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Determination of Fluvalinate Metabolite Residues in Cottonseed, Apples, Tomatoes, and Soil

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Fluvalinate is the active ingredient in Mavrik and Spur insecticides used to control insects on numerous field crops. Radiolabel experiments have shown that the residue of concern in most crops is the parent molecule. However, an analytical method was needed to determine the major hydrolytic metabolites of fluvalinate, 3-phenoxybenzoic acid (PBA) and 2-[2-chloro-4-(trifluoromethyl)anilino]-3-methylbutanoic acid (CAA), in selected crops. The method entails standard extraction and partition steps, basic hydrolysis to free conjugated metabolites, conversion to methyl esters, and determination by capillary gas chromatography/mass spectrometry. The methods were validated for the title crops with detection limits of 0.02 and 0.05 ppm, respectively, for CAA and PBA.

Fluvalinate [(*RS*)-α-cyano-3-phenoxybenzyl (*R*)-2-[chloro-4-(trifluoromethyl)anilino]-3-methylbutanoate; **1** in Figure 1] is the ingredient of Mavrik and Spur insecticides being developed by Sandoz Crop Protection Corp. for use on a variety of field crops (Henrick et al., 1980). Previous studies have demonstrated (Quistad et al., 1982) that the primary metabolic pathway of fluvalinate in plants is via ester hydrolysis and oxidation to yield 3-phenoxybenzoic acid (PBA, **2**) and 2-[2-chloro-4-(trifluoromethyl)anilino]-3-methylbutanoic acid (CAA, **3**). These metabolites exist in the plants in both free and conjugated forms.

In conjunction with field studies on the environmental fate of fluvalinate, analytical methods were needed for residues of PBA and CAA. Although PBA is a known metabolite from many synthetic pyrethroids, scant literature is available on determination of its residues. An early report on the high-pressure liquid chromatography behavior of PBA (Ram and Grushka, 1977) has not been expanded upon. The only method for PBA in the *Pesticide Analytical Manual* (FDA, 1985) was published as part of a method for the determination of permethrin and its metabolites. The method described herein is similar to the published permethrin method but is adapted to allow determination of the fluvalinate-specific acid CAA.

EXPERIMENTAL SECTION

Reagents. Fluvalinate and CAA were synthesized as previously described (Henrick et al., 1980). [*trifluoromethyl*-^{14}C]Fluvalinate was prepared as previously described (Quistad et al., 1982). PBA was purchased from Aldrich Chemical Co. The ethyl ester of CAA was synthesized from CAA by treatment with diazoethane. All solvents were high purity from Burdick and Jackson. Florisil (PR Grade, J. T. Baker) was heated at 150 °C overnight and deactivated with 6% water prior to use. Sodium sulfate (anhydrous, reagent grade) was slurried with anhydrous ethyl ether, treated with 0.5% by weight concentrated H_2SO_4, filtered, and dried at 100 °C prior to use.

Sample Preparation. Apples were chopped finely, and 20 g was treated with 5 mL of 0.5 N HCl. The sample was then Soxhlet extracted for 10 h with 200 mL of methanol containing 2.5% 0.5 N HCl. Tomatoes were chopped, and 20 g was blended with 50 mL of methanol and 5 mL of 0.5 N HCl. The extract was vacuum filtered and the residue reextracted as before. The final filter cake was washed with two additional 25-mL portions of methanol, and the filtrates were combined. Soil samples were ground and sifted through a No. 6 sieve. The soil was then sprinkled with water and allowed to equilibrate for 1 h. A 20-g portion of soil was then treated with acid and Soxhlet extracted. A separate 5-g sample of the moistened soil was dried to constant weight at 100 °C. Residues were corrected to a dry-weight soil basis. Cottonseed samples were ground in a coffee grinder. A 20-g cottonseed sample was then treated with acid and Soxhlet extracted.

