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On-line liquid chromatographic trace enrichment and high-performance liquid chromatographic determination of diquat, paraquat and difenzoquat in water

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Abstract

For the trace-level determination of diquat, paraquat and difenzoquat in drinking and surface water, a fully automated method using on-line trace enrichment, LC separation and UV detection is described. An automatic sample preparation device (OSP-2, Merck) was employed as the sample preparation unit. The three herbicides were trapped from the flowing water sample by adsorption on a silica cartridge placed in a circular cartridge magazine, then the load cartridge was moved to the elution side. On the elution side, the herbicides were transferred to the analytical column by the mobile phase. Detection limits for the three herbicides below 20 ng/l were obtained after preconcentrating 200 ml of drinking water. The total analytical procedure was applied for the monitoring of the three herbicides in drinking and surface water.

Keywords: Trace enrichment; Solid-phase extraction; Diquat; Paraquat; Difenzoquat; Pesticides

1. Introduction

The widespread use of bipyridylium herbicides in agricultural applications such as pre-harvest non-selective weed control means that these compounds may be present as residues in surface water owing to their persistence and polar character [1,2]. They are included in a priority list of herbicides of potential concern established for the Mediterranean countries by the European Union (EU), in which the selected criteria are based on the availability of usage data and the consideration of half-lives [3]. Some of these surface waters are supplied as drinking water, and because of this it is necessary to screen them for contamination by these herbicides.

The determination of trace amounts of diquat, paraquat and difenzoquat in natural and drinking waters requires a preconcentration step before high-performance liquid chromatographic (HPLC) analysis [4–6]. Solid-phase extraction (SPE) techniques are frequently used for the concentration of pesticides (including bipyridylium herbicides) from water prior to chromatographic analysis [7–9].

Nowadays, SPE methods used off-line can be converted into on-line SPE methods by direct connection of the precolumn to the analytical column via switching valves. The concentrated analytes are then directly desorbed and trans-

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ferred to the analytical column by a water–organic solvent gradient at the same time as they are separated [10–12]. Such systems often involve microprocessor control of the stages for sample switching and flushing of solvents and eluents through the concentrator and chromatographic columns.

The recent commercialization of automatic devices and sensitive variable- and programmable-wavelength detectors will certainly help in the development of on-line trace enrichment methods in environmental analysis [13]. In this study, a commercially available device for on-line coupling SPE to HPLC, and OSP-2A (Merck, Darmstadt, Germany), was applied to the determination of diquat, paraquat and difenzoquat in natural and drinking waters. Special attention was paid to the selection of the experimental parameters of the preconcentration step depending of the detection level required (less than 1 μg/l for each herbicide in surface water or 0.1 μg/l in drinking water) [14].

2. Experimental

2.1. Reagents

Diquat, paraquat and difenzoquat were supplied by Promochem (Ausburg, Germany). Individual standard solution were prepared by dissolving 100 mg of each compound in 100 ml of methanol. A composite standard solution was prepared by diluting 1000-fold 50-μl samples of each compound with distilled water to a final volume of 50 ml.

Tetramethylammonium hydroxide (TMAOH) was purchased from Fluka (Buchs, Switzerland) and ammonium sulphate from Panreac (Barcelona, Spain). Methanol (gradient grade) was obtained from Merck.

For HPLC, all the solutions and solvents were filtered through a 0.45-μm filter in a Waters–Millipore (Milford, MA, USA) system.

2.2. Apparatus

A LaChrom L-7100 two-pump system, an OSP-2A automatic sample preparatorion device, a LaChrom L-7400 programmable variable-wavelength detector and a D-2500 integrator, all from Merck–Hitachi, were used.

The preconcentration columns were LiChrospher 60 cartridges filled with 20–30-μm silica for the OSP-2A device and the analytical column was Spherisob SW3 (Pessac, France) (3 cm × 4.6 mm i.d.) filled with 3-μm particle size silica. A silica guard cartridge (1 cm × 4.6 mm i.d, 5 μm) was used to prevent the deterioration of the analytical column.

