A Gas Chromatographic Method for the Analysis of MBC in Plants and Soil

J. P. Rouchaud and J. R. Decallonne*

A method has been developed for the extraction, cleanup, and gas chromatographic analysis of MBC (methyl 2-benzimidazolecarbamate) in melon plants and soil previously treated with benomyl fungicide. Residual benomyl and MBC are extracted with benzene and partitioned into 0.1 *N* hydrochloric acid. The acidic layer is washed several times with chloroform and then neutralized. The single residual product MBC (present initially in the plant and soil or formed during the acidic cleanup by the quantitative hydrolysis of benomyl) is partitioned into ethyl acetate. MBC is then trifluoroacetylated giving MBC-TFA and the derivatized MBC is measured by glc using an electron capture detector. The lower limit of sensitivity for this method is 0.02 ppm. Overall recovery of benomyl residues obtained from fortified control samples ranged from 80 to 100%.

Benlate is the trade name of the systemic fungicide which has methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate as its active ingredient in the commercial formulation. Benomyl is the accepted common name for this chemical. This compound is a systemic fungicide having both preventive and curative properties as well as a mite ovicidal effect. It is useful at low dosage rates for control of a wide range of fungal diseases affecting fruits, vegetables, field crops, and ornamentals (Delp and Klopping, 1968). Benomyl hydrolyzes rapidly to methyl 2-benzimidazolecarbamate (MBC) (Clemons and Sisler, 1969). Pease and Gardiner (1969) and Pease and Holt (1971) have developed a method for the analysis of benlate in residues which involves extraction from the sample (vegetables, fruits, ...) and a time-consuming double hydrolysis of benomyl and MBC to 2-aminobenzimidazole which is, in turn, analyzed by fluorometry; the sensitivity of this method is about 0.1 ppm based on a 25-g sample. Erwin *et al.* (1968) and Peterson and Edgington (1969) have developed a sensitive but time consuming and rather approximative method for the estimation of benomyl and MBC using a bioautograph technique in which a thin-layer plate is sprayed with a mixture of agar and Penicillium spores; the diameter of the zone of inhibited growth around the fungitoxic spot is related to the amount of fungitoxic chemical; the sensitivity attains 10^{-7} g. White and Kilgore (1972) have developed a method for the analysis of benomyl and MBC residues in food crops involving two time-consuming steps for cleanup: solvent partitioning followed by thin-layer chromatography. The cleaned sample is then measured in an ultraviolet spectrophotometer at 287 nm, the lower limit of sensitivity being 0.05 ppm. The present work describes a gas chromatographic method for the analysis of benomyl and MBC in residues from melon plants and greenhouse soils; in fact only MBC is detected, with no benomyl being found in the residues. In any case, all benomyl present in the plant or in the soil would be transformed quantitatively in MBC during the cleanup. The lower limit of sensitivity is 0.02 ppm based on a 100-g sample.

CHEMICALS

Benzene, chloroform, hydrochloric acid, sodium hydroxide, ethyl acetate, and anhydrous sodium sulfate are analytical grade Merck products. Trifluoroacetic anhydride is the analytical grade Aldrich product. The glc liquid phase and the support are from Varian. The reference samples of benomyl and MBC were supplied by E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.

Laboratoire de Phytopathologie et de Mycologie Générale, Université Catholique de Louvain, de Croylaan 42, 3030 Heverlee, Belgium.

PROCEDURE

Extraction Method. Chopped is 100 g of fresh melon plants; to this is added 400 ml of benzene, and the mixture is blended at high speed (8000 rpm) for 15 min in a Sorvall omnimixer. The suspension is then centrifuged at 3000 rpm for 15 min and filtered through anhydrous sodium sulfate. The extract is stored in a cool dark area until analyzed. For absorption studies, melon plants were grown in 450-ml pots in the greenhouse. Wettable powder Benlate 50W was applied to the plants by drenching (150 mg of Benlate 50W/pot). For recovery studies, a representative sample of untreated plants was fortified directly with a known amount of benomyl or of MBC before adding the extracting solvent. Soil samples (100 g) and solvent (400 ml of benzene) were mixed together for 20 min on a Bühler shaker in a 1 l. erlenmeyer flask closed with a plastic stopper. The supernatant was filtered on Whatman paper no. 111 through anhydrous sodium sulfate. The measure of recovery is made in the same manner as described earlier.

