Abstract

The objective of this validation study was to demonstrate the applicability and repeatability of method D1304/02 for the determination of quizalofop-p-ethyl (BAS 9152 H) and its two metabolites quizalofop-p and 3-OH-quizalofop-acid in drinking (tap) and surface water using LC-MS/MS. All analyses of this independent laboratory validation were performed at Primera Analytical Solutions Corp. (PASC) in Princeton, New Jersey, USA.

Principle of the Method. For the analysis of quizalofop-p-ethyl, quizalofop-p, and 3-OH-quizalofop-acid, a water sample (10 g) was weighed out. An aliquot (0.5%) was diluted with acetonitrile:water (90:10, v/v) to determine the residues of quizalofop-p-ethyl and quizalofop-p using LC-MS/MS.

An additional aliquot (0.5%) was diluted with acetonitrile:water (55:45, v/v) to determine the residues of 3-OH-quizalofop-acid using LC-MS/MS.

Test Conditions. The method was validated at two fortification levels for each analyte: 0.001 mg/kg and 0.01 mg/kg for quizalofop-p-ethyl, quizalofop-p, and 3-OH-quizalofop-acid. For each fortification level and matrix, five replicates were analyzed. Additionally, at least two replicates of unfortified samples were analyzed with each sample set. For quizalofop-p-ethyl and quizalofop-p, two mass transitions (*m/z* 373→299 and *m/z* 375→301; *m/z* 345→299 and *m/z* 345→100) were evaluated using one chromatographic condition. For the metabolite 3-OH-quizalofop-acid, one mass transition (*m/z* 359→166) was used for both primary and confirmatory quantitation. A secondary chromatographic method was used for confirmation.

Matrix-matched standards and solvent-based standards were also analyzed and compared within this study to evaluate the matrix effects.

Limit of Quantitation (LOQ) and Limit of Detection (LOD). The limit of quantitation (LOQ) was defined as the lowest fortification level successfully tested. The LOQ is 0.001 mg/kg for quizalofop-p-ethyl, quizalofop-p, and 3-OH-quizalofop-acid (0.05 ng/mL). The limit of detection (LOD) is set at 20% of the LOQ (0.0002 mg/kg) for quizalofop-p-ethyl, quizalofop-p, and 3-OH-quizalofop-acid (0.01 ng/mL).

Selectivity. The method was able to determine residues of quizalofop-p-ethyl (BAS 9152 H) and its metabolites (quizalofop-p and 3-OH-quizalofop-acid) individually in water. No interfering peaks were found at the retention time for quizalofop-p-ethyl (BAS 9152 H) or for its metabolites individually (quizalofop-p and 3-OH-quizalofop-acid). No matrix-suppression or enhancement was found for quizalofop-p-ethyl (BAS 9152 H) or for its metabolites.

1. Introduction

1.1 Scope of the Method

Quizalofop-p-ethyl (BAS 9152 H) is an herbicide that was developed for use in a broad spectrum of crops in the US. Quizalofop-p and 3-OH-quizalofop-acid are two metabolites of interest for the compound.

An analytical method for the detection and quantitation of residues in water of the active ingredient quizalofop-p-ethyl (BAS 9152 H) and its two metabolites (quizalofop-p and 3-OH-quizalofop-acid) was needed for monitoring purposes with a limit of quantitation (LOQ) of 0.001 mg/kg.

As described below, BASF Method No. D1304/02 allows for the determination of the analytes in water with the required limit of quantitation. This method was developed at BASF Crop Protection in Research Triangle Park, NC and was validated at Primera Analytical Solutions Corp. (PASC) in Princeton, NJ. To demonstrate the validity of the method, recovery trials with fortified water samples were performed.

The method was validated at two fortification levels, LOQ and 10×LOQ (0.001 mg/kg and 0.01 mg/kg, respectively) in water samples. For each fortification level and matrix, five replicates were analyzed. Additionally, two replicates of unfortified samples were analyzed with each sample set. For quizalofop-p-ethyl and quizalofop-p, two mass transitions (*m/z* 373→299 and *m/z* 375→301; *m/z* 345→299 and *m/z* 345→100) were evaluated using one chromatographic condition, as outlined in Section 4.1.1. For 3-OH-quizalofop-acid, one mass transition (*m/z* 359→166) was evaluated using two chromatographic conditions, as outlined in Sections 4.1.2 and 4.1.3.

Matrix- and solvent-matched standards were also analyzed within this study to check for possible matrix effects.

1.2 Principle of the Method

For the analysis of quizalofop-p-ethyl, quizalofop-p, and 3-OH-quizalofop-acid, a water sample (10 g) was weighed out. An aliquot (0.5%) was diluted with acetonitrile:water (90:10, v/v) to determine the residues of quizalofop-p-ethyl and quizalofop-p using LC-MS/MS.

An additional aliquot (0.5%) was diluted with acetonitrile:water (55:45, v/v) to determine the residues of 3-OH-quizalofop-acid using LC-MS/MS.

1.3 Specificity

The method was able to accurately determine residues of quizalofop-p-ethyl, quizalofop-p, and 3-OH-quizalofop-acid. No interference was observed at the retention times for any of the three peaks. No matrix-suppression or enhancement was found to affect any of the analytes. The use of matrix-matched standards is not necessary as shown in this method validation study.

2. Materials and Methods

2.1 Test Systems

The following test systems were considered in this study of validation:

Test System 1: Tap Water (as Drinking water) (BASF Study 710640)

Test System 2: Surface Water (BASF Study 437860, RCN R130034)

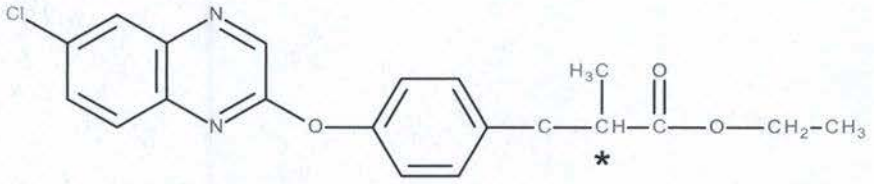
The characterization data for the water used is provided in the respective attached certificates (Appendix 5).

