434337-01

MORSE LABORATORIES, INC.
1525 Fulton Avenue
Sacramento, California 95825

SOP# Meth-62

Revision #4 Date 06/11/93

DETERMINATION OF THIOPHANATE METHYL (TM) AND ITS MAJOR METABOLITES, ALLOPHANATE (FH-432), DX-105 AND MBC IN/ON SOIL

METHOD REFERENCE:

HPLC Analytical Method for Simultaneous Determination of Thiophanate Methyl, Allophanate, DX-105 and MBC in/on Soils, 7/6/92, ELF-Atochem, North America.

PURPOSE OF REVISION:

1. To identify the HPLC gradient, mobile phase and column that are used to chromatograph MBC, FH-432, DX-105, and TM during a single analytical run. Specific changes are listed below.

   a. To identify specific brands of acetonitrile, hydrochloric acid and sodium phosphate that are used and to omit potassium hydroxide pellets and potassium hydroxide concentrated reagent from the Reagents and Materials section.

   b. To add the preparation of 6N hydrochloric acid and omit the preparation of 11.7M potassium hydroxide.

   c. Equipment and Apparatus section: To substitute the use of the DuPont Zorbax CN column for the Ultrasphere 60 RP-Select B column and Licrocart 4-4 guard column. To substitute the nylon 66 membrane filter for the cellulose acetate membrane filter used for the buffer preparation.

   d. To identify the new liquid chromatographic conditions in the HPLC Analysis section.

   e. To substitute the preparation of the 0.05M sodium phosphate buffer for the 0.025M potassium phosphate buffer as described in B. Preparation of Phosphate Buffer for Mobile Phase of the HPLC Analysis section.

   f. Omit C. Preparation of Mobile Phase of the HPLC Analysis section due to redundancy.

   Note: Method revisions 1a. through 1f. were previously used for Topsin set numbers 16 - 20. The analytical method used was referenced on

METHOD SOP DEVIATION

This is a true copy of the original document
By [signature] date 11/5/93

MORSE LABORATORIES PROJECT #: ML92-0299-ATO

PROTOCOL #: BR-92-30-1

METHOD #: SOP# Meth-[illegible]. Revision #4- "DETERMINATION OF THIOPHANATE METHYL (TM) AND ITS MAJOR METAPOLITES, ALLOPHANATE (FH-432), DX-105 AND MBC IN/ON SOIL"

DATE: October 14, 1993

SUBJECT: HPLC ANALYSIS: A. Liquid Chromatographic conditions: The detector wavelength setting is changed from 235 nm to 280 nm.

REASON: As of 10/08/93, the Study Director requested the analysis of only MBC for all future sampling intervals. To optimize the HPLC parameters for the MBC analysis, a detector wavelength setting of 280 nm will be used for the duration of the study. This was verbally authorized by the Study Director via telephone on 10/14/93 followed by written authorization on 10/15/93.

IMPACT ON STUDY: It is felt that this deviation may have a positive impact on the study; however, the final determination shall be made by the Study Director.

[signature]
Susan Clark
MORSE LABORATORIES, INC.
Analytical Project Coordinator
11-2-93
Date

[signature]
Gary L. Westberg
MORSE LABORATORIES, INC.
Laboratory Director
11/2/93
Date

[signature]
Jeannie Wright
ELF ATOCHEM NORTH AMERICA, INC.
Study Director
11-5-93
Date

Morse Laboratories, Inc. worksheets (ML120, 05/93) and Residue Data Summary Sheets (ML143, 04/91) as Morse Laboratories, Inc. SOP# Meth-62, Revision #3, with HPLC modifications.

2. The method deviation dated May 18, 1993 has been incorporated into this revision. Specifically, this deviation allows for the TM diluted fortification standard to be prepared on a bi-weekly basis as needed.

3. Step 6. of D. Solid Phase Extraction in the Sample Analysis section describes in greater detail how to evaporate the sample extract.

4. Added the word "immediately" to the start of step 7. of D. Solid Phase Extraction in the Sample Analysis section to assure there is no delay between step 6. and step 7.

