Residue Procedure for the Analysis of Benomyl, Carbendazim (MBC), and 2-Aminobenzimidazole (2AB) in Soils by Capillary Electrophoresis

George M. Adams, Jr.

Abstract

Benomyl, carbendazim (MBC), and 2-aminobenzimidazole (2-AB), can be analyzed in soils by capillary electrophoresis (CE). Parent benomyl, if present, is converted to MBC during the acidic extraction procedure. Analytes are extracted from 20-g samples into acetonitrile/ammonium hydroxide solution (90:10, v:v) and ethanol/Triton X-100/ammonium hydroxide/triethylamine (TEA)/water (70/15/10/2/3, v:v:v:v:v). Sample extract is concentrated and the aqueous extract is partitioned into ethyl acetate/triethylamine (8:2, v:v). The sample is then concentrated under nitrogen and analyzed by CE with ultraviolet detection (UV).

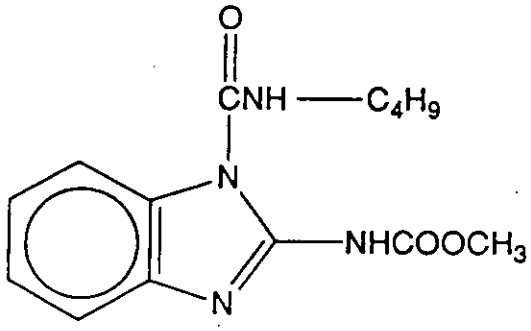
I. INTRODUCTION

This report describes a method developed for the analysis of residues of benomyl and its metabolites MBC and 2-AB in soil by capillary electrophoresis (CE).

Benomyl is the active ingredient for Benlate® Fungicide. To support reregistration of this compound, an alternate procedure was developed for the analysis of benomyl residues in treated soils.

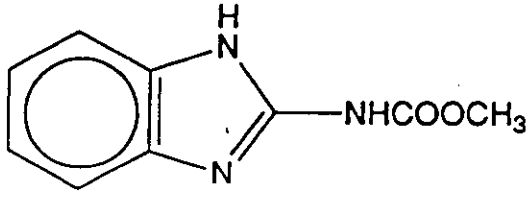
Previously, benomyl-derived residues in soil were analyzed by HPLC/UV for MBC and 2-AB (Reference 1). The methodology used for extraction (ACN/NH_4OH [90:10 v:v]) did not provide adequate extraction of greenhouse-incurred residues from soils. The current methodology encompasses the ACN/NH_4OH (90:10 v:v) solution in addition to Triton X-100 detergent extraction to extract incurred residues. Capillary electrophoresis provides easier sample preparation as well as improved analyte resolution and selectivity.

The *Chemical Abstracts* structure and name of benomyl, MBC, and 2-AB follow:



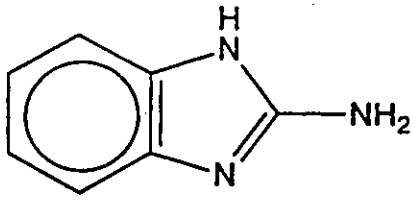
Benomyl

Methyl 1-[(butylamino)carbonyl]-1H-benzimidazol-2-ylcarbamate
CAS Registry No. 17804-35-2. Also known as DPX-T1991



carbendazim (MBC)

Methyl 1H-benzimidazol-2-ylcarbamate
CAS Registry No. 10605-21-7. Also known as IN-E965.



2-AB

2-Amine-1H-benzimidazole
CAS Registry No. 934-32-7. Also known as IN-B572.

II. TEST SOILS

The soils used in this analytical method were Chino (loamy soil) and Myaka (sandy soil). The Chino soil was collected from a site in California; the Myaka soil was collected from a site in Florida. Complete soil characterization data are presented in Table I.

III. ANALYTICAL PROCEDURE

A capillary electrophoresis (CE) method has been developed for the analysis of residues of benomyl, MBC, and 2-AB in soil. Exact procedures are described below.

