The Determination of α-(4-Chloro-2-methylphenoxy)-propionic Acid in Commercial Acid of this Name

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A method is described for the determination of α-(4-chloro-2-methylphenoxy)propionic acid in the presence of α-(4:6-dichloro-2-methylphenoxy)-propionic acid, α-(6-chloro-2-methylphenoxy)propionic acid and α-(2-methylphenoxy)propionic acid by chromatographic separation of the active acid and its subsequent measurement by ultra-violet absorption. The chromatographic separation is similar to that used by Freeman and Gardner in the examination of chloromethylphenoxyacetic acid.

To improve the accuracy of the method, the ultra-violet absorption of the main band is measured against a known similar concentration of 4-chloro-2-methylphenoxypropionic acid; in this way, a small difference in ultra-violet absorption can be measured and a more precise reading can be made with a spectrophotometer.

Some of the substituted β-phenoxypropionic acids, which are known to be present in a few chloromethylphenoxypropionic acid formulations, have been examined; these were found to have similar ultra-violet absorption characteristics to the α-analogues. β-(4-Chloro-2-methylphenoxy)propionic acid and β-(4:6-dichloro-2-methylphenoxy)propionic acid do not interfere, as they are eluted from the column before any α-acids appear in the eluate.

Selective weedkillers containing α-(4-chloro-2-methylphenoxy)propionic acid as active constituent have recently been introduced. Technical chloromethylphenoxypropionic acid (CMPP), from which the formulations are prepared, is manufactured by processes similar to those used to prepare chloromethylphenoxyacetic acid (MCPA), and, apart from the active constituent, will be found to contain α-(6-chloro-2-methyl)-, α-(4:6-dichloro-2-methyl)- and α-(2-methylphenoxy)propionic acids. If the α-chloropropionic acid used in the manufacturing process contains any of the β-isomer, a small portion of the corresponding β-acids can also be expected in the final product.

It must be noted that α-(4-chloro-2-methylphenoxy)propionic acid can exist in two optically active forms, only one of which has herbicidal properties; however, since no change in the ratio of D to L isomers can occur under normal conditions of manufacture, a determination of DL-(4-chloro-2-methylphenoxy)propionic acid can be regarded as a measure of biological activity.

The determination of the amount of active acid present in technical CMPP may seem a problem analogous to the analysis of MCPA, and similar methods to this end were first considered, *i.e.*, differential refractometry, infra-red and ultra-violet spectrophotometry and liquid - liquid partition chromatography.

Differential refractometry, although it gave satisfactory results for synthetic mixtures of the four principal acids, gave low results with most commercial acids. The cause of this trouble was found to be the presence of small amounts of tarry material in the extracted

carboxylic acids; this tar was non-volatile when the acids were heated *in vacuo* at 150° C, and its strong interference with the measurement of mixtures of synthetic acids by this means was also noted. Direct measurement of the active acid by ultra-violet absorption was ruled out by the strong interference of several of the major impurities.

An infra-red absorption method has been developed, but, in our opinion, its accuracy is not at present as good as that of the proposed method.

A gas-chromatographic method has also been examined. In this technique, a portion of the extracted acids was esterified with methanol. The yield of ester was about 95 per cent. of the theoretical, and it was estimated that all the acids were esterified to the same extent. A small volume of the resulting mixture of esters was examined by using a 6-foot column of 35 to 80-mesh Chromosorb containing 20 per cent. of the sodium salt of dodecyl benzenesulphonic acid as static phase and heating at 205° C. Two methods of measurement were possible, a direct determination of the 4-chloro-2-methyl acid could be made by measuring the height or area of the main elution peak, or, by placing a larger sample on the column, the percentage of each impurity detected could be measured and the sum of these figures subtracted from 100. Both methods of measurement were liable to error, either because unidentified acids having the same retention time as the main component were present or because impurities were not detected by the column conditions used. This meant that the result, although it was often correct, had, in the absence of supporting evidence, to be regarded as a maximum figure only. This reservation was subsequently borne out by experience.

