

1.0 INTRODUCTION

1.1 Purpose of the Study

The purpose of this study is to perform an Independent Method Validation of Analytical Method R0034/01: Method for the Quantitation of the Diastereomeric Forms of BAS 311 I (Reg. 127266) and Its Metabolites 3-Phenoxybenzoic Acid (Reg. No. 130213) and DCVA (Cis and Trans Isomers, Reg. No. 180011) in Soil by LC-MS/MS"

1.2 Summary of the Results

The independent laboratory validation of the BASF method was successfully completed in the first trial.

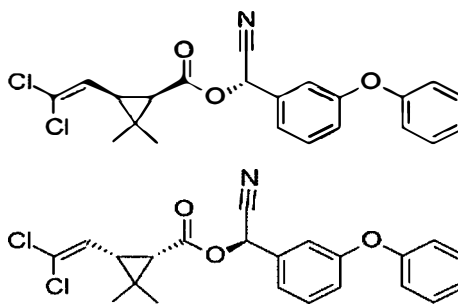
2.0 REFERENCE SUBSTANCE AND SAMPLE HISTORY

2.1 Reference Materials

Reference substances Reg. No 4111341, Reg. No 4111342, 3-Phenoxybenzoic Acid, and DCVA isomers were used for the fortifications and the LC-MS/MS calibration. Concentrated (stock), fortification, and calibration standards were prepared according to the analytical method. Examples of standard solution preparations are presented in **Table 11.8**. A brief description of the reference standards used in this study are presented below.

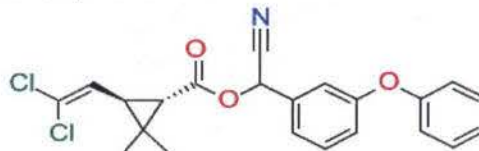
Common Name:	Alpha-cypermethrin
BASF Code Name:	BAS 310 I
BASF Registry Number:	4078193
CAS Number:	67375-30-8
Molecular Formula:	C ₂₂ H ₁₉ Cl ₂ NO ₃
Molecular Weight:	416.3
Batch Number:	AC9575-006
Purity:	99.8% ± 1.0%
Expiration Date:	September 1, 2016

Structural Formula:

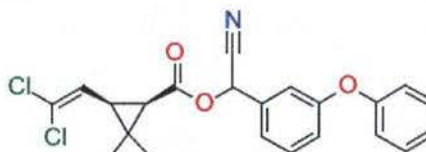


Common Name:	Trans III/ Trans IV Isomers =43.5: 56.5
BASF Registry Number:	4111342
CAS Number:	211504-94-8
Molecular Formula:	C ₂₂ H ₁₉ Cl ₂ NO ₃

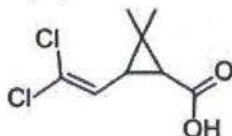
Molecular Weight: 416.3
Batch Number: AC8949-77
Purity: 99.8% ± 1.0%
Expiration Date: June 1, 2020
Structural Formula:



Common Name: Cis I / Cis II Isomers = 45:55
BASF Registry Number: 4111341
CAS Number: 211504-93-7
Molecular Formula: $C_{22}H_{19}Cl_2NO_3$
Molecular Weight: 416.3
Batch Number: AC8949-76
Purity: 99.8% ± 1.0%
Expiration Date: August 1, 2015
Structural Formula:

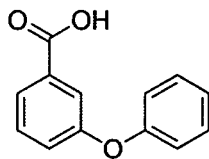


Common Name: DCVA (Mixture of Cis/Trans Isomers) = 51.5:48.5
BASF Registry Number: 180011
CAS Number: 55701-05-8
Molecular Formula: $C_8H_{10}Cl_2O_2$
Molecular Weight: 209.1
Batch Number: AC9966-87
Purity: 99.0% ± 1.0%
Expiration Date: May 1, 2023
Structural Formula:



Common Name: 3-Phenoxybenzoic acid (3-PBA)
BASF Registry Number: 130213
CAS Number: 3739-38-6
Molecular Formula: $C_{13}H_{10}O_3$
Molecular Weight: 214.2
Batch Number: AC12251-34
Purity: 100.0% ± 1.0%
Expiration Date: December 1, 2020

Structural Formula:



The performance of the instrument was evaluated during the injection set. Standard solutions prepared for this study were stored under refrigerated conditions in refrigerator LETS#118 which had a temperature range of 2°C – 8°C during the course of the study.

