CHROM. 4727

NOVEL DETERMINATIONS OF PICLORAM BY GAS–LIQUID CHROMATOGRAPHY*

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(Received March 23rd, 1970)

SUMMARY

The herbicide 4-amino-3,5,6-trichloropicolinic acid (Picloram, Tordon) can be analyzed by gas–liquid chromatography both in trimethylsilylated and decarboxylated forms. The latter compound, 4-amino-3,5,6-trichloropyridine, may be obtained either directly from soil samples or from suitable extracts. It is easily purified by column chromatography on silica gel and can be found in a minimum amount of three picograms by Ni-63 electron capture detection. Using the decarboxylation of soil extracts, linear calibration curves can be established for the range between 10 and 1000 p.p.b. The minimum residue level detectable by this method is less than 5 p.p.b., starting from a 1 g sample.

INTRODUCTION

The herbicide Picloram is widely used in agricultural and military applications. Its main target in the United States is the control of woody plants and broad-leaved weeds. While most grasses are unaffected by high levels of Picloram, certain crops such as beans or tomatoes are extremely susceptible. Consequently, a reliable and highly sensitive method for the determination of Picloram residues in soil is of some importance.

Most present methods use electron capture gas–liquid chromatography (EC–GLC) of the Picloram methyl ester. This approach has obvious advantages, since Picloram responds strongly in the EC detector. Esterification then confers the volatility required for GLC and also allows a more efficient column chromatographic clean-up.

* Contribution from the Experiment Station Chemical Laboratories, Journal Series No. 5932. Approved by the director. This paper was presented at the 5th Midwest Regional ACS Conference in Kansas City, Mo., October, 1969. It was supported by Public Health Science Research Grant No. FD-00262, former CC-00314, and by Grant No. 12-14-100-9146 (34) of the Crops Research Division, Agricultural Research Service, United States Department of Agriculture

** Data in this paper are taken from Doctoral Thesis.

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LEAHY AND TAYLOR[1] extracted soil with 0.05 *N* KOH in 15% aqueous KCl, acidified and partitioned into ethyl acetate. They esterified the extract prior to a Florisil clean-up and analyzed by EC–GLC. Alkaline extraction is quite effective; the free acid Picloram can be recovered from various types of soils by 0.1 *N* NaOH up to 97.5%, as YOUNGSON *et al.*[2] have shown with labeled material. BJERKE *et al.*[3] used a similar extraction technique, but cleaned up the free acid on alumina before esterification and EC–GLC. MERKLE *et al.*[4] and SAHA AND GADALLAH[5] extracted Picloram from soil with acidic acetone and analyzed the methyl ester by EC–GLC without prior column clean-up.

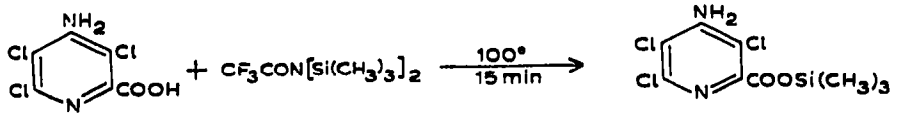
In our hands, these methods became difficult to operate, and ambiguous to interpret, at Picloram levels between 10 and 100 p.p.b., a concentration range of considerable importance. Not only was good quantitation difficult to achieve, but positive identification of peaks as originating from Picloram demanded additional amounts of work, expertise, and/or faith.

Consequently, we investigated methods for the analysis of Picloram by GLC other than the ones based on the methyl ester. The trimethylsilyl ether (TMS) derivative and the decarboxylated Picloram presented themselves as obvious possibilities. The latter compound had been found and characterized by PLIMMER AND KEARNEY[6] as a by-product in the extraction of Picloram containing soils with acetonitrile. (In two recent notes, HALL *et al.*[7,13] reported the use of a pyrolysis technique for on-column decarboxylation of Picloram.) The following paragraphs describe our attempts to develop these possibilities into methods suitable for the determination of Picloram in standard solutions and soil extracts.

