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DETERMINATION OF 2-CHLOROETHYLPHOSPHONIC ACID (ETHEPHON) IN PALM OIL BY THIN-LAYER CHROMATOGRAPHY

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SUMMARY

A thin layer chromatography system is described for the estimation of 2-chloroethylphosphonic acid (Ethephon), a constituent of Ethrel (a proprietary plant stimulant) in palm oil. A sample of Ethrel is dissolved in benzene and extracted with dilute acetic acid. The acid extract is spotted on a plate precoated with Silica Gel (ohne F) Merck and the chromatogram developed in a solvent mixture of *tert.*-butanol, water and glacial acetic acid. The Ethephon spot is detected by spraying with a chromogenic reagent, sodium molybdate–hydrazine sulphate. Quantitative estimation of Ethephon is based on measurement of the area of the spot. The quantity of Ethrel in palm oil can be determined from the Ethephon content; the mean coefficient of variation is ±2.28 % and the average recovery 95.47 %.

INTRODUCTION

Work at the Rubber Research Institute of Malaya has shown that Ethrel, a proprietary plant stimulant, is very effective for increasing the latex yield of rubber plants[1] (*Hevea brasiliensis*). Recently, ABRAHAM[2] showed that the optimum concentration level for application was 10 % of Ethrel in a palm oil formulation. Palm oil is used as a carrier for the stimulant and the active ingredient is 2-chloroethylphosphonic acid which decomposes to produce free ethylene gas as the plant hormone. Earlier samples of Ethrel manufactured by Amchem Products, Inc., Ambler, Pa., U.S.A., comprised three components[3] *viz.* 2-chloroethylphosphonic acid (45–48 %), 2-chloroethylphosphonic anhydride (11–14 %) and mono-2-chloroethyl ester (34–38 %). However, currently produced Ethrel for use as a latex stimulant consists of 39.56 % of 2-chloroethylphosphonic acid which has been given the name "Ethephon". As this later form of Ethrel is likely to be used on a large scale in the natural rubber industry, a precise method is required for estimation of the Ethrel content in palm oil formulations, for purposes of quality control and the study of possible changes on storage.

EXPERIMENTAL

Apparatus

Graduated separation funnels (50 ml)
Shandon spray gun

Disposable micro-pipettes — Drummond (3 μl)
Camag developing chamber
Precoated silica gel plates (ohne F) Merck.

Reagents

Benzene (Analar)
Glacial acetic acid (Analar)
Concentrated sulphuric acid (sp. gr. 1.84)
Palm oil (Hew Pharmacy)
Ethrel (Amchem)
2-Chloroethylphosphonic acid (Amchem)
tert.-Butanol (reagent grade)
Sodium molybdate (reagent grade)
Hydrazine sulphate (reagent grade).
Dilute acetic acid, pH = 3. 3 ml of glacial acetic acid added to 250 ml of distilled water.
Ethephon reference solutions were prepared by dissolving a series of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 g of Ethrel in 10 ml of dilute acetic acid. 3 μl aliquots of these reference solutions were spotted on a plate to give a range of 23.7–166.2 μg Ethephon (60–420 μg Ethrel).

Chromogenic reagent

The sodium molybdate–hydrazine sulphate reagent, similar to the one used by HAHN AND LUCKHANS[4] for the colorimetric determination of phosphate and arsenate, was prepared by dissolving 3.5 g of sodium molybdate and 0.2 g of hydrazine sulphate in 25 ml of distilled water. 25 ml of concentrated sulphuric acid were added slowly to this solution. After cooling in water, the whole solution was diluted to 250 ml with distilled water and kept as spraying reagent.

Extraction of Ethephon from Ethrel/palm oil mixture

A 5 g portion of a representative sample of Ethrel in palm oil was dissolved in about 30 ml of benzene in a 50 ml graduated separation funnel. Benzene was in slight excess to ensure complete dissolution. 10 ml of dilute acetic acid with a pH of 3 were then pipetted into the separation funnel. Ethephon is stable in aqueous solutions below a pH of 3.5. Above this pH value, disintegration of the molecule would take place and free ethylene gas, chloride and phosphate ions would be liberated[5]. The mixture was shaken for 3 min and left to stand for half an hour. Two distinct fractions were obtained, an upper yellow-brown layer which consisted of palm oil in benzene and a clear bottom portion which contained Ethephon in acetic acid. The bottom fraction was drained off and kept for TLC spotting.