2.2 Test System

Soil matrix was provided by BASF. Soil matrix was sent from BASF Crop Protection, Inc. on August 07, 2013 and received by PASC on August 09, 2013.

The extracted solutions were stored under refrigerated conditions in refrigerator LETS#118 which had a temperature range of 2°C – 8°C during the course of the study.

3.0 PROCEDURE - METHOD SYNOPSIS

Method Number R0034/01:

Method for the Quantitation of the Diastereomeric Forms of BAS 311 I (Reg. 127266) and Its Metabolites 3-Phenoxybenzoic Acid (Reg. No. 130213) and DCVA (Cis and Trans Isomers, Reg. No. 180011) in Soil by LC-MS/MS.

3.1 Summary of Analytical Procedure

For the extraction and analysis of diastereomeric forms of BAS 311 I, a 5 g sample of soil was weighed into a 250 mL wide-mouth powder bottle for each condition. Conditions included one reagent blank, two matrix blanks, five samples at LOQ, and five samples at 10x LOQ. Each sample had 50 mL of extraction solvent consisting of Acetonitrile with 0.1% Formic Acid added before being shaken at approximately 300 RPM for 30 minutes. A 20 mL aliquot was taken from each sample and then each sample aliquot was centrifuged for 5 minutes at 3600 RPM. 10 mL of the supernatant was transferred volumetrically to a culture tube. These samples were then evaporated to dryness in a 50°C water bath using nitrogen. Samples were then reconstituted in 2 mL of final volume solvent containing water/acetonitrile (1:1, v/v) with 0.1% Formic Acid, sonicated for 1 minute, vortexed for 15 seconds, and then transferred to a HPLC vial.

For the extraction and analysis of metabolites 3-PBA and DCVA isomers, a 5 g sample of soil was measured into a glass centrifuge tube for each condition. Conditions included one reagent blank, two matrix blanks, five samples at LOQ, and five samples at 10x LOQ. Each sample had 25 mL of extraction solvent, consisting of acetonitrile/water (70:30, v/v) added before being shaken at 300 RPM for 30 minutes. Samples were then centrifuged for 5 minutes at 3600 RPM at 0°C and the supernatant was decanted into a separate glass centrifuge tube. Another 25 mL of extraction solvent was added to the soil matrix. The samples were then vortexed, shaken for 30 minutes, and finally centrifuged at 3600 RPM at 0°C. The supernatant was decanted into the same centrifuge tube containing the first 25 mL extract. The combined extracts were vortexed and centrifuged at 3600 RPM at 0°C. A 5 mL aliquot of the mixed extract was transferred to a culture tube and evaporated to dryness in a 50°C water bath using nitrogen. Once dry, samples were reconstituted in 1 mL of final volume solvent containing methanol/water (20:80, v/v) with 0.1% Formic Acid. Samples were sonicated for 1 - 2 minutes, vortexed for 15 seconds, and

transferred to an HPLC vial. Results were determined by HPLC. Instrument parameters are described in the table below.

Residue results were determined by LC-MS/MS. Five different LC-MS/MS methods were validated. During the validation, a Shimadzu HPLC was used instead of an Agilent 1200 for all of the HPLC/MS/MS methods. An AB Sciex 4000 was used in Method E and Method F instead of an AB Sciex 5500 with good sensitivity and selectivity. An AB Sciex 5000 was used in Method B (BAS 311 I method) and Method I instead of an AB Sciex 5500 with good sensitivity and selectivity. Method G was modified to Method I to improve peak shape. All the detailed instrument parameters for Method B, Method C, Method E, Method F, and Method I are listed in **Table 11.9**.

