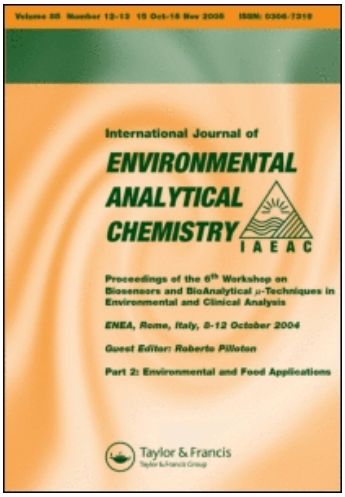

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Simultaneous Flow Injection Determination of Diquat and Paraquat in Foodstuffs, Natural Waters and Biological Fluids

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SIMULTANEOUS FLOW INJECTION DETERMINATION OF DIQUAT AND PARAQUAT IN FOODSTUFFS, NATURAL WATERS AND BIOLOGICAL FLUIDS

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The reduction of diquat and paraquat with alkaline sodium dithionite has been applied to the simultaneous determination of both herbicides using a flow injection system. A dual-channel manifold with two detectors in parallel was used. The sample bolus is split into two subplug, one of the sub-boluses merges with a 0.1% sodium dithionite stream and routes to a spectrofluorimeter where diquat is determined. The other sub-bolus merges with a 0.5% sodium dithionite stream and routes to a spectrophotometer for the measurement of paraquat. The system allows 70 samples to be analysed per hour. Applications of the method to the determination of diquat and paraquat in real samples are reported.

KEY WORDS: Diquat, paraquat, flow injection analysis, spectrophotometry, spectrofluorimetry.

INTRODUCTION

Diquat (6,7-dihydrodipyrido(1,2-a:2',1'-c) pyrazine-diium, dibromide) and paraquat (1,1'-dimethyl-4,4'-bipyridylium dichloride) are nonselective, quick acting herbicides and desiccants, which are nonresidual because of their very rapid inactivation by irreversible adsorption on contact with the soil. When they are not used for direct plant spraying (e.g., for weed control before crop sowing or emergence), no significant residues are likely to be found[1]. On the other hand, direct treatment with these herbicidal sprays is often carried out for preharvest desiccation of various plants and consequently some residues of active ingredients may be present.

The determination of single paraquat or single diquat in herbicide formulations[2], water[3], blood[4–6], urine[7,8] and agricultural products[9–11] has been reported. These methods include spectrophotometry[2], spectrofluorimetry[12,13], thin-layer chromatography[14], gas chromatography[15], liquid chromatography[7,16], polarography[17–19] and gas chromatography-mass spectrometry[20]. Other methods include direct potentiometry with ion selective electrodes[21,22], electron spin resonance spectroscopy[23], flow

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injection analysis[24], photokinetic methods[25,26], radioimmunoassay[27], fluoroimmunoassay[28] and enzymoimmunoassay[29].

These herbicides are extremely toxic to man[30] and, consequently, cases of accidental, suicidal and homicidal poisonings[31,32], have been encountered. Some commercial products contain mixtures of both compounds and hence rapid methods capable of analysing diquat and paraquat simultaneously are required for forensic and clinical purposes. High-performance liquid chromatography[8,11,33,34], gas-liquid chromatography[5,35] and spectrophotometric methods[2] have been used for the determination of the mixture of both herbicides in different samples.

The flow injection (FI) analysis has proved to be a suitable technique for multiple detection, generally focusing on the resolution of mixtures of different species[36]. Both series[37] and parallel[38] detection systems have been used for this purpose.

This paper describes, for the first time, the simultaneous FI determination of diquat and paraquat by using two detectors in parallel. The method is based on the reduction of both herbicides by sodium dithionite. The reduced diquat is very fluorescent and the reduced paraquat does not fluoresce at all. The sample is injected into the distilled water stream. The sample bolus is split into two subplugs. One of the sub-boluses routes to a spectrofluorimeter, where diquat is determined and the other to a spectrophotometer for the measurement of paraquat.

