Journal of Chromatography, 391 (1987) 338–342
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 19 294

Note

Determination of fenpropimorph in citrus fruit by reversed-phase high-performance liquid chromatography

J. L. TADEO* and M. T. LAFUENTE
Instituto Valenciano de Investigaciones Agrarias (I.V.I.A.) Moncada, Valencia (Spain)
(Received November 26th, 1986)

Fenpropimorph, 4-{3-[4-(1,1-dimethylethyl)phenyl]-2-methyl}propyl-2,6-dimethylmorpholine, is a new ergosterol inhibitor fungicide[1,2] with high activity against fungi causing citrus decay, including resistant strains to benzimidazole fungicides[3,4].

After application, post-harvest fungicides remain mainly in the citrus peel with a low penetration into the fruit[5]. The determination of residue levels in different parts of the fruit is of great importance in studies of fungicide efficiency in disease control and also for toxicological reasons.

A gas chromatographic method for the determination of fenpropimorph residues using a nitrogen–phosphorus detector[6] has recently been developed. This paper describes an alternative method for determining fenpropimorph in whole fruit, peel, albedo or pulp of citrus by high-performance liquid chromatography (HPLC) with UV detection.

EXPERIMENTAL

Reagents and chemicals

All reagents and solvents employed were analytical-reagent grade (Panreac, Spain). Hexane and methanol were redistilled in glass. A standard of fenpropimorph (94.5% purity) was obtained from Maag (Dielsdorf, Switzerland).

Apparatus

A Hewlett-Packard Model 1084B liquid chromatograph equipped with a 20-μl loop injector, a variable-wavelength UV detector (HP 79875A) and a Model 79850B recorder was used. HPLC was performed at ambient temperature on an RP-18 (10 μm) stainless-steel column (20 × 0.46 cm I.D.). The mobile phase was methanol–water (87:13) plus 0.25% of ammonia. The flow-rate and chart speed were set at 1.3 ml/min and 0.25 cm/min, respectively. The attenuation was 32 AU · 10^{-4}/cm and the wavelength was fixed at 215 nm. The UV spectrum of fenpropimorph was recorded on a Perkin-Elmer 550 S spectrophotometer. A DuPont Sovall Omni-Mixer was used as a homogenizer.

Sample preparation

Fruit of two citrus varieties, "Washington Navel" oranges and "Hernandina"

0021-9673/87/$03.50

clementines, were used. A representative ground sample (25 g) was homogenized three times in 75 ml of hexane, after addition of 5 g of anhydrous sodium sulphate and 3 ml of 2 *M* sodium hydroxide solution. The homogenates were filtered under vacuum through a Whatman No. 1 filter-paper and the filtrate was vacuum concentrated. The remaining solution was transfered into a separating funnel and washed with 20 ml of sodium carbonate–sodium hydrogen carbonate buffer (pH 9.2), which washings were subsequently extracted with additional hexane. The organic layers were filtered through anhydrous sodium sulphate, evaporated to dryness, and the resulting residue was dissolved in an adequate volume of methanol for HPLC analysis. The fungicide concentration was calculated by comparing the peak areas obtained for samples with those obtained for standards.

Preparation of standard solutions

A 100 mg amount of fenpropimorph was dissolved in 100 ml of methanol. Standard solutions containing 0.3–10 μg/ml of fenpropimorph in methanol were prepared by taking a suitable aliquot from the concentrated standard.

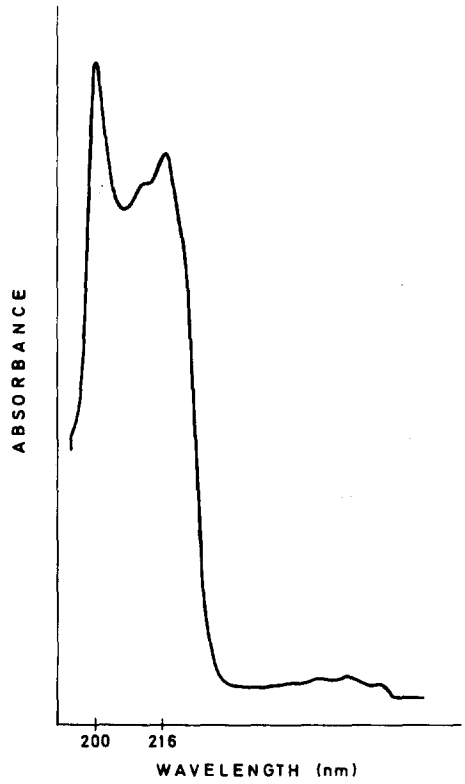

Fig. 1. UV spectrum of fenpropimorph standard solution in the mobile phase [methanol–water (87:13) plus 0.25% NH_3].

