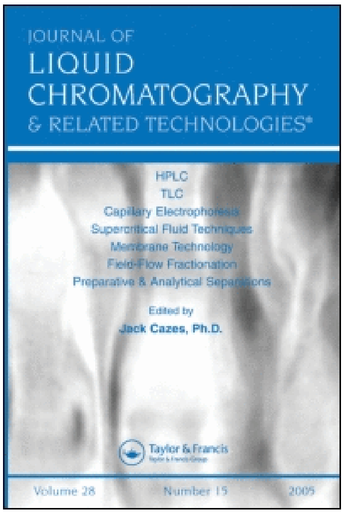

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

HPLC Determination of Pesticide Residues Widely Employed in Sugar-Cane Cultures in River Water Samples

S. G. Brondi[a]; F. M. Lanças[a]
[a] Institute of Chemistry of São Carlos, University of São Paulo, São Carlos, SP, Brazil

To cite this Article Brondi, S. G. and Lanças, F. M.(2004) 'HPLC Determination of Pesticide Residues Widely Employed in Sugar-Cane Cultures in River Water Samples', Journal of Liquid Chromatography & Related Technologies, 27: 1, 171 — 179

To link to this Article: DOI: 10.1081/JLC-120027093

URL: http://dx.doi.org/10.1081/JLC-120027093

JOURNAL OF LIQUID CHROMATOGRAPHY & RELATED TECHNOLOGIES®
Vol. 27, No. 1, pp. 171–179, 2004

HPLC Determination of Pesticide Residues Widely Employed in Sugar-Cane Cultures in River Water Samples

S. H. G. Brondi and F. M. Lanças*

University of São Paulo, Institute of Chemistry of São Carlos, São Carlos, SP, Brazil

ABSTRACT

Pesticides are substances used in agriculture, to increase the quantity and quality of agricultural products. They are toxic, cause environmental contamination, as well as generating public health problems. In the face of their increasing use, the present work aims at the determination of the multi-residues present in pesticides, which are widely used in sugar cane culture, focusing on the herbicides tebuthiuron; hexazinone; diuron; 2,4-*D*; and ametrine in water samples. Liquid–liquid extraction (LLE) and solid phase extraction (SPE), with subsequent analysis by high performance liquid chromatography (HPLC), with ultraviolet detection using acetonitrile/water as mobile phase were used in this study. Two extraction techniques were evaluated, one being solid phase extraction, using C18 as sorbent and acetonitrile as both the solvent to condition the

---

*Correspondence: F. M. Lanças, University of São Paulo, Institute of Chemistry of São Carlos, 13.560-970, São Carlos, SP, Brazil; E-mail: flancas@iqsc.sc.usp.br.

DOI: 10.1081/JLC-120027093
1082-6076 (Print); 1520-572X (Online)

www.dekker.com

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

solid phase and for the elution of the analytes; the other technique was liquid–liquid extraction using dichloromethane as the extracting solvent. Both methods presented good recovery values, above 94%. In the analyses of water samples from Ribeirão das Cruzes, Ribeirão das Anhumas, and Córrego do Paiól, none of the studied compounds were registered, neither in the summer nor in the winter seasons.

Key Words: Pesticides; Water; Contamination; Extraction; Chromatography.

INTRODUCTION

During past years, the pollution and contamination of the earth has increased considerably. This is due mainly to the increase in population, industrial activity, and the modernization of agricultural practices, such as the increasingly widespread use of pesticides, resulting in an increase of the amount of effluents thrown into the air, water, and soil. This environmental contamination by organic micropollutants has been concerning society, and several countries are carrying on research activities dealing with the presence, monitoring, and toxicity of these compounds in surface, underground, and water for human consumption.[1] Among the ecosystems, the aquatic ones work as temporary or final reservoirs of a large variety and quantity of pollutants.[2]

Pesticides arrive in the water directly or through drainage waters from the cultures, but their indiscriminate use can cause serious environmental and public health problems. When the application of the pesticides is accomplished appropriately there are few chances of contamination or accumulation in the environment.[3]

Sugar-cane culture is one of the most abundant of the São Paulo State in Brazil, being predominant in the studied area, the municipal district of Araraquara, occupying extensive areas and using large amounts of pesticides, mainly herbicides. According to Poltronieri,[4] the largest incidence of contamination caused by pesticides in the referred state occur in the sugar-cane, corn, citrus, and banana plantations.

