

PLANT PROTECTION DIVISION RESIDUE ANALYTICAL METHOD No. 1B

THE DETERMINATION OF RESIDUES OF PARAQUAT IN CROPS

- a spectrophotometric method

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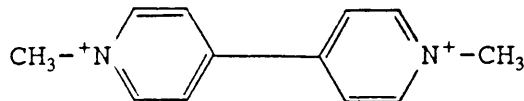
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1. SCOPE

The analytical procedures described are suitable for the determination of residues of paraquat (I, 1, 1'-dimethyl-4,4'-bipyridinium ion) in crops.



The method can also be used for the determination of residues of paraquat in plant juice ie. sugar cane crusher juice. The limit of quantitation of the method is 0.01 to 0.05 mg/kg depending on the crop analyzed.

2. SUMMARY

The sample is boiled under reflux in a 0.5M sulfuric acid solution. The filtered digest is percolated through a column of cation-exchange resin which retains the paraquat and some of the natural crop constituents. The column is washed with 2.5% ammonium chloride solution and water; the paraquat is eluted with saturated ammonium chloride solution. A portion of the column eluent is treated with sodium hydrosulfite in alkali. This reduces paraquat to a free radical, the light absorption of which is measured with a spectrophotometer.

3. REAGENTS

- (a) Sodium chloride solution - saturated
- (b) Ammonium chloride solution - 2.5% (w/v) and saturated
- (c) Sulfuric acid - concentration 18M
- (d) Cation exchange resin: 4R cation exchange resin, strong acid (Na), chromatographic grade, particle size 0.150-0.300 mm (52-100 mesh). BDH chemicals, Catalog No. 55171. Available from Gallard-Schlesinger Industries, Inc. 584 Mineola Ave. Carle Place, NY 11514-1731. Telephone: (516) 333-5600. 800 645-3044
- (e) Standard paraquat solutions: Paraquat dichloride standard available from ICI Americas Inc. Western Research Center, 1200 S. 47th Street, Box 4023, Richmond, California, 94804-0023. Attention: Dr. Norman. T. Nelson.
 - (i) Stock Solution (250 µg/mL of paraquat): Dissolve 0.08633 grams of pure paraquat dichloride, C₁₂H₁₄N₂Cl₂ (molecular weight, 257.2; 72.4% cation), in saturated ammonium chloride solution and make up to 250 mL with saturated ammonium chloride solution. Paraquat salts are hygroscopic; they should be dried at 100° C for 5 hours and cooled in a desiccator before use.
 - (ii) Working Solutions: Make dilutions of the stock solution to give a range of working solutions. Concentrations of these working solutions will vary depending on the level of residues in the samples analyzed. These solutions are stable under normal laboratory conditions provided that they are not exposed to sunlight for long periods.
- (f) Sodium hydrosulfite solution, 0.2% (w/v) in 0.3M sodium hydroxide. Dissolve 0.1 g of sodium hydrosulfite in 15 mL of 1M sodium hydroxide in a 50 mL volumetric flask. Dilute this solution to a final volume of 50 mL with water. Gently mix this solution by carefully inverting the flask. DO NOT SHAKE THIS SOLUTION. This solution should be used immediately, and must not be used more than 30 minutes after preparation. Solid sodium hydrosulfite is unstable in the presence of moisture, and should therefore be stored in a tightly sealed plastic container.

(g) Octan-2-ol (2-Octanol)

4. SAFETY COMMENTS

The following information is included as an indication to the analyst of the nature and hazards of the reagents used in this procedure. If in doubt, consult the appropriate safety manual containing recommendations and procedures for handling chemicals.

(a) OCTAN-2-OL

Harmful vapor
Harmful if taken internally
Highly flammable
Avoid breathing vapor
Avoid contact with skin/eyes

(b) SULFURIC ACID - concentrated

corrosive - causes burns
Prevent contact with skin and eyes
Do not put water into container
TLV 1 mg/m³

(c) PARAQUAT

Toxic by ingestion
Harmful dust
Avoid contact with eyes, skin and mouth. Avoid breathing dust.
Wash hands and exposed skin before meals and after work.
Ingestion of paraquat should be regarded as a dire emergency and action taken immediately. Details of remedial action/antidotes should be available in the laboratory. For medical emergency information telephone (1-800) 327-8633.

5. APPARATUS

(a) Equipment which can be used for the initial preparation of samples i.e. for chopping or dicing vegetables and fruit, grinding grain and chopping grass.

(b) Heating mantles of 1 liter capacity. a heating unit containing six mantles which operates to a maximum of 250° C is suitable (eg. Electromantle ME, Electrothermal Engineering Ltd., available through VWR Scientific.)

It is important that the heating equipment allows the sample to boil without local overheating or charring.

