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Short Report

Development and Validation of a Multi-Residue Analytical Methodology to Determine the Presence of Selected Pesticides in Water Through Liquid Chromatography

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No presente trabalho uma metodologia analítica é descrita para determinar a presença de multiresiduos de pesticidas utilizados na cultura da cana de açúcar (tebuthiuron, hexazinona, diuron, 2,4-D e ametrina), em fontes de água de abastecimento. Os analitos foram isolados da matriz água, através das seguintes técnicas: extração liquido–liquido (LLE), utilizando diclorometano como solvente; extração em fase sólida (SPE), utilizando C18 como suporte sólido e acetonitrila como solvente para condicionamento da fase e eluição dos analitos; e extração com fluido supercrítico (SFE), utilizando C18 como suporte sólido e CO_2 no estado supercrítico como solvente de extração. Análise cromatográfica foi feita por cromatografia liquida de alta eficiência (HPLC) com detector ultravioleta, utilizando acetonitrila/água como fase móvel. Dentre as três técnicas de extração avaliadas, os melhores resultados foram obtidos com extração liquido–liquido e extração em fase sólida.

In the present work an analytical methodology is described to determine the presence of multiresidues of selected pesticides used in sugar-cane cultures (tebuthiuron, hexazinone, diuron, 2,4-D and ametrine), in source of drinking water. The analytes were isolated from the water matrix through the following techniques: liquid-liquid extraction (LLE) using dichloromethane as solvent; solid phase extraction (SPE) using C_{18} as solid support and acetonitrile as solvent to condition the solid phase and elution of the analytes; and supercritical fluid extraction (SFE) using C18 as solid support and CO_2 in supercritical state as extractant solvent. The chromatographic analysis was done by high performance liquid chromatography (HPLC) with an ultraviolet detector and using acetonitrile/water as mobile phase. Among the three evaluated extraction techniques, the best results were obtained with both liquid-liquid extraction and solid phase extraction.

Keywords: pesticides, water, extraction, chromatography

Introduction

Pesticides are substances used in agriculture, in order to increase the quantity and quality of agricultural products. They are toxic, cause environmental contamination as well as generating public health problems. The analysis of pesticide residues in water is difficult, since these compounds occur at very low concentration levels. They are usually present in high diversity, under different physical-chemical properties and with high concentrations of interfering compounds. According to Biziuk and Pryjazny,[1] most analytical steps in pesticide analysis involve sampling, pre-treatment of the sample, analysis and treatment of the data. The pre-treatment, including isolation and preconcentration of the analytes usually occupies over half of the total analysis time.

The three isolation and preconcentration methods used in this work, liquid-liquid extraction (LLE), solid phase extraction (SPE) and supercritical fluid extraction (SFE) are often applied in pesticide residue analysis in water. LLE is a simple and convenient technique used to separate organic compounds from solutions or aqueous suspensions where they are present. The substances contained in the solution will distribute among two solvents in accordance to their relative solubility. The organic substances are, in general, soluble in organic solvents and only slightly soluble in water.[2] LLE presents as its main disadvantage a high consumption of high purity solvents if not used properly, besides the costs associated with their use and discard.[3] SPE is used in the analysis of both polar and non polar analytes where the matrix and the analyte of interest are usually dissolved in a liquid. It is applied to pesticides analysis in water samples since it is an easy and fast process.[4] According to Font *et al.*,[5] the recovery of pesticides from water samples through SPE depends on the type of water, pH and treatment

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of the sorbent. Supercritical fluid extraction is a more recent technique, also used in the extraction of pesticide residues, presenting as advantages in relation to the other techniques the economy of samples, solvents, reagents and time. It also usually presents a larger recovery in the analysis of real samples.[6] Levy [7] claims that there is little literature available about supercritical fluid extraction of organic pollutants in aqueous matrix, due to the mechanical difficulty of retaining the liquid and polar matrix in the extraction cell. Carbon dioxide has been the most widely used solvent due to its mild critical conditions, high volatility, low viscosity, high diffusivity, low cost,[8-10] among other favorable properties.

