

STUDIES ON THE MICROCOLUMN SAMPLE CLEANUP
TECHNIQUES USED IN THE ANALYSIS OF
MEXACARBATE RESIDUES FROM CONIFER FOLIAGE

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ABSTRACT

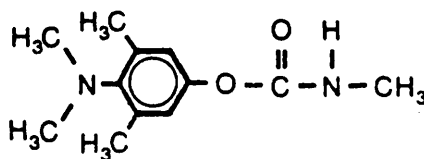
A new chromatographic column cleanup technique has been developed for the cleanup of foliar extracts containing mexacarbate residues. The technique involves the use of aluminum oxide "90 active" (neutral, activity grade I) as the adsorbent and ethyl acetate as the eluting solvent. Prior to the cleanup procedure, the foliar sample was extracted with either ethyl acetate or acetonitrile followed by liquid-liquid partitioning using acetonitrile and hexane to remove some of the co-extractive impurities. The percent recovery of the insecticide from fortified balsam fir (*Abies balsamea*) foliage samples were quantified.

INTRODUCTION

Mexacarbate (4-dimethylamino-3,5-xylyl N-methylcarbamate) (Fig. 1) was introduced by Union Carbide under the trade name Zectran® in 1961¹. The chemical was field tested for control of spruce budworm (*Choristoneura fumiferana*, Clemens) larvae in different eastern provinces of Canada during the 1972-73 spray seasons². Because of its desirable properties such as selectivity³, low mammalian toxicity (LD₅₀ 20 mg/kg)⁴, and low persistence in the environment^{5,3}, the chemical is being re-examined at present by the Forest Pest Management Institute (FPMI), Canadian Forestry Service, for large scale forestry use in Canada.

One of the primary requirements with such extensive operational use patterns, is to have sensitive and reliable residue methods to study the distribution, persistence and metabolic fate of the sprayed material found in various forestry substrates. The purpose of this report is to study and develop the necessary analytical techniques for the identification and quantification of mexacarbate in balsam fir foliage. The technique included (1) sample preparation, (2) foliar extraction, (3) column cleanup required to remove coextractive impurities and finally (4) chromatographic analysis of the mexacarbate residues.

Figure 1.



Mexacarbate: 4-dimethylamino-3,5-xylyl
(Zectran®) N-methylcarbamate
(C₁₂H₁₈N₂O₂)

MATERIALS AND METHODS

Standard Solution:

The carbamate insecticide used in this study is mexacarbate (Zectran®, 4-dimethylamino-3,5-xylyl N-methylcarbamate) (99.9%, Union Carbide).

Apparatus:

Homogenizer - Polytron PT-20 (Brinkman Instruments Canada Ltd.)

Rotary Evaporator - Buchler

Evaporation apparatus - Meyer N-evap® Model III (Organomation Associates Inc.)

Sample mixer - Thermolyne maxi-mix (Fisher Scientific)

Gas Chromatograph (GC) - Hewlett Packard Model HP5710A equipped with a nitrogen-phosphorous flame ionization detector (NP-FID).

Solvents:

Ethyl acetate, acetonitrile, hexane, dichloromethane and acetone are pesticide grade solvents obtained from Fisher Scientific and Caledon Laboratories Ltd.

Adsorbents:

Charcoal - Nuchar® SN (Fisher Scientific, Cat. No. C-177).

Cellulose - CF-11 (Whatman).

Florisil - 60-100 mesh (Fisher Scientific, Cat. No. F-100).

Aluminum Oxide - Activity Stage I (BDH Chemicals Canada Ltd.).
"90 Active"

Accessories:

Liquid Chromatographic Columns - 4 mL Pasteur pipet (Fisher Scientific, Cat. No. 13-678-8).

Filters - (i) Millipore Model XX1002500 or XX1007400 (Millipore Ltd.).

(ii) Mitex® (Teflon) membrane, 5.0 µm pore size (Millipore Ltd.).

(iii) Glass Fibre Filter (3.7 cm diam) (Gelmar Instruments Co.).

GC Column Packing - 1.5% OV-17 + 1.95% OV-210 on Chromosorb W HP, 80-100 mesh (Chromatographic Specialties Ltd.)