1.0 Materials and Reagents

1.1 *Materials*

Polypropylene centrifuge bottle, 250 mL, Nalgene® #16129-028 (VWR, Bridgeport, NJ), or equivalent

Polypropylene centrifuge tubes, 15 mL, Corning® Catalog #21008-044 (VWR, Bridgeport, NJ), or equivalent

Branson Ultrasonic Cleaner, Model 3200 (Branson Instruments, Danbury, CT), or equivalent

Sorvall® Centrifuge, Model RC2-B, with Model GSA rotor (4 positions, each 250-mL) (Sorvall Instruments, Wilmington, DE), or equivalent

Model Phi50 pH meter, Catalog #BK3984C (Beckman® Instrument Co., Fullerton, CA), or equivalent

Magnetic stirrer unit, Model 320, Catalog #58935-410 (VWR, Bridgeport, NJ), or equivalent

Mixer, Vortex Genie 2, Catalog #58815-178 (VWR, Bridgeport, NJ), or equivalent

EDP® Pipettors, volume range 10 µL to 10 mL (Rainin Instrument Co., Woburn, MA), or equivalent

Concentrator, TurboVap 500® (Zymark Corp., Hopkinton, MA), or equivalent

Refrigerated circulating water bath, Model 1160 (VWR, Bridgeport, NJ), or equivalent

N-Evap® Analytical Evaporator, Model 111 (Organomation Associates, Inc., South Berlin, MA), or equivalent

Capillary Electrophoresis instrument, Model ^{3D}CE diode-array UV absorbance detector (Hewlett-Packard Instruments, Valley Forge, PA), or equivalent

Hewlett-Packard Extended Light Path Capillaries, 75-µm i.d., 56-cm eff. length or 72-cm eff. length, Catalog #G1600-61331 (Hewlett-Packard Instruments, Valley Forge, PA), or equivalent

Polypropylene 100-L vials, Catalog #9301-0978 (Hewlett-Packard Instruments, Valley Forge, PA), or equivalent

Nalgene® Tissue Culture Filter Unit, 0.45 µm nylon, 150 mL, Catalog #150-0045 (VWR, Bridgeport, NJ), or equivalent

Spartan-3 Disposable Syringe Filter, Nylon 0.45-µm, Catalog #00710 (VWR, Bridgeport, NJ), or equivalent

Disposable syringe, 1-cc, Catalog #BD309602 (VWR, Bridgeport, NJ), or equivalent

Polypropylene centrifuge tubes, 50 mL, Corning® Catalog #21008-124 (VWR, Bridgeport, NJ), or equivalent

Polypropylene microcentrifuge tubes, 1.5 mL, Catalog #20172-698 (VWR, Bridgeport, NJ), or equivalent

Beckman® Microfuge B, Catalog #330720 (Beckman Instruments, Palo Alto, CA), or equivalent

L.2 *Reagents*

Water, HPLC-grade, EM Science Catalog #WX0004-1 (VWR, Bridgeport, NJ), or equivalent

Hydrochloric acid (HCl), 36 to 38% concentrated reagent, J. T. Baker Catalog #9535-01 (VWR, Bridgeport, NJ), or equivalent

Triethylamine (TEA), sequanal-grade, Catalog #25108 (Pierce, Rockford, IL), or equivalent

Acetonitrile (ACN), HPLC-grade, EM Science Catalog #AX0142-1 (VWR, Bridgeport, NJ), or equivalent

Ethyl Acetate, HPLC-grade, EM Science Catalog #E196-4 (VWR, Bridgeport, NJ), or equivalent

Prepared Buffer Solution for HPCE, 100 mM sodium Triethanolamine-Phosphoric Acid, Catalog #05100-TP (MicroSolv™ CE from Scientific Resources, Inc., Eatontown, NJ) (CE buffer)

Methyl 2-benzimidazole carbamate (MBC, carbendazim), DuPont #E0965 (DuPont Agricultural Products, Wilmington, DE)

2-Aminobenzimidazole carbamate (2-AB), DuPont #B0572 (DuPont Agricultural Products, Wilmington, DE)

Benzimidazole, technical, Catalog #19412-3 (Aldrich Chemical Company, Milwaukee, WI)

