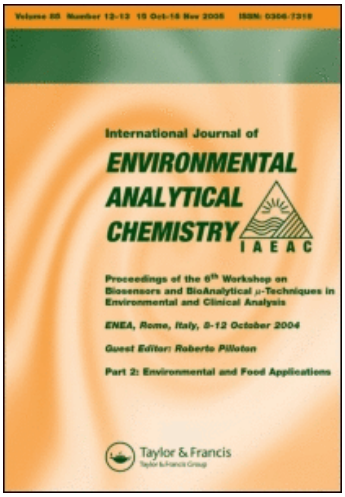

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Determination of disulfoton in surface water samples by cloud-point extraction and gas chromatography

Anizio M. Faria[a]; Raquel P. Dardengo[a]; Claudio F. Lima[a]; Antonio A. Neves[a]; Maria Eliana L. R. Queiroz[a]
[a] Departamento de Química, Universidade Federal de Viçosa, Viçosa, MG, Brazil

To cite this Article Faria, Anizio M. , Dardengo, Raquel P. , Lima, Claudio F. , Neves, Antonio A. and Queiroz, Maria Eliana L. R.(2007) 'Determination of disulfoton in surface water samples by cloud-point extraction and gas chromatography', International Journal of Environmental Analytical Chemistry, 87: 4, 249 — 258
To link to this Article: DOI: 10.1080/03067310601068841
URL: http://dx.doi.org/10.1080/03067310601068841

Intern. J. Environ. Anal. Chem.
Vol. 87, No. 4, 10 April 2007, 249–258

Taylor & Francis
Taylor & Francis Group

Determination of disulfoton in surface water samples by cloud-point extraction and gas chromatography

ANIZIO M. FARIA, RAQUEL P. DARDENGO, CLAUDIO F. LIMA, ANTONIO A. NEVES and MARIA ELIANA L. R. QUEIROZ*

Departamento de Química, Universidade Federal de Viçosa, 36571-000, Viçosa, MG, Brazil

(*Received 8 June 2006; in final form 13 October 2006*)

This article proposes an alternative method, using cloud-point extraction and gas chromatography, for extraction and determination of disulfoton in water samples. For cloud-point extraction, the nonionic surfactant Triton X-114 was used. Before gas chromatography, a cleanup stage for surfactant removal from the extracts was optimized. Cleanup used two columns, in series, containing silica gel and Florisil, with methanol:hexane (1 : 1) as eluent, resulting in the removal of more than 95% of the Triton X-114. Factors such as ionic strength (>0.5 mol L^{-1}) and surfactant concentration (1.0% w/v) increased the extraction efficiency of the cloud-point methodology, yielding disulfoton recoveries of almost 100%. Compared with liquid–liquid extraction, the cloud-point methodology was more efficient, with a better detectability, and resulted in a significant reduction in solvent volume.

Keywords: Cloud-point extraction; Disulfoton; Organophosphorus pesticides; Gas chromatography

1. Introduction

Disulfoton [*O*,*O*-diethyl-*S*-[2-(ethylthio)ethyl]-phosphorodithioate] (figure 1) is a systemic insecticide and acaricide, also marketed as Di-Syston. It is used mainly as emulsifiable concentrates for foliage treatment and as granules for soil application to protect plants from insect attack [1, 2]. In Brazil, disulfoton is commonly used on several types of crops, but mainly for the production of coffee, and has an efficient action in controlling the Bicho Mineiro (*Perileucoptera cooffeella*), one of the main pests of coffee cultivation [3, 4]. The large amounts of disulfoton applied can lead to leaching to surface and underground waters, and so there is a need to monitor its residues in such waters.

Usually, the monitoring of pesticide residues is performed by an appropriate extraction technique followed by an analytical separation technique, e.g. gas chromatography (GC) or high-performance liquid chromatography (HPLC).