The liquid - liquid chromatographic method described by Freeman and Gardner,[1] which was later adopted by the Joint Herbicides Committee of the Association of British Insecticide Manufacturers and the Ministry of Agriculture, Fisheries and Food, was tried on a mixture of the principal CMPP acids. The components of the mixture could not be separated, but it became evident that some degree of separation would be possible if the column conditions were modified. Such modifications were therefore examined in more detail.

Experimental

Samples of the pure α and β mono and dichloro acids were prepared, and the purity of each was checked by gas chromatography of its methyl ester. Ultra-violet measurements were made in 4-cm silica cells with a Unicam SP500 spectrophotometer.

An attempt (based on Freeman and Gardner's work[1] on MCPA) was made to separate the mixed acids on a chromatographic column.

With a 0·25 *M* aqueous phosphate buffer supported on kieselguhr as static phase and an equilibrated diethyl ether - chloroform mixture as moving phase, it was found that α-(2-methylphenoxy)propionic acid was easily separated from the other main constituents, as it was more soluble in the static phase and was therefore retained on the column for a longer time. It was observed that, whereas chloroform tended to separate the 4:6-dichloro-2-methyl acid from a band containing the 4-chloro-2-methyl and 6-chloro-2-methyl α-acids, diethyl ether tended to increase the rate of elution of all acids and to elute the 4:6-dichloro-2-methyl and 4-chloro-2-methyl acids as one band, 6-chloro-2-methylphenoxypropionic acid being the isolated component in this instance.

The effect of changing the pH of the static phase is such that an increase renders the acids more soluble, and, although the degree of separation is better, the elution peaks are less sharp and the time of elution is longer.

It was hoped that, by using a suitable solvent mixture and static phase, complete separation of the four acids might be possible, but it was soon found that the pH required to achieve this would be so high as to make the procedure lengthy and impracticable.

Ultra-violet absorption measurements of solutions of each acid showed that, in the wavelength region around 287 mμ, the 4-chloro-2-methyl acid absorbed strongly and 6-chloro-2-methylphenoxypropionic acid had negligible absorption.

It was then decided to separate the four components into three main bands containing (*i*) 4:6-dichloro-2-methylphenoxypropionic acid, (*ii*) 4-chloro-2-methyl- and 6-chloro-2-methylphenoxypropionic acids, and (*iii*) 2-methylphenoxypropionic acid, and to measure the ultra-violet absorption of the second band at 287 mμ. From our experience, all light absorption at this wavelength could be attributed to the 4-chloro-2-methyl acid. The most rapid way of effecting the chromatographic separation was found to necessitate a

static phase at pH 7·3 and a moving phase consisting of a mixture of equal volumes of diethyl ether and chloroform. A typical elution curve for this system is shown in Fig. 1, the columns having been calibrated by applying a synthetic mixture and then titrating the eluate with 0·01 *N* sodium hydroxide.

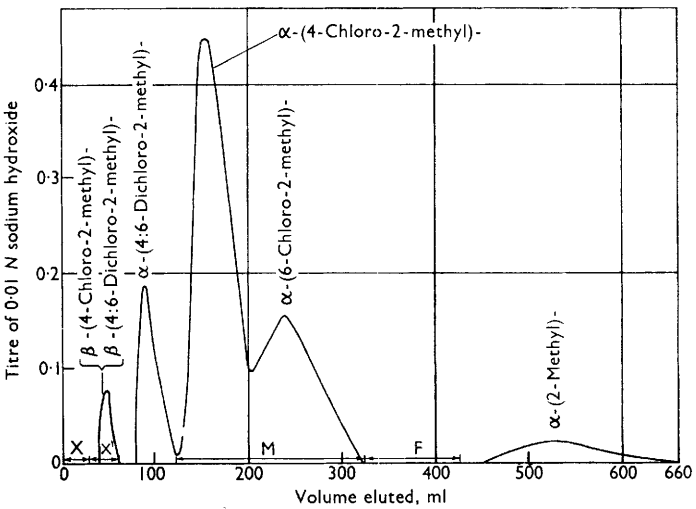

Fig. 1. Elution of phenoxypropionic acids. The static phase was 12·5 ml of 0·25 *M* phosphate buffer solution (pH 7·3) on 25 g of Hyflo Super-Cel, the moving phase was an equilibrated mixture of ether and chloroform (1 + 1), the flow rate was 2 ml per minute (3 lb per sq. inch) and the column loading was 8 mg of acids