Partitioning of Extract. For each substrate, the extract was diluted to 200 mL and a 4-g equivalent portion (40 mL) transferred for further processing to a 500-mL separatory funnel. To the funnel were added 200 mL of 0.1 M $NaHCO_3$, 5 mL of saturated NaCl, and 50 mL of isooctane that had been saturated with methanol. After 1 min of extraction, the upper isooctane layer was discarded. The isooctane partitioning was repeated three times to ensure complete removal of all fluvalinate.

Hydrolysis. The aqueous layer from the partitioning was treated with 5 mL of 5 N KOH and heated at reflux for 1 h. The basic solution was then cooled in ice and

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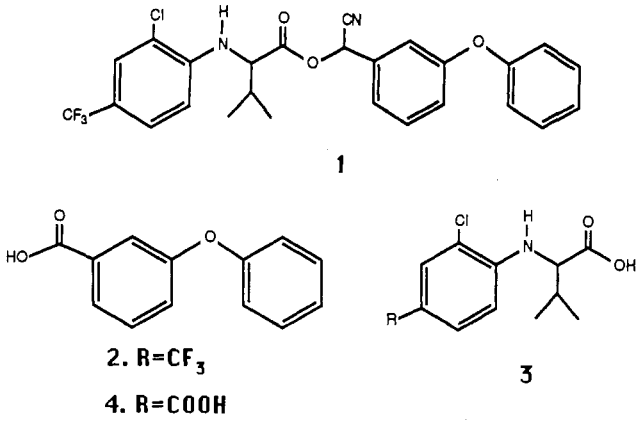

Figure 1. Structures of fluvalinate and metabolites.

adjusted to a pH <2 with concentrated HCl. The acidic solution was extracted with three 50-mL portions of 30/70 (v/v) ether/pentane. The combined organic layers were back-extracted with three 100-mL portions of 0.1 M $NaHCO_3$ adjusted to pH 10 with 5 N KOH. Finally, the aqueous layer was reacidified with concentrated HCl to pH 2 and extracted with two 50-mL portions of 30/70 ether/pentane. The extract was dried by passing it through 10 g of acidified sodium sulfate, and the separatory funnel and sodium sulfate were rinsed with an additional 50 mL of ether. The combined extracts were evaporated to 5 mL on a 40 °C water bath with a gentle stream of nitrogen.

Derivatization. The sample was transferred to a 50-mL centrifuge tube, 1 mL of methanol added, and the sample reevaporated to 1 mL. BF_3/methanol (3 mL) was added and the sample heated at 60–65 °C for 20 min. After cooling, the sample was partitioned between isooctane (8 mL) and 30 mL of 5% aqueous Na_2SO_4. A 4.0-mL portion of the isooctane (2 g equiv of original sample) was added to 0.2 μg of internal standard (CAA ethyl ester) and evaporated to a final volume of 2 mL.

Optional Florisil Cleanup. Extracts from cottonseed and apples required an additional Florisil cleanup. A 2 g equiv portion of the isooctane above (before addition of internal standard) was placed on a minicolumn prepared from 0.5 mL of acidified Na_2SO_4, 1 mL of Florisil, and 0.5 mL of acidified Na_2SO_4. Any eluted isooctane was discarded, and the desired compounds were eluted with 12 mL of ethyl ether. Internal standard (0.2 μg in 2.0 mL isooctane) was added to the eluate and the extract carefully evaporated to 2.0 mL with a gentle stream of nitrogen.

Gas Chromatography/Mass Spectrometry (GC/MS). Determination was performed by selected ion monitoring GC/MS using a Hewlett-Packard 5996 instrument. The column was a Hewlett-Packard 30-m narrow bore, methylsilicone fused silica capillary. Samples were injected splitless at 90 °C after which the oven was programmed at 30 °C/min to 180 °C and then at 2 °C/min to 210 °C. Under these conditions CAA methyl ester eluted at 6.7 min, CAA ethyl ester at 7.2 min, and PBA methyl ester at 9.2 min. The mass spectral ions scanned were m/z 250 for the CAA esters and m/z 228 and 197 for PBA methyl ester.

