

Analytical method for the enantiomers of metconazole [BAS 555 F; M555F000-RR (Reg.No. 5836048), M555F000-RS (Reg.No. 5836046), M555F000-SR (Reg.No. 4677200), and M555F000-SS (Reg.No. 5836047)] in water

Reports: ECM: EPA MRID No.: 50200402. Andrews, R.S. 2015. Validation of Method D1501/01 (L0273/01): Method for the determination of Enantiomers of BAS 555 F (Reg.No. 5836046, Reg.No. 4677200, Reg.No. 5836047 and Reg.No. 5836048) in Surface and Drinking Water by LC-MS/MS. BASF Study No.: 427928. BASF Registration Document No.: 2015/7000428. Report prepared, sponsored and submitted by BASF Corporation, Research Triangle Park, North Carolina; 136 pages. Final report issued April 27, 2015.

ILV: EPA MRID No. 50200401. Perez, S., M. Marshall. 2015. Independent Laboratory Validation of Residue Method D1501/01 (L0273/01): "Method for the determination of Enantiomers of BAS 555 F (Reg.No. 5836046, Reg.No. 4677200, Reg.No. 5836047 and Reg.No. 5836048) in Surface and Drinking Water by LC-MS/MS". BASF Study ID No.: 698022. BASF Registration Document No.: 2015/7000429. ADPEN Study No.: 2K14-903-698022. Report prepared by ADPEN Laboratories, Inc., Jacksonville, Florida, sponsored and submitted by BASF Corporation, Research Triangle Park, North Carolina; 147 pages. Final report issued April 28, 2015.

Document No.: MRIDs 50200402 & 50200401

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practice (GLP) standards, 40 CFR, Part 160 (p. 3 of MRID 50200402). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5).

ILV: The study was conducted in accordance with USEPA FIFRA GLP standards, 40 CFR, Part 160 (p. 3 of MRID 50200401). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5).

Classification: This analytical method is classified as **acceptable**. The method generally satisfied the repeatability and reproducibility criteria, with RSDs \leq 20% and mean recoveries in the range of 70 – 120%, except for some analyte peak overlapping and poor linearity associated with the confirmation ion (M555F000-RR) in the ILV.

PC Code: 125619

EFED Final Reviewer: William Gardner, Ph.D.,
Environmental Scientist

Signature: **William Gardner**
Date: 8/24/18

Digitally signed by William Gardner
Date: 2018.09.24 15:55:44 -04'00'

CDM/CSS- Dynamac JV Reviewers: Lisa Muto,
Environmental Scientist

Signature: *Lisa Muto*
Date: 8/14/17

Reviewers: Kathleen Ferguson, Ph.D.,
Environmental Scientist

Signature: *Kathleen P. Ferguson*
Date: 8/14/17

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel.

Executive Summary

The analytical method, BASF Method D1501/01 (L0273/01), is designed for the quantitative determination of the enantiomers of metconazole [BAS 555 F; M555F000-RR (Reg.No. 5836048), M555F000-RS (Reg.No. 5836046), M555F000-SR (Reg.No. 4677200), and M555F000-SS (Reg.No. 5836047)] in water at the LOQ of 25 ng/L (25 ppt) using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in water for the four enantiomers of metconazole (0.0029 mg ai/L; USEPA 2015). The ECM validated the method using characterized surface and drinking water. The ILV validated the method in the first trial with insignificant modifications to the analytical instrumentation using uncharacterized surface and drinking water which was provided by the Sponsor.

Water samples were fortified with a mixture of cis- and trans-metconazole. In the ECM, analyte identification in the chromatograms was performed by comparison of the retention times of three of the four peaks observed in the mixed calibration standards compared to individual solutions of the M555F000-RS, M555F000-SR and M555F00-RR reference materials; the fourth peak in the chromatograms was identified as M555F000-SS by the process of elimination since a reference compound was not included for the analyte M555F000-SS. In the ILV, analyte identification in the chromatograms appeared to be based on the ECM assignment.

