Analytical method for ipconazole in saltwater

Reports:	ECM: EPA MRID No. 50771001. Wang, J. 2018. Method Validation for Analysis of Ipconazole in Salt Water. Study No.: 85887. Kureha Reference No.: 317_2017/012. Report prepared by Analytical Bio-Chemistry Laboratories, Inc. (a wholly owned subsidiary of EAG, Inc.), Columbia, Missouri, and sponsored and submitted by Kureha Corporation, Tokyo, Japan, and Kureha America Inc., Houston, Texas; 60 pages. Final report dated June 13, 2018; Revision No. 1 dated June 21, 2018. ILV: EPA MRID No. 50771002. MacGregor, J.A., and R.L. Van Hoven. 2018. Independent Laboratory Validation of a Method for the Determination of Ipconazole in Aqueous Matrices by LC-MS/MS. Project No.: 556K-101. Kureha Reference No.: 317_2018/001. Report prepared by EAG Inc., Easton, Maryland, and sponsored and submitted by Kureha Corporation, Tokyo, Japan, and Kureha America Inc., Houston, Texas; 124 pages. Final report issued December 14, 2018.
Document No.:	MRIDs 50771001 & 50771002
Guideline:	850.6100
Statements:	ECM: The study was not conducted in accordance with USEPA Good Laboratory Practices (GLP; 40 CFR Part 160; 1989), with the exceptions that the water characterization was non-GLP and the employed evaporator and applied vacuum were not recorded (p. 3 of MRID 50771001). The report noted that US EPA GLP were compatible with OECD GLP (1997). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). A statement of the authenticity of the study report was included with the Quality Assurance statement. ILV: The study was conducted in accordance with USEPA and OECD (1998) GLP standards (p. 3 of MRID 50771002). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). A statement of the authenticity of the study report was not included.
Classification:	This analytical method is classified as supplemental pending submission of a revised ECM that addresses the changes advised in the ILV and the additional matrices. Since the reported method LOQ were not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest levels of method validation (LLMV) rather than LOQ. The ILV was not performed independently of the ECM. The ILV validated the method in saltwater, as well as surface, ground, and drinking water matrices; however, the ECM only validated the method in saltwater.
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EFED Final Reviewer:	Zoe Ruge, M.S., Physical Scientist Signature: Zoe Ruge Date: June 30, 2020
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This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac Joint Venture personnel. The CDM/CSS-Dynamac JV role does not include establishing Agency policies.

Executive Summary

This analytical method, Analytical Bio-Chemistry Laboratories Study No. 85887, is designed for the quantitative determination of total ipconazole (sum of ipconazole cc and ipconazole ct isomers) in saltwater at the LOQ of 0.05 µg/L, using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in saltwater (1.03 µg/L chronic NOAEC for Sheepshead minnow). Based on the performance data submitted by the ILV and ECM, the LLMV was equivalent to the reported method LOQ for ipconazole in saltwater. The ILV validated the method in characterized saltwater, as well as characterized surface, ground, and drinking water matrices; however, the ECM only validated the method in partially characterized saltwater. The ECM considered the saltwater matrix validation to be applicable to all types of water since saltwater is a more complex matrix.

The method was validated by the ILV with the first trial for the surface, ground, and drinking water matrices and with the second trial for the saltwater matrix. The method was validated as written with insignificant modifications of analytical instrumentation and parameters; however, the ILV noted a critical step of regulating the SPE flow rate to *ca*. 1 drop/second or less. The SPE flow rate was only specified as gravity in the ECM, and the failure of the first ILV trial for saltwater (low recoveries) was presumed to be due to an inappropriate interpretation of the specified SPE flow rate during analyte elution. An updated ECM with a more precisely (nominally) defined SPE elution flow rate was advised by the ILV and required since it was necessary for the successful validation of the method for saltwater. The ILV validations in surface, ground, and drinking water matrices were performed before the ILV validation in saltwater and were performed without difficulty due to the SPE extraction step; however, ILV study author noted that close regulation of the SPE flow rate during potential repeats of the validations in ground and drinking water matrices may have yielded higher recoveries (some recoveries were <70% even though overall acceptable results were obtained).

The ILV was not performed independently of the ECM since technical communication occurred between the ECM and ILV (via the Kureha Corporation Study Monitor), the communicated technical communication was necessary for the successful validation of the method for salt water, and the ILV required multiple method clarifications of essential details from the ECM.

All ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for ipconazole in tested water matrices. The two isomers of ipconazole, ipconazole cc and ipconazole ct, were quantified separately, then the results for each isomer were

summed to quantify total ipconazole.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Ipconazole[1]	50771001[2]	50771002[4]		Saltwater	13/06/2018 (Final report) 21/06/2018 (Rev. No. 1)	Kureha America, Inc.	LC/MS/MS	0.05 µg/L
	None submitted[3]			Drinking, Surface and Ground Water	14/12/2018 (ILV)[5]			

1 Ipconazole was a mixture of cis/cis ipconazole (ipconazole cc) and cis/trans ipconazole (ipconazole ct). Ipconazole cc: (l*RS*,2*SR*,5*RS*)-2-(4-chlorobenzyl)-5-isopropyl-l-(1*H*-1,2,4-triazol-1-ylmethyl) cyclopentanol; and ipconazole ct: (l*RS*,2*SR*,5*SR*)-2-(4-chlorobenzyl)-5-isopropyl-l-(1*H*-1,2,4-triazol-l-ylmethyl) cyclopentanol.

2 In the ECM, the saltwater (pH 8.10; target salinity 20 ± 3%) was prepared by mixing commercial sea salt mix (Crystal Sea Marinemix from Marine Enterprises International, Inc., Baltimore, Maryland) with laboratory freshwater (on-site well water blended with well water that was demineralized by reverse osmosis to yield water with total hardness 130-160 mg $CaCO_3$/L; p. 12; Appendix B, pp. 41-43 of MRID 50771001). The non-GLP water characterization provided by the performing laboratory quantified levels of metals and pesticides.

3 The ECM only validated the method in saltwater; the ILV validated the method in saltwater, as well as surface, ground, and drinking water matrices (p. 12; Appendix B, pp. 41-43 of MRID 50771001; pp. 13-14; Appendices III-VI, pp. 105-108 of MRID 50771002). According to the ILV communication log, the ECM considered "saltwater…[to be]…"a more complex matrix and could be used as an enforcement method for all types of water" (September 13, 2018 email; Appendix X, p. 120 of MRID 50771002).

4 In the ILV, the saltwater (ID Code: SLW-IR-090718; salinity *ca.* 20%, pH 8.0, hardness 4875 mg equivalent $CaCO_3$/L, conductivity 30.30 mmhos/cm) was natural seawater collected by EAG Laboratories-Easton from the Indian River in Delaware (pp. 13-14; Appendices III-VI, pp. 105-108 of MRID 50771002). The surface water (ID Code: SFW-TL-080718; pH 7.5, hardness 61 mg equivalent $CaCO_3$/L, conductivity 0.17 mmhos/cm) was collected by EAG Laboratories-Easton from Tuckahoe Lake in Tuckahoe State Park in Ridgely, Maryland. The ground water (ID Code: GRW-WL-080718; pH 8.2, hardness 153 mg equivalent $CaCO_3$/L, conductivity 0.34 mmhos/cm) was collected from a collected from a well at EAG Laboratories-Easton testing facility. The drinking water (ID Code: DRW-TP-080718; pH 8.3, hardness 27 mg equivalent $CaCO_3$/L, conductivity 0.25 mmhos/cm) was collected from a tap at EAG Laboratories-Easton testing facility. The water characterization performed by Agvise Laboratories (Northwood, North Dakota).

5 The ILV method date was reported for the method date of surface, ground, and drinking water matrices since these matrices were not included in the ECM.

I. Principle of the Method

Water samples (20 mL) were transferred into 20-mL culture tubes and fortified, as necessary, via exchanging the fortification volume with an equal volume of the sample (pp. 12-13; Appendix C, pp. 49-50 of MRID 50771001). The samples were mixed with 1 mL with acetonitrile, then applied to a Waters Sep-pak C_{18} solid phase extraction (SPE) cartridge (6 cc, 1.0 g; pre-conditioned with 5 mL of acetonitrile then 5 mL of HPLC water). The sample flask was rinsed with 5 mL of HPLC water, and the rinse was applied to the SPE cartridge. After residual water was removed from the SPE cartridge via airflow, the analytes were eluted via gravity (flow rate

not specified) with 8 mL of acetonitrile. A 20-µL volume of octan-1-ol was added to the eluate, and the sample was reduced to near dryness under a steady stream of nitrogen at *ca*. 45°C in an evaporator. After reconstitution with 1 mL of methanol via vortex-mixing and ultra-sonication, the sample was diluted to 2 mL using HPLC water and analyzed via LC/MS/MS.

Samples were analyzed using a Waters Acquity UPLC coupled to an AB Sciex API 5500 Q Trap MS (pp. 12-14 of MRID 50771001). The following LC conditions were used: Phenomenex Biphenyl (100 mm x 4.6 mm column; 2.6 µm particle size; column temperature 40°C), mobile phase of (A) 0.1mM formic acid + 0.1mM aqueous ammonium formate and (B) methanol [percent A:B (v:v) at 0.00-1.00 min. 30:70, 8.00 min. 25:75, 8.01-10.0 min. 5:95, 10.1-12.5 min. 30:70], and injection volume of 2.00 or 5.00 µL (ipconazole cc) and 10.0 or 20.0 µL (ipconazole ct). The following MS/MS conditions were used: positive mode (source temperature 500°C), turboion spray ionization interface, and multiple reaction monitoring (MRM). Two ion pair transitions were monitored for each isomer of ipconazole (quantitation and confirmation, respectively): *m/z* 334.100→70.100 and *m/z* 334.100→125.000 for ipconazole cc and *m/z* 334.101→70.100 and *m/z* 334.101→125.000 for ipconazole ct. Retention times were *ca*. 5.46 minutes for ipconazole cc and *ca*. 5.86 minutes for ipconazole ct (Figure 4, p. 33).

