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Comparison of solid-phase extraction and solid-phase microextraction for carbofuran in water analyzed by high-performance liquid chromatography–photodiode-array detection

M.C. López-Blanco, B. Cancho-Grande, J. Simal-Gándara*

Nutrition and Bromatology Group, Analytical and Food Chemistry Department, Faculty of Food Science and Technology, University of Vigo, Ourense Campus, 32004 Ourense, Spain

Abstract

In this study a direct solid-phase microextraction (SPME) procedure has been developed for the determination of carbofuran in water. Experimental parameters such as selection of SPME coating, effect of temperature, effect of salt addition and solvent desorption were studied and optimized. Analytical parameters such as linearity, precision, detection and quantitation limits, and matrix effects for solid-phase extraction (SPE) and SPME methods were evaluated for comparison purposes with the aim of selecting the most appropriate depending on the detection capabilities required. SPE and SPME were followed by high-performance liquid chromatography with diode-array detection, using a 50×4.6 mm I.D. guard column and a 150×4.6 mm I.D. analytical column, both packed with C_{18} silica. Both methods can be applied to real samples and give the same results, but SPE allows the detection of lower carbofuran concentrations (0.06 μg/L) as compared to SPME (8.9 μg/L).

Keywords: Extraction methods; Water analysis; Carbofuran

1. Introduction

Carbofuran is used extensively in agriculture as an insecticide to control a broad spectrum of insects on potatoes, corn, rice, alfalfa, grapes and other foodstuffs. Its implications as a potential endocrine disrupter have been demonstrated [1]. Carbofuran is highly mobile in soils and appears in waters because of its high solubility [2,3]. It has a high potential for groundwater contamination of aquifers via leaching from treated fields, and also from surface waters which enter as a result of runoff from treated fields.

The current US Environmental Protection Agency (EPA) maximum contaminant level (MCL) for carbofuran in drinking water is 40 μg/L. However, the current California MCL is 18 μg/L and the Office of Environmental Health Hazard Protection Agency (OEHHA) of the California Environmental Protection Agency proposes a public health goal of 1.7 μg/L. The European Union has set a maximum admissible concentration of 0.5 μg/L for the sum of all pesticides and 0.1 μg/L for an individual compound in drinking water [4].

The aim of this study was to develop methods based on solid-phase extraction (SPE) and microextraction (SPME) followed by HPLC–photodiode-array detection (DAD) for the determination of carbofuran in water. No commercial interface is used for coupling SPME with HPLC equipment and high reproducibility was achieved. Five commercially

*Corresponding author. Tel.: +34-988-387-000; fax: +34-988-387-001.

E-mail address: jsimal@uvigo.es (J. Simal-Gándara).

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available SPME fibers were considered. The experimental parameters that affect the adsorption and desorption processes were evaluated. Once the SPME–HPLC–DAD procedure had been optimized, quality parameters such as linearity, precision, and limits of detection and quantification were determined and compared with those obtained by the solid-phase extraction (SPE)–HPLC–DAD method in order to select the most suitable depending on the detection capabilities required.

2. Experimental

2.1. Chemicals, disposables and materials

Carbofuran (98%) was purchased from Dr. Ehrenstorfer's Laboratory (Germany). Other reagents used were methanol and acetonitrile of analytical grade from Merck (Darmstadt, Germany); ammonium acetate and sodium chloride ACS-ISO for analysis were from Panreac (Spain). Ultrapure water was from a Milli-Ro Waters purification system (Milford, MA, USA).

Waters Sep-Pak C_{18} Plus cartridges were used as SPE minicolumns for purification and concentration. A Visiprep SPE vacuum manifold (Supelco, Bellefonte, PA, USA) was used to simultaneously process up to 24 SPE tubes. A Visidry drying attachment (Supelco) was used to dry up to 24 SPE tubes at one time, and can be used with any inert gas supply. It is also useful for evaporating and concentrating recovered samples. Nitrogen C-50 of analytical quality was supplied by Carburos Metálicos (Spain). SPE extracts were placed in 2-mL vials (Supelco). Homogenization of SPE extracts was achieved by vortex agitation (Heidolph Reax Top, Germany). Other small apparatus, such as an ultrasonic bath (Selecta, Spain), were used.

