Appendix 6. Analytical Method

HW-001-S09-01

METHOD TITLE

Analytical Method For The Determination of Residues of Tebuconazole In Soil Using LC/MS/MS

Report Author
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DATE

14 May, 2009

Guideline Requirement

OPPTS 860.1340

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Residue Analytical Method Number

HW-001-S09-01

Total Number of Pages

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Appendix 6. Analytical Method (continued)

Bayer CropScience **HW-001-S09-01**

1. SUMMARY

This method is suitable for the determination of the total extractable residues of Tebuconazole in soil. The limit of quantitation (LOQ) of the method is 10.0ng/g.

Tebuconazole is extracted from soil using microwave extraction. An isotopic internal standard is added to the extract, which is evaporated to dryness, reconstituted in water:acetonitrile with acetic acid and analyzed by LC/MS/MS for tebuconazole. One MRM transition was monitored for tebuconazole: m/z 308→70.

This method is suitable for the analysis of tebuconazole in soil at an LOQ of 10.0ng/g.

2. BACKGROUND

This analytical method was developed for the analysis of tebuconazole in soil, and based on *method 107704* "Validation of an analytical *method for* the determination of Tebuconazole in Soil and turf by Electrospray HPLC/MS-MS"[1] and issued on June 3, 1997. In the original method a sample aliquot was extracted using a Soxtec™ Extractor followed by analysis on a TSQ7000 MS/MS. In this updated method microwave extraction was used in place of the Soxtec Extractor, while samples were analyzed on an API 4000 MS/MS.

The structure of tebuconazole is presented in Section 4.

Typical recovery results are presented in Appendix 3. This data was obtained during the development of this method and also includes data from the reanalysis of a sample previously analyzed using the original method (107704) to confirm that the updated extraction procedure gives similar results. The limit of quantitation (LOQ) of the method is 10.0ng/g.

3. PRINCIPLE

Tebuconazole is extracted from soil by adding 7:3 v/v methanol:water to an aliquot of soil followed by microwave extraction. An isotopic internal standard is added to the extract and further diluted using 7:3 v/v methanol:water. An aliquot of this sample is analyzed by tandem mass spectrometry (LC/MS/MS). Quantification was based on the use of internal standards and comparison of peak areas with those of known standards.

The detection by MS/MS was performed on a triple-quadrupole tandem mass spectrometer, equipped with a Turbo IonSpray (ESI) interface operated in positive ion mode and multiple reaction monitoring (MRM).

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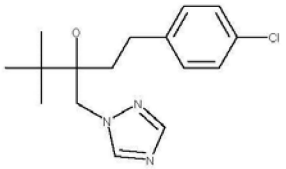
4. COMPOUNDS

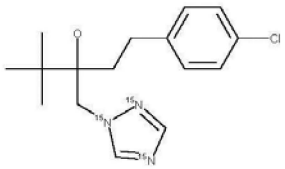
The structures for Tebuconazole and the associated internal standards are presented below:

Code Name: Tebuconazole
(Parent Molecule)



CAS Name: α-[2-(4-Chlorophenyl)ethyl]-α-(1,1-dimethylethyl)-1*H*-1,2,4-triazole-1-ethanol
CAS Number 107534-96-3
Molecular Formula: $C_{16}H_{22}ClN_3O$
Molecular Weight: 308g/mol

Code Name: Tebuconazole -$^{15}N_3$
(Parent Molecule, Isotopic Internal Standard)



CAS Name: α-[2-(4-Chlorophenyl)ethyl]-α-(1,1-dimethylethyl)-1*H*-1,2,4-triazole-*1,2,4*-$^{15}N_3$-1-ethanol
Molecular Formula: $C_{16}H_{22}Cl^{15}N_3O$
Molecular Weight: 311g/mol

5. APPARATUS

Use as a guide; equivalent apparatus may be substituted.

