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## Note

# Gas chromatographic determination of bupirimate in apples and pears

G. F. ERNST, R. HEUTINK and S. Y. VERVELD-RÖDER
*Food Inspection Service, Nijenoord 6-3552 AS Utrecht (The Netherlands)*
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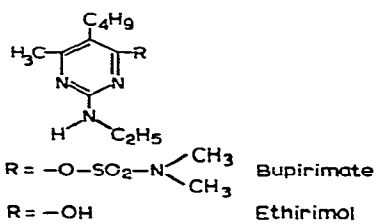
Bupirimate, a substituted pyrimidine (a sulphamate ester of ethirimol, 5-butyl-2-ethylamino-6-methylpyrimidin-4-yl-dimethylsulphamate), is a local systemic fungicide that is effective in controlling mildew in roses and apples. It has no significant insecticidal properties and is not poisonous to mites[1].

Bupirimate formulations are commercially available in the Netherlands as ICI Ivosta Anti-Meeldauw (6826 N), Nimrod spuitpoeder (6827 N) and Nimrod vloeibaar (6834 N)*. The Dutch tolerance for residues of bupirimate in apples and pears is 0.2 mg/kg, of which at the most 0.1 mg/kg is the metabolite ethirimol (5-butyl-2-ethyl-amino-4-hydroxy-6-methylpyrimidine)[2].




Up to now a direct method to determine bupirimate in apples has not been described in the literature. This paper describes such a method, which includes prior clean-up by column chromatography followed by gas chromatography using an alkali flame ionization detector (AFID) as well as a flame photometric detector equipped with a sulphur filter (394 nm).

## EXPERIMENTAL

### *Chemicals and reagents*

Aluminium oxide (Woelm, Eschwege, G.F.R., W 200 neutral, activity super I, particle size 70–290 mesh) was activated at 200° for *ca.* 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 sufficient amount to make the ratio of aluminium oxide to water 95:5

* Registered at the Ministry of Agriculture and Fishery of the Netherlands (reg. Nos. 10321T, 9903T and 9902T, respectively).

(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 × 8 mm I.D.) were used, to which quartz-wool, 5 g of freshly prepared aluminium oxide and 1 g of sodium sulphate were added successively.

Bupirimate (Dr. S. U. I. Ehrenstorfer, Augsburg, G.F.R.) was dissolved in ethyl acetate; dilution with ethyl acetate yielded standards solutions. All of the reagents were of analytical reagent grade.

*Apparatus*

*Sulphur mode.* A Tracor MT-220 gas chromatograph was equipped with a Melpar 200 AT flame photometric detector (394 nm sulphur filter) and a Honeywell FB 80 recorder with a sensitivity of 1 mV. The column (pyrex U-shape, 75 × 0.3 cm I.D.) was packed with 10% OV-101 on Gas-Chrom Q 60–80 mesh. The chromatographic conditions were as follows: injector temperature, 225°; detector temperature, 200°; column temperature, 215°; carrier gas (nitrogen) flow-rate, 50 ml/min; hydrogen flow-rate, 150 ml/min; oxygen flow-rate, 15 ml/min; air flow-rate, 40 ml/min; chart speed, 5 mm/min.

*Nitrogen mode.* A Hewlett-Packard 5710A gas chromatograph was equipped with a dual nitrogen–phosphorus flame ionization detector model 18789A and a Honeywell FB 80 recorder with a sensitivity of 1 mV. The column (pyrex, 50 × 0.3 cm I.D.) was packed with 10% OV-101 on Gas-Chrom Q 60–80 mesh. The chromatographic conditions were as follows: injector temperature, 250°; detector temperature, 300°; column temperature, 200°; carrier gas (helium) flow-rate, 30 ml/min; hydrogen flow-rate, 3 ml/min; air flow-rate, 50 ml/min; chart speed 5 mm/min.

*Extraction*

The sample was cut in a food cutter and extracted by macerating 50 g of cut sample with 100 ml of dichloromethane and 50 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 25-ml volume of the extract was evaporated to dryness in a rotary vacuum evaporator. The residue was reconstituted in 1 ml of ethyl acetate.

*Clean-up (optional)*

A 25-ml volume of the extract was evaporated to dryness in a rotary vacuum evaporator. The residue was transferred quantitatively into an aluminium oxide column, using *ca.* 2 ml of hexane–dichloromethane (1:1). The column was eluted with 80 ml of hexane–dichloromethane (4:1) and the eluate (eluate I) was discarded. The column was then eluted with 80 ml of dichloromethane, the eluate (eluate II) was evaporated to dryness and the residue was dissolved in 1 ml of ethyl acetate.

*Gas chromatography*

A 5-$\mu$l of the solution was injected into the gas chromatograph. Standard solutions containing 1–10 ng of bupirimate per 5 $\mu$l were also injected.

