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Gas Chromatographic Determination of Picloram in Human Urine

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Picloram (4-amino-3,5,6-trichloropicolinic acid) is used to control broadleaf weeds and woody plants. It is used in forestry for site preparation and to help release pine seedlings from broadleaf competition. The use of any pesticide requires that we monitor not only its effectiveness but also the exposure of human beings to the compound. Methods for analyses of exposure and the fate of compounds differ for individual pesticides. Although methods have been developed for analysis of picloram from urine, none have been reported using boron trifluoride methylation with C18 cartridge cleanup and electron capture detection.

Nolan et al. (1984) have reported that 88-94% of picloram that had been orally ingested by human volunteers was excreted unchanged in the urine within 72 hours. In the same paper they report an analytical method for picloram in urine using GC/MS with a limit of quantification of 10 ppb.

Tonder and Daugherty (1981) have reported an analytical screening method for acidic toxic substances involving diazo-methane derivatization followed by florisil cleanup. The percent recovery for picloram was 60-63% at 40 ppb. Draper (1982) also used diazomethane derivatization with a recovery of 104 $\pm$ 6% for 4 replications fortified at 100 ppb. Libich et al. (1984) analyzed for picloram with a Hall detector in the chlorine mode following base hydrolysis and methylation with boron trifluoride in methanol. The mean recovery was 80% with a range of 76% to 90%.

In order to complete one of our research projects, we needed an analytical method for picloram in urine using election capture detection. We also wished to avoid the use of diazomethane due to the potential health hazards associated with it. Our method affords a limit of quantification of 10 ppb.

MATERIALS AND METHODS

All solvents were pesticide grade. Sodium hydroxide, hydrochloric acid, and boron trifluoride in methanol were reagent grade.

A Perkin-Elmer Sigma 1 equipped with a ^{63}Ni electron capture

detector and a 366 cm x 2 mm id 1.5% SP-2250/1.95% SP-2401 on 100/120 Supelcoport column (available from Supelco Inc.) was used. The temperatures of the injector, oven, and detector, respectively, were 250°C, 215°C, and 350°C. The carrier gas was 5% methane in argon at 50 ml/min.

Confirmation of picloram methyl ester was by injecting the sample on a 366 cm x 2 mm id 3% OV-225 column and comparing the retention time to that of a standard obtained from E.I. du Pont de Nemours and Company.

Confirmation for 10 urine samples was also done by preparing the butyl ester using boron trifluoride-butanol and comparing the retention times to butyl ester standards on both a 200 cm x 2 mm id 1.5% SP-2250/1.95% SP-2401 on 100/120 Supelcoport column and a 200 cm x 2 mm id 3% SP-1000 on 100/120 Supelcoport column.

Ten mL of urine was combined with 2 ml of concentrated hydrochloric acid in a 125 mm x 20 mm culture tube equipped with a Teflon®-lined screw cap. The cap was secured and the tube was placed in a 95°C oven for 60 min. The tube was removed, cooled in an ice bath, and the solution was extracted with three 4-ml portions of ether, shaking for 1 min each time. After each extraction the tube was centrifuged for 5 min at 750 X g to break up the emulsion. The ether portions were combined in a clean culture tube and extracted with 3 ml of 0.1 N NaOH by shaking for 1 min each time. The ether layer was discarded and the aqueous layer was acidified with 0.4 ml concentrated hydrochloric acid. The acidified aqueous solution was extracted with three 5-ml portions of ether, shaking for 1 min each time. The ether portions were combined in a clean culture tube and evaporated to dryness in a 35-40°C water bath under a stream of nitrogen. Boron trifluoride-methanol (0.5 mL) was added; the tube was capped and placed in a boiling water bath for 15 min. The tube was removed and cooled and then 4.5 ml of deionized water was added.

