MRID: 50406055

Title

Method of Analysis of BAS 850 H Metabolite in Water with Limit of Determination (LOD) Calculation (Method R0048/01)

Guidelines Covered

U.S. EPA Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation
EC Guidance document:
SANCO/3029/99 rev 4 (11/07/2000); SANCO/825/00 rev 8.1, (16/11/2010)

FINAL REPORT

Study Title

Validation of Method R0048/01:
Method for the Determination of M850H040 (Reg.No. 6095223)
in Surface and Drinking Water by LC-MS/MS

Guidelines Covered

US EPA Ecological Effects Test Guideline, OCSPP 850.6100,
Environmental Chemistry Methods and Associated Independent Laboratory Validation;
SANCO/3029/99 rev 4 (11/07/2000); SANCO/825/00 rev 8.1 (Nov 16, 2010)

1. INTRODUCTION

1.1 Background and Purpose of Study

The objective of this validation study was to demonstrate the applicability and repeatability of BASF Analytical Method No. R0048/01 used for the determination of M850H040 residues – a metabolite of the herbicide trifludimoxazin - in water by LC-MS/MS.

2. MATERIALS AND METHODS

2.1 Test Systems

The water samples used in this study were drinking (well) water and surface (lake) water samples, which were characterized by AGVISE Laboratories. The GLP water characterization reports are provided in Appendix K. The samples were held under refrigeration during the experimental period. Each analysis set was uniquely identified with a Master Sheet Number, which consisted of the study number plus a unique number (e.g., 858175-02). The test system samples were assigned unique numbers and these were recorded in each analytical set or “Master Sheet” (e.g., water fortification sample 858175-02-04, from Master Sheet No. 858175-02). The actual sample numbers used for the analysis were identified in the raw data and in this final report.

2.2 Test and Reference Substance

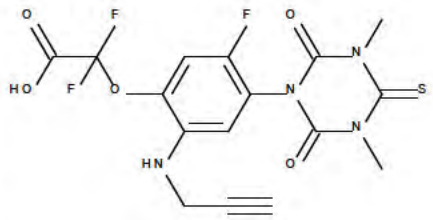
The test/reference standard, shown below, was synthesized by BASF Aktiengesellschaft (Limburgerhof, Germany) and was maintained in a freezer until use in this study. BASF Aktiengesellschaft determined characterization and purity prior to the substance being used in this study. Details of these determinations are available to BASF and are located at Landwirtschaftliche Versuchsstation der BASF, Limburgerhof, Germany. BASF has retained a reserve sample of the chemical and has documentation at BASF Agricultural Solutions (Research Triangle Park, North Carolina, USA).

The test/reference substance are the same substance. The substance was used in solution to generate data for both instrument and method performance. Quantitation of residues in all samples was achieved using calibration curves calculated by linear regression (1/x weighting) of instrument responses for the reference substance. The performance of the instrument was evaluated during each injection set.

2.2.1 M850H040

Common Name	None assigned
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	$C_{16}H_{13}F_3N_4O_5S$
Molecular Weight	430.4
Lot No.	L2017-109
Purity:	87.2%
Expiration Date	December 01, 2019

Chemical structure:



Stock solutions of M850H040 are prepared in methanol and the intermediate (fortification) solutions containing M850H040 are prepared by diluting an aliquot of stock solution using methanol. Solvent-based calibration standards are prepared by serial dilution of the intermediate standards in water. The stability of M850H040 in standard solutions was determined in conjunction with this study. During the course of this study, the test/reference substance solutions were stored under refrigeration and were used within the demonstrated period of stability. Preparation and dilution data forms pertaining to the stock and working solutions are located in the test facility data and are archived periodically. Example standard dilution and use information, as performed in the subject study, are provided in Appendix L.

2.3 Route of Administration

In this method validation study, the test substance was applied to the test system as analytical standard solutions (in methanol) by micropipette to ensure precise delivery of a small amount of the test substance.

2.4 Analytical Method

2.4.1 Principle of the Method

Using BASF Analytical Method No. R0048/01, M850H040 residues in both surface and drinking water were filtered and then quantified using LC-MS/MS. The method procedures validated in this study are provided in Appendix B.

2.4.2 Specificity/Selectivity

The M850H040 residues are determined by HPLC-MS/MS with electrospray ionization monitoring ion transitions *m/z* 429→335 (primary) and *m/z* 429→296 (secondary) in the negative mode. The results are calculated by direct comparison of the sample peak responses to those of external standards. Two mass transitions are available for quantitation of M850H040 using the tested LC-MS/MS method, as described above. Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique is not necessary. The multiple reaction monitoring (MRM) transitions used to identify M850H040 were determined by product ion scan (see Appendix J).

2.5 Validation of Method

For validation, untreated drinking (well) water and surface (lake) water samples were fortified with M850H040 and analyzed according to the established method validation guidelines. To test the repeatability of the method, the analytical sets typically consisted of a reagent blank, and for each matrix, two controls, five replicates fortified with M850H040 at the method limit of quantitation, 30ng/L (ppt/, and five replicates fortified at a higher level, corresponding to 10X the limit of quantitation, 300 ng/L (ppt). For M850H040, the two mass transitions available [*m/z* 429→335 (primary) and *m/z* 429→296 (secondary)]were evaluated.

2.6 Influence of Matrix Effects on Analysis

In conjunction with the subject study, matrix-matched standards and solvent-based standards were analyzed in a separate analysis set to evaluate any potential matrix effects on LC-MS/MS analysis. This involved comparing calibration standards prepared with control matrix against calibration standard solutions prepared with water. The matrix-matched standards were prepared, using control sample material worked up through the method, to final concentration levels approximating 1/2XLOQ, 1XLOQ, and 2XLOQ. Each set of matrix-matched standards (for each water type) was bracketed by a block of calibration standards with additional injections of tested standard levels occurring as appropriate during the run.

The data generated were evaluated by comparing the average area response of the standards for typically three injections of each type (with and without matrix) for each of the three standard concentration levels. Acceptability (i.e., matrices had no significant influence on the analysis) requires a difference in area of <20%, calculated as the "Mean Area Change (%)". For each matrix/ion transition, an overall average "Mean Area Change (%)" across the three tested concentrations was calculated to make a general assessment of acceptability with respect to matrix effects.

2.7 Stability of Extracts

The stability of M850H040 residues in stored extract solutions was determined in conjunction with the subject method validation study. To establish stability, one control and multiple method validation recovery samples for each fortification level (n=5 total recovery samples) that had been stored under refrigeration at the final volume stage were re-analyzed. Quantification of M850H040 in the stored samples for this experiment was performed using the primary ion transition.

3. RESULTS

3.2 Influence of Matrix Effects

In conjunction with the subject study, matrix-matched standards and solvent-based standards were analyzed in a separate experiment to evaluate any potential matrix effects on LC-MS/MS analysis. The results of the testing on each water matrix (for both ion transitions) demonstrated that the matrix load in the water samples had no significant influence on the analysis. Matrix effects, calculated as the overall mean percent area count difference between matrix-matched standards and solvent-based standards, at three standard concentration levels, were less than 20% (Table 2).

3.3 Storage Stability of Solutions

Standards. In this study, M850H040 was shown to be stable in stock or intermediate (fortification) standard solutions prepared in methanol and in calibration standards prepared by serial dilution of intermediate standards using water for at least 1 month (32 days), the longest interval tested, each when held under refrigeration (Table 3). 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.

Extracts. The method validation fortification samples were analyzed on the same day as sample preparation. The acceptable method recoveries obtained during analysis demonstrate the storage stability of M850H040 residues in the extracts in the brief period prior to analysis. In addition, recoveries from stored fortified samples indicated that M850H040 is stable in water extracts (final volume, both surface and drinking water) for approximately 1 week, the longest interval tested, sufficient to support the storage intervals and conditions incurred by the extracts in the subject study, as shown in Table 4.

4. CALCULATIONS AND RAW DATA

An example calculation is included in Appendix C (page 43). Detailed analytical data such as supporting raw data necessary for re-calculations, standards and calibration curve data are provided in Appendix D (page 45). Example standard curves are provided in Appendix H (page 59). Example chromatographs are provided in Appendix I (page 63).

