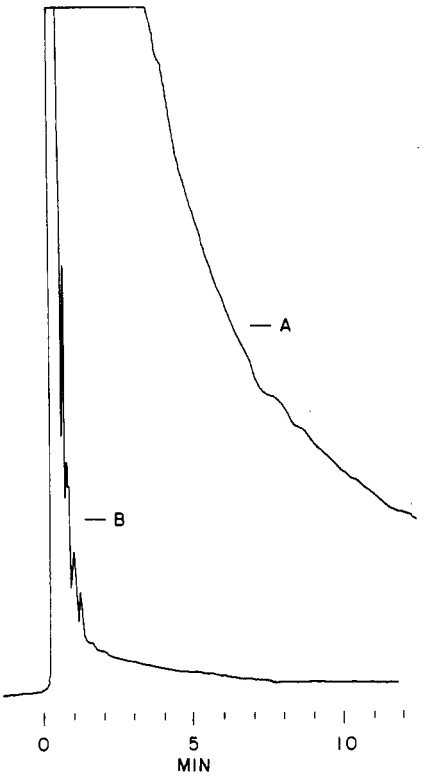


**Figure 1.** Chromatograms of ion-pair alkylation and diazomethane reagent blanks using GC/ECD: A, diazomethane reagent blank; B, ion-pair alkylation reagent blank.

without further cleanup.

## CONCLUSION

These experiments have shown that the ion-pair alkylation procedure is equivalent to the diazomethane procedure for giving high yields and good reproducibility for methylating CPA's and PCP in food residues. The alkylation procedure is better than the diazomethane procedure, because it gives cleaner reagent blanks and involves the handling of less toxic more stable reagents. The ion-pair alkylation procedure can be used as an alternate methylation procedure to the diazomethane procedure. Further methylation studies on other compounds using this alkylation procedure are in progress and will be reported in the future.

## ACKNOWLEDGMENT

I thank J. Kevin Cline for his assistance in the intralaboratory evaluation.

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Received for review March 28, 1986. Accepted October 9, 1986. Reference to any commercial material, equipment, or process does not, in any way, constitute endorsement or recommendation by the Food and Drug Administration.

# Gas Chromatographic Analysis of Fluazifop-butyl (Fusilade) in Potatoes, Soybeans, and Soil

B. Stephen Clegg

Fluazifop-butyl, (+)-butyl 2-[4-[[5-(trifluoromethyl)-2-pyridyl]oxy]phenoxy]propionate, is the active ingredient in Fusilade, a new highly selective systemic postemergence grass herbicide introduced by ICI for use in broadleaf crops. A specific gas chromatographic procedure is described for the determination of residues of fluazifop-butyl in potatoes, soybeans, and soil samples. Following an initial alkaline hydrolysis, residues are extracted with dichloromethane, methylated with diazomethane, subjected to Florisil column cleanup, and determined by capillary gas–liquid chromatography with a nitrogen-specific detector. The method is suitable for the determination of fluazifop, fluazifop-butyl, and its conjugates in various crops and soil samples. Recoveries of fluazifop-butyl, determined as its methyl ester derivative, were greater than 70% following fortification levels of 0.05–1.0 $\mu g\ g^{-1}$. Residues in potatoes and soybeans treated with Fusilade at recommended rates were below 0.05 $\mu g\ g^{-1}$ when harvested 90 days after herbicide application.

Fluazifop-butyl, (+)-butyl 2-[4-[[5-(trifluoromethyl)-2-pyridyl]oxy]phenoxy]propionate, is the active ingredient of the herbicide Fusilade, and the structure is presented in Figure 1.

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Fusilade is a highly selective systemic postemergence grass herbicide for use in broadleaf crops (Bates et al., 1982; Wagner, 1983). Currently, fluazifop-butyl under the trade name Fusilade is temporarily registered in Canada for use on field crops such as flax, sugar beets, and sunflowers. Legume forage crops include alfalfa, red clover, and birdsfoot trefoil, while potato is the only vegetable listed (OMAF, 1985).

