MRID: 51207301

FINAL REPORT

Study Title

Validation of BASF Analytical Method R0067/01: “Method for the Determination of Residues of BAS 850 H (Reg. No. 5654329) and its Metabolite M850H001 (Reg. No. 5749359) in Soil with LOQ of 0.1 µg/kg using LC-MS/MS”

Guidelines Covered

US EPA Ecological Effects Test Guideline, OCSPP 850.6100 Environmental Chemistry Methods and Associated ILV
SANCO/3029/99 Rev 4 (11/07/2000)
SANCO/825/00 Rev 8.1 (16/11/2010)

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. R0067/01, used for the determination of residues of BAS 850 H and its metabolite M850H001 in soil with LOQ of 0.1 µg/kg by LC-MS/MS. The soil method (D1401/02, Reference 1) for the analysis of BAS 850 H and M850H001 with other relevant analytes was originally developed with LOQ of 0.001 mg/kg to support the terrestrial field dissipation study (Reference 2). The current method is now developed to fulfil the LOQ requirements supporting the ecotoxicology end points (seedling emergence studies, Reference 3).

2. MATERIALS AND METHODS

2.1 Test Systems

The untreated soil samples used as the test systems were provided by the Sponsor. The clay loam soil sample was sourced from BASF Study 834740 (Trial R180181; California, 12-18" untreated control soil, Reference 4). The sandy loam soil sample was sourced from BASF Study 780082 (Trial R170053; Iowa, 0-3" untreated control soil, (Reference 5). Both test systems were characterized by AGVISE Laboratories, and the GLP soil characterization reports are provided in the corresponding study reports (References 4 and 5). C

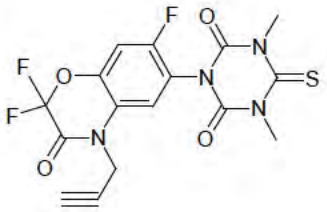
Soil samples were shipped frozen to ADPEN Laboratories, Inc. in Jacksonville, Florida and stored frozen (c.a. -20 °C) for the duration of the study unless needed for analyses. Each sample was assigned a unique number (e.g., 200409001-001) through ADPEN's Laboratory Information Management System (LIMS), and each analysis set was uniquely identified with a Work Order Number (e.g., WO-20052010). The test system samples were assigned unique numbers and these were recorded in each analytical set or "work order" (e.g., soil fortification sample 20052010-Recovery1-1, from Work Order Number WO-20052010). The actual sample numbers used for the analysis were identified in the raw data and in this final report.

2.2 Test and Reference Substances

The test/reference standards, shown below, were provided by the Sponsor and stored frozen (c.a. < -5 °C) upon receipt at the testing facility. Characterization and stability data for the substances is maintained by the Sponsor, and a reserve sample of these standards is retained at BASF, Research Triangle Park, North Carolina.

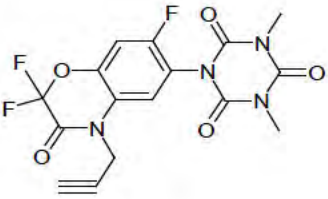
2.2.1 BAS 850 H

Common Name	Trifludimoxazin
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 g/mol
Lot No.	L84-130
Purity:	99.2%
Expiration Date	January 01, 2028



2.2.2 M850H001

Common Name	Not assigned
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 g/mol
Lot No.	L85-52
Purity:	98.7%
Expiration Date	March 01, 2022



The test/reference substances in solution were used in the study 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 substances. The performance of the instrument was evaluated during each injection set.

Standard Solution Stability

Stock solutions of BAS 850 H and its M850H001 metabolite were prepared in methanol with 0.1% formic acid. Mixed fortification solutions containing each analyte were prepared by combining aliquots of the stock solutions for each analyte and diluting with methanol with 0.1% formic acid. Precursor calibration standard solutions containing each analyte were prepared by diluting the mixed fortification solutions with methanol-water with 0.1% formic acid (20:80, v/v).

The stability of the analytes in standard solutions has been determined in related studies on BAS 850 H and its metabolites in soil (Reference 6). During the course of this study, the test/reference substance solutions were stored under refrigeration (< 10 °C). Preparation and dilution data forms pertaining to the stock and working solutions are located in the analytical

facility data and are archived periodically. Example standard dilution and use information, as performed in the subject study, are provided in Appendix C.

2.3 Route of Administration

In this method validation study, the test substances were applied to the test system as analytical standard solutions (in methanol with 0.1% formic acid) by micropipette to ensure precise delivery of a small amount of the test substances as described below.

For each sample, 5 g (± 0.1 g) of soil was weighed into a 50 mL Teflon centrifuge tube and fortified with the appropriate fortification solution using a calibrated micropipette. The following scheme was used for fortification of the samples.

Sample Type	Sample Weight	Concentration of Spiking Solution [µg/mL]	Volume of Spiking Solution [mL]	Level of Fortification [µg/kg]
Control	5 g	-	-	-
Fortification (LOQ)	5 g	0.01	0.050	0.10*
Fortification (10×LOQ)	5 g	0.10	0.050	1.0

* Limit of quantitation

2.4 Analytical Method

2.4.1 Principle of the Method

Using BASF Method No. R0067/01, residues of BAS 850 H and M850H001 in soil were extracted from clay loam soil and sandy loam soil. The method procedures validated in this study are provided in Appendix D. Briefly, residues of BAS 850 H in soil samples (5 g each) were extracted twice by shaking with methanol-water with 0.1% formic acid (70:30, v/v). Residues in an aliquot of the combined extracts were concentrated to aqueous and partitioned into cyclohexane-ethyl acetate (90:10, v/v). An aliquot of the organic layer was then evaporated to dryness and re-dissolved in methanol-water with 0.1% formic acid (20:80, v/v). Final extracts were analyzed by LC-MS/MS using matrix-matched calibration standards.

2.4.2 Specificity/Selectivity

Residues of BAS 850 H and M850H001 were determined by LC-MS/MS. For BAS 850 H, positive ion transitions m/z 413→74 (primary) and m/z 413→134 (confirmatory) were monitored and validated. For M850H001, positive ion transition m/z 397→114 was monitored and validated using two analytical columns (primary column; Phenomenex Kinetex EVO C18, and confirmatory column; Waters Xbridge BEH C18 XP). The results were calculated from direct comparison of the sample peak responses to those of external, matrix-matched calibration standards.

As LC-MS/MS is regarded as a highly-specific detection method when two ion transitions have been validated, an additional confirmatory method or technique was not necessary for BAS 850 H. Since a single transition was available for the M850H001 metabolite, an additional confirmatory LC-MS/MS method was validated using a different HPLC column.

