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Determination of Methiocarb and Its Degradation Products, Methiocarb Sulfoxide and Methiocarb Sulfone, in Bananas Using QuEChERS Extraction

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ABSTRACT: The present work describes the development of an analytical method for the determination of methiocarb and its degradation products (methiocarb sulfoxide and methiocarb sulfone) in banana samples, using the QuEChERS (quick, easy, cheap, effective, rugged, and safe) procedure followed by liquid chromatography coupled to photodiode array detector (LC-PAD). Calibration curves were linear in the range of 0.5−10 mg L^{-1} for all compounds studied. The average recoveries, measured at 0.1 mg kg^{-1} wet weight, were 92.0 (RSD = 1.8%, n = 3), 84.0 (RSD = 3.9%, n = 3), and 95.2% (RSD = 1.9%, n = 3) for methiocarb sulfoxide, methiocarb sulfone, and methiocarb, respectively. Banana samples treated with methiocarb were collected from an experimental field. The developed method was applied to the analysis of 24 samples (peel and pulp) and to 5 banana pulp samples. Generally, the highest levels were found for methiocarb sulfoxide and methiocarb. Methiocarb sulfone levels were below the limit of quantification, except in one sample (not detected).

KEYWORDS: *pesticides, methiocarb, degradation products, bananas, QuEChERS*

INTRODUCTION

Fruits and vegetables provide biologically active substances as well as nutrients including vitamins, minerals, and digestible and nondigestible carbohydrates. However, they can also be a source of toxic substances, such as pesticides.[1] To ensure the safety of food and regulate international trade, legislation, such as the European Union (EU) directives, lay down maximum residue limits (MRLs) for pesticides in foodstuffs.[2]

Bananas are the main fruit in international trade and one of the most consumed fruits around the world, having high nutritional and energetic values,[3] and are often the first solid food given to infants. In terms of volume, banana is the first exported fruit. Banana production plays a very important role in economic, social, environmental, and political causes. According to Food and Agriculture Organization of the United Nations (FAO) statistics estimations, world total exports of banana accounted for 17.3 million tons in 2009.[4] India, China, The Philippines, Brazil, and Ecuador alone produced >60% of the total world banana production, and the four leading banana exporting countries in 2009 (Ecuador, Costa Rica, The Philippines, and Colombia) accounted for 64.4% of world exports, with Ecuador alone providing >32.9% of global banana exports.[4]

In Portugal, bananas are produced in Madeira and the Azores islands. Grown in a relatively warm and humid climate, the size of the fruit is smaller when compared with other countries, but the pulp is more consistent and tasty.

Snails attack a wide variety of agricultural and horticultural plants including bananas, various beans and peas, peanuts, cultivars of *Brassica* (e.g., broccoli, cabbage), carrot, various citrus species, lettuce, sweet potato, and tomato.[5] Pesticides belonging to different chemical classes are commonly applied. Carbamate pesticides are often used because they constitute a very versatile class of compounds that can act as insecticides, herbicides, fungicides, nematocides, acaracides, molluscicides, or sprout inhibitors[6] and replace the organochlorine and organophosphorous pesticides, due to their low environmental persistence and low toxic effects on mammalians.[7] However, carbamate pesticides are suspected carcinogens and mutagens because they are acetylcholinesterase inhibitors.[8] Thus, it is necessary to quantify their residue amounts in foods to prevent harmful effects on animals, humans, and the environment.[9]

Methiocarb (3,5-dimethyl-4-methylthiophenyl-*N*-methylcarbamate) is a carbamate pesticide that has been found useful in the control of snails[5,10,11] in a wide range of agricultural situations.[11] Because methiocarb converts to methiocarb sulfoxide (3,5-dimethyl-4-methylsulfinylphenyl-*N*-methylcarbamate) and methiocarb sulfone (3,5-dimethyl-4-methylsulfonylphenyl-*N*-methylcarbamate) (Figure 1), which have an equivalent toxicological profile to methiocarb,[12] the levels of all three compounds need to be evaluated in crop studies.[13]

Commission Regulation (EC) No. 901/2009 sets out a coordinated European Community program aimed at verifying compliance with MRLs for pesticide residues in and on food of plant and animal origin. The program also aims at assessing exposure of consumers to such pesticide residues. Member States shall, during the years 2010, 2011, and 2012 have to take