The determination involves collecting the appropriate volumes of eluate shown in Fig. 1 as X, X′, M and F. Solutions X and X′ always consist of the first and second 30-ml portions (0 to 30 ml and 30 to 60 ml), respectively, but the range collected for solutions M and F depends on the calibration curve. The volume collected for solution M depends on how sharply the acids are being eluted; normally, 200 ml suffices, and, even if the volume in which the main band is eluted is smaller, it is convenient to collect it in a 200-ml fraction. Solution F always has a volume of 100 ml and is collected immediately after elution of the main band and collection of solution M.

The background optical density of the eluate (uncontaminated by acids) was fairly constant, and any difference in optical density (x) between solutions X and F is assumed to have arisen from a shift in value, the shift changing linearly with the volume eluted.

The optical density of solution M can be measured against solution F, and, after correction for background optical density, the amount of active acid can be read directly from a calibration graph.

The reliability of the method depends on the accuracy of this reading: the reading is usually large (about 1), so that it is not easily determined with any certainty.

In order to increase the accuracy of the method, the optical density of solution M is not measured directly against solution F, but against a solution, G, which consists of pure 4-chloro-2-methylphenoxypropionic acid in solution F. Solution G has such a concentration that, neglecting all changes in background optical density, it would correspond exactly to solution M if the latter were 200 ml in volume and the acid put on the column had been 8 mg of pure 4-chloro-2-methylphenoxypropionic acid.

The relatively low optical density (h) can be accurately measured with a Unicam SP500 spectrophotometer.

The optical density of solution G against solution F (z) is large, but the accuracy of measurement is adequate to permit its use in equation (1).

If, then, it is valid to assume that any background optical-density drift is linear with respect to volume eluted, it can be shown by considering the geometry of the problem that the percentage of α-(4-chloro-2-methylphenoxy)propionic acid is given by—

$$\frac{z + \frac{x}{3} - h}{5z} \times \frac{\text{Volume of solution M, ml}}{\text{Weight of sample, g}} \quad .. \quad .. \quad .. \quad (1)$$

(where the weight of sample is as described under "Method," *i.e.*, fifty times greater than that applied to the column).

All these values, except z, can be reliably determined. Since z appears in both numerator and denominator, however, and as x is always small and h is usually small by comparison, any small inaccuracy is not significant.

INTERFERENCE FROM OTHER CONSTITUENTS IN CMPP FORMULATIONS—

Other constituents known to be present in a few CMPP formulations are certain substituted β-phenoxypropionic acids. Some of these acids have been examined, and their ultra-violet absorption spectra were found to be similar to those of the α-analogues.

The β-(4-chloro-2-methyl)- and β-(4 : 6-dichloro-2-methylphenoxy)propionic acids absorb significantly at 287 mμ and have been examined chromatographically under the conditions described previously. They have been found not to interfere with the determination, as they are eluted from the column before any α-acids appear in the eluate.

METHOD

The chromatographic separation is similar to that used in the examination of MCPA.[1] The four acids, 4 : 6-dichloro-2-methyl-, 4-chloro-2-methyl-, 6-chloro-2-methyl- and 2-methylphenoxypropionic acids, are separated by partition chromatography, kieselguhr and phosphate buffer being used as static phase and diethyl ether - chloroform mixture as moving phase; separation of the 2-methyl and 4 : 6-dichloro-2-methyl acids is complete, but the 4-chloro-2-methyl and 6-chloro-2-methyl acids are only partly separated. The column is standardised with pure acids, and the 4-chloro-2-methyl acid is then determined in a sample by collecting the fraction of the eluate containing the mixed 4-chloro-2-methyl and 6-chloro-2-methyl acids and measuring its ultra-violet absorption at 287 mμ, at which wavelength the 4-chloro-2-methyl acid absorbs strongly, but the 6-chloro-2-methyl acid has negligible absorption. The packing of the column does not appear to be so critical as in the method for MCPA.

