Gas Chromatographic Method for Determination of Residues of Tebuconazole in Crops, Processed Products, Soil and Water

1.0 Summary

The analytical method for determining the residue of tebuconazole [FOLICUR®, HWG 1608, α-[2-(4-chlorophenyl)ethyl]-α-(1,1-dimethylethyl)-1H-1,2,4,-triazole-1-ethanol] in plant material, soil and water (Miles Ag Report No. 94295) has been revised based on suggestions following analysis of field-treated peanut meats and hay and minor modifications which were needed during an independent laboratory validation of the method. These revisions involve extraction, cleanup and gas chromatography procedures. In addition, during the analysis of tebuconazole residue in processed peanut products, different extraction procedures were needed. The extraction procedures developed for peanut processed products (refined and crude oil, soapstock) are included in this revised analytical method.

Extraction of residue of tebuconazole from grain (green foliage, kernels, and straw) and peanuts (foliage, hay, and kernels) involves an initial homogenization of the samples in acetone/water (3:1). In the case of peanut shells, the shells are not homogenized in acetone/water, but are first finely ground and then subjected to an overnight reflux in acetone/water (3:1). Plant material containing a high water content or aqueous samples (i.e. wine) are extracted with acetone only. The resultant acetone/water extract is saturated with sodium chloride, and the tebuconazole residue is partitioned into dichloromethane.

Extraction of the tebuconazole residue from refined and crude peanut oil involves extraction with hexane followed by partitioning the residue of tebuconazole into acetonitrile. Soapstock is extracted with ethyl acetate. The ethyl acetate is partitioned sequentially against 1*N* aqueous HCl and water. Residue of tebuconazole partitioning into ethyl acetate are further purified using a hexane/acetonitrile cleanup.

With the exception of peanut shells, all plant extracts are purified using gel permeation chromatography and silica gel column chromatography. Peanut shell extracts are subjected to an acetone precipitation procedure. With the exception of peanut oil, extracts are further purified using either an SPS hplc column or a C-18 Bond Elut® column prior to gas chromatography with a nitrogen/phosphorus detector.

The residue of tebuconazole is extracted from water samples using dichloromethane and from soil by refluxing with methanol/water (4:1). Water and soil samples are purified using gel permeation chromatography. In the case of extracts of soil and water, the silica gel column cleanup can be eliminated.

2.0 Introduction

The analytical residue method devised by Maasfeld[1] (Miles Ag Report No. 94295) for the gas chromatographic determination of tebuconazole [FOLICUR®, HWG 1608, α-[2-(4-chlorophenyl)ethyl]-α-(1,1-dimethylethyl)-1H-1,2,4,-triazole-1-ethanol] in plant material, soil and water has been revised and rewritten.

During the analysis of tebuconazole residue in crops[2,3] and during an independent laboratory validation (PR Notice 88-5) of the method[4], minor modifications were suggested to both the cleanup and gas chromatography procedures.

In a study using aged radioactive residues from [^{14}C] tebuconazole metabolism studies to determine the extraction efficiency of the procedures described in Miles Ag Report No. 94295, the extraction of [^{14}C] tebuconazole was found to be adequate from wheat straw, peanut forage, and peanut kernels but was marginal from peanut shells.[5] An overnight reflux in acetone/water (3:1) was found to improve the extraction of aged [^{14}C] tebuconazole residue from peanut shells. The residue method for peanut shells which includes an overnight reflux has been validated.[6]

Analysis of tebuconazole residue in processed peanut products has also been conducted using the analytical method described in Miles Ag Report No. 94295. Modifications of the crop method which were used to analyze for tebuconazole residue in peanut oil involved adding a hexane/acetonitrile partitioning followed by gel permeation chromatography and silica gel column chromatography.[6] Modifications of the crop method when used to analyze for tebuconazole residue in soapstock involved extracting with ethyl acetate, partitioning with hexane/acetonitrile followed by gel permeation chromatography and silica gel column chromatography, and using an SPS hplc column for final cleanup.[6] Earlier residue analyses of soapstock were performed using either a Lobar low pressure reverse phase column or a C-18 Bond Elut® column with adequate recovery.[7,8] The current residue method for soapstock was validated when using the SPS hplc column for final cleanup.[6]

All of the modifications referred to above have been included in this revised method.

