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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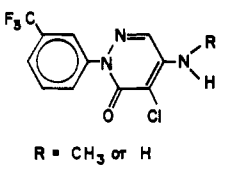

Figure 1. Norflurazon and *N*-desmethylnorflurazon.

column and a Tracor Model 910 A, variable-wavelength, absorbance detector. The mobile phase was acetonitrile–water (30:70 v/v) and total flow rates were 2.0 mL/min. The column oven was held at 30 °C. The chromatograph was equipped with a 200-μL injection loop. Absorption spectra were recorded on a Varian Model 219 UV–visible spectrophotometer.

Extraction/Partitioning. Each sample (20 g) was added to a blender jar with 50 mL of water, 2 mL of 2% aqueous sodium laurel sulfate, and 100 mL of dichloromethane. The mixture was homogenized for 2 min and transferred to a 200-mL centrifuge bottle. phase separation was complete after 5 min of low-speed centrifugation. The lower layer was removed with a 100-mL transfer pipet and added to a separator. The sample was returned to the blender jar with 100 mL of fresh solvent and the extraction/partition process repeated. The combined extract was dried by passage through anhydrous sodium sulfate (~1 tsp retained in a funnel with filter paper) and collected in a 500-mL round-bottom flask. The separator was rinsed with 50 mL of dichloromethane and the rinsate used to wash the drying agent. The extract was reduced in volume to ~5 mL on a rotary vacuum evaporator, made to ~50 mL with petroleum ether, and again concentrated to ~5 mL; the water bath did not exceed 40 °C.

Florisil Fractionation. An activated Florisil column (100 mm; 60–100 mesh; PR grade) was prepared in a 21-mm (i.d.) glass column and topped with ~25 mm of anhydrous sodium sulfate. Florisil was activated by storage at 130 °C prior to use. The column was rinsed with 50 mL of petroleum ether and the sample transferred to the column quantitatively by rinsing the flask with 2 × 5 mL of petroleum ether. The column was eluted with the following series and fraction A discarded: for fraction A, 50 mL of petroleum ether, 50 mL of 6% diethyl ether in petroleum ether, 50 mL of 15% diethyl ether in petroleum ether, 50 mL of 50% diethyl ether in petroleum ether, and 50 mL of diethyl ether; for fraction B, 75 mL of acetone. The eluant flow rate did not exceed 3 mL/min.

Fraction B, containing norflurazon and desmethylnorflurazon, was taken just to dryness on a rotary evaporator and redissolved in 1.5 mL of acetonitrile with thorough agitation. Immediately prior to analysis the sample volume was adjusted to 5.0 mL by addition of water.

Quantitation. A 200-μL aliquot of the sample was subjected to analysis by HPLC. Absolute calibration was accomplished by comparison of peak areas (or heights) with those of a nanogram per microliter mixed standard prepared in acetonitrile–water (30:70 v/v); detector responses for I and II were linear between 100 ng and 10 μg by peak height or area. Detection was by UV absorption at 234, 254, or 285 nm.

Recovery Determination. Weighed samples were added directly to blender jars. Fortification was accomplished by addition of microliter volumes of milligram per milliliter or 0.1 mg/mL standard solutions of I and II in acetone. These "spiked" samples and duplicate untreated samples were subjected to the analytical procedure. Recovery values reflect subtraction of any interference appearing in the sample blank; this correction was only required occasionally for the desmethyl compound. Some samples were fortified and stored for 5 months at −5 °C to determine the stability of residues during prolonged storage.