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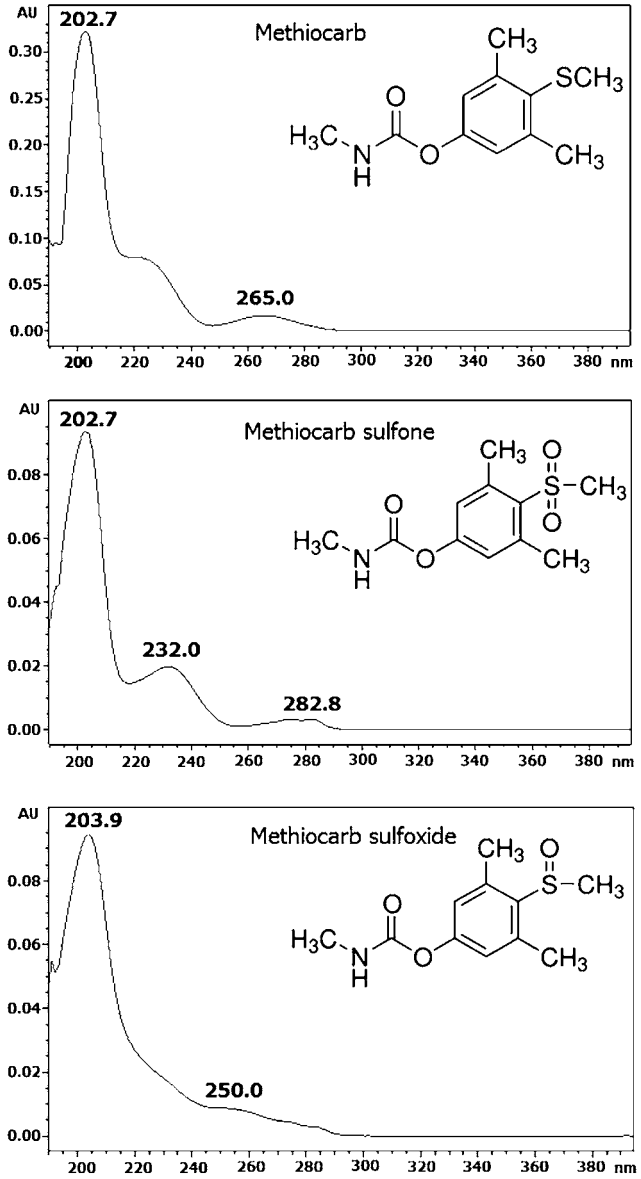

Figure 1. Structures and UV spectra of methiocarb, methiocarb sulfone, and methiocarb sulfoxide.

and analyze samples for the product and pesticide residue combinations. In 2012, methiocarb and the two degradation products should be analyzed on aubergines (eggplants), bananas, cauliflower, table grapes, orange juice, peas (fresh/frozen, without pod), peppers (sweet), and wheat.[13]

The QuEChERS (quick, easy, cheap, effective, rugged and safe) method is a sample preparation approach used for the extraction of pesticides in fruits and vegetables.[14] It has already received worldwide acceptance because of its simplicity and high throughput, enabling a laboratory to process a high number of samples in a short period of time.[15] The three primary QuEChERS methods were (i) the original, using acetonitrile (ACN) as extraction solvent and sodium chloride (NaCl) in QuEChERS content to enhance extraction and to reduce polar interferences;[14] (ii) dispersive AOAC 2007.01, which employs 1% acetic acid in ACN and anhydrous sodium acetate (CH_3COONa) buffer in QuEChERS content to protect base-sensitive analytes from degradation and provides superior recovery for pH-sensitive compounds;[16] and (iii) European method EN 15662, which also uses ACN as extraction solvent and includes NaCl to limit polar interferences and several buffering reagents with citrate salts to preserve base-sensitive analytes.[17]

Chromatographic techniques, mainly gas chromatography (GC) and high-performance liquid chromatography (LC), have usually been applied for the determination of pesticide residues in food samples. In recent years, LC has emerged as an excellent alternative technique, especially for the analyses of polar and thermolabile pesticides such as carbamates that are not readily amenable to GC or require derivatization before GC analysis.[2]

To our knowledge, four studies using QuEChERS as extraction methodology for the analysis of pesticides in bananas[15,18,19] and banana leaves[20] have been described. The first work referring to the application of the QuEChERS approach for pesticide extraction from banana samples was described by Wang et al.[18] In this study, a matrix solid-phase dispersion (MSPD) method using C18-bonded silica as dispersant sorbent and ethyl acetate as eluting solvent was proposed. The method compared favorably to the QuEChERS method based on the use of 6 g of anhydrous $MgSO_4$ and 1.5 g of NaCl, followed by cleanup with 50 mg of primary−secondary amine (PSA) and 150 mg of anhydrous $MgSO_4$. Fifteen fungicide residues were studied in apple, orange, banana, grape, lettuce, and tomato samples.