The independent laboratory performed the ECM as written, except for insignificant modifications of analytical instrumentation and parameters (pp. 16-18; Table 1, p. 26; Appendix X, p. 118 of MRID 50771002). The same SPE cartridge was used; however, the ILV noted a critical step of regulating the SPE flow rate to *ca*. 1 drop/second or less (flow rate only specified as gravity in the ECM). An AB Sciex API 5000 MS coupled with an Agilent 1200 Infinity series LC was used. The following LC conditions were used: Phenomenex Biphenyl (100 mm x4.6 mm column; 2.6 µm particle size; column temperature 40°C), mobile phase of (A) 0.1mM formic acid + 0.1mM aqueous ammonium formate and (B) methanol [percent A:B (v:v) at 0.00-1.00 min. 25.0:75.0, 8.00 min. 20.0:80.0, 8.01-10.0 min. 5.00:95.0, 10.10-12.50 min. 25.0:75.0], and injection volume of 10.0 µL (ipconazole cc) or 25.0 µL (ipconazole ct). All MS parameters were generally the same as the ECM. The same two ion pair transitions were monitored for each isomer of ipconazole (quantitation and confirmation, respectively): *m/z* 334.1→70.1 and *m/z* 334.1→125; these monitored ions were similar to those monitored in the ECM. Retention times were *ca*. 5.1 minutes for ipconazole cc and *ca*. 5.5 minutes for ipconazole ct.

The method Limit of Quantification (LOQ) for ipconazole was reported as 0.05 µg/L in water in the ECM and ILV (pp. 15-19; Tables 1-2, pp. 24-25 of MRID 50771001; pp. 12, 18, 21 of MRID 50771002). The method Limit of Detection (LOD) for ipconazole in water was reported as the Minimum Quantifiable Limit (MQL) in the ECM or ILV, which equated to 0.01 µg/L for ipconazole cc and 0.001 µg/L for ipconazole ct in the ECM and 0.005 µg/L for ipconazole cc and 0.001 µg/L for ipconazole ct in the ILV (the ILV MQLs were reviewer-calculated). The Method Detection Limit (MDL) and Practical Quantitation Limit (PQL) were calculated for each isomer of ipconazole in the ECM and ILV. In the ECM, the MDL and PQL for ipconazole cc in saltwater were calculated as 0.0132 and 0.0660 µg/L, respectively, for the quantitation ion transition and 0.0306 and 0.153 µg/L, respectively, for the confirmation ion transition. The MDL and PQL for ipconazole ct in saltwater were calculated as 0.00216 and 0.0108 µg/L, respectively, for the quantitation ion transition and 0.00240 and 0.0120 µg/L, respectively, for the confirmation ion transition. In the ILV, the MDL and PQL for ipconazole cc in water were

calculated as 0.00400 and 0.0200 µg/L, respectively, for the quantitation ion transition and 0.00461 and 0.0231 µg/L, respectively, for the confirmation ion transition. The MDL and PQL for ipconazole ct in water were calculated as 0.00161 and 0.00807 µg/L, respectively, for the quantitation ion transition and 0.00110 and 0.00552 µg/L, respectively, for the confirmation ion transition. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

II. Recovery Findings

ECM (MRID 50771001): Mean recoveries and relative standard deviations (RSDs) met requirements (mean 70-120%; RSD ≤20%) for analysis of ipconazole in one prepared saltwater matrix at the LOQ (0.05 µg/L) and 10×LOQ (0.50 µg/L; Table 3, p. 26). Results were based on total ipconazole, ipconazole cc summed with ipconazole ct. Two ion pair transitions were monitored, one quantitation and one confirmation; quantitation and confirmation recovery results were comparable. The saltwater (pH 8.10; target salinity 20 ± 3%) was prepared by mixing commercial sea salt mix (Crystal Sea Marinemix from Marine Enterprises International, Inc., Baltimore, Maryland) with laboratory freshwater (on-site well water blended with well water that was demineralized by reverse osmosis to yield water with total hardness 130-160 mg $CaCO_3$/L; p. 12; Appendix B, pp. 41-43 of MRID 50771001). The non-GLP water characterization provided by the performing laboratory quantified levels of metals and pesticides.