3.0 Experimental

3.1 Materials

3.1.1 Apparatus

Beaker, 1-liter metal
Chromatography column: 19 mm id, length 340 mm, elongated discharge with Teflon cock or equivalent
Gas chromatograph with thermionic N/P detector, Varian 3700 or HP 5890 or equivalent
Gel permeation chromatograph GPC Autoprep 1002 (Analytical Biochemistry Laboratories, Inc., Columbia, MO) or equivalent, equipped with a chromatography column (25 mm id, length 600 mm) packed with 60 g of Bio Beads® S-X 3, 200-400 mesh, pretreated in cyclohexane/ethyl acetate 1:1 (v/v) to allow for swelling. Elution rate: 5.0 ml/min. (For each new gel column or after approx. 500 samples have been run, the elution profile should be determined, see 3.2.1.1)
Glass powder funnel, 100 mm diameter
Graduated cylinders, 100 ml, 250 ml, 500 ml
Graduated pipets, 1 ml, 3 ml, 5 ml, 10 ml
Heating mantels, for 1000 ml round-bottomed flasks, or equivalent
High rpm blender, Polytron (KINEMATICA, Kriens, Switzerland) or equivalent
Injection syringe with Luer-Lock connection, 10 ml

Injection syringe, 10 µl
Integrator, Spectra Physics 4100 or LAS on HP 1000 A900 or equivalent
Magnetic stirrer/hot plate or equivalent.
N-Evap analytical evaporator (Organomation Association, Inc., South Berlin, MA) or equivalent.
Porcelain Buchner funnel, 110 mm id
Round-bottomed flasks with ground-glass joints, 25 ml, 100 ml, 125 ml, 250 ml, 500 ml, 1000 ml
Reflux condenser
Rotary vacuum evaporator with water bath, bath temperature 30°C
Separatory funnels with ground-glass stopper, 500 ml, 1000 ml
Test tubes with ground-glass joints, 10 ml
Vacuum filter flasks, 500 ml, 1000 ml
Volumetric flasks with ground-glass joints, 25 ml, 50 ml, 100 ml

3.1.2 Reagents/Chemicals/Supplies

Acrodisc® CR PTFE, non-sterile, 0.45 µm and 1.0 µm pore size, No. 4219 and No. 4226, respectively (Gelman Sciences, product suppled by Baxter Scientific Product, McGaw Park, IL)
Bond Elut® C-18 octadecyl disposable column, 500 mg capacity (Varian, Harbor City, CA)
Filter aid, Celite® 545, not acid washed (Fisher Scientific, Fair Lawn, NJ) or equivalent
Filter paper, No. 41, 90 mm (Whatman, Hillsboro, OR)
Gases: helium, air, nitrogen, hydrogen.
Glass microfibre filters, GF/A, 90 mm (Whatman, Hillsboro, OR), for use on soapstock only
Glass wool
Hydrochloric acid (concentrated), reagent grade
Polystyrene gel: Bio Beads® S-X3, 0.037 to 0.074 mm, 200 to 400 mesh, No. 152-2750 (Analytical Biochemistry Laboratories, Inc., Columbia, MO)
Silica gel 60: 0.063 to 0.200 mm, 70 to 230 mesh (MERCK No. 7734), activated overnight at 130 to 150°C, then 5% (95 g silica gel + 5 g water) deactivated with water. (For each new batch of silica gel, the elution profile must be determined see 3.2.1.2)
Sodium chloride, reagent grade
Sodium sulfate (anhydrous), reagent grade
Solvents, pesticide grade: acetonitrile, acetone, cyclohexane, dichloromethane, ethyl acetate, n-hexane, methanol, toluene, water (hplc grade)
Solvent mixtures, acetone/water 3:1 (v/v), hexane/ethyl acetate 1:4 (v/v), hexane/ethyl acetate 1:1 (v/v), cyclohexane/ethyl acetate 1:1 (v/v), methanol/water 7:3 (v/v), methanol/water 9:1 (v/v), 1*N* HCl (v/v)

3.1.3 Standard Solutions Required

Analytical standards of tebuconazole may be obtained from Miles Inc., Agriculture, Metabolism and Residue Methodology, Miles Research Park, 17745 S. Metcalf Ave, Stilwell, Kansas 66085.

Prepare a standard solution of tebuconazole at 250 µg/ml in ethyl acetate. From this standard solution prepare 0.0625 µg/ml, 0.125 µg/ml, 0.25 µg/ml, 0.625 µg/ml, 1.25 µg/ml, 2.50 µg/ml, 25 µg/ml solutions of tebuconazole in ethyl acetate.

Store standards under refrigerated conditions (2°C). Stable under refrigerated conditions for at least 1 month.

3.2 Method

3.2.1 Calibrations

3.2.1.1 GPC Elution Profile Determination

1. Process a control sample through the method to the gpc step (3.2.3).

2. Fortify this control with 2 ml of the 250 µg/ml standard of tebuconazole.

3. Evaporate the solvent from the fortified control sample using a rotary vacuum evaporator, and re-dissolve the dry residue in 10 ml of the gpc solvent, cyclohexane/ethyl acetate (1:1, v:v).