EXPERIMENTAL

Trimethylsilylation

Using a closed-vial technique similar to one described by GEHRKE *et al.*[8], optimum time and temperature conditions were determined for the reaction of Picloram with bis(trimethylsilyl)trifluoroacetamide (BSTFA, ref. 9). The optimum conditions were 100° for 15 min, resulting in a single, symmetrical GLC peak by flame ionization detection. The structure of the compound formed was not further investigated; however, we assumed it to represent Picloram trimethylsilylated at the carboxyl group. This assumption was based on the fact that under similar trimethylsilylation conditions 4-amino-3,5,6-trichloropyridine (decarboxylated Picloram) remained unaffected. This assumption seems further justified when steric hindrance by the chlorine atoms vicinal to the amino group is considered. The reactivity of the amino group is further reduced by the inductive effects of the three chlorine atoms and the carboxyl group.



At optimized conditions, varying amounts of Picloram from 5 to 500 μg were derivatized with 0.2 ml of BSTFA, which functioned both as a reagent and as a solvent. Injecting 2 μl onto a 10% OV-17 on Chromosorb W-HP, 100/120 mesh column, the

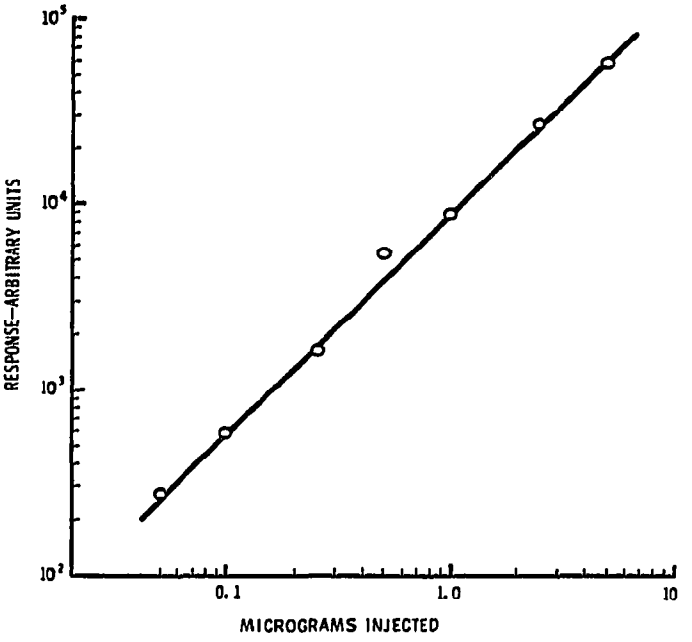


Fig. 1. Standard curve for Picloram-BSTFA. Reaction conditions. 0 2 ml BSTFA, 100°, 15 min. Injection: 2 μl. Column: 10% OV-17 on 100/120 mesh Chromosorb W-HP, 2.0 m × 4 mm I.D. Pyrex. Oven temperature 220° isothermal. N_2 flow rate. 60 ml/min. MicroTek model MT-220, FID.

standard curve shown in Fig. 1 was obtained. It indicates that the reaction *per se* is suitable for application in a quantitative analysis.

Extracts of natural materials, however, often present serious GLC background problems after trimethylsilylation. BSTFA in particular is an extremely efficient reagent, encompassing a wide spectrum of possible substrates. Unless a rigorous clean-up is possible, low residue levels can often not be determined in complex matrixes (*e.g.* see ref. 10).

Consequently, the detection of the TMS derivative by a selective means, electron capture, was investigated. The great excess of BSTFA, however, seriously disturbed the Ni-63 EC detector used in this study. An attempt to remove the excess BSTFA and dissolve the derivative in an aprotic, non-polar solvent met with failure, since the derivative hydrolyzed very easily and serious errors in quantitation resulted.

At present, the use of the trimethylsilyl derivative of Picloram for analytical GLC is confined to the hydrogen flame detector. Due to the success of the decarboxylation method as described in the following paragraphs, no further studies concerning the trimethylsilylation of Picloram present in biological extracts were attempted.