Five different coated fibers, 7-μm poly(dimethylsiloxane) (7-PDMS), 100-μm poly(dimethylsiloxane) (100-PDMS), 85-μm polyacrylate (PA), 50-μm Carbowax-template resin (CW–TPR) and 60-μm poly(dimethylsiloxane)–divinylbenzene (PDMS–DVB), were used. The commercially available SPME device and fibers were purchased from Supelco. Fibers were initially conditioned prior to use in methanol at room temperature for 1 h. For SPME, water samples were placed in 40-mL EPA vials (Wheaton, USA) equipped with stir bars and sealed with a PTFE-faced silicone septa; SPME desorbed extracts were placed in 150-μL inserts inside the 2-mL vials prior to chromatographic analysis.

2.2. Standard solutions

A stock standard solution (1000 mg/L) of carbofuran was prepared in methanol by exactly weighing about 0.01 g of analyte into a 10-mL volumetric flask and diluting to volume. A secondary standard solution (10 mg/L) was prepared by dilution in water of the primary standard solution. Stock and secondary solutions were stored at 0–4 °C in the dark. Ultrapure water solutions were prepared by spiking with different volumes of the secondary standard solution and were used for calibration.

2.3. Extraction procedure

2.3.1. Solid-phase extraction (SPE)

The 360 mg C_{18} Sep-Pak cartridge was previously conditioned with 5 mL of methanol followed by 10 mL of ultrapure water without allowing the cartridge to dry out. The aqueous sample (1 L) was passed through the cartridge at a rate of 4 mL/min. The cartridge was dried by blowing N_2 for 10 min. Adsorbed carbofuran was eluted by 3 mL of methanol. This methanolic eluate was evaporated to 0.4 mL under a gentle stream of nitrogen and diluted to 1 mL with ultrapure water. Homogenization of the final extract was achieved with vortex agitation.

Calibration was performed by direct injection into the HPLC column of appropriate standard solutions.

2.3.2. Direct solid-phase microextraction (SPME)

For direct SPME, water samples (40 mL) were placed in a 40-mL glass sample vial. To each sample solution, 9 g of sodium chloride was added before SPME sampling. The vial was sealed with a PTFE-faced septum cap. PDMS–DVB fiber was introduced directly into the aqueous sample. During extraction

the sample was stirred at room temperature (22 °C) for 30 min. The extracted analytes were immediately transferred to a glass vial with a 150-μL insert containing methanol (40%)–0.1 *M* ammonium acetate aqueous solution (60%), where the desorption process was produced at 70 °C for 15 min.

Calibration was performed by direct injection into the HPLC column of desorbed extracts obtained from the SPME fiber used in the analysis of standard spiked waters.

2.4. Analytical instrumentation and operating conditions

The analysis was performed with a Thermo HPLC system equipped with an AS1000 autosampler, a P2000 binary pump and a UV6000LP diode-array detector linked to a personal computer running the software programme ChromQuest, version 2.51 (ThermoQuest, Italy).

The analytical column (150×4.6 mm I.D.) used was a Waters Symmetry 5 μm C_{18}. The guard column (50×4.6 mm I.D.) was packed with dry 40 μm Pelliguard LC-18 (Supelco). For HPLC analysis, an aliquot (100 μL) was injected into the column and eluted at room temperature, with a constant flow-rate of 1.5 mL under the following isocratic conditions: methanol (40%) and ammonium acetate 0.1 *M* aqueous solution (60%). Detection was carried out at wavelengths between 200 and 380 nm, and quantification was performed at 278 nm.

3. Results and discussion

3.1. SPE method characterization

The linearity of the method was determined by regressing the areas vs. concentrations of the pesticide aqueous standards submitted to the SPE procedure (Table 1). Analysis of blank ultrapure water did not give any response at the retention time of carbofuran after applying the SPME method. The linearity test developed by Wells and coworkers [5] was applied.

