VALIDATION OF METHOD 244-93-3 FOR MK-0244 AND ITS 8,9-Z ISOMER IN/ON SOIL

Project Description:

ADC will validate Merck method 244-93-3 for the analysis of MK-0244 and its metabolite in soil. The validated method will be used for subsequent sample assays. MK-0244 (4"-deoxy-4"-epi-methylamino-avermectin B1 benzoate salt) is extracted from fortified control soil and derivatized with trifluoroacetic anhydride (TFAA) in the presence of 1-methylimidazole. The derivatized samples and standards are quantitated by using an HPLC equipped with a C-18 column and fluorescence detection.

The B1a and B1b components are quantitated via the derivatized B1a standard. The 8,9-Z isomer forms the same derivative as the B1a component and is also quantitated by comparison with the B1a standard curve. The limit of detection (LOD) is defined as 0.2 ng/g and results below that level are reported as not detected (ND); the limit of quantitation (LOQ) is 0.4 ng/g and results below the LOQ, but greater than or equal to the LOD, are reported as not quantitated (NQ).

The original report, chromatograms, and notebooks will be archived at the Animal Science Research Communications Center, presently located at Merck and Co., Inc., Metropolitan Corporate Plaza, 485C Route 1, Iselin, NJ 08830, location WBC-125. ADC will maintain all facility specific information along with certified copies of the notebooks, chromatograms, and the final report in archival storage. Samples and archive samples will be retained frozen by ADC until the sponsor specifies their disposition.

Sample Procurement and Processing:

The control validation samples from trials 001-93-3003R (samples 999 and 547), 001-92-6010R (samples 2 & 5), and 001-92-6011R (sample 3A) were received from Merck on 11/18/93 and assigned Master Log (ML) # 93-922-1385 . A second set of soil samples from trial 001-92-6011R (samples 5 and 92) was received 2/1/94 and logged in as ML # 94-076-1385. The samples were received processed and stored frozen until needed. Portions of the 001-92-6010R samples were combined and mixed by hand for use in the validation.

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Validation set	Date extracted	Date injected
MKLE11A	12/27/93	12/28/93
MKLE12	12/29/93	12/30/93
MKLE12C	12/29/93	1/3/94
MKLE13A	1/4/94	1/6/94
MKLE14	1/17/94	1/20/94
MKLE15B	1/20/94	1/24/94
MKLE15D	1/20/94	1/26/94
MKLE16	1/24/94	1/26/94
MKLE17	1/27/94	1/28/94
MKLE18	2/2/94	2/3/94
MKLE19	2/9/94	2/10/94

Sample Analysis:

Merck Method 244-93-3[1], modified as necessary, was used for the analysis of MK-0244 in soil samples. MK-0244 is extracted from soil with methanol followed by 1% ammonium acetate in methanol in a heated sonicator (85-95 °C). The combined extract is evaporated to 1 mL, water is added, and the solution is extracted with ethyl acetate. The ethyl acetate supernatant is loaded onto a conditioned PRS SPE column and eluted with 1% ammonium acetate in methanol. The eluate is evaporated to 1 mL, water is added, and the aqueous phase is partitioned against ethyl acetate. The ethyl acetate is evaporated to 0.5 mL and acetonitrile is added. The samples and standards are derivatized with trifluoroacetic anhydride (TFAA) in the presence of 1-methylimidazole. The derivatized samples and standards are quantitated by monitoring fluorescence after injection on a HPLC equipped with a C-18 column.

Soil moistures were determined in duplicate by the procedure given in ADC SOP #48.2.

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[1] Payne, L.D., Wehner, T.A., and Tway, P.C., "HPLC-Fluorescence Method to Determine the Residues of MK-0244 and Its 8,9-Z Isomer in/on Soil", Merck Analytical Research Method 244-93-3, 9/3/93 (draft).

Standards:

MK-0244 B1a:

An MK-0244 B1a stock standard, at a concentration of 100 µg/mL, was prepared on 11/22/93 by diluting 5.34 mg of standard MK-0244 (L-656,748-052S003, 93.6% B1a, 3.8% B1b; received from Merck and logged in as 93-532-1355) to 50 mL with acetonitrile. The 100 µg/mL B1a standard also contains 4.1 µg/mL MK-0244 B1b. Working standard solutions were prepared at 1200, 1000, 100, and 10 ng/mL by dilution of the stock.