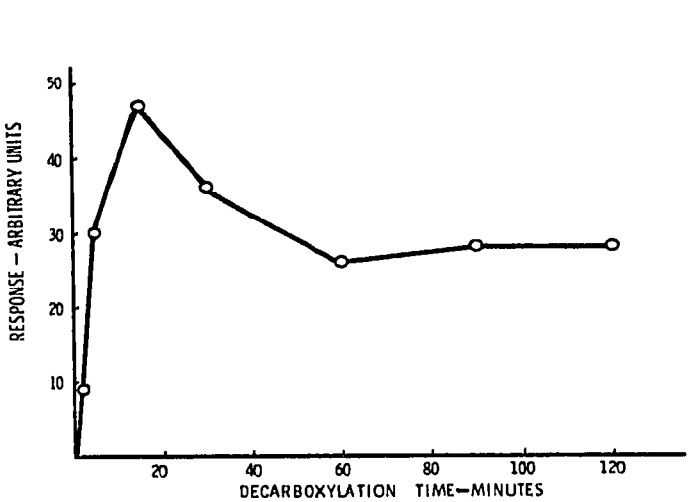
Decarboxylation

The decarboxylated product, 4-amino-3,5,6-trichloropyridine, possesses unique advantages for low-level residue analysis. It responds strongly in the EC detector and can be synthesized and kept in reasonable purity as a standard. It is not prone to hydrolysis like many other types of derivatives and is formed in a very specific reaction. Easily purified by column chromatography, it chromatographs well on a variety of

GLC columns. At least in our hands, however, difficulties associated with the decarboxylation reaction required careful control of the reaction conditions.

The decarboxylation of Picloram was conducted at different temperatures and reaction times, with or without added catalysts, in culture tubes with teflon-lined screw-caps. About 3 h at 150° were necessary to effect a maximum of decarboxylated product. With 5 to 10 μl of concentrated HCl added per ml acetonitrile, maximum decarboxylation was achieved in 15 min at 150°. Fig. 2 shows a representative yield study in this series of experiments.

Under these conditions, aqueous HCl hydrolyzed the solvent acetonitrile to acetamide and finally acetic acid and ammonium chloride. Small amounts of these products, however, did not seriously interfere with the GLC analysis. In contrast to the trimethylsilylation reaction, the solvent used in decarboxylation could be evaporated under a stream of dry nitrogen without loss of decarboxylated Picloram. The compound was then redissolved in hexane or a suitable non-polar solvent and injected into the gas chromatograph. This change of solvent resulted in much better performance of the EC detector. With pure, recrystallized material, the standard curve shown in Fig. 3 was obtained. It illustrates the range of linear response of the EC detector. The minimum detectable amount was approximately 3 pg injected. For undetermined reasons, the extrapolated calibration curve does not go through the origin.



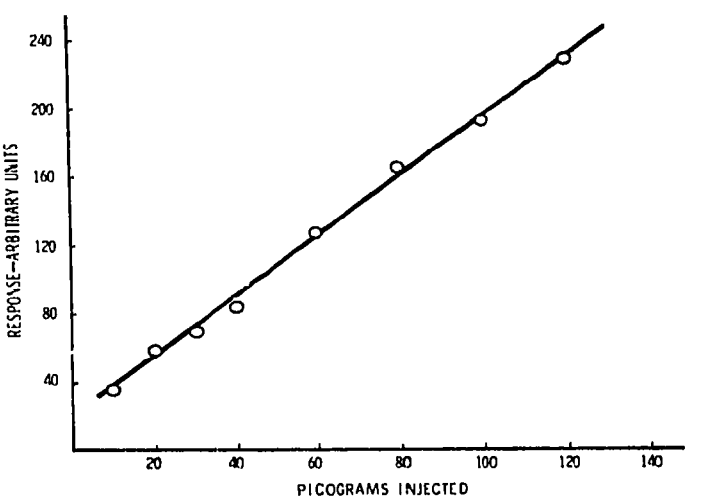


Fig. 2. Decarboxylation of 10 ng of Picloram as a function of time. Reaction conditions: 1 ml of acetonitrile, 10 μl of conc. HCl, 150°. Injection: 5 μl hexane solution. Column: 1.5% OV-17 + 1.95% QF-1 on 100/120 mesh Chromosorb W-HP, 1.8 m × 4 mm I.D. Pyrex. Oven temperature: 155°. N_2 flow rate: 45 ml/min. MicroTek model MT-220, Ni-63 detector, d.c. mode.

