

AVENTIS CROPSCIENCE

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Title : **Thidiazuron:** Analytical Method for the Determination of 1,2,3-thiadiazol-5-ylurea (AE F132345) residues in Crop Matrices Using LC/MS/MS.

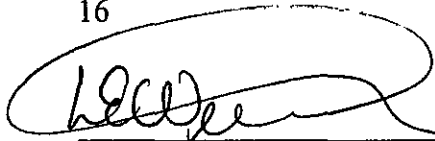
Analytes : AE F132345.

Substrates : Crop Matrices

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Author :



Lee E. Williams, Ph.D.
Senior Research Scientist

August 17, 2001
Date

Approval :



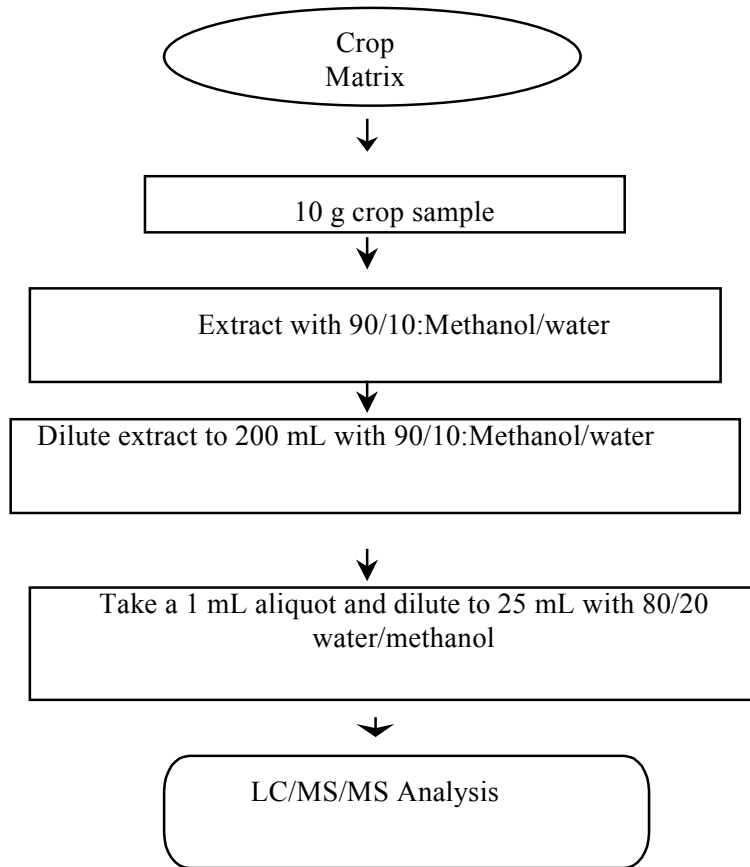
J. R. Hudson, Ph.D.
Residue Methods Section Leader

August 17, 2001
Date

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Summary Flowchart of Analytical Method



Thidiazuron: Analytical Method for the Determination of 1,2,3-thiadiazol-5-ylurea (AE F 132345) residues in Crop Matrices Using LC/MS/MS.

I. Introduction

This method has been developed for the analysis of AE F 132345 in crop matrices (wheat forage, radish roots, and radish tops). This method has been verified during the method development stage at the spike level of fifty parts per billion (50 ppb).

A. Scope

This method sets forth the procedure for determining the residues of AE F 132345 in crop matrices. Recovery data collected during the method development stage are summarized at the end of this method.

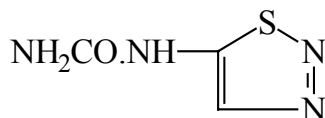
B. Principle

Residues of AE F 132345 are extracted from crop matrices by blending with 90/10 methanol/water. The extracts are filtered, into a 250 mL graduated mixing cylinder and brought to a volume of 200 mL with 90/10:methanol/water. A 1 mL aliquot is transferred to 25 mL volumetric flask and diluted with 80/20:water/methanol to the mark. This solution is then injected on to a LC/MS/MS. Quantification of AE F 132345 is accomplished by high performance liquid chromatography using an MS/MS detector.