RESULTS

Spectral data

The UV spectrum of fenpropimorph in the mobile phase shows two intense bands at 200 nm (ε_{max} = 11 400 l mol^{-1} cm^{-1}) and 216 nm (ε_{max} = 9618 l mol^{-1} cm^{-1}) (Fig. 1). Fenpropimorph was determined at 216 nm because at this wavelength a better signal-to-noise ratio and a lower level of possible interfering substances were obtained.

Selection of mobile phase

Fig. 2 shows the changes in the retention time of fenpropimorph with variation in the proportion of methanol in the mobile phase. The elution of fenpropimorph is clearly delayed when the proportion of methanol in the mobile phase decreases. The

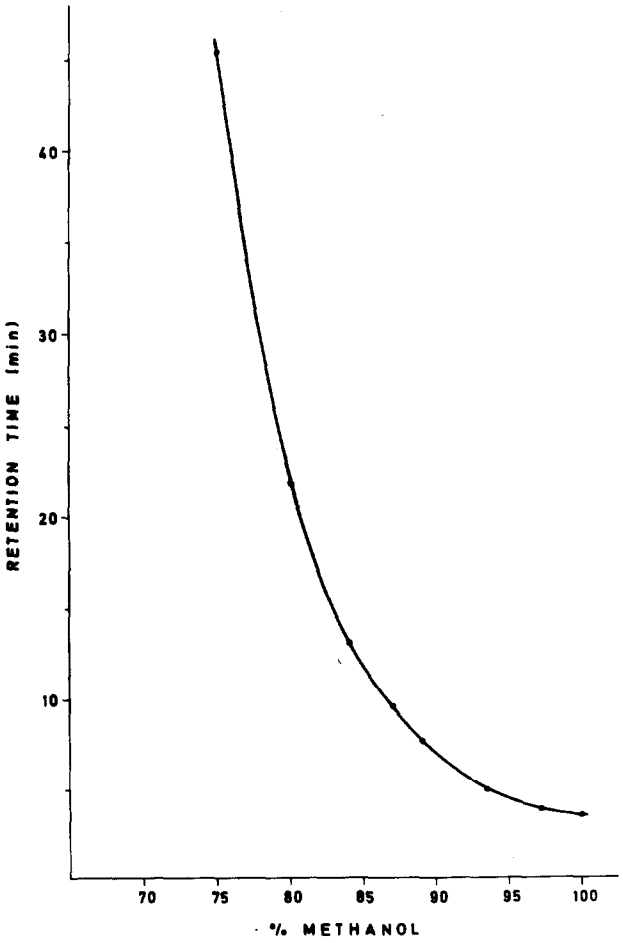

Fig. 2. Effect of percentage of methanol in the mobile phase on the retention time of fenpropimorph. Column, 20 × 0.46 cm I.D. RP-18; mobile phase, methanol–water plus 0.25% NH_3.

retention time varies from nearly 4 min (100% methanol) to 40 min (76% methanol). As interfering substances are not so strongly retained, the different elution pattern can be used to avoid laborious purification procedures. Under the proposed chromatographic conditions, using methanol–water (87:13) at a flow-rate of 1.3 ml/min, the retention time of fenpropimorph was 9.6 min. If the proportion of methanol in the mobile phase was higher than 89% the time of analysis decreased, but it was impossible to separate fenpropimorph from interfering substances.