PRINCIPLE:

Soil samples are extracted twice with 100 mL of an acetic acid:methanol mixture followed by two extractions with 100 mL of an ammonium hydroxide:methanol mixture. Following each extraction, the samples are centrifuged and the supernatants are collected. The pH of the combined supernatant is adjusted to 6.80 with ammonium hydroxide. To avoid losses, the pH change is conducted in an ice bath. The aqueous extract is then partitioned three times with methylene chloride (CH_2Cl_2). The CH_2Cl_2 extract is retained, concentrated by rotary evaporation and passed through a 1', 2' amino solid phase extraction column. The compounds are then eluted with a cyclohexane:ethyl acetate:acetonitrile mixture. This eluate is evaporated to dryness and redissolved in 5 mL of 15% methanol: 85% 0.025M potassium phosphate (KH_2PO_4). Quantitation and detection is by use of a high pressure liquid chromatograph equipped with a variable wavelength, ultraviolet detector. The limit of quantitation is 0.05 ppm for all analytes.

NOTE: During all analyses, equivalent apparatus, solvents, glassware, or techniques (such as sample concentration) may be substituted for those specified in the method unless otherwise noted. In the event an equivalent piece of equipment or an equivalent technique is used, its use will be documented in the study records.

REAGENTS AND MATERIALS:

Solvents:

NOTE:	**Solvents should be distilled in glass, residue grade and degassed by purging with nitrogen.**
Methanol:	"Resi-analyzed" J.T. Baker Chemical Co., Phillipsburg, NJ
Methylene Chloride:	"Omnisolve", EM Science, Gibbstown, NJ
Cyclohexane:	"Resi-analyzed" J. T. Baker Chemical Co., Phillipsburg, NJ
Acetonitrile:	"Resi-analyzed" J. T. Baker Chemical Co., Phillipsburg, NJ
Acetonitrile (HPLC use):	B & J Brand High Purity Solvent, Baxter Healthcare Corp., Burdick and Jackson Division, Muskegon, Michigan, 49442
Ethyl Acetate:	"Resi-analyzed" J. T. Baker Chemical Co., Phillipsburg, NJ

Reagents:

NOTE:	**Reagents should be ACS grade or better.**
Acetic Acid, Glacial:	"concentrated", EM Science, Gibbstown, NJ (do not substitute)
Ammonium Hydroxide:	"concentrated, reagent", Fisher Scientific, Fairlawn, NJ (do not substitute)
Hydrochloric Acid:	"concentrated reagent", J.T. Baker Chemical Co., Phillipsburg, NJ
Potassium Phosphate:	"monobasic", J. T. Baker Chemical Co., Phillipsburg, NJ
Phosphoric Acid:	"concentrated", J. T. Baker Chemical Co., Phillipsburg, NJ
o-Phosphoric Acid 85% (0.1N Phosphoric acid):	"HPLC-Grade", Fisher Scientific, Fairlawn, NJ

Water:	B & J Brand High Purity Solvent, Baxter Healthcare Corp., Burdick and Jackson Division, Muskegon, Michigan, 49442 (do not substitute)
Sodium Phosphate:	"monobasic, monohydrate", J.T. Baker Chemical Co., Phillipsburg, NJ
Sodium Sulfate:	anhydrous (granular), Mallinckrodt, Paris, KY
	NOTE: The sodium sulfate must be washed with methylene chloride and dried prior to use.
Dry Ice:	for ice baths (crushed) and rotary evaporation

High purity helium and nitrogen gas for sparging solvents

Preparation of Reagents:

0.05N Phosphoric Acid:	Add 1.1 mL of phosphoric acid to a 1 liter volumetric flask filled to approximately three-quarters with HPLC water. Dilute to 1 liter with HPLC water.
0.1N Phosphoric Acid:	Add 2.2 mL of phosphoric acid to a 1 liter volumetric flask filled to approximately three-quarters with HPLC water. Dilute to 1 liter with HPLC water.
0.5N Phosphoric Acid:	Add 11.2 mL of phosphoric acid to a 1 liter volumetric flask filled to approximately three-quarters with HPLC water. Dilute to 1 liter with HPLC water.
6N Hydrochloric Acid:	Add 50.0 mL of HPLC water to a 100 mL volumetric flask. Dilute to 100 mL with concentrated hydrochloric acid.
3N Acetic Acid:	Add 172.5 mL of concentrated acetic acid to a 1 liter volumetric flask filled to approximately three-quarters with HPLC water. Dilute to 1 liter with HPLC water.
1N Ammonium Hydroxide:	Add 69.0 mL of concentrated ammonium hydroxide to a 1 liter volumetric flask filled to approximately three-quarters with HPLC water. Dilute to 1 liter with HPLC water.

Preparation of Phosphate Buffer (for the 15% Methanol:85% 0.025M Potassium Phosphate Transfer Solution)

1. Weigh 3.40 g of monobasic potassium phosphate into a weighing vessel.

2. Transfer the potassium phosphate (KH_2PO_4) into a 1000 mL beaker (containing a stir bar) with rinses of HPLC water. Bring to a final volume of 1000 mL and place on a stir plate until dissolved.

3. Adjust to pH 3.50 (± 0.05) with 0.5N phosphoric acid.

Transfer Solution

15% Methanol:85% 0.025M Potassium Phosphate: 170 mL prepared 0.025M potassium phosphate (pH 3.5) mixed with 30 mL methanol (also used for the preparation of HPLC standard dilutions).

Solvent systems

NOTE: All solvent systems must be degassed by sparging with nitrogen prior to each use.

Extraction Solvent 1 - 3:1 Methanol:3N Acetic Acid (v/v)

Extraction Solvent 2 - 1:1 Methanol:3N Acetic Acid (v/v)

Extraction Solvent 3 - 3:1 Methanol:1N Ammonium Hydroxide (v/v)

1',2' Amino Solid Phase Extraction Column Eluting solvent:
50% cyclohexane:30% Ethyl Acetate:20% Acetonitrile

EQUIPMENT AND APPARATUS:

HPLC:	Spectra 100 UV/VIS detector SP8800 Ternary Gradient Pump equipped with SP4400 (ChromJet) integrator and SP8880 Autosampler (all manufactured by Spectra-Physics Analytical, Fremont, CA)
Column:	DuPont Zorbax CN (5 µm) 250 mm x 4.6 mm i.d. MAC-MOD Analytical, Inc., Chadds Ford, PA

Cold Finger Rotary Evaporation System:	Brinkmann Instruments, Inc., Westbury, NY or equivalent.
pH Meter:	capable of accurately measuring 0-14 pH
1',2' Amino Solid Phase Extraction Column:	Mega Bond Elut™ Reservoirs fitted with frits, Analytichem Bondesil PSA, 2 g, (prepacked by Varian), Varian, Harbor City, CA
Vac Elut™ SPS 24:	solid phase extraction vacuum collection unit or equivalent.
Laboratory platform (reciprocating) shaker:	Eberbach Model 6000, Eberbach Corp., Ann Arbor, MI
Glass funnel:	100 mm
Centrifuge:	equipped to accept 200 mL centrifuge bottles and capable of operating at 2000 rpm.
Centrifuge bottles:	glass 200 mL capacity with teflon-lined screw caps, Pyrex or equivalent.
Glass separatory funnel:	1000 mL capacity equipped with polyethylene stoppers, size 27.
Evaporation flasks:	1000 mL, 250 mL
Graduated cylinders:	assorted

Hot plate equipped with stirring mechanism

Magnetic stir bars

Ice baths

Filters:

For Buffer: Nylon 66 membrane filters, 0.45μ, Alltech Associates, Deerfield, IL 60015

For filtration of final sample extract: Millipore 0.45μ HV, Millipore Corp., So. San Francisco, CA

Beakers: 600 and 1000 mL capacity

Graduated, Class A pipets

Disposable Pasteur pipets

Volumetric flasks, Class A, assorted sizes

Syringes, (microliter) assorted sizes, Hamilton Co., Reno, NV

Assorted laboratory glassware

Aluminum foil

Amber screw-cap autosampler vials, Alltech Associates, Inc., Deerfield, IL

Aluminum weigh tins

Glass wool, VWR Scientific, Bridgeport, NJ

Whatman #4, 9 cm filter paper, VWR Scientific, Bridgeport, NJ

Balances:

Analytical balance: capable of weighing 0.0000 g for weighing analytical standards

Top-loading balance: capable of weighing 0.00 g for weighing samples and soil moistures.