5. STATISTICS AND DATA INTEGRITY

Statistical treatment of the data included simple descriptive statistics, such as determinations of averages, standard deviation and/or RSD for the procedural recoveries and area counts and calculation of the calibration curve and correlation coefficient (r) by linear regression of the instrument responses for the reference standards. The statistical calculations throughout this report were performed using an automated computer spreadsheet (Microsoft Excel®) and were rounded for presentation purposes. Slight differences may be noted in hand calculations using the recoveries presented in the tables. These are due to rounding and have no effect on the scientific conclusions presented in this report. The detailed analytical data may be consulted for confirmation of the calculated results.

Several measures were taken to ensure the quality of the study results. The quality assurance unit at BASF inspected the analytical procedures for compliance with Good Laboratory Practices that included adherence to the protocol. The dates inspected are detailed in the quality assurance unit statement. Study samples and test and reference items were maintained in secured (i.e. pad-locked) storage with limited access. Freezer and refrigerator temperatures were continuously monitored by electronic means.

6. SUMMARY OF METHOD

Summaries of the method parameters and characteristics are provided in Table 5 and Table 6.

7. INDEPENDENT LABORATORY VALIDATION

The independent laboratory validation of BASF method (R0048/01) was successfully completed for M850H040 in drinking water and surface water in two trials. The first trial was repeated due to insufficient instrument performance during initial analysis. Reinjection was not possible due to lapse of extract storage time (greater than one week). The recoveries obtained from the second trial in surface and drinking water was used for reporting ILV results [Reference 1].

Table 5. Summary Parameters for the Analytical Method Used for the Quantitation of M850H040 Residues in Surface and Drinkinng 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/Detector	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).
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 (minutes)	M850H040, ~1.7 minutes

Table 6. Characteristics for the Analytical Method Used for the Quantitation of M850H040 Residues in Surafce and Drinking Water

Analyte	M850H040 residues in drinking and surface water
Equipment ID	Waters Aquity UPLC system equipped with Sciex Instruments API 5500
Limit of quantitation (LOQ)	30 ng/L (30 ppt), for M850H040
Limit of detection (LOD)	6 ng/L (6 ppt), for M850H040 f
Reliability of the Method/ [ILV]	A successful independent laboratory validation [ILV] has been conducted for BASF Analytical Method No. R0048/01 for the determination of M850H040 residues in water. The values obtained are indicative of the reliability of Method No. R0048/01.
Linearity	The method-detector response was linear over the 0.006 to 0.15 ng/mL range ($r \geq 0.9981$ for method validation sets).
Specificity/ Selectivity	The control chromatograms generally have no peaks above the chromatographic background and the spiked sample chromatograms contain only the analyte peak of interest. Peaks were well-defined and symmetrical. There appeared to be no carryover to the following chromatograms.
	An experiment to evaluate any potential matrix effects showed that the matrix load in the samples from each water matrix had no significant influence on analysis (matrix effects <20%).
Confirmatory technique	Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique is not necessary.
Time required	A set of 13 samples (one reagent blank, two controls, and 10 fortified samples for recovery experiments) requires about 4 hours of work (calculation of the results included).

Appendix B. BASF Analytical Method No. R0048/01

We create chemistry

Method Procedure:

Method for the determination of M850H040 (Reg.No. 6095223) in Surface and Drinking Water by LC-MS/MS

BASF Method Number R0048/01

Definitions and acronyms

Sample Set:	A group of samples that are extracted and cleaned up at the same time using the same method represented.
Untreated Sample:	A sample that has not been treated with the test substance.
Control Sample:	Usually an untreated sample used for fortification experiments (it can be acquired from the same study or from a different source e.g. a different study or commercially purchased).
Unknown Sample:	A sample with unknown residues.
Treated Sample:	A sample that has been treated with the test substance.
(Solvent) Blank:	Solvent, solution or mobile phase injected together with a sample set during the analytical run.
Reagent Blank:	A sample consisting of solvents and reagents only in the absence of any sample matrix subjected to complete analysis according to the method. This type of sample is also known as "blank of reagents" or "procedural blank," and is analyzed within the sample set in order to evaluate possible contamination from chemicals/reagents.
Procedural Recovery: **(Fortification Sample)**	A control sample to which a known amount of analyte has been added before sample work up. This sample is then extracted and analyzed according to the method along with, or "concurrently", with the unknown samples in order to determine the performance of the method.
Instrument Recovery:	A control sample carried through the method to which a known amount of analyte has been added directly before injection. This sample is analyzed within the sample set in order to evaluate the matrix effect on the instrument response. The instrument recovery is also known as a "matrix-matched standard."
Analytical Run:	A group of samples that undergo a determinative measurement on an analytical instrument in a defined and continuous sequence under identical instrumental conditions.
Limit of Quantitation (LOQ):	Lowest tested concentration of the analyte in a sample that can be determined with acceptable accuracy and precision according to the analytical method. This is also known as reporting limit.
Limit of Detection (LOD):	Concentration of analyte equivalent to a defined percentage of the limit of quantitation of the method (e.g 20% of LOQ). At this concentration, the analyte must be qualitatively detectable in sample matrix (analyte peak height at least 3x baseline noise).

1 Introduction

BAS 850 H is a PPO herbicide, and is developed by BASF to be used for a broad spectrum of crops in the US. The analytical method R0048/01 allows for the determination of M850H040 residues in surface and drinking water.

BASF Method Number R0048/01 was successfully tested during method development in surface and drinking water.

This method was developed at BASF Agricultural Solutions, Research Triangle Park, NC, USA.

History of the method

TP Version	Release Date	Change
01	April 5, 2018	New method
01	August 8, 2018	Added stability information (sections 2.6.5, 3.1), updated matrix effects (section 3.6), corrected typos

2 Materials

2.1 Safety

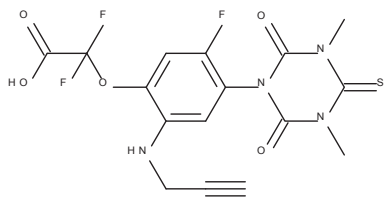
The test and reference items, as well as the chemicals required for this analysis, should be handled in accordance with good industrial hygiene and safety practice. Avoid contact with the skin, eyes and clothing. Wearing of closed work clothing is recommended. Remove contaminated clothing. Store work clothing separately. Keep away from food, drink and animal feed stuffs. No eating, drinking, smoking or tobacco use at the place of work. Hands and/or face should be washed before breaks and at the end of the shift. All procedures involving organic solvents should be performed in a well-ventilated hood.

Safety details are given in the Safety Data Sheets (SDS) of the individual substances. The SDS should be considered before start of a study/handling the substance.

Disposal of samples and chemicals must be done in compliance with on-site safety policies and procedures.

2.2 Test and Reference Items

Test and reference items should be stored according to the information provided in the certificate of analysis.

BAS-Code	M850H040	
IUPAC 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	
Reg. No.	6095223	
CAS-No.	N/A	
Molecular Formula	$C_{16}H_{13}F_3N_4O_5S$	
Molecular Weight	430.4 g/mol	

2.3 Equipment:

Equipment	Size, Description	Manufacturer	Catalog No.
Balance, Analytical	Model AT100	Mettler	----
Balance, Top Loader	Model PJ3600	Mettler DeltaRange	----
Beakers	Various sizes	PYREX Brand, VWR Scientific Products	13922-029
Bottle, Amber glass	Qorpak, 2 oz and 4 oz with Teflon®-lined cap	VWR Scientific Products Boston Round, Amber	89042-908
Culture tube caps	16 mm	VWR	60828-768
Culture Tubes	Glass, disposable, 16x100mm size	Fisher	14-961-29
Cylinder, Graduated	Various sizes	Various	----
Flasks	Various sizes	Various	----
Plastic syringe	1 mL	Various	----
Positive Displacement Pipette and tips	1000 µL, 250 µL, 25 µL	Gilson Microman Fisher Scientific	----
Repeater Pipette and tips	50 mL	BrandTec Scientific	----
Syringe filter	PTFE Acrodisc® 0.45 µm pore size	Pall Gelman	4543
Volumetric, pipettes	0.5 mL, 1 mL, 2.5 mL, 5 mL, 10mL, 20 mL	Various – Class A	----
Volumetric flask	Various sizes	Various – Class A	----
Vortex	Genie 2	VWR Scientific Products	14216-184
LC System	Acquity UPLC I-Class System	Waters	----
Mass Spectrometer	Sciex 5500 Mass Spectrometer	Sciex	----
HPLC Column	Acquity BEH C18, 2.1x50 mm, 1.7 um	Waters	186002350

Note: The equipment and instrumentation listed above represents typical laboratory equipment and can be substituted by equipment of similar technical specifications. Suitability of the entire set of equipment is confirmed if the recoveries of the fortification experiments are in the expected concentration range.

