Independent Lab Validation of BASF Analytical Method D1304/02: "Analytical Method for the Determination of Residues of Quizalofop-p-ethyl and its Metabolites Quizalofop-p and 3-OH-Quizalofop-acid in Water by LC-MS/MS"

ABSTRACT

The purpose of the study was to demonstrate that BASF Analytical Method D1304/02 "Analytical Method for the Determination of Residues of Quizalofop-p-ethyl and its Metabolites Quizalofop-p and 3-OH-Quizalofop-acid in Water by LC-MS/MS" could be performed successfully at an outside facility with no prior experience with the method. This method was originally developed in BASF Corporation, RTP, NC and was validated at Primera Analytical Solutions Corporation (PASC) in Princeton, New Jersey (Reference 1).

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 (0.001 and 0.01 mg/kg) for all analytes in water. For each fortification level, five replicates were analyzed. Additionally, at least two replicates of unfortified samples were examined.

The final determination of quizalofop-p-ethyl was performed by LC-MS/MS. For quizalofop-p-ethyl the transition at *m/z* 373.0→299.0 was monitored in positive mode for primary quantification; the transition at *m/z* 375.0→300.9 was monitored in positive mode for confirmation. For quizalofop-p, the transition *m/z* 345.0→299.0 was monitored in positive mode for primary quantification; the transition at *m/z* 345.0→100.0 was monitored in positive mode for confirmation. 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.

Limit of Quantitation (LOQ) and Limit of Detection (LOD): The limit of quantification (LOQ) was defined as the lowest fortification level successfully tested. The LOQ is 0.001 mg/kg for all analytes in all matrices. The limit of detection in water was estimated at 20% of the LOQ, equivalent to 0.0002 mg/kg.

Selectivity: The method determines residues of quizalofop-p-ethyl and its metabolites in water matrices. No interfering peaks were found at the retention times for each analyte. No matrix suppression or enhancement was found for quizalofop-p-ethyl or for its metabolites.

Linearity: For both of the mass transitions of quizalofop-p-ethyl and quizalofop-p in the mixed standard calibration solutions, good linearity (r^2 > 0.98) was observed in the range of 0.010 ng/mL to 0.20 ng/mL. Good linearity (r^2 > 0.99) was also observed for quizalofop-p-ethyl and quizalofop-p in the range of 0.010 ng/mL to 1.0 ng/mL. For the single mass transition under both chromatographic conditions for 3-OH-quizalofop-acid in the single-standard calibration solutions, good linearity (r^2 > 0.99) was observed in the range of 0.010 ng/mL to 0.20 ng/mL. Good linearity (r^2 > 0.99) was also observed for 3-OH-quizalofop-acid in the range of 0.010 ng/mL to 1.0 ng/mL.

1. Introduction

1.1 Scope of the Method

BASF Method D1304/02 was developed to determine the residues of BAS 9152 H in water using LC-MS/MS at BASF Crop Protection in Research Triangle Park, North Carolina. This method was validated at Primera Analytical Solutions Corporation (PASC) in Princeton, New Jersey (Reference 1) and was independently validated at EPL Bio Analytical Services (EPL).

The independent lab validation was conducted using two fortification levels (0.001 and 0.01 mg/kg) for water. 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

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

To demonstrate the specificity of the analytical method, one additional mass transition was monitored simultaneous to the primary detection transition for analysis of quizalofop-p-ethyl and quizalofop-p. 3-OH-Quizalofop-acid used a different column for confirmatory detection. 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.

2. Materials and Methods

2.1 Test Systems

The test systems considered in this study were surface water (BASF Study 437860; RCN R130034) and tap water (BASF reference number 22014).

The control samples were provided by BASF. The water samples were received on November 4, 2014. Upon arrival at the laboratory, the samples were opened, inspected, and checked against enclosed shipping forms. The test systems were received frozen and were stored under frozen conditions at all times, unless necessary for laboratory analysis. The test systems were characterized at AGVISE Laboratories (604 Highway 15 West, Northwood, ND 58267). The characterization for the water samples used is provided in the respective attached certificates of analysis (Appendix J).

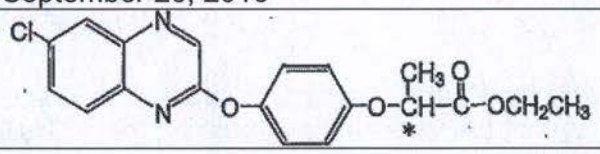
2.2 Test and Reference Substances

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

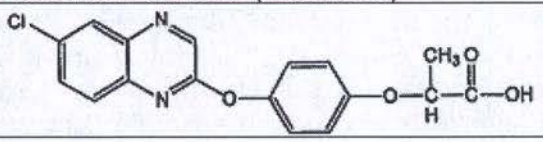
characterization information for each of these compounds available at BASF, Research Triangle Park, North Carolina.