All ILV data regarding repeatability, accuracy, precision, and linearity were satisfactory for all four enantiomers of metconazole in both matrices, based on the quantitation ion results. The confirmation ion analysis in the ILV was insufficient for the linearity of M555F000-RR and the performance data of M555F000-SR in drinking water, but a confirmatory method is not typically required where GC/MS and/or LC/MS methods are used as the primary method(s) to generate study data. All ECM data regarding repeatability, accuracy, precision, and linearity were satisfactory for all four enantiomers of metconazole in both matrices. However, the specificity of the method was not supported by the ECM and ILV representative chromatograms because the analyte peak pairs of M555F000-RS and M555F000-SR and M555F00-RR and M555F000-SS overlapped at the bottom which interfered with peak integration of each analyte; larger overlaps were observed in the ILV chromatograms versus the ECM chromatograms.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide ¹	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Metconazole (BAS 555 F) Enantiomers: M555F000-RR (Reg.No. 5836048) M555F000-RS (Reg.No. 5836046) M555F000-SR (Reg.No. 4677200) M555F000-SS (Reg.No. 5836047)	50200402	50200401		Water ^{2,3}	27/04/2015	BASF Corporation	LC/MS/MS	25 ng/L

1 M555F000-RR = (1R,5R)-5-(4-Chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol;

M555F000-RS = (1R,5S)-5-(4-Chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol;

M555F000-SR = (1S,5R)-5-(4-Chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol; and

M555F000-SS = (1S,5S)-5-(4-Chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol.

2 In the ECM, drinking (well) water (20141105-drinking-Well; pH 7.4; 25 mg equiv. CaCO₃/L hardness; 0.7 ppm total organic carbon) and surface (lake/pond) water (20141104-surface-JL; pH 6.9; 32 mg equiv. CaCO₃/L hardness; 6.1 ppm total organic carbon) were used in the study (p. 14; Appendix H, pp. 127-131 of MRID 50200402). The water samples were collected locally and characterized by Agvise Laboratories, Northwood, North Dakota; water sources were not further specified.

3 In the ILV, the drinking and surface water matrices were provided by the Sponsor (p. 17 of MRID 50200401). The water matrices were not characterized; the specific sources of the water matrices were not reported.

I. Principle of the Method

Samples (10 ± 0.01 g or 10 mL) were transferred to culture tubes for analysis (pp. 19-21; Figure 1, p. 25 of MRID 50200402). Samples were fortified, as necessary, with 0.025 mL of 10 or 100 ng/mL of a mixed fortification solution containing cis- and trans-BAS 555 F, mixed well and analyzed directly by LC/MS/MS. Higher fortifications or cases of significant matrix effects may require appropriate dilution with water. The method noted that the analytes in water were stable when held under refrigeration.

Samples were analyzed for analytes using a Waters UPLC System [operated under HPLC conditions (<400 bar)] coupled to an AB Sciex 5500 mass spectrometer (pp. 23-24 of MRID 50200402). The LC/MS conditions consisted of a Chiralpak AD-3R column (2.1 x 150 mm, 3- μ m; column temperature 40°C), a mobile phase of (A) water:formic acid (1000:1, v:v) and (B) methanol:formic acid (1000:1, v:v) [percent A:B (v:v) at 0-14 min. 15:85] and MS/MS detection in ESI positive ion mode (ionization temperature not reported). Injection volume was 100 μ L. Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: *m/z* 320→70 and *m/z* 322→70 for the enantiomers of metconazole. Retention times were *ca.* 7.8, 8.6, 10.6, and 11.5 minutes for M555F000-RS, M555F000-SR, M555F00-RR and M555F000-SS, respectively. The assignment of three of the four peaks in the chromatograms was based on the retention times observed in the mixed calibration standards compared to individual solutions of the M555F000-RS,

M555F000-SR and M555F00-RR reference materials; the fourth peak in the chromatograms was identified as M555F000-SS by the process of elimination (p. 30).

The method noted the following potential problem: the glassware which is used for the method should be thoroughly rinsed with methanol to prevent contamination (p. 31 of MRID 50200402). The method also noted the following comment/recommendation from the ILV: a new analytical column may require extensive conditioning (2 to 6 hours) to sufficiently separate the trans-metconazole isomeric peaks (also reported in the ILV; p. 32 of MRID 50200402; p. 21 of MRID 50200401).