Cleanup. Concentrate 400 ml of extract to approximately 50 ml by the use of a rotary vacuum concentrator (Büchi, Switzerland) at 40°. Transfer the concentrated benzene extract into a 250-ml separatory funnel. Add 50 ml of 0.1 *N* hydrochloric acid to the funnel and shake the contents vigorously for 1 min. Frequently relieve the internal pressure generated within the funnel during the initial partitioning step. Allow the phases to separate and repeat the acid extraction once more, using another 50 ml of 0.1 *N* hydrochloric acid. Collect the combined acid fractions in a 250-ml separatory funnel and discard the benzene layer. Wash the aqueous phase three times with 50-ml portions of chloroform and discard the chloroform after each phase separation. The process may be interrupted at this point since MBC is relatively stable to further chemical degradation.

Neutralize the aqueous phase to pH 2.0–3.0 with 1 *N* sodium hydroxide and then adjust the pH to 7.8–8.2 with 0.1 *N* sodium hydroxide. Partition the resultant methyl 2-benzimidazole carbamate into three 50-ml portions of ethyl acetate. Discard the neutralized aqueous phase following the final ethyl acetate extraction. Filter the ethyl acetate extracts through anhydrous sodium sulfate and wash the sodium sulfate filter cake with an additional 50 ml of ethyl acetate. All the decantations must be done carefully, otherwise the final sample is not sufficiently clean.

Derivatization. Evaporate to dryness the combined ethyl acetate eluates by use of a rotary vacuum concentrator at 40°. Add 5 ml of ethyl acetate, dried by filtration

Table I. Recovery of MBC by the Extraction Method from Plants and Soils Fortified with Varying Amounts of the Chemical

MBC added, ppm	Number of analyses	Range of percentages of MBC recovered from	
		Melon plants	Soils
0.02	3	77–103	76–98
0.1	4	78–111	73–105
1.0	3	82–102	81–98
10.0	4	81–95	84–98
20.0	3	83–100	89–102
50.0	4	85–99	84–99

through potassium carbonate, and transfer quantitatively to a 15-ml Pyrex tube; add 0.6 ml of trifluoroacetic anhydride; seal the tube; let 35 min at 100°; cool to 25° and break the opening of the tube; transfer quantitatively into a 20-ml glass flat-bottomed flask (diameter 2.5 cm); evaporate to dryness by a flow of dry nitrogen and heating at 40°. A kinetic study of the trifluoroacetylation of MBC has shown by using glc and mass spectrometry that by applying these conditions of derivatization, MBC is converted to MBC-TFA with a yield and a conversion rate of 100%. These results are observed both with samples of extracts fortified with MBC and with solutions of pure MBC. Mass spectrometry and glc indicate that MBC-TFA is the monotrifluoroacetyl derivate of MBC at the 1 position of the benzimidazole ring. The reproducibility observed by glc for the derivatization of known amounts of MBC is within ±1%, corresponding to the reproducibility of the glc.

Glc. The amount of MBC-TFA is determined by injecting a 1.5-μl "plug" of the final glc sample into a gas chromatograph with a series 7005 N Hamilton syringe under the conditions given below. Instrument is a Varian Aerograph model 2700 equipped with a ^3H capture detector. Recorder is a Varian model A-25, 1 mV. Chart speed is 50 cm/hr. Column is glass, 1.50 m × 2.2 mm internal, packed with 5% SE-30 on 80–100 mesh Chromosorb R. Injection temperature is 250°. Column temperature is 140°; after 1 day of chromatography (about ten analyses), the column temperature is elevated to 210° during the night. In this way, the high boiling natural products, unrelated to MBC, but which are not eliminated by the cleanup, are eluted. This procedure allows one to keep a good baseline, a good sensitivity at the detector, and a good accuracy. In order to restrict this pollution of the detector and of the column, the volume of sample that is injected is limited to 1.5 μl. Detector temperature is 225°. Carrier gas is nitrogen at 40 ml/min. Retention times are 3.1 min for MBC-TFA and 7.9 min for parathion. To the dry residue of derivatization is added 1 ml of acetone, dried by filtration through potassium carbonate. If necessary, this solution is quantitatively diluted in such a way that the concentration of MBC-TFA in the final solution for glc is not higher than 150 ng/μl. Parathion is the internal standard for glc. A calibration curve is plotted each day using standards containing 7.5, 15, 30, 50, 80, 110, and 150 ng/μl of MBC-TFA, each being fortified with parathion. By measuring the peak area with a planimeter, it has been found that there is a linear relationship between peak area and the amount of fungicide. The attenuation range at the electrometer is from 1×10^{-10} to $1\beta \times 10^{-10}$ A.

