HPLC ANALYSIS OF AVERMECTIN B1 AND ITS Δ 8,9 ISOMER IN SOIL

Soil Study: 001-86-559R

Project Description:

Avermectin B1 and its Δ 8,9 isomer are extracted from soil and derivatized to yield a residue that is detected by high-performance liquid chromatography (HPLC) using a reverse-phase column and a fluorescence detector. Avermectin B1a, B1b, and its Δ 8,9 isomer are quantitated from an avermectin B1a curve. Peak height measurements are used in a linear least-squares program.

Sample Procurement and Analysis Dates:

Study 001-86-559R (samples and protocols) was received in four shipments. The first set of samples (all depths day 0 and 0-2" day 7 intervals) was received, logged in, and given master log number 89-061-992 on 2/10/89. The second set of samples (2-4" day 7 interval) was received, logged in, and given master log number 89-102-992 on 3/3/89. The remainder of the day 7 interval (4-12") and days 14 and 28 intervals (all) were received 3/8/89 and given master log number 89-110-992. The treated samples of both the 42 and 60 day intervals were received 4/26/89 and given master log number 89-180-992. The samples were received previously processed and were stored frozen until analyzed.

sample numbers	date extracted	date analyzed
001-86-559R-[95(4-12),121-125(0-2)] [127,128,130,131 (0-2)]	2/13/89	2/15/89
001-86-559R-[92(0-12),93(6-12)] [94(0-12),95(0-4)]	2/15/89	2/16/89
001-86-559R-[88(4-12),89(0-12)] [91(0-12),93(6-12)]	2/17/89	2/21/89
001-86-559R-[85,86 (0-12),87(2-4)] [88(0-4),91(4-6)]	2/21/89	2/22/89
001-86-559R-[121,122,124,125,127, 128,130,131,132 (2-4)]	3/6/89	3/7/89
001-86-559R-[121,122 (4-12)] [123(6-12),124,125 (4-12)]	4/7/89	4/11/89

sample numbers		date extracted	date analyzed
001-86-559R-	[126(6-12),127,128 (4-12)] [130,131 (4-12)]	4/11/89	4/14/89
001-86-559R-	[133,134 (0-12)] [135(6-12)]	4/13/89	4/17/89
001-86-559R-	[135(2-4),136,137 (0-12)]	4/17/89	4/18/89
001-86-559R-	[138(4-6),139,140 (0-12)] [142(0-4)]	4/19/89	4/20/89
001-86-559R-	[140(0-2),142(4-12)] [143(0-12),144(0-2),145(0-12)]	4/21/89	4/24/89
001-86-559R-	[146(0-12),147(0-2)] [148(0-12),149(0-4)]	4/26/89	4/27/89
001-86-559R-	[149(4-12),150(6-12)] [151,152 (0-12)]	4/28/89	5/2/89
001-86-559R-	[153(6-12),154,155 (0-12)]	5/3/89	5/4/89
001-86-559R-	[153(4-6),181,182 (0-12)] [184(0-4)]	5/5/89	5/8/89
001-86-559R-	[150(4-6),184(4-12)] [185,187 (0-12)]	5/9/89	5/11/89
001-86-559R-	[147(6-12),188,190 (0-12)] [191(0-4)]	5/12/89	5/15/89
001-86-559R-	[150(2-4),157,158 (0-12)] [191(4-12)]	5/16/89	5/22/89
001-86-559R-	[156(2-4),157,158 (2-4)] [160,161 (0-12)]	5/18/89	5/24/89
001-86-559R-	[147(6-12),163,164 (0-12)]	5/23/89	5/25/89
001-86-559R-	[147(4-6),166,167 (0-12)]	5/26/89	5/31/89

Validation of Method:

Please refer to the report entitled "Method Validation for the HPLC Analysis of Avermectin B1 and Its Δ 8,9 Isomer in Soil", dated 11/1/88, prepared by Analytical Development Corporation (ADC).

Sample Analysis:

Merck method #8003[1] was used for these determinations. The 25.0-gram soil subsample was extracted in a Soxhlet apparatus, allowing the solvent (230 mL of acetonitrile:deionized water [1:1]) to wash the

[1]Tway, P.C. and Rosenthal, M., "HPLC Fluorescence Determination for Avermectin B1 and Its Δ 8,9 Isomer in Soil", Merck Method #8003, 10/12/88.

69

sample approximately every 6 minutes. The sample was cooled and 250 mL of deionized water was added to the flask. The aqueous solution was passed through a C-8 column. The column was eluted with acetonitrile; the eluant was concentrated and adjusted to be 20% in water. The solution was then partitioned with hexane. The combined hexane extract was passed through an aminopropyl column. The column was washed and eluted with 50% acetone/dichloromethane (DCM); the eluant was diluted to 10 mL with DCM. The sample was split, with one half saved for repeat analysis, if necessary, and the other half taken to dryness.

