

Analytical method for trifludimoxazin (BAS 850 H) metabolite M850H040 in water

- Reports:** ECM: EPA MRID No.: 50406055. Delinsky, D. 2018. Method of Analysis of BAS 850 H Metabolite in Water with Limit of Determination (LOD) Calculation (Method R0048/01). BASF Study Nos.: 858175 and 858175_01 (Appendix A, p. 8; Appendix B, p. 93). BASF Registration Document No.: 2018/7005812. Report prepared, sponsored and submitted by BASF Agricultural Solutions, Research Triangle Park, North Carolina, North Carolina; 146 pages. Final report issued August 14, 2018.
- ILV: EPA MRID No. 50406056. Hawkins, A. 2018. Independent Laboratory Validation of BASF Analytical Method R0048/01, "Method for the Determination of M850H040 (Reg.No. 6095223) in Surface and Drinking Water by LC-MS/MS". BASF Study ID No.: 858176. BASF Registration Document No.: 2018/7005395. OMIC USA Study No.: 18CB03. Report prepared by OMIC USA, Inc., Portland, Oregon, sponsored and submitted by BASF Agricultural Solutions, Research Triangle Park, North Carolina; 71 pages. Final report issued August 14, 2018.
- Tier II Summary and Evaluation: EPA MRID No. 50406045. Kleppe, C. 2018. Multi-Lateral Submission for the Evaluation of the Active Substance BAS 850 H. Chapters 4.5. BASF Registration Document No.: 2018/7000015 US. Report submitted by BASF Corporation, Research Triangle Park, North Carolina; 45 pages. Final report issued March 1, 2018.
- Document No.:** MRIDs 50406055 & 50406056 & 50406045
- Guideline:** 850.6100
- Statements:** ECM: The study was not conducted in accordance with Good Laboratory Practice (GLP) standards since it was not a study (p. 3 of MRID 50406055). Signed and dated No Data Confidentiality and GLP statements were provided; Quality Assurance and Authenticity statements were not provided (pp. 2-3). The ECM was a compilation of the study reports for an analytical method and LOD determination (p. 5). These two study reports (BASF Study # 858175 and 858175_01; Appendix A, p. 8; Appendix B, p. 93) were conducted in accordance with USEPA FIFRA GLP standards, 40 CFR, Part 160 (Appendix A, p. 10; Appendix B, p. 95). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (Appendix A, pp. 9-12; Appendix B, pp. 94-97).
- ILV: The study was conducted in accordance with USEPA FIFRA GLP standards, 40 CFR, Part 160 (p. 3 of MRID 50406056). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5).
- Tier II Summary and Evaluation: Signed and dated No Data Confidentiality statement was provided (p. 2 of MRID 50406045). No GLP, Quality Assurance, and Authenticity statements were provided.

Classification: This analytical method is classified as **acceptable**. All ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory.

PC Code: 080800

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

Signature:
Date: 8/20/20

CDM/CSS-Dynamac JV Reviewers: Lisa Muto,
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Signature: 

Date: 11/26/2019

Mary Samuel, M.S.,
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Signature: 

Date: 11/26/2019

This Data Evaluation Record is a modification of the Tier II DER submitted to the Environmental Fate and Effects Division (EFED) by the registrant. Statistical analyses have been performed according to EFED guidance, and the Executive Summary has been revised. The DER may have been altered by EFED personnel subsequent to signing by CDM/CSS-Dynamac JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.

Executive Summary

This analytical method, BASF Method R0048/01, is designed for the quantitative determination of M850H040 in water at the LOQ of 30 ng/L using LC/MS/MS. Eco-toxicology endpoints in water for this metabolite have not been determined. However, the LOQ is less than the lowest toxicological level of concern for the parent molecule (0.17 µg/L) and all other metabolites in water. The ECM and ILV performed the method with two characterized water matrices: surface water and drinking (well) water; the ILV surface water matrix was the same as that of the ECM. The ILV validated BASF method (R0048/01) in drinking water and surface water in the second trial with modifications to the analytical instrument and parameters. All ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory.

