

Independent Lab 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"

ABSTRACT

The purpose of this study was to demonstrate that the 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", could be performed with acceptable recoveries at an outside laboratory with no prior experience with the method. This method was originally developed in BASF Corporation, RTP, NC and was validated at Primera Analytical Solutions Corp. (PASC) in Princeton, New Jersey (Reference 1).

Principle of the Method: Soil samples (5 g) were extracted by shaking twice with acetonitrile-6% phosphoric acid (80:20, v/v) in deionized (DI) water. An aliquot (8%) from the extract was diluted with acetonitrile-water (90:10, v/v) for the residue determination of quizalofop-p-ethyl and quizalofop-p using LC-MS/MS. A separate aliquot (40%) from the original extract was diluted with water for the residue determination of 3-OH-quizalofop-acid using LC-MS/MS.

Test Conditions: The method was validated at two fortification levels (0.005 and 0.05 mg/kg for quizalofop-p-ethyl and quizalofop-p, 0.001 and 0.01 mg/kg for 3-OH-quizalofop-acid) for soil. 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 LOQ was defined as the lowest fortification level tested. LOQ for quizalofop-p-ethyl and metabolite, quizalofop-p is 0.005 mg/kg. The method limit of detection was estimated at 20% of the LOQ, equivalent to 0.001 mg/kg. The LOQ for 3-OH-quizalofop-acid is 0.001 mg/kg and the LOD is 0.0002 mg/kg.

Selectivity: The method determines residues of quizalofop-p-ethyl and its metabolites in soil. No interfering peaks were found at the retention times of 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 ($r^2 > 0.99$) was observed in the range of 0.010 ng/mL to 0.2 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 also observed in the range of 0.010 ng/mL to 1.0 ng/mL.

1. Introduction

1.1 Scope of the Method

BASF method D1303/02 was developed to determine the residues of BAS 9152 H in soil 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.

The independent lab validation was conducted using two fortification levels (0.005 and 0.05 mg/kg for quizalofop-p-ethyl and quizalofop-p, 0.001 and 0.01 mg/kg for 3-OH-quizalofop-acid) for soil. For each fortification level, five replicates were analyzed. Additionally, one reagent blank and two replicates of unfortified samples were examined.

1.2 Principle of the Method

Soil samples (5 g) were extracted by shaking twice with acetonitrile-6% phosphoric acid (80:20, v/v) in DI water. An aliquot (8%) from the extract was diluted with acetonitrile-water (90:10, v/v) for the residue determination of quizalofop-p-ethyl and quizalofop-p using LC-MS/MS. A separate aliquot (40%) from the original extract was diluted with water for the residue determination 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 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 System

The test system considered in this study was soil (silty clay loam, BASF Study 437860; RCN130034, depth 12-18 inches).

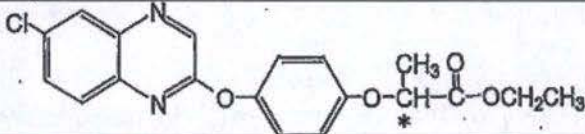
The control sample was provided by BASF. The soil sample was received on November 4, 2014. Upon arrival at the laboratory, the sample was opened, inspected, and checked against enclosed shipping forms. The test system was received frozen and was stored under frozen conditions at all times, unless necessary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

2.2 Test and Reference Substances

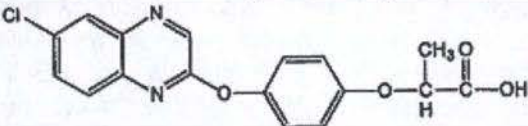
Standard substances were stored in a freezer ($\leq -5^{\circ}\text{C}$) until use. BASF has retained reserve samples of these chemicals, and has documentation specifying the location of the synthesis and characterization information for each of these compounds available at BASF, Research Triangle Park, 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 ₂ O 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 ₂ O 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	3-OH-quizalofop-p (also: 3-OH-quizalofop-acid)
BASF Reg. No.	N/A
CAS No.	N/A
Molecular Formula	C ₁₇ H ₁₃ ClN ₂ O ₅
Molecular Weight	360.8 g/mol

IUPAC Name	(R)-2-[4-(6-chloro-3-hydroxyquinoxalin-2-ylloxy)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 specific equipment used in this study was documented in the raw data. Maintenance files and applicable SOPs for the equipment are retained at the testing facility.