C. Method Limits

The target level for the limit of quantitation (LOQ) is 50 ppb for AE F 132345 in all crop matrices. Mean percent recoveries at the LOQ carried out during the method development were 88 percent for all substrates.

D. Chemical Structure



AE F132345

C.A. Name: 1,2,3-thiadiazol-5-ylurea

IUPAC Name: 1,2,3-thiadiazol-5-ylurea

E. Safety

There are no particular safety hazards with this method. The method should be used by trained laboratory personnel. The user should review appropriate MSD sheets and observe normal laboratory safety procedures.

II. Materials

Reagents and solvents were used as received from supplier, unless otherwise noted.

Equivalent reagents, solvents and equipment may be substituted where appropriate.

A. Reagents

1. Acetic acid , glacial, 99.9%, JT Baker, Cat. N° 9507-05

B. Solvents

1. Methanol, B&J ACS/HPLC, Burdick and Jackson, Cat. N° BJAH230-4
2. Water, Milli-Q

C. Solutions

1. 90/10:methanol/water solution

Add 900 mL of methanol to a 1 liter graduated cylinder. Add 100 mL of water and shake gently until it is mixed thoroughly.

2. 0.1% acetic acid in water solution

Add 1 mL of glacial acetic acid to 999 mL of Milli-Q water. Shake gently until it is mixed thoroughly.

- 3 80/20:Water/methanol solution

Add 800 mL of water to a 1 liter graduated cylinder. Add 200 mL of methanol and shake gently until it is mixed thoroughly

D. Equipment

1. Analytical Balance, Mettler PM2000 or PG2002, VWR Cat. N° 11273-722
2. Professional Level Balance, Ohaus portable
3. Spoons, and/or spatula
4. Beakers, appropriate sizes

5. Blender, Sorvall Omni-Mixer, Omni International
6. Blender Blade Assembly, Omni International
7. Blending jars (Mason pint size canning)
8. Glass fiber filter paper, Whatman 934-AH (Cat. No 1827-90)
9. Kimax graduated mixing cylinders, 250 mL, Kimble, VWR Cat. No 24763-109
10. Volumetric flasks, and stoppers appropriate sizes, class A, VWR, eg. Cat. No 29619-6xx
11. Volumetric Pipets, appropriate sizes, class A, VWR, eg. Cat. No 53046-xxx
12. Eppendorf pipettors and tips, appropriate sizes
13. Disposable Pasteur Pipets, sizes 5 ¾ inch and 9 inch, VWR, Cat. No 14673-010, 72050-900
14. Autosampler Vials, 1.5 mL, clear, Sun, Cat No. 200 250
15. Vial caps with split septa, Sun, Cat No. 500 061
16. Amber bottles, 4oz, VWR Cat. No 16153-135
17. Porcelain Büchner funnels, 83 mm, Coors USA, VWR Cat. N° 300310-109
18. Graduated cylinders, and stoppers appropriate sizes, VWR, eg. Cat. N° 34795-0xx
19. Optional : Vacuum adaptor, #27 stopper joint top and bottom, top female stopper joint is unground and measures 60 mm from the top of the joint to the top of the inner flair, (used for filtration directly into mixing cylinders), Chem. Glass Inc., VWR Part N°. VWR-9794-27H

E. Analytical Standards

Analytical Standard available from Aventis CropScience

CAS Name

AE F 132345 : 1,2,3-thiadiazol-5-ylurea

III. Fortification and Calibration Standard Solutions

A. Preparation

The stated concentrations of standard solutions should be adjusted to account for the purity of the neat solid standards. After preparation, standards should be transferred from the volumetric flasks into screw-capped amber bottles. Store standard solutions in the refrigerator at less than 5°C when not in use.

The following is provided as an example of how standard solutions may be prepared. Other concentrations may be used as appropriate. Number of digits advises for precision.