The quizalofop-p-ethyl (lot number 302D-S110926), quizalofop-p (lot number 302D-ACID-S050325), and 3-OH-quizalofop-acid (lot number 3-OH-302D-ACID-M941088) reference substances were provided by the sponsor and were received on November 4, 2014. The reference substances were stored in frozen conditions when not in use. The certificates of analysis are presented in Appendix G. Summaries of the reference substances are presented below.

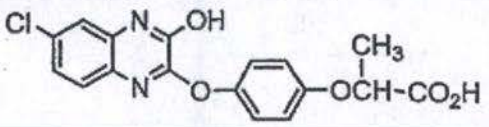
Quizalofop-p-ethyl

Common Name	Quizalofop-p-ethyl
BASF Reg. No.	N/A
CAS No.	100646-51-3
Molecular Formula	$C_{19}H_{17}ClN_2O_4$
Molecular Weight	372.8 g/mol
IUPAC Name	Ethyl(R)-2-[4-(6-chloroquinoxalin-2-yloxy)-phenoxy]propionate
Lot Number	302D-S110926
Purity	99.9 %
Storage	Dark and Cool (Below 10°C recommended)
Expiration Date	September 26, 2016
Chemical Structure	

Quizalofop-p

Common Name	Quizalofop-p
BASF Reg. No.	N/A
CAS No.	94051-08-8
Molecular Formula	$C_{17}H_{13}ClN_2O_4$
Molecular Weight	344.7 g/mol
IUPAC Name	(R)-2-[4-(6-chloroquinoxalin-2-yloxy)phenoxy]-propionic acid
Lot Number	302D-ACID-S050325
Purity	99.8%
Storage	Dark and Cool (Below 10°C recommended)
Expiration Date	December 5, 2017 (estimated)
Chemical Structure	

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 g/mol
IUPAC Name	(R)-2-[4-(6-chloro-3-hydroxyquinoxalin-2-yloxy)phenoxy]propionic acid
Lot Number	3-OH-302D-ACID-M941088
Purity	95.9%
Storage	Dark and Cool (Below 10°C recommended)
Expiration Date	March 3, 2017 (estimated)
Chemical Structure	

2.3 Materials

2.3.1 Equipment

The equipment used in this study was documented in the raw data. Maintenance files and applicable Standard Operating Procedures (SOPs) for the equipment are retained at the testing facility.

Class A volumetric glassware
Laboratory glassware (beakers, graduated cylinders, culture tubes, scintillation vials)
Volumetric pipettes, glass; various sizes
Analytical balance, capable of measuring to 0.01 mg
Air displacement pipette, various volumes with disposable tips
Vortex mixer
HPLC system: Agilent 1290
HPLC analytical column: Acquity BEH C18 (2.1 x 50 mm, 1.7 µm)
HPLC analytical column: Acquity BEH Phenyl (2.1 x 100 mm, 1.7 µm)
HPLC analytical column: Acquity HSS T3 (2.1 x 100 mm, 1.8 µm)
Mass spectrometer: AB Sciex 6500 Q-Trap
HPLC autosampler vials with screw-top, pre-slit caps

2.3.2 Reagents

Chemicals

Chemical	Manufacturer/ Supplier	Lot Number(s)
Acetonitrile	Fisher	144504, 146154, 145336, 146809, 135808
DI Water	In House	N/A
Methanol	EMD	54105, 54192
Formic Acid	Fisher	124119
Ammonium Formate	Fluka	BCBM5612V

Solutions and Solvent Mixtures

Description	Code	Composition
Final Volume Solvent (for determination of quizalofop-p-ethyl and quizalofop-p)	S1	Water-Acetonitrile, 10:90 (v/v)
Final Volume Solvent (for determination of 3-OH-quizalofop-acid)	S2	Water-Acetonitrile, 45:55 (v/v)
HP LC mobile phase C	LC3	4 mM Ammonium Formate with 0.1% Formic Acid in Water
HPLC mobile phase D	LC4	4 mM Ammonium Formate with 0.1% Formic Acid in Methanol

2.3.3 Standard Solutions

Stock Solutions

Individual 1 mg/mL stock solutions were prepared by weighing an appropriate amount of each analyte into a 10 mL flask and adding the required volume. The stock solutions for quizalofop-p-ethyl and quizalofop-p were made in acetonitrile and the stock solution for 3-OH-quizalofop-acid was made in methanol. Fortification and calibration standard solutions were prepared from one stock solution but in different dilution series.

Fortification Solutions

Quizalofop-p-ethyl and Quizalofop-p

Standard solutions (approximately 10, 1 and 0.1 µg/mL) for fortification were prepared by combining stock solutions of quizalofop-p-ethyl and quizalofop-p using the scheme in the table below and diluting volumetrically with acetonitrile. The use of sonication or vortexing was also employed for ensuring a complete homogeneous solution.