Benomyl, DuPont #DPX-T1991 (DuPont Agricultural Products, Wilmington, DE)

Ammonium Hydroxide, reagent grade or better, J. T. Baker Catalog #9721-01 (VWR, Bridgeport, NJ), or equivalent

Methanol (MeOH), spectrophotometry-grade, J. T. Baker Catalog #9069-03 (VWR, Bridgeport, NJ), or equivalent

Triton X-100, J. T. Baker Catalog #X198-07, or EM Science Catalog #TX1568-3 (VWR, Bridgeport, NJ), or equivalent

Ethanol (EtOH), spectrophotometry-grade, J. T. Baker Catalog #9229-03 (VWR, Bridgeport, NJ), or equivalent

Acetone, spectrophotometry-grade, EM Science Catalog #AX0116-1 (VWR, Bridgeport, NJ), or equivalent

Sodium Hydroxide Solution for HPCE, Catalog #5062-8576 (Hewlett-Packard Instruments, Valley Forge, PA), or equivalent

2.0 *Preparation of Solutions*

2.1 *Extraction Solution (Extraction A)*

Composition: acetonitrile:ammonium hydroxide (30% solution reagent grade) (90:10, v:v).

Procedure:
Prepare an adequate volume of extraction solution by mixing ACN (900 mL) and ammonium hydroxide (100 mL) in the ratio of 90:10.

Storage:
Keep solution at room temperature; prepare as needed.

2.2 *Extraction Solution (Extraction B)*

Composition:
70% ethanol: 15% of 0.5% Triton X-100 detergent: 10% water: 2% TEA: 3% ammonium hydroxide (v:v:v:v:v).

Procedure:

Prepare a dilute solution of Triton X-100 (0.5% Triton X-100) by weighing Triton X-100 (1.0 g) and diluting with 200 mL of water.

Prepare an adequate volume of extraction solution by mixing EtOH, dilute detergent solution (from the previous step), water, TEA, and ammonium hydroxide in the ratio of 70:15:10:2:3.

Storage:
Keep solution at room temperature; prepare as needed.

2.3 *Acidified Methanol (MeOH)*

Composition:
Acidified MeOH

Procedure:
Acidified MeOH - 1000 mL of MeOH and adjust pH to 2.5 with the addition of concentrated HCl.

Storage:
Solution may be kept at room temperature up to 3 months.

2.4 *Ethyl Acetate/TEA Solution (ET Solution)*

Composition:
80% Ethyl Acetate:20% TEA (v:v)

Procedure:
Prepare solution by mixing 800 mL of ethyl acetate and 200 mL of TEA.

Storage:
Prepare solution fresh as needed.

2.5 *Stock MBC Standard Solution*

Composition:
50 ppm MBC in acidified MeOH

Procedure:
Weigh 5.0 mg of MBC standard into a 100-mL volumetric flask. Dissolve in 75 mL of acidified MeOH. Dilute to volume (100 mL) with acidified MeOH.

Storage:
Keep solution refrigerated until needed. Solution is stable for up to 3 months, if MeOH is prevented from evaporating.

2.6 *Stock 2-AB Standard Solution*

Composition:
50 ppm 2-AB in acidified MeOH

Procedure:
Weigh 5.0 mg of 2-AB standard into a 100-mL volumetric flask. Dissolve in 75 mL of acidified MeOH. Dilute to volume (100 mL) with acidified MeOH.

Storage:
Keep solution refrigerated until needed. Solution is stable for up to 3 months, if MeOH is prevented from evaporating.

2.7 *CE Injection Standard Solution*

Composition:
500 ppm benzimidazole

Procedure:
Weigh 25 mg of benzimidazole into 50-mL volumetric flask and dilute with acidified MeOH.

Storage:
Keep solution at room temperature; prepare solution monthly.

2.8 *CE Running Buffer*

Composition:
95% 50 mM Triethanolamine phosphoric acid (TEAP) pH 2.5: 5% ACN

Procedure:
Prepare solution by mixing 50 mL of TEAP buffer (100 mM solution) and 50 mL of water. Mix 95 mL of 50 mM Triethylamine solution and 5 mL of ACN. Or prepare by mixing 47.5 mL water, 47.5 mL TEAP buffer and 5 mL of ACN (total 100 mL).

