Extraction, Cleanup, and Electron-Capture Gas Chromatographic Analysis of Fenac Residues in Soils

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Two methods for the extraction of fenac from soils are described. In both methods (Soxhlet and shake-flask), soil samples were adjusted to pH 2.0 with HCl then extracted with a mixture of benzene and methyl ethyl ketone. Prior to electron-capture gas chromatographic analysis, the crude extracts were cleaned up by partitioning fenac between CCl_4 and H_2O through pH adjustment. After cleanup, extracted fenac was esterified with BCl_3 and methanol. The shake-flask extraction method gave recoveries averaging 95% of the amount applied (0.5 to 8.0 p.p.m.) from six soils. The Soxhlet method gave poorer recoveries, particularly from fine-textured soils.

Fenac, an isomeric mixture of polychlorinated phenylacetic acids (marketed as sodium salts), is an effective herbicide for the control of several annual and perennial terrestrial and aquatic weeds. At present, USDA registration limits its use on food crops to that of a preemergence herbicide in sugar cane. This herbicide is known to persist in soils for extended periods under certain environmental and soil conditions (*1–3*). In some cases, however, it is less persistent; and at low rates loss or inactivation may occur within a few weeks or months. These conclusions are based on plant bioassays.

Extraction methods for fenac residues in sugar cane juice have been devised, and the amounts present determined by electron-capture gas chromatography (*5*), but little work has been done with residues in soils. To study the behavior of fenac in soils, a method of analysis for soil residues was needed.

Two extraction methods, cleanup of extracts, methylation of extracted fenac, and gas chromatography are described. The use of ^{14}C-labeled fenac facilitated the development of extraction and cleanup procedures. Labeled fenac was also used in determining methylation yields. The 2,3,6-trichloro-substituted isomer, which accounts for approximately 50% of the mixture of isomers in formulated fenac herbicide, is the most phytotoxic component. Therefore, attention was focused on this isomer during gas chromatographic analysis throughout the present investigations.

Materials and Apparatus

Apparatus. Soxhlet apparatus equipped with 125-ml. flasks. Variable-speed reciprocating shaker. Microsyringe (10-μl.). Gas chromatograph (Research Specialties Model 600) equipped with an electron-capture detector (10-mc. ^{90}Sr source) and a Westronics 5.5-mv. recorder.

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Reagents. The following reagent grade solvents were used: benzene, methyl ethyl ketone, carbon tetrachloride, and methanol. Hydrochloric acid (0.2*N*, 0.5*N*, and 2.0*N*), 1*N* NaOH, and saturated NaCl solutions were prepared as needed. Boron trichloride (C.P. grade) was obtained in a lecture bottle from the Matheson Co., Inc., East Rutherford, N. J. Carboxyl-^{14}C-labeled fenac (mixed isomers) (specific activity 4.9 μc. per mg.) and 2,3,6-trichlorophenylacetic acid (high purity) were obtained through Amchem Products, Inc., Ambler, Pa.

Soils. Properties and characteristics of soils used in this investigation are shown in Table I. The initial extraction and cleanup studies, utilizing ^{14}C-labeled fenac, were conducted with Wehadkee silt loam. Recovery of the purified, nonradioactive 2,3,6-isomer from each of the six soils was later determined by gas chromatography.

Methods and Results

Treatment of Soil Samples Prior to Extraction. Thirty-gram samples of air-dry Wehadkee silt loam that had previously passed through a 2-mm. screen were placed in 100-ml. beakers. To each soil sample was added 1 ml. of a methanolic solution containing 0.1 μc. of fenac-^{14}C and nonradioactive fenac to give a final concentration of 1 p.p.m. in the soil (a total of 30 μg. of fenac per 30 grams of soil). After the solution

Table I. Soil Properties

Soil Type	pH	Clay, %	Organic Matter, %	Cation Exchange Capacity, Meq./100 Grams
Lakeland sandy loam	6.2	10.5	3.3	2.9
Christiana loam	4.4	24.4	1.0	5.6
Hagerstown silty clay loam	7.4	32.4	2.3	8.8
Chester loam	4.9	23.9	2.9	5.2
Garland clay	7.7	40.6	1.1	23.2
Wehadkee silt loam	5.6	25.2	1.9	10.2

penetrated into the soil, the samples were mixed thoroughly with a glass rod. The soil samples were allowed to dry for approximately 2 hours, brought to field capacity with tap water, and stored for several days. Moisture loss was not restricted; therefore, the samples were almost air-dry by the time of extraction.