2.2 Test and Reference Items

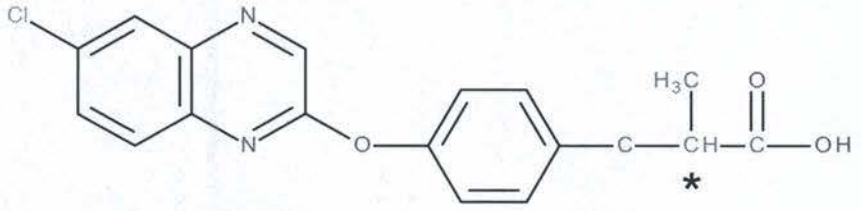
Standard substances were stored in a freezer (≤ -5°C) until use.

BASF has retained reserve samples of these chemicals, and has documentation specifying the location of the synthesis and characterization information for each of these compounds available at BASF, Research Triangle Park, North Carolina. The certificate of analysis for the reference standards are shown in Appendix 5.

2.2.1 Quizalofop-p-ethyl (BAS 9152 H)

Common Name	Quizalofop-p-ethyl (QPE)
BASF Reg. No.	N/A
CAS No.	100646-51-3
Molecular Formula	$C_{19}H_{17}ClN_2O_4$
Molecular Weight	372.8
IUPAC Name	ethyl (R)-2-[4-(6-chloroquinoxanlin-2-yloxy)-phenoxyl]propionate
Batch No.	302D-S110926
Purity (%)	99.9%
Test Substance Type	PAI
Storage Advice	Dark and Cool (below 10°C is recommended)
GLP	Yes
Expiration Date	September 26, 2016
Chemical Structure	

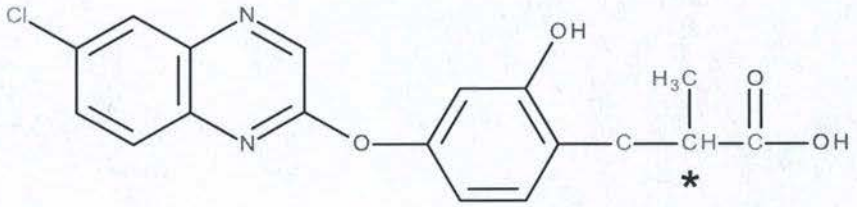
2.2.2 Quizalofop-p

Common Name	Quizalofop-p (QP)
BASF Reg. No.	N/A
CAS No.	94051-08-8
Molecular Formula	$C_{17}H_{13}ClN_2O_4$
Molecular Weight	344.7
IUPAC Name	(R)-2-[4-(6-chloroquinoxanlin-2-yloxy)-phenoxyl]propionic acid
Batch No.	302D-ACID-S050325
Purity (%)	99.8%
Test Substance Type	Metabolite
Storage Advice	Dark and Cool (below 10°C is recommended)
GLP	Yes
Expiration Date	December 5, 2017
Chemical Structure	

2.2.3 R(+)-3-OH-quizalofop-acid

Common Name	R(+)-3-OH-quizalofop-acid (3-OH-QA)
BASF Reg. No.	N/A
CAS No.	N/A
Molecular Formula	$C_{17}H_{13}ClN_2O_5$
Molecular Weight	360.8
IUPAC Name	(R)-2-[4-(6-chloro-3-hydroxyquinoxanlin-2-yloxy)-phenoxyl]propionic acid
Batch No.	3-OH-302D-ACID-M941088
Purity (%)	95.9%
Test Substance Type	Metabolite
Storage Advice	Dark and Cool (below 10°C is recommended)
GLP	Yes
Expiration Date	March 3, 2017

Chemical Structure



2.2.4 Stability of Test and Reference Items

2.2.4.1 Stability of Fortification and Calibration Standard Solutions

Results obtained during BASF validation study 437873 demonstrated that stock standard solutions of quizalofop-p-ethyl, quizalofop-p, and 3-OH-quizalofop-acid were stable for 95 days when stored refrigerated. Calibration solutions were stable for one month when stored refrigerated.

The results of the standard solution stability tests are documented in the report, "*Validation of BASF Analytical Method (D1303/02): 'Analytical Method for the Determination of Residues of Quizalofop-p-ethyl (BAS 9152 H) and its two Metabolites Quizalofop-p and 3-OH-Quizalofop-acid in Soil by LC-MS/MS,*" BASF Registration Document Number 2014/7003590. [3]

2.2.4.2 Stability of Extracts

In order to store the sample solutions before final measurement, the stability of the analytes in extracts after initial extraction and in final solutions before measurement was tested. The sample extracts used for storage stability were fortification samples at 0.001 mg/kg (LOQ).

Data were obtained from the refrigerated (4°C) stored extract solutions and final solutions after 7 days. The concentrations of quizalofop-p-ethyl, quizalofop-p, and 3-OH-quizalofop-acid were measured against freshly prepared standards within one analytical queue.

Quantitation of the analytes was done for both mass transitions for quizalofop-p-ethyl and quizalofop-p, and for both chromatographic conditions for 3-OH-quizalofop-acid. The recoveries of procedural fortifications were used to prove the stability of the analytes in the water extracts. Extract stability data for each matrix and analyte/mass transition are presented in Appendix 1, Tables 1.1 - 1.12.

