ABSTRACT

The purpose of this study was to demonstrate that BASF Analytical Method D1705/01 “Method for the Determination of S(Br-OH)-8007 (Reg. No. 5959595), AB-Oxa (Reg. No. 5959600), and MFBA (Reg. No. 6088668) in Surface and Drinking Water by LC-MS/MS”, can be performed with acceptable recoveries at an outside facility without having any prior experience with the method.

Principle of the method. For the determination of the residues of S(Br-OH)-8007 (Reg. No. 5959595), AB-Oxa (Reg. No. 5959600), and MFBA (Reg. No. 6088668) in surface and drinking water, 10 mL methanol is added to a 10 mL water sample and mixed. After filtration, the sample is ready for analysis by LC-MS/MS. Drinking water samples were analyzed using solvent based standards whereas matrix matched calibration standards were used to compensate the matrix effect of surface water.

Test conditions. For validation, untreated water samples were fortified with S(Br-OH)-8007 (Reg. No. 5959595), AB-Oxa (Reg. No. 5959600), and MFBA (Reg. No. 6088668) and analyzed according to the established method validation guidelines. The analytical sets for each matrix typically consisted of a reagent blank, two control samples, five replicates fortified with each analyte at the method limit of quantitation of 25 ppt (LOQ) and five replicates fortified at 250 ppt (10×LOQ). The transitions for S(Br-OH)-8007 at *m/z* 601 → 256 and at *m/z* 601 → 581 were monitored in positive mode for primary and confirmation quantification, respectively. The transitions for AB-Oxa at *m/z* 583 → 444 and at *m/z* 583 → 494 were monitored in positive mode for primary and confirmation quantification, respectively. The transition for MFBA at *m/z* 274 → 254 was monitored in positive mode for primary quantification. A secondary chromatographic technique using a different stationary phase was used for MFBA confirmatory technique.

Limit of Quantification (LOQ) and Limit of Detection (LOD). The LOQ of the method was set at 25 ng/L (25 ppt) in water for S(Br-OH)-8007, AB-Oxa, and MFBA which was lower than the lowest relevant endpoint in water ecotoxicology(NOEC is 6.3 ng/L for the parent, BAS 450 I) and also defined as the lowest fortification level for each analyte. The limit of detection (LOD) in water was set at 5 ng/L which was 20% of the defined LOQ. The LOD for each analyte was shown to be detectable as the absolute amount of analyte injected.

Selectivity. The method determines residues of S(Br-OH)-8007 and AB-Oxa in water by LC-MS/MS using two transitions. MFBA was determined with two different columns as only one frigment was strong enough for determination. No interfering peaks were found at the retention times for all analytes.

Linearity. Acceptable linearity was observed for the solvent-based and matrix-matched standard range. The method-detector response was linear over the 0.0025-0.25 ng/mL range for S(Br-OH)-8007, AB-Oxa and MFBA, in water analysis.

Standard Stability Standards, stock and fortification solutions of S(Br-OH)-8007, AB-Oxa, and MFBA are prepared in acetonitrile, and calibration standard solutions were prepared by serial dilution of the intermediate standard solutions using 50/50 methanol/water (v:v). Matrix-matched standard solutions were prepared in 50/50 methanol/ surface water (v:v). During the course of this study, the test/reference substance solutions were stored at temperature of 2°C - 6°C and all solutions were used within the demonstrated time period of stability.

All analytes were shown to be stable in stock and fortifcation solutions prepared in acetonitrile for at least 65 days when stored under refrigeration. Each analyte was shown to be stable in calibration standard solutions prepared by serial dilution of the intermediate standard solutions with 50/50 methanol/water (v:v) and held under refrigeration for at least 62 days for all analytes.

Extract Stability.
Water extracts have been shown to be stable, when stored under refrigeration, for at least 7 days for all anlytes.

Recovery and Repeatability. The independent laboratory validation (ILV) was performed successfully for each water matrix using both primary and secondary quantiataion techniques available for the method, using solvent-based and matrix-matched standards. using both primary and secondary quantiataion techniques

Apparent residues of S(Br-OH)-8007, AB-Oxa, and MFBA were below the method limit of detection in all of the control water samples.

1. INTRODUCTION

1.1 Scope of the Method

BASF Analytical Method No. D1705/01 was developed to determine the residues of S(Br-OH)-8007, AB-Oxa and MFBA in surface and drinking water matrices by LC-MS/MS at BASF Crop Protection in Research Triangle Park, North Carolina. This method was validated at BASF Crop Protection in Research Triangle Park, North Carolina (Reference 1) and was independently validated at Primera Analytical Solution Corp.