1. For the fortification standards:
 - 1.1 Weigh 25.0 ± 0.1 mg of AE F132345 into a 25 mL volumetric flask. Dilute in 25 mL of methanol. The concentration of this standard is 1000 $\mu\text{g/mL}$.
 - 1.2 Withdraw a 1.0 mL aliquot from the 100 $\mu\text{g/mL}$ standard and add to a 100 mL volumetric flask. Dilute to final volume with methanol and mix well. The concentration of this standard is 10.0 $\mu\text{g/mL}$.
 - 1.3 Withdraw a 10.0 mL aliquot from the 10.0 $\mu\text{g/mL}$ standard and add to a 100 mL volumetric flask. Dilute to volume with methanol and mix well. The concentration of this standard is 1.00 $\mu\text{g/mL}$.
 - 1.4 Withdraw a 10.0 mL aliquot from the 1.00 $\mu\text{g/mL}$ and add to a 100.0 mL volumetric flask. Dilute to volume with methanol and mix well. The concentration of this standard is 100 ng/mL .
 - 1.5 Withdraw a 10.0 mL aliquot from the 100 ng/mL and add to a 100.0 mL volumetric flask. Dilute to volume with methanol and mix well. The concentration of this standard is 10.0 ng/mL .
2. For the calibration standards:
 - 2.1 Transfer 0.200 mL aliquot from the 10 ng/mL standard to a 100.0 mL volumetric flask. Dilute to volume with 80:20 water/methanol to obtain a 0.02 ng/mL standard.
 - 2.2 Transfer 0.400 mL aliquot from the 10 ng/mL standard to a 100.0 mL volumetric flask. Dilute to volume with 80:20 water/methanol to obtain a 0.04 ng/mL standard
 - 2.3 Transfer 0.600 mL aliquot from the 10 ng/mL standard to a 100.0 mL volumetric flask. Dilute to volume with 80:20 water methanol to obtain a 0.06 ng/mL standard.
 - 2.4 Transfer 1.00 mL aliquot from the 10 ng/mL standard to a 100.0 mL volumetric flask. Dilute to volume with 80:20 water methanol to obtain a 0.10 ng/mL standard

- 2.5 Transfer 2.0 mL aliquot from the 10 ng/mL standard to a 100.0 mL volumetric flask. Dilute to volume with 80:20 water/methanol to obtain a 0.20 ng/mL standard

B. Stability

Calibration standards and fortification solutions of AE F 132345 in methanol or water/methanol have been shown to be stable for a period of at least three months when stored below 5°C.

IV. Method of Analysis

A. Method Tips and Observations

1. For convenience, samples can be weighed initially and stored in a freezer for future use, since it takes several hours for some samples to thaw.
2. Dispense solvents into an appropriate container for usage. Placing pipets into main bottles could lead to contamination.

B. Analytical Method

The diamond symbol (◆) indicates a stopping point in the method. Overnight storage in a refrigerator is recommended. Samples should be allowed to warm to ambient conditions prior to continuation of the method.

1. Extraction

- 1.1 Weigh 10.0 ± 0.1 g of sample into a pint mason jar.
- 1.2 Perform sample fortification at this point if appropriate. For example, fortify 10 g of crop with 0.50 mL (Eppendorf pipet) of the 1.0 $\mu\text{g/mL}$ fortification standard for a 50 ppb fortification. Let fortified samples sit for 10 minutes.
- 1.3 Add 150 mL of 90/10:methanol/water solution to each sample. Blend samples at speed of 4-5 on the omni mixer.
- 1.4 Before filtration rinse the blending blade with ≈ 5 mL of methanol, such that the rinse is collected into the mason jar. Filter entire contents of extract under suction through a Büchner funnel containing glass fiber filter paper into a 250 mL graduated mixing cylinder.
- 1.5 Rinse mason jar with an additional 50 mL of methanol. Swirl the methanol to rinse the walls of the mason jar. Pour the rinse onto the filter cake from step 1.4 collecting the rinse in the same 250 mL graduated mixing cylinder.