Example Preparation of mixed Fortification solutions (Quizalofop-p-ethyl and Quizalofop-p)

Take solution (µg/mL)	Volume (mL)	Dilute with acetonitrile to a final volume of (mL)	Concentration (µg/mL)
1000 (of both analytes)	0.25	25	10.0
10.0	2.5	25	1.0
1	2.5	25	0.10

3-OH-Quizalofop-acid

Standard solutions (10, 1 and 0.1 µg/mL) for fortification were prepared for 3-OH-quizalofop-acid using the scheme in the table below and diluting volumetrically with methanol. The use of sonication or vortexing was also employed for ensuring a complete homogeneous solution.

Example Preparation of mixed Fortification solutions (3-OH-Quizalofop-acid)

Take solution (µg/mL)	Volume (mL)	Dilute with methanol to a final volume of (mL)	Concentration (µg/mL)
997	0.25	25	10.0
10.0	2.5	25	1.0
1	2.5	25	0.10

Calibration Standard Solutions

Standard calibration solutions for LC-MS/MS analysis were prepared using the solutions, which were prepared in the previous section "Stock Solutions", and by diluting them with the appropriate solvents as exemplified in the tables above. Calibration standard solutions were prepared in a separate dilution series from fortification solutions. The use of sonication or vortexing was also used to ensure a complete homogeneous solution.

Example Preparation of standard solutions for calibration (Quizalofop-p-ethyl and Quizalofop-p)

Take solution (ng/mL)	Volume (µL)	Dilute with S1 final volume of (mL)	Concentration (ng/mL)
100	0.25	25	1.0
1.0	10	50	0.20
0.20	25	50	0.10
0.10	25	50	0.050
0.050	20	50	0.020
0.020	25	50	0.010

Example Preparation of standard solutions for calibration (3-OH-Quizalofop-acid)

Take solution (ng/mL)	Volume (µL)	Dilute with S2 final volume of (mL)	Concentration (ng/mL)
100	0.25	25	1.0
1.0	10	50	0.20
0.20	25	50	0.10
0.10	25	50	0.050
0.050	20	50	0.020

0.020	25	50	0.010

3. Analytical Procedure

For the analysis of quizalofop-p-ethyl, quizalofop-p, and 3-OH-quizalofop-acid a water sample (10 ± 0.1 g) was weighed into a glass culture tube. An aliquot was diluted with acetonitrile-water (90:10, v/v) and acetonitrile to determine the residues of quizalofop-p-ethyl and quizalofop-p using LC-MS/MS. An additional aliquot was diluted with acetonitrile-water (55:45, v/v) to determine the residues of 3-OH-quizalofop-acid using LC-MS/MS.

3.1 Weighing and Fortification

For control samples, 10 g of water was weighed into a glass culture tube. For fortified samples, 10 ± 0.1 g of the matrix was also weighed into a glass culture tube. Then, fortification solutions were added to the matrix as shown in the following table:

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

* Limit of quantification (LOQ)

3.2 Extraction of Sample Material

0.1 mL acetonitrile and 0.1 mL methanol was added to each control sample to ensure that all samples had the same solution proportions as the fortified samples. Samples were vortexed to ensure homogenization.

3.3 Preparation for Measurement

3.3.1 Preparation for measurement of Quizalofop-p-ethyl and Quizalofop-p

A 0.051 mL aliquot of the sample solution was pipetted into an autosampler vial and 0.449 mL of acetonitrile and 0.5 mL of 10:90 DI Water:Acetonitrile were added to the vial. Samples were vortexed and submitted for analysis.

3.3.2 Preparation for measurement of 3-OH-Quizalofop-acid

A 0.051 mL aliquot of the sample solution was pipetted into an autosampler vial. 0.949 mL of 45:55 DI Water:Acetonitrile was added to the vial. Samples were vortexed and submitted for analysis.

3.4 Method Modifications

The following method modifications were necessary for the implementation of method D1304/02 at EPL.

Reagent blanks were extracted with each validation set. EPL DI water was weighed in place of study sample water to prepare a reagent blank.

Step 3.6.1 Instructed (for the analysis of quizalofop-p-ethyl and quizalofop-p) to dilute residues above the LOQ with S1 as needed to fit into the calibration curve. At EPL, the 10X LOQ fortifications were diluted by a factor of 5 with S1 prior to analysis. Step 3.6.2 instructed (for the analysis of 3-OH-quizalofop-acid) to dilute residues higher than the LOQ with S2 as needed to fit into the calibration curve. At EPL, the 10X LOQ fortifications were diluted by a factor of 5 with S2 prior to analysis. Further, samples in Steps 3.6.1 and 3.6.2 were prepared directly in HPLC autosampler vials instead of glass culture tubes.

Section 4.2 Detailed the instrumental analysis. The chromatographic system and detection system identified in the analytical method were different from the chromatographic 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 analytical 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. Instrument parameters are described in Section 4.

The HPLC gradients identified in Section 4.2 included 0.4 minutes of equilibrium time at the end of each gradient. During the ILV it was found that additional equilibrium 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 equilibrium time to compensate for available analytical equipment. The quizalofop-p was measured with 3.0 additional minutes of equilibrium time at the end, and the 3-OH-quizalofop-acid was measured with 2.0 additional minutes of equilibrium time added to the end of each run.