The independent lab validation was conducted using two fortification levels: limit of quantitation (LOQ) 25 ng/L (25 ppt) and 250 ng/L (250 ppt) in water for S(Br-OH)-8007, AB-Oxa, and MFBA.

For each fortification level and matrix, five replicates were analyzed. Additionally, one reagent blank and two replicates of unfortified samples were examined.

1.2 Principle of the Method

The samples (10 mL) fortified with the compounds and then extracted with 10 mL methanol. The extracts were then filtered and analyzed with LC-MS/MS.

1.3 Specificity

To demonstrate the specificity of the analytical method, one additional mass transition (*m/z* 601 → 581) was monitored simultaneously to the primary quantitation transition (*m/z* 601 → 256) for analysis of S(Br-OH)-8007. One additional mass transition (*m/z* 583 → 494) was monitored simultaneously to the primary quantitation transition (*m/z* 583 → 444) for analysis of AB-Oxa. For MFBA, a secondary chromatographic technique using a different stationary phase was included for verification. The method was able to accurately determine residues of S(Br-OH)-8007, AB-Oxa and MFBA. No interference was observed at the retention times of the analyte peaks.

2. REFERENCE SUBSTANCE AND SAMPLING HISTORY

2.1 Test Systems

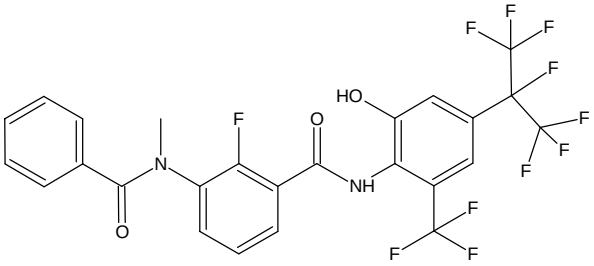
The test systems considered in this study were surface water and drinking water. The water matrices were characterized by AGVISE Laboratories. A copy of the characterization data is provided in **Appendix D**.

The control samples were provided by BASF. The samples were received on June 8, 2017. Upon arrival at the laboratory, the samples were opened, inspected, and checked against enclosed shipping forms and assigned a unique laboratory analysis code
(e.g.,170744-1).
The test systems were received frozen and were stored under frozen conditions at all times until sample analysis.

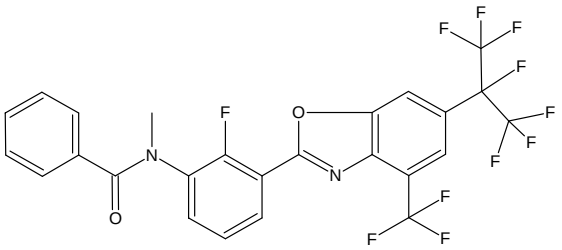
2.2 Test and Reference Substances

The standard substance was stored at room temperature. BASF has retained a reserve sample of this chemical, and has documentation specifying the location of the synthesis and characterization information available at BASF Crop Protection, Research Triangle Park, North Carolina.
S(Br-OH)-8007 (Lot No.296-012-016-1), AB-Oxa (Lot No. 296-012-012-1) and MFBA (Lot No. N4145911-146), reference substances were provided by the sponsor and received on June 8, 2017. The certificates of analysis of all substances are presented in Appendix A. A detailed summary of the reference substances is presented below.

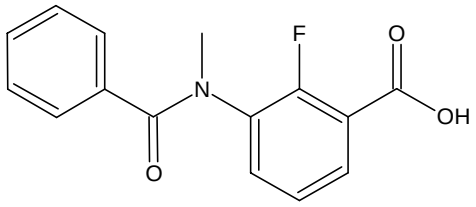
BAS Code Name	None
Common Name	S(Br-OH)-8007
Chemical Name	2-fluoro-*N*-[2-hydroxy-4-(perfluoropropan-2-yl)-6-(trifluoromethyl)phenyl]-3-(*N*-methylbenzamido) benzamide
BASF Reg. No.	5959595
CAS-No.	None
Molecular Formula	C25H15F11N2O3
Molecular Weight	600.4
Lot Number	296-012-016-1
Purity	98.38
Expiration Date	June 28, 2017
Storage Condition	Room T, light-protection



Common Name	AB-Oxa
Chemical Name	N-{2-fluoro-3-[6-perfluoropropan-2-yl)-4-(trifluoromethyl)-1,3-benzooxazol-2-yl]phenyl}-N-methylbenzamide
BASF Reg. No.	5959600
CAS-No.	None
Molecular Formula	C24H13F11N2O2
Molecular Weight	582.4
Common Name	AB-Oxa
Lot #	296-012-012-1
Purity	98.89%
Expiration Date	June 28, 2017
Storage Condition	Room T, light-protection