*Corresponding author. Fax: +55-31-3899-3065. Email: meliana@ufv.br

International Journal of Environmental and Analytical Chemistry
ISSN 0306-7319 print/ISSN 1029-0397 online © 2007 Taylor & Francis
DOI: 10.1080/03067310601068841

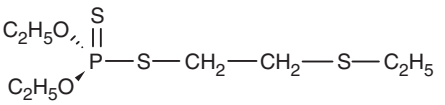

Figure 1. Structural formula of disulfoton [*O*,*O*-diethyl *S*-[2-(ethylthio)ethyl]phosphorodithioate].

For disulfoton, GC is the preferred analytical separation technique due to its high volatility. Thus, extraction with organic solvents from environmental samples has been recommended by the principal regulatory agencies. However, the large amounts of organic solvents required by these techniques for efficient extractions and the need for detection of pesticide residues at trace-level concentrations [5, 6], commonly found in waters, have been driving forces for the development of alternative methodologies. Several techniques have been proposed in recent years with this goal [7–11]. Among these, the use of cloud-point phase separation with a surfactant in the aqueous samples for determination of trace organic compounds has become a focus of considerable interest ([12–21] and references therein).

The tendency of several nonionic or zwitterionic surfactants to separate into two liquid phases (a surfactant rich-phase and aqueous phase) when their aqueous solutions are heated above a given temperature offers an interesting alternative to organic solvent-based extractions. The small volume of the surfactant rich phase stems from the quest to develop rapid, simple, sensitive, and efficient sample-preparation procedures for trace environmental assays, yielding enhanced detection due to the large concentration factors.

In spite of the growth of interest in recent years, the use of cloud-point phase separation as an extraction and concentration step prior to GC has found very few applications, due to the need to remove the surfactant molecules, which can block the analytical capillary column [22, 23]. In addition, there is apparently no literature on the extraction of pesticides from water samples by cloud-point methodology prior GC analysis. In the present work, we describe the optimization and validation of a cloud-point methodology using the nonionic surfactant Triton X-114 as an efficient alternative for the extraction and concentration of disulfoton from surface water prior to its determination by GC-FID. To this end, a cleanup system for the removal of the Triton X-114 from the surfactant-rich phase was developed and optimized.

2. Experimental

2.1 *Chemicals*

Analytical standards of disulfoton were purchased from Bayer (Wuppertal, Germany). *n*-Hexane, methanol, chloroform, and dichloromethane, obtained from Tedia (Rio de Janeiro, Brazil), were of residue analysis grade. As column materials, silica gel 60 (0.063–0.200 and 0.040–0.063 mm), Florisil (0.150–0.250 mm), and microcrystalline cellulose were obtained from Merck (Darmstadt, Germany). The surfactant Triton X-114 was purchased from Sigma (Milwaukee, WI). Solutions of NaCl (Reagen, Rio de Janeiro, Brazil) with different concentrations, to vary the ionic strength of the samples, were prepared in distilled water.

2.2 *Apparatus*

For qualitative and quantitative analysis of the disulfoton, a Shimadzu GC-17A gas chromatograph (Kyoto, Japan) with flame-ionization detection was used with a fused silica capillary column (30 m × 0.32 mm) coated with 1.0 μm of 5% poly(methylphenylsiloxane) and 95% poly(dimethylsiloxane) (BP-5) (Agilent, Wilmington, DE). The temperature of the injection port was held at 250°C. The injection was done in the split mode (split ratio of 1 : 5). The temperature programme for the GC was 200°C, 20°C min^{-1} to 280°C and 280°C for 6 min. The flame ionisation detector was maintained at 300°C.

A Fanem Excelsa II 206 MP model centrifuge (Guarulhos, Brazil) was used for phase separation with the cloud-point methodology. A Tecnal TE-184 model thermostated bath (Piracicaba, Brazil) was also used. A Hitachi UI 1100 model UV/Vis photometer (Tokyo) was used to quantify the surfactant from extracts after being eluted from the cleanup system.

2.3 *Cloud-point methodology*

2.3.1 Ratio of phases. The ratio between the volume of aqueous phase and surfactant-rich phase (V_{SRP}), was determined by measuring the volume of both phases obtained for solutions containing different Triton X-114 concentrations (0.2–10%) using 10 mL of surfactant solution, at the cloud-point temperature. All the solutions were heated for a period of 10 min in a thermostated bath at 40°C and centrifuged at 3500 rpm for approximately 5 min. Then, the surfactant-rich phase was measured in calibrated tubes.

