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STUDY REPORTS:

MRID No. 46801809 Lam, C.K., and Qadri, S.S. 9 January 2006. Validation of Bayer CropScience Method AI-004-A05-01. Analytical Method for the Determination of Residues of AE 0317309 in Animal Tissues and Milk Using LC/MS/MS. Bayer CropScience Study Number: RAAIX006. 100 pages.

MRID No. 46801810 Billian, P. and Wirkner, H. 4 November 2005. Independent Method Validation of the Analytical Method AI-004-A05-01 for the Determination of Residues of AE 0317309 in Animal Tissues and Milk Using LC/MS/MS. Bayer CropScience Report Number: MR-122/05. 56 pages.

MRID No. 46801811 Lam, C. K., and Qadri, S.S. 8 March 2006. Extraction Efficiency of Bayer CropScience Method AI-004-A05-01. Analytical Method for the Determination of Residues of AE 0317309 in Animal Tissues and Milk Using LC/MS/MS. Bayer CropScience Study Number: RAAIX010. 47 pages.

EXECUTIVE SUMMARY:

Bayer CropScience developed a high-performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS), method AI-004-A05-01, as the data gathering and enforcement method for residues of pyrasulfotole in/on livestock tissues including milk. Briefly, livestock tissues are extracted using acetonitrile (ACN)/water (H_2O) (2/1, v/v). Milk samples are diluted with water and filtered. In the case of whipping cream, the samples are extracted with ACN. The tissue sample extracts are heated to 60°C for at least 30 minutes; afterwards the samples are cooled down and centrifuged (only liver). The stable isotopic internal standard is added to sample extracts and mixed. An aliquot is purified by C18 solid phase extraction (SPE). Milk and whipping cream samples are syringe filtered or partitioned with n-hexane. The solvent is removed from the samples and the residues are reconstituted for analysis using HPLC-MS/MS.

The limit of quantitation (LOQ) is 0.010 ppm for bovine muscle, liver, kidney, and fat; and 0.005 ppm for bovine milk. The proposed enforcement method was adequately validated in bovine tissues and milk matrices. A successful independent laboratory validation (ILV) was completed with samples of kidney, liver, whipping cream and whole milk. Extraction efficiency data demonstrated that the enforcement method can account for incurred residues of pyrasulfotole in kidney, liver and whole milk.

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

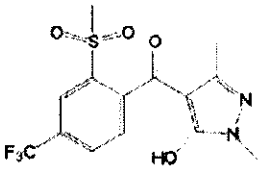
Compound	Chemical Structure
	
Common name	Pyrasulfotole
Company Experimental name	AE 0317309
IUPAC name	(5-hydroxy-1,3-dimethylpyrazol-4-yl)(α,α,α-trifluoro-2-mesyl-*p*-tolyl)methanone
CAS name	(5-hydroxy-1,3-dimethyl-1*H*-pyrazol-4-yl)[2-(methylsulfonyl)-4-(trifluoromethyl)phenyl] methanone
CAS #	365400-11-9
End-use product/(EP)	Herbicide: suspo-emulsion AE 0317309 02 SE06 A1

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
n-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:

The livestock tissues are extracted using ACN/H_2O (2/1, v/v). The sample extracts are heated to 60°C for at least 30 minutes, afterwards the samples are cooled down and centrifuged (only liver). The stable isotopic internal standard is added to the sample extract and mixed. An aliquot is purified by C18 SPE. The solvent is removed from the samples and the residues are reconstituted for analysis using HPLC-MS/MS.

Milk samples are diluted with water and the stable isotopic internal standard is added. The diluted samples are syringe filtered and analyzed by HPLC-MS/MS. For whipping cream, the samples are extracted with ACN and centrifuged. The supernatant is decanted into a separatory funnel and the stable isotopic internal standard is added. The extract is partitioned with n-hexane. The ACN layer is drained into glass vial; and the n-hexane phase is discarded. An aliquot of the ACN phase is concentrated to dryness and reconstituted for analysis using HPLC-MS/MS.

Residues of pyrasulfotole were monitored and quantitated in selected reaction monitoring (SRM) mode by a tandem MS operated in positive electrospray ionization $(M + H)^+$ from m/z of 363.1 to 251.1. The confirmation ion was monitored in negative electrospray ionization $(M - H)^-$ from 361.1 to 79.1. Quantitation of the native analyte was based on duplicate, six level calibration curves with concentrations of 2.5, 5.0, 10, 50, 250 and 500 ppb. The peak area ratio of native to internal standard of the compound was plotted against its nominal standard concentrations.

TABLE B.1.1. Summary Parameters for the Analytical Method Used for the Quantitation of Pyrasulfotole (AE 0317309) Residues in Beef Tissues and Milk Matrices.