Sodium sulfate - anhydrous (Caledon Laboratory Ltd.).

Glass wool - silanized, (Chromatographic Specialties Ltd.).

Pipetman - 1000 µL (Gilson).

Microsyringe - 10 µL (Hamilton Co.).

Preparation of Foliage

Balsam fir foliage was obtained from the greenhouse at the Great Lakes Forest Research Centre, Sault Ste. Marie, Ontario. The needles from the previous year's growth period were removed from the branches with scissors and used in the study. The needles were thoroughly mixed and 50 g aliquots were fortified with 50 µg of mexacarbate giving a concentration of 1.0 ppm in the foliage.

Extraction

Two solvents (ethyl acetate and acetonitrile) were used separately to extract the spiked mexacarbate from foliage samples. The extraction procedure was as follows: aliquots of foliage (50 g) and anhydrous sodium sulfate (50 g) were successively homogenized for 5 min in presence of 100, 50 and 50 mL of either ethyl acetate or acetonitrile using a Polytron PT-20 set at moderate speed. The homogenates were filtered under gentle aspiration through a Millipore filtration unit containing in sequence 50 g of anhydrous sodium sulfate, three glass fibre filters, and one Teflon membrane. The unit was prewashed with 30 mL of extracting solvent. The residue in the filtration unit was rinsed with 30 mL of extracting solvent. All the extracts and the rinse were pooled, transferred quantitatively to a 500 mL round bottom flask and flash evaporated at 30°C to approximately 10 mL. The concentrate was quantitatively transferred to a 50 mL graduated tube and the final volume adjusted to 20 mL with the extracting solvent.

Liquid-Liquid Partitioning

The extract was dissolved in 100 mL acetonitrile and partitioned twice with 50 mL hexane to separate the mexacarbate residues from the plant lipids and terpene materials present in the foliage. The hexane layers were discarded. The acetonitrile layer was passed through a column containing 30 g of anhydrous sodium sulfate into a 500 mL round bottom flask followed by a 20 mL acetonitrile rinse. The extract was then flash evaporated gently to dryness at 30°C. The residue was taken in 10 mL of ethyl acetate and quantitatively transferred to a 50 mL graduated tube. The volume was adjusted to 50 mL with ethyl acetate, to give a foliar concentration of 1.0 g/mL. The stock solutions thus obtained were used in the microcolumn cleanup techniques discussed below.

Column Cleanup

Column Packings Various microcolumn loadings were examined using different eluting solvent systems.

The types of packings tested were (1) microcolumns with (a) 15%, (b) 20% and (c) 40% Nuchar® SN in cellulose⁶, (2) microcolumns with alternating layers of florisil and 20% Nuchar® SN in cellulose, (3) disposable Baker Octadecyl extraction columns and (4) microcolumns with (a) alkaline, (b) acidic, and (c) netural (activated and non-activated) aluminum oxide "90 active".

Column Procedure Four different systems of column cleanup were investigated during the study.

System 1

Aliquots of fortified foliar extract containing 1.0 µg of mexacarbate/1.0 g of foliage were transferred using a 1000 µL Pipetman to double microcolumn assemblies prewashed with 7 mL of ethyl acetate. Each assembly consisted of two microcolumns joined by a Tygon® sleeve. The top column contained 5.0 cm of Nuchar® SN (acid-washed)⁷ cellulose mixture topped with 1.0 cm of anhydrous sodium sulfate; the bottom column contained 5.0 cm of Florisil and 1.0 cm of anhydrous sodium sulfate (Fig. 2).

Each microcolumn assembly was eluted separately with the following solvent or solvent systems (a) 12 mL of ethyl acetate, (b) 20 mL of 20% methanol in ethyl acetate, (c) 25% methanol in ethyl acetate, (d) 30% methanol in ethyl acetate, and (e) 35% methanol in ethyl acetate. The eluates were collected in graduated centrifuge tubes and concentrated to 1.0 mL using a gentle stream of nitrogen on the N-evap® analytical evaporator for GC analysis.