- VWR Pyrex® Brand volumetric pipets, glass class A (Assorted Volumes)
- Eppendorf Reference Series 2000 pipettes (Cat. No.: 05-402-48 and 05-402-50)
- VWR Pyrex® Brand volumetric flasks, glass class A (Assorted Volumes)
- VWR Pyrex® Brand disposable Pasteur pipets (Cat. No.: 53283-910 & 53283-914)

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- National Scientific LC vials, Snap-Its (Cat. No.: C4011-5)
- National Scientific LC vial Snap-It Seals, (Cat. No.: C4011-55)
- Phenomenex Prodigy 50 x 2mm 5µm particle size(Part Number 287559-4)
- Milestone Ethos E Microwave Labstation, equipped with a Model 320 Touch Screen Controller, a PRO-16 High throughput sampler rotor and automatic temperature control with fiber optic sensor.
- Milestone 100mL Pressure Reactors
- Applied Biosystems PE Sciex 4000 LC/MS/MS System with Analyst Software Version 1.4.1 or higher installed.
- Shimadzu LC-10AD VP HPLC pumps (two), Shimadzu SCL-10A VP Controller with a Shimadzu HIL-20A autosampler
- VICI Cheminert Valve and 2 position actuator Controller.
- Disposable, 1"-long, 5/16"-diameter magnetic stir bars (Fisher catalog # 1451394)
- Fisherbrand 125-mL glass jars (Cat. No. 02-911-455)
- Fisherbrand 50-mL polypropylene disposable centrifuge tube (Cat. No. 06-443-18)
- Fisherbrand 1.5mL micro centrifuge tubes (Cat. No: 02-681-238)
- Eppendorf centrifuge 5810.

6. REAGENTS

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

- Methanol, Fisher Scientifc Optima
- Acetonitrile, Fisher Scientifc Optima
- Deionized Water filtered through a Milli-Q water system or Water, Fisher Scientific Optima
- Ammonium Acetate, Aldrich
- Certified analytical reference standards of Tebuconazole.
- Certified internal standards of Tebuconazole-triazole-$^{15}N_3$.

7. PREPARATION OF ANALYTICAL STANDARDS

NOTE: The following procedure is an example description of how standard solutions may be prepared. Standards may be prepared as mixed solutions by dilution from individual stock solutions or prepared individually. Alternate or additional standards of appropriate weight and volume may be prepared as needed.

Class "A" volumetric glassware or calibrated pipets should be used in the preparation of all analytical standards. All standard solutions should be stored in a refrigerator in amber glass bottles when not in use. Solutions should be allowed to warm to room temperature prior to use.

Appendix 6. Analytical Method (continued)

7.1 Primary Stock Standard Solutions

Prepare an individual stock solution of tebuconazole (~100µg/mL) by transferring a known amount of the analyte into a 100mL volumetric flask, diluting to an appropriate volume with acetonitrile and mix well.

NOTE: Corrections for standard purities should be applied when expressing standard concentrations.

7.2 Fortification Standard Solutions

Prepare a 1.0µg/mL (1000ng/mL) fortification solution by taking a 1.0mL aliquot of the 100µg/mL stock solution and diluting to 100mL with acetonitrile.

Further dilutions of this fortification solution may be made as needed.

7.3 Isotopic Internal Standard Solutions

Prepare an individual 100µg/mL stock solution of tebuconazole-triazole-$^{15}N_3$ by transferring a known amount of tebuconazole-triazole-$^{15}N_3$into a 50mL volumetric flask, diluting to an appropriate volume with acetonitrile and mix well.

Prepare a 1.0µg/mL (1000ng/mL) internal standard solution by taking a 1.0mL aliquot of the 100µg/mL stock solution and diluting to 100mL with acetonitrile.

Further dilutions of this mixed fortification solution may be made as needed.

7.4 Calibration Standard Solutions

Prepare working calibration solutions consisting of 0.0, 2.5, 5, 10, 20, 50, 100, and 200ng/mL for tebuconazole in 70/30 methanol:water as shown in the table below. Further calibration solutions may be prepared as needed.

When refrigerated, the calibration solutions are stable for at least 4 months.

