Journal of Chromatography, 174 (1979) 269–271
© Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 11,792

Note

High-performance liquid chromatographic analysis of ethoxyquin in apples

G. F. ERNST and S. Y. VERVELD-RÖDER
Food Inspection Service, Nijenoord 6, 3552 AS Utrecht (The Netherlands)
(Received February 13th, 1979)

Ethoxyquin (1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline) is used as a post-harvest dip to prevent scald on apples and pears. The acute oral LD_{50} to rats is 0.8–1.0 g/kg bodyweight[1]. Most of the methods described for the determination of ethoxyquin in feed and food products are based on fluorimetric principles adapted to paper, column and thin-layer chromatography or spectrophotometric equipment[2,3]. Dahle and Skaare[4] reported a gas chromatographic method for the determination of ethoxyquin.

A direct method, avoiding prior clean-up, has obvious advantages for routine analysis and the development of such a method is described here. This method is based on high-performance liquid chromatography with fluorimetric detection.

EXPERIMENTAL

Chemicals and reagents

Aluminium oxide (Woelm, W 200 basic, activity super I, particle size 70–290 mesh) was activated at 200° for about 2 h, then cooled to room temperature in a desiccator. It was then deactivated with water as follows. Water was distributed on the inside of a glass-stoppered bottle, activated aluminium oxide was added in an amount to make the proportions of aluminum oxide and water 95:5 (w/w) and the bottle was shaken for several minutes. The aluminium oxide could be used after 1 h, with occasional shaking prior to use.

For column chromatography, glass columns (500 × 6 mm I.D.) were used, to which quartz-wool, 10 g of aluminium oxide freshly prepared as described above and 1 g of anhydrous sodium sulphate were added successively.

Ethoxyquin (Koch-Light, Colnbrook, Great Britain) was dissolved in acetone (the stock solution obtained was stable for several weeks in a refrigerator in a brown bottle); dilution with methanol yielded standard solutions containing 50–200 ng of ethoxyquin per 20 µl. All of the reagents were of analytical-reagent grade. Methanol (Nanograde; Mallinckrodt, St. Louis, Mo., U.S.A.) and distilled water, used as eluents in liquid chromatography, were pre-filtered through a G-1 glass filter.

Apparatus

A Hewlett-Packard Model 1084A liquid chromatograph equipped with a variable-wavelength fluorimeter, Model Schoeffel FS 970 L.C., was used. The column

(stainless steel, 250 × 4.6 mm I.D.) was packed with Spherisorb 10 ODS (10 μm). The chromatographic conditions were as follows: mobile phase, water–methanol (20:80); flow-rate, 2.5 ml/min; temperature ambient; detection wavelength, 345 nm (excitation) and 470 nm (emission).

Extraction

Apple samples were cut in a food cutter and extracted by macerating 100 g of apple with 200 ml of *n*-hexane and 25 g of anhydrous sodium sulphate in an Ultra Turrax at moderate speed. The macerate was centrifuged for 5 min at 2500 *g* and the extract was collected. A 100-ml volume of the extract was evaporated to dryness by means of a rotary vacuum evaporator. The residue was dissolved in 2 ml of methanol, warmed to 50° and immediately cooled to remove waxes. The solution was filtered through a G-2 glass filter.

Clean-up (optional)

A 100-ml volume of the extract was evaporated to dryness by means of a rotary vacuum evaporator. The residue was transferred quantitatively into an aluminium oxide column, using about 2 ml of diethyl ether. The column was eluted with 60 ml of diethyl ether–acetonitrile (95:5), the eluate was evaporated to dryness and the residue dissolved in 2 ml of methanol. The solution was warmed to 50°, immediately cooled to remove waxes and filtered through a G-2 glass filter.

High-performance liquid chromatography

A 20-μl volume of the solution was injected into the liquid chromatograph. Standard solutions containing 50–200 ng of ethoxyquin per 20 μl were also injected.

