Analytical method for etoxazole and its metabolites R-8 and R-13 in water

Reports:	ECM: EPA MRID No.: 50539904. Perez, R. 2018. Method Validation of Analytical Method Number RM-37S-3: The Determination of Residues of Etoxazole and its Metabolites, R-8 and R-13 in Sediment and Water Matrices using LC-MS/MS. Laboratory Project ID: VP-41072. Report prepared by ADPEN Laboratories, Inc., Jacksonville, Florida, and sponsored and submitted by Valent USA, LLC, Dublin, California; 200 pages. Final report issued January 22, 2018. ILV: EPA MRID No. 50539906. Perez, S. 2018. Independent Laboratory Validation of Valent Analytical Method RM-37S-3: "The Determination of Residues of Etoxazole and its Metabolites, R-8 and R-13 in Sediment and Water Matrices Using LC-MS/MS". Laboratory Project ID: V-17-200100. Report prepared by ADPEN Laboratories, Inc., Jacksonville, Florida, and sponsored and submitted by Valent USA, LLC, Dublin, California; 135 pages. Final report issued January 30, 2018. See Reviewer's Comment #1 regarding study author citation.
Document No.:	MRIDs 50539904 & 50539906
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 50539904). 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 50539906). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5).
Classification:	This analytical method is classified as unacceptable. The study cannot be used to fill guideline requirements. The LOQ exceeded the lowest toxicological level of concern in water for chronic risk to invertebrates. It could not be determined that ILV MRID 50539906 was conducted independently of ECM MRID 50539904. The specificity of the method for R-8 was not supported by ILV and ECM chromatograms. ILV linearity was not satisfactory for etoxazole.
PC Code:	107091
EFED Final Reviewers:	Zoe Ruge, M.S., Physical Scientist — Signature: Zoe Ruge; Date: 7/3/19 Dena Barrett, PhD., Chemist — Signature: Dena Barrett; Date: 7/3/19
CDM/CSS-Dynamac JV	Lisa Muto, M.S., Environmental Scientist — Signature: Lisa Muto; Date: 10/09/2018

Reviewers:

Mary Samuel, M.S.,
Environmental Scientist

Signature: 

Date: 10/24/2018

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.

Executive Summary

The analytical method, Valent Analytical Method RM-37S-3, is designed for the quantitative determination of etoxazole and its metabolites R-8 and R-13 in water at the LOQ of 0.01 mg/L using LC/MS/MS. The LOQ is greater than the lowest toxicological level of concern in water for etoxazole and its metabolites R-8 and R-13 (0.13 µg/L). The ECM and ILV validated the method using characterized surface water; ILV water matrix was the same one which was used in the ECM. The ILV validated the method for surface water in the first trial with insignificant modifications to the final dilution ratio and LC/MS injection volume; however, it could not be determined that ILV MRID 50539906 was conducted independently of ECM MRID 50539904 since both validations were conducted at the same facility (ADPEN Laboratories, Inc., Jacksonville, Florida) and insufficient evidence was provided to support the independence of the two laboratories. The communication between the staff of the initial and independent validations was not addressed. The analytical instrumentation and all other analytical parameters were the exact same as those in the ECM. It was not confirmed that no interactions between staff and no sharing of equipment and both validations occurred at the same address. All ILV and ECM data regarding repeatability, accuracy, precision, and linearity were satisfactory for etoxazole and its metabolites R-8 and R-13, except for the ILV linearity for etoxazole. The specificity of the method for etoxazole and R-13 was supported by ILV and ECM representative chromatograms, but specificity of the method for R-8 was not supported due to one or more nearby contaminants which were 30x-80x larger (peak ht. ≥ LOQ peak ht.) in R-8 chromatograms compared to etoxazole and R-13 chromatograms.

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						
Etoxazole	50539904[1]	50539906[2]	Unacceptable	Water	22/01/2018	Valent USA, LLC	LC/MS/MS	0.01 mg/L
R-8								
R-13								

1 In the ECM, the surface water (Lab Code #170110002-019; pH 7.4; 62 mg/L as $CaCO_3$ hardness; 1312 ppm total dissolved solids) obtained from Jacksonville, Florida, was used in the study (pp. 14-15; Appendix A, p. 42 of MRID 50539904). The water sample was characterized by Agvise Laboratories, Northwood, North Dakota.

