Prothioconazole in Surface Water/ECM0220W1-W4/RCC Ltd/722417

ENVIRONMENTAL CHEMISTRY METHOD REVIEW EVALUAION

Data Requirement: PMRA Data Code: NA
EPA DP Barcode: - D319586
OECD Data Point: NA
EPA Guideline: ECM Method Review

Test material:
Common name: Prothioconazole
Chemical name: 2-[2-(1-chlorocycloprypy)-3-(2-chlorophenyl)-2-hydroxypropy]-1,2-Dihydro-3H-1,2,4-triazole-3-thione (CAS); 2-[2-(1-chlorocyclo-propyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-1,2,4-triazole-3-thione
IUPAC: (RS)-2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxy-proyl]-1,2-dihydro-2H-1,2,4-trizaole-3-thione

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ANALYTICAL METHOD: Lam, C.K., 2004. Analytical Method JA002-W04-01 for the Determination of JAU 6467, Desthio, S-methyl, and JAU 6476-thiazocine in Water. Unpublished method created by Bayer CropScience Laboratory, 16745 S. Metcalf Ave, Stilwell, KS 66085-9104 and submitted by Bayer CropScience Company, 2 T.W. Alexander Drive, Research Triangle Park, NC 27709. Study ID: 200517, Method Effective Date: March 4, 2004. Independent laboratory validation study completed on June 18, 2003.

EXECUTIVE SUMMARY

The method is applicable for the quantitative determination of residues of JAU 6467 and 3 degradates in surface water. The method was submitted to EPA to support studies performed to seek registration for Prothioconazole. The method was created by Bayer Company in Research Triangle Park, NC in the spirit of EPA's good laboratory Practice Standards, Title 40, Code of the Federal Regulations Part 160. An independent laboratory validation was submitted with this method. It was entitled, "Determination of

JAU 6476, Desthio (SXX 0665), S-methyl, and thiazocine in Water by LC/MS/MS". It was performed by Battelle Memorial Institute, Columbus, Ohio.

Method Summary: An analytical method was developed to quantify JAU 6476, Desthio (SSX 065), S-methyl and thiazocine in water using high-performance liquid chromatography electrospray tandem mass spectrometry (LC-MS/MS). The method was validated from control water obtained from New Port (Arkansas) at 0.3 ppb. Isotopic internal standards of JAU 6476, Desthio (SSX 0665), S-methyl and thiazocine were to the water sample (50 mL). The water samples were extracted with C_{18} solid phase extraction (SPE) cartridge and eluted by LC-MS/MS, and quantitation was done against known amount of isotopic internal standards.

The average recoveries of JAU 6476, Desthio, S-methyl and JAU 6478-thiazocine fortified at the LOQ (0.3 ppb) and the 10 x LOQ (3.0 ppb) were all within the acceptable range.

METHOD ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS

Under the conditions and parameters set in the Ecological Effects Test Guidelines, OPPTS 850.7100, Data Reporting for Environmental Chemistry Methods; "Public Draft." (U.S. Environmental Protection Agency. Office of Prevention, Pesticides, and Toxic Substances (7101). U.S. Government Printing Office: Washington, DC, 1996, EPA-712-C-96-348), this method was acceptable for recoveries of all analytes.

COMPLIANCE

Although there were no signed and dated statements that this method was conducted in accordance with the requirements for Good Laboratory Practice Standards, 40 CFR 160, it was stated that the work was done in the spirit of GLP. It was also stated that although there was no protocol for the validation of this method, the method was validated under GLP and recoveries used for the validation of this method were conducted under GLP. Also stated was that no QA inspections were performed for the sample analysis portions of the studies conducted by Bayer for purposes of registration. A statement of non-confidentiality on the basis of the method falling within the scope of FIFRA Section 10 (d)(1)(A)(B), or (C) was signed and dated along with information on the Quality Assurance inspection dates and signatures.

A. BACKGROUND INFORMATION

Prothioconazole is an experimental fungicide being developed by Bayer CropScience for use on wheat, corn, peanuts, barley, canola and vegetables. It belongs to the class of Triazlinthione. It is effective against leaf spots, ear stem, fusarium, bunt, smut, powdery mildew and sheath blight.

TABLE A.1. Test Compound Nomenclature

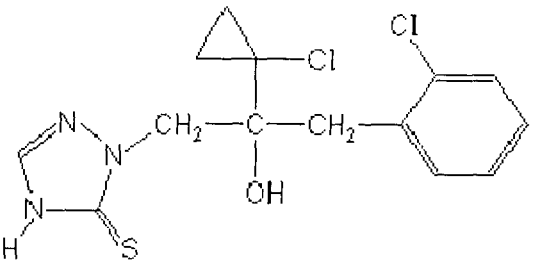
Compound	 Chemical Structure: JAU 6476
Common name	Prothioconazole
Company experimental name	JAU 6476
IUPAC name	(RS)-2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-1,2,4-triazole-3-thione
CAS Name	2-[2-(1-chlorocyclopropy)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3H-1,2,4-triazole-3-thione
CAS #	178928-70-6