System 2

A column assembly consisting of alternating layers (1.5 cm) of florisisil and 20% Nuchar® SN in cellulose with 1.0 cm of anhydrous sodium sulfate on top was tested (Fig. 3). The column was prewashed with 7 mL of ethyl acetate. The foliar extract (1.0 mL) was added to the column and then eluted with a 25 mL mixture of acetonitrile and acetone (1:1 v/v).

The eluate was collected in a graduated centrifuge tube and concentrated to 1.0 mL under a gentle flow of nitrogen for GC analysis.

FIG. 2

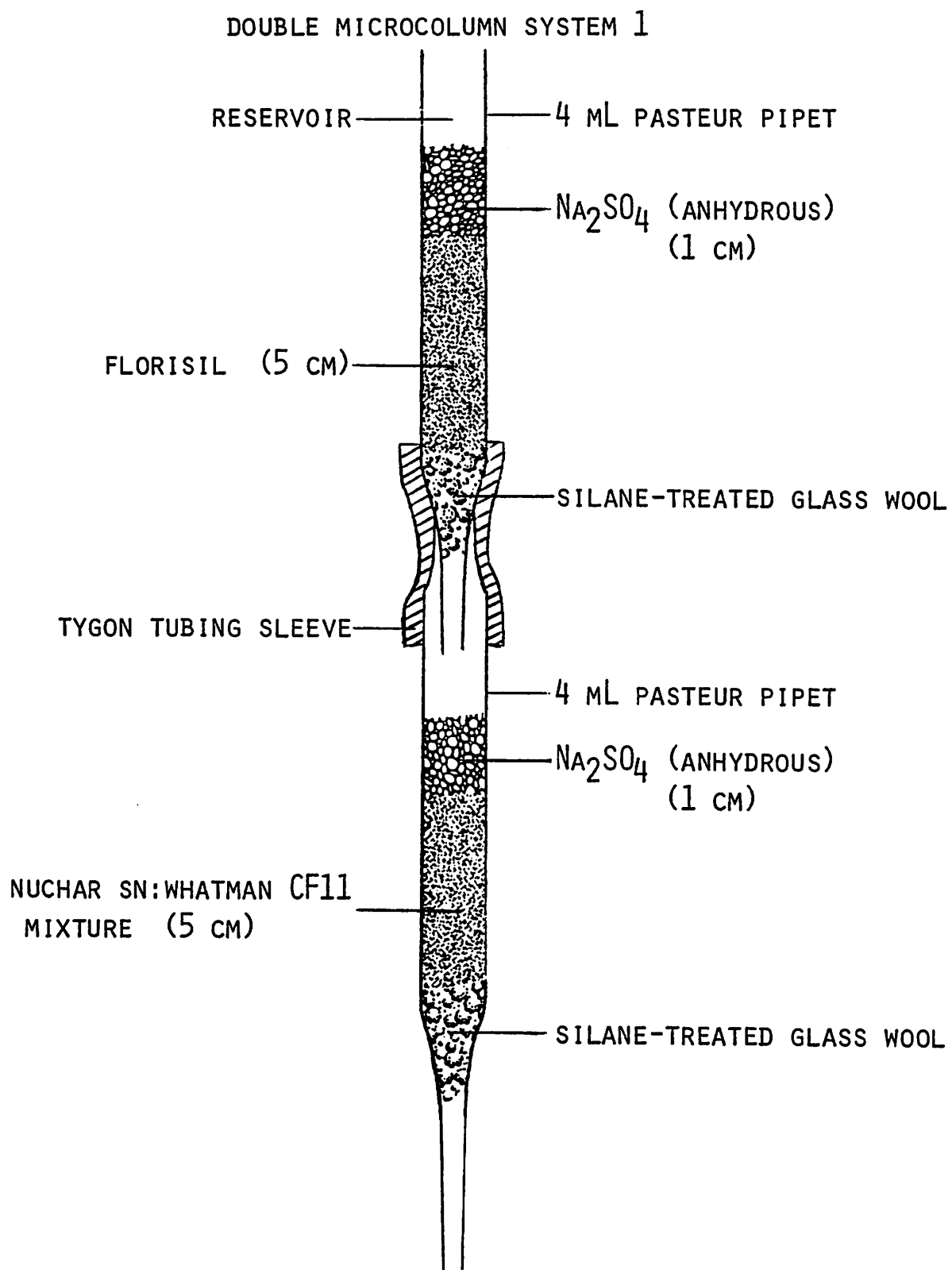