Fluazifop-butyl, a (pyridyloxy)phenoxy compound is related structurally to a new series of herbicides often

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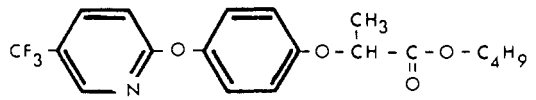
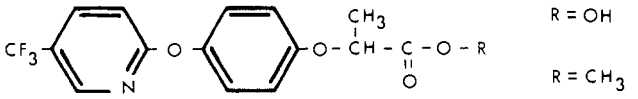


**FUSILADE**

**Fluazifop–butyl**



**Fluazifop**

**Fluazifop–methyl**

**Figure 1.** Structures of Fusilade: fluazifop-butyl ester, fluazifop, and fluazifop-methyl ester.

designated as phenoxyphenoxys such as diclofop-methyl and haloxyfop-methyl. A direct method of analysis of these compounds by HPLC has been described (Atreya et al., 1981; Stringham and Bennett, 1983), and although a gas chromatographic method has been published for diclofop-methyl (Johnstone et al., 1985), none has been reported for fluazifop-butyl. The present paper describes a sensitive gas chromatographic method suitable for the determination of fluazifop, fluazifop-butyl, and potential conjugates in potatoes, soybeans, and soil.

It has been shown that fluazifop-butyl, when applied under field conditions, degrades rapidly to the free acid (fluazifop), which is conjugated in the plant (Atreya et al., 1981). Following an alkaline hydrolysis, residues from fluazifop-butyl are released and methylated with diazomethane, and the fluazifop-methyl ester is determined by capillary gas–liquid chromatography with a nitrogen-specific detector. The use of a nitrogen- specific detector eliminates interferences often observed when electron-capture detectors are used in residue analysis.

Further assessments of the applicability of this method for determination of residues of other new herbicides such as flamprop-methyl, fenoxaprop-ethyl, fenthiaprop-ethyl, sulfometuron, and haloxyfop-methyl are being completed.

## EXPERIMENTAL SECTION

**Chemicals.** All solvents used were pesticide grade (Caledon Laboratories Ltd., Georgetown, Ontario, Canada). Florisil (Floridin Co.), 60–100 mesh, was heated 220 °C for 24 h prior to use. Diazomethane was generated from 1-methyl-3-nitro-1-nitrosoguanidine (Aldrich Chemical Co., Milwaukee, WI) by safe laboratory practices.

**Sample Extraction.** A representative homogenized sample (100 g of potatoes, 30 g of soybean, 50 g of soil) was added to a 500-mL centrifuge bottle with 200 mL of 0.1 N NaOH. The bottle was placed in a 60 °C water bath and shaken for 60 min and, after cooling, was centrifuged for 5 min at 1500 rpm. The alkaline extract was decanted into a layer of Hyflo Supercel in a Buchner funnel and filtered under vacuum. An additional 100 mL of 0.1 N NaOH was added to the centrifuge bottle and the process repeated (30 min in water bath). After being rinsed with 20 mL of water, the entire alkaline extract was transferred to a 500-mL separatory funnel and extracted with 50 mL of hexane, which was discarded. The aqueous alkaline fraction was then acidified with ca. 2.5 mL of $H_2SO_4$ to pH <2, and 100 mL of saturated NaCl was added. The phenoxyalkanoic acids were extracted twice with 50 mL of dichloromethane by shaking for 5 min. The dichloromethane extracts were drained through dichloromethane prewashed and dried cotton into a 250-mL receiver. Isooctane (0.5 mL) was added, and the samples were concentrated on a rotary evaporator (50 °C) to insipient dryness.

**Table I. Major Fragmentation Ions and Percent Intensity for Esters of Fluazifop**

| compound | $M^+$ | $m/z$ (% intensity) |
|---|---|---|
| fluazifop-methyl ester | 341 | 342 (12), 341 (71), 283 (14), 282 (100), 255 (31), 254 (90), 238 (22), 227 (44), 226 (30), 146 (71) |
| fluazifop-butyl ester | 383 | 383 (28), 283 (15), 282 (100), 255 (27), 254 (51), 238 (18), 227 (27), 226 (9), 146 (34), 126 (11), 91 (21) |

