

A PRELIMINARY STUDY ON THE MICROCOLUMN
SAMPLE CLEANUP TECHNIQUE IN PESTICIDE
RESIDUE ANALYSIS

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INTRODUCTION

Chemical pesticide residues arising from forestry spray programs have been analyzed from many different types of substrates such as forest litter, soil, conifer foliage, stream and lake sediments and natural waters. Sample preparation and purification for instrumental analysis have often been a problem in terms of efficiency and cost.

In our laboratory (Szeto and Sundaram 1980) we often use the liquid-liquid partitioning technique to clean up the sample extract. This technique has received the general recognition in the analytical chemistry profession, due to its reliability in recovery and a relatively pure final product. The drawback to this technique is that it is rather tedious, especially when applied to more complex substrates. The cost of various organic solvents, and the amounts used are quite considerable. Our laboratory recently initiated a study to look for a more economical, simple method to substitute for the conventional liquid-liquid partitioning techniques. In this context, the method developed by Brown (1975) was examined and modified to suit our purpose.

This report presents the details of the development of a micro-column liquid chromatographic technique, along with the selection of adsorbent and its use in analysing the residues of fenitrothion and aminocarb (Matacil®) present in conifer foliage and fingerling trout.

MATERIALS AND METHODS

Both treated and untreated balsam fir foliage samples were obtained from New Brunswick during the 1981 Matacil® spray program (Sundaram 1981). An extract of fingerling rainbow trout (*Salmo gairdneri* Richardson) (Na_2SO_4) dried in ethyl acetate was obtained from the 1981 Ice Water Creek spray program (Sundaram 1981). Analytical standards of fenitrothion (99.99% pure) and aminocarb (99.3% pure) were supplied by Sumitomo Chemical Company and Mobay Chemical Corporation, respectively. Nuchar SN (Fisher Scientific) was acid washed according to the method of Brown (1975). Whatman CF-11 cellulose powder, Florisil (Fisher Scientific) and Silane-treated glass wool (Chromatographic Specialties) were used in the micro-column cleanup.

Preparation of Sample Extract

Five grams of shredded foliage samples were weighed in a 50 ml Tefzel Nalgene round centrifuge tube (Nalge-Sybron Corp.) and homogenized with 15 ml of ethyl acetate for 1 min in a Polytron homogenizer (Type PT20). The homogenate was centrifuged at 2000 x g for 5 min. The supernatant was carefully decanted into a 50 ml volumetric flask. The residue was re-extracted twice as described above. The combined supernatant was made up to 50 ml with ethyl acetate and filtered through ca. 10 g of Na_2SO_4 column to remove moisture.

Fortification of Sample Extract

Ten ml of foliage extract was fortified separately with either 2 µg of aminocarb or 0.385 µg of fenitrothion. Ten ml of blank rainbow trout extract which contained neither of the two insecticides was fortified with either 0.385 µg of fenitrothion or 2 µg of aminocarb.

Preparation of Microcolumn

A large 4 ml Pasteur pipet (Fisher Scientific, Cat. No. 13-678-8) was plugged with a small wad of silane-treated glass wool and a 3-cm layer of Florisil or Nuchar SN-Whatman CF11 cellulose powder (4:10, w/w) was added (Fig. 1). The packing was topped with a small plug of glass wool and pre-washed with ca. 2 ml of ethyl acetate. The single microcolumn containing the Nuchar SN-cellulose was used for foliage extract cleanup. The double column system consisted of a Florisil column first, followed by a Nuchar SN-cellulose column. These two columns were joined by a sleeve of Tygon tubing and were used for fish extract cleanup. A control column was prepared, similar to that used by Brown (1975), with a 5 cm layer of Nuchar SN-cellulose in a regular 2 ml Pasteur pipet.

Cleanup Procedure

1) Fenitrothion in foliage extract

An aliquot of 10 ml extract (1.0 g of foliage) in ethyl acetate was transferred to the pre-washed single microcolumn containing Nuchar SN-cellulose. A further 10 ml of ethyl acetate was added to strip the column. All elutants were collected in a round bottom flask and flash-evaporated to a small volume. The concentrate was transferred into a graduated centrifuge tube and made up to 2 ml with ethyl acetate for GC analysis.

2) Fenitrothion in rainbow trout extract

An aliquot of 10 ml extract (2 g of fish) in ethyl acetate was transferred to the double column system. The column stripping and subsequent sample treatment were similar as described above.