- 1.6 After filtration, remove the Büchner funnel containing the Whatman 934-AH filter paper and filter cake (discard). Rinse the filter adapter with 3 – 5 mL of methanol while attached to the 250 ml graduated mixing cylinder.
- 1.7 Dilute the extract to 200.0 mL with 90/10:methanol/water. Mix well. This is Extract A.

2. Dilution

- 2.1 Transfer a 1.0 mL aliquot of Extract A to a 25 mL volumetric flask.
- 2.2 Dilute to volume with 80/20:water/methanol and mix well.
- 2.3 If further dilution of samples is necessary to get the sample onto the curve for quantitation, dilute with same solution stated in step 2.2 as needed. ♦
- 2.4 Vial the samples and standards for LC/MS/MS analysis.

V. Liquid Chromatography

A. Instrumentation

LC/MS/MS System:	Perkin Elmer Sciex API 3000 LC/MS/MS system with PE Sciex TurboIonSpray Electrospray Interface; Shimadzu LC-10AD VP HPLC pumps (2) with 250 µL high pressure mixer and SCL-10A VP Pump Controller; Gilson Series 215 autosampler
Ionization and MS Mode:	Electrospray (TurboIonSpray) - positive ion mode
MS Mode:	MS/MS with multiple reaction monitoring (MRM)
Ion Spray:	+5200V
Nebulizer Setting:	5 (Air)
Curtain Gas Setting:	7 (Nitrogen)
TurboIonSpray Settings:	Heated air at ~8.5L/min, 375°C
Collision Gas Setting:	8 (Nitrogen)

Mass Transitions, Collision Energy, Declustering and Focusing Potential, CXP:

AE F 132345: 145.1/102.0 amu, 19V 26V 80V 8V

(Dwell Times 150ms)

Column:	Thermo Hypersil Keystone Scientific Operation, Nucleosil C18 100 x 2mm 5µ particle size with an inline filter	
Mobile Phase Flow Rate:	0.300 mL/minute, no split	
Mobile Phase Composition:	0% Methanol/100% Water(0.1% acetic acid) initial conditions	
Gradient Profile:	Methanol/Water(0.1% acetic acid)	Time(min.)
	(linear gradient between time points)	
	0/100	2.0
	80/20	6.0
	80/20	9.0
	0/100	9.5
	0/100	12.5
Injection Volume:	50µL, (can be increased if needed)	
Retention times:	4.9 - 5.0 min	

Note the indicated LC-MS-MS parameters are guidelines and should be optimized for the instrument and column actually used. Instrument parameters and mobile phase compositions may be adjusted to improve separation from interfering peaks.

VI. Calculations

A. Calibration Curves

1. Linear regression should be used to generate calibration curves for the analyte. At least four different standard concentrations should be run with each set of samples. Extracts should be diluted such that the peak areas obtained are within the area range between the lowest and highest standards injected.

2. Linear regression coefficients should be calculated from 'peak area' (or 'peak height') versus 'ng / mL injected'. Data from the analytical standards should be fit to the linear equation : $y = a + bx$.

where: y = peak area or height

a = calibration line intercept

b = calibration line slope

x = concentration of analyte in injected solution

B. Quantification of Residues

1. AE F 132345 can be quantitated by comparison to the standard curves obtained from a linear regression analysis of the data.

2. Equations

- 2.1 Concentration of analyte in sample in ppb (parts per billion=ng/g).

$$\text{ppb} = \frac{(y - a)}{b} \times \frac{c}{d \times e}$$

where : y = peak area, response of analyte of interest

a = intercept of the linear regression curve (area)

b = slope of the linear regression curve (response per ng/mL)

c = final volume of sample (mL)

d = sample weight (g)

e = aliquot correction factor ($e=0.005$)

ppb = conc of analyte in sample (ppb)

- 2.2 Percent recovery

$$\text{Recovery (\%)} = \frac{\text{analyte found in sample (ppb)} - \text{amount found in control (ppb)}}{\text{fortification level (ppb)}} \times 100$$

VII. Recovery Data

The following tables represent recoveries obtained during method verification.