In the ILV, the ECM was performed as written for the enantiomers of metconazole, except for the use of a different LC/MS/MS system (pp. 18-19; Table 1, p. 25; Table 19, p. 43 of MRID 50200401). A mixture of cis- and trans-BAS 555 F was used to prepare the mixed fortification solution. An Agilent 1290 UPLC System coupled to an AB Sciex 5500 mass spectrometer was used. All LC/MS conditions were the same; ionization temperature was reported as 550°C. The same two ion transitions were monitored as in the ECM. Retention times were *ca.* 10.1, 11.3, 13.9, and 14.7 minutes for M555F000-RS, M555F000-SR, M555F00-RR and M555F000-SS, respectively. No other modifications to the ECM were reported. All four enantiomers were present in the calibration solutions; therefore, peak assignment was based on retention time (see Reviewer's Comment #5).

The Limit of Quantification (LOQ) and Limit of Detection (LOD) for the enantiomers of metconazole in water were 25 ng/L (25 ppt) and 5 ng/L (5 ppt), respectively, in the ECM and ILV (pp. 7, 26 of MRID 50200402; pp. 11, 19 of MRID 50200401).

II. Recovery Findings

ECM (MRID 50200402): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD \leq 20%) for analysis of the four enantiomers of metconazole [BAS 555 F; M555F000-RR (Reg.No. 5836048), M555F000-RS (Reg.No. 5836046), M555F000-SR (Reg.No. 4677200), and M555F000-SS (Reg.No. 5836047)] in two water matrices at fortification levels of 25 ng/L (25 ppt; LOQ) and 250 ng/L (250 ppt; 10 \times LOQ; Table 1, pp. 27-28). Metconazole enantiomers, M555F000-RR, M555F000-RS, M555F000-SR, and M555F000-SS, were identified using two ion transitions; performance data (recovery results) from primary and confirmatory analyses were comparable, except for M555F000-SS in surface water at the LOQ which were less comparable. The assignment of three of the four peaks in the chromatograms was based on the retention times observed in the mixed calibration standards compared to individual solutions of the M555F000-RS, M555F000-SR and M555F00-RR reference materials; the fourth peak in the chromatograms was identified as M555F000-SS by the process of elimination (p. 30). The drinking (well) water (20141105-drinking-Well; pH 7.4; 25 mg equiv. CaCO₃/L hardness; 0.7 ppm total organic carbon) and surface (lake/pond) water (20141104-surface-JL; pH 6.9; 32 mg equiv. CaCO₃/L hardness; 6.1 ppm total organic carbon) were used in the study (p. 14; Appendix H, pp. 127-131). The water samples were collected locally and characterized by Agvise Laboratories, Northwood, North Dakota; water sources were not further specified.

ILV (MRID 50200401): Mean recoveries and RSDs were within guideline requirements for analysis of M555F000-RR, M555F000-RS, M555F000-SR, and M555F000-SS in two water matrices at fortification levels of 25 ng/L (25 ppt; LOQ) and 250 ng/L (250 ppt; 10×LOQ), except for the LOQ confirmation ion analysis of M555F000-SR in drinking water (RSD 20.9%; pp. 12-15; Tables 2-17, pp. 26-41). A confirmatory method is not usually required when LC/MS and GC/MS is the primary method. M555F000-RR, M555F000-RS, M555F000-SR, and M555F000-SS were identified using two ion transitions; performance data (recovery results) from primary and confirmatory analyses were comparable, except for M555F000-SR in drinking water at the LOQ which were less comparable. All four enantiomers were present in the calibration solutions; therefore, peak assignment was based on retention time (see Reviewer's Comment #5). The drinking and surface water matrices were provided by the Sponsor (p. 17). The water matrices were not characterized; the specific sources of the water matrices were not reported. The method was validated in the first trial with insignificant modifications to the analytical instrumentation (pp. 11, 18-19, 21-22, Table 19, p. 43).

