1.0 Introduction

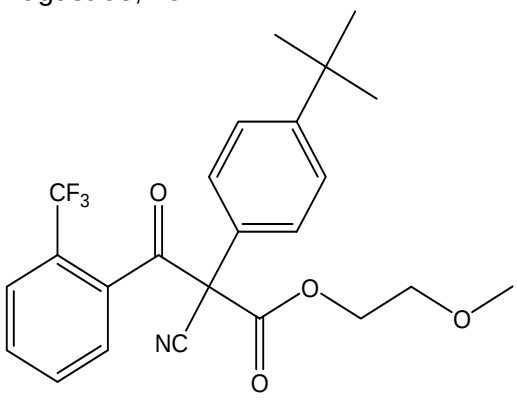
1.1 Objectives of the Study

The objective of this study was to validate BASF method D1002 for the analysis of residues of BAS 9210 I (5465430) and its metabolites in soil samples at a limit of quantitation (LOQ) of 0.01 ppm for four different soil types including sandy loam (0–3”depth), silt loam (12–18” depth), loamy sand (high pH, 12–18” depth), and sandy loam (German soil).

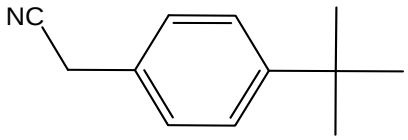
2.0 Materials

2.1 Test and Reference Substances

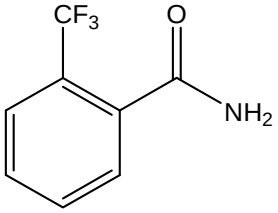
The reference standards shown below were used in the analytical portion of this study. The reference substances were stored in freezer E-109 until they were utilized in this study. Freezer E-109 had a temperature range of −21 to −17 °C.

BASF Code Name:	BAS 9210 I
Common Name:	Cyflumetofen
Source:	Otsuka Chemical Co., Ltd.
BASF Registry Number:	5465430
CAS Number:	400882-07-7
IUPAC Name:	2-methoxyethyl-(*R*,*S*)-2-(4-*tert*-butylphenyl)-2-cyano-3-oxo-3-(α,α,α-trifluoro-*o*-tolyl) propionate
Molecular Formula:	$C_{24}H_{24}F_3NO_4$
Molecular Weight:	447.5 g/mol
Lot Number:	006005
Purity:	99.5%
Date Assayed:	August 04, 2009
Expiration Date:	August 03, 2012
Chemical Structure:	

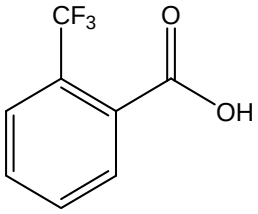
BASF Code Name: A-2
Source: BASF, Limburgerhof, Germany
BASF Registry Number: 133276
CAS Number: 3288-99-1
IUPAC Name: (4-*tert*-butylphenyl) acetonitrile
Molecular Formula: $C_{12}H_{15}N$
Molecular Weight: 173.3 g/mol
Lot Number: L84-64
Purity: 98.5%
Date Assayed: December 14, 2010
Expiration Date: December 1, 2012
Chemical Structure:



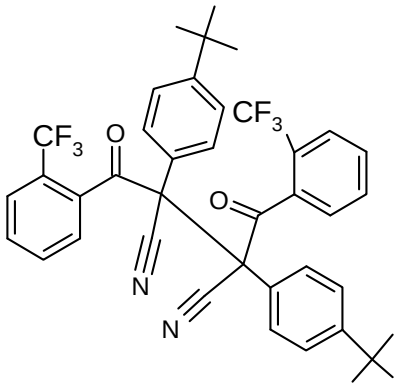
BASF Code Name: B-3
Source: Sigma–Aldrich
BASF Registry Number: 4288294
CAS Number: 360-64-5
IUPAC Name: 2-(trifluoromethyl) benzamide
Molecular Formula: $C_8H_6F_3NO$
Molecular Weight: 189.13 g/mol
Lot Number: 08721EB
Purity: 99.9%
Date Assayed: N/A
Expiration Date: January 7, 2012
Chemical Structure:



BASF Code Name: B-1
Common Name: trifluoro-*o*-toluic acid
Source: Sigma–Aldrich
BASF Registry Number: 104415
CAS Number: 433-97-6
IUPAC Name: 2-(trifluoromethyl) benzoic acid
Molecular Formula: $C_8H_5F_3O_2$
Molecular Weight: 190.12 g/mol
Lot Number: MKBB8135
Purity: 99.4%
Date Assayed: December 2008
Expiration Date: December 7, 2011
Chemical Structure:



BASF Code Name: AB-1 Dimer
Source: BASF, Limburgerhof, Germany
BASF Registry Number: 5756389
CAS Number: N/A
Molecular Formula: $C_{40}H_{34}F_6N_2O_2$
Molecular Weight: 688.7 g/mol
Lot Number: L84-54
Purity: 92.9%
Date Assayed: December 13, 2010
Expiration Date: December 1, 2012
Chemical Structure:



Reference substances for BAS 9210 I, A-2, B-3, B-1, and AB-1 dimer were used for fortifications and LC-MS/MS calibration. The stock solutions were prepared as 1.0 mg/mL in acetonitrile, except for AB-1 dimer, which was prepared as 0.1 mg/mL in methanol. The subsequent dilutions of BAS 9210 I, B-1, B-3, and A-2 stock solutions were prepared in 0.1% formic acid in acetonitrile and AB-1 dimer was prepared in acetonitrile/water (85:15, v/v).

The fortification solutions of BAS 9210 I, B-1, and B-3 were diluted in 0.1% formic acid in acetonitrile, A-2 in 0.1% formic acid in acetonitrile, and AB-1 dimer in acetonitrile/water (85:15, v/v).

The calibration solutions of BAS 9210 I, B-1, and B-3 were diluted in 0.1% formic acid in acetonitrile, A-2 in 0.1% formic acid in water/acetonitrile (75:25, v/v), and AB-1 dimer in acetonitrile/water (85:15, v/v).

Standard solutions prepared for this study were stored under refrigerated conditions in refrigerator E-51, which had a temperature range of 2–4 °C during the course of the study.

Standard Solution and Extract Stability

The stability of fortification and calibration standard solutions was established during the soil storage stability study (BASF study 350786). Stability data for calibration standard and fortification solutions is presented in the Method D1002 (Appendix C). Extract stability was established in BASF study number 350786 (Reference 2).

2.2 Sampling Storage and Handling

Control sandy loam (0–3”depth), silt loam (12–18” depth), loamy sand (high pH, 12–18” depth) were obtained from BASF Corporation on September 16, 2011. The German sandy loam soil sample was obtained from BASF Corporation on September 28, 2011.

Upon receipt, samples were logged in and stored in freezer E-16, which had a temperature range of −25 to −7 °C during the course of this study. The Laboratory Information Management System (LIMS) provided a unique laboratory analysis code (e.g., 110929001-001) and is cross-referenced on the detailed residue reports to the assigned unique sample number. Sample extracts awaiting LC-MS/MS analysis were stored in refrigerator E-20, which had a temperature range of 6–7 °C during the course of this study.

2.3 Experimental Design

This study was conducted in compliance with US Environmental Protection Agency (EPA) Good Laboratory Practices (GLP) standards, 40 CFR 160 (Reference 3), EPA EFATE Guidelines Series 835.6100 (Terrestrial Field Dissipation) (Reference 4), 860.1340 Residue Analytical Method (Reference 5), and EC Guidance documents, SANCO/3029/99 rev.4 (2000, pre-registration residue methods) (Reference 6), and SANCO/825/00 rev.6 (2000, post-registration residue methods) (Reference 7).

This report contains information on reference materials, experimental details, summary of the analytical method, calculations, results and discussion, residue data, procedural recovery data, and representative chromatograms.

The method has a limit of quantitation (LOQ) of 0.01 ppm (0.01 mg/kg) for BAS 9210 I and its metabolites in soil matrices. The method LOD was set at 20% of the LOQ for each analyte, which is equivalent to 0.002 ppm.

For the validation, an analytical set consisted of a reagent blank, two unfortified matrix controls, five control samples fortified at the LOQ (0.01 ppm), and five control samples fortified at 10× LOQ (0.1 ppm). Recoveries were corrected for residues found in control samples.

The validation was conducted in six analytical sets consisting of sandy loam (0–3”depth), silt loam (12–18” depth), loamy sand (high pH, 12–18” depth), and sandy loam (German soil).