Appendix 6. Analytical Method (continued)

Concentration of Standard Solution used for dilution (ng/mL)	Concentration of Internal Standard Solution used for dilution (ng/mL)	Aliquot Native mix Taken (mL)	Aliquot Internal Standard Taken (mL)	Dilution Volume (mL)	Concentration of Calibration Solution (ng/mL)
1000	1000	20.0	0.5	100.0	200.0
1000	1000	10.0	0.5	100	100.0
1000	1000	5.0	0.5	100	50.0
1000	1000	2.0	0.5	100	20.0
1000	1000	1.0	0.5	100	10.0
1000	1000	0.5	0.5	100	5.0
1000	1000	0.25	0.5	100	2.5
1000	-	-	0.5	100	0

8. ANALYTICAL PROCEDURE FOR ANALYSIS OF SOIL

A summary of the analytical method parameters is presented in Table 1.

Stopping points in the analytical method are designated by the following symbol: §

8.1 Sample Preparation

Treated samples of soil should be thoroughly homogenized and stored frozen until sampled for extraction.

8.2 Extraction

Note: This method uses an internal standard to determine the concentration of Tebuconazole in soil. If the Tebuconazole concentration is above the range of the calibration curve the analyses will have to be repeated using either a reduced sample weight or by further diluting the sample extract prior to the addition of the internal standard. If a further dilution is made to the final extract, adjust the concentration of internal standard added in step 8.2.8 so that the final concentration of internal standard present in the final sample is 5.0ng/mL.

8.2.1 Weigh 20 ± 0.05 grams of soil into the Milestone Ethos E Teflon pressure reactor vessel (alternatively, the sample may be weighed into a 50mL disposable centrifuge tube if the extraction is not being performed immediately, and transferred to the pressure reactor vessel just prior to extraction). §

8.2.2 Fortify the recovery samples at the desired fortification level with the appropriate standard solution prepared in acetonitrile. For a fortification at the LOQ of 10ng/g,

Appendix 6. Analytical Method (continued)

add 0.20mL of the 1.0µg/mL fortification solution to the sample aliquot. (see Section 7.2 Fortification Stock Solutions). Let the fortified samples sit for about 5 minutes.

8.2.3 Add approximately 50mL of 70:30 methanol:water to each sample.

Note: If the sample was initially weighed into a disposable centrifuge tube, rinse the tube with 50mL of solvent, before adding it to the sample.

8.2.4 Add a magnetic stirrer to each reactor vessel, and insert the reactor vessel into the outer safety shield. Place the Teflon cover over the pressure reactor vessel, and cap the outer safety shield with the 30 bar safety valve. Hand tighten the safety valve.

Note: The microwave extraction system monitors the reaction temperature using an automatic fiber optic temperature control system. The temperature sensor is directly inserted into one of the reactor vessels through a modified Teflon cover and safety valve. It is recommended that the reaction temperature is monitored using the UTC sample.

8.2.5 Load the samples into the polypropylene rotor body, and ensure that the self releasing pressure valves in each of the reactor vessels are pointing away from the rotor. Insert the fiber optic temperature control probe into the UTC pressure reactor vessel, and manually rotate the rotor body to check that the temperature control probe cable does not catch on any part of the rotor body.

8.2.6 Close the microwave door, and program the microwave with the following method:

Step Number (Nr)	Time Duration (t)	Temperature set point at end of step (T1)	Power Limit to maintain/ control temperature (E)	Comments
1	10 min.	100 °C	800 Watts Max	Ramp from ambient to 90 °C
2	15 min.	100 °C	350 Watts Max	Maintain 90 °C

Ventilation time:	1 minute
QP limit:	60-80% (Shut off limit in case vapors in oven become too concentrated)
Stirrer (speed setting):	Value not used. Manual control overrides program setting.
Rotor control:	On (Rotor rotation is on)
Twist control:	On (Rotor rotates clockwise and then counterclockwise to keep probe cable from twisting)

8.2.7 Extract the samples using the above method. Once the reaction vessels have cooled, remove the pressure reactor vessels from the microwave and release excess pressure from the reaction vessel by introducing the relief valve tool into the hole of the self-releasing valve on the pressure reactor. Ensure the external hole of the releasing valve is pointing into a fume hood.

