

# Influence of organic matter and surfactants on solid-phase extraction of diquat, paraquat and difenzoquat from waters<sup>1</sup>

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## Abstract

Analysis of diquat, paraquat and difenzoquat in water is performed using solid-phase extraction (SPE) on silica cartridges followed by high-performance liquid chromatographic (HPLC) analysis with a normal-phase column. UV detection with variable wavelength was used to determine each compound at its maximum of absorption providing an excellent sensitivity. Recoveries of these herbicides extracted from 1 l of drinking water spiked at levels between 0.1 and 25  $\mu\text{g/l}$  were higher than 85%. The limits of detection were under 0.1  $\mu\text{g/l}$ . The effect that the presence of organic matter and surfactants in natural waters has on the extraction procedure was studied. Both, surfactants and organic matter (as humic material), show a negative effect on the recoveries of the herbicides and so their content in natural waters must be controlled.

**Keywords:** Water analysis; Environmental analysis; Solid-phase extraction; Pesticides; Diquat; Paraquat; Difenzoquat

## 1. Introduction

Bipyridylum herbicides diquat (1,1'-ethylene-2,2'-bipyridylum), paraquat (1,1'-dimethyl-4,4'-bipyridylum) and difenzoquat (1,2-dimethyl-3,5-diphenyl-1H-pyrazolium), are important cationic pesticides used in agriculture. They are usually supplied as dibromide, dichloride and methyl sulphate salts, respectively. These quaternary ammonium compounds are cationic species in aqueous solution which are biologically active when they are applied

to plant foliage. After application, these chemicals can be adsorbed into the soil or transported to the aquatic environment by runoff or leaching.

These quaternary ammonium compounds show high aqueous solubility and low volatility. Because of these physical properties, paraquat, diquat and difenzoquat are usually determined by high-performance liquid chromatographic (HPLC) techniques. Most of these methods use  $\text{C}_{18}$  [1–4] or  $\text{C}_8$  [5] analytical columns and ion-exchange resins [6]. The use of silica analytical columns [7,8] has also been reported. This type of stationary phase is not often used for the analysis of these compounds because it requires organic solvents, such as hexane or dichloromethane as mobile phase.

Some of these HPLC methods are not sensitive enough to comply with the EU Directive (80/778/EC) which sets a maximum admissible individual

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concentration at 0.1  $\mu\text{g/l}$  for pesticides in drinking water.

To identify and quantify these pesticides in aqueous matrices, the aqueous samples must be extracted in order to minimize interferences and to improve the detection limits of the method. For these two purposes solid-phase extraction (SPE) of paraquat, diquat and difenzoquat with  $\text{C}_{18}$  [4], cyanopropyl [9] and silica columns [1,7,8] has been used.

At the extraction step, competitive interaction processes occur between the selected herbicides and the active site on the solid phase or the components of the sample matrix. The adsorption of analytes on sediments or on dissolved particles of the sample matrix may affect the efficiency of their preconcentration from water samples, especially when the samples are natural or waste waters.

Among the factors that may affect the SPE of paraquat, diquat and difenzoquat, organic matter and surfactants present in water samples must be considered. Although non-living organic materials in aquatic environments are composed of carboxylic acids, amino acids, carbohydrates, hydrocarbons and trace elements, humic substances are the most abundant [10]. Divalent cationic bipyridylium herbicides can react with two negatively charged sites (phenolic or carboxylic groups) of humic substances while the phenyl groups of difenzoquat can react with the non-polar part of the humic acids. Cationic, anionic, zwitterionic and non-ionic surfactants can be found in water as ubiquitous pollutants.

Humic substances and surfactants can affect the extraction process of the selected pesticides. The purpose of this work is to study the influence of these interfering compounds in the determination of these three cationic herbicides.

## 2. Experimental

### 2.1. Reagents and standards

The selected bipyridylium pesticides paraquat, diquat and difenzoquat were supplied by Promochem (Augsburg, Germany). Standards solutions (1000  $\mu\text{g/ml}$ ) were prepared in methanol and stored at 4°C. A working solution of 1  $\mu\text{g/ml}$  was prepared

by mixing 50  $\mu\text{l}$  of each standard solution and diluting to 50 ml with distilled water.

