Analytical method for trifludimoxazin (BAS 850 H) and its metabolites M850H001, M850H002, M850H003, M850H004, M850H012, and M850H035 in water

Reports: ECM: EPA MRID No.: 50406039. Delinsky, D. 2018. Methods of Analysis of BAS 850 H and its Relevant Metabolites in Water with Limit of Determination (LOD) Calculation (Method D1724/01). BASF Study Nos.: 784160 and 784160_1 (Appendix A, p. 8; Appendix B, p. 258). BASF Registration Document No.: 2017/7017089. Report prepared, sponsored and submitted by BASF Corporation, Research Triangle Park, North Carolina, North Carolina; 317 pages. Final report issued February 14, 2018.

ILV: EPA MRID No. 50406040. Perez, S. 2018. Independent Laboratory Validation of BASF Analytical Method D1724/01: “Method for the determination of BAS 850 H (Reg.No. 5654329) and M850H001 (Reg.No. 5749359), M850H002 (Reg.No. 5757725), M850H003 (Reg.No. 5757726), M850H004 (Reg.No. 5833884), M850H012 (Reg.No. 5797901), and M850H035 (Reg.No. 6070203) in Surface and Drinking Water by LC-MS/MS”. BASF Study ID No.: 826949. BASF Registration Document No.: 2017/7008201. ADPEN Study No.: 17K0204. Report prepared by ADPEN Laboratories, Inc., Jacksonville, Florida, sponsored and submitted by BASF Corporation, Research Triangle Park, North Carolina; 457 pages. Final report issued February 15, 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 50406039 & 50406040 & 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 50406039). 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 # 784160 and 784160_1) were conducted in accordance with USEPA FIFRA GLP standards, 40 CFR, Part 160 (Appendix A, p. 10; Appendix B, p. 260). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (Appendix A, pp. 9-12; Appendix B, pp. 259-262).

ILV: The study was conducted in accordance with USEPA FIFRA GLP standards, 40 CFR, Part 160 (p. 3 of MRID 50406040). 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 **unacceptable**. The ILV was not independent. Otherwise, the method satisfied the repeatability and reproducibility criteria, with RSDs generally < 20% and mean recoveries in the range of 70 – 120%, except ILV performance data was not acceptable for the quantitation analysis of M850H035 in surface water.

PC Code: 080800

EFED Final Reviewer:	William Gardner, Ph.D., Environmental Scientist	Signature: Date: 11/2/2020	William Gardner Digitally signed by William Gardner Date: 2020.11.02 15:08:17 -05'00'
CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, Environmental Scientist	Signature:	Lisa Muto
		Date:	11/26/2019
	Mary Samuel, M.S., Environmental Scientist	Signature:	MarySamuel
		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 D1724/01, is designed for the quantitative determination of trifludimoxazin (BAS 850 H), M850H001, M850H002, M850H003, M850H004, M850H012, and M850H035 in water at the LOQ of 0.03 µg/L using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern (for BAS 850 H, 0.115 µg/L); in water for trifludimoxazin and its six metabolites. The ECM and ILV performed the method with two characterized water matrices: surface water and drinking (well) water; the ILV water matrices were the same as those of the ECM. The ILV validated BASF method (D1724/01) for all analytes in drinking water and surface water within two attempts, except M850H004 in surface water. M850H004 in surface water required three attempts before the method could be successfully validated, due to some unforeseen, avoidable experimental circumstances, but also the need for minor modifications including slight changes to the LC mobile phase gradient (alternate LC-MS/MS conditions were used) as well as changes to the matrix-matched standards. The ILV recommended modifications were included in the final submitted ECM. The ILV was not conducted independently of the ECM since the BASF Study Monitor for the ILV was also the ECM study author and technical communication occurred. All ILV and ECM data regarding repeatability, accuracy, precision, and specificity were satisfactory for all seven analytes, except for ILV performance data for M850H035 in surface water. ILV linearity was not satisfactory for all analytes in both matrices, except for M850H004 in drinking water. ECM linearity was not satisfactory for BAS 850 H in surface water, for M850H002 in both matrices, and for M850H003 in drinking water.

In a separate study, the method LOD of BASF Analytical Method No. D1724/01 was validated in accordance with the methodology set forth in 40 CFR Ch. 1 Part 136 Appendix B. BASF analytical method D1724/01 has two preparations/cleanups (simple dilution for BAS 850 H, M850H002, M850H003, M850H004, M850H012, and M850H035; and a liquid-liquid partition / concentration for M850H001). An MDL calculation and subsequent LOD evaluation were conducted for each preparation/cleanup based on the least sensitive analyte. Based on the calculated MDLs for M850H012 and M850H001, the LOD was calculated as 6 ng/L for both the simple dilution preparation and the liquid-liquid partition preparation.

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

All referenced page numbers for MRID 50406039 refer to those reported on the right-handed margin of the document pages.

Table 1. Analytical Method Summary – BASF Analytical Method D1724/01

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						
Trifludimoxazin (BAS 850 H; Reg. No. 5654329)	50406039[1]	50406040		Water[2,3]	14/02/2018[4]	BASF Corporation	LC/MS/MS	30 ng/L
M850H001 (Reg.No. 5749359)								
M850H002 (Reg.No. 5757725)								
M850H003 (Reg.No. 5757726)								
M850H004 (Reg.No. 5833884)								
M850H012 (Reg.No. 5797901)								
M850H035 (Reg.No. 6070203)								

1 A compilation of BASF Study #s 784160 and 784160_1 (Appendix A, pp. 8, 23-25; Appendix B, p. 258 of MRID 50406039).

2 In the ECM, drinking (well) water (Sample No. CM15-030; pH 7.5, 0.8 ppm total organic carbon, 42 ppm calcium, 116 mg equiv. $CaCO_3$/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. $CaCO_3$/L hardness) obtained Golden Lake, North Dakota, were used (Appendix A, Appendix K, pp. 248-250 of MRID 50406039). Test systems were characterized by Agvise Laboratories as part of previous studies, drinking water under BASF Study # 433575 (DocID: 2015/7001125) and surface water under BASF Study # 805401 (DocID: Not yet available).

3 In the ILV, drinking (well) water (Sample No. CM15-030; pH 7.5, 0.8 ppm total organic carbon, 42 ppm calcium, 116 mg equiv. $CaCO_3$/L hardness) and surface (lake) water (Sample No. CM17-052; Golden Lake Water; pH 8.9, 18.6 ppm total organic carbon, 93 ppm calcium, 657 mg equiv. $CaCO_3$/L hardness) were provided by BASF and used in the study (p. 16; Appendix A, pp. 386-387 of MRID 50406040). The test systems were the same as those of the ECM.

4 Method dates were February 14, 2018 for BASF Study # 784160 and January 26, 2018 for BASF Study # 784160_1 (Appendix A, p. 8; Appendix B, p. 258 of MRID 50406039).

I. Principle of the Method

Method D1724/01

BAS 850 H and its metabolites M850H001, M850H002, M850H003, M850H004, M850H012, and M850H035 in surface and in drinking water are diluted with methanol containing 0.5% formic acid and filtered. In a separate analysis, M850H001 residues in water samples are acidified, partitioned with ethyl acetate:cyclohexane (10:90, v/v), and centrifuged; residues in an aliquot of the organic layer are then evaporated to dryness, re-dissolved in a final volume of methanol:water with 0.1% formic acid (20:80 v/v), and filtered. Following clean-up by filtration or liquid/liquid partitioning and filtration, as described above, residues of trifludimoxazin in water are determined by liquid chromatography (LC) electrospray ionization tandem mass spectrometry (ESI-MS/MS), monitoring ion transitions m/z 413→74 for parent trifludimoxazin; m/z 397→141 and 397→134 for M850H001; m/z 373→323 and 373→193 for M850H002; m/z 357→307 and 357→193 for M850H003; m/z 387→131 and 387→74 for M850H004; m/z

257→163 and 257→116 for M850H012; or m/z 371→257 and 371→163 for M850H035. In lieu of a secondary (alternate) ion transition for parent trifludimoxazin, confirmatory analysis is performed using a separate chromatographic technique with a different LC column and gradient. 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 each analyte 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 analyte 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 each analyte, the two mass transitions or chromatographic techniques described 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 Residues of Trifludimoxazin in Water

