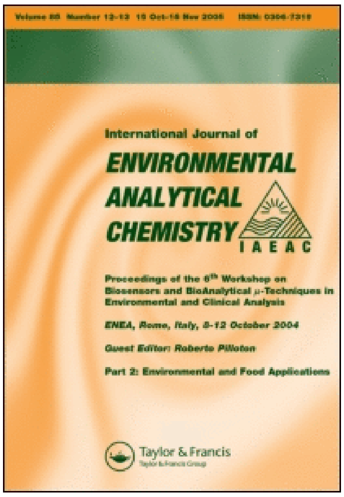

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Multiresidue Analysis of Pesticides in Water from Rice Cultures by On-line Solid Phase Extraction Followed by LC-DAD

Teresa C. R. Santos[a]; Julio C. Rocha[b]; Damia Barceló[c]
[a] Department of Chemistry, Fed Univ. of Maranhão (UFMA), São Luís - MA, Brazil [b] Department of Analytical Chemistry, Univ. Est. Paulista (IQ/UNESP), Araraquara - SP, Brazil [c] Department of Environmental Chemistry, CID-CSIC, C/Jordi Girona, Barcelona, Spain

To cite this Article Santos, Teresa C. R. , Rocha, Julio C. and Barceló, Damia(1998) 'Multiresidue Analysis of Pesticides in Water from Rice Cultures by On-line Solid Phase Extraction Followed by LC-DAD', International Journal of Environmental Analytical Chemistry, 70: 1, 19 — 28

To link to this Article: DOI: 10.1080/03067319808032601

URL: http://dx.doi.org/10.1080/03067319808032601

Intern. J. Environ. Anal. Chem., Vol. 70(1-4), pp. 19-28
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MULTIRESIDUE ANALYSIS OF PESTICIDES IN WATER FROM RICE CULTURES BY ON-LINE SOLID PHASE EXTRACTION FOLLOWED BY LC-DAD

TERESA C. R. SANTOS[a], JULIO C. ROCHA[b] and DAMIA BARCELÓ[c*]

[a] *Department of Chemistry, Fed Univ. of Maranhão (UFMA), Av. Portugueses, s/n°., 65. 080–040, São Luís – MA, Brazil,* [b] *Department of Analytical Chemistry, Univ. Est. Paulista (IQ/UNESP), Cx. Postal 355, 14.800–400, Araraquara – SP, Brazil and* [c]*Department of Environmental Chemistry, CID-CSIC, C/ Jordi Girona, 18–26, 08034, Barcelona, Spain*

(Received 5 July, 1997; In final form 16 October, 1997)

An automated on-line solid phase extraction procedure followed by liquid chromatography with diode array detection was investigated for the determination of different classes of pesticides in water samples containing varied amount of humic substances. The different pesticides used were: carbendazin, carbofuran, atrazine, diuron, propanil, molinate, alachlor, parathion-ethyl, diazinon, trifluralin and the degradation products deisopropylatrazine and deethylatrazine. Humic substances extracted from a Brazilian sediment were used from 5 to 80 mg/l and their influence on recoveries was evaluated in neutral and acidic media. Recoveries higher than 70 % were obtained for all the pesticides, from the preconcentration of 75 ml of aqueous sample fortified at 2 ng/ml using precolumns packed with PLRP-S. Good recoveries were obtained at neutral pH for most of the analytes up to 40 mg/l of humic acid. Only at 80 mg/l the recoveries were significantly affected, both at acidic and neutral pH. The method was applied to the determination of pesticides in river water spiked at 0.1 to 1 ng/ml. Detection limits obtained for water containing 10 mg/l of humic acid were between 0.05 and 0.3 ng/ml.