The LC-MS/MS analysis was conducted as shown in the following tables.

Instrument & Transition Monitored	BAS 311 I isomers			
	Cis II	Cis I	Trans III	Trans IV
UPLC (Method B)				
Primary Quantification	m/z 433→191	m/z 433→191	m/z 433→191	m/z 433→191
Confirmatory Quantification	m/z 435→193	m/z 435→193	m/z 435→193	m/z 435→193
HPLC (Method C)				
Primary Quantification	m/z 433→191	m/z 433→191	m/z 433→191	m/z 433→191
Confirmatory Quantification	m/z 435→193	m/z 435→193	m/z 435→193	m/z 435→193
Instrument & Transition Monitored	Metabolites			
	PBA	DCVA Cis	DCVA Trans	
UPLC				
Primary Quantification	m/z 213→93 (Method I)	m/z 207→207 (Method I)	m/z 207→207 (Method I)	
Confirmatory Quantification	m/z 213→93 (Method F)	m/z 209 (Method E)	m/z 209 (Method E)	
HPLC				
Primary Quantification	m/z 213→93 (Method E)	m/z 207 (Method E)	m/z 207 (Method E)	
Confirmatory Quantification	m/z 213→93 (Method F)	m/z 207 (Method F)	m/z 207 (Method F)	

4.0 LIMIT OF QUANTITATION AND DETECTION

The method limit of quantitation (LOQ) for residues per analyte in soil was 0.001 mg/kg and the method limit of detection (LOD) was set at 0.0002 mg/kg (20% of LOQ).

Sub-samples were fortified with BAS 800 H and its metabolites at the LOQ and at 10 times the LOQ and analyzed using Analytical Method R0034/01.

5.0 CALIBRATION, CALCULATIONS, AND STATISTICS

Quantitation of residues in all samples was achieved using an external calibration curve calculated using a linear regression of instrument responses for the reference substances at multiple concentrations.

A standard curve was generated by injecting standard solutions at appropriate concentrations for each analyte. Calibration standard concentrations for diastereomeric forms of BAS 311 I and its metabolites 3-PBA and DCVA isomers ranged from 0.10 – 10.0 ng/mL. Each calibration standard was injected in duplicate. Analyst® 1.4.2 and 1.5.1 software created the standard curve based on linear regression using 1/x. The regression functions were used to calculate the best-fit line by plotting the analyte found (ng) on the x-axis versus the detector's peak response (peak area) on the y-axis. The calibration curves are presented in **Figures 12.1 - 12.7** for BAS 311 I and isomers, 3-PBA, and DCVA isomers, respectively. Representative chromatograms of calibration standards for BAS 311 I and isomers, 3-PBA, and DCVA isomers are presented in **Figures 12.8 - 12.14**, respectively. The performance of the instrument was evaluated during each injection set.

Peak integration and quantitation were performed within Analyst® 1.4.2 and 1.5.1 software using the calibration curve equation to determine the amount of analyte found (ng/mL) during sample analysis. Recovery results were calculated for each set of samples using Microsoft® Office Excel spreadsheets which are presented in **Appendix A**.

