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Note

Analysis of the pesticide flufenoxuron in apples and kiwifruit by high-performance liquid chromatography

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Flufenoxuron, 1-{4-[2-chloro-4-(trifluoromethyl)phenoxy]-2-fluorophenyl}-3-(2,6-difluorobenzoyl)-urea, is an acylurea compound currently being evaluated for control of insects and mites attacking a range of important crops[1,2]. The compound is a slow-acting growth regulator[3] which inhibits the synthesis of chitin[1,4] in these pests causing death without producing a detrimental effect on important predatory mites[2].

As part of efficacy trials, we were required to measure residues on fruit harvested at varying times after spray application.

The methods available were laborious[5–7]. Sample extraction was followed by a multi-step clean-up involving three or four evaporations plus either multiple partitions or a solid-phase clean-up cartridge, plus fractionation by reversed-phase high-performance liquid chromatography (HPLC). Final analysis was achieved using an alternative HPLC system.

This report describes a very simple and rapid method which limits clean-up to a single liquid–liquid partition and evaporation. This has been achieved by changing the extraction solvent and by using a more selective partition solvent. The extracts are analysed by reversed-phase HPLC producing good recoveries (81–117%) and low detection limits ($\leqslant$ 0.005 mg/kg).

EXPERIMENTAL

Chemical standards

A sample of flufenoxuron (97.6%) was obtained from Shell Research (Sittingbourne, U.K.). A stock solution of the standard was made accurately in methanol at about 200 μg/ml, and stored at 2°C. Dilute solutions at 10 and 1 μg/ml in methanol were made and stored similarly at 2°C. These dilute solutions were used for spiking samples and for the preparation of analytical standards which were prepared in methanol–water (80:20).

Reagents

Solvents were HPLC grade. Water was distilled in glass then passed through a Millipore Milli-Q water purifier.

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Apparatus

HPLC was performed using a Shimadzu LC-6A gradient liquid chromatography system with variable-wavelength UV absorption detector, set to 254 nm, and autosampler. Data aquisition was by peak height from a Shimadzu Chromatopac C-R3A. The analytical column, a 5-μm Zorbax ODS (25 cm × 4.6 mm I.D.) (DuPont, Wilmington, DE, U.S.A.), was preceded by a 2-μm in-line filter (Rheodyne, Cotati, CA, U.S.A.) and an MPLC RP-8 guard column (Brownlee Labs., Santa Clara, CA, U.S.A.).

Sample extraction and clean-up

Samples (fresh fruit) were stored deep frozen (−15°C) prior to analysis. The thawed fruit was finely diced and mixed in a Hobart food chopper. A subsample (50 g) was macerated with methanol (100 ml) for 4 min and filtered through a Buchner funnel and glass fibre filter paper (Whatman GFA). An aliquot (15 ml) of the filtrate (which is in approximately 10:4 methanol–water due to the water contribution from fresh fruit) was pipetted into a clean test tube. Water (10 ml) and hexane (10 ml) were added to the test tube which was stoppered and shaken. An aliquot (4 ml) of the upper hexane layer was blown to dryness with nitrogen at 50°C. The residue was dissolved in methanol (0.8 ml) and water added (0.2 ml).

The calculation factor required inclusion of both the water contribution from the fresh fruit (mean dry matter content 16%, range 13–19%), and the volume contractions (*ca.* 2.8%) caused by mixing methanol and water. The final analysis solution was thus calculated to be equivalent to 2.17 g of fresh fruit per ml of solution.

HPLC conditions

The mobile phase was acetonitrile–water (74:26) run at 1 ml/min. The detector was set at 254 nm. Injection size was 100 μl for standards and samples. Flufenoxuron retention time was 11.3 min (capacity factor, $k' = 4.5$), and a 0.1 μg/ml standard solution gave 20% of full scale deflection at 0.005 a.u.f.s. Kiwifruit samples were run in groups of three with injections at 12.5-min intervals; the third followed by a solvent programme to flush late eluting peaks from the column. In this case the solvent was changed to 100% acetonitrile over 5 min and held there for 5 min until reset to initial conditions. The apple samples had no late eluting peaks and were run isocratically.