The recovery and repeatability of carbofuran from water were measured at a level of 1 μg/L by the analysis of five samples (1 L) of fortified ultrapure water. These samples were quantified with carbofuran standard solutions injected directly into the HPLC column (Table 1). The absolute recovery value for the whole calibration range was obtained from the ratio between the slope of the line corresponding to the spiked waters ($y = 295\,650x + 95\,215$) and the slope of the line for direct injection into the analytical column ($y = 291\,550x + 136\,902$); the ratio shows an absolute recovery of 101.4% for the calibration range 0.1–50 μg/L (SPE concentration factor 1000). Since carbofuran was recovered quantitatively with SPE, calibration and quantification is easily performed by regressing the carbofuran area vs. concentration of the pesticide aqueous standards injected directly into the column (0.1–50 mg/L).

The reproducibility of the SPE method was as-

Table 1
Repeatability, reproducibility, linear dynamic ranges, determination coefficients (r^2) and limits of detection (LOD) and quantification (LOQ) of the SPE and SPME techniques followed by HPLC–DAD

Extraction technique	Recovery (%) ±repeatability[a] (RSD, %)	Reproducibility[b] (RSD, %)	Linearity[c] range	Determination coefficient (r^2)	LOD[a] (μg/L)	LOQ[a] (μg/L)
SPE	90 (absolute recovery at 1 μg/L), ±3.2	7.0	0.1–50 μg/L, method calibration	0.999	0.06	0.08
			0.1–50 mg/L, direct calibration	0.999		
SPME	100 (relative recovery at 10 μg/L), ±7.7	5.1	10–50 μg/L	0.980	8.9	10.0

[a] $n = 5$.
[b] $n = 9$.
[c] $n = 10$.

sessed by analyzing a total of nine water samples spiked at a concentration of 1 μg/L on three different days. Limits of detection (LOD) and quantitation (LOQ) were evaluated on the basis of the signal obtained by the analysis of unfortified water samples ($n=7$). LOD and LOQ (Fig. 1a) were defined as the concentrations of the analyte that produced signal-to-noise ratios of 3 and 5, respectively.

The optimized method was applied for the determination of carbofuran by spiking samples of ultrapure water and samples of groundwater at 1 μg/L. Duplicate samples of ultrapure and real waters were analyzed. Standard deviations and mean values obtained were compared using, respectively, the Fischer *F*-test (95% probability) and the Student two-tailed *t*-test (95% probability) [6]. No significant differences between the matrices were obtained. This method can then be applied to real groundwater samples.

3.2. SPME method characterization

3.2.1. Optimization

Several parameters related to the extraction and desorption processes were evaluated.

3.2.1.1. Selection of SPME coating

Five SPME fiber coatings were evaluated to select the most suitable for the direct SPME–HPLC–DAD determination of carbofuran. The performance of commercially available 7-PDMS, 100-PDMS, PA, CW–TPR and PDMS–DVB was studied. The analysis of each fiber prior to re-exposure confirmed that carbofuran was completely removed and the mobile phase was a good desorption solvent. The most suitable coating was PDMS–DVB (Table 2). The porous polar polymer (divinylbenzene) modifies the selectivity towards polar compounds, improving the extraction efficiency with respect to PDMS fibers. PDMS–DVB fiber can be used repeatedly for 60 extraction cycles, obtaining reproducible and consistent results.

3.2.1.2. Effect of temperature

The effect of temperature on the adsorption process was evaluated with aqueous samples spiked at 10 mg/L which were extracted at 22, 45 and 60 °C by holding the sample in a water bath. It was observed that carbofuran extraction decreased with temperature. This can be explained because the solubility of carbofuran in water increases with temperature [7]. Further experiments were performed at room temperature.

3.2.1.3. Effect of salt addition

The effect of the addition of salt was also evaluated with an aqueous sample spiked at 10 mg/L carbofuran and salted with NaCl (1–9 g). Experiments were performed in duplicate. Salt increases the ionic strength of the aqueous sample and enhances the extraction from water because it reduces analyte solubility [8]. Greater DAD areas were registered when larger amounts of NaCl were added. Further experiments were performed by the addition of 9 g of NaCl.

3.2.1.4. Sorption and desorption time profiles

Duplicate ultrapure water samples (spiked at 10 mg/L) were analyzed. The extraction time profile was obtained by plotting the DAD response vs. the extraction times evaluated (Fig. 2a). The extraction time profile shows that carbofuran reaches a maximum extraction yield in 30 min. The desorption time is also an important parameter to ensure that carbofuran is completely desorbed from the fiber to attain the highest sensitivity and to avoid carryover. Fig. 2b shows that 15 min is enough to guarantee total desorption.