2.3 Materials and Methods

2.3.1 Equipment

Equipment	Size, Description	Manufacturer/ Supplier	Catalog Number
Balance, top loading	Model PE 3600	Mettler	---
Balance, analytical	Model MT 5	Mettler	---
Beakers	100 mL	Various Suppliers	---
Bottle, amber glass	Qorpak, 2 oz and 4 oz with Teflon®-lined screw cap	Various Suppliers	---
Culture tubes	Glass, disposable, 16x100 mm	Various Suppliers	---
Cylinders, graduated	Various sizes	Various Suppliers	---
Flask, Erlenmeyer, 24/40	1000 mL	Various Suppliers	---
Mechanical Pipette	1000 µL 250 µL 50 µL	Various Suppliers	---
Pipettes, volumetric	2.5 mL 5 mL 20 mL 25 mL	Various Suppliers	---
Vortex	Model Vortex Genie 2	VWR	---
Liquid Chromatograph	UPLC Acquity system	Waters	---
MS/MS Detector	API 5000 system	AB Sciex	---
HPLC Column	Acquity UPLC® BEH C18, 1.7 µm, 2.1 x 50 mm	Waters	186002885
HPLC Column	Acquity UPLC® BEH Phenyl, 1.7 µm, 2.1x100 mm	Waters	186006067
HPLC Column	Acquity UPLC® HSS T3, 1.8 µm, 2.1x100 mm	Waters	176001132

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.

2.3.2 Reagents

2.3.2.1 Chemicals

Chemical	Grade	Manufacturer/ Supplier	Catalog Number
Acetonitrile	HPLC Grade	Fisher Scientific, *PHARMCO AAPER	30721 300000HPLC
Water	HPLC Grade	BDH ARISTAR PLUS *Millipore Milli-Q Gradient A10	87003-652 ---
Methanol	HPLC Grade	EMD *J.T. Baker	MX0475-P1 9093-03
Formic Acid	98% GR ACS	EMD *Sigma	FX-0440-7 27001
Ammonium formate	ACS	*Fluka	09735

*Chemicals used in the validation at PASC

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

2.3.2.2 Solutions and Solvent Mixtures

Description	Code	Composition
Final volume solvent (quizalofop-p-ethyl and quizalofop-p)	S1	Water:Acetonitrile (10:90, v/v) Add 100 mL of water and 900 mL of acetonitrile into a 1-L Erlenmeyer flask and mix well to ensure a complete homogeneous solution.
Final volume solvent (3-OH-quizalofop-acid)	S2	Water:Acetonitrile (45:55, v/v) Add 450 mL of water and 550 mL of acetonitrile into a 1-L Erlenmeyer flask and mix well to ensure a complete homogeneous solution.
LC mobile phase A	LC1	0.1% Formic Acid in Water (by volume) Add 999 mL of water and 1 mL of concentrated formic acid into a 1-L Erlenmeyer flask and mix well to ensure a complete homogeneous solution.
LC mobile phase B	LC2	0.1% Formic Acid in Acetonitrile (by volume) Add 999 mL of acetonitrile and 1 mL of concentrated formic acid into a 1-L Erlenmeyer flask and mix well to ensure a complete homogeneous solution.
LC mobile phase C	LC3	4 mM Ammonium Formate with 0.1% Formic Acid in Water (by volume) Add 0.25 g of ammonium formate to 500 mL of water in a 1-L graduated cylinder. Once fully dissolved, add 1 mL of concentrated formic acid and bring the volume to 1 L with water.
LC mobile phase D	LC4	4 mM Ammonium Formate with 0.1% Formic Acid in Methanol (by volume) Add 0.25 g of ammonium formate to 500 mL of methanol in a 1-L graduated cylinder. Once fully dissolved, add 1 mL of concentrated formic acid and bring the volume to 1 L with methanol.

Note: If necessary, the solutions may also be prepared in different volumes as long as the proportions are not modified.
Only LC3 and LC4 were used in this validation.

2.4 Standard Solutions

Amber bottles with Teflon-lined screw caps were used as storage containers for all standard solutions.

2.4.1 Stock Solutions

Stock standard solutions containing 1 mg/mL of Quizalofop-p-ethyl (BAS 9152 H) and its metabolites individually (Quizalofop-p and 3-OH-Quizalofop-acid) were prepared by weighing an appropriate amount of reference item or standard into a volumetric flask and adding the required volume.

For example, 5 mg of Quizalofop-p-ethyl was weighed into a 5 mL volumetric flask. The reference substance was dissolved and diluted to mark with acetonitrile. A completely homogeneous solution was ensured with a combination of sonication and vortexing.

The stock solutions for Quizalofop-p-ethyl and Quizalofop-p were made in acetonitrile and the stock solution for 3-OH-Quizalfop-acid was made in methanol.

A correction for purity was not performed in this study. A correction for purity is done if the purity is ≤ 95%. If the purity is > 95 %, correction is optional.

Note: Standard calibration and fortification solutions should initially be confirmed to show correct preparation of the solutions. This can be achieved by 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.

2.4.2 Fortification Solutions

Samples were analyzed for quizalofop-p-ethyl and quizalofop-p in a different injection than for 3-OH-quizalofop-acid. Therefore, two different sets of fortification solutions were required for analysis.

Fortification Solutions for Quizalofop-p-ethyl and Quizalofop-p
Fortification solutions containing both quizalofop-p-ethyl and quizalofop-p were prepared at concentrations of 10, 1, and 0.1 µg/mL using the example dilution scheme in the table below. Solutions were diluted volumetrically with acetonitrile and were vortexed to ensure a completely homogeneous solution.

Preparation of Mixed Fortification Standard Solutions (quizalofop-p-ethyl and quizalofop-p)

Take Solution Concentration of Each Analyte (µg/mL)	Volume (mL)	Dilute with Acetonitrile to a Final Volume (mL)	Final Concentration of Each Analyte (µg/mL)
1000	0.25	25	10.0
10.0	2.5	25	1.0
1.0	2.5	25	0.10

Fortification Solutions for 3-OH-quizalofop-acid
Fortification solutions containing 3-OH-quizalofop-acid were prepared at concentrations of 10, 1.0, and 0.1 µg/mL using the example dilution scheme in the table below. Solutions were diluted volumetrically with methanol and were vortexed to ensure a completely homogeneous solution.