Common Name	MFBA
Chemical Name	2-fluoro-3-(N-methylbenzamido) benzoic acid
BASF Reg. No.	6088668
CAS-No.	None
Molecular Formula	C15H12FNO3
Molecular Weight	273.26
Common Name	MFBA
Lot Number	N4145911-146
Purity	99.87%
Expiration Date	Oct 11, 2019
Storage Condition	Room T, light-protection



3. ANALYTICAL METHOD

BASF Analytical Method D1705/01 of "Method for the Determination of S(Br-OH)-8007 (Reg. No. 5959595), AB-Oxa (Reg. No. 5959600), and MFBA (Reg. No. 6088668) in Surface and Drinking Water by LC-MS/MS" was used for sample analysis for this study.

The primary (quantitative) and secondary (confirmatory) transition ions monitored are presented below:

Analyte	**Transition (*m/z*)**		**Ionization Mode**	**Retention Time (min)**
	Primary	**Secondary**		
S(Br-OH)-8007	601 →256	601 →581	Positive	2.6
AB-Oxa	583 →444	583 →494	Positive	3.3
MFBA	274 →254	N/A	Positive	1.9
	274 →254	N/A	Positive	1.4*

*with a different column in confirmation method

5. SUMMARY OF METHOD

Type of Method LC-MS/MS

Test Systems Surface water and drinking water

Selected mass transitions (m/z)

	Quantitation	Confirmation
S(Br-OH)-8007	601 →256	601 →581
AB-Oxa	583 →444	583 →494
MFBA	274 →254	

Analytical Procedure BASF Analytical Method D1705/01 of "Method for the Determination of S(Br-OH)-8007 (Reg. No. 5959595), AB-Oxa (Reg. No. 5959600), and MFBA (Reg. No. 6088668) in Surface and Drinking Water by LC-MS/MS" was used for sample analysis for this study.

Confirmatory Technique A secondary MRM transition for S(Br-OH)-8007 at *m/z* 601 →581 and at 583 →494 for AB-Oxa in positive mode monitored for confirmation quantification. For MFBA, a secondary chromatographic technique using a different stationary phase was included for verification.

Method of Quantitation The quantitation is based on the monitoring of two mass transitions for S(Br-OH)-8007, AB-Oxa and one transition for MFBA. Recovery data was reported for each mass transition considered.

LOQ 25 ng/L (25 ppt) in water for S(Br-OH)-8007, AB-Oxa and MFBA.

LOD 5 ng/L in water for S(Br-OH)-8007, AB-Oxa and MFBA.

Levels of Fortification 25 ppt and 250 ppt for S(Br-OH)-8007, AB-Oxa and MFBA in surface and drinking water.

Time Required A set of 13 samples requires approximately 4 hours of work (calculation of the results included).

Justification of Ions The ions used to conduct the ILV were determined in the validation (Reference 1) and are shown in Appendix C.

7. RECOMMENDATIONS/ CONCLUSIONS FROM ILV

This independent laboratory validation was successfully completed on the second try at Primera Analytical Solution Corp for both surface water and drinking water due to analyst error. In the ILV normal standards were used for drinking water while matrix matched standards were used for surface water. Recovery results and statistical data demonstrate BASF Analytical Method D1705/01 can be performed successfully for quantitation of S(Br-OH)-8007 (Reg. No. 5959595), AB-Oxa (Reg. No. 5959600), and MFBA (Reg. No. 6088668) in surface and drinking water.

The method is well-written and contains a fair amount of comments to guide the analyst through the procedure for the first time.There are no recommendations for BASF method D1705/01.

8. PROTOCOL, AMENDMENTS, AND DEVIATIONS

No amendments or deviations were made during the ILV study.

9. COMMUNICATION

Communications between the Study Director and the BASF study monitor and personnel are documented. At no time during the course of the study was anyone from BASF allowed to visit the testing facility.

The study monitor was informed of the successful completion of the study on 08/11/2017

10. REFERENCE

1. Delinsky, D. (2017) "Validation of Method D1705/01: Method for the Determination of S(Br-OH)-8007 (Reg. No. 5959595), AB-Oxa (Reg. No. 5959600), and MFBA (Reg. No. 6088668) in Surface and Drinking Water by LC-MS/MS" BASF Study Number 838397. BASF Reg. Doc. No. 2017/7012333.