APPARATUS—

Chromatographic tube—A glass tube 50 cm in length having an internal diameter of between 1·55 and 1·65 cm. The tube is constricted at its lower end and has a B19 socket at its upper end, into which a tap funnel fits.

Packer—A stainless-steel disc of diameter 1 mm less than the internal diameter of the chromatographic tube. The disc has six holes $\frac{1}{16}$-inch in diameter and a centrally located rod ($\frac{1}{8}$-inch in diameter and about 60 cm in length).

Burette—A 5-ml microburette fitted with a soda-lime - asbestos guard-tube. The burette should be calibrated in 0·01 or 0·02-ml divisions and should be capable of delivering a 0·02-ml drop.

Pipette—To deliver a volume of 1 ml between two graduations.

Unicam SP500 *spectrophotometer*.

REAGENTS—

All materials must be of recognised analytical-reagent grade.

Kieselguhr—Hyflo Super-Cel (obtained from Johns-Manville Ltd., Artillery Row, London, S.W.1).

Buffer solution A, pH 7·3—Mix 165 ml of 0·25 *M* disodium hydrogen orthophosphate and 35 ml of 0·25 *M* sodium dihydrogen orthophosphate. Check the concentration of the disodium hydrogen orthophosphate solution by titration against 0·25 *N* hydrochloric acid

(use bromocresol green as indicator and the 0·25 *M* sodium dihydrogen orthophosphate as a standard for the end-point). Check the concentration of the sodium dihydrogen orthophosphate solution by titration against 0·25 *N* sodium hydroxide (use thymol blue as indicator and the 0·25 *M* disodium hydrogen orthophosphate as a standard for the end-point).

Alternatively, dissolve 5·857 g of anhydrous disodium hydrogen orthophosphate and 1·366 g of hydrated sodium dihydrogen orthophosphate, $NaH_2PO_4.2H_2O$, in distilled water, and dilute the solution to 200 ml. Check the purity of the sodium dihydrogen orthophosphate by titration against *N* sodium hydroxide with thymol blue as indicator (1 ml of *N* sodium hydroxide ≡ 0·156 g of hydrated sodium dihydrogen orthophosphate), and adjust the weight used if necessary. Check the purity of the disodium hydrogen orthophosphate by titration against *N* hydrochloric acid with bromocresol green as indicator (1 ml of *N* hydrochloric acid ≡ 0·142 g of disodium hydrogen orthophosphate).

Diethyl ether - chloroform mixture—Mix equal volumes of diethyl ether and chloroform, cool to room temperature, and keep the mixture protected from strong light. This is solution B.

Solvent mixture—Equilibrate solutions A and B at room temperature by shaking 1 litre of solution B and 50 ml of solution A in a separating funnel. Cool the mixture to room temperature, and filter the lower layer through cotton-wool to remove traces of suspended aqueous layer. This is solution C.

Sodium hydroxide solution (*free from carbon dioxide*), 0·01 N—Prepare this solution in the way described by Davies and Nancollas,[2] and store it in an aspirator bottle protected from atmospheric carbon dioxide. Alternatively, 0·01 *N* barium hydroxide, which must also be protected from atmospheric carbon dioxide, can be used.

Solution D—Dissolve 0·1 g of 4:6-dichloro-2-methylphenoxypropionic acid, 0·5 g of 4-chloro-2-methylphenoxypropionic acid and 0·2 g each of 6-chloro-2-methylphenoxypropionic acid and 2-methylphenoxypropionic acid in 100 ml of solution B.