Humic acids were from Aldrich (Milwaukee, WI, USA) and the working solution was 10 mg/l in distilled water.

Cetrimide was obtained from Panreac (Barcelona, Spain), sodium dodecyl sulphate from Merck (Darmstadt, Germany) and lauryl sulphate (sodium tetradecyl sulphate), lauryl sulphobetaine [dodecyldimethyl(3-sulphopropyl)ammonium hydroxide inner salt], Brij 35, Triton X-100 and benzalkonium chloride from Aldrich. Tetramethylammonium hydroxide pentahydrate (TMAOH) from Fluka (Buchs, Switzerland) and ammonium sulphate, ammonium hydroxide, potassium permanganate from Panreac. HPLC-grade methanol, sodium oxalate and sulphuric acid were obtained from Merck.

HPLC mobile phase, distilled water and methanol were filtered through a 0.45- $\mu\text{m}$  filter in a Waters-Millipore system (Milford, MA, USA).

Other solutions used were 0.5 M sulphuric acid (solution A); 2% (v/v) concentrated ammonium hydroxide in water (solution B) and 2 g of TMAOH with 30 g of ammonium sulphate in 1 l of water and adjusted to pH 3 with 5 M sulphuric acid (solution C).

### 2.2. Extraction

Commercial silica Sep-Pak cartridges (Waters-Millipore) were successively washed with 2.5 ml of solution A, 5 ml of distilled water, 2.5 ml of solution B and again with 5 ml of distilled water. Water samples (250–1000 ml) were passed through the Sep-Pak cartridges, which were then inverted and eluted with 1 ml of a mixture of solution C–methanol (90:10). A 20- $\mu\text{l}$  aliquot of the eluate was injected onto the HPLC system.

### 2.3. HPLC analysis

Liquid chromatography was carried out with a pump system Model L-7100, a programmable variable-wavelength detector Model L-4250 and an integrator Model D-2500, all from Merck-Hitachi.

The analytical column was a Spherisorb SW3 (Pessac, France) (3 cm $\times$ 4.6 mm I.D. filled with

3- $\mu\text{m}$  particle size silica). A silica guard cartridge (1 cm $\times$ 4.6 mm I.D., 5  $\mu\text{m}$ ) was used to prevent damage of the analytical column.

The separation of the selected herbicides was performed by gradient elution. The initial mobile-phase composition was 100% solution C, linearly programmed to a mixture with 50% methanol after 15 min and maintained during 10 min. The flow-rate of the mobile phase was 0.5 ml/min. The eluted compounds were monitored with a 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 concentrations of paraquat, diquat and difenzoquat in the final extract were calculated by comparing the peak areas for each compound with those obtained from a solution containing 1  $\mu\text{g/l}$  in solution C.

### 3. Results and discussion

#### 3.1. High-performance liquid chromatography

To determine cationic herbicides, normal-phase chromatography with UV detection has been proposed [7,8]. The exact mechanism involved in the chromatographic process is not well defined. In this way, interaction between the normal phase and the herbicides can include ion-pair mechanisms as has been reported when using sodium chloride and sodium perchlorate [7]. The herbicide fixed to the silica can be liberated by using displacing agents in the mobile phase as TMAOH and ammonium sulphate [8].

The effect of the percentage of methanol in the mobile phase on the separation of paraquat, diquat and difenzoquat is examined in this study. An acceptable resolution between paraquat and diquat is reached with 10% of methanol in the mobile phase. A larger amount of methanol reduces the resolution between the peaks. In these conditions difenzoquat has a very long retention time. Therefore, an isocratic elution could not be used for the simultaneous determination of the three cationic herbicides. To determine the three selected herbicides with a single and fast run, the use of a gradient increasing the percentage of methanol was necessary. Narrow and

reproducible peaks were generated by using the proposed elution gradient. Detection was performed at the absorption maximum wavelength for each herbicide in order to improve the sensitivity and selectivity of the method.

Using the above mentioned conditions, the retention times obtained were 4.43 min, 8.66 min and 14.86 min for diquat, paraquat and difenzoquat, respectively.