According to Grosser et al.[5] the Environmental Protection Agency (EPA) recognizes the application of high performance liquid chromatography (HPLC) for the analysis of non volatile, thermally labile, and polar materials. The use of biodegradable pesticides in agriculture is making HPLC the favorite analytical technique in this area.

The two isolation and preconcentration methods used in this work, liquid–liquid extraction (LLE) and solid phase extraction (SPE), are usually applied in the analysis of pesticide residues in water. Liquid–liquid extraction is a simple and convenient technique, which is broadly used to separate organic compounds from solutions or aqueous suspensions where they exist. It also presents disadvantages, because it tends to consume large volumes of high purity solvents, which can present a danger to health, besides the costs associated with its use and disposal.[6] Solid phase extraction is used in the analysis of both polar and non polar analytes. However, in this technique, both the sample and the analyte should be dissolved in a liquid. According to Bengtsson and Ramberg,[7] this technique is appropriate for pesticide analysis, particularly due to the fact that SPE is a fast technique, which consumes only small volumes of organic solvents.

The aim of this work was to monitor the water supply of the city of Araraquara, an important industrial and agricultural region in São Paulo State, Brazil, related to the presence of relevant pesticides used in sugar-cane culture (tebuthiuron, hexazinone, diuron, 2,4-*D*, and ametrine), employing LLE and SPE as extraction techniques, followed by liquid chromatography.

EXPERIMENTAL

Chemicals

All the analytical standards were obtained from Chem Service (West Chester, PA). They presented purities around 99%. The stock and working solutions were prepared in HPLC grade acetonitrile, obtained from Mallinckrodt (Phillipsbourg, NJ), and stored at 8°C, remaining stable for several months. The stock solutions were prepared in the concentration of 100 mg/mL, and was later diluted to obtain different working solutions, at concentrations of 10.0; 1.0; 0.5; and 0.1 mg/mL.

The real water samples came from the reservoirs of the three rivers that supply Araraquara city, namely Ribeirão das Cruzes, Ribeirão das Anhumas, and Córrego do Paiól, all located in the center east of the São Paulo State, Brazil, among the parallel of 48°00′–48°15′ longitude W and 21°40′–21°55′ latitude S. This area contains extensive sugar-cane culture, and these pesticides are widely used in their culture as stated by the local authorities. The samples were collected during the summer and winter seasons of 1999 in amber flasks, being immediately taken to the laboratory and conditioned at low temperature. They had the pH decreased to 2, inhibiting the activity of the microorganisms and were kept under refrigeration until the moment of the analysis.

Extraction

For the development of both extraction methods LLE and SPE, 100 mL of water purified in a Milli-Q system (Millipore, Eschborn, Germany) were enriched with the mixture of analytical standards at a concentration of 1 mg/mL. In the liquid-liquid extraction technique, 60 mL of dichloromethane (Merck, Darmstadt, Germany) were used to remove analytes from the water matrix, repeating this process three times. In SPE, 1.0 g of C18-bonded phase, from Supelco (Bellefonte, PA), was used as a solid support, while acetonitrile was used for both conditioning the solid phase and for the elution of the compounds.[8]

After optimizing the extraction techniques LLE and SPE, using water enriched with the analytical standards, the real water samples were analyzed using both LLE and SPE techniques for comparative purposes.

LC-UV Analysis

Pesticides were analyzed by HPLC. A Shimadzu 10 A liquid chromatograph, from Shimadzu (Kyoto, Japan), equipped with an ultraviolet detector and a 250 mm × 4 mm × 5 μm RP-8 column, acquired from Supelco (Bellefonte, PA), were used. The mobile phase was acetonitrile/water (2.8 : 7.2 v/v), at 1 mL/min, the detection being done at 254 nm for tebuthiuron, hexazinone, diuron, and at 238 nm for 2,4-*D*. The sample volume injected was 20 μL.