Hernández-Borges et al.[15] assessed the residues of 11 pesticides (10 of them organophosphorus pesticides) in 57 banana samples taken from the local markets and supermarkets of the Canary Islands (Spain). Analyses were carried out by the QuEChERS approach, using GC with nitrogen−phosphorus detection (NPD). Chlorpyrifos was detected in 50 samples (0.03−0.65 mg/kg), malathion in 5 samples (0.16−0.17 mg/kg), fenitrothion in 4 samples (0.02−0.10 mg/kg), and buprofezin in 1 sample (0.15 mg/kg). Because of the high occurrence of chlorpyrifos, its distribution between the pulp and the peel was also investigated. Results showed that most of the pesticide remained in the peel and that amounts between only 0.07 and 0.12 mg/kg occur in the pulp even at concentrations in the peel as high as 0.87 mg/kg.

Wang and Leung[19] developed and validated a QuEChERS method for the determination of 142 pesticides in fruit- and vegetable-based infant foods, including apples, apples and bananas, pears, bananas, apple juice, peas, sweet potatoes, creamed corn, squash, and carrots for use in the Canadian National Chemical Residues Monitoring Program. It is the only study that reports QuEChERS recovery tests for methiocarb and the two degradation products.

A group of eight insecticides (seven organophosphorus pesticides, ethoprophos, diazinon, chlorpyrifos-methyl, fenitrothion, malathion, chlorpyrifos and fenamiphos; and one thiadiazine, buprofezin) were analyzed by González-Curbelo et al.,[20] for the first time, in banana leaves that were currently being used to feed cattle and hogs. A modified version of the QuEChERS method was used. The developed method was also applied to investigate pesticide occurrence in different banana leaf samples collected in different cultivars of the Canary Islands to ensure their safe consumption by animals. Residues of chlorpyrifos were found in 10 of 12 treated banana leaf samples.

The main purpose of present study was to optimize the QuEChERS extraction of methiocarb and its degradation products from banana samples. Samples treated with methiocarb were collected from an experimental field and

were not intended for human consumption. The levels of methiocarb and its degradation products in pulp and in the pulp and peel (whole) of banana were also investigated.

MATERIALS AND METHODS

Reagents, Solvents, and Materials. ACN and methanol (MeOH) of HPLC grade were obtained from Merck (Darmstadt, Germany), and glacial acetic acid (purity ≥ 99.7%) was obtained from Carlo Erba (Rodano, Italy). Methiocarb, methiocarb sulfoxide, and methiocarb sulfone were obtained from Sigma-Aldrich (Steinheim, Germany).

Purified water (18.2 MΩ cm) (Millipore, Molsheim, France) was used to prepare mobile phases in liquid chromatography.

Stock standard solutions of individual compounds (methiocarb, methiocarb sulfoxide, and methiocarb sulfone) were prepared by dissolving 10 mg of the powder of each compound in 10 mL of MeOH and stored at −20 °C. Working standard solutions containing the three compounds were prepared daily from the individual stock standard solutions, using MeOH as solvent, and kept at 4 °C prior to analysis. Amber glassware was used to prevent light degradation of the compound, because methiocarb is unstable in light.[11]

All standard solutions and sample extracts were filtered through a 0.20 μm PTFE syringe filter (Teknokroma, Barcelona, Spain) and homogenized using a VWR vortex mixer (Radnor, DE, USA). All chromatographic solvents were filtered through a 0.20 μm nylon membrane filter (Supelco, Bellefonte, PA, USA) using a vacuum pump (Dinko D-95, Barcelona, Spain) and degassed for 15 min in an ultrasonic bath Sonorex Digital 10P (Bandelin Electronic, Berlin, Germany).

Three different types of QuEChERS were tested. Their corresponding content in the 50 mL Teflon centrifuge tube and the manufacturers were the following: QuEChERS 1 (4 g of $MgSO_4$ and 1 g of NaCl) and QuEChERS 2 (6 g of $MgSO_4$ and 1.5 g of CH_3COONa), obtained from Unit Chemical Technologies (UCT), Inc. (Bristol, PA, USA); QuEChERS 3 (4 g of $MgSO_4$, 1 g of NaCl, 1 g of sodium citrate dehydrate (NaCit), and 0.50 g of sodium citrate sesquihydrate (Na_2Cit)), obtained from Agilent Technologies (Santa Clara, CA, USA).

Sample Collection. For each banana sample, a total mass of 1 kg was collected.[21] Each sample was divided into two parts, one containing entire bananas (peel and pulp) and the other, only the pulp (the peel was removed). All samples were chopped and homogenized, and subsamples of 50 g were collected in plastic flasks and kept at −20 °C until analysis.

Liquid Chromatography. Extracts and working standard solutions were analyzed in triplicate using a Waters 2795 Alliance HT system (Milford, MA, USA) equipped with an automatic injection valve and a Waters 2996 photodiode array detector (PAD). Separation of the analytes was performed at 25 °C in a C18 column (Waters Spherisorb ODS2, 250 × 4.6 mm; 5 μm particle size). The injected volume was 20 μL.

