Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Paraquat

MRID #: 422174-03

Matrix: Soil

Analysis: Spectrophotomtr

Paraquot 3/3 9 40

Found in Ref
42802-01
field 164-5

Actually
171-4(C)
in Document

EPA

MRID NUMBER

422174-03

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VOLUME 3 422174-03

Study Title

The Determiniation of Residues of Paraquat in Soil A Spectrophotometric Method

Data Requirement

Guideline 171-4(C)
158.125 Residue Chemistry

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Study Completed On

January 23, 1986

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Plant Protection Division Residue
Analytical Method No. 2B

133

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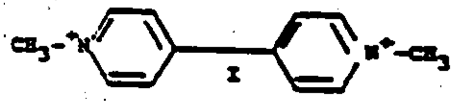
422174-03

This method cancels and replaces PPRAM 2A dated November 1980.

1. SCOPE

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


I

The limit of determination of the method is 0.05 mg kg^{-1}.

2. METHOD SUMMARY

The sample is boiled in 6M sulphuric acid solution. The filtered digest is percolated through a column of cation-exchange resin which retains the paraquat and some of the natural soil constituents. The column is washed with dilute hydrochloric acid, 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 dithionite 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) Sulphuric acid - concentrated (13M)

(d) Hydrochloric acid solution (2M)

(e) Cation-exchange resin: Duolite C225 (SRC 14) chromatographic grade resin, 52-100 mesh, 0.68-0.85 water regain, sodium form.

(f) Standard paraquat solutions:

(i) Stock solution (250ug/ml of paraquat)

Dissolve 0.0864 g pure paraquat dichloride, $C_{12}H_{14}N_2Cl_2$ (mol.wt., 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 analysed.

These solutions are stable under normal laboratory conditions provided that they are not exposed to sunlight for long periods.

(g) Sodium dithionite solution, 0.2% (v/v) in 0.3M sodium hydroxide. This solution should, if possible be used immediately, and must not be used more than half an hour after preparation. When preparing this solution do not shake vigourously.

Solid sodium dithionite is unstable in the presence of moisture, and should therefore be stored in a tightly sealed plastic container.

(h) Octan-2-ol

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 (eg ICI Laboratory Safety Manual) containing recommendations and procedures for handling chemicals, and a monograph such as 'Hazards in the Chemical Laboratory', edited by G D Muir, The Chemical Society, London.

(a) HYDROCHLORIC ACID

Irritant vapour
Corrosive - causes burns
Avoid breathing vapour.
Prevent contact with eyes and skin
(TLV 7 mg m^{-3} as HCl).

(b) SULPHURIC ACID - concentrated

Corrosive - causes burns
Prevent contact with skin and eyes
Do not put water into container
(TLV 1 mg m^{-3})

(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.

(d) **OCTAN-2-OL**

Harmful vapour
Harmful if taken internally
Highly flammable
Avoid breathing vapour or contact with skin and eyes
(TLV 2.60 mg m^{-3})

5. APPARATUS

(a) Equipment which can be used for the initial preparation of samples ie, metal trays for drying soils.

(b) Heating mantles for the extraction procedure (e.g Electrothermal Heating Unit available from Electrothermal Engineering Ltd, London E7 UK).

(c) Boiling flasks - 500 ml round bottom flasks with B34 necks fitted with water cooled reflux condensers.

(d) Glass columns for chromatography of 1.0 cm i.d. and 50 cm long (25 ml burettes are suitable).

(e) Scanning Spectrophotometer eg, Perkin Elmer Lambda 5 UV/VIS Spectrophotometer.

(f) Sieve, 2-3 mm mesh size.

6. PROCEDURE

6.1 Extraction and Chromatographic Separation

(a) Thoroughly mix the soil sample and weigh about 100 g into a shallow metal tray or glass petri dish. Dry the soil sample at room temperature for several days.

(b) Grind the dry soil sample to pass through a 2-3 mm sieve. Weigh an aliquot (25 g) of the ground soil into a 500 ml round bottomed flask and add water (65 ml) followed by 18M sulphuric acid (35 ml).