RESULTS AND DISCUSSION

As the goal of this method was the determination of metabolite concentrations in the presence of an expected excess of the parent compound, it was necessary to take special precautions to prevent fluvalinate hydrolysis during extraction and partitioning. Under mildly basic conditions (Fitch et al., 1984) or during methanol extraction, fluvalinate is readily hydrolyzed. Methanol Soxhlet extraction of unacidified soil can cause up to 20% conversion of fluvalinate to CAA. Acidification of the sample prior to hydrolysis minimizes the hydrolysis. Experiments with ^{14}C-labeled fluvalinate indicated that less than 3% of the compound was hydrolyzed under the standard conditions. In addition less than 0.2% of an initial fluvalinate concentration remained in the aqueous layer after the exhaustive aqueous methanol/isooctane partitioning steps.

Table I. Recovery of CAA and PBA from Spiked Samples

matrix	spike level, ppm	CAA recovery, % N	CAA recovery, % av	CAA recovery, % range	PBA recovery, % av	PBA recovery, % range
cottonseed	0.05	2	80	76–83	96	91–100
	0.1	2	75	74–76	101	92–110
soil	0.1	2	60	54–65	88	77–100
apples	0.1	2	84	78–89	94	92–95
tomatoes	0.1	2	80	79–80	87	85–89

Although it was not the purpose of this method, these techniques could form the basis of a total residue determination method. With the exception of oily crops or tissues (where less polar solvents are required), methanol is a good extracting solvent for fluvalinate as well as the more polar metabolites. The combined initial isooctane partitions contain a quantitative yield of any parent fluvalinate residue, which could be further determined with existing methods (Fitch et al., 1984). In soil the total fluvalinate residue is complicated by the further metabolism of CAA to 2-chloro-4-(trifluoromethyl)aniline (Staiger and Quistad, 1983).

Prior work (Quistad et al., 1982) has shown that CAA and PBA conjugates are readily hydrolyzed under basic conditions. Too violent a hydrolysis, however, can lead to conversion of CAA to the dicarboxylic acid 4. The large quantities of extraneous compounds freed during the hydrolysis led to the need for a long partitioning cleanup sequence and, in some cases, a Florisil cleanup prior to determination.

The chemistry of the fluvalinate-specific metabolite CAA led to several complications in the method. Although an amino acid, CAA is sufficiently nonbasic to allow ready partitioning of the compound into organic solvents at low pH. However, it was necessary to use acidified sodium sulfate to avoid irreversible adsorption during drying operations. In addition, CAA methyl ester is very volatile and easily lost during solvent concentration steps.

In preliminary experiments, we showed that CAA methyl ester could be easily detected by GC with electron capture detection. However, a method for both CAA and PBA was desired, so GC/MS was chosen for the final determination step. The GC/MS behavior of both derivatives was adequate. However, it was found advantageous to add a quantitation standard to the solutions to eliminate irreproducibility in the GC/MS injection system. The standard used, CAA ethyl ester, could be monitored at the same ion as that used for CAA methyl ester, the m/z 250 ion which is characteristic of all CAA esters (including fluvalinate). This ion in the mass spectra of these esters is attributed to α cleavage of the amino acid with charge retention on nitrogen. The m/z 228 ion used for PBA quantitation is the molecular ion for this methyl ester. In certain cases it is advantageous to use the m/z 197 ion for PBA, when there are interferences at m/z 228.