Recoveries are corrected for amounts found in untreated control (UTC).

ND means not detected.

A. Wheat Forage

Sample ID	Fortification (ppb)	Recovery (%) AE F 132345
UTC 10	ND	ND
UTC 11 + 50	50 ppb	. 89
UTC 12 + 50	50 ppb	92

B. Radish roots

Sample ID	Fortification (ppb)	Recovery (%) AE F 132345
UTC 1	ND	ND
UTC 2 + 50	50 ppb	. 90
UTC 3 + 50	50 ppb	95

C. Radish Tops

Sample ID	Fortification (ppb)	Recovery (%) AE F 132345
UTC 4	ND	ND
UTC 5 + 50	50 ppb	. 81
UTC 6 + 50	50 ppb	81

VIII APPENDIX I Typical Data and Example Chromatograms

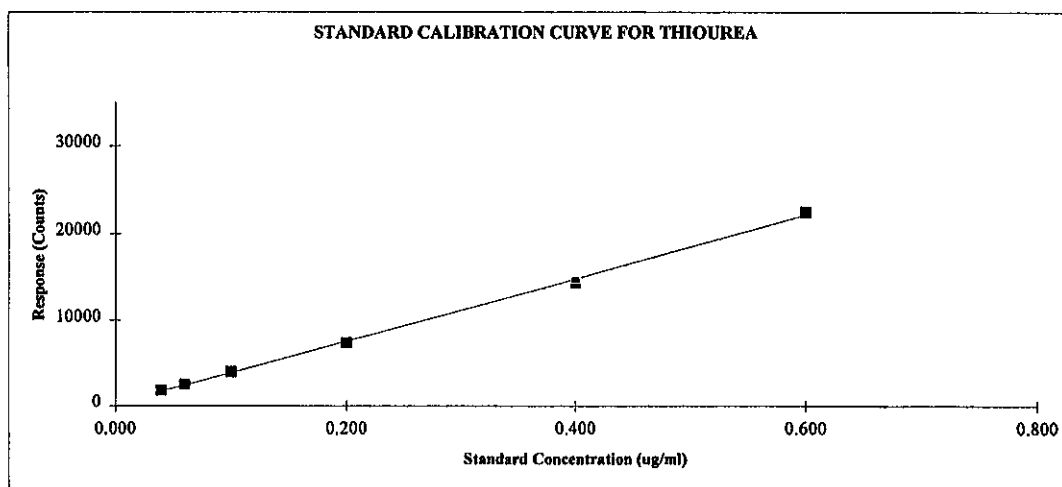
Appendix I

AVENTIS Chemical Company Residue Chemistry Calculation Spreadsheet

Study No.: 31219
 Inject Date: 5/10/2001
 Substrate: Mustard Green
 Compound: Thiourea

Standard Soln. Ref. #	Standard Conc. (ng/ml)	Response (Cts.)	Calculated Line
	0.040	1850	1726
	0.060	2540	2456
	0.100	4020	3916
	0.200	7380	7565
	0.400	14400	14864
	0.600	22500	22163