Appendix 6. Analytical Method (continued)

8.2.8 Add by pipet 500µL of the 1.0µg/mL internal standard solution prepared in acetonitrile (see Section 7.3 Internal Standard Solutions) to the contents of each reaction vessel and shake well. §

8.2.9 Transfer ~1.5mL of the extraction solution to a 2mL HPLC vial. Centrifuge the sample to remove fine particles of soil. (recommended conditions: ~ 3500 rpm for a minimum of 10 minutes).

8.2.10 Transfer ~1mL of this solution to a HPLC vial and dilute with ~ 1mL of 70:30 methanol: water. Cap the vial and mix well. Await analysis by LC/MS/MS. §

9. LC/MS/MS ANALYSIS

9.1 Sample Analysis

Tebuconazole is analyzed by LC/MS/MS using an isotopic internal standard.

Inject an aliquot of each test sample (or fortified sample matrix) from step 8.2.10 onto the LC/MS/MS under the conditions presented in Appendix I.

Variations in equipment or sample characteristics may require different injection volumes or slight modifications in the chromatographic or detector conditions listed in order to obtain adequate chromatographic peak shapes or sensitivity.

It is often beneficial to make several 'priming' injections of standards and/or samples prior to starting the LC/MS/MS analysis. Typically 4 to 6 priming injections are made. The results from these injections are not included in any calculations used in residue determinations. These injections help stabilize the LC/MS/MS response prior to running the analytical set.

Example chromatograms using each of the three sets of LC/MS/MS conditions are shown in Appendix 4.

9.2 LC/MS/MS Standard Calibration and Residue Calculations

Standardize the LC/MS/MS response under the conditions outlined in Appendix 1 by injecting an aliquot of each LC/MS/MS calibration solution both before and after the sample solutions.

Tebuconazole residues were quantified using internal standard linear regression analysis. A separate calibration curve was produced for each set of samples analyzed on the LC/MS/MS. A calibration curve was generated by 1/x weighted linear regression of the ratio of standard peak/internal standard peak areas versus the standard concentrations in ng/mL using Applied Biosystems Analyst Software (Version 1.4.1), a computer-programmed data capturing system. The Analyst Software uses the MS/MS standard responses to calculate the regression coefficients for slope, M, and intercept, B, for each analytical set.

The standards were fit to the linear equation: Y = MX + B

Appendix 6. Analytical Method (continued)

where: X is the concentration of the reference standard in ng/mL[a]
M is the calibration line slope
B is the calibration line intercept
Y is the native peak area:isotopic peak area ratio

[a] As the reference standards and samples contain the same internal standard concentrations this value may be omitted from the calculation

The equation shown below is used to calculate of tebuconazole residues.

After regression coefficients were calculated, residues in ng/g(ppb) are determined using the following equation,

$$\text{Tebuconazole found (ng/g)} = \frac{(Y-B) \times D}{M}$$

$$\text{Where Dilution Factor (D)} = \frac{\text{Initial volume}(V_1)}{\text{Initial sample wt. (W)}} \times \frac{\text{Final dilution volume } (V_3)}{\text{Aliquot taken } (V_2)}$$

Where: W = 20g
V_1 = 50mL
V_2 = 1mL
V_3 = 2mL

Analyst software was used to calculate the amount of tebuconazole in ng/g for each sample and the percent recovery for the fortified samples.

9.3 Fortification Experiments

Note: Fortification experiments may be performed as needed to monitor method efficiency and reproducibility, but 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.