3.0 Analytical Methods

3.1 Description of the Analytical Procedure

The method validation was conducted using Analytical Method D1002 (Reference 1) Entitled “Determination of Cyflumetofen (BAS 9210 I) and its Metabolites in Soil using LC-MS/MS”.

The LC-MS/MS method conditions used for this validation study are described in Method D1002 (Appendix C). LC-MS/MS Methods H, I, J, and K were used for the analysis of BAS 9210 I, B-1 and B-3, analysis of B-1 for confirmation purposes, analysis of A-2, and analysis of AB-1 dimer, respectively.

The flow diagram of the analytical procedure is provided in Figure 1 (Appendix B).

The technical procedure of this method is attached to this report as Appendix C. A brief description for the technical procedure is provided below:

Residues of cyflumetofen (BAS 9210 I), B-1, B-3, A-2, and AB-1 dimer are extracted from soil matrices with acetonitrile by vortexing, shaking, and centrifuging. Acetonitrile/water (60:40, v/v) is added and the above sample steps are repeated. The combined extract is diluted with 1:1 (v/v) with 0.1% formic acid in acetonitrile for the residue determination of BAS 9210 I, B-1, and B-3. Similarly the extracts are diluted (1:1, v/v) with 0.1% formic acid in water and with acetonitrile, for the analysis of A-2 and AB-1 dimer, respectively. The residues are determined by LC-MS/MS.

All soils were analyzed using a sample size of 0.1 g (Section 3.3 of the technical procedure) except for German soil which used a 5 g sample size (Section 3.2) and LC-MS/MS methods for all analytes in HPLC mode.

3.2 Limits of Quantitation and Detection

The limit of quantitation (LOQ) is defined as the lowest fortification level successfully tested. During this study, an LOQ of 0.01 ppm for BAS 9210 I and its metabolites was confirmed in

different soil matrices. The limit of detection (LOD) was not determined during this study, but was set at 20% of the LOQ for each analyte; equivalent to 0.002 ppm. In addition, a minimum signal to noise ratio (S/N) of 3:1 was used for the lowest standard in the calibration curves.

3.3 Calibration, Calculations, and Statistics

A standard curve was prepared by injecting standard solutions at appropriate concentrations ranging from 0.125–10.0 ng/mL for all analytes. A calibration standard was typically injected every two to four sample injections. Analyst® 1.5.1 created the standard curve based on linear regression, using 1/x weighting. The regression functions were used to calculate the best-fit line by plotting the standard concentrations (ng) on the x-axis versus the detector's peak response (peak area) on the y-axis. Typical calibration curves are presented in Figure 3–7 (Appendix B) and representative chromatograms of calibration standards for BAS 9210 I, A-2, B-3, B-1, and AB-1 dimer are presented in Figures 8–17, respectively (Appendix B). The performance of the instrument was evaluated during each injection set.

Peak integration and quantitation were performed within Analyst 1.5.1 software; using the calibration curve equation to determine sample concentrations of the analyte found during sample analysis. Recovery results and additional sample concentrations were calculated for each set of samples within Microsoft® Office Excel and reported in the analytical data which are presented in Appendix D. Recoveries were corrected for residues detected in the control sample, if applicable. Typical residue (ng found) and percent recovery calculations for LC-MS/MS quantitation are shown below:

The equation used for quantitation is: $y = mx + b$

Where:
- y = peak area
- x = ng found for peak of interest
- m = slope
- b = y-intercept

a) Solving for x: $x = \frac{y - b}{m}$

These equations were used for residue and recovery calculations within Microsoft® Excel:

b) Amount of sample injected (mg) = $\frac{\text{sample weight (g)}}{\text{final volume (mL)}} \times \text{injection size } (\mu\text{L})$

c) ppm = $\frac{\text{ng found}}{\text{mg injected}}$

d) Percent recovery = $\frac{(\text{ppm in the sample} - \text{ppm in the control})}{\text{ppm added}} \times 100$

As an example, calculations to obtain BAS 9210 I recovery results in sandy loam using Lab Code 110929001-001C) from work order WO-11101001 are shown below:

a) ng found = $\frac{11772-288}{2.89e+006} = 0.003971\,\text{ng}$

b) mg of sample injected = $\frac{100.0\,\text{mg}}{1.6\,\text{mL}} \times 0.007\,\text{mL} = 0.4375\,\text{mg}$

c) ppm = $\frac{0.003971\text{ng}}{0.4375\,\text{mg}} = 0.00908\,\text{ppm}$

Average residue found in the control samples (ADPEN Lab Code: 110929001-001A and 110929001-001B) = 0.00000 ppm.

d) Percent recovery = $\frac{(0.00908 - 0.00000)\ \text{ppm}}{0.01\ \text{ppm}} \times 100 = 90.8\%$

Statistical treatment of the data included calculation of means, standard deviations (SD) and percent relative standard deviations (% RSD) and calculations for statistical outliers (Dixon and Grubb's Tests). These calculations were performed using Excel. Results were rounded only for reporting purposes, and no calculations were made with rounded numbers.