Storage:
Store at room temperature up to 1 month.

2.9 *Stock Benomyl Standard Solution*

Composition:
50 ppm benomyl in acidified MeOH

Procedure:
Weigh 5.0 mg of benomyl standard into a 100-mL volumetric flask. Dissolve in 75 mL of MeOH. Dilute to volume (100 mL) with MeOH.

Storage:
Prepare solution fresh before fortifications.

2.10 *CE Sample Solution*

Composition:
50% water/50% acetone.

Procedure:
Prepare 50% water by mixing 50 mL of water and 50 mL of acetone.

Storage:
Keep solution at room temperature. Prepare solution monthly.

3.0 *Extraction Procedure*

3.1 Weigh soil sample (20 g) into a 250-mL plastic centrifuge bottle.

3.2 Fortify the sample at the desired level(s) with stock standard solution(s).

3.3 Add 50 mL Extraction Solution A (ACN/NH_4OH) to the solid. Sonicate the sample for at least 30 minutes in the ultrasonic bath. Every 15 minutes vent the bottle by removing the bottle cap, then replace cap and manually shake the bottle for approximately 1 minute.

3.4 Centrifuge sample for about 5 minutes at 7000 rpm.

3.5 Decant soil extract into a TurboVap 200-mL evaporation vessel. Repeat steps 3.3 through 3.5 once more on the pellet from step 3.4.

3.6 Add 50 mL Extraction Solution B (Ethanol/Triton X-100/NH_4OH/TEA/Water) to the pellet. Sonicate the sample for at least 30 minutes in the ultrasonic bath. Every 15 minutes, vent the bottle by removing the bottle cap, then replace cap and manually shake the bottle for approximately 1 minute.

3.7 Centrifuge sample for 5 minutes at 7000 rpm.

3.8 Decant soil extract into evaporation vessel. Repeat steps 3.6 through 3.7.
NOTE: After this step, stop if necessary. Samples may be stored overnight at room temperature.

4.0 *Concentration of Crude Extract*

4.1 Concentrate combined Solution A and Solution B to less than 10 mL. The TurboVap water bath should be set at 60°C and the condenser bath at 5°C by using a TurboVap equipped with a refrigerated circulating bath.

4.2 Transfer the concentrated solution from the concentration vessel into a 50-mL centrifuge tube. Rinse the concentration vessel with ethyl acetate/TEA (ET) solution and add to the transfer solution in the centrifuge tube.

NOTE: After this step, stop if necessary. Samples may be stored at room temperature.

5.0 *Ethyl Acetate Partitioning*

5.1 Add 5 mL of TEA and 10 mL of ET solution to the centrifuge tube. Vortex sample and centrifuge for at least 2 minutes. Two layers will form: bottom-aqueous and top-ethyl acetate/TEA. Pipette top-ethyl acetate layer into a clean 50-mL centrifuge tube. (Benomyl-derived residues will partition into ethyl acetate layer at high pH.)

5.2 Add 5 mL TEA and 10 mL of ET solution to aqueous layer. Repeat step 5.1 twice more. Combine ethyl acetate fractions and evaporate almost to dryness (N-Evap bath @ 50-60°C).
NOTE: After this step, stop if necessary. Samples may be stored in the refrigerator.

5.3 Dissolve sample in 0.50 mL of CE sample solution. Pipette 0.010 mL CE Injection Standard to each sample (injection standard volume may vary with final volume; 0.010 mL is based on 0.50 mL final volume). Vortex and centrifuge sample, if needed.

5.4 Refrigerate sample until analysis.

6.0 *Preparation of CE Standards*

Prepare standards containing MBC and 2-AB. Standards are prepared and evaporated under nitrogen to dryness and reconstituted in 1.0 mL of CE sample solution.