Fig. 3. Standard curve for 4-amino-3,5,6-trichloropyridine. Injection: 5 μl hexane solution. Column: 1.5% OV-17 + 1.95% QF-1 on 100/120 mesh, Chromosorb W-HP, 1.8 m × 4 mm I.D. Pyrex. Oven temperature: 155°. N_2 flow rate: 45 ml/min. MicroTek model MT-220, Ni-63 detector, d.c. mode.

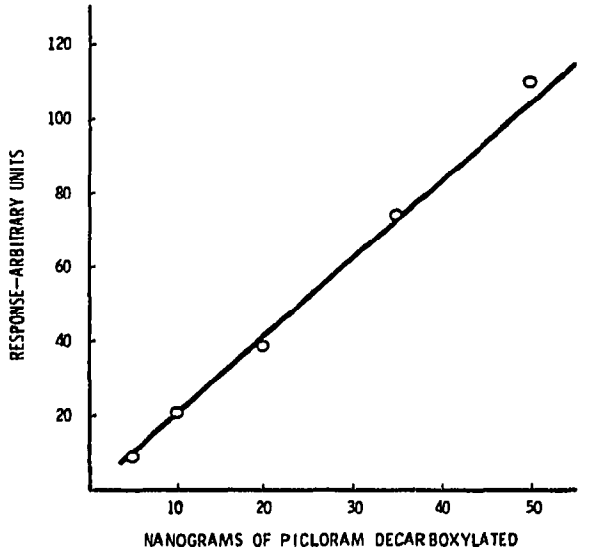


Fig. 4. Standard curve for the decarboxylation of Picloram. Reaction conditions 1 ml acetonitrile, 10 μl conc. HCl. 150° for 15 min, 5 μl injection. Column: 1.5% OV-17 + 1.95% QF-1 on 100/120 mesh Chromosorb W-HP, 1.8 m × 4 mm I.D. Pyrex. Oven temperature 155°. N_2 flow rate: 45 ml/min. MicroTek model MT-220, Ni-63 detector, d.c. mode.

Varying amounts of the free acid Picloram were decarboxylated under identical conditions with the results shown in Fig. 4. These, and all other response data in this paper, represent peak heights. The curve indicates that—even though the yield of decarboxylated product is less than theoretical—the reaction can serve as the basis for an analytical method.

Decarboxylation on soil

The decarboxylation of Picloram on soil was tested with Menfro silt loam, a soil of medium organic content. 5 ml of acetonitrile, 50 μl of conc. HCl, and 1 g of soil were put into a 16×75 mm culture tube (Corning Glass Company), and the tube capped with a teflon-lined plastic screw-cap and heated at 150° for ½ h in an oil bath behind a safety shield. After cooling, the sample was centrifuged, the supernatant decanted, and the residue resuspended twice in 5 ml portions each of acetonitrile and centrifuged. The combined extracts were evaporated to dryness and the residue taken up in hexane for injection into the gas chromatograph. All organic solvents used were Mallinckrodt, Nanograde. In this method, the right amount of HCl depends, of course, on the type of soil. While the method is fast, it extracts more interfering material than the following one, and as a result, the minimum detectable limit is only 0.1 p.p.m.

Decarboxylation of a soil extract

This more sensitive, albeit somewhat lengthier, method uses a modification of extraction and partition steps described in the literature[2]. The solvent is then changed from ethyl acetate to acetonitrile, and the Picloram decarboxylated and purified on a silica gel column. The minimum detectable limit is 5 p.p.b., and the minimum limit of quantitation about 10 p.p.b. Column clean-up is necessary only at these extremely low levels; from 30 p.p.b. upward this step can be omitted and the analysis consider-

