

US EPA ARCHIVE DOCUMENT



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STUDY REPORTS:

MRID No. 46801806 Gould, T. Timberlake, B. and Brungardt, J. 2006. Validation of Bayer CropScience Method AI-001-P04-01 An Analytical Method for the Determination of Residues of AE 0317309, AE 1073910, and AE B197555 in Wheat, Corn, and Soybean Matrices Using LC/MS/MS. Unpublished Bayer CropScience Report No.: RAAIX005. 206 pages.

MRID No. 46801807 Billian, B. 2005. Independent Laboratory Validation of the Analytical Method AI-001-P04-01 for the Determination of Residues of AE 0317309, AE 1073910 and AE B197555 in Plant Material. Unpublished Bayer CropScience, Monheim, Germany Study No.: P612050574. Bayer CropScience Report No.: MR-097/05. 72 pages.

MRID No. 46801808 Gould, T. and Brungardt J. 2006. Extraction Efficiency of AE B197555, AE 1073910, and AE 0317309 by Method AI-0001-P04-01. Unpublished Bayer CropScience Report No.: RAAIX011. 60 pages.



EXECUTIVE SUMMARY:

Bayer CropScience has developed a high-performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS), Method AI-004-A05-01, as the data collection and enforcement method for residues of pyrasulfotole (AE 0317309), pyrasulfotole-desmethyl (AE 1073910), and pyrasulfotole-benzoic acid (AE B197555) in crop matrices.

Briefly, the crop matrices are extracted with a mixture of acetonitrile (ACN)/water (H₂O)/concentrated hydrochloric acid (HCl) (30:15:3, v/v). The sample extract is heated to 60°C for at least 30 minutes then cooled and a mixture of isotopic internal standards is added to the sample extract. A small aliquot is purified by C18 solid phase extraction (SPE). The solvent is removed from the sample and the residue is reconstituted for analysis by HPLC-MS/MS.

The limit of quantitation (LOQ) is 0.010 ppm for each analyte in each matrix. The proposed enforcement method was adequately validated in soybean grain, corn grain, corn stover, wheat forage, barley hay, and barley grain. A successful independent laboratory validation (ILV) was completed with samples of wheat grain and soybean grain. Extraction efficiency data demonstrated that the enforcement method can account for incurred residues of pyrasulfotole, pyrasulfotole-desmethyl, and pyrasulfotole-benzoic acid in plant matrices.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the analytical method test data are classified as scientifically acceptable.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document [DP# 333412], in Canada's Regulatory Decision Document, and in Australia's Residues Evaluation Report.

COMPLIANCE:

Signed and dated Good Laboratory Practice (GLP), Quality Assurance and Data Confidentiality statements were provided.

A. BACKGROUND INFORMATION

Pyrasulfotole, ((5-hydroxy-1,3-dimethyl-1*H*-pyrazol-4-yl)[2-(methylsulfonyl)-4-(trifluoromethyl) phenyl]methanone), is a postemergence dicot herbicide for use in cereal crops. Pyrasulfotole is an effective inhibitor of the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPDase), and consequently blocks the pathway of prenylquinone biosynthesis in plants. The end-use products are applied to the target weeds and act primarily through leaf uptake and translocation to the target site. The first symptoms appear three to five days after application. Bleaching and discoloration appear initially and symptoms progress to tissue necrosis and plant death within two weeks.