Class A volumetric glassware

Laboratory glassware (beakers, graduated cylinders, culture tubes, scintillation vials)

Glass centrifuge tubes, 50 mL with screw-top caps

Volumetric pipettes, glass; various sizes

Analytical balance, capable of measuring to 0.01 mg

Teflon® centrifuge tubes, 40 mL with screw-top lids

Air displacement pipette, various volumes with disposable tips

Mechanical shaker

Centrifuge, with rotors to accommodate 40 mL Teflon® centrifuge tubes

Whatman filter paper, 90 mm No. 4

Vortex mixer

HPLC system: Agilent 1290

HPLC analytical column: Acquity BEH C18 (2.1 x 100 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	Expiration Date
Acetonitrile	Fisher	145336, 144504, 146154, 146809	6/30/19
Deionized Water	EPL house DI water system	--	--
Methanol	EMD	54105	6/30/19

Phosphoric Acid	BDH	2011071543	6/30/17
Formic Acid	Fisher	124119	6/30/18
Celite® 545	Fisher	141444	6/30/19
Ammonium Formate	Fluka	BCBM5612V	6/30/19

Solutions and Solvent Mixtures

Description	Code	Composition
Extraction Solvent	S1	Acetonitrile-6% Phosphoric Acid in water, 80:20 (v/v)
Final Volume Solvent (for determination of Quizalofop-p-ethyl and Quizalofop-p)	S2	Water-Acetonitrile, 10:90 (v/v)
Final Volume Solvent (for determination of 3-OH-Quizalofop-acid)	S3	Water-Acetonitrile, 45:55 (v/v)
HPLC mobile phase C	LC3	4mM 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 1.0 mg/mL stock solutions of quizalofop-p-ethyl and quizalofop-p were prepared by weighing an appropriate amount of each reference standard into a 10 mL volumetric flask and adding the required volume of acetonitrile. A stock solution of 3-OH-quizalofop-acid was prepared by weighing an appropriate amount of the reference standard into a 10 mL volumetric flask and adding the required volume of methanol. The use of sonication or vortexing was also considered for ensuring a complete homogeneous solution. Fortification and calibration standard solutions were prepared from separate dilution series from the same stock.

ID	Purity	Weight (g)	Concentration (µg/mL)
Quizalofop-p-ethyl	99.9 %	0.01000	1000.000
Quizalofop-p	99.8 %	0.01000	1000.000
3-OH-Quizalofop-acid*	95.9 %	0.01040	997.360

*A correction for purity was required.

Fortification Solutions

Standard solutions for fortification were prepared by combining stock solutions of each analyte (0.25 mL) into a 25 mL volumetric flask. Afterwards, dilution series were made up by using

acetonitrile or methanol, as exemplified in the tables below. The use of sonication or vortexing was also considered for ensuring a complete homogeneous solution.

Preparation of mixed Fortification solutions – Quizalofop-p-ethyl and Quizalofop-p

Solution Used (µg/mL)	Volume Taken (mL)	Diluted with acetonitrile to a final volume of (mL)	Concentration (µg/mL)
1000	0.25	25	10.0
10.0	2.5	25	1.0
1	2.5	25	0.10

Preparation of mixed Fortification solutions – 3-OH-Quizalofop-acid

Solution Used (µg/mL)	Volume Taken (mL)	Diluted 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, and by diluting them with the appropriate solution as needed. These solutions were prepared according to the tables below.

Preparation of standard solutions for calibration – Quizalofop-p-ethyl and Quizalofop-p

Diluted to volume with 10:90 (v/v) Water-Acetonitrile

Solution Used (ng/mL)	Volume Taken (mL)	Final Volume (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

Preparation of standard solutions for calibration – 3-OH-Quizalofop-acid

Diluted to volume with 45:55 (v/v) Water-Acetonitrile

Solution Used (ng/mL)	Volume Taken (mL)	Final Volume (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

All standard solutions were stored refrigerated in amber bottles when not in use.