Test tubes:

15 mL, conical, graduated, glass

13 x 100 mm borosilicate glass calibrated at 5.0 mL

STANDARDS:

1. Thiophanate Methyl (TM), Dimethyl {(1,2-phenylene) bis(iminocarbonothioyl)}bis(carbamate)

2. MBC, Methyl-2-benzimidazolyl carbamate

3. FH-432 (allophanate) Dimethyl{(1,2-phenylene) bis(iminocarbonyl)}bis(carbamate)

4. DX-105, MethylN-{2-(N'-methoxycarbonylthioureido) phenylaminocarbonyl}carbamate

NOTE: Analytical standards are available from Elf Atochem North America, Bryan, Texas 77801.

Standard Preparation

Preparation of TM, DX-105, and FH-432 standards

1. Correcting for purity, weigh 0.0100 g of TM, into a glass weigh boat and transfer to a 100 mL volumetric flask using methanol. Prepare the standard solutions for DX-105 and FH-432 in the same manner.

   NOTE: **Avoid use of plastics for the weighing of compounds and storing of reagents. The small amounts of plasticizers leaching into the sample will have a detrimental effect on the analysis.**

2. Dilute to volume with methanol and mix by inverting several times. Final concentration = 100 µg/mL.

3. The concentrated standard solutions remain stable at 1 to 8 °C for two weeks. New solutions should be prepared bi-weekly.

Preparation of MBC standard

1. Prepare a 0.05N solution of phosphoric acid (H_3PO_4) to be used as the dilnent.

2. Correcting for purity, weigh 0.0050 g of MBC into a 50 mL beaker. Pulverize to a fine powder with a small glass rod. Rinse the rod with a small amount of the dilute phosphoric acid.

3. Place a small stir bar into a 50 mL beaker, add 10 -20 mL of the phosphoric acid diluent and place the beaker on a heated stir plate.

4. Using low heat (~45 °C), gently stir the MBC for approximately 15 to 30 minutes, or until completely dissolved, adding additional amounts of the dilute phosphoric acid as necessary.

5. Wash into a 100 mL volumetric flask with the 0.05N phosphoric acid. Final concentration = 50 µg/mL

6. The concentrated standard remains stable at 1 to 8 °C for four weeks. New standard solutions should be prepared on a monthly basis.

Preparation of diluted fortification standards

NOTE: Make subsequent dilutions in volumetric flasks with appropriate solvents as needed for fortification.

1. FH-432, DX-105 and TM diluted standards should be prepared on a bi-weekly basis by dilution of the concentrated standard solution with methanol. Store at 1 to 8 °C.

2. MBC diluted standard should be prepared on a bi-weekly basis by dilution of the concentrated standard solution in 0.05N phosphoric acid. Store at 1 to 8 °C.

Preparation of HPLC standards

HPLC standards should be prepared on a bi-weekly basis by dilution of the concentrated standard solution with the 15% methanol:85% 0.025M potassium phosphate (pH 3.5) transfer solution. Store at 1 to 8 °C.

HPLC ANALYSIS:

A. Liquid Chromatographic Conditions

1. Wavelength: 235 nm

Mobile Phase: B: 100% 0.05M NaH_2P
C: 100% Acetonitrile

Gradient Program:

T	% B	% C
0	80	20
4	80	20
5	75	25
12	75	25
15	65	35
23	65	35
24	80	20
31	80	20

Flow Rate: 0.8 mL/min.