Keywords: Pesticides; humic acids; water; solid phase extraction

INTRODUCTION

The potential contamination of source water by pesticides used in agriculture are often subject of research. Liquid chromatography (LC) associated to an enrichment step through solid phase extraction (SPE) sorbents is generally the method

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* Corresponding author. Fax: +34-93-4006100 . E-mail: dbcgam@cid.csic.es

of choice for the determination of thermolabile, non-volatile and polar pesticides that are not suitable for gas chromatography analysis[1–3]. The advantage of automation renders the method particularly attractive due to swiftness of complete analysis of the sample, no risk of losses or contaminations and higher stability of pesticides trapped on the sorbent when compared to pesticides stored in water[4–5]. In this work apolar copolymer PLRP-S, which was frequently used for a variety pesticide classes[1–3,6], have been used for the on-line preconcentration followed by LC – diode array detection (DAD) of pesticides commonly applied in rice tropical cultures. However, the non-selectivity of the sorbent permits the extraction of other substances which are present in the sample and the study of possible matrix interferences is imperative.

The humic substances present in natural water are the major contributors of dissolved organic carbon in the ecosystem. It has been shown that the preconcentration of pesticides by SPE can be significantly affected by the presence of humic substances[7–9]. The dissolved organic carbon (DOC) may saturate the sorptive sites of the sorbent, as in the case of preconcentrating trifluralin, diazinon and chlorpyrifos from water samples containing up to 10 mg/l of humic substances[10]. This was related to the log K_{oc} of these pesticides, that varied from 4.7 – 5. Recoveries were lower than 60% with water containing humics as compared with water without humics and consequently an interaction between pesticide and the humic acid complex that it is not efficiently extracted was postulated. In the case of on-line SPE systems interfering compounds are absorbed together with the analytes so that large interfering matrix peak appears somewhere in the chromatogram, depending on the gradient. In general, the hydrophobic adsorption of the pesticide depends on both the total organic carbon content of the sample and its desorption constant, which is proportional to the n-octanol water partition coefficient. In the present study we used compounds which have relatively high K_{oc} (trifluralin, diazinon, parathion), so the same hydrophobic interactions could be expected.

Most of the work reported in the literature thus far on the recovery of pesticides spiked in water containing humic substances has been carried out with commercially available humic acids. However, humic and fulvic acids have their structures directly dependent on the site in which they were conceived. The lack of studies with natural humic substances isolated from the environment prompted us to undertake the present work. The objectives of the present work were: (i) to investigate the effect of natural humic substances isolated from a Brazilian sediment spiked in water at levels varying from 5 up to 80 mg/l together with pesticides commonly used in rice agriculture, both common to Spain and Brazil, (ii) to find out the best working range of pesticide concentrations that are found in natural environment, volume and pH of the water at different concentrations of humic substances studied by using an automated on-line solid phase extraction system with OSP-2 cartridges filled with PLRP-S and followed by liquid chromatography with diode array detection (LC-DAD). To our knowledge such type of study has not been undertaken up till now. These studies not only help in understanding the role of humic substances in the aquatic environment but also contributes for a better understanding of their influence on the analysis of pesticides in natural water.

EXPERIMENTAL

Apparatus

A model HP 1090 liquid chromatography (Hewlett-Packard, Palo Alto, CA) equipped with a ternary solvent-delivery system and HP 1090 photodiode array detector was used for direct injection and precolumn elution. Trace enrichments were carried out on 10 × 2 mm I.D. disposable precolumns of the OSP-2 (Merck, Germany) connected to the L-6200A Intelligent Pump (Merck-Hitachi).

Stationary phases and chromatographic conditions

The analytical column was a 15 cm × 4,6 mm I.D. packed with 5 μm octadecylsilica Ultracarb 5 ODS-30 (Phenomenex, Torrance, California). The cartridges used for the extraction were manually prepacked with PLRP-S (Polymer Laboratories, Church Stretton, UK), a styrenedivinylbenzene copolymer with 20 μm of particle size, 300 Å porosity size, specific surface area around 500 $m^2.g^{-1}$ and porous volume around 0.62 $ml.g^{-1}$, providing high porosity and adsorption capacity as expected, besides having the wide pH range, from 1 to 13.