A linear gradient (MeOH (A)−purified water (B)) starting from 35% of A programmed to 45% in 10 min with a hold of 2 min and raised to 85% of A in 8 min with a hold of 3 min was used. Initial conditions were reached in 2 min and maintained for 5 min before the next run. Total time of analysis was 30 min. The flow rate was 1.0 mL min^{-1}, and chromatograms were analyzed at 202 nm (Figure 1). Empower software (Shimadzu Corp., Kyoto, Japan) was used for control and data processing.

Extraction Procedure. An aliquot of homogenized banana sample was placed into a 50 mL centrifuge Teflon tube (QuEChERS) with a screw cap, which keeps the tube closed for most of the process of sample preparation, thus avoiding losses in some stages.

For recovery studies, samples were fortified by addition of the working standard solution at 0.1 mg kg^{-1} wet weight.[22] For blanks, pure MeOH was added. A gentle stream of nitrogen was placed on the top of the QuEChERS tube to ensure that all MeOH present in the fortified and in the blank tests evaporated and that only the analytes remained in contact with the sample.

Three different QuEChERS contents were tested in whole banana samples (pulp and peel). The packet with the QuEChERS content was taped on a flat surface and shaken to homogenize the salts that are at the bottom, and it was opened by tearing straight across at the precut slit. The packet was slowly poured over the sample.

The influence of pH was evaluated using ACN without pH adjustment and with 1% of acetic acid. Maximum speed in vortex device was needed for good homogenization during a tested time. After centrifugation in a 2.16 Sartorius centrifuge (Sigma, Goettingen, Germany), for 10 min at 3000 rpm, an aliquot (8 mL) was transferred to a dark vial, evaporated with a gentle stream of nitrogen to dryness, and redissolved with 300 μL of MeOH. The vial was shaken vigorously in the vortex and filtered through a 0.20 μm syringe filter. The extract was placed into an insert inside a 1.5 mL vial in the autosampler for LC-PAD analysis.

Method Validation. To ensure that a new analytical method generates reliable and interpretable information, it must undergo a validation process.[23] Once the best conditions for the analysis of methiocarb, methiocarb sulfoxide and methiocarb sulfone were defined, the validation of the method was carried out according to parameters described below.

The analytical curves and linearity of the detector response for methiocarb, methiocarb sulfoxide, and methiocarb sulfone were evaluated by injecting five calibration working standard solutions at concentration levels in the range 0.5−10 mg L^{-1} (calibration curve 1) and three replicate injections per concentration. Similarly, two additional analytical curves were built. Matrix-matched calibrations of i) blank matrix extract of banana pulp sample fortified at concentration levels between 0.5 and 10 mg L^{-1} (calibration curve 2), and ii) blank matrix extract of whole banana (peel and pulp) sample fortified at concentration levels between 0.5 and 10 mg L^{-1} (calibration curve 3), respectively.

Limits of detection (LOD) and quantification (LOQ) were calculated, respectively, as 3 and 10 times the standard deviation (SD) estimated for each regression equation ($S_{Y/X}$) dividing by the slope of the calibration equation for each compound.[24]

The precision, in terms of repeatability, and the accuracy of the analytical method, in terms of recovery, were obtained by carrying out the extraction and analysis of fortified samples (0.1 mg kg^{-1}) in triplicate. Each extract was injected three times.

Matrix effect (ME) can lead to a significant increase or decrease in the response of an analyte in a sample compared to a pure standard solution. Therefore, the evaluation of ME is required as part of quantitative method development.[25] One way to evaluate the ME is through the comparison of the slopes obtained in the calibration with matrix matched-standards with those obtained with calibration with standards diluted in the solvent.[26]

RESULTS AND DISCUSSION

Chromatographic Analysis. For detection and quantification of methiocarb and its degradation products, the programmed chromatographic conditions were optimized to separate the analytes in the shortest possible analysis time, to avoid peak tailing and to obtain good resolution, peak shape, and reproducibility. Different mobile phases comprising several combinations of MeOH and purified water were tested using isocratic and gradient elution. Under the described optimized experimental conditions, the retention times were 10.31 (RSD = 0.45%, n = 15), 11.03 (RSD = 0.43%, n = 15), and 21.31 min (RSD = 0.08%, n = 15) for methiocarb sulfoxide, methiocarb sulfone, and methiocarb, respectively.

Extraction Procedure. Several extraction parameters were optimized, namely, ratio of mass of sample per volume of extraction solvent, QuEChERS content, influence of pH in the extraction solvent (ACN), extraction time, and extraction process.