3.2.2. Validation

The linearity of the method was evaluated by regressing the carbofuran peak areas vs. the analyte concentration using standard fortified ultrapure water samples after applying the SPME method to all standards. The linearity test gave a linear concentration range between 10 and 50 μg/L. Since samples and fortified blank samples were analyzed using the same procedure, extraction yields are compensated and the relative recovery was about 100%.

The repeatability and reproducibility of the SPME–HPLC–DAD method were assessed by analyzing five spiked ultrapure samples (10 μg/L) on the same day and a total of three samples per day for two weeks, respectively. The results are reported

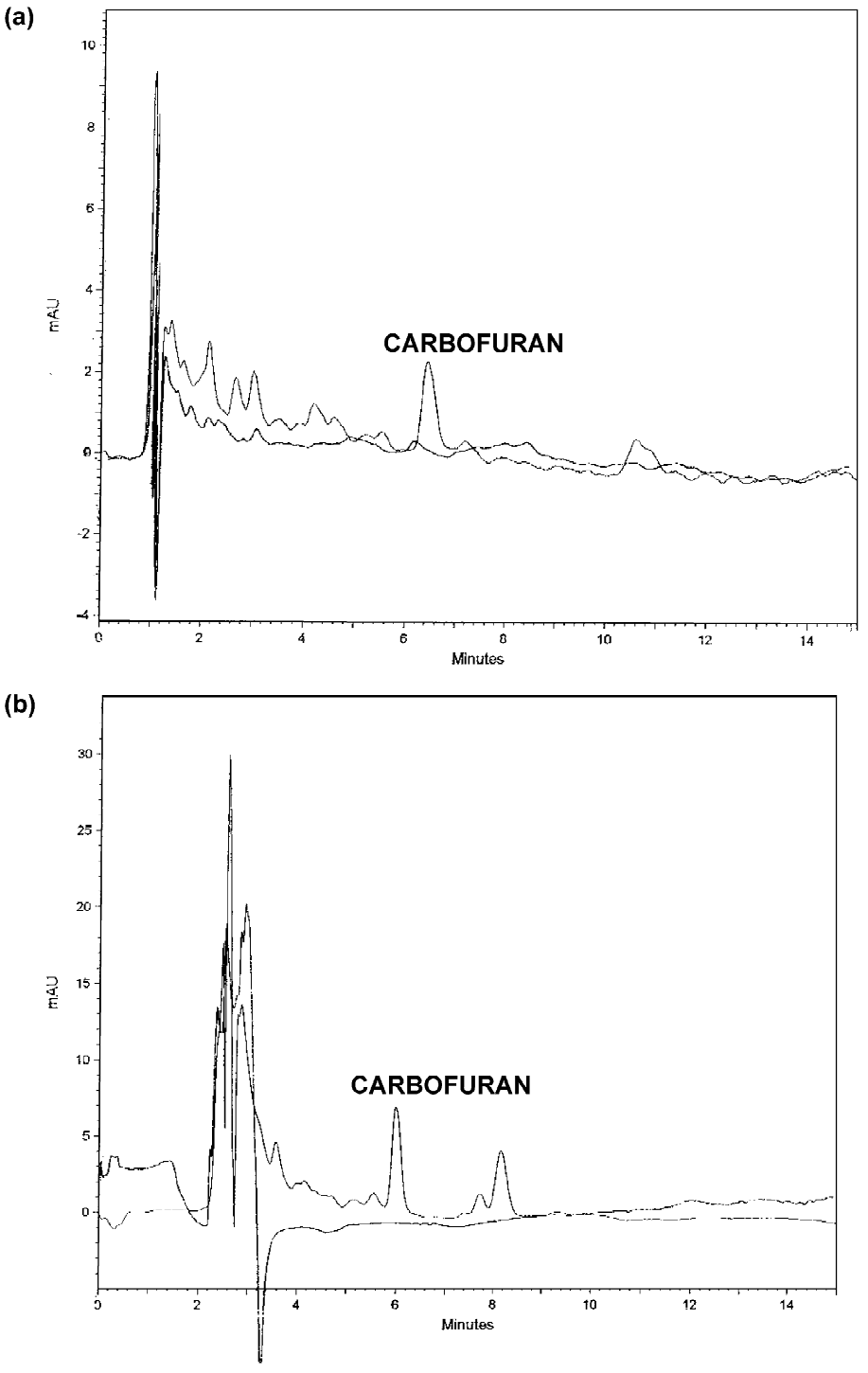

Fig. 1. HPLC chromatograms of blank and spiked water samples at LOQ levels analyzed by (a) SPE–HPLC–DAD and (b) SPME–HPLC–DAD.