Chemicals

Solvents were HPLC-grade (Merck, Darmstadt) filtered through 0.45 μm membrane filters (Millipore, MA, USA) and degassed for 15 minutes. Stock solutions of 1000 ng/μl in methanol were prepared and stored at 4°C using pesticides with minimum purity of 95% obtained from Riedel de Häen (Hannover, Germany), Dr. Ehrenstorfer (Augsburg, Germany), PolyScience (IL, USA) or Chem Service (West Chester, Pennsylvania). Diluted solutions were prepared in acetonitrile and were found to be stable at least for a week, under refrigeration. Anionic resin Amberlite IRA-400 and cationic resin Dowex 50X used to purify humic substances were obtained from Merck. The resins were repeatedly used after cleaning as follow: heating in KOH 0.5 mol/l up to 70°C and addition of H_2O_2 30 % (5 ml) with stirring during 30 minutes; after achieving ambient temperature the resins were continuosly washed with water and stored in HCl 0.5 mol/l (cationic) or NH_4OH 2 mol/l (anionic) until their use.

Humic acid extraction

The humic substances were extracted from the sediments of a Brazilian estuary (Cananeia, São Paulo, Brazil). The extraction was carried out by shaking dried sediment in NaOH 0.5 mol/l for 4 hours under N_2. After centrifugation at 3000 rpm for 15 min to remove humin and other insoluble constituents the humic acid was precipitated by acidifying at pH 2 with HCl 6 mol/l and isolated by centrifugation at 3000 rpm for 30 min. The humic acid was then dissolved in dilute ammonium hydroxide and purified passing through anionic and cationic resins in sequence. The purification procedure was repeatedly carried out until ash content around 5 %. The humic acid was then freeze-dried. The elemental analysis showed 33.09 %C, 4.67 %H, 3.54 %N and 2.41 %S.

On-line SPE-HPLC procedure

The precolumns were first conditioned with 5 ml of methanol and 5 ml of water at a flow rate of 1 ml/min. A volume of 75 ml of the sample was then preconcentrated at a flow rate of 2 ml/min. After the extraction the precolumn was washed with 1 ml of HPLC-grade water at 1 ml/min. The desorption step was performed using a gradient with acetonitrile and water, both containing 0.1 % (v/v) of acetic acid while maintaining the pH 3 during the elution. The gradient applied to elution was 5 to 100 % of acetonitrile over 45 min to separate compounds with a broad range of polarity, back to initial conditions in 5 min and post-run of 10 min, with the flow rate at 1 ml/min.

RESULTS AND DISCUSSION

Breakthrough volumes and calibration conditions

The pesticides included in this study belong to different groups such as triazine, two of its degradation products which are very stable, carbamate, phenylurea, organophosphorous, dinitroaniline, benzimidazole and chloroacetanilide with distinct physical properties. In order to determine the appropriate volume that can be percolated through the precolumn without significant losses of these pesticides three replicates of different volumes of Milli-Q water spiked at a constant amount of each pesticide (200 ng) were percolated. A linear regression was carried out using recoveries versus percolated volumes. Afterwards a recovery of 80 % was used for determining the corresponding breakthrough volumes, as described in Ref. (11). The Table I presents the values obtained. As can be seen, early breakthrough volume was observed for deisopropylatrazine, which has been reported in previous works[1,12]. At the sample volume selected (75 ml) satisfactory recoveries for all pesticides were observed, although breakthrough values higher than 300 ml were obtained for the most of pesticides determined. The advantage in the use of on-line systems was observed by same-day precision, determined from five repetitions at the concentration of 1 ng/ml. Relative standard deviation (R.S.D.) less than 0.4 % for the retention times and less than 10 % for the peak areas were presented. The exception was diazinon, which shows a poor absorption at that wavelength used for detection, presenting a R.S.D. of 12 % for the peak areas. Direct injection of corresponding amount of pesticides showed R.S.D of 1.5 and 3 % for retention times and peak areas, respectively. Day-to-day repeatability were also determined and the R.S.D.obtained were less than 1% for the retention time and less than 11 % for the areas for reproducibility in four following days. Calibration plots with nine data points for spiked Milli-Q and estuarine water from Ebre River (Tarragona, Catalunya) were drawn. Linearities were observed within the range from 0.1 to 5 ng/ml, with typical correlation coefficients around 0.99, except for deisopropylatrazine and trifluralin, respectively the most polar and apolar selected compound, which showed R^2 of 0.96. Since limits of detection can suffer increase when using natural water due to interferences LODs at ratio signal to noise of 3 were calculated for five pesticide and 10 mg/l of purified humic acid. The LODs varied from 0.05 to 0.3 ng/ml and did not differ significantly from the results obtained for water without humic substances.

