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# DETERMINATION OF BENOMYL AND ITS METABOLITES BY CATION-EXCHANGE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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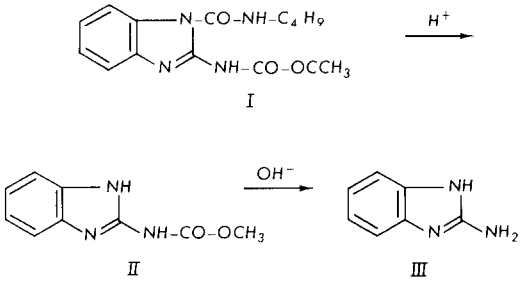
## SUMMARY

In the high-performance liquid chromatography determination of benomyl fungicide [methyl 1-(butyl carbamoyl)-2-benzimidazolecarbamate] quantitative conversion to the metabolite methyl 2-benzimidazolecarbamate (MCB) is effected by the addition of 0.25 *M* hydrochloric acid during the rotary evaporation at 50–60°C of the extraction solvents, methanol–ethyl acetate (1:9). This mild hydrolysis allows for the simultaneous determination of MBC and 2-aminobenzimidazole by cation-exchange chromatography in a variety of agricultural commodities, environmental samples, and worker exposure monitoring devices. The interferences usually occasioned by acid reflux hydrolysis are minimized. A Zipax SCX column, maintained at 60°C, was used with a mobile phase of 0.025 *M* tetramethylammonium nitrate–0.025 *M* nitric acid; and detection was by UV at 280 nm.

## INTRODUCTION

The fungicide benomyl [methyl 1-(butyl carbamoyl)-2-benzimidazolecarbamate] (Compound I in the scheme), also known as Benlate or Dupont 1991, continues to hold a strong position in plant protection schemes. Both the food and the ornamental industries depend heavily upon this material for control of certain diseases. Food-use tolerances for fruits vary from 1 ppm for bananas up to 35 ppm for pineapples, with allowable residues for animal-fed dried pomaces as high as 125 ppm. Corresponding vegetable tolerances range from 1 ppm for curcubits to 10 ppm for



mushrooms. Certain livestock feeding restrictions apply to many by-products of vegetable and grain production because of high residues[1]. Since benomyl's involvement in the RPAR (Rebuttable Presumption Against Registration) process several years ago, residue and regulatory data requirements for this material and its metabolites have increased both in number and in sensitivity. Accordingly, methods have had to be developed that are facile and versatile enough to cope with the variety of complex matrices encountered and to give accurate results not only for the parent compound, but also for the metabolites methyl 2-benzimidazole carbamate (MCB) and 2-aminobenzimidazole (2-AB) (II and III, respectively).

Fluorimetric and colorimetric analyses of benomyl entailed a two-step acid–base hydrolysis scheme that converted I to II and subsequently II to III[2,3]. Excitation at 285 nm and quantitation at 335 nm was susceptible to interference by other benzimidazole fungicides, and constant calibration and allowance for quenching factors had to be made. Bromination of 2-AB was required if the residues were to be analyzed colorimetrically. An UV spectrophotometric method employed the pH dependence of the benomyl absorption spectrum and determinations at 301.5 nm (pH 12.0) were reported for extracts of grapes, must and wine[4]. However, interference by tryptophan, indoleacetic acid, and other metabolic debris, coupled with the inherently high plant blank of the reference, limited this method to certain process applications. Direct UV measurements could be made on material that had first been extracted and purified by thin-layer chromatography[5]. Gas–liquid chromatographic (GLC) methods have included that of Rouchaud and Decallonne[6], in which, after extraction, acid hydrolysis, and clean-up, MBC residues are derivatized with trifluoroacetic anhydride and chromatographed with electron-capture detection. Pyysalo's[7] alternative approach employed high-resolution glass capillary GLC of MBC and 2-AB acetates with detection by N–P thermionic instrumentation. Sensitivity was reportedly better (0.00001 ppm), but derivatization and clean-up were still necessary.