The extracts were cleaned up with a Baker 10 Extraction System® (available from VWR Scientific) using a 3-ml C18 cartridge as follows: The cartridge was conditioned by drawing through one column length of methanol (2-3 ml) followed by two column lengths of deionized water. The 5-ml sample (0.5 ml boron trifluoride-methanol plus 4.5 ml deionized water) was drawn through the cartridge at 2-4 drops per sec. One ml of deionized water was drawn through, and then air was drawn through for approximately 1 min to dry the cartridge. The picloram methyl ester was eluted with 1.5 ml of toluene and collected in a 5-ml volumetric flask. The volume was adjusted to 5 ml with toluene and an aliquot was removed for GC analysis.

RESULTS AND DISCUSSION

Chromatograms for both fortified and unfortified samples are given in Figure 1. Recovery data are given in Table 1.

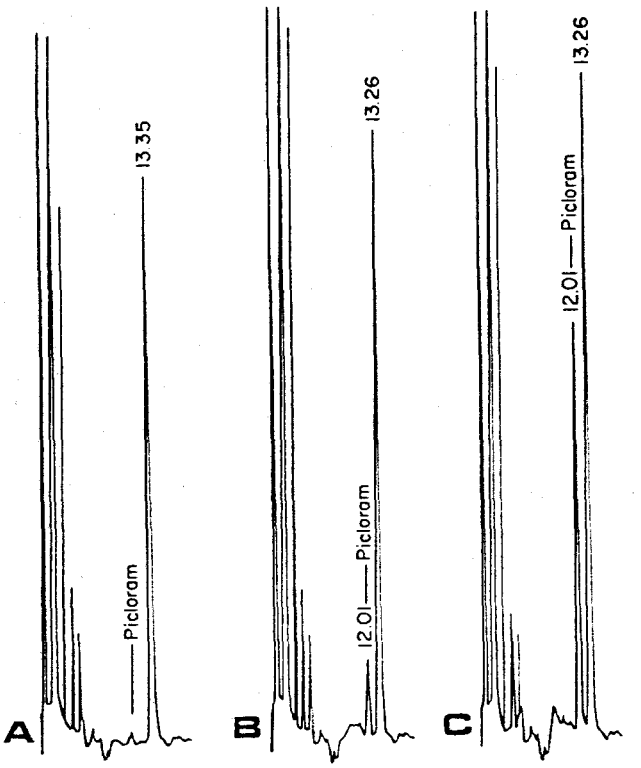

Figure 1. Chromatograms of Urine Samples for Picloram Methyl Ester A. Urine blank B. Urine containing 0.02 ppm picloram C. Urine containing 0.10 ppm picloram. Retention time in min.

Table 1. Recovery of Picloram from Urine with Electron Capture Detection.

Fortification (ppb)	% Recovery ± standard deviation[a]
0	not detected
10	79 ± 27
20	75 ± 4
100	76 ± 3
400	80 ± 4

[a]Seven replications except for 10 ppb for which there were nine replications.

A large peak typically eluted after the picloram methyl ester peak as seen in Figure 1; however, resolution was always such that this peak did not interfere with quantitation.

Urine from seven people was fortified with picloram and analyzed to see if variation in urine would affect recovery. The percent recovery was not found to be dependent on the source of the urine.

This method has been used to analyze urine from 53 different people in an applicator exposure study of workers applying TORDON 101R (trademark of the Dow Chemical Company). TORDON 101R contains a 3.7/1 ratio of 2,4-D (2,4-dichlorophenoxyacetic acid) to picloram.

Urine collected from each worker the day prior to application showed no detectable levels of picloram (10 ppb limit of detection), indicating that there were no artifacts in the urine of any of these 53 individuals that would give a false positive value.

The method provides excellent sensitivity and precision using electron capture detection which is commonly available in laboratories performing pesticide analyses. It avoids the use of diazomethane and, since the cleanup uses commercially available C18 cartridges with a vacuum manifold capable of holding ten cartridges at a time, 20-30 analyses can be performed each day.

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