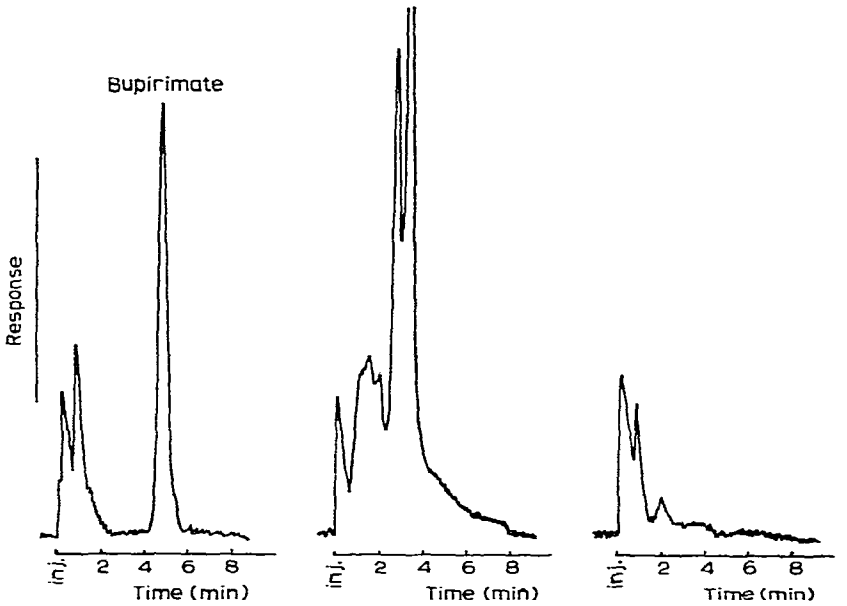



Fig. 1. Chromatogram of 20 ng of bupirimate standard. Column, 75 × 0.3 cm I.D., 10% OV-101 on Gas-Chrom Q 60–80 mesh; injector temp., 225°; detector temp., 200°; column temp., 215°; flow-rate, carrier gas (nitrogen), 50 ml/min, hydrogen, 150 ml/min, oxygen, 15 ml/min, air, 40 ml/min; chart speed, 5 mm/min; flame photometric detector (394 nm sulphur filter).

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

Fig. 3. Chromatogram of eluate II from blank apple. Conditions as in Fig. 1.

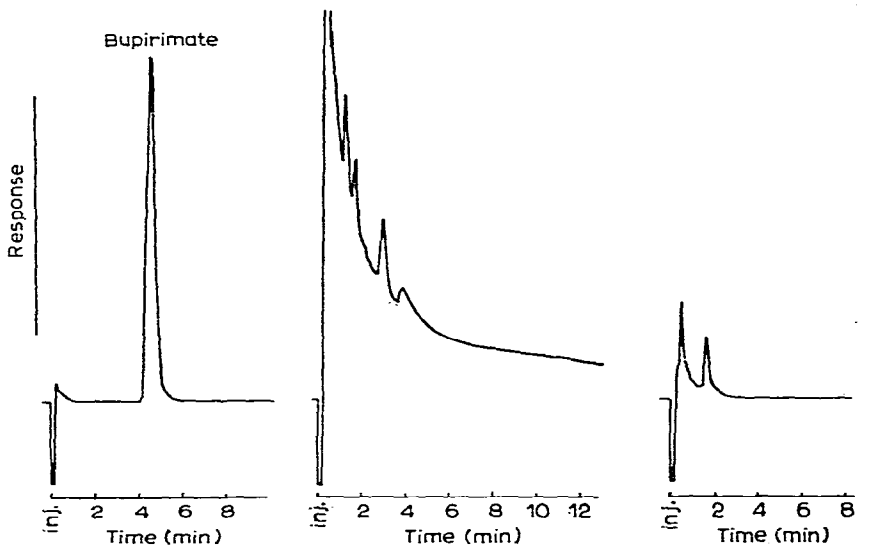



Fig. 4. Chromatogram of 10 ng of bupirimate standard. Column, 50 × 0.3 cm I.D., 10% OV-101 on Gas-Chrom Q 60–80 mesh; injector temp., 250°; detector temp., 300°, column temp., 200°; flow-rate, carrier gas (helium), 30 ml/min, hydrogen, 3 ml/min, air, 50 ml/min; chart speed ,5 mm/min; dual nitrogen–phosphorus flame ionization detector.

Fig. 5. Chromatogram of extract from blank apple without clean-up. Conditions as in Fig. 4.

Fig. 6. Chromatogram of eluate II from blank apple. Conditions as in Fig. 4.

## RESULTS AND DISCUSSION

Chromatograms of bupirimate standard and apple samples are shown in Figs. 1–6. The interference in the chromatograms of the uncleaned apple samples (Figs. 2 and 5) is dependent on the apple variety. For routine or screening work the optional clean-up can be omitted.

The flame photometric detector with sulphur filter as well as the AFID showed a linear response over a bupirimate range of 0.03–0.15 mg/kg (2–10 ng absolute).

The recovery of bupirimate was determined by analysing apple samples spiked with bupirimate at levels from 0.05 to 0.3 mg/kg. The results are shown in Table I. The limit of detection was *ca.* 0.02 mg/kg for both detectors.

TABLE I

RECOVERIES OF BUPIRIMATE FROM BLANK APPLE TREATED WITH BUPIRIMATE STANDARD SOLUTIONS FOLLOWING CLEAN-UP PROCEDURE

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

| *Bupirimate added to apple (ppm)* | *Average recovery (%)* |
|---|---|
| 0.05 | 98.4 |
| 0.10 | 98.6 |
| 0.30 | 97.4 |

The metabolite of bupirimate, ethirimol, could be determined gas chromatographically with AFID after derivatization with diazomethane[3].

## REFERENCES

1 *Gids voor ziekten- en onkruidbestrijding in land- en tuinbouw*, 6th ed., Consulentschappen voor Plantenziektenbestrijding, Wageningen, The Netherlands, 1977, p. 65.
2 *Bestrijdingsmiddelenwet*, Uitvoeringsvoorschriften (C II-4), Vermande Zonen, IJmuiden, The Netherlands, 1978.
3 M. D. Edwards, *Anal. Methods Pestic. Plant Growth Regul.*, 8 (1976) 291–297.