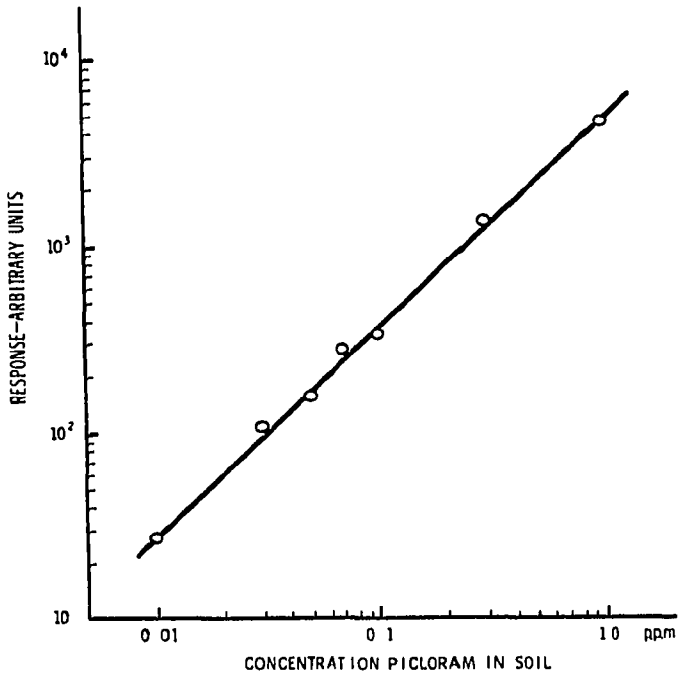


Fig. 5 Standard curve for the extraction of Picloram from soil. Column 1.5% OV-17 + 1.95% QF-1 on 100/120 mesh Chromosorb W-HP, 1.8 m × 4 mm I.D. Pyrex. Oven temperature 155° N_2 flow rate. 45 ml/min. MicroTek model MT-220, Ni-63 detector, d c mode.

ably accelerated. In repeated checks, recovery of decarboxylated Picloram from the silica gel column was always higher than 90%.

Fig. 5 shows a standard curve for Picloram recovered from Menfro silt loam spiked with 10 to 1000 p.p.b. of the herbicide. Quadruplicate analyses were done at the 10, 100, and 1000 p.p.b. levels, resulting in relative standard deviations of 23%, 15%, and 5%, respectively. The other points shown are single analyses. A 5 p.p.b. sample gave a clearly discernible peak for Picloram but could not be quantitated with reasonable accuracy. Fig. 6 shows two representative chromatograms from soils with low and high organic content spiked with 10 p.p.b. of Picloram. In the following paragraph a typical procedure of analysis is outlined in detail.

Typical soil analysis

Shake 1 g of soil for 3 min with 3 ml of 1 *N* KOH in a 16 × 75 mm culture tube on a Vortex mixer (Scientific Industries, Springfield, Mass). Centrifuge, decant the supernatant into a 50 ml tube, and repeat twice. Acidify the combined supernatants with 1 ml conc. HCl, and partition twice with 10 ml portions of ethyl acetate. Evaporate the combined ethyl acetate layers to dryness in a 16 × 75 mm screw-cap culture tube, using a 90° sand bath and a gentle stream of nitrogen. Add 1 ml acetonitrile and 5 μl conc. HCl, cap the tube securely and keep in an oil bath at 150° for 15 min. (This part of the procedure should be carried out behind a safety shield with due caution. Samples with leaking caps must be discarded.) Remove the tube from the oil bath, cool, open, and evaporate the acetonitrile under a stream of nitrogen with gentle heating, not exceeding 40°. Prepare a column for liquid chromatography by loosely plugging a 230 mm Pasteur disposable pipet (Fisher Scientific Company) with glass wool and fill

