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GAS CHROMATOGRAPHIC DETERMINATION OF DIQUAT AND PARAQUAT IN CROPS

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SUMMARY

A sensitive and reproducible gas chromatographic procedure for the determination of diquat and paraquat in potatoes and rapeseed was developed. The volatilization of analytes was carried out via their hydrogenation with sodium borohydride–nickel(II) chloride. After their isolation from the reaction mixture, the derivatives of bipiperidine were separated on a column packed with Apiezon L plus potassium hydroxide. Comparable detection limits (0.005 mg/kg) were achieved with a nitrogen–phosphorus detector and by mass fragmentography, however, the latter method was preferred for analyses of rapeseed extracts owing to its higher selectivity.

INTRODUCTION

The bipyridinium derivatives diquat (1,1′-ethylene-2,2′-bipyridinium dication) and paraquat (1,1′-dimethyl-4,4′-bipyridinium dication) are rapid-acting herbicides, killing green plants with which they come into contact. On reaching the soil they are inactivated.

When diquat and paraquat 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[1,2]. 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. As bipyridinium herbicides are toxic the monitoring of their residues in food crops is necessary.

Spectrophotometric methods based on the procedure developed by Calderbank[3] have been widely used[4,5]. Owing to the cationic nature of both diquat and paraquat, their displacement from binding sites of an organic matrix (mostly by boiling with strong sulphuric acid) has to be performed. Purification and preconcentration of analytes (usually by cation-exchange chromatography) is carried oud prior to quantitative analysis.

Various chromatographic methods for the determination of bipyridinium herbicides have also been described. A high-performance liquid chromatographic procedure with UV detection[6] was used for analyses of body fluids. After isolation from

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hydrolysed potato tubers, residues of diquat and paraquat as their heptanesulphonate ion pairs were separated on a reversed-phase column[7]. The gas chromatographic (GC) determination of bipyridinium herbicides is not possible unless they are converted into volatile products. Partly hydrogenated derivatives were reported to originate from the parent compounds (1,1′-ethyleneoctahydro-2,2′-bipyridine from diquat and 1,1′-dimethyloctahydro-4,4′-bipyridine from paraquat) when sodium borohydride was used for their reduction. GC methods with nitrogen–phosphorus detection (NPD)[8,9] utilizing this reaction for volatilization of analytes were applied to analyses of potatoes, and similarly a GC–mass spectrometric (MS) method [10] was used for the analysis of plasma samples. Another method of reducing bipyridinium compounds involves catalytic hydrogenation with platinium oxide as a catalyst. In this instance fully hydrogenated products (corresponding derivatives of bipiperidine) can be obtained. Procedures based on this principle have been used for the analysis of soil[11] vegetables[12] and body fluids[13]. Although most of the above methods involve acid hydrolysis, it has been demonstrated that this step can be omitted because efficient displacement of diquat and paraquat from an analysed matrix occurs in the course of hydrogenation[9]. Dequaternization of bipyridinium herbicides can also be achieved by pyrolysis. The main product formed, 2,2′-bipyridine from diquat and 4,4′-bipyridine from paraquat, are amenable to GC analysis. A pyrolysis GC–MS method for analyses of biological samples has been published[14].

The aim of this study was to develop a rapid and efficient method for the analysis of residues of bipyridinium herbicides in treated crops and to introduce it for routine checking purposes.

EXPERIMENTAL

Pesticide standards

Analytically pure diquat dibromide and paraquat dichloride were supplied by ICI Plant Protection Division; standard solutions (0.01 mg/ml, expressed as dication) in 0.2 *M* hydrochloric acid were prepared. Desmetryne was obtained from Supelco; a stock solution (7.2 μg/ml) in hexane was prepared.

Chemicals

All chemicals (Lachema, Brno, Czechoslovakia) were of analytical-reagent grade; solvents were distilled before use.