In a separate study, the method LOD of BASF Analytical Method No. R0048/01 was validated in accordance with the methodology set forth in 40 CFR Ch. 1 Part 136 Appendix B. Since the calculated MDL for M850H040 was too low for accurate quantitation, the LOD was set to 6 ng/L, the lowest calibration standard.

Some data obtained directly from Tier II Summary and Evaluation: EPA MRID No. 50406045 was not cited.

All referenced page numbers for MRID 50406055 refer to those reported on the right-handed margin of the document pages.

Table 1. Analytical Method Summary – BASF Analytical Method R0048/01

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						
M850H040 (Reg.No. 6095223)	50406055 ¹	50406056		Water ^{2,3}	14/08/2018 ⁴	BASF Corporation/ BASF Agricultural Solutions	LC/MS/MS	30 ng/L

1 A compilation of BASF Study #s 858175 and 858175_01 (Appendix A, pp. 8, 19; Appendix B, p. 93 of MRID 50406055).

2 In the ECM, drinking (well) water (Sample No. CM17-053; pH 8.0, 0.1 ppm total organic carbon, 36 ppm calcium, 101 mg equiv. CaCO₃/L hardness) obtained from Bahama, North Carolina, and surface (lake) water (Sample No. CM17-052; pH 8.9, 18.6 ppm total organic carbon, 93 ppm calcium, 657 mg equiv. CaCO₃/L hardness) obtained Golden Lake, North Dakota, were used (Appendix A, Appendix K, pp. 86-88 of MRID 50406055). Test systems were characterized by Agvise Laboratories: drinking water in conjunction with this study and surface water under BASF Study # 805401 (DocID: Not yet available).

3 In the ILV, drinking (well) water (Sample No. CM17-053; OMIC Sample ID 18CB03_DW; pH 8.0, 0.1 ppm total organic carbon, 36 ppm calcium, 101 mg equiv. CaCO₃/L hardness) and surface (lake) water (Sample No. CM17-052; OMIC Sample ID 18CB03_SW; Golden Lake Water; pH 8.9, 18.6 ppm total organic carbon, 93 ppm calcium, 657 mg equiv. CaCO₃/L hardness) were provided by BASF and used in the study (p. 12; Appendix 10.7, pp. 50-51 of MRID 50406056). The surface water was the same as that of the ECM.

4 Method dates were August 13, 2018 for BASF Study # 858175 and 858175_01 Appendix A, p. 8; Appendix B, p. 93 of MRID 50406055).

I. Principle of the Method

Method R0048/01

BAS 850 H metabolite M850H040 in surface and in drinking water are diluted with water (if needed), filtered and determined by liquid chromatography (LC) electrospray ionization tandem mass spectrometry (ESI-MS/MS), monitoring ion transitions m/z 429→335 (primary quantitation) and m/z 429→296 (secondary quantitation) in the negative mode. The results are calculated by direct comparison of the sample peak responses to those of external standards.

For validation, untreated drinking (well) water and surface (lake) water samples were fortified with M850H040 and analyzed according to the established method validation guidelines. The analytical sets for each water matrix typically consisted of a reagent blank, two controls, five replicates fortified with M850H040 at the method limit of quantitation, 30 ng/L (30 ppt), and five replicates fortified at a higher level, corresponding to 10X the limit of quantitation, 300 ng/L (300 ppt). For M850H040, the two mass transitions above were evaluated. In conjunction with the subject study, matrix- and solvent-matched standards were analyzed in a separate experiment to evaluate any potential matrix effects.

Summary parameters for the analytical method are listed in the table shown below (**Table 2**).