ILV (MRID 50771002): Mean recoveries and RSDs met requirements for analysis of ipconazole in four water matrices (surface, ground, and drinking water and natural saltwater) at the LOQ (0.05 µg/L) and 10×LOQ (0.50 µg/L; Tables 2-9, pp. 27-34). Results were based on total ipconazole, ipconazole cc summed with ipconazole ct. Two ion pair transitions were monitored, one quantitation and one confirmation; quantitation and confirmation recovery results were comparable. The saltwater (ID Code: SLW-IR-090718; salinity *ca.* 20%, pH 8.0, hardness 4875 mg equivalent $CaCO_3$/L, conductivity 30.30 mmhos/cm) was natural seawater collected by EAG Laboratories-Easton from the Indian River in Delaware (pp. 13-14; Appendices III-VI, pp. 105-108). The surface water (ID Code: SFW-TL-080718; pH 7.5, hardness 61 mg equivalent $CaCO_3$/L, conductivity 0.17 mmhos/cm) was collected by EAG Laboratories-Easton from Tuckahoe Lake in Tuckahoe State Park in Ridgely, Maryland. The ground water (ID Code: GRW-WL-080718; pH 8.2, hardness 153 mg equivalent $CaCO_3$/L, conductivity 0.34 mmhos/cm) was collected from a collected from a well at EAG Laboratories-Easton testing facility. The drinking water (ID Code: DRW-TP-080718; pH 8.3, hardness 27 mg equivalent $CaCO_3$/L, conductivity 0.25 mmhos/cm) was collected from a tap at EAG Laboratories-Easton testing facility. The water characterization was performed by Agvise Laboratories (Northwood, North Dakota). The method was validated by the ILV with the first trial for the surface, ground, and drinking water matrices and with the second trial for the saltwater matrix (pp. 16-18; Table 1, p. 26; Appendix X, pp. 117-118). The method was validated as written with insignificant modifications of analytical instrumentation and parameters; however, the ILV noted a critical step of regulating the SPE flow rate to *ca*. 1 drop/second or less. The SPE flow rate was only specified as gravity in the ECM, and the failure of the first ILV trial for saltwater (low recoveries) was presumed to be due to an inappropriate interpretation of "gravity" as the

specified SPE flow rate during analyte elution. An updated ECM with a more precisely (nominally) defined SPE elution flow rate was advised by the ILV since "regulation of the SPE flow rate will have a direct correlation to the magnitude of analyte recovery" (Appendix X, p. 118). Additionally, this update to the ECM was necessary for the successful validation of the method for saltwater; the ILV validations in surface, ground, and drinking water matrices were performed before the ILV validation in saltwater and were performed without difficulty due to the SPE extraction step.

Table 2. Initial Validation Method Recoveries for Ipconazole in Water[1,2,3]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[3]	Relative Standard Deviation (%)
Saltwater						
Quantitation ion transition						
Ipconazole	0.050 (LOQ)	5[4]	73-98	91	10.4	11.5
	0.50	5[5]	96-99	98	1	1.0
Confirmation ion transition						
Ipconazole	0.050 (LOQ)	5[4]	70-94	87	9.9	11.4
	0.50	5	96-98	97	1	1.0

Data (uncorrected recovery results; pp. 15-17) were obtained from Table 3, p. 26 of MRID 50771001. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

1 Ipconazole was a mixture of cis/cis ipconazole (ipconazole cc) and cis/trans ipconazole (ipconazole ct). Ipconazole cc: (l*RS*,2*SR*,5*RS*)-2-(4-chlorobenzyl)-5-isopropyl-l- (1*H*-1,2,4-triazol-1-ylmethyl) cyclopentanol; and ipconazole ct: (l*RS*,2*SR*,5*SR*)-2-(4-chlorobenzyl)-5-isopropyl-l-(1*H*-1,2,4-triazol-l-ylmethyl) cyclopentanol.

2 Two ion pair transitions were monitored for each isomer of ipconazole (quantitation and confirmation, respectively): *m/z* 334.100→70.100 and *m/z* 334.100→125.000 for ipconazole cc and *m/z* 334.101→70.100 and *m/z* 334.101→125.000 for ipconazole ct. The results for each isomer were summed to quantify total ipconazole (p. 17).

3 The saltwater (pH 8.10; target salinity 20 ± 3%) was prepared by mixing commercial sea salt mix (Crystal Sea Marinemix from Marine Enterprises International, Inc., Baltimore, Maryland) with laboratory freshwater (on-site well water blended with well water that was demineralized by reverse osmosis to yield water with total hardness 130-160 mg $CaCO_3$/L; p. 12; Appendix B, pp. 41-43). The non-GLP water characterization provided by the performing laboratory quantified levels of metals and pesticides.

4 Recovery results quantified as 70% and 73% were the original results, but the sample was re-diluted in duplicate and re-analyzed to confirm the original results.

5 Recovery results quantified for two of the five replicates (98% and 99% recoveries) were the result of the average of the duplicate re-analyses of the sample; the original results were not reported.