Table 14 Instrumentation and Conditions for S(Br-OH)-8007, AB-Oxa, and MFBA

	Parameter		
Chromatographic System	Waters Acquity		
Analytical-column	XBridge BEH Phenyl 2.5µm, 2.1x100mm		
Column Temperature	50°C		
Injection Volume	30 µL		
Mobile Phase A Mobile Phase B	Water / formic acid, 1000/1, v/v Methanol / formic acid, 1000/1, v/v		
Flow Rate	600 µL/min		
Gradient (including wash and equilibration)	Time (min)	Phase A	Phase B
	0.00	70	30
	0.10	70	30
	1.10	45	55
	1.20	30	70
	3.20	5	95
	4.20	5	95
	4.25	70	30
	5.00	70	30
Detection System	Sciex 6500		
Ionisation	Electrospray (ESI)		
Ionisation Temperature	700 °C		
Analyte	**Transitions (m/z)**	**Polarity**	**Expected Retention Time**
S(Br-OH)-8007 (Reg. No.5959595)	601 → 256* 601 → 581	positive	approx. 2.6 min
AB-Oxa (Reg. No. 5959600)	583 → 444* 583 → 494	positive	approx. 3.3 min
MFBA (Reg. No. 6088668)	274 → 254*	positive	approx. 1.9 min

* proposed as quantification transition. Any of these transitions could be used for quantitation in case interference is observed at the same retention time

Table 15 Confirmatory Instrumentation and Conditions for MFBA

	Parameter		
Chromatographic System	Waters Acquity		
Analytical-column	XBridge BEH C18 1.7µm, 2.1x50mm		
Column Temperature	50°C		
Injection Volume	30 µL		
Mobile Phase A Mobile Phase B	Water / formic acid, 1000/1, v/v Methanol / formic acid, 1000/1, v/v		
Flow Rate	600 µL/min		
Gradient (including wash and equilibration)	Time (min)	Phase A	Phase B
	0.00	90	10
	0.10	70	30
	1.10	45	55
	1.30	5	95
	1.90	5	95
	2.00	90	10
	2.50	90	10
Detection System	Sciex 6500		
Ionisation	Electrospray (ESI)		
Ionisation Temperature	700 °C		
Analyte	**Transitions (m/z)**	**Polarity**	**Expected Retention Time**
MFBA (Reg. No. 6088668)	274 → 254*	positive	approx. 1.4 min

* proposed as quantification transition. Any of these transitions could be used for quantitation in case interference is observed at the same retention time

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 must 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) is required, then methodology must 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.
The same applies to different mass transitions used: Validation of the methodology is required as described above (fortification and assessment of matrix effect).
Other parameters, such as ion source gas flows and voltages, are highly specific of the equipment used and therefore not listed. Those parameters may need to be adapted to the actual instrument.

Table 16 Typical Analytical Standards Dilutions and Use Record

Solution ID	Analyte[1]	Standard (Lot #) / Parent Solution ID	Amount Weighed / Volume	Final Dilution Vol. (mL)	Final Conc.	Solvent[2]	Prep. Date[3]
Stock solutions							
S20170627-1	AB-Oxa	296-012-012-1	10.017 mg	10	1.0 mg/ml	Acetonitrile	06/27/17
S20170627-2	S(Br-OH)-8007	296-012-016-1	19.930 mg	20	0.9965 mg/ml	Acetonitrile	06/27/17
S20170627-3	MFBA	N4145911-146	20.00 mg	20	1.0 mg/ml	Acetonitrile	06/27/17
Serial dilutions							
F20170627-1	Mix	S20170627-1 S20170627-2 S20170627-3	500 uL 500 uL 500 uL	10	50 µg/mL	Acetonitrile	06/27/17
F20170627-2	Mix	F20170627-1	50 uL	50	0.050 µg/mL	Acetonitrile	06/27/17
F20170627-3	Mix	F20170627-2	5000 uL	50	0.005 µg/mL	Acetonitrile	06/27/17
Calibration							
C20170627-1	Mix	F20170627-3	5.0	50	0.50 ng/mL	FV1	06/27/17
C20170627-2	Mix	C20170627-1	5.0	50	0.05 ng/mL	FV1	06/27/17
C20170627-3	Mix	C20170627-2	12.5	25	0.025 ng/mL	FV1	06/27/17
C20170627-4	Mix	C20170627-2	6.25	25	0.0125 ng/mL	FV1	06/27/17
C20170627-5	Mix	C20170627-2	2.5	25	0.005 ng/mL	FV1	06/27/17
C20170627-6	Mix	C20170627-2	1.25	25	0.0025 ng/mL	FV1	06/27/17
Fortification							

Sample Data		Fortification Data					Final Volume (mL)
Surface water sample	Vol. (mL)	ppt Fortified	Analyte	Vol. of Standard Used	Standard Conc.	Standard Number	
CM16-016	10	Control	None	NA	NA	NA	20
CM16-016	10	25	S(Br-OH)-8007, AB-Oxa, MFBA	50 µL	5.0 ng/mL	F20170808-3	20
CM16-016	10	250	S(Br-OH)-8007, AB-Oxa, MFBA	50 µL	50 ng/mL	F20170808-2	20

1. Mix= S(Br-OH)-8007, AB-Oxa and MFBA
2. FV1 = 50% Methanol and 50% water.
3. Stock solutions are stored for up to 3 months. Fortification and Calibration solutions are stored for up to 4 weeks.