RESULTS AND DISCUSSION

All samples were analyzed by liquid chromatography using an ultraviolet-visible detector (UV/Vis). The analytical conditions were optimized to obtain appropriate experimental conditions for the separation of all pesticides (tebuthiuron, hexazinone, diuron, ametrine, and 2,4-*D*) in a single run, as shown in Fig. 1.

Table 1 presents the recovery values percentage and their precision, as measured by the relative standard deviation percentage, obtained when the LLE and SPE were applied for the extraction of tebuthiuron, hexazinone, diuron, 2,4-*D*, and ametrine compounds, in river water fortified with known concentrations of the analytical standards.

The recovery results of both evaluated extraction techniques, LLE and SPE, were good, with all values within the 70 to 130% interval suggested by

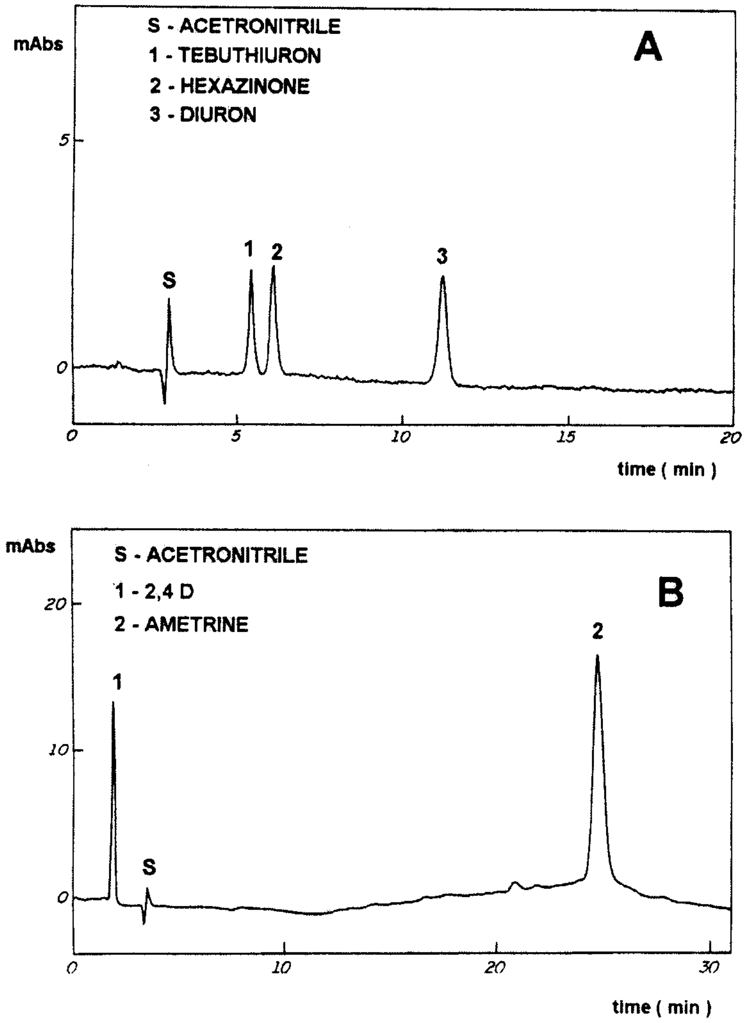

Figure 1. Typical HPLC UV chromatograms of a mixture containing (A) tebuthiuron, hexazinone, diuron at $\lambda = 254\,\text{nm}$; and (B) 2,4-*D*, ametrine at $\lambda = 238\,\text{nm}$. Concentration: $1.0\,\mu\text{g/mL}$; S = solvent (acetonitrile).

the USA-Environmental Protection Agency.[9] Using LLE with dichloromethane as extraction solvent, the recovery values varied from 94.3% for ametrine to 95.8% for hexazinone. Employing SPE using C_{18} as solid support and acetonitrile as the conditioning phase, as well as for the elution of the

Table 1. Recovery results (%) and relative standard deviation (RSD) of selected pesticides obtained employing liquid–liquid extraction (LLE) and solid phase extraction (SPE). Sample: river water enriched with 1.0 μg/mL of each analyte.

