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METHOD FOR THE DETERMINATION OF PARAQUAT RESIDUE IN OIL MATRIX

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Two experiments were carried out using the method developed by Imperial Chemical Industry (ICI) to determine paraquat residue in grains and crops The aim of these experiments was to evaluate the feasibility of the method in determining paraquat residue in palm oil and palm oil products. Paraquat free RBD palm olein was used in the study. The method involved three steps: extraction of residue from the oil, clean up procedure using two types of equivalent resin, Duolite and Amberlite, and spectrophotometric determination of the purified material. In Experiment I, in which the resin Duolite was used, the percentage recoveries were adequate ranging from 50%-83%. The percentage recoveries increased with increasing concentration of paraquat. The estimated limit of detection based on recovery data of this experiment was 0.01 μg g^{-1}. In experiment II, using Amberlite, the percentage recoveries were greater than 90% for both the 0.05 μg ml^{-1} and 0.50 μg ml^{-1} level of concentration. Results from the two experiments showed that the ICI method can be applied for determination of paraquat residue in palm oil and palm oil products, and that the method with the use of Amberlite resin in the clean up step can give better recoveries of the analyte.

INTRODUCTION

Paraquat is the common name for 1, 1'-dimethyl-4,4' bipyridinium ion (see molecular structure next page) and the commercial form of this ion is dichloride and di-(methyl

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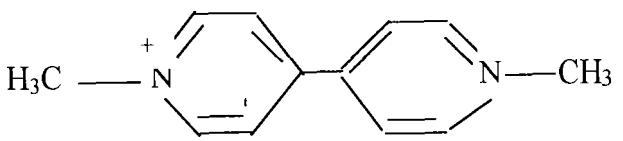
paraquat dichloride sulphate) sold under the trade name of Grammoxone. It is a colourless crystalline solid which is very soluble in water. As a herbicide, paraquat destroys green plant tissue through contact action with some translocation. On contact with soil, paraquat is inactivated rapidly and is therefore a good control for prolific weed growth in plantations without affecting the roots of the palm. Regular spraying of this herbicide in oil palm plantations is necessary when the palms are still young (< eight years old) and with less canopy, while mature palms need less spraying because their closed canopy helps to retard weed growth.



Bipyridium ion

In this paper, the method used for paraquat analysis is an adaptation of the Imperial Chemical Industry (ICI) United Kingdom method for oil containing crops such as rapeseed, sunflower seed, olives and grain, vegetables, fruits and others (Kennedy,1986). The method involves a cation exchange clean up and reduction of paraquat by sodium dithionite. This results in the formation of a free radical with an intense blue colour which absorbs strongly at 396 nm.

The objective of this investigation was to study the suitability of this method for the determination of paraquat residues in oil using two different cationic exchange resins.

EXPERIMENTAL

Materials

The column for cation exchange resin chromatography consisted of a 25 ml glass burette packed with suitable resin. The resin used were Duolite 225 (SRC 13) and Amberlite IR-120, each with mesh size of 14-52, particle diameter 0.3-1.18 mm and in the sodium form. Paraquat-free RBD palm olein was the edible oil used in this experiment. The reagents used were 98% concentrated sulphuric acid (Merck, Germany) sodium chloride AR grade (Merck, Germany), ammonium chloride AR grade (Merck, Germany), standard paraquat dichloride 98% purity, sodium dithionite (EM, West Germany) 0.2% (w/v) in 0.3 M sodium hydroxide and octan-2-ol AR grade (Merck, Germany) as anti-foaming agent

Apparatus

Heating was carried out using an electrothermal heating mantle of two litres capacity to hold a one litre round-bottomed boiling flask fitted with a water-cooled reflux condenser. The spectrophotometer used was a Hitachi 150–20. The cell was of 1 cm path length (recommended 4 cm) with measurement at wavelength of 396 nm.

Method

Paraquat standard solutions. A 250 $\mu g\ ml^{-1}$ stock solution of paraquat was prepared by dissolving 0.0881 g pure paraquat dichloride, $C_{12}H_{14}N_2Cl_2$, in 250 ml saturated ammonium chloride solution.

Working standard solutions. Working standard solutions of 10 $\mu g\ ml^{-1}$ and 5 $\mu g\ ml^{-1}$ were prepared by diluting the stock solution with appropriate volumes of saturated ammonium chloride solution.

Preparation of sample. Fifty grammes of oil were placed in a one litre boiling flask, followed by the addition of 250 ml water and 25 ml of concentrated sulphuric acid. Some anti-bumping granules and 1 ml of octan-2-ol were also added and the contents of the flask (with a reflux condenser attached) were heated on a heating mantle. Occasionally, the flask was swirled to minimize localized overheating until the solution started to boil. After the solution was boiled for five hours, it was allowed to cool. The condenser was rinsed with 50 ml water and the solution transferred into a one litre separating funnel. The oil layer was then separated from the aqueous solution.