Table 2. Initial Validation Method Recoveries for the Enantiomers of Metconazole [BAS 555 F; M555F000-RR (Reg.No. 5836048), M555F000-RS (Reg.No. 5836046), M555F000-SR (Reg.No. 4677200), and M555F000-SS (Reg.No. 5836047)] in Water^{1,2,3}

Analyte	Fortification Level (ng/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Drinking (Well) Water						
Quantitation ion						
M555F000-RR	25 (LOQ)	5	89-111	105	9	9
	250	5	99-103	101	2	2
M555F000-RS	25 (LOQ)	5	101-108	105	3	3
	250	5	97-103	100	3	3
M555F000-SR	25 (LOQ)	5	101-106	104	2	2
	250	5	95-101	99	2	2
M555F000-SS	25 (LOQ)	5	106-111	108	2	2
	250	5	97-105	100	4	4
Confirmatory ion						
M555F000-RR	25 (LOQ)	5	107-111	109	2	2
	250	5	99-105	101	2	2
M555F000-RS	25 (LOQ)	5	102-108	104	2	2
	250	5	96-101	98	2	2
M555F000-SR	25 (LOQ)	5	98-108	103	4	4
	250	5	94-102	98	3	3
M555F000-SS	25 (LOQ)	5	105-112	107	3	3
	250	5	96-111	103	6	6
Surface (Lake/Pond) Water						
Quantitation ion						
M555F000-RR	25 (LOQ)	5	94-109	102	10	9
	250	5	95-100	98	2	2
M555F000-RS	25 (LOQ)	5	105-116	108	4	4
	250	5	93-99	96	2	2
M555F000-SR	25 (LOQ)	5	100-112	105	5	4
	250	5	92-100	95	3	3
M555F000-SS	25 (LOQ)	5	102-112	107	4	4
	250	5	94-101	96	3	3
Confirmatory ion						
M555F000-RR	25 (LOQ)	5	104-114	108	4	3
	250	5	93-101	97	3	3
M555F000-RS	25 (LOQ)	5	104-113	108	4	3
	250	5	95-98	96	1	2
M555F000-SR	25 (LOQ)	5	100-109	102	4	4
	250	5	89-95	92	2	3
M555F000-SS	25 (LOQ)	5	87-118	101	13	12
	250	5	90-99	94	4	4

Data (uncorrected recovery results, pp. 24-25; Appendix B, p. 42) were obtained from Table 1, pp. 27-28 of MRID 50200402.

1 M555F000-RR = (1R,5R)-5-(4-Chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol;
M555F000-RS = (1R,5S)-5-(4-Chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol;
M555F000-SR = (1S,5R)-5-(4-Chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol; and
M555F000-SS = (1S,5S)-5-(4-Chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol.

- 2 The drinking (well) water (20141105-drinking-Well; pH 7.4; 25 mg equiv. CaCO₃/L hardness; 0.7 ppm total organic carbon) and surface (lake/pond) water (20141104-surface-JL; pH 6.9; 32 mg equiv. CaCO₃/L hardness; 6.1 ppm total organic carbon) were used in the study (p. 14; Appendix H, pp. 127-131). The water samples were collected locally and characterized by Agvise Laboratories, Northwood, North Dakota; water sources were not further specified.
- 3 Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: m/z 320→70 and m/z 322→70 for the enantiomers of metconazole. Retention times were *ca.* 7.8, 8.6, 10.6, and 11.5 minutes for M555F000-RS, M555F000-SR, M555F00-RR and M555F000-SS, respectively. The assignment of three of the four peaks in the chromatograms was based on the retention times observed in the mixed calibration standards compared to individual solutions of the M555F000-RS, M555F000-SR and M555F00-RR reference materials; the fourth peak in the chromatograms was identified as M555F000-SS by the process of elimination (p. 30).