Calculation of results is based on area measurements. The recoveries and residues of BAS 311 I and its isomers, 3-PBA, and DCVA isomers in ppm were calculated using the following formulas:

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

- V_{end} = Final volume of the extract after all dilution steps [mL]
 C_A = Concentration of analyte as read from the calibration curve [ng/mL]
 G = Weight of the sample extracted [g]
 A_F = Aliquotation factor
1000 = Factor remaining after all unit conversions

The recoveries of spiked compounds were calculated using the following equation:

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

As an example, calculations to obtain Alpha-cypermethrin (Cis II) UPLC Quantification primary transition LOQ-1 recovery results are shown below:

$$\text{Residue in fortified sample (mg/kg)} = \frac{2\text{mL} \times 0.515\text{ng/mL}}{5.0\text{g} \times 0.2 \times 1000} = 0.00103\text{mg/kg}$$

$$\text{Amount of analyte fortified (mg/kg)} = \frac{0.11\mu\text{g/mL} \times 0.05\text{mL}}{5.0\text{g}} = 0.0011\text{mg/kg}$$

$$\text{Recovery\%} = \frac{(0.0103\text{mg/kg} - 0\text{mg/kg}) \times 100}{0.0011\text{mg/kg}} \times 100 = 93.6\%$$

7.0 COMMUNICATION

The sponsor was contacted by the study director by phone and e-mail on 09/04/2013 regarding the MS detection of confirmatory transition of 3-PBA. Protocol amendment #1 was issued on 09/06/2013 to change the confirmatory UPLC method for PBA to Method B and the confirmatory HPLC method for PBA to Method F.

The sponsor contacted the study director on 09/18/2013 by e-mail to change the study title. Protocol amendment #2 was issued on 09/25/2013 to correct the study title.

The sponsor was contacted by the study director by phone and e-mail on 10/01/2013 regarding the LC-MS/MS methods used in the validation. Protocol amendment #3 was issued on 10/02/2013 to list all the changes in the LC-MS/MS method validation.

The independent laboratory method validation of Method R0034/01 was successfully completed and the Study Monitor was informed of the successful completion of the study at the end of the trial on 09/23/2013.

RECOMMENDATION

The following recommendations should be incorporated into the technical procedure:

Section 4.2.4: Calculation of Residues and Recoveries

1. The calculation of residues of the diastereomeric forms of BAS 311 I and metabolites 3-PBA and DCVA isomers should all be listed.
2. The aliquotation factor should be specified for both the BAS 311 I isomers and the metabolites.

8.0 PROTOCOL CHANGES

1. Protocol amendment #1 was issued on 09/06/2013 to change the confirmatory UPLC method for PBA to Method B and the confirmatory HPLC method for PBA to Method F.
2. Protocol amendment #2 was issued on 09/25/2013 to correct the study title.
3. Protocol amendment #3 was issued on 10/02/2013 to list all the changes in the LC-MS/MS method validation
4. Protocol amendment #4 was issued on 07/24/2014 to document study director change, and correction of the typo in the protocol title.

Table 11.8 Example Standard Solutions Preparation and Dilution Data

Typical Analytical Standards Dilutions and Use Records for BAS 311 I Isomers

Concentrated Standard Solutions (Solvent: Acetonitrile)							
Analytical Standard	Analyte	Isomer	Conc. Standard No.	Amount Weighed (mg)	Final Dilution Vol. (mL)	Final Conc. (mg/mL)	Prep. Date
AC8949-76	Cis I / Cis II Isomers	Cis I	S08281301	20.02	10	0.9009	08/28/13
		Cis II				1.101	
AC8949-77	Trans III / Trans IV Isomers	Trans III	S08281302	20.01	10	0.8704	
		Trans IV				1.131	

Intermediate Standard and Fortification Standard Solutions (Solvent: Acetonitrile + 0.1% Formic Acid)							
Intermediate Standard	Analyte	Conc. Standard No.	Conc. (mg/mL)	Aliquot Vol. (mL)	Dilution Vol. (mL)	Final Conc. (µg/mL)	Prep. Date
F08281301	Cis I	S08281301	0.9009	0.50	50	9.009	08/28/13
	Cis II		1.101			11.01	
	Trans III	S08281302	0.8704	0.50		8.704	
	Trans IV		1.131			11.31	
F08281302	Cis I	F08281301	0.009009	5	50	0.9009	
	Cis II		0.01101			1.101	
	Trans III		0.008704			0.8704	
	Trans IV		0.01131			1.131	
F08281303	Cis I	F08281302	0.0009009	5	50	0.009009	
	Cis II		0.001101			0.1101	
	Trans III		0.0008704			0.08704	
	Trans IV		0.001131			0.1131	

