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Note

Simplified method for the clean-up and reversed-phase high-performance liquid chromatographic determination of benomyl in mangoes*

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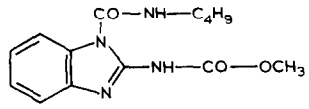
The fungicide benomyl (I)[1,2], which is methyl-1-(butylcarbamoyl)benzimidazol-2-ylcarbamate according to the International Union of Pure and Applied Chemistry (IUPAC) system, or methyl-1-[(butylamino)carbonyl]-1H-benzimidazol-2-ylcarbamate according to the Chemical Abstracts (C.A.) system, or commonly called methyl-1-(butylcarbamoyl)-2-benzimidazole carbamate controls a wide variety of diseases of fruits, nuts, vegetables, field crops, turf trees and ornamentals. Because of its relatively low mammalian toxicity coupled with effective fungitoxic action in a wide variety of plants, it has been a highly desired chemical for the agroindustries. Consequently, residue data are needed by the regulatory agencies for registration purposes.



I
Benomyl

Several methods[3–16] using fluorimetric, colorimetric, gas–liquid chromatographic (GLC) and high-performance liquid chromatographic (HPLC) procedures are available for benomyl determination in various substrates. The most widely used method has been the HPLC procedure[8,9,12] using a strong cation-exchange column. Since it has been tested on and applied to most common plant matrices, this would normally be the method of choice. However, in the absence of required facilities, specified column and associated reagents, it was desirable to develop an alternative HPLC method feasible with the existing materials and conditions of our laboratory within the given budget and time constraints. The present report describes an alternative HPLC method for determination of benomyl in mangoes.

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EXPERIMENTAL

Materials

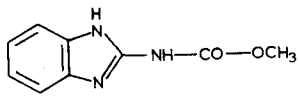
Benomyl was obtained from Pesticides and Industrial Chemicals Repository of U.S. Environmental Protection Agency. HPLC-grade solvents were used throughout.

Instrumentation

HPLC was performed on a Hewlett-Packard (Avondale, PA, U.S.A.), Model 1084A liquid chromatograph equipped with a Hewlett-Packard Model 1030B variable-volume injector. A stainless-steel column, 25 cm × 4.6 mm I.D. packed with a polar bonded packing, Permaphase® ETH (E. I. DuPont de Nemours & Co., Wilmington, DE, U.S.A.) was used. A mobile phase of water–methanol (99:1) was run isocratically at a flow-rate of 0.5 ml min^{-1}, and detection was monitored by UV at 207 nm. Both the column and solvent temperatures were maintained at 35°C.

Preparation of samples

Seeds were removed from whole mangoes and representative 50 g samples of chopped crops were extracted with 150 ml of methanol–ethyl acetate (1:1) using a Polytron® homogenizer for 2 min at medium speed. After filtration the cake was blended and filtered two more times with 150-ml portions of the extracting solvent. The filtrates were pooled, and 25 ml of 0.1 *M* hydrochloric acid were added, followed by rotary evaporation of the mixture at 40°C to reduce the volume to *ca.* 30 ml. Benomyl was converted to MBC(II) by this procedure. At this stage, some colouring matters and interfering coextractives were removed by partitioning with 3 ml hexane, and discarding the hexane top layer. The aqueous acidic mixture was adjusted to pH 7.5–8 with 0.1 *M* sodium hydroxide. MBC was recovered from this aqueous basic phase by partitioning with three 100-ml portions of ethyl acetate, and finally discarding the aqueous phase. The combined ethyl acetate phases were dried by adding anhydrous sodium sulfate, and concentrated to 3–5 ml by rotary evaporation at 35°C. Next, the contents were quantitatively transferred to a graduated centrifuge tube, and evaporated to near dryness using a gentle stream of nitrogen. Final adjustment of the volume was made to 2 ml in ethyl acetate–water (1:1). Suitable aliquots were injected for HPLC analysis.



