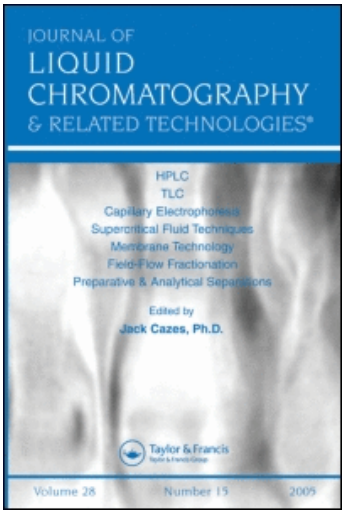

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Silvia H. G. Brondi[a]; José C. Rodrigues da Silva[b]; Fernando M. Lanças[b]
[a] Octávio Bastos Education Foundation, SP, Brazil [b] University of São Paulo, São Carlos, SP, Brazil

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Development and Validation of a Methodology for the Determination of Pesticides in Water by SPME–LC/DAD

Silvia H. G. Brondi
Octávio Bastos Education Foundation, São João da Boa, SP, Brazil

José C. Rodrigues da Silva and Fernando M. Lanças
University of São Paulo, São Carlos, SP, Brazil

Abstract: This study develops a method for solid-phase microextraction (SPME) coupled with high performance liquid chromatography with diode array detector (HPLC-DAD) of two pesticides widely used in sugar cane culture, tebuthiuron and diuron. The SPME-HPLC coupling was made by a home made interface. Parameters affecting the sorption and desorption of analyte, including sampling time, fiber type, stirring rate, pH, ionic strength, temperature, mobile phase composition, and desorption time were evaluated. The best conditions were obtained by a polyacrilate fiber, higher temperatures, sampling time of 50 minutes, and desorption time of 15 minutes.

Keywords: Pesticides, Water, Solid-phase microextraction, Liquid chromatography

INTRODUCTION

The most common methods to measure pesticides in drinking water involves either liquid-liquid extraction (LLE) or solid-phase extraction (SPE), followed by gas or liquid chromatography determination. Although LLE is a simple and appropriate separation technique in the analysis of residues, it presents certain disadvantages. LLE involves consumption of large volumes of high purity solvents, which can present a danger to health in addition to the high costs associated with their use.[1] SPE is applied in the pesticides analysis as it is an easy and fast extraction technique[2] and presents advantages such as

Address correspondence to Silvia H. G. Brondi, Octávio Bastos Education Foundation, 13870-159, São João da Boa, Vista, SP, Brazil. E-mail: shgb@uol.com.br

efficiency, economy, reproducibility, speed, safety, and selectivity. However, this technique involves several steps and requires a longer time of analysis.

During the last decade, the solid-phase microextraction technique (SPME) has been used as an alternative to LLE and SPE. This technique was developed and studied extensively by Pawliszyn and collaborators,[3–5] and has been applied in the extraction of organic compounds from different matrices, including air,[6] water,[7] and soil.[8]

According to Magdic and Pawliszyn,[9] SPME presents advantages over other conventional techniques as it spares the use of solvents, which are expensive and toxic and could be harmful to humans and the environment. This technique has been applied to the analyses of traces of organic pollutants at sub-ppt levels. It is considered an ideal technique in analyses of surface drinking water, thereby eliminating the use of organic solvents, simplifying the extraction, improving the precision, and saving time and money.[5]

SPME involves an apparatus composed of a microsyringe that contains a silica fiber coated with a known volume of a polymeric stationary phase that extracts and concentrates the analytes. The later is desorbed in the injector of a gas or liquid chromatograph and analyzed. The extraction of the sample by SPME can be carried out with the coated fiber immersed in the liquid sample, or in the headspace, where the extraction fiber is suspended above the sample, usually in a closed system.[10]

According to Chen & Pawliszyn,[11] a lot of organic compound classes used now are semi or not volatile, such as pharmaceutical products, drugs, proteins, and some pesticides and polycyclic aromatic hydrocarbons, and they are better separated by liquid chromatography. The main difference between SPME-GC and SPME-HPLC is the desorption process. In the analyses by GC, the fiber is introduced in the injector of the gas chromatography where the analytes are terminally desorbed from the fiber, while in the analyses by HPLC the thermal desorption may cause problems such as, degradation of the polymer and no adsorbed volatile compound remaining in the fiber, making the desorption with solvent the best alternative. To apply SPME in the analyses by liquid chromatography it is necessary to use an interface for desorption.

