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STUDY REPORTS:

MRID No. 46801812 Lam, C. K., and Qadri, S. S. 28 February 2006. Validation of Bayer CropScience Method AI-005-A05-01. Analytical Method for the Determination of Residues of AE B197555 in Poultry and Eggs Using LC/MS/MS. Unpublished Bayer CropScience Study Number: RAAIP012. 91 pages.

MRID No. 46801813 Nelson, S. 1 March 2006. Independent Method Validation of the Analytical Method AI-005-A05-01 for the Determination of Residues of AE 0317309 in Poultry and Eggs Using LC/MS/MS. Unpublished Enviro-Test Laboratories Report Number 06BAY27.REP. 78 pages.

EXECUTIVE SUMMARY:

Bayer CropScience developed a high-performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS) method (AI-005-A05-01) as the data gathering and enforcement method for residues of the metabolite pyrasulfotole-benzoic acid (AE B197555) in/on poultry tissues, including eggs.

Briefly, the poultry tissues are extracted twice using acetonitrile (ACN)/2M hydrochloric acid (HCl) (2/1, v/v). The samples are heated to 60°C for at least 30 minutes; afterwards the samples are cooled down and centrifuged. In the case of eggs, samples are extracted twice with ACN, and partitioned with n-hexane. The stable isotopic internal standard is added to the sample extract and mixed. An aliquot is purified by C18 solid-phase extraction (SPE). The solvent is removed from the samples and the residues are reconstituted in methanol/10mM ammonium acetate for analysis using HPLC-MS/MS.

The limit of quantitation (LOQ) is 0.010 ppm for poultry matrices, including eggs. The proposed enforcement method was adequately validated for the determination of pyrasulfotole-benzoic acid (AE B197555) in poultry liver, muscle, skin and eggs. A successful independent laboratory validation (ILV) was completed with samples of breast muscle and eggs.

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.

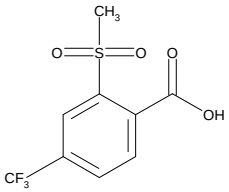
Compound	Chemical Structure
	
Common name	Pyrasulfotole-benzoic acid
Company Experimental name	AE B197555
IUPAC name	2-mesyl-4-trifluoromethylbenzoic acid
CAS name	2-(methylsulfonyl)-4-(trifluoromethyl)benzoic acid
CAS #	142994-06-7
End-use product/(EP)	Not applicable

TABLE A.2. Physicochemical Properties of Pyrasulfotole-benzoic acid

Parameter	Value	Reference
Dissociation constant (pK_a)	1.77	1
n-Octanol-water partition coefficient Log(K_{OW})	0.10 at pH 4.4	2

B. MATERIALS AND METHODS

B.1. Data-Gathering Method

B.1.1. Principle of the Method:

The poultry tissues are extracted twice using ACN/2M HCl (2/1, v/v). The samples are heated to 60°C for at least 30 minutes, and afterwards the samples are cooled down and centrifuged. The stable isotopic internal standard (IS) 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 in methanol/10mM ammonium acetate for analysis using HPLC-MS/MS.

Egg samples are extracted twice with ACN. The supernatants are combined in a separatory funnel and the stable isotopic internal standard is added. The extract is partitioned with n-hexane. An aliquot of the ACN phase is concentrated to dryness and reconstituted for analysis using HPLC-MS/MS.

The LOQ for pyrasulfotole-benzoic acid was 0.01 ppm for poultry tissues and eggs. Residues of pyrasulfotole-benzoic acid were monitored and quantitated in selected reaction monitoring (SRM) mode by a tandem MS operated in negative electrospray ionization $(M-H)^-$ from m/z of 267 to 159. The confirmation ion was monitored in negative electrospray ionization $(M-H)^-$ from m/z of 267 to 223. Quantitation of pyrasulfotole-benzoic acid was based on duplicate, five level calibration curves with concentrations of 5, 10, 20, 100 and 500 ppb.

TABLE B.1.1. Summary Parameters for the Analytical Method Used for the Quantitation of AE B197555 Residues in Poultry and Eggs.	
Method ID	AI-005-A05-01
Analyte(s)	Pyrasulfotole-benzoic acid (AE B197555) including deuterated internal standard
Extraction solvent/technique	Tissues: Extract twofold ACN:2M HCl (2:1)/ Heat to 60°C for tissues. Eggs: Extract twofold ACN
Cleanup strategies	Tissues: Extract, centrifuge, C-18 extraction and concentrate for HPLC-MS/MS. Eggs: Liquid-liquid partition with hexane and concentrate for HPLC-MS/MS.
Instrument/Detector	HPLC separation using a C-18 column (50mm x 2mm x 3µm). Tandem Mass Spectrometer with negative 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	≈ 5.1 minutes for AE B197555

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

Poultry metabolism studies conducted using [phenyl-UL-^{14}C]-AE 0317309 and [pyrazole-3-^{14}C]-AE 0317309 in laying hen indicated that the metabolism of pyrasulfotole (AE 0317309) was not extensive. The only residue that poultry would be exposed to as a result of a cereal grain diet from a pyrasulfotole treated cereal would be pyrasulfotole-benzoic acid (AE B197555). The results presented in TABLE C.1.1 demonstrate the method validation.