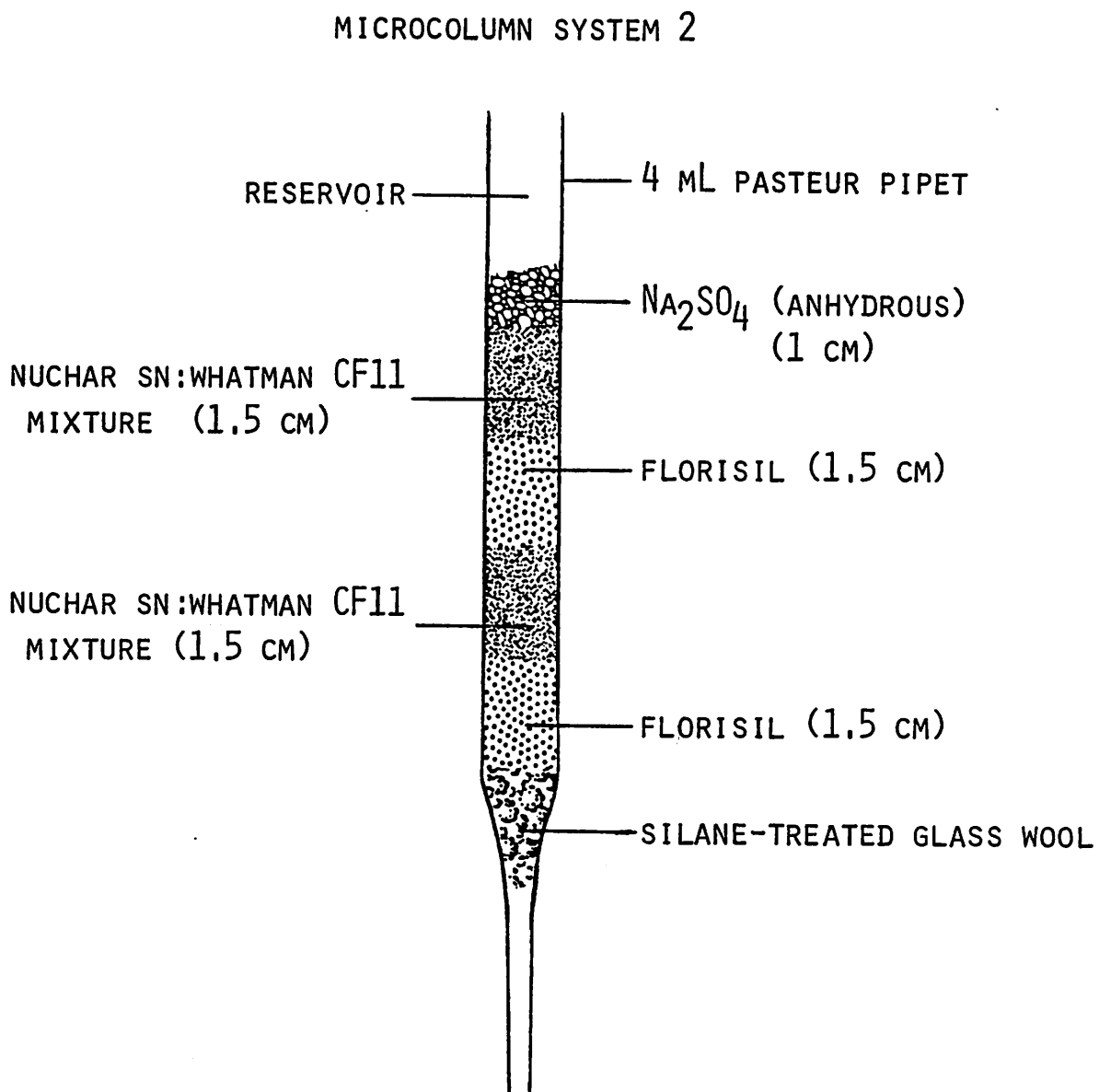


FIG. 3



System 3

A disposable non-polar Baker Octadecyl extraction column was tested for its efficiency in removing some of the coextractive impurities. It was washed first with 7 mL of ethyl acetate then the fortified foliar extract was transferred to the column by a 1000 μ L Pipetman. A few seconds following the addition of the sample, 20 mL of ethyl acetate was added to elute the column. The eluate was collected in a graduated centrifuge tube and concentrated to 1.0 mL for GC analysis.