EXPERIMENTAL

Apparatus

The flow injection system consisted of a Gilson Minipuls HP4 peristaltic pump and an Omnifit injection valve. Two detectors were used: a Hitachi F-3010 Spectrofluorimeter with a Hellma flow cell (inner volume 25 μl) and a Spectronic 100 spectrophotometer with a Hellma flow cell (inner volume 18 μl).

Reagents

All chemicals were of analytical-reagent grade. Doubly distilled water was used throughout. Aqueous 100 $\mu g\ ml^{-1}$ paraquat and diquat stock solutions were prepared from pure chemicals supplied by Serva and Dr. S. Ehrenstorfer, respectively. Less concentrated solutions were prepared by suitable dilution. A 0.1% and a 0.5% (w/v) sodium dithionite solutions were prepared every two hours.

Sample pretreatment

Potatoes About 2500 g of potato tubers were taken from the sample provided. They were washed from soil and dried with a cloth. Each tuber was cut into four approximately equal segments and the two opposite segments were rejected. The remaining segments were passed through a meat mincer and mixed the pulp well. A 100 g portion was placed in the macerator jar together with 10 ml of water and 20 ml

of 18 N sulphuric acid. After macerating for abut 3 min, the sample was transferred to a boiling flask and refluxed for 5 hours. After filtering through a sintered glass filter (no. 4), the filtrate was transferred to a 200 ml calibration flask. The standard-addition method with four additions for the determination of diquat and paraquat was used.

Serum To 1 ml of blood from a healthy volunteer, 1 ml of 5 $mg.l^{-1}$ of diquat and paraquat solution was added. The protein was separated with sulphosalicylic acid[39]. After filtering, diquat and paraquat were determined in the filtrate by the standard-addition method.

Urine The preparation of synthetic urine was performed according to the literature[40]. The sample was diluted with doubly distilled water to obtain the adequate concentration level and the standard-addition method to determine both herbicides was used.

RESULTS AND DISCUSSION

Spectral characteristics

Diquat and paraquat are reduced with alkaline sodium dithionite to coloured free radicals[41]:

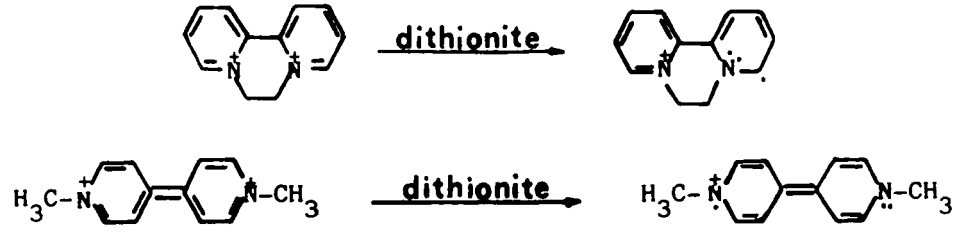

Figure 1 shows the spectra for the radicals from diquat and paraquat, formed with alkaline sodium dithionite. These radicals are stable only in an excess of the reducing reagent. The colour may fade on standing owing to depletion of the dithionite in the immediate vicinity of the radical ions which are then oxidised back to the parent cations. The maximum colour intensity for reduced paraquat and for reduced diquat can be restored immediately by gently swirling of the solution. Vigorous shaking of the reduced solution causes rapid discharge of colour, owing to oxidation of the radicals by atmospheric oxygen.

The diquat radical is very fluorescent but the paraquat radical does not fluoresce at all. The excitation and emission spectra for the reduced diquat are shown in Figure 2.