Figure 73 Calculation Formula and Example.

Sample Description: surface water LOQ-1-Fortified surface water (Results File: 20170808-surface water.rdb)

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

$$\text{Concentration of Analyte } (C_B)(\text{ppt}) = C_A * \text{dilution factor} * 1000 \text{ (dilution factor = 2)}$$

Analyte	**S(Br-OH)-8007**	**AB-Oxa**	**MFBA**
Peak Area =	14000	1490	13100
Intercept =	1460	-77	2820
Slope =	948000	134000	989000
C_A (ng/mL) =	0.0132	0.0116	0.0104
C_A (ppt) =	26.4	23.4	20.8

NOTE: Slight rounding differences with analyst® data may be noted when using a hand calculator. Full computer/calculator precision was used for any intermediate calculations. Only the final value was rounded.

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Working Procedure:

Method for the Determination of S(Br-OH)-8007 (Reg. No. 5959595), AB-Oxa (Reg. No. 5959600), and MFBA (Reg. No. 6088668) in Surface and Drinking Water by LC-MS/MS

BASF Method Number D1705/01

Final

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Date
August 29, 2017

Test Facility
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USA

Number of Pages
17

ABSTRACT

BASF Method D1705/01 is developed to determine the residues of BAS 450 I metabolites S(Br-OH)-8007 (Reg. No. 5959595), AB-Oxa (Reg. No. 5959600), and MFBA (Reg. No. 6088668) in surface and drinking water using LC-MS/MS at BASF Crop Protection, Research Triangle Park, N.C.

Brief description of the method:

10 mL methanol is added to a 10 mL water sample and mixed. After filtration, the sample is ready for analysis by LC-MS/MS.

The method has a limit of quantitation (LOQ) of 25 ng/L (25 ppt) in water. The limit of detection (LOD) in water is 5 ng/L.

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. Also known as blank of reagents or procedural blank. This sample is analyzed within the sample set in order to evaluate possible contamination on 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. 20% 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 INTRODUCTION

BAS 450 I is a new insecticide that will be used for various crops. The analytical method D1705/01 offers the possibility to determine residues of S(Br-OH)-8007 (Reg. No. 5959595), AB-Oxa (Reg. No. 5959600), and MFBA (Reg. No. 6088668), metabolites of BAS 450 I, in water. Method D1705/01 was successfully validated in surface and drinking water for all analytes.

This method was developed at BASF Crop Protection, Research Triangle Park, NC.

2 MATERIALS

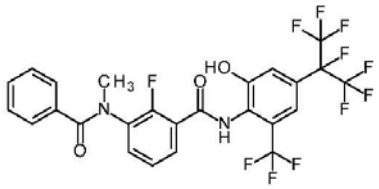
2.1 Safety

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. Ensure 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 Materials Safety Data Sheets (MSDS) 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.

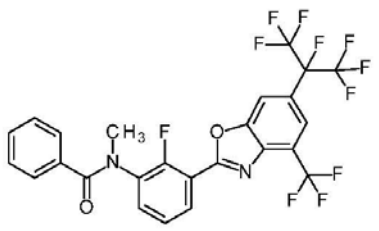
2.2 Test and Reference Items

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

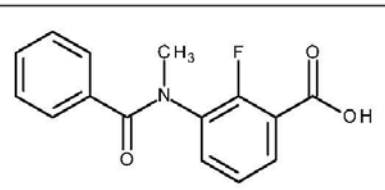
Common Name	S(Br-OH)-8007
Chemical Name	2-fluoro-*N*-[2-hydroxy-4-(perfluoropropan-2-yl)-6-(trifluoromethyl)phenyl]-3-(*N*-methylbenzamido) benzamide
BASF Reg. No.	5959595
CAS-No.	None
Molecular Formula	$C_{25}H_{15}F_{11}N_2O_3$
Molecular Weight	600.4



Common Name	AB-Oxa
Chemical Name	N-{2-fluoro-3-[6-perfluoropropan-2-yl)-4-(trifluoromethyl)-1,3-benzooxazol-2-yl]phenyl}-N-methylbenzamide
BASF Reg. No.	5959600
CAS-No.	None
Molecular Formula	$C_{24}H_{13}F_{11}N_2O_2$
Molecular Weight	582.4



