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ANALYTICAL METHOD

Department: Residue Chemistry

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Title: An Analytical Method for the Determination of Residues of Pyrimethanil (AE B100309) and Its Major Metabolites AE F132593 in Soil with Soxhlet Extraction using Gas Chromatography with Mass Selective Detection

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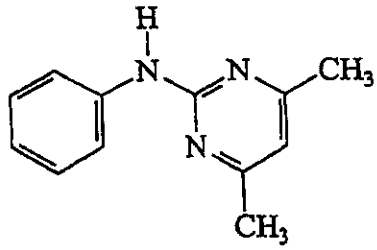
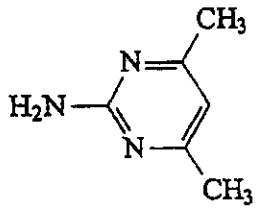
1. SCOPE

This method is suitable for the determination of the total extractable residues of Pyrimethanil (AE B100309) and its major metabolite AE F132593 in soil. The structures for AE B100309 and its metabolite AE F132593 are shown below. The lower limit of quantitation for each analyte has been set at 0.01 ppm in soil.



Common Name:	Pyrimethanil, (AN1), (AE B100309)
Molecular Formula:	$C_{12}H_{13}N_3$
Molecular Weight:	199.2
C.A. Name:	4,6-dimethyl-N-phenyl-2-pyridinamine
IUPAC Name:	2-anilino-4,6-dimethylpyrimidine
CAS Reg. No.:	[53112-28-0]



Common Name:	AE F132593, (AN7)
Molecular Formula:	$C_6H_9N_3$
Molecular Weight:	123.2
C.A. Name:	4,6-dimethyl-2-pyrimidinamine
IUPAC Name:	2-amino-4,6-dimethylpyrimidine
CAS Reg. No.:	[767-15-7]

2. PRINCIPLE

Studies on the metabolism of pyrimethanil in soil have shown the degradation of the parent compound to different metabolites.[1] Pyrimethanil and one metabolite AE F132593 was found in quantifiable concentrations. The method of analysis described here allows quantitation of both pyrimethanil (AE B100309) and AE F132593 in soil to a lower quantitative limit of 0.01 ppm.

Residues of pyrimethanil and AE F132593 were extracted from soil by Soxhlet extraction with 90:10 acetonitrile:water. The extracts are then evaporated to dryness, and re-dissolved in ethyl acetate. Residues are quantitated by gas chromatography using mass selective detection.

3. APPARATUS

Use as a guide; equivalent apparatus may be substituted.

- 500 mL flat bottomed glass boiling flasks with 24/40 joint (VWR, Cat # 29113-122)
- Adapter Bushings, 55/50 to 24/40 joints (Kimble Cat # 150750-2610)
- Aluminum Lab Frame for supporting soxhlet extraction apparatus (VWR Cat # 60075-009)
- Barnstead Nanopure water system
- Base deactivated 4 mm injection cyclosplitter sleeve (Restek Cat # 20707-210.5)
- Class A 50 and 100 mL volumetric flasks.
- Class A pipets, various volumes as needed.
- Dewar Vacuum trap, 1000 mL reservoir, (Kontes Cat # 926910-1000), used between rotary evaporators and vacuum source. Chilling either with dry ice/IPA or mechanically.
- Disposable Pasteur pipets, 5.75 in. and 9 in. (VWR Cat # 14672-200 and 14672-380)
- GC column, 0.25 mm x 30 meter DB-17MS, 0.25 µm film thickness (J&W Scientific, Krackeler Scientific Cat # 122-4732)
- Glass Wool, Pyrex (Krackler Scientific Cat # 1-3950)
- Heating Mantles (Model – Electromantle, ME), Electrothermal Engineering Ltd.
- Hewlett-Packard 5890 II gas chromatograph with capillary split/splitless inlet equiped with a Model 7673A autosampler and 5970 mass selective detector.
- Lauda Water circulation coolers (Model WK 3200) for soxhlet condensers. (Set at 10° (+/- 5°).
- Neslab CC-80 Cryocool immersion cooler, used to cool vacuum trap.
- Neslab CFT-75 re-circulating chiller used to cool rotary evaporator condensers to 5° C (+/- 5°).
- Reflux Condensers, 300 mm, (Kimble Cat # 18140-300)
- Rotary evaporators, Buchi B-481 water bath and R-124 Rotovapor. Water baths are generally maintained between 40° and 50° C for this procedure.
- Soxhlet Extractor body, 55/50 to 24/40 joints, (Kimble Cat # 586000-0023)
- Three Prong Swivel Clamps (VWR Cat # 21573-708)
- Vacuum pump, Welch 8920A-55, used with rotary evaporators
- Whatman Cellulose Extraction Thimbles, 33mm x 94mm, (Whatman Cat # 2800339)