RESULTS AND DISCUSSION

The analytical procedure is based upon the relative ease with which the parent compound benomyl degrades to the simpler and more stable compound MBC. This degradation product is the compound which is determined in this method. The recovery studies with untreated control samples reinforced with benomyl before the cleanup ensured

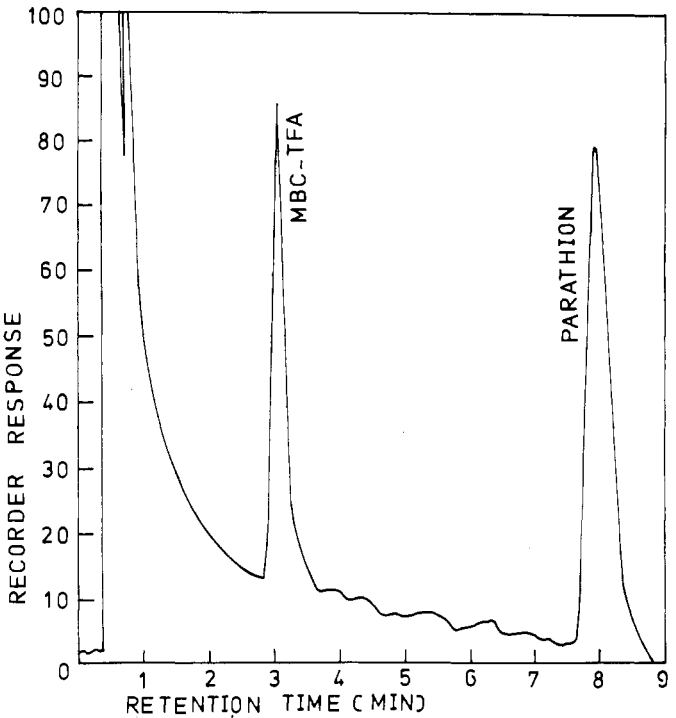

Figure 1. Glc tracing of MBC-TFA, with MBC being extracted from 100 g of melon plants containing 0.2 ppm of MBC, the final solution of the extract being 1.0 ml and the volume injected in the glc being 1.5 μl.

the quantitative *in vitro* degradation of benomyl during the cleanup and the total extraction of the degradation product. The limit of sensitivity of this method when gas chromatographing a 1.5-μl aliquot originating from 100 g of plant or soil sample in 1 ml of final solution is 0.02 ppm. With several other clean-up systems (Filtercel, Florisil, Sephadex, ...) we did not succeed in obtaining sufficiently clean samples. The alkali flame ionization detector proved to be not reliable. Recoveries were determined by adding known amounts of MBC (from 0.02 to 50 ppm relative to the weight of the fresh sample) to the untreated control in the Sorvall omnimixer for the plants or in the erlenmeyer or the Bühler shaker for the soils (Table I). The data in Table I show range of recoveries for each level of added MBC. The standard solutions used to fortify the untreated samples were prepared in benzene. Figure 1 is a tracing of the gc recorder response of 1.5 μl of an acetone solution of the residue extracted from 100 g of melon plants containing 0.2 ppm of MBC, the final volume of the extract being 1.0 ml. Gas chromatograms of numerous control samples of untreated soil or plants did not show any significant peaks (peak heights less than 0.2 cm) which interfered with that of MBC-TFA.

ACKNOWLEDGMENT

This work was supported by I.R.S.I.A. Grant 2035 A. Benomyl and MBC were supplied by the Chimac Society and Du Pont de Nemours (Belgium).

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Received for review May 1, 1973. Accepted November 26, 1973.