Common Name	MFBA
Chemical Name	2-fluoro-3-(N-methylbenzamido) benzoic acid
BASF Reg. No.	6088668
CAS-No.	None
Molecular Formula	$C_{15}H_{12}FNO_3$
Molecular Weight	273.26



2.3 Equipment

Equipment	Size, Description	Manufacturer	Catalog No.
Amber Bottles	60 mL, Boston Round bottle with PTFE-faced PE lined cap attached	VWR	89042-908
Balance, Top-Load	150 g, CP153	Sartorius	
Beakers	Various Sizes	PYREX Brand, VWR Scientific Products	13922-029
Centrifuge Tubes (disposable)	50 mL	VWR	89039-660
Filters, Syringe Tip	13mm Syringe Filter, 0.45 µm PTFE membrane	PALL Life Sciences	4555
Graduated Cylinder	10 mL, PYREX	VWR	89090-636
LC-MS/MS injection vials	1.5 mL, Target DP	VWR, Thermo Scientific	00162506
LC Column	XBridge BEH Phenyl, 2.5 µm, 2.1x100 mm	Waters	186006067
LC Column (confirmation)	Acquity BEH C18, 1.7 µm, 2.1x50 mm	Waters	186002350
LC System	Acquity	Waters	
Mass Spectrometer	API 5500	Sciex	
Microman Pipettes	1000 µL 250 µL 50 µL	Gilson	M1000 M250 M50
Microman Pipette tips	1000 µL tips 250 µL tips 50 µL tips	Gilson	CP1000 CP250 CP50
Pasteur Pipettes, disposable	2 mL, 14.6 cm Borosilicate Glass	VWR	14673-010
Scintillation Vials	20 mL	VWR	66022-060
Shaker	KS501 digital	IKA Labortechnik	0002526401
Spatula		Various	
Syringes, Disposable	1 mL	Thermo Scientific	S7510-1
Volumetric Flasks	10 mL, 50 mL, 100 mL	Various	
Volumetric Pipettes	Various, class-A	Various	
Vortex Mixer	Genie 2	Fisher Scientific Co	12-812
Vortexer	Multi-tube vortexer, VX-2500	VWR	444-7063

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	AX0145P-1
Methanol	HPLC Grade	EMD	MX0475P-1
Water	HPLC Grade	BDH ARISTAR PLUS	87003-652
Formic Acid	≥95%	Sigma-Aldrich	F0507

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

2.4.2 Solutions and Solvent Mixtures

Description	Code	Composition
Final Volume Solvent	FV1	**Methanol-water, 50:50, v/v** Add 500 mL of methanol and 500 mL of water into a 1L Erlenmeyer flask and mix well to ensure complete homogeneous solution.
HPLC mobile phase A	LC1	**0.1% Formic Acid in Water** Add 1000 mL of water and 1 mL of concentrated formic acid into a, e.g., 1L Erlenmeyer flask and mix well to ensure complete homogeneous solution.
HPLC mobile phase B	LC2	**0.1% Formic Acid in Methanol** Add 1000 mL of Methanol and 1 mL of concentrated formic acid into a, e.g., 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 of solvents are not modified.

2.4.3 Standard Solutions

Stock Solutions

Prepare a 1.0 mg/mL stock solution individually by weighing an appropriate amount of the analyte into a flask and add the required volume.
For example, to prepare 10 mL of 1.0 mg/mL stock solution of S(Br-OH)-8007 in acetonitrile, weigh 10 mg of S(Br-OH)-8007 into a 10 mL volumetric flask. Dissolve and dilute to mark with acetonitrile. Ensure a complete homogeneous solution (e.g. by sonication or vortexing). The stock solutions for all other analytes are made in a similar fashion.

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 done if the purity is ≤ 95%. If the purity is > 95 % correction is optional.

Fortification Solutions

Prepare standard solutions for fortification by dilution of the above stock solution. Dilute volumetrically with appropriate solvents as exemplified in the table below and ensure a complete homogeneous solution (e.g. by sonication or vortexing).

Preparation of mixed Fortification solutions

Take solution (µg/mL)	Volume (mL)	Dilute with acetonitrile to a final volume of (mL)	Concentration (µg/mL)
1000	0.5	10	50
50	0.05	50	0.050
0.050	5	50	0.005

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.

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". Dilute volumetrically with appropriate solvents as exemplified in the table below and ensure a complete homogeneous solution (e.g. by sonication or vortexing).

