COMMUNICATIONS

Analysis of Tordon Formulations by Ultraviolet Spectrophotometry

A simple method was developed for analysis of Tordon formulations. The active ingredients, 4-amino-3,5,6-trichloropicolinic acid (picloram) and 2,4-dichlorophenoxyacetic acid (2,4-D) or 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), are converted to methyl esters. These are separated on a partition column and the ultraviolet absorbance of the two fractions is compared with standard solutions of the methyl esters with absorption maxima at 295 nm (picloram methyl ester), 284 nm (2,4-D methyl ester), and 289 nm (2,4,5-T methyl ester).

Picloram was introduced in different formulations under the trade name of Tordon as a herbicide in 1963. Infrared spectrophotometric methods for analysis of Tordon formulations were developed within the Dow Chemical Company (Melcher, 1965) and an ultraviolet spectrophotometric method is mentioned as unpublished work (Hummel, 1965) by Ramsey (1967), making use of the solubility of picloram in strong acid and its ultraviolet absorbance at 285 nm. No methods for analysis of Tordon herbicide formulations have been published since.

The following method was mainly developed for Tordon formulations containing 5% picloram and 20% 2,4-D or 2,4,5-T. Other mixture proportions have been received for analysis by this laboratory, and the method is with slight modifications also suitable for them.

Picloram, 2,4-D, and 2,4,5-T may be present in the formulations as potassium salts, triisopropanolamine salts, triethylamine salts, isooctyl esters, or propylene glycol ether esters, all of which can be converted to methyl esters with boron trifluoride–methanol solution.

The method is based on the solubility of picloram methyl ester in hydrochloric acid. By using a partition column with hydrochloric acid as stationary phase, and a mixture of hexane and ether as mobile phase, 2,4-D methyl ester or 2,4,5-T methyl ester passes through the column before picloram methyl ester.

EXPERIMENTAL

Esterification. Weigh 1.0 g of sample to the nearest 1 mg into a test tube and add 5 ml of boron trifluoride–methanol solution (14% BF_3). Mix the tube contents and immerse the test tube to the depth of the solution in a beaker of warm water (50–60° C). Keep the solution boiling for 1 hr on a steam bath and, after cooling, transfer it with ether (30 ml) to a 100 ml separatory funnel. Wash the ether solution with water (2 × 25 ml), and the combined water washes with ether (2 × 20 ml) and hexane (1 × 10 ml). Filter all ether solutions and the hexane wash through cotton wool into a 100 ml volumetric flask and make up to volume with ether.

Column Preparation. Weigh 10 g of silicic acid (Mallinckrodt analytical reagent, 100 mesh, No. 2847, dried overnight at 105° C) into a 50 ml Erlenmeyer flask and add 7 ml of 5 *N* HCl. Stir and shake the mixture vigorously until a homogenous powder results. Slurry with 8% ether (v/v) in benzene-free hexane for ultraviolet purposes (mobile solvent). Into a chromatographic tube (1.6 cm i.d., fitted with fritted glass disk and Teflon stopcock) pour 5 ml of mobile solvent and add the homogenous silicic acid slurry. Remove air bubbles and ensure a smooth, even surface of the silicic acid.

This is most important for a good separation. Allow the silicic acid to settle, then place a 5 mm layer of acid-washed sand on top to prevent any disturbance of the support during further addition of solvent. Run off the mobile solvent until the level is just above the top surface of the sand.

Column Separation. Take an aliquot of the ether solution of the methylated herbicides containing approximately 5 mg picloram methyl ester and 20 mg 2,4-D methyl ester or 2,4,5-T methyl ester. In the case of the most common Tordon mixture (5% picloram and 20% 2,4-D or 2,4,5-T) this volume is 10 ml. Pipet this aliquot into a test tube and evaporate it to near dryness under a stream of dry nitrogen. Dissolve the residue in 0.25 ml ether, add 3 ml hexane, and transfer quantitatively to the top of the column. Wash the test tube with more mobile solvent, introducing the wash carefully into the column when the level of the solvent is just above the top of the sand. Collect the first 50 ml of eluate in a 50-ml volumetric flask. This fraction contains the 2,4-D methyl ester or 2,4,5-T methyl ester. To elute the picloram methyl ester, pass 100 ml of 30% ether in hexane (v/v) through the column, collecting this in a 150-ml round-bottomed flask. Evaporate the latter fraction to dryness in a water bath at approximately 40° C, using a rotary film evaporator. Transfer the residual white crystals with ethanol (ultraviolet grade) into a 50-ml volumetric flask and make up to volume with ethanol.