Method ID	**BASF method D1724/01**	
Analyte(s)	Residues of trifludimoxazin (BAS 850 H) and its metabolites M850H001, M850H002, M850H003, M850H004, M850H012 and M850H035 in drinking and surface water	
Extraction Solvent/technique	Briefly, residues of trifludimoxazin and metabolites (except M850H001) in water samples (10 mL each) are diluted with acidified methanol, filtered (0.45 µm PTFE) and analyzed by LC-MS/MS. M850H001 residues in a separate aliquot of the water samples are acidified, partitioned with ethyl acetate:cyclohexane (10:90, v/v), and centrifuged; residues in an aliquot of the organic layer are then evaporated to dryness, re-dissolved in a final volume of methanol:water with 0.1% formic acid (20:80 v/v), filtered (0.45 µm PTFE) and analyzed by LC-MS/MS.	
Cleanup Strategies	Centrifugation; liquid/liquid partition; filtration.	
Instrument	Analyses for most of the analytes are performed using a Waters Aquity UPLC system equipped with a Acquity HSS T3 column (100 x 2.1 mm, 1.8 µm particle size) and a detector and using a mobile phase gradient of water with 1% formic acid:methanol with 0.1% formic acid 85:15, 60:40, 30:70, 5:95, to 85:15, v/v, over 6.5 minutes (flow rate 500 uL/minute). For the analysis of M850H001 (both ion transitions) and confirmatory analysis for parent trifludimoxazin are conducted with a separate chromatographic technique, in separate injections for each analyte, using the same UPLC system and detector equipped with a Waters Acquity BEH C_{18} column (50 x 2.1 mm, 1.7 µm particle size) using a mobile phase gradient of water with 1% formic acid:methanol with 0.1% formic acid, 85:15 to 40:60, v/v, over 6.25 minutes (flow rate 600 uL/minute).	
Detector	Sciex Instruments API 6500 Mass Spectrometer	
Analyte	Quantitation (*m/z*)	Confirmation (*m/z*)
Parent trifludimoxazin	413→74	413→74*
M850H001	397→141	397→134
M850H002	373→323	373→193
M850H003	357→307	357→193
M850H004	387→131	387→74
M850H012	257→163	257→116
M850H035	371→257	371→163

Ionization Mode	Positive for trifludimoxazin, M850H001, M850H004, M850H012 and M850H035; negative for M850H002 and M850H003
Standardization Method	Linear regression (1/x 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 each analyte is stable in stock solutions prepared in 0.1% formic acid in methanol for at least 3 months (≥81 days), or acetone in the case of M850H035 for at least 1 month (43 days), when held under refrigeration. In addition, the data indicate that each analyte is stable in mixed intermediate (fortification) standards prepared by diluting combined aliquots of the stock solutions with 0.1% formic acid in methanol and in mixed calibration standards prepared by serial dilution of the intermediate standards using methanol:water (20:80, v/v) with 0.1% formic acid for at least 1 month (≥27 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)	Parent trifludimoxazin, ~5.0 (for alternate chromatographic technique, ~6.1); M850H002, ~4.7; M850H003, ~3.2; M850H004, ~4.7; M850H012, ~3.0; M850H035, ~4.4; M850H001, ~4.3

* For confirmation, the same mass transition is used with a separate chromatographic technique.

Table obtained from Table 1, p. 9 of MRID 50406045.

Instrument/Detector for Confirmatory Method: For each analyte, except as noted for trifludimoxazin, accurate quantitation is possible using one chromatographic method and two different mass transitions; therefore, no additional confirmatory techniques are required. In lieu of a secondary (alternate) ion transition for parent trifludimoxazin, confirmatory analysis is performed using a different LC column and gradient.

A Method Flow Chart was provided (Appendix A, p. 70 of MRID 50406039).

ILV

The ILV performed the ECM method as written with modifications of the analytical method; the ILV recommended some method modifications (p. 32 of MRID 50406040). The ILV parameters are reported in **Table 3** below.

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

Method ID	**BASF method D1724/01**
Analyte(s)	Residues of trifludimoxazin (BAS 850 H) and its metabolites M850H001, M850H002, M850H003, M850H004, M850H012 and M850H035 in drinking and surface water
Extraction Solvent/technique	Briefly, residues of trifludimoxazin and metabolites (except M850H001) in water samples (10 mL each) are diluted with acidified methanol, filtered (0.45 µm PTFE) and analyzed by LC-MS/MS. M850H001 residues in a separate aliquot of the water samples are acidified, partitioned with ethyl acetate:cyclohexane (10:90, v/v), and centrifuged; residues in an aliquot of the organic layer are then evaporated to dryness, re-dissolved in a final volume of methanol:water with 0.1% formic acid (20:80 v/v), filtered (0.45 µm PTFE) and analyzed by LC-MS/MS.
Cleanup Strategies	Centrifugation; liquid/liquid partition; filtration.
Instrument	Analyses for most of the analytes are performed using a Agilent 1290 UPLC system equipped with an HSS T3 column (100 x 2.1 mm, 1.8 µm particle size) and a ABSciex 6500 Triple Quad detector and using a mobile phase gradient of (A) water with 1% formic acid and (B) methanol with 0.1% formic acid 85:15, 60:40, 30:70, 5:95, to 85:15, v/v, over 5.75 minutes (flow rate 500 uL/minute); alternate conditions were used for M850H004 in surface water and consisted of similar

	parameters with a longer run time. For the analysis of M850H001 (both ion transitions) and confirmatory analysis for parent trifludimoxazin are conducted with a separate chromatographic technique, in separate injections for each analyte, using the same UPLC system and detector equipped with a BEH C_{18} column (50 x 2.1 mm, 1.7 µm particle size) using a mobile phase gradient of (A) water with 1% formic acid and (B) methanol with 0.1% formic acid, 85:15 to 40:60, v/v, over 7.5 minutes (flow rate 600 uL/minute).	
Detector	ABSciex 6500 Triple Quad Mass Spectrometer	
Analyte	Quantitation (*m/z*)	Confirmation (*m/z*)
Parent trifludimoxazin	413→74	413→74*
M850H001	397→141	397→134
M850H002	373→193	373→323
M850H003	357→307	357→137
M850H004	387→131	387→74
M850H012	257→163	257→116
M850H035	371→257	371→163
Ionization Mode	Positive for trifludimoxazin, M850H001, M850H004, M850H012 and M850H035; negative for M850H002 and M850H003	
Standardization Method	Linear regression (1/x 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 each analyte is stable in stock solutions prepared in 0.1% formic acid in methanol for at least 3 months (≥81 days), or acetone in the case of M850H035 for at least 1 month (43 days), when held under refrigeration. In addition, the data indicate that each analyte is stable in mixed intermediate (fortification) standards prepared by diluting combined aliquots of the stock solutions with 0.1% formic acid in methanol and in mixed calibration standards prepared by serial dilution of the intermediate standards using methanol:water (20:80, v/v) with 0.1% formic acid for at least 1 month (≥27 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.	
Retention times (approximate minutes)	Parent trifludimoxazin, ~5.7 (for alternate chromatographic technique, ~7.2); M850H002, ~5.6; M850H003, ~4.0; M850H004, ~5.5; M850H012, ~3.8; M850H035, ~5.2; M850H001, ~4.8	

* For confirmation, the same mass transition is used with a separate chromatographic technique.

Table obtained from Table 5, p. 24 of MRID 50406045.

Extract stability for water matrices was not established in this study. Extract stability in the final volume solution (20:80 methanol-water with 0.1% formic acid, v/v) was established in the method validation study, 7 days for surface water and 6 days for drinking water.

Instrument/Detector for Confirmatory Method: For each analyte, except as noted for trifludimoxazin, accurate quantitation is possible using one chromatographic method and two different mass transitions; therefore, no additional confirmatory techniques are required. In lieu of a secondary (alternate) ion transition for parent trifludimoxazin, confirmatory analysis is performed using a different LC column and gradient.