4. Fill the 5-ml loop of the gpc, and inject 5 ml of the sample onto the gpc column.

5. Collect twenty-three, 10-ml fractions, and evaporate a 1-ml aliquot from each fraction to dryness.

6. Re-dissolve the dry residue of each fraction in 10 ml of ethyl acetate, and analyze each fraction by gc (Gas Chromatographic Analysis 3.2.6) to determine the amount of tebuconazole in the gpc column eluate by comparing against a 2.5µg/ml tebuconazole standard (this standard represent 100% recovery of tebuconazole from the column). Note: the amount of tebuconazole in 1 ml is 1/10th of the fraction (1 ml from 10 ml).

7. Establish the "dump" fraction and "collect" fraction ranges by plotting the percent of tebuconazole present in each of the twenty-three, 10-ml fractions. Starting from the last 10-ml fraction which contained tebuconazole and moving toward the first fraction, total the percent recovered until at least 90% has been reached. This set of fractions represents the "collect" fraction. In this manner, the majority of the control sample matrix will be excluded from the "collect" fraction and will be in the "dump" fraction.

3.2.1.2 Silica Gel Column Profile Determination

1. Process a control sample through the method to the silica gel cleanup procedure (3.2.4).

2. Prepare the chromatography column in the following sequence: 10 ml toluene, glass wool plug, 20 g silica gel slurried in toluene (filling height approximately 170 mm), approximately 10 mm sodium sulfate layer. Drain the toluene until the solvent is just above the sodium sulfate.

3. Fortify the processed control sample with 1 ml of the 250 µg/ml standard of tebuconazole.

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4. Evaporate the solvent from the fortified control sample using a rotary vacuum evaporator, and re-dissolve the dry residue in 10 ml of toluene.

5. Transfer the dissolved sample to the column with a pipet, and allow the toluene to drain down to the sodium sulfate layer.

6. Rinse the flask with 20 ml of hexane/ethyl acetate (1:1, v/v), and transfer the solvent to the column.

7. Repeat Step 6 three times for a total of 80 ml of rinse solvent. Note: collect all of the eluate up to and including this step in a 250-ml graduated cylinder. This fraction represents the "discard" fraction. Adjust the volume of the eluate to 200 ml and retain for Gas Chromatographic Analysis, 3.2.7.

8. Elute tebuconazole from the column with 200 ml of hexane/ethyl acetate (1:4, v/v). This fraction represents the "collect" fraction. Adjust the volume of the eluate to 200 ml, and retain for Gas Chromatographic Analysis, 3.2.7.

9. Analyze each "collect" and "discard" fraction by gc (Gas Chromatographic Analysis, 3.2.7) to determine the amount of tebuconazole in each fraction. Compare each fraction to a 1.25 µg/ml tebuconazole standard (the 1.25 µg/ml standard represents 100% recovery of tebuconazole from the column).

10. If greater than 10% of the tebuconazole is found in the "discard" fraction, decrease the moisture content of the silica gel to 2.5% water (97.5 g silica gel + 2.5 g water), and repeat the elution profile. If less than 90% of the tebuconazole is found in the "collect" fraction, increase the moisture content of the silica gel to 7% or 10% water (e.g. 90 g silica gel + 10 g water), and repeat the elution profile.

3.2.1.3 SPS Hplc Column Profile Determination

1. Prepare a 100 µg/ml standard of tebuconazole in methanol/water (85:15).

2. Initiate the chromatography process using the following hplc parameters:

   Instrument: High performance liquid chromatograph (hplc), Beckman Model 338 (Fullerton, CA) or equivalent, capable of solvent gradient elution and equipped with a variable wavelength uv flow-through detector.

   Mobile phase solvents: Methanol and hplc grade water
   Solvent flow rate: 2 ml/min
   UV detector wavelength: 220 nm
   Solvent program: Start with a linear gradient of 60% methanol to 80% methanol in 20 min, followed by a linear gradient of 80% methanol to 100% methanol in 15 min. Maintain 100% methanol for 15 min. Reequilibrate at 60% methanol for 25 min before making a second injection.

Column: Semi-Permeable Surface (SPS), C8, 5 μm, 25 cm X 10 mm i.d. (Regis Chemical Company, Morton Grove, IL)
Pre-Column: Spheri-5, RP-18, 5 μm, 30 mm X 4.6 mm (Applied Biosystems, Inc., San Jose, CA)
Sample loop: 1.0 ml

3. Inject 100 μg of tebuconazole in 1 ml methanol/water (85:15).

4. Determine the retention time for tebuconazole in order to establish when to collect the column eluate. If the retention time for tebuconazole is about 23 min, the collection window should be about 22 to 25.5 min. (The "collection window" should be 1 min before and 2.5 min after the observed elution time for tebuconazole)

3.2.2 Extraction

3.2.2.1 Grain (green foliage, kernels, straw); Peanut (green foliage, kernels, hay)

1. Add 400 ml of acetone/water (3:1) to 50 g of green foliage, or 50 g of kernels, or 25 g of straw or hay, contained in a 1-liter beaker; homogenize the sample for 3 min with a Polytron or equivalent blender.