2.4 Chemicals

Chemical	Grade	Manufacturer/Supplier	Catalog No.
Formic acid (LC Mobile Phase Use)	95%	Sigma Aldrich	F0507-100 mL
Methanol	HPLC Grade	MilliporeSigma	MX0475P-1
Acetonitrile	HPLC Grade	MilliporeSigma	AX0156-1
Water	HPLC Grade	BDH Aristar Plus	87003-652

Note: Equivalent reagents and chemicals from other suppliers may be used.

2.5 Solvent Mixtures

Description	Code	Composition
Mobile Phase A	**LC1**	0.1% Formic Acid in Water Add 1 mL of formic acid to 1 L of water into an appropriate container and mix well to ensure complete homogeneous solution.
Mobile Phase B	**LC2**	0.1% Formic Acid in Acetonitrile Add 1 mL of formic acid to 1 L of acetonitrile into an appropriate container and mix well to ensure complete homogeneous solution.

Note: If necessary, the solutions may also be prepared in different volumes as long as the proportions are not modified.

2.6 Working Solutions

2.6.1 Stock Solutions

Prepare a 1.0 mg/mL stock solution individually by weighing an appropriate amount of analyte into a flask and add the required volume of methanol. To calculate the final true concentration, the actual weight (and if required purity see below) has to be documented and taken into account.

For example, to prepare 10 mL of 1.0 mg/mL stock solution of M850H040 in methanol, weigh 10 mg M850H040 into a 10 mL volumetric flask. Dissolve and dilute to mark with methanol. Ensure a complete homogeneous solution (e.g. by sonication or vortexing).

Independence of standard calibration and fortification solutions should initially be confirmed to show correct preparation of the solutions. This can be achieved for example using one of the following approaches:

- Two stock solutions are independently prepared. One is used for preparation of fortification solutions, the other for calibration standard solutions.

- Fortification and calibration standard solutions should be prepared from one stock solution in separate dilution series.

For subsequent preparations of solutions, freshly prepared solutions can be compared directly to previous standard solutions.

A correction for purity is mandatory if the purity is ≤ 95%. If the purity is > 95 % correction is optional.

2.6.2 Fortification Solutions

Prepare standard solutions in a flask. Dilute, e.g. volumetrically with appropriate solvents as exemplified in the table below and ensure a complete homogeneous solution (e.g. by vortexing).

Examples for Preparation of Fortification Solutions

Initial Concentration (µg/mL)	Aliquot Volume (mL)	Dilute with methanol to a final volume of (mL)	Final Concentration (µg/mL)
1000	0.25	25	10
10	1.0	10	1
1	0.75	25	0.03
0.03	2.5	25	0.003

Note: A different concentration scheme may be used. If necessary, the volume of solution prepared may be changed.

2.6.3 Calibration Standard Solutions

Prepare standard calibration solutions for LC-MS/MS analysis by using the solutions that were prepared in Section "stock solutions" or "fortification solutions" in flasks. Dilute e.g. volumetrically with appropriate solvents as exemplified in the table below and ensure a complete homogeneous solution (e.g. by vortexing).

Examples for Preparation of standard solutions for calibration

Initial Concentration (ng/mL)	Aliquot Volume (mL)	Dilute with water to a final volume of (mL)	Final Concentration (ng/mL)
30*	1.0	30	1.0
1.0	3.75	25	0.15†
1.0	1.5	25	0.06†
1.0	0.75	25	0.03†
1.0	0.375	25	0.015†
1.0	0.15	25	0.006†

* Solution is in methanol, prepared in section 2.6.2
† Proposed solutions to be used for calibration curve

Note: A Different concentration scheme may be used. If necessary, the volume of solution prepared may be changed.

2.6.4 Matrix matched Standards

In case matrix-matched standards (= instrument recovery samples) are needed, calibration standard solutions are prepared in matrix solution, i.e., using a final volume mixture from multiple control samples or using a large batch of sample, carried through the analytical procedure. Matrix-matched standards should be prepared in a way that the matrix load is at least 90% of the matrix load in the unknown samples.

Examples for Preparation of matrix-matched standards for calibration

Initial Concentration (ng/mL)	Aliquot Volume (mL)	Dilute with control water matrix to a final volume of (mL)	Final Concentration (ng/mL)
30*	0.10	3.0	1.0
1.0	0.30	2.0	0.15†
0.15	0.40	1.0	0.06†
0.15	0.20	1.0	0.03†
0.15	0.10	1.0	0.015†
0.15	0.040	1.0	0.006†

* Solution is in methanol, prepared in section 2.6.2
† Proposed solutions to be used for calibration curve

Note: A Different concentration scheme may be used. If necessary, the volume of solution prepared may be changed.

2.6.5 Stability of Standard Solutions

Stability of M850H040 solutions in methanol (stock and fortification solutions) is at least 32 days when stored under refrigerated conditions. Stability of calibration standard solutions in water is 32 days when stored under refrigerated conditions [Reference 1].

3 Analytical procedure

The methodology described below can be scaled up and/ or down as long as proportions are kept constant.

3.1 Sample Preparation

Sample homogenization is not needed for water samples. However, samples should be fully thawed and mixed before removing an aliquot for analysis.

3.2 Weighing and Preparation of Fortified / Treated Samples

For fortified samples, 5.0 ± 0.1 mL of control water sample are measured into a suitable glass container to allow proper mixing, e.g. a disposable culture tube. Appropriate amounts of fortification solutions are added to the matrix according to the table below.

The following fortification scheme may be used:

Sample Type	Sample Volume	Concentration of Spiking Solution	Volume of Spiking Solution	Level of Fortification
Control	5.0 mL	-	-	-
Fortification (LOQ)	5.0 mL	0.003 µg/mL	0.050 mL	30 ng/L *
Fortification (10xLOQ)	5.0 mL	0.03 µg/mL	0.050 mL	300 ng/L

* limit of quantitation

Note: Different concentration schemes can be used, if different fortification levels are required.

Total volume of solutions prepared can be changed if overall ratios are maintained. Volume of spiking solution added to generate the fortified sample should not exceed 10 % of sample weight or volume.

3.3 Extraction of Water Samples

No extraction is necessary for water matrices.

3.4 Sample Clean-up

No clean-up is necessary for water matrices.

3.5 Preparation for Measurement

Filter all samples using 0.45µm PTFE syringe filters directly into HPLC injection vials, sending the first approximately 0.1 – 0.2 mL to waste.

In case of high residues, an appropriate dilution with water may be necessary to remain in the linear range of the calibration curve.

3.6 Influence of matrix effects on analysis

During method validation, it was demonstrated that the matrix load in the samples from the water matrices had no significant influence on the analysis (i.e., matrix effects < 20%). Therefore, samples can be analyzed using calibration standard solutions prepared in purified water.

If significant matrix effects are observed, matrix-matched standards can be used.

3.1 Stability of Final Volumes

M850H040 has been shown to be stable in final volume for at least the time period tested, 7 days in drinking water and 8 days in surface water [Reference 1].

4 Quantification and calculation

4.1 Set-up of the analytical run

A sequence for measurement generally consists of:
- Calibration standards
- Control samples
- Procedural recovery samples
- Unknown samples
- Instrument recovery sample

Reagent blanks or blanks can also be injected if considered necessary. Each injection set should begin and end with an injection of a calibration standard. Standards should be interspersed with samples. Each calibration standard should at least be injected twice. At least 5 calibration levels are needed. At least one Instrument recovery sample has to be measured with each sequence if solvent calibration standards are used. In case of Matrix-matched calibration standards, it can be omitted.

4.2 Instrumental analysis

4.2.1 Instrumentation and Conditions for M850H040

	Parameter		
Chromatographic System	Waters Acquity UPLC system**		
Analytical-column	Acquity BEH C18, 2.1 x 50 mm, 1.7 µm particle size		
Column Temperature	50 ºC		
Injection Volume	10 µL (can be raised or lowered depending on sensitivity)		
Mobile Phase A Mobile Phase B	Water with 0.1% formic acid (**LC1**) Acetonitrile with 0.1% formic acid (**LC2**)		
Flow Rate	600 µL/min		
Gradient (including wash and equilibration)	Time (min)	Phase A	Phase B
	0.00 1.00 1.25 2.25 2.50 3.00	85 50 5 5 85 85	15 50 95 95 15 15
Detection System	AB Sciex 5500 Mass Spectrometer		
Ionization	Electrospray (ESI)		
Ionization Temperature	500 °C		
Analyte	**Transitions (m/z)**	**Polarity**	**Expected Retention Time**
M850H040 (Reg No 6095223)	429 → 335* 429 → 296	Negative	~ 1.7 min

* Proposed as quantification transition. Any of these transitions could be used for quantitation.