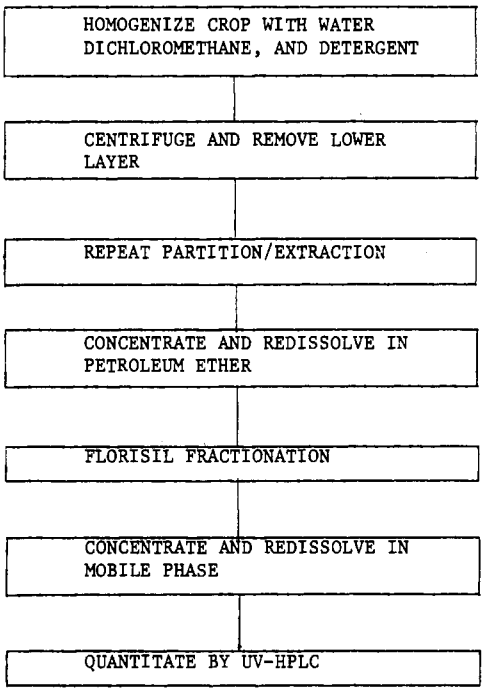

Figure 2. Analysis scheme.

RESULTS AND DISCUSSION

Extraction/Partitioning. The analytical procedure is outlined in Figure 2. The initial extraction made use of the favorable partitioning of I and II between water and dichloromethane. Sample maceration, dispersal, and partitioning were facilitated by the introduction of water and detergent. Emulsions developed during blending, but centrifugation resulted in complete phase separation within 5 min. Three layers were commonly observed: aqueous supernatant, sediment in the lower aqueous layer, dichloromethane layer. Pigments including anthocyanins and other flavonoids remained in the aqueous layer; carotenoids and chlorophyll were extracted. Nut and seed crops may require defatting (Brady et al., 1978) and therefore are not directly amenable to the extraction/partitioning process.

Florisil Separation. Certain crops were found to contain a natural product which interfered with the determination of I by reverse-phase HPLC. Sample cleanup by adsorption chromatography on Florisil eliminated the interfering component. Although the elution sequence is involved, the separation has advantages in that I and II elute in the same fraction, interferences for I are removed, and both residues are isolated in good yield. Prior to chromatography on Florisil the dichloromethane extractives were redissolved in petroleum ether. Precipitates often formed due to the large change in solvent polarity, but recoveries were not adversely effected.

Liquid Chromatography. After the sample eluate was stripped of acetone, an excellent UV absorber, the residue was resuspended in acetonitrile and diluted with water. A large sample aliquot was injected onto the HPLC to achieve low detection limits. Due to the large injection volume, column efficiency and band shape were adversely affected if the sample was not dissolved in a solvent of composition similar to that of the mobile phase. Some samples were clarified by filtration with a small plug of glass wool held in a disposable pipet. By use of the

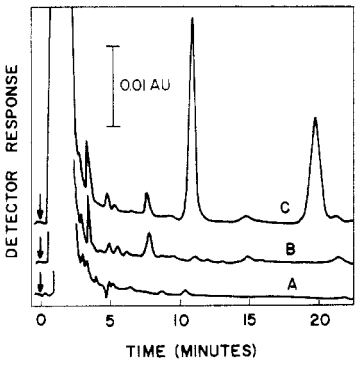

Figure 3. Representative chromatograms: (A) method blank; (B) untreated cherry sample; (C) cherry sample fortified with 1.0 μg/g norflurazon and desmethylnorflurazon. Detection by absorbance at 285 nm.

Table I. Recovery of Norflurazon and *N*-Desmethylnorflurazon from Various Crops

crop	fortified level, μg/g		recovery, %	
	norflurazon	desmethylnorflurazon	norflurazon	desmethylnorflurazon
apricot	1.0	1.0	104	76
blueberry	1.0	1.0	112	94
	0.10	0.10	75	66
cherry	1.0	1.0	100	81
cranberry	1.0	1.0	76	66
	0.10	0.10	96	85
nectarine	1.0	1.0	104	76
peach	1.0	1.0	109	84
plum	1.0	1.0	111	85
prune	1.0	1.0	75	58
prune[a]	1.0	1.0	104	100
blueberry[b]	0.10	0.0[c]	89	nd[d]
(freezer	0.10	0.0	89	nd
study)	1.0	0.0	80	nd
	1.0	0.0	90	nd

[a] 5-g sample. [b] Crop samples fortified and stored for 5 months at −5 °C. [c] Standard not available at the time of fortification. [d] Not detected.

chromatographic parameters described, norflurazon exhibited a retention time of ~20 min while the more polar desmethyl analogue eluted in 11 min.