System 4

The column packing used in this system contained separate quantities (5 cm length) of different types of aluminum oxide, namely (a) alkaline, (b) neutral (activated and non-activated), and (c) acidic, each topped with 1.0 cm of anhydrous sodium sulfate (Fig. 4).

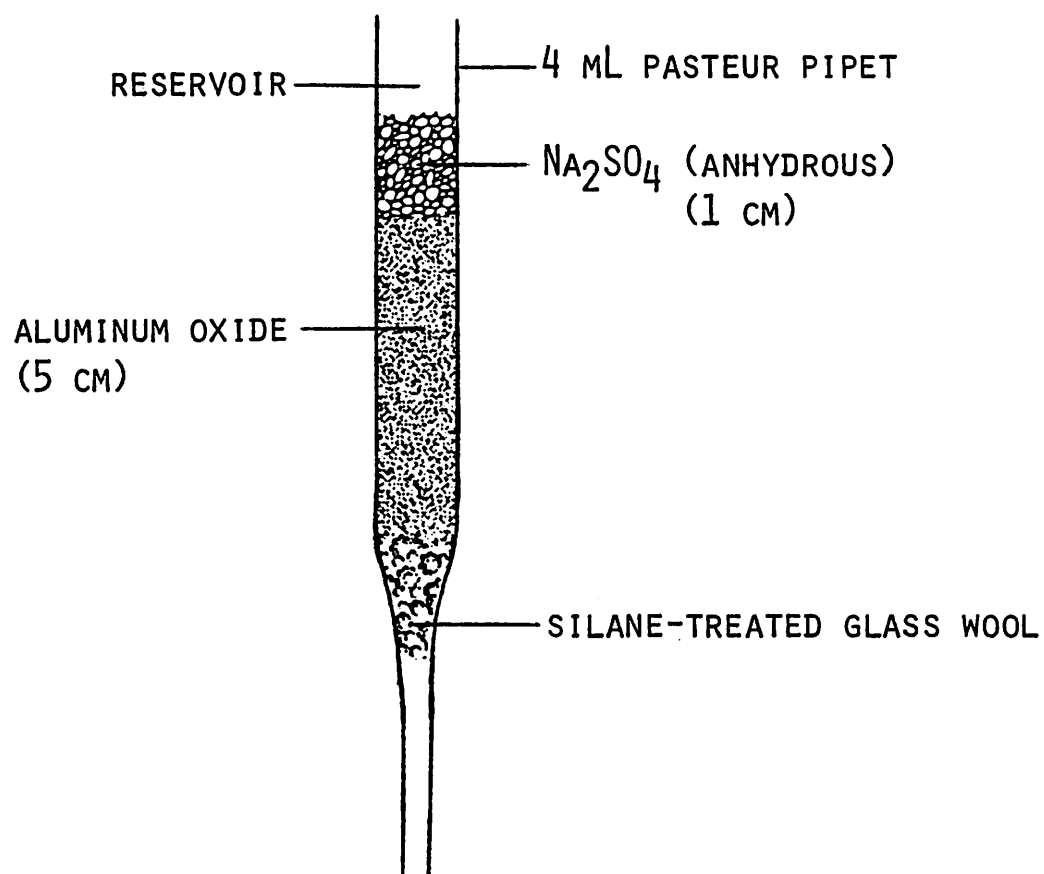
Each column was washed with 7 mL of ethyl acetate prior to the addition of the concentrated fortified foliar extract. The chemical was eluted with 10 mL of ethyl acetate. The eluate was collected in a 15 mL graduated centrifuge tube and concentrated to 1.0 mL for GC analysis.

