Spectrophotofluorometric Determination of the Hydrolyzed Residues of 2-Fluoroethyl-4-biphenylacetate (Fluenethyl) in Some Fruits

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A method has been developed for the determination of fluenethyl residues in apples, grapes, peaches, pears, and plums. This method is based on the extraction of the acaricide with methylene chloride, followed by liquid–liquid partitioning and thin-layer chromatographic cleanup, hydrolysis, and spectrophotofluorometric assay down to 1 ppb.

Fluenethyl (2-fluoroethyl-4-biphenylacetate) was synthesized for the first time by the Agricultural Research Institute of Montecatini–Edison and is characterized by a high level of toxicity to the winter eggs of mites, aphids, scales, and Psyllae when applied as a late winter treatment (De Pietri-Tonelli *et al.*, 1965, 1969; Salvaneschi and Carassai, 1968). It is a colorless solid, very slightly soluble in water but soluble in various solvents such as *n*-hexane, xylene, benzene, acetone, acetonitrile, chloroform, and methylene chloride. The use of fluenethyl has called for the development and the employment of a method for the determination of residues in crops. This method is based on the extraction of fluenethyl, by means of methylene chloride, from fruits previously homogenized in a blender, on subsequent cleanup from some interfering plant substances by liquid–liquid partitioning (aqueous methanol/*n*-hexane first and *n*-hexane/acetonitrile second), on thin-layer chromatography for further cleanup and finally on spectrophotofluorometric evaluation of the hydrolysis product (4-biphenylacetate sodium salt) of fluenethyl.

METHOD

Apparatus. Desaga, chromatographic chamber (22 × 10 × 22 cm, Pabisch, Milano, Italy) thin-layer applicator (Chemetron, Milano, Italy). Aminco–Bowman spectrophotofluorometer with fused quartz cells 1 cm². The source for the excitation monochromator was a Xenon lamp (American Instrument Co., Catalog No. 416-992). The detector for the emission monochromator was a 1P28 phototube. The apparatus, for 1 μg of quinine sulfate, under the conditions outlined in the Amino–Bowman No. 2392 D bulletin, shows a relative intensity of 87 instead of 94.

Reagents. Washed silica gel G with the washing to be done as follows. In a 750-ml flask with ground glass stopper, place 100 g of silica gel with 500 g of redistilled methylene chloride. Stir four times, each time for 5 min, and allow to stand for 2 min after each stirring operation. Then filter through a Gooch G 3 fritter with a glass frit, washing six times with 250 ml of redistilled methylene chloride. Keep in an oven for 30 min at 50° C.

Prepare the glass plates (20 × 20 cm) for chromatography as follows. Mix 50 g of washed silica gel with 85 ml of redistilled water in a flask and stir for 3 min. Coat the glass plate with a silica gel layer of 0.5 mm and place in an oven at 110° C for 90 min (for four plates 50 g of silica gel are generally sufficient).

Solvent system for thin-layer chromatography: cyclohexane (for chromatography) and redistilled methylene chloride (1 + 1 v/v). Developing distance 14 cm. Chromogenic reagent: solution of zirconium nitrate at 0.05% and sodium alizarin sulfonate at 0.05% in redistilled water (100 ml), plus 10 ml of hydrochloric acid at 37% (Stahl, 1965). Standard solution in redistilled methylene chloride of pure fluenethyl, obtained by repeated crystallization first from ligroin (reagent grade, boiling range 75 to 120° C) and then from *n*-hexane (at least two crystallizations for each solvent).

Procedure. PREPARATION OF LINEAR STANDARD GRAPHS. For obtaining data for the standard graphs, 0, 0.01, 0.05, 0.1, 0.15, 0.2, 0.3, and 0.4 μg amounts of fluenethyl are placed in 15 ml cone-shaped tubes. The solvent is then removed completely by dipping the test tubes in water at 35–40° C under a gentle stream of filtered air. The last traces of solvent are removed at room temperature, until a dry residue is obtained. 1 ml of NaOH *N* is added and the solution heated to 60° C for 10 min in order to get a thorough hydrolysis. After cooling, readings are taken with the spectrophotofluorometer under the following working conditions. Excitation: 260 nm (330 nm results in maximum fluorescence intensity). Photomultiplier: 0.001; sensitivity: 26 and 50. Slits: A = 3 mm; B = none; C = none; D = 1 mm; E = 0.5 mm; F = 1 mm; and G = 0.5 mm.

Excitation (260 nm) and slit program conditions were established with a view to obtaining a lower scatter effect and a greater fluenethyl response (relative intensity), taking due account of fruit residue interference.