4. Instrumentation and Conditions

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	20 µL		
Mobile Phase	A = 4 mM Ammonium Formate with 0.1% Formic Acid in Water B = 4 mM Ammonium Formate with 0.1% Formic Acid in Methanol		
Flow Rate	800 µL/minute		
Steps † (including wash and equilibration)	Time (min)	Composition	
		%A	%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		
Ionization	Turbo Ion Spray		
Analyte	**Transitions (*m/z*)‡ Positive mode**		**Expected Retention Time**
	Primary	**Secondary**	
quizalofop-p-ethyl	373.0-299.0	375.0-300.9	~3.3 min
quizalofop-p	345.0-299.0	345.0-100.0	~2.9 min

† Additional equilibration time may or may not be used at end of gradient.
‡ Exact transition masses may vary with instrument optimization.

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	20 µL		
Mobile Phase	A = 4 mM Ammonium Formate with 0.1% Formic Acid in Water B = 4 mM Ammonium Formate with 0.1% Formic Acid in Methanol		
Flow Rate	600 µL/minute		
Steps † (including wash and equilibration)	Time (min)	Composition	
		%A	%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		
Ionization	Turbo Ion Spray		
Analyte	**Transition Negative mode (*m/z*)‡**		**Expected Retention Time**
3-OH-quizalofop-acid	359.0-166.0		~3.15 min

† Additional equilibration time may or may not be used at end of gradient.
‡ Exact transition masses may vary with instrument optimization.

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	20 µL		
Mobile Phase	A = 4 mM Ammonium Formate with 0.1% Formic Acid in Water B = 4 mM Ammonium Formate with 0.1% Formic Acid in Methanol		
Flow Rate	600 µL/minute		
Steps † (including wash and equilibration)	Time (min)	Composition	
		%A	%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		
Ionization	Turbo Ion Spray		
Analyte	**Transition Negative mode (*m/z*)‡**		**Expected Retention Time**
3-OH-quizalofop-acid	359.0-166.0		~3.3 min

† Additional equilibration time may or may not be used at end of gradient.
‡ Exact transition masses may vary with instrument optimization.

4.1 Calibration Procedures

Calculation of results was based on peak area measurements using a calibration curve. A standard curve was prepared by injected standard solutions at appropriate concentrations for each analyte. Calibration standard concentrations for quizalofop-p-ethyl ranged from 0.010-1.000 ng/mL. Calibration standard concentrations for quizalofop-p ranged from 0.010-1.000 ng/mL for the LOQ and 0.010-0.200 ng/mL for the 10X LOQ. Calibration standard concentrations for 3-OH-quizalofop-acid ranged from 0.010-1.000 ng/mL for the LOQ and 0.010-0.200 ng/mL for the 10X LOQ. A calibration standard was typically injected every two to five sample injections. Analyst® 1.6 created the standard curve based on linear regression, typically using 1/x weighting. The regression functions were used to calculate the best-fit line by plotting the standard concentrations (ng/mL) on the x-axis versus the detector's peak response (peak area) on the y-axis. Typical calibration curves and representative chromatograms for calibration standards for quizalofop-p-ethyl are presented in Appendices C and D. Typical calibration curves and representative chromatograms for calibration standards for quizalofop-p are presented in Appendices A and B. Typical calibration curves and representative chromatograms for calibration standards for 3-OH-quizalofop-acid are presented in Appendices E and F.

4.2 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.

4.3 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 instrumentation. 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 coefficient of determination (r^2) by linear regression of the instrument responses for the reference standards.

4.4 Calculation of Residues and Recoveries

Peak integration and quantitation were performed within Analyst® 1.6 software using the calibration curve equation to determine sample concentrations of the analyte found during sample analysis. The data processing was completed in MultiQuant, which is a companion software program accessed via Analyst. Recovery results and additional sample concentrations were calculated for each set of samples within Microsoft Office Excel 2007 and reported in spreadsheet data reports, which are presented in Appendix H.

The calculations were performed in the following way:

Relative Error Accuracy (%) =

$$\frac{\text{(Calculated Standard Concentration (ng/mL) – Nominal Standard Concentration (ng/mL))} * 100}{\text{Nominal Standard Concentration (ng/mL)}}$$

mg/kg (ppm) Found =

$$\frac{\text{Amount Found (ng/mL)} * \text{Final Vol. (mL)} * \text{Extract Vol. (mL)} * \text{Dilution Factor}}{\text{Sample Weight (g)} * \text{Aliquot Vol. (mL)} * 1000\ \text{mg/g}}$$

Fortification Level (ppm) =

$$\frac{\text{Volume Spiking Solution (mL)} * \text{Conc. of Spiking Solution (µg/mL)}}{\text{Sample Weight (g)}}$$

Corrected Recovery (%) =

$$\frac{\text{[ppm Found in Spike – Average ppm Found in Control]} * 100}{\text{Fortification Level (ppm)}}$$