Preparation of ion-exchange column for clean up procedure. The ion-exchange column

was prepared by filling with a slurry containing 3.5 g of resin in water into a 25 ml burette. The resin was washed successively by eluting it with *20* ml saturated sodium chloride solution and then with 50 ml water at the rate of 5 ml min^{-1}. The aqueous sample solution was then allowed to percolate through the resin column at a flow rate of 5-10 ml min^{-1}. The column was then washed successively with the following solutions at a rate of 3-4 ml min^{-1}.

i) 25 ml water;
ii) 100 ml 2.5% (w/v> ammonium chloride solution; and
iii) 25 ml water.

The paraquat was then eluted from the column with saturated ammonium chloride solution at a flow rate of about 1 ml min^{-1}. The first 50 ml were collected in a 50 ml volumetric flask.

Two sets of experiment using two different types of resin for the clean up procedure were carried out. Duolite and Amberlite were used in Experiment I and II respectively.

Spectrophotometric measurement. Ten millilitres of eluant were pipetted into a test tube. Two millilitres of 0.2% sodium dithionite were added and the solution was mixed by gently inverting the tube once or twice. A portion was placed in a 1.0 cm cell and the absorbance at 396 nm was recorded. A blank (10 ml saturated ammonium chloride) was treated in the same way as the sample and its absorbance was recorded. The actual absorbance of the analyte was the difference between absorbance of the sample and that of the blank.

Calibration curve. The 5 $\mu g\ ml^{-1}$ of working standard was diluted accordingly with the appropriate volumes of saturated ammonium chloride solution to obtain solutions containing paraquat in the range of 0.1 to 2 $\mu g\ ml^{-1}$. These solutions were then treated with sodium dithionite and their absorbance values at 396 nm were recorded. A calibration curve of the absorbance at 396 nm of the solutions against the concentrations of paraquat ($\mu g\ ml^{-1}$) was drawn.

Recovery studies. RBD palm olein, previously analysed and found to be free of paraquat residue was fortified with 0.05-2.00 $\mu g\ ml^{-1}$ of paraquat solution made from the 10 μg ml-' working standard solution. Two recovery experiments, Experiments I and II, were carried out using the spiked RBD palm olein. All the samples were subjected to extraction, clean up procedure and spectrophotometric determination. The clean up procedure for Experiment I was performed using Duolite while Amberlite was used for Experiment II.

The concentration of paraquat in the oil sample ($\mu g\ g^{-1}$) was calculated as follows:

$$\frac{\text{Volume of eluent from column (ml)}}{\text{Weight of sample (g)}} \times \text{Concentration in eluent } (\mu g\ ml^{-1})$$

RESULTS AND DISCUSSION

Both the Food and Drug Administration (FDA) (PAM, 1987) and ICI (Kennedy, 1986) used the spectrophotometric method of analysis for the determination of paraquat residue in biological materials. In this method, sulphuric acid was used to free the paraquat from the adsorbed or bound state, followed by clean up through a resin column.

In most of the literature published, paraquat residue determination was carried out on oil seeds (Kennedy, 1986; PAM, 1987), crops (Worobey, 1987) and in water (Ahmad, 1983; Simon, 1991). In this experiment, the method was used to determine paraquat residue in palm oil.

Recovery of Paraquat Residue in Experiment I

Calibration data for resin Duolite. *Table 1* shows the values of the calibration data for the first experiment where the resin Duolite was used for clean up. The equation derived from the calibration data was Y = 0.1790x with an r^2 = 0.9989, where Y is the absorbance at 396 nm and x is the concentration in $\mu g\ ml^{-1}$ (ppm). The calibration data was used to calculate the following recoveries.

TABLE 1. DATA ON CALIBRATION CURVE FOR EXPERIMENT I

Paraquat concentration μg ml-'	Mean absorbance at 396 nm[a]	S.D.	C.V. (%)
0.1	0.013	0.00256	19.69
0.5	0.089	0.00415	4.68
1.0	0.187	0.00587	3.14
1.5	0.274	0.00467	1.70
2.0	0.350	0.00191	0.55
N	5	-	-
R^2	0.9989	-	-
Slope	0.1790	-	-

Note: "Mean of quintuplicate with three readings for each replicate.