Table 3. Independent Validation Method Recoveries for Ipconazole in Water[1,2]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Saltwater						
Quantitation ion transition						
Ipconazole	0.050 (LOQ)	5	96.6-113	104	5.98	5.74
	0.50	5	79.6-98.9	88.4	6.85	7.76
Confirmation ion transition						
Ipconazole	0.050 (LOQ)	5	94.4-109	100	5.55	5.53
	0.50	5	80.3-95.5	88.1	5.97	6.77
Surface Water						
Quantitation ion transition						
Ipconazole	0.050 (LOQ)	5	79.5-96.9	89.3	6.86	7.69
	0.50	5	79.4-92.0	85.8	5.93	6.91
Confirmation ion transition						
Ipconazole	0.050 (LOQ)	5	78.5-92.0	83.3	5.21	6.26
	0.50	5	80.3-98.0	88.4	6.70	7.57
Ground Water						
Quantitation ion transition						
Ipconazole	0.050 (LOQ)	5	66.0-78.5	72.3	5.78	7.99
	0.50	5	68.9-99.2	78.9	12.4	15.8
Confirmation ion transition						
Ipconazole	0.050 (LOQ)	5	65.3-85.1	73.5	7.84	10.7
	0.50	5	70.3-91.3	80.7	9.75	12.1
Drinking Water						
Quantitation ion transition						
Ipconazole	0.050 (LOQ)	5	68.9-80.1	74.0	4.56	6.16
	0.50	5	71.5-86.2	78.8	6.23	7.91
Confirmation ion transition						
Ipconazole	0.050 (LOQ)	5	68.0-87.4	75.9	7.33	9.66
	0.50	5	73.9-87.6	80.4	4.91	6.10

Data (uncorrected recovery results, pp. 18-20 and Tables 2-9, pp. 27-34) were obtained from Tables 2-9, pp. 27-34 of MRID 50771002. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

1 Ipconazole was a mixture of cis/cis ipconazole (ipconazole cc) and cis/trans ipconazole (ipconazole ct). Ipconazole cc: (l*RS*,2*SR*,5*RS*)-2-(4-chlorobenzyl)-5-isopropyl-l- (1*H*-1,2,4-triazol-1-ylmethyl) cyclopentanol; and ipconazole ct: (l*RS*,2*SR*,5*SR*)-2-(4-chlorobenzyl)-5-isopropyl-l-(1*H*-1,2,4-triazol-l-ylmethyl) cyclopentanol.

2 The same two ion pair transitions were monitored for each isomer of ipconazole (quantitation and confirmation, respectively): *m/z* 334.1→70.1 and *m/z* 334.1→125; these monitored ions were similar to those monitored in the ECM. The results for each isomer were summed to quantify total ipconazole (p. 20).

3 The saltwater (ID Code: SLW-IR-090718; salinity *ca.* 20%, pH 8.0, hardness 4875 mg equivalent $CaCO_3$/L, conductivity 30.30 mmhos/cm) was natural seawater collected by EAG Laboratories-Easton from the Indian River in Delaware (pp. 13-14; Appendices III-VI, pp. 105-108). The surface water (ID Code: SFW-TL-080718; pH 7.5, hardness 61 mg equivalent $CaCO_3$/L, conductivity 0.17 mmhos/cm) was collected by EAG Laboratories-Easton from Tuckahoe Lake in Tuckahoe State Park in Ridgely, Maryland. The ground water (ID Code: GRW-WL-080718; pH 8.2, hardness 153 mg equivalent $CaCO_3$/L, conductivity 0.34 mmhos/cm) was collected from a collected from a well at EAG Laboratories-Easton testing facility. The drinking water (ID Code: DRW-TP-080718; pH 8.3, hardness 27 mg equivalent $CaCO_3$/L, conductivity 0.25 mmhos/cm) was collected from a tap at EAG Laboratories-Easton testing facility. The water characterization performed by Agvise Laboratories (Northwood, North Dakota).