5.2 Summary of Method

Type of Method	LC-MS/MS
Test Systems	Surface Water and Drinking Water
Selected mass transitions (m/z)	Quizalofop-p-ethyl 373.0→299.0* 375.0→300.9 Quizalofop-p 345.0→299.0* 345.0→100.0 3-OH-Quizalofop-acid 359.0→166.0* 359.0→166.0 *Primary quantification transition
Analytical Procedure	BASF Analytical Method D1304/02: "Analytical Method for the Determination of

	Residues of Quizalofop-p-ethyl and its Metabolites Quizalofop-p and 3-OH-Quizalofop-acid in Water by LC-MS/MS"
Confirmatory Technique	Both transitions listed in Method D1304/02 were validated for quizalofop-p-ethyl and quizalofop-p. Secondary chromatographic method using one transition was validated for 3-OH-quizalofop-acid.
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 for 3-OH-quizalofop-acid. Recovery data was reported for each mass transition and chromatographic method considered, as shown in Appendix H.
LOD	0.0002 mg/kg
LOQ	0.001 mg/kg (lowest fortification level)
Levels of Fortification	0.001 mg/kg and 0.01 mg/kg
Time Required	A set of 13 samples requires approximately 12-14 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).

7. Conclusions/Recommendation from ILV

In summary, the independent laboratory validation of the BASF method (D1304/02) was successfully completed in surface water and drinking water. All average recoveries were within the acceptable range (70-120%).

Three method findings/recommendations were noted upon completion of the ILV:

a. 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 equilibrium 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. The quizalofop-p was measured with 3.0 additional minutes of equilibration time at the end, and the 3-OH-quizalofop-acid was measured with 2.0 additional minutes of equilibration time added to the end of each run. Additionally, the flow for the determination of quizalofop-p and quizalofop-p-ethyl was increased to 800 µL/minute, and the flow for the 3-OH-quizalofop-acid quantitation method was increased to 600 µL/minute.

b. 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 are shown in Section 4 Instrumentation and Conditions.

c. The stability information presented for stock solutions of quizalofop-p and quizalofop-p-ethyl in Section 2.5.4 of the reference method did not agree with the stability information documented in the method validation report (Reference 2). The method validation report found that stock solutions of quizalofop-p and quizalofop-p-ethyl were stable for 95 days when stored refrigerated (less than 10% decline), however Section 2.5.4 stated a stability of 59 days for these stock solutions when stored refrigerated.

8. Protocol, Amendments, and Deviations

There were no protocol amendments or noted deviations during the course of this study.

9. Communication

Communications between the Study Director and the BASF study monitor and personnel are documented and presented in Appendix I. 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 after the completion of all trials on February 5, 2015.

10. 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

11. References

1. Shaozhi (Chelsie) Zheng. Validation of BASF Analytical Method (D1304/02): "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 Water by LC-MS/MS" BASF Study 437862; BASF Registration document number 2014/7003589.

2. Xiaorong Shen. 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 Study 437873; BASF Registration document number 2014/7003590.

The following is a chronological summary of e-mail correspondence between BASF and EPL:

Abbreviations:

Abbreviation	Full Name	Company/Position
SS	Sara E. Sharp	EPL Bio Analytical Services (EPL), Study Director
JJ	John E. Jones	BASF Crop Protection, Study Monitor
MS	Manasi G. Saha	BASF Crop Protection, Project Coordinator
KB	Kim Brunner	EPL Bio Analytical Services (EPL), CFO
NP	Nancy Purdeu	EPL Bio Analytical Services (EPL), Standard & Solution
MH	Matthew Horowitz	BASF Crop Protection, Agricultural Research Chemist
MSm	Michelle Smith	EPL Bio Analytical Services (EPL), TFM
AD	Angela Dawson	EPL Bio Analytical Services (EPL), Quality Assurance

Pre-Study:	Prior to Study Director signing study protocol.
Analytical Phase:	After Study Director has signed study protocol and before completion of analytical labwork.
Report Phase:	After completion of analytical labwork.

Date	E-mail	Study Interval	Content/Subject	Attachment(s)
10/31/2014	JJ→ MS,SS	Pre-study	BASF provided protocol template.	BASF Protocol template for water ILV
11/5/2014	JJ→ MS,SS, KB	Pre-study	BASF confirmed that the guidelines for the water ILV will be 850.6100, US EPA GLP/s 40 CFR Part 160 and report format PR Notice 2011-3.	N/A
11/7/2014	SS→ JJ, MS, KB	Pre-study	EPL sent completed draft protocol for BASF review.	1st draft of water ILV protocol
11/10/2014	SS→ JJ, MS, NP	Pre-study	EPL confirmed receipt of the reference materials. The Certificate of Analysis (CoA) for quizalofop-p stated that the sample should be dried in the oven for 2 hours prior to use. EPL asked for clarification regarding drying of the reference material. The Technical Procedure (TP) does not instruct to dry the reference material prior to use.	CoA for Quizalofop-p
11/10/2014	MS→ JJ, SS, NP	Pre-study	BASF confirmed that the quizalofop-p reference material does not need dried and can be used as received.	N/A
11/13/2014	JJ→ MS, SS	Pre-study	BASF provided an updated protocol draft with comments from BASF Quality Assurance.	Updated protocol draft
11/14/2014	SS→ JJ, MS	Pre-study	EPL reported that the analytical columns cited in the TP are not consistent. Sections 2.4 (Equipment) and 4.2 (Instrumental Analysis) instruct to use different HPLC columns for analysis. EPL requested clarification on the correct HPLC columns to be used for analysis.	N/A

