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Note

Analysis of paraquat formulations by liquid chromatography*

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A number of methods for the analysis of residues of paraquat, a non-selective herbicide, are available which utilize either bioassay procedures[1], colorimetry[2–7], gas chromatography[8–11], or thin-layer chromatography[12,13]. However, there is only one acceptable method for the analysis of paraquat in commercial formulations: a colorimetric procedure developed by Yuen *et al.*[14] and later adopted as the official procedure by the Association of Official Analytical Chemists[15] following intensive collaborative studies by Carlstrom[16,17] and Ashley[18].

Recently, liquid chromatography (LC), using an ultraviolet detector, has been applied to the analysis of pesticides[19,20]. Since it was known that paraquat has a maximum ultraviolet absorption at 257 nm under buffered conditions, the LC technique was successfully applied to the analysis of paraquat formulations. The results of the study, including the optimum parameters for the analysis of paraquat by LC and comparative data obtained by the colorimetric procedure are the substance of this report.

EXPERIMENTAL

Equipment and materials

A Hewlett-Packard Model 1010-B high-pressure liquid chromatograph was used in this study, which included a Schoeffel SF-770 variable (200–700 nm) UV detector operated at ambient temperature and a Hewlett-Packard Model 5702-A, 10 mV, 0.5 in./min chart speed, recorder. The variable wavelength detector was adjusted to 264 nm. The LC column was stainless steel, 3.2 mm I.D. × 25 cm, packed with Vydac (The Separations Group, Hesperia, Calif., U.S.A.) cation exchange resin (sulfonic acid–solid core material; particle size 30–44 μm). The columns were obtained pre-packed from Hewlett-Packard. The mobile phase was 0.2 *M* dimethylamine hydrochloride in methanol. A flow-rate of 4–6 ml/min was the optimum rate for paraquat analysis. Pure (100%) paraquat dichloride was supplied by Dr. A. A. Carlstrom.

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Chevron Chemical Research Center, Ortho Chemical Division, Richmond, Calif., U.S.A. Samples of commercially formulated paraquat dichloride, containing 29.1% of active ingredient, were obtained from five different locations in California by agricultural inspectors of the State of California Department of Food and Agriculture. Each sample originated from a different manufacturer's batch mix.

Sample preparation

Five samples (each 70–80 mg) were taken from each commercial paraquat formulation mix. Each sample was weighed into a 10-ml volumetric flask, made to volume with absolute methanol and 10 μl of each was analyzed by LC. Similarly, five samples (1 g each) were taken from each commercial formulation for analysis by the official colorimetric procedure[15]. Each sample was weighed into a 500-ml volumetric flask and made to volume with distilled water and labeled the stock solution; 10 ml of the stock solution were transferred to a 100-ml volumetric flask, made to volume with distilled water, and 10 ml of this solution were used for analysis by colorimetry. Standard paraquat solutions were prepared from analytical grade paraquat dichloride previously dried for 3 h at 100–120°. A standard solution for LC was prepared with 24 mg of paraquat dichloride in 10 ml of absolute methanol; 10-μl aliquots were used for LC calibration curves. A second solution was prepared, containing 0.1728 g paraquat dichloride in a volume of 500 ml of distilled water; subsequent aqueous dilutions were made for use in the preparation of a standard curve in the range of 2–10 μg/ml of paraquat dichloride for the colorimetric procedure, to be measured in a Beckman DU spectrophotometer at 600 nm.

The mobile solvent is corrosive to stainless steel. Therefore, the LC system should be flushed with 200 ml of absolute methanol at the rate of 3 ml/min on completion of use of this particular mobile solvent. An exhausted cation–ion exchange column can be regenerated with 0.4 *M* aqueous phosphoric acid at the rate of 1.5 ml/min for 1 h followed by flushing the column with water and methanol.

Calculations

For the LC procedure, the peak height of the recorded curve of the paraquat sample was measured with a mm rule and compared to the peak height of the recorded curve of a known amount of a paraquat standard solution. The ratio value of the two measurements was determined and applied to subsequent volume and sample concentration calculated values to calculate the amount of paraquat dichloride in the sample.

For the colorimetric procedure, the absorption reading of the sample obtained at 600 nm was referred to a standard curve previously prepared under similar analytical conditions to determine the amount of paraquat dichloride in the analyzed sample.