2 In the ILV, the surface water (Lab Code #170110002-019; pH 7.4; 62 mg/L as $CaCO_3$ hardness; 1312 ppm total dissolved solids) obtained from Jacksonville, Florida, was used in the study (p. 14; Appendix A, p. 95 of MRID 50539906). The water sample was characterized by Agvise Laboratories, Northwood, North Dakota. The water matrix was the same one which was used in the ECM.

I. Principle of the Method

Samples (15 mL) were fortified, as necessary, and extracted with 15 mL of methanol:water, 90:10 (v:v), containing 10 mM ammonium bicarbonate (pp. 19-21 of MRID 50539904). The extraction was performed by shaking (vortexing) method for one minute. After centrifugation for 10 minutes at *ca*. 3500 rpm, a 0.5 mL aliquot of the supernatant was diluted to 40 mL using methanol:water, 50:50 (v:v). After sonication and vortexing for 30 seconds, a 1.5 mL aliquot was transferred to an autosampler vial, and the analytes residues were analyzed by LC-MS/MS. A flow chart was provided (Appendix C, p. 65).

Samples were analyzed for etoxazole and metabolites R-8 and R-13 by Agilent 1290 UPLC system coupled with an AB SCIEX 5500 QTrap MS (Acquity UPLC BEH C18 column, 2.1 mm x 50 mm, 1.7 µm column; column temperature 60°C) using a gradient mobile phase of A) 0.1% formic acid + 5 mM ammonium formate in HPLC water and B) 0.1% formic acid + 5 mM ammonium formate in methanol [percent A:B; 0.0-0.2 min. 90:10, 0.7-2.0 min. 5:95, 2.1-3.0 min. 90:10] with MS/MS-ESI (electrospray ionization; temperature 450°C) detection in positive ion mode and multiple reaction monitoring (MRM; pp. 19, 21-23 of MRID 50539904). Injection volume was 5.0 µL. Etoxazole and metabolites R-8 and R-13 were identified using two ion transitions; one for quantitation (Q) and one for confirmation (C). Ion transitions monitored were *m/z* 360.203→141.0 (Q) and *m/z* 360.203→177.1 (C) for etoxazole, *m/z* 238.087→165.1 (Q) and *m/z* 238.087→147.1 (C) for R-8, and *m/z* 358.145→140.8 (Q) and *m/z* 358.145→274.0 (C) for R-13. Approximate retention times were 1.3, 1.1, and 1.4 minutes for etoxazole, R-8 and R-13, respectively.

In the ECM, the following experimental precautions were listed: 1) the use of HPLC grade water for mobile phase solutions was important to lower background noise; 2) carry-over in the LC/MS analysis can be avoided by diluting high-recovery samples and including blank injections after high-level samples; and 3) additional needle washes and valve washes with organic solvent may help eliminate carry-over of the analytes (p. 24 of MRID 50539904).

In the ILV, the ECM was performed as written, except the final dilution ratio differed (0.5 mL aliquot diluted to 20 mL) and increase of the injection volume to 25.0 µL (pp. 16, 19, 24; Table 14, p. 40 of MRID 50539906). The analytical instrumentation and all other analytical parameters were the exact same as those in the ECM; monitored ion transitions matched those reported in the ECM. Approximate retention times were 1.7, 1.6, and 1.8 minutes for etoxazole, R-8 and R-13, respectively.

The Limit of Quantitation (LOQ) for etoxazole and its metabolites R-8 and R-13 in water was 0.01 mg/L in the ECM and ILV (pp. 26-27, 29 of MRID 50539904; pp. 20, 23-24 of MRID 50539906). In the ECM and ILV, the Limit of Detection (LOD) for water was set at 0.002 mg/L (20% of the LOQ) for all three analytes. In the ILV, the LOD was calculated as 0.001 mg/L for the quantification and confirmation analyses for all three analytes.

II. Recovery Findings

ECM (MRID 50539904): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of etoxazole and its metabolites R-8 and R-13 in one water matrix at fortification levels of 0.01 mg/L (LOQ) and 0.10 mg/L (10×LOQ; p. 26; Table 2, p. 34). Etoxazole and its metabolites R-8 and R-13 were identified using two ion transitions; performance data (recovery results) from primary and confirmatory analyses were comparable. The surface water (Lab Code #170110002-019; pH 7.4; 62 mg/L as $CaCO_3$ hardness; 1312 ppm total dissolved solids) obtained from Jacksonville, Florida, was used in the study (pp. 14-15; Appendix A, p. 42). The water sample was characterized by Agvise Laboratories, Northwood, North Dakota.