TABLE A.1. Test Compound Nomenclature

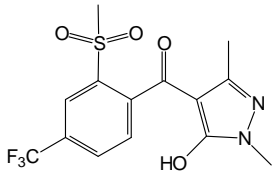
Compound	Chemical Structure 
Common name	Pyrasulfotole
Company Experimental name	AE 0317309
IUPAC name	(5-hydroxy-1,3-dimethylpyrazol-4-yl)(α,α,α -trifluoro-2-mesyl- <i>p</i> -tolyl)methanone
CAS name	(5-hydroxy-1,3-dimethyl-1 <i>H</i> -pyrazol-4-yl)[2-(methylsulfonyl)-4-(trifluoromethyl)phenyl]methanone
CAS #	365400-11-9
End-use product/(EP)	Herbicide; AE 0317309 02 SE06; AE 0317309 03 EC 23 A8

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound

Parameter	Value		Reference
Melting point	Pure: 201°C No boiling point, decomposition starts at 245°C		1
pH at 22.9°C	3.03		2
Density (g/cm ³)	1.53		3
Water solubility (g/L at 20°C)	2.3 4.2 69.1 49.0	pH 3.0 (distilled water) pH 3.9 (buffer pH 4.0) pH 5.4 (buffer pH 7.0)* pH 5.2 (buffer pH 9.0)* * exceeded buffer capacity	4
Solvent solubility (g/L at 20°C)	Ethanol n-Hexane Toluene Dichloromethane Acetone Ethyl acetate Dimethyl sulfoxide	21.6 0.038 6.86 120-150 89.2 37.2 ≥ 600	5
Vapour pressure at 20°C	2.7 X 10 ⁻⁷ Pa		6
Dissociation constant (pK _a)	4.2		7
<i>n</i> -Octanol-water partition coefficient Log(K _{OW}) at 23°C	0.276 -1.362 -1.580	pH 4.0 pH 7.0 pH 9.0	8
UV/visible absorption spectrum	λ_{max} = 264, 241, 216 nm in water, 0.1M HCl, 0.1M NaOH respectively.		9



B. MATERIALS AND METHODS

B.1. Data-Gathering Method

B.1.1. Principle of the Method:

A 2.0 g aliquot of the crop matrix was weighed into a 60-mL vial and a mixture of ACN/H₂O/concentrated HCl (30:15:3, v/v) was added. The sample extract was heated to 60°C for at least 30 minutes. The samples were cooled and a mixture of isotopically labeled internal standards (IS) was added to the sample extract and mixed (0.10 ppm of each IS). A small aliquot (about 1.25 mL) was purified by C18 SPE. The solvent was removed from the sample and the residue was reconstituted for analysis by HPLC-MS/MS.

TABLE B.1.1. Summary Parameters for the Analytical Method Used for the Quantitation of AE 0317309 Derived Residues in Plants.

Method ID	AI-001-P04-01		
Analyte(s)	Pyrasulfotole (AE 0317309), pyrasulfotole-desmethyl (AE 1073910) and pyrasulfotole-benzoic acid (AE B197555)		
Extraction solvent/technique	Acetonitrile: deionized water: HCL (30:15:3) at 60°C for at least 30 minutes		
Cleanup strategies	Solid phase extraction cartridge (C-18)		
Instrument/Detector	Phenomenex Gemini C-18, 50 x 2.0 mm, 5 µm Thermo Finnigan Quantum Ultra LC-MS/MS with aqueous 0.01 M NH ₄ OAc and methanol as mobile phases		
Standardization method	Multi-point calibration curve with isotopically labeled internal standards		
Stability of std solutions	Up to 274 days (~ 9 months) in acetonitrile:water (2:1 v/v)		
Retention times (approximate)	AE B197555 2.2 min AE 1073910 2.7 min AE 0317309 3.0 min		
Mass Spectrometer Data - Major daughter ion transitions monitored. AE B197555 was not detected in positive ion mode.	Analyte	Parent Ion	Daughter Ion
	AE B19755	266.95	158.95
	AE 1073910	346.95	266.95
	AE 0317309	360.95	78.95

B.2. Enforcement Method

The enforcement method is the same as the data-gathering method.



C. RESULTS AND DISCUSSION

C.1. Data-Gathering Method

TABLE C.1.1. Recovery Results from Method Validation of Soybean, Corn, Wheat, and Barley using the Data-Gathering Analytical Method.

