Application of a New Glass Capillary Chromatographic Technique in the Analysis of Phenoxyacetic Acid Herbicides

Marja Liisa Hattula[1] and Seppo Räisänen[2]

[1]*Department of Chemistry University of Jyväskylä Jyväskylä, Finland*

[2]*Technical Research Centre of Finland, Food Research Laboratory Otaniemi, Finland*

In this work the degradation of three herbicides, MCPA, 2,4-D and 2,4,5-T was studied in three different pH:s and a new high resolution glass capillary chromatographic technique was applied in the determination.

According to our experiences the analyzing of MCPA in small amounts in water and biological material has proved to be difficult because its response to EC-detector is weak. This fact is indispensable of the esterification techniques (preparing a trimethylsilyl derivative [COLLIER and GRIMES (1974)] or the methylation by boron trifluoride [HORNER et al. (1974)] in addition to the widely used methylation with diazomethane. The resolution of the packed column is not very good in separation of MCPA from other related substances, either. By using high resolution glass capillary column the amount of 100 pg (100×10^{-12}g) of the methyl derivative of MCPA can be detected by FID-detector. The compound can also easily be separated from the impurities of the biological material as well as from other phenoxyacetic acid herbicides. 2,4-D and 2,4,5-T both have a higher response to EC-detector and they can easily be separated from the compounds which are not sensitive to EC-detector. The analyzing methods of the phenoxyacetic acid herbicides have been widely described [YIP (1975)] and the techniques mostly used have consisted of GLC after different clean-up methods and the methylation [COLLIER and GRIMES (1974), GROSBY and WONG (1973), DEVINE and ZWEIG (1969), HENSHAW et al. (1975), LAROSE and CHAU (1973) and PURKAYASTHA (1974)]. In this work the experimental conditions were so arranged that FID-detector could be used. If, however, small amounts in biological samples have to be analyzed the use of EC-detector is more recommedable.

MATERIALS AND METHODS

a) The herbicides used were:

2,4-D (Fluka, purity 97 %)
2,4,5-T (")
MCPA (Kemisk Værk Køge A/S, Denmark 99,99 %)

Bulletin of Environmental Contamination & Toxicology, Vol. 16, No. 3 © 1976 by Springer-Verlag New York Inc.

b) The extraction solvent used was the mixture of diethylether and chloroform 1:1 v/v (Merck, p.a., the chloroform redestilled)

c) 0.1-n HCl was used for the adjustment of pH

d) The gas chromatographic equipment

The gas chromatograph used was Carlo Erba Model Fractovap 2300 equipped with FID-detector and a Grob-type split-less injection system. The column used was a glass capillary column of 30 m with the liquid phase of FFAP. The inside diameter of the column was 0.35 mm.

The column used was prepared by Mr. A Hesso and Räisänen in the University of Helsinki, Finland.

e) The analytical procedure

The stock solutions of the three herbicides were made 1 mg/ml and the final concentration in the test solutions was 20 ppm of each compound in destilled water. Two solutions of 1 ppm and two zeros in each temperature were also included in the experiment. The pH:s used were 5.5, 6.5 and 7.5 and the temperatures chosen were 0^{o} and $20^{o}C$. The oxygen content of the solutions was measured by the official method of A.P.H.A. [(Standard Methods... (1971)] and it remained ca. 95 % through the experiment.

The solutions were made in one liter Pyrex glasses and the liquid front was carefully marked. The evaporated water was replaced before the sample taking. Two aliquots of 50 ml were taken at a time and the extraction of the residues was carried out as follows: The solution was made acidic by 0.1-n HCl (pH under 2) and shaken three times with 15 ml of the extraction solvent. The combined extracts were then filtered through 5 g anhydrous sodiumsulphate (Merck, p.a.) and the solvent was evaporated in Büchi Rotavapor in $40^{o}C$. The residue was dissolved in ether and methylated with diazomethane for 10 minutes. The ether was then carefully evaporated with pure nitrogen and dissolved in n-hexane (Merck, Uvasol). To ascertain that the evaporation did not cause losses of the methyl-esters, a series of standard solutions was treated in the same way.