ILV (MRID 50539906): Mean recoveries and RSDs were within guideline requirements for analysis of etoxazole and its metabolites R-8 and R-13 in one water matrix at fortification levels of 0.01 mg/L (LOQ) and 0.10 mg/L (10×LOQ; p. 18; Tables 7-12, pp. 33-38). Etoxazole and metabolites R-8 and R-13 were identified using two ion transitions. Performance data (recovery results) from primary and confirmatory analyses were comparable; however, recoveries were consistently lower in the confirmatory analysis. The surface water (Lab Code #170110002-019; pH 7.4; 62 mg/L as $CaCO_3$ hardness; 1312 ppm total dissolved solids) obtained from Jacksonville, Florida, was used in the study (p. 14; Appendix A, p. 95). The water sample was characterized by Agvise Laboratories, Northwood, North Dakota. The water matrix was the same one which was used in the ECM. The method was validated for the surface water matrix in the first trial with insignificant modifications to the final dilution ratio and LC/MS injection volume (pp. 16, 18-19, 24; Table 14, p. 40). The analytical instrumentation and all other analytical parameters were the exact same as those in the ECM.

Table 2. Initial Validation Method Recoveries for Etoxazole and Its Metabolites R-8 and R-13 in Water[1,2]

Analyte	Fortification Level (mg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Surface Water					
	Quantitation ion					
Etoxazole	0.01 (LOQ)	5	84-91	87	3.5	4.0
	0.10	5	89-96	92	2.4	2.6
R-8	0.01 (LOQ)	5	101-112	105	4.7	4.4
	0.10	5	89-107	97	7.1	7.3
R-13	0.01 (LOQ)	5	73-78	76	2.3	3.0
	0.10	5	77-80	78	1.3	1.7
	Confirmatory ion					
Etoxazole	0.01 (LOQ)	5	85-96	90	4.6	5.1
	0.10	5	84-95	89	3.9	4.4
R-8	0.01 (LOQ)	5	102-110	107	3.1	2.9
	0.10	5	88-100	95	4.3	4.5
R-13	0.01 (LOQ)	5	72-83	80	4.7	5.9
	0.10	5	75-87	79	4.5	5.6

Data (uncorrected recovery results, Appendices D-E, pp. 68, 78-83) were obtained from p. 26; Table 2, p. 34 of MRID 50539904.

1 The surface water (Lab Code #170110002-019; pH 7.4; 62 mg/L as $CaCO_3$ hardness; 1312 ppm total dissolved solids) obtained from Jacksonville, Florida, was used in the study (pp. 14-15; Appendix A, p. 42). The water sample was characterized by Agvise Laboratories, Northwood, North Dakota.

2 Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: *m/z* 360.203→141.0 (Q) and *m/z* 360.203→177.1 (C) for etoxazole, *m/z* 238.087→165.1 (Q) and *m/z* 238.087→147.1 (C) for R-8, and *m/z* 358.145→140.8 (Q) and *m/z* 358.145→274.0 (C) for R-13.

Table 3. Independent Validation Method Recoveries for Etoxazole and Its Metabolites R-8 and R-13 in Water[1,2]

Analyte	Fortification Level (mg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Surface Water					
	Quantitation ion					
Etoxazole	0.01 (LOQ)	7	93-105	99	3.5	3.5
	0.10	7	112-123	117	4.4	3.7
R-8	0.01 (LOQ)	7	102-107	105	2.0	1.9
	0.10	7	102-114	106	4.0	3.7
R-13	0.01 (LOQ)	7	88-95	92	2.7	2.9
	0.10	7	90-102	94	3.9	4.1
	Confirmatory ion					
Etoxazole	0.01 (LOQ)	7	87-96	90	2.7	3.0
	0.10	7	103-111	107	3.0	2.8
R-8	0.01 (LOQ)	7	100-107	103	2.5	2.4
	0.10	7	100-114	104	4.5	4.3
R-13	0.01 (LOQ)	7	82-90	87	2.6 (2.7)[3]	2.9 (3.1)[3]
	0.10	7	85-96	89	3.3 (3.5)[3]	3.7 (3.9)[3]

Data (uncorrected recovery results, Figure 30, p. 92) were obtained from p. 18; Tables 7-12, pp. 33-38 of MRID 50539906.