Preparation of Fortification Standard Solutions (3-OH-quizalofop-acid)

Take Solution Concentration (µg/mL)	Volume (mL)	Dilute with Methanol to a Final Volume (mL)	Final Concentration (µg/mL)
1000	0.25	25	10.0
10.0	2.5	25	1.0
1.0	2.5	25	0.10

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.
If necessary, the fortification and calibration solution may be prepared separately as long as the same solutions and the proportions are not modified.
Do not prepare quizalofop-p-ethyl in methanol.

2.4.3 Calibration Standard Solutions

Calibration standards were prepared for LC-MS/MS analysis, in flasks, by using the solutions that were prepared in Section 2.4.2 (Fortification Solutions). Calibration solutions were diluted volumetrically with appropriate solvents. The solutions were vortexed to ensure a completely homogeneous solution.

The calibration solutions for quizalofop-p-ethyl and quizalofop-p were made and diluted with final volume solvent S1, while the calibration solutions for 3-OH-quizalofop-acid were made and diluted with final volume solvent S2. Solutions were prepared following the example dilution schemes in the tables below.

Preparation of Mixed Standard Solutions for Calibration (quizalofop-p-ethyl and quizalofop-p)

Take Solution Concentration of Each Analyte (ng/mL)	Volume (mL)	Dilute with S1 to a Final Volume (mL)	Final Concentration of Each Analyte (ng/mL)
100	0.25	25.0	1.0
1.0	10.0	50.0	0.20
0.20	25.0	50.0	0.10
0.10	25.0	50.0	0.050
0.050	20.0	50.0	0.020
0.020	25.0	50.0	0.010

Preparation of Standard Solutions for Calibration (3-OH-quizalofop-acid)

Take Solution Concentration (ng/mL)	Volume (mL)	Dilute with S2 to a Final Volume (mL)	Final Concentration (ng/mL)
100	0.25	25.0	1.0
1.0	10.0	50.0	0.20
0.20	25.0	50.0	0.10
0.10	25.0	50.0	0.050
0.050	20.0	50.0	0.020
0.020	25.0	50.0	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.

2.4.4 Matrix-Matched Calibration Standard Solutions

Matrix-matched standard solutions were prepared in both the drinking (tap) water matrix and in the surface water (RCN R130034) matrix in order to study the matrix effect on analyte response. Matrix-matched standards were prepared for standards at 0.5×LOQ, LOQ, and 2.5×LOQ in the following manner:

1. Fortification solutions, prepared in Section 2.4.2 above, were used to prepare the precursor matrix-matched solutions by following the example dilution schemes in the tables below.

Preparation of Mixed Matrix-Matched Standard Precursor Solutions (quizalofop-p-ethyl and quizalofop-p)

Take Solution Concentration of Each Analyte (ng/mL)	Volume (mL)	Dilute with S1 to a Final Volume (mL)	Final Concentration of Each Analyte (ng/mL)
100	0.625	50.0	1.25
1.0	25.0	50.0	0.50
0.50	25.0	50.0	0.25

Preparation of Matrix-Matched Standard Precursor Solutions (3-OH-quizalofop-acid)

Take Solution Concentration (ng/mL)	Volume (mL)	Dilute with S2 to a Final Volume (mL)	Final Concentration (ng/mL)
100	0.625	50.0	1.25
1.0	25.0	50.0	0.50
0.50	25.0	50.0	0.25

2. Three control solutions were prepared for each water matrix and were brought up to final solutions, as outlined in Section 3 (Analytical Procedure).
3. All three extracts for each matrix were combined and vortexed to homogenize.
4. The matrix-matched standards were prepared according to the example dilution schemes in the tables below, using the precursor standards from Step 1 and the combined control extracts from Step 3 to make the solutions.

Preparation of Mixed Matrix-Matched Standard Solutions (quizalofop-p-ethyl and quizalofop-p)

Final Concentration of Each Analyte in Matrix (ng/mL)	Volume of Combined Control Extract Taken (mL)	Precursor Standard Concentration of Each Analyte in S1 (ng/mL)	Volume of Precursor Standard (mL)
0.125 (2.5×LOQ)	0.9	1.25	0.1
0.05 (LOQ)	0.9	0.5	0.1
0.025 (0.5×LOQ)	0.9	0.25	0.1

Preparation of Matrix-Matched Standard Solutions (3-OH-quizalofop-acid)

Final Concentration in Matrix (ng/mL)	Volume of Combined Control Extract Taken (mL)	Precursor Standard Concentration in S2 (ng/mL)	Volume of Precursor Standard (mL)
0.125 (2.5×LOQ)	0.9	1.25	0.1
0.05 (LOQ)	0.9	0.5	0.1
0.025 (0.5×LOQ)	0.9	0.25	0.1

3. Analytical Procedure

3.1 Sample Preparation

Samples were sufficiently homogenized beforehand, in order to assure that the aliquot taken for residue analysis was representative of the whole sample.

3.2 Sample Storage

Water samples were kept frozen until analysis. Freezer storage stability of quizalofop-p-ethyl and its metabolites in water will be determined in BASF study 437859.

3.3 Weighing and Fortification

For all control, treated, untreated, and fortified samples, 10 ± 0.1 g of water was weighed into a glass culture tube.

For fortified recovery samples, fortification solutions were added to the matrix according to the table below. When analyzing all three compounds, two different fortification solutions were added. The fortification solution for quizalofop-p-ethyl and quizalofop-p was added to the matrix, followed by the fortification solution for 3-OH-quizalofop-acid.