2. Add approximately 15 g of filter aid to the beaker, and swirl the contents several times.

3. Filter the mixture through a porcelain Buchner funnel under gentle vacuum.

4. Rinse the beaker twice with 100-ml portions of acetone/water (3:1), and filter the rinse through the solids retained in the Buchner funnel under gentle vacuum.

5. Discard the filtered solids.

6. Transfer the filtrate to a 1000-ml separatory funnel.

7. Saturate the filtrate with approximately 40 g of sodium chloride, and partition the mixture with 100 ml of dichloromethane.

8. Allow the layers to separate, and discard the lower aqueous phase.

9. Transfer the organic phase to a 1000-ml boiling flask, and concentrate the organic phase to a volume of 40 ml or less using a rotary vacuum evaporator.

10. Add 100 ml of dichloromethane to the flask. While slowly swirling the flask, add 150 to 200 g of sodium sulfate to the flask.

11. Immediately filter the sample through a glass funnel containing a glass wool plug and approximately 3 cm of sodium sulfate into a 500-ml round-bottomed flask.

12. Rinse the 1000-ml boiling flask (Step 10) three times with 50-ml portions of dichloromethane, and pass the rinses through the sodium sulfate into the 500-ml round-bottomed flask in step 11.

13. Evaporate the filtrate plus rinses to dryness on a rotary vacuum evaporator.

14. Proceed to Gel Permeation Chromatography (3.2.3).

3.2.2.2 Peanut (shells)

1. Add 400 ml of acetone/water (3:1) to 25 g of finely ground shell contained in a 1000-ml boiling flask. Reflux the sample overnight (approx. 16 hours). Allow the sample to cool to room temperature prior to proceeding to Step 2.

2. Add approximately 15 g of filter aid (Celite or Hyflo-Super Cel) to the flask, and swirl the contents several times.

3. Vacuum filter the mixture through a Whatman #41 filter paper (Whatman, Hillsboro, OR) supported on a Buchner funnel into a 1000-ml separatory funnel equipped with a ground glass or Teflon stopcock.

4. Rinse the flask twice with 100-ml portions of acetone/H_2O (3:1), and vacuum filter the rinse through the solids retained on the Buchner funnel into the separatory funnel in Step 3.

5. Discard the filtered solids.

6. Saturate the filtrate with approximately 40 g of sodium chloride, and partition the mixture with 100 ml of dichloromethane.

7. Allow the layers to separate, and discard the lower aqueous layer.

8. Transfer the organic phase to a 1000-ml boiling flask, and concentrate the organic phase to dryness using a rotary vacuum evaporator. Use acetonitrile (approx. 150 to 300 ml) to form an azeotrope with any water present.

9. Add about 30 ml of acetone to the flask from Step 8, and sonicate (approx. 1 min) the flask to loosen the dry residues.

10. Slurry 10 g of filter aid (Celite 545®, Fisher Scientific, Fair Lawn, NJ) in acetone, and vacuum filter the slurry through a 5.5 cm Whatman #2 filter paper supported on a Buchner funnel. Discard the acetone.

11. Vacuum filter the sample from Step 9 through the Celite 545® prepared in Step 10 into a clean 500-ml boiling flask.

12. Repeat Step 9 two more times, and vacuum filter the acetone rinses into the 500-ml boiling flask in Step 11.

13. Evaporate the acetone filtrate to dryness using a rotary vacuum evaporator.

14. Dissolve the dry residues from Step 13 in methanol, and transfer the solution to a 13-ml centrifuge tube using methanol.

15. Adjust the volume of the methanol solution to 10.0 ml.

16. Filter the methanol solution through a 1.0 µm Acrodisc® CR PTFE (Gelman Sciences, Ann Arbor, MI) into a 13-ml centrifuge tube.

17. Proceed to SPS Hplc Column Cleanup (3.2.6).

3.2.2.3 Plant Material with High Water Content

1. Add 300 ml of acetone to 100 g of bananas, or 50 g of banana peel, or 100 g of grapes in a 1000-ml beaker; homogenize the sample for approximately 3 min with a blender.

2. Proceed to Step 2 of 3.2.2.1 for further processing of the sample.

3.2.2.4 Must and Wine

Note: Must is first mixed with approximately 15 g of filter aid and vacuum filtered through filter paper contained in a porcelain Buchner funnel to prevent emulsions.