Method ID	AI-004-A05-01
Analyte(s)	AE 0317309 including deuterated internal standard
Extraction solvent/technique	ACN:Deionized Water (2:1)/ Heat to 60°C for tissues. Dilution with water for milk
Cleanup strategies	Extract, centrifuge, C-18 extraction and concentrate for residues of AE 0317309 in tissues. No cleanup required for milk.
Instrument/Detector	HPLC separation using a C-18 column (50mm x 2mm x 5µm). Tandem Mass Spectrometer with positive electrospray ionization Confirmation ion was monitored by negative electrospray ionization
Standardization method	Multi-point linear regression curve versus stable isotopic internal standard.
Stability of std solutions	Up to 274 days (~9 months).
Retention times	≈ 3.8 minutes for AE 0317309

B.2. Enforcement Method

The enforcement method is the same as the data-gathering method described in Section B.1.

C. RESULTS AND DISCUSSION

C.1. Data-Gathering Method

TABLE C.1.1. Recovery Results from Method Validation of Beef Tissues and Milk Matrices using the Data-Gathering Analytical Method. Positive Ionization.

Matrix	Spiking level (ppm)	Recoveries (%)	Mean	Std. Dev	RSD
Fat	0.01	93, 93, 90, 88, 91, 94, 92	92	2	2.4
	0.1	118, 120, 115, 121, 122	119	3	2.5
Kidney	0.01	87, 84, 84, 84, 86, 86, 85	85	1	1.5
	0.1	112, 111, 113, 116, 111	113	2	2.0
Liver	0.01	88, 86, 87, 85, 82, 84, 86	85	2	2.0
	0.1	112, 112, 112, 113, 107	111	2	2.1
Muscle	0.01	90, 92, 91, 93, 88, 92, 94	92	2	2.1
	0.1	118, 117, 116, 111, 115	116	3	2.4
Skim milk	0.01	85, 84, 83, 84, 84, 87, 83	84	1	1.7
	0.1	114, 115, 114, 115, 115	115	1	0.5
Whole milk	0.01	90, 87, 88, 88, 86, 86, 86	87	2	1.8
	0.1	111, 112, 111, 113, 114	112	1	1.1
Whipping cream	0.01	72, 70, 71, 71, 75, 72, 74	72	2	2.3
	0.1	98, 91, 95, 94, 96	95	3	2.8

TABLE C.1.2. Characteristics for the Data-Gathering Analytical Method Used for the Quantitation of Pyrasulfotole Residues in Livestock.	
Analyte	Pyrasulfotole (AE 0317309)
Equipment ID	Phenomenex Gemini C-18, 50 x 2.0 mm, 5 µm Finnigan Quantum Discovery HPLC-MS/MS
Limit of quantitation (LOQ)	Fat, liver, kidney, muscle: 0.010 ppm; skim milk, whole milk, whipping cream: 0.005 ppm
Limit of detection (LOD)	whole milk: 0.00025 ppm; skim milk: 0.00023 ppm; whipping cream: 0.00026 ppm; beef muscle: 0.00061 ppm; beef liver: 0.00054 ppm; beef kidney: 0.00039 ppm, and beef fat: 0.00068 ppm.
Accuracy/Precision	Percent recoveries indicate acceptable accuracy/precision for livestock commodities in the range of the LOQ. The average recoveries, standard deviation and relative standard deviation (RSD) at the LOQ of 0.01 ppm (AE 0317309) from fat, kidney, liver and muscle were 92 ± 2% (RSD=2.4%), 85 ± 1% (RSD=1.5%), 85 ± 2% (RSD=2.0%) and 92 ± 2% (RSD=2.1%) respectively. The average recoveries, standard deviation and relative standard deviation (RSD) at the LOQ of 0.005 ppm (AE 0317309) from whole milk, skim milk and whipping cream were 87 ± 2% (RSD=1.8%), 84 ± 1% (RSD=1.7%), and 72 ± 2% (RSD=2.3%), respectively.
Reliability of the Method/ [ILV]	An independent laboratory method validation (ILV) (Bayer Study Report: MR-122/05) was conducted to verify the reliability of method no. AI-004-A05-01 for the determination of pyrasulfotole residues in livestock matrices. The values obtained are indicative that the method is reliable.
Linearity	The detector response was linear in solvent for pyrasulfotole from the range of 0.0025 to 0.50 ppm with coefficients of determination (r^2) greater than 0.99.
Specificity	The control chromatograms generally had no peaks above the chromatographic background and the spiked sample chromatograms contained only the analyte peak of interest within the retention window. Peaks were generally well defined and symmetrical. The precursor ion (m/z 363) from pyrasulfotole is formed from positive ionization of parent (MW = 362) in the first quadrupole analyzer. Thus MS/MS is extremely specific and selective, and detects only the ion with transition of m/z from 363 to 251. For confirmation, a secondary ion was monitored in negative electrospray ionization (M - H) from 361.1 to 79.1. The results are in agreement with the ion generated from primary positive ionization.