After preliminary extraction, cleanup, and methylation studies were completed, several 30-gram samples of all six soils were treated with purified 2,3,6-trichlorophenylacetic acid. These samples were used in further extraction studies involving gas chromatographic analysis. The soils, previously sifted through a 2-mm. screen, were air-dry at the time of adding the chemical. Appropriate samples received 1-ml. aliquots of methanolic solutions containing enough 2,3,6-trichlorophenylacetic acid to give 0, 0.5, 1.0, 2.0, and 8.0 p.p.m. in soil. Methanol was allowed to evaporate; and several hours after treatment, the samples were brought to field capacity with tap water. The soils were almost air-dry by the time of extraction.

Selection of Extraction Solvent. In preliminary experiments chloroform, methyl ethyl ketone (MEK), carbon tetrachloride, methylene chloride, benzene, methanol, trichloroethylene, and a 50–50 v./v. mixture of benzene and MEK were compared in Soxhlet extractors for efficiency in extracting ^{14}C-labeled fenac from acidified Wehadkee silt loam. The benzene-MEK system was superior to the other solvents and to benzene or MEK used alone. The benzene-MEK combination, therefore, was used throughout the remainder of this investigation.

Soxhlet Extraction. Thirty-gram samples of Wehadkee soil that had received ^{14}C-labeled fenac were placed in 33 × 65 mm. filter paper extraction thimbles. The upper 15 mm. of 33 × 80 mm. thimbles were removed to prevent loss of fenac by capillary movement into that portion of the thimble which extended above the siphon tube (the maximum solvent level in the Soxhlet extractor). It was observed that 6% or more of the fenac could migrate to the upper part of a long thimble during extraction. In the extraction thimbles samples were acidified to pH 2 by the addition of 10 ml. of 0.2*N* HCl. The concentration and volume of HCl necessary to lower the pH to 2 were determined for each soil type. Excess moisture is undesirable. Each sample was covered with a glass-wool plug and extracted for 2.5 to 3 hours with 100 ml. of the benzene-MEK solvent system.

After cooling, the extracts were made up to 100 ml. and 1-ml. aliquots were transferred to stainless steel planchets. The aliquots were allowed to dry slowly at room temperature, then ^{14}C was counted in a windowless gas flow proportional counter immediately after the planchets appeared dry. Recoveries of fenac from Wehadkee silt loam, based on counts of ^{14}C in the crude extracts, varied between 92.4 and 97.2% with an average of 94.7% for four observations. This method was later used to extract nonradioactive 2,3,6-trichlorophenylacetic acid from six different soil types.

Shake-Flask Extraction. The highly purified nonradioactive 2,3,6-isomer of fenac was used in the development of this method. Recovery of this isomer from six different soils was determined by gas chromatographic analysis. Thirty-gram soil samples were placed in 250-ml. Erlenmeyer flasks and enough HCl was added to each sample to lower the pH to 2 without excessively wetting the soil. Fifty milliliters of benzene-MEK (50–50 v./v.) were added to each flask, and the flasks were then placed on a mechanical shaker for about 2 hours (arbitrary time). The solvent was removed from each soil sample by carefully decanting it into a 125-ml. Erlenmeyer flask. Soil samples were extracted two more times with a total of 50 ml. of benzene-MEK (two 25-ml. extracts) by shaking for approximately 15 seconds each by hand. The three extracts from each sample were combined.

Cleanup Procedure. Prior to methylation and subsequent analysis in the gas chromatograph, the benzene-MEK extracts were carried through the following steps.

Each extract was made basic by the addition of 5 ml. of 1*N* NaOH and evaporated to dryness under an air stream.

Residue was dissolved in 5 ml. of 2*N* HCl and the solution transferred to a 125-ml. separatory funnel. The flask was rinsed three times with a total of 30 ml. of carbon tetrachloride and the rinses were added to the separatory funnel.

Funnel contents were shaken moderately and allowed to stand for at least 10 minutes. The bottom layer (organic phase) was drawn off into a second 125-ml. separatory funnel.

The extraction of the aqueous phase with 10 ml. of carbon tetrachloride was repeated once more and this final extract added to the second separatory funnel. The aqueous phase was discarded.

Forty milliliters of water and 10 ml. of 1*N* NaOH were added to the carbon tetrachloride in the separatory funnel. The mixture was shaken vigorously and allowed to stand until the bottom layer was clear. The bottom layer (organic phase) was discarded.

Ten milliliters of 2*N* HCl and 20 ml. of carbon tetrachloride were added to the aqueous phase remaining in the separatory funnel. The contents were shaken moderately and the lower layer (organic phase) was drawn off into a beaker after sufficient time for clearing was allowed. Adequate clearing is essential. The extraction of the aqueous phase was repeated twice more with 10-ml. portions of carbon tetrachloride, and the extracts were combined.