Calibration Standard Solutions (Solvent: ACN:H2O (1:1 v/v) + 0.1% Formic Acid)							
Working Standard	Analyte	Parent Standard No.	Conc. (ng/mL)	Aliquot Vol. (mL)	Dilution Vol. (mL)	Final Conc. (ng/mL)	Prep. Date
C08281301	Cis I	S08281301	900,900	0.05	50	900.9	08/28/13
	Cis II		1,101,100			1101	
	Trans III	S08281302	870,400	0.05		870.4	
	Trans IV		1,131,000			1131	
C08281302	Cis I	C08281301	900.9	0.5	50	9.009	
	Cis II		1101			11.01	
	Trans III		870.4			8.704	
	Trans IV		1131			11.31	
C08281303	Cis I	C08281301	900.9	0.25	50	4.505	
	Cis II		1101			5.506	
	Trans III		870.4			4.352	
	Trans IV		1131			5.653	
C08281304	Cis I	C08281302	9.01	5	50	0.9009	
	Cis II		11.01			1.101	
	Trans III		8.704			0.8704	
	Trans IV		11.31			1.131	
C08281305	Cis I	C08281303	4.505	5	50	0.4505	
	Cis II		5.506			0.5506	
	Trans III		4.352			0.4352	
	Trans IV		5.653			0.5653	
C08281306	Cis I	C 08281304	0.9009	5	50	0.09009	
	Cis II		1.101			0.1101	
	Trans III		0.8704			0.08704	
	Trans IV		1.131			0.1131	

Typical Analytical Standards Dilutions and Use Records for 3-PBA and DCVA Isomers

Concentrated Standard Solutions (Solvent: Methanol)							
Analytical Standard	Analyte	Isomer	Conc. Standard No.	Amount Weighed (mg)	Final Dilution Vol. (mL)	Final Conc. (mg/mL)	Prep. Date
AC9966-87	DCVA	Cis	S08281303	10.08	5.0	1.038	08/28/13
		Trans				0.9778	
AC12251-34	3-PBA	3-PBA	S08281304	5.08	5.0	1.016	

Intermediate Standard and Fortification Standard Solutions (Solvent: Methanol)							
Intermediate Standard	Analyte	Conc. Standard No.	Conc. (mg/mL)	Aliquot Vol. (mL)	Dilution Vol. (mL)	Final Conc. (µg/mL)	Prep. Date
F08291301	Cis DCVA	S08281303	1.038	0.50	50.0	10.38	08/29/13
	Trans DCVA		0.9778			9.778	
	3-PBA	S08281304	1.016			10.16	
F08291302	Cis DCVA	F08291301	10.38	5.0	50.0	1.038	
	Trans DCVA		9.778			0.9778	
	3-PBA		10.16			1.016	
F08291303	Cis DCVA	F08291302	1.038	5.0	50.0	0.1038	
	Trans DCVA		0.9778			0.09778	
	3-PBA		1.016			0.1016	