FLUENETHYL EXTRACTION FROM FRUITS. Finely chop the fruit sample (2 kg, unpeeled and unwashed) and homogenize in a blender. (This method was adopted for it allows an unquestionably representative average sample, resulting from a homogenized fruit sample of 2 kg, to be obtained.) In a 500-ml flask with ground glass stopper, weigh 50 g of the slurry and add 250 ml of methylene chloride. Shake vigorously on a vibrating platform for 40 min. Allow to stand, decant the solution, transfer onto a filter made from filter paper (Whatman No. 1) and collect the filtrate into a 750-ml flask. Wash with two 100-ml portions of methylene chloride.

Shake and transfer the solvent to the filter again by decan-

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tation. Transfer the fruit slurry to the filter quantitatively, wait until the aqueous phase has percolated completely, and finally wash the slurry with 50 ml of methylene chloride.

Pour the resulting solution into a 500-ml separatory funnel and allow the aqueous phase to separate. Transfer the methylene chloride phase to a 100-ml flask, extract the aqueous phase with two 30-ml portions of methylene chloride, shaking each time for 2 min, and add the extracts to the main methylene chloride solution.

PREPARATION OF FORTIFIED CONTROL SAMPLE. Curves resulting from the addition of fluenethyl to the residue obtained from 50 g of untreated fruits previously subjected to the analytical treatment outlined in the cleanup have been found to coincide with those resulting from fluenethyl alone. The fluorescence from a given concentration of fluenethyl was found to increase linearly with concentrations between 0 and 0.4 μg.

Cleanup. Concentrate the entire methylene chloride phase at 20–25° C by using a vacuum rotary evaporator until a slightly moist residue is obtained. Add 25 ml of redistilled water. Rinse the walls of the container and transfer the washings to a 150-ml centrifuge tube with ground-glass stopper. Repeat this operation with 5 ml of methanol. Rinse again with two 15-ml portions of *n*-hexane and add the liquid to the large centrifuge tube. (By using a series of solvents, we intended to guarantee the transfer of fluenethyl, if there were any retained by plant residues which are soluble in alcohol and water and may be insoluble in *n*-hexane.) Shake for 2 min and centrifuge for 30 min at 2000 rpm (650 $\times$ *g*). Syringe off the upper hexane phase and add it to a 250-ml rotary evaporator flask. Add a further 30 ml of *n*-hexane to the large centrifuge tube, then shake, centrifuge, syringe off the upper hexane phase, and collect it into the 250-ml graduated separatory funnel, as above. Again extract the aqueous alcoholic phase with 20 ml of *n*-hexane, shaking, centrifuging, and syringing off as above. The last portion of *n*-hexane is also added to the 250-ml separatory funnel.

Adjust to a volume of 100 ml with *n*-hexane. Extract with two 75-ml portions of *n*-hexane-saturated acetonitrile, shaking each time for 3 min. Transfer the clear acetonitrile phase to a 1000-ml flask and remove the solvent in a rotary evaporator completely at a maximum temperature of 40° C. Take up the residue in methylene chloride and transfer to a 15-ml cone-shaped tube. Remove the solvent by dipping the test tube in water at 35–40° C under a gentle stream of filtered air. Remove the last traces of solvent at room temperature until a dry residue is obtained. Take up this residue in a 15-ml cone-shaped tube with 1 ml of methylene chloride. Swirl to complete solution and draw off the greatest possible volume by using a 1-ml piston-type pipette (Bicasa, Sesto S. Giovanni, Milano, Italy), apply 0.9 ml of the solution onto the plate, 3 cm from its lower edge, as a uniform line 11 cm long. Apply, as a spot, the remaining volume of solution in the pipette, after the addition of 1000 μg of fluenethyl, as a spot onto the plate 3 cm from the left edge and 3 cm from the lower edge. 1000 μg are required to obtain a good chromogenic detection in the presence of plant residues.

After complete solvent evaporation, place the plate in the chromatographic tank. To ensure that the tank is saturated with solvent vapors, cover the walls of the chamber with filter paper sheets, their lower end dipped in the solvent mixture. Conduct the migration and repeat this operation twice, each