Table 2: Summary Parameters for the Analytical Method Used for the Quantitation of M850H040 Residues in Water

Method ID	BASF Analytical Method No. R0048/01	
Analyte(s)	M850H040 residues in drinking and surface water	
Extraction Solvent/technique	Briefly, M850H040 residues in water samples are diluted with water (if needed), filtered (0.45 µm PTFE) and analyzed by LC-MS/MS.	
Cleanup Strategies	No cleanup required.	
Instrument	Liquid chromatography (LC) electrospray ionization tandem mass spectrometry (ESI-MS/MS) monitoring ion transitions m/z 429→335 and 429→296 in the negative mode. Analyses are performed using a Waters Aquity UPLC system equipped with an Acquity BEH C ₁₈ column (50 x 2.1 mm, 1.7 µm particle size) and a Sciex Instruments API 5500 detector and using a mobile phase gradient of water with 0.1% formic acid:acetonitrile with 0.1% formic acid 85:15 to 50:50 to 5:95, v/v, over 2.5 minutes (flow rate 600 uL/minute).	
Detector	Sciex Instruments API 5500 Mass Spectrometer	
Analyte	Quantitation (m/z)	Confirmation (m/z)
M850H040	429→335	429→296
Ionization Mode	Negative	
Standardization Method	Linear regression (1/x weighting). Direct comparison of the sample peak area responses to those of external standards.	
Stability of Std Solutions	M850H040 is stable in stock or intermediate (fortification) solutions prepared in methanol and in calibration standards prepared by serial dilution of the intermediate standards using water for at least 1 month (32 days), each when held under refrigeration. During the course of this study, the test/reference substance solutions were stored under refrigeration and all solutions were used within the demonstrated time period of stability.	
Expected Retention times (approximate minutes)	M850H040, ~1.7 minutes	

Table obtained from Table 3, pp. 35-36 of MRID 50406045.

Instrument/Detector for Confirmatory Method: Accurate quantitation of M850H040 residues is possible using one chromatographic method and two different mass transitions; therefore, no additional confirmatory techniques are required. The MRM transitions used to identify M850H040 were determined by product ion scan, which were included in the report.

A Method Flow Chart was provided (Appendix A, p. 47 of MRID 50406055).

ILV

The ILV performed the ECM method as written with modifications of the analytical method (p. 19 of MRID 50406056). The ILV parameters are reported in **Table 3** below.

Table 3: Summary Parameters for the Analytical Method Used for the Quantitation of M850H040 Residues in Water

Method ID	BASF Analytical Method No. R0048/01	
Analyte(s)	M850H040 residues in drinking and surface water	
Extraction Solvent/technique	Briefly, M850H040 residues in water samples are diluted with water (if needed), filtered (0.45 µm PTFE) and analyzed by LC-MS/MS.	
Cleanup Strategies	No cleanup required.	
Instrument	Liquid chromatography (LC) electrospray ionization tandem mass spectrometry (ESI-MS/MS) monitoring ion transitions m/z 429→335 and 429→296 in the negative mode. Analyses are performed using a Waters Acquity UPLC system equipped with an Acquity BEH C ₁₈ column (50 x 2.1 mm, 1.7 µm particle size) and an AB Sciex Qtrap 6500 Mass Spectrometer detector and using a mobile phase gradient of water with 0.1% formic acid:acetonitrile with 0.1% formic acid 85:15 to 50:50 to 5:95, v/v, over 3.25 minutes (flow rate 600 µL/minute).	
Detector	AB Sciex Qtrap 6500 Mass Spectrometer	
	Analyte	Confirmation (m/z)
	M850H040	429→296
	Quantitation (m/z)	
	429→335	
Ionization Mode	Negative	
Standardization Method	Linear regression (no weighting). Direct comparison of the sample peak area responses to those of external standards.	
Stability of Std Solutions	The available storage stability data indicate that M850H040 is stable in stock or intermediate (fortification) solutions prepared in methanol and in calibration standards prepared by serial dilution of the intermediate standards using water for at least 1 month (32 days), each when held under refrigeration. During the course of the subject study, the test/reference substance solutions were stored under refrigeration and all solutions were used within the demonstrated time period of stability.	
Expected Retention times (approximate minutes)	M850H040, ~1.35 minutes	

Table obtained from Table 3, p. 43 of MRID 50406045.

Instrument/Detector for Confirmatory Method: Accurate quantitation of M850H040 residues is possible using one chromatographic method and two different mass transitions; therefore, no additional confirmatory techniques are required. The MRM transitions used to identify M850H040 were determined by product ion scan, which were included in a separate method validation report.