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Table 1. Calibration Curves, LOD, LOQ and Assessment of the Matrix Effect for Methiocarb Sulfoxide, Methiocarb Sulfone, and Methiocarb in Methanol, Whole Banana, and Banana Pulp

compound	matrix	regression eq[a]	$S_{Y/X}$	LOD (mg kg^{-1})	LOQ (mg kg^{-1})	ME[b]
methiocarb sulfoxide (A)	MeOH	(1) $y = 138869x - 34884$	1787	0.003	0.010	
	banana pulp	(2) $y = 139528x + 3456$	2404	0.004	0.013	99.5
	banana peel and pulp	(3) $y = 137196x + 35027$	2422	0.004	0.013	101.2
methiocarb sulfone (B)	MeOH	(1) $y = 114047x - 42091$	1584	0.003	0.010	
	banana pulp	(2) $y = 114407x - 28935$	2035	0.004	0.013	99.7
	banana peel and pulp	(3) $y = 114315x + 21822$	2229	0.004	0.015	99.8
methiocarb (C)	MeOH	(1) $y = 173313x - 20717$	2256	0.003	0.010	
	banana pulp	(2) $y = 172980x + 56389$	2579	0.003	0.011	100.2
	banana peel and pulp	(3) $y = 171825x + 82626$	3368	0.004	0.014	100.9

[a]Linear range 0.5−10 mg L^{-1}; y is the area and x the concentration (mg L^{-1}). [b]ME, matrix effect evaluated as ((slope (1))/(slope (2) or slope (3))) × 100.

Since the development and publication of the method in 2003 by Anastassiades et al.,[14] QuEChERS has been gaining significant popularity and provides not only high recoveries but also good reproducibility. Therefore, for QuEChERS optimization only one extraction for each test was performed.

In a previous work using QuEChERS extraction,[27] an optimized extraction time has been set at 3 min. In our preliminary studies 3 min was used. Subsequently, the extraction time was also studied.

Ratio of Mass of Sample per Volume of Solvent. Four ratios of sample per volume of extraction solvent were evaluated, namely, 1 (15 g per 15 mL and 10 g per 10 mL), 0.50 (5 g per 10 mL), 0.20 (2.0 g per 10 mL), and 0.17 (2.5 g per 15 mL), respectively. In order to obtain a good homogenization between the sample, the QuEChERS content and the extraction solvent, it was concluded that 5 g of sample are required for 10 mL of solvent (ratio 0.5). The use of 5 g of sample was a modification, since the original QuEChERS method [4] employs 10 g per 10 mL (ratio 1).

Fortified samples (0.1 mg kg^{-1} in each analyte) were prepared by adding 100 μL of working standard solution (5 mg L^{-1} in each analyte) to a portion of 5.0 ± 0.1 g of sample.

QuEChERS Content and Extraction Solvent. QuEChERS contents 1, 2, and 3 were studied using ACN or 1% acetic acid in ACN as extraction solvents. None of the compounds could be detected using QuEChERS 1 and ACN (original QuEChERS method [14]), and with 1% acetic acid in ACN only methiocarb sulfoxide was detected with a low recovery (37.7%, n=1).

Using QuEChERS 2, only methiocarb was detected and the recovery was similar with or without pH adjustment (30.1% and 29.8%, respectively, n = 1).

The recoveries for methiocarb sulfoxide, methiocarb sulfone, and methiocarb using QuEChERS 3 were: 92.0 (RSD = 1.8%, n = 3), 84.0 (RSD = 3.9%, n = 3), and 95.2% (RSD = 1.9%, n = 3) without pH adjustment in ACN and 86.56 (n = 1), 65.10 (n = 1), and 94.67% (n = 1) using 1% acetic acid in ACN, respectively. Higher results for methiocarb sulfone were obtained using ACN without pH adjustment. Therefore, QuEChERS 3 and ACN were used as QuEChERS content and extraction solvent, respectively.

The recoveries obtained are in agreement with the ones presented in the literature for the same analytes in other types of samples and using different extraction methods. Wang and Leung[19] reported recoveries for methiocarb sulfoxide, methiocarb sulfone, and methiocarb using QuEChERS extraction for fruit- and vegetable-based infant foods of 91.0, 92.8, and 99.5% (fruit based) and 95.1, 91.4, and 98.9% (vegetable based), respectively. Blasco et al.[28] obtained a recovery of 77% for methiocarb in peaches. The samples were extracted with ethyl acetate and anhydrous sodium sulfate. González-Rodríguez et al.[29] reported for methiocarb recoveries of 98% in Swiss chard, 85% in spinach, and 81% in lettuce. The chopped vegetable samples (10 g) were extracted in an ultrasound bath, for 10 min with 30 mL of ACN. Afterward, NaCl (3 g) and $MgSO_4$ (12 g) were added followed by vigorous shaking for 5 min. Hiemstra and de Kok[30] reported recoveries for methiocarb sulfoxide, methiocarb sulfone, and methiocarb, respectively, of 88, 81, and 88% for lettuce; 97, 91, and 97% for orange; 94, 96, and 103% for apples; 97, 90, and 91% for cabbage; 99, 94, and 99% for grapes; and 94, 97, and 97% for wheat flour. The samples were extracted with acetone (30 mL) with a probe blender for 20 s. Then, dichloromethane (30 mL) and light petroleum (30 mL) were added, and the sample extracts were partitioned using the probe blender for another 20 s.