The present paper evaluates the quality of the surface drinking water related to the presence of pesticides used in sugar cane culture, tebuthiuron, and diuron, by employing the solid-phase microextraction technique and subsequent analyses of the extract in a liquid chromatograph using a home-made interface for the desorption of the analytes.

EXPERIMENTAL

Chemicals

All analytical standards, tebuthiuron, and diuron were obtained from Chem Service (West Chester, PA, USA) and were about 99% pure. The stock and

working solutions were prepared in HPLC grade acetonitrile obtained from Mallinckrodt (Phillipsbourg, NJ, USA), stored in a freezer at $-21^{\circ}C$, and kept stable for several months. The stock solutions were prepared in the concentration of 100 mg/mL, being later diluted to obtain different working solutions. The working solutions used in SPME were prepared daily, and the stock solution was diluted in water purified in a Milli-Q Plus System from Millipore (Bedford, MA, USA).

The real water samples came from the reservoirs of the rivers that supply the cities of Araraquara (Ribeirão das Cruzes, Ribeirão das Anhumas and Córrego do Paiól) and São Carlos (Ribeirão do Feijão), areas with sugar cane and orange cultures. Those cities are all located in the central region of São Paulo State, Brazil.

Extraction

Solid-Phase Microextraction (SPME) Procedure

SPME was carried out using a holder and a fiber 10 cm long, acquired from Supelco (Bellefonte, PA, USA), already coated with a fine layer of polydimethylsiloxane (100 μm thick) or polyacrylate (85 μm thick) fiber.

Water samples purified in a Milli-Q System (Millipore, Eschborn, Germany) were enriched with a mixture of the pesticides analytical standards, in the concentration of 0.1 mg/L for diuron and 0.5 mg/L for tebuthiuron. An aliquot of 5 mL of the sample was transferred to a conical flask sealed with septum, with a magnetic mini bar inside. The extraction was performed through the positioning of the submerged fiber in the sample. After the extraction, the SPME fiber was removed from the sample and immediately inserted in the liquid chromatography interface for desorption.

The development of an interface which allows the SPME-LC is necessary, because it is not possible to install the fiber directly in the liquid chromatography injector similar to the gas chromatography procedure. The interface for HPLC is simple and similar to a traditional loop injection, it consists of a desorption camera and a six port valve injection.[12] The development of an interface being commercially available was recently accomplished by Pawliszyn,[13] but it has high cost and, according to Jinno et al.,[14] it presents problems of enlargement of the picks.

Figure 1 displays the interface model built at Chemistry Institute for the pesticide analyses, applying the solid-phase microextraction (SPME) followed by HPLC.[15]

LC-DAD Analyses

Pesticides were analyzed by liquid chromatography from Shimadzu (Kyoto, Japan), equipped with diode array detector (DAD), 250 mm × 4 mm × 5 μm