Hydrogenation of diquat and paraquat in model samples

An aliquot of a standard solution of bipyridinium herbicides was transferred into a separating funnel containing 2 ml of 0.2 *M* hydrochloric acid, 1 ml of 1 *M* nickel(II) chloride and 0.5 ml of toluene (antifoaming agent). Sodium borohydride (100 mg) was then carefully added, which resulted in the evolution of hydrogen and formation of a black precipitate of nickel boride. After standing for 30 min at room temperature the reaction was stopped by addition of 2 ml of 1 *M* sodium hydroxide solution. Extraction with three 5-ml portions of diethyl ether followed. To the combined extracts (dried by passing through anhydrous potassium carbonate), two drops of concentrated hydrochloric acid were added and the solvent was evaporated under reduced pressure. The residue in the flask was made alkaline with 0.2 ml of 5 *M*

sodium hydroxide solution and the analytes were extracted into 2 ml of hexane prior to GC analysis.

Analysis of potatoes

To 50 g of a representative sample of potatoes (several tubers were mixed in a blender) placed in a beaker, 50 ml of 0.2 *M* hydrochloric acid, 20 ml of 1 *M* nickel(II) chloride, 2.5 g of sodium borohydride and 5 ml of toluene were added. After 60 min, the reaction mixture was filtered through a layer of glass-wool and the filtrate was made alkaline with 30 ml of 1 *M* sodium hydroxide solution. Three portions (one 80 ml and two 50 ml) of diethyl ether were used for extraction of hydrogenated bipyridinium compounds; separation of organic and aqueous phases was achieved by centrifugation (carried out under cooling). The combined extracts were dried over anhydrous potassium carbonate and then evaporated in the presence of 0.1 ml of concentrated hydrochloric acid. To the residue in the flask, 0.3 ml of 5 *M* sodium hydroxide solution were added and the analytes were then transferred (with vigorous shaking) into a small volume (1–2 ml) of hexane containing desmetryne as an internal standard for the subsequent GC analysis.

Analysis of rapeseed

Either 5 g of ground rapeseed or 2 g of meal were placed in a beaker, to which 20 ml of distilled water, 20 ml of 0.2 *M* hydrochloric acid and 10 ml of nickel(II) chloride were added. The hydrogenation was started by addition of 1 g of sodium borohydride. After 60 min, the reaction mixture was filtered into a separating funnel (30 ml of 0.2 *M* hydrochloric acid were used for rinsing), and the pH of filtrate was adjusted to 1–2 with 50% hydrochloric acid. This solution was extracted with 25 ml of diethyl ether and the organic layer, after separation by centrifugation, was discarded. The aqueous phase was made alkaline (pH 11–12) and then extracted with three 25 ml portions of diethyl ether. The combined extracts were processed in the same way as for the analysis of potatoes.

Gas chromatography

All GC analyses were carried out on a Hewlett-Packard 5880 A chromatograph equipped with a nitrogen–phosphorus detector. The injector temperature was 230°C, the detector temperature was 300°C and the nitrogen carrier gas flow-rate was 30 ml/min. The following columns were used: (i) 2.4 m × 2 mm I.D. glass column packed with 5% Carbowax 20M plus 2% potassium hydroxide on Inerton Super (0.125–0.160 mm), with temperature programming from 160 to 220°C at 5°C/min; (ii) 2.4 m × 2 mm I.D. glass column packed with 5% Apiezon L plus 3% potassium hydroxide on Inerton Super, isothermal at 200°C.

Gas chromatography–mass spectrometry

GC–MS analyses were performed on a Shimadzu QP 1000 gas chromatograph–quadrupole mass spectrometer equipped with a 3.2 m × 1.6 mm I.D. glass column packed with 5% Apiezon L plus 3% potassium hydroxide on Inerton Super. The column temperature was 220°C, ion source temperature 280°C and ionization energy 70 eV. The flow-rate of helium carrier gas was 30 ml/min. For mass fragmentography, version 9.1 of the original Shimadzu "MF" software was used.

RESULTS AND DISCUSSION

GC methods for the analysis of residues of diquat and paraquat are mostly based on the determination of volatile dienes originating as the main products from the parent compounds by the reduction with sodium borohydride. In our experience, however, and in agreement with another study[8], the degree of hydrogenation depends on many factors (*e.g.*, reaction time, reaction temperature), which can result in a poor reproducibility. It therefore seemed more reasonable to convert bipyridinium compounds into their perhydrogenated products. This reaction was carried out with a sodium borohydride–nickel(II) chloride reduction system similar to that used by Kawase *et al.*[15] for the hydrogenation of diquat and paraquat precipitated from body fluids by reinecke reagent. However, they encountered problems with incompleteness of reduction of analytes, which they attributed to the poisoning of the catalyst by the sulphide ion released from thiocyanate contained in reineckate complexes.