Methodology to Evaluate MDL and LOD

Evaluation of LOD of BASF Analytical Method No. D1724/01required the experimental determination of MDL as defined by 40 CFR Ch.1 Part 136 Appendix B (Reference 2). Method

D1724/01 has one limit of quantitation (LOQ); however, it has two preparations/clean-ups: one for BAS 850 H and metabolites M850H002, M850H003, M850H004, M850H012, and M850H035; and one for M850H001. Consequently, two independent LOD determinations were conducted within this study. A brief description of the methodology to determine MDL is as follows:

1. Injections of standards containing all analytes were injected using LC-MS/MS parameters from D1724/01. All transitions were monitored according to the method. The least sensitive transition of the least sensitive analyte for each preparation/clean-up was determined qualitatively through visual inspection of factors such as peak height, relative background level, area count, etc. Once the appropriate analytes and transitions were selected, an estimation was made to what level a sample in matrix would produce a S/N of 2-10.
2. Using BASF Analytical Method D1724/01, seven control sample aliquots (5 mL) were acidified (0.05 mL water with 10% formic acid). 10 mL cyclohexane-ethyl acetate (90:10, v/v) was added to each sample, mixed and centrifuged. An aliquot (8 mL) of the organic layer was evaporated to dryness and reconstituted with 0.2 mL of a standard (methanol with 0.1% formic acid at a concentration 5 times the desired final concentration, determined in step 1), 0.2 mL methanol with 0.1% formic acid, and 0.6 mL water with 0.1% formic acid to make the post-extraction fortified control samples for LOD determination.

   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 14 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:

$$\text{MDL} = \text{S x } t_{(\text{N-1,1-}\infty=.99)}$$

   MDL = Method detection limit

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

   $t_{(\text{N-1,1-}\infty=.99)}$ = 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 post-extraction 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. 278 of MRID 50406039).

LOQ/LOD

The LOQ was defined by the lowest fortification level successfully tested. The validated LOQ for residues of BAS 850 H and its metabolites in water is 30 ng/L for each analyte (p. 26 of MRID 50406045). The limit of determination is set to be 6 ng/L (0.006 µg/L) for each analyte in water.

II. Recovery Findings

Method D1724/01

Tier II Summary and Evaluation (MRID 50406045)

ECM (MRID 50406039/Tier II): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of trifludimoxazin (BAS 850 H; Reg.No. 5654329), M850H001 (Reg.No. 5749359), M850H002 (Reg.No. 5757725), M850H003 (Reg.No. 5757726), M850H004 (Reg.No. 5833884), M850H012 (Reg.No. 5797901), and M850H035 (Reg.No. 6070203) in two water matrices at fortification levels of 30 ng/L (LOQ) and 300 ng/L (10×LOQ; Table 2, pp. 13-15 of MRID 50406045). Analytes were 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 corrections were made (Appendix A, p. 69; Appendix A, Appendix D, pp. 76-105 of MRID 50406039). Matrix-matched calibration standards were used. Drinking (well) water (Sample No. CM15-030; pH 7.5, 0.8 ppm total organic carbon, 42 ppm calcium, 116 mg equiv. $CaCO_3$/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. $CaCO_3$/L hardness) obtained Golden Lake, North Dakota, were used (Appendix A, Appendix K, pp. 248-250 of MRID 50406039). Test systems were characterized by Agvise Laboratories as part of previous studies, drinking water under BASF Study # 433575 (DocID: 2015/7001125) and surface water under BASF Study # 805401 (DocID: Not yet available).

ILV (MRID 50406040/Tier II): Mean recoveries and RSDs were within guideline requirements for analysis of trifludimoxazin (BAS 850 H; Reg.No. 5654329), M850H001 (Reg.No. 5749359), M850H002 (Reg.No. 5757725), M850H003 (Reg.No. 5757726), M850H004 (Reg.No. 5833884), and M850H012 (Reg.No. 5797901) in two water matrices at fortification levels of 0.03 µg/L (LOQ) and 0.3 µg/L (10×LOQ), except for the LOQ confirmation analysis of M850H001 in drinking water (RSD 22.3%; Table 6, pp. 27-30 of MRID 50406045). Mean recoveries and RSDs were within guideline requirements for analysis of M850H035 (Reg.No. 6070203) in drinking water at fortification levels of 0.03 µg/L (LOQ) and 0.3 µg/L (10×LOQ);

however, the quantitation analysis in surface water was unacceptable (RSD 21.1% LOQ, 22.1% 10×LOQ; confirmation analysis was acceptable). Analytes were identified using two ion transitions; performance data (recovery results) from primary and confirmatory analyses were comparable. Matrix-matched calibration standards were used. Drinking (well) water (Sample No. CM15-030; pH 7.5, 0.8 ppm total organic carbon, 42 ppm calcium, 116 mg equiv. $CaCO_3$/L hardness) and surface (lake) water (Sample No. CM17-052; Golden Lake Water; pH 8.9, 18.6 ppm total organic carbon, 93 ppm calcium, 657 mg equiv. $CaCO_3$/L hardness) were provided by BASF and used in the study (p. 16; Appendix A, pp. 386-387 of MRID 50406040). The test systems were the same as those of the ECM. The ILV validated BASF Method D1724/01 in surface water for BAS 850 H, M850H002, M850H003, M850H012 and M850H035 analytes during the first trial (pp. 9, 32 of MRID 50406040). The M850H004 analyte did not run during the first trial due to an unresolved issue. The first trial was repeated, and the recovery results were high. After removing matrix effects with minor modifications, low recoveries were observed and failed the second trial (presumed due to compromised formic acid). It was then successfully performed in the third trial after additional minor modifications. The independent laboratory validation was performed successfully for M850H001 in surface water during the second trial. The ILV validated BASF Method D1724/01 in drinking water for BAS 850 H, M850H002, M850H003, and M850H012 during the first trial. The independent laboratory validation was performed successfully for M850H001, M850H004 and M850H035 in drinking water during the second trial. The ILV recommended the following modifications to the ECM: 1) changes to the organic rinse and equilibrium times to reduce matrix components at the time of analyte elution and allow for full analytical column re-equilibration for some LC systems; and 2) slight changes to the formic acid concentration in the matrix-matched calibration standards for each analyte (except M850H001) were recommended to match the concentration in control and recovery samples (p. 29 of MRID 50406039; p. 32 of MRID 50406040; p. 26 of MRID 50406045). The recommended changes were included in the final report for the subject study and the separate method validation study (BASF Study 784160, BASF Reg. Doc. # 2017/7008199).

Table 1: Method Validation Recoveries for BAS 850 H and Metabolites in Fortified Control Water Samples Using BASF Analytical Method D1724/01[1]

Analyte	Matrix	Fortification Levels (ppt)	n	Recovery (%)	Average Rec. (%)	Standard Deviation	%RSD[a]
Trifludi-moxazin (BAS 850 H)	Surface water Matrix-matched Standards	Primary Transition (m/z 413→74)					
		30	5	85, 98, 96, 82, 77	88	9	10
		300	5	81, 87, 91, 91, 85	87	4	5
		Overall	10	Range, 77 - 98	87	7	8
		Confirmatory Technique – C18 Column (m/z 413→74)					
		30	5	98, 99, 94, 91, 95	95	3	3
		300	5	86, 86, 86, 85, 82	85	2	2
		Overall	10	Range, 82 - 99	90	6	7
Trifludi-moxazin (BAS 850 H)	Drinking water Matrix-matched Standards	Primary Transition (m/z 413→74)					
		30	5	106, 104, 96, 104, 102	102	4	4
		300	5	105, 92, 114, 90, 101	100	10	10
		Overall	10	Range, 90 - 114	101	7	7
		Confirmatory Technique – C18 Column (m/z 413→74)					
		30	5	91, 93, 87, 87, 93	90	3	3
		300	5	86, 90, 89, 90, 99	91	5	5
		Overall	10	Range, 86 - 99	91	4	4
M850H001	Surface water Matrix-matched Standards	Primary Transition (m/z 397→141)					
		30	5	98, 99, 92, 103, 97	98	4	4
		300	5	87, 90, 89, 95, 94	91	3	4
		Overall	10	Range, 87 - 103	94	5	5
		Confirmatory Transition (m/z 397→134)					
		30	5	89, 94, 92, 94, 98	93	3	4
		300	5	87, 90, 90, 98, 95	92	4	5
		Overall	10	Range, 87 - 98	93	4	4
M850H001	Drinking water Matrix-matched Standards	Primary Transition (m/z 397→141)					
		30	5	92, 89, 88, 86, 88	89	2	2
		300	6	97, 93, 95, 94, 88, 90	93	3	4
		Overall	11	Range, 86 - 97	91	4	4
		Confirmatory Transition (m/z 397→134)					
		30	5	92, 97, 89, 82, 91	90	5	6
		300	6	102, 90, 98, 98, 87, 88	94	6	7
		Overall	11	Range, 82 - 102	92	6	6