Methodology to Evaluate MDL and LOD

Evaluation of LOD of BASF Analytical Method No. R0048/01 required the experimental determination of MDL as defined by 40 CFR Ch.1 Part 136 Appendix B (Reference 2). A brief description of the methodology to determine MDL is as follows:

1. Standard solutions containing M850H040 were injected using LC-MS/MS parameters from R0048/01. All transitions were monitored according to the method. The least sensitive transition was determined qualitatively through visual inspection of factors such as peak height, relative background level, area count, etc. Once the appropriate transition was selected, an estimation was made to what level a sample in matrix would produce a signal to noise (S/N) ratio of 2-10.

2. Using BASF Analytical Method R0048/01, seven (7) control aliquots (2.88 mL) were diluted with a standard (0.12 mL, 0.15 ng/mL in control water – this concentration is 25 times the desired final concentration, determined in step 1) to make the fortified control samples for MDL determination. This procedure is similar to the method section for preparation of matrix matched standards (section 2.6.4).

All samples were then filtered using a 0.45µm PTFE syringe filter directly into HPLC injection vials, passing the first approximately 0.1 - 0.2 mL to waste.

These 7 matrix spiked samples were injected with appropriate bracketing calibration standards on the LC-MS/MS system for quantitation.

3. Using the standard curve to calculate the concentrations of the seven matrix-spiked samples, the results were put into the equation shown below:

$$\text{MDL} = S \times t_{(N-1, 1-\alpha=0.99)}$$

MDL = Method detection limit

S = Standard deviation of the matrix-spiked sample set concentrations

$t_{(N-1, 1-\alpha=0.99)}$ = Critical t value from a student t-test table at 99% confidence

The acceptance criteria for the MDL calculation were:

- a. The calculated MDL must be able to be seen on the instrument with S/N of ≥ 2 .
 - b. The concentration of the matrix-spiked samples must be no greater than 10X the calculated MDL.
4. A fortified control sample at the MDL was injected on the LC-MS/MS (no standard curve required) to verify that the MDL can be seen with a S/N ≥ 2 .

Surface water (Sample No. CM17-052) was used for the LOD determination (Appendix B, Appendix A, p. 108 of MRID 50406055).

LOQ/LOD

The LOQ was defined by the lowest fortification level successfully tested. The validated LOQ for residues of M850H040 in water is 30 ng/L for each analyte (p. 37 of MRID 50406045). The limit of determination is set to be 6 ng/L (0.006 µg/L).

II. Recovery Findings

Method R0048/01

Tier II Summary and Evaluation (MRID 50406045)

ECM (MRID 50406055/Tier II): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD \leq 20%) for analysis of trifludimoxazin metabolite M850H040 (Reg.No. 6095223) in two water matrices at fortification levels of 30 ng/L (LOQ) and 300 ng/L (10 \times LOQ; Table 4, p. 38 of MRID 50406045). M850H040 was identified using two ion transitions; performance data (recovery results) from primary and confirmatory analyses were comparable. Recovery results were corrected for residues quantified in the controls; however, no residues were quantified in the controls (Appendix A, p. 69; Appendix A, Appendix D, pp. 53-56 of MRID 50406055). Solvent-based calibration standards were used. The drinking (well) water (Sample No. CM17-053; pH 8.0, 0.1 ppm total organic carbon, 36 ppm calcium, 101 mg equiv. CaCO₃/L hardness) obtained from Bahama, North Carolina, and surface (lake) water (Sample No. CM17-052; pH 8.9, 18.6 ppm total organic carbon, 93 ppm calcium, 657 mg equiv. CaCO₃/L hardness) obtained Golden Lake, North Dakota, were used (Appendix A, Appendix K, pp. 86-88 of MRID 50406055). Test systems were characterized by Agvise Laboratories: drinking water in conjunction with this study and surface water under BASF Study # 805401 (DocID: Not yet available).