In our experiments we found hydrogenation to have been completed in 30 min. Perhydrogenated paraquat and two isomers originating from diquat were the only products obtained. Although these compounds could be separated on a column packed with Carbowax 20M plus potassium hydroxide, an impurity present in the blank sample had a retention time identical with that of the peak corresponding to paraquat (see Fig. 1). This problem was overcome by the use of Apiezon L plus potassium hydroxide stationary phase (see Fig. 2). The response of the nitrogen–phosphorus detector was linear within the range tested, *i.e.*, 0.5–50 ng (injected amount). For precise quantification, both peaks corresponding to diquat must be considered, because in freshly prepared samples the peak-area ratio (first peak to the second) was 1.9 and gradually increased, reaching 3.0 within 2 days (at laboratory temperature). This phenomenon was probably due to the establishment of thermodynamic equilibrium between the *cis* and *trans* isomers of the reaction product.

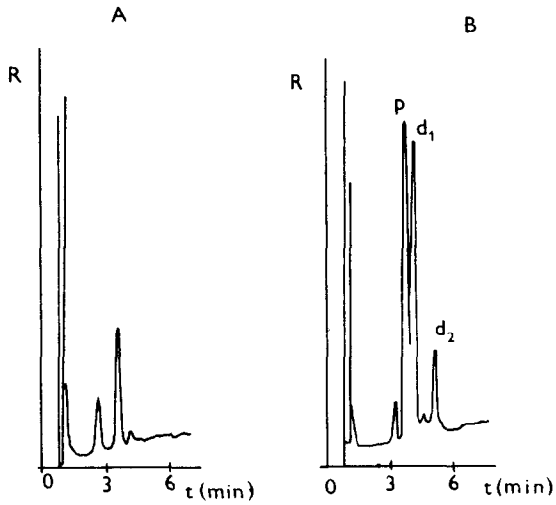


Fig. 1. Gas chromatogram of hydrogenated diquat (peaks d_1 and d_2) and paraquat (peak p) on Carbowax 20M plus KOH. (A) Blank sample; (B) model mixture (injected amount of analytes, 10 ng).

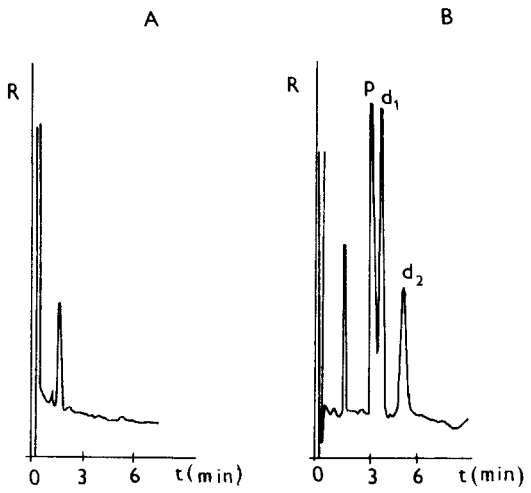


Fig. 2. Gas chromatogram of hydrogenated diquat (peaks d_1 and d_2) and paraquat (peak p) on Apiezon L plus KOH. (A) Blank sample; (B) model mixture (injected amount of analytes, 10 ng).

Almost the same procedure as that employed for the preparation of model samples was used for analyses of potato tubers. In this instance, however, a greater amount of sodium borohydride was necessary for the "direct" reduction of potato homogenate, and the reaction time also had to be extended. When products of reduction were extracted (with diethyl ether) from the reaction mixture, stable emulsions were formed. Their separation could be carried out only by centrifugation. The evaporation of an excess of solvent from the combined extracts was performed in the presence of several drops of hydrochloric acid to prevent the loss of volatile analytes.

