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Note

Gas–liquid chromatographic method for the determination of dodine in fruit

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Dodine (*n*-dodecylguanidine acetate, $CH_3(CH_2)_{10}CH_2NH{-}C({=}NH){-}NH_3^+CH_3COO^-$) is a fungicide widely used for the control of apple and pear scab, leaf spot in cherry, olive and other crops and many other diseases. Its residues decrease only slowly during storage of treated crops[1], therefore it is necessary to have a suitable method for their determination.

Several methods for dodine determination in biological materials have been developed. The spectrophotometric procedure of Steller *et al.*[2] is very laborious and its sensitivity is relatively low. Dodine, as with other monosubstituted guanidine-containing compounds[3], undergoes hydrolysis in alkaline media yielding the corresponding amine, *n*-dodecylamine[4], which is amenable to gas–liquid chromatography (GLC) after trifluoroacetylation. The disadvantage of this procedure is the long period necessary to complete hydrolysis. In the method developed by Newsome[5], dodine is determined as its volatile substituted pyrimidine, the product of reaction with hexafluoroacetylacetone.

In the procedure described in this paper, dodine is converted into a volatile compound suitable for determination by GLC via reaction with trifluoroacetic anhydride. In comparison with the method mentioned above[5], the derivatization step is very quick and no further clean-up of the reaction mixture is necessary.

EXPERIMENTAL

Chemicals

Dodine analytical standard was supplied by Serva (Feinbiochemica, Heidelberg, F.R.G.). All other chemicals were of reagent grade.

Procedure

A 50-g amount of a representative fruit sample was homogenized for 2 min with 200 ml of methanol–chloroform (2:1, v/v). The homogenate was then filtered through Whatman No. 1 paper on a Büchner funnel using a gentle vacuum. The filter was rinsed with 50 ml of the mixture used for extraction. The filtrate was acidified with 1 ml of concentrated hydrochloric acid and its volume was reduced to 30–40 ml using a vacuum evaporator (water-bath temperature 40°C). The pH of this

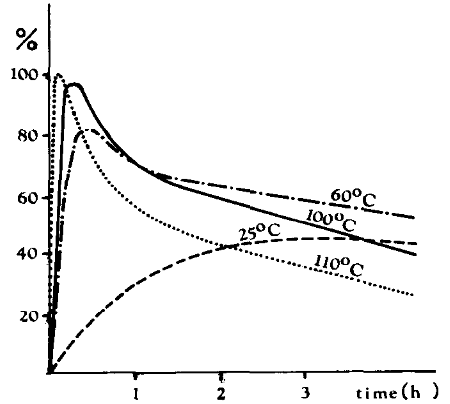

Fig. 1. The influence of the reaction temperature on the yield of dodine derivative.

concentrate was adjusted to 2 by means of hydrochloric acid, followed by extraction with two 25-ml portions of chloroform and one 25-ml portion of hexane. The organic layer was discarded and the aqueous phase was adjusted to pH 11 using sodium hydroxide (4 mol/l). Dodine was then extracted with three 25-ml portions of chloroform. The collected extracts were dried over anhydrous sodium sulphate and then evaporated to dryness under vacuum at 40°C. The residue was dissolved in 0.2 ml of hexane, 0.2 ml of trifluoroacetic anhydride were added and the flask was tightly stoppered. The reaction mixture was heated for 10 min in an oil-bath held at 100°C. After cooling, a solution of hexadecane (internal standard) in hexane was added.

GLC analyses were performed (using an Hewlett-Packard 5880 gas chromatograph) under the following conditions: glass column (1200 × 2 mm I.D.) packed with 3% OV-101 on Chromaton N-AW-DMCS (0.125–0.160 mm); column temperature programmed from 100 to 250°C at 10°C/min; injection port held at 250°C; carrier gas (nitrogen) flow-rate, 30 ml/min; flame ionization detector.

RESULTS AND DISCUSSION

The formation of a volatile derivative of dodine as the result of its reaction with trifluoroacetic anhydride was utilized as described. In order to obtain the max-

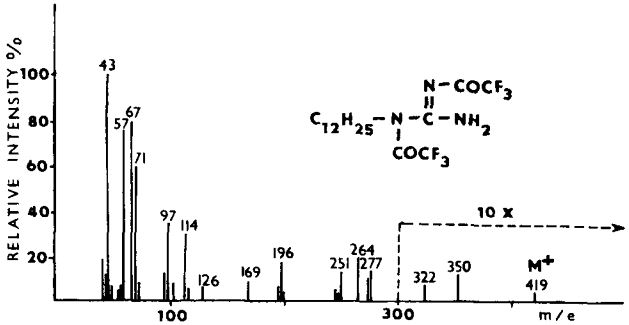

Fig. 2. Mass spectrum of 1-dodecyl-1,2-bis(trifluoroacetyl)guanidine (ionization voltage 70 eV).

imum yield of this substance, various conditions for the derivatization step were studied, see Fig. 1. It is obvious that the compound decomposed when heating (at 100°C) was prolonged, *i.e.*, greater than 10 min. The stability of the dodine derivative at room temperature was satisfactory; in the presence of an excess of trifluoroacetic anhydride, the decrease in the initial content of a model mixture (1 mg of dodine was used for derivatization) was found to be less than 1% after 1 h. Removal of the derivatizing agent resulted in relative rapid decomposition of the dodine derivative.