With each sample set, analyze an untreated control sample and one or more fortified control samples. Calculate recoveries using the following equation:

$$\text{Recovery (\%)} = \frac{(R - S)}{T} \times 100$$

Where: R = ng/g of target analyte found in fortified sample
S = ng/g of target analyte found in control sample, real or apparent
T = theoretical ng/g in fortified sample

Appendix 6. Analytical Method (continued)

Recoveries are determined by analyzing fortified control samples alone or in conjunction with a sample set. Samples may be fortified prior to extraction at the LOQ of 10ng/g in soil or other appropriate level with fortification solutions. Calculate the final residue R for the control (S) and fortified control (R) samples.

10. DISCUSSION

10.1 Method Validation

The method verification data performed during the development of this method is summarized in Appendix 3.

10.2 Analytical stopping points *(IF NEEDED)*

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

11. REFERENCES

No.	Doc. No.	**Author(s).** Title. Year.
1	Method-No. 10774	**Mattern, G. C., Nuessle, C.I., Greene, D.L., Leimkuehler, W.M.,** Validation of an analytical method for the determination of Tebuconazole in Soil and turf by Electrospray HPLC/MS-MS, 1997
2	FR022115	**Lee, R.,** Terrestrial Field Dissipation Of Tebuconazole in a Canadian Soil, 2000

Appendix 6. Analytical Method (continued)

Bayer CropScience **HW-001-S09-01**

Table 1 Analytical Method Summary Parameters (DER Table B.1.1)

Summary Parameters for the Analytical Method For The Determination of Residues of Tebuconazole In Soil Using LC/MS/MS	
Method ID	HW-001-S09-01
Analyte(s)	Tebuconazole
Extraction solvent / Technique	70:30 methanol:water. microwave extraction
Instrument Detector Column	Shimadzu LC-10AD VP HPLC pumps (two), Shimadzu SCL-10A VP Controller with a Shimadzu SIL-20A autosampler Mass Spectrometer, Applied Biosystems API 4000 Phenomenex Prodigy 50 x 2mm 5µm particle size
Standardization Method	Linear regression with 1/x weighting for tebuconazole
Stability of Standard Solutions	The calibration solutions are stable for at least 4 months when stored under refrigerated conditions
Retention times	Tebuconazole (≈ 3.6 min.)

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Appendix 6. Analytical Method (continued)

Bayer CropScience **HW-001-S09-01**

Table 2 Characteristics for the Analytical Method (DER Table C.1.2)

Characteristics for the Analytical Method Used for the Quantitation of f Tebuconazole In Soil Using LC/MS/MS	
Analyte	Tebuconazole
Equipment ID	Applied Biosystems API 4000 LC/MS/MS, Shimadzu LC-10AD VP HPLC pumps (two), Shimadzu SCL-10A VP Controller with a Shimadzu SIL-20A autosampler
Limit of quantitation (LOQ)	10 ng/g for tebuconazole
Accuracy/Precision[a] (%) Mean Recovery ± SD(RSD)	
Linearity	The method/detector response was linear
Specificity	Some apparent residues were detected in the untreated control samples but in all instances these residues were no more than 10% of the LOQ (1ng/g). The fortified sample chromatograms contain only the analyte peak of interest. Peaks were well defined and symmetrical. There appeared to be no carryover to the following chromatograms.

[a] Data obtained during development of method. See Appendix 3

Appendix 6. Analytical Method (continued)

Bayer CropScience **HW-001-S09-01**

Appendix 1 Instrument Conditions For Tebuconazole

NOTE: Variations in equipment or sample characteristics may require slight modifications in the chromatographic or detector conditions listed in order to obtain adequate chromatographic peak shapes or sensitivity. Therefore, the given LC/MS/MS parameters listed below are guidelines and may be modified. These parameters should be optimized for the instrument and column actually used. Also, instrument parameters and mobile phase may be adjusted to improve separation from any observed interfering peaks.

Equipment with equivalent or better sensitivity and performance may be substituted.