RESULTS AND DISCUSSION

A chromatogram of ethoxyquin is shown is shown in Fig. 1. Although the

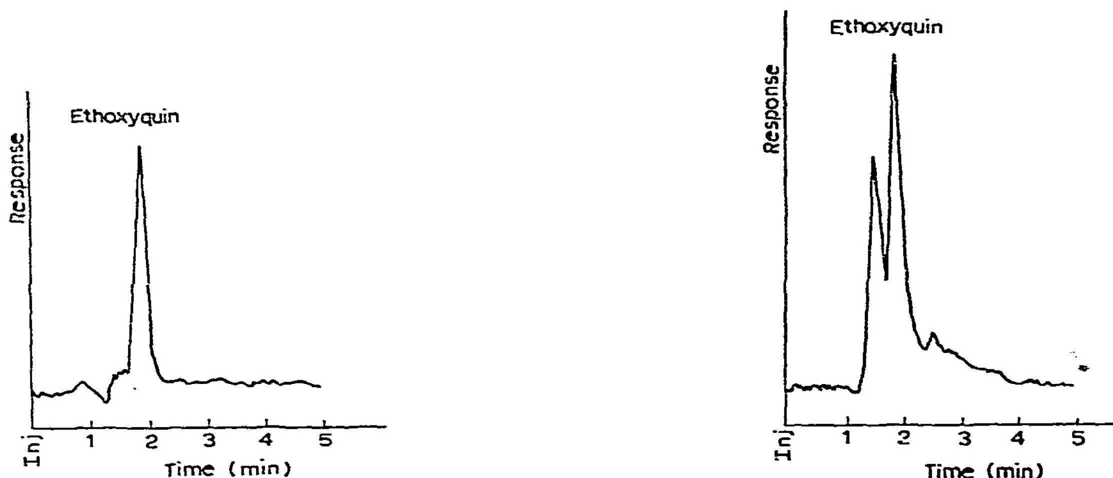

Fig. 1. Chromatogram of 50 ng of ethoxyquin standard. Column, 250 × 4.6 mm I.D., Spherisorb 10 ODS, 10 μm; mobile phase, water–methanol (20:80); flow-rate, 2.5 ml/min; temperature, ambient, injection volume, 20 μl; detection, excitation at 345 nm, emission at 470 nm.

Fig. 2. Chromatogram of ethoxyquin from apple without clean-up. Conditions as in Fig. 1.

chromatogram is free from interference at the point of elution of ethoxyquin, some apple varieties give an interfering peak just in front of the ethoxyquin peak (Fig. 2). The aluminium oxide column clean-up eliminates this interference.

Experiments with market samples containing ethoxyquin showed that extraction with dichloromethane and methanol is as efficient as with *n*-hexane.

The method was shown to give a linear detector peak height response over an ethoxyquin range of 0.05–0.25 mg/kg, and a concentration of 0.05 mg/kg gave a peak height of 10% full-scale deflection at a detector sensitivity of 0.1 a.u.f.s.

The recovery of ethoxyquin was determined by analysing apple samples spiked with ethoxyquin at levels from 0.1 to 1.0 mg/kg; the results are shown in Table I. The limit of detection was about 0.05 mg/kg.

TABLE I

RECOVERY OF ETHOXYQUIN FROM BLANK APPLE TREATED WITH ETHOXYQUIN STANDARD SOLUTIONS WITH CLEAN-UP PROCEDURE

Average values are given for three recovery experiments, each carried out with five concentrations of ethoxyquin.

Ethoxyquin added to apple (ppm)	*Average recovery (%)*
0.1	97.6
0.2	98.2
0.4	98.1
0.8	98.8
2.0	98.2

REFERENCES

1 D. E. H. Frear, *Pesticide Index*, State College Science Publishers, Pa., 4th ed., 1969, p. 218.
2 H. R. Weilenmann, J. Hurter, K. Stoll and A. Temperli, *Lebensm. Wiss. Technol.*, 5 (1972) 106.
3 S. C. Witt, E. M. Bickhoff and G. O. Kohler, *J. Ass. Offic. Anal. Chem.*, 56 (1973) 167.
4 H. K. Dahle and J. U. Skaare, *J. Agr. Food Chem.*, 23 (1975) 1093.