Figure 1. Flow Diagram of Analytical Method Number D1002 – Soil Matrices

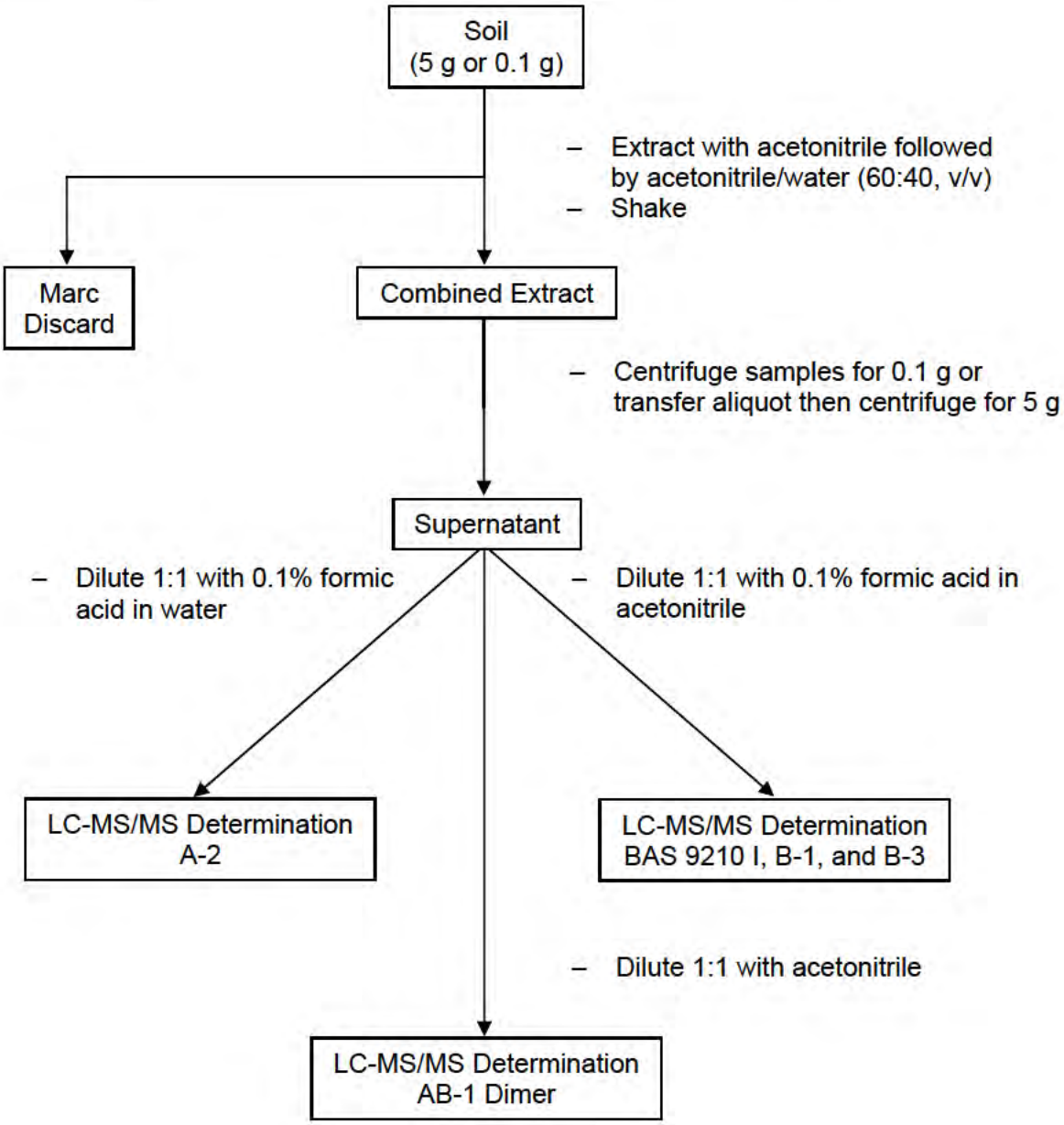


Figure 2. Instrument Conditions and Parameters

LC-MS/MS conditions for the Analysis of BAS 9210 I, B-1, B-3

Agilent 1200 HPLC Conditions				
Column:	Acquity UPLC, HSST3: 1.8 μm × 2.1 mm × 50 mm			
Temperature:	60 °C			
Gradient:	Time (min)	Flow (μL/min)	A (%)	B (%)
	0.00	500	99.0	1.0
	0.10	500	99.0	1.0
	0.40	500	60.0	40.0
	1.35	500	0.0	100
	2.25	500	0.0	100
	2.30	500	99.0	1.0
	3.60	500	99.0	1.0
Mobile Phase A:	0.1% Formic acid in water			
Mobile Phase B:	0.1% Formic acid in acetonitrile			
Injection Volume:	7.0 µL			

MS/MS Conditions						
Interface	AB SCIEX 5500 Triple Quad					
Polarity	Positive (Negative for B-1)					
Curtain gas (CUR)	Nitrogen set at 40.0 (arbitrary units)					
Temperature (TEM)	500 °C					
Collision gas setting (CAD)	10.0					
GS1	45.0					
GS2	45.0					
Entrance potential (EP)	10.0 (B-1: −12.0)					
Scan type	MRM					
MRM Conditions	**BAS 9210 I**		**B-1**		**B-3**	
	Primary	**Secondary**	**Primary**	**Secondary**	**Primary**	**Secondary**
Q1 m/z	448.2	448.2	189.1	189.1	190.0	190.0
Q3 m/z	173.0	145.1	69.0	145.1	130.0	102.0
Dwell Time (msec)	200.0		200.0		200.0	
Expected Retention (min.)	2.40		1.88		1.77	
Declustering potential (DP)	60.0		−110.0		95.0	
Collision energy (CE)	24.0	70.0	−46.0	−18.0	26.0	46.0
Collision cell exit potential	8.0		−10.0	−7.0	14.0	12.0

Figure 2. Instrument Conditions and Parameters (continued)