II
MBC

Standard solution

A standard solution was prepared by taking 1.30 mg analytical benomyl standard in 5 ml methanol–ethyl acetate (1:1), followed by hydrolysis to MBC with addition of 25 ml of 0.1 *M* hydrochloric acid, and keeping the mixture in a water bath at 40°C for *ca.* 10 min. Upon cooling to room temperature, the aqueous acidic mixture was adjusted to pH 7.5–8 with 0.1 *M* sodium hydroxide, and partitioned three

times with 100-ml portions of ethyl acetate. Combined ethyl acetate fractions were concentrated in a rotary evaporator at 40°C. Final volume was adjusted to 150 ml in the same solvent. The resulting solution was used as the standard stock solution of MBC derived from 1.30 mg benomyl/150 ml ethyl acetate, *i.e.* 8.66 μg ml^{-1}. Whenever necessary, appropriate dilutions were made in ethyl acetate.

Fortification

Recoveries of benomyl as MBC were determined by extraction of the chopped crops fortified at different mg kg^{-1} level of the chemical. To 50-g samples of chopped control crops, standard solutions of benomyl converted to MBC (as outlined above in *Standard solution*) representing known weights of the chemical in 5.0 ml of ethyl acetate to correspond to the desired mg kg^{-1} fortification level. The fortified samples were mechanically shaken, and allowed to stand for 1 h prior to extraction. The entire contents were extracted without any sub-sampling. Three replicates at each fortification level were analyzed.

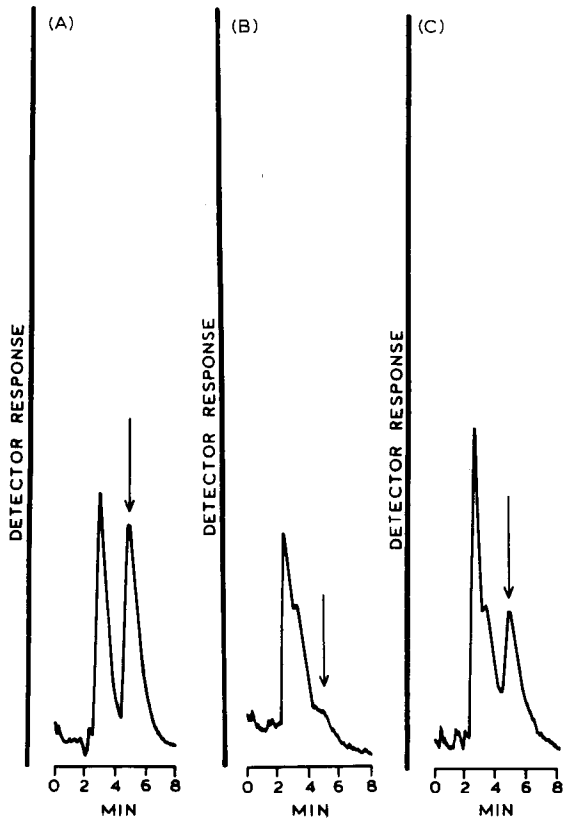


Fig. 1. Chromatogram of (A) 100 ng of a standard benomyl analyzed as MBC. Integrator attenuation 2^3 and detector range 0.04; (B) an unfortified control sample of mangoes. Integrator attenuation 2^3 and detector range of 0.04; (C) a control sample of mangoes fortified with benomyl at 0.30 mg kg^{-1} level and analyzed as MBC. Integrator attenuation 2^3 and detector range 0.04.

RESULTS AND DISCUSSION

Fig. 1A shows a typical chromatogram of a standard benomyl analyzed as MBC. Fig. 1B and C represent the chromatograms of an unfortified control sample and a control sample fortified with standard benomyl, respectively. The limit of detection of benomyl residues by this method is 0.1 mg kg^{-1}. Recoveries were in the range 75–92%, and the results are given in Table I.

The scheme outlined in the present communication is based on the earlier reports[3,4,9,12] on the quantitative hydrolysis of benomyl to MBC (methyl benzimidazol-2-ylcarbamate according to IUPAC or methyl 1H-benzimidazol-2-ylcarbamate according to C.A. or commonly, methyl 2-benzimidazolecarbamate) which is then determined by HPLC. The extraction and clean-up steps involved in this procedure are different from those reported in the published HPLC procedures.