Calibration and Absorbance Reading. 2,4-D or 2,4,5-T. Weigh 10 mg (to the nearest 0.01 mg) 2,4-D methyl ester or 2,4,5-T methyl ester into a 100-ml volumetric flask, dilute the ester in 1.6 ml ether, and make up to volume with hexane. With a burette transfer 10, 12, 14, 16, 18, and 20 ml of this solution to 20 ml volumetric flasks and make up to volume with 1.6% ether in hexane (v/v). Measure the ultraviolet absorbance in stoppered, matched silica cells (1 cm path length) against 1.6% ether in hexane (v/v) at 284 nm (2,4-D methyl ester) or 289 nm (2,4,5-T methyl ester). Draw a calibration curve of absorbance vs. concentration.

Transfer 10 ml of the 2,4-D or 2,4,5-T fraction into a 50-ml volumetric flask and make up to volume with hexane. Measure the absorbance of this solution as described for the calibration procedure and read the concentration of 2,4-D methyl ester or 2,4,5-T methyl ester from the calibration curve.

PICLORAM. Weigh 10 mg (to the nearest 0.01 mg) picloram

methyl ester into a 100-ml volumetric flask, and make up to volume with ethanol (ultraviolet grade). With burette transfer 10, 12, 14, 16, 18, and 20 ml of this solution to 20 ml volumetric flasks and make up to volume with ethanol. Measure the ultraviolet absorbance in stoppered matched silica cells (1 cm path length) against ethanol as reference at 2.95 nm. Draw a calibration curve of absorbance *vs.* concentration. Measure the absorbance of the picloram fraction as described for the calibration procedure and read the concentration of picloram methyl ester from the calibration curve.

RESULTS AND DISCUSSION

The sample was weighed instead of measured volumetrically because of precision reasons. If the final result is wanted in terms of a w/v the SG has to be determined by any recognized method. It was not possible to elute the picloram methyl ester from the column in a reasonable volume of mobile phase; therefore it was found necessary to increase the polarity of the solvent mixture after 2,4-D methyl ester or 2,4,5-T methyl ester has been removed from the column.

The degree of separation was tested by collecting 5 ml eluate between the two fractions, running a spectrum of this solution, evaporating it down and testing it by tlc chromatography. No 2,4-D methyl ester, 2,4,5-T methyl ester, and picloram methyl ester could be detected down to the limits of detection. If 2,4-D or 2,4,5-T was not eluted completely before collection of the picloram fraction, this can easily be seen by scanning this fraction. The picloram methyl ester peak becomes then distorted by the 2,4-D or 2,4,5-T methyl ester with two maxima.

The absorbance of the 2,4-D methyl ester or 2,4,5-T methyl ester fraction is read directly after dilution, whereas the ultraviolet absorbance of picloram methyl ester proved to be very sensitive to the solvent composition. Small differences in solvent composition (for example in a mixture of hexane and ether) result in shifting of the peak and also influence the peak height. This difficulty has been overcome by evaporating the fraction to dryness and using ethanol as solvent for picloram methyl ester. Therefore it is not necessary to use benzene-free hexane for removal of this fraction from the column. This, however, should be verified for the batch of hexane by evaporating 70 ml of hexane to dryness, dissolving the residue in ethanol (ultraviolet grade), and testing if there is no ultraviolet absorption down to 250 nm.

For general analytical purposes the described volumetric dilution of the standard solutions is precise enough for the construction of the calibration curves. Gravimetric dilution, however, improves the precision of the curves, which obey Beer's Law in the appropriate region.

The molar absorptivity was found to be $e = 1920$ for 2,4-D methyl ester, $e = 2510$ for 2,4,5-T methyl ester, and $e = 2240$ for picloram methyl ester. These were determined on a Uvispek spectrophotometer with a band width of 0.5 nm (slit width 0.3 mm).

After recoveries of 2,4-D, 2,4,5-T, and picloram in self-prepared mixtures were satisfactory, six determinations, as described in the procedure, were made on one Tordon sample. The results were as follows:

	Picloram	*2,4-D*
Mean percentage	5.14	20.63%
Standard deviation	0.052	0.434
Relative standard deviation	1.0%	2.1%

ACKNOWLEDGMENT

The author thanks D. J. Hamilton for valuable suggestions in the preparation of this manuscript.

Irmtraud Müller

Agricultural Chemical Laboratory Branch
Department of Primary Industries
Brisbane, Queensland
Australia 4000

LITERATURE CITED

Hummel, R. A., Special Services Laboratory, The Dow Chemical Co., Midland, Mich., unpublished work, 1965.
Melcher, G. R., Spectroscopy Service Laboratory, The Dow Chemical Co., Midland, Mich., unpublished work, 1965.
Ramsey, J. C., "Pesticides, Plant Growth Regulators and Food Additives," Gunter Zweig, Ed., Vol. V, pp 507–25, Academic Press, New York, 1967.

Received for review July 10, 1970. Accepted January 22, 1971.