(c) Boiling flasks - 1 liter round bottom flasks with B34 necks fitted with water cooled reflux condensers. The size of the flask should be large enough to allow complete coverage of the sample with the sulfuric acid solution.

(d) Glass columns for chromatography of 10 mm internal diameter and 500 mm length (25 mL burettes are suitable).

(e) Scanning spectrophotometer, eg. Shimadzu UV-265, UV-visible scanning spectrophotometer.

6. METHODS

6.1 Extraction and Chromatographic Separation

- (a) Thoroughly mix the diced, chopped or crushed sample and weigh a representative aliquot (See Table below) into a 1 liter round bottomed flask together with the required volumes (See Table below) of water and 18M sulfuric acid.

Sample	Weight for Analysis (grams)	Treatment	Volume (mL) to be added ¹	
			Water	Acid
Vegetables and fruit	250	Mince the sample before weighing	250	15
Grain and seed	50	Grind to fine powder before weighing	450	15
Grass, straw, hay, etc.	100 (fresh)	Cut to 2.5 cm lengths prior to weighing	500	15
	25 (dry)	Grind to a fine powder before weighing	450	15
Oil seed crops - rape, sunflowers, soybeans	50	Grind to a fine powder and extract oil using hexane in soxhlet extractor. Analyze cake only	450	15
Other oil crops - Olives	250	Extract oil from fruit using soxhlet extractor as above	250	15
Sugar-cane crusher juice	100 (mL)	Shake well before sampling	250	10

Notes: (1) Caution! The sulfuric acid must be added to the aqueous solution with care.

- (2) Important Samples such as potatoes etc. must be rinsed with water to remove any adhering soil before preparation for analysis.

(b) After adding anti-bumping granules and 2-octanol (0.3 mL) which acts as an anti-foaming agent, place the 1 liter flask containing the sample on a heating mantle. Attach a water-cooled reflux condenser and heat to boiling. Swirl the flask contents occasionally to minimize local overheating and charring until the solution is boiling steadily.

(c) Boil under reflux for 5 hours and allow to cool. It is important that the sample boils steadily and evenly without local overheating or charring. The solution can be left overnight at this stage.

(d) While the samples are being boiled the ion-exchange columns are prepared as follows. Plug the glass chromatography column with glass wool at the stopcock end of the column. Wash 3.5 g of cation exchange resin into the glass column with saturated sodium chloride solution (25 mL). Wash the column at a flow rate of 5 mL/min with water (50 mL). Fill the column to the top with water and close the stopcock. The column is now ready to be used. Prepare a separate column for each sample.

- (e) Wash the reflux condenser attached to the boiling flask with water (50 mL) into the cooled contents of the round bottomed flasks from 6.1 (c) above. Filter the sample by suction through 2 Whatman No. 5 filter papers. Suction the filter pad dry and wash the filter twice with water (100 mL), allowing the first 100 mL to pass through the filter cake before adding the second.
- (f) Transfer the filtrate to a 1 liter separatory funnel. Attach the separatory funnel to the chromatographic column with rubber tubing. Allow the filtered sample solution to percolate through the prepared ion-exchange column from 6.1(d) above at a flow rate of 5-10 mL/min. Close the stopcock when the sample solution has reached the top of the resin bed.
- (g) Remove the separatory funnel from the column. Rinse the column with water (25 mL) with the stopcock full open. Reattach the separatory funnel to the column. Wash the column with 100 mL of 2.5% (w/v) ammonium chloride solution at a flow rate of 3-5 mL/min by filling the column and placing the remaining wash solution in the funnel above the column. When the ammonium chloride solution has drained to the top of the column remove the separatory funnel and add 2 X 5 mL portions of water to the column. Then add an additional 10 mL portion of water to the column to reduce the concentration of the ammonium chloride solution in the column. At this point also increase the column flow rate to 5 - 10 mL/min. Allow the wash to drain to the top of the resin then rinse the column with 25 mL of water with the stopcock full open. Close the stopcock and add 20 mL water to the column and let it sit for at least 30 minutes. The column may be left overnight at this point.
- (h) Drain the water off the column then elute the paraquat from the column with saturated ammonium chloride solution at a flow rate of about 1 mL/min. Collect the first 50 mL of the eluent in a 50 mL volumetric flask and mix by shaking. *NOTE: The recovery of the paraquat from the resin column will be adversely affected if the flow rate of the eluent exceeds 1 mL/min. The use of Celite as a filter aid is not recommended as it can reduce paraquat recovery. However, with samples of ground grain and straw celite may be needed as filtration will be difficult without it.*