2.5 Validation of Method

For validation, untreated soil samples were fortified with each analyte and analyzed according to the established method validation guidelines. To test the repeatability of the method, the analytical sets consisted of a reagent blank, and for each matrix, two controls, five replicates fortified with each analyte at the method limit of quantitation, 0.1 µg/kg (ppb), and five replicates fortified at a higher level, corresponding to 10X the limit of quantitation, 1.0 µg/kg. For BAS 850 H, the two mass transitions described above were evaluated. For M850H001, a single mass transition was quantified with the two methods described above (primary column; Phenomenex Kinetex EVO C18, and confirmatory column; Waters Xbridge BEH C18 XP).

2.6 Influence of Matrix Effects on Analysis

In conjunction with the subject study, the influence of the matrix effects was determined using the responses of each analyte in the matrix that were compared to the standards in calibration solution [methanol-water with 0.1% formic acid (20:80, v/v). The matrix-fortified solutions equivalent to approximate sample concentrations of ½ LOQ, LOQ and 2xLOQ (0.05 ng/mL, 0.10 ng/mL, and 0.20 ng/mL, respectively) were prepared and analyzed with solvent-based standard curve.

The data generated were evaluated by comparing the average calculated concentration (expressed in ng/mL) to the nominal standard concentration (ng/mL) at each of the three concentration levels examined. Acceptability (i.e., matrices had no significant influence on the analysis) required a % difference <20%, calculated as the "Percent Difference (%)". For each matrix/ion transition/method, an "Overall Mean Difference (%)" 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 each analyte in stored extract solutions was determined in conjunction with the subject method validation study. To establish stability, reserved initial extracts from several control and method validation recovery soil samples that had been stored under refrigeration were cleaned-up and analyzed against freshly-prepared matrix-matched standards according to the method. Additionally, stability in the final volume solution was established for each matrix by reanalyzing several control and method validation recovery extracts, as described above, which had been stored under refrigeration at the final volume stage. Quantification of BAS 850 H in the stored samples for these experiments was performed for the primary mass transition, and quantification of M850H001 in stored samples for these experiments were performed for the primary analysis method.

3. RESULTS

3.2 Influence of Matrix Effects

The experiment to evaluate matrix effects showed that the matrix load in the samples from each soil type had significant influence on analysis (matrix effects > 20% for at least one transition or confirmatory method); therefore, the validation samples were analyzed using matrix-matched calibration standards. For clay loam soil, the overall mean difference (%) for each transition or method evaluated ranged from 3-24%, and the highest overall mean difference of 24% was found for the BAS 850 H secondary transition (m/z 413→134). For sandy loam soil, the overall mean difference (%) for each transition or method evaluated ranged from 7-35%, and the highest overall mean difference of 35% was found for the M850H001 confirmatory method (m/z 397→114 analyzed on a Waters Xbridge BEH C18 XP analytical column). Matrix effects were calculated as the overall concentration differences between the nominal standard concentrations of matrix-fortified standards [prepared at three standard concentration levels: LOQ, ½ LOQ and 2 x LOQ] and the actual concentrations observed from these samples using solvent-based standard calibration curves for quantitation.

3.3 Extract Storage Stability

The method validation sample extracts were analyzed within 1 day of extraction. The acceptable method recoveries obtained during analyses demonstrate the storage stability of residues of BAS 850 H in the extracts in the brief period prior to analysis. To assess storage stability of the initial extracts, selected extracts from each method validation set were retained and stored refrigerated (< 10 °C) and then re-subjected to method clean-up and re-prepared for measurement against freshly-prepared matrix-matched calibration solutions after a minimum of 3 days of refrigerated storage. To assess the storage stability of the final volume solutions, selected samples extracts at final volume stage in autosampler vials from each method validation set were retained and stored refrigerated before re-analysis using freshly-prepared matrix-matched calibration solutions after at least 3 days of refrigerated storage. The recoveries from stored solutions generated during extract stability experiments confirmed the stability of BAS 850 H residues in soil extracts for at least 3 days of refrigerated storage, as shown in Table 3. Detailed data from extract storage stability experiments is shown in Appendix H.

3.4 Evaluation of Limit of Determination (LOD)

The LOD for each matrix was estimated statistically using the standard deviation (STDEV) of the calculated concentrations from recovery measurements for five replicate samples fortified at the LOQ of 0.1 µg/kg (ppb). In clay loam soil, the LOD's for BAS 850 H [(m/z 413→74 (primary) and m/z 413→134 (confirmatory)] and M850H001 (using transition m/z 397→114 for primary and confirmatory chromatographic methods) were calculated as 0.033, 0.045, 0.023 and 0.040 µg/kg, respectively. In sandy loam soil, the LOD's for BAS 850 H [(m/z 413→74 (primary) and m/z 413→134 (confirmatory)] and M850H001 (using transition m/z 397→114 for primary and confirmatory chromatographic methods) were calculated as 0.030, 0.038, 0.023 and 0.024 µg/kg, respectively. The experimental LOD of 0.026 µg/kg was based on the signal-to-noise (S/N) ratio of the BAS 850 H secondary transition, which was lowest of all analytes considering primary and secondary transitions/method (S/N ratio for BAS 850 H secondary transition in lowest calibration standard in sandy loam soil was 4). The detailed Limit of Detection

calculations are shown in Table 4. The detailed data used to complete the Limit of Detection calculations is shown in Appendix F.

4. CALCULATIONS AND RAW DATA

Example calculations are included in Appendix I.

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 ADPEN Laboratories, Inc. 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 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, respectively. Instrument parameters used for the method validation are summarized in Table 7. A flow chart of the method is included as Appendix L.

8. DISCUSSION

The method validation was performed successfully for each soil matrix and the ion transitions (LC-MS/MS; primary and secondary transitions for BAS 850 H) and methods (chromatographic

columns; primary and secondary methods for M850H001) available, using matrix-matched standards. The overall results are summarized below.

Linearity:	Acceptable linearity was observed for the standard range and the two mass transitions (BAS 850 H) or two instrumental methods (M850H001) tested. The method-detector response for the method validation sets was linear over the calibration range tested (0.025-0.5 ng/mL, r ≥0.9916).
Specificity:	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.
Limit of Quantification:	The LOQ was defined by the lowest fortification level successfully tested. The validated LOQ for residues of BAS 850 H and M850H001 in soil was 0.10 µg/kg.
Limit of Detection	The LOD was defined as 0.026 µg/kg (26% of the LOQ) when based on the estimated signal-to-noise ration of the M850H001 confirmatory method. In addition, the method LOD was calculated using the standard deviation of the LOQ fortifications in each matrix. The calculated method LOD's for BAS 850 H (both transitions) and M850H001 (both methods) in clay loam soil were 0.03, 0.04, 0.02 and 0.04 µg/kg, respectively. The calculated LOD's for BAS 850 H (both transitions) and M850H001 (both methods) in sandy loam soil were 0.03, 0.04, 0.02 and 0.02 µg/kg, respectively.
Selectivity	The multiple reaction monitoring (MRM) transitions used to identify BAS 850 H and M850H001 were determined by product ion spectra. The experiment to evaluate any potential matrix effects showed that the matrix load in the samples from crop matrix had a significant influence on analysis (matrix effects ≥20% for at least one transition or analyte). The validation samples were analyzed using matrix-matched calibration standard solutions.
Confirmatory Techniques	Two mass transitions are available for BAS 850 H, therefore an additional confirmatory technique was not necessary for parent quantitation. One mass transition was available for M850H001, therefore a confirmatory instrumental method with a different analytical column was validated for M850H001.