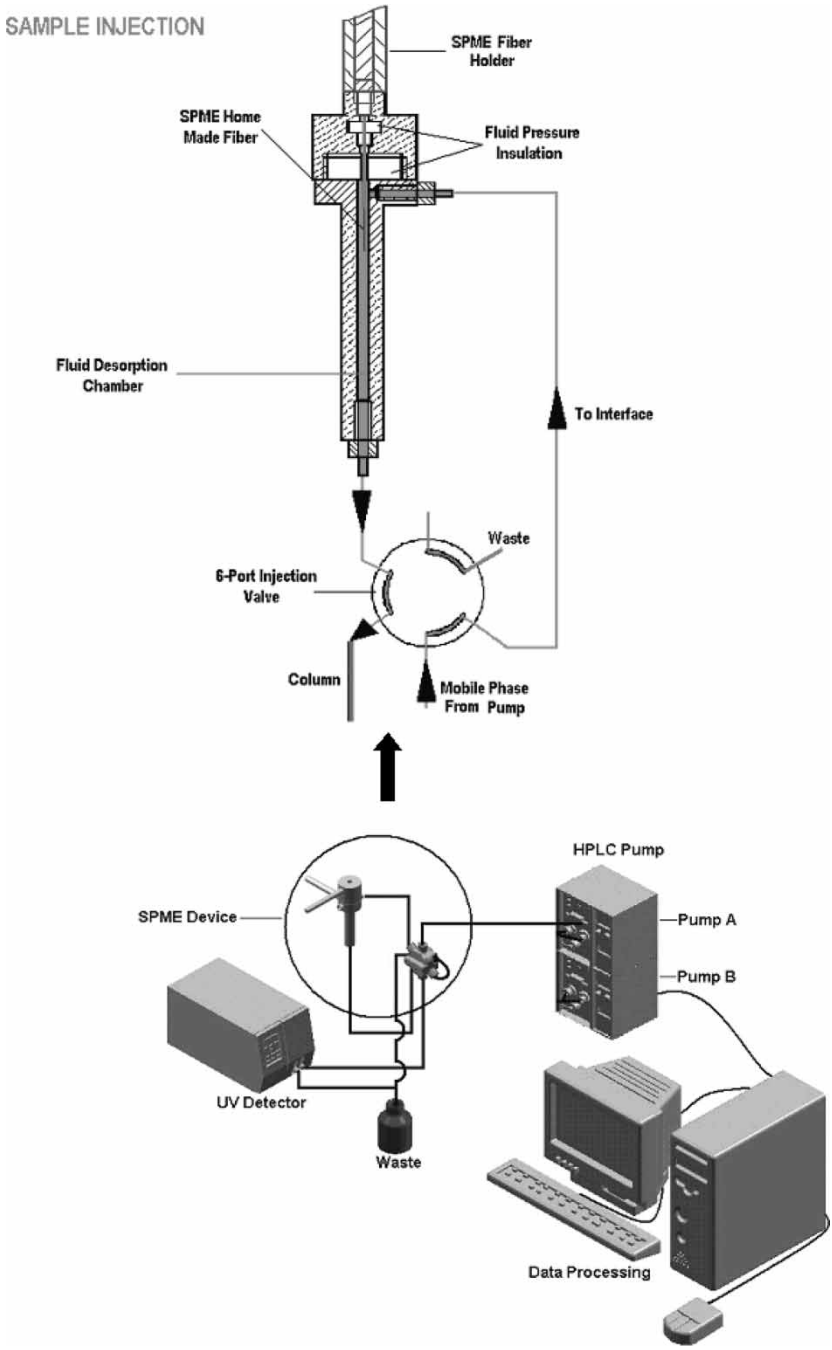

Figure 1. Home-made interface model through it is possible analyses SPME-LC.

RP-8 column acquired from Supelco (Bellefonte, PA), and using a 40°C oven temperature. The mobile phase was acetonitrile/water (3.1 : 6.9 v/v), at 1 mL/min, than detection at 254 nm.

Method Validation

To check the credibility of the data in the quantitative analyses, the analytical validation of the method should be performed. In the present paper some factors were considered in the validation of the present method, including relative standard deviation (RSD), correlation coefficient (r), linearity, limit of detection (LOD), and limit of quantification (LOQ).

RESULTS AND DISCUSSION

SPME consists of the adsorption of the analytes present in the aqueous matrix by the polymeric fiber, through its immersion in the sample and the transfer of the concentrated analytes to the chromatograph, where their desorption, separation, and quantification will occur.[16] Different parameters can affect the SPME process, including time of fiber exposure in the aqueous sample, choice of the type of fiber, stirring speed, pH, ionic strength, temperature of adsorption and desorption time. Figure 2 presents the chromatogram obtained by SPME for the compounds analyzed by HPLC, using a polyacrylate (PA) fiber, 50 minutes of extraction, temperature of 45°C, without addition of salt and stirring.

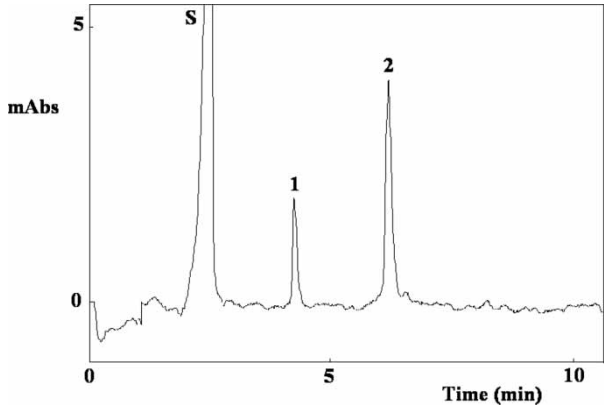

Figure 2. Typical HPLC-DAD chromatogram of a mixture containing tebuthiuron (1) and diuron (2) at 254 nm. Concentration: 0.1 μg/mL.