3. Analytical Procedure

3.1 Weighing and Fortification

5 g +/- 0.1 g of the soil sample was weighed into 40 mL Teflon centrifuge tubes. Fortification samples were spiked with the appropriate standard solution to obtain five fortifications at the LOQ (0.005 mg/kg for quizalofop-p-ethyl and quizalofop-p and 0.001 mg/kg for 3-OH-quizalofop-acid) and five fortifications at 10 x LOQ (0.05 mg/kg for quizalofop-p-ethyl and quizalofop-p and 0.01 mg/kg for 3-OH-quizalofop-acid). The following scheme was used:

Matrix	Sample Type	Ideal Sample Weight (g)	Concentration of Spiking Solution (µg/mL)	Volume of Spiking Solution (mL)	Level of Fortification (mg/kg)
Soil (quizalofop-p-ethyl and quizalofop-p)	Control	5	-	-	0.00
	Fortification (LOQ)	5	0.100	0.250	0.005
	Fortification (10x LOQ)	5	1.000	0.250	0.05
Soil (3-OH-quizalofop-acid)	Control	5	-	-	0.00
	Fortification (LOQ)	5	0.100	0.050	0.001
	Fortification (100x LOQ)	5	0.997	0.050	0.01

3.2 Extraction of Sample Material

Approximately 1.5 g (1.45-1.55 g) of Celite® was pre-weighed and added to the samples. Exactly 15 mL of S1 was added to each sample then shaken at approximately 300 rpm for approximately 30 minutes. Samples were centrifuged for 5 minutes at 4000 rpm. Using a 50 mL glass centrifuge tube, 29 mL of acetonitrile was added then set aside. Into a separate culture tube, 7.5 mL of the supernatant was transferred and the tube was capped. In the weighed sample tube, 15 more mL of S1 was added. The sample was shaken again for approximately 30 minutes at approximately 300 rpm then centrifuged for 5 minutes at 2000 rpm.

From the culture tube holding the supernatant, 7 mL was taken and transferred to the weighed sample tube. The supernatant culture tube was washed by adding 4.5 mL of acetonitrile (taken from the 50 mL glass centrifuge tube). From that rinse, 4 mL was transferred to the weighed sample tube. The rinse process was repeated one more time with 4.5 mL of acetonitrile, bringing the total volume of the weighed sample tube to 37.5 mL. The samples were vortexed for 10 seconds, then centrifuged for 5 minutes at 2000 rpm. 30 mL of the supernatant was transferred into the 50 mL glass centrifuge tube containing acetonitrile then vortexed.

3.3 Preparation for Measurement

To prepare for analysis of BAS 9152 H and quizalofop-p, 0.10 mL of the extract was diluted with 0.7 mL of S2 in an autosampler vial. High concentrations were further diluted using S2. To prepare for analysis of 3-OH-quizalofop-acid, 0.50 mL of the extract was diluted with 0.3 mL of DI water in an autosampler vial. High concentrations were further diluted using S3.

3.4 Method Modifications

The following modifications were necessary in order to independently validate the procedure:

Step 3.4.3: Instructed to add 1.5 g of Celite® to each sample. During the course of the ILV, the Celite® was pre-weighed (1.45 – 1.55 g) into glass scintillation vials. The vials were stored ambient in the main laboratory until needed.

Step 3.6.3: (Analysis of quizalofop-p and BAS 9152 H) instructed that any residues above the LOQ were to be diluted with S2 to be bracketed by the calibration curve. During the course of the ILV, the 10 x LOQ fortifications were diluted by a factor of 5 with S2 prior to analysis. For analysis of 3-OH-quizalofop-acid, this step also instructed to dilute residues higher than the LOQ with S3 to be bracketed by the calibration curve. During the course of the study, the 10 x LOQ fortifications were diluted by a factor of 2 with S3 prior to analysis.

Section 4: Addressed the instrumental analysis. The analytical instrumentation used at EPL varied from the equipment listed in the analytical method. Instrumental analysis was completed using an Agilent 1290 LC system and an AB Sciex 6500 Q-Trap MS/MS detector. Exact instrumental conditions were optimized for the available instrument at EPL and recorded in the raw data with each data set.