Once introduced, high-performance liquid chromatography (HPLC) rapidly assumed the dominant role in benomyl analyses. Kirkland's initial utilization of strong cation-exchange chromatography of animal tissue hydrolysates to determine mammalian metabolites was followed rapidly by the extension of these techniques to include soil and plant tissues[8,9]. While adequate, and, in fact, necessary in order to free known conjugates in soil samples, the acidic hydrolysis/extraction step liberated too many interfering products when performed on plant tissues. Accordingly, a neutral extraction of plant tissues with ethyl acetate was adopted —conditions similar to those for fluorimetric determination— and hydrolysis could either be stopped at the MBC stage, or taken all the way to 2-AB. It was through modification and refinement of this general technique that our current scheme evolved.

More recently, reversed-phase HPLC methods have appeared. While Munitz and Pedersen[10] used an acidic extraction for determinations in cucumbers and cherries, they relied on a $C_{18}$ Sep-Pak clean-up to produce a solution suitable for reversed-phase chromotography. The reported sensitivity of their method was 0.2 ppm from a 50 g sample. For those situations where the relative amounts of benomyl and MBC were necessary data, Chiba and Veres'[11] low-temperature extraction in the presence of *n*-alkyl isocyanates, followed by adsorption chromatography on a silica gel column, was developed. It was limited to surface stripping of apple leaves at a sensitivity of 0.2 ppm. Zweig and Gao[12] have reported the quantitative conversion

of surface-stripped benomyl to MBC in ambient acetonitrile in 3 h, followed by reversed-phase HPLC determination. The broad applicability of this potentially valuable method remains to be established.

## EXPERIMENTAL

### *Instrumentation*

We used a Micromeritics Model 7000B liquid chromatograph with heated column oven, maintained at 60°C and equipped with a Tracor Model 970A UV–VIS variable-wavelength detector and a Rheodyne Model 7120 valve loop (100 μl) injector, and a DuPont Zipax SCX strong cation-exchange column with dimensions of 1 m × 2.1 mm I.D., eluted with 0.025 *M* tetramethylammonium nitrate–0.025 *M* nitric acid carrier at a flow-rate of 0.5 ml/min.

### *Reagents*

All solvents were of spectral or liquid chromatography grade: hexane and acetone were glass-redistilled. Benomyl, MBC and 2-AB standards were obtained from DuPont (Wilmington, DE, U.S.A.).

### *Plant tissue samples*

Chopped plant samples (50 g) were blended with 200 ml of methanol–ethyl acetate (1:9) for 5 min. After filtration, the cake was blended and filtered two more times with 150-ml portions of the extracting solvent. To the 500 ml of combined filtrate was added 25 ml of 0.1 *M* hydrochloric acid. The volume of this solution was reduced to 25 ml on a rotary evaporator at a bath temperature of 50°C. During this process benomyl was quantitatively converted to MBC. After transferring the aqueous phase to a 250-ml separatory funnel with a 15-ml hot water rinse, it was extracted three times with 50-ml portions of hexane and the organic layers were discarded. Saturated sodium sulfate (30 ml) was added to the remaining water solution along with 25 ml of 6.5 *M* sodium hydroxide. This basic solution immediately received four successive extractions (2-min shaking intervals) with 75-ml portions of methanol–ethyl acetate (1:9). Each extract was filtered through a bed of anhydrous sodium sulfate granules and the combined solutions were concentrated to *ca.* 10 ml on the rotary evaporator. After transferring the ethyl acetate to a small separatory funnel with two 5-ml rinses, 15 ml of 0.1 *M* hydrochloric acid was added. After phase separation the extraction was repeated. To the combined acid extracts was added sufficient 6.5 *M* sodium hydroxide (*ca.* 2 ml) to make it strongly basic to PHydrion paper. Water-saturated ethyl acetate (50 ml) was added to the funnel along with 10 ml of saturated sodium sulfate. Following shaking and phase separation, the basic aqueous phase was extracted three more times with successive 50 ml portions of water-saturated ethyl acetate, each extract being filtered through a bed of granular sodium sulfate. The combined extract was concentrated to about 10 ml on a rotary evaporator, transferred to a 30-ml beaker with ethyl acetate rinses, and evaporated to 3–5 ml with an air stream. A volume of 1 ml of 0.033 *M* phosphoric acid was added to the beaker, and the evaporation was continued until all ethyl acetate was removed. The final volume was adjusted in a 2.0-ml volumetric flask with 0.033 *M* phosphoric acid for HPLC injection. Results for a variety of commodities we have so analyzed are found in Table I.