11/17/2014	MH→ JJ, MS, SS	Pre-study	BASF clarified the HPLC columns to be used for analysis.	N/A
11/19/2014	JJ→ MS, SS	Pre-study	BASF provided a partially-signed protocol for the water study.	Protocol draft signed by BASF only
11/21/2014	SS→ JJ, MS	Analytical Phase	EPL received the original signature pages for the study protocol containing BASF signatures. The protocol was signed at EPL, and a copy of the fully-signed protocol sent to BASF.	Signed Study Protocol
12/3/2014	SS→ JJ	Analytical Phase	EPL notified BASF that the System Suitability testing for water matrices was unsuccessful (recovery < 70%). EPL will verify standard preparation and communicate with BASF as method develops.	Set T001R Quiz BASF Gradient Analyte Report
12/4/2014	JJ→ SS	Analytical Phase	JJ reviewed the unsuccessful System Suitability report and expressed concerns about the calibration curve: the relationship of the standards to each other did not look correct and the 1.0 ng/mL standard listed in the method may be decreasing the confidence in the LOQ-area of the calibration curve.	N/A
12/8/2014	SS→ JJ	Analytical Phase	EPL repeated the System Suitability testing with new calibration standards and evaluted the chromatography. With the 500 µL/min flow cited in the method, the chromatography for quizalofop-p and quizalofop-p-ethyl was poor. With the flow increased to 800 µL/min the chromatography improved. EPL provided example chromatograms with both flow rates for comparison and recommended the use of the higher flow rate for both the quizalofop-p/quizalofop-p-ethyl method as well as the 3-OH-quizalofop-acid method.	Set T001R Quiz New Stds BASF Gradient chromatography and Set T001R with New Stds Flow 800 chromatography
12/12/2014	JJ→ MS,SS	Analytical Phase	JJ acknowleged that EPL may need to modify the method gradient to compensate for EPL instrumentation and instructed to incorporate necessary gradient modifications in the study data.	N/A
12/12/2014	SS→ JJ, MS	Analytical Phase	EPL notified that the first trial was completed for both water matrices. Acceptable results were obtained for quizalofop-p and 3-OH-quizalofop-acid, but the recovery for quizalofop-p-ethyl was low	Excel summaries of Set V001 (Surface Water) and V002 (Drinking Water)
12/15/2014	SS→ JJ, MS	Analytical Phase	EPL notified that the samples from V001 and V002 were re-analyzed for quizalofop-p-ethyl using the alternate instrumental conditions listed in Section 4.2.1 of the TP. However, the average recoveries for quizalofop-p-ethyl were still < 70%. EPL provided all Excel summaries and Analyst PDF reports for Sets V001 and V002.	Updated Excel summaries for Sets V001 and V002, Analyst PDF Reports for Sets V001 and V002
12/15/2014	JJ→ MS,SS	Analytical Phase	Regarding V001/V002, BASF questioned how long after spiking the samples were allowed to sit prior to removing the aliquot for dilution with 90/10 acetonitrile/water and what type of extraction vessel was used.	N/A