TABLE I Some characteristics of the pesticides and degradation products. Solubility according to ref. (14) and (15); octanol-water partition coefficient. log K_{ow}. according (5) and (13); sorption soil coefficient. log K_{oc}. and hydrolisis half life in soil (a) or water (b), DT_{50}, according ref. (14) and (15). Breakthrough volumes using PLRP-S were calculated from 200 ng of each pesticides and compared by direct injection of the same amounts of the compounds

Pesticides	*Class*	*Wavelength (nm)*	*Log K_{ow}*	*Log K_{oc}*	*Solubility ($mg.l^{-1}$)*	*DT_{50} (days)*	*V_b (ml)*
1-Carbendazin (F)	**benzimidazole**	210	1.40 – 1.52	2.11	8	5.2 (a)350 (b)	> 300
2- Deisopropylatrazine	**triazine's DP**	210	0.30 – 1.10	-	3200	-	35
3- Deethylatrazine	**triazine's DP**	210	0.70 – 1.50	1.69	670	-	> 300
4- Carbofuran (I)	**carbamate**	220	1.30 – 1.63	1.34	320	50 (a)121 (b)	200
5- Atrazine (H)	**triazine**	220	2.20 – 2.75	2.09	33	50 (b)	> 300
6- Diuron (H)	**phenylurea**	210	2.68	2.66	42	64 (b)	> 300
7- Propanil (H)	**propionalide**	210	2.80	2.17	130	1 (a)365 (b)	> 300
8- Molinate (H)	**thiocarbamate**	215	3.21	2.28	88	21 (a)	260
9- Alachlor (H)	**chloroacetanilide**	220	2.80	2.07	242	>30 (a)	180
10- Parathion-ethyl (I)	**organophosphorous**	220	2.15 – 3.93	3.70	11	14 (a)260 (b)	> 300
11- Diazinon (I)	**organophosphorous**	220	3.11 – 3.81	2.43	60	23 (a)185 (b)	> 300
12- Trifluralin (H)	**dinitroaniline**	220	3.97 – 5.10	3.81	0.22	170 (a)	> 300

(H) Herbicide; (I) Inseticide; (F) Fungicide; (DP) Degradation Product**(a) half life in soil(b) half life in water at pH 7.0 and 22°C**

Influence of humic substances

The analysis of pesticides by SPE/LC can be seriously affected by the presence of humic substances and the alteration can be dramatic when using SPE systems in which the amount of sorbent is limited. The available sites of the sorbent can interact either with the pesticide molecule and/or the humic substance. So, the competition will generally depends on the pH and concentration of humic substances. Since the large humic molecules have different configurations depending on the pH, the possible effect of co-extraction was evaluated with 75 ml of water samples containing 10 mg/l of humic acid and spiked at 2 ng/ml of each pesticide at different pH (Figure 1). The water samples had been adjusted to pH 3, 5 and 7 with diluted $HClO_4$ or NaOH, when it was necessary. As can be seen at pH 3 the co-extraction was more intense and this can be ascribed to the fact that at pH 7 the humic molecule has a larger charge (pK_a of humic acid between 3 and 5) and this makes difficult the extraction of the humic substance by the sorbent[9]. At pH 5, the commonly presented pH value for natural water containing high humic concentration, the co-extraction effect was also less accentuated. The Table II shows the recoveries at pH 3 and pH 7 and the results suggest that at acidic pH the co-extraction of humic substances makes difficult the determination of various pesticides cited in this work.