Repeatability	Overall relative standard deviations (%RSD) for all fortification levels were below 20%.
Reproducibility	Reproducibility of the method was not determined within this validation study.
Extractability	Extractability was tested in a separate study (Reference 8). The results from this study showed that the extraction solvent and procedure used in this method are adequate to extract incurred residues from field samples.

9. CONCLUSIONS

The results of this method validation study demonstrate that BASF Analytical Method R0067/01 fulfils the requirements with regard to specificity, repeatability, limit of quantification, and recoveries and is; therefore, applicable to correctly determine residues of the herbicide BAS 850 H and its M850H001 metabolite in clay loam and sandy loam soils with an LOQ of 0.1 µg/kg.

Table 5. Summary Parameters for the Analytical Method Used for the Quantitation of Residues of BAS 850 H in Soil

Method ID	BASF Analytical Method No. R0067/01
Analyte(s)	Residues of BAS 850 H and the M850H001 metabolite
Extraction solvent/technique	Residues of BAS 850 H and M850H001 in soil samples (5 g each) are extracted by shaking (twice) with methanol-water with 0.1% formic acid (70:30, v/v).
Cleanup strategies	An aliquot of the sample extract is concentrated to aqueous and subjected to liquid-liquid clean-up with a mixture of cyclohexane-ethyl acetate (90:10, v/v). An aliquot of the organic layer is then evaporated to dryness, reconstituted in methanol with 0.1% formic acid and diluted with water with 0.1% formic acid. The final extract was filtered through 0.45 µm PTFE syringe filter and vialed for instrumental analysis.
Instrument/Detector	All analyses were performed on an Agilent 1290 LC-MS/MS system (Sciex 6500 with electrospray ionization). The analysis of BAS 850 H and M850H001 (primary method) was completed with a Phenomenex Kinetex EVO C18 column (50 x 3.0 mm, 2.6 µm), using a mobile phase gradient of water/acetonitrile, each acidified with 0.1% formic acid (80:20 to 1:99, v/v, over ~7 minutes, flow rate 500 uL/minute), and monitoring ion transitions m/z 413→74 and m/z 413→134 for BAS 850 H; m/z 397→114 for M850H001. The confirmatory method for M850H001 was completed with a Waters Xbridge BEH C18 XP column (50 x 4.6 mm, 2.5 µm), using a mobile phase gradient of water/acetonitrile, each acidified with 0.1% formic acid (80:20 to 1:99, v/v, over ~8 minutes, flow rate 800 uL/minute), and monitoring ion m/z 397→114 for M850H001.
Standardization method	Linear regression (1/x weighting). Direct comparison of the sample peak responses to those of external standards.
Stability of standard solutions	Stock solutions of BAS 850 H and M850H001 prepared in methanol with 0.1% formic acid have been demonstrated stable, when held under refrigeration, for at least 235 days. In addition, calibration solutions of BAS 850 H and M850H001 prepared by serial dilution of the fortification solutions with methanol-water with 0.1% formic acid (20:80, v/v) have been demonstrated stable, when stored refrigerated, for at least 83 days (Reference 6).
Retention times (minutes)	BAS 850 H ~6.5 minutes M850H001 (primary method) ~ 4.5 minutes M850H001 (confirmatory method) ~5.9 minutes

Table 6. Characteristics for the Analytical Method Used for the Quantitation of Residues of BAS 850 H in Soil Matrices

Analyte	Residues of BAS 850 H, including the M850H001 metabolite
Equipment ID	Agilent 1290 HPLC chromatographic system with a Phenomenex Kinetex EVO C18 column (50 x 3.0 mm, 2.6 µm), using a mobile phase gradient of water/acetonitrile, each acidified with 0.1% formic acid (80:20 to 1:99, v/v, over ~7 minutes, flow rate 500 uL/minute), used for primary LC-MS/MS method. Agilent 1290 HPLC chromatographic system with a Waters Xbridge BEH C18 XP column (4.6 x 50 mm, 2.5 µm), using a mobile phase gradient of water/acetonitrile, each acidified with 0.1% formic acid (80:20 to 1:99, v/v, over ~8 minutes).
Limit of quantitation (LOQ)	The validated LOQ for residues of BAS 850 H in soil was 0.1 µg/kg for each analyte, which corresponds to a concentration in the final volume of 0.095 ng/mL.
Limit of detection (LOD)	0.026 µg/kg (26% of the LOQ).
Reliability of the Method/ [ILV]	An independent laboratory validation [ILV] of BASF Analytical Method No. R0067/01 was successfully conducted at JRF America. The ILV was completed in the first trial using clay loam soil (typically the more difficult soil type) with no alterations to the method.
Linearity	The method-detector response was linear over the 0.025-0.5 ng/mL range ($r \geq 0.992$) for the analysis of each analyte in soil for the 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 the each commodity had a significant influence on analysis (matrix effects ≥20% for at least one analyte).
Confirmatory technique	Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique was not necessary for BAS 850 H. Since only one ion transition was available for M850H001, a confirmatory instrumental method was validated on an alternate analytical column.
Time required	A set of 13 samples (one reagent blank, two controls, and 10 fortified samples for recovery experiments) requires about 8 hours of work (calculation of the results not included).