4. Instrumentation and Conditions

Quizalofop-p-ethyl and Quizalofop-p

	Parameter		
Chromatographic System	Agilent 1290		
Analytical-column	Acquity BEH C18; (2.1 x 100 mm, 1.7 µm)		
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	0.600 mL/min		
Detection System	AB Sciex 6500 Q Trap		
Ionization	ESI ⁺		
Ionization Temperature	500 °C		
Analyte	Transitions (m/z)	Polarity	Approximate Retention Time
Quizalofop-p-ethyl	373.0 → 299.0* 375.0 → 300.9	Positive	3.3 minutes
Quizalofop-p	345.0 → 299.0* 345.0 → 100.0	Positive	2.9 minutes

*Primary quantification transition.

3-OH-Quizalofop-acid (Primary Chromatographic Method)

	Parameter
Chromatographic System	Agilent 1290

Analytical-column	Acquity BEH Phenyl; (2.1 x 100 mm, 1.7 µm)		
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	0.600 mL/min		
Detection System	AB Sciex 6500 Q Trap		
Ionization	ESI ⁻		
Ionization Temperature	550 °C		
Analyte	Transitions (m/z)	Polarity	Approximate Retention Time
3-OH-Quizalofop-acid	359.0 → 166.0*	Negative	3.15 minutes

3-OH-Quizalofop-acid (Secondary Chromatographic Method)

	Parameter		
Chromatographic System	Agilent 1290		
Analytical-column	Acquity HSS T3 ; (2.1 x 100 mm, 1.8 µm)		
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	0.600 mL/min		
Detection System	AB Sciex 6500 Q Trap		
Ionization	ESI ⁻		
Ionization Temperature	550 °C		
Analyte	Transitions (m/z)	Polarity	Approximate Retention Time
3-OH-Quizalofop-acid	359.0 → 166.0	Negative	3.15 minutes

4.1 Calibration Procedures

Calculation of results was based on peak area measurements using a linear calibration curve (weighted 1/x). The calibration curve was obtained by direct injection of the calibration standards containing known amounts of each quizalofop-p-ethyl and quizalofop-p in the range of 0.010 ng/mL to 0.20 ng/mL. The calibration curve was obtained by direct injection of the calibration standards containing known amounts of 3-OH-quizalofop-acid in the range of 0.010 ng/mL to 1.0 ng/mL.

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

Data was acquired with validated Analyst software. The data processing was completed in MultiQuant, which is a companion software program accessed via Analyst.

Relative Error Accuracy (%) =

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

Amount Found (mg/kg, ppm) =

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

Fortification Level ($\mu\text{g/g}$, ppm) =

$$\frac{\text{Volume Spiking Solution (mL)} * \text{Concentration of Spiking Solution } (\mu\text{g/mL})}{\text{Sample Weight (g)}}$$

Recovery (%) =

$$\frac{\text{ppm Found in Spike} * 100}{\text{Fortification Level (ppm)}}$$

5.2 Summary of Method

Type of Method	LC-MS/MS
Test Systems	Soil
Selected mass transitions (m/z)	BAS 9152 H 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 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"
Confirmatory Technique	A confirmatory transition was monitored for quizalofop-p-ethyl and quizalofop-p. A different column was used for confirmatory analysis of a 3-OH-quizalofop-acid.
Method of Quantitation	The quantitation was 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 H.
LOD	0.001 mg/kg quizalofop-p-ethyl and quizalofop-p 0.0002 mg/kg 3-OH-quizalofop-acid
LOQ	0.005 mg/kg quizalofop-p-ethyl and quizalofop-p 0.001 mg/kg 3-OH-quizalofop-acid
Levels of Fortification	0.005 and 0.05 mg/kg quizalofop-p-ethyl and quizalofop-p

