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Determination of Carbofuran and 3-Hydroxycarbofuran Residues in Coconut Water by Solid-Phase Extraction and Liquid Chromatography with UV Detection

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Determination of Carbofuran and 3-Hydroxycarbofuran Residues in Coconut Water by Solid-Phase Extraction and Liquid Chromatography with UV Detection

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Abstract: A simple method was developed to determine carbofuran and 3-hydroxycarbofuran in coconut water. The procedure involved solid-phase extraction using C₁₈ cartridges with acetonitrile for elution. The analysis of these compounds was carried out by liquid chromatography with UV detection at 275 nm using a gradient solvent system. The method was validated with fortified samples at different concentration levels (0.01–2.5 µg/mL). Average recoveries ranged from 81 to 95% with relative standard deviation between 1.6 and 12.5%. Each recovery analysis was repeated at least five times. Detection limits ranged from 0.008 to 0.01 µg/mL. The analytical procedure was applied to coconut water samples from palms submitted to treatment with commercial formulation under field conditions.

Keywords: Coconut water, Carbofuran, 3-Hydroxycarbofuran, Pesticides

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INTRODUCTION

The coconut fruit (*Cocos nucifera* L.) is subject to attack by a number of phytophagous insects such as *Rhinostomus barbirotris*, *Rhynchophorus palmarum*, *Homalinotus coriaceus*, *Amerrhinus ynca*, and *Brassolis sophorae*.^[1] Among the different products used for the pest control, the insecticide carbofuran, belonging to the chemical class of the *N*-methylcarbamates, is commonly employed in the coconut culture. However, carbofuran is speedily metabolized in plants, via the carbofuran conversion to 3-hydroxycarbofuran,^[2] which can translocate it into the coconut fruit and contaminate the coconut water. This fact becomes a serious problem, once the coconut water has been consumed *in natura* as a refreshing drink by the population.

Some methods for determining carbofuran in water, fruit, and vegetable matrices have been described in the literature. They include liquid–liquid extraction (LLE), solid-phase extraction (SPE), and solid-phase microextraction (SPME) procedures. Analyses have been carried out by liquid chromatography with UV diode array or mass spectrometric detection and gas chromatography with mass spectrometric detection.^[3–7] On the other hand, studies on determination of pesticide residues in coconut water have not been published, except in our previous work.^[8]

The present work reports on a simple methodology for simultaneous determination of carbofuran and 3-hydroxycarbofuran in coconut water by solid-phase extraction and liquid chromatography, using ultraviolet detection (HPLC/UV) and confirmation by gas chromatography with mass spectrometric detection (GC-MS).

EXPERIMENTAL

Chemicals and Materials

Acetonitrile, methanol, dichloromethane, and ethyl acetate (Mallinckrodt Baker Inc., Paris, KY, USA) was nanograde. HPLC grade water was obtained by filtering deionized water through a 0.45 μm filter with a Waters Millipore (Milford, MA, USA) system. Acetonitrile and water were degassed using a Branson 5200 (Branson Ultrasonic Corporation, Danbury, CT, USA) ultrasonic bath.

Reference standards of carbofuran and 3-hydroxycarbofuran were purchased from Dr. Ehrenstorfer (Augsburg, Germany). The standards were at least 95% pure. The individual stock solutions of the analytes were prepared by diluting 1.0 mg of the standards in 10.0 mL of acetonitrile to obtain a concentration of 100 $\mu\text{g}/\text{mL}$. The working standard solutions were prepared by diluting the stock solutions as required.

Anhydrous sodium sulfate was analytical grade reagent (Mallinckrodt Baker Inc., Paris, KY, USA).

Sep-Pak Vac C₁₈ cartridges were purchased from Waters (Milford, MA, USA). These cartridges were of 3 mL capacity packed with 500 mg of solid phase. The vacuum manifold from Supelco (Bellefonte, PA, USA) was used to perform the SPE.

