ATTACHMENT I Method for the Determination of Me_2BA in Water

Principle

Me_2BA is extracted from water by passing the water through a solid phase extraction (SPE) SCX column and eluting with ammonium hydroxide and methanol. Concentrations of Me_2BA are determined by high pressure liquid chromatography (HPLC) using an instrument equipped with a C_8 column and a UV detector at 265 nm. Results are calculated using linear regression from external standards. The validated sensitivity of the method is 1.0 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) SCX SPE Columns: J.T. Baker Chemical Co., 6 cc, 1 g

(5) Reservoirs: 60-mL capacity, J.T. Baker Chemical Co.

(6) HPLC: Shimadzu 6A

(7) HPLC Column: Shiseido Capcell Pak C_8, 25 cm x 4.6 mm i.d., 5-μm mesh size

(8) Assorted Glassware: Flat bottom flasks, beakers, assorted volumetric flasks, pipets, syringes, etc.

(9) Scientific Software System: Vax^{TM} MULTICHROM^{TM}, VG Data Systems Ltd.

Reagents

(1) Analytical Standards: Me_2BA, Lot No. 3, purity 99.8%, Kumiai

(2) Methanol: Pesticide Grade, Burdick & Jackson

(3) Glacial Acetic Acid: Sigma Chemical Co.

(4) Millipore Water: ABC Laboratories

(5) Ammonium Hydroxide: J.T. Baker Chemical Co.

(6) Hydrochloric Acid: Fisher Scientific

(7) Diethylene Glycol: Sigma Chemical 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 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 1.0 mg/mL of Me_2BA.

(2) Standard Solutions

One milliliter of the 1000 µg/mL stock solution was brought to volume using 10:90 methanol:water in a 100-mL volumetric flask yielding a 10 µg/mL final concentration. One milliliter of the 10 µg/mL solution was brought to volume using 10:90 methanol:water in a 100-mL volumetric flask yielding a 1 µg/mL final concentration. From these solutions, standard curve points were diluted in 10:90 methanol:water as indicated in the following table:

Initial Concentration µg/mL	Aliquot mL	Dilution Volume mL	Final Concentration µg/mL
10.0	5.0	100	0.5
1.0	25.0	100	0.25
1.0	5.0	50	0.1
0.5	15.0	100	0.075
0.5	10.0	100	0.05
0.25	10.0	100	0.025
1.0	1.0	100	0.01

(3) Fortification Solutions

Sample fortification was performed using 2.5 and 0.5 µg/mL solutions of Me_2BA to obtain levels of 1, 5, 10, and 50 ppb. Fifty milliliters of water were placed into flasks. One milliliter of the appropriate fortification solution was pipeted

into the water for the 10 and 50 ppb levels. One hundred microliters of the appropriate fortification solution were added to the water for the 1 and 5 ppb levels. The water sample was swirled for mixing. The fortification solutions were diluted with water in the following manner:

Initial Concentration µg/mL	Aliquot mL	Dilution Volume mL	Final Concentration µg/mL
1000.0	0.25	100	2.5
1000.0	0.05	100	0.5

Extraction of Me_2BA in Water

(1) The SCX SPE column was prepped by allowing approximately 20 mL of 6 N ammonium hydroxide to pass through the column at a moderate rate. This was followed by 30 mL of Millipore water and 20 mL of 6 N hydrochloric acid, then 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.

(2) The sample was poured into the reservoir above the column and acidified with 0.1 mL of 6 N HCl. One milliliter was allowed to drain onto the top of the column with the vacuum off. The sample was pulled through the column at a moderate rate using a vacuum. Once the sample had passed completely through the reservoir and was near the top of the column packing material, the reservoir and column were rinsed with Millipore water. The column was not allowed to go dryness at any time. The rinse water was stopped approximately 2 mm above the top of the column packing material.

(3) Ten milliliters of 6 N ammonium hydroxide were drained through the column into appropriately labeled flat bottom flasks in the vacuum box. The column was not allowed to go to dryness. Again, approximately 2 mm of liquid was left above the column.

(4) Ten milliliters of methanol were drained through the column into the same flat bottom flask containing the ammonium hydroxide from step 3. The column was allowed to go to complete dryness.

(5) Approximately 0.2 mL of 5% diethylene glycol in 10:90 methanol:Millipore water was added to the flat bottom and the sample was taken to dryness on the rotoevaporator with the water temperature at approximately 35 °C.

(6) The sample was brought to volume with 2 mL of 10:90 methanol:Millipore water and sonicated for 2 min. The sample was then transferred to a screw-top HPLC vial for injection.

Instrumentation

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

Column: Shiseido Capcell Pak C_8
25 cm x 4.6 mm i.d.
5-μm mesh size

Mobile Phase: 10:90:0.05 MeOH:H_2O:HAc

Sample Wash: 50:50 MeOH:H_2O

Flow Rate: 1.0 mL/min

Absorbance Wavelength: 265 nm

Injection Volume: 100 μL

Sample Run Time: 13 min

The retention time of the Me_2BA using the described HPLC parameters was approximately 6.23 minutes.

Data Acquisition and Calculations

Peak height values were obtained using the Vax™ MULTICHROM™ System. MULTICHROM™ automatically calculates the slope, y-intercept, correlation coefficient, and 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 MULTICHROM™ using previously entered sample volumes in milliliters and the final dilution volume in milliliters.

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

Recoveries from fortified samples were determined by the following formula:

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

Notes on Extraction

(1) Samples should not be allowed to sit for long periods of time (over 1 hr) in the basic solution.

(2) SCX SPE columns can be regenerated by following step 1 of the extraction method. Used columns should be discarded if the integrity of the column has been compromised by usage or by the sample run through it.

(3) New columns are treated by step 1 of the extraction procedure to eliminate any interference peaks experienced when the columns were not treated as previously used columns.