For the separation of the bipyridylium herbicides selected, gradient elution was performed. Solvent A was a solution of 2 g of TMAOH and 30 g of ammonium sulphate in 1 l of water, adjusted to pH 3 with 5 *M* sulphuric acid; solvent B was methanol. The initial mobile phase composition was 100% solvent A, linearly programmed to 50% A after 15 min and maintained there for 10 min. The eluted compounds were monitored with the UV detector set initially at 310 nm for diquat, after 6 min at 260 nm for paraquat and after 12 min at 255 nm for difenzoquat. The flow-rate of the mobile phase was 0.5 ml/min.

2.3. Operation

A scheme of the system detailing each component is shown in Fig. 1. Basic operation conditions were valve 1 and 2 in position one and the clamp open. One of the HPLC pumps provided time-controlled no-voltage switching contacts (time events). The pump time programme controlled all OSP-2A functions and the HPLC pump. Two independent controllable switching valves (valves 1 and 2) and the clamp device for the extraction cartridge were operated pneumatically. The circular cartridge magazine can hold up to 72 extraction columns.

The trace-enrichment cartridges do not need to be preconditioned. Different volumes of water samples (depending of the kind of water) were passed through the cartridge in the enrichment side. Interfering matrix compounds of the samples were removed with 5 ml of distilled water. Desorption was performed by coupling the cartridge on-line with the analytical column and starting the gradient. Gradient elution was per-

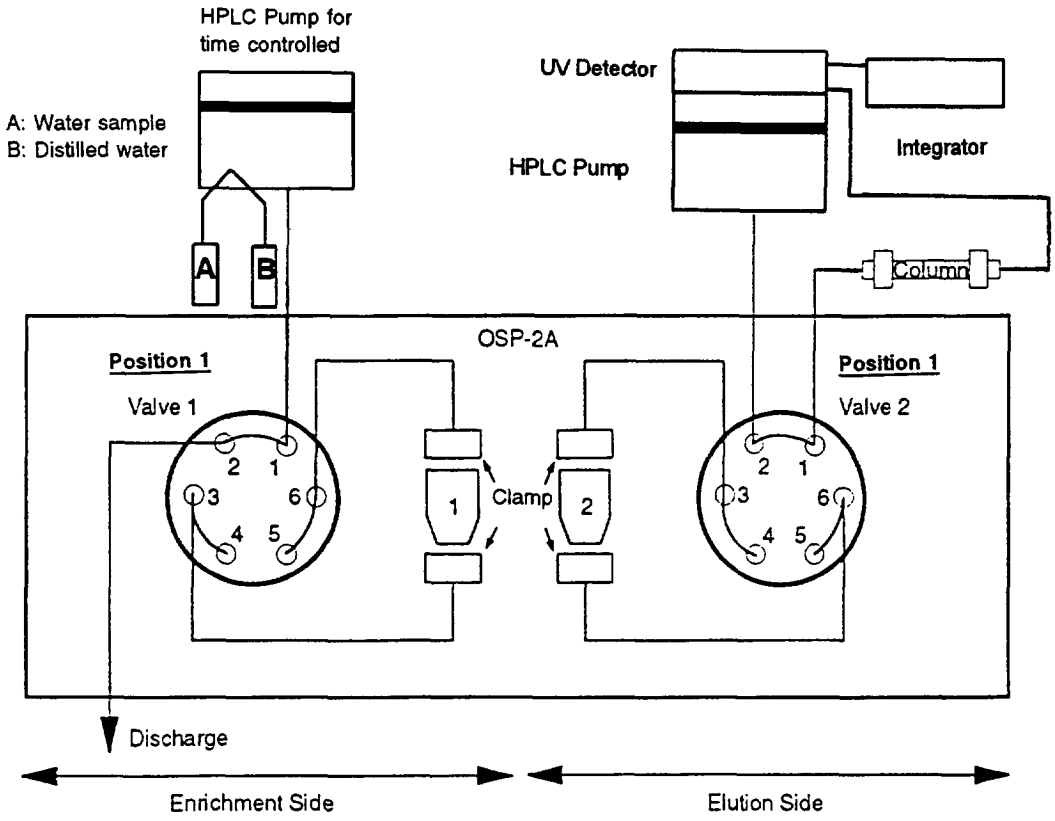

Fig. 1. Scheme of the system used.

formed in the opposite direction to the sample preconcentration. The OSP-2A device allows sample concentration through the cartridge of the enrichment side, while the previously prepared cartridge on the elution side is integrated into the HPLC analysis. The same cartridge was used in all the recovery experiments and no accumulation effects were observed. In Table 1 the sample preparation programmes are listed.