** The above gradient is appropriate for the hardware profile listed. Different instrument combinations may require additional time for column rinse (high organic) or additional equilibration time at the end of the LC conditions to prepare the system and/or column for the next injection.

Note: Instruments with similar specifications may substitute the equipment listed above. The instruments used are applicable for analysis if the recoveries of the fortification experiments are in the acceptable range.

A divert valve can be used to reduce the matrix load on the detection system. Instrument conditions, e.g. injection volume, column, gradient steps may be modified; however, changes have to be documented in the raw data. Changes are acceptable, if the recoveries of the fortification experiments are in the acceptable range of the required guidelines.

If the use of different analytical columns (different stationary phase) or mass transitions is required, then methodology has to be validated by analyzing at least five replicates of fortified samples prepared at e.g. LOQ and 10xLOQ. Assessment of matrix impact by preparation of at least one concentration level of a matrix matched standard is also required.

Other parameters such as ion source temperature, gas flows, and voltages, are highly specific to the equipment used and therefore not listed. Those parameters need to be adapted to the actual instrument used. To obtain stable measurements, it is recommended to condition the instrument properly.

4.2.2 Calibration procedures

Calculation of results is based on peak area measurements using a calibration curve. At least 5 calibration levels need to be injected (e.g., required for enforcement).

The calibration curve is obtained by direct injection of solvent or matrix standards into LC-MS/MS in the range of e.g. 0.006 ng/mL to 0.15 ng/mL. In a given injection run, the same injection volume is used for all samples and standards.

Linear calibration functions are preferred for evaluation. If other functions are used (e.g. quadratic), this should be fully justified.

4.2.3 Calculation of Residues and Recoveries

Calculation of results is based on area measurements.

For procedural recoveries, the sample volume will be considered to be 5.0 mL in the final calculation of residues [ng/L]. This approach requires that the sample volume has to be within a measurement precision of +/- 0.1 mL for fortification samples (matrix). The recovery is the percentage of the fortified amount of the analyte (µg or ng), which is recovered after the entire sample work-up steps.

Outside of this range, the actual sample weight must be used of the calculation, for this approach the amount of fortified analyte(s) as to be calculated individually, depending on the sample weight.

Calculation is described by the equation given below:
The residues of all analytes in ng/L are calculated as shown in equations **I** and **II**:

I. **Concentration [ng/mL]** $= \frac{\text{Response} - \textit{Intercept}}{\textit{Slope}} = C_A$

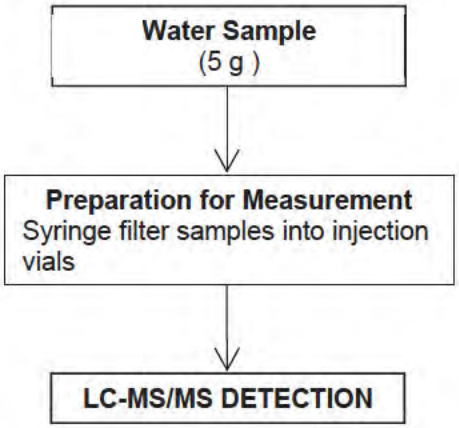
II. **Residue [ng/L]** $= \frac{V_{End} \times C_A \times 1000}{G \times A_F}$

V_{end}	=	Final volume of the extract after all dilution steps [mL]
C_A	=	Concentration of analyte as read from the calibration curve [ng/mL]
G	=	Volume of the sample extracted [mL]
A_F	=	Aliquotation factor
1000	=	Factor remaining after all unit conversions

The recoveries of spiked compounds are calculated according to equation **III**:

III. **Recovery %** $\frac{(\text{Residue in fortified sample} - \text{Residue in control}) \times 100}{\text{Amount of analyte fortified}}$

5 Flowchart


Water Sample
(5 g)
Preparation for Measurement
Syringe filter samples into injection vials
LC-MS/MS DETECTION

6 Method management and time requirements

Description of the amount of time required for sample preparation, analysis and data reduction.
The analysis of one series of samples (= 13 unknown samples, 2 fortified samples for recovery experiments, 1 blank sample) requires 0.5 working days (4 hours) per laboratory assistant. This time includes the calculation of the results, the preparation of the equipment as well as the reporting of all raw data under GLP.

7 Conclusion and method capabilities

Limit of Quantification (LOQ) and Limit of Detection (LOD)
The limit of quantification is defined as the lowest fortification level successfully tested.
The limit of quantification is 30 ng/L.
The limit of detection is 20% of the limit of quantification.
The lowest standard for each analyte in the calibration curve has good detectability (signal to noise ratio greater than 3:1).

Selectivity
The tested untreated surface and drinking water samples showed no significant interferences (< 20 %) at the retention time of the analyte of interest.

Confirmatory Techniques
The LC-MS/MS determination for M850H040 is a highly selective detection technique. Quantitation is possible at two different mass transitions. Therefore, no additional confirmatory technique is required.

Potential Problems
M850H040 has been observed to be unstable in acidic aqueous solutions. Therefore, acid should be avoided for stored solutions.

9 Appendix

9.1 Example of Calculation

Example: M850H040, 429 → 335; surface water sample fortified at 30 ng/L:

Concentration in the final volume [ng/mL]

Concentration [ng/mL]:

$$= \frac{Response\ -Intercept}{Slope} = C_A$$

Residue in the sample [mg/kg]

Residue [ng/L]:

$$= \frac{V_{End} \times C_A \times 1000}{G \times A_F}$$

Recovery %:

$$= \frac{(\boldsymbol{Residue\ in\ fortified\ sample\ -Residue\ in\ control}) \times \mathbf{100}}{\boldsymbol{Amoun}\text{t of analyte fortified}}$$

The following values were used in this calculation:

Response of fortified sample	31645
Response of control sample	0
Slope:	1120000
Intercept:	-63.1
Sample Volume (G):	5.0 mL
Final Volume (V_{end}):	5.0 mL
Aliquotation factor A_F:	1 (= 100%)
Conversion factor mL → L:	1000

Concentration (ng/mL):

$$= \frac{31645\ -(-63.1)}{1120000} = 0.0282\ ng/mL$$

Residue (ng/L):

$$= \frac{5.0\ mL \times 0.0282\ ng/mL \times 1000}{5.0\ mL \times 1} = 28.2\ ng/L$$

Recovery %:

$$= \frac{(28.2\ \text{ng/L}\ -0.00\ \text{ng/L}) \times 100}{30\ \text{ng/L}} = 94.0\%$$

Appendix C. Example Calculations

Typical Recovery Calculation for LC-MS/MS Quantitation

Sample No. 858175-02-04. Control surface water sample fortified with M850H040 at the LOQ (30 ppt), Master Sheet No. 858175-2.

$$\text{Concentration of analyte (ng/mL)} = \frac{\text{peak area - intercept}}{\text{slope}}$$

	M850H040
Peak Area =	12842
Intercept =	-1.49E+01
Slope =	4.97E+05
Conc. (ng/mL) =	0.0259

The concentration of analyte in µg/kg (ppt) is calculated as shown in equation:

$$\text{Residue [ppt]} = \frac{V_{end} \times C_A \times 1000}{G \times A_F}$$

Where:

V_{end} = Final volume [mL] after all dilution steps
C_A = Concentration of analyte as read from the calibration curve [ng/mL]
G = Volume of the sample extracted [mL]
A_F = Aliquotation factor
1000 = Factor remaining after all unit conversions

	M850H040
V_{end} =	5 mL
A_F =	100%
G =	5.00
Conc. (ng/mL) =	0.0259
Residue (ppt) =	25.9

Net residue (ppt of analyte) = Residue (ppt of analyte) - Residue in Control (ppt)

$$\text{Recovery of analyte (\%)} = \frac{\text{Residue (ppt of analyte) - Residue in Control (ppt)}}{\text{Amount Fortified (ppt)}} \times 100$$

Use full calculator precision in any intermediate calculations. Round only the final value.