**Table II. Recovery Data of Fusilade at Various Fortification Levels for Substrates**

| substrate | fortif level, ppm | sample wt, g | no. of replicates | % rec (±SD) |
|---|---|---|---|---|
| potato | 0.05 | 50 | 6 | 72 ± 12 |
| | 0.10 | 50 | 6 | 69 ± 7.6 |
| | 0.20 | 25 | 4 | 84 ± 2.9 |
| | | 50 | 3 | 70 ± 10 |
| | 1.0 | 50 | 5 | 71 ± 0.04 |
| total | | | 24 | 72 ± 12 |
| soybean | 0.05 | 50 | 6 | 73 ± 8.8 |
| | 0.10 | 50 | 3 | 78 ± 6.1 |
| | 1.0 | 50 | 3 | 74 ± 3.5 |
| total | | | 12 | 74 ± 7.0 |
| soybean plant | 0.05 | 50 | 4 | 74 ± 7.4 |
| | 0.10 | 50 | 3 | 71 ± 6.0 |
| | 1.0 | 50 | 3 | 72 ± 4.0 |
| total | | | 10 | 72 ± 5.5 |
| soil | 0.05 | 50 | 4 | 77 ± 6.8 |
| | 0.10 | 50 | 4 | 83 ± 5.1 |
| | 1.0 | 50 | 4 | 82 ± 8.7 |
| total | | | 12 | 81 ± 7.0 |

**Esterification.** Diazomethane, prepared from 1-methyl-3-nitro-1-nitrosoguanidine, was used to prepare the methyl ester of fluazifop. Caution must be taken when preparing diazomethane; i.e., the preparation should be carried out in a fume hood with adequate protection to prevent contact of nitrosoguanidine or the diazoalkane solution with skin, as skin rashes may develop. The diazoalkanes are toxic and potentially explosive. Etched or scratched glassware and strong light should be avoided (Stanley, 1966). Major fragmentation ions and percent intensity for esters are given in Table I.

**Florisil Column Cleanup.** Activated Florisil (15 g) was added to a 19-mm i.d. × 300-mm length glass column, and a layer (2.5 cm) of anhydrous sodium sulfate was placed on top of the Florisil. The column was then prewashed with 50 mL of dichloromethane followed by a 200-mL wash of petroleum ether (to remove all traces of dichloromethane), and each was discarded. The sample residue was dissolved in 5.0 mL of dichloromethane/petroleum ether (1:4) and the resultant mixture transferred quantitatively to the column. The transfer process was repeated with an additional 5.0 mL of 20% dichloromethane in hexane, and the column was then eluted with an additional 100 mL of eluate, which was discarded. Final elution was with 200 mL of 5% diethyl ether in methylene chloride, and this fraction was concentrated on a rotary evaporator (50 °C) to insipient dryness. The solvent was exchanged with an additional 10.0 mL of isooctane, and the final extract was made up to 5.0 mL with isooctane prior to GC analysis.

**Fortification.** Recoveries were determined by fortifying plant tissue and soil with 0.05, 0.10, and 1.0 $\mu g\ g^{-1}$ with

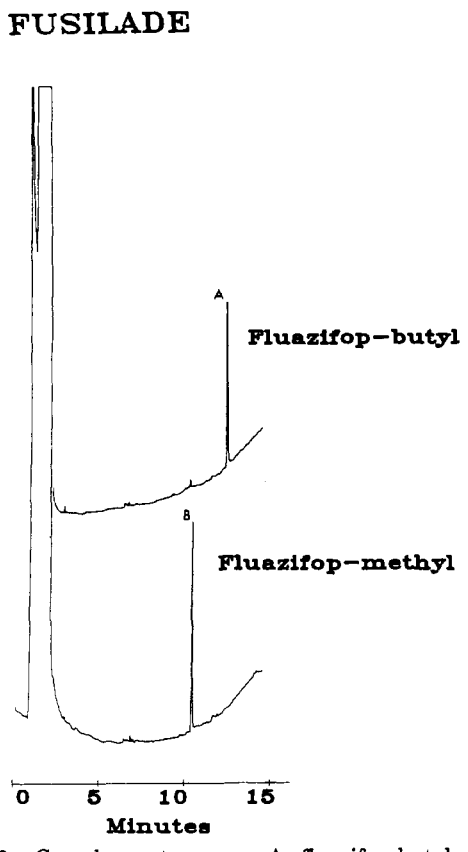



**Figure 2.** Gas chromatograms: A, fluazifop-butyl ester; B, fluazifop-methyl ester.

fluazifop-butyl and were greater than 70% in all cases. Recovery data for various substrates are given in Table II. Quantitation was accomplished by use of fluazifop-methyl, prepared by the hydrolysis and methylation of fluazifop-butyl (100 $\mu$g mL$^{-1}$) and serially diluted.