The following abbreviations are used in the LC/MS/MS acquisition parameters listed below:

MRM	Multiple Reaction Monitoring
MCA	Multiple Channel Acquisition
DP	Declustering Potential
EP	Entrance Potential
CE	Collision Energy
CXP	Collision Cell Exit Potential
CAD:	Collision gas (Collision Activated Dissociation)
CUR:	Curtain gas
GS1:	Ion Source Gas 1
GS2:	Ion Source Gas 2
IS:	Ion Spray Voltage
TEM:	Temperature
ihe:	Interface Heater
CEM	Channel Electron Multiplier
DF	Deflector

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Appendix 6. Analytical Method (continued)

Bayer CropScience **HW-001-S09-01**

Appendix 1 (Continued)

LC/MS/MS Conditions

MS/MS parameters

Sample Acq Duration:	6min0sec
Number of Scans:	356
Periods in File:	1
Synchronization Mode:	LC Sync
Auto-Equilibration:	Off

Period 1:

Scans in Period:	356
Relative Start Time:	0.00 msec
Experiments in Period:	1

Period 1 Experiment 1:

Scan Type:	MRM (MRM)
Polarity:	Positive
Ion Source:	Turbo Spray
Resolution Q1:	Unit
Resolution Q3:	Unit
Intensity Thres.:	0.00 cps
Settling Time:	0.0000 msec
MR Pause:	5.0070 msec
MCA:	No
Step Size:	0.00 amu

Tebuconazole (~3.6 minutes)

Q1 Mass (amu)	Q3 Mass (amu)	Dwell(msec)	Param	Start	Stop
308.00	70.00	500.00	DP	61.00	61.00
			CE	79.00	79.00
			CXP	16.00	16.00

Tebuconazole-$^{15}N_3$ (~3.6 minutes)

Q1 Mass (amu)	Q3 Mass (amu)	Dwell(msec)	Param	Start	Stop
311.00	73.00	500.00	DP	76.00	76.00
			CE	53.00	53.00
			CXP	14.00	14.00

Parameter Table (Period 1 Experiment 1)

CAD:	7.00
CUR:	30.00
GS1:	40.00
GS2:	50.00
IS:	4500.00
TEM:	500.00
ihe:	ON
EP	10.00

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Appendix 6. Analytical Method (continued)

Bayer CropScience **HW-001-S09-01**

Appendix 1 (Continued)

Instrument Parameters
Detector Parameters (Positive):
CEM 2200.0
DF -50.0

HPLC Parameters

Pumps Used:	Two Shimadzu LC-10ADVP (High Pressure Mixer) pumps with a Shimadzu SCL-10 controller	
Minimum Pressure:	0.0 psi	
Maximum Pressure:	4000 psi	
Shutdown Time:	999.9 min.	
Column Temperature:	Ambient	
Column:	Manufacturer:	Phenomenex
	Type:	Prodigy
	Particle Size:	5 µm
	Diameter:	2 mm
	Length:	50 mm
	Mobile Phase A:	Water with 5mM ammonium acetate
	Mobile Phase B:	Methanol

Gradient Program:

Step	Time (min.)	Module	Flow Rate (mL/min)	A(%)	B(%)
0	0.00	Pumps	0.20	80	20
1	0.01	Pumps	0.20	80	20
2	0.50	Pumps	0.20	80	20
3	2.00	Pumps	0.20	5	95
4	4.00	Pumps	0.20	5	95
5	4.01	Pumps	0.20	80	20
8	6.00	System Controller	Stop		

Divert Valve Program:

Step	Total Time (min.)	Divert Location
1	0.0	To Waste
2	2.0	To LC/MS
3	4.5	To Waste

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Appendix 6. Analytical Method (continued)

Bayer CropScience **HW-001-S09-01**

Appendix 1 (Continued)

Autosampler parameters

Model: SIL-HTA
Shimadzu LC system Injection Volume = 1.00 uL
Rinse Volume: 200 uL
Needle Stroke: 50 mm.
Rinse Speed: 35 uL/sec.
Sampling Speed: 15.0 uL/sec.
Purge Time: 1.0 min.
Rinse Dip Time: 0 sec.
Rinse Mode: No rinsing

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Appendix 6. Analytical Method (continued)

Bayer CropScience **HW-001-S09-01**

Appendix 2 Example Calculation and representative calibration curves

An example calculation for tebuconazole in soil which was analyzed during the development of this method. Sample MEDHP019-B1-014-1UT1-LOQ was fortified with 1.0ng/g tebuconazole. The chromatogram used in this example is presented in Appendix 4 (Chromatogram 4).