The precursor ion (m/z 267) from AE B197555 is formed from the negative ionization of parent (MW = 268) in the first quadrupole analyzer. The product ion (m/z 159) is formed by collision-induced dissociation (CID) of the precursor ion with argon gas in the collision cell (Q2) and is analyzed in the third quadrupole analyzer (Q3). In addition, a second confirmation ion may be monitored using the negative ionization mode from m/z of 267 to 223. Consequently, the method is selective and specific when HPLC separation is coupled with the two possible ion transitions.

TABLE C.1.1. Recovery Results from Method Validation of Poultry Tissues and Eggs Using the Data-Gathering Analytical Method.

Matrix	Spike Level (ppm)	Recoveries, %	Mean	Std Dev	RSD
Eggs	0.01	85, 97, 92, 100, 87, 79, 84	89	7.6	8.6
	0.10	100, 101, 99	100	1.4	1.4
Liver	0.01	112, 105, 110, 107, 106, 111, 105	108	3.0	2.8
	0.10	112, 115, 110	112	2.4	2.2
Muscle	0.01	112, 100, 102, 107, 116, 106, 112	108	5.7	5.3
	0.10	114, 113, 111	112	1.4	1.3
Skin	0.01	111, 104, 102, 100, 109, 109, 103	106	4.2	4.0
	0.10	113, 112, 113	113	0.4	0.4

TABLE C.1.2. Characteristics for the Data-Gathering Analytical Method Used for the Quantitation of AE B197555 Residues in Poultry and Eggs.

Analyte	Pyrasulfotole-benzoic acid (AE B197555)
Equipment ID	Phenomenex Gemini C-18, 50 x 2.0 mm, 3 µm; Finnigan Quantum Discovery LC-MS/MS
Limit of quantitation (LOQ)	Tissues = 0.010 ppm; Eggs = 0.010 ppm
Limit of detection (LOD)	The LODs of AE B197555 from the chicken egg, liver, muscle (dark and white meat combined) and skin were 0.00271, 0.00095, 0.00182 and 0.00139 ppm respectively.
Accuracy/Precision	The recoveries from validation tests varied from 79% to 116% over all the spike levels and sample matrices. The average recoveries, standard deviation and relative standard deviation (RSD) for 0.01 ppm of AE B197555 from chicken egg, chicken liver, chicken muscle and chicken skin were 89 ± 8% (RSD=8.6%), 108 ± 3% (RSD=2.8%), 108 ± 6% (RSD=5.3%) and 106 ± 4% (RSD=4.0%) respectively.
Reliability of the Method/ [ILV]	An ILV was conducted to verify the reliability of method No. AI-005-A05-01 for the determination of AE B197555 residues in poultry matrices. The values obtained are indicative that the method is reliable.
Linearity	The detector response was linear in solvent for AE B197555 in the range of 0.005 to 0.50 ppm with coefficients of determination (r^2) greater than 0.99.
Specificity	The method employs a highly specific and selective detector (HPLC-MS/MS). The control chromatograms generally often have no peaks above the chromatographic background and the spiked sample chromatograms contain only the analyte peak of interest within the retention window. Peaks were well defined and symmetrical.

C.2. Enforcement Method

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

C.3. Independent Laboratory Validation

An ILV was performed to evaluate of the ruggedness, usability, and potential weaknesses of the method of analysis for AE B197555 residue in poultry tissues and eggs. The matrices selected for the ILV were chicken breasts (boneless and skinless) and eggs.

The accuracy of the method is considered to be acceptable as the mean recoveries for all sample materials and all spiking levels are in the range of 70–114%. The results of the ILV are presented in TABLE C.3.1.

TABLE C.3.1. Recovery Results Obtained by an Independent Laboratory Validation of the Enforcement Method for the Determination of AE B197555 in Poultry Matrices

Sample Material	Spiking Level [ppm]	Recovery Values [%]					Mean Value [%]	RSD [%]
Eggs	0.01	96	91	92	77	82	88	8.9
	0.02	114	90	91	95	85	95	11.8
	0.10	88	88	99	91	100	93	6.3
Breast	0.01	111	72	112	105	106	101.2	16.4
	0.02	105	102	93	98	88	97	7.0
	0.10	88	92	96	85	100	92	6.5

D. CONCLUSION

Adequate method validation data have been submitted for the HPLC-MS/MS method (AI-005-A05-01) for the determination of residues of the metabolite pyrasulfotole-benzoic acid in poultry tissues and egg matrices. The validation data are representative of the expected residue levels for the poultry commodities. The LOQ for pyrasulfotole-benzoic acid is 0.010 ppm for poultry liver, muscle, skin and eggs.

E. REFERENCES

1. Mills, E.A.M. (2003).The determination of the pKa for the isoxaflutole metabolite RPA203328. Unpublished Bayer CropScience Document No. C036496.

2. Certon, A. and Cousin, J. (1994). RPA202248, RPA203328, RPA205834 Octanol/water Partition coefficients (Summary). Unpublished Bayer CropScience Document No. C016455.

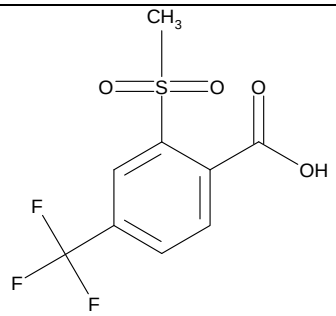
F. DOCUMENT TRACKING

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-benzoic acid AE B197555	2-(Methylsulfonyl)-4-(trifluoromethyl)benzoic acid	
[phenyl-$^{13}C_6$]AE B197555 AE B197555-IS	2-(Methylsulfonyl)-4-(trifluoromethyl)benzoic-*1,2,3,4,5,6-13C6* acid