Analysis of 2,4-D and 2,4,5-T in Lingonberries, wild Mushrooms, Birch and Aspen Foliage

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SUMMARY

A method was developed for residue analysis of 2,4-D and 2,4,5-T in lingonberries, wild mushrooms, birch and aspen foliage. The method involves the extraction of the residues with diethyl ether followed by alkali hydrolysis, purification by column chromatography, methylation of the free acids and their determination by gas chromatography. The limit of detection is 0.05 ppm for 2,4-D and 0.02 ppm for 2,4,5-T for a 20 g sample with a recovery of 75 % ± 11 %. Results of some analyses are also reported.

INTRODUCTION

Phenoxy herbicides containing 2,4-D (2,4-dichlorophenoxyacetic acid) and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) are widely used in Finland for weed and brush control. These compounds are applied as formulations of esters, amines or other salts. In this study a new method was developed for residue analysis of 2,4-D and 2,4,5-T in lingonberries, wild mushrooms and foliage. The method is suitable for the determination of free acids, esters and conjugates of 2,4-D and 2,4,5-T.

Several methods for determining residues of chlorophenoxy acids have been reported. LØKKE (1975) developed a method for analyzing free and bound chlorophenoxy acids in cereals. ALLEBONE and HAMILTON (1975) analyzed 2,4-D in plant tissues and MUNRO (1972) 2,4-D and 2,4,5-T in tomato plants and other vegetables. LE ROY BJERKE et al. (1972) described a method for detecting four phenoxy acids in milk and cream. The determination of chlorophenoxy acids in soil and water samples and tissues of sheep and cattle has also been reported (CLARK et al. 1975, SUFFET 1973, PURKAYASTHA 1974, RENBERG 1974).

EXPERIMENTAL

Procedure. The diagram in Fig. 1 shows the different steps i the analytical procedure. The acidified sample is first extracted with diethyl ether. The extract is then hydrolyzed with sodium hydroxide to convert the herbicide residues into free acid form. Before the methylation with diazomethane, the extract is purified by passage

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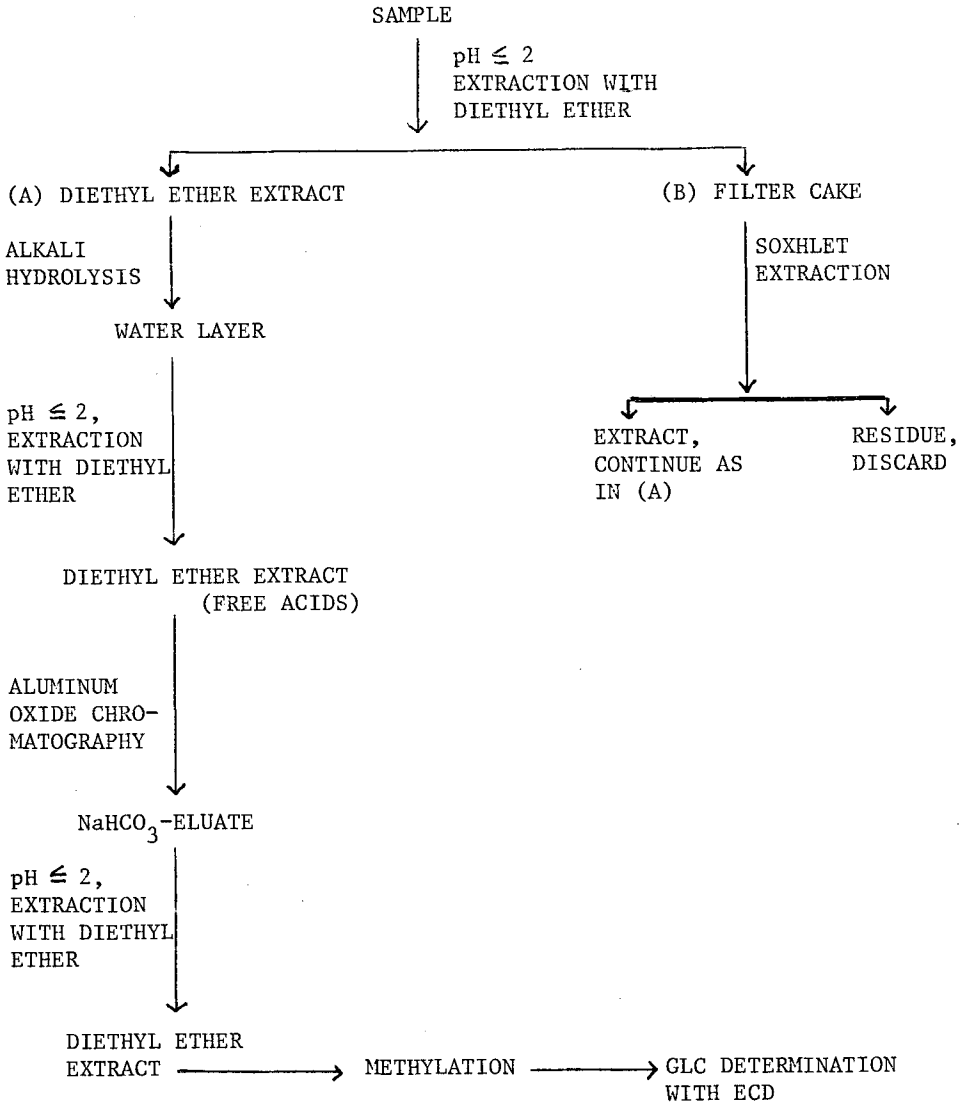