12/15/2014	SS→ JJ, MS	Analytical Phase	The Study Director confirmed that the spiked sample extracts were not allowed to sit prior to dilution and that the extraction vessel was a polypropylene centrifuge tube to match the composition of the control sample storage vessels.	137G966 Method Modifications Summary
12/15/2014	JJ→ MS,SS	Analytical Phase	BASF stated that the quizalofop-p-ethyl was likely sticking to the plastic tubes and requested that the trials be repeated with glass culture tubes/test tubes.	N/A
12/15/2014	SS→ JJ, MS	Analytical Phase	EPL asked if the repeat trials were to be analyzed for quizalofop-p-ethyl only or for all 3 analytes (since Sets V001/V002 gave acceptable data for quizalofop-p and 3-OH-quizalofop-acid).	N/A
12/16/2014	JJ→ MS,SS	Analytical Phase	BASF confirmed that the repeat trials were to be analyzed for all 3 analytes. Thus, no data from V001/V002 was accepted by sponsor. Further, since Sets V001/V002 did not follow the method as written (plastic tubes were used instead of glass culture tubes), the repeat trials (V001R/V002R) would be considered the first ILV attempt.	N/A
12/17/2014	SS→ JJ, MS	Analytical Phase	EPL provided data for Sets V001R/V002R. When the validation sets were repeated in glass tubes (per sponsor instruction) multiple analytes did not show acceptable average recoveries at various fortification levels. EPL asked the sponsor to review the draft data summaries for data acceptance.	Excel summaries of Sets V001/V001R and V002/V002R.
12/18/2014	JJ→ MS,SS	Analytical Phase	BASF requested the Analyst PDF reports for Sets V001R/V002R to review the chromatography prior to data acceptance.	N/A
12/18/2014	SS→ JJ, MS	Analytical Phase	EPL provided all Analyst PDF reports for V001/V001R and V002/V002R for sponsor to review chromatography.	Analyst PDF reports for V001, V002, V001R and V002R
12/18/2014	SS→ JJ, MS	Analytical Phase	The Study Director, Study Monitor and Project Coordinator had a conference call on 12/18/2014 to discuss the results collected to date. A record of the phone conference was documented by the Study Director and sent to BASF for review.	Memo to document key points from EPL/BASF conference call on 12/18/2014
12/18/2014	MS→ JJ, SS	Analytical Phase	MS returned a copy of the Conference Call memo with her edits.	Memo to document key points from EPL/BASF conference call on 12/18/2014 updated by BASF
12/22/2014	SS→ JJ, MS	Analytical Phase	The Study Director had re-optimized the MS/MS for quizalofop-p and reanalyzed the samples from V001R/V002R for quizalofop-p only as V001RAJ/V002RAJ.	Excel summaries and Analyst PDF reports for V001RAJ and V002RAJ
12/24/2014	SS→ JJ, MS	Analytical Phase	The Study Director reanalyzed the sample extracts from V001R/V002R ("B" aliquots) using the confirmatory method for 3-OH-quizalofop-acid. The data was provided to BASF for review.	Updated Excel Summaries for V001R/V002R that include the 3-OH-quizalofop-acid confirmation method

1/6/2015	SS→ JJ, MS	Analytical Phase	The Study Director sent a complete summary of all Surface Water data collected to date (Set V001R)	Updated Excel summary and Analyst PDF reports for Set V001R
1/6/2015	SS→ JJ, MS	Analytical Phase	The Study Director sent a complete summary of all Drinking Water data collected to date (Set V002R)	Updated Excel summary and Analyst PDF reports for Set V002R
1/7/2015	JJ→ MS,SS	Analytical Phase	The Study Monitor asked for the 3-OH-quizalofop-acid (quantitation method) data from Set V001R to be reprocessed with the low standard included and the 1.0 ng/mL standard excluded.	N/A
1/7/2015	SS→ JJ, MS	Analytical Phase	The Study Director provided re-processed data for V001R and V002R (the low standard was included and the high standard excluded for 3-OH-quizalofop-acid quantitation method.	Updated Excel summaries and Analyst PDF reports for V001R and V002R.
1/7/2015	JJ→ MS,SS	Analytical Phase	The Study Monitor asked for the 0.02 ng/mL standard to be included in the data for the drinking water validation trial (V002R).	N/A
1/8/2015	SS→ JJ, MS	Analytical Phase	The Study Director re-processed Set V002R and V001R. The 3-OH-quizalofop-acid confirmation method data was revised to use a calibration curve that ranged from 0.01- 0.20 ng/mL.	Updated Excel summaries and Analyst PDF reports for V001R and V002R.
1/16/2015	SS→ JJ, MS	Analytical Phase	The Study Director and Study Monitor had a conference call on 1/16/2015 to discuss the results collected to data. A record of the phone conference was documented by the Study Director and sent to BASF for review. The Study Director also confirmed that the Dixon's Q-test did not identify 966-W002-S4RBJ as a stastical outlier, so Set V002S will include both the LOQ and 10x LOQ fortification levels for the 3-OH-quizalofop-acid confirmation method.	Memo to document key points from EPL/BASF conference call on 1/16/2015
1/16/2015	JJ→ MS,SS	Analytical Phase	The Study Monitor confirmed that the documentation for the phone conference on 1/16/2015 was accurate.	N/A
1/18/2015	SS→ JJ, MS, MSm	Analytical Phase	The Study Director provided data for Sets V001S and V002S. The quizalofop-p data was not acceptable due to poor calibration.	Excel summaries and Analyst PDF reports for V001S and V002S.
1/18/2015	SS→ JJ, MS	Analytical Phase	The Study Director re-analyzed the samples extracts from Sets V001S and V002S for quizalofop-p only. The data satisfied protocol requirements for average recovery, but the calibration standards used were two days past their stated expiration.	Updated Excel summaries and Analyst PDF reports for V001S and V002S.