Table 1: Method Validation Recoveries for BAS 850 H and Metabolites in Fortified Control Water Samples Using BASF Analytical Method D1724/01[1] (continued)

Analyte	Matrix	Fortification Levels (ppt)	n	Recovery (%)	Average Rec. (%)	Standard Deviation	%RSD[a]
M850H002	Surface water Matrix-matched Standards	Primary Transition (m/z 373→323)					
		30	5	97, 105, 100, 105, 104	102	4	3
		300	5	110, 113, 113, 120, 120	115	5	4
		Overall	10	Range, 97 - 120	109	8	7
		Confirmatory Transition (m/z 373→193)					
		30	5	95, 105, 97, 108, 103	102	5	5
		300	5	112, 114, 112, 117, 118	115	3	2
		Overall	10	Range, 95 - 118	108	8	7
M850H002	Drinking water Matrix-matched Standards	Primary Transition (m/z 373→323)					
		30	5	110, 113, 112, 105, 111	110	3	3
		300	5	120, 111, 116, 119, 119	117	4	3
		Overall	10	Range, 105 - 120	114	5	4
		Confirmatory Transition (m/z 373→193)					
		30	5	111, 113, 110, 106, 111	110	3	2
		300	5	119, 109, 118, 120, 120	117	5	4
		Overall	10	Range, 106 - 120	114	5	5
M850H003	Surface water Matrix-matched Standards	Primary Transition (m/z 357→307)					
		30	5	94, 98, 97, 97, 97	97	2	2
		300	5	92, 88, 89, 93, 93	91	2	3
		Overall	10	Range, 88 - 98	94	3	4
		Confirmatory Transition (m/z 357→193)					
		30	5	101, 101, 95, 94, 95	97	3	4
		300	5	88, 84, 88, 90, 94	89	4	4
		Overall	10	Range, 84 - 101	93	6	6
M850H003	Drinking water Matrix-matched Standards	Primary Transition (m/z 357→307)					
		30	5	97, 96, 96, 93, 98	96	2	2
		300	5	108, 98, 103, 106, 107	104	4	4
		Overall	10	Range, 93 - 108	100	5	5
		Confirmatory Transition (m/z 357→193)					
		30	5	93, 96, 95, 89, 100	95	4	4
		300	5	101, 101, 105, 106, 112	105	5	4
		Overall	10	Range, 89 - 112	100	7	7
M850H004	Surface water Matrix-matched Standards	Primary Transition (m/z 387→131)					
		30	5	71, 66, 68, 70, 73	70	3	4
		300	5	78, 75, 79, 78, 83	79	3	4
		Overall	10	Range, 66 - 83	74	5	7
		Confirmatory Transition (m/z 387→74)					
		30	5	72, 67, 69, 73, 74	71	3	4
		300	5	79, 73, 78, 80, 85	79	4	5
		Overall	10	Range, 67 - 85	75	5	7
M850H004	Drinking water Matrix-matched Standards	Primary Transition (m/z 387→131)					
		30	5	90, 88, 90, 84, 85	87	3	3
		300	5	106, 98, 100, 105, 107	103	4	4
		Overall	10	Range, 84 - 107	95	9	9
		Confirmatory Transition (m/z 387→74)					
		30	5	92, 87, 90, 82, 85	87	4	5
		300	5	107, 98, 101, 101, 110	103	5	5
		Overall	10	Range, 82 - 110	95	10	10

Table 1: Method Validation Recoveries for BAS 850 H and Metabolites in Fortified Control Water Samples Using BASF Analytical Method D1724/01[1] (continued)

Analyte	Matrix	Fortification Levels (ppt)	n	Recovery (%)	Average Rec. (%)	Standard Deviation	%RSD[a]
M850H012	Surface water Matrix-matched Standards	Primary Transition (m/z 257→163)					
		30	5	91, 94, 93, 95, 95	94	2	2
		300	5	94, 88, 90, 93, 91	91	2	3
		Overall	10	Range, 88 - 95	92	2	3
		Confirmatory Transition (m/z 257→116)					
		30	5	96, 101, 95, 94, 101	97	3	3
		300	5	101, 91, 98, 101, 99	98	4	4
		Overall	10	Range, 91 - 101	98	4	4
M850H012	Drinking water Matrix-matched Standards	Primary Transition (m/z 257→163)					
		30	5	98, 100, 99, 88, 99	97	5	5
		300	5	104, 96, 103, 102, 103	102	3	3
		Overall	10	Range, 88 - 104	99	5	5
		Confirmatory Transition (m/z 257→116)					
		30	5	102, 98, 93, 92, 97	96	4	4
		300	5	110, 101, 100, 104, 103	104	4	4
		Overall	10	Range, 92 - 110	100	5	5
M850H035	Surface water Matrix-matched Standards	Primary Transition (m/z 371→257)					
		30	5	91, 100, 90, 94, 95	94	4	4
		300	5	95, 91, 90, 95, 95	93	2	3
		Overall	10	Range, 90 - 100	94	3	3
		Confirmatory Transition (m/z 371→163)					
		30	5	93, 103, 90, 95, 101	96	5	6
		300	5	95, 91, 90, 97, 98	94	4	4
		Overall	10	Range, 90 - 103	95	4	5
M850H035	Drinking water Matrix-matched Standards	Primary Transition (m/z 371→257)					
		30	5	99, 98, 98, 94, 97	97	2	2
		300	5	106, 97, 101, 103, 103	102	3	3
		Overall	10	Range, 94 - 106	100	4	4
		Confirmatory Transition (m/z 371→163)					
		30	5	97, 98, 97, 95, 100	97	2	2
		300	5	105, 95, 100, 102, 104	101	4	4
		Overall	10	Range, 95 - 105	99	4	4

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

Table obtained from Table 2, pp. 13-15 of MRID 50406045. Recovery results were corrected for residues quantified in the controls; however, no corrections were made (Appendix A, p. 69 of MRID 50406039).

[1] Drinking (well) water (Sample No. CM15-030; pH 7.5, 0.8 ppm total organic carbon, 42 ppm calcium, 116 mg equiv. $CaCO_3$/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. $CaCO_3$/L hardness) obtained Golden Lake, North Dakota, were used (Appendix A, Appendix K, pp. 248-250 of MRID 50406039). Test systems were characterized by Agvise Laboratories as part of previous studies, drinking water under BASF Study # 433575 (DocID: 2015/7001125) and surface water under BASF Study # 805401 (DocID: Not yet available).