#### 3.2. Recovery studies

In a previous method for determination of diquat and paraquat, the silica extraction cartridge was eluted with a 0.05 M ammonium formate solution in methanol–water (25:75), and adjusting the pH to 2.19 with sulphuric acid. From the different assayed eluents it was observed that passing water at pH < 3 through the inverted cartridge was enough to elute both diquat and paraquat quantitatively. The other constituents of the eluent solution were added to assimilate its composition to the mobile phase used in the HPLC determination and to avoid problems and interferences.

The mobile phase when starting a run (2 g of TMAOH and 30 g of ammonium sulphate in 1 l water at pH 3) was initially chosen to elute the three herbicides from the silica Sep-pak. Under these conditions, about 50% of difenzoquat remained in the silica cartridge. The partial loss of difenzoquat could be explained by its less polar character in comparison with the other two herbicides. This problem was solved by adding 10% methanol to the eluent. Further increasing the methanol percentage did not improve the efficiency of the method.

The ability of the silica cartridge to quantitatively retain the cationic herbicides on passing through increasing volumes (0.25, 0.5 and 1 l) of distilled and tap water spiked with 1 ml of the working solution was not modified.

The recovery and the within-run precision of the proposed method with various concentrations of diquat, paraquat and difenzoquat were assessed. Samples of distilled and tap water were spiked with the analytes at levels of 25, 1 and 0.1  $\mu\text{g/l}$ . The results are summarized in Table 1 and show that the recovery of the three compounds was independent of the level of the spike.

Table 1  
Recoveries of cationic herbicides extracted from 1 l of spiked distilled and tap waters

Compound	Recovery (%) <sup>a</sup>					
	Distilled water			Tap water		
	25 µg/l	1 µg/l	0.1 µg/l	25 µg/l	1 µg/l	0.1 µg/l
Diquat	91±10	89±8	85±14	86±9	89±8	88±13
Paraquat	95±12	91±9	89±15	88±10	93±11	89±14
Difenzoquat	92±8	95±6	90±12	89±8	91±12	92±11

<sup>a</sup> Mean values±relative standard deviation (R.S.D.) obtained from quintuplicate determinations.

After the extraction of 1 l of water with the HPLC conditions previously selected the detection limit (signal-to-noise ratio=3) for the three compounds studied was 25 ng/l (four times lower than the EU level permitted for drinking water).

Fig. 1 shows chromatograms obtained by the

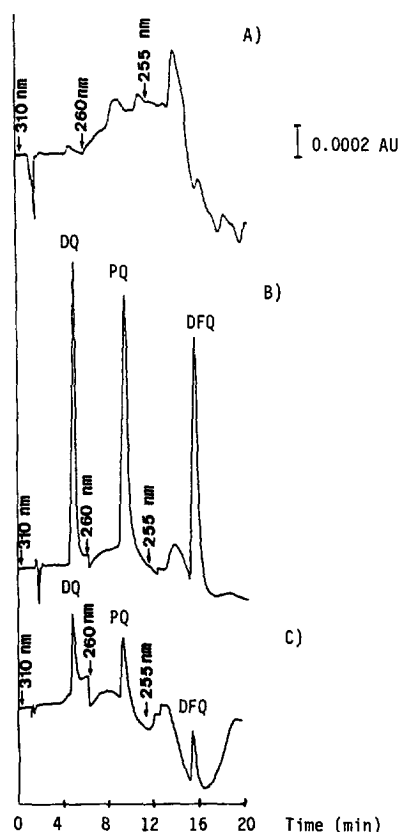


Fig. 1. HPLC chromatograms from (A) tap water, (B) tap water spiked with the selected herbicides at 1 µg/l, and (C) tap water spiked with the selected herbicides at 0.1 µg/l. Peak identification: DQ=diquat; PQ=paraquat; and DFQ=difenzoquat.

analysis of a tap water sample (Fig. 1A) and the same tap water spiked with different concentrations of diquat, paraquat, and difenzoquat (Fig. 1B). The 0.1 µg/l level is given in Fig. 1C. All three analytes showed up well, indicating that real trace level analysis is possible.