METHOD SUMMARY FOR WATER ANALYSIS

TESTING LABORATORY: **AGVISE LABORATORIES, INC.**
P.O. BOX 510; 604 HIGHWAY 15
NORTHWOOD, ND 58267-0510
(701) 587-6010

The following is a summary of analytical methods used by AGVISE Laboratories, Inc. in the determination of water characteristics and nutrient content. Analytical data of some or all of these analytical methods are presented based upon the testing requested by the firm submitting the water specimens.

Alkalinity – Determined by titration with 1N Sulfuric Acid (NUT.02.30).

Anions NO3-N, NO2-N, Sulfate-S, Phosphate-P, Chloride, Fluoride, Bromide – Determined using ION Chromatography (NUT.02.112).

Carbonate and Bicarbonate – Determined by titration using 1N Sulfuric Acid and 0.25% Phenophthalieum in 50% Ethanol (NUT.02.26).

Cations Ca, Na, Mg, K, Fe, Zn, Mn, and Cu – The cations are determined by using the ICP (NUT.02.97).

Chemical Oxygen Demand – Chemical Oxygen Demand is determined by measuring the portion of the organic matter susceptible to oxidation by a strong oxidant (NUT.02.38).

Conductivity – Determined by using a Conductivity Meter (NUT.02.22).

Hardness – Calculated from the Ca & Mg content in a water specimen (NUT.02.18).

Nitrogen, Total – Determined by the Kjeldahl procedure (NUT.02.28).

Organic Carbon, Total & Dissolved – Determined by using an Elementar Vario TOC Cube Analyzer (NUT.02.111)

Oxygen, Dissolved – Determined by using the Azide modification of the Winkler titration method (NUT.02.37).

pH – Determined by using a pH Electrode (NUT.02.17).

Phosphorus, Total, Organic & Reactive – Determined colorimetrically using a Potassium Persulfate Molybdovanadate method (NUT.02.64)

Appendix B:

Evaluation of the Limit of Detection (LOD) for Method R0048/01, “Method for the Determination of M850H040 (Reg. No. 6095223) in Surface and Drinking Water by LC-MS/MS"

BASF Study Number:
858175_01

BASF Registration Document Number:
2018/7005523

1. INTRODUCTION

1.1 Background

BAS 850 H is an herbicide used in multiple crops. To analyze for M850H040, BASF analytical method R0048/01, “Method for the Determination of M850H040 (Reg. No. 6095223) in Surface and Drinking Water by LC-MS/MS" was validated (**Reference 1**). The purpose of this study is to evaluate the LOD for this validated method.

1.2 Definitions

Method Detection Limit (MDL):	The method detection limit (MDL) is the lowest level that the instrument can reliably differentiate from a blank or non-detect sample.
Limit of Detection (LOD):	The limit of detection (LOD) is the lowest level that can be reliably brought through the method and quantitated.
Limit of Quantitation (LOQ):	The limit of quantitation (LOQ) is the lowest level of fortification tested of an analyte in the matrix and is determined by the proposed tolerance.

2. MATERIALS AND METHODS

2.1 Test Systems

The test system considered in this study was surface water. The surface water was previously characterized at AGVISE Laboratories (604 Highway 15 West, Northwood, ND 58267).

The matrix effects were evaluated in the validation of method R0048/01 (**Reference 1**). No significant (≥20%) matrix effects were shown for either water matrix. Surface water was chosen as the matrix to use when conducting the MDL and LOD evaluation.

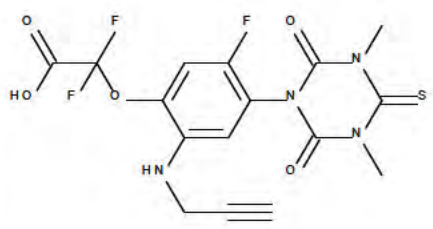
2.2 Test and Reference Substances

The test/reference substance, shown below, was synthesized by BASF Aktiengesellschaft (Limburgerhof, Germany) and was maintained frozen until use in this study. BASF Aktiengesellschaft determined characterization and purity prior to the substance being used in this study. Details of this determination is available to BASF and is located at Landwirtschaftliche Versuchsstation der BASF, Limburgerhof,

.

Common Name	None assigned
BAS Code Name	M850H040
IUPAC 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	C16H13F3N4O5S
Molecular Weight	430.4
Lot No.	L2017-109
Purity:	87.2%
Expiration Date	December 01, 2019

Chemical structure:



The test/reference substance are the same substance. The substance was used in solution to generate data for both instrument and method performance.

2.3 Route of Administration

In this study, the test substance was applied to the test system as analytical standard solutions by micropipette to ensure precise delivery of a small amount of the test items.

3. METHODOLOGY TO EVALUATE MDL and LOD

3.1 Method Synopsis

For method R0048/01, M850H040 residues in water samples are filtered (0.45 µm PTFE) and analyzed by LC-MS/MS.

The storage stabilities of the extracts and final volume were established in the method validation (**Reference 1**).

3.2 Methodology to Determine MDL

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. See Appendix G. 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 No. 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 are put into the equation below:

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

MDL = Method detection limit
S = Standard deviation of the matrix-spiked sample set concentrations
$t_{(N-1,1-\infty=.99)}$ = Critical t value from a student t-test table at 99% confidence

Acceptance criteria for MDL:

a. The determined MDL must 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 determined MDL.

4. A fortified control sample at the MDL was injected on the LC-MSMS (no standard curve required) to verify that the MDL can be seen with a S/N ≥ 2.

3.3 Methodology to Determine LOD

Based on an evaluation of the susceptibility of the analyte of interest to instrument variability, LC-MS/MS drift, unexpected contamination, and untested matrix effects, the MDL was raised to an appropriate value that will mitigate the anticipated issues. This new value will be the LOD.

4.5 Specificity/Selectivity

Quantitation of M850H040 was accomplished by LC-MS/MS, monitoring in the negative ionization mode. The ion transitions used are as follows:

Analyte	Ionization Mode	Transition (*m/z*)	Primary or Confirmatory Quantitation
M850H040	Negative	429 → 335	Primary
		429 → 296	Confirmatory

All of these transitions were considered when choosing the best candidates for the evaluation of LOD.

6. STATISTICS AND DATA INTEGRITY

Statistical treatment of the data included simple descriptive statistics, such as determinations of standard deviation for the matrix-spiked samples and area counts and calculation of the calibration curve and correlation coefficient (r) by linear regression of the instrument responses for the reference standards. The statistical calculations throughout this report were performed using an automated computer spreadsheet (Microsoft Excel® or Analyst®) and were rounded for presentation purposes. Slight differences may be noted in hand calculations using the recoveries presented in the tables. These are due to rounding and have no effect on the scientific conclusions presented in this report. The detailed analytical data may be consulted for confirmation of the calculated results.

All signal to noise (S/N) calculations were conducted by the following equation in Analyst®:

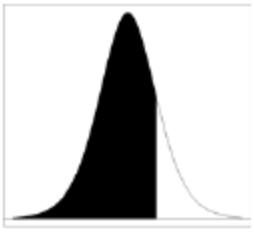
$$S/N = \frac{\Delta\, Height\ of\ Analyte\ Peak}{\Delta\, Height\ of\ Appropriate\ Noise\ Region}$$

Several measures were taken to ensure the quality of the study results. The quality assurance unit at BASF inspected the analytical procedures for compliance with Good Laboratory Practices that included adherence to the protocol. The dates inspected are detailed in the quality assurance unit statement. Study samples and test and reference items were maintained in secured (i.e. pad-locked) storage with limited access. Freezer temperatures were continuously monitored by electronic means.