Fig. 1. Scheme of residue analysis.

through an aluminum oxide column. Conjugates are dissolved by extracting the filter cake in a Soxhlet-apparatus with a mixture of diethyl ether, acetone and ethanol (1+2+2). The Soxhlet-extract is then treated as described for the diethyl ether extract. The final determination is made by gas chromatography using an electron capture detector. This method gives the sum of the free acids, esters and conjugates of both acids.

Reagents and apparatus. Diethyl ether (commercial grade) was purified by distillation over potassium hydroxide pellets. Acetone (commercial grade) was purified by double distillation. Ethanol, n-propanol, toluene, sodium chloride, sodium hydroxide, sodium bicarbonate, sulfuric acid and phosphoric acid were of reagent grade. Sodium sulfate (Ph. Nord.) was heated at 250°C for 4 hours before use. Aluminum oxide (Woelm, acid) was used without purification. Diazomethane was prepared from Diazald (Aldrich Chemicals) according to the method of DE BOER and BACKER (1954). Millipore Milli-Q (without Twin-90 Filter Unit) purified water was used for all aqueous reagents.
Varian Aerograph gas chromatographs equipped with an electron capture detector (^{63}Ni and ^{3}H) were used with the following columns: 4 % GE XE-60 on Gas Chrom Z, 100/120 mesh (glass, 1.5 m x 3 mm), 5 % D.C. 200 + 7.5 % QF-1 on Varaport 30, 100/120 mesh (glass, 1.5 m x 3 mm), SE-30 (glass capillary, 15 m x 0.3 mm, isothermal, split 1:15) and FFAP (glass capillary, 25 m x 0.4 mm, neutral, isothermal, split 1:20).

Extraction and hydrolysis. 20-100 g of a finely chopped sample was acidified to pH 2 or less with 0.5 ml of 9 N sulfuric acid. Diethyl ether was added to cover the sample. The lingonberry and mushroom samples were set aside for 16-20 hours. The foliage samples were first shaken in a mechanical shaker for 2 hours before setting aside for 16-20 hours. The samples were then homogenized and after the addition of sodium sulfate the supernatant was filtered through a layer of sodium sulfate. The residues were washed with diethyl ether and filtered. The combined diethyl ether extracts were concentrated in a rotary evaporator at about 25°C and quantitatively transfered to a 150 ml evaporation flask. The extract was evaporated until just dry. The residue was then dissolved in 3 ml of 0.1 N sodium hydroxide and 5 ml of n-propanol. The flask was covered with a watch-glass and hydrolyzed for half an hour in a boiling water bath. After evaporating the n-propanol in a rotary evaporator the solution was acidified with sulfuric acid (0.15 ml 9 N) and 10 ml of a saturated aqueous solution of sodium chloride added to it. The solution was extracted with diethyl ether (15 ml x 3) and the combined diethyl ether extracts were dried with anhydrous sodium sulfate. The extract was evaporated in a rotary evaporator and the volume adjusted to 10 ml.