It has been observed[16] that humic acid had less effect than salinity when using Empore disks for the extraction of some of the pesticides used in this work. Previously, Johnson[10] showed the influence of Aldrich humic substances in the recovery of diazinon and parathion with a recovery less than 60 % using C18 cartridges. The difference in the recoveries could be attributed not to the amount but preferably to the type of sorbent used, since C18 can offer free silanols that can be much more easily saturated by the humic substances than the polymeric material PLRP-S. Our results agree in many instances to those found out by Senseman et al[17], that studied the effect of Aldrich humic substances up to 25 mg/l for common pesticides studied in this paper like atrazine, propanil and trifluralin, at pH 6 and 8. The data of Senseman showed that at lower pH the more non polar environmental permit that the more hydrophobic form of the polymer can bind hydrophobic compounds more effectively through association with uncharged protons of the humic polymer. The data found by Senseman and also our data suggest that when the water sample exhibits a pH 7 the extraction efficiency probably would not be significantly reduced. In this paper we have shown that even the addition of up to 40 mg/l does not affect the recovery of the pesticides studied. Concentrations of humic acid at 80 mg/l were also used because in tropical rivers of the Amazon area the amounts can be high a enough. In that case the determination of certain pesticides depends highly on the humic material. At lower pH the recoveries are generally very poor. So the use of higher pH is recommended as compared to lower pH to avoid interferences with humic substances and even at much high concentration of humics, the recoveries of six out of twelve pesticides is above 73%.