Application of the acid extract to the absorbent

A 3 μl aliquot of the dilute acetic acid extract was deposited on the starting line of the precoated silica gel plate, by means of a disposable micro-pipette. Simultaneously a series of standard spots from the Ethephon reference solutions in dilute acetic acid containing 23.7–166.2 μg Ethephon (60–420 μg Ethrel) were transferred onto the same plate. Care was taken to make the spot areas as uniform as possible.

Development of the chromatogram

The spotted plate was placed in the developing chamber containing a solvent mixture of *tert.*-butanol–water–glacial acetic acid (6:3:1, v/v). It took about 5 h for the solvent to reach the finish line (10 cm from the starting line), at which time the plate was removed and the solvent allowed to evaporate. The locations of the various spots were then detected by the HAHN AND LUCKHANS[4] reagent which made the colourless spots visible after baking the plate in an oven at 100° for 10 min. The spots at R_F 0.56 indicated the presence of Ethephon which formed a blue phosphomolybdate complex with molybdate solution. A slightly blue background was also formed by the sodium molybdate solution from the indicator, but this could be easily blown off by a stream of cool air from a hair dryer or removed by cooling the chromatogram in the air. The blue spot was stable for two days.

Quantitative estimation of spots

As the spots developed on the chromatogram were sharp and well defined, both semi-quantitative and quantitative estimation could be successfully carried out as follows.

For semi-quantitative analysis, the size of the unknown spot was compared visually with that of the standards and the percentage of the unknown was calculated from the following expression:

$$E_p = 100 \times \frac{E_s \times V_e}{V_t \times W_s}$$

where E_p = % Ethephon in palm oil
E_s = Ethephon in sample spot estimated from standards expressed in g
V_e = volume of dilute acetic acid extract in ml
V_t = volume of sample transferred onto the plate in ml
W_s = weight of the sample taken in g.

For quantitative analysis, the areas of the spots were measured by placing a sheet of transparent paper marked in mm² over the chromatogram and tracing the outline of the spots. A standard calibration curve was drawn by plotting the square root of the spot area against the logarithmic weight of Ethephon by running a series of Ethephon standards and by using the following expression[6]:

$$A = m \log W + c$$

where A = area of Ethephon spot; W = weight of Ethephon applied; and m and c = constants.

RESULTS AND DISCUSSION

A typical chromatogram with Ethrel solutions developed on a precoated silica gel plate is shown in Fig. 1. The sharp, compact and well defined blue spots at R_F 0.56 were confirmed as being Ethephon by comparison with chromatograms of the pure compound. Chromatographic data obtained for Ethrel standards on three different plates are tabulated in Table I. It can be seen that spot area values vary significantly from plate to plate, thus requiring that standards and unknowns should be developed on the same plate. The square root of the spot area was linearly related

to the logarithm of the weight of Ethephon applied (Fig. 2). Typical results obtained from 5 samples of palm oil containing Ethrel are also given in Table I. The average recovery of Ethrel in palm oil based on the Ethephon content was 95.47 %.

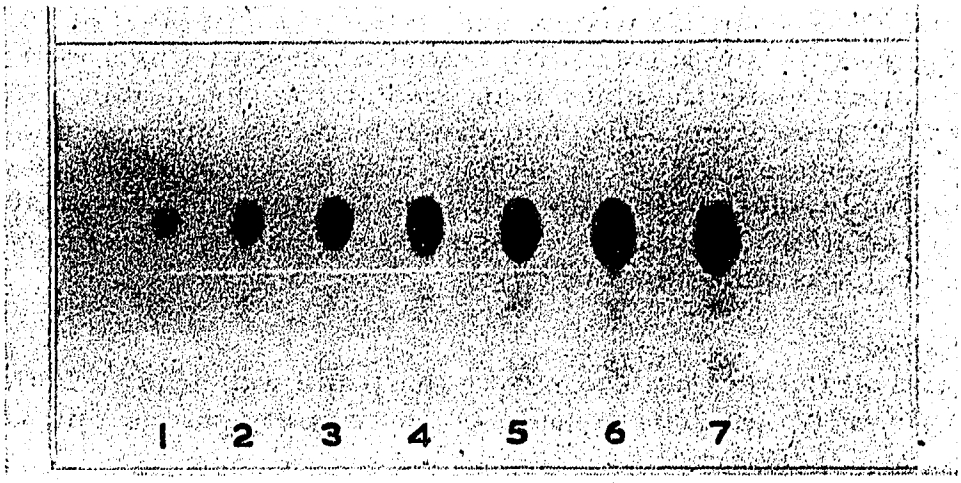

Fig. 1. Typical chromatogram of Ethephon on precoated silica gel plate from Ethrel solutions. 1 = 23.7 μg; 2 = 47.5 μg; 3 = 71.2 μg; 4 = 94.9 μg; 5 = 118.7 μg; 6 = 142.4 μg and 7 = 166.2 μg.