**Gas Chromatography.** A Perkin-Elmer Model 8320B capillary gas chromatograph, equipped with a nitrogen–phosphorus detector, and a Perkin-Elmer GP 100 plotting integrator were used. The column used was a J&W fused silica capillary column, 30 m × 0.25 mm, with a 0.25-$\mu$m coating of SE54 (Chromatographic Specialties Ltd., Brockville, Ontario, Canada). Chromatographic conditions were as follows. Temperatures: injector, 225 °C; detector, 300 °C; column oven, initial temperature of 90 °C programmed at 20 °C min$^{-1}$ to 150 °C and then 10 °C min$^{-1}$ to 250 °C with a hold time of 7 min to allow elution of late compounds. Gas flows: helium carrier head pressure of 70 kPa, 2 mL min$^{-1}$; air, 20 mL min$^{-1}$; hydrogen, 8 mL min$^{-1}$. Injections were 2 $\mu$L with capillary inlet system configured in splitless mode with vent valve open for 0.5–1 min. Under the described conditions, retention times of 12.3 and 14.6 min were obtained for fluazifop-methyl and fluazifop-butyl, respectively.

**Gas Chromatography–Mass Spectrometry.** A Hewlett-Packard 5790 capillary gas chromatograph interfaced with a Hewlett-Packard 5970A mass-selective detector (MSD) was used for mass spectrometric determinations of the butyl and methyl esters of fluazifop. The chromatographic column was similar to that in the Perkin-Elmer gas chromatograph. The capillary column was connected to the MSD with a direct capillary interface operated at 260 °C with a carrier gas flow of 2 mL min$^{-1}$. The following oven temperature profile was used: initial temperature of 90 °C programmed for 1-min hold, then 10 °C min$^{-1}$ ramp to 270 °C, and a final hold at this temperature to allow elution of late compounds.

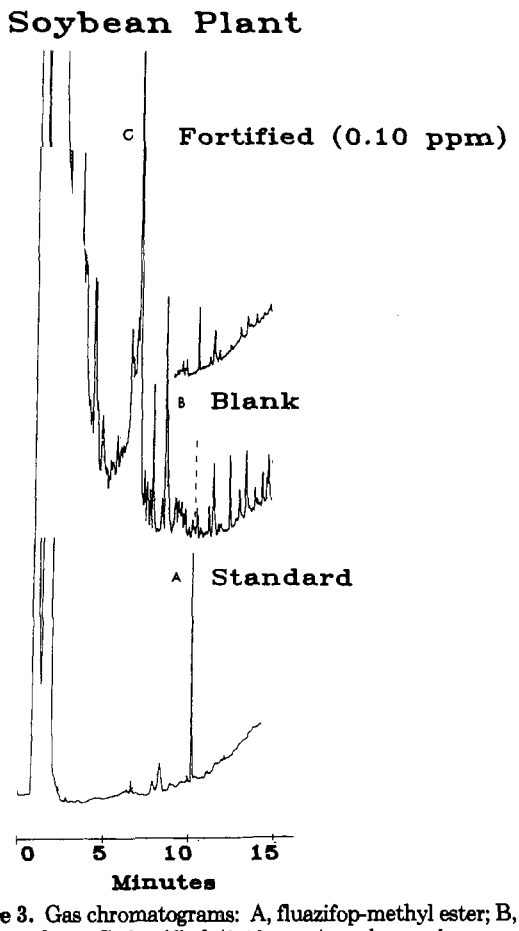



**Figure 3.** Gas chromatograms: A, fluazifop-methyl ester; B, blank soybean plant; C, fortified (0.10 ppm) soybean plant.