4. REAGENTS

Use as a guide; equivalents or different manufactures (brands) may be substituted.

- Acetone (PG or better)
- Acetonitrile (PG of better)
- Deionized Water
- Ethyl Acetate (PG or better)
- AE B100309 and AE F132593: Analytical stock standards made up in acetone (nominally 1000 µg/mL).

- Fortification solutions may be made individually or in combinations. All dilutions should be made into acetone.
- AE B100309 and AE F132593 calibration standards prepared in ethyl acetate. (0.005, 0.01, 0.02, 0.05, and 0.10 µg/mL).
- Other standards may be prepared as necessary.

5. PROCEDURE

5.1 Sample Preparation

Grind, chop or mix soil samples well. Samples should be stored frozen at ≤ 20 °C until extraction.

5.2 Extraction

Note: Fortification experiments (see Section 6.3) are not required when analysis of samples is performed for tolerance enforcement. Fortification experiments can be used for data collection methods or establishing & validating method efficiency as required.

5.2.1 Weigh 20.0 grams (W, ± 0.05 g) of soil into a cellulose soxhlet extraction thimble. Fortify the recovery samples at the desired level with AE F132593 and/or AE B100309 (Pyrimethanil) metabolites prepared in acetone (see Section 6.1.2). Plug the top of the cellulose extraction thimble with glass wool.

5.2.2 Assemble a 500 mL boiling flask containing 250 mL of 90:10 acetonitrile: water followed by the soxhlet extractor containing the extraction thimble with sample, and a condenser. Place soxhlet in a heating mantle and secure the apparatus. Place the heating mantles on 70% power and allow the samples to cycle (extract) for 6 hours. The sample analysis may be paused at this point to resume analysis at a later time.

Note: The heating mantles may be placed on a timer to allow the soxhlet extraction to proceed unattended for 6 hours.

5.2.3 Once the extraction is complete, rinse out the condenser with a small volume of acetonitrile and allow it to collect in the soxhlet extractor.

5.2.4 Disassemble the soxhlet apparatus and decant any residual solvent into the 500 mL boiling flask. Discard the extraction thimble containing the soil.

Note: When rotary evaporating the acetonitrile:water extracts, care should be excersised to prevent the sample from "bumping" and being aspirated into the rotary evaporator condenser. This chance may be reduced by applying a reduced vacuum first, allowing the sample to evaporate for a period of time, then apply the full vacuum to complete evaporation.

5.2.5 Rotary evaporate the acetonitrile:water extracts between 40 and 50 °C to dryness. Remove any residual water if required by adding 10 to 20 mL of acetonitrile to the flask swirling and re-rotary evaporating. (Repeat if necessary until flask is dry). Reconstitute in 20 mL of ethyl acetate, stopper, and shake well to await analysis by GC/MSD.

6. ANALYSIS

6.1 Analytical Standard Solution Preparation

6.1.1 Stock Standard Solutions

Prepare 1000 µg/ml stock solutions of AE F132593 and AE B100309 (Pyrimethanil) separately in acetone.