Table 7. Instrument Parameters for Method Validation

Method A (HPLC Mode): BAS 850 H and M850H001

	Parameter		
Chromatographic System	Agilent 1290 system		
Analytical-Column	Phenomenex Kinetex EVO C18, 3.0×50 mm, 2.6 µm		
Column Temperature	50 °C		
Injection Volume	100 µL (can be lower if sensitivity allows)		
Mobile Phase A Mobile Phase B	Water 1.0% formic acid Acetonitrile 0.1% formic acid		
Flow Rate*	500 µL/min		
Gradient (including wash and equilibration)	Time (min)	Phase A	Phase B
	0.00 0.05 6.00 7.00 7.90 7.91 8.00	80 80 50 1 1 80 80	20 20 50 99 99 20 20
Detection System	AB Sciex 6500 Mass Spectrometer		
Ionization	Electrospray (ESI)		
Ionization Temperature	200 °C		
Analyte	**Transitions *(m/z)***	**Polarity**	**Expected Retention Time**
BAS 850 H	413 → 74** 413 → 134	positive	~ 6.53 min
M850H001	397 → 114	positive	~ 4.50 min

*System pressure is approximately 2000 PSI using this method
**Proposed as quantification transition. Any of these transitions could be used for quantitation in case interference is observed at the same retention time

Table 7. Instrument Parameters for Method Validation (continued)

Method B (HPLC Mode): M850H001 Confirmatory method)

	Parameter		
Chromatographic System	Agilent 1290 system		
Analytical-Column	Waters Xbridge BEH C18 XP 2.5 µm 4.6×50mm		
Column Temperature	50 °C		
Injection Volume	100 µL (can be lower if sensitivity allows)		
Mobile Phase A Mobile Phase B	Water 1.0% formic acid Acetonitrile 0.1% formic acid		
Flow Rate*	800 µL/min		
Gradient (including wash and equilibration)	Time (min)	Phase A	Phase B
	0.00 0.05 6.00 8.00 8.90 9.00 10.00	80 80 50 1 1 80 80	20 20 50 99 99 20 20
Detection System	AB Sciex 6500 Mass Spectrometer		
Ionization	Electrospray (ESI)		
Ionization Temperature	200 °C		
Analyte	**Transitions *(m/z)***	**Polarity**	**Expected Retention Time**
M850H001	397 → 114	positive	~ 5.90 min

*System pressure is approximately 2000 PSI using this method

Typical Analytical Standards Dilution and Use Records for BAS 850 H and M850H001

Standard Number	Analyte[1]	Standard (Lot # used)	Amount Weighed / Volume[2]	Final Dilution Vol. (mL)	Final Conc.[3]	Solvent[4]	Prep. Date	Expiry Date
Stock Solutions								
C9527	P	L84-130	10.23744 mg	10	1.023744 mg/mL	MIX-1	1-Jun-2020	1-Sept-2020
C9529	1	L85-52	10.19571 mg	10	1.019571 mg/mL	MIX-1	1-Jun-2020	1-Sept-2020
Serial Dilutions								
I10182	All	C9527 C9529	0.488 mL 0.490 mL	50	10 µg/mL	MIX-1	1-Jun-2020	1-Sept-2020
W16158-1	All	I10182	5 mL	50	1.0 µg/mL	MIX-1	1-Jun-2020	1-Sept-2020
W16158-2	All	W1568-1	5 mL	50	0.10 µg/mL	MIX-1	1-Jun-2020	1-Sept-2020
W16158-3	All	W1568-2	5 mL	50	0.010 µg/mL	MIX-1	1-Jun-2020	1-Sept-2020
Calibration Precursor Standards								
W16159-1	All	W16158-1	1.25 mL	25	50 ng/mL	MIX-2	2-Jun-2020	2-July-2020
W16159-2	All	W16158-1	0.625 mL	25	25 ng/mL	MIX-2	2-Jun-2020	2-July-2020
W16159-3	All	W16158-2	2.5 mL	25	10 ng/mL	MIX-2	2-Jun-2020	2-July-2020
W16159-4	All	W16158-2	1.25 mL	25	5 ng/mL	MIX-2	2-Jun-2020	2-July-2020
W16159-5	All	W16158-2	0.625 mL	25	2.5 ng/mL	MIX-2	2-Jun-2020	2-July-2020
Matrix-Matched Calibration Standards								
W16174-1	All	W16159-1	0.010 mL	1	0.50 ng/mL	CNTRL	9-Jun-2020	2-July-2020
W16174-2	All	W16159-2	0.010 mL	1	0.25 ng/mL	CNTRL	9-Jun-2020	2-July-2020
W16174-3	All	W16159-3	0.010 mL	1	0.10 ng/mL	CNTRL	9-Jun-2020	2-July-2020
W16174-4	All	W16159-4	0.010 mL	1	0.050 ng/mL	CNTRL	9-Jun-2020	2-July-2020
W16174-5	All	W16159-5	0.010 mL	1	0.025 ng/mL	CNTRL	9-Jun-2020	2-July-2020

1. P= BAS 850 H; 1 = M850H001 Metabolite
2. Corrected for purity of reference material.
3. The concentration for each analyte.
4. MIX-1 = methanol with 0.1% formic acid. MIX-2 = methanol-water with 0.1% formic acid (20:80, v/v). CNTRL = Control Matrix extracted through method

Example fortification scheme used for analysis (Work Order WO-2052010)

Sample Type	Weight (g)	Compounds Fortified	Fortification Solution	Conc. of Fortification Solution	Volume Added	Level of Fortification
Reagent Blank	N/A	None	None	None	None	-
Control	5.0	None	None	None	None	-
Control	5.0	None	None	None	None	-
Fortification at LOQ	5.0	BAS 850 H and M850H001	W16158-3	0.010 µg/mL	0.050 mL	0.10 µg/kg
Fortification at 10x LOQ	5.0	BAS 850 H and M850H001	W16158-2	0.10 µg/mL	0.050 mL	1.0 µg/kg

Appendix D. Procedure Used for Method Validation

We create chemistry

Technical Procedure:

Method for the Determination of Residues of BAS 850 H (Reg. No. 5654329) and its metabolite M850H001 (Reg. No. 5749359), in Soil with LOQ of 0.1 µg/kg using LC-MS/MS

BASF Method Number:

R0067/01

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 (can be acquired from same study or from a different source).
Unknown Sample:	The samples with unknown residues.
Treated Sample:	A sample that has been treated with the test substance.
Blank:	Solvent, solution or mobile phase injected together with a sample set.
Reagent Blank:	A complete analysis conducted using solvents and reagents only in absence of any sample (known as blank or reagents or procedural blank). This sample is analyzed within the sample set in order to evaluate possible contamination of chemicals/reagents.
Procedural Recovery:	A control sample to which a known amount of analyte has been added before sample work up. This sample is then carried through the method and analyzed with the unknown samples in order to determine the reliability of the method.
Instrument Recovery:	A control sample which is carried through the method and to which a known amount of analyte has been added before injection. This sample is analyzed within the sample set in order to evaluate the matrix effect in the instrument.
Analytical Run:	A group of samples that undergo a determinative measurement on an analytical instrument (such as GC, HPLC, CE, GC/MS, or LC/MS/MS) 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 method.
Limit of Detection (LOD):	Concentration of analyte equivalent to a defined percentage of the limit of quantitation of the method (e.g. 26% of LOQ). At this concentration, the analyte must be qualitatively detectable in sample matrix (analyte peak height at least 3-5 x baseline noise).