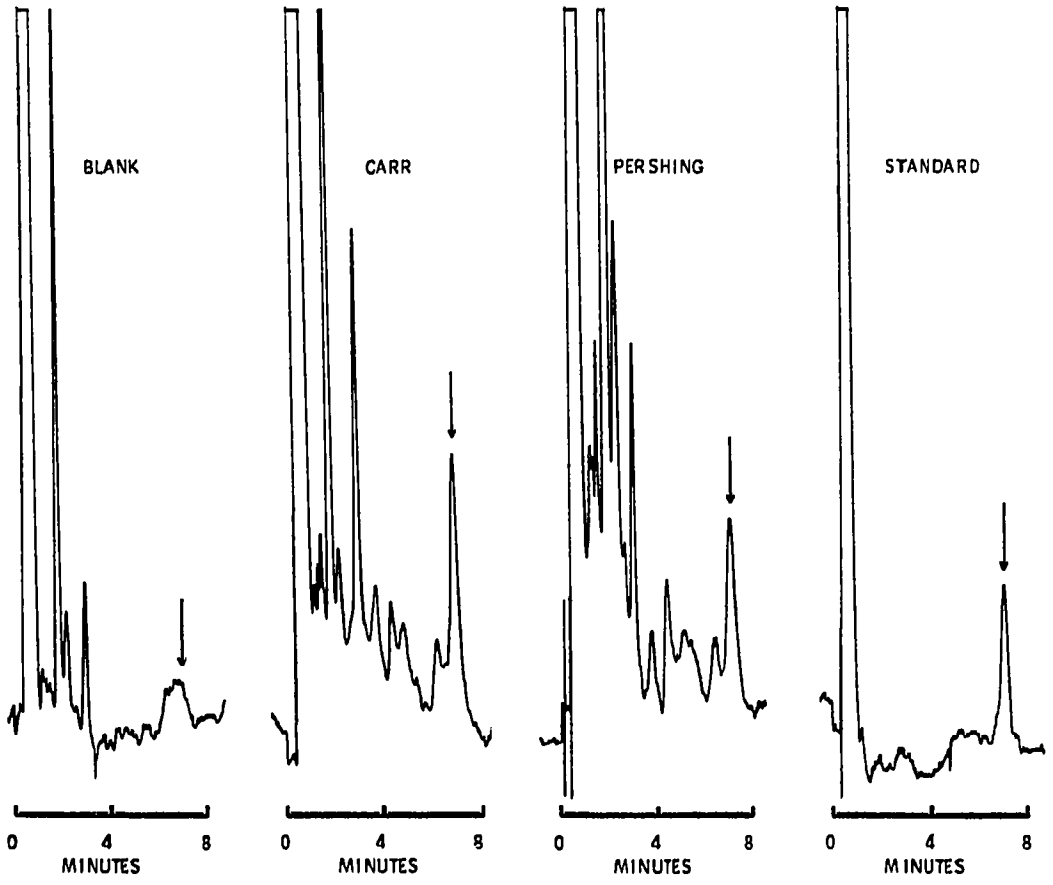


Fig. 6. Chromatograms of Pershing and Carr soils spiked with 0.01 p.p.m Picloram. Extracts cleaned up on silica gel. Injection: 4 μl out of 1 ml Column 3.5% OV-17 + 4.5% QF-1 on 80/100 mesh Chromosorb W-HP, 1.8 m × 4 mm I.D Pyrex. Oven temperature 160°. N_2 flow rate: 40 ml/min. MicroTek model MT-220, Ni-63 detector, R F. mode, 60 V, 270 μsec interval, 10 μsec width.

it about three fourths full with a slurry of No. 923 Silica Gel (Fisher Scientific Company) in hexane. (Pre-extract the silica gel with acetone for 3–4 h in a soxhlet to remove interfering materials.) Remove air bubbles from the column and introduce the residue dissolved in hexane. Elute with 15 ml of 5% acetone in hexane, evaporate the eluate to dryness under a stream of nitrogen, redissolve in hexane and inject a suitable aliquot into the gas chromatograph. The following GLC conditions have been used in our study, but are by no means the only ones possible: MicroTek MT 220 gas chromatograph with 1.8 m × 4 mm I.D. Pyrex U-tube column filled with 3.5% OV-17 + 4.5% QF-1 (1.5% OV-17 + 1.95% QF-1 in some experiments) on Chromosorb W-HP, 80/100 mesh[11,12]. Column temperature 155°, injector 235°, detector 290°; nitrogen flow rate 45 ml/min; d.c. voltage at 90% of standing current, or R.F. potential 60 V, pulse interval 270 μsec, pulse width 10 μsec. Run samples interspersed with standards to account for changes in detector sensitivity. Calculate the amounts of Picloram present from peak heights.

