

**Prothioconazole in soil/ECM0220S1-S4/Bayer CropScience /
ENVIRONMENTAL CHEMISTRY METHOD REVIEW EVALUATION**

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-Chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-
1,2-dihydro-3H-1,2,4-triazole-3-thione

IUPAC: (RS)-2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-
-1,2-dihydro-2H-1,2,4-triazole-3-thione

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Peer Reviewer: Charles Kennedy **Date:** 2/7/06
Charles Kennedy, Chemist, EPA/OPP/BEAD/ECB

QA Officer: Christian Byrnes **Date:** 02/07/06
Dr. Christian Byrnes, EPA/OPP/BEAD/ECB

ANALYTICAL METHOD: Lam, C.K., March 3, 2004. Analytical Method for the Determination of JAU 6476, Desthio, S-methyl and JAU 6476-thiazocine in Soil and Sediment, MRID # 462465-31, Unpublished method created and submitted by Bayer CropScience Study ID: 200515 Method Effective Date: March 3, 2004

EXECUTIVE SUMMARY

The method is applicable for the quantitative determination of residues of JAU 6476, Desthio, S-methyl and JAU 6476-thiazocine in soil.

The method was submitted to EPA by Bayer CropScience to support studies performed to seek registration for prothioconazole. The method was created and validated by Bayer CropScience of Stilwell, KS and of RTP, NC in accordance with EPA's Good Laboratory Practice Standards, Title 40 Code of Federal Regulations Part 160. An independent laboratory validation was submitted with this method. It was entitled, "Independent Laboratory Validation of "Analysis of JAU 6476, Desthio, S-methyl, and JAU 6476-thiazocine in Soil".

Soil samples (15 g) are extracted with acetonitrile/water/cysteine hydrochloride, then centrifuged. An aliquot of the supernatant is then transferred to culture tubes and isotopic internal standards are added. An aliquot of the extract was added to a HPLC vial and diluted with water. The resultant solution was analyzed by LC-MS/MS, and

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quantitation was done against known amount of isotopic internal standards. The limit of quantitation (LOQ) in soil for all four analytes is 10 ppb.

The average recoveries of JAU 6476, Desthio, S-methyl and thiazocine fortified at the LOQ (10 ppb) and the 10 X LOQ (100 ppb) were all within the acceptable range.

METHOD ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS

Based on the 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) ECB finds this method acceptable as submitted.

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 specifically performed for this method, however, there were inspections 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

JAU 6476 is an experimental fungicide being developed by the Bayer CropScience for use on wheat, corn, peanuts, barley, canola and vegetables. It belongs to the class of Triazolinthione. It is effective against leaf spots, ear stem, fusarium, bunt, smut, powdery mildew and sheath blight.

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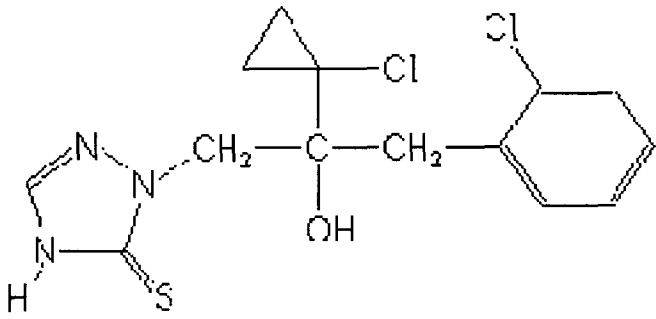
TABLE A.1. Test Compound Nomenclature	
Compound	 <p>Chemical Structure</p>
Common name	Prothioconazole
Company experimental name	JAU 6476
IUPAC name	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-3H-1,2,4-triazole-3-thione
CAS Name	2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3H-1,2,4-triazole-2-thione
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
pH	
Density	
Water solubility (g substance at 20°C)	at pH 9 = 2 at pH 8 = 0.3 at pH 4 = 0.005
Solvent solubility (g substance at 20°C)	n-heptane <0.1 xylene 8 1-octanol 58 2-propanol 87 Ethyl acetate >250 Polyethylene glycol >250 Acetonitrile 69 Acetone >250 Dimethylsulfoxide 126 Dichloromethane 88
Vapour pressure at °C	<<4x10 ⁻⁷ Pa
Dissociation constant (pK _a)	pK _a =6.9
Octanol/water partition coefficient	Unbuffered 4.05 log P _{ow}
UV/visible absorption spectrum	In acetonitrile – shows a single peak maximum at 257 nm