1.0 INTRODUCTION

BAS 850 H is a PPO herbicide, and is developed by BASF to be used for broad a spectrum of crops in US. For registration of this herbicide and for establishing the DT50/90 values from field dissipation studies for these use patterns, a residue analytical method D1401/02 for the active ingredient and its relevant metabolites in soil was developed by BASF (Reference 1). The current method, R0067, is developed in soil with the required LOQ of 0.1 µg/kg, which is below the ecotox end points from a seedling emergence study (References 2 and 3)

BASF Method Number R0067 was successfully tested during method development in clay and sandy loam soil types.

History of the method

TP Version	Release Date	Change
01	11 March 2020	New method

The method has a limit of quantitation of 0.1 µg/kg in soil for BAS 850 H and its metabolite, M850H001 with the limit of detection of 0.026 µg/kg (26% of LOQ). All analytes are determined individually.

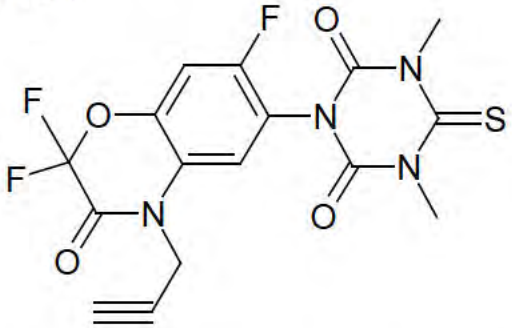
2.0 MATERIALS

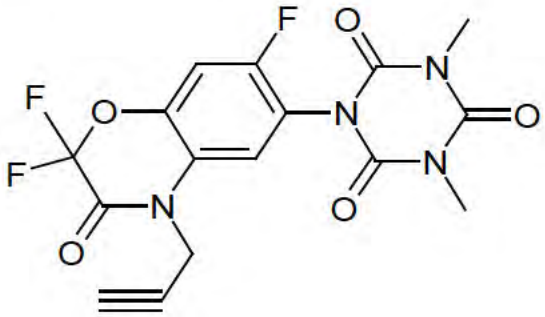
2.1 Safety

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. Ensure that work clothing is stored 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. Details are given in the Safety Data Sheets (SDS) of the individual substances. All procedures involving organic solvents should be performed in a well-ventilated hood. 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.

Chemical Name (IUPAC):	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
CAS Registry No.:	1258836-72-4
Code No.:	BAS 850 H
BASF Reg. No.:	5654329
Molecular Formula:	$C_{16}H_{11}F_3N_4O_4S$
Molecular Weight:	412.3
Molecular Structure:	

Chemical Name (IUPAC):	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
CAS Registry No.:	None
Code No.:	M850H001
BASF Reg. No.:	5749359
Molecular Formula:	$C_{16}H_{11}F_3N_4O_5$
Molecular Weight:	396.3
Molecular Structure:	

2.2.1 Reference Standards (used for calibration)

Same as fortification compounds.

2.3 Equipment

Equipment	Size, Description	Manufacturer	Catalog No.
Balance, Top Loader	Model PJ3600	Mettler DeltaRange	--
Balance, Analytical	Model AT100	Mettler	--
Beakers	Various sizes	PYREX Brand, VWR Scientific Products	13922-029
Bottle, Amber glass	Qorpak , 2 oz and 4 oz with Teflon®-lined screw cap	VWR Scientific Products Boston Round, Amber	89042-908
Centrifuge	Allegra 6	Bechman Coulter	--
Centrifuge Tubes (Teflon®)	40 mL	VWR	21009-477
Culture Tube caps	PTFE Lined Cap	Various	--
Culture Tube caps	16 mm	VWR	60828-768
Culture Tube, Graduated	10 mL	Various	--
Culture Tubes	Glass, disposable, 16x100mm size	Fisher	14-961-29
Cylinder, Graduated	Various sizes	Various	--
Flask, Erlenmeyer, 24/40	1000 mL	Various	--
Glass Centrifuge Tubes	50 mL	VWR	8422-50
HPLC Column	Kinetex EVO C18 3.0×50 mm, 2.6 µm	Phenomenex	00b-4725-y0
HPLC Column	X-Bridge BEH C18 XP Column, 4.6×50 mm, 2.5 µm	Waters	186006037
HPLC	1290	Agilent	--
Mass Spectrometer	AB Sciex 6500	AB Sciex	--
Mechanical shaker	KS501 Digital	IKA Labortechnik	--
Nitrogen evaporator	TurboVap	Biotage	--
Repeater Pipette	1000 µL 250 µL 25 µL	Gilson Microman Fisher Scientific	F148506G
Volumetric, pipettes	0.5 mL, 1 mL, 2.5 mL, 5 mL, 10mL, 20 mL, 25 mL	Fisher Scientific – Class A	13-650-2A
Vortex	Genie 2	VWR Scientific Products	14216-184

Note: The equipment and instrumentation listed above may be substituted by that of similar specifications. The applicability is confirmed if the recoveries of the fortification experiments are in the expected concentration range.

2.4 Reagents

2.4.1 Chemicals

Chemical	Grade	Manufacturer/Supplier	Catalog No.
Acetonitrile	HPLC Grade	EMD	AX0145-P1
Cyclohexane	GR Grade	EMD	CX2290-3
Ethyl Acetate	HPLC Grade	EMD	EX0245-1
Formic acid	98% GR ACS	EMD	FX0440-7
Methanol	HPLC Grade	EMD	MX0475-P1
Water	HPLC Grade	BDH ARISTAR PLUS	87003-652

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

2.4.2 Solutions and Solvent Mixtures

Description	Code	Composition
Solution	S1	Methanol with 0.1% Formic Acid Add 1 mL of formic acid to 1000 mL of methanol into a, e.g., 1L Erlenmeyer flask and mix well to ensure complete homogeneous solution.
Solution	S2	Water with 0.1 % Formic Acid Add 1 mL of formic acid to 1000 mL of water into a, e.g., 1L Erlenmeyer flask and mix well to ensure complete homogeneous solution.
Extraction Solvent	S3	Methanol-Water mixture with 0.1% Formic Acid (70:30,v/v) Add 700 mL of S1 to 300 mL of S2 into a, e.g., 1L Erlenmeyer flask and mix well to ensure complete homogeneous solution.
Final Volume Dilution Solution	S4	Methanol-Water mixture with 0.1% Formic Acid (20:80, v/v) Add 200 mL of S1 to 800 mL of S2 into a, e.g., 1L Erlenmeyer flask and mix well to ensure complete homogeneous solution.
Clean up Solution	S5	Cyclohexane-ethyl acetate mixture (90:10, v/v) Add 450 mL of Cyclohexane and 50 mL of Ethyl Acetate into a 500 mL flask and mix well to ensure complete homogenous solution.
Mobile Phase A	LC1	1% Formic Acid in Water Add 990 mL of water to 10 mL of concentrated formic acid into a 1L Erlenmeyer flask and mix well to ensure complete homogeneous solution.
Mobile Phase B	LC2	0.1% Formic Acid in Acetonitrile Add 999 mL of acetonitrile, to 1 mL of concentrated formic acid into a 1L Erlenmeyer flask 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.