High performance liquid chromatography (HPLC) is the method often used when the compounds to be analyzed are polar, non volatile and thermally labile.[11] The use of biodegradable pesticides is making HPLC the favorite analytical technique, allowing large volumes of injection of aqueous samples.[12]

The aim of this work is the development and validation of analytical methodologies through liquid-liquid extraction, solid-phase extraction and supercritical fluid extraction, to simultaneously determine the pesticides tebuthiuron, hexazinone, diuron, 2,4-D and ametrine in water matrices, employing HPLC for the separation and quantification of the analytes studied. Although widely used in agriculture these compounds were selected because of their extensive use in sugar cane plantations in Brazil, then presenting a risk of reaching water bodies, including drinking water resources.

Experimental

Chemicals

All the analytical standards used in this work were obtained from Chem Service (West Chester, PA, USA) with purity around 99%. The stock and working solutions were prepared in HPLC grade acetonitrile as solvent, obtained from Mallinckrodt (Phillipsbourg, NJ, USA). They were stored in a refrigerator at 8°C until their use. Tests demonstrated that the solutions stayed stable for several months. The stock solutions were prepared in the concentration of 100 mg mL^{-1} and were diluted in different working solutions, to achieve the following concentrations: 10.0; 1.0; 0.5 and 0.1 mg mL^{-1}.

Extraction

For all three extraction methods investigated, liquid-liquid extraction (LLE), solid phase extraction (SPE) and supercritical fluid extraction (SFE) 100 mL of water purified in a Milli-Q system (Millipore, Eschborn, Germany) was enriched with the mixture of analytical standards at concentration of 1mg/mL^{-1}. Several solvents (hexane, dichloromethane, acetonitrile, methanol) in different quantities (10, 20, 40, 60 mL), solid phases of different chemical compositions (C18, florisil, chromossorb, alumina, sílica gel, XAD 7), different modifiers for supercritical fluid (hexane, acetonitrile, methanol) and various temperature (50, 60 °C) and pressure (120, 250, 300 atm) conditions were tested. The best results obtained for each one for the three extraction methods are described below. In LLE, 60 mL of dichloromethane (Merck, Darmstadt, Germany) were used to remove analytes from the aqueous matrix, being the process repeated three times. In SPE, 1.0 g of the C18 – bonded silica phase obtained from Supelco (Bellefonte, PA, USA) was used as solid support; acetonitrile was used to condition the phase and elution of the compounds. In SFE, the C18 – bonded silica obtained from Supelco (Bellefonte, PA, USA) was used as support for the sample and CO_2 in supercritical state modified with acetone (Merck, Darmstadt, Germany) in the extraction of the compounds at 60° C and pressure of 300 atm.

LC – UV analysis

Pesticides were analyzed through high performance liquid chromatography (HPLC) using a Shimadzu 10A liquid chromatograph (Shimadzu, Kyoto, Japan) equipped with ultraviolet detector and a 250 mm x 4 mm x 5 μm RP - 8 column from Supelco (Bellefonte, PA, USA). The mobile phase used was acetonitrile/water (2.8: 7.2 v/v) at 1 mL min^{-1}, and the detection was done at 254 nm for the analytes tebuthiuron, hexazinone, diuron and at 238 nm for ametrine and 2,4-D. The volume of the sample injected was 20 μL in all cases.

Validation

The quality and credibility of an analytical work is based on careful procedures that produce data that express the real value of the obtained measurement. To validate an analytical method, statistical techniques are required in order to evaluate the quality of the data according to standardized criteria. Some factors were considered in the validation of the present method, including the precision measured by the standard deviation (S), estimated standard deviation (ESD), correlation coefficient (r), linearity, recovery, limit of detection (LOD) and limit of quantification (LOQ). The external standard method was used to quantify the analytes through analytical curve.