Injection Volume: 50 µL

B. Preparation of the 0.05M Sodium Phosphate Buffer for the Mobile Phase:

1. Weigh 6.90 g of monobasic, monohydrate sodium phosphate into a weighing vessel.

2. Transfer the sodium phosphate (NaH_2PO_4) into a 1000 mL beaker (containing a stir bar) with rinses of HPLC water. Bring to a final volume of 1000 mL and place on a stir plate until dissolved.

3. Adjust to pH 3.50 (± 0.05) with 6N hydrochloric acid added dropwise.

4. Filter the buffer through a nylon 66 membrane 0.45µ filter (using a millipore vacuum filter system).

SAMPLE ANALYSIS:

A. Extraction - Rapid processing is essential to the success of this analysis due to the labile nature of the analytes. Care must be taken to exclude light, heat, and oxygen from the sample extracts at all times. All clear flasks/beakers containing compounds should be kept wrapped in aluminum foil. All flasks/beakers containing samples should be stored under refrigeration when not in use. Exclusion of oxygen can be accomplished by purging all air spaces with N_2 as appropriate. All solvents and HPLC water used for the extraction, organic partition and solid phase extraction steps must be sparged with nitrogen for approximately 5 minutes prior to use.

1. Weigh 10.0 g of randomly sampled soil into a tared centrifuge bottle (200 mL capacity).

2. For the preparation of analytical recovery samples, fortify control samples in the extraction bottle by applying a known volume and concentration of the fortification standard onto the sample matrix.

3. Place the extraction bottle into an ice bath (approximately 4 °C or less). Add 100 mL of extraction solvent 1 (3:1 Methanol:3N Acetic Acid).

4. Purge any air from the extraction bottle (~5-10 seconds) by carefully directing a stream of nitrogen into the bottle. Avoid splashing contents or inserting stream directly into soil. Withdraw the N_2 stream and quickly cap the extraction bottle tightly to form a good seal.

5. Shake the sealed extraction bottle (placed on its side) for ten minutes using a reciprocating platform shaker.

6. Centrifuge the extraction bottle in chilled centrifuge cups for 5 minutes at approximately 2000 rpm.

7. Decant the supernatant to a 600 mL beaker.

8. Place the extraction bottle back into the ice bath. Add 100 mL of extraction solvent 2 (1:1 Methanol:3N Acetic Acid).

   NOTE: After adding 10-20 mL of extraction solvent, tap bottle gently to resuspend the pellet.

9. Repeat the extraction and centrifugation as outlined in steps 4 through 7, combining the supernatants into the 600 mL beaker.

10. Repeat steps 8 and 9 two more times using extraction solvent 3 (3:1 Methanol:1N Ammonium Hydroxide).

E. pH Adjustment

1. Place the 600 mL sample beaker containing the combined supernatants and a magnetic stir bar in an ice bath on a stir plate and stir rapidly. The ice bath temperature should be <4 °C during pH adjustment.

Appendix V

Copies of:

Morse Laboratories, Inc. Method SOP# Meth-62, Original, "Determination of Thiophanate Methyl (TM) and its Major Metabolites, Allophanate (FH-432), DX-105 and MBC in/on Soil"

Morse Laboratories, Inc. Method SOP# Meth-62, Revision #2, "Determination of Thiophanate Methyl (TM) and its Major Metabolites, Allophanate (FH-432), DX-105 and MBC in/on Soil"

Morse Laboratories, Inc. Method SOP# Meth-62, Revision #3, "Determination of Thiophanate Methyl (TM) and its Major Metabolites, Allophanate (FH-432), DX-105 and MBC in/on Soil"

Morse Laboratories, Inc. Method SOP# Meth-62, Revision #4, "Determination of Thiophanate Methyl (TM) and its Major Metabolites, Allophanate (FH-432), DX-105 and MBC in/on Soil"

Authentic Copy of Method (SOP) Deviation, dated 05/18/93 to Morse Laboratories, Inc. SOP# Meth-62, Revision #3

Authentic Copy of Method SOP Deviation, dated 10/14/93, to Morse Laboratories, Inc. SOP# Meth-62, Revision #4