2.3.2 Cleanup stage. A cleanup system using small glass chromatographic columns (115 × 13 mm) for removal of surfactant from the surfactant-rich phase was optimized. Different adsorbents and eluents were evaluated. For this, 1.0 mL of Triton X-114 (10% w/v) solution was added to 9.0 mL samples of water fortified with disulfoton. The mixtures were placed in a thermostated bath for 10 min at 40°C (±0.1°C) to induce phase separation. This temperature was used to prevent any slight cooling of the mixture (which could revert to an isotropic phase again). Afterwards, the solutions were centrifuged at 3500 rpm for 10 min. The supernatant was removed from the tube, with only the surfactant-rich phase remaining. One hundred microlitres of this phase was added to the top of the columns containing the different adsorbents and eluted with 10 mL of eluent, using a flow rate of ca 5 mL min^{-1}. The eluate was dried in a stream of nitrogen and dissolved in 1.0 mL of methanol. The absence or presence of Triton X-114 in the eluates, after the cleanup step, was confirmed by UV/Vis spectrophotometry.

2.3.3 Optimized procedure applying cloud-point extraction (CPE) with gas-chromatographic analysis. Forty-millilitre aliquots of aqueous solutions containing disulfoton in the presence of 1.0% (w/v) Triton X-114 and 0.5 mol L^{-1} NaCl, dissolved in water, were kept in a thermostated bath at 40°C for 10 min. Afterwards, the solutions were centrifuged at 3500 rpm for 10 min to accelerate phase separation. One hundred

microlitres of the separated surfactant-rich phase was withdrawn using a micro-syringe after the dissolution of the surfactant-rich phase in methanol. This small volume was applied to the optimized cleanup system, and the eluates were analysed by GC, using the previously cited conditions.

2.3.4 Evaluation of the methodology using conventional liquid–liquid extraction (LLE). Aqueous samples (10.0 mL) of disulfoton-spiked water were placed into a separation funnel and submitted to three sequential extractions with 10.0 mL of dichloromethane, shaking for 2 min each time. The organic portion was removed and left in contact with ~5 g of Na_2SO_4 for 20 min. Following filtration, the extract was concentrated in a rotary evaporator to close to dryness. The residues were dissolved in methanol, the volume made up to 10.0 mL, and 1.0 µL analysed by GC, using the same conditions described above.

3. Results and discussion

3.1 *Phase ratio and diagrams*

The study of the influence of the surfactant concentration on the ratio phases indicates that, as surfactant concentration increases, the surfactant-rich phase volume increases, which leads to a lower concentration factor. The concentration factor is approximately 100 times for solutions at 0.2% (w/v) and 20 times at 1.0% (w/v) Triton X-114 solutions (figure 2).

An adequate relation between the concentration of surfactant in the solution and the volume of surfactant-rich phase (455 µL) was obtained using 10.0 mL of 1.0% (w/v) Triton X-114 solution. Part of this volume of surfactant-rich phase, 100.0 µL, was used

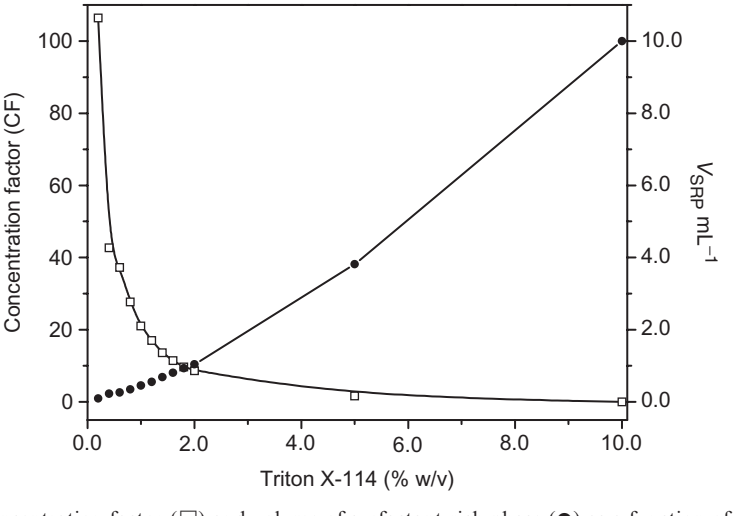

Figure 2. Concentration factor (□) and volume of surfactant-rich phase (●) as a function of Triton X-114 concentration.