Table 5: Independent Laboratory Validation Summary Results of Method D1724/01: Residues of BAS 850 H in Drinking and Surface Water[1]

Analyte	Fortification Levels (ppb)	n	Recovery (%)[b]	Average Recovery (%)	Standard Deviation	%RSD[a]
Surface Water						
BAS 850 H	Quantitation (*m/z* 413→74)					
	0.03	5	72, 98, 96, 90, 102	91	11.8	12.9
	0.3	5	(66), 86, 84, 86, 90	82	9.5	11.5
	Overall	10	Range: 66-102	87	11.1	12.8
	Confirmation (*m/z* 413→74)					
	0.03	5	75, 79, 81, 85, 88	82	5.2	6.3
	0.3	5	(65), 73, 82, 82, 81	77	7.4	9.6
	Overall	10	Range: 65-88	79	6.6	8.3
M850H001	Quantitation (*m/z* 397→141)					
	0.03	5	104, 101, 99, 93, 94	98	4.8	4.8
	0.3	5	82, 87, 93, 83, 73	84	7.2	8.7
	Overall	10	Range: 73-104	91	9.7	10.7
	Confirmation (*m/z* 397→134)					
	0.03	5	115, 115, 106, 92, 84	102	13.8	13.5
	0.3	5	86, 88, 86, 81, 80	84	3.4	4.1
	Overall	10	Range: 80-115	93	13.5	14.5
M850H002	Quantitation (*m/z* 373→193)					
	0.03	5	83, 101, 102, 97, 103	97	8.6	8.8
	0.3	5	85, 96, 95, 91, 92	92	4.2	4.5
	Overall	10	Range: 83-103	95	7.0	7.4
	Confirmation (*m/z* 373→323)					
	0.03	5	88, 91, 103, 102, 105	98	8.0	8.1
	0.3	5	81, 88, 91, 91, 96	89	5.5	6.2
	Overall	10	Range: 81-105	93	7.9	8.4
M850H003	Quantitation (*m/z* 357→307)					
	0.03	5	95, 105, 102, 102, 109	103	5.2	5.0
	0.3	5	92, 96, 93, 97, 103	96	4.2	4.4
	Overall	10	Range: 92-109	100	5.6	5.6
	Confirmation (*m/z* 357→137)					
	0.03	5	102, 101, 107, 96, 108	103	4.7	4.6
	0.3	5	107, 99, 105, 117, 103	106	6.8	6.4
	Overall	10	Range: 96-117	104	5.8	5.5
M850H004	Quantitation (*m/z* 387→131)					
	0.03	5	(61), 80, 78, 70, 78	73	7.9	10.7
	0.3	5	73, 78, 76, 78, 81	77	2.9	3.7
	Overall	10	Range: 61-81	75	5.9	7.9
	Confirmation (*m/z* 387→74)					
	0.03	5	(64), 76, 79, 71, 71	72	5.8	8.0
	0.3	5	74, 81, 80, 82, 83	80	3.7	4.6
	Overall	10	Range: 64-83	76	6.0	7.9

Table 5: Independent Laboratory Validation Summary Results of Method D1724/01: Residues of BAS 850 H in Drinking and Surface Water[1] (continued)

Analyte	Fortification Levels (ppb)	n	Recovery (%)	Average Recovery (%)	Standard Deviation	%RSD[a]
Surface Water						
M850H012	Quantitation (*m/z* 257→163)					
	0.03	5	88, 92, 94, 89, 97	92	3.8	4.1
	0.3	5	81, 93, 93, 91, 91	90	5.2	5.8
	Overall	10	Range: 81-97	91	4.4	4.9
	Confirmation (*m/z* 257→116)					
	0.03	5	92, 103, 99, 91, 93	95	5.2	5.4
	0.3	5	85, 90, 95, 89, 116	95	12.2	12.8
	Overall	10	Range: 85-116	95	8.8	9.3
M850H035	Quantitation (*m/z* 371→257)					
	0.03	5	(54), 90, 91, 97, 95	86	18.1	**21.2**
	0.3	5	(50), 87, 93, 93, 87	82	18.1	**22.1**
	Overall	10	Range: 50-97	84	17.2	**20.5**
	Confirmation (*m/z* 371→163)					
	0.03	5	(65), 104, 92, 98, 108	93	17.0	18.3
	0.3	5	(63), 75, 80, 97, 101	83	15.7	18.8
	Overall	10	Range: 63-108	88	16.3	18.5
Drinking Water						
BAS 850 H	Quantitation (*m/z* 413→74)					
	0.03	5	72, 80, 82, 96, 91	84	9.7	11.5
	0.3	5	74, 98, 92, 93, 95	90	9.4	10.4
	Overall	10	Range: 72-98	87	9.6	11.0
	Confirmation (*m/z* 413→74)					
	0.03	5	71, 73, 75, 111, 90	84	16.7	19.9
	0.3	5	(69), 85, 81, 81, 71	78	6.8	8.8
	Overall	10	Range: 69-111	81	12.5	15.5
M850H001	Quantitation (*m/z* 397→141)					
	0.03	5	72, 97, 108, 81, 83	88	14.1	16.0
	0.3	5	86, 78, 81, 85, 85	83	3.4	4.1
	Overall	10	Range: 72-108	86	10.0	11.7
	Confirmation (*m/z* 397→134)					
	0.03	5	(134), 103, 96, 78, 84	99	22.0	**22.3**
	0.3	5	79, 87, 79, 80, 87	82	3.9	4.7
	Overall	10	Range: 78-134	91	17.3	19.1
M850H002	Quantitation (*m/z* 373→193)					
	0.03	5	84, 103, 88, 94, 98	93	7.9	8.4
	0.3	5	86, 98, 92, 100, 107	97	8.0	8.3
	Overall	10	Range: 84-107	95	7.7	8.1
	Confirmation (*m/z* 373→323)					
	0.03	5	73, 92, 93, 96, 100	91	10.3	11.4
	0.3	5	84, 92, 95, 91, 100	92	5.7	6.2
	Overall	10	Range: 73-100	92	7.9	8.6

Table 5: Independent Laboratory Validation Summary Results of Method D1724/01: Residues of BAS 850 H in Drinking and Surface Water[1] (continued)

Analyte	Fortification Levels (ppb)	n	Recovery (%)[b]	Average Recovery (%)	Standard Deviation	%RSD[a]
Drinking Water						
M850H003	Quantitation (*m/z* 357→307)					
	0.03	5	93, 114, 109, 109, 110	107	8.4	7.9
	0.3	5	93, 100, 104, 108, 105	102	5.8	5.7
	Overall	10	Range: 93-114	105	7.3	7.0
	Confirmation (*m/z* 357→137)					
	0.03	5	88, 116, 109, 115, 117	109	12.2	11.2
	0.3	5	110, 111, 100, 111, 111	109	4.9	4.6
	Overall	10	Range: 88-117	109	8.8	8.0
M850H004	Quantitation (*m/z* 387→131)					
	0.03	5	98, 105, 88, 115, 85	98	12.2	12.5
	0.3	5	96, 91, 103, 111, 93	99	8.1	8.2
	Overall	10	Range: 85-115	98	9.8	9.9
	Confirmation (*m/z* 387→74)					
	0.03	5	106, 101, 90, 108, 98	101	7.0	6.9
	0.3	5	90, 83, 105, 106, 83	93	11.2	12.0
	Overall	10	Range: 83-108	97	9.6	9.9
M850H012	Quantitation (*m/z* 257→163)					
	0.03	5	81, 88, 96, 94, 101	92	7.6	8.2
	0.3	5	79, 91, 95, 108, 107	96	11.9	12.5
	Overall	10	Range: 79-108	94	9.6	10.2
	Confirmation (*m/z* 257→116)					
	0.03	5	82, 93, 89, 96, 87	89	5.5	6.2
	0.3	5	77, 98, 95, 109, 107	97	12.7	13.0
	Overall	10	Range: 77-109	93	10.1	10.9
M850H035	Quantitation (*m/z* 371→257)					
	0.03	5	96, 87, 89, 106, 81	92	9.6	10.4
	0.3	5	91, 81, 93, 97, 84	89	6.5	7.3
	Overall	10	Range: 81-106	91	7.9	8.7
	Confirmation (*m/z* 371→163)					
	0.03	5	92, 86, 89, 105, 83	91	8.2	9.0
	0.3	5	93, 82, 94, 94, 86	90	5.6	6.2
	Overall	10	Range: 82-105	90	6.7	7.4

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

[b] Individual recovery results in parenthesis are outside the acceptable range; however, the average recovery results are within the acceptable range.

Table obtained from Table 6, pp. 27-30 of MRID 50406045. Uncorrected recovery results were reported (Appendix C, pp. 397-424 of MRID 50406040).

[1] Drinking (well) water (Sample No. CM15-030; pH 7.5, 0.8 ppm total organic carbon, 42 ppm calcium, 116 mg equiv. $CaCO_3$/L hardness) and surface (lake) water (Sample No. CM17-052; Golden Lake Water; pH 8.9, 18.6 ppm total organic carbon, 93 ppm calcium, 657 mg equiv. $CaCO_3$/L hardness) were provided by BASF and used in the study (p. 16; Appendix A, pp. 386-387 of MRID 50406040). The test systems were the same as those of the ECM.