Recovery results using resin Duolite. Sensitivity and recovery of the method were determined by analysis of RBD palm olein spiked with paraquat. The spiked RBD palm olein was subjected to the following procedures: extraction, clean up procedure and spectrophotometric determination. Results of triplicate analysis, each solution measured nine times, are summarized in *Table 2.* Recoveries ranged from 50% to 83% which were satisfactory for residue analysis It was observed that at higher levels of concentration the percentage recovery was higher compared to that at a lower level of concentration. Sensitivity for the paraquat was found to be 0.05 μg ml^{-1} of paraquat in the spiked sample. The sensitivity of the spectrophotometer was such that a level of 0.0558 μg g^{-1} would be the minimum quantitative detectable level for paraquat using this technique. The estimated limit of detection based on recovery data of this experiment was 0.01 μg g^{-1}.

Recovery of Paraquat Residue in Experiment II

In this experiment, Amberlite IR120, a resin equivalent to Duolite, was used in clean up process. The same procedure as that in Experiment I was repeated to determine the efficiency of this resin.

Calibration data for resin Amberlite. *Table* 3 shows the calibration data used in the recovery of paraquat in Experiment II. The equation derived was Y = 0.45297x (S.D. 0.0056) with an r^2 of 0.9988, where Y was the absorbance measured at 396 nm and x the concentration in μg ml-" (ppm).

TABLE 2. RECOVERY OF PARAQUAT RESIDUE USING DUOLITE RESIN

Amount added to spiked samples (μg g^{-1})	Absorbance at 396 nm[a]	Amount recovered (μg g^{-1})	Recovery (%)	S.D.	C.V. (%)
0.05	0.005	0.0279	55.9	9.60	17.2
0.10	0.009	0.0502	50.2	0.00	0.0
1.50	0.066	0.3707	74.1	1.58	2.0
1.00	0.143	0.8009	80.1	0.30	0.4
1.50	0.224	1.2535	83.6	0.40	0.5

Note: [a]Mean of triplicate with three readings for each replicate.

TABLE 3. DATA ON CALIBRATION CURVE FOR EXPERIMENT II

Paraquat concentration (μg ml-9	Mean absorbance at 396 nm[a]	S.D.	C.V. (%)
0.000	**0.000**	0.00184	0
0.025	0.010	0.00176	17.60
0.05	0.020	0.00169	8.45
0.10	0.050	0.00157	3.14
0.20	0.082	0.00148	1.80
0.30	0.140	0.00158	1.13
0.40	0.181	0.00186	1.03
0.50	0.227	0.00225	0.99
N	8	-	
R^2	0.9988		
Slope	0.4530 (± 0.0056)	-	-

Note: "Mean of duplicate with three readings for each replicate.

Recovery results using resin Amberlite. Two levels of concentration, 0.05 and 0.5 μg g^{-1} of paraquat, were evaluated to test suitability of Amberlite IR-120 resin in the determination of paraquat residue in RBD palm olein. The results obtained are shown in ***Table 4.***

CONCLUSION

The ICI method, using Duolite resin, can be used to determine paraquat residue in edible oil. The percentage recoveries obtained (ranging from 55.9%-83.6%, starting from low level of

TABLE 4. RECOVERY OF PARAQUAT RESIDUE USING AMBERLITE RESIN

Spiked RBDPOo (μg g^{-1})	Absorbance at 396 nm[a]	Amount found (μg g^{-1})	Recovery (%)	Average (%)	S.D.	C.V. (%)
0.05	0.023	0.050	100	95.0	7.07	7.44
0.05	0.020	0.045	90		-	-
0.50	0.223	0.490	98	98.5	0.71	0.72
0.50	0.225	0.495	99	-	-	

Note: 'Mean of two with three readings for each sample.

The recovery of paraquat using Amberlite was nearly quantitative, as can be seen in *Table* 4. For a concentration of 0.05 μg g^{-1}, the recovery was 95% and for 0.50 μg g^{-1}, the recovery was 98.5%. This indicates that Amberlite IR-120 resin efficiency was comparable, if not superior, to Duolite and could be used as an alternative to the latter resin.

0.05 μg g^{-1} to 1.50 μg g^{-1}) were generally acceptable. The coefficient of variation was less than 20% for the concentration level of 0.05 μg g^{-1} and < 2% for all the other concentrations tested.

The resin Amberlite IR-120 can be used to replace the resin Duolite in the clean up procedure. In fact, the efficiency of the Amberlite resin was found to be much better than Duolite

as shown by the recovery of greater than 90% for concentration levels of 0.50 and 0.05 $\mu g\ g^{-1}$. The coefficient of variation for low (0.05 $\mu g\ g^{-1}$) and high (0.5 $\mu g\ g^{-1}$) level of concentrations was lower for resin Amberlite IR-120 than for resin Duolite. The recovery was also more consistent for this resin.

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