trapping the milk and milk product nonvolatile substances and protecting the column. The application of this method is limited by the lower boundary of concentrations of the analysed compounds occurring in the sample. It depends on the sensibility of the apparatus used. In our case for example even 0.08 mg% diacetyl, 0.04 mg% *n*-propanol, 0.02 mg% isopropanol and 0.1 mg% acetone could be determined.

The work suggests the use of this method also for the determination of other compounds as described and shows the possibility of its application to other materials than milk products. For this purpose it is necessary to change the analytical conditions and to use respectively some other combination of column packing polymer materials.

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The determination of picloram in fescue by gas–liquid chromatography*

Several residue methods for the determination of picloram (4-amino-3,5,6-trichloropicolinic acid) in soil and a variety of crops employ extraction with aqueous base and electron capture gas–liquid chromatography (GLC) of the methyl ester or the pyrolysis product of this herbicide. Recently we have reported a method for the determination of picloram in soil, which uses a closed-tube decarboxylation[1].

Picloram is extensively used to eradicate brush and other broad-leaved plants on grazing ranges[2]. We therefore decided to apply our method to the analysis of fescue. However, several serious analytical difficulties became apparent.

Basic extracts of fescue caused emulsions of considerable stability. The formerly used silica gel column failed to separate larger amounts of co-extractants from the

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decarboxylated picloram and there was danger of its loss in the prior and subsequent evaporation steps. Finally, the yield of decarboxylated product decreased as the culture tubes were used repeatedly in this investigation.

To cope with these problems, an organic extraction system, an alumina column, and two "keepers" were introduced. A special study traced the inconsistencies in decarboxylation efficiency to the catalytic activity of the tube walls, which could be reduced by acid treatment and silylation. (In our work on soil, decarboxylation tubes had repeated prior exposure to silylation reagents which—unrealized then—contributed to the success of the method.)

Experimental

Pretreatment of glassware. Screw cap culture tubes (16 × 75 mm) for the decarboxylation of picloram were soaked in *aqua regia* overnight and thoroughly rinsed with distilled water. After drying, the tubes were filled with a 10% solution of trimethylchlorisilane (TMCS) or dimethyldichlorosilane (DMCS) in toluene, capped and allowed to stand for 48 h. Then the tubes were rinsed with a few small portions of toluene and filled with methanol. After standing for about 30 min, the methanol was discarded and the tubes were rinsed with several portions of acetone and dried.

Sample preparation. The fescue was frozen in liquid nitrogen and finely chopped in a blender. Care was taken to maintain the sample in a frozen state by keeping a small amount of dry ice in the blender. The chopped fescue was stored in a deep freeze and samples were weighed out and spiked as needed. Picloram was added to 2 g fescue samples in 22 × 150 mm tubes with 19/38 ground glass tops in 0.1 ml or less of acetone. The solvent was allowed to evaporate and the samples were thoroughly mixed.

Extraction and partitioning. The samples were shaken for 3 min on a Vortex mixer with three 15 ml portions of acetonitrile–ammonia (95:5) and filtered through a plug of glass wool into a 100 ml round bottom flask. The extract was taken to dryness on a rotary evaporator, and transfered to a 125 ml separatory funnel with 50 ml of 0.1 *N* KOH. This solution was shaken with two 25 ml portions of ethyl acetate. After separation, the ethyl acetate fractions were discarded and the aqueous solution remaining in the separatory funnel was acidified with 10 ml of 1 *N* HCl. Two 25 ml portions of ethyl acetate were used to extract the acidic solution. The combined ethyl acetate layers were taken to dryness on a rotary evaporator and the residue transferred to a 16 × 75 mm screw cap culture tube (previously treated with *aqua regia* and TMCS) with several small portions of ethyl acetate, and evaporated to dryness under a gentle stream of nitrogen.

Decarboxylation. One ml of acetonitrile containing 5 μl of 12 *N* HCl was added to culture tubes containing samples and standards and capped tightly. The tubes were placed in a 150° oil bath for 30 min. After this time, the tubes were removed, cooled in a beaker of water and opened. Ten μl of ethylene glycol were added to each tube and the solutions carefully evaporated just to dryness under a gentle stream of nitrogen. Two ml of hexane were added to each tube.

Column clean-up. A column of basic alumina (Brockman activity grade I) was prepared by adding a hexane slurry of alumina to a disposable Pasteur pipet plugged with a small amount of glass wool. The column was washed with 10 ml of hexane and one half (1 ml) of the sample was placed on the column. The column was then washed with 5 ml of benzene, the benzene discarded, and the decarboxylated picloram was