Reliability studies

The described method was tested on four different soils: (1) Carr loamy fine

TABLE I

PERCENT RECOVERY OF PICLORAM FROM FORTIFIED SOILS

Average of three determinations

Soil type	*Organic content*	*100 p p b*	*10 p p b.*
Pershing silt loam	High	94	68
Menfro silt loam	Medium	94	62
Leta silty clay loam	Medium	93	74
Carr loamy fine sand	Low	101	72

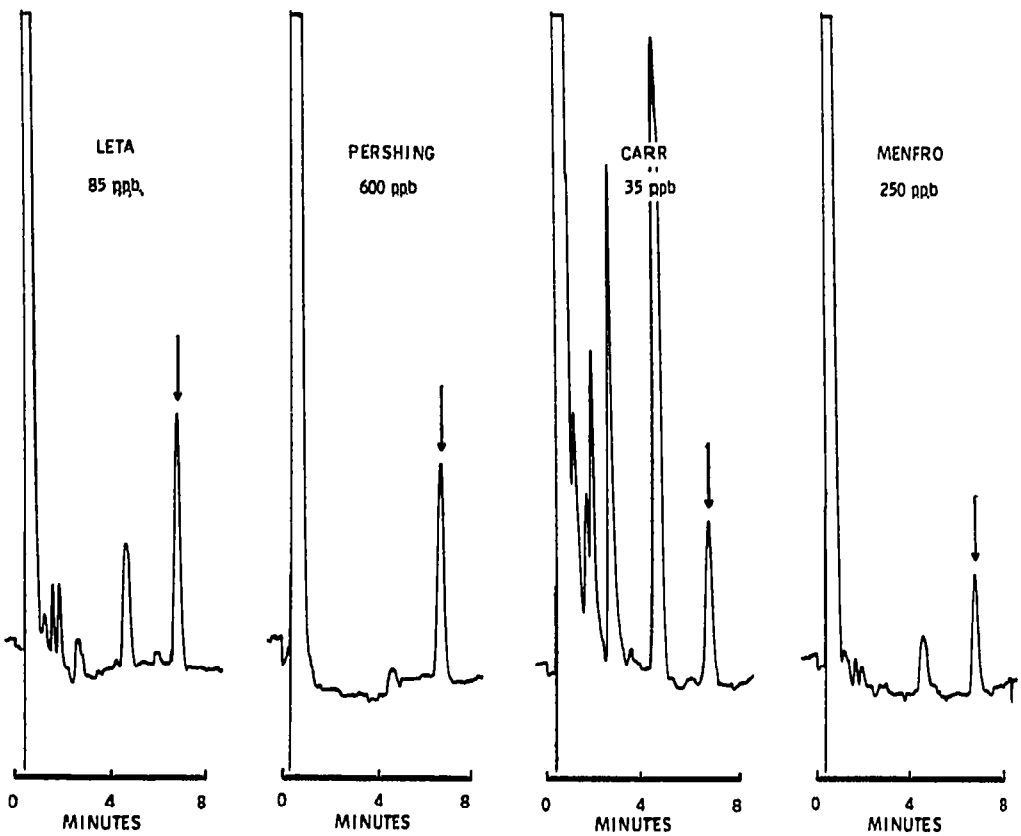


Fig 7. Chromatograms of Pershing, Menfro, Leta and Carr soils spiked at levels unknown to the analyst. Extracts not cleaned up on silica gel. Column 3.5% OV-17 + 4.5% QF-1 on 80/100 mesh Chromosorb W-HP, 1.8 m × 4 mm I.D. Pyrex. Oven temperature 160°. N_2 flow rate 40 ml/min. MicroTek model MT-220, Ni-63 detector, R F. mode, 60 V, 270 μsec interval, 10 μsec width. Sample volume and injection size adjusted as required.

TABLE II

ANALYSIS OF SOILS OF UNKNOWN PICLORAM CONTENT

Soil	*p.p.b. present*	*p.p.b. found*	
Pershing silt loam	600	600	[a]
Menfro silt loam	250	190	190
Leta silty clay loam	85	85	67
Carr loamy fine sand	35	44	38

[a] Sample lost during decarboxylation due to leaking cap

sand (low in organic matter) from the Missouri river bottom; (2) Leta silty clay loam (moderate organic matter) from the Missouri river bottom; (3) Menfro silt loam, courtesy of the Plant Pathology Department, University of Missouri; (4) Pershing silt loam (moderately high organic matter) from an upland area. All four soils were collected in Boone County, Mo.

The results of the analyses at 10 and 100 p.p.b. levels are shown in Table I. Clean-up on the silica gel column was used only for the 10 p.p.b. samples.

