	97.5 (7.2)	107.8 (2.7)	95.3 (4.2)	103.9 (6.4)	122.9 (3.6)	90.9 (2.0)	104.1(1.3)	208.6 (6.5)
Endosulfan alpha	106.4 (0.9)	95.0 (1.0)	97.8 (5.7)	82.6 (0.7)	86.3 (0.5)	79.4 (0.3)	81.8 (0.9)	89.2 (1.6)	83.1 (1.9)	82.5 (1.1)	88.3 (1.8)	87.2 (7.2)
Endosulfan beta	103.2 (2.7)	89.0 (4.6)	95.8 (2.2)	83.8 (0.6)	89.2 (0.3)	82.2 (2.1)	83.2 (1.4)	96.7 (1.6)	90.5 (3.6)	89.2 (2.0)	94.4 (1.1)	91.0 (11.1)
Endosulfan sulphate	104.4 (3.9)	88.2 (5.2)	108.2 (5.5)	88 (3.4)	93.3 (2.0)	89.7 (0.6)	93.1 (2.0)	104.1 (3.1)	115.9 (3.8)	95.1 (1.5)	97.3 (2.8)	150.6 (0.5)
Endrin	109.8 (5.3)	93.8 (7.2)	112.8 (11.2)	80.8 (4.9)	82.6 (2.3)	78.0 (4.9)	81.4 (0.7)	90.2 (3.2)	89.3 (3.0)	83.4 (0.5)	89.5 (91.6)	96.1 (1.5)
Fenitrothion	137.0 (1.9)	101.6 (13.1)	134.6 (7.5)	102.5 (6.0)	93.5 (1.8)	102.5 (3.2)	98.8 (0.2)	111.7 (6.6)	118.8 (3.4)	99.4 (2.9)	110.2 (3.9)	166.0 (2.9)
Lindane	113.4 (2.9)	105.6 (1.3)	117.2 (2.5)	89.7 (1.9)	95.3 (1.6)	88.8 (0.7)	91.8 (0.4)	99.8 (2.6)	99.0 (1.6)	91.0 (0.3)	97.7 (1.0)	104.5 (10.4)
Malathion	138.8 (0.6)	119.2 (4.0)	119.4 (5.1)	100.4 (5.4)	96.1 (0.9)	99.2 (2.4)	90.9 (2.0)	104.7 (4.2)	109.2 (2.4)	83.3 (3.3)	99.2 (1.1)	142.8 (1.5)
Parathion-ethyl	122.8 (3.6)	88.4 (13.0)	141.9 (0.3)	98.4 (3.4)	82.2 (2.4)	96.8 (5.2)	93.4 (2.3)	106.6 (6.6)	103.6 (2.5)	91.1 (3.4)	103.6 (6.1)	169.6 (1.0)
Permethrin-cis	119.0 (11.9)	82.6 (11.2)	117.7 (9.2)	75.1 (5.7)	75.9 (3.5)	80.6 (8.5)	71.0 (2.1)	79.7 (1.0)	77.1 (5.3)	70.5 (0.7)	85.1 (0.8)	104.7 (4.4)
Permethrin-trans	101.6 (3.4)	95.6 (8.6)	114.9 (2.8)	78.2 (2.9)	87.4 (5.9)	80.8 (4.8)	75.0 (0.5)	86.3 (3.1)	81.6 (2.7)	75.7 (0.6)	86.3 (1.1)	97.2 (7.0)
Pirimifos-methyl	134.2 (4.4)	93.4 (3.0)	126.2 (4.0)	97.3 (5.7)	93.8 (2.6)	93.9 (8.4)	89.2 (2.8)	98.1 (4.0)	98.8 (5.2)	89.8 (2.0)	93.9 (2.2)	112.2 (7.0)
Tetradifon	106.0 (3.3)	101.8 (0.7)	100.6 (0.3)	85.4 (3.1)	85.7 (1.2)	81.7 (0.1)	84.9 (1.2)	93.1 (2.8)	83.7 (3.2)	87.5 (0.3)	92.9 (0.8)	99.9 (5.2)

Table 4
Pesticide levels (mg kg^{-1}) found in real samples.

Pesticides	MRL[a] (mg kg^{-1})		Wheat A	Wheat B	White flour A	White flour B	Bran A
	Brazil	EU					
Bifenthrin	0.6	0.5	0.02	<LOD	0.03	0.03	0.11
Chlorpyrifos-ethyl	0.2	0.05	n.d.	n.d.	n.d.	<LOQ	0.01
Chlorpyrifos-methyl	–	3.0	n.d.	n.d.	n.d.	0.01	0.04
Cypermethrin	–	2.0	<LOQ	0.01	0.01	0.01	0.02
Dicofol	–	0.5	0.01	n.d.	<LOQ	n.d.	n.d.
Fenitrothion	1.0	0.5	<LOQ	<LOQ	0.01	0.07	0.47
Malathion	8.0	8.0	n.d.	<LOQ	n.d.	<LOQ	0.01
Pirimifos-methyl	10.0	5.0	0.16	0.01	0.20	3.57	0.28

[a] Brazilian and European Union MRL values for wheat; n.d.: not detected.

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