III. Method Characteristics

The LOQ was defined as the lowest fortification level tested (p. 26 of MRID 50406045). The LOQ for residues of BAS 850 H (including parent compound and its metabolites M850H001, M850H002, M850H003, M850H004, M850H012, and M850H035) in water was 30 ng/L (30 ppt, 0.030 µg/mL, 0.030 ppb; p. 20 of MRID 50406045). 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 for each analyte was shown to be detectable as the absolute amount of analyte injected into the LC-MS/MS when the lowest calibration standard (0.005 ng/mL for all analytes except 0.025 ng/mL for M850H001) was analyzed with the acceptable signal to noise ratio (S/N > 3:1).

BASF analytical method D1724/01 has two preparations/cleanups (simple dilution for BAS 850 H, M850H002, M850H003, M850H004, M850H012, and M850H035; and a liquid-liquid partition / concentration for M850H001). An MDL calculation and subsequent LOD evaluation were conducted for each preparation/cleanup. The transitions (both primary and confirmatory) used for quantitation in this method were determined using product ion spectra. Results for both LOD determinations are detailed below.

The least sensitive analyte/transition for Method D1724/01 was determined by the injection of solvent standards. By comparison of the chromatograms of calibration solutions used, it was determined qualitatively that the MS/MS ion transition for M850H012 (*m/z* 257 → *m/z* 116) was the least sensitive and, therefore, the best candidate to conduct the MDL/LOD evaluation.

The MDL was calculated to be 0.00011 ng on-column for M850H012. To determine this value, the seven (7) control samples were run through the extraction procedure and were fortified with standard solution prior to the LC-MS/MS determination step to achieve a concentration of 0.005 ng/mL, and 0.10 mL (100 µL, 0.0005 ng on-column) of each sample was injected on the LC-MS/MS system according to method D1724/01. Calculation of MDL for M850H012 was conducted according to the table provide in 40 CFR Ch. 1 Part 136 Appendix B.

Based on this calculated MDL, the LOD for M850H012 was set at 0.00048 ng on-column (i.e. 0.0048 ng/mL injected at 0.10 mL). The LOD values on-column correspond to a LOD of 6 ng/L (ppt) for M850H012 (based on the workup of the method, e.g. aliquot actor, final volume, etc.). This increase from the MDL to the LOD is to account for variability in the residue method, natural drift of the LC-MS/MS instrumentation, potential contamination issues, untested matrix effects, and potential unseen background interferences. Detailed calculations for MDL determination is shown in the following table.

Table 6: Calculation of MDL for M850H012 (m/z 257 → m/z 116) for Method D1724/01, using the Methodology Set Forth in 40 CFR Ch 1 Part 136 Appendix B

0.005 ng/mL Matrix-Spike Replicate	Calculated Concentration (ng/mL)
1	0.00446
2	0.00526
3	0.00425
4	0.00459
5	0.00439
6	0.00434
7	0.00432
Standard Deviation (S) =	0.000346
N-1 =	6
Critical t value (t) =	3.143
Injection Volume (µL)	100
MDL (ng/mL) =	0.0011
MDL (ng on-column) =	0.00011

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

Table obtained from Table 3, p. 17 of MRID 50406045.

For M850H001, 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 D1724/01. It was determined qualitatively that the MS/MS ion transition for M850H001 (m/z 397 → m/z 141) was the least sensitive transition and therefore the best candidate to conduct the LOD evaluation for this cleanup.

The MDL was determined to be 0.00099 ng on-column for M850H001. To determine this value, the seven (7) control samples were run through the extraction procedure and were fortified with standard solution prior to the LC-MS/MS determination step to achieve a concentration of 0.025 ng/mL and 0.1 mL (100 µL, 0.0025 ng on-column) of each sample was injected on the LC-MS/MS system according to method D1724/01. Calculation of MDL for M850H001 was conducted according to the table provide in 40 CFR Ch. 1 Part 136 appendix B.

To verify this MDL, one of the seven spiked control samples was injected in triplicate using an injection volume appropriate to inject 0.001 ng on-column. The resultant chromatogram peak had a signal to noise ratio ≥ 2. An example chromatogram of a matrix-spiked sample at MDL was provided in the report.

Based on this calculated MDL, the LOD for M850H001 was set at 0.0024 ng on-column (i.e. 0.024 ng/mL injected at 0.1 mL). The LOD values on-column correspond to a LOD of 6 ng/L (ppt) for M850H001 (based on the workup of the method, e.g. aliquot actor, final volume, etc.). This increase from the MDL to the LOD is to account for variability in the residue method, natural drift of the LC-MS/MS instrumentation, potential contamination issues, untested matrix effects, and potential unseen background interferences. Detailed calculations for MDL determination is shown in the following table.

Table 7: Calculation of MDL for M850H001 (m/z 397 → m/z 141) for Method D1724/01, using the Methodology Set Forth in 40 CFR Ch 1 Part 136 Appendix B

0.025 ng/mL Matrix-Spike Replicate	Calculated Concentration (ng/mL)
1	0.0205
2	0.0254
3	0.0291
4	0.0290
5	0.0257
6	0.0243
7	0.0227
Standard Deviation (S) =	0.00314
N-1 =	6
Critical t value (t) =	3.143
Injection Volume (µL)	100
MDL (ng/mL)=	0.0099
MDL (ng on-column) =	0.00099

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

Table obtained from Table 4, p. 18 of MRID 50406045.

Table 8. Method Characteristics - Analytical Method D1724/01

Analyte		**Trifludimoxazin (BAS 850 H; Reg.No. 5654329)**	**M850H001 (Reg.No. 5749359)**	**M850H002 (Reg.No. 5757725)**	**M850H003 (Reg.No. 5757726)**	**M850H004 (Reg.No. 5833884)**	**M850H012 (Reg.No. 5797901)**	**M850H035 (Reg.No. 6070203)**
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)[1,5]	ECM	r = **0.9934** (SW, Q) r = 0.9975 (SW, C) r = 0.9977 (DW, Q) r = 0.9991 (DW, C)	r = 0.9980 (SW, Q) r = 0.9983 (SW, C) r = 0.9979-0.9996 (DW, Q) r = 0.9984-0.9993 (DW, C)	r = **0.9899** (SW, Q) r = **0.9902** (SW, C) r = **0.9940** (DW, Q) r = **0.9933** (DW, C)	r = 0.9984 (SW, Q) r = 0.9974 (SW, C) r = **0.9922** (DW, Q) r = **0.9904** (DW, C)	r = 0.9996 (SW, Q) r = 0.9998 (SW, C) r = 0.9979 (DW, Q) r = 0.9981 (DW, C)	r = 0.9996 (SW, Q) r = 0.9993 (SW, C) r = 0.9991 (DW, Q) r = 0.9990 (DW, C)	r = 0.9989 (SW, Q) r = 0.9982 (SW, C) r = 0.9983 (DW, Q) r = 0.9978 (DW, C)
	ILV	r = 0.9989 (SW, Q) r = 0.9971 (SW, C) r = 0.9992 (DW, Q) r = **0.9932** (DW, C)	r = 0.9982 (SW, Q) r = 0.9991 (SW, C) r = 0.9972 (DW, Q) r = 0.9984 (DW, C)	r = 0.9982 (SW, Q) r = 0.9991 (SW, C) r = 0.9991 (DW, Q) r = 0.9995 (DW, C)	r = 0.9998 (SW, Q) r = 0.9961 (SW, C) r = 0.9988 (DW, Q) r = 0.9987 (DW, C)	r = 0.9973 (SW, Q) r = 0.9978 (SW, C) r = **0.9946** (DW, Q) r = 0.9968 (DW, C)	r = 0.9984 (SW, Q) r = 0.9991 (SW, C) r = 0.9958 (DW, Q) r = 0.9965 (DW, C)	r = 0.9988 (SW, Q) r = 0.9996 (SW, C) r = 0.9993 (DW, Q) r = 0.9985 (DW, C)
	Range	0.005-0.125 ng/mL	0.025-0.5 ng/mL	0.005-0.125 ng/mL				