ILV (MRID 50406056/Tier II): Mean recoveries and RSDs were within guideline requirements for analysis of trifludimoxazin metabolite M850H040 (Reg.No. 6095223) in two water matrices at fortification levels of 0.03 μ g/L (LOQ) and 0.3 μ g/L (10 \times LOQ; Table 4, p. 45 of MRID 50406045). M850H040 was identified using two ion transitions; performance data (recovery results) from primary and confirmatory analyses were comparable. Recovery results were corrected when residues were quantified in the controls (p. 21 of MRID 50406056). Solvent-based calibration standards were used. Drinking (well) water (Sample No. CM17-053; OMIC Sample ID 18CB03_DW; pH 8.0, 0.1 ppm total organic carbon, 36 ppm calcium, 101 mg equiv. CaCO₃/L hardness) and surface (lake) water (Sample No. CM17-052; OMIC Sample ID 18CB03_SW; Golden Lake Water; pH 8.9, 18.6 ppm total organic carbon, 93 ppm calcium, 657 mg equiv. CaCO₃/L hardness) were provided by BASF and used in the study (p. 12; Appendix 10.7, pp. 50-51 of MRID 50406056). The surface water was the same as that of the ECM. The ILV validated BASF Method R0048/01 in surface and drinking water for M850H040 during the second trial (p. 19 of MRID 50406056; p. 44 of MRID 50406045). The first ILV trial was unsuccessful due to insufficient instrument performance. In the successful second attempt at validation, acceptable sensitivity for M850H040 was obtained using a Sciex QTrap 6500 mass spectrometer (LOD equivalent to 0.006 ng/mL in the sample), and the injection volume was increased from 10 to 50 μ L for both primary and confirmation transitions. No recommended ILV modifications to the method were reported.

Table 1: Method Validation Recoveries for M850H040 Residues in Fortified Control Water Samples Using BASF Analytical Method R0048/01¹

Analyte	Matrix	Fortification Levels (ppt)	n	Recovery (%)	Average Rec. (%)	Standard Deviation	%RSD ^a
M850H040	Surface water	Primary Transition (<i>m/z</i> 429→335)					
		30	5	86, 86, 78, 88, 92	86	5	6
		300	5	100, 104, 110, 102, 105	104	4	4
		Overall	10	Range, 78 - 110	95	10	11
		Secondary Transition (<i>m/z</i> 429→296)					
		30	5	86, 87, 87, 86, 88	87	1	1
		300	5	96, 111, 109, 105, 106	105	6	5
		Overall	10	Range, 86 - 111	96	11	11
M850H040	Drinking water	Primary Transition (<i>m/z</i> 429→335)					
		30	5	98, 107, 109, 106, 101	104	5	4
		300	5	109, 104, 117, 103, 105	108	6	5
		Overall	10	Range, 98 - 117	106	5	5
		Secondary Transition (<i>m/z</i> 429→296)					
		30	5	103, 112, 106, 109, 111	108	4	3
		300	5	109, 112, 119, 105, 105	110	6	5
		Overall	10	Range, 103 - 119	109	5	4

^a Relative Standard Deviation = (Standard Deviation ÷ Average Recovery) × 100

Table obtained from Table 4, p. 38 of MRID 50406045. Recovery results were corrected for residues quantified in the controls; however, no corrections were made (Appendix A, p. 49 of MRID 50406055).

¹ Drinking (well) water (Sample No. CM17-053; pH 8.0, 0.1 ppm total organic carbon, 36 ppm calcium, 101 mg equiv. CaCO₃/L hardness) obtained from Bahama, North Carolina, and surface (lake) water (Sample No. CM17-052; pH 8.9, 18.6 ppm total organic carbon, 93 ppm calcium, 657 mg equiv. CaCO₃/L hardness) obtained Golden Lake, North Dakota, were used (Appendix A, Appendix K, pp. 86-88 of MRID 50406055). Test systems were characterized by Agvise Laboratories: drinking water in conjunction with this study and surface water under BASF Study # 805401 (DocID: Not yet available).