in subsequent steps for quantification of disulfoton, obtaining a concentration factor of 10 times under these conditions. For determination of disulfoton in real samples, using 40.0 mL of the water sample, the concentration factor was 40-fold.

3.2 *Cleanup stage*

A cleanup stage is essential for analysis by GC using the cloud-point methodology. The presence of surfactant molecules can lead to rapid deterioration of the analytical column. Several adsorbents (silica gel, florisil, activated carbon, and cellulose) in small columns (115×13 mm) and various eluents (chloroform, hexane, methanol, and dichloromethane) were evaluated for Triton X-114 removal without loss of disulfoton. All extracts were analysed before and after passage through these adsorbents at the wavelength of maximum absorption of Triton X-114 (275 nm) and quantified by an appropriate surfactant analytical curve. The eluates were also analysed by GC, after verifying that the Triton X-114 was at a sufficiently low concentration, to quantify the recovery of disulfoton. The best results for Triton X-114 removal ($95 \pm 5\%$) were obtained with two columns coupled in series: the first containing silica gel (2.0 g) and the second containing Florisil (1.0 g). The most efficient disulfoton recoveries, about $93 \pm 4\%$, were obtained with a methanol:hexane (1 : 1) mixture as eluent. All experiments were performed with six replicates.

3.3 *Optimization of disulfoton recovery*

The extraction of the disulfoton from surface water samples using the cloud-point methodology was optimized by studying the parameters that influence this process, such as: cloud-point temperature, surfactant concentration, pH, and ionic strength. First, cloud-point temperature was determined for the separation of phases in different Triton X-114 concentrations (figure 3). The cloud-point temperature of Triton X-114 solutions in aqueous samples varies between 23.6 and 29.1°C for surfactant

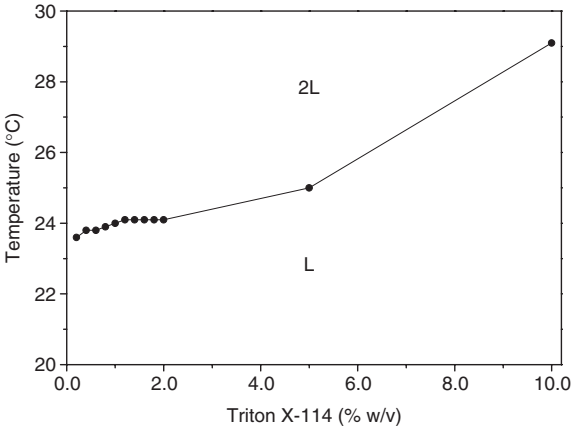

Figure 3. Variation of the cloud-point temperature with Triton X-114 concentration (% w/v). (L) denotes the single isotropic solution region, whereas (2L) indicates the region where the two isotropic phases coexist.

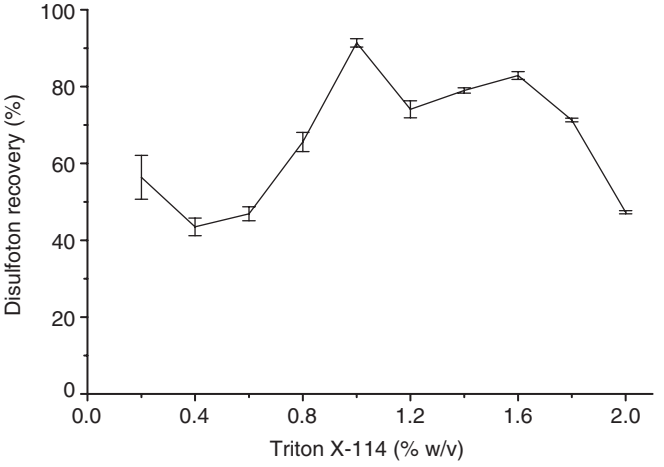

Figure 4. Effect of the surfactant concentration on the recoveries by cloud-point extraction of disulfoton from water. Initial concentration of disulfoton: 12.0 μg L^{-1}.