Fortification of Recovery Samples

Sample Type	Sample Weight (g)	Analytes	Concentration of Spiking Solution [µg/mL]	Volume of Spiking Solution [mL]	Level of Fortification [µg/g]
Control	10	-	-	-	0.00
Fortification (LOQ*)	10	Quizalofop-p-ethyl Quizalofop-p	0.1	0.1	0.001
		3-OH-quizalofop-acid	0.1	0.1	0.001
Fortification (10×LOQ)	10	Quizalofop-p-ethyl Quizalofop-p	1.0	0.1	0.01
		3-OH-quizalofop-acid	1.0	0.1	0.01

*Limit of quantitation (LOQ)

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

3.4 Extraction of Sample Material

Added 0.1 mL of acetonitrile and 0.1 mL of methanol to all samples (control, untreated and treated) except the fortified samples. These additional solutions were added to ensure that all samples would have the same solution proportions as the fortified samples. The samples were vortexed and mixed thoroughly to ensure a homogeneous solution. For the preparation of samples for measurement of quizalofop-p-ethyl and quizalofop-p, the method was continued with Section 3.6.1. For the preparation of samples for measurement of 3-OH-quizalofop-acid, the method was continued with Section 3.6.2.

3.5 Sample Clean-up

No sample clean-up was necessary.

3.6 Preparation for Measurement

3.6.1 Preparation for Measurement of Quizalofop-p-ethyl and Quizalofop-p

For all samples, exactly 0.051 mL of the extracted sample was transferred to a glass culture tube. Exactly 0.449 mL of acetonitrile and 0.5 mL of S1 (code from section 2.3.2.2) were added to the tube. Each sample was vortexed to mix well, and an aliquot of the sample was transferred to an HPLC vial for LC-MS/MS analysis.

In case of residues higher than the LOQ level, the samples were diluted with S1 as needed to fit into the calibration curve.

The LC-MS/MS conditions for the analysis of quizalofop-p-ethyl and quizalofop-p are presented in Section 4.1.1.

Note: The method may be interrupted at this point.

3.6.2 Preparation for Measurement of 3-OH-quizalofop-acid

For all samples, exactly 0.051 mL of the extracted sample was transferred to a glass culture tube. Exactly 0.949 mL of S2 (code from section 2.3.2.2) was added to the tube. Each sample was vortexed to mix well, and an aliquot of the sample was transferred to an HPLC vial for LC-MS/MS analysis.

In case of residues higher than the LOQ level, the samples were diluted with S2 as needed to fit into the calibration curve.

The primary and confirmatory LC-MS/MS conditions for the analysis of 3-OH-quizalofop-acid are presented in Sections 4.1.2 and 4.1.3.

Note: The method may be interrupted at this point.

3.7 Influence of Matrix Effects on Analysis

In order to test the influence of the matrix effects on the analysis, the response of each analyte in the matrix was compared to the response of solvent-based standards. Therefore, calibration standard solutions (prepared in S1 or S2 solvent) were compared against their respective calibration standards prepared in blank matrix extracts (matrix-matched standards).

The matrix-matched standards were made using the procedure as outlined in Section 2.4.4 of this report. Comparable solvent-based standards were prepared using the same procedure as outlined in that section. Both of the primary and secondary mass transitions for quizalofop-p-ethyl and quizalofop-p were evaluated. Both of the chromatographic conditions for 3-OH-quizalofop-acid were evaluated. The resultant peak areas were compared between the matrix-matched standards and the solvent-based standard using the following equation:

$$\text{Matrix Effect (\% Area)} = \frac{\text{Avg Area}_{(\text{solvent standards})} - \text{Avg Area}_{(\text{matrix-matched standards})}}{\text{Avg Area}_{(\text{solvent standards})}} \times 100$$

Reagent blanks or blanks were injected as necessary. Matrix-matched standards and solvent-based standards were interspersed throughout the sequence. Three calibration levels were injected (0.5×LOQ, LOQ, and 2.5×LOQ).

Summaries of the matrix-matched standard data are presented in Appendix 3, Tables 3.1 - 3.12, while more detailed raw data tables are presented in Appendix 6.

If significant matrix interference necessitates the use of matrix matched standards, testing of the individual standards to construct the calibration curve is required.

4. Instrumental Analysis

4.1 Instrumentation and Conditions

4.1.1 Instrumentation and Conditions for Quizalofop-p-ethyl and Quizalofop-p

	Parameter		
Chromatographic System	Waters UPLC Acquity system		
Analytical-column	Acquity UPLC BEH C18 1.7 µm, 2.1x50 mm		
Column Temperature	50°C		
Injection Volume	20 µL		
Mobile Phase A	4 mM Ammonium Formate with 0.1% Formic Acid in Water		
Mobile Phase B	4 mM Ammonium Formate with 0.1% Formic Acid in Methanol		
Flow Rate	500 µL/min		
Gradient (including wash and equilibration)	Time (min)	% Phase A	% Phase B
	0.0	95	5
	0.5	95	5
	1.0	50	50
	3.0	5	95
	3.5	5	95
	3.6	95	5
	4.0	95	5
Detection System	AB Sciex 5000 Mass Spectrometer		
Ionization	Electrospray (ESI)		
Ionization Temperature	500°C		
Analyte	**Transitions (*m/z*)**	**Polarity**	**Expected Retention Time**
Quizalofop-p-ethyl	373→299* 375→301	positive	approx. 2.86 min
Quizalofop-p	345→299* 345→100	positive	approx. 2.46 min

*Proposed as quantitation transition. Either of these transitions could be used for quantitation in case interference was observed at the same retention time.