Table 5: Independent Laboratory Validation Recoveries for M850H040 Residues in Fortified Control Water Samples Using BASF Analytical Method R0048/01¹

Matrix	Fortification Levels (ng/L)	n	Recovery (%)	Average Recovery (%)	Standard Dev.	% RSD ^a
Drinking Water	Primary Transition (<i>m/z</i> 429 → 335)					
	30	5	96.2, 120, 107, 115, 121	112	10.3	9.19
	300	5	101, 125, 94.7, 138, 99.4	112	18.8	16.8
	Overall	10	Range: 94.7 – 138	112	14.3	12.8
	Confirmation Transition (<i>m/z</i> 429 → 296)					
	30	5	97.6, 121, 106, 113, 122	112	10.3	9.18
	300	5	109, 116, 94.0, 134, 94.5	110	16.8	15.4
Overall	10	Range: 94.0 – 134	111	13.2	11.9	
Surface Water	Primary Transition (<i>m/z</i> 429 → 335)					
	30	5	86.3, 85.2, 82.8, 86.3, 88.4	85.8	2.02	2.35
	300	5	90.0, 90.4, 87.9, 88.6, 96.1	90.6	3.26	3.60
	Overall	10	Range: 82.8 – 96.1	88.2	3.58	4.06
	Confirmation Transition (<i>m/z</i> 429 → 296)					
	30	5	87.5, 90.7, 86.3, 87.5, 90.0	88.4	1.88	2.12
	300	5	87.7, 88.1, 88.3, 87.8, 92.5	88.9	2.05	2.31
Overall	10	Range: 86.3 – 92.5	88.6	1.87	2.11	

^a Relative Standard Deviation = (Standard Deviation ÷ Average Recovery) × 100

Table obtained from Table 4, p. 45 of MRID 50406045. Recovery results were corrected when residues were quantified in the controls (p. 21 of MRID 50406056).

¹ Drinking (well) water (Sample No. CM17-053; OMIC Sample ID 18CB03_DW; pH 8.0, 0.1 ppm total organic carbon, 36 ppm calcium, 101 mg equiv. CaCO₃/L hardness) and surface (lake) water (Sample No. CM17-052; OMIC Sample ID 18CB03_SW; Golden Lake Water; pH 8.9, 18.6 ppm total organic carbon, 93 ppm calcium, 657 mg equiv. CaCO₃/L hardness) were provided by BASF and used in the study (p. 12; Appendix 10.7, pp. 50-51 of MRID 50406056). The surface water was the same as that of the ECM.

III. Method Characteristics

The LOQ was defined as the lowest fortification level tested (p. 37 of MRID 50406045). The LOQ for residues of BAS 850 H metabolite M850H040 in water was 30 ng/L (30 ppt, 0.030 µg/mL, 0.030 ppb). The method LOD was defined as the lowest analyte concentration injected as a calibration solution, resulting in an LOD of 6 ng/L (0.006 µg/L, 20% of the LOQ). The report stated that the LOD was shown to be detectable as the absolute amount of analyte injected (1.2 pg on column) into the LC-MS/MS when the lowest calibration standard was analyzed (6 ng/L) with acceptable signal to noise ratio (S/N) greater than 3:1.

To determine the least sensitive transition for the purpose of LOD determination, solvent based standard solutions were injected using the validated analytical LC-MS/MS method (all transitions were evaluated) from method R0048/01. It was determined qualitatively that the MS/MS ion transition for M850H040 (*m/z* 429 → *m/z* 296) was the least sensitive transition and

therefore the best candidate to conduct the LOD evaluation.

The MDL was calculated to be 0.0000232 ng on-column for M850H040. To determine this value, the seven (7) control samples were prepared according to method R0048/01 and were fortified with standard solution prior to the LC-MS/MS determination step to achieve a concentration of 0.006 ng/mL. 0.010 mL (10 μ L, 0.00006 ng on-column) of each sample was injected on the LC-MS/MS system according to method R0048/01. Calculation of MDL for M850H040 was conducted according to the table provided in 40 CFR Ch. 1 Part 136 Appendix B.