Solution E—Dissolve exactly 0·2 g of 4-chloro-2-methylphenoxypropionic acid in 100 ml of diethyl ether.

Ethanol, absolute.

Bromothymol blue indicator solution.

PREPARATION OF COLUMN—

Place 25 g of Hyflo Super-Cel in a mortar, and add, with careful mixing, 12·5 ml of buffer solution A. Triturate gently for several minutes. Add about 250 ml of equilibrated solvent mixture C, and again triturate gently for 3 to 4 minutes.

Close the lower end of the chromatographic tube (which should be cut square and not obliquely) by means of a cork, and hold it loosely in two clamps. For convenience, place the lower clamp below the constriction and the upper clamp near the top of the column. The tube can now be rotated easily, but will remain firm under vertical pressure.

Pack a firm wad of cotton-wool that has been extracted with diethyl ether to form a base for the column, and add solvent to fill about two-thirds of the tube. Maintain this depth of liquid above the packed solid throughout the packing operation. Add a little of the slurried Hyflo Super-Cel, and vigorously agitate with the packer disc to remove any entrained air. Pack the first 0·5 cm of the tube firmly, so as to form a sound base for the column.

Pack the remainder of the column at a sufficient pressure, as found by experience. Hold the packer disc about 3 to 4 cm from the surface of the packed material, and slowly push down so as to impact 1 to 2 mm of material. Press firmly down, and then, with short rapid strokes, consolidate the edges of the column by holding the disc against the side of the tube and rotating both tube and packer. The rapid motion of the packer disc during this operation prevents the impacting of the new material. Repeat this process until all the Hyflo Super-Cel has been packed in 1 to 2-mm sections.

The consolidation of the edges of the column after each section has been packed is most important. Large amounts of thick slurry must not be pushed down and impacted quickly, as this leads to entrainment of air in the column. Build up the column slowly and uniformly, the entire operation should take 1 hour; each addition of slurry, with subsequent packing, results in a uniformly packed column. The length of the packed column should be 26 to 34 cm. If the length of the packing is not within this range, then either the trituration or the packing has not been done correctly. Longer and more vigorous trituration or harder packing, or both, will produce a shorter column.

STANDARDISATION OF COLUMN—

Assemble the apparatus as shown in Fig. 2. There will be about 20 to 30 ml of solvent above the packed Hyflo Super-Cel. This may be used to ascertain the pressure of nitrogen (applied through A) required to give the necessary flow rate of 2 to 2·5 ml per minute. The pressure required should be 3 to 5 lb per sq. inch (as shown on the gas-cylinder gauge). If the pressure required is much greater than this, the column has probably been packed too tightly.