2.4.2.1 Standard Solutions

Use amber bottles with Teflon-lined screw caps as storage containers for all standard solutions.

Stock Solutions (BAS 850 H and M850H001)

Prepare a stock solution containing 1 mg/mL by weighing an appropriate amount of each reference item or standard into a volumetric flask and adding the required volume of **S1**.

For example, weigh 10 mg BAS 850 H into a 10 mL volumetric flask. Dissolve and dilute to mark with **S1**. This creates a solution containing 1 mg/mL of BAS 850 H in **S1**. Ensure a complete homogeneous solution (e.g. by sonication and/or vortexing).

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:

1. Two stock solutions are independently prepared. One is used for preparation of fortification solutions, the other for calibration standard solutions.
2. 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 done if the purity is ≤ 95%. If the purity is > 95 % correction is optional.

Fortification Solutions Preparation

Mixed Fortification Solutions (BAS 850 H and M850H001)

Prepare 10, 1 , 0.1, and 0.01 µg/mL fortification solutions in **S1** from the 1 mg/mL (1000 µg/mL) stock solutions prepared in **S1** from section “Stock Solutions (BAS 850 H and M850H001)” using the scheme in table below. Combine all stock solutions together to make fortifications with all analytes. Dilute volumetrically with appropriate solvents as described in the table below and ensure a complete homogeneous solution (e.g. vortexing).

Preparation of Mixed Fortification Solutions

Take solution (µg/mL)	Volume (mL)	Dilute with S1 to a final volume of (mL)	Concentration (µg/mL)
1000	0.50	50	10.0
10.0	5	50	1.0
1	5	50	0.10
0.10	5	50	0.010

Note: A different concentration scheme may be used, if other fortification levels are needed for the analysis.

If necessary, the volume of solution prepared may be changed as long as the proportions are not modified.

Precursor Calibration Standard Solutions (BAS 850 H and M850H001)

Prepare mixed standard calibration solutions in **S4** for LC-MS/MS analysis, in flasks, by using the 1.0 µg/mL (1000 ng/mL) and 0.1 µg/mL (100 ng/mL) solutions prepared in **S1** from section "Mixed Fortification Solutions (BAS 850 H and M850H001)". Dilute volumetrically with appropriate solvents as exemplified in the table below and ensure a complete homogeneous solution (e.g. by vortexing). These standard solutions will be used to prepare matrix calibration standard solutions.

Preparation of Mixed Standard Solutions

Take solution (ng/mL)	Volume (mL)	Dilute with S4 to a final volume of (mL)	Concentration (ng/mL)
1000 (in S1)	1.25	25	50.0
1000 (in S1)	0.625	25	25.0
100 (in S1)	2.5	25	10.0
100 (in S1)	1.25	25	5.0
100 (in S1)	0.625	25	2.5

Note: A different concentration scheme may be used, if other fortification levels are needed for the analysis.

If necessary, the volume of solution prepared may be changed as long as the proportions are not modified.

2.4.2.2 Matrix Matched Calibration Standards (BAS 850 H and M850H001)

In case matrix-matched standards (and 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.

Preparation of Mixed Matrix Matched Standard Solutions for Calibration

Take solution (ng/mL)	Volume (mL)	Dilute with Control Matrix to a final volume of (mL)	Concentration (ng/mL)
50	0.010	1.0	0.5
25	0.010	1.0	0.25
10	0.010	1.0	0.10
5	0.010	1.0	0.05
2.5	0.010	1.0	0.025

Note: A different concentration scheme may be used, if other fortification levels are needed for the analysis.

If necessary, the volume of solution prepared may be changed as long as the proportions are not modified.

Additional Information:

Use amber bottles with Teflon-lined screw caps as storage containers for all standard solutions.

2.4.3 Stability of Standard Solutions

BASF recommends that stock solutions (1 mg/mL) of all analytes be made fresh every three months. Pre-calibration solutions should be prepared every month.

The stability of BAS 850 H and M850H001 in standard solutions stored at an average temperature of 3°C has been determined in a separate study as noted below.

Stock and intermediate (fortification) standards solutions of BAS 850 H and its metabolites were prepared in methanol with 0.1% formic acid and exhibited stability up to 235 days for BAS 850 H and M850H001 (Reference 4).

Calibration solutions of BAS 850 H and M850H001 are prepared monthly by serial dilution of the fortification standards solutions of BAS 850 H and M850H001 with methanol/water with 0.1% formic acid (20:80, v/v). Calibration solutions exhibited stability up to 83 days for BAS 850 H and M850H001 (Reference 4).

If solutions are stored at different conditions or/and for a longer time, the stability of the reference items must be confirmed.

3.0 ANALYTICAL PROCEDURE

3.1 Sample Preparation

Samples must be sufficiently homogenized prior to analysis in order to ensure that the aliquot taken for residue analysis is representative for the whole sample.
In case of small sample sizes (micro-extraction), special emphasis must be put on sample homogenization, e.g. cryo-milling or additional homogenization steps.

3.2 Weighing and Fortification

For control and treated samples, weigh 5 ± 0.1 g of soil sample into a 50 mL Teflon centrifuge tube.

For fortified samples, weigh 5 ± 0.1 g of control sample into a 50 mL Teflon centrifuge tube and add fortification solutions volumetrically according to the table below and then proceed to Section **3.3**. Extraction BAS 850 H, M850H001.

Sample Type	Sample Weight	Concentration of Spiking Solution	Volume of Spiking Solution	Level of Fortification
Control	5 g	--	--	0.00 µg/kg
Fortification (LOQ)*	5 g	0.01 µg/mL	0.05 mL	0.1 µg/kg
Fortification (10xLOQ)	5 g	0.10 µg/mL	0.05 mL	1.0 µg/kg
Fortification (100xLOQ)	5 g	1.0 µg/mL	0.05 mL	10.0 µg/kg
Treated	5 g	--	--	--

***** Limit of quantification

3.3 Extraction (BAS 850 H and M850H001)

Add exactly 25 mL of **S3** to the pre-weighed sample from Section **3.2**. Shake the sample using a mechanical shaker, at about 300 rpm, for approximately 45 minutes and centrifuge for about 5 min at approximately 4000 rpm. Decant the supernatant into a 50 mL volumetric flask.