After reducing the paraquat and diquat solutions with alkaline sodium dithionite the resolution of the mixtures can be carried out by measuring the fluorescence

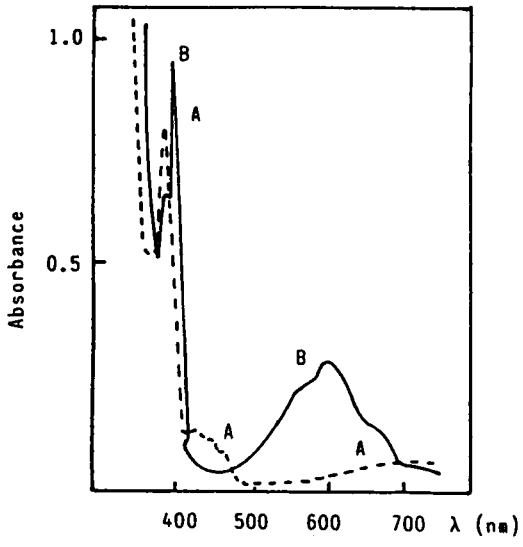

Figure 1 Absorption spectra of diquat and paraquat in reduced solution: curve A, diquat 5.10^{-5}M in 0.1% $Na_2S_2O_4$; and curve B, paraquat 5.10^{-5}M in 0.1% $Na_2S_2O_4$.

intensity (with excitation and emission wavelengths of 428 and 497 nm, respectively) and the absorbance (at 605 nm). The content of diquat is directly obtained from the fluorescence measurement. To calculate the content of paraquat the absorbance of the solution must be corrected because diquat absorbs slightly at 605 nm.

Design of the two-channel analyzer

The two-channel Flow Injection Analyzer (Figure 3) consisted of a diquat line (top), injection and split line (middle) and paraquat line (lower). Following the sample

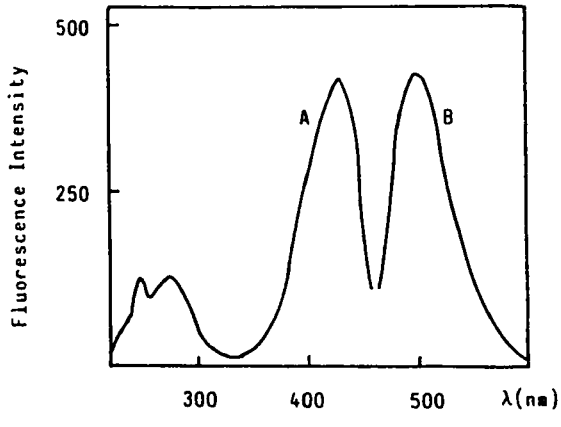

Figure 2 Fluorescence excitation (A) and emission spectra (B) of reduced diquat (5.10^{-7}M) in the presence of $Na_2S_2O_4$ 0.05% and borax buffer pH 8.

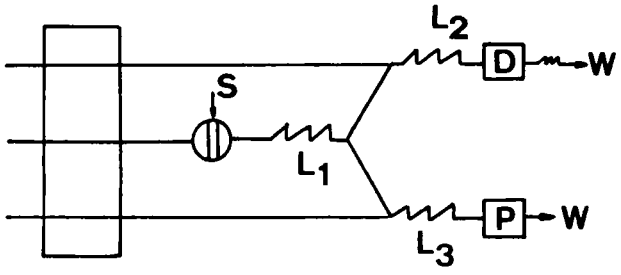

Figure 3 Flow diagram of the dual channel manifold used in the simultaneous determination of diquat and paraquat by flow injection. S is the point of sample injection, D and P represent the detectors, spectrofluorimeter and spectrofotometer respectively. R_1 and R_2 = reagent solution which are propelled at 0.6 ml/min by the peristaltic pump.

injection point (s), a 60-cm long split delay coil was inserted to avoid the effect of variations of the injection speed on the sample splitting. In this way, the sample zone reaches the split point only when the flow rates have been reestablished after the injection, and the splitting is therefore affected only by pumping action. The sample, split in the ratio 4:1 (paraquat:diquat) at the split point proceeds through a short transmission line (5 cm) into the diquat and paraquat branches. The ratio of splitting is achieved by enlarging the diameter of the coil in the paraquat line and by increasing the flow resistance of the diquat line by placing a brake coil behind the diquat flow cell.