Note: <u>CAUTION!</u> The sulphuric acid must be added to the aqueous solution with care.

(c) After adding anti-bumping granules and octan-2-ol (1 ml) which acts as an anti-foaming agent, place the 500 ml flask containing the sample on a heating mantle. Attach a water-cooled reflux condenser and heat to boiling. Swirl the flask contents occasionally to minimise local overheating and charring until the solution is boiling steadily.

It is important to avoid local overheating and charring and this can be done by ensuring that only the bottom quarter of the flask makes contact with the heating mantle (which should be oversize).

(d) Boil under reflux for 5 hours and allow to cool. The solution can be left overnight at this stage.

(e) While the samples are being refluxed the ion-exchange columns are prepared as follows: Wash 5.0 g of resin with water into a burette (25 ml) containing a glass wool plug placed near the stopcock. Pass successively through the column at the rate of 5 ml/min. saturated sodium chloride solution (20 ml) and water (50 ml). Prepare a separate column for each sample.

(f) Wash the reflux condenser attached to the boiling flask with water (50 ml) into the cooled contents of the 500 ml round-bottomed flasks from 5.1 (d) above. Dilute the flask contents to near 500 ml by the addition of water and filter the solution by suction through 2 Whatman No.5 filter papers. Suck the filter pad dry and wash the filter twice with water (100 ml), allowing the first portion to be sucked through before adding the second.

(g) Transfer the filtrate to a 1 litre separating funnel. Allow the solution to percolate through a prepared resin column from 5.1 (e) above at a flow rate of 5-10 ml/min.

(h) Remove the funnel and wash the column at a flow rate of 3-4 ml/min successively with water (25 ml), 2N-hydrochloric acid (100 ml), water (25 ml), 2.5% (w/v) ammonium chloride solution (50 ml) and water (25 ml). (The process can be left overnight provided the resin column has been covered with water).

(i) Elute the paraquat from the column with saturated ammonium chloride solution at a flow rate of about 1ml/min. Collect the first 50 ml of the eluant in a 50 ml volumetric flask and mix.

N.B The recovery of the paraquat from the resin column will be adversely affected if the flow rate of the eluant exceeds 1.0 ml/min.

6.2 Determination

(a) Pipette an aliquot (10.0 ml) of the eluent into a 15 ml glass-stoppered test-tube. Add by pipette 0.2% (w/v) sodium dithionite solution (2.0 ml) and mix by gently inverting the tube once.

(b) Within 5 minutes of adding the sodium dithionite, use a recording spectrophotometer to record the spectrum of the solution in a 4 cm path length cell over the range 360-430 nm, against a reference solution prepared from saturated ammonium chloride (10.0 ml) and sodium dithionite (2.0 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 at 396 nm.

(d) Draw a calibration curve relating the peak height at 396 nm (mm above the baseline) to the concentration of paraquat in μg/ml.

N.B 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

Read off from the prepared calibration curve, using the peak height measured at 396 nm, the concentration (ug/ml) of paraquat in the eluent.

N.B It has been found useful to use the linear regression function on an electronic calculator to produce the calibration curve.

Then paraquat concentration in sample (mg/kg)

$$= \frac{\text{Volume of eluent, ml from column}}{\text{Weight of sample, (g)}} \text{ X } \text{Concentration in eluent (mg/ml)}$$

To correct the paraquat concentration for the experimental recovery multiply by 100 and divide by the % of recovery figure.

8. RECOVERIES AND LIMIT OF DETERMINATION

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

In these laboratories using this procedure recoveries of between 80% and 95% of the added paraquat are expected with a limit of determination of 0.05 mg/kg of the compound (25 g sample).