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

Parameter	Value
Melting point/range	139.1°C – 144.5°C
Color and physical state	White to light-beige crystalline powder
Volatility	$<<3 \times 10^{-5}$ Pa x m^3/mol
Partition coefficient in octanol/water	Unbuffered 4.05 log P_{ow}
Solubility	(g substance in 1000 mL at 20°C)
Vapor pressure at 20°C	$<<4 \times 10^{-7}$ Pa
Solubility in water	At pH 9 2 At pH 8 0.3 At pH 4 0.005
Solubility in organic solvents	n-heptane <0.1

	xylene	8
	1-octanol	58
	2-propenol	87
	Ethyl acetate	>250
	Polyethylene glycol	>250
	Acetonitrile	69
	Acetone	>250
	Dichloromethane	88
	Dimethylsulfoxide	126
Dissociation constant	pKa = 6.9	

MATERIALS AND METHODS

B.1. Principle of Method

The analytical methods for determination of Prothioconazole (determined as JAU 6467) as well as its metabolites Desthio (SSX0665), S-methyl, and JAU 6476-thiazocine were extracted from 50 mL of control water. The water was then fortified as appropriate with the test substances. The water samples were extracted with C_{18} solid phase extraction (SPE) cartridge and eluted with 4 milliliter of acetonitrile : water(9:1, v/v). The resultant solution was analyzed by LC-MS/MS, and quantitation was done against known amount of isotopic internal standards.

TABLE B.1.1.	**Summary Parameters for the Analytical Method Used for the Quantitation of Chemical Residues in Matrices Studied**
Method ID	ECM0220W1-W4
Analyte(s)	JAU 6476, Desthio (SSX 0665), S-methyl, JAU 6476-Thiazocine
Extraction solvent/technique	Water extraction accomplished by passing through preconditioned 500-mg C_{18} SPE cartridge
Cleanup strategies	None
Instrument/Detector	HPLC Conditions: ThermoFinnigan P-400 quaternary pump with a ThermoFinnigan degasser and A3000 autosampler. MS Conditions: ThermoFinnigan TSQ 7000 quadrupole Atmospheric pressure API II in electrospray ionization (ESI) mode.

C. RESULTS AND DISCUSSION

C.1. Recovery Results Summary

TABLE C.1.1. Recovery Results from Method Validation of [matrices]

Matrix Surface Water	Spiking Level ppb	Average Recovery Obtained (%)	Relative Standard Deviation
JAU 6476	0.3 3.0	96 99	17.8 3.9
Desthio	0.3 3.0	107 99	4.3 2.2
S-methyl	0.3 3.0	104 99	1.1 1.1
Thiazocine	0.3 3.0	103 99	4.7 1.3

C.1.2. Method Characteristics

TABLE C.1.2. Method Characteristics

Analyte	JAU 6467, Desthio, S-methyl, JAU 6467-Thiazocine
Limit of Quantitation	0.3 ppb, 0.3 ppb, 0.3 ppb, 0.3 ppb
Limit of Detection (LOD)	0.160 ppb, 0.044 ppb, 0.011 ppb, 0.046 ppb
Accuracy/Precision at LOQ	See above chart
Reliability of the Method/ [ILV]	An independent laboratory method validation [ILV], (MRID No.462465-27), was conducted to verify the reliability of method (MRID No. 462465-35) for the determination of Prothioconazole and it's degradates in water. The values obtained indicated that the registrant method is acceptable according to OPPTS 850.7100 Guidelines.
Linearity	All method responses were linear (coefficient of determination for all compounds was greater than $r^2 = 0.9910$.
Specificity	The method is specific for the determination of Prothioconazole and its degradates by virtue of the chromatographic separation and selective detection system used. According to recently published guidelines, when detection is performed by tandem mass spectrometry methods, confirmation of the presence of the analyte should require the observation of a precursor ion plus one structurally significant product ion observed at the same retention time. Further confirmation is not necessary due to the highly specific nature of the MS/MS transitions monitored.

C.2. Independent Laboratory Validation (ILV)

The ILV was conducted in accordance with the *OPPTS 850.7100 Guidelines.*

TABLE C.2.1. Average Recovery Results Obtained by an Independent Laboratory Validation of the Method for the Determination of JAU 6476, Desthio (SXX 0665), S-methyl, and JAU 6467-Thiazocine in Water by LC/MS/MS.

Matrix Surface Water	Spiking Level (ppb)	Average Recoveries Obtained (%)	Relative Standard Deviation
JAU 6467	0.3 3.0	102 104	7.8 3.5
Desthio	0.3 3.0	102 100	9.6 3.3
S-Methyl	0.3 3.0	98 96	12.4 3.3
JAU 6476-Thiazocine	0.3 3.0	101 100	9.4 3.4

D. CONCLUSION

From a review of the method, C. K. Lam "Analytical Method for the Determination of JAU 6476, Desthio, S-methyl and JAU 6476-Thiazocine in Water", ECB concludes that the method appears scientifically sound and capable of determining the residues of Prothioconazole and its degradates in water.