Table 1. Calibration equation showing the linear regression analysis; correlation coefficient (r); limit of detection (LOD); and limit of quantification (LOQ) for all investigated compounds as obtained by liquid chromatography

Pesticides Investigated	Concentration Range (μg mL^{-1})	Calibration Equation	Correlation Coefficient	LOD (μg L^{-1})	LOQ (μg L^{-1})
Tebuthiuron	0.1 – 1.0	3.03×10^5 X + 1.45×10^4	0.99934	10	35
Hexazinone	0.1 – 1.0	2.17×10^5 X + 5.99×10^3	0.99841	15	50
Diuron	0.1 – 1.0	2.39×10^5 X – 4.01×10^3	0.99971	20	65
2,4-D	0.1 – 1.0	2.50×10^5 X – 8.71×10^3	0.99897	25	85
Ametrine	0.1 – 1.0	2.28×10^5 X + 6.32×10^3	0.99702	30	100

The concentrations of each analyte, used in the acquisition of the analytical curve were 1.0; 0.5; 0.25 and 0.1 mg mL^{-1}; Linear Regression Method (LRL) was also applied.

Results and Discussion

After the optimization of the analytical conditions the pesticides of interest were analysed by liquid chromatography using an ultraviolet detector, in order to obtain the separation of tebuthiuron, hexazinone, diuron, ametrine and 2,4-D (Figure 1). As it can be observed, the compounds analyzed are baseline separated being the mobile phase and the chromatographic conditions used appropriate to the objective of the work. The ultraviolet detector (UV) response was satisfactory for the analytical conditions used.

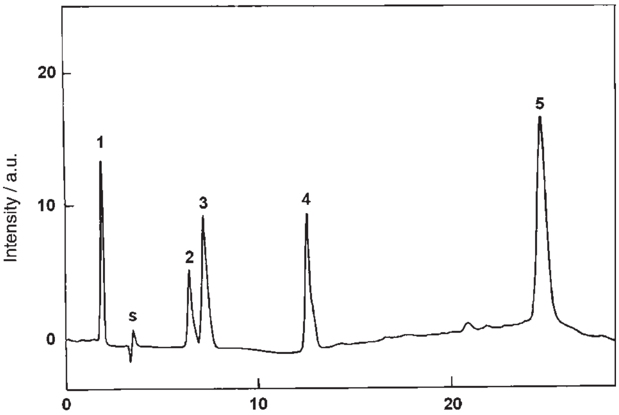

Figure 1. Typical HPLC – UV chromatograms of analytical standards mixture, λ = 238 nm, concentration of 1.0 μg mL^{-1}. S = acetonitrile solvent; 1 = 2,4-D; 2 = tebuthiuron; 3 = hexazinone; 4 = diuron; 5 = ametrine.

The external standard method was used to quantify the analytes, with an analytical curve of four points for each analyte, calculated in four concentrations, applying the linear regression method. The correlation coefficients r were all higher than 0.99, indicating that the detector answer is linear for the concentrations of the analytes present in the injected samples, 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[13] and the limit of quantification as being ten times the average value of the noise in this same region.[14]

Table 1 presents the figures of merit corresponding to the analytical curve, correlation coefficient, limit of detection, limit of quantification, for all studied compounds. The extraction recovery values (%) with their estimated standard deviation RSD (%) obtained through liquid-liquid extraction (LLE), solid phase extraction (SPE) and supercritical fluid extraction (SFE) for tebuthiuron, hexazinone, diuron, 2,4-D and ametrine are presented in Table 2.