The splitting of sample at the above cited ratio is advisable in order to be able to analyse larger ratios of these analytes. This is a consequence of the higher fluorimetric sensitivity for diquat than the photometric sensitivity for paraquat.

Optimization studies

The optimization of the different variables influencing the system (FI and chemical) was carried out using the univariate method. The effect of pumping rate, loop size and length coil was studied over the range shown in Table 1 and the optimum values are also summarized by each analyte. The selected values for these variables are: flow-rate = 0.6 ml/min; injected sample = 240 μl; coil length (L_2) = 60 cm and bore = 0.5 mm for the diquat line; coil length (L_3) = 60 cm and bore 0.7 mm for paraquatline. These values allow the reduced form of paraquat and diquat to be

Table 1 Results of the optimization of FIA variables.

Variable	*Range studied*	*Optimum value*	
		Diquat	*Paraquat*
Flow rate, ml min^{-1}	0.4–3	0.6	0.6
Sample volume, μl	40–300	100–300	240–300
Coil length, cm	10–120	30–120	30–120

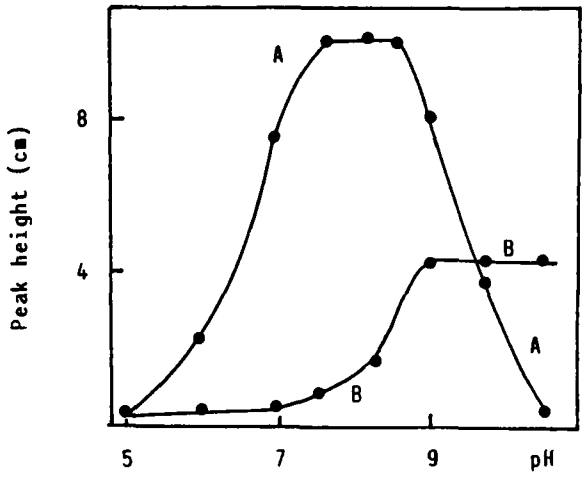

Figure 4 Influence of pH on the peak height. Curve A: diquat (0.5 mg l^{-1}). Curve B: paraquat (0.5 mg l^{-1}).

formed with the least dispersion by the time the sample plugs pass through the detectors.

The study showed that pH and the concentration of sodium dithionite affected the peak height (Figures 4 and 5) as a consequence of the influence of these chemical variables on the fluorescence of the reduced diquat and on the absorbance of the reduced paraquat. Maxima peak heights are obtained using two reagent streams formed by 0.1% sodium dithionite in 0.5 M borax buffer pH 8 for the diquat line (R_1) and 0.5% sodium dithionite in 0.5 M ammonia buffer pH 9 for the paraquat line (R_2).

Since temperature changes in the range 20–40°C had little effect on the signals of diquat and paraquat, the experiments were performed at room temperature.

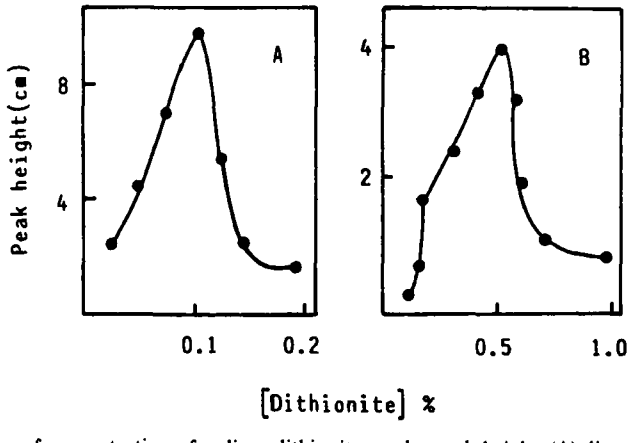

Figure 5 Influence of concentration of sodium dithionite on the peak height. (A) diquat (0.5 mg l^{-1}); (B) paraquat (0.5 mg l^{-1}).

