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Determination of the Insecticide/Acaricide Formetanate in Fresh Fruit by Reversed-Phase Liquid Chromatography

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Formetanate [*m*-[[(dimethylamino)methylene]amino]phenyl methylcarbamate] was extracted from fresh samples of oranges, apples, plums, peaches, and pears with acidified acetonitrile. An aliquot of the filtered extract was evaporated to an aqueous residue, which was cleaned up by alternate acidic and basic aqueous–organic partitions. The final residue was dissolved in distilled water for analysis by liquid chromatography on an RP-18 reversed-phase column with a mobile phase consisting of 35% acetonitrile in 0.01 M ammonium phosphate, monobasic (pH 8.0), and UV detection at 254 nm. Average recoveries at ≥0.5 ppm were over 80%. At the 0.1-ppm level average recoveries were generally 70% or higher. Minimum detectable levels were estimated to be ~0.02–0.05 ppm in the fruits studied.

Formetanate [*m*-[[(dimethylamino)methylene]amino]-phenyl methylcarbamate], in the form of its hydrochloride salt, is used as an insecticide/acaricide for control of spider mites, rust mites, certain aphids, thrips, lygus bugs, leaf hoppers, slugs, and snails on a variety of orchard fruit (Jenny and Kossmann, 1978). Interest in this compound has recently arisen as a result of questionable toxicology studies which were used to secure its registration for use in the United States and Canada. No data have been reported up to now on residue levels of this pesticide in the food supply, in particular, orchard fruits such as citrus, apples, peaches, pears, and plums. In the past, analytical techniques for formetanate residues involved hydrolysis of the pesticide to yield m-aminophenol which was diazotized and coupled with *N*-(1-naphthalene)diamine dihydrochloride to produce a reddish blue azo dye which could be quantitated by colorimetry (Jenny and Kossman, 1978). An improved method involving gas chromatography (GC) was also devised by Jenny and Kossman (1978) which made use of hydrolysis of the pesticide to *m*-aminophenol followed by bromination which yielded 2,4,6-tribromophenol. This product was then determined by GC with electron-capture detection. Both of these methods are indirect, requiring chemical reactions and the measurement of a derivative rather than the parent compound. Other carbamates have been directly determined in foods by LC (Lawrence, 1977; Lawrence and Leduc, 1977, 1978; Nelsen and Cook, 1979; Robinson and Chapman, 1980; Thean et al. 1978); however, no applications to formetanate were included. We have developed a direct method for the analysis of formetanate which makes use of reversed-phase liquid chromatography (LC) and UV-absorbance detection. As a result, the technique is more rapid and involves less sample manipulation than the previously described colorimetric or GC methods.

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The method has been applied to a limited survey of fruits from various regions of Canada.

EXPERIMENTAL SECTION

Reagents. All organic solvents used for sample extractions, cleanup, and liquid chromatography were distilled-in-glass grade. The stock solution of formetantate hydrochloride was prepared in acetonitrile at a concentration of 1.0 mg/mL. Dilutions of this for spiking purposes were made with acetonitrile. Chromatography solutions were prepared by appropriately diluting the the stock with distilled deionized water. These were stored in a refrigerator when not in use and were stable for at least 1 week. Chromatography standards were never prepared in the LC mobile phase due to the degradation of the pesticide at pH 8–9, which caused losses of ~20% after 1 day.

The fruits were purchased in five different regions of Canada and consisted of both domestic and imported goods. Recovery studies were carried out on locally purchased samples of oranges, plums, and apples.

Sample Extraction. Twenty-five grams of homogenized fruit was blended in a Sorvall Omnimixer for 3 min at medium speed with 70 mL of acetonitrile containing 0.5% concentrated HCl. The mixture was filtered with suction through a medium-porosity sintered glass funnel and the filtrate collected in a 100-mL volumetric flask. The residue in the funnel was rinsed with a small volume of acetonitrile and the volume of the total filtrate adjusted to 100 mL.