Add exactly another 25 mL of extraction solvent **S3** to the soil marc, vortex to dislodge the soil and shake the sample using a mechanical shaker, at about 300 rpm, for approximately 45 minutes followed by centrifuging for 5 minutes at approximately 4000 rpm. Decant the supernatant into the 50 mL volumetric flask that already contains the first extract. Bring the extract to 50 mL final volume, then vortex the combined extract thoroughly and proceed to Section **3.4** for sample clean-up.

Note:	Only Teflon or glass equipment should be used for analysis of BAS 850 H and its metabolites. Plastics have been confirmed to cause interference and suppression on LC-MS/MS.

Extractability

The exact ^{14}C-metabolism extraction solvents are used to demonstrate extractability in this method (Reference 5).

3.4 Sample Clean-up (BAS 850 H, M850H001)

a) Transfer exactly 25 mL of extract from Section **3.3** (50% aliquot) into a graduated glass tube.
b) Concentrate to just below the 7.5 mL mark (~7.4 mL) under nitrogen at 50 °C. All methanol must be removed.
c) Add 10 mL of **S5** to the aqueous extracts in tube (Step **3.4[b]**).
d) Secure sample with green Teflon-lined screw cap and then mix thoroughly using shaking gently by hand followed by vertexing. Allow layers to separate.
e) Remove exactly 9.5 mL of the top organic layer into a clean culture and evaporate to dryness under nitrogen at 50 °C.
f) Proceed to Section **3.5** to prepare the sample for measurement on the LC-MS/MS.

3.5 Preparation for Measurement (BAS 850 H, M850H001)

Add exactly 0.5 mL of **S1** to the samples from Step **3.4[f]**. Vortex and then sonicate for 2 minutes to dissolve the residue at the bottom and from the side of the glass culture tube. Add exactly 2.0 mL of **S2.** Vortex thoroughly to ensure a homogenous solution.

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 (e.g. 50 µL + 950 µL) with control extract is necessary to remain in the range of the calibration curve.

See Section **4.2** for LC-MS/MS conditions.

Note:	Method could be interrupted at this point

3.6 Influence of Matrix Effects on Analysis

For the analysis of BAS 850 H and M850H01, significant matrix effects were observed during method development in all soil matrices tested. Therefore, the use of matrix matched standards is recommended (see Section **2.4.2.2**).

3.7 Stability of Extracts and Final Volumes

Stability of extracts/final volumes will be conducted as part of the method validation study.

Procedural recoveries can also be used to prove the stability over a longer time interval, if necessary.

3.8 Moisture Determination

The procedural recoveries will not be corrected for moisture content of the sample. Results of soil analysis are reported on a “dry weight” basis for residue determination. Therefore, field treated soil sample weights must be corrected for moisture content by any method the laboratory customarily uses. The moisture determination will be conducted for the treated samples with residue value above at or above LOD.

An example of a moisture determination procedure is provided below:

The percent moisture is determined using automated moisture determination equipment (e.g. Mettler Toledo HR83) or any other standard process using the formula below:

$$\text{Moisture content [\%]} = \frac{\text{Weight moist soil - Weight dry soil}}{\text{Weight moist soil}} \times 100$$

The dry residue (ppm) is then calculated in an excel sheet using the following formula:

$$\text{Dry Residue (ppm)} = \frac{\text{Wet Sample Residue (ppm)}}{(100 - \text{Percent Moisture}) / 100}$$

4.0 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 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 be at least injected twice. At least 5 calibration levels need to be injected.

4.2 Instrumental Analysis

4.2.1 Instrumentation and Conditions (BAS 850 H & M850H001)

Method A (HPLC Mode): BAS 850 H and M850H001

	Parameter		
Chromatographic System	Agilent 1290 system		
Analytical-Column	Phenomenex Kinetex EVO C18, 3.0×50 mm, 2.6 µm		
Column Temperature	50 °C		
Injection Volume	100 µL (can be lower if sensitivity allows)		
Mobile Phase A Mobile Phase B	Water 1.0% formic acid Acetonitrile 0.1% formic acid		
Flow Rate*	500 µL/min		
Gradient (including wash and equilibration)	Time (min)	Phase A	Phase B
	0.00 0.05 6.00 7.00 7.90 7.91 8.00	80 80 50 1 1 80 80	20 20 50 99 99 20 20
Detection System	AB Sciex 6500 Mass Spectrometer		
Ionization	Electrospray (ESI)		
Ionization Temperature	200 °C		
Analyte	**Transitions *(m/z)***	**Polarity**	**Expected Retention Time**
BAS 850 H	413 → 74** 413 → 134	positive	~ 6.53 min
M850H001	397 → 114	positive	~ 4.50 min

*System pressure is approximately 2000 PSI using this method
*Proposed as quantification transition. Any of these transitions could be used for quantitation in case interference is observed at the same retention time

4.2.1 Instrumentation and Conditions (BAS 850 H & M850H001) continued

Method B (HPLC Mode): M850H001 Confirmatory method)

	Parameter		
Chromatographic System	Agilent 1290 system		
Analytical-Column	Waters Xbridge BEH C18 XP 2.5 µm 4.6×50mm		
Column Temperature	50 °C		
Injection Volume	100 µL (can be lower if sensitivity allows)		
Mobile Phase A Mobile Phase B	Water 1.0% formic acid Acetonitrile 0.1% formic acid		
Flow Rate*	800 µL/min		
Gradient (including wash and equilibration)	Time (min)	Phase A	Phase B
	0.00 0.05 6.00 8.00 8.90 9.00 10.00	80 80 50 1 1 80 80	20 20 50 99 99 20 20
Detection System	AB Sciex 6500 Mass Spectrometer		
Ionization	Electrospray (ESI)		
Ionization Temperature	200 °C		
Analyte	**Transitions *(m/z)***	**Polarity**	**Expected Retention Time**
M850H001	397 → 114	positive	~ 5.90 min

*System pressure is approximately 2000 PSI using this method

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.

In general, a divert valve is used to reduce the matrix load on the detection system.

Instrument conditions, e.g. injection volumes, columns, gradient steps or mass transitions may be modified, but any changes must be recorded in the raw data. Changes are acceptable, when the recoveries of the fortification experiments are in the acceptable range.

Other parameters like gas flows and voltages are depended of the equipment used and therefore not listed. Those parameters may need to be adapted for the used instrument.

4.2.2 Calibration Procedures

Calculation of results is based on peak area measurements using a calibration curve. The calibration curve is obtained by direct injection of BAS 850 H and M850H001 standards containing a known amount of analytes in the range of 0.025 ng/mL to 0.50 ng/mL.

Linear calibration functions with 1/x weighting are recommended for evaluation.

4.2.3 Calculation of Residues and Recoveries

Calculation of results is based on area measurements.

For the procedural recoveries, the sample weight will be considered 5 g in the final calculation of residues [mg/kg]. The method requires that the sample weight to be 5 ± 0.1 g for fortification samples. The recovery is the percentage of the fortified amount (µg or ng), which is recovered through the method and the weights cancels out, as shown in the equation below, during the final calculation step.