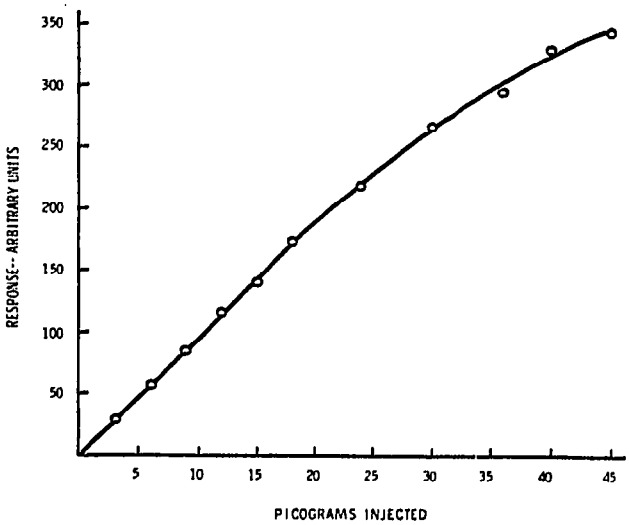
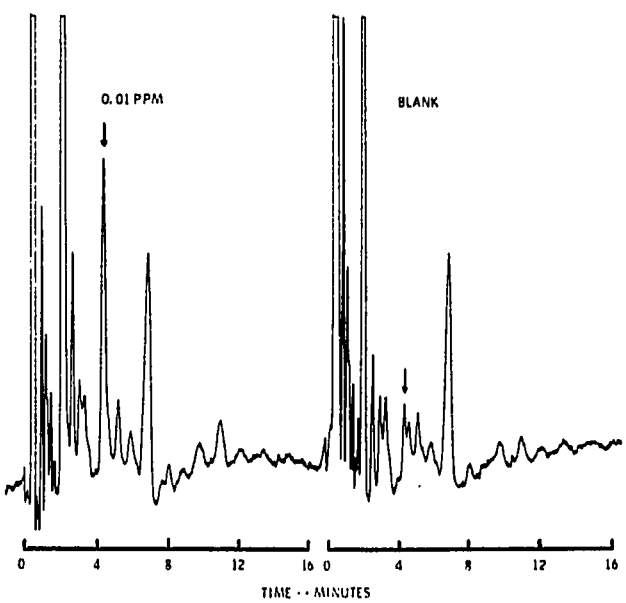


Fig. 1. Standard curve for decarboxylated picloram. Column: 3.5% OV-17 + 4.5% QF-1 on 80/100 mesh, Chromosorb W-HP, 1.8 m × 4 mm I.D. Pyrex. Oven temperature: 200°. N_2 flow rate: 40 ml/min. Micro Tek model MT-220, Ni-63 detector, pulse mode, 60 V, 250 μsec rate, 6 μsec width.



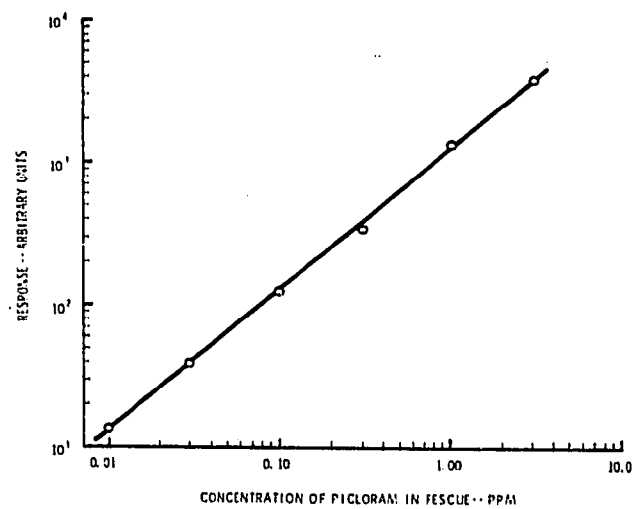


Fig. 2. Chromatograms of a fescue sample spiked with 0.01 p.p.m. picloram, and of a blank. Column: 3.5% OV-17 + 4.5% QF-1 on 80/100 mesh Chromosorb W-HP, 1.8 m × 4 mm I.D. Pyrex. Oven temperature: 200°. N_2 flow rate 40 ml/min. Micro Tek model MT-220, Ni-63 detector, pulse mode, 60 V, 250 μsec rate, 6 μsec width.

Fig. 3. Calibration curve for the extraction of picloram from fescue. 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: 200°. N_2 flow rate: 40 ml/min. Micro Tek model MT-220, Ni-63 detector, pulse mode, 60 V, 250 μsec rate, 6 μsec width.

eluted from the column with 15 ml of 5% ethyl ether in benzene. This solution was adjusted to contain a concentration of picloram suitable for injection into the gas chromatograph (see below). If this meant concentrating the solution, 10 μl of tetradecane were added prior to evaporation under a stream of nitrogen.

Gas–liquid chromatography. At the time of this work, the linear range of our ^{63}Ni electron capture detector was between 3 and 18 pg of decarboxylated picloram injected (Fig. 1). The various fescue samples were adjusted to a final volume such that a 5 μl injection would produce a peak for decarboxylated picloram within this linear range of the detector. Representative chromatograms of a blank and of a 10 p.p.b. sample are shown in Fig. 2. A calibration curve for fescue samples spiked with 10 to 3000 p.p.b. picloram is shown in Fig. 3.

Results and discussion

Our original method for the determination of picloram[1] was developed for soil—the use of grass as a substrate therefore required extensive modification. Emulsion problems encountered when fresh crop samples were extracted with aqueous base could be circumvented by using a basic acetonitrile solution for extraction. Much of the interfering coextracted material was subsequently eliminated in the regular partitioning step[3]. The remaining interferences were further reduced by chromatography on alumina. As a result, relatively "clean" chromatograms could be obtained from fescue samples containing as little as 10 p.p.b. picloram.

Deactivation of the culture tubes used for decarboxylation was found to aid greatly the long-term reproducibility of results. Apparently, traces of metals and/or active sites in the glass caused decomposition. In line with this assumption, a 30 min decarboxylation time gave a better yield than the previously reported 15 min, provided deactivated tubes were used.

Ethylene glycol and tetradecane, the two "keepers" employed in the method, helped to minimize losses of decarboxylated picloram during evaporation. Neither of these compounds interfered with the final analysis by GLC.

The described method offers an alternate—and confirmatory—route to the determination of picloram residues in fescue and possibly other plant materials. It shows a wide linear range down to picloram concentrations of 10 p.p.b. and is thus well suited for residue analysis.

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