concentrations ranging between 0.2 and 10.0% (w/v). The cloud-point temperature is roughly constant (23.6–24.1°C) over a range of concentrations from 0.2 to 2.0% (w/v) (figure 3). From an analytical point of view, the cloud-point temperature of Triton X-114 is very interesting, because it does not need a drastic alteration to obtain the separated phases.

The effect of surfactant concentration on disulfoton recovery was evaluated by extracting the pesticide from surface water samples at different Triton X-114 concentrations, 0.2–2.0% (w/v). The eluates were analysed by GC using flame-ionization detection. The results show the highest efficiency for disulfoton extraction (91.4%) at a concentration of 1.0% (w/v) of Triton X-114 (figure 4). At both lower and higher concentrations, the efficiencies of the CPE were lower than 70%.

The pH of the aqueous samples also plays an important role in CPE. Depending on the pH of sample, some organic compounds can assume an ionizable form, resulting in a lower solubility inside the micelle and, consequently, lower recoveries. Disulfoton recovery was evaluated from pH 2.0 to 12.0. The pH of water samples was adjusted with H_3PO_4 or NaOH diluted solutions, using a pH meter. The results are presented in figure 5 and show that between pH 4.0 and 7.0, disulfoton recovery is maximized. Coincidentally, these are the pH conditions of greatest stability for the insecticide [24]. Under more extreme conditions, disulfoton recovery decreases considerably to values below 40%, as the pesticide is partly decomposed. According to the literature [24], disulfoton hydrolyses in alkaline medium and has a greater stability in an acidic medium, which supports the results presented in figure 5.

The ionic strength effect was observed by addition of different NaCl concentrations to the disulfoton spiked surface water samples. According to figure 6, an increase in the ionic strength has a positive influence on the efficiency of CPE of disulfoton from water. At an ionic strength of 1.0 mol L^{-1}, 100% extraction is observed. The increase in ionic strength of an aqueous surfactant solution increases the aggregation number (n, amount of surfactant monomers) in the Triton X-114 micelles, to values higher than 200 [25]. A typical Triton X-114 micelle possesses approximately $n = 120$. This implies

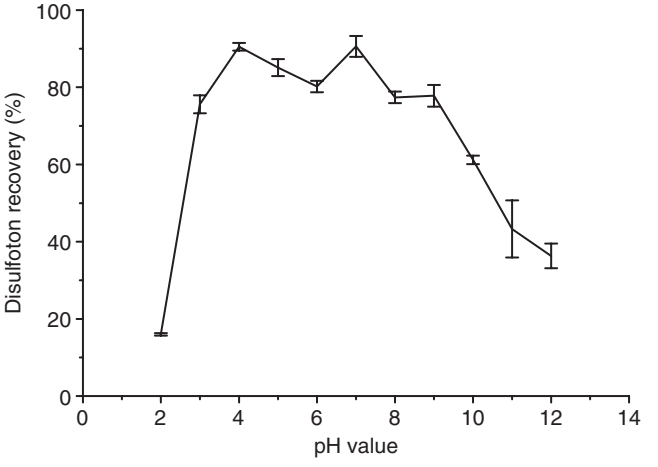

Figure 5. Effect of the ionic strength on the recoveries by cloud-point extraction of disulfoton from deionized water samples. Ionic strength modified with NaCl. Initial disulfoton concentration: 12.0 μg L^{-1}, $n = 3$.