4.1.2 Instrumentation and Conditions for 3-OH-quizalofop-acid

Method A: Used as the primary chromatographic method

	Parameter		
Chromatographic System	Waters UPLC Acquity system		
Analytical-column	Acquity UPLC BEH Phenyl 1.7 µm, 2.1x100 mm		
Column Temperature	50°C		
Injection Volume	30 µL		
Mobile Phase A	4 mM Ammonium Formate with 0.1% Formic Acid in Water		
Mobile Phase B	4 mM Ammonium Formate with 0.1% Formic Acid in Methanol		
Flow Rate	500 µL/min		
Gradient (including wash and equilibration)	Time (min)	%Phase A	% Phase B
	0.0	95	5
	0.5	95	5
	3.0	5	95
	3.5	5	95
	3.6	95	5
	4.0	95	5
Detection System	AB Sciex 5000 Mass Spectrometer		
Ionization	Electrospray (ESI)		
Ionization Temperature	550°C		
Analyte	**Transition (*m/z*)**	**Polarity**	**Expected Retention Time**
3-OH-quizalofop-acid	359→166	negative	approx. 3.02 min

4.1.3 Instrumentation and Conditions for 3-OH-quizalofop-acid

Method B: Used as the secondary chromatographic method

	Parameter		
Chromatographic System	Waters UPLC Acquity system		
Analytical-column	Acquity UPLC HSS T3 1.8 µm, 2.1x100 mm		
Column Temperature	50°C		
Injection Volume	30 µL		
Mobile Phase A	4 mM Ammonium Formate with 0.1% Formic Acid in Water		
Mobile Phase B	4 mM Ammonium Formate with 0.1% Formic Acid in Methanol		
Flow Rate	600 µL/min		
Gradient (including wash and equilibration)	Time (min)	% Phase A	% Phase B
	0.0	95	5
	0.5	95	5
	3.0	5	95
	3.5	5	95
	3.6	95	5
	4.0	95	5
Detection System	AB Sciex 5000 Mass Spectrometer		
Ionization	Electrospray (ESI)		
Ionization Temperature	550°C		
Analyte	**Transition (*m/z*)**	**Polarity**	**Expected Retention Time**
3-OH-quizalofop-acid	359→166	negative	approx. 3.02 min

Note: Instruments with similar specifications may be substituted for 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 in 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 dependent of the equipment used and are therefore not listed. Those parameters may need to be adapted for the instrument used.

4.2 Calibration Procedures

Calculation of results was based on peak area measurements using a calibration curve. The calibration curve was obtained by direct injection of quizalofop-p-ethyl and its metabolites, quizalofop-p and 3-OH-quizalofop-acid, at five known concentration levels across the range of 0.010 ng/mL to 0.20 ng/mL. In all injection runs, the same injection volume was used for all samples and standards.

The residues of quizalofop-p-ethyl, quizalofop-p, and 3-OH-quizalofop-acid were evaluated by linear regression with 1/x weighting.

4.3 Rounding Numbers

Numerical values in this report are frequently rounded to a smaller degree of precision (number of digits) than were used in the actual calculation to increase readability and to indicate the approximate precision of the reported results. Minor differences in the results obtained with such "rounded" values in comparison to those obtained with higher precision values are well within the limits of the experimental accuracy and therefore of no practical concern.

4.4 Statistical Analysis of Data

Mean recoveries were calculated on the data generated where appropriate. Full computer/calculator precision was used in any intermediate calculations, and only the final value was rounded. Slight differences may be noted in hand calculations versus calculations in the individual data tables presented in this report due to rounding and significant figures presented in calibration curve data provided by the mass spectroscopy laboratory. Simple descriptive statistics were performed on the data (average and/or standard deviation), as considered appropriate. Statistical treatment of the data included simple descriptive statistics, such as determinations of averages 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.

4.5 Calculation of Residues and Recoveries

For the procedural recoveries, an approximate sample weight of 10 g was used in calculating the final concentration of residues (mg/kg).

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

Note: The concentration in ng/mL is automatically calculated by the analyst software using the formula above.

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

Where, 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 = Aliquot Factor
1000 = Factor remaining after unit conversions

c) $\text{Recovery (\%)} = \frac{(\text{Residue in Fortified Sample})-(\text{Residue in Control Sample})}{\text{Amount fortified}} \times 100$

Example: Quizalofop-p-ethyl (BAS 9152 H) in tap water fortified at 0.001 mg/kg, quantitated at the primary mass transition (*m/z* 373→299) (Sample ID RTP-tap water LOQ-1 in batch QP_QPE_water_0Day_20141003.dab)

a) Calibration curve: $y = 8.24e^{+005}x + 420$

$$\text{Concentration (ng/mL)} = \frac{39600-420}{8.24e^{+005}} = 0.0475 \text{ ng/mL}$$

b) $$\text{Residue (mg/kg)} = \frac{(10.2 \text{ mL}) \times (0.0475 \text{ ng/mL})}{(10.0 \text{ g}) \times (0.051 \text{ mL}) \times 1000} = 0.000950 \text{ mg/kg}$$

c) $$\text{Recovery (\%)} = \frac{0.000950 \text{ mg/kg} - 0.000000 \text{ mg/kg}}{0.001 \text{ mg/kg}} \times 100 = 95.0\%$$

Note:	Slight rounding differences may be noted when using a hand calculator. Full computer/calculator precision was used in any intermediate calculations. Only the final value was rounded.

5.5 Summary of Method

Type of Method: LC-MS/MS

Test Systems:

1. Drinking (Tap) Water (BASF Study 710640)
2. Surface Water (BASF Study 437860, RCN R130034)

Analytes and Selected Mass Transitions (*m/z*):

Quizalofop-p-ethyl	373→299*
	375→301
Quizalofop-p	345→299*
	345→100
3-OH-quizalofop-acid	359→166**

*Used as the primary transition for quantitation
**The same mass transition was used with a primary and secondary chromatographic method

Analytical Procedure: The analytes were extracted from the sample using acetonitrile and methanol.

Confirmatory Technique: Due to the high selectivity and specificity of LC-MS/MS, an additional confirmatory technique is not necessary.

Method of Quantitation: The quantitation is based on the monitoring of two mass transitions for quizalofop-p-ethyl and quizalofop-p, and one mass transition and two chromatographic methods for 3-OH-quizalofop-acid. Recovery data was reported for each mass transition and chromatographic method considered, as shown in Appendix 6.