Table I shows the recovery data for spiked samples of several crops taken through this procedure. In general the recoveries for PBA were higher than those for CAA. These lower and more variable recoveries of CAA were attributed to variable losses during partitioning, hydrolysis, solvent

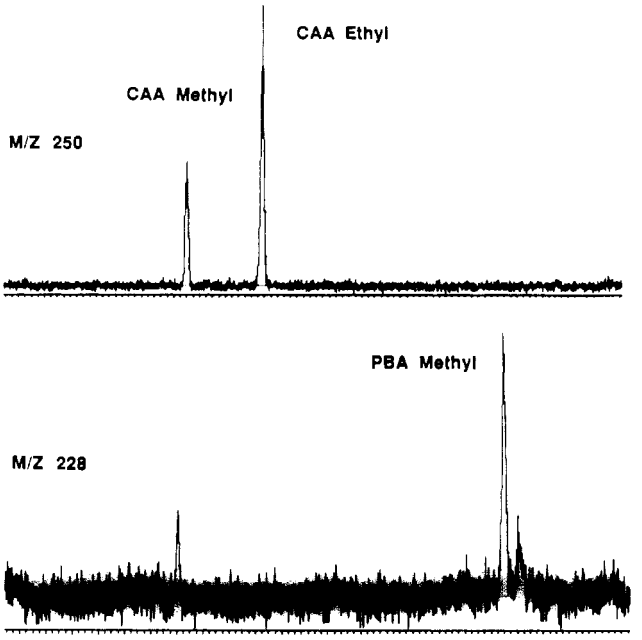

Figure 2. GC/MS chromatograms for a cottonseed sample spiked with CAA and PBA at 0.05 ppm. The lower trace shows m/z 228 detection of the PBA methyl ester. The upper trace shows m/z 250 detection of the CAA methyl and ethyl esters.

drying, and extract evaporation.

Figure 2 shows the reconstructed and single-ion chromatograms from the analysis of a cottonseed sample. The method detection limit is estimated at 0.02 ppm for CAA and 0.05 ppm for PBA. The high detection limits are due to the presence of interfering compounds in many of the matrices tested and to variable GC/MS sensitivities.

CONCLUSION

The method described herein allows reliable determination of trace quantities of the fluvalinate metabolites CAA and PBA in a variety of matrices. The methods have been used in these laboratories to analyze samples of crops that have been field treated with fluvalinate. The sensitivity of fluvalinate to hydrolysis and the difficult analytical characteristics of CAA and its methyl ester require a careful observance of several method details.

Registry No. 2, 3739-38-6; 3, 74971-63-4.

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Extraction and Quantitation of Soy Protein in Sausages by ELISA

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An indirect ELISA procedure was applied to detect and measure soy protein in sodium carbonate buffer extracts (pH 9.8) of cooked and uncooked sausages without prior delipidation or protein isolation. The method was evaluated on frankfurters prepared in our laboratory containing 0, 1, 2, 3, 4, and 5% soy isolate. With food-grade soy isolate as standard, results showed that experimental values were 94% in agreement with all levels of added soy isolate and had 95% confidence limits of 80–107%. When the extraction and ELISA procedures were applied to 23 commercial samples, results were negative on 18 products with no soy additive label while four lots of one product showed <2.5% soy protein and another product had 2.6% (±0.4, $p < 0.05$). This procedure provides a more simple, rapid, and direct analysis of soy additives suitable for monitoring adherence to the legal restrictions regarding use of soy protein additives in processed meat products.

Regulatory agencies in the United States, Canada, and EEC (European Economic Community) member countries are concerned about illegal addition of vegetable protein in meat products. Soy protein isolate, concentrate, and flour are commonly used as meat additives. In the United States, up to 2% soy protein isolate and up to 3.5% soy flour or concentrate can be added to sausage products, loaves, stews, or soups (*Code of Federal Regulations*, 1987).

To date, there is no satisfactory routine method used to measure the quantity of soy protein in meat products. Nonimmunochemical methods for detection of soy and vegetable proteins, reviewed by Llewellyn (1979, 1982), Olsman and Hitchcock (1980), Eldridge and Wolfe (1980), and Eldridge (1981), lack sensitivity and specificity. However, immunochemical methods that offer high sensitivity, specificity, and large sample throughput merit further investigation. Immunochemical methods devel-

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