TABLE I

ANALYSES OF VARIOUS COMMODITIES FOR BENOMYL

Only MBC found and determined: reported as benomyl.

| *Commodity* | *Detection limit (ppm)** | *Recovery (%)* | | *Residue found (ppm)* |
|---|---|---|---|---|
| | | *MBC* | *2-AB* | |
| Water | 0.02 | 86 | 114 | 0.10 |
| Brussels sprouts | 0.02 | 95 | 65 | <0.02–9.4 |
| Snap beans | 0.002 | 114 | 67 | <0.002–0.26 |
| Grapes | 0.02 | 102 | 57 | <0.02 |
| Endive | 0.01 | 106 | 57 | 0.04–11 |
| Bok choy | 0.02 | 75 | 70 | <0.02–6.4 |
| Cauliflower | 0.02 | 100 | 65 | 0.06–1.7 |
| Beet tops | 0.02 | 80 | 67 | <0.02–0.78 |

* 0.02 ppm = 8% full scale deflection (F.S.D.): 0.01 ppm = 5% E.S.D.; 0.002 ppm = 2% F.S.D.

*Worker exposure monitoring materials*

A 26-cm$^2$ circle was cut from the center of each 16-layer gauze dermal exposure assessment patch and was subsequently extracted for 20 min in 50 ml of acetone on a wrist action shaker. A volume of 50 ml of 0.1 *M* hydrochloric acid was added to the decanted acetone and the mixture was evaporated to *ca.* 50 ml on a rotary evaporator at 50°C. Sodium hydroxide (20 ml, 6.5 *M*) was added. The benomyl having been hydrolyzed to MBC, the residues were then extracted from the basic solution with four successive 50-ml portions of water-saturated ethyl acetate, each of which was passed through a pad of granular sodium sulfate. The volume of the combined ethyl acetate fractions was reduced by rotary evaporation, followed by air-stream evaporation to 3–5 ml. Following the addition of 1 ml of 0.033 *M* phosphoric acid, the remaining ethyl acetate was evaporated, and the remaining solution was transferred to a 2.0-ml volumetric flask and diluted to volume for injection. Recoveries for several monitoring fabrics, gauze patches, cotton gloves, and Tyvex disposable coveralls, averaged 110% for MBC; the detection limit for deposited benomyl was 0.005 $\mu$g/cm$^2$.

## RESULTS AND DISCUSSION

2-AB had not been reported as a metabolite of plant metabolism[2], and in several years of subsequent studies we have not found it. However, at the insistence of the Environmental Protection Agency, recovery samples and determinations of 2-AB were required on all benomyl petitions submitted for tolerance. The neutral extraction with ethyl acetate, which had been adequate for MBC and benomyl, did not give adequate recoveries of 2-AB[9]. In response to this, we added 10% methanol to the initial blending/extraction step while expendiently conducting the hydrolysis in the rotary evaporator, and we supplemented the clean-up with an optional, second acid–base–neutral partitioning scheme. When both methods were compared for MBC and 2-AB recovery from beet tops, the original extraction gave 90% and 7%, respectively, whereas our modifications yielded 80% and 67% for the corresponding

materials. Clearly, the 2-AB recovery is improved at minor expense to the MBC recovery. All subsequent determinations employed these modifications, as seen in Table I. No attempts were made to optimize results for individual commodities.

This analysis scheme has proven to be reliable and versatile in producing acceptable regulatory and environmental data on benomyl and its metabolites. Although recoveries vary among matrices, they are consistent within a series, and sample-to-sample reproducibility is generally within 4%. Retention times for MBC and 2-AB are identical to previous reports of 18 and 22 min, respectively[9]. Simplified schemes, such as that given for exposure monitoring devices are readily developed to handle large numbers of relatively clean samples.

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