3) Aminocarb in foliage extract

The procedure was the same as described in (1) above, except that after loading the sample, the column was washed with 5 ml of ethyl acetate and all of the elutant was discarded. The column was stripped with 25 ml of either 20% or 10% methanol in dichloromethane.

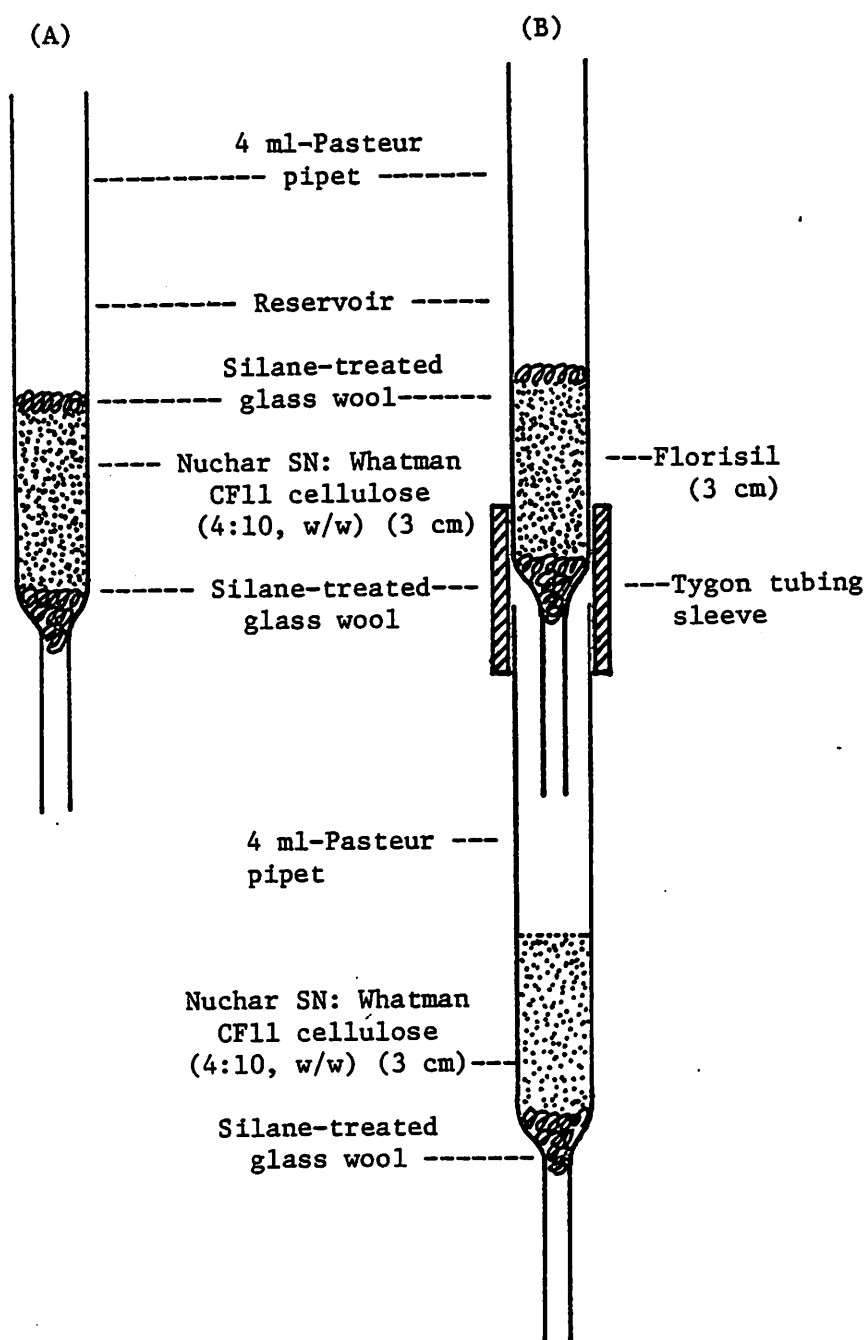


Figure 1. Microcolumns: (A) The Single Column System
(B) The Double Column System

4) Aminocarb in rainbow trout extract

The procedure was the same as described in (3) above, except that the Double Column System, consisting of a Florisil and a Nuchar SN-cellulose column, was employed.

Gas Chromatography

A Hewlett Packard 5710A GC/NPD was used for both fenitrothion and aminocarb residue analysis. The GC conditions were:

Column: 4 mm x 4 ft glass column, packed with 1.5% OV-17 + 1.95% OV-210 on chromosorb W, H.P., 80/100 mesh.