LC-MS/MS conditions for the Analysis of A-2

Agilent 1200 HPLC Conditions				
Column:	Acquity UPLC, BEH C_{18}: 1.7 μm × 2.1 mm × 50 mm			
Temperature:	50 °C			
Gradient:	Time (min)	Flow (μL/min)	A (%)	B (%)
	0.00	600	70.0	30.0
	0.05	600	70.0	30.0
	0.90	600	20.0	80.0
	1.50	600	1.0	99.0
	2.45	600	1.0	99.0
	2.50	600	70.0	30.0
	3.50	600	70.0	30.0
Mobile Phase A:	1% formic acid in water			
Mobile Phase B:	0.1% formic acid in acetonitrile			
Injection Volume:	40.0 µL			

MS/MS Conditions		
Interface	AB SCIEX 5500 Triple Quad	
Polarity	Positive	
Curtain gas (CUR)	Nitrogen set at 30.0 (arbitrary units)	
Temperature (TEM)	400–500 °C	
Collision gas setting (CAD)	8.0	
GS1	40.0	
GS2	40.0	
Entrance potential (EP)	10.0	
Scan type	MRM	
MRM Conditions	**A-2**	
	Primary	**Secondary**
Q1 m/z	174.1	174.1
Q3 m/z	147.1	117.1
Dwell Time (msec)	200.0	
Expected Retention (min.)	1.80	
Declustering potential (DP)	65.0	
Collision energy (CE)	15.0	45.0
Collision cell exit potential	15.0	14.0

Figure 2. Instrument Conditions and Parameters (continued)

LC-MS/MS conditions for the Analysis of AB-1 Dimer

Agilent 1200 HPLC Conditions				
Column:	Acquity UPLC, BEH C_{18}: 1.7 μm × 2.1 mm × 50 mm			
Temperature:	50 °C			
Gradient:	Time (min)	Flow (μL/min)	A (%)	B (%)
	0.00	500	70.0	30.0
	0.05	500	70.0	30.0
	0.90	500	20.0	80.0
	1.50	500	1.0	99.0
	2.45	500	1.0	99.0
	2.50	500	70.0	30.0
	3.00	500	70.0	30.0
Mobile Phase A:	1% formic acid in water			
Mobile Phase B:	0.1% formic acid in acetonitrile			
Injection Volume:	20.0 µL			