Matrix	Analyte	Spiking Level (ppm)	Recoveries (%)	Mean Recovery \pm SD (%)	
Soybean Grain	AE B197555	0.010	96, 91, 93, 87, 99, 96, 94	94 \pm 4.0	
		0.250	94, 96, 97, 98, 97, 102, 98	97 \pm 2.5	
	AE 1073910	0.010	92, 91, 93, 88, 93, 98, 90	92 \pm 3.1	
		0.250	100, 98, 101, 100, 101, 101, 100	100 \pm 1.0	
	AE 0317309	0.010	88, 86, 80, 82, 75, 85, 89	84 \pm 5.8	
		0.250	99, 104, 99, 98, 100, 100, 98	100 \pm 2.0	
Corn Grain	AE B197555	0.010	90, 92, 85, 91, 95, 91, 86	90 \pm 4.0	
		0.250	99, 97, 98, 96, 97, 96, 94	97 \pm 1.4	
	AE 1073910	0.010	89, 91, 86, 87, 86, 86, 90	88 \pm 2.2	
		0.250	100, 100, 100, 101, 102, 99, 97	100 \pm 1.8	
	AE 0317309	0.010	75, 81, 76, 85, 76, 88, 80	80 \pm 6.1	
		0.250	95, 95, 97, 95, 95, 95, 92	95 \pm 1.5	
	Corn Stover	AE B197555	0.010	90, 98, 81, 97, 90, 93, 88	91 \pm 6.3
			0.250	98, 98, 100, 97, 96, 97, 98	98 \pm 1.3
AE 1073910		0.010	89, 92, 90, 98, 85, 91, 93	91 \pm 4.3	
		0.250	98, 99, 100, 99, 96, 96, 99	98 \pm 1.6	
AE 0317309		0.010	84, 86, 84, 90, 79, 84, 83	84 \pm 4.0	
		0.250	100, 101, 102, 99, 104, 100, 100	101 \pm 1.7	
Wheat Forage		AE B197555	0.010	93, 98, 94, 96, 93, 91, 90	94 \pm 2.8
			0.250	99, 99, 97, 99, 99, 101, 94	98 \pm 2.2
	AE 1073910	0.010	89, 95, 92, 94, 95, 93, 92	93 \pm 2.3	
		0.250	101, 100, 102, 101, 104, 105, 102	102 \pm 1.6	
	AE 0317309	0.010	80, 87, 89, 86, 85, 84, 88	86 \pm 3.3	
		0.250	97, 96, 97, 100, 98, 99, 99	98 \pm 1.4	
	Barley Grain	AE B197555	0.010	100, 88, 96, 96, 89, 91, 99	94 \pm 5.2
			0.250	96, 95, 99, 97, 99, 98, 99	98 \pm 1.9
AE 1073910		0.010	94, 92, 91, 95, 92, 96, 93	93 \pm 1.8	
		0.250	102, 104, 104, 101, 102, 104, 103	103 \pm 1.2	
AE 0317309		0.010	87, 91, 88, 86, 91, 90, 92	89 \pm 2.6	
		0.250	97, 97, 100, 96, 99, 97, 98	98 \pm 1.2	
Barley Hay		AE B197555	0.010	104, 90, 93, 101, 96, 85, 104	96 \pm 7.7
			0.250	94, 98, 94, 99, 94, 94, 95	96 \pm 2.0
	AE 1073910	0.010	95, 91, 93, 93, 93, 93, 92	93 \pm 1.3	
		0.250	99, 101, 98, 101, 99, 100, 101	100 \pm 1.1	
	AE 0317309	0.010	90, 88, 86, 89, 89, 86, 92	89 \pm 2.6	
		0.250	99, 100, 100, 101, 101, 99, 100	100 \pm 0.9	



TABLE C.1.2. Characteristics for the Data-Gathering Analytical Method Used for the Quantitation of AE 0317309, AE 1073910, and AE B197555 in Soybean, Corn, Wheat, and Barley.

