Principle

Me_2BA is extracted from soil by shaking the soil with 50:50 acetonitrile:water. The sample is filtered and the acetonitrile:water is taken to dryness using rotoevaporation. The residue is solubilized with 0.05 N sodium hydroxide and passed through a C_8 solid phase extraction (SPE) column. The Me_2BA is eluted from the column using 0.05 N sodium hydroxide, neutralized with acid, and then taken to dryness. The residue is dissolved in water and analyzed using high-pressure liquid chromatography (HPLC). Chromatography is performed on a C8 column using methanol:water:acetic acid as the mobile phase and a wavelength of 265 nm. The limit of quantitation is 5.0 ppb.

Apparatus (Items from other manufacturers may be used provided they are functionally equivalent.)

(1) Balance: Analytical, Sartorius

(2) Balance: Pan, Mettler Model PM200

(3) Vacuum Manifold: Supelco

(4) Shaker Bath: Eberbach, reciprocating

(5) Roto Evaporator: Rinco with variable temperature water bath

(6) C_8 SPE Columns: 1 g Mega Bond Elute, 6 cc, Varian

(7) Reservoirs: 60-mL capacity, J.T. Baker

(8) Filter Paper: Glass fiber, 11 or 12.5 cm, Whatman

(9) HPLC: Shimadzu 6A

(10) HPLC Column: Supelcosil LC-8-DB, 25 cm x 4.6 mm i.d., 5-μm mesh size.

(11) Assorted Glassware: Flat bottom flasks, beakers, assorted volumetric flasks, pipets, syringes, glass jars, Büchner funnels, etc.

(12) Scientific Software System: Vax MULTICHROM, VG Data Systems Ltd.

Reagents

(1) Analytical Standards: Me_2BA, Lot No. 003, purity 99.8%, Kumiai

(2) Methanol: Pesticide Grade, Burdick & Jackson

(3) Acetonitrile: Pesticide Grade, Burdick & Jackson

(4) Glacial Acetic Acid: Sigma Chemical Co.

(5) Millipore Water: ABC Laboratories, Inc.

(6) Sodium Hydroxide: J.T. Baker Co

Preparation of Reference Solutions

(1) Stock Solution

A 5-g glass weigh boat was tared to 0.0000 g on an analytical balance. Exactly 25.1 mg of Me_2BA, 99.8% purity, was weighed into the weigh boat. The compound was rinsed with Millipore water from the weigh boat into a 25-mL volumetric flask. The volumetric flask was brought to volume with water and the solution was well mixed. The prepared solution contained 1000 µg/mL of Me_2BA.

(2) Standard Solutions

One milliliter of the 1000 µg/mL stock solution was brought to volume in a 100-mL volumetric flask using Millipore water yielding a final concentration of 10 µg/mL. From this solution, standard curve points were diluted in water as indicated in the following table:

Initial Concentration µg/mL	Aliquot mL	Dilution Volume mL	Final Concentration µg/mL
10.0	15.0	100	1.5
10.0	5.0	50	1.0
10.0	5.0	100	0.5
1.0	5.0	50	0.1
0.5	5.0	50	0.05
1.0	1.0	50	0.02

(3) Fortification Solutions

Sample fortification was performed using 1.0 and 10 µg/mL solutions of Me_2BA to obtain levels of 1, 2, 5, 10, and 50 ppb. Twenty-five grams of soil were placed in glass jars. A volume of 25, 50, 125, and 250 µL of the 1.0 µg/mL concentration was used for the 1, 2, 5, and 10 ppb respective fortifications. A volume of 125 µL of the 10.0 µg/mL concentration was used for the 50 ppb level. The fortification solutions were prepared in Millipore water in the following manner:

Initial Concentration µg/mL	Aliquot mL	Dilution Volume mL	Final Concentration µg/mL
1000.0	1.0	100	10.0
10.0	5.0	50	1.0

Extraction of Me_2BA in Soil

(1) Twenty-five grams of soil were weighed into a glass jar. Sample fortifications were made at this point by adding the appropriate aliquot of fortification solution to the sample. Seventy-five milliliters of 50:50 acetonitrile:water were added to the sample and the jar was capped with an aluminum foil-lined cap. The sample was placed on a reciprocating shaker and shaken for 15 minutes.