Simultaneous determination of diquat and paraquat

For practical applications, the FI system depicted in Figure 3 was first calibrated with standard solutions of diquat and paraquat. Linear plots were obtained for two ranges: 0.06–0.6 and 0.6–10 $\mu g\ ml^{-1}$ for diquat and 0.2–2 and 2–20 $\mu g\ ml^{-1}$ for paraquat. The detection[42] limits were 7 $\mu g\ l^{-1}$ for diquat and 36 $\mu g\ l^{-1}$ for paraquat. Figure 6 shows some recorder outputs for the standards of diquat and paraquat, each sample solution being injected in triplicate.

On the basis of the spectral results, and with regard to the FI simultaneous determination of the two herbicides, the following can be concluded: the fluorescence peak is due solely to diquat and is independent of the paraquat concentration, whereas the absorbance peak is a function of the total amount of both. Expressed in terms of measured absorbance at 605 nm the calibration function for the height of this peak can be summarized by the following equation:

$$A = a + k_D C_D + k_P C_P$$

where a is the blank value and k_D, C_D, k_P and C_P are the partial sensitivities and concentrations of diquat and paraquat respectively. As k_D is very little, the correction is negligible at diquat concentrations less than 2 $\mu g\ ml^{-1}$.

The results for the simultaneous determination of diquat and paraquat in a series of synthetic mixtures are given in Table 2.

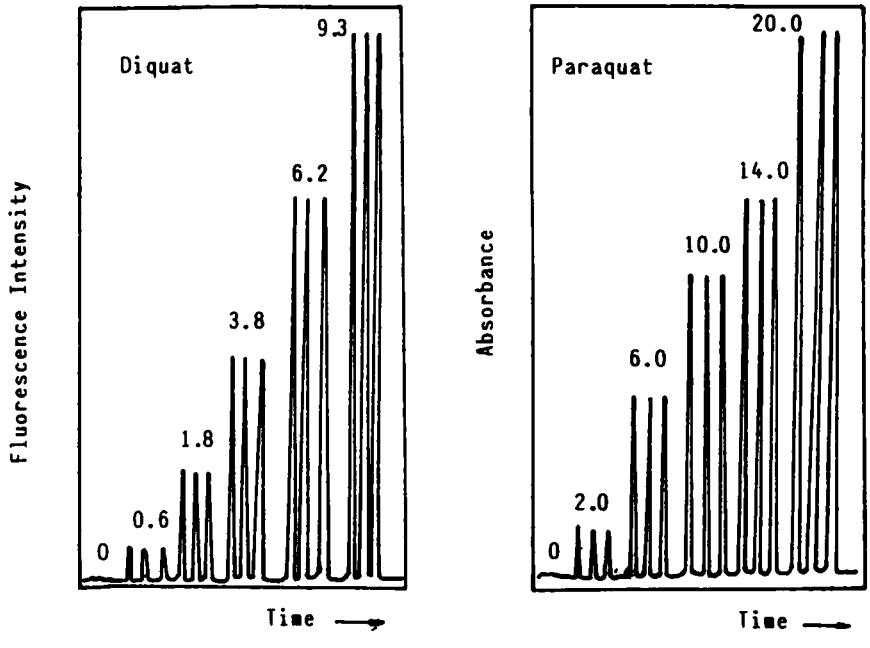

Figure 6 Recorder signal for standard solutions. Values above peaks are concentrations in $mg\ l^{-1}$.

Table 2 Simultaneous determination of diquat and paraquat in synthetic mixtures using a two-line manifold with two detectors in parallel.

Added ($\mu g\ ml^{-1}$)		*Found** ($\mu g\ ml^{-1}$)	
Diquat	*Paraquat*	*Diquat*	*Paraquat*
0.093	1.86	0.090 ± 0.5	1.83 ± 0.2
0.46	1.86	0.48 ± 0.4	1.87 ± 0.3
0.93	1.86	1.01 ± 0.4	1.91 ± 0.2
1.86	1.86	1.84 ± 0.3	1.82 ± 0.2
1.86	0.93	1.78 ± 0.3	0.97 ± 0.4
1.86	0.46	1.89 ± 0.2	0.42 ± 0.5
1.86	0.18	1.79 ± 0.3	0.22 ± 0.5

* Mean ± r.s.d. of four determinations.

