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A High-Performance Liquid Chromatographic Method and Extraction for the Determination of Benomyl Metabolite 2-AB from Soil

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INTRODUCTION AND SUMMARY

Scope

An analytical method is described for determining benomyl metabolite 2-Aminobenzimidizole (2-AB) in soil. The method has been written to replace "Determination of Benomyl Residues in Soils and Plant Tissues by High Speed Cation Exchange Liquid Chromatography", which yielded low, erratic recoveries for the determination of 2-AB (Reference 1). The chemical structure for 2-AB is given in Figure 1.

This method is based on extracting 2-AB from soil in a mixture of acetonitrile/ammonium hydroxide, removing the solvent by rotary evaporation, and dissolving the residue in mobile phase. The analyte is then determined by liquid chromatography (LC). Three soil types have been examined in this method development: Rochelle, Fargo, and Hanford. The characteristics of these soils are prsented in Table I. The lower limit of detection is 10 ppb and the lower limit of quantitative measurement is 0.05 ppm. The mean recovery for 2-AB is 90.63% (Std. Dev. = 14%, N = 18) (See Table II).

MATERIALS AND METHODS

The following sections include a list of suggested equipment and reagents and recommendations for preparing stock solutions and standards.

Equipment

1. Tekmar Tissuemizer, Model #SDT182EN (Tekmar Co., Cincinnati, OH).
2. Sorvall® Centrifuge, Model #RC3B (Du Pont Medical Products, Wilmington, DE).

3. Brinkmann Rotavapor RE11 rotary evaporator (Brinkmann Instruments, Inc., Westbury, NY).
4. Hewlett Packard Liquid Chromatograph, Model 1090 (Hewlett Packard, Avondale Division, Avondale, PA) equipped with a diode array detector.

Reagents and Standards

1. The 2-AB standard (compound #B572) was supplied by Kodak Chemical Co. Its chemical purity is 95%.
2. Reagent-grade ammonium hydroxide (J. T. Baker, Inc., Phillipsburg, NJ).
3. Glass-distilled acetonitrile, Omnisolv (E. M. Science, Gibbstown, NJ).
4. Milli-Q® water (Millipore Corp., Bedford, MA).

Stock Solution

To prepare a stock solution of 2-AB (100 ppm or µg/mL), accurately weigh out 0.01 g of 2-AB. Dissolve the compound with acetonitrile, bringing it to volume in a 100-mL volumetric flask.

To prepare a standard curve (0.01 to 0.4 ppm), dissolve appropriate quantities of the stock solution in mobile phase to yield 0.01, 0.05, 0.1, 0.2, 0.3, and 0.4 ppm. To fortify control samples (0.01, 0.05, and 0.2 ppm), combine 20 mL of stock solution at the appropriate concentration with 20 g of control soil. When not in use, all standard solutions should be stored in a refrigerator at 4 °C.

Analytical Procedure

Soil Extraction and Fortification. Prepare extraction solvent on a daily basis as follows: Add 900 mL of analytical-grade, glass-distilled acetonitrile and 100 mL of Baker reagent-grade ammonium hydroxide to a 1500-mL beaker. Stir the solution with a glass rod, and cover the beaker with aluminum foil. Use this mixture within two hours.

To determine 2-AB in composited soil samples, weigh approximately 20 g of a representative sample into a 500-mL plastic centrifuge bottle and spike it with the desired level of 2-AB standard (0.1 - 1.0 ppm). Add 50 mL of the extracting solvent, and blend

the contents in a Tekmar Tissuemizer at 50% of its maximum speed for 30 seconds. Rinse the shaft with pure acetonitrile three times, and centrifuge the sample for 10 minutes at 3000 rpm. Following centrifugation, decant the extract into a 600-mL beaker, labeling each sample appropriately. Repeat the extraction and centrifugation procedure three more times and pool the extracts. Evaporate the solvent to dryness using a rotoevaporator at 40 °C. Either refrigerate the residue overnight or dissolve it in 0.05M sodium phosphate (pH 3.5) containing 8% acetonitrile to obtain the desired dilution.