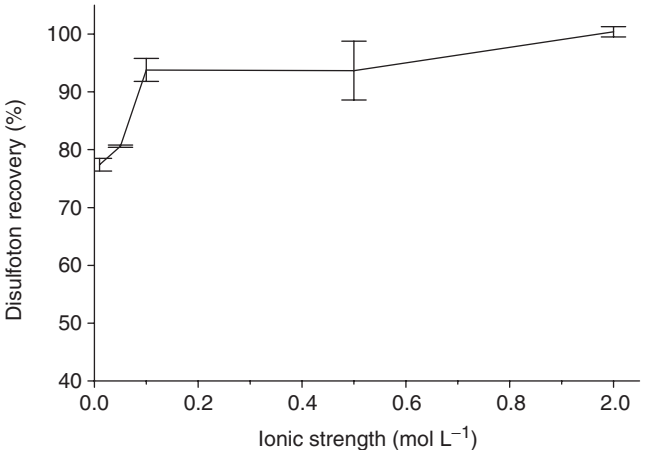

Figure 6. Effect of pH on the recoveries of disulfoton from surface water samples using cloud-point extraction. Initial concentration of disulfoton: 12.0 μg L^{-1}, $n = 3$.

an increase in the number of surfactant molecules in a cluster and, consequently, an increase in the micelle capability to solubilize larger amounts of disulfoton in their interior.

3.4 *Analytical characteristics of the cloud-point methodology*

Extraction using the cloud-point methodology was carried out using a total sample volume of 40.0 mL with 1.0% (w/v) Triton X-114 and 0.5 mol L^{-1} NaCl. After phase separation, a 100.0-μL aliquot was submitted to cleanup with the silica gel and Florisil columns in series, eluting with 10 mL of 1 : 1 methanol : hexane. After evaporation of the solvent from the eluate, the residue was taken up in 1.0 mL of methanol.

Table 1. Figure of merits for the liquid–liquid extraction and cloud-point extraction of disulfoton from surface water samples ($n = 3$).

	LLE	CPE
Sample volume (mL)	10	40
Eluent volume (mL)	30	10
Eluents	Dichloromethane	Methanol : hexane (1 : 1)
LOD ($\mu g\,L^{-1}$)[a]	50	1.2
LOQ ($\mu g\,L^{-1}$)[a]	165	3.9
Sensitivity ($ng\,L^{-1}$)	–	18.2
Recovery (%)	90.8	93.8
Repeatability (%RSD)[b]	–	3.4
Intermediate precision (%RSD)[c]	–	4.9

[a]After concentration factor.
[b]Performed at three different disulfoton concentrations, one, three, and ten times the LOQ.
[c]Waters samples fortified with disulfoton concentrations of three times the LOQ.

An analytical curve of disulfoton was constructed in the range from 3.9 to 150 $\mu g\,L^{-1}$. A linear relation between peak area and disulfoton concentration was obtained with a correlation coefficient (r) of 0.998. The precision of the method, evaluated as the relative standard deviation (RSD) over three different days with three replicates per day, showed acceptable values for disulfoton recovery (RSD = 4.9%). The repeatability for six samples on the same day, for samples fortified with three different disulfoton concentrations, one, three, and ten times LOQ (4.0, 12.0, and 40.0 $\mu g\,L^{-1}$), was RSD = 3.4% (table 1). The accuracy of the method was evaluated by comparison of the results obtained with CPE and with liquid–liquid extraction (LLE) (table 1). The figures of merit for the cloud-point methodology were obtained using the parameters proposed by the International Conference on Harmonisation (ICH) [26, 27].

To obtain a disulfoton recovery by LLE equivalent to that obtained by CPE, using an aqueous sample volume of 40 mL, the volume of organic extractant should be 120 mL (three times 40 mL). This value is 12 times higher than that employed in CPE (10 mL of methanol : hexane), reducing the use of toxic solvent with this technique, in relation to LLE.

The limit of detection (LOD) was calculated as three times the signal-to-noise ratio. The LOD encountered was 1.2 $\mu g\,L^{-1}$. The limit of quantification (LOQ), calculated as ten times the signal-to-noise ratio, was 3.9 $\mu g\,L^{-1}$. The high concentration factor of the CPE provides excellent detectability for disulfoton determination, despite the fact that the use of a flame ionization detector (FID) yields a lower sensitivity for these structures. These LOD and LOQ values are much lower than those obtained using LLE, 50 and 165 $\mu g\,L^{-1}$ for LOD and LOQ, respectively, with the same GC-FID system. The disulfoton standard solution stability was evaluated during the process and no changes in potency were noted after 2 weeks for refrigerated solutions, as no new impurities, at levels equal to or greater than the LOD, were noted in the chromatograms.