	Recovery ± RSD (%)	
Pesticides	LLE	SPE
Tebuthiuron	95.70 ± 0.75	103.97 ± 6.02
Hexazinone	95.84 ± 0.46	98.09 ± 4.67
Diuron	94.51 ± 0.86	102.68 ± 1.37
2,4-*D*	95.11 ± 4.57	101.20 ± 13.14
Ametrine	94.32 ± 4.26	102.80 ± 0.25

analytes, the recovery values varied from 98.0% for hexazinone to 103.9% for tebuthiuron.

Although, LLE is often considered as the conventional extraction method, it presents disadvantages, because it consumes large volumes of organic solvents which are expensive, and represents a danger to health and the environment. Majors,[10] comparing LLE with SPE, concluded that SPE presents advantages, since it reduces the consumption of solvents, it has fewer steps, is less laborious, more efficient, it avoids emulsions, and it is more appropriate for automation. According to Albanis and Hela,[11] SPE is widely applied in pesticides analysis in water samples, being an easy and fast process.

Relative standard deviation (RSD) values of the extraction recovery were good for all the studied compounds, being inside of the acceptance range.[12,13] Except for 2,4-*D* and tebuthiuron, extracted by SPE, all other RSD values are lower than 5%. In average, LLE presents RSD values lower than SPE.

The real water samples were collected at the following rivers that supply the city of Araraquara, an important city located in São Paulo State: Ribeirão das Cruzes, Ribeirão das Anhumas, and Córrego do Paiól. They were submitted to both liquid-liquid and solid phase extraction techniques, using the developed and validated methodology. Both summer and winter seasons were studied; they registered compounds with retention times of 12.10 and 14.80 min, detection at 254 nm (Fig. 2), far different from the retention time of the studied compounds, determined as 5.82 (tebuthiuron); 6.20 (hexazinone) and 11.50 (diuron) minutes. Therefore, none of the studied compounds were detected in the three environment water samples in either summer or winter seasons.