III. Method Characteristics

The method LOQ was reported as 0.05 µg/L in water in the ECM and ILV (pp. 15-19; Tables 1-2, pp. 24-25 of MRID 50771001; pp. 12, 15-16, 18, 21 of MRID 50771002). In the ECM, the method LOQ was defined as the lowest fortification level in the method validation. No calculations or comparisons to background levels were reported to justify the LOQ for the method in the ECM or ILV. The method LOD for ipconazole in water was reported as the MQL in the ECM or ILV, which was calculated as the product of the lowest standard concentration and the volume for analysis divided by the volume sampled. The MQL equated to 0.01 µg/L for ipconazole cc and 0.001 µg/L for ipconazole ct in the ECM and 0.005 µg/L for ipconazole cc and 0.001 µg/L for ipconazole ct in the ILV (the ILV MQLs were reviewer-calculated). The MDL and PQL were calculated for each isomer of ipconazole in the ECM and ILV. In the ECM and ILV, the MDLs for ipconazole in water were calculated as 3.143 (which was the one-tailed t-statistic at the 99% confidence level for n-1 replicates, $t_{0.99}$) multiplied by the standard deviation of the measured concentrations of seven replicates fortified at the lowest calibration standard for each isomer. The PQL was calculated as five times the MDL. In the ECM, the MDL and PQL for ipconazole cc in saltwater were calculated as 0.0132 and 0.0660 µg/L, respectively, for the quantitation ion transition and 0.0306 and 0.153 µg/L, respectively, for the confirmation ion transition. The MDL and PQL for ipconazole ct in saltwater were calculated as 0.00216 and 0.0108 µg/L, respectively, for the quantitation ion transition and 0.00240 and 0.0120 µg/L, respectively, for the confirmation ion transition. In the ILV, the MDL and PQL for ipconazole cc in water were calculated as 0.00400 and 0.0200 µg/L, respectively, for the quantitation ion transition and 0.00461 and 0.0231 µg/L, respectively, for the confirmation ion transition. The MDL and PQL for ipconazole ct in water were calculated as 0.00161 and 0.00807 µg/L, respectively, for the quantitation ion transition and 0.00110 and 0.00552 µg/L, respectively, for the confirmation ion transition.

Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

Table 4. Method Characteristics

		Ipconazole	
Matrix		**Saltwater**	**Drinking/Surface/Ground Water**
Limit of Quantitation (LOQ)*	ECM	0.05 µg/L	**Not performed**
	ILV		0.05 µg/L
Limit of Detection (LOD)	ECM	0.01 µg/L (cc)[1] 0.001 µg/L (ct)[1]	**Not performed**
	ILV	0.005 µg/L (cc)[1,2] 0.001 µg/L (ct)[1,2]	
Linearity (calibration curve r and concentration range)	ECM	r = 0.99973052 (Q, cc) r = not reported (C, cc)[3] r = 0.99989135 (Q, ct) r = not reported (C, ct)[3] 0.100-10.0 ng/mL (cc) 0.01-1.00 ng/mL (ct)	**Not performed**
	ILV	r = 0.9994286 (Q, cc) r = 0.9996541 (C, cc) r = 0.9975403 (Q, ct) r = 0.9990707 (C, ct) 0.05-10.0 ng/mL (cc) 0.01-5.00 ng/mL (ct)	
Repeatable	ECM[4]	Yes for LOQ and 10×LOQ in one characterized saltwater matrix (*ca.* 20% salinity).	**Not performed**
	ILV[5,6]		Yes for LOQ and 10×LOQ in characterized surface, ground, and drinking water matrices.
Reproducible		Yes for 0.05 µg/L (LLMV)* and 0.50 µg/L	
Specific	ECM	Yes, no matrix interferences were observed; however, some minor baseline noise was observed near the RT of the ipconazole ct peak.[3]	
	ILV	Yes, no matrix interferences were observed.	

Data were obtained from pp. 15-19; Tables 1-2, pp. 24-25 (LOQ/LOD); Table 3, p. 26 (recovery data); p. 19 (linearity data); Figure 1, p. 30 (calibration curves); Figures 2-6, pp. 31-35 (chromatograms) of MRID 50771001; pp. 12, 21 (LOQ/LOD); Tables 2-9, pp. 27-34 (recovery data); pp. 15-16 (linearity data); Figures 1-2, pp. 35-36 (calibration curves); Figures 3-22, pp. 37-56 (chromatograms) of MRID 50771002; DER Attachment 2. Q = quantitative ion transition; C = confirmatory ion transition. cc = cis/cis ipconazole (ipconazole cc); ct = cis/trans ipconazole (ipconazole ct).

* Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ. The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV.

1 The method LOD for ipconazole in water was reported as the Method Quantification Limit (MQL) in the ECM or ILV, which was calculated as the product of the lowest standard concentration and the volume for analysis divided by the volume sampled (p. 19; Table 3, p. 26 of MRID 50771001; pp. 15-16, 18 of MRID 50771002).

2 ILV MQLs were reviewer-calculated since only the calculation formula was reported in the ILV (the product of the lowest standard concentration and the volume for analysis divided by the volume sampled; pp. 15-16, 18 of MRID 50771002). The ILV MQL results of the calculation formula were not reported in the study.

3 Values and figures only provided for the quantitation ion transition. A confirmatory method is not usually required when LC/MS/MS or GC/MS is used as the primary method to generate study data.

4 In the ECM, the saltwater (pH 8.10; target salinity 20 ± 3%) was prepared by mixing commercial sea salt mix (Crystal Sea Marinemix from Marine Enterprises International, Inc., Baltimore, Maryland) with laboratory freshwater (on-site well water blended with well water that was demineralized by reverse osmosis to yield water

with total hardness 130-160 mg $CaCO_3$/L; p. 12; Appendix B, pp. 41-43 of MRID 50771001). The non-GLP water characterization provided by the performing laboratory quantified levels of metals and pesticides.