RESULTS AND DISCUSSION

The results of the analyses for paraquat in commercial formulations determined by both LC and colorimetry are given in Table I. An example of a LC chromatogram is illustrated in Fig. 1: the retention time for the compound was 1.5 min. It was later suggested that the tailing effect noted in the LC curve could be minimized by adding about 5 drops of conc. ammonium hydroxide to each liter of the mobile phase. The linear range for paraquat dichloride, using the LC instrument range setting

TABLE I

AMOUNT OF PARAQUAT DICHLORIDE FOUND IN COMMERCIAL FORMULATIONS BY TWO METHODS OF ANALYSIS

Manufacturer's label indicated that commercial formulation contained 29.1 % paraquat dichloride.

	Sample No.				
	82864	*83039*	*83059*	*83160*	*83324*
	Paraquat dichloride (%)				
Liquid chromatographic method					
1	29.4	29.6	30.5	29.0	29.6
2	29.4	29.0	30.0	29.2	29.8
3	29.2	29.2	30.0	29.3	29.6
4	29.5	29.6	30.0	29.5	30.1
5	29.0	29.8	30.2	29.6	30.4
Mean	29.3	29.4	30.1	29.3	29.9
Colorimetric method					
1	30.2	30.7	30.2	30.6	30.2
2	30.7	30.9	30.8	30.0	30.6
3	30.4	30.7	30.6	30.3	30.1
4	30.3	30.8	30.5	30.2	30.6
5	30.7	30.9	30.7	30.8	30.0
Mean	30.4	30.8	30.5	30.3	30.3

of 1.0, was 10–35 μg; minimum detectability was 100 ng with an instrument setting of 0.02.

The manufacturer's label for the commercial formulations indicated a paraquat dichloride content of 29.1 %. The mean analytical value of 25 determinations by LC

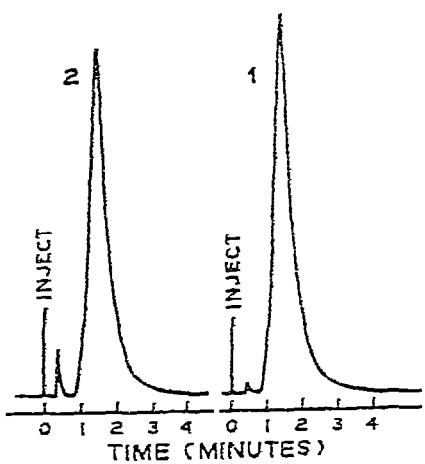

Fig. 1. LC curves of paraquat dichloride. 1, Analytical-grade paraquat, 24.2 μg/10 μl. 2, Commercial paraquat formulation. Conditions: Hewlett-Packard Model 1010-B liquid chromatograph equipped with variable wavelength UV detector; UV range 264 nm; range setting 1.0; LC column of stainless steel, 25 cm × 3.2 mm I.D., packed with Vydac cation ion-exchange resin; flow-rate 4 ml/min; mobile phase 0.2 *M* dimethylamine hydrochloride in absolute methanol; observed pressure 600 p.s.i.

TABLE II

ANALYSIS OF VARIANCE ON METHODS AND SAMPLES FROM THE OBSERVED VALUES

Source of variation	*Degrees of freedom*	*Sum of squares*	*Mean square*
Method (LC and colorimetric)	1	9.68	9.68*
Samples (5 locations)	4	1.66	0.42
Interaction (samples × method)	4	1.96	0.49**
Individuals	40	2.68	0.067
Total	49	15.98	

* Significant at 95% level.
** Significant at 99% level.

was 29.5% with a standard deviation of 0.37%. The mean analytical value for a similar number of determinations obtained by the official colorimetric procedure was 30.5% with a standard deviation of 0.28%. Statistical analysis indicated a significant difference between the two methods of analysis (Table II); the results obtained by the LC procedure related more closely to the active ingredient label designation of the product than did the results obtained by the colorimetric method. Although the coefficient of variation was slightly higher (1.25%) for the LC data compared to that for the colorimetric data (0.91%), the LC method suggested a more reliable procedure for the acquisition of consistent and uniform results. The LC procedure is less tedious and less time-consuming when compared to the colorimetric method.

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