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

- a. The HPLC columns identified in Section 2.3 of the reference method did not agree with the HPLC columns identified in Section 4.2. The sponsor provided clarification that the columns identified in Section 4.2 were the correct HPLC columns for instrumental analysis.
- b. 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. During the ILV, an additional 3.0 minutes (BAS 9152 H and quizalofop-p) or 2.0 minutes (3-OH-quizalofop-acid) of equilibration time was added at the end of the gradient.
- c. The HPLC column identified by the reference method for the determination of BAS 9152 H and quizalofop-p was an Acquity UPLC BEH C18 (1.7 μ m, 2.1 x 50 mm). During the ILV, it was found that the chromatography for quizalofop-p was poor with the 50 mm HPLC column. The quantitation transition chromatography showed a tailing interference peak, and the confirmation transition chromatography showed a poor peak shape. The HPLC column used during the ILV was an Acquity UPLC BEH C18 (1.7 μ m, 2.1 x 100 mm), and the additional theoretical plates from the longer column resolved the chromatographic issues observed for both transitions.
- d. The chromatographic system and detection system identified in the reference method were different from the 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.
- e. 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 1). 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 January 16, 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. Reference

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

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)
11/5/2014	SS→ MS,JJ, KB	Pre-study	The Study Director asked for clarification regarding the scope of the ILV. BASF had provided a draft template which stated that both sample levels (5 g and 0.1 g) would be tested.	N/A
11/5/2014	JJ→ MS,SS, KB	Pre-study	The Study Monitor notified EPL that the ILV protocol guidelines will be 850.6100 and US EPA GLP standards (40 CFR Part 160). The report format will be PR Notice 2011-3. The study monitor also confirmed that the ILV will only include one validation set at the 5 g level.	N/A
11/7/2014	SS→ JJ, MS	Pre-study	The Study Director provided the draft protocol to BASF for sponsor review.	Draft 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
11/12/2014	JJ→ MS,SS	Pre-study	The Study Monitor provided an updated protocol draft that was reviewed by BASF Quality Assurance.	Updated draft protocol (reviewed by BASF)
11/13/2014	SS→ JJ, MS	Pre-study	The Study Director addressed BASF comments in the draft protocol and provided the updated protocol draft to the sponsor for final review.	Updated draft protocol (Sponsor changes reviewed by CRO)
11/14/2014	JJ→ MS, SS	Pre-study	The Study Monitor sent a copy of the final study protocol with signature pages signed by BASF.	Updated draft protocol with BASF signatures.

11/18/2014	SS→ JJ, MS	Analytical Phase	The Study Director sent BASF a copy of the fully signed final protocol.	Final protocol with all signatures.
12/3/2014	SS→ JJ	Analytical Phase	EPL notified BASF that the System Suitability testing for soil 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
12/12/2014	SS→ JJ, MS	Analytical Phase	The soil method did not pass the System Suitability testing, which was completed at EPL twice.	Set T001RA Ran with New Stds Flow 800 Analyte Report
12/18/2014	SS→ JJ, MS	Analytical Phase	At the request of the sponsor, the soil System Suitability was repeated with glass tubes. The quizalofop-p confirmatory ion chromatography did not show adequate sensitivity to meet the LOD of the method. EPL will work to re-optimize the MS/MS parameters for quizalofop-p.	967 Soil Try Out 121814 Analyte Report
12/29/2014	SS→ JJ, MS	Analytical Phase	The first trial of the soil ILV was completed and the data was sent to BASF for review.	Excel summary and Analyst PDF reports for set V001S.
12/29/2014	MS→ JJ,SS	Analytical Phase	BASF reviewed the data from Set V001S and suspects significant instrument signal suppression. The Analytical Monitor suggested that matrix-matched standards may be needed.	N/A
12/29/2014	SS→ JJ, MS	Analytical Phase	The Study Director informed BASF that the sample extracts were beyond the stability stated in the Technical Procedure (TP), so the extracts did not afford re-evaluation with matrix-matched standards. Further, the TP does not instruct to dilute the high fortifications with control matrix in the event that matrix-matched standards are needed. A draft Method Modification Memo for Trial #2 (Set V001T) was sent to the sponsor for review/approval.	137G967 Method Modifications memo for Set V001T
12/29/2014	MS→ JJ,SS	Analytical Phase	The Analytical Monitor provided feedback on the method modifications memo proposed for Set V001T. The memo states that in Step 3.4.3 the centrifuge speed would be reduced from 4000 rpm to 2000 rpm to prevent the glass tubes from breaking. The Analytical Monitor felt that this change was a significant change to the method and could be the ultimate cause of the low recovery values seen for Set V001S if the centrifuge speed was 2000 rpm during the extraction of that set.	N/A