Calibration standards at 0.25, 0.5, 1.25, 2, and 2.5 ng/mL were prepared by aliquoting 0.1, 0.2, 0.5, 0.8, and 1.0 mL of the 10 ng/mL working standard solution to separate tubes and derivatizing along with the samples. The derivatized standards were diluted to 4.0 mL with acetonitrile and injected on the HPLC before and after the samples.

8,9-Z Isomer:

An MK-0244 B1a 8,9-Z stock standard, at a concentration of 100 µg/mL, was prepared on 11/22/93 by diluting 5.38 mg of standard 8,9-Z (L-695,638-001C001, 92.9% B1a; received from Merck and logged in as 93-533-1355) to 50 mL with acetonitrile. Working standard solutions were prepared at 1000, 100, and 10 ng/mL by dilution of the stock.

Fortifications:

Control soil samples for study 001-93-3003R were fortified at 0.4, 10, and 120 ppb MK-0244 B1a; the 10 and 120 ppb samples also contain 0.41 and 4.9 ppb B1b, respectively. Samples were also fortified with 8,9-Z at 0.4 and 10 ppb. Additional samples were fortified with both B1a and 8,9-Z at 0.4 and 10 ppb each. The samples were analyzed in quadruplicate; controls were analyzed in quintuplicate.

Control soil samples for study 001-92-6010R were fortified at 0.4 and 10 ppb MK-0244 B1a; the 10 ppb sample also contains 0.41 ppb B1b. Samples were also fortified with 8,9-Z at 0.4 ppb. Additional samples were fortified with both B1a and 8,9-Z at 10 ppb each. The samples were analyzed at least in triplicate; controls were analyzed in duplicate.

Control soil samples for study 001-92-6011R were fortified at 0.4 and 10 ppb MK-0244 B1a; the 10 ppb sample also contains 0.41 ppb B1b. Samples were also fortified with 8,9-Z at 0.4 ppb. Additional samples were fortified with both B1a and 8,9-Z at 10 ppb each. The samples were analyzed at least in triplicate; controls were analyzed in triplicate.

Instrumentation and Operating Conditions:

Injector:	Spectra-Physics 8775 to deliver 50 µL.
Pump:	Spectra-Physics 8810 at 1.0 mL/min.
Mobile Phase:	7% deionized water in methanol.
Column:	ES Industries Chromegabond MC-18, 150 x 4.6 mm, 3 µm particle size column at ambient temperature.
Precolumn:	Brownlee Spheri-5 RP-18, 15 x 3.2 mm, 7-µm particle size
Detector:	Emission 470. Excitation λ = 365 nm. Emission λ = 470 nm. Time constant = 4 sec. Gain = 1000x.
Integrator:	Nelson Analytical System 2600 software (version 5.1.5 M3) on a LAN.

Calculations:

Calculations were done using a linear least-squares fit program. Concentration in ng/mL was assigned to the x-axis and peak height was assigned to the y-axis. The unknown sample peak heights were entered along with the standard values; the program solved for the unknown concentrations. The concentration in ng/g was determined in the following manner: $concentration = \frac{(X)*(FV)}{SW}$, where X = ng/mL as determined from the curve; FV = the final volume of the sample (4.0 mL x dilution); and SW = the sample weight. The peak heights for all standards were plotted except where noted. The final concentrations and recoveries were based on the entire number provided by the Nelson System. The final result reported was rounded to the appropriate number of significant digits.

The % moisture was calculated as follows:

$\%\ moisture = \frac{(W_2-W_3)}{(W_2-W_1)} \times 100$, where W_1 is the weight of the empty, dry vessel, W_2 is the weight of the wet soil and vessel, and W_3 is the weight of the dried soil and vessel. The average results from duplicate determinations are given later in the report. The recoveries given in Table I are <u>not</u> corrected for moisture because the criteria for acceptance or rejection of the data were not based on the corrected result.