Cleanup. A 20-mL aliquot of the filtrate (equivalent to 5.0 g of sample) was transferred to a 100-mL round-bottom flask and evaporated under vacuum at 30 °C to an aqueous residue (~2 mL). To this was added 3 mL of H_2O and the contents were quantitatively transferred to a 125-mL separatory funnel containing 5 mL of 0.2 N H_2SO_4 and 10 mL of methylene chloride. The funnel was shaken by hand for 1 min and the layers were permitted to separate. The methylene chloride layer was discarded. Following this 10 mL of saturated sodium chloride solution

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was added to the aqueous phase and the solution was adjusted to pH 8–9 (pH paper) by the addition of ~3 g of sodium bicarbonate (the initial addition was made slowly in order to keep the resulting effervescence to a minimum). The solution was immediately extracted with 3 × 40 mL of methylene chloride. The organic extracts were combined and transferred to a 250-mL separatory funnel containing 50 mL of 0.2 N H_2SO_4. The contents were shaken and the methylene chloride layer was removed to a second 250-mL separatory funnel and the extraction was repeated twice more with fresh 50-mL volumes of 0.2 N H_2SO_4. To the combined acidic extracts were added 50 mL of saturated sodium chloride solution followed by ~8 g of sodium bicarbonate (added slowly as described above) to render the pH to 8–9. This solution was immediately partitioned with 3 × 60 mL of methylene chloride which were combined and passed through a small filter funnel containing a wad of glass wool and ~5 g of anhydrous sodium sulfate and collected in a 500-mL round-bottom flask. The dried extract was evaporated to dryness at 30 °C under vacuum. The flask was then flushed with a gentle stream of nitrogen in order to remove the last traces (including vapors) of methylene chloride. The residue was dissolved in an appropriate volume of distilled water for LC analysis.

Liquid Chromatography. An Altex Model 110A pump was used for solvent delivery. A Pye Model LC-3 variable-wavelength UV detector set at 254 nm (absorbance maximum for formetanate) and 0.8 absorbance unit full scale (AUFS) was used for detection. The 10-mV detector output was connected to a 1.0-mV recorder which resulted in a ×10 scale expansion. Thus the recorder full-scale pen deflection represented 0.008 AUFS. The column (25 cm × 4.6 mm i.d.) was packed with LiChrosorb RP-18 (10 μm). The mobile phase was 35% acetonitrile in 0.01 N $NH_4H_2PO_4$ (pH 8.0) filtered through a 0.45-μm filter and degassed by using a water aspirator vacuum for ~3 min before use. The flow rate was 1.0 mL/min. After the system stablized (20–30 min from initial warm-up), samples (normally 100 μL) were injected via a Rheodyne syringe-loop injector (100-μL loop) for analysis. The retention time of formetanate was ~8 min.

RESULTS AND DISCUSSION

Although formetanate is a methylcarbamate insecticide, its chemical and physical properties are different enough to exclude it from routine procedures for detecting other carbamates. Formetanate is a weakly basic compound which hydrolyses slowly in acidic media but considerably faster in neutral and basic aqueous solutions. Because of this, certain precautions in the extraction and cleanup procedure must be taken to ensure high recoveries. The hydrochloric acid (0.5%) in the initial extracting solvent facilitates the organic solubility of the formetanate and thus its removal from the fruit matrix. During the aqueous–organic partitioning the pH is adjusted to 8–9 before extracting the compound into methylene chloride. This stage must be carried out as quickly as possible (e.g., within 30 min) in order to reduce formetanate hydrolysis to an insignificant level. It was found that formetanate at 1 μg/mL in aqueous solution at pH 8 decomposed by 20% after 1 day at room temperature. Cooling the separatory funnel and its contents at this stage was used by Jenny and Kossman (1978); however, the present authors found this unnecessary if the extractions were carried out quickly. Also, we found that sodium carbonate (Jenny and Kossman, 1978) used for pH adjustment was less satisfactory than sodium bicarbonate due to the stronger basic nature of the former which resulted in poorer reproducibilities and slightly lower recoveries due to hydrolysis.