As a final test, each of the four soils was fortified with an amount of Picloram unknown to the analyst. Duplicate determinations were run on each soil according to the described procedure. The analyst decided in each case that silica gel clean-up was not required. The results are shown in Table II, and representative chromatograms appear in Fig. 7. The average time spent by the analyst in this study was 1.25 h per sample (= total time spent divided by number of samples).

DISCUSSION

The use of two derivatives of the herbicide Picloram for determination by GLC has been investigated. The trimethylsilylated product can be determined by flame ionization, but causes appreciable difficulties when the EC detector is used. If a good clean-up method for biological extracts could be found and the detection difficulties could be circumvented with another type of selective detector, this approach should develop into a suitable analytical method. A procedure to remove the excess silylation reagent before GLC would permit the use of the EC detector and thus provide an additional, sensitive means of confirming the presence or absence of Picloram.

The decarboxylation of Picloram in its two versions proved far more successful. Of these methods, the decarboxylation on soil is faster and eliminates the need for separate extraction and partition steps. The present minimum level of quantitation, however, is only around 100 p.p.b. The method depends to a great degree on the nature of the matrix (the soil) and suitable modifications must be made for each particular type of sample.

The most careful control of parameters during the analytical procedure is called for in the decarboxylation step. If standards, samples, and blanks are not treated exactly alike, the reliability of the data is bound to suffer.

Using the steps of extraction and partition as described in the literature and adding a silica gel column clean-up prior to decarboxylation allows quantitation at the 10 p.p.b. level. We have obtained much "cleaner" chromatograms by decarboxylation than by formation of the methyl ester according to reported procedures. This may, however, be due to our greater familiarity with decarboxylation. If positive identification of a suspected residue in soil is required, concurrent determinations of Picloram both as the methyl ester and as the decarboxylated product provide, in our opinion, the best test for its presence.

Thus, the described method should offer attractive alternatives to the common analysis of Picloram as the methyl ester. The use of the decarboxylated compound allows a fast and reliable determination of Picloram and unequivocal confirmation of results obtained by other procedures.

ACKNOWLEDGEMENTS

The authors are pleased to acknowledge the competent technical assistance of Mr. ERNEST LEAKE and want to thank Dow Chemical Company, Midland, Mich., U.S.A., for several samples of analytical grade Tordon.

They also gratefully recognize the valuable discussions with the Department of Agronomy, University of Missouri, Columbia, Mo.

REFERENCES

1 J. S. LEAHY AND T A. TAYLOR, *Analyst*, 92 (1967) 371.
2 C R. YOUNGSON, C. A. GORING, R W MERKLE, H H SCOTT AND J D GRIFFITH, *Down to Earth*, 23 ,No. 2 (1967) 3.
3 E. L. BJERKE, A. H. KUTSCHINSKI AND J C. RAMSEY, *Agr. Food Chem* , 15 (1967) 469
4 M. G. MERKLE, R. W. BOVEY AND R. HALL, *Weeds*, 14 (1966) 161.
5 J G SAHA AND L A GADALLAH, *J. Assoc Offic. Anal. Chemists*, 50 (1967) 637
6 J. R. PLIMMER AND P. C. KEARNEY, *Paper presented at the 1968 Meeting of the Weed Society of America, New Orleans, La., February, 1968.*
7 S. D. ABBOT, R. C HALL AND C. S. GIAM, *J. Chromatog* , 45 (1969) 319.
8 C. W. GEHRKE, H. NAKAMOTO AND R W. ZUMWALT, *J Chromatog.*, 45 (1969) 24.
9 D. L. STALLING, C. W. GEHRKE AND R. W. ZUMWALT, *Biochem. Biophys. Res Commun* , 31 (1968) 616.
10 G. T. FLINT AND W. A. AUE, in preparation.
11 R. S. HENLEY, R. F. KRUPPA AND W. R. SUPINA, *J. Agr Food Chem.*, 14 (1966) 667
12 J. F. THOMPSON, A. C WALKER AND R. F. MOSEMAN, *J. Assoc. Offic. Anal. Chemists*, 52 (1969) 1263.
13 R. C. HALL, C. S. GIAM AND M. G. MERKLE, *Anal. Chem.*, 42 (1970) 423.