6.1.2 Fortification Stock Solutions

Prepare a 10 µg/mL fortification solution containing a mixture of AE F132593 and AE B100309 by taking a 1 mL aliquot of each stock solution and diluting to 100 mL with acetone. Further dilutions of this mixed fortification solution may be made as needed.

6.1.3 GC Calibration Solutions

Prepare a 10 µg/mL fortification solution containing a mixture of AE F132593 and AE B100309 by taking a 1 mL aliquot of each stock solution and diluting to 100 mL with ethyl acetate. Prepare working calibration solutions in the range of 0.005 µg/ml to 0.100 µg/mL in ethyl acetate. Other ranges may be prepared as needed; but it is recommended that a 20X range not be exceeded.

6.2 GC-MSD Analysis

6.2.1 Sample Analysis

Inject a 2 µL aliquot of each test sample (or fortified sample matrix) from Steps 5.2.5 into the GC/MSD under the conditions stated in Appendix 1. Variations in equipment or sample characteristics may require different injection volumes or slight modifications in the chromatographic conditions listed in order to obtain adequate chromatographic peak shapes or sensitivity. Make dilutions as necessary to maintain the response within the upper range of the standard curve (see Appendices 2 and 3).

It is often beneficial to make several 'priming' injections of standards and/or samples prior to starting the GC/MSD analysis. This helps stabilize the GC/MSD response.

6.2.2 GC/MSD Standard Calibration (See Appendix 2 and 3)

Standardize the GC response under the conditions outlined in Appendix 1, GC conditions, by injecting a 2 µL aliquot of each GC calibration solution interspersed

with samples. Construct a standard curve for AE F132593 and AE B100309 (Pyrimethanil) by plotting the response vs the standard concentration. Obtain the least square regression line of this data.

Compare the peak areas of the analyzed sample with the standard curve. Calculate the total residue concentration (R) using *Equation 1* as follows:

$$R\ (ppm) = \frac{(Y - b)/m}{C} \times F \qquad \textit{(Equation 1)}$$

where:

Y = peak area response (cts.)
b = Y-intercept of standard regression line (cts.)
m = slope of standard regression line (cts mL/µg)
C = crop/solvent ratio (g/mL)
F = molecular weight conversion factor

Note: No molecular weight conversion is required in this case for either AE B100309 (Pyrimethanil) or AE F132593. The value is 1.00

The crop/solvent ratio *"C"* is defined by the concentration of sample in g/mL at injection using either *Equation 2*. This factor incorporates all aliquots taken and dilutions made during sample work-up. Further dilutions may be required to maintain the response within the range of the standard calibration curve as indicated by the variable *D*.

$$C = W/V_1 \times D \qquad \textit{(Equation 2)}$$

where: W = 20.0 grams for Soil
V_1 = 20.0 mL for Soil

The dilution factor *D* is defined by *Equation 3* below:

$$D = V_3/V_3 \qquad \textit{(Equation 3)}$$

where: V_2 = Aliquot taken in mL at the final volume
V_3 = Total volume in mL of the dilution

6.3 Fortification Experiments (Optional for Tolerance Enforcement)

Note: *Fortification experiments are not required when analysis of samples is performed for tolerance enforcement. Fortification experiments are intended to be used for data collection methods or establishing & validating method efficiency.*

6.3.1 With each sample set, analyze an untreated control sample and one or more fortified control samples. Calculate recoveries by *Equation 4* as follows:

$$Recovery\ (\%) = \frac{R - S}{T} \times 100 \qquad \textit{(Equation 4)}$$

where: R = ppm of target analyte found in fortified sample
S = ppm of target analyte found in control sample
T = theoretical ppm in fortified sample

Note: If fortification experiments are required, untreated control samples must be analyzed using the same analytical method described to verify that any co extracted substances present in the samples do not interfere with the final determination of the analytes of interest. (See Appendix 4 for typical control chromatograms of representative soil matrices.)