Table 3. Independent Validation Method Recoveries for the Enantiomers of Metconazole [BAS 555 F; M555F000-RR (Reg.No. 5836048), M555F000-RS (Reg.No. 5836046), M555F000-SR (Reg.No. 4677200), and M555F000-SS (Reg.No. 5836047)] in Water^{1,2,3}

Analyte	Fortification Level (ng/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Drinking (Well) Water						
Quantitation ion						
M555F000-RR	25 (LOQ)	5	73-90	77	7.0	9.0
	250	5	69-80	75	4.2	5.6
M555F000-RS	25 (LOQ)	5	74-86	80	5.3	6.6
	250	5	72-83	77	4.7	6.1
M555F000-SR	25 (LOQ)	5	78-92	84	6.0	7.1
	250	5	71-84	76	5.4	7.1
M555F000-SS	25 (LOQ)	5	70-81	74	5.4	7.3
	250	5	72-80	75	3.5	4.6
Confirmatory ion						
M555F000-RR	25 (LOQ)	5	77-91	82	5.7	7.0
	250	5	72-81	78	3.3	4.2
M555F000-RS	25 (LOQ)	5	71-85	78	5.8	7.4
	250	5	73-82	79	3.6	4.6
M555F000-SR	25 (LOQ)	5	71-114	85	17.8	20.9
	250	5	69-79	75	4.3	5.7
M555F000-SS	25 (LOQ)	5	70-83	74	5.5	7.5
	250	5	72-86	79	5.3	6.8
Surface (Lake/Pond) Water						
Quantitation ion						
M555F000-RR	25 (LOQ)	5	80-97	87	6.3	7.2
	250	5	81-89	86	3.1	3.5
M555F000-RS	25 (LOQ)	5	82-94	87	4.3	4.9
	250	5	82-90	85	3.4	4.0
M555F000-SR	25 (LOQ)	5	83-95	87	4.8	5.5
	250	5	82-95	89	4.9	5.5
M555F000-SS	25 (LOQ)	5	83-90	88	3.1	3.5
	250	5	83-90	87	3.1	3.6
Confirmatory ion						
M555F000-RR	25 (LOQ)	5	84-94	90	4.3	4.8
	250	5	81-86	84	2.3	2.7
M555F000-RS	25 (LOQ)	5	83-91	88	3.1	3.6
	250	5	83-93	87	4.3	5.0
M555F000-SR	25 (LOQ)	5	85-99	92	4.9	5.4
	250	5	84-90	88	2.4	2.8
M555F000-SS	25 (LOQ)	5	81-97	88	5.9	6.7
	250	5	83-95	86	4.7	5.5

Data (uncorrected recovery results, pp. 19-20; Appendix B, pp. 116-147) were obtained from pp. 12-15; Tables 2-17, pp. 26-41 of MRID 50200401.

1 M555F000-RR = (1R,5R)-5-(4-Chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol;
M555F000-RS = (1R,5S)-5-(4-Chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol;
M555F000-SR = (1S,5R)-5-(4-Chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol; and
M555F000-SS = (1S,5S)-5-(4-Chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol.

- 2 The drinking and surface water were provided by the Sponsor (p. 17). The water matrices were not characterized; the specific sources of the water matrices were not reported.
- 3 Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: m/z 320→70 and m/z 322→70 for the enantiomers of metconazole. Retention times were *ca.* 10.1, 11.3, 13.9, and 14.7 minutes for M555F000-RS, M555F000-SR, M555F00-RR and M555F000-SS, respectively. All four enantiomers were present in the calibration solutions; therefore, peak assignment was based on retention time (see Reviewer's Comment #5).

III. Method Characteristics

The LOQ and LOD for the enantiomers of metconazole in water were 25 ng/L (25 ppt) and 5 ng/L (5 ppt), respectively, in the ECM and ILV (pp. 7, 26, 31, 33 of MRID 50200402; pp. 11, 19 of MRID 50200401). In the ECM and ILV, the LOQ was defined as the lowest fortification level successfully tested. The LOD was set at 20% of the LOQ in the ECM and ILV. In the ECM, the LOD was justified by the fact that the lowest standard for each analyte in the calibration curve (5 ng/L) had a signal-to-noise ratio > 3:1. No calculations or comparisons to background levels were reported to justify the LOQ for the method in the ECM and ILV; no calculations were reported to justify the LOD for the method in the ECM and ILV.