Regression	Output
Slope =	36494.37
Y- Int. =	266.31
Corr. =	0.999

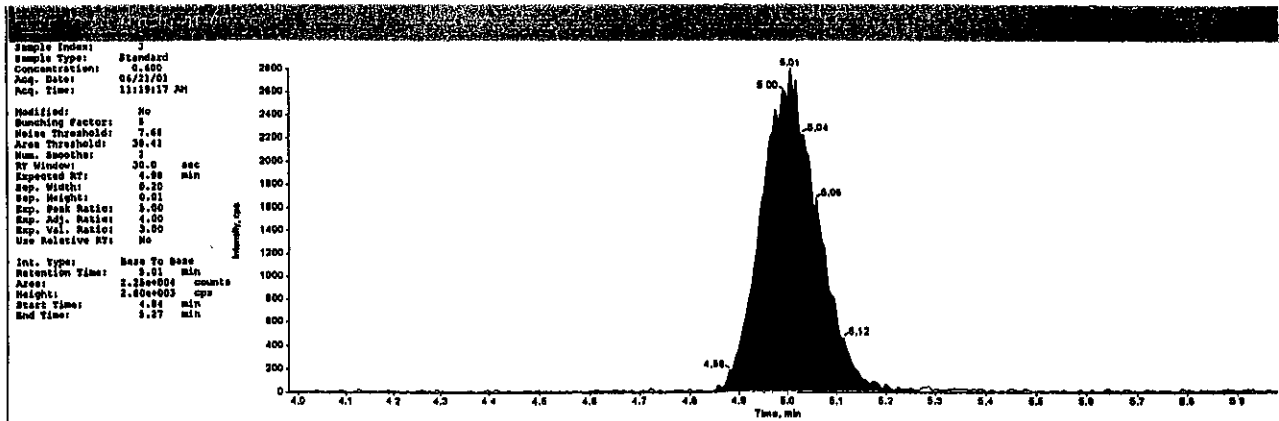
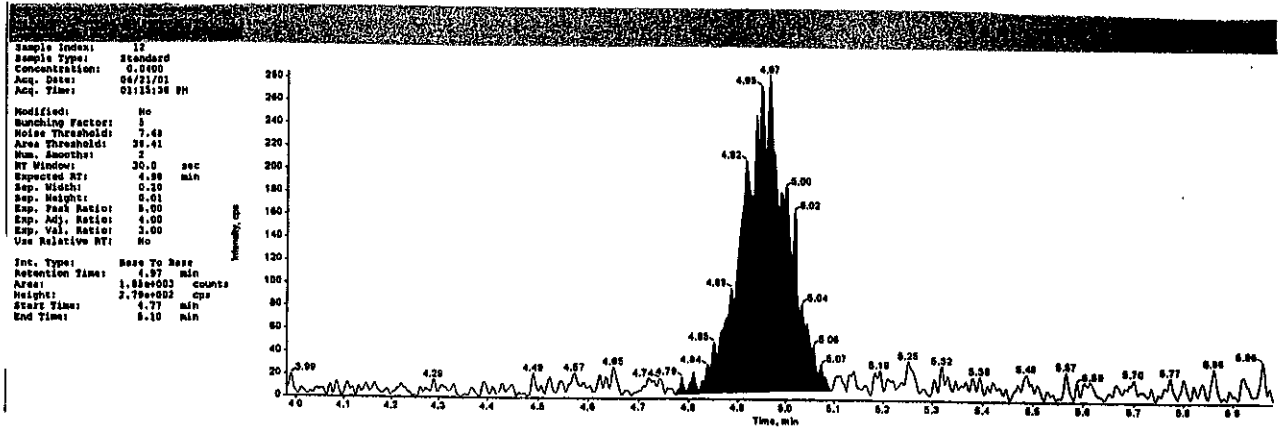


AGREVO Sample #	Sample Weight (g)	Amount Analyte Added (ng)	Recovery Level (ppb)	Response (Cts.)	Det. Amt. of Analyte (ug/ml)	Crop / Solv. Ratio (g/ml)	Determined Residue (ppb)	Percent Recovery
<i>Section 1: Control Samples</i>								
UTC 10 Mustard Greens	10.00	0.0	0.00	ND	#VALUE!	0.002	#VALUE!	
<i>Section 2: Recovery Samples (Corrected For Control Residues)</i>								
UTC 11 + 0.05	10.00	500.00	50.00	3510	0.089	0.002	44.4409	89%
UTC 12 + 0.05	10.00	500.00	50.00	3630	0.092	0.002	46.0850	92%
UTC 10S + 0.05	10.00	500.00	50.00	2910	0.072	0.002	36.2205	72%

Chromatograms of Standard

0.04 ng/mL Standard – top chromatogram

0.60 ng/mL Standard – bottom chromatogram



Chromatograms of Wheat Forage Samples
 Untreated Control – top chromatogram
 0.05 ppm Fortification – bottom chromatogram