3.5 *Cloud-point extraction of disulfoton from surface waters*

The procedure developed was used for extraction and determination of disulfoton content in fortified surface water samples (collected from the São Bartolomeu River,

Table 2. Disulfoton recoveries from real surface water samples ($n = 3$).

	Disulfoton amount ($\mu g\,L^{-1}$)		
Sample	Added	Found	Recovery (%)
São Bartolomeu river	0	nd	–
	5.0	4.7 ± 0.1	94.0
	15.0	14.3 ± 0.1	95.3

[a]nd: not detected or concentration level below LOD.

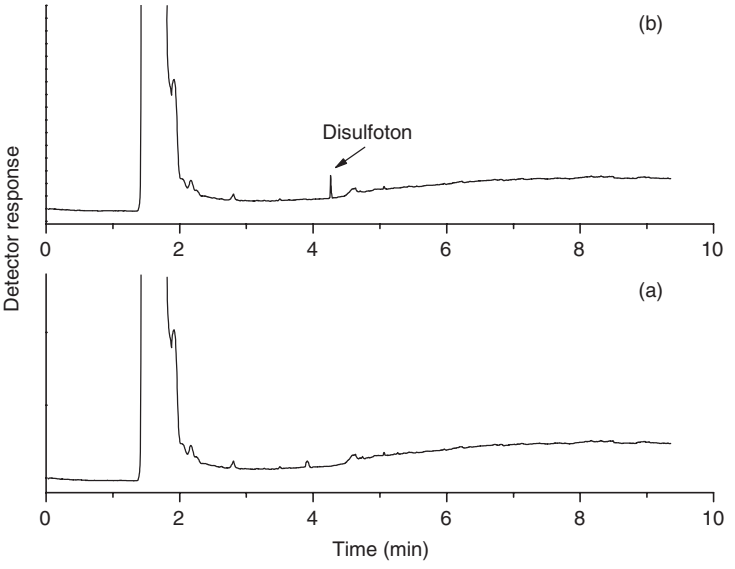

Figure 7. Chromatograms of (a) non-fortified and (b) fortified ($5.0\,\mu g\,L^{-1}$ of disulfoton standard solution) real water samples after extraction by the cloud-point methodology (1.0% Triton X114).

Viçosa, MG, Brazil) in order to test its applicability. The results are described in table 2, and chromatograms of extracts of a fortified ($5.0\,\mu g\,L^{-1}$ disulfoton standard solution) and a non-fortified water sample are presented in figure 7. The added disulfoton can be quantitatively recovered from water samples by the proposed procedure. Recoveries (%) of spiked concentrations (5.0 and $15.0\,\mu g\,L^{-1}$) from water samples were quantitative. These results demonstrate the applicability of the procedure for disulfoton determination in surface water samples.

4. Conclusions

This work showed that is possible, using an appropriate cleanup stage, to apply CPE as a step prior to pesticide analysis by GC without harming the capillary column and to obtain a good recovery. The parameters evaluated demonstrate that the cloud-point methodology is more efficient than conventional extraction methods, such as LLE. A principal advantage offered by the CPE technique is the use of significantly lower organic solvent quantities during the extraction process. Thus, besides reducing the cost

of the analyses, it also reduces impacts on the environment by such solvents. In addition, CPE is simple, easy to carry out, and economically viable.

Based on the success obtained with disulfoton, other classes of insecticides, fungicides, herbicides, etc. are currently under study using CPE and GC in our laboratory.

Acknowledgments

The authors thank Fundação de Amparo à Pesquisa do Estado de Minas Gerais and Coordenação e Aperfeiçoamento de Pessoal de Nível Superior, for financial support, and Prof. Carol H. Collins, for assistance with language.