Five milliliters of 1*N* NaOH were added to the extract and the contents evaporated to dryness under an air stream.

Carbon-14–labeled fenac was used throughout the development of this cleanup procedure. All phases that were to be discarded during the cleanup were dried and monitored for ^{14}C. No radioactivity was detected in discarded phases, a fact which indicated that, within limits of ^{14}C detection, all of the fenac was recovered during cleanup of the crude benzene-MEK extract. During the development of this cleanup procedure, carbon tetrachloride and diethyl ether were compared for efficiency as cleanup solvents. When carbon tetrachloride was used, recoveries of ^{14}C-labeled fenac (previously extracted from Wehadkee silt

loam with benzene-MEK) ranged from 98 to 104% of the amount in the crude extract. Lower recoveries, ranging from 79 to 84%, were obtained with ether. Ether also extracted considerably more undesirable colored material.

Methylation. After the final solution in the cleanup procedure was evaporated to dryness, the residue was dissolved in 5 ml. of 2*N* HCl, and the solution was quantitatively transferred to a 25-ml. Erlenmeyer flask containing 10 ml. of methanol. Boron trichloride was bubbled into the solution at such a rate that reflux began after about 2 minutes. Boron trichloride injection was stopped 5 minutes after initiation, and the flask was heated in a water bath at 80° C. for 5 minutes. After injection of BCl_3 into the flask at the same rate as before for 1 minute, the flask was again heated for 5 minutes at the same temperature. The 1-minute injection of BCl_3 and the 5-minute heating were repeated once more. This methylation procedure was suggested by Segal (*4*).

The methylated fenac solution was transferred to a 125-ml. separatory funnel with two 10-ml. rinses of benzene. Approximately 2 ml. of saturated sodium chloride solution were added (to break the emulsion and cause phase separation) and the funnel contents were shaken. The lower (aqueous plus methanol) phase was drained back into the 25-ml. methylation flask and the benzene drained into a 50-ml. volumetric flask. Extraction of the aqueous methanol phase was repeated once more without the NaCl addition. The benzene extracts were combined and made to volume in the 50-ml. flask. Five-microliter aliquots were used routinely for injection into the gas chromatograph.

To determine the methylation yield, three ^{14}C-labeled fenac samples were subjected to the above methylation procedure and the final benzene solutions concentrated to about 3 ml. by using a Kuderna-Danish concentrator. Approximately 100 μl. of the concentrated solution were applied to $1^1/_2 \times 20$ inch Whatman No. 1 paper strips and chromatographed ascendingly in butanol–ammonium hydroxide–methanol (2:1:1 by volume) overnight. The chromatograms were then scanned in a 4-pi strip scanner. Average R_f values for three replications were 0.66 and 0.82 for the acid and ester forms, respectively. Radioactive spots on the chromatograms were counted by using a Savant Model HS-2 hand flow window counter in conjunction with the scaler-timer of a proportional counter. Methylation yield, based on the amount of radioactive fenac in the ester form compared to the total activity in both ester and acid forms, ranged from 67.2 to 70.1% with an average of 69.0% for three replications.

In subsequent studies of methylation procedures it was found that elimination of water from the reaction mixture improved the yield of methylation. Woolson and Harris (*6*) obtained yields approaching 100% when fenac (essentially anhydrous methanolic solutions) was heated at 70° C. for approximately 1 hour in the presence of BF_3-methanol (10 to 14% w./v.) reagent.

Gas Chromatography. A 4-foot borosilicate glass column ($^1/_8$-inch i.d.) packed with 1.3% Versamid 900 on Diatoport S, 60 to 80 mesh, was used in the quantitative analysis of nonradioactive fenac (2,3,6-isomer). The flow rate of the nitrogen carrier gas was 120 cc. per minute, as measured at the detector exhaust port. Column temperature was isothermal at 155° C., while the injection block and detector were maintained at 220° C. The potential across the electron-capture detector was maintained at 3 volts. The retention time for the 2,3,6-isomer of fenac (methyl ester) was 3.3 minutes.

Known amounts of purified 2,3,6-trichlorophenylacetic acid were esterified, diluted, and used as standards in gas chromatographic determinations. Peak heights from the recording charts were used to establish standard curves and, by comparison, to determine the concentration of fenac in unknown samples.