Calibration Standard Solutions (Solvent: MeOH:H2O (4:1 v/v) + 0.1% Formic Acid)							
Working Standard	Isomer	Parent Standard No.	Conc. (ng/mL)	Aliquot Vol. (mL)	Dilution Vol. (mL)	Final Conc. (ng/mL)	Prep. Date
C08291301	Cis DCVA	S08281303	1,038,000	0.05	50.0	1,038	08/29/13
	Trans DCVA		978,000			977.8	
	3-PBA	S08281304	1,016,000			1,016	
C08291302	Cis DCVA	C08291301	1,038	0.50	50.0	10.38	
	Trans DCVA		977.8			9.778	
	3-PBA		1,016			10.16	
C08291303	Cis DCVA	C08291301	1,038	0.25	50.0	5.191	
	Trans DCVA		977.8			4.889	
	3-PBA		1,016			5.080	
C08291304	Cis DCVA	C08291302	10.38	5.0	50.0	1.038	
	Trans DCVA		9.778			0.9778	
	3-PBA		10.16			1.016	
C08291305	Cis DCVA	C08291302	10.38	2.5	50.0	0.5191	
	Trans DCVA		9.778			0.4889	
	3-PBA		10.16			0.5080	
C08291306	Cis DCVA	C08291303	5.191	2.5	50.0	0.2596	
	Trans DCVA		4.889			0.2444	
	3-PBA		5.080			0.2540	
C08291307	Cis DCVA	C08291304	1.038	5.0	50.0	0.1038	
	Trans DCVA		0.9778			0.0978	
	3-PBA		1.016			0.1016	

Table 11.9. Instrument Conditions and Parameters

Primary and Confirmatory Quantification Method B (UPLC Mode) for the Analysis of BAS311 I

Waters UPLC			
Column:	Acquity UPLC HSS T3 1.8µm 150 × 2.1 mm		
Temperature:	62 °C ^a		
Flow (µL/min):	350		
Gradient:	Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
	0.0	90.0	10.0
	1.0	90.0	10.0
	6.0	22.0	78.0
	18.0	22.0	78.0
	18.1	2.0	98.0
	19.0	0.0	98.0
	19.1	90.0	10.0
22.1	90.0	10.0	
Mobile Phase A:	0.1% formic acid, 4mM ammonium formate in water		
Mobile Phase B:	0.1% formic acid, 4mM ammonium formate in MeOH		
Injection Vol.:	20 µL ^b		

MS/MS Conditions		
Interface	AB SCIEX 5000 Triple Quad	
Polarity	positive	
Temperature (TEM)	600 °C	
Scan type	MRM	
MRM Conditions	Alpha-cypermethrin/Cis I Isomer/Trans III Isomer/ Trans IV Isomer	
	Primary	Confirmatory
Q1 m/z	433.0	191.0
Q3 m/z	435.0	193.0
Retention Time (min)	Alpha-cypermethrin ~13.3 Cis Isomer ~13.8 Trans III Isomer ~13.1 Trans IV Isomer ~12.9	

^a The original temperature in method B was 60°C.

^b The original Injection volume in method B was 30µL.

Primary and Confirmatory Quantification Method C (HPLC Mode) for the Analysis of BAS311 I

Shimadzu LC-10ADvp			
Column:	XSelect HSS T3 2.5µm 150 × 2.1 mm		
Temperature:	60 °C		
Flow (µL/min):	288		
Gradient:	Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
	0.0	95.0	5.0
	1.0	95.0	5.0
	6.0	22.0	78.0
	27.0	22.0	78.0
	27.1	2.0	98.0
	28.1	2.0	25.0
	28.2	95.0	5.0
	31.2	95.0	5.0
Mobile Phase A:	0.1% formic acid, 4mM ammonium formate in water		
Mobile Phase B:	0.1% formic acid, 4mM ammonium formate in MeOH		
Injection Vol.:	20 µL		

MS/MS Conditions		
Interface	AB SCIEX 4000 Triple Quad	
Polarity	positive	
Temperature (TEM)	400 °C ^a	
Scan type	MRM	
MRM Conditions	Alpha-cypermethrin/Cis I Isomer/Trans III Isomer/ Trans IV Isomer	
	Primary	Confirmatory
Q1 m/z	433.3	191.2
Q3 m/z	435.2	193.2
Retention Time (min)	Alpha-cypermethrin ~16.5 Cis Isomer ~17.0 Trans III Isomer ~16.2 Trans IV Isomer ~15.9	

^a The original temperature in method C was 500°C.