5 In the ILV, the saltwater (ID Code: SLW-IR-090718; salinity *ca.* 20%, pH 8.0, hardness 4875 mg equivalent $CaCO_3$/L, conductivity 30.30 mmhos/cm) was natural seawater collected by EAG Laboratories-Easton from the Indian River in Delaware (pp. 13-14; Appendices III-VI, pp. 105-108 of MRID 50771002). The surface water (ID Code: SFW-TL-080718; pH 7.5, hardness 61 mg equivalent $CaCO_3$/L, conductivity 0.17 mmhos/cm) was collected by EAG Laboratories-Easton from Tuckahoe Lake in Tuckahoe State Park in Ridgely, Maryland. The ground water (ID Code: GRW-WL-080718; pH 8.2, hardness 153 mg equivalent $CaCO_3$/L, conductivity 0.34 mmhos/cm) was collected from a collected from a well at EAG Laboratories-Easton testing facility. The drinking water (ID Code: DRW-TP-080718; pH 8.3, hardness 27 mg equivalent $CaCO_3$/L, conductivity 0.25 mmhos/cm) was collected from a tap at EAG Laboratories-Easton testing facility. The water characterization performed by Agvise Laboratories (Northwood, North Dakota).

6 The ILV validated the method with the first trial for the surface, ground, and drinking water matrices and with the second trial for the saltwater matrix (pp. 16-18; Table 1, p. 26; Appendix X, pp. 117-118 of MRID 50771002). The method was validated as written with insignificant modifications of analytical instrumentation and parameters; however, the ILV noted a critical step of regulating the SPE flow rate to *ca*. 1 drop/second or less. The SPE flow rate was only specified as gravity in the ECM, and the failure of the first ILV trial for saltwater (low recoveries) was presumed to be due to an inappropriate interpretation of "gravity" as the specified SPE flow rate during analyte elution. An updated ECM with a more precisely (nominally) defined SPE elution flow rate was advised by the ILV since "regulation of the SPE flow rate will have a direct correlation to the magnitude of analyte recovery" (Appendix X, p. 118). Additionally, this update to the ECM was necessary for the successful validation of the method for saltwater; the ILV validations in surface, ground, and drinking water matrices were performed before the ILV validation in saltwater and were performed without difficulty due to the SPE extraction step.

IV. Method Deficiencies and Reviewer's Comments

1. Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than LOQ (pp. 15-19; Tables 1-2, pp. 24-25 of MRID 50771001; pp. 12, 15-16, 18, 21 of MRID 50771002). The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV. Based on the performance data submitted by the ILV and ECM, the LLMV was equivalent to the reported method LOQ for ipconazole in water.

2. The ILV was not performed independently of the ECM since technical communication occurred between the ECM and ILV (via the Study Monitor), the technical communication was necessary for the successful validation of the method for saltwater, and the ILV required multiple method clarifications of essential details (Appendix X, pp. 117-122 of MRID 50771002). The communication between the ILV Study Author (Jon MacGregor of EAG, Inc., Easton, MD), Eric S. Bodle (General Manager of EAG, Inc., Easton, MD), and Study Monitor (Keiichi Sudo of Kureha Corporation) were detailed. Communications involved ILV Study Author (Jon MacGregor) relaying needs for method clarification of essential details, the addition of saltwater matrix to the ILV validation, ILV proposed causes for low recoveries in the first saltwater trial to be forwarded to the ECM (October 8, 2018 email), ECM response to ILV proposed causes indicating the SPE flow rate (October 11, 2018 email), ILV identification of the SPE flow rate as a critical step with required an ECM modification with more specifics (October 17 and 18, 2018 emails), and ILV results.

   The ECM and ILV laboratories were part of the same company (EAG, Inc.; p. 1 of MRID 50771001; p. 1 of MRID 50771002). The provided lists of ILV and ECM study personnel were distinct, but Keiichi Sudo served as the Sponsor Representative for the ECM and ILV and facilitated communication between the ECM and ILV laboratories (pp. 1, 3, 5 of MRID 50771001; pp. 1, 3, 6; Appendix X, pp. 117-122 of MRID 50771002).

   OCSPP 850.6100 guidance states that, if the laboratory that conducted the validation belonged to the same organization as the originating laboratory, 1) the analysts, study director, equipment, instruments, and supplies of the two laboratories must have been distinct and operated separately and without collusion, and 2) the analysts and study director of the ILV must have been unfamiliar with the method both in its development and subsequent use in field studies.