Table 2
Extraction efficiency of different SPME fiber coatings for sampling water spiked with carbofuran at 10 mg/L by direct SPME followed by HPLC–DAD

Fiber type	DAD response (area counts)
PDMS–DVB	451 496
CW–TPR	73 100
100-PDMS	23 072
7-PDMS	6575
PA	5560

in Table 1, together with limits of detection and quantitation (Fig. 1b).

The optimized method was also applied for the determination of carbofuran by spiking samples of ultrapure water and samples of groundwater at 20 μg/L. Duplicate samples of ultrapure and real water were analyzed. Standard deviations and mean values obtained were compared using, respectively, the Fischer *F*-test (95% probability) and the Student two-tailed *t*-test (95% probability) [6]. No significant differences between matrices were obtained. This method can then be applied to real groundwater samples.

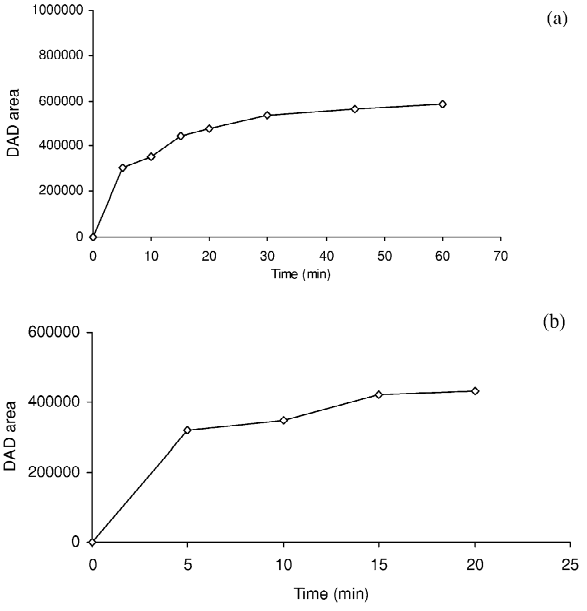

Fig. 2. Time profiles for carbofuran determination by SPME–HPLC–DAD: (a) extraction and (b) desorption profiles.

3.3. Comparison of direct SPME and SPE

Ultrapure water samples spiked at 25 μg/L were analyzed, in triplicate, by conventional SPE–HPLC–DAD and by optimized SPME–HPLC–DAD in order to compare experimental results. Quantification of both methods was performed using the calibration curve for each extraction technique. No significant differences were found between the results given by these two techniques.

SPME provides a precision comparable to SPE, with the added advantages of requiring no solvent and being more rapid because it eliminates the intensive manual labor of the SPE method. This technique can be applied to real samples, avoiding the problems of C_{18} cartridge obstruction observed for SPE. The limit of detection obtained with SPE is, however, lower (0.06 μg/L) with respect to that obtained with SPME (8.9 μg/L), although this latter value is similar to other detection values (between 1.0 and 15.0 μg/L) obtained for other carbamates with SPME followed by HPLC [9–11].

4. Conclusions

SPE and SPME combined with HPLC–DAD was found to be suitable for the determination of carbofuran in water samples. SPE allows the detection of concentrations greater than 0.06 μg/L, matrix effects do not interfere with the quantitation process, it can be automatized and the calibration can be performed using aqueous standards injected directly into the column, due to the absolute recovery of about 100%. For the SPME method, PDMS–DVB fiber is proposed for extracting this pesticide. The main advantages of this method are that it avoids the use of organic solvents and the intensive manipulation compared with the SPE method, and matrix effects do not interfere with the quantitation process. However, this method is less sensitive than the other method (8.9 μg/L). The proposed method allows the detection of carbofuran levels in drinking water established by the EPA.

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