1 The surface water (Lab Code #170110002-019; pH 7.4; 62 mg/L as $CaCO_3$ hardness; 1312 ppm total dissolved solids) obtained from Jacksonville, Florida, was used in the study (p. 14; Appendix A, p. 95). The water sample was characterized by Agvise Laboratories, Northwood, North Dakota. The water matrix was the same one which was used in the ECM.

2 Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: *m/z* 360.203→141.0 (Q) and *m/z* 360.203→177.1 (C) for etoxazole, *m/z* 238.087→165.1 (Q) and *m/z* 238.087→147.1 (C) for R-8, and *m/z* 358.145→140.8 (Q) and *m/z* 358.145→274.0 (C) for R-13.

3 Reported values for standard deviation and relative standard deviation differed slightly on p. 18 and Table 12, p. 38. Values on parentheses are from Table 12.

III. Method Characteristics

The LOQ for etoxazole and its metabolites R-8 and R-13 in water was reported to be 0.01 mg/L in the ECM and ILV (pp. 26-27, 29 of MRID 50539904; pp. 20, 23-24 of MRID 50539906). In the ECM and ILV, the LOQ was defined as the lowest fortification level successfully tested. In the ECM and ILV, the LOD for water was set at 0.002 mg/L (20% of the LOQ) for all three analytes. In the ECM and ILV, the LOD was set as the lowest calibration standard with an acceptable signal-to-noise ratio (S:N, >3:1). In the ILV only, the LOD was calculated as 0.001 mg/L for the quantification and confirmation analyses for all three analytes using the data of the seven LOQ recovery samples. The LOD was calculated for each analyte using the following equation:

$LOD = (t_{0.99} \times SD)$

Where, $t_{0.99}$ is the one-tailed t statistic for n = 5 (3.747) and SD is the standard deviation of the analyte recovery measurements at the target LOQ. The calculated LODs supported the method LOD. No calculations or comparisons to background levels were reported to justify the LOQ for the method in the ECM or ILV. The LOD calculations were provided for the ILV only; no calculations were reported to justify the LOD for the method in the ECM.

Table 4. Method Characteristics

Analyte[1]			**Etoxazole**	**R-8**	**R-13**
Limit of Quantitation (LOQ)	ECM		0.01 mg/L		
	ILV				
Limit of Detection (LOD)	ECM	Method	0.002 mg/L		
		Calculated	Not calculated		
	ILV	Method	0.002 mg/L		
		Calculated	0.001 mg/L (Q & C)		
Linearity (calibration curve r^2 and concentration range)[1]	ECM		r^2 = 0.9986 (Q) r^2 = 0.9992 (C)	r^2 = 0.9980 (Q) r^2 = 0.9960 (C)	r^2 = 0.9990 (Q) r^2 = 0.9988 (C)
			0.00005-0.005 ng (equivalent to 0.01-1.0 ng/mL on column)		
	ILV		r^2 = **0.9945** (Q) r^2 = 0.9989 (C)	r^2 = 0.9976 (Q & C)	r^2 = 0.9983 (Q) r^2 = 0.9994 (C)
			0.00025-0.025 ng		
Repeatable	ECM[2]		Yes at LOQ and 10×LOQ (characterized surface water matrix)		
	ILV[3,4]				
Reproducible			Yes at LOQ and 10×LOQ		
Specific	ECM		Yes, no matrix interferences were quantified. More baseline noise was noted in the C ion.	**No**, no matrix interferences were quantified; however, elevated baseline and nearby contaminants [(RT 1.16-1.3) peak ht. ≥ LOQ (RT 1.09) peak ht.] were noted.[5]	Yes, no matrix interferences were quantified.
	ILV		Yes, matrix interferences were <1% of the LOQ (based on peak area).	**No**, matrix interferences were <2% of the LOQ (based on peak area); however, nearby contaminant [(RT 1.58-1.67) peak ht. ≈ LOQ (RT 1.42) peak ht.] was noted.[5]	Yes, matrix interferences were <1% of the LOQ (based on peak area).

Data were obtained from pp. 26-27, 29 (LOQ/LOD); p. 26; Table 2, p. 34 (recovery data); Appendix E, pp. 78-83 (calibration coefficients); Appendix J, Figures 17-25, pp. 188-196 (chromatograms) of MRID 50539904; pp. 20, 23-24 (LOQ); p. 18; Tables 7-12, pp. 33-38 (recovery data); Figure 17, pp. 77-79 (calibration curves); Appendix D, pp. 126-131 (calibration coefficients); Figures 18-29, pp. 80-91 (chromatograms) of MRID 50539906; DER Attachment 2. Q = Quantitation ion transition; C = Confirmation ion transition. **Red values** indicate items not in compliance with guidelines.