The mass spectrum of this derivative was obtained by means of gas chromatography–mass spectrometry of a standard solution and as expected, see Fig. 2, 1-dodecyl-1,2-bis(trifluoroacetyl)guanidine was identified. Trifluoroacetic anhydride reacts in a similar way with the guanidino group contained in arginine[6].

Typical gas chromatograms of dodine in apples and pears are shown in Fig. 3. Under the described conditions the time of dodine elution was 8.8–8.9 min; no interfering substances were present in the analysed samples. Efficient clean-up of raw extracts was achieved after their acidification by means of solvent extraction. After making basic the aqueous phase, dodine (in the form of its free base) could be immediately extracted; because of its limited stability under these conditions, pH values higher than 11 should be avoided.

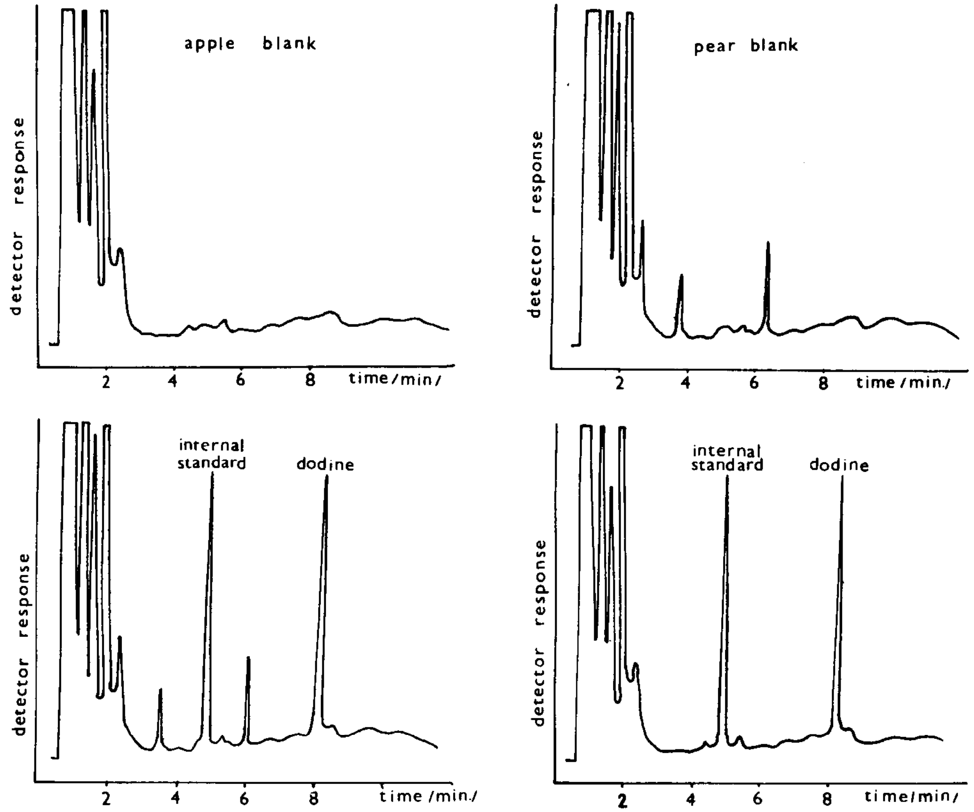

Fig. 3. Gas–liquid chromatograms of dodine in apples (left) and pears (right). Each injection represents the equivalent of 1 mg of sample (0.05 mg/kg of dodine were added).

TABLE I

RECOVERIES OF DODINE FROM APPLES AND PEARS

Apples		*Pears*	
Dodine added (mg/kg)	*Recovery** (%)	*Dodine added (mg/kg)*	*Recovery** *(%)*
0.01	96.5	0.01	94.4
0.05	97.0	0.05	97.6
0.10	99.0	0.10	95.0
0.50	98.6	0.50	98.0
Mean recovery (%)	97.8		96.3
Standard deviation	0.5		0.8

* Values calculated from five parallel determinations.

The average recoveries in the range 0.01–0.5 mg/kg are summarized in Table I. It is evident that a linear relationship exists between the amount of contaminant added to fruits and that determined.

The minimum detectable amount of dodine (signal to noise ratio 2:1) was found to be 0.005 mg/kg when using a flame ionization detector; comparable concentrations could be detected by means of a nitrogen–phosphorus detector. Because of the large amount of trifluoroacetic anhydride present, it was not possible to employ an electron-capture detector.

REFERENCES

1 J. Davídek, J. Hajšlová and Z. Svobodová, *Prum. Potravin*, 9 (1982) 490.
2 W. A. Steller, K. Klotsas, E. J. Kuchar and M. V. Norris, *J. Agric. Food Chem.*, 8 (1960) 460.
3 J. H. Hingstmann, F. C. Falkner, J. T. Throck Watson and J. Oates, *Anal. Chem.*, 46 (1974) 34.
4 N. N. Melnikov, in *Chemistry of Pesticides*, Springer, New York, Heidelberg, Berlin, 1971, pp. 238.
5 W. H. Newsome, *J. Agric. Food Chem.*, 24 (1976) 997.
6 F. Hušek and K. Macek, *J. Chromatogr.*, 113 (1975) 139.