1. Add 200 ml of acetone to 100 g must or wine contained in a 1000-ml separatory funnel.

2. Saturate the water solution with approximately 40 g of sodium chloride.

3. Add 100 ml of dichloromethane to the separatory funnel, and shake the separatory funnel for approximately 3 min.

4. Allow the layers to separate, and discard the lower aqueous phase.

5. Proceed to Step 9 of Section 3.2.2.1 for further processing of the sample.

3.2.2.5 Soil

1. Place 50 g of soil in a 1000-ml boiling flask containing 300 ml of methanol/water (7:3).

2. Attach a condenser to the boiling flask, and heat the flask contents at reflux for 4 hours.

3. Allow the sample to cool to room temperature; filter the sample using a gentle vacuum through filter paper contained in a Buchner funnel containing approximately 15 g of filter aid.

4. Rinse the flask twice with 50-ml portions of methanol/water (7:3), and filter the rinse through the solids retained in the Buchner funnel.

5. Concentrate the filtrate to the aqueous residue (approximately 100 ml) on a rotary vacuum evaporator.

6. Transfer the aqueous residue to a 250-ml separatory funnel.

7. Partition the aqueous residue with 100 ml of dichloromethane.

8. Allow the layers to separate, and drain the dichloromethane through sodium sulfate (glass funnel with glass wool plug and a 3 cm deep layer of sodium sulfate) into a 500-ml boiling flask.

9. Repeat Steps 7 and 8 twice with 50-ml portions of dichloromethane.

10. Rinse the sodium sulfate contained in the glass funnel three times with 25-ml portions of dichloromethane.

11. Evaporate the dichloromethane to dryness on a rotary vacuum evaporator.

12. Proceed to Gel Permeation Chromatography (3.2.3). Note: The silica gel column cleanup is not necessary.

3.2.2.6 Water

Note: For other volumes of water, the volumes of dichloromethane must be adjusted accordingly.

1. Add 200 ml of water to a 500-ml separatory funnel, and partition the water twice with 100-ml portions of dichloromethane.

2. Allow the layers to separate, and drain the dichloromethane through sodium sulfate (glass funnel with glass wool plug and approximately 3 cm deep layer of sodium sulfate) into a 500-ml boiling flask.

3. Repartiton the water layer with 50 ml of dichloromethane, and drain the dichloromethane through the sodium sulfate into the 500-ml boiling flash from Step 2.

4. Rinse the sodium sulfate with approximately 25 ml of dichloromethane.

5. Evaporate the dichloromethane to dryness on a rotary vacuum evaporator.

6. Proceed to Gel Permeation Chromatography (3.2.3). Note: The silica gel column cleanup is not necessary.

3.2.2.7 Refined and Crude Peanut Oil

1. Weigh 25 g of peanut oil into a 500-ml beaker.

2. Dissolve the oil in 200 ml of hexane and transfer to a 500-ml separatory funnel with two, 25-ml portions of hexane.

3. Partition the hexane solution with 100 ml of acetonitrile pre-saturated with hexane. Collect the acetonitrile phase in a 500-ml flask.

4. Repeat Step 3. Combine both acetonitrile fractions in the 500-ml flask, and evaporate the acetonitrile to dryness at room temperature using a rotary vacuum evaporator.

5. Proceed to Gel Permeation Chromatography (3.2.3).

3.2.2.8 Peanut Soapstock

1. Weigh 12.5 g of soapstock into a 1-liter beaker.

2. Add 200 ml of ethyl acetate to the beaker, and extract the soapstock using a Polytron for 30 sec.

3. Add 10 to 15 g of Celite® to the extract, and filter the mixture through a Whatman GF/A filter into a 500-ml separatory funnel. Rinse the beaker twice with 20-ml portions of ethyl acetate, and filter the rinse through the filter cake.

4. Partition the ethyl acetate filtrate with 100 ml of 1*N* HCl. Discard the aqueous layer.

5. Partition the filtrate two times with 200-ml portions of water (pre-saturated with ethyl acetate). Discard the aqueous layer.

6. Drain the ethyl acetate layer into a 500-ml boiling flask through a powder funnel containing a plug of glass wool and filled with sodium sulfate. Rinse the sodium sulfate three times with 20-ml portions of ethyl acetate.

7. Evaporate the sample to dryness using a rotary vacuum evaporator.

8. Transfer the sample to a 1000-ml separatory funnel using two, 25-ml portions of acetonitrile (pre-saturated with hexane) and two, 100-ml portions of hexane.

9. Shake the separatory funnel for 30 sec, and drain the lower acetonitrile phase into a 250-ml boiling flask.

10. Partition the hexane fraction with an additional 50 ml of acetonitrile (pre-saturated with hexane), and drain the acetonitrile into the same 250-ml boiling flask from Step 9.