Limit of Detection (LOD): 0.0002 mg/kg for all three analytes

Limit of Quantitation (LOQ): 0.001 mg/kg (lowest fortification level for all three analytes) corresponding to a concentration of 0.05 ng/mL in the final extract

Levels of Fortification: 0.001 mg/kg (LOQ, 0.05 ng/mL in the final extract)
0.01 mg/kg (10×LOQ, 0.5 ng/mL in the final extract)

Time Required: A set of 13 samples requires about 24 hours of work (LC-MS/MS determination and calculation of the results included)

8. Conclusion and Method Capabilities

It was demonstrated that the method D1304/02 fulfils the requirements with regard to specificity, repeatability, limit of quantitation, and recovery, and is therefore applicable to correctly determine residues of the herbicide quizalofop-p-ethyl (BAS 9152) and its metabolites quizalofop-p and 3-OH-quizalofop-acid in drinking (tap) and surface water.

The method recoveries and relative standard deviations were within the acceptance range (70 - 120% and < 20%, respectively). Recovery results are presented in Tables 1 - 3.

Representative chromatograms of reference standard solutions, reagent blanks, control samples, and samples fortified at LOQ and 10×LOQ are presented in Appendix 4.

Correlation coefficients (r) for all of the standard curves were ≥ 0.99. Representative calibration curves are presented in Appendix 2.

Detailed analytical tables containing all of the information of the analyses in this validation study are presented in Appendix 6.

9. Potential Problems

1. The glassware used for the method should be thoroughly rinsed with acetonitrile to prevent contamination.
2. Certain LC vials with silicon polymer may introduce interference peaks during LC-MS/MS analysis of Quizalofop-p-ethyl and its metabolites.
3. If matrix suppression or enhancement is observed, matrix-matched standards should be used.

10. Recommendations from Independent Laboratory Method Validation (ILV)

The independent laboratory validation of the BASF method (D1304/02) was successfully completed in surface water and drinking water. Quizalofop-p-ethyl was successfully quantitated in surface and drinking water in the first trial. Quizalofop-p was successfully quantitated at the LOQ fortification level in surface and drinking water in the first trial, and successfully quantitated at the 10×LOQ fortification level in the second trial for both matrices. 3-OH-quizalofop-acid was successfully quantitated at the LOQ fortification level in surface water in the first trial, and at the 10×LOQ fortification level in the second trial (both primary and confirmatory chromatographic methods). 3-OH-quizalofop-acid was successfully quantitated at the LOQ fortification level in drinking water (primary method only) in the first trial, and at the 10×LOQ fortification level and LOQ confirmatory method in the second trial. Some minor method modifications and clarifications were required for successful completion of the method validation. Two method modifications/findings were noted upon completion of the ILV:

1. The HPLC gradients identified in Section 4.2 included 0.4 minutes of equilibration time at the end of each gradient. During the ILV it was found that additional equilibration time at the end of the method improved calibration linearity for all monitored transitions. It is suggested from the ILV that language be added to the method to allow for additional

equilibration time to compensate for available analytical equipment. Quizalofop-p was measured with 3.0 additional minutes of equilibration time at the end, and 3-OH-quizalofop-acid was measured with 2.0 additional minutes of equilibration time added to the end of each run.

2. The chromatographic system and detection system identified in the reference method were different from the chromatograph system and detection system used during the ILV. The reference method was completed using a Waters UPLC Acquity System as the chromatographic system, but at the ILV facility an Agilent 1290 chromatographic system was used. Further, the reference method was completed using an AB Sciex 5500 Mass Spectrometer detection system, but at the ILV facility an AB Sciex 6500 Q-trap Mass Spectrometer detection system was used. The summary of the instrument parameters used in ILV are shown below :

Instrumentation and Conditions:

Method A : Determination of Quizalofop-p-ethyl and Quizalofop-p

	Parameter		
Chromatographic System	Agilent 1290		
Analytical-column	Acquity BEH C18; 1.7 µm, 2.1 X 50 mm		
Column Temperature	50°C		
Injection Volume	Typically, 20 µL		
Mobile Phase A	4 mM Ammonium Formate with 0.1% Formic Acid in Water		
Mobile Phase B	4 mM Ammonium Formate with 0.1% Formic Acid in Methanol		
Flow Rate	600 µL/min		
Gradient	Time (min)	% Phase A	% Phase B
	0.0	95	5
	0.5	95	5
	1.0	50	50
	3.0	5	95
	3.5	5	95
	3.6	95	5
	7.0	95	5
Detection System	AB Sciex Instruments 6500 Q-Trap		
Software Version:	Analyst 1.6		
Analyte	**Transition (*m/z*)**	**Polarity**	**Expected Retention Time**
Quizalofop-p-ethyl	373.0→299.0* 375.0→300.9	Positive	Approx. 3.3 min
Quizalofop-p	345.0→299.0* 345.0→100.0	Positive	Approx. 2.9 min

*This is the primary ion, which is used as default; any of these transitions could be used for quantitation in case interference is observed at the same retention time

Method B : Determination of 3-OH-Quizalofop-acid (Primary Chromatographic Method)

	Parameter		
Chromatographic System	Agilent 1290		
Analytical-column	Acquity BEH Phenyl; 1.7 µm, 2.1 X 100 mm		
Column Temperature	50°C		
Injection Volume	Typically, 20 µL		
Mobile Phase A	4 mM Ammonium Formate with 0.1% Formic Acid in Water		
Mobile Phase B	4 mM Ammonium Formate with 0.1% Formic Acid in Methanol		
Flow Rate	600 µL/min		
Gradient	Time (min)	% Phase A	% Phase B
	0.0	95	5
	0.5	95	5
	3.0	5	95
	3.5	5	95
	3.6	95	5
	6.0	95	5
Detection System	AB Sciex Instruments 6500 Q-Trap		
Software Version:	Analyst 1.6		
Analyte	**Transition (*m/z*)**	**Polarity**	**Expected Retention Time**
3-OH-quizalofop-acid	359.0→166.0	Negative	Approx. 3.15 min