Analyte	Soybean Grain	Corn Grain	Corn Stover	Wheat Forage	Barley Grain	Barley Hay
AE 0317309 (Pyrasulfotole)						
LOQ (ppm)	0.010	0.010	0.010	0.010	0.010	0.010
LOD (ppm)	0.0015	0.0020	0.0011	0.0010	0.0009	0.0009
Accuracy/ Precision	75% to 104%	75% to 97%	79% to 104%	80% to 100%	86% to 100%	86% to 101%
AE 1073910 (Pyrasulfotole-desmethyl)						
LOQ (ppm)	0.010	0.010	0.010	0.010	0.010	0.010
LOD (ppm)	0.0014	0.0012	0.0017	0.0008	0.0008	0.0008
Accuracy/ Precision	88% to 101%	86% to 102%	85% to 100%	89% to 105%	91% to 104%	91% to 101%
AE B197555 (Pyrasulfotole-benzoic acid)						
LOQ (ppm)	0.010	0.010	0.010	0.010	0.010	0.010
LOD (ppm)	0.0012	0.0014	0.0020	0.0009	0.0019	0.0029
Accuracy/ Precision	87% to 102%	85% to 99%	81% to 100%	90% to 101%	88% to 100%	85% to 104%
Equipment ID	ThermoFinnigan Surveyor HPLC with Quantum Ultra LC-MS/MS					
Method Reliability	An independent laboratory validation was conducted with wheat grain and soybean grain. Recoveries ranging from 90% to 115% were obtained.					
Linearity	The detector response for all analytes from 0.005 ppm to 2.50 ppm was linear with a correlation coefficient (R^2) of >0.999.					
Specificity	Control samples had residue levels of 0.0012 ppm or below. Peaks for all three analytes were well defined and symmetrical. There appeared to be no carryover to subsequent chromatograms.					

C.2. Enforcement Method

The enforcement method is the same as the data-gathering method. The extraction efficiency of method AI-001-P04-01 for the determination of the total radioactive residue (TRR) of pyrasulfotole was tested using samples obtained from metabolism studies treated with [phenyl- $U-^{14}C$]-pyrasulfotole^{10,11}. The analytical residue method effectively extracted 96%, 110% and 104% of the TRR from aged wheat forage, wheat grain, and wheat hay, respectively.

Concurrent recovery data demonstrated that the method can effectively recover pyrasulfotole equivalent residues spiked from 0.01 to 0.250 ppm. The method adequately bracketed the expected residues in wheat, barley, soybean, and corn commodities. The LC-MS/MS was specific for the analysis of analytes of interest. No interferences were noted above the LOD.



TABLE C.2.1. Distribution of Residues Found in Wheat Forage, Hay and Grain by Residue Method as Compared with Those Found in Metabolism Studies.

Matrices	Amount of AE 0317309 equivalents				Extraction Efficiency (%)
	Analytical Residue Method		Metabolism Studies		
	% TRR	ppm	% TRR	ppm	
Wheat Forage		1.472, 1.660, 1.488			
Average	59.9	1.540	67.1	1.61 ^a	95.7
Wheat Hay		0.0527, 0.0504, 0.0496			
Average	80.3	0.0509	80.2	0.049 ^b	103.9
Wheat Grain		0.1915, 0.1661, 0.1389			
Average	79.8	0.1655	97.6	0.15 ^a	110.3

a Value from the wheat metabolism study (phenyl-label) with safener.

b Value from the confined rotational crop study (phenyl-label) with safener.

C.3. Independent Laboratory Validation

An ILV was performed using wheat grain and soybean grain. The individual recoveries for all analytes were between 70% and 120%, with a relative standard deviation (RSD) below 20%. The laboratory performing the ILV suggested no modifications to the method.



TABLE C.3.1. Recovery Results Obtained by an Independent Laboratory Validation of the Enforcement Method for the Determination of AE 0317309, AE B197555, and AE 1073910 in Plant Matrices.