11. Evaporate the combined acetonitrile fractions to dryness on a rotary vacuum evaporator.

12. Proceed to Gel Permeation Chromatography (3.2.3).

3.2.3 Gel Permeation Chromatography (gpc)

1. Dissolve the dry residue in 10 ml of cyclohexane/ethyl acetate (1:1, v/v).

2. Using a 10-ml glass syringe, inject 7 to 8 ml into a 5-ml sample loop on the gel chromatograph.

3. Based on the elution profile determined in 3.2.1.1, collect tebuconazole from the gpc column in a 125-ml boiling flask, and evaporate the solution to dryness on a rotary vacuum evaporator.

4. Proceed to Silica Gel Column Chromatography (3.2.4). Note: For soil and water samples proceed to Gas Chromatographic Analysis (3.2.7).

3.2.4 Silica Gel Column Chromatography

1. Use the column as prepared in 3.2.1.2.

2. Dissolve the dry residue in 10 ml of toluene, transfer this solution to the column with a pipet, and allow the toluene to drain down to the sodium sulfate layer.

3. Elute the column as determined in 3.2.1.2.

4. Collect the tebuconazole fraction in a 250-ml round-bottomed flask, and evaporate the solution to dryness on a rotary vacuum evaporator.

5. Proceed to C-18 Bond Elute Cleanup (3.2.5). Note: For peanut oil proceed to Gas Chromatographic Analysis (3.2.7), and for soapstock samples proceed to the SPS Hplc Column Cleanup (3.2.6).

3.2.5 C-18 Bond Elut® Cleanup

1. Add 1 ml of methanol to the dry residue, and swirl the flask.

2. Add 9 ml of water to the sample, and swirl the flask.

3. Condition a C-18 Bond Elut® column with approximately 2 ml of methanol followed by approximately 2 ml of water.

4. Add the aqueous sample to the C-18 column using a pipet, and elute the column dropwise. Discard the eluate.

5. Wash the column with 1 ml of 10% methanol in water, and elute the column dropwise. Discard the eluate.

6. Add 10 ml of methanol/water (7:3), and elute the column dropwise. Collect the eluate.

7. Evaporate the methanol/water fraction to dryness on a rotary vacuum evaporator. Add acetonitrile to form an azeotrope with the water.

8. Transfer the dry residue to a 13-ml centrifuge tube with acetone.

9. Evaporate the residue to dryness using a gentle stream of nitrogen.

10. Proceed to Gas Chromatographic Analysis (3.2.6).

3.2.6 SPS Hplc Column Cleanup

1. Establish a "collection window" as described in 3.2.1.3 (SPS Column Profile Determination).

2. Sample preparation.

A. Peanut shells

(1). Pipet 5.0 ml of the methanol filtrate from Step 16 of 3.2.2.2 into a 13-ml centrifuge tube, and evaporate the methanol filtrate to 1.7 ml using a gentle stream of nitrogen.

(2) Add 300 μl of distilled water to bring the volume in the tube to 2.0 ml, and mix the contents of the tube well and centrifuge if necessary.

(3) Reserve the sample for hplc injection (Step 3).

B. Soapstock

(1) Transfer the dry residues from Step 4 of 3.2.4 to a 5-ml centrifuge tube.

(2) Evaporate the sample to the oily residue using a gentle stream of nitrogen.

(3) Pipet 1.7 ml of methanol to the sample and mix the contents of the centrifuge tube to dissolve the residue.

(4) Pipet 0.3 ml of distilled water to the sample and mix the contents again.

(5) Centrifuge the contents of the centrifuge tube for 10 min at approximately 1200 rpm.

(6) Reserve the sample for hplc injection (Step 3).

3. Inject 1.2 ml of the sample solution from Step 2 into the 1.0 ml sample loop of the hplc.

4. Collect the eluate during the "collection window" established in Section 3.2.1.3 (SPS Column Profile Determination) in a 125-ml pear-shaped flask.

5. Evaporate the solvent in the 125-ml flask to an aqueous solution using a rotary vacuum evaporator.

6. Add 30 ml of acetonitrile to the flask, and evaporate the solution to dryness using a rotary vacuum evaporator.

7. Dissolve the dry residues in ethyl acetate, and transfer the solution to a 13-ml centrifuge tube.

8. Refer to Table 1 for the correct sample volume needed for gc analysis.

9. Proceed to Gas Chromatographic Analysis (3.2.7)

3.2.7 Gas Chromatographic Analysis

3.2.7.1 Standard Procedure

A. Instrument Conditions

Primary:
Instrument: Varian 3700
Data Acquisition: Hewlett-Packard 1000 Mini-Computer with Beckman CALS System Software
Detector: TSD (Nitrogen-Phosphorous)

Column: 6 ft x 0.25 inch x 2 mm id 3% OV-101 on 80/100 Supelcoport
Gases:
Carrier gas: Helium, 12 ml/min
Makeup: Helium, 17 ml/min
Combustion: Hydrogen, 5 ml/min; Air, 125 ml/min