Figure 1 shows a GLC trace obtained from commercial fenac herbicide after methylation. The predominant peak (2,3,6-isomer) is shown accompanied by lesser peaks representing other polychlorinated components. Also shown in this figure are GLC traces obtained from untreated and commercial fenac-treated Wehadkee soil. The initial extracts were obtained by the shake-flask method. The treated soil represented by trace *C* in Figure 1 was obtained from a field plot to which fenac had been applied several months before extraction. The relative amounts of isomers found in the field-plot soil, when compared to original material applied, were similar; some differences can be noted. however. Peaks from extraneous compounds in the 2,3,6-isomer region did not pose an interference problem.

Several 30-gram samples of each soil shown in Table

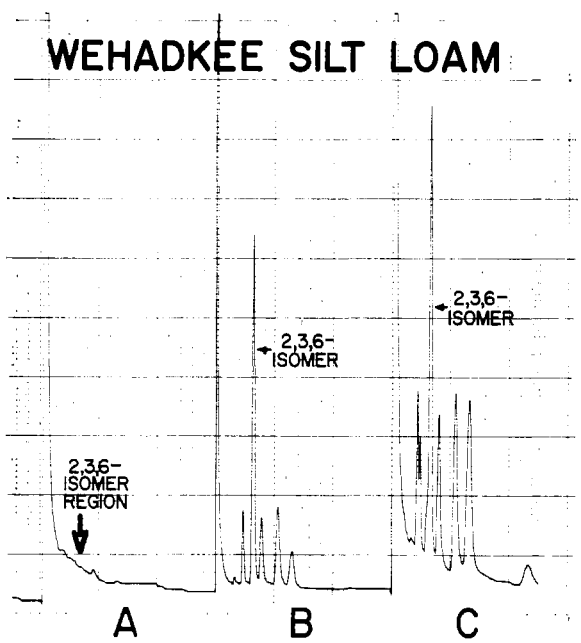

Figure 1. GLC recorder traces of methylated extracts from Wehadkee silt loam

A. Untreated
B,C. Commercial fenac-treated
B obtained from soil treated in laboratory and extracted within a few days. *C* obtained from a field sample treated several months before extraction. Initial amounts applied differed

I were treated with 0.0, 0.05, or 2.0 p.p.m. of 2,3,6-trichlorophenylacetic acid and extracted by the shake-flask method. After the extracts were carried through the cleanup and methylation procedures, 5-μl. samples were injected into the gas chromatograph. Figure 2 shows typical GLC traces obtained from Lakeland sandy loam, Wehadkee silt loam, Hagerstown silty clay loam, and Garland clay. Peak heights obtained from 5-μl. injections of methylated extracts from 0.05 p.p.m.-treated soils represent approximately 2% of full-scale deflection of the recorder. This concentration, therefore, approaches the lower limit of detection by these methods and volumes.

Recovery of Fenac (2,3,6-Isomer) from Six Soils. Thirty-gram samples of the six soils listed in Table I were treated with fenac (2,3,6-isomer) in concentrations ranging from 0 to 8.0 p.p.m. (see Treatment of Soil Samples Prior to Extraction). The Soxhlet and shake-flask methods were then compared for efficiency in extracting this isomer. Crude extracts of the six soils were subjected to cleanup and methylation and injected into the gas chromatograph for quantitative analysis. Table II shows the recoveries obtained.

Table II. Per Cent Recovery of Fenac (2,3,6-Isomer) from Six Soils[a]

	Amount Originally Applied, P.P.M.						
	Soxhlet Extraction Method			Shake-Flask Extraction Method			
	0.5	1.0	2.0	0.5	1.0	2.0	8.0
Lakeland	78	...	84	93	90	84	98
Christiana	85	80	68	107	94	105	92
Hagerstown	85	...	77	92	84	93	83
Chester	74	82	63	100	96	103	107
Garland	19	...	29	88	98	104	90
Wehadkee	77	...	82	93	...	94	...

[a] Recoveries based on comparison of peak heights with those obtained from methylated fenac (2,3,6-isomer) standards, using gas-liquid chromatographic analysis.