3. The ILV validated the method in saltwater, as well as surface, ground, and drinking water matrices; however, the ECM only validated the method in saltwater (p. 12; Appendix B, pp. 41-43 of MRID 50771001; pp. 13-14; Appendices III-VI, pp. 105-108 of MRID 50771002). The reproducibility of the method in surface, ground, and drinking water matrices could not be evaluated since only one set of performance data was provided for those matrices. According to the ILV communication log, the ECM considered "saltwater…[to be]…"a more complex matrix and could be used as an enforcement method for all types of water" (September 13, 2018 email; Appendix X, p. 120 of MRID

50771002). The communication log indicated that the ILV was originally tasked with only including surface, drinking, and ground water matrices, but the ILV protocol was amended to include saltwater as a matrix after concern that the enforcement method may fail agency review if the enforcement method was not tested directly as written (September 22, 2018 email; Appendix X, pp. 121-122).

4. The ILV validations in surface, ground, and drinking water matrices occurred first and were successful in the first trial (Appendix X, pp. 117-118, 122 of MRID 50771002). SPE flow rate was determined as a critical step in the saltwater trial, and the close regulation of the SPE flow rate did not occur during the validations in surface, ground, and drinking water matrices. The ILV study author noted that close regulation of the SPE flow rate during potential repeats of the validations in ground and drinking water matrices may have yielded higher recoveries (some recoveries were <70% even though overall acceptable results were obtained).

5. Only quantitation ion transition linearity data/calibration curves and representative chromatograms were included in the ECM (Figures 1-6, pp. 30-35 of MRID 50771001). These omissions did not affect the linearity or specificity assessment of the method since a confirmatory method is not usually required when LC/MS/MS or GC/MS is used as the primary method to generate study data.

6. ILV calculations indicated that recovery results were corrected for residues quantified in the controls; however, no residues were quantified so the results were uncorrected (pp. 18-20 and Tables 2-9, pp. 27-34 of MRID 50771002).

7. The determinations of the LOD and LOQ in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 15-19; Tables 1-2, pp. 24-25 of MRID 50771001; pp. 12, 15-16, 18, 21 of MRID 50771002). In the ECM, the method LOQ was defined as the lowest fortification level in the method validation. No calculations or comparisons to background levels were reported to justify the LOQ for the method in the ECM or ILV. The method LOD for ipconazole in water was reported as the MQL in the ECM or ILV, which was calculated as the product of the lowest standard concentration and the volume for analysis divided by the volume sampled. Detection Limit should not be based on the arbitrarily selected lowest concentration in the spiked samples.

   Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

   The MDL and PQL were calculated for each isomer of ipconazole in the ECM and ILV. In the ECM and ILV, the MDLs for ipconazole in water were calculated as 3.143 (which was the one-tailed t-statistic at the 99% confidence level for n-1 replicates, $t_{0.99}$) multiplied by the standard deviation of the measured concentrations of seven replicates fortified at the lowest calibration standard for each isomer (pp. 15-19; Tables 1-2, pp. 24-

25 of MRID 50771001; pp. 12, 15-16, 18, 21 of MRID 50771002). The PQL was calculated as five times the MDL.

8. In the ECM, stability of ipconazole in saltwater at 0.0500 and 0.500 µg/L were stored at 5 ± 3°C and determined to be stable for up to 8 days for ipconazole cc and up to 12 days for ipconazole ct (pp. 17, 19; Tables 5-6, pp. 28-29 of MRID 50771001). Ipconazole calibration solutions were determined to be stable for up to 25 days for ipconazole cc (0.100 and 1.00 µg/L) and ipconazole ct (0.0200 and 0.200 µg/L).

9. In the ECM and ILV, no significant matrix effects were observed (<20%; p. 19; Table 4, p. 27 of MRID 50771001; pp. 18, 21 of MRID 50771002). Solvent-based calibration standards were used in the ECM and ILV.

10. The revisions to the ECM were listed, which included the addition of equipment details, sample preparation steps, and analytical condition details (p. 10 of MRID 50771001).

11. In the ILV, the time requirement for the method was reported as *ca*. 1.5 days to complete one sample set (13 samples), with *ca*. 4 hours for preparation, *ca*. 4 hours for sample processing, and *ca*. 16 hours for LC/MS/MS analysis and processing (Appendix X, pp. 118-119 of MRID 50771002).

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.

40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319, and Revision 2; 1994 and 2016.

Attachment 1: Chemical Names and Structures

Ipconazole

IUPAC Name: Ipconazole cc: (l*RS*,2*SR*,5*RS*)-2-(4-chlorobenzyl)-5-isopropyl-l-(1*H*-1,2,4-triazol-1-ylmethyl) cyclopentanol
Ipconazole ct: (l*RS*,2*SR*,5*SR*)-2-(4-chlorobenzyl)-5-isopropyl-l-(1*H*-1,2,4-triazol-l-ylmethyl) cyclopentanol

CAS Name: Not reported

CAS Number: Ipconazole (CAS [125225-28-7])
Ipconazole cc (CAS [115850-69-6])
Ipconazole ct (CAS [115937-89-8])

SMILES String: c1cc(Cl)ccc1CC2CCC(C(C)C)C2(O)Cn3ncnc3