Sampling Time in the Aqueous Sample

The amount of analytes adsorbed increases with increasing the time of extraction to reach equilibrium. Compounds with larger molecular mass tend to present slower mass transport and require more time to reach equilibrium when compared to analytes of smaller molecular mass.

The time of adsorption was investigated in this study by monitoring the area values as a function of the exposure time of the fiber in the standard solution containing the analytes, in an interval from 20 to 55 minutes. Fifty minutes were the time of balance, as a longer period did not increase the area values.

According to Arthur and Pawliszyn[17] high sample concentrations present a smaller capacity of the fiber to adsorption/desorption of the analytes and the equilibrium might not happen.

Selection of the Fiber

SPME is an equilibrium process that involves the partition of the analytes between the liquid or gaseous sample with the polymeric phase, in agreement with the partition coefficient K.[18] The selection of an appropriate stationary phase is extremely important,[19] usually made by taking into account the polarity of the analytes. Polar compounds are extracted mainly for polar coatings and vice-versa.[20] The large majority of the study applying SPME was performed using polydimethylsiloxane (PDMS) and polyacrylate (PA) fibers. The polydimethylsiloxane fiber is used in the analysis of non-polar compounds and the polyacrylate fiber is utilized for moderately polar compounds.

The compounds were analyzed using a polydimethylsiloxane fiber (100 μm film thickness) and a polyacrylate fiber (85 μm film thickness), exposing the fiber 50 minutes to the aqueous sample. The best results were obtained with the polyacrylate fiber for the compounds studied.

Stirring Rate

According to Arthur et al.[3] the analyte accumulation in the fiber is controlled by its diffusion in both the matrix and the fiber. In the static case, the transport of the analyte from the aqueous solution to the fiber is limited, due to the aqueous layer that is formed on the surface of the fiber, limiting the adsorption speed. However, in the dynamic mode, under stirring, a fine aqueous layer forms on the surface of the polymer, facilitating the diffusion.

An intense agitation of the aqueous solution reduces the necessary time to reach the equilibrium between the aqueous sample and the fiber.[21] Using polyacrylate fiber, the highest values were obtained with maximum stirring.

pH

Since the initial pH of the solution (5.8) was slightly acid, an aliquot was adjusted with addition of hydroxide of sodium until becoming basic (pH = 8.5) and another acidified until reaching acid medium (pH = 3.5). The highest area values for the analytes studied using polyacrylate fiber were obtained with pH 5.8.

Ionic Strength

The addition of salt (usually sodium chloride or sodium sulfate) increases the ionic strength of the solution, making the non-polar organic compounds less soluble and increasing the partition coefficient several times.

The addition of salt and its effect on SPME was studied, since previous studies had shown that for some compounds, the increase in the ionic force increased their retention in the polymer,[3,10] or, decreased it.[19,22] According to Natangelo et al.,[23] the use of high concentrations of salt can cause an accumulation of salt particles on the surface of the fiber after analytes desorption in the chromatograph injector. For the studied compounds, there was an area increase related to 20% addition of salt.

Adsorption Temperature

The temperature of adsorption has an opposite effect when SPME is applied. Increasing the temperature of the aqueous solution, the diffusion coefficient of the analytes increases. On the other hand, since the adsorption is an exothermic process, the constant of distribution of the analytes decreases when the temperature rises.[24]

High temperatures might interfere in the results of the analysis; as the extraction is made with the submerged fiber in the aqueous matrix, the analytes may suffer degradation. In this study the temperature varied from 26°C (room temperature) to 50°C, where the area values began to decrease. The best results were obtained using the PA fiber at near 45°C.

Desorption Time

The desorption happens in the interface with the solvent passing through the fiber of SPME without occurring thermal desorption. The desorption process is extremely important in HPLC, and it needs to be optimized for each application with different compositions of solvents adjusted by the solubility of the analytes in the mobile phase.

The mobile phase used for the desorption of the analytes was acetonitrile/water (6.0 : 4.0 v/v). Three different desorption times were tested in this study (10, 15, 20 minutes), using polyacrylate fiber. Fifteen minutes were enough for the desorption of all the compounds and no memory effect was observed.