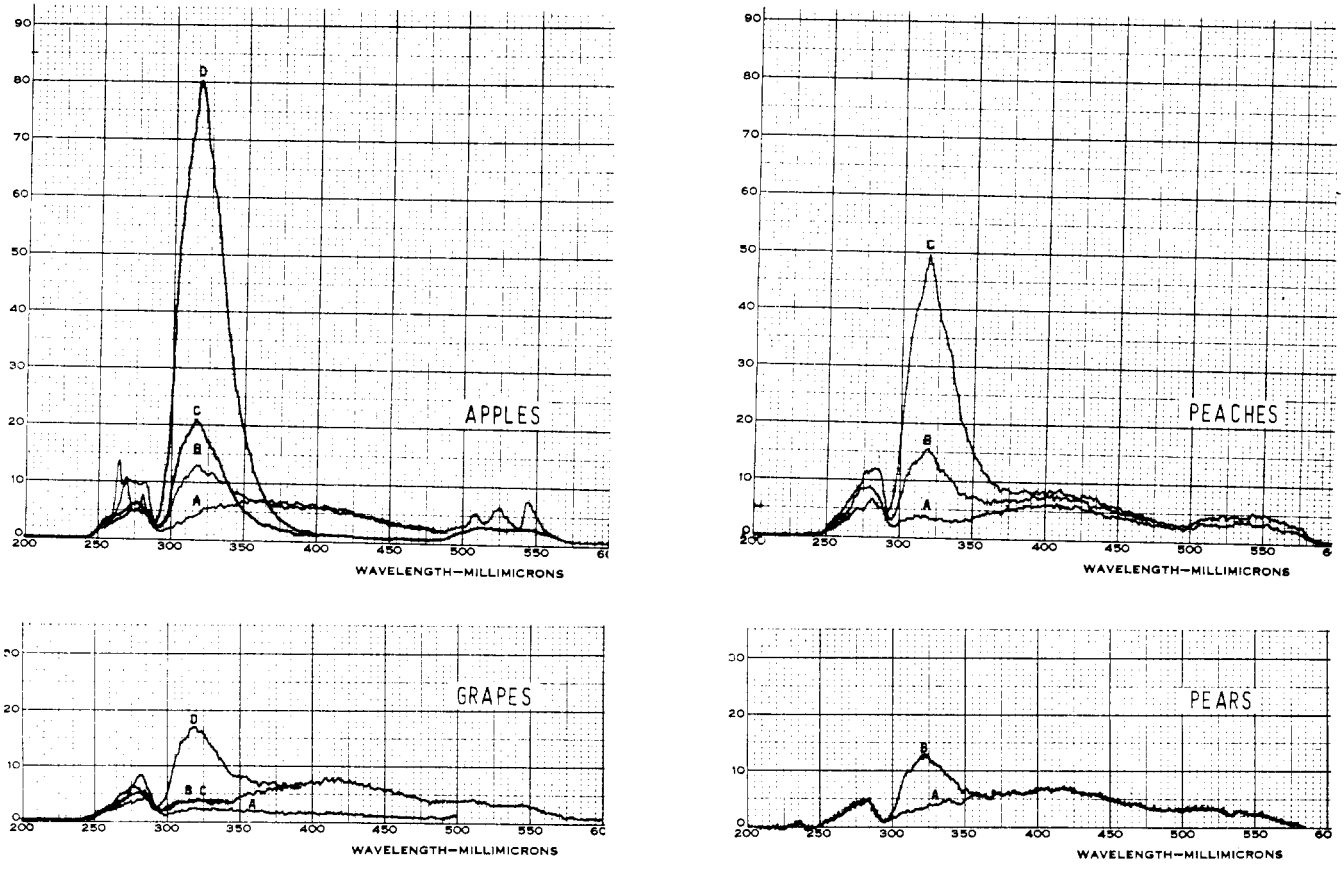

Figure 1. Uncorrected fluorescence spectra of fluenethyl (ordinate: relative intensity). Apples: A = untreated fruit (P.B.); B = P.B. + 1 ppb; C = fluenethyl 0.05 μg in NaOH; D = fluenethyl 0.2 μg in NaOH *N*. Peaches: A = P.B.; B = P.B. + 1 ppb; C = P.B. + 4 ppb. Grapes: A = NaOH *N*; B and C = P.B.; D = P.B. + 1 ppb. Pears: A = P.B.; B = P.B. + 1 ppb

Table I. Final Recoveries of Fluenethyl[a]

Fluenethyl (ppb) added to crop homogenate, 50 g	Recoveries corrected for background, %	Fluenethyl (ppb) added to crop homogenate, 50 g	Recoveries corrected for background, %
Apples		**Pears**	
1	82.0	1	78.0
1	58.0	1	64.0
1	58.0	1	70.0
1	68.0	4	57.0
1	68.0	4	60.0
4	66.0	4	61.5
4	66.0	4	61.5
4	66.5	4	71.0
4	71.0	4	71.0
4	71.5	**Peaches**	
4	72.5	1	66.0
4	76.0	1	66.0
8	58.0	1	90.0
8	62.0	4	59.5
8	64.3	4	65.0
Grapes		4	75.0
1	66.0	**Plums**	
1	66.0	4	62.5
1	66.0	4	75.0
1	68.0		
4	63.0		

[a] Some extraction efficiency tests carried out with methylene chloride by adding 1 ppm and 0.01 ppm of fluenethyl (labeled with ^{14}C on the carbon atom of the carboxylic group) to the slurry, permitted a recovery of 95% after 2 hr contact. The extraction efficiency determination was not prolonged for more than 2 hr because fluenethyl decomposes when it comes into contact with plant material.