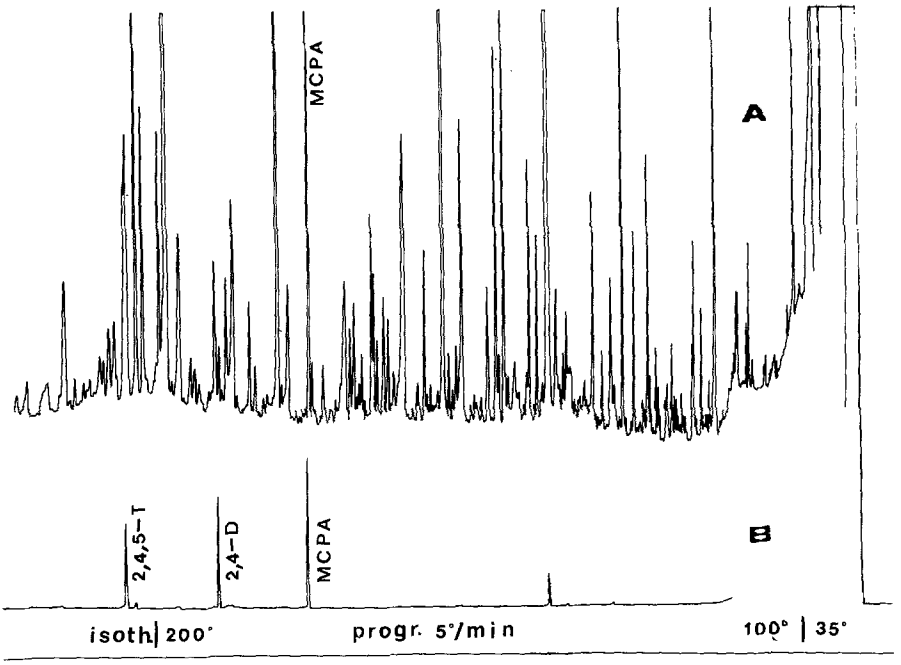

Figure 1. A. A well water sample contaminated by herbicides. Column 40 m/0.04 mm FFAP, 4,8 ml H_2/min, attenuation 1 x 16.

B. The chromatogram of three herbicides. Attenuation 1 x 64.

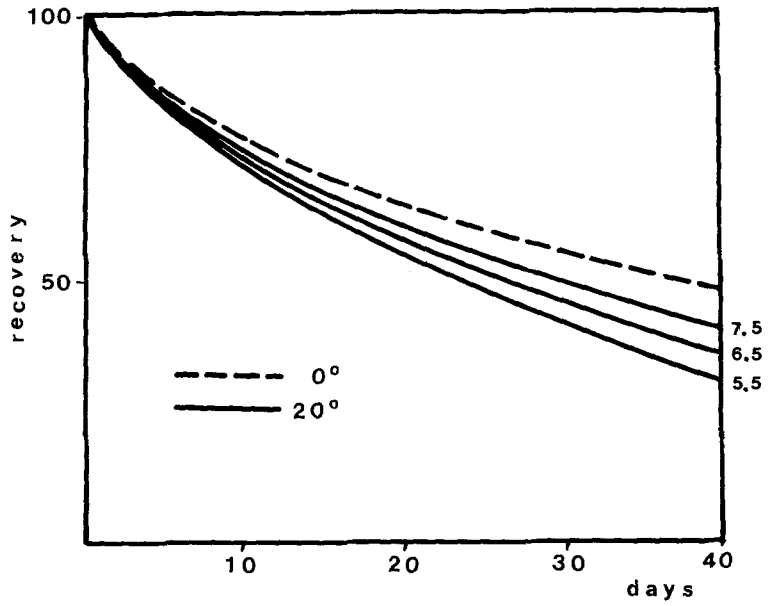

Figure 2. The degradation of herbicides in two temperatures (0° and 20°C) and in three pH:s (5.5, 6.5 and 7.5)