1 Correlation coefficients (r^2) values were reviewer-calculated from r values provided in the study report (Appendix E, pp. 78-83 of MRID 50539904; Appendix D, pp. 126-131 of MRID 50539906; DER Attachment 2). Solvent-based calibration standards were used (p. 18 of MRID 50539904; p. 20 of MRID 50539906). For the ILV correlation coefficients, the reviewer limited the value to four significant figures, even though seven significant figures were provided in the study report.

2 In the ECM, the surface water (Lab Code #170110002-019; pH 7.4; 62 mg/L as $CaCO_3$ hardness; 1312 ppm total dissolved solids) obtained from Jacksonville, Florida, was used in the study (pp. 14-15; Appendix A, p. 42 of MRID 50539904). The water sample was characterized by Agvise Laboratories, Northwood, North Dakota.

3 In the ILV, the surface water (Lab Code #170110002-019; pH 7.4; 62 mg/L as $CaCO_3$ hardness; 1312 ppm total dissolved solids) obtained from Jacksonville, Florida, was used in the study (p. 14; Appendix A, p. 95 of MRID

50539906). The water sample was characterized by Agvise Laboratories, Northwood, North Dakota. The water matrix was the same one which was used in the ECM.

4 The ILV validated the method for the surface water matrix in the first trial with insignificant modifications to the final dilution ratio and LC/MS injection volume (pp. 16, 18-19, 24; Table 14, p. 40 of MRID 50539906). The analytical instrumentation and all other analytical parameters were the exact same as those in the ECM.

5 Based on Appendix J, Figures 20-22, pp. 191-193 of MRID 50539904; and Figures 22-25, pp. 84-87 of MRID 50539906.

Linearity is satisfactory when $r^2 \geq 0.995$.

IV. Method Deficiencies and Reviewer's Comments

1. It could not be determined that ILV MRID 50539906 was conducted independently of ECM MRID 50539904 since both validations were conducted at the same facility (ADPEN Laboratories, Inc., Jacksonville, Florida) and insufficient evidence was provided to support the independence of the two laboratories (p. 1 of MRID 50539904; p. 1 of MRID 50539906). According to OCSPP guidelines, if the laboratory that conducted the validation belonged to the same organization as the originating laboratory, the analysts, study director, equipment, instruments, and supplies of the two laboratories must have been distinct and operated separately and without collusion. Furthermore, 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. In order to support their independence claim, ADPEN Laboratories, Inc., showed that the study director and validation performers (chemists and other technical staff) of each validation was different; however, some laboratory personnel were the same, including the Project Coordinator, Sample Custodian, and Laboratory Coordinator (pp. 1-6, 13 of MRID 50539904; pp. 1-6, 11 of MRID 50539906). The communication summary reported that the ILV was conducted independently of the Study Sponsor (Valent USA, LLC) and that "laboratory personnel performing the tests were not familiar with the method" (p. 24 of MRID 50539906). The communication between the staff of the initial and independent validations was not addressed. Additionally, the reported analytical equipment was the same in the ECM and ILV, although retention times differed slightly (pp. 22-23 of MRID 50539904; Table 14, p. 40 of MRID 50539906). Analytical instrument numbers were not reported in the ECM or ILV. The ILV water matrix was the same one which was used in the ECM (pp. 14-15; Appendix A, p. 42 of MRID 50539904; p. 14; Appendix A, p. 95 of MRID 50539906). The registrant needs to provide additional information to confirm no interactions between staff and no sharing of equipment when both validations occur at the same address.

   The following significant typographical error was noted in the ILV: the study author was reported as "Perez R" when the signature pages reported the study director as Steven Perez (pp. 1-6 of MRID 50539906). Additionally, the ILV was cited in the reference section of the ECM, and "Perez, S." was reported as the author (p. 31 of MRID 50539904). For this review, it was concluded that the study director of the ECM and ILV were two different people and corrected the typographical error when reporting the citations of the DER.