3. Results and discussion

The choice of the HPLC system is very important to obtain the optimum sensitivity and selectivity. The HPLC system used here was selected in previous work [15] and the basis of the method is the compatibility of the enrichment column with the analytical column.

The linearity of the UV detector response for diquat, paraquat and difenzoquat was tested by injecting 20 μl of solutions of a mixture of the three compounds at the same concentrations, in which the amount of herbicides varied from 5 to 4000 ng. The response of the UV detector to the herbicides was linear up to at least 4000 ng with a minimum detectable amount of ca. 1 ng without trace enrichment.

The breakthrough is the key parameter in SPE because it indicates the sample volume and the amount of analyte that can be preconcentrated. Two factors can be responsible for breakthrough: insufficient retention of the analytes by the sorbent and overloading of the sorbent. An experimental method for determining breakthrough volume has been developed [13]. The method consists in percolating through the column of a small volume (V_p) spiked with a known concentration (C_p) of each herbicide, then the sample volume is increased and the concentration decreased in order to have a constant value of $CpVp$ for the analytes in each sample volume

Table 1
Time schedule for automated SPE of aqueous samples

Time (min)	V_1[a]	V_2[a]	Flow-rate (ml/min)	Comment
0.0	1[b]	1	0.000	Cartridge clamp closed
0.1	1	1	2.500	Start HPLC water pump; flush capillaries with water
1.0	2[b]	1	2.500	Flush the silica column with HPLC water
2.0	2	1	–	Start to load sample; the time of loading sample depends on the sample volume passed and the flow-rate selected (this time can be called F)
F.1.	2	1	2.500	Flush the silica column with HPLC water to remove interfering compounds
F.1 + 2	1	1	0.000	Finish sample extraction and open the clamp
F.2 + 2	1	1	0.000	The magazine advances one position
F.3 + 2	1	2	0.000	The clamp closes. The cartridge is integrated into the analytical cycle. The initiates the actual analysis and starts the data analysis

[a] V_1 and V_2 indicate valves 1 and valve 2.
[b] Numbers 1 and 2 indicate the valve positions; position 1 is as in Fig. 1.

percolate. The results are plotted as peak area versus sample volume for diquat, paraquat and difenzoquat in Fig. 2; no breakthrough was observed up to a volume of 200 ml for a total amount of 20 ng.

A recovery study was conducted with laboratory-spiked samples. The working standard samples were prepared by diluting the composite standard solution with distilled water to concentrations between 0.1 and 20 μg/l. The sample volumes were varied from 5 to 200 ml. Recovery data are given in Table 2.

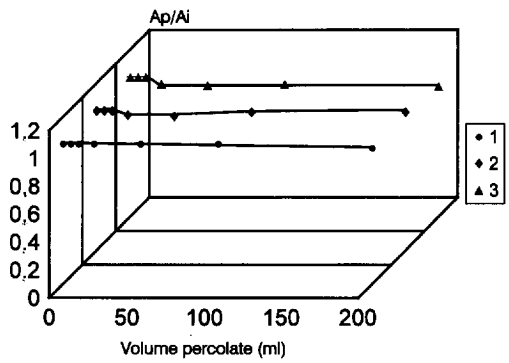

Fig. 2. Experimental breakthrough curve recorded with an enrichment cartridge filled with 80 mg of silica. Sample solution spiked with 1 μg/l of (1) diquat, (2) paraquat and (3) difenzoquat. Ap peak area of one analyte when percolating Vp; Ai peak area of one analyte in the first chromatogram without breakthrough.

The linearity of the total procedure was tested by trace enrichment of three selected herbicides from 50 ml of water over the concentration range 0.1–20 μg/l (four data points). The regression coefficients obtained were diquat 0.9990, paraquat 0.9984 and difenzoquat 0.9976. The precision found on preconcentrating the three herbicides at the concentrations shown in Table 2 ranged from 1.8% to 8.0% (R.S.D., $n = 12$).