Table II. Residues (ppb) of Fluenethyl in Treated Fruits[a]

Concentration of spray (% fluenethyl)	Date treated	Date harvested	Residues of fluenethyl as ppb	Country of origin
Apples				
0.06	3/15/67	7/20/67	<1	Italy
0.06	2/26/69	9/25/69	<1	France
0.06	5/8/69	9/18/69	<1	Canada
0.06	4/3/69	10/1/69	<1	Bulgaria
0.06	9/12/69	3/16/70	<1	New Zealand
0.05	7/7/69	9/8/69	1	Lebanon
0.1	7/7/69	9/8/69	2.5	Lebanon
0.05	8/8/69	9/8/69	4	Lebanon

[a] All other analyses of apples, grapes, peaches, pears, and plums grown variously in Belgium, Bulgaria, Canada, France, Italy, and New Zealand, and when also winter-treated with 0.06% fluenethyl sprays, produced residues of less than 1 ppb if the date of harvest was more than 3 months following the date of treatment.

time allowing a drying interval of 20 min. Let the plate air-dry for at least 30 min and then spray only the side of the plate previously spotted with 1000 μg of fluenethyl. After 5 min, mark the corresponding R_f region on the unsprayed side of the plate, on the basis of the chromogenic detection information. (The mean R_f value is 0.67. The size of fluenethyl area to be removed ranges from 7.5 to 10.5 cm to be measured from the point of application, when the solvent front reaches 14 cm in height.)

Using a microscope slide, completely remove all the silica gel from around the considered area. Clean the uncovered glass carefully, first with a wadding pad moistened with methylene chloride and then with filter paper (Whatmann No. 1) wetted with the same solvent. Scrape off the silica gel from the area containing fluenethyl and transfer to a 10 $\times$ *g* 4 Jena fritted glass funnel mounted on a 50-ml Kjeldahl flask. Elute the silica gel with methylene chloride portions to a total volume of 40 ml, taking care to stir thoroughly, by means of a glass rod, the silica gel prior to each washing operation. Concentrate the filtrate to the rotary evaporator until a volume of about 5 ml is obtained, then transfer to a cone-shaped centrifuge tube, washing with methylene chloride. Remove the solvent by immersing the tube in water heated to 40° C under a slight jet of filtered air. After complete solvent removal, add 1 ml of NaOH *N*, shake, and place the tube in a water bath at 60° C for 10 min. Allow to cool and centrifuge for 15 min at 5000 rpm (4080 $\times$ *g*). Finally pipet directly into the cell, taking care not to stir the clear liquid. Take readings from the spectrophotofluorometer under the working conditions outlined in "PREPARATION OF LINEAR STANDARD GRAPHS."

FLUENETHYL RESIDUE DETERMINATION. The 4-biphenylacetate sodium salt resulting from alkaline hydrolysis of fluenethyl gives a fluorescence relative intensity response which does not vary with time. The concentration of fluenethyl in the substrate should be calculated as follows

$$\text{ppm} = \frac{\mu g \cdot I_{Rx}}{I_{Ri} \cdot g}$$

where μg = μg of fluenethyl added to the 50 g untreated slurry at the beginning of the analysis work. I_{Rx} = relative intensity value of the sample being examined; I_{Ri} = relative intensity value of the fortified control sample with added fluenethyl. g = weight of substrate.

RESULTS AND DISCUSSION

The use of the spectrophotofluorometric method for the determination of fluenethyl is specific against possible fluorescent metabolites (*e.g.*, the 4-biphenylacetic acid, diphenyl) resulting from fluenethyl, as these are eliminated during cleanup. The treatment with NaOH *N* at 60° C induces the formation of 4-biphenylacetate sodium salt which, unlike most fruit residues, is soluble in the alkaline solution. This method is suitable for the determination of fluenethyl in apples, grapes, peaches, plums, and pears. 45-g samples of untreated fruits do not normally have background response exceeding the equivalent of 0.3 ppb of fluenethyl.

Table I gives the recovery values of fluenethyl added to the different fruit substrates, whereas Table II reports the results of the analyses conducted on fruits spray-treated in the field with formulations of fluenethyl containing 2% mineral oil. Figure 1 reproduces some fluorescence spectra resulting from fluenethyl alone and from fluenethyl added to apples, peaches, grapes, and pears.

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