B. MATERIALS AND METHODS

B.1. Principle of Method

Fifteen grams of soil are extracted with 30 ml of solvent (acetonitrile/water/cysteine hydrochloride), shaken, and centrifuged; then an aliquot of the supernatant is transferred to a culture tube and isotopic internal standards added. An aliquot of extract is added to HPLC vial and diluted with water. Analysis was performed using high-performance liquid chromatography tandem mass spectrometry (LC-MS/MS).

TABLE B.1.1.	Summary Parameters for the Analytical Method Used for the Quantitation of Chemical Residues in Matrices Studied
Method ID	ECM0220S1-S4
Analyte(s)	Prothioconazole
Extraction solvent/technique	Soil samples are extracted with acetonitrile/water/cysteine hydrochloride followed by centrifugation. A deuterated isotope internal standard is added to an aliquot of the extract.
Cleanup strategies	No cleanup
Instrument/Detector	LC/MS/MS – ThermoFinnigan P-4000 HPLC and ThermoFinnigan TSQ 7000 triple quadrupole

C. RESULTS AND DISCUSSION

C.1. Recovery Results Summary

TABLE C.1.1. Recovery Results from Method Validation of [matrices]				
Compound Name	LOQ = 10 ppb		10 x LOQ = 100 ppb	
	Avg. Rec.	RSD	Avg. Rec.	RSD
Prothioconazole	9.83	10	97.72	4.16
Desthio	10.31	5.6	95.62	3.94
S-Methyl	10.14	1.3	93.48	1.29
Thiazocine	9.97	4.3	96.95	1.61

C.1.2. Method Characteristics

TABLE C.1.2. Method Characteristics	
Analyte	Prothioconazole
Limit of Quantitation	10 ppb
Limit of Detection (LOD)	MDLs were ≤ 3.093 ppb for the parent and all degradates
Accuracy/Precision at LOQ (10 ppb)	See above table for details

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Reliability of the Method/ [ILV]	An independent laboratory method validation [ILV], (MRID No. 462465-29), was conducted to verify the reliability of method for the determination of prothioconazole and three degradates in soil. The values obtained indicated that the registrant method is acceptable according to <i>OPPTS 850.7100 Guidelines</i> .
Linearity	No raw calibration data was supplied but calibration curves with equations were given. ($r^2 > 0.990$)
Specificity	Peaks were well defined and symmetrical. There appeared to be no carryover to the following chromatograms.

C.2. Independent Laboratory Validation (ILV)

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

Compound	Spiking Level (ug/L)	Average Recoveries Obtained (%)	Relative Standard Deviation
JAU 6476	10 ppb – LOQ	88	9
	100 ppb – 10 X LOQ	81	10
Desthio	10 ppb – LOQ	93	5
	100 ppb – 10 X LOQ	91	2
S-Methyl	10 ppb – LOQ	93	2
	100 ppb – 10 X LOQ	90	3
JAU 6476-Thiazocine	10 ppb – LOQ	96	9
	100 ppb – 10 X LOQ	88	2

D. CONCLUSION

From a review of the method “Analytical Method for the Determination of JAU 6476, Desthio, S-methyl and JAU 6476-thiazocine in Soil and Sediment” and the ILV data presented in the “Independent Laboratory Validation of “Analysis of JAU 6476, Desthio, S-methyl, and JAU 6476-thiazocine in Soil”, ECB finds this ECM acceptable.