2. The specificity of the method for R-8 was not supported by ILV and ECM representative chromatograms. Q LOQ chromatograms of metabolite R-8 showed one or more nearby significant contaminants: ILV [(RT 1.58-1.67) peak ht. ≈ LOQ (RT 1.42) peak ht.] (Figures 22-25, pp. 84-87 of MRID 50539906); and ECM [(RT 1.16-1.3) peak ht. ≥ LOQ (RT 1.09) peak ht.] (Appendix J, Figures 20-22, pp. 191-193 of MRID 50539904). The Certificate of Analysis reported a purity of 98.9% for R-8, so the contaminants did not originate from the test material (Appendix B, p. 45 of MRID 50539904; Appendix B, p. 98 of MRID 50539906). These contaminants were seen in the chromatograms of other analytes, but the peak heights of the contaminants were *ca*. 1000 cps compared to 30000-80000 cps in the R-8 chromatograms (Appendix J, Figures 17-25, pp. 188-196 of MRID 50539904; Figures 18-29, pp. 80-91 of MRID 50539906). Also, there was no apparent loss of R-8 since recoveries were *ca*. 100%. So, these contaminants should have been identified and/or the method modified to eliminate these contaminants for R-8 identification and quantification. This reviewer also notes that baseline interferences were most significant in the ECM R-8 confirmatory chromatograms (Appendix J, Figures 20-22, pp. 191-193 of MRID 50539904).

3. ILV linearity was not satisfactory for the quantitation ion analysis of etoxazole in surface water, $r^2 = 0.9945$ (Appendix D, p. 126 of MRID 50539906; DER Attachment 2). Linearity is satisfactory when $r^2 \geq 0.995$.

4. The estimations of LOQ and LOD in ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 26-27, 29 of MRID 50539904; pp. 20, 23-24 of MRID 50539906). In the ECM and ILV, the LOQ was defined as the lowest fortification level successfully tested. In the ECM and ILV, the LOD for water was set at 0.002 mg/L (20% of the LOQ) for all three analytes. In the ECM and ILV, the LOD was set as the lowest calibration standard with an acceptable signal-to-noise ratio (S:N, >3:1). In the ILV, the LOD was calculated as 0.001 mg/L for the quantification and confirmation analyses for all three analytes using the data of the seven LOQ recovery samples. The LOD was calculated for each analyte using the following equation: LOD = ($t_{0.99}$ x SD), where $t_{0.99}$ is the one-tailed t statistic for n = 5 (3.747) and SD is the standard deviation of the analyte recovery measurements at the target LOQ. The calculated LODs supported the method LOD. No calculations or comparisons to background levels were reported to justify the LOQ for the method in the ECM or ILV; no calculations were reported to justify the LOD for the method in the ECM. Detection limits should not be based on arbitrary values.

5. The ILV and ECM reported that no significant matrix suppression or enhancement was observed (p. 24; Table 3, pp. 35-36 of MRID 50539904; p. 20 of MRID 50539906).

6. In the ECM, the stability of the sample extracts was determined to be acceptable after 7 days of storage in a refrigerator (p. 28; Table 5, p. 39 of MRID 50539904).

7. The time required to complete the method for a validation set (13 samples) was reported as *ca*. 4-6 hours in the ILV, not including the instrument time and the calculation of results (p. 20 of MRID 50539906).

V. References

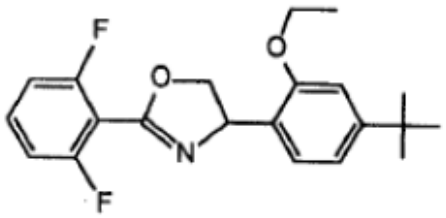
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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

Attachment 1: Chemical Names and Structures

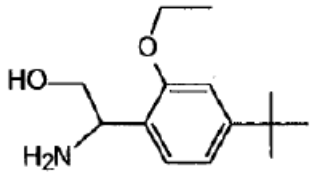
Etoxazole

IUPAC Name: Not reported
CAS Name: 2-(2,6-difluorophenyl)-4-[4-(1,1-dimethylethyl)-2-ethoxyphenyl]-4,5-dihydrooxazole
CAS Number: 153233-91-1
SMILES String: Not found



R-8

IUPAC Name: Not reported
CAS Name: 2-amino-2-(4-tert-butyl-2-ethoxyphenyl)ethanol
CAS Number: 153281-81-3
SMILES String: Not found



R-13

IUPAC Name: Not reported
CAS Name: 4-(4-tert-butyl-2-ethoxyphenyl)-2-(2,6-difluorophenyl)oxazol
CAS Number: Not reported
SMILES String: Not found