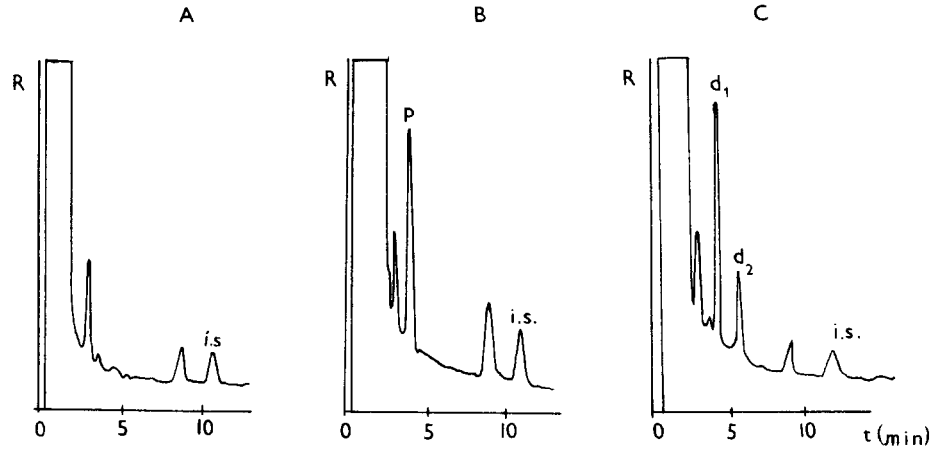


Fig. 3. Determination of bipyridinium herbicides in potatoes (column ii, see Experimental). Each injection represents 150 mg of sample. (A) Blank sample; (B) potatoes spiked with paraquat at 0.1 mg/kg; (C) potatoes spiked with diquat at 0.1 mg/kg.

TABLE I

RECOVERIES OF DIQUAT AND PARAQUAT FROM POTATOES

Compound	*Spiking level*[a] *(mg/kg)*	*Recovery*[b] *(%)*	*Relative standard deviation (%)*
Diquat	0.01	86.1	12.7
	0.04	92.3	5.6
	0.10	95.0	4.2
	1.50	96.6	3.8
Paraquat	0.04	109.8	8.2
	0.10	101.7	6.9
	1.50	100.1	4.0

[a] Expressed as dication.
[b] Mean value of five replicates.

These were released from their protonated form by alkalinization and then immediately extracted into a small volume of *n*-hexane containing desmetryne, an internal standard for GC (see Fig. 3). As can be seen form Table I, high recoveries and good reproducibility of the results were achieved by our procedure. We found that the recovery of residues did not depend on the time of post-spiking incubation, which means that the interaction of bipyridinium compounds with the plant matrix did not result in the formation of non-extractable residues. This fact favours the use of a procedure based on the "total" hydrogenation because, in contrast to our findings, Worobey and Panopio[8], who determined diquat and paraquat as partly hydrogenated products (dienes), encountered serious problems with low recoveries, the value of

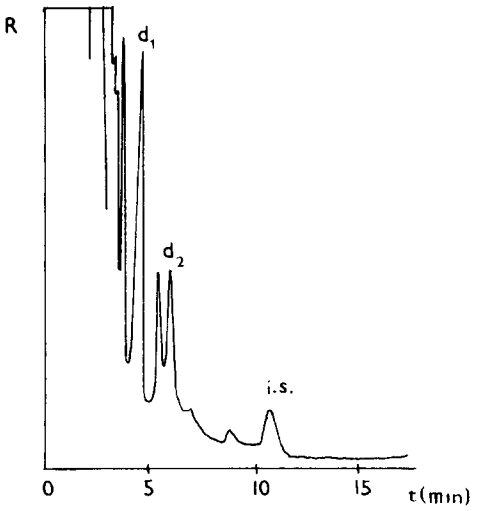


Fig. 4. GC analysis of rapeseed (column ii, see Experimental). Injection represents 15 mg of sample.