It has been reported [11] that the surfactants most widely used in detergent formulations are anionic and zwitterionic surfactants, mainly linear alkyl sulphonates (LAS) such as sodium tetradecylsulphate, lauryl sulphate and phenyl positional isomers. Although the maximum level established by EU law is 300 µg/l, reported concentrations of LAS in surface waters range from 5 to 50 µg/l [12].

Before applying the proposed method to natural water, a study was made of the effect that the presence of different patterns of surfactants and organic matter have in the extraction step.

Aliquots of 250 ml of distilled water were spiked with the herbicides at 4 µg/l, and different concentrations of the selected surfactants were added. The recoveries reported in Table 2 show that the quantitative extraction by the silica cartridge of diquat, paraquat and difenzoquat is affected by the presence of anionic, zwitterionic and non-ionic surfactants when they are present in water at a level up to 50 µg/l. At a level of 300 µg/l about 60% of bipyridylum cations was lost but at higher levels of surfactants (3000 µg/l) the percentage lost was of the same order. When cationic surfactants (such as cetrimide and benzalkonium chloride) were added, the extraction efficiency was not reduced at any concentration of surfactants studied.

The chromatograms obtained by the analysis of spiked water to which 3000 µg/l cetrimide (Fig. 2A), lauryl sulphate (Fig. 2B) and Brij 35 (Fig. 2C) were added, are shown in Fig. 2. Comparing the

Table 2  
Recoveries of cationic herbicides (4  $\mu\text{g/l}$ ) from 0.25 l of water samples containing various concentrations of different surfactants

Surfactants	Concentration ( $\mu\text{g/l}$ )	Recovery (%) <sup>a</sup>		
		Diquat	Paraquat	Difenzoquat
Cetrimide	5	98	99	90
	50	93	92	92
	300	95	91	93
	3000	87	89	92
Benzalkonium chloride	5	101	114	94
	50	102	107	93
	300	108	102	95
	3000	109	105	103
Sodium tetradecylsulphate	5	99	102	87
	50	98	95	93
	300	41	47	41
	3000	34	30	37
Lauryl sulphate	5	84	85	92
	50	109	100	93
	300	42	35	41
	3000	34	30	39
Lauryl Sulphobetaine	5	89	83	79
	50	91	94	84
	300	47	57	42
	3000	54	55	48
Brij-35	5	85	89	92
	50	83	101	91
	300	45	33	37
	3000	36	30	42
Triton X-100	5	86	89	92
	50	102	101	91
	300	45	33	37
	3000	36	30	42

<sup>a</sup> Average recovery calculated from four determinations.

chromatogram of Fig. 2A with the one of Fig. 1B (corresponding to the water analysis with the same concentration of herbicides in the final extract), it appears that the presence of cetrimide improves the herbicide peaks (see Fig. 2A). The reduction in the herbicide peaks caused by the presence of lauryl sulphate or Brij 35 is evident.

The surfactant content of water is an important parameter to control the application of the method to natural waters.

The organic matter dissolved in water consists for about 50% of humic substances and for 50% of small organic molecules, such as carboxylic acids, amino acids, carbohydrates, etc. [10].

Extraction efficiency of pesticides from natural

waters with high dissolved organic carbon content (DOC) can be lower than expected [13–15]. The DOC can interfere with the analysis of diquat, paraquat and difenzoquat in two ways. First, the DOC can saturate sorptive sites of the silica cartridge. This effect is produced by non-specific organic matter and by humic material. The second source of losses consists of interactions between humic acids and cationic herbicides.

The organic content of natural water samples, calculated as chemical oxygen demand (COD), frequently occurs at levels ranging between 1.4 and 17.2 mg O<sub>2</sub>/l [16] which are equivalent to humic acid concentrations ranging between 2.5 and 25 mg/l.