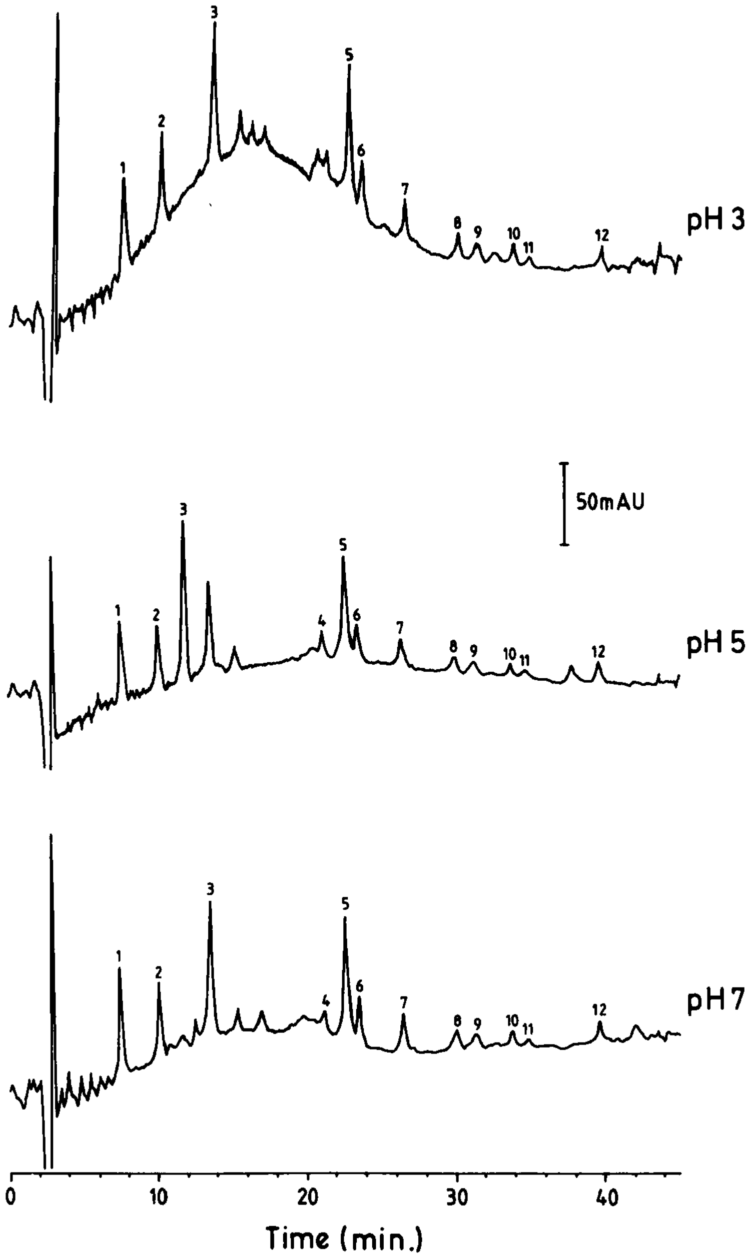

FIGURE 1 Effect of the co-extraction of humic acid (10 mg/l) at different pH on the preconcentration of 75 ml of water spiked at 2 ng/ml of each pesticide. For peak numbers, see tables

TABLE II Percent recovery of the pesticides at 2 ng/ml preconcentrated in water containing humic acid at different pH samples and limits of detection obtained (a) without and (b) with 10 mg/l of humic acid

Humic Acid	*5 mg/l*		*10 mg/l*		*40 mg/l*		*80 mg/l*		*LOD(a) (ng/ml)*	*LOD(b) (ng/ml)*
Pesticide	*pH 3.0*	*pH 7.0*	*pH 3.0*	*pH 7.0*	*pH 3.0*	*pH 7.0*	*pH 3.0*	*pH 7.0*		
1-Carbendazim	70	95	75	94	34	94	70	82	0. 2	0.3
2-Deisopropilatrazine	47	68	54	50	nq	40	40	45	0.1	0.3
3-Deethylatrazine	100	95	87	95	62	58	78	95	0.05	0.01
4-Carbofuran	72	59	120	119	nq	nq	nq	117	0.2	0.3
5-Atrazine	87	87	93	101	64	73	91	101	0.05	0.03
6-Diuron	89	99	73	86	nq	100	80	104	0.2	0.2
7-Propanil	89	117	75	113	101	44	59	87	0.05	0.05
8-Molinate	96	85	109	123	nq	51	117	70	0.05	0.07
9-Alachlor	86	103	90	85	nq	47	107	54	0.05	0.08
10-Parathion-ethyl	94	101	102	91	nq	105	104	98	0.1	0.1
11-Diazinon	84	120	83	100	nq	94	95	119	0.2	0.3
12-Trifluralin	78	69	83	69	nq	88	74	113	0.2	0.1

nq: not quantified

CONCLUSIONS

The recoveries of a variety of pesticides used in rice cultures were studied for different amounts of humic substances extracted from a Brazilian environment from 5 up to 80 mg/l and using varied pH of 3, 5 and 7. The use of neutral pH is recomended in all cases and showed good recovery values up to 40 mg/l of humic substance. Only when the contents of humic acid are 80 mg/l the recoveries were lower but which were acceptable for 50% of the pesticides. However, such high amounts of humic acid of 80 mg/l is very unlikely to occur in many natural environments. So the methodology presented in this paper allows the determination of distinct classes of pesticide in the presence of humic acid up to 40 mg/l, with detection limits between 0.05 and 0.3 ng/ml for water containing 10 mg/l of humic acid.

Acknowledgements

This work was supported by the Commission of the European Community (Contract No. ENVY - CT96 – 0333) and CICYT (AMB97 – 1597 – CE). T. C. Santos acknowledges the grant from CAPES (BEX – 1843/95–6), a Brazilian research support.

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