HPLC/UV Analysis

A Waters high performance liquid chromatograph (Waters Assoc., Milford, MA, USA) equipped with two solvent delivery pumps (Model 501), injector (Model U6K), UV-Vis absorbance detector (Model 486), and a reporting integrator (Model 746) was used for the determination of carbofuran and 3-hydroxycarbofuran. A stainless steel analytical column LiChrospher 100 RP₁₈ (250 × 4.6 mm i.d., 5 μm; Merck, Darmstadt, Germany) connected to a 100 RP₁₈ guard column (20 × 4.6 mm i.d., 5 μm; Merck, Darmstadt, Germany) was used. Carbofuran and 3-hydroxycarbofuran were analyzed by a linear binary gradient consisting of acetonitrile-water: 20:80 (0 min) up to 50:50 (20 min) and equilibrated at initial conditions for 5 min, at a flow rate of 1 mL/min using UV absorption at 275 nm.

GC-MS Analysis

A Varian system consisting of a Saturn ion trap mass spectrometer equipped with a GC-3800 gas chromatograph with a split/splitless injector, was used for the quantification and confirmation of the pesticides studied. A fused silica column DB-1 (100% polydimethylsiloxane; 30 m × 0.25 mm i.d., 0.25 μm), supplied by J&W Scientific (Folsom, CA, USA), was employed with helium (purity 99.999%) as carrier gas, at flow rate of 1.8 mL/min. The column temperature was programmed as follows: 80°C for 1 min, increasing to 240°C at 10°C/min, and directly to 265°C at 10°C/min, and holding for 10 min. The solvent delay was 3 min. The injector port was maintained at 250°C and 1 μL volume was injected in splitless mode (0.7 min). The data were acquired and processed by the Varian software. The total analysis time was 10 min. The eluent from the GC column was transferred via a transfer line held at 280°C.

Field Trials

The trials were carried out in a coconut grove at the city of Aracaju (state of Sergipe, Brazil) owned by the Embrapa-Tabuleiros Costeiros, on the coconut (*Cocos nucifera* L.) cultivar. Treatments were carried out with a sprayer, with the following commercial product: Marshal 200 SC[®] (200 g/L of carbosulfan) at the dose of 50 mL/100 L of water.

Twenty coconut samples were collected from dried plants. The collection was done before the Marshal 200 SC[®] application and it was repeated 2, 5, and 120 h after the application. The coconut water was collected from five different coconut palms (one coconut fruit per palm). The coconut water was withdrawn from the coconut fruit, put into bottles, and frozen immediately.

Sample Preparation and Control Coconut Water Samples

Coconut water samples were filtered in Whatman 40 and 0.45 μm filters, respectively, before the solid phase extraction. For the recovery tests, coconut water control samples were used. Coconut water samples used for developing and validating the proposed method were bought from local market in the city of Aracaju, state of Sergipe, Brazil.

For the validation method, fortified samples were prepared by adding standard solutions (500 μL) to 100.0 mL of coconut water, followed by homogenization on a mechanical shaker.

Method

A Sep-Pak Vac C₁₈ cartridge was preconditioned with 1.0 mL of acetonitrile followed by 2.5 mL of water. It was placed on top of a vacuum block. An analytical aliquot of 100 mL coconut water was transferred to the cartridge at a flow rate of ca. 0.5 mL/min and the solid phase allowed to dry for 15 min. The pesticides were eluted with 5.0 mL of acetonitrile and concentrated to 1 mL with nitrogen stream. The 20 μL aliquot of eluate was analyzed by HPLC/UV.

RESULTS AND DISCUSSION

Study of the Analytical Variables

A previous method^[8] was optimized for the determination of carbofuran and 3-hydroxycarbofuran in coconut water by HPLC-UV. Liquid chromatographic analyses of carbofuran and 3-hydroxycarbofuran were performed on octadecyl reversed-phase. It was found, that acetonitrile-water gradient elution as a mobile phase, at 1.0 mL/min and the detection at 275 nm, was shown to be the best condition with respect to the analysis of these compounds. Figures 1 and 2 show chromatograms of the control sample of coconut water and coconut water sample fortified with standard solutions of carbofuran and 3-hydroxycarbofuran at 4.0 $\mu\text{g}/\text{mL}$, respectively. There are few peaks in the chromatogram of the control sample, but retention times of these endogenous compounds were not matched with that of the compound