Method C : Determination of 3-OH-Quizalofop-acid (Secondary Chromatographic Method)

	Parameter		
Chromatographic System	Agilent 1290		
Analytical-column	Acquity HSS T3; 1.8 µm, 2.1 X 100 mm		
Column Temperature	50°C		
Injection Volume	Typically, 20 µL		
Mobile Phase A	4 mM Ammonium Formate with 0.1% Formic Acid in Water		
Mobile Phase B	4 mM Ammonium Formate with 0.1% Formic Acid in Methanol		
Flow Rate	600 µL/min		
Gradient	Time (min)	% Phase A	% Phase B
	0.0	95	5
	0.5	95	5
	3.0	5	95
	3.5	5	95
	3.6	95	5
	6.0	95	5
Detection System	AB Sciex Instruments 6500 Q-Trap		
Software Version:	Analyst 1.6		
Analyte	**Transition (*m/z*)**	**Polarity**	**Expected Retention Time**
3-OH-quizalofop-acid	359.0→166.0	Negative	Approx. 3.15 min

11. Protocol Changes

No changes or adjustments were made to the protocol.

12. Data Retention and Archiving

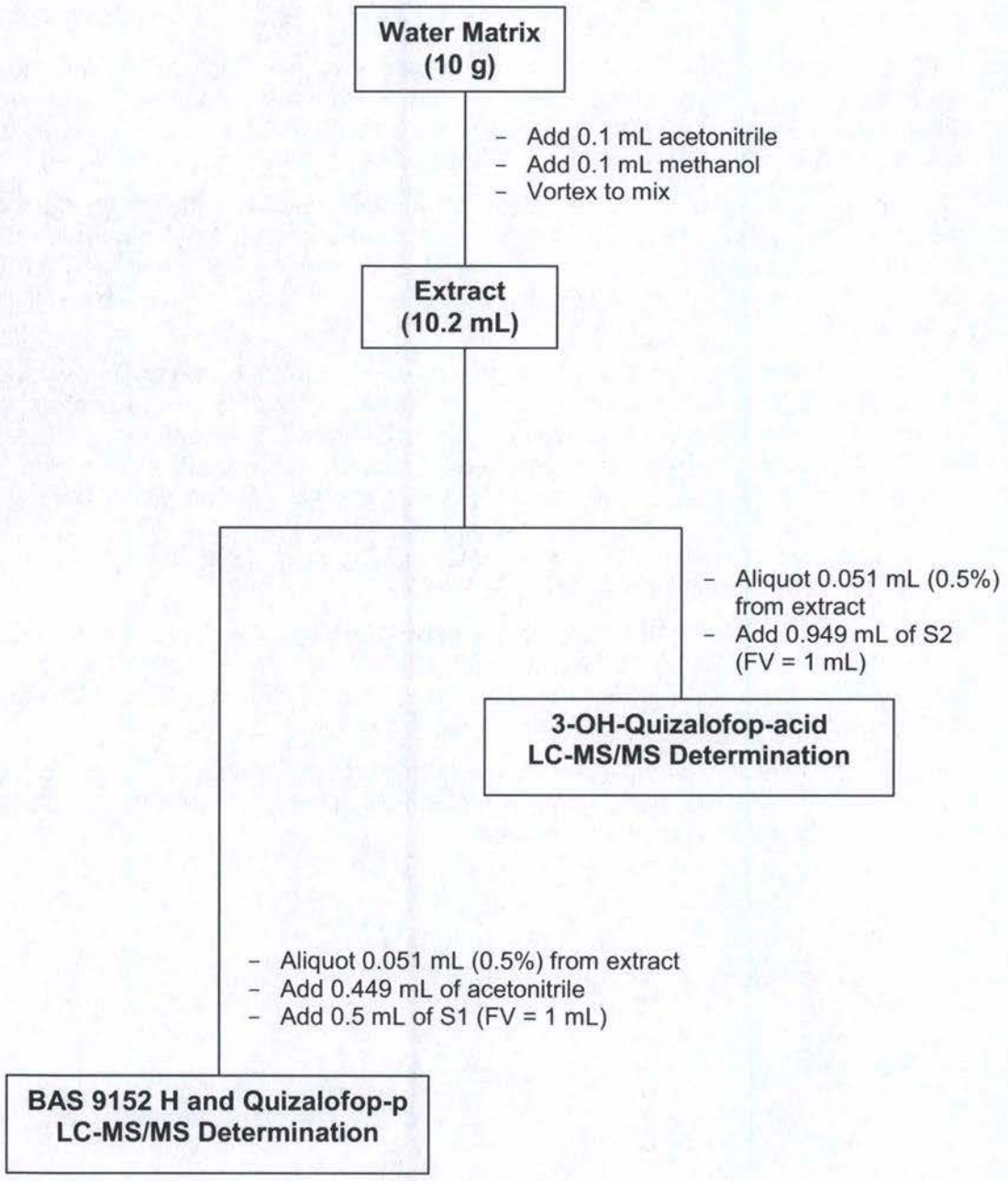
The raw data, analytical phase report, and all study related records pertaining to the analytical phase of the application verification samples will be archived at:

BASF Crop Protection
26 Davis Drive
Research Triangle Park, NC 27709

References

[1] Shen, Xiaorong. *Validation for BASF Analytical Method D1303/02: "Analytical Method for the Determination of Residues of Quizalofop-p-ethyl (BAS 9152 H) and its two Metabolites Quizalofop-p and 3-OH-Quizalofop-acid in Soil by LC-MS/MS."* BASF Registration Document Number 2014/7003590, September 2014.

Figure 1: Analysis of Quizalofop-p-ethyl (BAS 9152 H) and its Metabolites, Quizalofop-p and 3-OH-quizalofop-acid in Drinking (Tap) and Surface Water



S1 = Water-acetonitrile (10:90, v/v)
S2 = Water-acetonitrile (45:55, v/v)