APPENDIX 1

Spectrophotometer conditions and spectra for
absorbance and derivative modes

BEST AVAILABLE COPY

ABSORBANCE MODE

```
PERKIN-ELMER
LAMBDA 5 UV/VIS SPECTROPHOTOMETER

SAMPLE ID ..............................
OPERATOR ...............................
METHOD .................................
                            SCAN/MANUAL
 01 ORDINATE MODE
                                    ABS
 02 SLIT
                                   2 NM
 03 SCAN_SPEED
                             120 NM/MIN
 04 RESPONSE
                                    2 S
 05 LAMP
                               332.0 NM
 06 CYCLES/TIME
                         1 /  0.05 MIN
 07 PEAK THRESHOLD
                                0.02  A
 08 RECORDER
                       SERIAL / DASH 1
 09 ORD MIN/MAX
                        -0.050 / 0.250
 10 ABSC MIN/MAX
                         360.0 / 430.0
 11 ABSCISSA FORMAT
                              10 NM/CM
 12 PRINTER    GRID/SCALE/      /
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DERIVATIVE MODE

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PERKIN-ELMER
LAMBDA 5 UV/VIS SPECTROPHOTOMETER

SAMPLE ID ..............................
OPERATOR ...............................
METHOD                      SCAN/MANUAL

 01 ORDINATE MODE                   D2

 02 SLIT                          2 NM

 03 SCAN SPEED              120 NM/MIN

 04 RESPONSE                       2 S

 05 LAMP                      332.8 NM

 06 CYCLES/TIME          1 /  0.05 MIN

 07 DELTA WAVELENGTH              2 NM

 08 PEAK THRESHOLD              0.2 D2

 09 RECORDER          SERIAL / DASH 1

 10 ORD MIN/MAX        -2.200 / 1.000

 11 ABSC MIN/MAX        360.0 / 430.0

 12 ABSCISSA FORMAT          10 NM/CM

 13 PRINTER    GRID/SCALE/      /