Gas Chromatographic Determination

A Hewlett Packard 5710A GC/NPD was used for the mexacarbate residue analysis. The GC conditions were:

FIG. 4

MICROCOLUMN SYSTEM 4



Column: 2 mm ID x 1.8 m glass column, packed with 1.5% OV-17 + 1.95% OV-210 on Chromosorb W, HP, 80-100 mesh.

Oven Temp.: 185°C

H₂ Flow: 4 mL/min.

Injector Temp.: 250°C

Air Flow: 70 mL/min.

Detector Temp.: 300°C

He Flow: 40 mL/min.

Average recoveries of spiked mexacarbate are expressed in percentages with appropriate standard deviation. Results are given in Table 1.

RESULTS AND DISCUSSION

The various microcolumn cleanup techniques tested gave a very wide range of recovery levels for mexacarbate. Only five of the fifteen methods that were employed showed a recovery level > 80%. The five satisfactory results were obtained by using aluminum oxide as the column packing. The other microcolumns showed poor mexacarbate recovery levels ranging from 0% to 65%.

The most efficient microcolumn cleanup and recovery was obtained by using activated aluminum oxide (neutral) with ethyl acetate as the eluting solvent (100% recovery).

Comparison of ethyl acetate and acetonitrile as extracting solvents for mexacarbate from fir needles showed that ethyl acetate gave a cleaner sample with few coextractive impurities resulting in higher recoveries of mexacarbate. The use of acetonitrile as an extractant of mexacarbate from the forest foliage samples caused additional problems in the cleanup operations. Since it is more polar than ethyl acetate (μ 3.37 D vs. 1.81 D)⁸, an increased amount of pigments, waxes, terpenes

Table 1. Recovery of total mexacarbate from fortified foliar extracts (Original spiking level of 1.0 ppm).

System	Column Packing	Extracting Solvent System	Eluting Solvent System	Vol. (mL)	% Recovery	S.D. (±)
1 (i)	15% Nuchar® SN column assembly	ethyl acetate	ethyl acetate	12	43	-
		ethyl acetate	20% methanol in ethyl acetate	20	28	-
(ii)	20% Nuchar® SN column assembly	ethyl acetate	ethyl acetate	12	0 ⁺	0
		ethyl acetate	20% methanol in ethyl acetate	20	65 ⁺	5.1
		ethyl acetate	30% methanol in ethyl acetate	20	53	-
		ethyl acetate	35% methanol in ethyl acetate	20	0 ⁺	0
(iii)	40% Nuchar® SN column assembly	ethyl acetate	ethyl acetate	12	0	-
2	20% Nuchar® SN and florisil column assembly	ethyl acetate	acetonitrile and acetone (1:1)	25	0 ⁺	0
3	Baker Octadecyl extraction column	ethyl acetate	ethyl acetate	20	1	-
4 (i)	Aluminum Oxide (acidic)	ethyl acetate	ethyl acetate	10	3	-
(ii)	Aluminum Oxide (alkaline)	ethyl acetate	ethyl acetate	10	80*	4.0
(iii)	Aluminum Oxide (neutral)					
	(a) activated	ethyl acetate	ethyl acetate	10	100*	4.1
		acetonitrile	ethyl acetate	10	88*	4.7
	(b) non-activated	ethyl acetate	ethyl acetate	10	99*	5.1
		acetonitrile	ethyl acetate	10	94	-

*given value is the mean of triplicates.

⁺given value is the mean of duplicates.

and other polar plant constituents were coextracted causing problems in column cleanup.

SUMMARY AND CONCLUSIONS

Several microcolumn procedures were examined using various adsorbents and eluting solvent systems in the cleanup of mexacarbate from Balsam fir foliage extracts. A column cleanup technique utilizing aluminum oxide (neutral) as the adsorbent and ethyl acetate as the eluting solvent provided the highest mexacarbate recoveries which ranged from 95 to 106%.

A comparative study between ethyl acetate and acetonitrile in the extraction of mexacarbate from foliage illustrated that the ethyl acetate extraction procedure produced a much cleaner extract than that of acetonitrile. Also, the ethyl acetate method yielded better mexacarbate recoveries compared to acetonitrile which gave only about 89%.

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