Table 4. Method Characteristics

Analyte ¹		M555F000-RR (Reg.No. 5836048)	M555F000-RS (Reg.No. 5836046)	M555F000-SR (Reg.No. 4677200)	M555F000-SS (Reg.No. 5836047)
Limit of Quantitation (LOQ)	ECM	25 ng/L (25 ppt)			
	ILV				
Limit of Detection (LOD)	ECM	5 ng/L (5 ppt)			
	ILV				
Linearity (calibration curve r^2 and concentration range) ²	ECM	$r^2 = 0.9991$ (Q) $r^2 = 0.9984$ (C)	$r^2 = 0.9986$ (Q) $r^2 = 0.9982$ (C)	$r^2 = 0.9986$ (Q) $r^2 = 0.9976$ (C)	$r^2 = 0.9984$ (Q) $r^2 = 0.9982$ (C)
	ILV	$r^2 = 0.9959$ (Q) $r^2 = 0.9946$ (C)	$r^2 = 0.9960$ (Q) $r^2 = 0.9972$ (C)	$r^2 = 0.9966$ (Q) $r^2 = 0.9967$ (C)	$r^2 = 0.9951$ (Q) $r^2 = 0.9988$ (C)
	Concentration Range	0.005-0.1 (ng/mL)			
Repeatable	ECM ³	Yes at LOQ and 10×LOQ (characterized drinking and surface water matrices)			
	ILV ^{4,5}	Yes at LOQ and 10×LOQ (uncharacterized drinking and surface water matrices)	Yes at LOQ and 10×LOQ (uncharacterized drinking and surface water matrices), except for LOQ C ion analysis in drinking water (RSD 20.9%) ⁶	Yes at LOQ and 10×LOQ (uncharacterized drinking and surface water matrices)	
Reproducible	Yes at LOQ and 10×LOQ				
Specific	ECM	No matrix interferences were observed or quantified; however, the analyte peak pairs of M555F000-RS and M555F000-SR and M555F00-RR and M555F000-SS overlapped at the bottom which interfered with peak integration of each analyte. Overlaps were <i>ca.</i> 10% for M555F000-RS and M555F000-SR and <i>ca.</i> 20% for M555F00-RR and M555F000-SS.			
	ILV	No matrix interferences were observed or quantified; however, the analyte peak pairs of M555F000-RS and M555F000-SR and M555F00-RR and M555F000-SS overlapped at the bottom which interfered with peak integration of each analyte. Overlaps were <i>ca.</i> 5-10% for M555F000-RS and M555F000-SR and <i>ca.</i> 50-70% for M555F00-RR and M555F000-SS.			

Data were obtained from pp. 31, 33; Table 1, pp. 27-28 (recovery data); Appendix E, pp. 66-69 (calibration curves); Appendix F, pp. 96-123 (chromatograms) of MRID 50200402; pp. pp. 12-15; Tables 2-17, pp. 26-41 (recovery data); Figure 1, pp. 45-52 (calibration curves); Figures 6-12, pp. 83-110 (chromatograms) of MRID 50200401. Q = Quantitation ion transition; C = Confirmation ion transition.

1 M555F000-RR = (1R,5R)-5-(4-Chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol; M555F000-RS = (1R,5S)-5-(4-Chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol; M555F000-SR = (1S,5R)-5-(4-Chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol; and M555F000-SS = (1S,5S)-5-(4-Chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol.

2 Correlation coefficients (r^2) values were reviewer-calculated from r values provided in the study report (Appendix E, pp. 66-69 of MRID 50200402; Figure 1, pp. 45-52 of MRID 50200401; DER Attachment 2). Solvent-based calibration standards were used.

3 In the ECM, drinking (well) water (20141105-drinking-Well; pH 7.4; 25 mg equiv. CaCO₃/L hardness; 0.7 ppm total organic carbon) and surface (lake/pond) water (20141104-surface-JL; pH 6.9; 32 mg equiv. CaCO₃/L hardness; 6.1 ppm total organic carbon) were used in the study (p. 14; Appendix H, pp. 127-131 of MRID 50200402). The water

- samples were collected locally and characterized by Agvise Laboratories, Northwood, North Dakota; water sources were not further specified.
- 4 In the ILV, the drinking and surface water matrices were provided by the Sponsor (p. 17 of MRID 50200401). The water matrices were not characterized; the specific sources of the water matrices were not reported.
 - 5 The method was validated by the ILV in the first trial with insignificant modifications to the analytical instrumentation (pp. 11, 18-19, 21-22, Table 19, p. 43 of MRID 50200401).
 - 6 A confirmatory method is not usually required when LC/MS and GC/MS is the primary method. Linearity is satisfactory when $r^2 \geq 0.995$.