Temperatures:
Injector: 250°C or 280°C
Detector: 330°C
Column program: 220°C isothermal

Alternate:
Instrument: 5890A Hewlett Packard gas chromatograph
Data Acquisition: 3393A Integrator
Detector: N/P (nitrogen/phosphorous)

Column: Fused silica HP-5, 10 m x 0.53 mm id with 2.65 μm film thickness
Gases:
Carrier gas: Helium, 5 ml/min
Makeup: Helium, 25 ml/min
Combustion: Hydrogen, 3.5 ml/min; Air, 110 ml/min

Temperatures:
Injector: 280°C
Detector: 300°C
Column program: 60°C for 1 min
60°C to 180°C at 30°C/min
180°C to 240°C at 15°C/min
240°C for 15 min
240°C to 280°C at 10°C/min*
280°C for 5 min*
(*To be used with peanut meat if late eluting peaks are present.)
Alternate column temperature: 220°C

Alternate:
Instrument: Varian 3400 gas chromatograph
Data Acquisition: Perkin-Elmer Nelson System
Detector: TSD (nitrogen/phosphorous)

Column: HP-1, 25 m x 0.32 mm id with 0.17 μm film thickness

Gases:
Carrier gas: Helium, 2 ml/min
Makeup: Nitrogen, 26 ml/min
Combustion: Hydrogen, 4.5 ml/min; Air, 170 ml/min

Temperatures:
Injector: 250°C
Detector: 300°C
Column program: 180°C for 1 min
180°C to 230°C at 10°C/min
230°C for 15 min

B. Standard Curve (e.g. peanut oil, weight following gpc = 12.5 g, final volume = 1 ml)

1. Inject 3 to 5μl of each tebuconazole standard, 0.0625 μg/ml, 0.125 μg/ml, 0.25 μg/ml, 0.625 μg/ml, 1.25 μg/ml and 2.50 μg/ml prepared in ethyl acetate and in control matrix. The six standard concentrations above represent 0.005 ppm, 0.01 ppm, 0.02 ppm, 0.05 ppm, 0.1 ppm, and 0.2 ppm based on a final weight of 12.5 g following gpc and a final volume of 1 ml.

2. Plot area/height versus concentration to confirm a linear response of tebuconazole. The 1.25 μg/ml tebuconazole standard represents a 0.1 ppm standard based on a sample size of 12.5 g and a final volume of 1 ml.

C. Procedure:

1. Dissolve the dry residue in an appropriate volume of ethyl acetate (see Table 1).

2. Inject a 5 μl aliquot of the 0.1 ppm equivalent standard solution of tebuconazole (1.25μg/ml), and determine the area of the tebuconazole peak.

3. Inject a 5 µl aliquot of the sample, and determine the area of any peak at the retention time of tebuconazole.

4. Repeat sequence of standard, sample, standard, sample, etc. for injections.

5. For the purpose of calculating the ppm recovery in a sample, use the average value of the peak area of the two standards analyzed before and after the sample.

6. If the content of tebuconazole is greater than the response for the highest linearity value, dilute the sample appropriately with ethyl acetate to be within the linear range for tebuconazole, and re-inject the diluted sample.

D. Calculations

Example calculation for determining residues in peanut oil:

Extracts are diluted to a final volume of 1.0 ml and quantitated versus a 1.25 µg/ml tebuconazole standard. The 1.25 µg/ml tebuconazole standard is equivalent to a 0.1 ppm sample residue as shown below.

$$1.25\mu g/ml \times \frac{\text{(Sample Volume) } 1.0\text{ ml}}{\text{(Sample Wt) } 25\text{ g}} \times \frac{\text{(Pre-GPC Vol) } 10\text{ ml}}{\text{(GPC Aliquot) } 5\text{ ml}} = 0.1\ \mu g/g\ (0.1\text{ ppm})$$

Example calculation for determining residues in peanut shell:

Extracts are diluted to a final volume of 0.5 ml and quantitated versus a 1.25 µg/ml tebuconazole standard. The 1.25 µg/ml tebuconazole standard is equivalent to a 0.1 ppm sample residue as shown below:

$$1.25\ \mu g/ml \times \frac{\text{(Sample Volume) } 0.5\text{ ml}}{\text{(Sample Wt) } 25\text{g}} \times \frac{\text{(Pre-Filtration) } 10\text{ ml}}{\text{(Filtrate Aliquot) } 5\text{ ml}} \times \frac{\text{(Pre-Hplc Vol) } 2}{\text{(Hplc Aliquot) } 1} = 0.1\mu g/g\ (0.1\text{ppm})$$

Example calculation for determining residues in peanut kernels:

Extracts are diluted to a final volume of 2.0 ml and quantitated versus a 1.25 µg/ml tebuconazole standard. The 1.25 µg/ml tebuconazole standard is equivalent to a 0.1 ppm sample residue as shown below:

$$1.25\ \mu g/ml \times \frac{\text{(Sample Volume) } 2.0\text{ ml}}{\text{(Sample Wt) } 50\text{ g}} \times \frac{\text{(Pre-Filtration) } 10\text{ ml}}{\text{(Filtrate Aliquot) } 5\text{ ml}} = 0.1\mu g/g\ (0.1\text{ppm})$$

Residue levels in sample extracts are calculated by the following equation.

$$\frac{\text{Peak Response (Sample)}}{\text{Avg Peak Response (Standard)}} \times \text{Std Conc (0.1 ppm)} \times \text{Dilution Factor} = \text{ppm in sample}$$

3.2.7.2 Confirmatory Procedure

A. Instrument Conditions

Instrument: Varian 6000
Data Acquisition: Nelson Data Integration System (Model 2600 Chromatography Software, Rev 4.1, Nelson Analytical)
Detector: TSD (Nitrogen-Phosphorous)

Column: Rtx-200 15 m x 0.53 mm with a 1.0 µm trifluoropropylmethyl film thickness (Restek Corporation, Bellefonte, PA)

Gases:
Carrier gas: Nitrogen, 3 ml/min
Makeup: Nitrogen, 22 ml/min
Combustion: Hydrogen, 4.6 ml/min; Air, 170 ml/min

Temperatures:
Injector: 300°C
Detector: 300°C
Column: 210°C isothermal

B. Standard Curve - see Standard Procedure (3.2.7.1)

C. Procedure - see Standard Procedure (3.2.7.1)

D. Calculations - see Standard Procedure (3.2.7.1)

4.0 Conclusion

The analytical residue method (Miles Ag Report No. 94295) for determining residues of tebuconazole in plant material, soil and water has been rewritten to include modifications in extraction procedures, cleanup procedures, and gas chromatography analysis. In addition, extraction procedures for peanut processed products (refined and crude oil, soapstock) have also been included in this revised method.

5.0 Bibliography

1. Maasfeld, W. 1986. Method for gas chromatographic determination of residues of the fungicide FOLICUR (HWG 1608) in plant material, soil and water. Miles Ag Report No. 94295. (EPA MRID 40700963)

2. Williams, B. 1988. FOLICUR - Magnitude of the residue on peanuts 3.6F. Miles Ag Report No. 99129. (EPA MRID 51263316)

3. Koch, D. A. and B. B. Williams. 1988. FOLICUR (1.2 EC and 45 DF) - Magnitude of the residues on peanuts. Miles Ag Report No. 96728. (EPA MRID 40995936)

4. Erhardt-Zabik, S. and L. O. Ruzo. 1989. Validation of Miles Method No. 94295 - method for gas chromatographic determination of residues of the fungicide FOLICUR (HWG 1608) in plant materials, soil and water. Miles Ag Report No. 99624. (EPA MRID 41263319)

5. Minor, R. G. and P. L. Freeseman. 1991. Extraction efficiency of the analytical residue method for tebuconazole in plants. Miles Ag Report No. 101342.

6. Bajzik, M. 1992. The independent laboratory method validation for the analysis of tebuconazole (FOLICUR®) in crops and processed products. Miles Ag Report No. 101991.

7. Koch, D. A. and B. B. Williams. 1989. FOLICUR - Magnitude of the residue on peanut and peanut processed products. Miles Ag Report No. 98541. (EPA MRID 41068501)

8. Leslie, W. L. 1988. FOLICUR - Magnitude of the residue on peanut processed products. Miles Ag Report No. 98308. (EPA MRID 40995945)

Table 1. Final volumes for sample prior to gas chromatographic analysis. (Samples are compared to a 1.25 µg/ml tebuconazole standard)

Matrix	Sample Wt. (g)	Post-GPC Sample Wt. (g)	Post-Hplc Sample Wt. (g)	Final Vol. (ml)
Wheat				
Straw	25	12.5		1[1]
Green Forage	50	25		2[1]
Kernels	50	25		2[1]
Peanuts				
Hay	25	12.5		1[1]
Green Forage	50	25		2[1]
Kernels	50	25		2[1]
Peanut shell	25	-	6.25	0.5[1]
Peanut oil	25	12.5		1.0[1]
Soapstock	12.5	6.25	3.125	0.25[1]
Bananas				
Bananas	100	50		4[1]
Banana Peels	50	25		2[1]
Grapes				
Grapes	100	50		4[1]
Others				
Soil	50	25		2[1]
Wine/Must	100	50		0.4[2]
Water/Leachate	200	100		0.8[2]

[1]Based on the sample weight and final volume of the sample the 1.25 µg/ml standard represents a 0.1 ppm standard

[2]Based on the sample weight and final volume of the sample the 1.25 µg/ml standard represents a 0.01 ppm standard