Analyte		**Trifludimoxazin (BAS 850 H; Reg.No. 5654329)**	**M850H001 (Reg.No. 5749359)**	**M850H002 (Reg.No. 5757725)**	**M850H003 (Reg.No. 5757726)**	**M850H004 (Reg.No. 5833884)**	**M850H012 (Reg.No. 5797901)**	**M850H035 (Reg.No. 6070203)**
Repeatable	ECM[2]	Yes at LOQ and 10×LOQ (two characterized water matrices)						
	ILV[3,4]	Yes at LOQ and 10×LOQ (two characterized water matrices).	Yes at LOQ and 10×LOQ (two characterized water matrices; DW LOQ C RSD 22.3%).[5]	Yes at LOQ and 10×LOQ (two characterized water matrices).				Yes at LOQ and 10×LOQ (characterized DW). No at LOQ (RSD 21.2%) and 10×LOQ (RSD 22.1%; characterized SW; C data was acceptable).[5]
Reproducible		Yes at LOQ and 10×LOQ in DW and SW.						DW: Yes at LOQ and 10×LOQ. SW: No at LOQ and 10×LOQ.
Specific	ECM	Yes, no matrix interferences were observed.		Yes, matrix interferences were <5% of the LOQ (based on quantified residues).		Yes, no matrix interferences were observed.		
		Some minor baseline noise near the analyte peak and non-uniform peak integration was noted in several LOQ and 10×LOQ representative chromatograms.						
	ILV[7]	Yes, matrix interferences were <6% of the LOQ (based on peak area).	Yes, no Q matrix interferences were observed; however, a significant contaminant near the analyte peak was observed (RT 5.15 min.; peak height/area ≈ LOQ).[6]	Yes, matrix interferences were <5% of the LOQ (based on peak area).		Yes, no matrix interferences were observed.		

Analyte		**Trifludimoxazin (BAS 850 H; Reg.No. 5654329)**	**M850H001 (Reg.No. 5749359)**	**M850H002 (Reg.No. 5757725)**	**M850H003 (Reg.No. 5757726)**	**M850H004 (Reg.No. 5833884)**	**M850H012 (Reg.No. 5797901)**	**M850H035 (Reg.No. 6070203)**
		Minor baseline noise interfered with analyte peak attenuation and integration.	Analyte peak was very small at LOQ compared to baseline noise.	Minor baseline noise interfered with analyte peak attenuation and integration. Analyte peak was small at LOQ compared to baseline noise in many representative chromatograms.				

Data were obtained from MRID 50406045; Appendix A, Appendix D, pp. 76-105 (raw data sheets); Appendix A, Appendix H, pp. 139-145 (calibration curves) and Appendix A, Appendix I, pp. 154-239 (chromatograms) of MRID 50406039; Section A, pp. 74-383 (calibration curves and chromatograms) and Appendix C, pp. 397-424 (raw data sheets) of MRID 50406040. Q = Quantitation ion transition; C = Confirmation ion transition or method. DW = Drinking water; SW = surface water.

1 Matrix-matched calibration standards were used in the ECM and ILV.

2 In the ECM, drinking (well) water (Sample No. CM15-030; pH 7.5, 0.8 ppm total organic carbon, 42 ppm calcium, 116 mg equiv. $CaCO_3$/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. $CaCO_3$/L hardness) obtained Golden Lake, North Dakota, were used (Appendix A, Appendix K, pp. 248-250 of MRID 50406039). Test systems were characterized by Agvise Laboratories as part of previous studies, drinking water under BASF Study # 433575 (DocID: 2015/7001125) and surface water under BASF Study # 805401 (DocID: Not yet available).

3 In the ILV, drinking (well) water (Sample No. CM15-030; pH 7.5, 0.8 ppm total organic carbon, 42 ppm calcium, 116 mg equiv. $CaCO_3$/L hardness) and surface (lake) water (Sample No. CM17-052; Golden Lake Water; pH 8.9, 18.6 ppm total organic carbon, 93 ppm calcium, 657 mg equiv. $CaCO_3$/L hardness) were provided by BASF and used in the study (p. 16; Appendix A, pp. 386-387 of MRID 50406040). The test systems were the same as those of the ECM.

4 The independent laboratory validation of BASF method (D1724/01) was successfully completed for all analytes in drinking water and surface water within two attempts, except M850H004 in surface water. M850H004 in surface water required three attempts before the method could be successfully validated, due to some unforeseen, avoidable experimental circumstances (see report for details), but also the need for minor modifications including slight changes to the LC mobile phase gradient (alternate LC-MS/MS conditions were used) as well as changes to the matrix-matched standards.

5 A confirmation method is not usually required when LC/MS or GC/MS is the primary method to generate study data; therefore, deficiencies in the confirmation analysis of the performance data, linearity, and supporting chromatograms do not affect the validity of the method.

6 Based on Section A, pp. 102-105, 259-262 of MRID 50406040. The reviewer noted that C ion matrix interferences were *ca*. 20-30% of the LOQ (based on peak area), based on Section A, pp. 114-115, 271-272; however, this did not affect the specificity of the method since it was isolated to the C analysis.

Linearity is satisfactory when r ≥0.995 [updated DER acceptance criteria (11/2019); Linearity criterion is consistent with Superfund analytical methods for inorganic analytes (National Functional Guidelines for Inorganic Superfund Methods Data Review, EPA-540-R-2017-001, January 2017. **https://www.epa.gov/sites/production/files/201701/documents/national_functional_guidelines_for_inorganic_superfund_methods_data_review_01302017.pdf**)].

IV. Method Deficiencies and Reviewer's Comments

1. The BASF Study Monitor for the ILV (David Delinsky) was also the ECM study author and study director for BASF Study Nos.: 784160 and 784160_1 (p. 1; Appendix A, pp. 8, 21; Appendix B, pp. 258, 265 of MRID 50406039). The ILV study author briefly summarized that communications between the ILV and BASF Study Monitor (David Delinsky) involved communication of successful trials and technical communication via email and tele-conference regarding failed trials and future trials (pp. 15, 32-33 of MRID 50406040). Also, full communication details should be included for review. In this case, the role of the study monitor was not clearly defined.

2. The ILV performance data was not acceptable for the quantitation analysis of M850H035 (Reg.No. 6070203) in surface water (RSD 21.2% LOQ, 22.1% 10×LOQ; Table 6, pp. 27-30 of MRID 50406045). OCSPP guidelines state that RSD values are ≤20%. The reviewer also noted that the confirmation analysis of M850H035 (Reg.No. 6070203) in surface water was acceptable and the LOQ confirmation analysis of M850H001 in drinking water was unacceptable (RSD 22.3%); however, results/deficiencies in the confirmation analysis do not affect the validity of the method since a confirmation method is not usually required when LC/MS or GC/MS is the primary method to generate study data. The reviewer noted that the use of the confirmation ion transition as the quantitation ion transition for quantifying M850H035 could be proposed if recovery data are more robust with that transition.

3. ILV linearity (quantitation ion analysis) was not satisfactory for M850H004 in drinking water (r = 0.9946; Appendix C, pp. 397-424 of MRID 50406040). Linearity is satisfactory when r ≥0.995. The linearity of the confirmation analysis calibration curve was not satisfactory for BAS 850 H in drinking water; however, deficiencies in the confirmation analysis do not affect the validity of the method since a confirmation method is not usually required when LC/MS or GC/MS is the primary method to generate study data.

   ECM linearity (quantitation ion analysis) was not satisfactory for BAS 850 H (r = 0.9934 for surface water), M850H002 (r = 0.9899 for surface water and r = 0.9940 for drinking water), and M850H003 (r = 0.9922 for drinking water; Appendix A, Appendix D, pp. 76-105 of MRID 50406039). Linearity is satisfactory when r ≥0.995. The linearity of the confirmation analysis calibration curves was not satisfactory for M850H002 in both matrices and M850H003 in drinking water; however, deficiencies in the confirmation analysis do not affect the validity of the method since a confirmation method is not usually required when LC/MS or GC/MS is the primary method to generate study data.