The dried residue was derivatized with 1-methylimidazole and TFAA in DMF followed by reaction with methanolic ammonia. The mixture was dissolved in chloroform and passed through a silica column to remove the excess unreacted derivatizing reagents. The sample was dried, reconstituted with methanol, and analyzed by HPLC using a 3-μm particle size reversed-phase column and fluorescence detection.

A curve with 1.0, 3.0, 5.0, 7.0, and 10.0 ng/mL avermectin B1a standards was run at the beginning and the end of each sample set. Extracts with results greater than the highest 10.0 ng/mL avermectin B1a standard were diluted using methanol and reinjected. Results that were less than 0.5 ppb were reported as not detected (ND). Result that were less than 1.0 ppb, but greater than or equal to 0.5 ppb, were reported as not quantitated (NQ). The average B1a recovery for the sample sets was 96% (n=21, range 72%-105%).

Instrumentation and Operating Conditions:

Injector:	Hewlett-Packard or Spectra Physics 8875 autoinjector to deliver 50 μL.
Pump:	Hewlett-Packard 1090 or Spectra Physics 8810 at 1.5 mL/min.
Column:	ES Industries Chromegabond MC-18, 150 x 4.6 mm, 3-μm particle size analytical column at ambient temperature.
Precolumn:	Brownlee Newguard RP-18, 15 x 3.2 mm, 7-μm particle size.
Detector:	Kratos/Schoeffel Model 950 fluorescence detector. Excitation λ = 365 nm with FSA 403 excitation filter and coated mercury vapor lamp. Emission filter was 418 nm FSA 426. Sensitivity = 550-650 volts. Range = 0.02 μamps. Time constant = 6.
Integrator:	Nelson Analytical System 2600 software (version 5) for the IBM PC on a Zenith Z-159 (IBM PC compatible) linked to a LAN.
Manifold:	Applied Separations Spe-ed Mate 30.

Calculations:

Calculations were done using a linear least-squares fit program. Concentration in ng/mL was assigned to the x-axis and peak height to the y-axis. The sample peak heights were entered along with the standard values; the program solved for the unknown concentrations. The concentration in ng/g was determined as follows: x(FV)(2)/SW, where x = ng/mL found in the final volume, FV = final volume (5.0 mL), 2 = fraction split, and SW = sample weight (25.0 g). This corresponds to x/2.5 for soil, and this value (2.5) was placed in the sample amount of the peak summary table for samples (sets 3a-12a) to give results in ng/g, rather than ng/mL. Also, any dilution factors (final volume/5) were entered into the sequence file and were then used in determining the final result. The peak heights for all points were plotted, except where noted on the curves. Soil moisture calcnlations were as follows: wet weight = bottle weight + net weight (wet), net weight (dry) = dry weight - bottle weight, percent moisture = ((wet weight - bottle weight) - net weight (dry))/(wet weight - bottle weight) x 100.

Standards:

A 25 µg/mL stock standard was prepared 2/10/89 from avermectin B1 in glycerol formal solution (reference standard L-676,863-038A002, 0.956% w/w B1a, 0.071% w/w B1b; received from Merck 10/28/88 and logged in as 88-605-992) by taking 26.2 mg of the glycerol formal solution to 10 mL with acetonitrile. The 500 ng/mL and 2 µg/mL avermectin B1 standards were prepared by dilution of the stock standard. The 50 ng/mL and 100 ng/mL standards were prepared by dilution of the 500 ng/mL standard.

Calibration standards for each analysis set were prepared by aliquoting 0.1, 0.3, 0.5, 0.7, and 1.0 mL of the 50 ng/mL standard solution into separate silylated tubes and diluting to 5.0 mL, yielding 1.0, 3.0, 5.0, 7.0, and 10.0 ng/mL avermectin B1a, respectively, as specified in Merck method #8003.

Fortification at 2.0 ppb was prepared by adding 0.5 mL of the 100 ng/mL standard to a 25.0-g portion of the untreated control. Fortification at 10.0 ppb was prepared by adding 0.5 mL of the 500 ng/mL standard to a 25.0-g portion of the untreated control. Fortification at 40.0 ppb was prepared by adding 0.5 mL of the 2 µg/mL standard to a 25.0-g portion of the untreated control.

Sample Set Size and Composition:

An analysis set was comprised of five standards, one fortification,

and nine, ten, eleven, or twelve samples. The standards were injected at the beginning and at the end of the set.

Method Deviations:

The method was followed as written, but with the following exceptions, which are not expected to have a significant impact on the study:

1. The HPLC system had no in-line filter and the Brownlee Newguard RP-18 precolumn was filled with 7-μm packing.
2. Whatman 33 x 94 mm cellulose extraction thimbles were used, rather than 25 x 80 mm thimbles.
3. The column temperature was ambient, rather than 30 °C.