RESULTS AND DISCUSSION

The method for sample extraction and clean-up was chosen after several options were tested, including the use of different absorbants in clean-up columns and alternative solvent combinations for extraction and single step partition. The resultant chromatograms after column clean-up were unsatisfactory due to impurities interfering with the peak of interest. They also contained a number of late eluting peaks.

The best results were obtained from a methanol extraction with the addition of water and partition of the flufenoxuron into hexane. The ratio of water to methanol was found to be a critical factor in determining the percentage of flufenoxuron recovered in the hexane layer. Spiked solutions were made with an increasing volume of water to methanol, enabling the percentage recovery to be manipulated from 3.7%

TABLE I

PERCENTAGE RECOVERIES OF FLUFENOXURON INTO HEXANE PARTITION FROM SPIKED METHANOL–WATER MIXTURES

Ratio of methanol–water (v/v)	*Recovery of flufenoxuron in hexane partition (%)*
9:1	4
3:1	26
2:1	49
1:1	98
1:2	103

(methanol–water, 9:1) to near 100% (methanol–water, 1:1) as illustrated in Table I. Substituting water with saline solution (10%, w/v) did not improve the recoveries, and chromatograms of fruit extracts showed no reduction of co-extracted components. The chosen partition conditions employ a methanol–water ratio of approximately 1:1.4.

The HPLC conditions elute flufenoxuron clear of co-extracted components for both apple and kiwifruit extracts. Recoveries for replicate spiked samples of both apples and kiwifruit from several analytical runs were between 81–93% (0.1 mg/kg), 86–101% (0.05 mg/kg) and 84–117% (0.01 mg/kg).

Fig. 1 illustrates the analysis of apple extracts. Analysis of a field-treated sample with 0.005 mg/kg of flufenoxuron is shown in Fig. 1c. This peak represents a solution

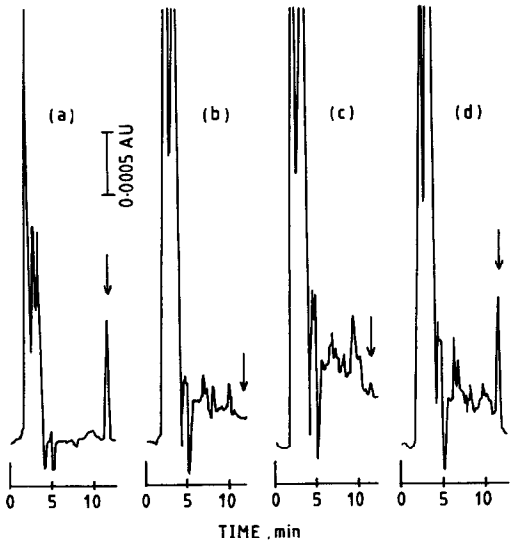

Fig. 1. Analysis of flufenoxuron in apple extracts. (a) Standard solution of flufenoxuron (0.1 μg/ml) in methanol–water (8:2); (b) extract of untreated apple; (c) extract of field-treated apple containing 0.005 mg/kg of flufenoxuron; and (d) extract of untreated apple spiked at 0.05 mg/kg flufenoxuron (95% recovery). Chromatographic conditions as in text including detection at 254 nm and 0.005 a.u.f.s. The arrow indicates the retention time of flufenoxuron.

concentration of 0.01 μg/ml and is close to the detection limit of the method (ca 2% f.s.d. at 0.005 a.u.f.s.; signal-to-noise ratio of at least 10), although estimates of lower levels were generally possible. Fig. 1b shows the analysis of apples untreated with flufenoxuron.

This procedure provides the basis for a sensitive, rapid and versatile method for the analysis of flufenoxuron in fruit. It illustrates the use of a simplified extraction and clean-up which should be suitable for residue analysis of similar acylurea compounds.

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