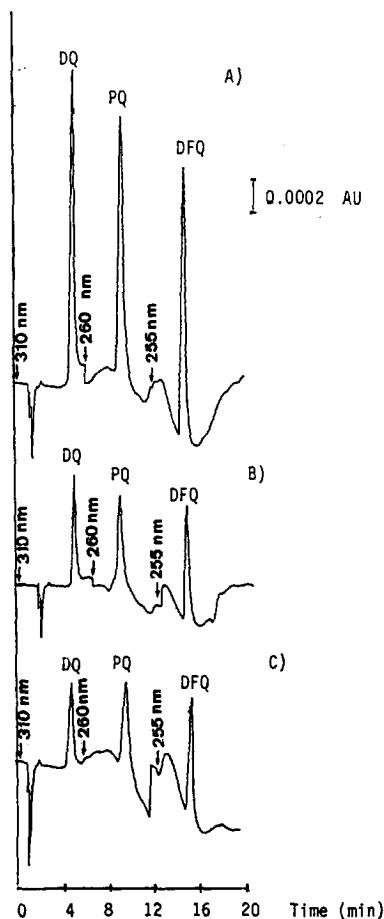


Fig. 2. HPLC-chromatograms from distilled water spiked with 4  $\mu\text{g/l}$  of diquat, paraquat and difenzoquat and 3000  $\mu\text{g/l}$  of the following surfactants: (A) cetrimide, (B) sodium tetradecyl sulphate, and (C) Brij 35. Peaks identification as in Fig. 1.

The effect of organic matter being present in water on the extraction and isolation of diquat, paraquat and difenzoquat using silica Sep-Paks was evaluated. In these experiments, simulated surface water samples were prepared from distilled water by adding, 2.5, 5, 10, 20, 50, 84, 100 mg/l of humic acid or 5.2, 10.2, 20.5, 41, 102, 172, 205 mg/l of saccharose (concentrations corresponding respectively to the same COD). The COD was determined by permanganate oxidation [17].

Fig. 3A shows that, in the presence of humic acids, the extraction of diquat, paraquat and difenzoquat was affected to some extent. The inter-

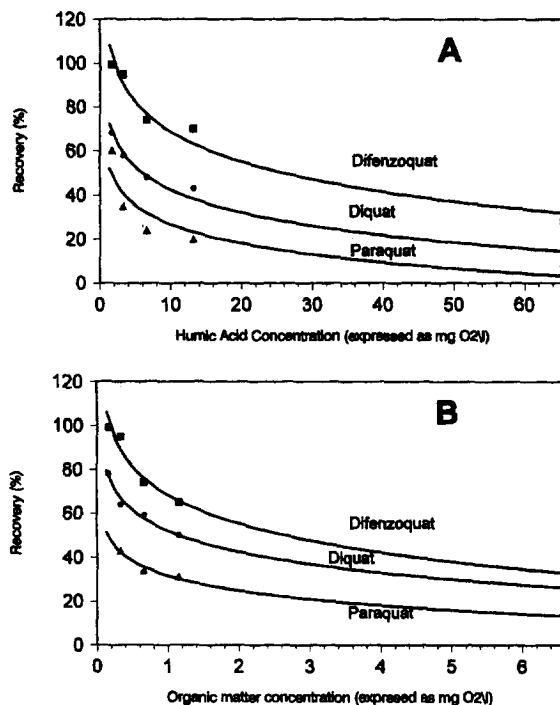


Fig. 3. Dependence of the recoveries of diquat, paraquat and difenzoquat (A) on the humic acid concentration of a synthetic water and (B) on the organic matter of a natural water.

action of humic acids with paraquat is stronger than with diquat, and with diquat stronger than with difenzoquat. The results can be adjusted to a logarithmic curve. However, when the same experiment was performed with saccharose, the extraction efficiency was unaffected.

These results are in agreement with those obtained by other authors [13,14], who observed a diminution of the recoveries for diquat and paraquat extracted from a humic acid solution using a C<sub>18</sub> cartridge and suggested that the interaction of humic acid with paraquat is stronger than with diquat. The hypothesis suggested by these authors is that it is very improbable that humic material was sorbed into the cartridges during the extraction because no coloration of the cartridge was observed during the extraction of the sample and because the silica cartridges used in this work are very polar while the C<sub>18</sub> cartridges are apolar. Another explanation is the existence of some kind of association between humic acids and the cationic herbicides considered. Binding of organic

molecules with humic acids has already been observed in waters [11–15].