IV. Method Deficiencies and Reviewer's Comments

1. The specificity of the method was not supported by the ECM and ILV representative chromatograms because the analyte peak pairs of M555F000-RS and M555F000-SR and M555F00-RR and M555F000-SS overlapped at the bottom which interfered with peak integration of each analyte (Appendix F, pp. 96-123 of MRID 50200402; Figures 6-12, pp. 83-110 of MRID 50200401). For M555F000-RS and M555F000-SR, peak overlaps were *ca.* 5-10% in the ECM and ILV. For M555F00-RR and M555F000-SS, peak overlaps were *ca.* 20% in the ECM and *ca.* 50-70% in the ILV. Peak integrations for all analytes in the overlapping areas were performed by bisecting in the middle; however, this method produced an inaccurate peak integration since the peaks were curved on both sides (although more curved on the tail side). The inevitable omission of a portion of the peak, especially for the first peak of each pair for which the peak tail was truncated, was amplified with larger peak overlaps, such as those observed in the ILV.

No discussion of the peak overlap or LC/MS/MS optimization was found in the ECM or ILV. Additionally, the mobile phase was isometric. Therefore, the reviewer could not determine if the peak separation of the enantiomers was optimized in the method.

2. A reference compound was not included for the analyte M555F000-SS in the ECM (pp. 15-16, 30 of MRID 50200402). Additionally, no identification with chemical name or structure was provided, including the assigned BASF Reg.No. The reviewer assigned M55F000-SS as Reg.No. 5836047 based on process of elimination with the method title and the isomers listed in the M55F000-RR Certificate of Analysis (pp. 1, 38). However, the reviewer noted that the only assignment of the Reg.No. to M555F00-SS was either an apparent typographical error or a reference to the fact that the M555F000-RR reference standard was used to assign the M555F000-SS peak in the chromatograms since it was Reg.No. 5836048 (p. 23).
3. The water matrices were not characterized in the ILV. The ILV reported that the drinking and surface water matrices were provided by the Sponsor, but the specific sources of the water matrices were not reported (p. 17 of MRID 50200401). It could not be determined if the ILV was provided with the most difficult matrices with which to validate the method. The reviewer suspects that the ILV water matrices were most likely the same as those used in the ECM, but no supporting evidence was provided.
4. The confirmation ion analysis in the ILV was insufficient for the linearity of M555F000-RR ($r^2 = 0.9946$) and the performance data of M555F000-SR in drinking water (RSD 20.9%; pp. 12-15; Tables 2-17, pp. 26-41; Figures 1-8, pp. 66-73 of MRID 50200401). Linearity is

satisfactory when $r^2 \geq 0.995$; OCSPP Guideline 850.6100 criteria for precision and accuracy is mean recoveries for replicates at each spiking level between 70% and 120% and relative standard deviations (RSD) $\leq 20\%$ at the stated LOQ and at higher concentrations. However, the reviewer noted that a confirmatory method is not typically required where GC/MS and/or LC/MS methods are used as the primary method(s) to generate study data. Therefore, the deficiencies of the LOQ confirmation ion analysis of these enantiomers of metconazole did not affect the acceptability of the method.