Recovery Determination. Norflurazon and desmethylnorflurazon were successfully analyzed in eight fruit crops without modification of the method described. Representative chromatograms are shown in Figure 3. The recovery of I was consistently higher than that of II (Table I), although both were acceptable. II may be extracted less efficiently due to its polarity and may also be subject to chemisorption on the chromatographic adsorbent. Recovery of I and II from the dried fruit sample (prune) was improved significantly by reducing the sample size subjected to extraction. Norflurazon residues were not diminished by extended storage at reduced temperature; no data are available on the storage stability of the desmethyl analogue.

Limits of Detection. The sensitivity with which a compound can be detected spectrophotometrically is determined by the compound's extinction coefficient. I and II each exhibited absorption maxima at 234 and 285 nm (Figure 4). Extinction coefficients were large in the near-UV due to the presence of a highly conjugated, auxochromic substituent on the aromatic ring; log ε values for I and II at 285 nm were 4.18 and 4.16, respectively. Although sensitivity was adequate when monitoring the column effluent at 254 nm, optimum detector response was

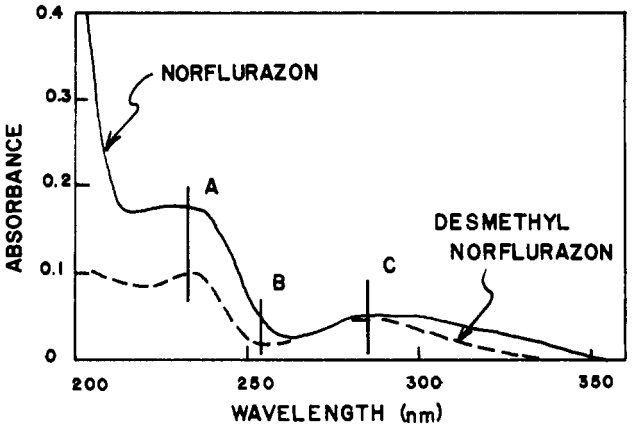

Figure 4. Absorption spectra of norflurazon and desmethylnorflurazon in methanol: (A) 234 nm; (B) 254 nm; (C) 285 nm.

Table II. Background Response for *N*-Desmethylnorflurazon in Various Crops at 234, 254, and 285 nm

crop	background[a] at		
	234 nm	254 nm	285 nm
apricot	0.02	0.1	0.01
blueberry	0.04	nd[b]	nd
cherry	0.007	0.1	0.01
cranberry	0.01	0.04	0.06
nectarine	0.01	nd	0.01
peach	0.02	0.06	0.02
plum	0.01	0.1	nd
prune	0.007	0.1	0.03

[a] Detector response converted to micrograms per gram. [b] Not detected.

attained at the absorption maxima (Figure 4).

Unlike norflurazon, endogenous compounds were detected in some fruits which interfered with desmethylnorflurazon. The background absorbance was weak, however, and could be minimized or eliminated by monitering the optimum wavelength (Table II). For blueberries and nectarines, no interference was detected at 254 nm. Blueberries and plums were "blank" at 285 nm. In the remaining crops the matrix response was between 0.007 and 0.02 μg/g at the optimum wavelength. These values are considerably lower than the working detection limit for compound II, 0.1 μg/g.

The limit of detection for compounds I and II was below 0.1 μg/g in blueberries and cranberries (Table I); detection limits were not tested experimentally in the remaining crops.

CONCLUSION

The analytical procedure described has been shown to be useful for the analysis of norflurazon and *N*-desmethylnorflurazon on eight fruit crops on which the herbicide is registered. Recoveries for each residue were high and their detection limits were below 0.1 μg/g. The need for a time-consuming Soxhlet extraction and discrete solvent partitioning step has been eliminated, considerably decreasing analysis time relative to an existing method.

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