7. CONCLUSIONS

Within the scope of BASF Analytical Method No. R0048/01, the MDL was successfully determined to be 0.000024 ng on-column for M850H040. After an evaluation of the analyte of interest to instrument variability, LC-MS/MS drift, unexpected contamination, and untested matrix effects, the LOD was determined to be 0.00006 ng on-column for M850H040. The results of this LOD evaluation study demonstrate that BASF Analytical Method No R0048/01 fulfills the requirements

TABLE A-2. CRITICAL VALUES OF STUDENT'S-*t* DISTRIBUTION



$t_{1-\alpha}$

Degrees of Freedom	1 - α								
	0.70	0.75	0.80	0.85	0.90	0.95	0.975	0.99	0.995
1	0.727	1.000	1.376	1.963	3.078	6.314	12.706	31.821	63.657
2	0.617	0.816	1.061	1.386	1.886	2.920	4.303	6.965	9.925
3	0.584	0.765	0.978	1.250	1.638	2.353	3.182	4.541	5.841
4	0.569	0.741	0.941	1.190	1.533	2.132	2.776	3.747	4.604
5	0.559	0.727	0.920	1.156	1.476	2.015	2.571	3.365	4.032
6	0.553	0.718	0.906	1.134	1.440	1.943	2.447	3.143	3.707
7	0.549	0.711	0.896	1.119	1.415	1.895	2.365	2.998	3.499
8	0.546	0.706	0.889	1.108	1.397	1.860	2.306	2.896	3.355
9	0.543	0.703	0.883	1.100	1.383	1.833	2.262	2.821	3.250
10	0.542	0.700	0.879	1.093	1.372	1.812	2.228	2.764	3.169
11	0.540	0.697	0.876	1.088	1.363	1.796	2.201	2.718	3.106
12	0.539	0.695	0.873	1.083	1.356	1.782	2.179	2.681	3.055
13	0.538	0.694	0.870	1.079	1.350	1.771	2.160	2.650	3.012
14	0.537	0.692	0.868	1.076	1.345	1.761	2.145	2.624	2.977
15	0.536	0.691	0.866	1.074	1.34	1.753	2.131	2.602	2.947
16	0.535	0.690	0.865	1.071	1.337	1.746	2.120	2.583	2.921
17	0.534	0.689	0.863	1.069	1.333	1.740	2.110	2.567	2.898
18	0.534	0.688	0.862	1.067	1.330	1.734	2.101	2.552	2.878
19	0.533	0.688	0.861	1.066	1.328	1.729	2.093	2.539	2.861
20	0.533	0.687	0.860	1.064	1.325	1.725	2.086	2.528	2.845
21	0.532	0.686	0.859	1.063	1.323	1.721	2.080	2.518	2.831
22	0.532	0.686	0.858	1.061	1.321	1.717	2.074	2.508	2.819
23	0.532	0.685	0.858	1.060	1.319	1.714	2.069	2.500	2.807
24	0.531	0.685	0.857	1.059	1.318	1.711	2.064	2.492	2.797
25	0.531	0.684	0.856	1.058	1.316	1.708	2.060	2.485	2.787
26	0.531	0.684	0.856	1.058	1.315	1.706	2.056	2.479	2.779
27	0.531	0.684	0.855	1.057	1.314	1.703	2.052	2.473	2.771
28	0.530	0.683	0.855	1.056	1.313	1.701	2.048	2.467	2.763
29	0.530	0.683	0.854	1.055	1.311	1.699	2.045	2.462	2.756
30	0.530	0.683	0.854	1.055	1.310	1.697	2.042	2.457	2.750
40	0.529	0.681	0.851	1.050	1.303	1.684	2.021	2.423	2.704
60	0.527	0.679	0.848	1.046	1.296	1.671	2.000	2.390	2.660
120	0.526	0.677	0.845	1.041	1.289	1.658	1.980	2.358	2.617
∞	0.524	0.674	0.842	1.036	1.282	1.645	1.960	2.326	2.576

Note: The last row of the table (∞ degrees of freedom) gives the critical values for a standard normal distribution (*Z*), e.g., $t_{\infty, 0.95} = z_{0.95} = 1.645$.

Example Calculation (M850H040)

Method Detection Limit (MDL)

The MDL is the lowest limit the instrument can reliably distinguish from a blank of the least sensitive analyte/transition, preparation for analysis, and injection on LC-MS/MS.

The calculation is based on the methodology described in 40 CFR Ch 1, part 136 appendix B (**Reference 2**). Seven (7) unique control samples were brought through the method and fortified at the end of the procedure at a concentration of 0.006 ng/mL. These samples have a representative amount of matrix, indicative of an incurred residue sample at the same concentration. The samples were injected along with a bracketing standard curve at an injection volume of 10 µL.

The MDL was calculated to be 0.0000232 ng on-column using the following equations:

Equation 1: $$\mathrm{MDL\ (ng/mL)} = \mathrm{S} \times \mathrm{t}_{(\mathrm{N-1,1-\infty=.99})}$$

S = standard deviation of the 7 matrix spiked samples
$t_{(N-1,1-\infty=.99)}$ = critical t value from student t-test table (Appendix C)

Equation 2: $$\mathrm{MDL\ on\text{-}column\ (ng)} = \mathrm{MDL\ (ng/mL)} \times \mathrm{Injection\ Volume\ (mL)}$$

Equation 3: $$\mathrm{Concentration\ (ng/mL)} = \frac{\mathrm{Response-Intercept}}{\mathrm{Slope}}$$

Example Calculation (continued)

Standard Curve Info

Intercept =	51.7
Slope =	5.94E+04
r =	0.9969

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 (µL)	**10**
MDL (ng/mL) =	**0.00232**
MDL (ng on-column) =	**0.0000232**

We create chemistry

Method Procedure:

Method for the determination of M850H040 (Reg.No. 6095223) in Surface and Drinking Water by LC-MS/MS

BASF Method Number R0048/01

Definitions and acronyms

Sample Set:	A group of samples that are extracted and cleaned up at the same time using the same method represented.
Untreated Sample:	A sample that has not been treated with the test substance.
Control Sample:	Usually an untreated sample used for fortification experiments (it can be acquired from the same study or from a different source e.g. a different study or commercially purchased).
Unknown Sample:	A sample with unknown residues.
Treated Sample:	A sample that has been treated with the test substance.
(Solvent) Blank:	Solvent, solution or mobile phase injected together with a sample set during the analytical run.
Reagent Blank:	A sample consisting of solvents and reagents only in the absence of any sample matrix subjected to complete analysis according to the method. This type of sample is also known as "blank of reagents" or "procedural blank," and is analyzed within the sample set in order to evaluate possible contamination from chemicals/reagents.
Procedural Recovery: **(Fortification Sample)**	A control sample to which a known amount of analyte has been added before sample work up. This sample is then extracted and analyzed according to the method along with, or "concurrently", with the unknown samples in order to determine the performance of the method.
Instrument Recovery:	A control sample carried through the method to which a known amount of analyte has been added directly before injection. This sample is analyzed within the sample set in order to evaluate the matrix effect on the instrument response. The instrument recovery is also known as a "matrix-matched standard."
Analytical Run:	A group of samples that undergo a determinative measurement on an analytical instrument in a defined and continuous sequence under identical instrumental conditions.
Limit of Quantitation (LOQ):	Lowest tested concentration of the analyte in a sample that can be determined with acceptable accuracy and precision according to the analytical method. This is also known as reporting limit.
Limit of Detection (LOD):	Concentration of analyte equivalent to a defined percentage of the limit of quantitation of the method (e.g 20% of LOQ). At this concentration, the analyte must be qualitatively detectable in sample matrix (analyte peak height at least 3x baseline noise).

1 Introduction

BAS 850 H is a PPO herbicide, and is developed by BASF to be used for a broad spectrum of crops in the US. The analytical method R0048/01 allows for the determination of M850H040 residues in surface and drinking water.

BASF Method Number R0048/01 was successfully tested during method development in surface and drinking water.

This method was developed at BASF Agricultural Solutions, Research Triangle Park, NC, USA.

History of the method

TP Version	Release Date	Change
01	April 5, 2018	New method
01	August 8, 2018	Added stability information (sections 2.6.5, 3.1), updated matrix effects (section 3.6), corrected typos

2 Materials

2.1 Safety

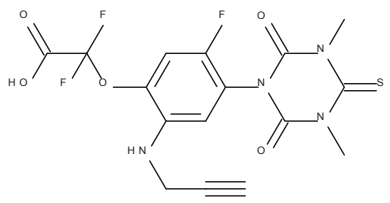
The test and reference items, as well as the chemicals required for this analysis, should be handled in accordance with good industrial hygiene and safety practice. Avoid contact with the skin, eyes and clothing. Wearing of closed work clothing is recommended. Remove contaminated clothing. Store work clothing separately. Keep away from food, drink and animal feed stuffs. No eating, drinking, smoking or tobacco use at the place of work. Hands and/or face should be washed before breaks and at the end of the shift. All procedures involving organic solvents should be performed in a well-ventilated hood.

Safety details are given in the Safety Data Sheets (SDS) of the individual substances. The SDS should be considered before start of a study/handling the substance.

Disposal of samples and chemicals must be done in compliance with on-site safety policies and procedures.

2.2 Test and Reference Items

Test and reference items should be stored according to the information provided in the certificate of analysis.