Primary Quantification Method I (UPLC Mode) for the Analysis of 3-PBA and DCVA Isomers

Agilent 1200			
Column:	Acquity UPLC HSS T3 1.8µm 50 × 2.1 mm		
Temperature:	60 °C		
Flow (µL/min):	400		
Gradient: ^a	Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
	0.0	90	10
	0.5	90	10
	8.5	35	65
	8.6	95	5
	11.0	95	5
	11.1	90	10
	14.0	90	10
Mobile Phase A:	DI water		
Mobile Phase B:	Acetonitrile		
Injection Vol.:	50 µL		

MS/MS Conditions			
Interface	AB SCIEX 5000 Triple Quad		
Polarity	Negative		
Temperature (TEM)	550 °C ^b		
Scan type	MRM		
MS Conditions	3-PBA	DCVA Cis	DCVA Trans
	Primary	Primary	Primary
Q1 m/z	213	207	207
Q3 m/z	93	207	207
Retention Time (min)	~4.2	~4.6	~5.5

^a Gradient was modified based on method G due to the peak shape issue.

^b The original temperature in Method G was 600°C.

Primary and Confirmatory Quantification Method E (HPLC Mode) for the Analysis of 3-PBA and DCVA Isomers

Shimadzu LC-10ADvp			
Column:	BEH Phenyl 2.5µm 100 × 2.1 mm		
Temperature:	60 °C		
Flow (µL/min):	350		
Gradient:	Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
	0.0	100.0	0.0
	0.5	100.0	0.0
	14.0	25.0	75.0
	14.1	100.0	0.0
	17.0	100.0	0.0
Mobile Phase A:	DI water		
Mobile Phase B:	Acetonitrile		
Injection Vol.:	50 µL		

MS/MS Conditions					
Interface	AB SCIEX 4000 Triple Quad				
Polarity	Negative				
Temperature (TEM)	550 °C ^a				
Scan type	MRM for 3-PBA, Q 1 for DCVA				
MS Conditions	3-PBA	DCVA Cis		DCVA Trans	
	Primary	Primary	Confirmatory	Primary	Confirmatory
Q1 m/z	213	207	209	207	209
Q3 m/z	93	N/A	N/A	N/A	N/A
Retention Time (min)	~8.8	~10.3	~10.3	~9.7	~9.7

^aThe temperature in original method E was 600°C.

Confirmatory Quantification Method F (HPLC Mode) for the Analysis of 3-PBA and DCVA Isomers

Shimadzu LC-10ADvp				
Column:	XSelect HSS T3 2.5µm 150 × 2.1 mm			
Temperature:	60 °C			
Gradient:	Time (min)	Flow Rate (µL/min)	Mobile Phase A (%)	Mobile Phase B (%)
	0.0	500	100.0	0.0
	15.0	500	30.0	70.0
	15.1	700 ^a	5.0	95.0
	17.0	700 ^a	5.0	95.0
	17.1	500	100.0	0.0
	20.0	500	100.0	0.0
Mobile Phase A:	DI water			
Mobile Phase B:	Acetonitrile			
Injection Vol.:	50 µL			

MS/MS Conditions			
Interface	AB SCIEX 4000 Triple Quad		
Polarity	Negative		
Temperature (TEM)	550 °C ^b		
Scan type	MRM for 3-PBA, Q 1 for DCVA		
MS Conditions	3-PBA	DCVA Cis	DCVA Trans
	Confirmatory	Confirmatory	Confirmatory
Q1 m/z	213	209	209
Q3 m/z	93	N/A	N/A
Retention Time (min)	~10.0	~12.0	~11.0

^a The flow rate was changed to 700 µL/min instead of 800 µL/min due to the high system pressure. The gradient from 15.1min to 17.0 min was used for column wash.

^b The temperature in the original method was 600°C.