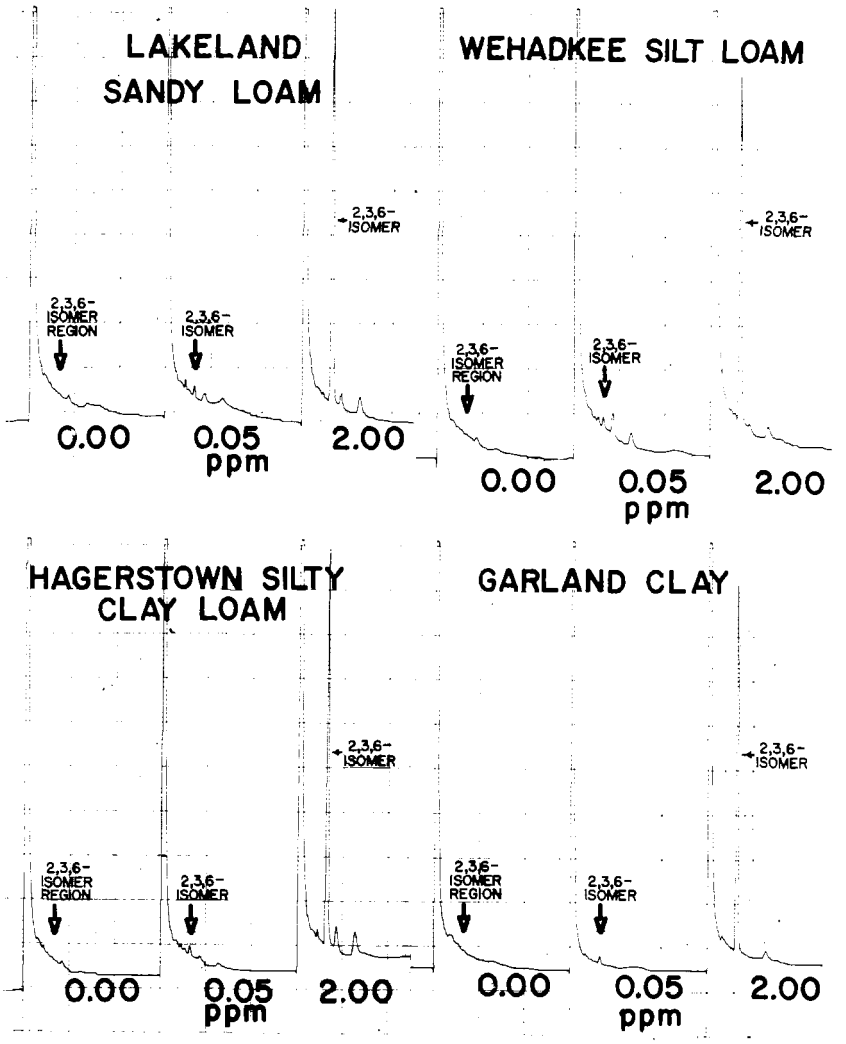

Figure 2. Typical GLC recorder traces of fenac (purified 2,3,6-isomer) after extraction, cleanup, and methylation

P.p.m. concentrations refer to amounts originally applied to soil samples prior to extraction

Greater recoveries were obtained from all soils by the shake-flask method than by the Soxhlet method. If the extremely low recovery average of 24% for Garland clay is disregarded, the average recovery from the other five soils was 78% in the 0.5- to 2.0-p.p.m. range with the Soxhlet extraction method. Through the same concentration range, the shake-flask method resulted in an average recovery of 95% from all six soils. Recoveries with the shake-flask method averaged the same (95%) throughout the range of 0.5 to 8.0 p.p.m.

Discussion

The use of benzene-MEK as an extraction solvent evolved from preliminary extraction work with the Soxhlet apparatus. The 50–50 v./v. mixture of these two solvents was tried because both were among the more efficient solvents that were tested individually, and the boiling points were sufficiently close to minimize differential rates of distillation in the Soxhlet apparatus. The boiling points of benzene and MEK are 80.0° and 79.6° C., respectively.

Although good recoveries were obtained from most soils with the Soxhlet method, higher recoveries were obtained with the shake-flask method. With the Soxhlet method, lower recoveries from all soils, and especially the very low recovery from Garland clay, were probably due to the addition of HCl which affected dispersion of the soil and restricted percolation of solvent through the sample. Acidification of the soil, however, is desirable since it enhances the solubility of fenac in the organic solvents and hence improves extraction.

The effect of extraction time on recovery has not been adequately studied. The 2- to 3-hour extraction period, which gave good recovery of fenac for both the Soxhlet and shake-flask methods, was arbitrarily chosen. It probably could be reduced without adversely affecting the recovery of fenac. Since our fenac reference standards as well as our "unknowns" were esterified in the same manner by the same worker, the methylation yield, although incomplete, was reliable by virtue of the reproducibility obtained. Further work (*6*) has shown that fenac methylation yield can be greatly increased with a concentrated BF_3-methanol procedure under anhydrous conditions. The latter procedure offers the advantages of greater sensitivity and probably less chance of yield variation among workers.

Acknowledgment

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