Calculating recoveries as defined by *Equation 4* monitors the method efficiency. Recoveries are determined by analyzing fortified control samples in conjunction with each sample set. Samples are fortified prior to extraction at the lower limit of quantitation of 0.01 µg/g with standard solutions prepared in acetone. Calculate the final residue R for the control and fortified control samples. Correct the results of the recovery samples by subtracting the final determined residue (real or apparent) detected in the control sample from the value determined for the fortified control sample. (See Appendix 4 for typical untreated controls and fortified control chromatograms of representative soil samples.)

7. DISSCUSION

7.1 General

7.1.1 Time Considerations

For a set of 12 samples, the samples may be extracted and readied for instrumental analysis in the course of a normal 8 hour working day. Instrumental analysis for a set will take approximately 6 hours for the GC/MSD. This assumes that all reagents and standards have been prepared prior to initiating the procedure.

7.1.2 Analytical Stopping Points

As noted in the method, the procedure may be paused after extraction. This should allow flexibly to accommodate the analyst's normal working day or schedule. It is assumed that the analysis will resume during the next working period.

8. REFERECNCES

1. Aerobic Degradation of ZK 100 309 in a Loamy Sand Soil at 20 °C
Identification of a Degradation Product.
UPSR 76/91 – PA 100 309.7/17 dated 11.03.1992, Registration Reference W26

2 Analytical Method for the Determination of Residues of the Pyrimehanil Metabolite ZK 512 723 in Soil by GLC/MSD.
U/R 15/93 – PA 100 309.5/16 dated 07.01.1993, Registration Reference W42

3. An Analytical Method for the Determination of Residues of Pyrimethanil (AE B100309) and Its Major Metabolites AE F132593 Soil Using Mass Selective Detection.
RAM: AN/01/00 dated May 10, 2000.

4. Krieger, M.S., Cook, W.L.., and Kennard, Extraction of Tricyclazole from Soil and Sediment with Subcritical Water, J. Agric. Food Chem. 2000, 48, 2178-2183

Appendix 1 Instrument Conditions

GC Conditions (GC/MSD)

Instrument:	Hewlett-Packard 5890 with 5970 MSD	
Column:	Fused silica megabore DB-17MS bonded phase 30 m x 0.25 mm i.d., 0.25 µm film thickness. (J & W Cat No. 122-4732)	
Carrier Gas:	Helium (Ultrapure 99.999%)	
	Carrier Pressure:	10 psi
Temperatures:	Injection Port:	250 °C
	Transfer Line:	250 °C
	Oven: Programmed	
	Initial:	55 °C for 2.0 min.
	Ramp 1:	25 °C/min to 165 °C hold 1.0 min.
	Ramp 2:	25 °C/min to 265 °C hold 3.6 min.

Injection Parameters

Autosampler:	HP 7673	
Splitless Injection:	Split On Time:	0.00 Minute
	Split Off Time:	1.00 Minute
Liner:	Base Deactivated 4mm i.d. Restek Cyclosplitter	
	Sample Washes:	3
	Sample Pumps:	3
	Injection Volume:	2.0 µL
	Post Injection Washes A:	4
	Post Injection Washes B:	4
	Viscosity Delay:	1 seconds
	Plunger Speed:	Fast
Mass Spectrometry:	Solvent Delay:	6.0 minutes
	EM Offset:	200 mV
Analyte	AE F132593	
SIM Ion:	123 (Quantitation)	
	96, 123, or 124 (Selected Ions for Confirmation if Needed)	
Resolution:	Low	
Dwell Time:	100 ms	

Appendix 1 (continued)

Time Window:	6.00 to 7.50 minutes
Retention Time:	6.4 minutes
Analyte	AE B100309
SIM Ion:	198
	198, 199, and 200 (Selected Ions for Confirmation if Needed)
Resolution:	Low
Dwell Time:	100 ms
Time Window:	11.00 to 12.00 minutes
Retention Time:	11.3 minutes