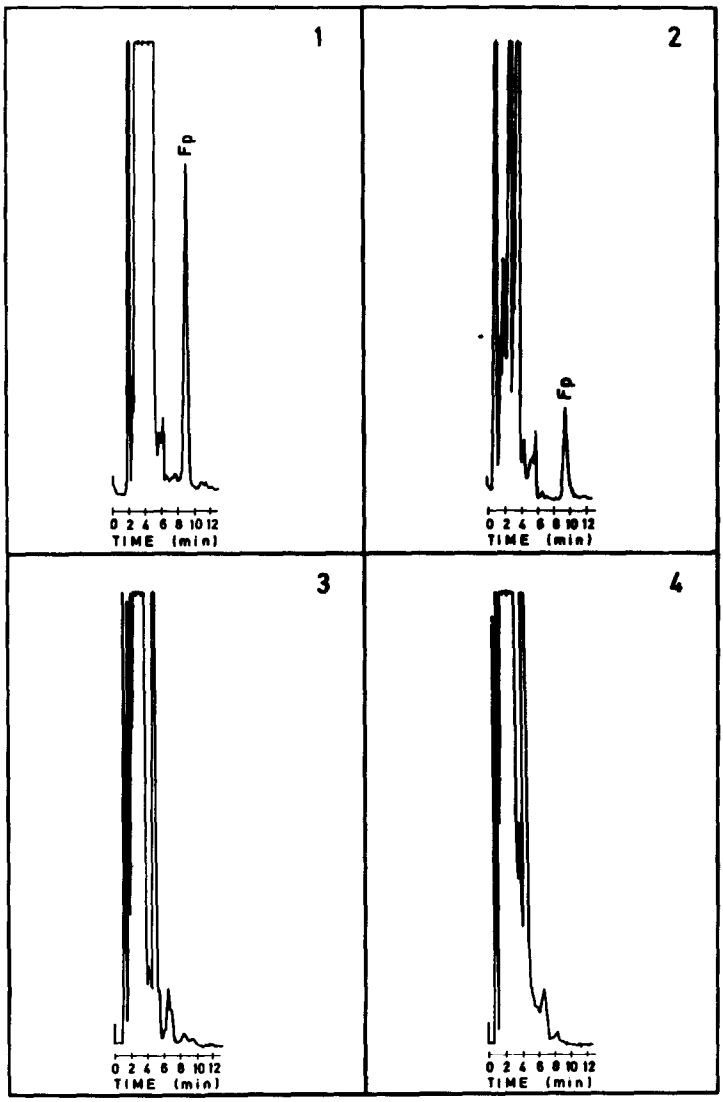

Fig. 3. HPLC chromatograms of fenpropimorph: 1, albedo of treated Washington Navel fruits (0.47 mg/kg); 2, pulp of treated Hernandina fruits (0.08 mg/kg); 3, whole fruit of control Hernandina fruits; 4, peel of control Washington Navel fruits.

TABLE I

RECOVERY OF ADDED FENPROPIMORPH FROM DIFFERENT PARTS OF CITRUS FRUIT

Sample	*Added (μg/g)*	*No. of analyses*	*Recovery ± standard deviation (%)*
Peel	2	4	84 ± 4
Whole fruit	1	4	74 ± 2
Albedo	0.5	3	91 ± 4
Pulp	0.05	4	78 ± 3

In a neutral mobile phase, fenpropimorph showed peak tailing. In order to solve this problem, probably due to the basicity of the morpholine ring, the mobile phase was modified by adding 0.25% ammonia. Fig. 3 shows some representative chromatograms of control and treated samples obtained in this study.

Linearity

The detector response was linear from 0.3 up to at least 10 μg/ml of fenpropimorph. The linearity of the method was checked by injecting three times each standard containing 0.3, 1, 2, 4, 7 and 10 μg/ml of fenpropimorph dissolved in methanol. The calibration graph showed a good correlation coefficient (r = 0.9998) between peak area and fungicide concentration. The slope was 2.70 and the intercept 0.139.

Recovery and limit of detection

Table I shows the fungicide recoveries from different parts of citrus fruit obtained using the proposed method. To study the recovery, known amounts of fenpropimorph in the range of 0.05–2 μg/g fresh weight were added to control samples, followed by analysis using the proposed procedure. The average recovery was always higher than 70% with a standard deviation, within each kind of sample, lower than 5%.

The limit of detection, defined as that producing a signal-to-noise ratio of 2, was 0.03 μg of fenpropimorph per gram of sample, based on a 25 g sample.

CONCLUSION

The proposed HPLC method is simple, rapid, reproducible and sensitive. The limit of detection allows the determination of fenpropimorph in citrus fruit at residue levels.

REFERENCES

1 R. I. Baloch, E. I. Mercer, T. E. Wiggings and B. C. Baldwin, *Phytochemistry*, 20 (1984) 2219.
2 P. Leroux and M. Gredt, *Agronomie*, 3 (1983) 123.
3 J. W. Eckert, B. F. Bretschneider and M. Ratnagake, *Proc. Int. Soc. Citricult.*, 2 (1981) 804.
4 Y. Gutter, *Proc. Int. Soc. Citricult.*, 2 (1981) 810.
5 M. T. Lafuente and J. L. Tadeo, *Int. J. Environ. Anal. Chem.*, 22 (1985) 99.
6 M. T. Lafuente, J. L. Tadeo and J. J. Tuset, *J. Assoc. Off. Anal. Chem.*, 69 (1986) 859.