1/26/2015	JJ→ MS,SS	Analytical Phase	The Study Monitor confirmed that the 3-OH-quizalofop-acid data from sets V001S and V002S was accepted. However, the quizalofop-p data was not accepted by the Study Monitor since the method had a 5 point calibration curve with one standard excluded- which does not agree with BASF "internal guidelines" for data acceptance. An additional trial of quizalofop-p was requested with calibration standards injected at least in duplicate and a suggested injection order was provided by the Study Monitor.	N/A
1/27/2015	SS→ JJ, MS	Analytical Phase	The Study Director requested clarification on BASF "Internal Guidelines" used to not accept the quizalofop-p data collected in Sets V001S and V002S. No such documentation was provided to EPL.	N/A
1/28/2015	JJ→ MS,SS	Analytical Phase	The Study Monitor requested that the upcoming quizalofop-p repeat validation sets be considered as the second trial for quizalofop-p.	N/A
1/28/2015	MS→JJ,SS	Analytical Phase	The Project Coordinator provided report templates to be used to draft the analytical report.	Water ILV Report Template
2/5/2015	SS→ JJ, MS, MSm, AD, KB	Analytical Phase	The Study Director completed Sets V001T/V002T for quizalofop-p only. All data met protocol requirements for recovery and precision. The Study Director asked for additional clarificiation as to the documentation of Sets V001S/V002S. The analytical phase of the study was completed.	Excel summaries and Analyst PDF reports for V001T and V002T.
2/23/2015	SS→ JJ, MS	Report Phase	The Study Director sent a draft report to BASF for review.	Draft Report

EPL Bio Analytical Services (EPL BAS)

EPL BAS Study No.: 137G966/ BASF 437863

Subject: Phone Conversation between S. Sharp, J. Jones and M. Saha 12/18/14

The Study Director and Study Monitor discussed the status of the ILV study to date. The following key points summarize the phone conversation:

- The first data sets collected (V001- Surface Water, V002- Drinking Water) will not be accepted. The sets were extracted in polypropylene centrifuge tubes instead of glass culture tubes. Since the method was modified with the use of polypropylene extraction tubes, these data sets will not be considered the first trial of the ILV.

- The second data sets collected (V001R- Surface Water, V002R- Drinking Water) were extracted in glass culture tubes and are considered the first trials of the ILV. Sets V001R and V002R gave acceptable results for quizalofop-p-ethyl and 3-OH-quilzalofop-acid. The data for quizalofop-p did not satisfy protocol requirements for recovery (70-120%) in confirmatory ion surface water and both confirmatory and primary ion at 10 X LOQ for drinking water .

- The MS/MS parameters will be re-optimized for quizalofop-p to improve sensitivity for both the quantitation and confirmatory transitions. Specifically, the ionization temperature will be re-optimized. If a 50-100% sensitivity increase is realized after MS/MS re-optimization, extracts from Sets V001R and V002R will be re-analyzed. Set V001R extracts will be re-analyzed for only the quizalofop-p confirmatory transition. Set V002R extracts will be re-analyzed for both the quantitation and confirmatory transitions of quizalofop-p.

- The secondary chromatographic method listed for 3-OH-quizalofop-acid is a requirement of the ILV. The Study Director will locate the appropriate HPLC column and analyze the sample extracts from Sets V001R and V002R with the secondary instrumental conditions listed in Section 4.2.4 of the analytical method.

EPL Bio Analytical Services (EPL BAS)

EPL BAS Study No.: 137G966/ BASF 437863

Subject: Phone Conversation between S. Sharp and J. Jones, 1/16/15

The Study Director and Study Monitor discussed the status of the ILV study to date. The following key points summarize the phone conversation:

- For Set V001R (Surface Water) the data for quizalofop-p-ethyl is accepted for both fortification levels. For quizalofop-p and 3-OH-quizalofop-acid, the data at the LOQ fortification level is accepted. The 10X fortification data for these analytes is not accepted (recovery ≥ 120%). An additional trial is needed for surface water. Set V001S will only include a blank, two unfortified controls and 5 fortifications at the 10X LOQ. Set V001S will only be analyzed for quizalofop-p and 3-OH-quizalofop-acid.

- For Set V002R (Drinking Water) the data for quizalofop-p-ethyl is accepted for both fortification levels. For quizalofop-p, the data at the LOQ fortification level is accepted. The 10X fortification data for quizalofop-p and both fortification levels for 3-OH-quizalofop-acid is not accepted (recovery ≥ 120% and/or % RSD ≥ 20%). An additional trial is needed for drinking water. Set V002S will include a blank, two unfortified controls, 5 fortifications at the LOQ and 5 fortifications at the 10X LOQ. Set V002S will only be analyzed for quizalofop-p and 3-OH-quizalofop-acid.

- For trials V001S and V002S, the following statements shall apply:

    - The calibration range will include five standards and range from 0.01 to 0.20 ng/mL. The 1.0 ng/mL calibration standard will not be analyzed.

    - The 10X LOQ fortifications will be diluted by a factor of 5 so that the nominal instrumental response will be 2X LOQ response.

    - The glass extraction tubes used in Sets V001R and V002R will be used according to the reference method.

    - Additional equilibration time will be added to the end of the HPLC gradient to ensure that the system is properly equilibrated prior to sample analysis.