The solutions were chromatographed in n-hexane and 2 µl of the solution was introduced directly without stream-splitting on to the solumn at 35°C. After the solvent peak had appeared the solution was programmed 30°/min to 190°C. This method has been described by GROB and GROB (1974). The concentrations of the compounds were calculated according to the peak areas. In this work the peak areas expressed as mm^2 for 2 µl and the standard deviations for each compound were as follows: 2,4-D, (15.4 ± 0.3), 2,4,5-T, (8.9 ± 0.2) and MCPA, (25.9 ± 0.52). The first water samples were taken immediately after applying the herbicides in the water and the following samples were taken after 10, 20, 30 and 40 days.

A typical chromatogram of the herbicides in well water sample containing 0.01 ppm MCPA (contaminated by accident) as well as reference chromatogram is presented in figure 1 (A and B).

RESULTS AND DISCUSSION

The external conditions of the experiment, although purposed to resemble the conditions of the lakes in some respects cannot be compared to the natural conditions because of the lack of irradiation, micro-organisms and the movement of the water. The results of the experiment expressed as the precent of recovery, are presented in figure 2. In this experiment no statistically significant difference could be observed in the degradation of the three compounds studied. There were slight difference in 20°C in different pH:s but the behaviour of the compounds was similar in 0°C. After 40 days the recovery %:s were as follows in pH 5.5 30, pH 6.5 35 and in pH 7.5 40 %, respectively. According to our results the degradation was the most rapid in the lowest pH.

In another experiment carried out by the authors 2,4-D, 2,4,5-T and MCPA were kept in oxygen-free destilled water both in 0° and 22°C in pH 7, 6 and 5. No degradation could be observed after 8 months in 0°C in pH 7 and in pH 6 and 5 there was left MCPA 85 and 70 %, respectively. 2,4-D and 2,4,5-T showed a little faster decomposition. At room temperature the degradation-rate was faster.

No attempt was made in this study for identifying the metabolites of the herbicides studied. The metabolites are easily detectable by the method used, either as free phenols or their methyl derivatives. The chromatographic technique was mainly

developed for the detection of MCPA and in optimal conditions the lowest concentration detected by the authors was 50 ppt MCPA in water. In these conditions the amount of water analyzed was 0.5 l which was shaken three times with 50 ml of chloroform-ether. The column used was a glass capillary column of 40 m of FFAP.

As to the gas chromatographic conditions the liquid phase, FFAP, although originally developed phenoxyacetic acid herbicides. If the compounds are analyzed as their trimethylsilyl-derivatives the use of OV-1, SE-30, OV-101 or SF-96 is more recommendable. If the herbicides are applied as iso-octyl esters in the nature the methylation by borontrifluoride is most recommendable.

In this paper only the analyzing of water samples has been described but the same gas chromatographic conditions can be used for the analyzing of soil extracts and different biological materials contaminated by phenoxyacetic acid herbicides without any clean-up process.

REFERENCES

COLLIER, R.H. and GRIMES, G.S., J.A.O.A.C., 57, 781 (1974).

CROSBY, D.G. and WENG, A.S., J. Agr. Food Chem. 21, 6 (1973).

DEVINE, J.M. and ZWEIG, G., J.A.O.A.C., 52, 187, (1969).

GROB, K. and GROB, K. Jr. Chromatogr. 94, 53 (1974).

HENSHAW, B., QUE HEE, S.S., SUTHERLAND, R.G. and LEE, C.C., J. Chromatog. 106, 33 (1975).

HORNER, J., QUE HEE, S.S. and SUTHERLAND, R.G., Anal. Chem. 46, 110 (1974).

PURKAYHSTHA, R., J. Agr. Food Chem. 22, 453 (1974).

Standard methods for the examination of water, A.P.H.A., vol III, Washington DC, 1971.

YIP, G., Chromatog. Sci. 13, 225 (1975).