Matrix	Analyte	Spiking Level (ppm)	Recoveries (%)	Mean Recovery \pm SD (%)
Wheat Grain	AE B197555	0.010	115, 104, 114, 100, 108	108 \pm 5.9
		0.200	112, 104, 101, 98, 107	104 \pm 5.2
		0.100	107, 104, 104, 106, 102	105 \pm 1.9
	AE 1073910	0.010	103, 105, 107, 109, 99	105 \pm 3.7
		0.200	106, 102, 100, 94, 102	101 \pm 4.4
		0.100	100, 102, 100, 101, 100	101 \pm 0.9
	AE 0317309	0.010	100, 99, 90, 93, 97	96 \pm 4.4
		0.200	96, 98, 96, 98, 94	96 \pm 1.7
		0.100	97, 95, 101, 97, 98	98 \pm 2.2
Soybean Grain	AE B197555	0.010	101, 100, 105, 94, 100	100 \pm 3.9
		0.200	103, 104, 97, 101, 104	102 \pm 2.9
		0.100	98, 100, 101, 94, 101	99 \pm 3.0
	AE 1073910	0.010	99, 97, 97, 96, 93	96 \pm 2.3
		0.200	97, 95, 94, 96, 95	95 \pm 1.2
		0.100	96, 95, 94, 95, 92	94 \pm 1.6
	AE 0317309	0.010	96, 103, 100, 96, 104	100 \pm 3.8
		0.200	103, 95, 99, 99, 100	99 \pm 2.9
		0.100	102, 98, 98, 99, 100	99 \pm 1.7

D. CONCLUSION

Adequate method validation data have been submitted for the LC-MS/MS method (AI-001-P04-01) for the determination of residues of pyrasulfotole, pyrasulfotole-desmethyl, and pyrasulfotole-benzoic acid in plant commodities. The validation data are representative of the expected residue levels for the plant commodities. The method is adequate to quantitate incurred residues of pyrasulfotole, pyrasulfotole-desmethyl, and pyrasulfotole-benzoic acid in plant matrices.

E. REFERENCES

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F. DOCUMENT TRACKING

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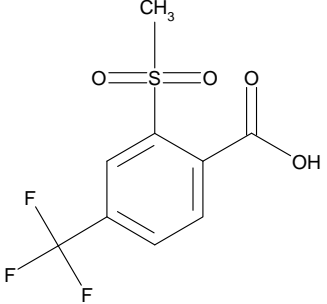
APPENDIX 1

Reference standards.

Common name/code	Chemical name	Chemical structure
pyrasulfotole AE 0317309	(5-hydroxy-1,3-dimethyl-1 <i>H</i> -pyrazol-4-yl)[2-(methylsulfonyl)-4-(trifluoromethyl)phenyl] methanone	
<i>d</i> ₃ -pyrasulfotole <i>d</i> ₃ -AE 0317309	(5-Hydroxy-1,3-dimethyl-1 <i>H</i> -pyrazol-4-yl)[2-[(methyl- <i>d</i> ₃)sulfonyl]-4-(trifluoromethyl)phenyl]methanone	
pyrasulfotole-desmethyl AE 1073910	(5-hydroxy-1 <i>H</i> -pyrazol-4-yl)[2-mesyl-4-(trifluoromethyl)phenyl]methanone	
[phenyl- ¹³ C ₆]AE 107391 AE 1073910-IS	(5-Hydroxy-3-methyl-1 <i>H</i> -pyrazol-4-yl)[2-(methylsulfonyl)-4-(trifluoromethyl)-phenyl- ¹³ C ₆]methanone	



Reference standards continued.

Common name/code	Chemical name	Chemical structure
pyrasulfotole-benzoic acid AE B197555	2-(Methylsulfonyl)-4-(trifluoromethyl)benzoic acid	
[phenyl- ¹³ C ₆]AE B197555 AE B197555-IS	2-(Methylsulfonyl)-4-(trifluoromethyl)benzoic-1,2,3,4,5,6- ¹³ C ₆ acid	