C.2. Enforcement Method

The enforcement method is the same as the data-gathering method described in Section C.1. Ruminant metabolism studies[10,11] conducted using [phenyl-UL-^{14}C]-pyrasulfotole and [pyrazole-3-^{14}C]-pyrasulfotole indicated that pyrasulfotole was the predominant residue in beef tissues and dairy products. The results presented in TABLE C.2.1. demonstrate the method extraction efficiency. The residue analytical method, AI-004-A05-01, has been shown to effectively extract incurred radioactive residues of pyrasulfotole from goat kidney (71.4%), liver (95.8%) and whole milk (106.4%).

TABLE C.2.1. Distribution of Residues Found in Goat Kidney, Liver and Whole Milk by the Residue Method as Compared with Those Found in Metabolism Studies.

Matrices	Amount of Pyrasulfotole				Extraction Efficiency (%)
	Analytical Residue Method		Metabolism Studies		
	% TRR	ppm	% TRR	ppm	
Kidney					
Samples	69.2, 80.0, 64.1	0.391, 0.452, 0.362			
Average	71.1	0.402	99.6	0.531	71.4
Liver					
Samples	91.1, 95.2, 88.1	1.277, 1.335, 1.235			
Average	91.5	1.282	95.5	1.411	95.8
Whole milk					
Samples	43.5, 52.2, 39.7, 26.4, 54.2, 31.8	0.0187, 0.0225, 0.0163, 0.0108, 0.0228, 0.0133			
Average	41.3	0.0174	38.8	0.017	106.4

C.3. Independent Laboratory Validation

An ILV study was performed to evaluate the ruggedness, usability, and potential weaknesses of the method of analysis for the pyrasulfotole residue in livestock tissues and whole milk. The matrices selected for the ILV were beef liver, beef kidney, whole milk and whipping cream.

The accuracy of the method is considered to be acceptable, as the mean recoveries for all matrices and all spiking levels are in the range of 70 110%. The results of the ILV are presented in TABLE C.3.1.

The analytical method for the determination of residues of pyrasulfotole in livestock tissues and whole milk was successfully validated by an independent laboratory.

TABLE C.3.1. Recovery Results Obtained by an Independent Laboratory Validation of the Enforcement Method for the Determination of Pyrasulfotole (AE 0317309) in Livestock Matrices.

Sample Material	Spiking Level [ppm]	Single Values [%]					Mean Value [%]	RSD [%]
Liver	0.01	85	87	86	82	80	84	3.5
	0.02	107	103	107	112	106	107	3.0
	0.10	99	99	119	95	106	104	9.1
Kidney	0.01	74	76	73	79	77	76	3.1
	0.02	91	89	94	95	98	93	3.8
	0.10	98	102	104	99	102	101	2.4
Whole milk	0.005	103	108	104	108	104	105	2.3
	0.01	107	107	104	107	106	106	1.2
	0.05	110	108	109	109	110	109	0.8
Whipping Cream	0.005	108	109	105	106	108	107	1.5
	0.01	106	110	109	109	108	108	1.4
	0.05	107	109	109	108	109	108	0.8

D. CONCLUSION

Adequate method validation data have been submitted for the HPLC-MS/MS method (AI-004-A05-01) for the determination of residues of pyrasulfotole in beef tissues and whole milk matrices. The validation data are representative of the expected residue levels for the livestock commodities. The method is adequate to quantitate incurred residues of pyrasulfotole in livestock matrices. The LOQ for pyrasulfotole is 0.010 ppm for bovine fat, kidney, liver, muscle and at 0.005 ppm in milk, including cream.

E. REFERENCES

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10. Rupprecht, J. K. and Ying, S. L. (2006). Metabolism of [phenyl-UL-^{14}C]-AE 0317309 in the Lactating Goat. Unpublished Bayer CropScience Report No. MEAIM009.

11. Rupprecht, J. K. and Ying, S. L. (2006). Metabolism of [pyrazole-3-^{14}C]-AE 0317309 in the Lactating Goat. Unpublished Bayer CropScience Report No. MEAIM010.

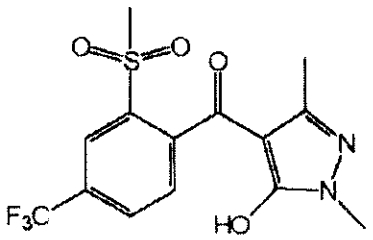
F. DOCUMENT TRACKING

RDI: Louise G Croteau (6 September 2006); RAB1 Chemists (29 November 2006); George Kramer (29 November 2006)
Petition Number: 6F7059
DP#: 333412

Template Version June 2005.

APPENDIX 1

Reference standards.

Common name/code	Chemical name	Chemical structure
pyrasulfotole AE 0317309	(5-hydroxy-1,3-dimethyl-1*H*-pyrazol-4-yl)[2-(methylsulfonyl)-4-(trifluoromethyl)phenyl] methanone	
d_3-pyrasulfotole d_3-AE 0317309	(5-Hydroxy-1,3-dimethyl-1*H*-pyrazol-4-yl)[2-[(methyl-d_3)sulfonyl]-4-(trifluoromethyl)phenyl]methanone