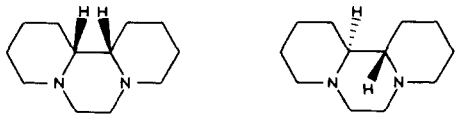
TABLE II

MASS SPECTRA OF PERHYDROGENATED PARAQUAT (p) AND ISOMERS OF DIQUAT (d_1 AND d_2)

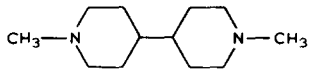
Compound	*M.W.*	*m/z (%)*
1,1'-Dimethyl-4,4'-bipiperidine (p)	196	96(100), 44(76), 45(65), 43(61) 42(50), 58(45), 70(39), 97(33), 98(32), 196(30)
cis-1,1'-Ethylene-2,2'-bipiperidine (d_1)	194	83(100), 111(50), 98(42), 55(41), 42(38), 194(23), 43(22), 96(17), 44(15), 69(14)
trans-1,1'-Ethylene-2,2'-bipiperidine (d_2)	194	83(100), 111(47), 110(44), 42(41), 55(40), 56(40), 98(34), 44(28), 45(27), 43(25), 194(21)

which moreover depended on the way in which the spiked samples were treated (intensity of mixing, incubation time, etc.).

Rapeseed is another crop that is commonly desiccated with preparations containing bipyridinium compounds (especially diquat). Owing to the higher content of volatile nitrogen-containing compound in this plant material, an additional clean-up step had to be introduced in the sample procedure, but nevertheless some impurities were still left in the final extract (see Fig. 4). Although the gas chromatogram of an "unspiked" sample is presented here, two peaks, d_1 and d_2, with retention times identical with those of isomers of perhydrogenated diquat can be seen. As it was not known whether the rapeseed sample had been treated with diquat, mass spectrometry was therefore employed for the identification of these compounds. Table II gives electron impact mass spectrometric data for our analytes, confirming the expected structures:



d_1 and d_2
originating from diquat



p
originating from paraquat

No significant differences existed (the sole exception was the presence of an ion of *m/z* 110 in the spectrum of d_2) in the fragmentation pathways of isomers originating from diquat; however, their absolute configuration could not be determined by this method. In all the spectra distinct molecular ions were present. Four ions were selected for mass fragmentographic analysis of rapeseed extract (see Fig. 5). The

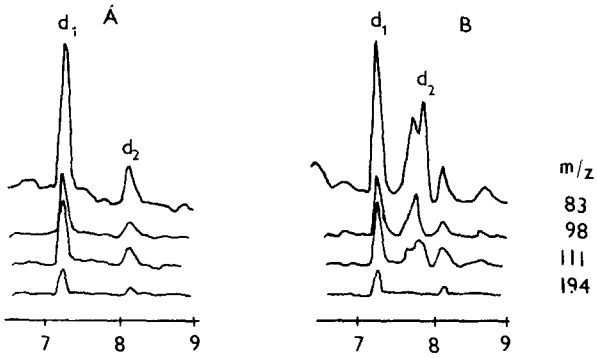


Fig. 5. Mass fragmentographic analysis of rapeseed. (A) Standard solution of hydrogenated diquat, injected amount 5 ng; (B) rapeseed, injection represents 5 mg of sample.

presence of diquat residues (at a level of 2.8 mg/kg) was found in this sample. As can be seen, the most selective ion was that of *m/z* 194 (molecular ion), but unfortunately its abundance in the spectrum was not high. Based on the monitoring of the most intense ion of *m/z* 83, the sensitivity of MS detection was comparable to that achieved by NPD. In the latter instance, however, the use of a more efficient capillary column for better resolution of the analytes (especially for levels lower than 1 mg/kg) is recommended. The recovery of diquat from both rapeseed and meal was high, *viz.*, 90.3 ± 5 and 92.8 ± 3%, respectively, at a spiking level 2 mg/kg.

We were also interested in the distribution of diquat residues between the rapeseed oil and remaining meal. A rapeseed sample containing 2.8 mg/kg of diquat was extracted with light petroleum (b.p. 40–70°C) in a Soxhlet apparatus. Nearly all the residues (99.3% of the original content) were found in the defatted rapeseed sample; the analysis of isolated oil did not reveal the presence of diquat. This finding is in agreement with the above-outlined physico-chemical properties of diquat. This bipyridinium compound is firmly bonded to the hydrophilic portion of the plant matrix and hence is not accessible for extraction with an apolar solvent.

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