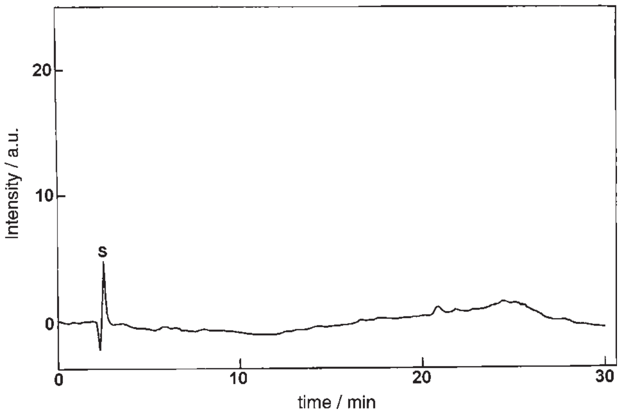

Figure 2. Representative HPLC – UV chromatogram of river water samples after SPE. Sample was collected at Ribeirão das Cruzes, in summer season. λ = 238 nm. S = acetonitrile solvent.

Table 2. Extraction recovery (%) and corresponding estimated standard deviation (ESD) obtained through liquid-liquid extraction (LLE), solid phase extraction (SPE) and supercritical fluid extraction (SFE), in water samples enriched with 1 mg mL^{-1} of each analyte

Pesticides	Recovery ± ESD (%)		
	LLE	SPE	SFE
Tebuthiuron	95.7 ± 0.8	104.0 ± 6.0	39.9 ± 15.1
Hexazinone	95.8 ± 0.5	98.1 ± 4.7	28.0 ± 8.0
Diuron	94.5 ± 0.9	102.7 ± 1.4	56.9 ± 8.4
2,4-D	95.1 ± 4.6	101.2 ± 13.1	58.1 ± 10.1
Ametrine	94.3 ± 4.3	102.8 ± 0.3	n.r.

n.r. - not recovered.

The three extraction techniques evaluated, liquid-liquid extraction (LLE), solid phase extraction (SPE) and supercritical fluid extraction (SFE) are appropriate for pesticide analysis in water samples. The best recovery results were obtained using LLE and SPE, with recovery values comprised between 94.32% and 103.97%, well within the recovery interval of 70 to 130% suggested by the USEPA.[15] In SFE, the recovery values obtained were low for all studied compounds, suggesting a need to alter some parameters such as temperature, pressure, modifier and extraction time, for a possible analysis improvement, since previous work developed in our group, applying SFE for other pesticides in different matrices, presented excellent recovery results, besides sample, solvent, reagent and time saving.[16-19]

Levy [7] observed that supercritical fluid extraction a pressure increase at a constant temperature, or a temperature increase at a constant pressure, usually improves the extraction. The efficiency of the extraction depends on its condition, such as, pressure and temperature, but many times it is necessary the addition of a modifier.

In the liquid-liquid extraction due to the high amount of solvents it is used, it presents some inconvenience like: costly solvent, environmental contamination and work safety conditions due to the manipulation of organic solvents.[20-22]

SPE has some advantages over the conventional extraction techniques for example liquid-liquid extraction such as simplicity, quick extraction, purer extracts free from interference, lower operational price, selective extraction, small volume consumption of high purity solvents and it allows the extraction and sampling in the field for later transport and storage.[23] According to Luque-García and Luque de Castro,[24] other advantages of SPE are the decrease of contamination problems and the easy automation of the process.

Conclusion

Among the three evaluated extraction techniques, liquid-liquid extraction (LLE), solid phase extraction (SPE) and supercritical fluid extraction (SFE), the best recoveries for the pesticides tebuthiuron, hexazinone, diuron, 2,4-D and ametrine in water were obtained using LLE and SPE. SPE is a very appropriate technique for pesticides analysis, due to its simplicity, to being fast and consuming very small volumes of high purity solvents. The results obtained in this work using SFE were still unsatisfactory, requiring to be further optimized, since it is a technique that has shown evidence to be quite appropriate in pesticides residues analysis in solid and semi-solid matrices and still scarcely studied for liquid ones.

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