Preparation of standard solutions for calibration

Take solution (ng/mL)	Volume (mL)	Dilute with FV1* to a final volume of (mL)	Concentration (ng/mL)
5.0	5.0	50	0.50 †
0.50	10	100	0.05
0.05	25	50	0.025
0.05	12.5	50	0.0125
0.05	5.0	50	0.005
0.05	2.5	50	0.0025

† Not intended to be a calibration standard but needed to prepare subsequent calibration standards.
* In case matrix-matched standards (= instrument recovery samples) are needed for successful analysis, calibration standard solutions are prepared in matrix solution, i.e., final volume of a control 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. In addition, the matrix load should be the same in all calibration standard solutions.

Note: A different concentration scheme may be used and additional standards may be prepared as needed. If necessary, the volume of solution prepared may be changed.

Additional Information:

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

2.4.4 Stability of Standard Solutions

Stability for solutions in acetonitrile (stock and fortification solutions) is 65 days for all analytes when stored under refrigerated conditions. Stability of calibration standard solutions (methanol-water, 50:50, v:v) is 62 days for all analytes when stored under refrigerated conditions.

3 ANALYTICAL PROCEDURE

3.1 Sample Preparation

Sample homogenization is not needed for water samples.

S(Br-OH)-8007, AB-Oxa, and MFBA have the potential to adhere to container walls. As a result, any water samples to be analyzed (of unknown volume) must be transferred to a new container (while measuring sample volume), such as a graduated cylinder. An equal volume of methanol should be added to the original container; shake methanol in containers for 15 minutes at 300 rpm on a mechanical shaker, ensuring that solvent contacts all interior surfaces of the container. The methanol should then be transferred to the new container that is holding the sample. (Be sure the new container used has adequate capacity to contain both the sample and the methanol to be added as well as allow adequate mixing.) The diluted sample should then be mixed, filtered, and analyzed as specified below.

3.2 Sample Storage

Water samples are to be kept frozen until analysis.

3.3 Weighing and Fortification

For treated samples and control samples, measure 10 ± 0.1 g (or 10 mL) of water sample into a disposable tube (such as 50 mL plastic centrifuge tube).

For fortification samples, measure 10 ± 0.1 g (or 10 mL) of water sample into a disposable tube (such as 50 mL plastic centrifuge tube). Fortify the solution with analytes and shake/vortex for approximately 1 minute to ensure sample homogeneity.

The following scheme may be used:

Sample Type	Sample Weight	Concentration of Spiking Solution†	Volume of Spiking Solution	Level of Fortification†
Control	0.010 L	-	-	0.00 ng/L
Fortification (LOQ*)	0.010 L	5.0 ng/mL	0.05 mL	25 ng/L (ppt)
Fortification (10xLOQ)	0.010 L	50 ng/mL	0.05 mL	250 ng/L (ppt)
Treated	0.010 L	-	-	-

* limit of quantification

Note: Volume of spiking solution added to generate the fortified sample should not exceed 10% of sample weight or volume.

For fortified samples, 0.05 mL solvent is added that is not added to control or treated samples. This additional volume is considered insignificant and will not be considered in recovery calculations.

3.4 Preparation for Measurement

Add 10 mL methanol to all samples and shake for 30 minutes at 300 rpm on a mechanical shaker to ensure homogeneity. Syringe filter all samples using 0.45µm PTFE syringe filters directly into HPLC injection vials, passing the first approximately 0.2 – 0.3 mL to waste. Samples are ready for injection.

High fortification and high residue samples - further dilute with **FV1** (methanol-water, 50:50, v/v) as necessary, to fit in the calibration curve.

3.5 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 solvent FV1 (see 2.4.3).

3.6 Stability of Extracts / Final Volumes

Each analyte has been shown to be stable in extracts for at least the time period tested, 7 days for all analytes in surface water.

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 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 for S(Br-OH)-8007, AB-Oxa, and MFBA

	Parameter		
Chromatographic System	Waters Acquity		
Analytical-column	XBridge BEH Phenyl 2.5µm, 2.1x100mm		
Column Temperature	50°C		
Injection Volume	20 µL		
Mobile Phase A Mobile Phase B	Water / formic acid, 1000/1, v/v Methanol / formic acid, 1000/1, v/v		
Flow Rate	600 µL/min		
Gradient (including wash and equilibration)	Time (min)	Phase A	Phase B
	0.00	70	30
	0.10	70	30
	1.10	45	55
	1.20	30	70
	3.20	5	95
	4.20	5	95
	4.25	70	30
	5.00	70	30
Detection System	Sciex 5500		
Ionisation	Electrospray (ESI)		
Ionisation Temperature	700 °C		
Analyte	**Transitions (m/z)**	**Polarity**	**Expected Retention Time**
S(Br-OH)-8007 (Reg. No.5959595)	601 → 256* 601 → 581	positive	approx. 2.6 min
AB-Oxa (Reg. No. 5959600)	583 → 444* 583 → 494	positive	approx. 3.3 min
MFBA (Reg. No. 6088668)	274 → 254*	positive	approx. 1.9 min