12/29/2014	SS→ JJ, MS	Analytical Phase	The Study Director confirmed that the centrifuge speed in Step 3.4.3 was reduced to 2000 rpm for Set V001S. Since the sponsor had specifically requested that Set V001S be extracted in glass tubes, it was not possible to centrifuge the glass tubes at 4000 rpm without the tubes shattering. Thus, the centrifuge speed had to be lowered to accommodate the glass tubes requested by sponsor. Since the sponsor has expressed that polypropylene tubes are not to be used and now expressed that the centrifuge speed of 4000 must also be maintained, the CRO requested that the sponsor send Teflon centrifuge tubes.	N/A
12/30/2014	MS→ JJ,SS	Analytical Phase	The sponsor will be sending Teflon tubes for soil analysis. Teflon tubes are the required extraction vessel.	N/A
12/31/2014	MS→ JJ,SS	Analytical Phase	The sponsor stated that the next trial (V001T) will now be considered as Trial #1 since the previous trial (V001S) did not follow the method exactly (the centrifuge speed was reduced to allow for the use of glass tubes as the extraction vessel).	N/A
1/6/2015	SS→ JJ, MS	Analytical Phase	The Study Director confirmed receipt of the Teflon tubes from the sponsor.	N/A
1/6/2015	SS→ JJ, MS	Analytical Phase	The Study Director provided the sponsor with the proposed method modifications memo for Set V001T for review/approval prior to proceeding.	Updated method modifications memo for Set V001T.
1/13/2015	SS→ JJ, MS	Analytical Phase	The Study Director provided the sponsor with a summary of the quizalofop-p and quizalofop-p-ethyl data from Set V001T. The samples were analyzed with the sponsor gradient in Set V001TAJ. The sample extracts were also analyzed in Set V001TAK with additional equilibration time added to the end of the gradient (to compensate for the different LC systems used during validation and ILV). The calibration linearity improved significantly in Set V001TAK compared to Set V001TAJ.	Excel summaries and Analyst PDF reports for Sets V001TAJ and V001TAK
1/14/2015	SS→ JJ, MS	Analytical Phase	The Study Director provided the sponsor with a summary of the 3-OH-quizalofop-acid data from Set V001T. The LC gradient was modified to add an additional 2 minutes of equilibration time at the end of the run.	Excel summaries and Analyst PDF reports for Sets V001TBL and V001TBM
1/15/2015	SS→ JJ, MS	Analytical Phase	The Study Director notified the sponsor of an additional method change for quizalofop-p and quizalofop-p-ethyl determination. The column the CRO was instructed to use was a 50 mm column, but the Study Director used a 100 mm column. The Study Director sent chromatography from a method development experiment that demonstrated with the 50 mm column the quizalofop-p peak shapes were poor (quantitation peak had interference peak and confirmatory peak was a poorly resolved doublet). The use of the 100 mm column resolved the chromatography issues by providing additional theoretical plates for separation.	Analyst PDF of chromatography with both 50 mm and 100 mm column for peak shape comparison
1/16/2015	SS→ JJ, MS	Analytical Phase	At the request of the sponsor, the Study Director reprocessed the quizalofop-p and quizalofop-p-ethyl data from Set V001TAK to exclude the 1.0 ng/mL calibration standard.	Updated Excel summaries and Analyst PDF reports for quizalofop-p and quizalofop-p-ethyl in Set V001TAK.

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 date. A record of the phone conference was documented by the Study Director and sent to BASF for review. The analytical work for this ILV study is now completed.	Memo to document key points from EPL/BASF conference call on 1/16/2015
2/23/2015	SS→ JJ, MS	Report Phase	The Study Director sent BASF a copy of the draft study report (reviewed by EPL Quality Assurance).	Draft Report

EPL Bio Analytical Services (EPL BAS)

EPL BAS Study No.: 137G967/ BASF 437875

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 V001T, the 3-OH-quizalofop-acid data is accepted from Sets V001TBL and V001TBM.
- For Set V001T, the quizalofop-p and quizalofop-p-ethyl data is accepted from Set V001TAJ.
- No additional trials will be needed for this ILV study.