Oven Temp.:	180°C	H ₂ flow:	4 ml/min
Injector temp.:	200°C	Air flow:	70 ml/min
Detector temp.:	250°C	He flow:	35 ml/min

RESULTS AND DISCUSSION

Recoveries of fenitrothion, using the Micro-Column System from fortified foliage and fish extract, are shown in Table 1. In the control set, the recovery (%) ranged from 67 to 100. Only 4 out of the 13 samples were lower than 80% and they contributed largely to the low mean value in recovery and large variation. The % recovery would be improved to 89% (SD = 7), if these 4 samples were discarded.

Aminocarb residues in both untreated and treated foliage samples were analyzed and used as background. The range of aminocarb concentrations was from 0 to 2.5 ppm. These values did not contribute to any interference in the recovery studies. Total recoveries of aminocarb using the Single Column eluted with 20% methanol in dichloromethane are shown in Table 2. One batch of 4 samples exhibited low recoveries ranging from 57% to 67% and we are unable to offer any explanation at present. By discarding this batch, the % recovery would be improved to 93% (SD = 8). The control set consisting of 14 samples showed an excellent and consistent recovery.

In the case of Micro-Column eluted with 10% methanol in dichloromethane, both foliage and rainbow trout exhibited a high % recovery showing a rather large variation (Table 2). The control set had a lower recovery of 81.4%.

The small column (5 cm Nuchar SN-cellulose in 2 ml-Pasteur pipet) technique of Brown was used as comparison for aminocarb recovery. The average recoveries with this technique for the two samples, when using 10% methanol in dichloromethane as eluent, were 99% for fortified foliage and 89% for control samples. Recovery level was the same when methanol concentration was increased to 20%, but was reduced to 55% when methanol concentration was increased to 30%.

Fenitrothion was stripped completely by ethyl acetate from the column at 15 and 20 ml volume levels. Aminocarb was retained in the column when washed with ethyl acetate but was removed completely with 25 to 30 ml of methanol-dichloromethane solvent system (10/90 or 20/80). The methanol-dichloromethane elutant obtained from the single Nuchar SN-cellulose column was very clean, and the GC response was good without having any interference peak, showing that all the impurities in the foliar samples were completely removed by ethyl acetate wash. In the rainbow trout samples, there was a large interference peak found immediately following aminocarb peak (R.T. 3.5 min) when only the Nuchar column was used. This interfering compound was completely removed by adding a Florisil column on top of the Nuchar column.

Fenitrothion had a retention time of 5 min. It was well resolved from the contaminants co-eluted by ethyl acetate.

The drawbacks to Brown's small Pasteur pipet column technique have been that the flow rate (gravity flow) is extremely low (ca. 6 ml/hr) and, that the void volume above column packing, which served as solvent reservoir, is very small (ca. 0.5 ml). These problems were overcome by means of the modified large Pasteur pipet column described here.

CONCLUSION

The modified microcolumn technique provides a new tool for cleaning forestry substrates in pesticide residue analysis. The method is simple, easy to follow and economical. The amount of solvent and time saved per analysis is considerable when compared to the conventional methods. Consequently, the efficiency in terms of daily output is also increased by at least 3 fold. Although the results seem to have high variation, the recoveries of both aminocarb and fenitrothion from foliage and fish are all above 80%. There is a great potential in this new-technique especially when we are faced with a large number of samples to analyse following an operational spray program. Further studies are required to identify the causes of low recoveries in certain cases and to verify other factors, such as faster flow rate under pressure, shorter column packing (Gatz and Hill 1980), pH of sample extract and proper % of Nuchar SN in the packing mixture so as to improve the reliability and efficiency of the technique reported here.

Table 1. % Recovery of fenitrothion using ethyl acetate as eluent.

Sample	Replication	Mean % Recovery	S.D.
Foliage	3	93.7	2.9
Fish	3	105.0	11.3
Control*	13	84.4	9.3

*0.385 μ g of fenitrothion standard in 10 ml of ethyl acetate.

Table 2. % Recovery of Aminocarb using solvent systems (A) 20% or (B) 10% methanol in dichloromethane as eluent followed ethyl acetate wash.

Solvent system	Sample	Replication	Mean % Recovery	S.D.
(A)	Foliage	8	82.2	17.0
	Control*	14	89.9	5.6
(B)	Foliage	6	98.0	10.4
	Fish	7	88.3	19.2
	Control*	8	81.4	12.3

* 2 μ g of Aminocarb standard in 10 ml ethyl acetate

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