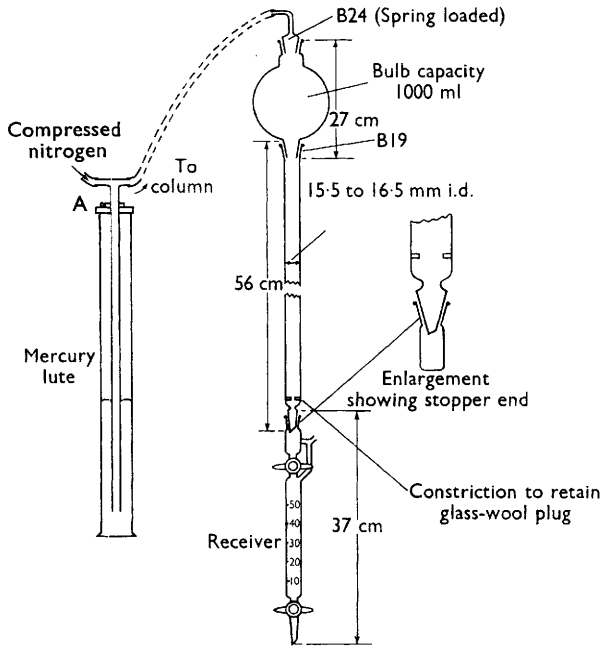

Fig. 2. Column assembly

Allow the level of the liquid above the column to fall until only an extremely thin layer of liquid remains above the Hyflo Super-Cel. Remove the separating funnel, and place 1 ml of solution D on the surface by means of a 1-ml pipette. Force this liquid through the column by pressure of nitrogen until the 1-ml portion of solution D has just been absorbed. Follow the same procedure with two successive 1-ml portions of solution C. Finally, replace the separating funnel containing solution C, and pass about 1 litre of this solution through the column. Collect successive 10-ml portions of the eluate, and remove the solvent by evaporation on a water bath in the presence of 3 to 4 ml of water. When all the diethyl ether - chloroform mixture has been removed, titrate each fraction with 0·01 *N* alkali. Pass a rapid stream of carbon dioxide-free air or nitrogen through the liquid during titration. The stream of gas should pass through the liquid for at least 2 minutes before the titration is begun in order to remove any dissolved carbon dioxide. From the results of these titrations, standardise the column by plotting volume eluted against titration of last 10-ml fraction (see Fig. 1). If the troughs between the peaks are not well defined, the column has not been properly packed and another must be prepared.

Non-uniform packing of the Hyflo Super-Cel will cause poor separation of the acids, and variation of the concentrations of buffer salts from those required will affect the resolution and positions of the peaks.

Provided that the standardisation curve is satisfactory, the column may be run "blind" at the same flow rate. It is advisable to check the column after four determinations and after storage. Take care to prevent the column from becoming dry, and, if a column is set aside for longer than 2 days, flush it with 50 ml of freshly equilibrated solvent before use.

PROCEDURE—

Prepare enough solution C to last the whole determination, and mix thoroughly. (Equilibrate the solvent mixture and the buffer solution immediately before use.) Pass

50 ml of solution C through the column, and allow the level of the liquid to fall to the level of the Hyflo Super-Cel.

Weigh accurately about 0·4 g of acids extracted from CMPP, and make up to 50 ml with diethyl ether. By pipette, carefully place 1 ml of this solution on the column, and wash it on with two successive 1-ml portions of solution C. Elute with the freshly prepared solution C (pass about 1 litre to ensure that the unchlorinated 2-methylphenoxypropionic acid is removed from the column). Collect the first 30 ml of eluate, the next 30 ml (*i.e.*, the 30 to 60-ml fraction) and the main band (solution M), which is in the appropriate volume determined by calibration (usually 200 ml). Collect and titrate a 10-ml fraction immediately before the main band is eluted as a check that the main band includes all the 4-chloro acid.

Collect the next 100 ml of eluate after elution of the main band (this is solution F).

Dilute 1 ml of solution E to 50 ml with solution F (this is solution G).

At 287 mμ, measure the optical densities (in 4-cm silica cells) of solution F against the first 30-ml portion of eluate, solution G against solution F and solution G against solution M. This gives the values of x, z and h, respectively, in equation (1). If x is greater than 0·05, measure the optical density of solution F against the 30 to 60-ml fraction of eluate, and, if x is still greater than 0·05, repeat the determination.

Calculate the percentage by weight of 4-chloro-2-methylphenoxypropionic acid in the sample by using equation (1), p. 97.

Results

Application to synthetic mixtures—

Five synthetic mixtures of the α-acids containing from 75 to 95 per cent. of α-(4-chloro-2-methylphenoxy)propionic acid were examined; the results are shown in Table I.

Replicate determinations on each sample gave results which were satisfactorily reproducible.

Table I

Analysis of synthetic mixtures of α-acids

Mixture	Amount of 4-chloro-2-methylphenoxypropionic acid present, %	Amount of acid found, %	Average, %
A*	75	75·4, 75·7, 76·1, 77·1	76·1
B†	80	81, 80·1, 78·0	79·7
C‡	85	87·1, 85·3, 83·2, 84·6	85·1
D§	90	91·0, 89·8, 89·8, 90·0	90·2
E‖	95	96·3, 95·9, 95·0, 94·3	95·4

* Mixture A also contained 10 per cent. each of 4:6-dichloro-2-methylphenoxypropionic acid and 6-chloro-2-methylphenoxypropionic acid and 5 per cent. of 2-methylphenoxypropionic acid.