The determinative step consists in reversed-phase HPLC analysis on an ETH column (25 cm × 4.6 mm I.D.) using a mobile phase of water–methanol (99:1), and UV detection at 207 nm. Under these conditions MBC elutes at 5.4 min at a flow-rate of 0.5 ml min^{-1}. The widely used HPLC method[8,9,12] employs a larger column (1 m × 2.1 mm I.D. Zipax SCX, strong cation-exchange of DuPont) maintained at 60°C, a mobile phase of 0.025 *M* tetramethylammonium nitrate–0.025 *M* nitric acid, and UV detection at 280 mm. Retention time reported is 18 min at a flow-rate of 0.5 ml min^{-1}. Another method[11] which involves reversed-phase HPLC determination uses a 25 cm × 2 mm I.D. RP-18 Spheri 5 column with a mobile phase of acetonitrile–water (1:1) and UV detection at 286 nm. MBC is reported to elute at 3.5 min at a flow-rate of 1.5 ml min^{-1}. An HPLC procedure[13] recently published employs a Regis Hi-chrom reversed-phase column, 5-μm Spherisorb ODS (C_{18}) of dimensions 15 cm × 4.6 mm I.D., mobile phase of acetonitrile–water–buffer pH 7 (60:30:10) and UV detection at 280 nm. MBC is reported to elute at 4.6 min at a flow-rate of 0.8 ml min^{-1}. In-depth studies concerning the relative merits and demerits of our method as compared to those of others have not been undertaken. However, the results presented herein clearly demonstrate the speed, accuracy and adequacy of the present procedure as applied to the determination of benomyl as MBC in mangoes.

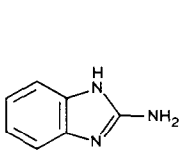
It is worthwhile to mention that in addition to MBC, several decomposition products and/or metabolites of benomyl are known. They are commonly named as 2-AB(III), STB(IV) and BBU(V) respectively. However, no evidence of any of them

TABLE I

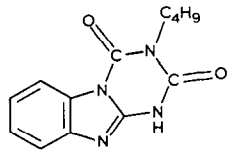
RECOVERY OF BENOMYL (ANALYZED AS MBC) FROM FORTIFIED SAMPLES OF MANGOES

Fortification level (mg kg^{-1})	*Recovery* (%)*
0.05	75 ± 8.2
0.10	81 ± 6.4
0.30	80 ± 7.1
0.50	92 ± 7.1

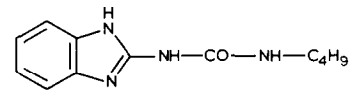
* Average of three replicates with standard deviations.



III
2-AB



IV
STB



V
BBU

in plant matrices has so far been reported. Furthermore, several years of research[12] over a wide range of crops for residues of 2-AB did not demonstrate any detectable level of 2-AB residues in any of the plant materials investigated. The present report excludes any residue determination of the aforsaid chemical entities.

ACKNOWLEDGEMENT

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REFERENCES

1 *The Agrochemicals Handbook*, The Royal Society of Chemistry, Nottingham, 1983.
2 G. L. Berg (Editor), *Farm Chemicals Handbook*, Meister, Willoughby, 1984, p. C29.
3 H. L. Pease and J. A. Gardiner, *J. Agr. Food Chem.*, 17 (1969) 267.
4 H. L. Pease and R. F. Holt, *J. Ass. Offic. Anal. Chem.*, 54 (1971) 1399.
5 E. Lemperle and E. Kerner, *Z. Anal. Chem.*, 254 (1971) 117.
6 J. P. Rouchaud and J. R. Decallonne, *J. Agr. Food Chem.*, 22 (1974) 259.
7 H. Pyysalo, *J. Agr. Food Chem.*, 25 (1977) 995.
8 J. J. Kirkland, *J. Agr. Food Chem.*, 21 (1973) 171.
9 J. J. Kirkland, R. F. Holt and H. L. Pease, *J. Agr. Food Chem.*, 21 (1973) 368.
10 M. Chiba and D. F. Veres, *J. Ass. Offic. Anal. Chem.*, 63 (1980) 1291.
11 G. Zweig and R. Gao, *Anal. Chem.*, 55 (1983) 1448.
12 T. D. Splitter, R. A. Marafioti and L. M. Lahr, *J. Chromatogr.*, 317 (1984) 527.
13 R. P. Singh and M. Chiba, *J. Agric. Food Chem.*, 33 (1985) 63, and references therein.
14 G. W. Tjan and J. T. A. Jansen, *J. Ass. Offic. Anal. Chem.*, 62 (1979) 769.
15 M. Maeda and A. Tsuji, *J. Chromatogr.*, 120 (1976) 449.
16 N. Aharonson and A. Ben-Aziz, *J. Ass. Offic. Anal. Chem.*, 56 (1973) 1330.