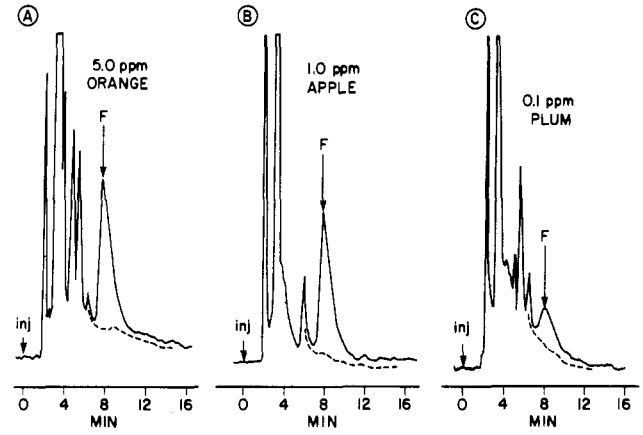

Figure 1. Chromatograms of various spiked fruit extracts. (A) Orange, 20 mg of equivalent sample injected. (B) Apple, 100-mg sample injected. (C) Plum, 250-mg sample injected. F indicates the response due to formetanate. The dashed line represents response from blank samples.

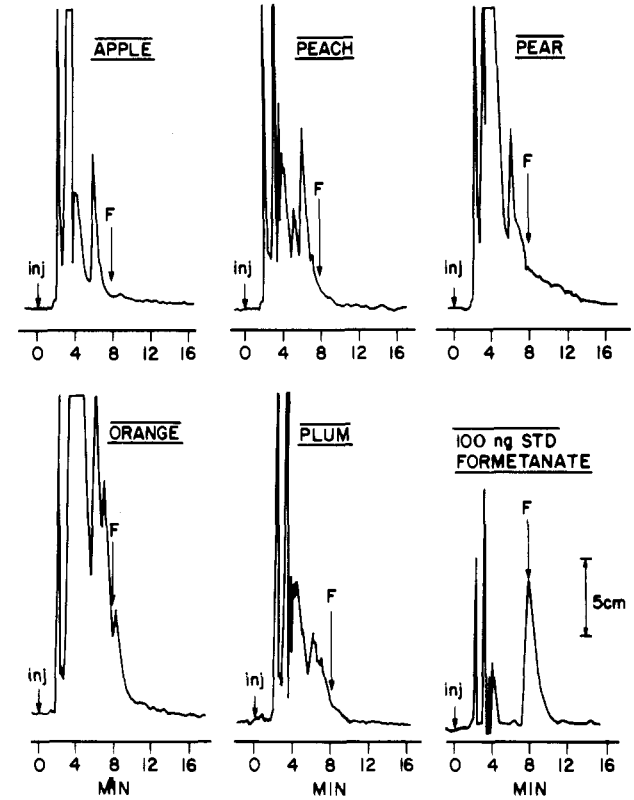

Figure 2. Typical chromatograms obtained during a limited formetanate survey. Quantities of sample injected were equivalent to 250 mg in all cases. The lower right chromatogram represents 100 ng of formetanate standard, which would be equivalent to a response in the samples of 0.4 ppm. Arrow indicates retention time of formetanate.

Because of its weakly basic nature, two possibilities were available for the analysis of formetanate by LC, namely, ion-pair chromatography and reversed-phase chromatography with ion suppression. The latter was chosen by us since all that was required was pH control of the mobile phase. We found that the chromatography was very reproducible although the peaks were somewhat broad. Standard solutions for LC were not prepared in the mobile phase since the instability of formetanate was such that ~20% was lost upon storage for 1 day.

The extraction procedure worked well for all of the fruit samples studied. It was found that for levels below 0.5

Table I. Recovery of Formetanate from Fresh Fruit

sample	recovery, %[a] 0.1 ppm	0.5 ppm	1.0 ppm	5.0 ppm	10.0 ppm
orange	69.3[b]	88.4	84.2	87.9	85.5
plum	81.4	93.8	95.0	91.0	-[c]
apple	79.2	83.5	89.5	89.6	85.2

[a] Average of duplicates. [b] Determination carried out with a mobile phase of 25% acetonitrile in 0.01 M monobasic ammonium phosphate. [c] No analyses carried out.

ppm the pH adjusted aqueous-organic partitions had to be repeated a second time in order to improve cleanup so that quantitative determinations could be made down to the 0.1-ppm level. In the final extract all traces of methylene chloride had to be removed from the residue since it interfered in the chromatography.