The samples volume required to met the EU Directive of 0.1 μg/ml is 50 ml. Limits of detection (LODs) of diquat, paraquat and difenzoquat using on-line LC trace enrichment and HPLC for 200-ml water samples were about 20 pg/l, even when using natural waters. The water sample volume could easily be increased up to 2 l, improving the LODs even more.

Although the volume percolate for water samples fortified with 20 μg/l must be lower than 50 ml to obtain a 100% recovery, the volume percolate for water samples fortified with 0.1 μg/l of the three bipyridylium herbicides can be up to 200 ml to obtain 100% recovery. This indicates overloading of the cartridge with amounts up to 3 μg of a three-compound mixture.

Some workers [11–13] have reported that with concentrations of the order of μg/l the occurrence of breakthrough due to overloading of the sorbent capacity is improbable, but they always

Table 2
Recoveries of diquat, paraquat and difenzoquat from model solutions in distilled water

Pesticides in water (μg/l)	Flow-rate (ml/min)	Sample volume concentrated (ml)	Pesticide amount concentrated (ng)	Recovery (%) (mean of twelve determinations)		
				Diquat	Paraquat	Difenzoquat
20	2.5	5	100	102	100	101
		10	200	99	94	99
		20	400	96	99	84
		50	1000	87	86	63
		100	2000	79	55	32
		200	4000	30	25	18
4	2.5	5	20	102	101	100
		10	40	99	98	97
		20	80	98	101	99
		50	200	100	99	89
		100	400	87	87	89
		200	800	82	60	56
1	2.5	5	5	101	103	101
		10	10	99	98	98
		20	20	100	102	102
		50	50	98	99	100
		100	100	92	95	92
		200	200	89	87	84
0.1	2.5	50	5	100	103	102
		100	10	101	100	101
		200	20	99	99	95

referred the results to the use of apolar sorbents for the preconcentration step. No data were found referring to silica.

To study the effect of the flow-rate on the extraction yield, the rate in the trace enrichment column was varied from 2.5 to 7.5 ml/min. No differences were found in this range and a flow-rate of 5 ml/min was selected.

To demonstrate the feasibility of this method, a surface water sample from L'Albufera and a drinking water sample from València city were spiked at 1 and 0.1 μg/l, respectively. The water samples were kept for 24 h to allow the equilibration to different processes which take place in natural waters, such as the binding of bypiridylium herbicides with organic matter present [9]. They were then filtered and analysed as described above. The recoveries obtained from drinking water were similar to those obtained with distilled water. In surface waters a small decrease in the recoveries (ca. 8%) was observed. The cause could be the strong influence of the organic matter content on the efficacy of the enrichment process, as has been reported for these herbicides [9,15]. Fig. 3 shows the chromatograms obtained from drinking and surface waters and a comparison with those obtained from non-spiked water and with standards with the same amounts. Some band broadening of the analytes can be observed after preconcentration, but the peak shapes are still acceptable and allow precise quantitation. This is in agreement with data reported previously by several workers [11–13], who attributed the cause to the transfer of the analytes from the enrichment cartridge to the analytical column. Although in order to avoid this problem they recommended smaller dimensions of the preconcentration column than those of the analytical column, the particle size of the preconcentration column should be the same.