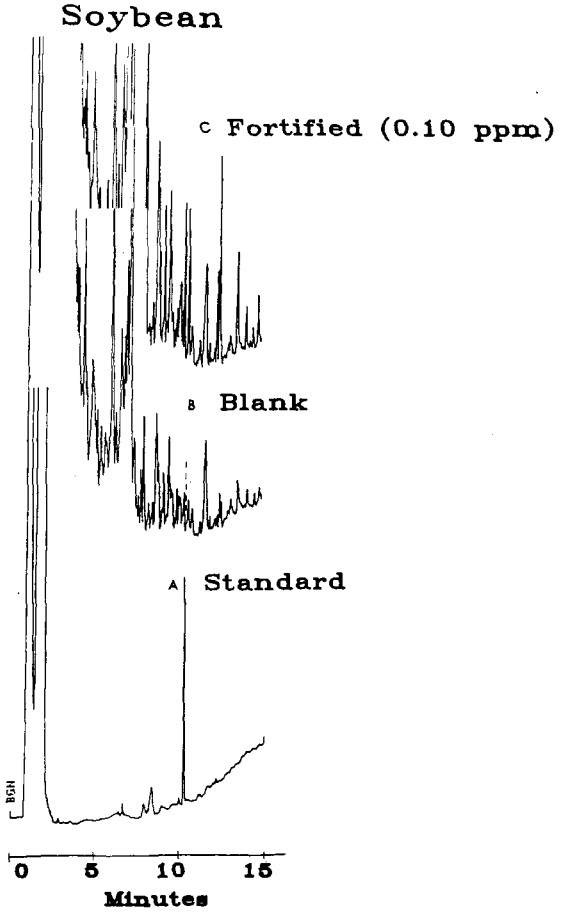



**Figure 4.** Gas chromatograms: A, fluazifop-methyl ester; B, blank soybean; C, fortified (0.10 ppm) soybean.

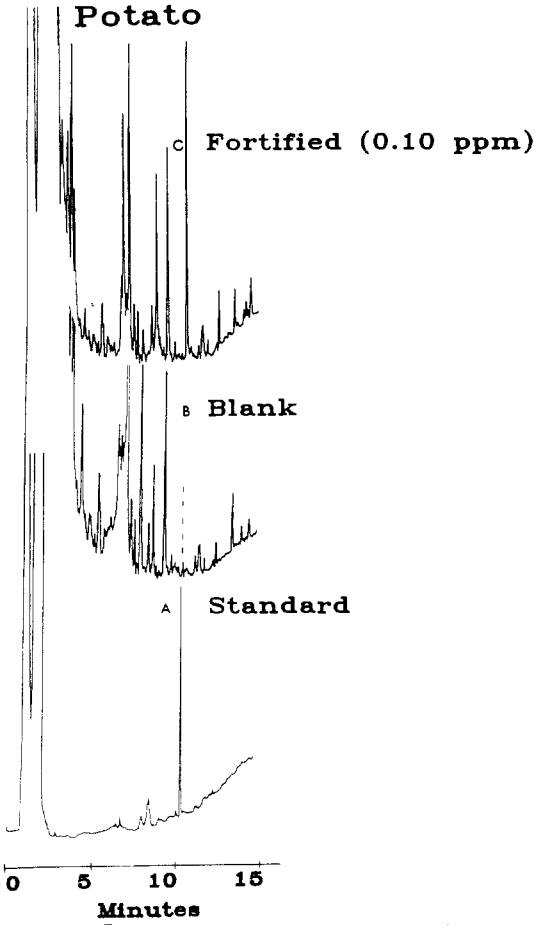



**Figure 5.** Gas chromatograms: A, fluazifop-methyl ester; B, blank potato; C, fortified (0.10 ppm) potato.

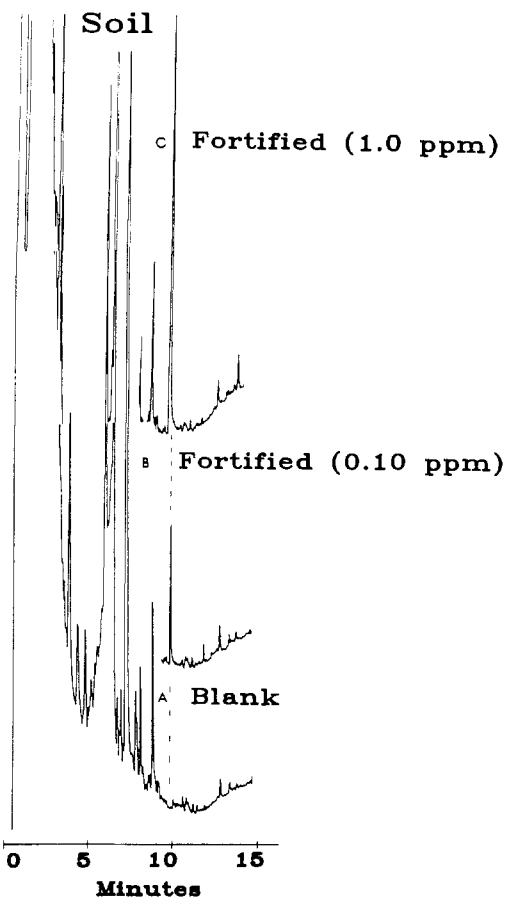



**Figure 6.** Gas chromatograms: A, blank soil; B, fortified (0.10 ppm) soil; C, fortified (1.0 ppm) soil.

The MSD was optimized by using a Hewlett-Packard disk software under AUTOTUNE conditions with PFTBA (perfluorotributylamine) calibration. Mass spectra were acquired over the 40–800 amu range at 380 amu $s^{-1}$ and normalized to DFTPP (decafluorotriphenylphosphine). Single- or multiple-ion monitoring was conducted with Hewlett-Packard software with up to 20 ions being monitored. Retention times: fluazifop-methyl, 12.4 min; fluazifop-butyl, 14.5 min.

## RESULTS AND DISCUSSION

A nitrogen–phosphorus detector was used to detect the methyl ester of fluazifop, taking advantage of the presence of the single nitrogen atom in the molecule and increased selectivity of this type of detector. Since the fluazifop molecule also contains a trifluoromethyl substituent, use of an electron-capture detector was examined, but it was found to be unsuitable for the determination of fluazifop-butyl and fluazifop-methyl. Poor response was observed, and interfering peaks were often present in crop extracts as previously described (Yip, 1971).