Extraction Time. The influence of extraction time was evaluated for 1 (time used in the original QuEChERS extraction[4]), 2, 3, 4, and 5 min using QuEChERS 3 and ACN. Recoveries improved 28.4, 31.8, and 16.6% for methiocarb sulfoxide, methiocarb sulfone, and methiocarb, respectively, when the extraction time was increased from 1 to 2 min, being constant thereafter. QuEChERS extraction using the vortex device for homogenization was subsequently carried out for 2 min.

QuEChERS Procedure. The order of QuEChERS content addition to the sample was also studied. Two tests were performed using QuEChERS 3. QuEChERS powder, sample (banana peel and pulp), and ACN was the order of addition for the first test and sample (banana peel and pulp), QuEChERS powder and ACN for the second test. At the end of the extraction in the first test, we observed that a portion of QuEChERS powder remained at the bottom of the Teflon tube. The sample was poured over the QuEChERS powder and did not allow a good mixture inside the Teflon tube.

Recovery results in the second test, when compared with the first, were between 16 and 22% higher (for the three analytes) due to a good homogenization between sample, QuEChERS powder, and ACN. This order of addition to the QuEChERS tube was important to define the final procedure for the QuEChERS extraction of the analytes in banana samples.

Table 2. Pesticide Concentrations (± SD, mg kg^{-1}) Determined in Whole Banana Samples (Pulp and Peel) (n = 2)

sample	methiocarb sulfoxide	methiocarb sulfone	methiocarb	$\Sigma_{methiocarb}$ (A + B + C)[a]
1	<LOQ	<LOQ	<LOQ	<LOQ
2	0.062 ± 0.001	<LOQ	0.033 ± 0.007	0.095 ± 0.008
3	0.056 ± 0.004	<LOQ	0.078 ± 0.009	0.134 ± 0.013 (>**MRL**)
4	0.047 ± 0.005	<LOQ	0.079 ± 0.001	0.126 ± 0.006 (>**MRL**)
5	nd[b]	<LOQ	0.060 ± 0.003	0.060 ± 0.003
6	0.050 ± 0.013	<LOQ	0.040 ± 0.013	0.090 ± 0.026
7	0.118 ± 0.012	<LOQ	0.065 ± 0.009	0.183 ± 0.021 (>**MRL**)
8	0.014 ± 0.001	<LOQ	<LOQ	0.014 ± 0.001
9	<LOQ	<LOQ	0.020 ± 0.002	0.020 ± 0.002
10	0.016 ± 0.001	<LOQ	0.049 ± 0.002	0.065 ± 0.003
11	<LOQ	<LOQ	0.023 ± 0.002	0.023 ± 0.002
12	0.023 ± 0.002	<LOQ	0.021 ± 0.001	0.044 ± 0.003
13	0.023 ± 0.001	<LOQ	0.050 ± 0.002	0.073 ± 0.003
14	0.018 ± 0.002	<LOQ	<LOQ	0.018 ± 0.002
15	0.029 ± 0.003	<LOQ	0.029 ± 0.001	0.058 ± 0.004
16	0.035 ± 0.003	<LOQ	0.035 ± 0.002	0.070 ± 0.005
17	0.023 ± 0.002	<LOQ	<LOQ	0.023 ± 0.002
18	0.015 ± 0.001	<LOQ	0.014 ± 0.001	0.029 ± 0.002
19	0.022 ± 0.002	<LOQ	0.056 ± 0.002	0.078 ± 0.004
20	0.091 ± 0.008	<LOQ	0.062 ± 0.007	0.153 ± 0.015 (>**MRL**)
21	nd	<LOQ	0.026 ± 0.001	0.026 ± 0.001
22	0.016 ± 0.001	<LOQ	0.024 ± 0.001	0.040 ± 0.002
23	<LOQ	nd	0.017 ± 0.001	0.017 ± 0.001
24	0.024 ± 0.001	<LOQ	<LOQ	0.024 ± 0.001

[a]A, methiocarb sulfoxide; B, methiocarb sulfone; C, methiocarb. [b]nd, not detected.