* proposed as quantification transition. Any of these transitions could be used for quantitation in case interference is observed at the same retention time

4.2.2 Confirmatory Instrumentation and Conditions for MFBA

	Parameter		
Chromatographic System	Waters Acquity		
Analytical-column	Acquity UPLC BEH C18 1.7µm, 2.1x50mm		
Column Temperature	50°C		
Injection Volume	20 µL		
Mobile Phase A Mobile Phase B	Water / formic acid, 1000/1, v/v Methanol / formic acid, 1000/1, v/v		
Flow Rate	600 µL/min		
Gradient (including wash and equilibration)	Time (min)	Phase A	Phase B
	0.00	90	10
	0.10	70	30
	1.10	45	55
	1.30	5	95
	1.90	5	95
	2.00	90	10
	2.50	90	10
Detection System	Sciex 5500		
Ionisation	Electrospray (ESI)		
Ionisation Temperature	700 °C		
Analyte	**Transitions (m/z)**	**Polarity**	**Expected Retention Time**
MFBA (Reg. No. 6088668)	274 → 254*	positive	approx. 1.4 min

* proposed as quantification transition. Any of these transitions could be used for quantitation in case interference is observed at the same retention time

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 must 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) is required, then methodology must 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.

The same applies to different mass transitions used: Validation of the methodology is required as described above (fortification and assessment of matrix effect).

Other parameters, such as ion source gas flows and voltages, are highly specific of the equipment used and therefore not listed. Those parameters may need to be adapted to the actual instrument.

4.2.3 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 standards (in the range of 0.05 ng/mL to 0.0025 ng/mL) for LC-MS/MS. 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.

4.2.4 Calculation of Residues and Recoveries

Calculation of results is based on area measurements.
For the procedural recoveries, the sample volume of 10 g (or 10 mL) will be considered in the final calculation of residues [ng/L]. This approach requires that the sample volume has to be within a measuring precision of 10 ± 0.1 g (or 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.

The residues of S(Br-OH)-8007, AB-Oxa, and MFBA in mg/L are calculated as shown in equations I and II:

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

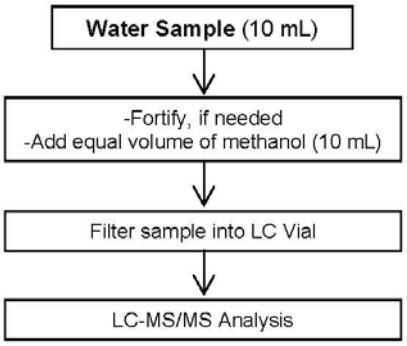
II. **Residue [ng/L]** $= \dfrac{V_{end} \times C_A}{G \times A_F}$

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 in L
A_F	=	Aliquot factor (1 for this method)

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

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

5 FLOWCHART



6 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 0.5 working day (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

Recoveries, Chromatograms, and Calibration Curves
Recovery data will be provided in the validation report of the analytical method D1705/01.

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 25 ng/L (25 ppt) for all analytes. The limit of detection is estimated to be 20% of the limit of quantification, equivalent to 5 ng/L 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 surface and drinking water samples showed no significant interferences (< 20%) at the retention time of the analytes.

Confirmatory Techniques
The LC-MS/MS final determination for S(Br-OH)-8007 and AB-Oxa is a highly selective detection technique and quantitation is possible at two different mass transitions. For MFBA, a secondary chromatographic technique using a different stationary phase is included for confirmation.

Potential Problems
A PVDF filter is not suitable for use with this method, however, GHP and nylon filters may be found to be acceptable.

The glassware used for the method should be thoroughly rinsed with methanol followed by acetone to prevent contamination.

S(Br-OH)-8007, AB-Oxa, and MFBA have the potential to adhere to container walls. As a result, any water samples to be analyzed (of unknown volume) must be transferred to a new container (while measuring sample volume), such as a graduated cylinder. An equal volume of methanol should be added to the original container; shake methanol in containers for 15 minutes at 300 rpm on a mechanical shaker, ensuring that solvent contacts all interior surfaces of the container. The methanol should then be transferred to the new container that is holding the sample. (Be sure the new container used has adequate capacity to contain both the sample and the methanol to be added as well as allow adequate mixing.) The diluted sample should then be mixed, filtered, and analyzed as specified below.