5. In the ECM, analyte identification in the LC/MS/MS chromatograms was performed by comparison of the retention times of three of the four peaks observed in the mixed calibration standards compared to individual solutions of the M555F000-RS, M555F000-SR and M555F00-RR reference materials; the fourth peak in the chromatograms was identified as M555F000-SS by the process of elimination (pp. 15-16, 30 of MRID 50200402). In the ILV, analyte identification in the LC/MS/MS chromatograms was not reported; however, the reviewer suspects that the assignment was based on the ECM assignment since only cis- and trans-metconazole were included as test materials (pp. 16-17 of MRID 50200401).
6. Although it was not clearly stated in the ECM, the reviewer suspects that the mixed fortification solutions were prepared using cis- and trans-metconazole (pp. 15-16, 18-21 of MRID 50200402).
7. The reviewer noted the following significant typographical error in ILV: 100×LOQ was reported instead of 10×LOQ in the high fortification chromatogram titles (Figure 9, pp. 95-98; Figure 12, pp. 107-110 of MRID 50200401).
8. In the ECM and ILV, the LOQ was defined as the lowest fortification level successfully tested. The LOD was set at 20% of the LOQ in the ECM and ILV. In the ECM, the LOD was justified by the fact that the lowest standard for each analyte in the calibration curve (5 ng/L) had a signal-to-noise ratio $> 3:1$. No calculations or comparisons to background levels were reported to justify the LOQ for the method in the ECM and ILV; no calculations were reported to justify the LOD for the method in the ECM and ILV. The reported limit of quantitation (LOQ) was determined as the lowest level of method validation (LLMV). Further work could have been done to explore the actual LOQ. This means that concentrations can be reliably quantified at the LOQ (*i.e.*, LLMV), but whether lower concentrations may also be reliably quantified is uncertain.
9. In the ECM, the storage stability of the standard solution of metconazole (all enantiomers) in DI water was determined to be 31 days under refrigeration ($< 5^\circ\text{C}$; p. 29; Appendix D, pp. 61-64 of MRID 50200402). In the drinking and surface water matrices, metconazole (all enantiomers) was determined to be stable for 7 days under refrigeration ($< 5^\circ\text{C}$). Stability was determined as acceptable recoveries (70-120%), not percent agreement with the original recovery.
10. The matrix effects were found to be insignificant for the enantiomers of metconazole in the ECM since procedural recoveries of the LOQ samples were acceptable and comparable to those of the 10×LOQ samples (pp. 29-30 of MRID 50200402).

11. The ILV study author reported that no communications between the ILV and ECM or study sponsor occurred other than to inform the Study Monitor of the successful completion of the ILV first trial (p. 21 of MRID 50200401).
12. It was reported for the ILV that one sample set of 13 samples required *ca.* 3 hours, not including LC/MS/MS analysis time (p. 21 of MRID 50200401). The reported time requirement was 2 hours for a set of 13 samples in the ECM (p. 32 of MRID 50200402).

V. References

- U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.
- U.S. Environmental Protection Agency. 2015 Metconazole: Preliminary Problem Formulation for Environmental Fate, Ecological Risk, Endangered Species, and Drinking Water Exposure Assessments in Support of Registration Review. Office of Pesticide Programs, Environmental Fate and Effects Division. DP 428303.
- 40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

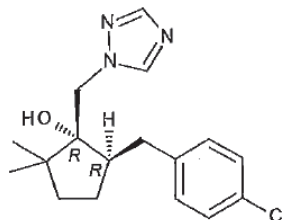
Attachment 1: Chemical Names and Structures**M55F000-RR (Reg. No. 5836048)**

IUPAC Name: (1R,5R)-5-(4-Chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol

CAS Name: Not reported

CAS Number: 115850-28-7

SMILES String: Not found

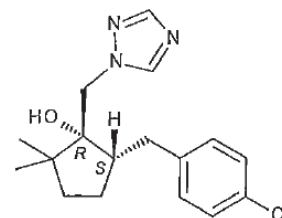
**M55F000-RS (Reg. No. 5836046)**

IUPAC Name: (1R,5S)-5-(4-Chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol

CAS Name: Not reported

CAS Number: 127307-20-4

SMILES String: Not found

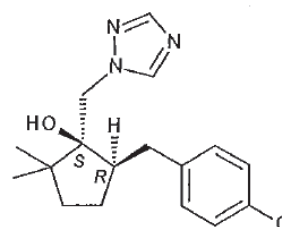
**M55F000-SR (Reg. No. 4677200)**

IUPAC Name: (1S,5R)-5-(4-Chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol

CAS Name: Not reported

CAS Number: 115850-27-6

SMILES String: Not found



M55F000-SS (Reg. No. 5836047)

IUPAC Name: (1S,5S)-5-(4-Chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol

CAS Name: Not reported

CAS Number: Not reported

SMILES String: Not found

(Structure not reported)