Table 3. Levels (mg kg^{-1}) of Methiocarb and Its Degradation Products in Banana Samples (Peel and Pulp vs Pulp Only) (n = 2)

	whole banana (peel and pulp)				banana pulp				
sample	methiocarb sulfoxide	methiocarb sulfone	methiocarb	$\Sigma_{methiocarb}$ (A + B + C)[a]	methiocarb sulfoxide	methiocarb sulfone	methiocarb	$\Sigma_{methiocarb}$ (A + B + C)[a]	ratio
2	0.062	<LOQ	0.033	0.095	0.022	<LOQ	0.019	0.041	2.32
3	0.056	<LOQ	0.078	0.134	0.035	<LOQ	0.023	0.058	2.31
4	0.047	<LOQ	0.079	0.126	0.036	<LOQ	0.021	0.057	2.21
7	0.118	<LOQ	0.065	0.183	0.032	<LOQ	0.061	0.093	1.97
20	0.091	<LOQ	0.062	0.153	0.051	<LOQ	0.017	0.068	2.25

[a]A, methiocarb sulfoxide; B, methiocarb sulfone; C, methiocarb.

Validation of the Method. Linearity, sensitivity, recovery, precision, and matrix effect were considered as the criteria for the validation of the developed analytical methodology.

Detailed analytical quality assurance data are shown in Table 1. Calibration curves generated using linear regression analysis and over the established concentration range (0.5−10 mg L^{-1}) gave good fits (determination coefficient (R) > 0.999). With regard to sensitivity, LOD and LOQ were 0.003 and 0.010 mg kg^{-1}, respectively, for calibration curves prepared in MeOH, ranging from 0.003 to 0.004 mg kg^{-1} and from 0.011 to 0.013 mg kg^{-1} for calibration curves prepared in banana pulp extract and were 0.004 mg kg^{-1} and ranged from 0.013 to 0.014 mg kg^{-1} for calibration curves prepared in whole banana (peel and pulp) extract. To ensure correct quantification, precision of the method was studied by analyzing five replicates of a 1 mg L^{-1} standard solution. Results for methiocarb sulfoxide, methiocarb sulfone, and methiocarb showed precisions of 0.10, 0.90, and 0.55% for intraday and 1.83, 1.77, and 1.44% for interday analyses, respectively.

In this work, the ME were evaluated by preparing calibration curves in whole banana (peel and pulp) and in banana pulp extracts and comparing the slopes with the one achieved for the standards prepared in MeOH. Calibration curves obtained in spiked banana pulp and in whole banana (peel and pulp) extracts and in solvent (MeOH) were similar; therefore, no matrix effect was observed (Table 1).

Application to Banana Sample Analysis. The extraction method was applied for the analysis of 24 banana samples (peel and pulp). The results are shown in Table 2. Generally, the highest levels were found for methiocarb sulfoxide and methiocarb. Methiocarb sulfone levels were below the LOQ, except in one sample (nd). Methiocarb sulfoxide was not detected in two samples and was <LOQ in four samples. Methiocarb was <LOQ in five samples.

Methiocarb sulfoxide and methiocarb were present simultaneously in 58.3% of the samples. The levels of each analyte in each of these samples were between 24.6 and 65.3% for methiocarb sulfoxide (46.6% on average) and between 34.7 and 75.4% for methiocarb (53.4% on average). Methiocarb