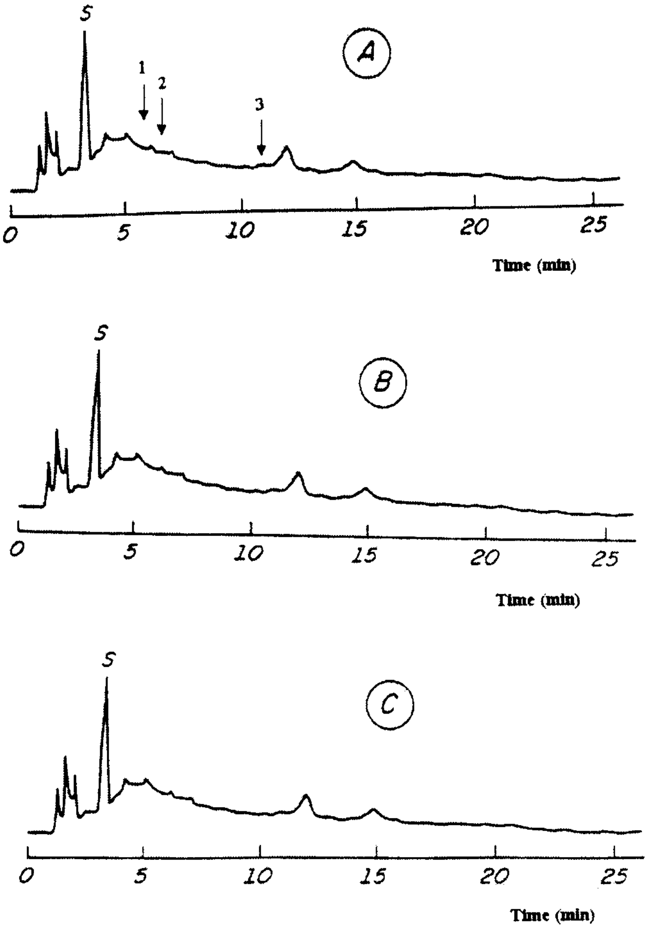

Figure 2. Representative HPLC-UV chromatograms of river water samples after SPE. Samples (summer season) were collected at (A) Ribeirão das Cruzes; (B) Ribeirão das Anhumas; and (C) Córrego do Paiól. $\lambda = 254\,\text{nm}$. Conditions same as for Fig. 1. Arrows indicate the retention time corresponding to: 1-tebuthiuron, 2-hexazinone, 3-diuron.

CONCLUSION

The two extraction techniques, LLE and SPE, evaluated in this work to recover pesticides from river, showed good recovery values. Solid phase extraction was shown to be the most appropriate technique for the pesticide analysis in water. This technique is simple, fast, and consumes a very small volume of high purity solvents. None of the compounds analyzed in the present work by liquid chromatography were detected in either summer or winter seasons in the water samples collected at Ribeirão das Cruzes, Ribeirão das Anhumas, and Córrego do Paiól rivers. These rivers supply water to the city of Araraquara, Brazil, an important industrial city surrounded by sugar cane plantations, a culture that uses large amounts of the investigated pesticides.

ACKNOWLEDGMENTS

The authors thank FAPESP (São Paulo State Research Foundation) for financial support and for a fellowship to SHGB (Proc. 96/4347-8).

REFERENCES

1. Ruocco, J., Jr.; Aiba, C.J.; Hokama, P.C.; Pires, J.M.; Mendes, C.A. Identification of organic pollutants. Ambiente **1987**, *1*, 147–149.
2. Lee, C.M. *Aquatic Toxicity Seminar*; In Course, Cetesb: São Paulo, 1980.
3. Mullison, R.W. Effects of herbicides on water and its inhabitants. Weed Sci. **1970**, *18*.
4. Poltronieri, L.C. The "Paulista" Map of Agrochemicals. http://www.unesp.br/noticia/main15.html (accessed March 2000).
5. Grosser, Z.A.; Ryan, J.F.; Dong, M.W. Environmental chromatographic methods and regulations in the united states of america. J. Chromatogr. **1993**, *642*, 75–87.
6. Hatrík, S.; Tekel, J. Extraction methodology and chromatography for the determination of residual pesticides in water. J. Chromatogr. A **1996**, *733*, 217–233.
7. Bengtsson, S.; Ramberg, A. Solid-phase extraction of pesticides from surface water using bulk sorbents. J. Chromatogr. Sci. **1995**, *33*, 554–556.
8. Brondi, S.H.G.; Lanças, F.M. Bull. Environ. Contam. Toxicol. **2003**, *in press*.
9. Tolosa, I.; Readman, J.W.; Mee, L.D. Comparison of the performance of solid-phase extraction techniques in recovering organophosphorus and organochlorine compounds from water. J. Chromatogr. A **1996**, *725*, 93–106.
10. Majors, R.E. A review of modern solid-phase extraction. LC-GC **1998**, *8*, 15–22.

11. Albanis, T.A.; Hela, D.G. Multi-residue pesticide analysis in environmental water samples using solid-phase extraction discs and gas chromatography with flame thermionic and mass-selective detection. J. Chromatogr. A **1995**, *707*, 283–292.
12. GARP (Pesticides Residues Analysis Group); ANDEF (Vegetal Defense National Association). *Validation of Analytical Methodologies for Determination of Pesticides Residues*; Ministery of Agriculture: Brazilian, 1997.
13. Gou, J.; Tragas, C.; Lord, H.; Pawliszyn, J. On-line of in-tube solid phase microextraction (SPME) to HPLC for analysis of carbamates in water samples: comparison of two commercially available autosamplers. Microcol. Sep. **2000**, *12*, 125–134.

Received April 15, 2003
Accepted June 10, 2003
Manuscript 6150

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016