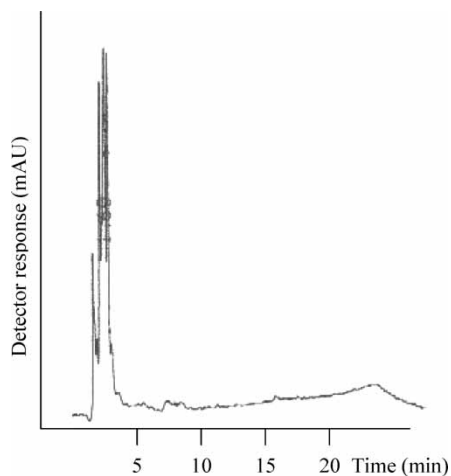


Figure 1. HPLC/UV chromatogram of coconut water control. For chromatographic conditions, see text.

peaks; so, they did not interfere with the determination of the carbofuran and 3-hydroxycarbofuran. Their identification was realized by the retention times obtained when the standard solution of these pesticides was injected into the liquid chromatograph. Under these chromatographic conditions, the retention times for 3-hydroxycarbofuran and carbofuran were 8.6 min and 16.2 min, respectively. The total running time of HPLC-UV analysis was 25 min. The

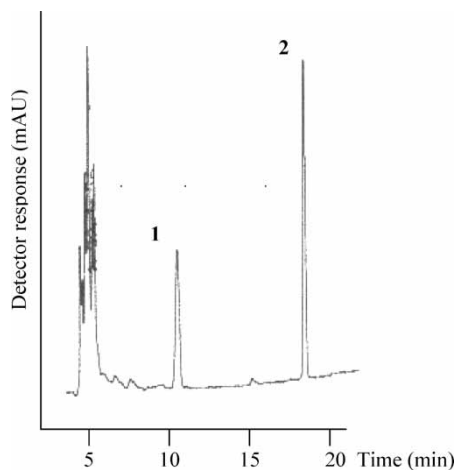


Figure 2. HPLC/UV chromatogram of spiked extract of untreated coconut water: 3-hydroxycarbofuran at 4.0 $\mu\text{g}/\text{mL}$ (1) and carbofuran at 4.0 $\mu\text{g}/\text{mL}$ (2). For chromatographic conditions, see text.

Sep-Pak Vac C₁₈ cartridges were satisfactory for the extraction of the pesticides in the coconut water samples. The acetonitrile was selected for the experiments because it provided higher or acceptable recoveries for the two compounds, in comparison with other solvents such as methanol, dichloromethane, and ethyl acetate. During the optimization of the solid phase extraction procedure to determine the elution volume of acetonitrile (2.0, 3.0, or 5.0 mL), lower recoveries for carbofuran (30%) and 3-hydroxycarbofuran (40%) were obtained when the elution was performed with 2.0 or 3.0 mL of acetonitrile. This problem was solved using 5.0 mL. To determine extraction efficiency, untreated coconut water samples were fortified at four levels, 0.01, 0.05, 0.50, and 2.5 µg/mL; the mean recoveries of fortified coconut water samples ranged from 81% to 95% with RSD values of 1.6% to 12.5% (Table 1). Each recovery analysis was repeated 5–7 times. Standard solutions were injected after every ten samples to monitor changes in chromatographic conditions. The Brazilian legislation^[9] establishes the maximum residue limits (MRL) for carbosulfan for the coconut matrix. The MRL value is 0.01 mg/Kg. This value should be the sum of carbofuran and 3-hydroxycarbofuran to accommodate residues obtained resulting from the use of carbosulfan or carbofuran. The precision and accuracy were considered adequate for the validation of the method according to the validation criteria.^[10] Under the chromatographic conditions described, good linearity and determination coefficients were achieved for the compounds studied. Replicates (n = 3) of the standard pesticide solutions of different concentrations were analysed, and the detector response (peak area) was plotted against concentrations. The correlations were found to be linear in the range from 0.01 to 5.00 µg/mL. The determination coefficients obtained for the pesticides were higher than 0.997. The criteria established by Thier and Zeumer^[11] to find LOD and LOQ was used in this study. The LOD for carbofuran and 3-hydroxycarbofuran were 0.008 µg/mL, which were calculated considering the sensitivity of the method and the standard deviation values obtained from recovery experiments with the lowest fortification level and the blank analysis. The LOQ was determined as the lowest concentration of