The standards were fit to the linear equation: $Y = MX + B$

where: X is the concentration of the reference standard in ng/mL
M is the calibration line slope
B is the calibration line intercept
Y is the native peak area:isotopic peak area ratio

After regression coefficients were calculated, the residue in ng/g was determined. The ng/g of tebuconazole in the soil was calculated using the following equation,

$$\text{Tebuconazole found (ng/g)} = \frac{(Y-B) \times D}{M}$$

$$\text{Where Dilution Factor (D)} = \frac{\text{Initial volume}(V_1)}{\text{Initial sample wt. (W)}} \times \frac{\text{Final dilution volume } (V_3)}{\text{Aliquot taken } (V_2)}$$

W	V_1	V_2	V_3	Native Peak Area	IS Peak Area	Y	M	B
20g	50mL	1mL	2mL	16186.7	102592.8	0.1578	0.07536	0.003302

The slope and intercept were obtained from the tebuconazole calibration curve generated by Analyst, and is presented on the next page.

From the above equations:

$$\text{Dilution Factor (D)} = \frac{50}{20} \times \frac{2}{1} = 5$$

$$\text{Tebuconazole found} = \frac{(0.1578 - (0.003302) \times 5}{0.07536} = 10.25\text{ng/g}$$

Therefore sample MEDHP019-B1-014-1UT1-LOQ contains 10.25ng/g tebuconazole.

Note: The above calculations were performed using rounded numbers and may vary slightly from the results presented in the raw data.

Appendix 6. Analytical Method (continued)

Bayer CropScience **GM-002-S07-01**

Appendix 5 Revision History

Method #	Revision	Description
GM-002-S07-01	01	Method based on Method Number 010774[1]. Method 010774 was revised to substitute a microwave extraction system in place of Soxtec extraction, and use of an API4000 MS/MS in place of a TSQ7000 MS/MS

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Appendix 7. Analytical Method, Modification

Method Modification HW-001-S09-RTP 01

Analytical Method For The Determination of Residues of Tebuconazole In Soil Using LC/MS/MS

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Date

2017-19-04

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6 Pages

Appendix 7. Analytical Method, Modification (continued)

Modification to Method Number HW-001-S09-01-RTP 01:Analytical Method For The Determination of Residues of Tebuconazole In Soil Using LC/MS/MS

The method was modified to incorporate a confirmatory MRM transition for tebuconazole. Changes made to method HW-001-S09-01 [1] in Appendix 1 are shown below:

Changes to original method are in bold and italics.

Appendix 1

NOTE: Variations in equipment or sample characteristics may require slight modifications in the chromatographic or detector conditions listed in order to obtain adequate chromatographic peak shapes or sensitivity. Therefore, the given LC/MS/MS parameters listed below are guidelines and may be modified. These parameters should be optimized for the instrument and column actually used. Also, instrument parameters and mobile phase may be adjusted to improve separation from any observed interfering peaks.

Equipment with equivalent or better sensitivity and performance may be substituted.

The following abbreviations are used in the LC/MS/MS acquisition parameters listed below:

MRM	Multiple Reaction Monitoring
MCA	Multiple Channel Acquisition
DP	Declustering Potential
EP	Entrance Potential
CE	Collision Energy
CXP	Collision Cell Exit Potential
CAD:	Collision gas (Collision Activated Dissociation)
CUR:	Curtain gas
GS1:	Ion Source Gas 1
GS2:	Ion Source Gas 2
IS:	Ion Spray Voltage
TEM:	Temperature
ihe:	Interface Heater
CEM	Channel Electron Multiplier
DF	Deflector