Both acid and base hydrolyses were examined for efficiency in releasing conjugated fluazifop-butyl; and the base hydrolysis produced fewer interferences with comparable recovery. In particular, the acid hydrolysis of potatoes produced an interference directly in the chromatographic region of fluazifop-methyl. For convenience of derivatization, boron trifluoride was examined as a methylating agent, but it proved to be unsuitable for methylating soybean plant and seed extracts. Poor recoveries were observed and attributed to incomplete methylation. Diazomethane corrected this problem; however, all extracts required Florisil cleanup prior to analysis.

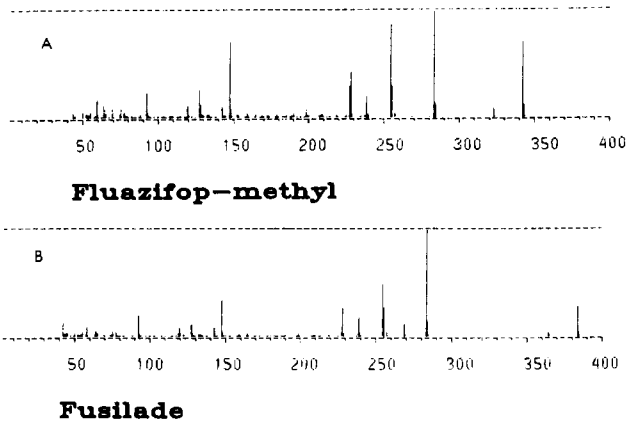



**Figure 7.** Mass spectra: A, fluazifop-methyl ester; B, Fusilade (fluazifop-butyl ester).

Chromatograms of the butyl ester (A) and methyl ester (B) of fluazifop are given in Figure 2. Retention times of the methyl ester and butyl ester were 12.3 and 14.6 min, respectively. Figures 3 and 4 give chromatograms of soybean plant and soybean material, respectively, as well as chromatograms of fortified samples for each. Chromatograms of blank and fortified potato are given in Figure 5, and blank and fortified soil samples are given in Figure 6. As indicated in each of these figures, no fluazifop-butyl is present in blank or fortified samples.