Aluminum oxide chromatography and methylation. The diethyl ether extract was transfered to a column containing 1 g of acidic aluminum oxide (600°C, 4 h, act. grade I) suspended in diethyl ether (LE ROY BJERKE 1972). The column was washed with 60 ml of diethyl ether,

eluted to dryness and the diethyl ether extract then discarded. The chlorphenoxy acids were eluted with 30 ml of 0.25 % aqueous sodium bicarbonate solution. The aqueous eluate was acidified with phosphoric acid (0.5 ml, 85 %) and 9 g of sodium chloride added to it. The solution was extracted with diethyl ether (15 ml x 3) and the combined extracts were dried over anhydrous sodium sulfate. After adding 1 ml of toluene, diethyl ether was evaporated to 1 ml and 2 ml of diazomethane reagent added to it. After half an hour the reagent was evaporated with nitrogen in a fume cupboard. The residue was dissolved in hexane and the volume adjusted to 10 ml.

Gas chromatographic determination. The concentration of the phenoxy methyl esters was determined by comparing the peak heights of the samples with those of reference standards.

Soxhlet-extraction. The filter cake was extracted in a Soxhlet-apparatus with a mixture of diethyl ether, acetone and ethanol (1+2+2) for 4 hours. The Soxhlet extract was analyzed separately from the diethyl ether extract by first concentrating it in a rotary evaporator and then hydrolyzing it according to the method described above.

RESULTS AND DISCUSSION

Recovery tests were made by adding known amounts of 2,4-D and 2,4,5-T as free acids to control samples collected from unsprayed areas. The levels ranged from 2 µg to 200 µg. The average recovery was 75 % with a range of 60 % - 94 %. The limit of detection was 0.05 ppm for 2,4-D and 0.02 ppm for 2,4,5-T for a 20 g sample.
Acetonitril and a mixture of diethyl ether, acetone and ethanol (1+1+1) were tested for extraction efficiency. Anhydrous sodium sulfate was not used with these solvents before filtration. In both cases the amounts of residue were about 50 % lower when compared with those obtained with diethyl ether extraction.

Soxhlet extraction of the filter cake with diethyl ether-acetone-ethanol solvent mixture increased the total residue amount by 15 % for both 2,4-D and 2,4,5-T. If diethyl ether alone was used as solvent in the Soxhlet-apparatus, the increase was less than 10 %. Both acid and alkali hydrolysis of the filter cake were also tested. The results were, however, so varied that no conclusions could be reached. Direct hydrolysis of the sample was also tested. A low recovery of 10-20 % was obtained.

Both silica gel (10 g) and silicic acid (2 g) were tested for purifying the samples after methylation. The results were not satisfactory. The best chromatograms were obtained when the samples were purified with 1 g of acidic aluminum oxide before methylation.

Thus, the best results were obtained when the sample was homogenized and extracted with diethyl ether and the filter cake extracted in a Soxhlet-apparatus with a mixture of diethyl ether, acetone and ethanol (1+2+2). It was shown that the Soxhlet extraction reproducibly increased the residue amount by 15 %. It was concluded that this step could be omitted and the resulting loss accounted for in the final result.
In this way the time required for analysis could be halved making it possible for 12 samples a week to be analyzed by one technician.

In 1974-76 forests in the north-eastern part of Finland were submitted to aerial phenoxy herbicide spraying for brush control. Lingonberry and wild mushroom samples were collected after 2-13 weeks and foliage samples after 13-43 weeks of spraying. The samples were stored at -25°C for 1-6 months before analysis. 32 lingonberry, 26 mushroom and 37 foliage samples were analyzed.