Appendix 7. Analytical Method, Modification (continued)

Appendix 1 (Continued)

LC/MS/MS Conditions

MS/MS parameters

Sample Acq Duration:	6min0sec
Number of Scans:	356
Periods in File:	1
Synchronization Mode:	LC Sync
Auto-Equilibration:	Off

Period 1:

Scans in Period:	356
Relative Start Time:	0.00 msec
Experiments in Period:	1

Period 1 Experiment 1:

Scan Type:	MRM (MRM)
Polarity:	Positive
Ion Source:	Turbo Spray
Resolution Q1:	Unit
Resolution Q3:	Unit
Intensity Thres.:	0.00 cps
Settling Time:	0.0000 msec
MR Pause:	5.0070 msec
MCA:	No
Step Size:	0.00 amu

Tebuconazole Quantitation MRM(~3.6 minutes)

Q1 Mass (amu)	Q3 Mass (amu)	Dwell(msec)	Param	Start	Stop
308.00	70.00	500.00	DP	61.00	61.00
			CE	79.00	79.00
			CXP	16.00	16.00

Tebuconazole Confirmatory MRM (~3.6 minutes)

Q1 Mass (amu)	***Q3 Mass (amu)***	***Dwell(msec)***	***Param***	***Start***	***Stop***
308.00	***125.00***	***500.00***	***DP***	***100.00***	***100.00***
			CE	***27.00***	***27.00***
			CXP	***14.00***	***14.00***

Tebuconazole-$^{15}N_3$ (~3.6 minutes)

Q1 Mass (amu)	Q3 Mass (amu)	Dwell(msec)	Param	Start	Stop
311.00	73.00	500.00	DP	76.00	76.00
			CE	53.00	53.00
			CXP	14.00	14.00

Appendix 7. Analytical Method, Modification (continued)

Appendix 1 (Continued)

Parameter Table (Period 1 Experiment 1)
CAD: 7.00
CUR: 30.00
GS1: 40.00
GS2: 50.00
IS: 4500.00
TEM: 500.00
ihe: ON
EP 10.00

Instrument Parameters
Detector Parameters (Positive):
CEM 2200.0
DF -50.0

HPLC Parameters

Pumps Used:	Two Shimadzu LC-10ADVP (High Pressure Mixer) pumps with a Shimadzu SCL-10 controller	
Minimum Pressure:	0.0 psi	
Maximum Pressure:	4000 psi	
Shutdown Time:	999.9 min.	
Column Temperature:	Ambient	
Column:	Manufacturer:	Phenomenex
	Type:	Prodigy
	Particle Size:	5 µm
	Diameter:	2 mm
	Length:	50 mm
	Mobile Phase A:	Water with 5mM ammonium acetate
	Mobile Phase B:	Methanol

Gradient Program:

Step	Time (min.)	Module	Flow Rate (mL/min)	A(%)	B(%)
0	0.00	Pumps	0.20	80	20
1	0.01	Pumps	0.20	80	20
2	0.50	Pumps	0.20	80	20
3	2.00	Pumps	0.20	5	95
4	4.00	Pumps	0.20	5	95
5	4.01	Pumps	0.20	80	20
8	6.00	System Controller	Stop		

Appendix 7. Analytical Method, Modification (continued)

Appendix 1 (Continued)

Step	Total Time (min.)	Divert Location
1	0.0	To Waste
2	2.0	To LC/MS
3	4.5	To Waste

Autosampler parameters

Model: SIL-HTA
Shimadzu LC system Injection Volume = 1.00 uL
Rinse Volume: 200 uL
Needle Stroke: 50 mm.
Rinse Speed: 35 uL/sec.
Sampling Speed: 15.0 uL/sec.
Purge Time: 1.0 min.
Rinse Dip Time: 0 sec.
Rinse Mode: No rinsing

Reference

1. Netzband, D.J., Bayer CropScience Method HW-001-S09-01 Analytical Method For The Determination of Residues of Tebuconazole In Soil Using LC/MS/MS