The mass spectra of the methyl and butyl esters of fluazifop are given in Figure 7, with major fragmentation patterns and percent intensity of each being given in Table I. Fluazifop-butyl is formulated at 125 and 250 g $L^{-1}$ of active ingredient, and these products are assigned the numbers TF1195 and TF1169, respectively. The 250 g $L^{-1}$

formulation (TF1169) is a racemic mixture of the enantiomeric pair of isomers designated at *R,S* or (±)-fluazifop-butyl. On the other hand, TF1195 is formulated at 125 g $L^{-1}$ since it contains only the *R* or + enantiomer (active isomer) of fluazifop-butyl.

Soybean samples were analyzed separately as plant and seeds. Soybean plant or seed samples field-treated with Fusilade showed no detectable fluazifop-butyl residues greater than 0.05 $\mu g\ g^{-1}$. The soybeans analyzed were treated at two rates (1.0 and 2.0 L $ha^{-1}$), with the period between herbicide application and harvest being greater than 90 days. Although no residues were detected in beans, it may be of future interest to examine soy oil for fluazifop-butyl residues.

The results of the analyses on potato samples were more remarkable, although the recovery data given in Table II suggested better recoveries when a 25-g sample was used rather than a 50-g sample. The method was verified for a 50-g sample in each substrate and various fortification levels. Potatoes analyzed were obtained from two separate trials, each being treated with Fusilade, with the two different formulations, i.e., TF1169 and TF1195, being used in these studies. Potatoes treated at two rates (1.0 and 2.0 L $ha^{-1}$) postemergence of TF1169 (250 g $L^{-1}$) showed no detectable residue (<0.01 $\mu g\ g^{-1}$) when harvested 90 days after application. On the other hand, potatoes treated two rates of TF1195 (125 g $L^{-1}$) showed residues of 0.16 and 0.47 $\mu g\ g^{-1}$ (fluazifop-methyl ester) for the 1.0 and 2.0 L $ha^{-1}$ rates, respectively. It must be noted that these potatoes were sampled only 42 days after the application, which is considerably shorter than the label recommendation of 90 days prior to harvest.

Currently, this method is being examined for its suitability in determining fluazifop-butyl residues in other crops and vegetables. The method shows potential as a multiresidue method for determination of the phenoxyphenoxy class of herbicides, and efforts are being made to assess its applicability in the analysis of other similar herbicides such as flamprop-methyl, fenoxaprop-ethyl, fenthiaprop-ethyl, sulfometuron, and haloxyfop-methyl.

ACKNOWLEDGMENT

I acknowledge the technical assistance of Young Ja Paik and the cooperation of C. G. Halliday (Chipman Inc., Stoney Creek, Ontario) for supplying potato samples and information regarding Fusilade formulations. Thanks are also extended to Glen Anderson (Crop Science Department, University of Guelph, Ontario) for providing the field treatments and collection of samples.

**Registry No.** Fluazifop-butyl, 69806-50-4.

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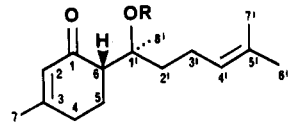
# The Intensely Sweet Sesquiterpene Hernandulcin: Isolation, Synthesis, Characterization, and Preliminary Safety Evaluation

César M. Compadre, Raouf A. Hussain, R. Lilia Lopez de Compadre, John M. Pezzuto, and A. Douglas Kinghorn*

Details are provided of the isolation of an intensely sweet compound, hernandulcin, from *Lippia dulcis* Trev. (Verbenaceae), in addition to its synthesis, stereochemical assignation, spectroscopic and chromatographic characterization, derivatization, and thermal stability. This compound, as well as its analogue, epihernandulcin, and its products of thermal degradation, 3-methyl-2-cyclohexen-1-one and 6-methyl-5-hepten-2-one, exhibited $LD_{50}$ values in excess of 1 g/kg of body weight, when tested for acute toxicity in mice. All four compounds were also found to be nonmutagenic in forward mutation assays utilizing *Salmonella typhimurium* strain TM677.

In a preliminary report (Compadre et al., 1985), we described the occurrence of the intensely sweet compound hernandulcin (1), a constituent of the herb *Lippia dulcis*




1 R = H
7 R = Ac

Trev. (Verbenaceae), grown in Mexico. The racemic form of hernandulcin has been efficiently synthesized by a di-

Program for Collaborative Research in the Pharmaceutical Sciences and Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612.

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