Figure 1 shows chromatograms of samples spiked at various levels. Table I lists the recoveries obtained at various spiking levels in three fruits. Of the samples studied, orange proved to have the most coextractives, although they did not interfere with the quantitation of formetanate near the tolerance level of 4.0 ppm (in both Canada and the United States). However, at 0.1 ppm the mobile phase was reduced to 25% acetonitrile in 0.01 M monobasic ammonium phosphate in order to better resolve the insecticide from proximate peaks. The plum samples occasionally contained a substance eluting ~30 s later than formetanate. In these instances the analyses were repeated with coinjection of 50 ng of formetanate standard in order to ensure that the unknown peak was not the pesticide.

Figure 2 shows typical results obtained from a limited survey. No formetanate was detected above the detection limit of 0.02-0.05 ppm in any of the 92 samples analyzed. Since tolerances in both Canada and the United States are in the range of 2-10 ppm for the fruits studied with the exception of plums which is 0.5 ppm in Canada, the described method is more than adequate for formetanate analysis in these foods.

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Determination of Norflurazon and Desmethylnorflurazon in Plant Tissue by High-Pressure Liquid Chromatography

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A general method for the determination of norflurazon and its major metabolite, *N*-desmethylnorflurazon, is described. The procedure was applied to the analysis of residues in eight major fruit crops on which the herbicide is registered. Mean recoveries were 94% for norflurazon and 79% for desmethylnorflurazon. A time-consuming Soxhlet extraction, utilized in existing methods, has been eliminated and extraction achieved by blending/partitioning. Residues are isolated by adsorption chromatography, and quantitative analysis is accomplished with a liquid chromatograph fitted with a reverse-phase column and UV-absorbance detector. Minimum detectable quantities for each residue were below 0.1 μg/g (ppm) in the crops tested.

The phenylpyridazinone herbicide, norflurazon, is effective in surface-applied, preemergence control of grasses, sedges, and broadleaf weeds in orchards (Sandoz, Inc., 1977). The compound is available in an 80% wettable powder formulation which is registered for use in apricots, cherries, nectarines, peaches, plums, prunes, and several varieties of nuts. Other formulations are marketed for cotton and cranberries.

Analytical methods have been described for the analysis of norflurazon and its desmethyl metabolite (Figure 1) in various matrices (Brady et al., 1978). Green crop samples and fruit are subjected to an overnight Soxhlet extraction, followed by solvent partitioning. Further purification by chromatography on alumina yields a sample suitable for quantitative analysis by electron capture gas chromatography.

The objective of the present work was to simplify the analysis of norfluazon and its desmethyl analogue in important edible crop matrices. Specifically, it was desirable to eliminate the time-consuming Soxhlet extraction step and to avoid a distinct solvent partitioning. Finally, the applicability of high-pressure liquid chromatography in the determinative step was evaluated. It was anticipated that the extended chromophore present in each residue would confer large extinction coefficients, allowing sensitive detection by ultraviolet absorbance.

EXPERIMENTAL SECTION

Chemicals. Authentic standards of norflurazon [4-chloro-5-(methylamino)-2-(α,α,α-trifluoro-*m*-tolyl)-3-(2*H*)-pyridazinone, I] and desmethylnorflurazon [4-chloro-5-amino-2-(α,α,α-trifluoro-*m*-tolyl)-3(2*H*)-pyridazinone, II] were provided by the U.S. Environmental Protection Agency (Research Triangle Park, NC) and Sandoz, Inc. (East Hanover, NJ), respectively. Commercially available, pesticide residue grade solvents were used; water for extraction purposes and chromatography was distilled and demineralized. Other chemicals were reagent grade.

Apparatus. High-pressure liquid chromatography (HPLC) was performed on a Varian Model 5021 instrument fitted with a 10-μm, reverse-phase, octadecylsilane

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