References

[1] S.Y. Szeto, M.J. Brown. *J. Agric. Food. Chem.*, **30**, 1082 (1982).
[2] S.Y. Szeto, R.S. Vernon, M.J. Brown. *J. Agric. Food. Chem.*, **31**, 217 (1983).
[3] D.B. Fragoso, P. Jusselino-Filho, A. Pallini-Filho, C.A. Badji. *Neotrop. Entomol.*, **31**, 463 (2002).
[4] S.A. Mourão, E.F. Vilela, J.C. Zanuncio, L. Zambolim, E.S. Tuelher. *Neotrop. Entomol.*, **32**, 103 (2003).
[5] D. Sicilia, S. Rubio, D.P. Bendito, N. Maniasso, E.A.G. Zagatto. *Anal. Chim. Acta*, **392**, 29 (1999).
[6] P. Parrilla, J.L.M. Vidal. *Anal. Lett.*, **30**, 1719 (1997).
[7] M. Letellier, H. Budzinski. *Analusis*, **27**, 259 (1999).
[8] W.F. Ng, M.J.K. Teo, H.A. Lakso. *Fresenius J. Anal. Chem.*, **363**, 673 (1999).
[9] M. Correia, C. Delerue-Matos, A. Alves. *J. Chromatogr. A*, **889**, 59 (2000).
[10] D.A. Lambropoulou, V.A. Sakkas, D.G. Hela, T.A. Albanis. *J. Chromatogr. A*, **963**, 107 (2002).
[11] K. Mastovska, S.J. Lehotay. *J. Chromatogr. A*, **1040**, 259 (2004).
[12] W.L. Hinze, E. Pramauro. *Crit. Rev. Anal. Chem.*, **24**, 133 (1993).
[13] R.C. Martinez, E.R. Gonzalo, M.G.G. Jimenez, C.G. Pinto, J.L.P. Pavon, J.H. Mendez. *J. Chromatogr. A*, **754**, 85 (1996).
[14] C.G. Pinto, J.L.P. Pavon, B.M. Cordero. *Anal. Chem.*, **67**, 2606 (1995).
[15] S.R. Sirimanne, J.R. Barr, D.G. Patterson, L. Ma. *Anal. Chem.*, **68**, 1556 (1996).
[16] B. Froschl, G. Stangl, R. Niessner. *Fresenius J. Anal. Chem.*, **357**, 743 (1997).
[17] F.H. Quina, W.L. Hinze. *Ind. Eng. Chem. Res.*, **38**, 4150 (1999).
[18] R.C. Martinez, E.R. Gonzalo, B.M. Cordero, J.L.P. Pavon, C.G. Pinto, E.F. Laespada. *J. Chromatogr. A*, **902**, 251 (2000).
[19] R.C. Martinez, E.R. Gonzalo, J.D. Alvarez, C.G. Pinto, J.H. Mendez. *J. Chromatogr. A*, **1005**, 23 (2003).
[20] C.P. Sanz, R. Halko, Z.S. Ferrera, J.J.S. Rodriguez. *Anal. Chim. Acta*, **524**, 265 (2004).
[21] R. Halko, C.P. Sanz, Z.S. Ferrera, J.J.S. Rodriguez. *Chromatographia*, **60**, 151 (2004).
[22] P.D. Zygoura, E.K. Paleologos, K.A. Riganakos, M.G. Kontominas. *J. Chromatogr. A*, **1093**, 29 (2005).
[23] T.I. Sikalos, E.K. Paleologos. *Anal. Chem.*, **77**, 2544 (2005).
[24] www.inchem.org/(accessed 13 November 2005).
[25] G.K. Hiller, N. Calkins, R. Wandruzka. *Langmuir*, **12**, 916 (1996).
[26] International Conference on Harmonisation (ICH). *Validation of Analytical Procedures: Definitions and Terminology*, Q2A (CPMP/ICH/381/95) (1995).
[27] International Conference on Harmonisation (ICH). *Validation of Analytical Procedures: Methodology*, Q2B (CPMP/ICH/281/95) (1995).