50 ppm MBC Working Standards	50 ppm 2-AB Working Standards	500 ppm Injection Standard	Final Volume	µg/mL of Either Standard
10 µL	10 µL	20 µL	1.0 mL	0.5
20 µL	20 µL	20 µL	1.0 mL	1.0
50 µL	50 µL	20 µL	1.0 mL	2.5
200 µL	200 µL	20 µL	1.0 mL	10
1000 µL	1000 µL	20 µL	1.0 mL	50

7.0 *Calculations*

Standards run during analysis of samples were plotted to define a response curve. The peak response for each fortified or field sample was calculated using the following equation:

$$peak\ response = \frac{(peak\ area_{\ analyte})}{(peak\ area_{\ benzimidazole})}$$

Least squares regression analysis ($y = mx + b$, where $b = 0$, line forced through origin) was used to determine the ppm injected. The following equations were used to calculate the ppm found:

$$ppm\ found = \frac{Corrected\ Peak\ Response \times Final\ Volume\ (mL)}{Slope \times Sample\ Weight\ (g)}$$

The ppm sample injected was determined from a calibration curve. Nonrounded values were used for the calculations.

Fortification percent recovery, reported to the nearest whole number, was calculated by finding the analyte concentration, as described in the previous equations, correcting for background detected in the control sample if any, dividing by the fortification level, and then multiplying by 100%, as shown below:

$$\%\ recovery = \frac{benomyl\ equivalents\ found\ (ppm)}{fortification\ level\ (ppm)} \times 100\%$$

Note: Residue levels in <u>treated</u> samples were <u>not</u> corrected by subtracting residues in control samples. This policy resulted in maximum reportable residues for all treated samples.

7.1 *Sample Calculations*

The concentration of benomyl as MBC in Chino (loamy soil) at a 50 ppm fortification level is calculated as follows:

$$ppm\ found = \frac{0.82 \times 0.51\ mL}{0.0643 \times 20\ g} \times 1.52^{*} = 0.50\ ppm\ benomyl\ equivalents$$

The MBC peak area corresponds to 0.326 ppm MBC from the standard curve (equation of the standard curve = $y = 0.064$; $r^2 = 0.999$). The internal standard (benzimidazole) peak area was 419.16.

Fortification percent recovery, reported to the nearest whole number, was calculated as follows:

$$\%\ recovery = \frac{0.50\ ppm}{0.50\ ppm} \times 100\% = 99.89\%^{\dagger}$$

The concentration of benomyl as 2-AB in Chino (loamy soil) at a 50 ppm fortification level is calculated as follows:

$$ppm\ found = \frac{10.645 \times 0.51\ mL}{0.1268 \times 20\ g} = 2.141\ ppm$$

The 2-AB peak area corresponds to 2.141 ppm 2-AB from the standard curve (equation of the standard curve = $y = 0.126$; $r^2 = 0.999$). The internal standard (benzimidazole) peak area was 42.79.

Fortification percent recovery, reported to the nearest whole number, was calculated as follows:

$$\%\ recovery = \frac{2.141\ ppm}{2.5\ ppm} \times 100\% = 85.64\%$$

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* 1.52 = MW benomyl (290.32)/MW MBC (191.19). Molecular weight data were taken from the Merck Index, 10th ed.

† The discrepancy in the % recovery is a result of rounding.

8.0 *Analysis*

8.1 *CE Conditions*

Instrument:	Hewlett-Packard Model ^{3D}CE
Capillary:	Extended Path Capillary 75 µm i.d.
Cartridge Temperature:	40°C
Detector:	200 nm
Voltage:	30 kV (72 effective length)
Injection:	10 second/50 mbar
CE Buffer:	95% 50 mM Triethanolamine-phosphoric acid pH 2.5/5% ACN

Migration Time:

	Migration Time (min)	Relative Migration Time (min)
Benzimidazole	8.1	(—)
2-AB	8.5	(.4)
MBC	12.1	(4.0)

8.2 *Capillary Conditioning*

Flush a new capillary sequentially for at least 10 minutes with water, 20 minutes with 1.0 N NaOH, 10 minutes water and 30 minutes CE buffer.

After each injection, flush capillary for 1 minute with water, 1 minute with 1N NaOH, 1 minute with water, and 5 minutes with CE buffer. In addition, reservoir buffers should be changed after every 5-6 samples.