To verify this MDL, a matrix spiked control sample was intended to be injected in triplicate at the calculated MDL to verify a signal to noise ratio ≥ 2 . This test was actually performed at 0.000024 ng on-column and produced a signal to noise ratio of approximately 2. As the tested concentration yielded a reasonable signal for an MDL and it meets all other acceptance criteria, the more conservative MDL of 0.000024 ng on-column is being reported. Chromatograms of a matrix-spiked sample at MDL of 0.000024 ng were provided in the report 6.

The instrument signal at MDL is too low for accurate quantitation. For quantitative analysis, the LOD will be set at the lowest calibration standard (6 ng/mL) analyzed during method. This LOD corresponds to 0.00006 ng injected on-column (i.e. 0.006 ng/mL injected at 0.01 mL).

Table 6: Calculation of MDL for M850H040 (m/z 429 \rightarrow m/z 296) for Method R0048/01, using the Methodology Set Forth in 40 CFR Ch 1 Part 136 Appendix B

0.006 ng/mL Matrix-Spike Replicate	Calculated Concentration (ng/mL)
1	0.00547
2	0.00407
3	0.00481
4	0.00352
5	0.00478
6	0.00549
7	0.00417
Standard Deviation (S) =	0.000737
N-1 =	6
Critical t value (t) =	3.143
Injection Volume (μ L)	10
MDL (ng/mL) =	0.00232
MDL (ng on-column) =	0.0000232

$$\text{MDL on-column (pg)} = \text{MDL (ng/mL)} \times \text{Injection Volume (\mu L)}$$

Table obtained from Table 5, p. 39 of MRID 50406045.

Table 7. Method Characteristics - Analytical Method R0048/01

Analyte		M850H040 (Reg.No. 6095223)
Limit of Quantitation (LOQ)	ECM	30 ng/L (0.030 µg/mL)
	ILV	
Limit of Detection (LOD)	ECM	6 ng/L (0.006 µg/L, 20% of the LOQ)
	ILV	
Linearity (calibration curve r and concentration range) ¹	ECM	r = 0.9995 (SW, Q) r = 0.9981 (SW, C) r = 0.9998 (DW, Q & C)
	ILV	r = 0.99820 (SW, Q) r = 0.99881 (SW, C) r = 0.99902 (DW, Q) r = 0.99876 (DW, C)
	Range	0.006-0.15 ng/mL
Repeatable	ECM ²	Yes at LOQ and 10×LOQ (two characterized water matrices)
	ILV ^{3,4}	
Reproducible		Yes at LOQ and 10×LOQ in DW and SW.
Specific	ECM	Yes, no matrix interferences were observed.
	ILV	Yes, no matrix interferences were observed; however, a nearby minor, broad peak (RT <i>ca.</i> 1.55 min.) interfered with analyte peak attenuation and integration.

Data were obtained from MRID 50406045; Appendix A, Appendix D, pp. 53-56 (raw data sheets); Appendix A, Appendix H, pp. 67-68 (calibration curves) and Appendix A, Appendix I, pp. 72-83 (chromatograms) of MRID 50406055; Appendix 10.2, pp. 23-26 (detailed data); Appendix 10.5, pp. 32-48 (calibration curves and chromatograms) of MRID 50406056. Q = Quantitation ion transition; C = Confirmation ion transition. DW = Drinking water; SW = surface water.

1 Solvent-based calibration standards were used in the ECM and ILV.

2 In the ECM, drinking (well) water (Sample No. CM17-053; pH 8.0, 0.1 ppm total organic carbon, 36 ppm calcium, 101 mg equiv. CaCO₃/L hardness) obtained from Bahama, North Carolina, and surface (lake) water (Sample No. CM17-052; pH 8.9, 18.6 ppm total organic carbon, 93 ppm calcium, 657 mg equiv. CaCO₃/L hardness) obtained Golden Lake, North Dakota, were used (Appendix A, Appendix K, pp. 86-88 of MRID 50406055). Test systems were characterized by Agvise Laboratories: drinking water in conjunction with this study and surface water under BASF Study # 805401 (DocID: Not yet available).