† Mixture B also contained 5 per cent. each of 6-chloro-2-methylphenoxypropionic acid and 2-methylphenoxypropionic acid and 10 per cent. of 4:6-dichloro-2-methylphenoxypropionic acid.

‡ Mixture C also contained 5 per cent. each of 4:6-dichloro-2-methylphenoxypropionic acid, 6-chloro-2-methylphenoxypropionic acid and 2-methylphenoxypropionic acid.

§ Mixture D also contained 5 per cent. each of 4:6-dichloro-2-methylphenoxypropionic acid and 6-chloro-2-methylphenoxypropionic acid.

‖ Mixture E also contained 2·5 per cent. each of 4:6-dichloro-2-methylphenoxypropionic acid and 6-chloro-2-methylphenoxypropionic acid.

These results were used to calculate the standard deviation of a single determination. From this value, the limits of 95 per cent. confidence, *i.e.*, the range in which nineteen out of twenty results of single determinations would be expected to lie, were calculated; the limits were $\pm$2·8 per cent.

Comparison with other methods—

In addition to partition chromatography, commercial formulations have also been examined by gas chromatography of the esters, infra-red analysis and a differential refractometric method. Ten samples were examined, and the results by different methods are shown in Table II.

These results show that, in general, agreement between the proposed and the infra-red methods was good. In all but one instance, results obtained by gas chromatography of the

esters were higher; it must be remembered, however, that chromatographic methods tend to give a maximum value, since the presence of any unknown material having the same retention time as the main band will lead to a positive error.

The differential refractometric method has been shown, in certain instances, to be considerably affected by small amounts of tarry material occurring in formulations, and hence it is not always reliable for the analysis of CMPP.

TABLE II

ANALYSIS OF COMMERCIAL CMPP FORMULATIONS BY VARIOUS METHODS

Sample	Amount of α-(4-chloro-2-methylphenoxy)propionic acid found by— proposed method, %	infra-red analysis, %	differential refractometry, %	gas chromatography, %
A	85·0, 85·2 (85·1)	86	84	90
B	80·1, 80·3 (80·2)	83·7	77	87·6
C	87·0, 86·1 (86·5)	86·7	80	90
D	82·5, 82·5 (82·5)	81·2	85·6	83·4
E	81·8, 82·8 (82·3)	—	79·5	90
F	96·1, 96·2 (96·1)	97·1	93·2	—
G	90·7, 92·5 (91·6)	91·6	85·6	93·9
H	94·3, 94·6 (94·4)	93·7	90·9	93·5
I	93·3, 92·2 (92·8)	—	92·6	95·4
J	96·5, 95·8 (96·2)	—	94·0	97·0

These results were also used to calculate the limits of 95 per cent. confidence, which, for commercial samples, were $\pm 1{\cdot}5$ per cent.

CONCLUSIONS

The results for synthetic mixtures indicate that, for samples containing α-(4-chloro-2-methyl)-, α-(6-chloro-2-methyl)-, α-(4:6-dichloro-2-methyl)- and α-(2-methylphenoxy)propionic acids, the proposed method is reliable for samples of mixed acids containing at least 75 per cent. or more of active constituent.

The procedure, although it may seem lengthy, is simple to carry out. Interference by an unknown substance would occur only if that substance had the same retention time (under the conditions used) as α-(4-chloro-2-methylphenoxy)propionic acid and an appreciable ultra-violet absorption at 287 mμ. This possibility is remote.

REFERENCES

1. Freeman, F., and Gardner, K., *Analyst*, 1953, **78**, 205.
2. Davies, C. W., and Nancollas, G. H., *Nature*, 1950, **165**, 237.

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