Validation of 2-AB Recoveries Using Radiolabeled 2-AB. Recovery of ^{14}C-labeled 2-AB was determined prior to the development of the LC method. The radiolabeled 2-AB was synthesized at Du Pont Agricultural Products Department. The fortified soil extraction procedure described above yielded recoveries of 2-AB ranging from 90 to 93% in fortified soil (Keyport, OR) are shown in Table III.

LC Procedure for Determining 2-AB in Soil. After 2-AB is extracted from the soil with acetonitrile containing 10% ammonium hydroxide, evaporate the solvent to dryness on a rotoevaporator at 40 °C. Bring the residue to volume (20 mL) with mobile phase.

Filter 2 mL of the sample through a 0.45-µ Gelman Acrodisc filter assembly (CR #4219) into a 2-mL autosampler vial using a 5-mL syringe. Seal the vial using a crimper (use Teflon® caps). The samples are then ready for analyses.

Instrument :	HP Liquid Chromatograph 1090A equipped with a photo diode array detector
Columns:	Two Zorbax® CN (25 cm x 4.6 mm) columns in series.
Column Temperature:	40 °C
UV Wavelength	280 nm
Mobile Phase:	8:92 (acetonitrile:0.05M sodium phosphate made to pH 3.5)
Flow Rate:	1 mL/min

Gradient:	Isocratic
Injection Volume:	200 µl
Attenuation:	2 x 2

Standard Curve. Prepare a standard curve for 2-AB as follows: Dilute 2-AB standard stock solution with mobile phase to prepare concentrations of 0.01, 0.05, 0.1, 0.2, 0.3, and 0.4 ppm. Inject each standard through an autosampler and obtain separate chromatograms. Plot peak height (mm) on the *x*-axis and 2-AB concentration (ppm) on the *y*-axis. Construct a curve using RS/1 computer programming.

Methods of Calculation. Determine residues of 2-AB in soil samples by comparing the chromatographic peak height of the analyte with the corresponding peak height for standards of known concentration. Calculate µg/g for each analyte in soil samples using Equation 1. For fortified control samples, calculate the percent recovery for 2-AB using Equation 2. The response factor (R_f) for 2-AB is simply the slope of its standard curve through the origin.

(1) µg/g of Analyte in Soils = $$\frac{[PK]_s \times VS \times DF}{R_f \times VI \times SW}$$

(2) Percent Recovery of Analyte from Soil= $$\frac{100\% \times [PK]_R \times VS \times DF}{R_f \times VI \times SP}$$

Variables for Equations 1 and 2 are defined as follows:

R_f = $\frac{[PK]_{STD}}{VI \times C}$ = Instrument response factor for the analyte in µvolts/µg.

C = Concentration of 2-AB in the standard solution in µg/mL.

DF = Dilution factor for samples requiring sample dilution. DF =1.0 if no dilution is required.

$[PK]_R$ = Chromatographic peak height (in mm) for the analyte in a fortified recovery sample.

$[PK]_S$ = Chromatographic peak height (in mm) for the analyte in an unknown sample.

$[PK]_{STD}$ = Chromatographic peak height (in mm) for analyte in a standard solution.

SW = Sample weight in g.

SP = Weight of analyte (in µg) fortified into a sample.

VI = Injection volume (in mL) of the sample or standard solution.

VS = Volume (in mL) of sample solution before injection or dilution.

Calibration Procedures. Prepare several standard solutions in mobile phase at concentrations that span the concentration ranges expected for the analyte in the sample solutions. Analyze the standards frequently during a series of sample analyses to provide data for standard curves. If the analyte peak height for a sample falls outside the working range of the standard curve, quantitatively dilute the sample and reinject it to keep the detector response within the working range of the standard curve.

To determine recovery efficiency, prepare and analyze at least one control sample and three control samples fortified with known quantities of the analytes before sample extraction. Fortification levels should approximate the residue concentration of the samples being analyzed.