BAS-Code	M850H040	
IUPAC 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	
Reg. No.	6095223	
CAS-No.	N/A	
Molecular Formula	$C_{16}H_{13}F_3N_4O_5S$	
Molecular Weight	430.4 g/mol	

2.3 Equipment:

Equipment	Size, Description	Manufacturer	Catalog No.
Balance, Analytical	Model AT100	Mettler	----
Balance, Top Loader	Model PJ3600	Mettler DeltaRange	----
Beakers	Various sizes	PYREX Brand, VWR Scientific Products	13922-029
Bottle, Amber glass	Qorpak, 2 oz and 4 oz with Teflon®-lined cap	VWR Scientific Products Boston Round, Amber	89042-908
Culture tube caps	16 mm	VWR	60828-768
Culture Tubes	Glass, disposable, 16x100mm size	Fisher	14-961-29
Cylinder, Graduated	Various sizes	Various	----
Flasks	Various sizes	Various	----
Plastic syringe	1 mL	Various	----
Positive Displacement Pipette and tips	1000 µL, 250 µL, 25 µL	Gilson Microman Fisher Scientific	----
Repeater Pipette and tips	50 mL	BrandTec Scientific	----
Syringe filter	PTFE Acrodisc® 0.45 µm pore size	Pall Gelman	4543
Volumetric, pipettes	0.5 mL, 1 mL, 2.5 mL, 5 mL, 10mL, 20 mL	Various – Class A	----
Volumetric flask	Various sizes	Various – Class A	----
Vortex	Genie 2	VWR Scientific Products	14216-184
LC System	Acquity UPLC I-Class System	Waters	----
Mass Spectrometer	Sciex 5500 Mass Spectrometer	Sciex	----
HPLC Column	Acquity BEH C18, 2.1x50 mm, 1.7 um	Waters	186002350

Note: The equipment and instrumentation listed above represents typical laboratory equipment and can be substituted by equipment of similar technical specifications. Suitability of the entire set of equipment is confirmed if the recoveries of the fortification experiments are in the expected concentration range.

2.4 Chemicals

Chemical	Grade	Manufacturer/Supplier	Catalog No.
Formic acid (LC Mobile Phase Use)	95%	Sigma Aldrich	F0507-100 mL
Methanol	HPLC Grade	MilliporeSigma	MX0475P-1
Acetonitrile	HPLC Grade	MilliporeSigma	AX0156-1
Water	HPLC Grade	BDH Aristar Plus	87003-652

Note: Equivalent reagents and chemicals from other suppliers may be used.

2.5 Solvent Mixtures

Description	Code	Composition
Mobile Phase A	**LC1**	0.1% Formic Acid in Water Add 1 mL of formic acid to 1 L of water into an appropriate container and mix well to ensure complete homogeneous solution.
Mobile Phase B	**LC2**	0.1% Formic Acid in Acetonitrile Add 1 mL of formic acid to 1 L of acetonitrile into an appropriate container and mix well to ensure complete homogeneous solution.

Note: If necessary, the solutions may also be prepared in different volumes as long as the proportions are not modified.

2.6 Working Solutions

2.6.1 Stock Solutions

Prepare a 1.0 mg/mL stock solution individually by weighing an appropriate amount of analyte into a flask and add the required volume of methanol. To calculate the final true concentration, the actual weight (and if required purity see below) has to be documented and taken into account.

For example, to prepare 10 mL of 1.0 mg/mL stock solution of M850H040 in methanol, weigh 10 mg M850H040 into a 10 mL volumetric flask. Dissolve and dilute to mark with methanol. Ensure a complete homogeneous solution (e.g. by sonication or vortexing).

Independence of standard calibration and fortification solutions should initially be confirmed to show correct preparation of the solutions. This can be achieved for example using one of the following approaches:

- Two stock solutions are independently prepared. One is used for preparation of fortification solutions, the other for calibration standard solutions.

- Fortification and calibration standard solutions should be prepared from one stock solution in separate dilution series.

For subsequent preparations of solutions, freshly prepared solutions can be compared directly to previous standard solutions.

A correction for purity is mandatory if the purity is ≤ 95%. If the purity is > 95 % correction is optional.

2.6.2 Fortification Solutions

Prepare standard solutions in a flask. Dilute, e.g. volumetrically with appropriate solvents as exemplified in the table below and ensure a complete homogeneous solution (e.g. by vortexing).

Examples for Preparation of Fortification Solutions

Initial Concentration (µg/mL)	Aliquot Volume (mL)	Dilute with methanol to a final volume of (mL)	Final Concentration (µg/mL)
1000	0.25	25	10
10	1.0	10	1
1	0.75	25	0.03
0.03	2.5	25	0.003

Note: A different concentration scheme may be used. If necessary, the volume of solution prepared may be changed.

2.6.3 Calibration Standard Solutions

Prepare standard calibration solutions for LC-MS/MS analysis by using the solutions that were prepared in Section "stock solutions" or "fortification solutions" in flasks. Dilute e.g. volumetrically with appropriate solvents as exemplified in the table below and ensure a complete homogeneous solution (e.g. by vortexing).

Examples for Preparation of standard solutions for calibration

Initial Concentration (ng/mL)	Aliquot Volume (mL)	Dilute with water to a final volume of (mL)	Final Concentration (ng/mL)
30*	1.0	30	1.0
1.0	3.75	25	0.15†
1.0	1.5	25	0.06†
1.0	0.75	25	0.03†
1.0	0.375	25	0.015†
1.0	0.15	25	0.006†

* Solution is in methanol, prepared in section 2.6.2
† Proposed solutions to be used for calibration curve

Note: A Different concentration scheme may be used. If necessary, the volume of solution prepared may be changed.

2.6.4 Matrix matched Standards

In case matrix-matched standards (= instrument recovery samples) are needed, calibration standard solutions are prepared in matrix solution, i.e., using a final volume mixture from multiple control samples or using a large batch of sample, carried through the analytical procedure. Matrix-matched standards should be prepared in a way that the matrix load is at least 90% of the matrix load in the unknown samples.

Examples for Preparation of matrix-matched standards for calibration

Initial Concentration (ng/mL)	Aliquot Volume (mL)	Dilute with control water matrix to a final volume of (mL)	Final Concentration (ng/mL)
30*	0.10	3.0	1.0
1.0	0.30	2.0	0.15†
0.15	0.40	1.0	0.06†
0.15	0.20	1.0	0.03†
0.15	0.10	1.0	0.015†
0.15	0.040	1.0	0.006†

* Solution is in methanol, prepared in section 2.6.2
† Proposed solutions to be used for calibration curve

Note: A Different concentration scheme may be used. If necessary, the volume of solution prepared may be changed.

2.6.5 Stability of Standard Solutions

Stability of M850H040 solutions in methanol (stock and fortification solutions) is at least 32 days when stored under refrigerated conditions. Stability of calibration standard solutions in water is 32 days when stored under refrigerated conditions [Reference 1].

3 Analytical procedure

The methodology described below can be scaled up and/ or down as long as proportions are kept constant.

3.1 Sample Preparation

Sample homogenization is not needed for water samples. However, samples should be fully thawed and mixed before removing an aliquot for analysis.

3.2 Weighing and Preparation of Fortified / Treated Samples

For fortified samples, 5.0 ± 0.1 mL of control water sample are measured into a suitable glass container to allow proper mixing, e.g. a disposable culture tube. Appropriate amounts of fortification solutions are added to the matrix according to the table below.

The following fortification scheme may be used:

Sample Type	Sample Volume	Concentration of Spiking Solution	Volume of Spiking Solution	Level of Fortification
Control	5.0 mL	-	-	-
Fortification (LOQ)	5.0 mL	0.003 µg/mL	0.050 mL	30 ng/L *
Fortification (10xLOQ)	5.0 mL	0.03 µg/mL	0.050 mL	300 ng/L

* limit of quantitation

Note: Different concentration schemes can be used, if different fortification levels are required.

Total volume of solutions prepared can be changed if overall ratios are maintained. Volume of spiking solution added to generate the fortified sample should not exceed 10 % of sample weight or volume.

3.3 Extraction of Water Samples

No extraction is necessary for water matrices.

3.4 Sample Clean-up

No clean-up is necessary for water matrices.

3.5 Preparation for Measurement

Filter all samples using 0.45µm PTFE syringe filters directly into HPLC injection vials, sending the first approximately 0.1 – 0.2 mL to waste.

In case of high residues, an appropriate dilution with water may be necessary to remain in the linear range of the calibration curve.