Validation: Linearity, Correlation Coefficients, Limit of Detection (LOD), Limit of Quantification (LOQ), and Relative Standard Deviation (RSD)

The external standard method was used to quantify the analytes through a calibration curve. The concentrations of each analyte, used in the acquisition of the calibration curve were 0.1, 0.05, 0.02, 0.01 mg/L for diuron and 0.5, 0.25, 0.1, 0.05 mg/L for tebuthiuron. The Linear Regression Method (LRM) was applied. The correlation coefficients (r) were all above 0.99, indicating that the detector answer is linear for the concentrations of the analytes under study, within the concentration range investigated.

The limit of detection (LOD) was calculated, multiplying by three the average value of the noise sampled at the retention time of each analyte,[25] and the limit of quantification (LOQ) was ten times the average value of the noise in this same region.[26]

After establishing the best analysis conditions for the studied compounds, using a polyacrylate fiber, time of extraction of 50 minutes and temperature of 45°C, the precision of the proposed methodology was calculated by the relative standard deviation (RSD).

According to GARP—Group of Analyst of Residues of Pesticides,[27] the acceptable values of variation coefficient are around 15%. On the other hand, the US-EPA[28] accepts values of variation coefficient up to 30%.

Table 1 presents the values corresponding correlation coefficient (r), limit of detection (LOD), limit of quantification (LOQ), and relative standard deviation (RSD) of the proposed methodology. The combination of SPME-LC/DAD allows the determination of the diuron and tebuthiuron in water

Table 1. Values of limit of detection (LOD), limit of quantification (LOQ), correlation coefficient (r) and relative standard deviation (RSD) for the studied compounds

Pesticides	LOD (μg/L)	LOQ (μg/L)	r	RSD (%)
Diuron	10	30	0.9955	1.9
Tebuthiuron	50	160	0.9999	4.7

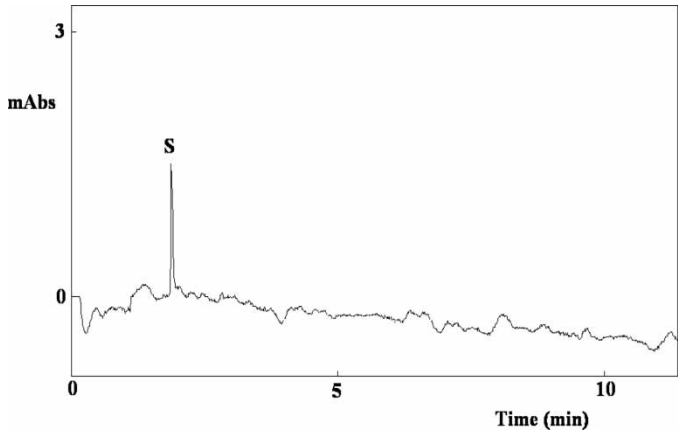

Figure 3. Chromatogram obtained by liquid chromatograph, diode array detector, after the solid-phase microextraction technique (SPME), of water sample from Ribeirão das Cruzes, in the winter season. $\lambda = 254$ nm. S = solvent (acetonitrile).

at the low detectable concentration, between 10–50 μg/L and the relative standard deviations in the range 1–5%.

Analysis of Water Samples

The water samples were collected at the dams of reception of rivers that supply the cities of Araraquara (Ribeirão das Cruzes, Ribeirão das Anhumas and Córrego do Paiól) and São Carlos (Ribeirão do Feijão), all located in the interior of São Paulo State, Brazil, in both summer (rainy) and winter (dry) seasons.

The samples were analyzed by solid-phase microextraction (SPME), followed by liquid chromatography (LC), with diode array detector (DAD). None of the compounds studied in the different monitored environments was registered (see Figure 3 for a representative example).

CONCLUSION

In this study SPME demonstrated to be an appropriate technique for the analysis of tebuthiuron and diuron pesticides in water, since they were detected at low concentrations, below 50 μg/L. The best results were obtained using polyacrylate fiber, 50 minutes of extraction, temperature of 45°C, without addition of salt and stirring. Another advantage of the technique is that it reduces the consumption of organic solvents that are associated with risks to the health and environment, including the costs with their use and discard.

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