3 In the ILV, drinking (well) water (Sample No. CM17-053; OMIC Sample ID 18CB03_DW; pH 8.0, 0.1 ppm total organic carbon, 36 ppm calcium, 101 mg equiv. CaCO₃/L hardness) and surface (lake) water (Sample No. CM17-052; OMIC Sample ID 18CB03_SW; Golden Lake Water; pH 8.9, 18.6 ppm total organic carbon, 93 ppm calcium, 657 mg equiv. CaCO₃/L hardness) were provided by BASF and used in the study (p. 12; Appendix 10.7, pp. 50-51 of MRID 50406056). The surface water was the same as that of the ECM.

4 The independent laboratory validation of BASF Method R0048/01 in surface and drinking water for M850H040 during the second trial with modifications to the analytical instruments and parameters (p. 19 of MRID 50406056; p. 44 of MRID 50406045). No recommended ILV modifications to the method were reported.

IV. Method Deficiencies and Reviewer's Comments

1. Although the ILV studies were conducted at a separate testing facility, there was a large amount of communication with the original laboratory (Appendix 10.8 p. 52 of MRID 50406056). These correspondences do not suggest collusion; however, detailed communications should be provided for review. There was no evidence that the analysts, study director, equipment, instruments, and supplies of the two laboratories were not distinct.
2. It could not be determined if the ILV was performed with the most difficult matrices with which to validate the method since the ILV surface water matrix was the same as that of the ECM.
3. The reviewer believed that more details regarding the failed ILV trial should be provided to assess the requirement for an updated ECM, including the analytical instrument and parameters and the unacceptable recovery data. The first ILV trial was unsuccessful due to insufficient instrument performance (p. 19 of MRID 50406056; p. 44 of MRID 50406045). In the successful second attempt at validation, acceptable sensitivity for M850H040 was obtained using a Sciex QTrap 6500 mass spectrometer (LOD equivalent to 0.006 ng/mL in the sample), and the injection volume was increased from 10 to 50 μ L for both primary and confirmation transitions. No recommended ILV modifications to the method were reported, and analytical optimization is a normal laboratory process; however, ILV modifications which were required to perform a successful trial are usually considered significant by the reviewer.
4. The specificity of the method was not well-supported by ILV representative chromatograms due to a nearby minor, broad peak (RT *ca.* 1.55 min.) that interfered with analyte peak attenuation and integration (Appendix 10.5, pp. 32-48 of MRID 50406056).
5. It was reported for the ILV that one sample set of 13 samples required *ca.* 4-6 hours of work, including LC/MS/MS and calculation of results (p. 17 of MRID 50406056).
6. The eco-toxicology endpoints in water (NOEC or LC50) for M850H040 have not been determined. However, toxicological levels of concern for the parent molecule and all other relevant metabolites of trifludimoxazin are greater than the LOQ of the method (p. 33 of MRID 50406045).

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.

MRID 50406045. Description of methods for analysis of water (parent and metabolites), Summary and evaluation (Tier II), BASF Reg. Doc. # 2018/7000015 US. BASF Corporation 26 Davis Drive P.O. Box 13528 Research Triangle Park, NC 27709.

Attachment 1: Chemical Names and Structures**2.2.1 M850H040**

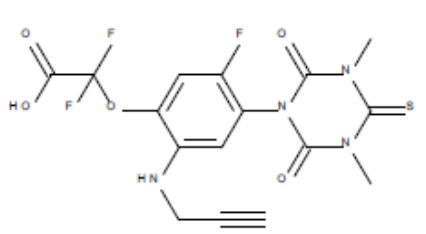
Common Name	None assigned	<p>Chemical structure:</p> 
BAS Code Name	M850H040	
Chemical Name	{4-(3,5-dimethyl-2,6-dioxo-4-sulfanylidene-1,3,5-triazinan-1-yl)-5-fluoro-2-[(prop-2-yn-1-yl)amino]phenoxy}(difluoro)acetic acid	
BASF Reg. No.	6095223	
Molecular Formula	C ₁₈ H ₁₃ F ₃ N ₄ O ₅ S	
Molecular Weight	430.4	
Lot No.	L2017-109	
Purity:	87.2%	
Expiration Date	December 01, 2019	

Figure from p. 19 of MRID 50406055.