3.6 Influence of matrix effects on analysis

During method validation, it was demonstrated that the matrix load in the samples from the water matrices had no significant influence on the analysis (i.e., matrix effects < 20%). Therefore, samples can be analyzed using calibration standard solutions prepared in purified water.

If significant matrix effects are observed, matrix-matched standards can be used.

3.1 Stability of Final Volumes

M850H040 has been shown to be stable in final volume for at least the time period tested, 7 days in drinking water and 8 days in surface water [Reference 1].

4 Quantification and calculation

4.1 Set-up of the analytical run

A sequence for measurement generally consists of:
- Calibration standards
- Control samples
- Procedural recovery samples
- Unknown samples
- Instrument recovery sample

Reagent blanks or blanks can also be injected if considered necessary. Each injection set should begin and end with an injection of a calibration standard. Standards should be interspersed with samples. Each calibration standard should at least be injected twice. At least 5 calibration levels are needed. At least one Instrument recovery sample has to be measured with each sequence if solvent calibration standards are used. In case of Matrix-matched calibration standards, it can be omitted.

4.2 Instrumental analysis

4.2.1 Instrumentation and Conditions for M850H040

	Parameter		
Chromatographic System	Waters Acquity UPLC system**		
Analytical-column	Acquity BEH C18, 2.1 x 50 mm, 1.7 µm particle size		
Column Temperature	50 ºC		
Injection Volume	10 µL (can be raised or lowered depending on sensitivity)		
Mobile Phase A Mobile Phase B	Water with 0.1% formic acid (**LC1**) Acetonitrile with 0.1% formic acid (**LC2**)		
Flow Rate	600 µL/min		
Gradient (including wash and equilibration)	Time (min)	Phase A	Phase B
	0.00 1.00 1.25 2.25 2.50 3.00	85 50 5 5 85 85	15 50 95 95 15 15
Detection System	AB Sciex 5500 Mass Spectrometer		
Ionization	Electrospray (ESI)		
Ionization Temperature	500 °C		
Analyte	**Transitions (m/z)**	**Polarity**	**Expected Retention Time**
M850H040 (Reg No 6095223)	429 → 335* 429 → 296	Negative	~ 1.7 min

* Proposed as quantification transition. Any of these transitions could be used for quantitation.

** The above gradient is appropriate for the hardware profile listed. Different instrument combinations may require additional time for column rinse (high organic) or additional equilibration time at the end of the LC conditions to prepare the system and/or column for the next injection.

Note: Instruments with similar specifications may substitute the equipment listed above. The instruments used are applicable for analysis if the recoveries of the fortification experiments are in the acceptable range.

A divert valve can be used to reduce the matrix load on the detection system. Instrument conditions, e.g. injection volume, column, gradient steps may be modified; however, changes have to be documented in the raw data. Changes are acceptable, if the recoveries of the fortification experiments are in the acceptable range of the required guidelines.

If the use of different analytical columns (different stationary phase) or mass transitions is required, then methodology has to be validated by analyzing at least five replicates of fortified samples prepared at e.g. LOQ and 10xLOQ. Assessment of matrix impact by preparation of at least one concentration level of a matrix matched standard is also required.

Other parameters such as ion source temperature, gas flows, and voltages, are highly specific to the equipment used and therefore not listed. Those parameters need to be adapted to the actual instrument used. To obtain stable measurements, it is recommended to condition the instrument properly.

4.2.2 Calibration procedures

Calculation of results is based on peak area measurements using a calibration curve. At least 5 calibration levels need to be injected (e.g., required for enforcement).

The calibration curve is obtained by direct injection of solvent or matrix standards into LC-MS/MS in the range of e.g. 0.006 ng/mL to 0.15 ng/mL. In a given injection run, the same injection volume is used for all samples and standards.

Linear calibration functions are preferred for evaluation. If other functions are used (e.g. quadratic), this should be fully justified.

4.2.3 Calculation of Residues and Recoveries

Calculation of results is based on area measurements.

For procedural recoveries, the sample volume will be considered to be 5.0 mL in the final calculation of residues [ng/L]. This approach requires that the sample volume has to be within a measurement precision of +/- 0.1 mL for fortification samples (matrix). The recovery is the percentage of the fortified amount of the analyte (µg or ng), which is recovered after the entire sample work-up steps.

Outside of this range, the actual sample weight must be used of the calculation, for this approach the amount of fortified analyte(s) as to be calculated individually, depending on the sample weight.

Calculation is described by the equation given below:
The residues of all analytes in ng/L are calculated as shown in equations **I** and **II**:

I. **Concentration [ng/mL]** $= \frac{\text{Response} - \textit{Intercept}}{\textit{Slope}} = C_A$

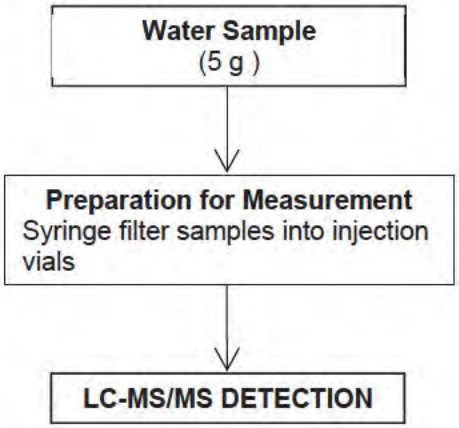
II. **Residue [ng/L]** $= \frac{V_{End} \times C_A \times 1000}{G \times A_F}$

V_{end}	=	Final volume of the extract after all dilution steps [mL]
C_A	=	Concentration of analyte as read from the calibration curve [ng/mL]
G	=	Volume of the sample extracted [mL]
A_F	=	Aliquotation factor
1000	=	Factor remaining after all unit conversions

The recoveries of spiked compounds are calculated according to equation **III**:

III. **Recovery %** $\frac{(\text{Residue in fortified sample} - \text{Residue in control}) \times 100}{\text{Amount of analyte fortified}}$

5 Flowchart



6 Method management and time requirements

Description of the amount of time required for sample preparation, analysis and data reduction.
The analysis of one series of samples (= 13 unknown samples, 2 fortified samples for recovery experiments, 1 blank sample) requires 0.5 working days (4 hours) per laboratory assistant. This time includes the calculation of the results, the preparation of the equipment as well as the reporting of all raw data under GLP.

7 Conclusion and method capabilities

Limit of Quantification (LOQ) and Limit of Detection (LOD)
The limit of quantification is defined as the lowest fortification level successfully tested.
The limit of quantification is 30 ng/L.
The limit of detection is 20% of the limit of quantification.
The lowest standard for each analyte in the calibration curve has good detectability (signal to noise ratio greater than 3:1).

Selectivity
The tested untreated surface and drinking water samples showed no significant interferences (< 20 %) at the retention time of the analyte of interest.

Confirmatory Techniques
The LC-MS/MS determination for M850H040 is a highly selective detection technique. Quantitation is possible at two different mass transitions. Therefore, no additional confirmatory technique is required.

Potential Problems
M850H040 has been observed to be unstable in acidic aqueous solutions. Therefore, acid should be avoided for stored solutions.

9 Appendix

9.1 Example of Calculation

Example: M850H040, 429 → 335; surface water sample fortified at 30 ng/L:

Concentration in the final volume [ng/mL]

Concentration [ng/mL]:

$$= \frac{Response\ -Intercept}{Slope} = C_A$$

Residue in the sample [mg/kg]

Residue [ng/L]:

$$= \frac{V_{End} \times C_A \times 1000}{G \times A_F}$$

Recovery %:

$$= \frac{(\boldsymbol{Residue\ in\ fortified\ sample\ -Residue\ in\ control}) \times \mathbf{100}}{\boldsymbol{Amoun}\text{t of analyte fortified}}$$

The following values were used in this calculation:

Response of fortified sample	31645
Response of control sample	0
Slope:	1120000
Intercept:	-63.1
Sample Volume (G):	5.0 mL
Final Volume (V_{end}):	5.0 mL
Aliquotation factor A_F:	1 (= 100%)
Conversion factor mL → L:	1000

Concentration (ng/mL):

$$= \frac{31645\ -(-63.1)}{1120000} = 0.0282\ ng/mL$$

Residue (ng/L):

$$= \frac{5.0\ mL \times 0.0282\ ng/mL \times 1000}{5.0\ mL \times 1} = 28.2\ ng/L$$

Recovery %:

$$= \frac{(28.2\ \text{ng/L}\ -0.00\ \text{ng/L}) \times 100}{30\ \text{ng/L}} = 94.0\%$$