A chromatogram of a lingonberry sample containing 0.2 ppm of 2,4-D and 0.05 ppm of 2,4,5-T is shown in Fig. 2. A chromatogram of a lingonberry control sample is also presented.

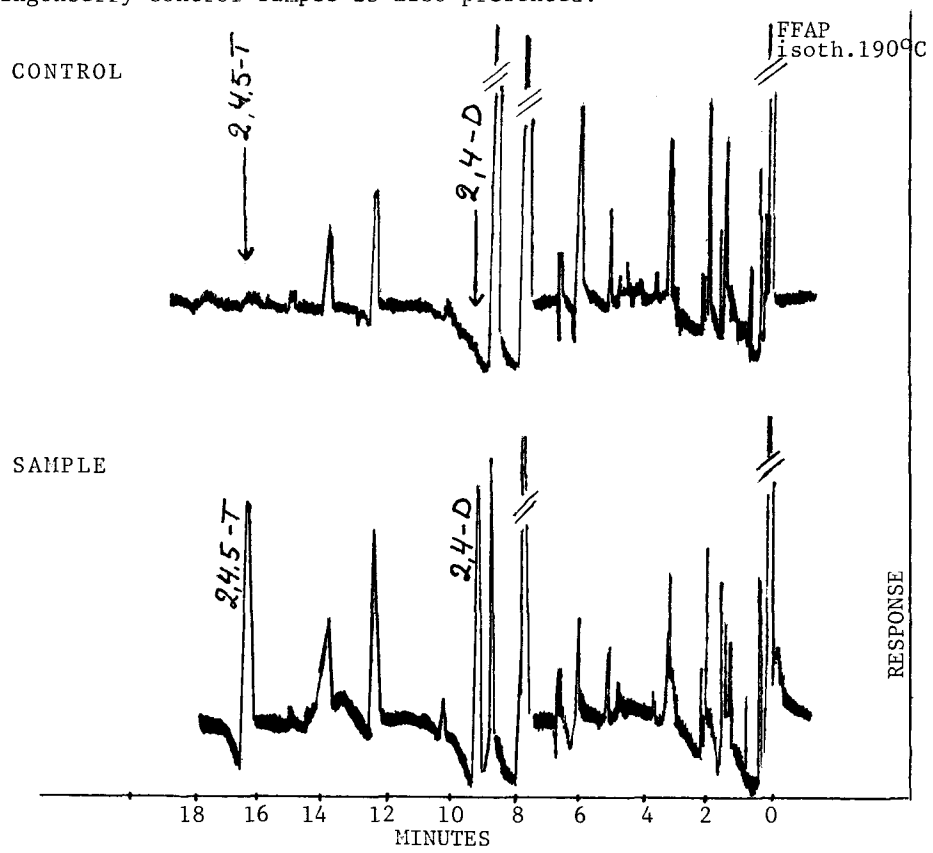

Fig. 2. Gas chromatograms of a lingonberry control sample and lingonberry sample containing 0.2 ppm of 2,4-D and 0.05 ppm of 2,4,5-T.

In lingonberries the residue of 2,4-D varied from < 0.05 to 7.0 ppm and that of 2,4,5-T from 0.07 to 15 ppm, and wild mushrooms from <0.05 to 1.2 ppm and <0.02 to 1.8 ppm respectively. In foliage the residue of 2,4-D varied from 0.15 to 31 ppm and that of 2,4,5-T from 0.1 to 30 ppm.

Some lingonberry samples collected after 2-3 weeks of spraying, were analyzed by omitting the hydrolysis step after the diethyl ether extraction. On average the results were 85 % less when compared with those obtained with hydrolyzed samples. This indicates that the ester formulations of the chlorophenoxy acids do not significantly hydrolyze to free acids during the first weeks after spraying. The results will be published in full elsewhere.

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