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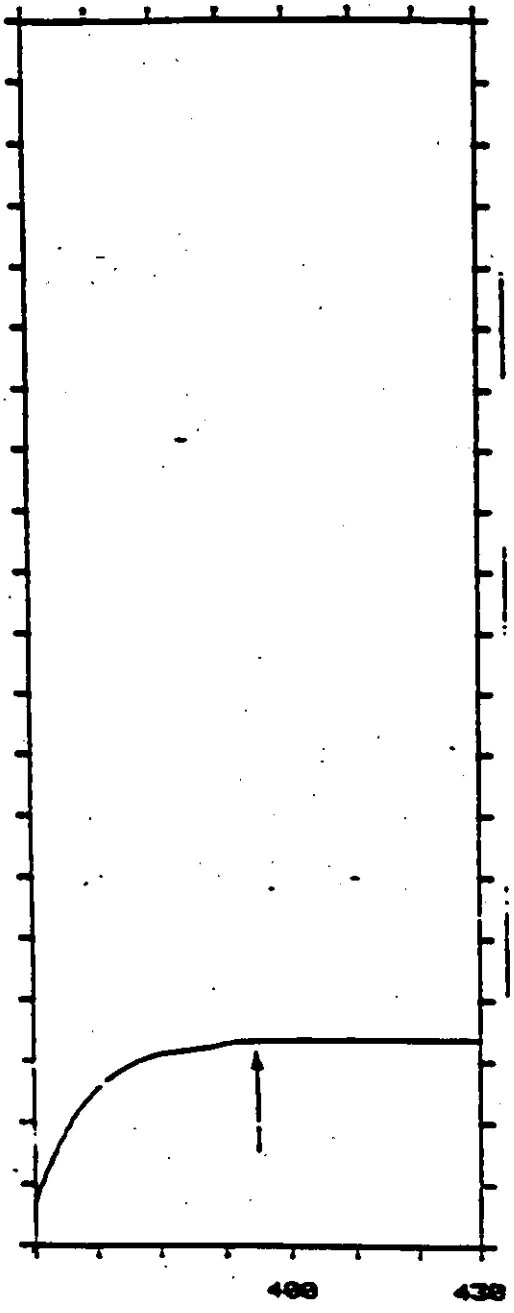
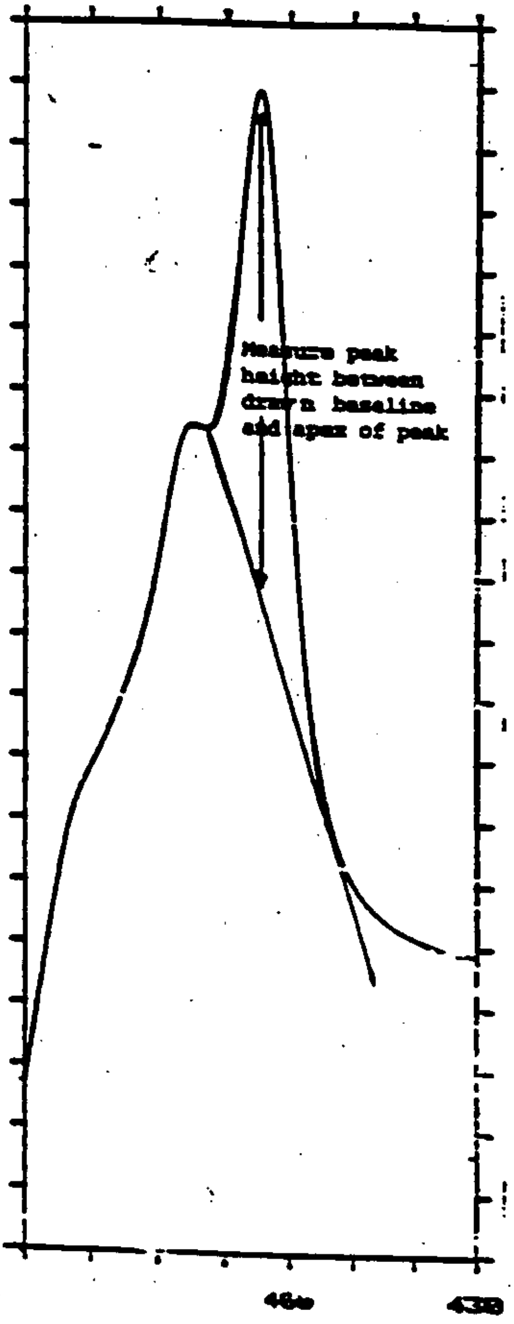
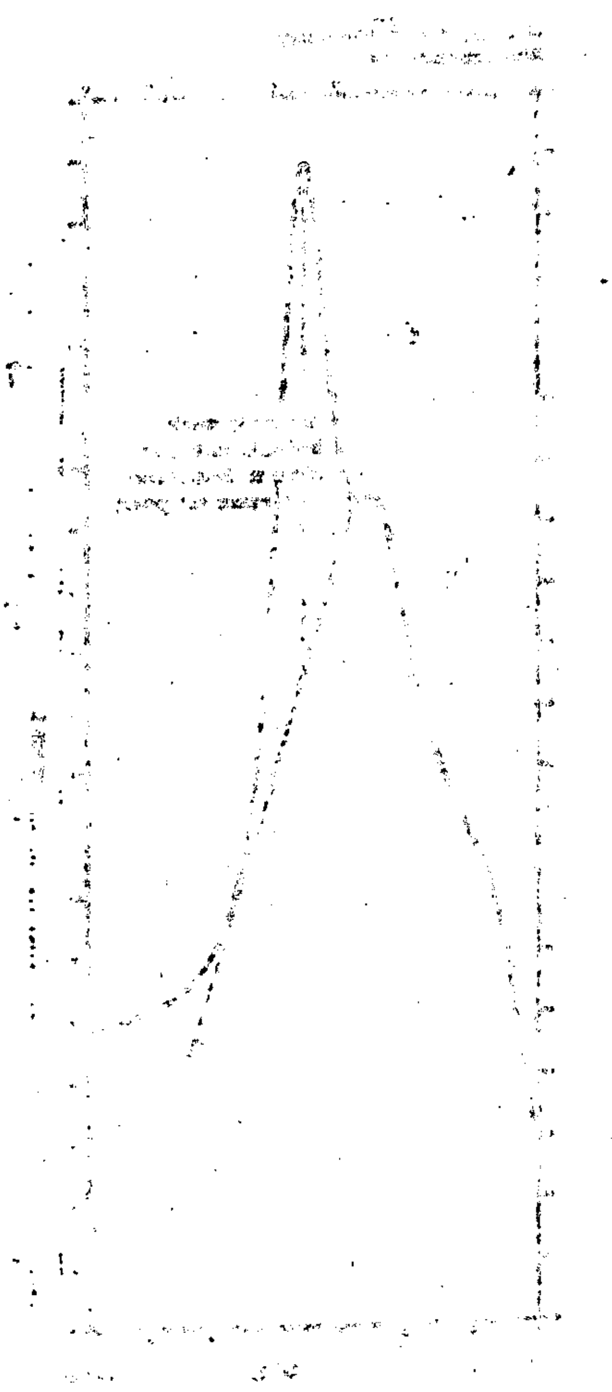
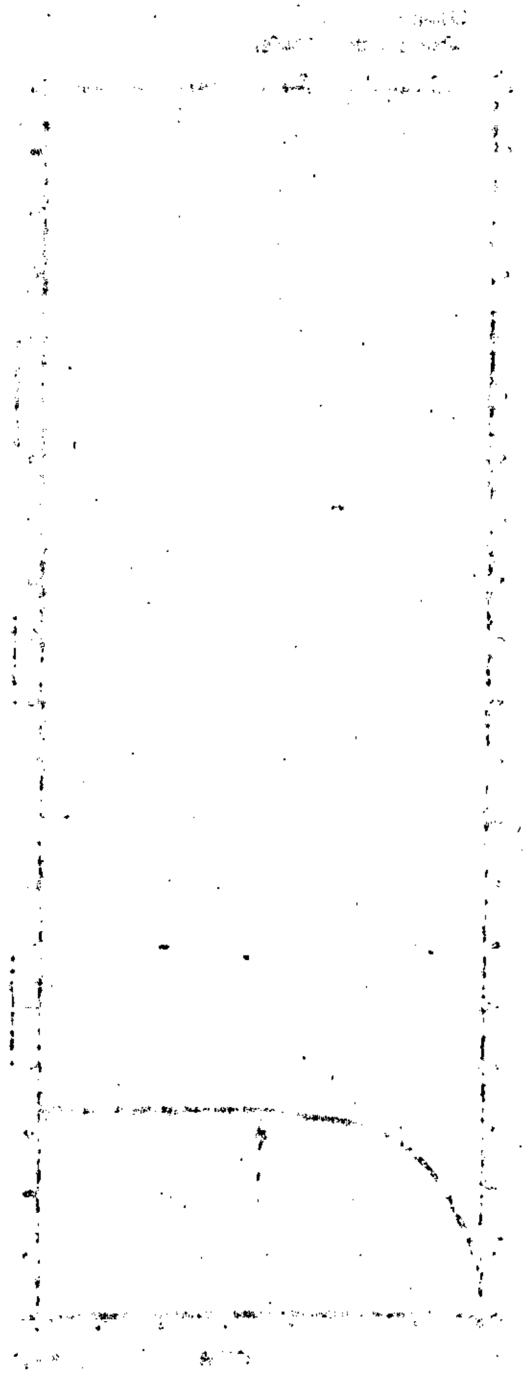
Absorbance Mode



0.3 μg cm^{-3} Paraquat
Absorbance Mode


Measure peak height between drawn baseline and apex of peak





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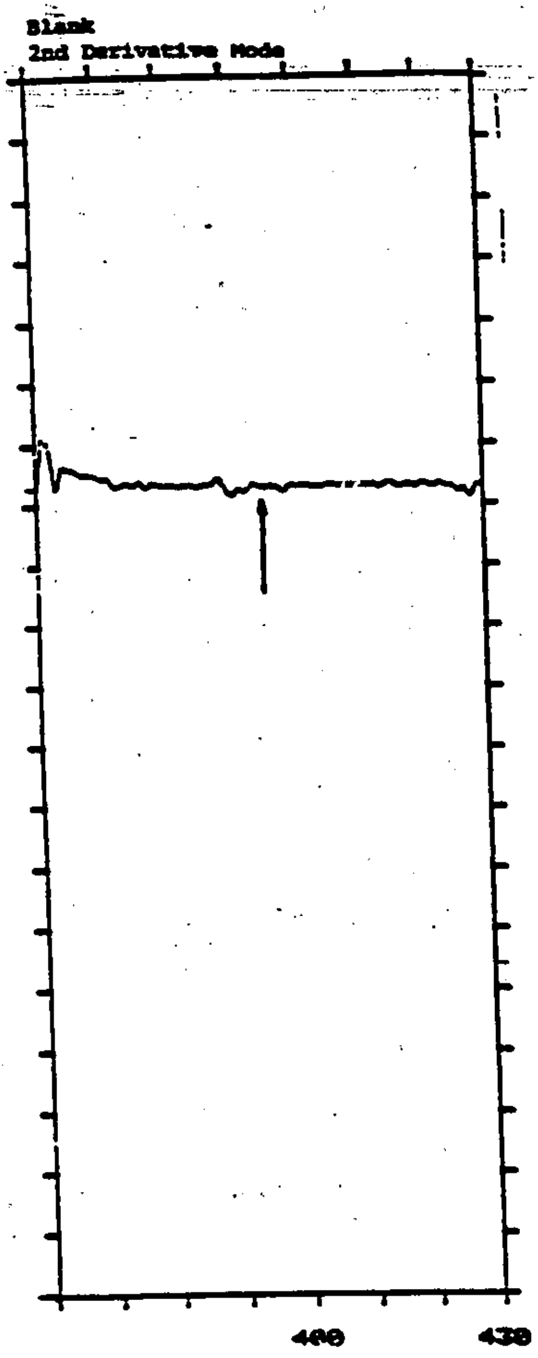
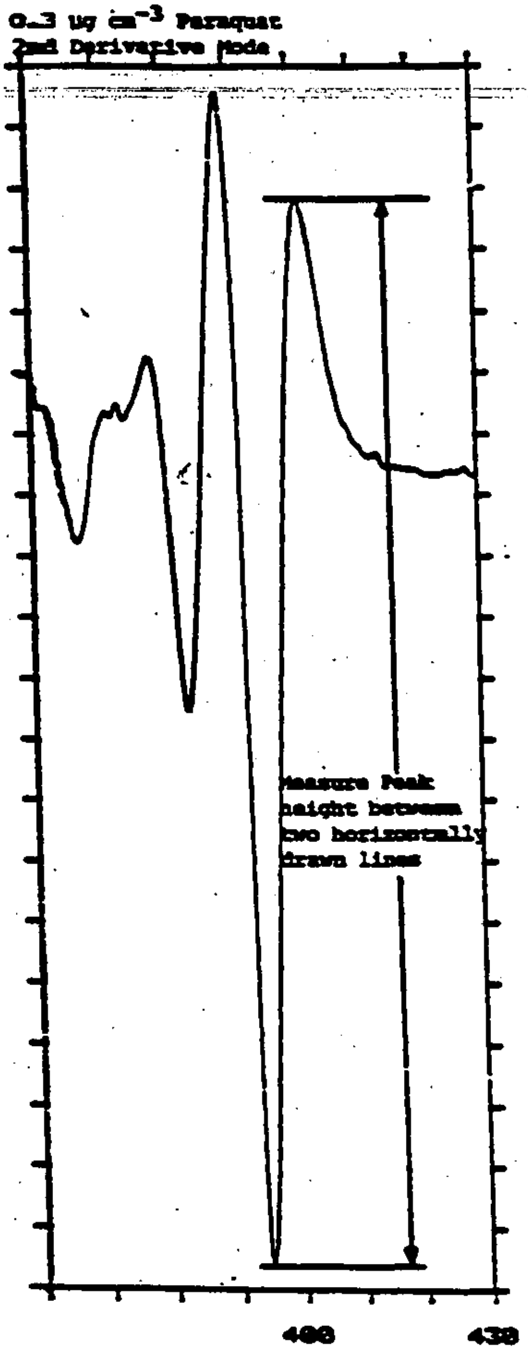

Blank
2nd Derivative Mode
400
430


0.3 ug cm-3 Paraquat
2nd Derivative Mode
Measure Peak height between two horizontally drawn lines
400
430