6.2 Determination

- (a) Pipette a 10.0 mL aliquot of the eluent into a 15 mL glass test tube. Add by pipette 2 mL of 0.2% (w/v) sodium hydrosulfite solution. Do not shake or agitate. Pouring this solution into the cuvette for analysis will mix the sample sufficiently.
- (b) Within 5 minutes of adding the sodium hydrosulfite use a recording spectrophotometer to record the spectrum of the solution in a 4 - 5 cm path length cell over the range 430 - 360 nm, against a reference solution prepared from saturated ammonium chloride (10 mL) and sodium hydrosulfite solution (2 mL).
- (c) Draw a baseline as a tangent to the curve from the valley in the region of 390 nm. Measure the height of the peak above the baseline.
- (d) Draw a calibration curve relating the peak height at 396 nm (mm above the baseline) to the concentration of paraquat in $\mu\text{g/mL}$. Using a linear regression function on a calculator or a computer calculate the linear regression of the calibration curve. From the curve calculate the slope of the line and the intercept. *NOTE: When using a spectrophotometer with a derivative function, operation in the 2nd derivative mode will give an enhanced response to paraquat. See appendix 1 for comparison of spectra and methods of measuring peak heights.*

7. CALCULATION

Using the equation: $\frac{y - B}{A} = x$ where: y = height of response in mm
 A = slope of regression curve
 x = concentration of sample in $\mu\text{g/mL}$
 B = intercept of regression curve

Calculate the concentration of the sample solution in $\mu\text{g/mL}$.

The paraquat concentration in the sample $\mu\text{g/g}$ (mg/kg) may be calculated using the following equation.

$$\mu\text{g/g} = \frac{\text{volume of eluent (mL) from the column}}{\text{weight of sample (g)}} \times \text{concentration of eluent } y \text{ (ug/mL) from above}$$

8. RECOVERIES AND LIMIT OF QUANTITATION

Recovery experiments should be carried out by adding known amounts of paraquat to untreated samples prior to the acid digestion of the sample. The amount added should be similar to the amounts that are expected in the treated samples.

A summary of the percentage recoveries and limits of determination obtained in these laboratories using this procedure is presented in the table below.

The limits of determination were calculated by taking into account the average apparent content of paraquat in control samples, the size of sample taken for analysis and the minimum amount of pure paraquat that can be determined with certainty.

Sample	Sample Size	Limit of Determination mg/kg	% Recovery Expected
Vegetables and fruit	250 g	0.01	70 - 85
Grain and Seed	50 g	0.05	65 - 75
	100 g	0.02	60 - 75
Grass and Straw	100 g	0.02	70 - 85
	25 g	0.05	80 - 95
Sugar-cane juice	100 mL	0.02	80 - 95
	100 mL	0.01	80 - 95

APPENDIX 1

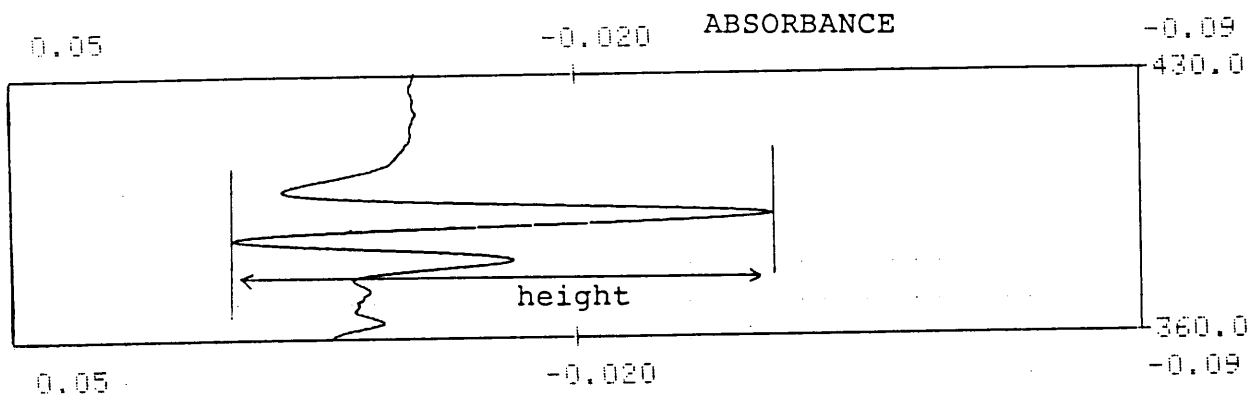
spectrophotometer conditions and spectra for
absorbance and derivative modes

RANGE	LOW	HIGH	START WL(NM)	END WL(NM)
ABS.	-0.09	0.05	430	360

SCALE(NM/CM)	SPEED	SLIT	CYC.T.(MIN)
20	FAST	2	0

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RANGE	LOW	HIGH	START WL(NM)	END WL(NM)
ABS.	0	0.14	430	360

SCALE(NM/CM)	SPEED	SLIT	CYC.T.(MIN)
20	FAST	2	0

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ABSORBANCE