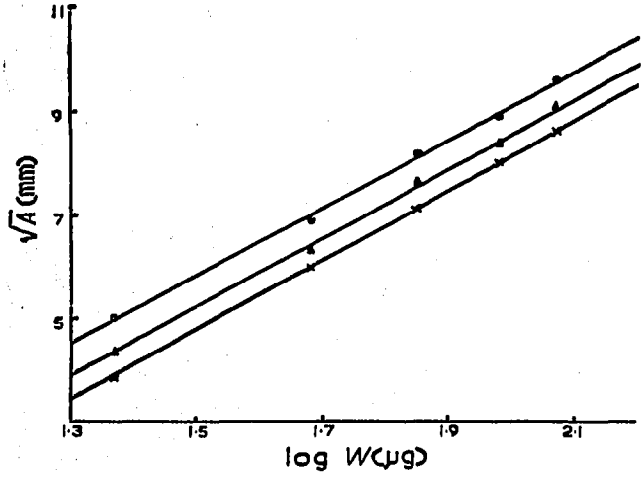

Fig. 2. Standard calibration curves: Plots of $\sqrt{A}$ against $\log_{10} W$ for Ethephon standards on different precoated silica gel plates (A = area of spot, W = weight of Ethephon).

No trace of Ethephon was detected in a second extraction indicating that one extraction of the palm oil is sufficient. It was also observed that the TLC chromatogram of a dilute acetic acid extract of palm oil alone did not contain any material which would interfere with the estimation of Ethephon.

The above results show that the determination of the Ethrel content in palm oil by means of TLC of Ethephon is reproducible and accurate. Factors which affect the accuracy of the determination are the homogeneity and thickness of the absorbent layer, the uniformity of the starting spots and the constancy of the vapour phase in the developing tank. A disadvantage of using these precoated silica gel plates is their relatively low sensitivity, the limit of detection being 7.9 μg Ethephon (20 μg

TABLE I

TYPICAL RESULTS OF STANDARDS AND SAMPLES OBTAINED FROM DIFFERENT PRECOATED SILICA GEL PLATES

Precoated plate No.	*Standards*					*Samples*					
	Ethrel (μg)	*Ethephon[a] content (W) (μg)*	*$\log_{10} W$*	*Area of spot on TLC (mm^2)*	*$\sqrt{A}$*	*Ethrel added (μg)*	*Ethephon added[a] (μg)*	*Area of spot on TLC (mm^2)*	*$\sqrt{A}$*	*Ethephon recovered (μg)*	*% recovery*
1	60	23.7	1.37	25	5.00	150	59.3	55	7.42	55.9	94.2
	120	47.5	1.68	48	6.93	150	59.3	55	7.42	55.9	94.2
	180	71.2	1.85	69	8.31	150	59.3	56	7.48	56.8	95.9
	240	94.9	1.98	80	8.94	150	59.3	57	7.55	58.2	98.1
	300	118.7	2.07	93	9.64	150	59.3	57	7.55	58.2	98.1
2	60	23.7	1.37	19	4.36	150	59.3	48	6.93	57.1	96.3
	120	47.5	1.68	41	6.36	150	59.3	47	6.86	55.1	93.1
	180	71.2	1.85	59	7.68	150	59.3	48	6.93	57.1	96.3
	240	94.9	1.98	71	8.43	150	59.3	46	6.78	54.3	91.6
	300	118.7	2.07	84	9.17	150	59.3	46	6.78	54.3	91.6
3	60	23.7	1.37	14	3.74	150	59.3	41	6.40	56.4	95.1
	120	47.5	1.68	36	6.00	150	59.3	42	6.48	57.1	96.3
	180	71.2	1.85	51	7.14	150	59.3	42	6.48	57.1	96.3
	240	94.9	1.98	65	8.06	150	59.3	42	6.48	57.1	96.3
	300	118.7	2.07	78	8.68	150	59.3	43	6.56	58.5	98.6
										Mean recovery	95.47%
										C.V.	±2.28%

[a] Based on 39.56% in Ethrel.

Ethrel). Also, the time for development of the chromatogram is relatively long. However, the procedure can be used for routine assay of commercial formulations of Ethrel in palm oil, provided standards and unknowns are determined on the same plate.

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