   The ECM was performed under the following guidelines, in addition to OCSPP 850.6100: SANCO/3029/99 rev 4 (11/07/2000) and SANCO/825/00 rev 8.1, (16/11/2010; p. 1 of MRID 50406039); the ILV was performed under the following guidelines, in addition to OCSPP 850.6100: ILV SANCO/825/00 rev.8.1 (Nov. 16, 2010; p. 1 of MRID 50406040). Under these guidelines, the requirement for the linear correlation coefficient (r) is ≥0.99. The only deviation from these linearity requirements was the ECM linearity (quantitation ion analysis) for M850H002 (r = 0.9899 for surface water; Appendix A, Appendix D, pp. 76-105 of MRID 50406039).

4. It could not be determined if the ILV was performed with the most difficult matrices with which to validate the method since the ILV water matrices were the same as those of the ECM.

5. The specificity of the method was not well-supported by ILV representative chromatograms of M850H001 due to significant contaminant near the analyte peak was observed (RT 5.15 min.; peak height/area ≈ LOQ (Section A, pp. 102-105, 259-262 of MRID 50406040. The reviewer noted that confirmation ion matrix interferences were *ca*. 20-30% of the LOQ (based on peak area), based on Section A, pp. 114-115, 271-272; however, this did not affect the specificity of the method since it was isolated to the confirmation analysis.

6. The reviewer assumed that the ILV MRID 50406040 contained a typographical error in the Abstract where the statement "independent laboratory validation was performed successfully for M850H001, M850H004 and M850H035 in **surface water and drinking water** during the second trial" should have been written as "independent laboratory validation was performed successfully for M850H001, M850H004 and M850H035 in **drinking water** during the second trial" since the paragraph was discussing drinking water results and M850H035 in surface water was successfully validated in the first trial (p. 9 of MRID 50406040).

7. It was reported for the ILV that one sample set of 13 samples required *ca*. 2-3 hours of work, excluding LC/MS/MS and calculation of results (p. 29 of MRID 50406040).

V. References

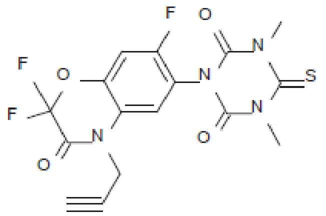
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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

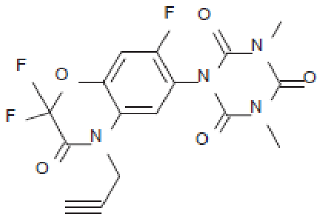
MRID 50406045. Description of methods for analysis of water (parent and metabolites), Summary and evaluation (Tier II), BASF Reg. Doc. # 2018/7000015 US. BASF Corporation 26 Davis Drive P.O. Box 13528 Research Triangle Park, NC 27709.

Attachment 1: Chemical Names and Structures

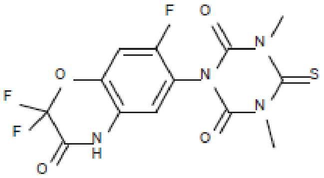
2.2.1 Trifludimoxazin

Common Name	Trifludimoxazin	Chemical structure: 
BAS Code Name	BAS 850 H	
IUPAC Name	1,5-dimethyl-6-thioxo-3-[2,2,7-trifluoro-3-oxo-4-(prop-2-yn-1-yl)-3,4-dihydro-2H-1,4-benzoxazin-6-yl]-1,3,5-triazinane-2,4-dione	
BASF Reg. No.	5654329	
Molecular Formula	$C_{16}H_{11}F_3N_4O_4S$	
Molecular Weight	412.3	
Lot No.	L84-130	
Purity:	99.2%	
Expiration Date	February 01, 2020	

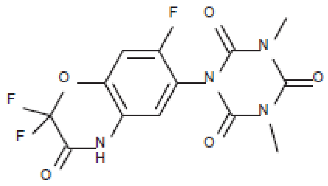
2.2.2 M850H001

Common Name	None	Chemical structure: 
BAS Code Name	M850H001	
IUPAC Name	1,3-dimethyl-5-[2,2,7-trifluoro-3-oxo-4-(prop-2-yn-1-yl)-3,4-dihydro-2H-1,4-benzoxazin-6-yl]-1,3,5-triazinane 2,4,6-trione	
BASF Reg. No.	5749359	
Molecular Formula	$C_{16}H_{11}F_3N_4O_5$	
Molecular Weight	396.3	
Lot No.	L85-52	
Purity:	98.7%	
Expiration Date	April 01, 2018	

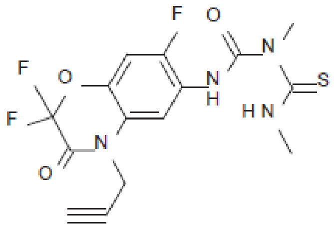
2.2.3 M850H002

Common Name	None	Chemical structure: 
BAS Code Name	M850H002	
IUPAC Name	1,5-dimethyl-6-thioxo-3- (2,2,7-trifluoro-3-oxo-3,4-dihydro-2H-1,4-benzoxazin-6-yl)-1,3,5-triazinane-2,4-dione	
BASF Reg. No.	5757725	
Molecular Formula	$C_{13}H_9F_3N_4O_4S$	
Molecular Weight	374.3	
Lot No.	L84-162	
Purity:	96.8%	
Expiration Date	February 01, 2020	

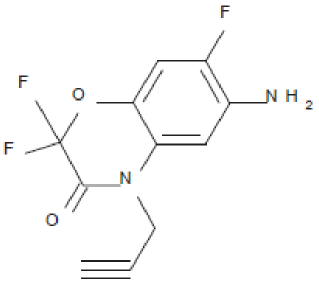
2.2.4 M850H003

Common Name	None	Chemical structure: 
BAS Code Name	M850H003	
IUPAC Name	1,3-dimethyl-5-(2,2,7-trifluoro-3-oxo-3,4-dihydro-2H-1,4-benzoxazin-6-yl)-1,3,5-triazinane-2,4,6-trione	
BASF Reg. No.	5757726	
Molecular Formula	$C_{13}H_9F_3N_4O_5$	
Molecular Weight	358.2	
Lot No.	L85-70	
Purity:	99.4%	
Expiration Date	April 01, 2018	

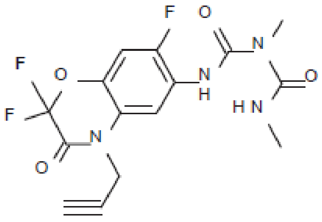
2.2.5 M850H004

Common Name	None	Chemical structure: 
BAS Code Name	M850H004	
IUPAC Name	N,N-dimethyl-N'-[2,2,7-trifluroro-3-oxo-4-(prop-2-yn-1-yl)-3,4-dihydro-2H-1,4-benzoxazin-6-yl] dicarbonimidothioicdiamide	
BASF Reg. No.	5833884	
Molecular Formula	$C_{15}H_{13}F_3N_4O_3S$	
Molecular Weight	386.4	
Lot No.	L85-50	
Purity:	99.5%	
Expiration Date	April 01, 2018	

2.2.6 M850H012

Common Name	None	Chemical structure: 
BAS Code Name	M850H012	
IUPAC Name	6-amino-2,2,7-trifluoro-4-(prop-2-yn-1-yl)-2H-1,4-benzoxazin-3(4H)-one	
BASF Reg. No.	5797901	
Molecular Formula	$C_{11}H_7F_3N_2O_2$	
Molecular Weight	256.2	
Lot No.	L85-66	
Purity:	98.9%	
Expiration Date	September 01, 2018	

2.2.7 M850H035

Common Name	None	Chemical structure: 
BAS Code Name	M850H035	
IUPAC Name	N,N-dimethyl-N'-[2,2,7-trifluroro-3-oxo-4-(prop-2-yn-1-yl)-3,4-dihydro-2H-1,4-benzoxazin-6-yl]-2-imidodicarbonic diamide	
BASF Reg. No.	6070203	
Molecular Formula	$C_{15}H_{13}F_3N_4O_4$	
Molecular Weight	370.3	
Lot No.	L2017-007	
Purity:	100.0%	
Expiration Date	February 01, 2019	

Figures from pp. 16-18 of MRID 50406039.