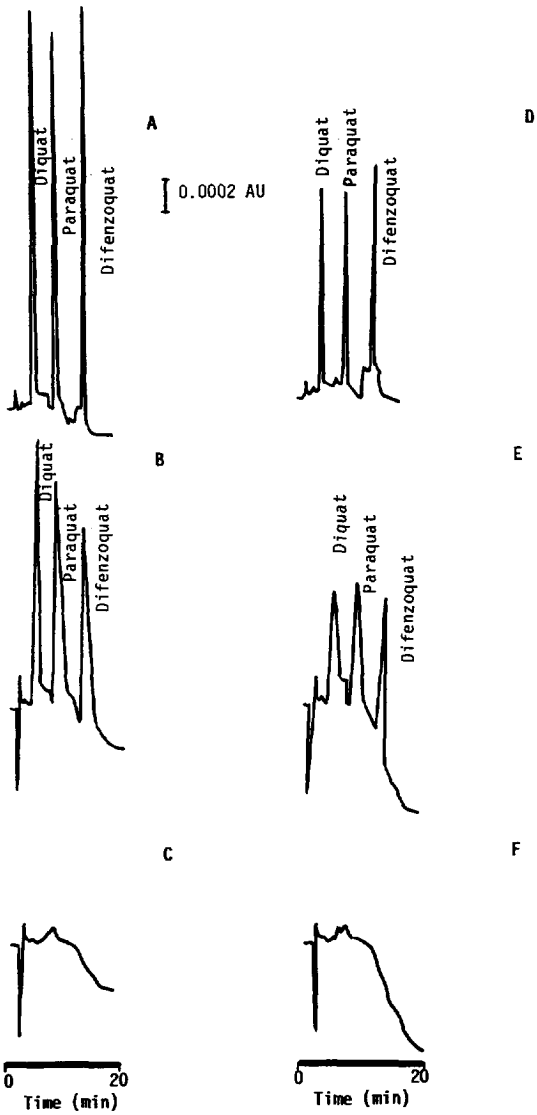

Fig. 3. SPE–LC–UV traces for (A) standard injection of 20-μl loop of a 1 μg/l solution, (B) 20 ml of concentrate of spiked natural water from L'Albufera lake at a level of 1 μg/l, (C) non-spiked natural water from L'Albufera lake, (D) standard injection of 20-μl loop of a 0.5 μg/l solution, (E) 100 ml of concentrate of spiked drinking water from València city at a level of 0.1 μg/l and (F) non-spiked drinking water from València city.

Unfortunately, it was impossible to obtain preconcentration columns with a smaller particle size and increasing the dimensions of the analytical column extends the analysis time considerably.

Table 3
Diquat, paraquat and difenzoquat levels found in the analysis of twelve surface water samples from L'Albufera lake

Sample No.	Pesticide found	Level (μg/l)
1	Diquat	0.8
	Paraquat	0.4
5	Paraquat	0.5
10	Paraquat	0.4
12	Difenzoquat	0.6

Twelve surface water samples from the Natural Park of L'Albufera (València, Spain) and four drinking water samples from different cities (València, Bétera, Villamartxant, Xilxes) were taken during the first week of April 1995. Volumes of 20 ml for surface water and 100 ml for drinking water were analysed by the standard procedure. Table 3 gives the pesticide concentrations in surface waters and Fig. 4 illustrates some of the chromatograms obtained; matrix interferences were not present.

The presence of these herbicides was detected in some of the surface water samples. Although the concentration was lower than 1 μg/l, their appearance demonstrates a real environmental impact. Diquat, paraquat and difenzoquat were not detected in drinking water.

All these experiments allowed us to check the performance of the system during a 2-month period. Within this period, the system was continuously switched on for 10–12 analyses per day. Hence in total over 600 samples were analysed, and during time the only problem encountered was when using non-filtered surface water. The analytical column did not deteriorate during the test period.

4. Conclusions

The method proposed represents an alternative procedure for determining bipyridylium herbicides in water and allows automation. The average of the on-line system to the off-line method is that it provides rapid access to information on water quality and allows a rela-

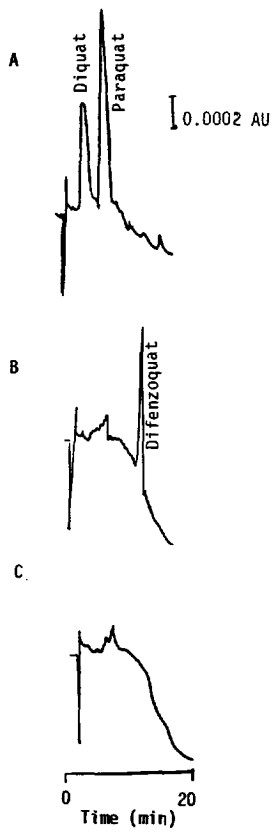

Fig. 4. Chromatograms obtained after concentrating 20 ml of (A) sample 1, (B) sample 12 and (C) negative sample (for sample concentrations, see Table 3).

tively high frequency of sampling. The system can be used for monitoring purposes.

Further work is in progress dealing with the use of other solid sorbents and with the study of the effects of the characteristics of the aqueous matrix and the presence in it of probably interfering compounds on the capability of a reconcentration cartridge to extract quantitatively the bipyridylium herbicides.

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