The reproducibility of the method was studied with eleven replicate injections of a sample with 0.27 and 0.93 $\mu g\ ml^{-1}$ of diquat and paraquat, respectively; the relative standard deviation was ±1.42% for diquat and 1.02% for paraquat.

A systematic study of the effect of foreign species on the simultaneous determination of 0.5 $\mu g\ ml^{-1}$ of diquat and 0.5 $\mu g\ ml^{-1}$ of paraquat was undertaken. The criterion for interference was fixed at ±3% variation of the average peak height, calculated for the established levels of the diquat and paraquat. The results are given in Table 3.

Applications

The proposed FI method was applied to the simultaneous determination of diquat and paraquat in real samples: potable water, potatoes, urine and blood serum. The

Table 3 Tolerance of different species in the simultaneous determination of 0.5 $\mu g\ ml^{-1}$ of diquat and 0.5 $\mu g\ ml^{-1}$ of paraquat.

Species added	*Tolerance ratio (w/w)*	
	Diquat	*Paraquat*
Glucose; urea; lactic, uric, ascorbic, hipuric acids; amino acids; vitamins.	5000*	5000*
Cl^-, Br^-, I^-, NO_3^-, Na^+,K^+, CO_3^{2-},	5000*	5000*
PO_4^{3-}, SO_4^{2-},	1000	2000
Ca^{2+}, Mg^{2+}, Ba^{2+}, Zn^{2+}	1000	1000
Co^{2+}, Fe^{3+}	100	500
Ni^{2+}	100	50

* Maximum ratio tested.

Table 4 Simultaneous determinations of diquat and paraquat in real samples.

Sample Waters	*Content**		*Found†*	
	Diquat	*Paraquat*	*Diquat*	*Paraquat*
Sample 1 ($\mu g\ ml^{-1}$)	0.46	0.93	0.49 ± 0.9	0.96 ± 1.0
Sample 2 ($\mu g\ ml^{-1}$)	1.49	0.62	1.39 ± 0.8	0.64 ± 0.8
Potatoes				
Sample 1 ($\mu g\ g^{-1}$)	7.40	9.30	7.33 ± 0.2	9.30 ± 0.2
Sample 2 ($\mu g\ g^{-1}$)	1.86	5.02	1.93 ± 0.5	4.87 ± 0.3
Blood serum				
Sample 1 ($\mu g\ ml^{-1}$)	4.65	6.50	4.55 ± 0.2	6.52 ± 0.3
Sample 2 ($\mu g\ ml^{-1}$)	6.50	3.72	6.31 ± 0.2	3.81 ± 0.2
Synthetic urine				
Sample 1 ($\mu g\ ml^{-1}$)	1.86	3.72	1.91 ± 0.4	3.62 ± 0.3
Sample 2 ($\mu g\ ml^{-1}$)	6.21	9.30	6.25 ± 0.2	9.15 ± 0.2

* Synthetic values.
† Average ± r.s.d. for five separate determinations.

results shown in Table 4 were in good agreement with the amounts added in the preparation of the synthetic samples.

Conclusions

The results obtained clearly demonstrate the suitability of the FI method as a continuous flow technique for the sensitive, rapid and reproducible simultaneous determination of diquat and paraquat.

When this method is compared with the high-performance liquid chromatographic analysis of diquat and paraquat proposed by Simon and Taylor[34], it can be concluded that the last method provides the best limits of detection (0.5 $\mu g\ l^{-1}$ for both herbicides) but the FI method has the advantages of a greater sampling rate (70 samples h^{-1}) and simplicity.

The FI method is suitable for the quality control of waters, edible products, and commercial herbicides and for routine determinations in forensic and clinical applications, as it is very simple and much less time consuming than chromatographic[5,8,11,33–35] and manual[2] methods.

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