The residues of BAS 850 H in mg/kg are calculated as shown in equations **a)** and **b)**:

a) Concentration [ng/mL] = $\frac{\text{Response} - \text{Intercept}}{\text{Slope}} = C_A$

b) Residue [mg/kg] = $\frac{V_{end} \times C_A}{G \times A_F \times 1000}$

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 = Weight of the sample extracted [g]
A_F = Aliquotation factor
1000 = Factor remaining after all unit conversions

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

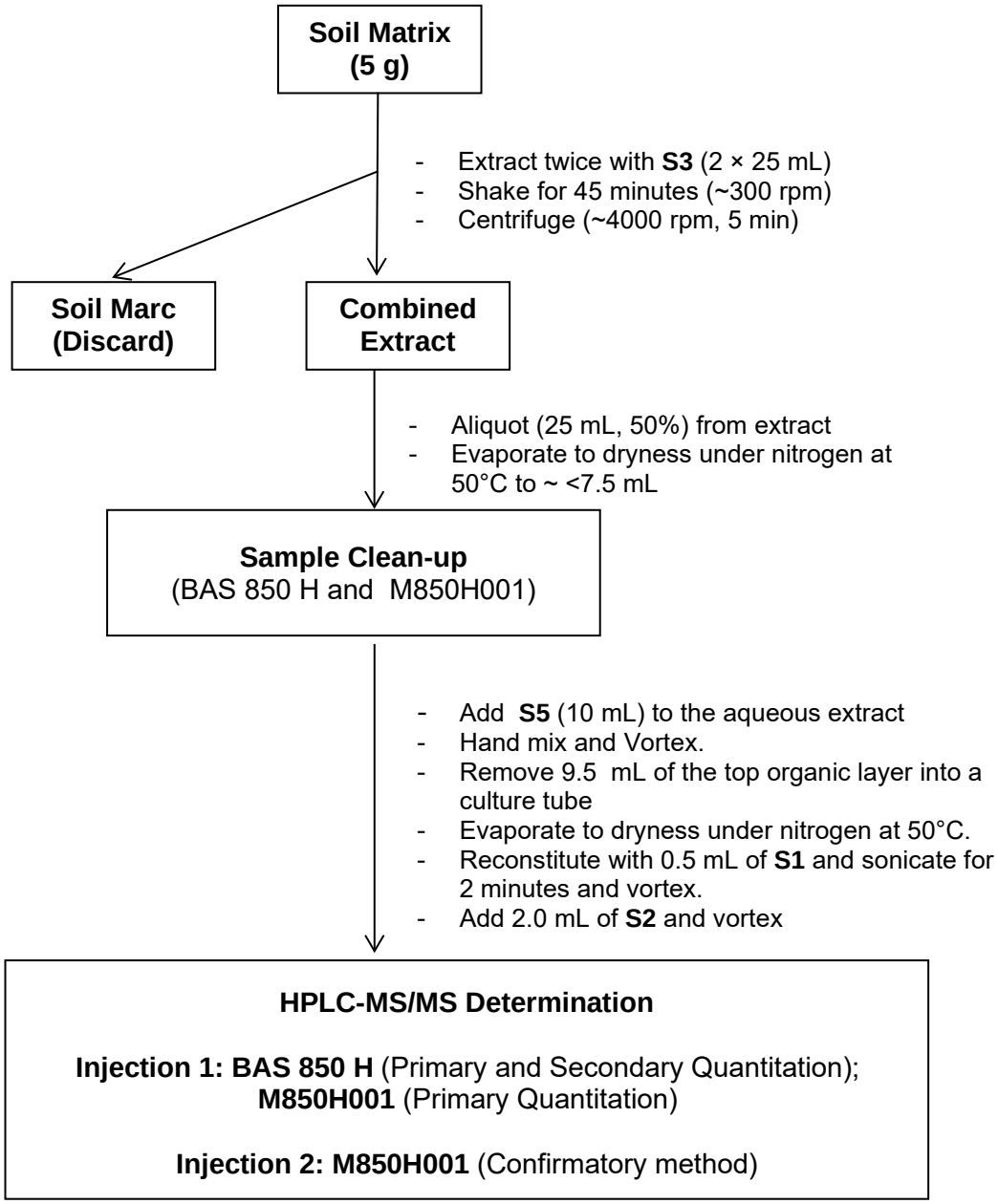
c) Recovery % = $\frac{\text{Residue in fortified sample} - \text{Residue in control}}{\text{Amount of analyte fortified}} \times 100$

Soil residues based on soil dry weight

d) Residue [mg/kg] (Dry residue) = $\frac{\text{Wet Sample Residue (ppm)}}{(100 - \text{Percent Moisture})} \times 100$

5.0 FLOWCHART

5.1 Analysis of BAS 850 H and M850H001 in Soil



S1 = Methanol with 0.1% formic acid
S2 = Water with 0.1% formic acid
S3 = (70:30, v/v) S1:S2
S5 = (90:10, v/v) Cyclohexane: Ethyl Acetate

6.0 METHOD MANAGEMENT AND TIME REQUIREMENTS

The analysis of one series of samples (13 unknown samples, 2 fortified samples for recovery experiments, 1 blank sample) requires 1 working day (8 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.

7.0 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 0.1 µg /kg for BAS 850 H and its metabolite, M850H001, and a limit of detection of 0.026 µg/kg. All analytes are determined individually. The limit of detection was estimated at 26% of the limit of quantification for all analytes. 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 samples showed no significant interferences (< 30%) at the retention time of the analyte of interest.

Confirmatory Techniques
The HPLC-MS/MS final determination is a highly selective detection technique. For BAS 850 H, quantitation is possible using two different transition ions, which allows for confirmation using a secondary transition. For M850H001, only one transition ion provides enough sensitivity for quantitation; therefore, confirmation must be conducted using a secondary HPLC column and instrument conditions.

No additional confirmatory technique is required.

Potential Problems
The glassware used for the method should be thoroughly rinsed with acetonitrile to prevent contamination.

Plastic containers should not be used for this method. Only Teflon® or glass can be used.

The compounds are also sensitive to matrix build up in the instrument. The analyte response and signal to noise ratios over time should be closely monitored. It is recommended to clean the orifice plate regularly, and more thorough cleaning of the hardware as needed, as well as a gradient system to flush the column on a routine basis.

Matrix matched standards are likely to be required for analysis. Care should be taken to ensure an adequate number of control samples are extracted for the preparation of matrix matched standards and dilution of higher concentration samples. Furthermore, controls should be extracted at the same time as samples to ensure that controls have the same matrix load as samples. Variable matrix effects have been observed when controls for matrix matched standards are extracted separately from fortified samples.