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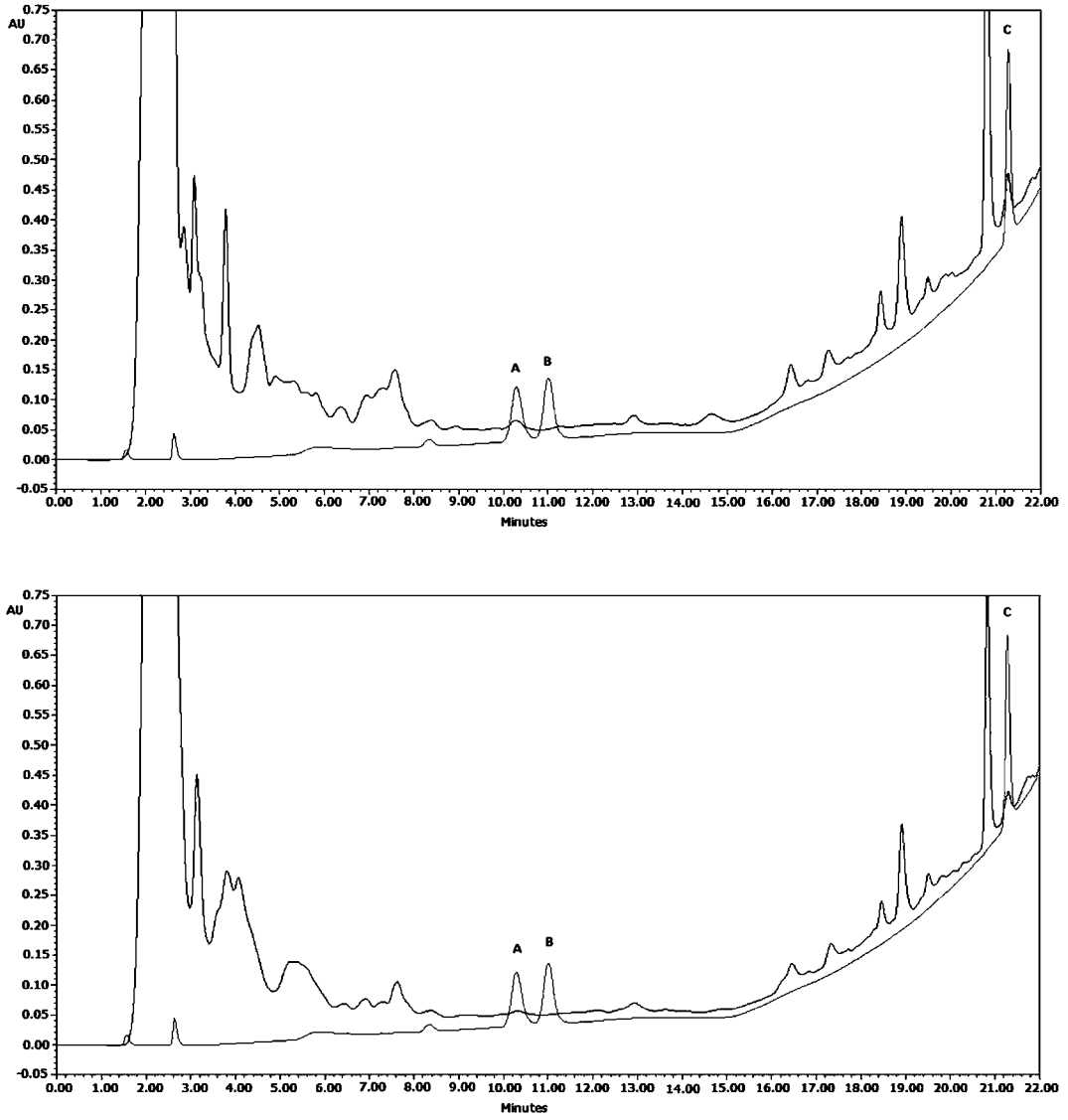

Figure 2. Overlay LC-PAD chromatograms of a standard mixture and (1) whole banana and (2) banana pulp extracts (sample 7).

sulfoxide was the only compound quantified in 16.7% of the samples and 20.8% for methiocarb.

Four samples presented methiocarb concentrations ($\Sigma_{methiocarb}$ = methiocarb sulfoxide + methiocarb sulfone + methiocarb) in whole banana (peel and pulp) higher than the value allowed by Commission Regulation (EC) No. 901/2009, that is, 0.1 mg kg^{-1},[22] in the range of 0.126−0.183 mg kg^{-1}.

Pesticide Levels in Banana Samples (Peel and Pulp vs Pulp Only). The concentrations of methiocarb sulfoxide, methiocarb sulfone, and methiocarb in whole banana and in banana pulp were studied. The analysis of the pulp was performed in the five samples with the highest methiocarb concentration ($\Sigma_{methiocarb}$) in whole banana (peel and pulp) (Table 3). Representative chromatograms obtained for sample 7 are illustrated in Figure 2.

In the pulp, methiocarb concentrations ($\Sigma_{methiocarb}$) were between 0.041 and 0.093 mg kg^{-1}. The ratio for the concentration in whole banana and banana pulp was in the range from 1.97 (sample 7) to 2.32 (sample 2), with an average value of 2.21. With regard to the analysis of banana pulp, none of the five samples exceeded the MRL.

The present study comprises the optimization and validation of an analytical methodology for the determination of methiocarb and its degradation products, methiocarb sulfoxide, and methiocarb sulfone, in banana samples based on the QuEChERS approach.

Methiocarb concentrations are reported in whole banana (peel and pulp) for a set of 24 samples and in banana pulp for 5 samples. All samples were treated with methiocarb in an experimental field. The results revealed that the methiocarb concentration ($\Sigma_{methiocarb}$) is about double (average ratio of 2.21) when whole bananas were analyzed compared with banana pulp. Methiocarb sulfoxide and methiocarb were the main compounds found, whereas methiocarb sulfone levels were below the LOQ in all samples except one (nd).

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Notes

The authors declare no competing financial interest.

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