Table 1. Recovery of pesticides from fortified coconut water by HPLC/UV

Matrix	Spiked level (µg/mL)	% Range of Recovery ^a (%mean; %RSD)	
		Carbofuran	3-Hydroxy carbofuran
Coconut water	0.01	95–98 (92; 1.6)	78–95 (87; 9.5)
	0.05	80–98 (94; 10.7)	82–100 (95; 12.5)
	0.50	78–83 (81; 8.6)	80–85 (82; 4.8)
	2.50	70–86 (80; 8.8)	79–92 (89; 8.9)

^an = 5–7 replicates.

the compounds that gives a response that could be quantified with a RSD of less than 20% and a recovery at least 70%. Thus, the LOQ values for these compounds were between 0.01 $\mu\text{g}/\text{mL}$. On the other hand, the results obtained from coconut water corroborate the fact that the parent carbosulfan is too lipophilic ($K_{ow} = 10^{5.5}$) to be translocated within plants and, hence, its systemic action is attributable to their metabolites. The lipophilicity of carbofuran ($K_{ow} = 10^{1.5}$) is close to the optimum value required for systemicity in the transpiration stream of the plants.^[2]

Field Treatment

The proposed methodology was applied to a preliminary decay study of carbosulfan in coconut fruit. This decay study was carried out under field conditions. The coconut palms were sprayed with an aqueous suspension of Marshal 200 SC[®] (dose: 50 mL/100 L of water). Samples were taken in duplicate at different times: 2, 5, and 120 h after the treatment. Carbofuran residues were not detected in the 2, 5, and 120 h. The 3-hydroxycarbofuran was detected in all of the coconut water samples until 120 h after the treatment. Table 2 shows the results obtained from this experiment. The amounts of 3-hydroxycarbofuran obtained were corrected with the following factor to convert to the amounts of carbosulfan: [mol. wt. of carbosulfan (380.5)/mol. wt. of 3-hydroxycarbofuran (238.3) = 1.59]. A typical HPLC/UV chromatogram of real coconut water samples obtained from field trials collected after Marshal 200 SC[®] application (120 h), is demonstrated in Figure 3. Residues of 3-hydroxycarbofuran were confirmed by the use of GC-MS.

Table 2. Residues of carbofuran and 3-hydroxycarbofuran in coconut water after Marshal 200 SC[®] application in coconut palms from field trials

Pesticide	Time after application (h)	Residues ($\mu\text{g}/\text{mL}$) ^a
		Coconut water
Carbofuran	2	nd
	5	nd
	120	nd
3-Hydroxycarbofuran	2	0.15
	5	0.12
	120	0.03

nd = Not detected.

^aResidue values are the means of duplicate analysis from five replicates.

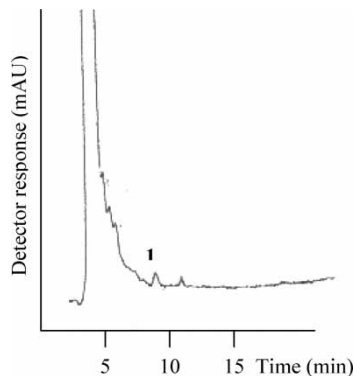


Figure 3. HPLC/UV chromatogram of real coconut water sample from coconut palms after Marshal 200 SC[®] application (120 h): 3-hydroxycarbofuran (1) at 0.03 µg/mL. For conditions, see text.

CONCLUSIONS

The proposed method allows a simple and rapid determination of carbofuran and 3-hydroxycarbofuran residues, the degradation product of carbofuran, in coconut water. The method yields mean recoveries that ranged between 81–95%. The limit of quantification was in good agreement with MRL established by Brazilian legislation, which is indicative that the used method is valid for determination of residual levels of carbofuran and 3-hydroxycarbofuran derivatives in this matrix. Further investigation should also be performed to better assess the behaviour of these compounds after scheduled treatments.

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