(2) After shaking, the sample was filtered through glass-fiber filter paper contained in a Büchner funnel. The sample container and foil-lined cap were rinsed with approximately 25 mL of 50:50 acetonitrile:water and the rinse was passed through the sample. All filtrates were collected in a 500-mL T flask, then taken to dryness using rotoevaporation and a water bath with a temperature of approximately 30 °C. The remaining residue was dissolved in 5 mL of 0.05 N sodium hydroxide and then transferred to a C8 SPE column. If particulate matter was present in the solution, it was first transferred to a 12-mL screw-cap culture tube, centrifuged, and the liquid transferred to the SPE column.

(3) The C_8 SPE column was prepared by allowing approximately 10 mL of Millipore water to pass through the column at a moderate rate. This was followed by 10 mL of acetonitrile and an additional 30 mL of Millipore water. The column was not allowed to go to dryness. The final water portion was stopped approximately 2 mm above the top of the column packing.

(4) The sample was poured onto the column and allowed to pass through the column at approximately 1 drop/sec using a vacuum of 8-15 inches of mercury. Once the sample had passed completely through the column, the column was washed with an additional 5 mL of 0.05 N sodium hydroxide. This 5 mL of wash was first added to the original sample flask. The solution was mixed and if necessary transferred to a 12-mL cultured tube and centrifuged prior to adding the wash to the column. The column was allowed to go to dryness and the vacuum was left on for 2 additional minutes to ensure that no water remained in the column. The original sample aliquot plus the five additional milliliters of 0.05 N sodium hydroxide were collected in a screw-cap culture tube.

(5) The 10 mL of solution collected in the culture tube was transferred to a 125-mL T flask, the tube rinsed with 1 mL of Millipore water, and the pH of the combined solutions adjusted to the acid side with 1 + 1 acetic acid:water. The solution was then taken to dryness using rotoevaporation and a water bath maintained at approximately 30 ° C. The residue was dissolved in 1 mL of Millipore water, mixed, and transferred to a liquid chromatographic sample vial. The sample was either analyzed immediately, or placed in a refrigerator until analysis was performed.

Instrumentation

Instrumentation used for the chromatography of the Me_2BA was a Shimadzu 6A with an ultraviolet detector set at a wavelength of 265 nm. General chromatography parameters are as follows:

Column:	Supelcosil LC-8-DB 25 cm x 4.6 mm i.d. 5-μm mesh size
Mobile Phase:	5:95:0.1 Methanol:H2O:HAc 3.5:96.5:0.1 Methanol:H_2O:HAc
Sample Wash:	5:95 Methanol:H_2O
Flow Rate:	1.3 to 1.9 mL/min
Absorbance Wavelength:	265 nm
Injection Volume:	100 μL
Sample Run Time:	17 min

The retention time of Me_2BA ranged from 7 to 10 minutes.

Data Acquisition and Calculations

Peak height values were obtained using the VaxTM MULTICHROMTM System. MULTICHROMTM automatically calculates the slope, y-intercept, correlation coefficient, and it plots all pertinent standards and the linear regression line. From the linear regression line, concentrations of sample residues were automatically interpolated. These values were converted to parts per billion by MULTICHROMTM using previously entered sample volumes in milliliters and the final dilution volume in milliliters.

$$ppb\ residue = \frac{ng/mL\ detected \times final\ volume\ (mL)}{sample\ weight\ (g)}$$

Recoveries from fortified samples were determined by the following formula:

$$\%\ recovery = \frac{ppb\ residue\ found - average\ ppb\ residue\ in\ control}{ppb\ residue\ added} \times 100$$

Method Comments

All sample solutions and reference standards should be refrigerated when not in use or being analyzed. Degradation of the Me_2BA is significantly retarded with refrigeration.

Soils that are difficult to filter can be centrifuged prior to the initial filtration step. If this is necessary, the extraction can be performed in a round plastic bottle (Nalgene), the bottle

centrifuged, and the resulting supernatant transferred to a flat bottom flask for rotoevaporation. Rinsing the residue remaining in the plastic bottle is not necessary. Recovery of Me_2BA should be determined if this procedure is to be used.

Solutions should not be left in a basic medium for extended periods of time. The 0.05 N sodium hydroxide should be added to the sample just before the column cleanup step is initiated and the basic sample obtained after the column cleanup should be taken to dryness as soon as possible.

Occasional cleaning of the analytical column may be beneficial. This can be accomplished by pumping pure methanol through the column to wash it. This should then be followed by pumping the mobile phase used for the analysis through the column for several minutes to equilibrate it.