A natural surface water taken from Albufera de Valencia (Spain), which showed a COD of 12 mg O<sub>2</sub>/l was analyzed and diquat, paraquat and difenzoquat were not detected. Aliquots of this natural water were diluted with distilled water to 1/2, 1/4 and 1/8 to equalize the organic matter content of the simulated surface water prepared with humic acid and saccharose. Several volumes of 250 ml of the samples prepared in this way were spiked with the herbicides at a 4 µg/l level.

Fig. 3B shows the recoveries obtained by the analysis of natural water and its dilutions. It can be observed that the recoveries increase when the natural COD is reduced. Although the COD values for the natural water and its dilutions are lower than those in the experiment performed with humic acids (it is difficult to find a natural water with higher COD), Fig. 3B shows the same tendency to a logarithmic curve as Fig. 3A. The recoveries of the three herbicides added to the natural water are a bit higher than those obtained with a pure solution of humic acids. This fact could be expected since the dissolved organic matter is composed not only of humic substances.

These experiments show that the diminution in the recoveries of the cationic herbicide extraction is mainly due to humic materials.

### 3.3. Application to environmental samples

To show the applicability of the method in routine analysis of natural waters, three samples were taken from three different sites of the Comunitat Valenciana (Spain): from irrigation channels of 'Ribarroja del Turia', 'El Palmar' and 'Puçol' with COD values of 1.4, 3.9 and 12.0 mg O<sub>2</sub>/l, respectively. Diquat was found in the 'El Palmar' and 'Ribarroja del Turia' samples at concentrations of 6 µg/l and 13 µg/l, respectively.

Fig. 4 shows the chromatograms corresponding to the analysis of three environmental water samples. It can be observed that the peaks obtained for diquat are wider than those obtained in the chromatograms of the standards (see Fig. 1 and Fig. 2). This peak enlargement is caused by the ageing of the analytical column (at the moment of the injection of these

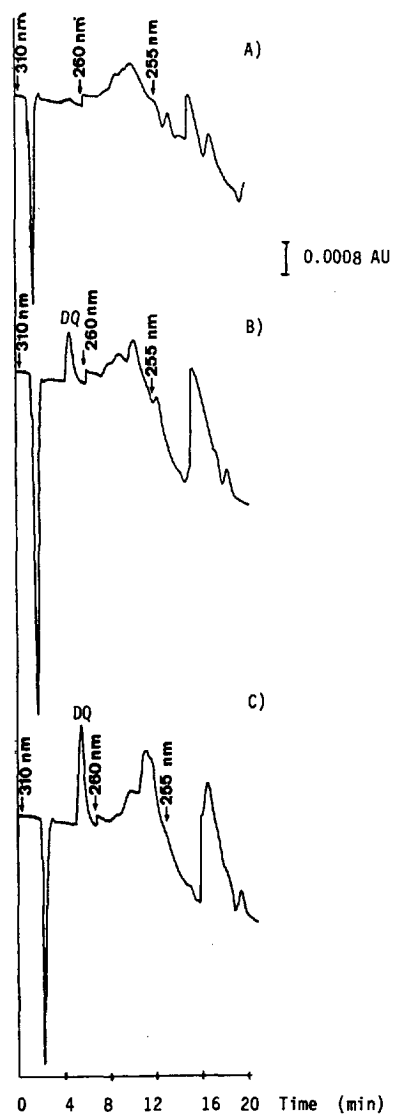


Fig. 4. HPLC chromatograms from environmental water samples of irrigation channels from (A) Puçol, (B) El Palmar and (C) Ribarroja del Turia. (For concentration of diquat see the text.) Peak identification as in Fig. 1.

samples, more than two hundred injections had been performed).

In this work, common normal solid-phase extraction efficiency for isolation of traces of the three studied herbicides from drinking water has been demonstrated and for monitoring diquat, paraquat and difenzoquat in water, it is also advantageous to

use a normal phase in the HPLC determination since one obtains well defined narrow peaks.

In conclusion, although some compounds such as surfactants and humic acids, which are common contaminants of natural waters, have a negative effect on the recoveries of the three cationic herbicides, the method is useful for monitoring their presence in natural water and the effect that those substances have on the herbicides studied.

Further work will be devoted to the determination of different mechanisms to avoid the losses of diquat, paraquat and difenzoquat due to the presence of humic acids and surfactants, to widen the scope of the method here described.

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