MS/MS Conditions		
Interface	AB SCIEX 5500 Triple Quad	
Polarity	Positive	
Curtain gas (CUR)	Nitrogen set at 30.0 (arbitrary units)	
Temperature (TEM)	500 °C	
Collision gas setting (CAD)	8.0	
GS1	40.0	
GS2	40.0	
Entrance potential (EP)	10.0	
Scan type	MRM	
MRM Conditions	**AB-1 Dimer**	
	Primary	**Secondary**
Q1 m/z	689.4	689.4
Q3 m/z	288.2	268.2
Dwell Time (msec)	200.0	
Expected Retention (min.)	2.70	
Declustering potential (DP)	100.0	
Collision energy (CE)	20.0	37.0
Collision cell exit potential	8.0	

Figure 2. Instrument Conditions and Parameters (continued)

LC-MS/MS conditions for the Analysis of BAS 9210 I, B-1, B-3 in HPLC mode (Sandy Loam, German soil)

Agilent 1200 HPLC Conditions				
Column:	Gemini C_{18}: 3.0 µm × 4.6 mm × 100 mm			
Temperature:	50 °C			
Gradient:	Time (min)	Flow (µL/min)	A (%)	B (%)
	0.00	800	95.0	5.0
	0.10	800	95.0	5.0
	0.60	800	60.0	40.0
	2.80	800	25.0	75.0
	7.00	800	0.0	100.0
	7.50	800	0.0	100.0
	7.60	800	95.0	5.0
	10.60	800	95.0	5.0
Mobile Phase A:	0.1% Formic acid in water			
Mobile Phase B:	0.1% Formic acid in acetonitrile			
Injection Volume:	20.0 µL			

MS/MS Conditions						
Interface	AB SCIEX 5500 Triple Quad					
Polarity	Positive (Negative for B-1)					
Curtain gas (CUR)	Nitrogen set at 40.0 (arbitrary units)					
Temperature (TEM)	500 °C					
Collision gas setting (CAD)	10.0					
GS1	45.0					
GS2	45.0					
Entrance potential (EP)	10.0 (B-1: −12.0)					
Scan type	MRM					
MRM Conditions	**BAS 9210 I**		**B-1**		**B-3**	
	Primary	**Secondary**	**Primary**	**Secondary**	**Primary**	**Secondary**
Q1 m/z	448.2	448.2	189.1	189.1	190.0	190.0
Q3 m/z	173.0	145.1	69.0	145.1	130.0	102.0
Dwell Time (msec)	200.0		200.0		200.0	
Expected Retention (min.)	6.41		4.12		3.35	
Declustering potential (DP)	60.0		−110.0		95.0	
Collision energy(CE)	24.0	70.0	−46.0	−18.0	26.0	46.0
Collision cell exit potential	8.0		−10.0	−7.0	14.0	12.0

Figure 2. Instrument Conditions and Parameters (continued)

LC-MS/MS conditions for the Analysis of A-2 in HPLC mode (Sandy Loam, German soil)

Agilent 1200 HPLC Conditions				
Column:	Gemini C_{18}: 3.0 µm × 4.6 mm × 100 mm			
Temperature:	50 °C			
Gradient:	Time (min)	Flow (µL/min)	A (%)	B (%)
	0.00	800	70.0	30.0
	0.10	800	70.0	30.0
	3.00	800	20.0	80.0
	4.00	800	1.0	99.0
	5.00	800	1.0	99.0
	5.10	800	70.0	30.0
	8.00	800	70.0	30.0
Mobile Phase A:	1% formic acid in water			
Mobile Phase B:	0.1% formic acid in acetonitrile			
Injection Volume:	40.0 µL			

MS/MS Conditions		
Interface	AB SCIEX 5500 Triple Quad	
Polarity	Positive	
Curtain gas (CUR)	Nitrogen set at 30.0 (arbitrary units)	
Temperature (TEM)	400 °C	
Collision gas setting (CAD)	8.0	
GS1	40.0	
GS2	40.0	
Entrance potential (EP)	10.0	
Scan type	MRM	
MRM Conditions	**A-2**	
	Primary	**Secondary**
Q1 m/z	174.1	174.1
Q3 m/z	147.1	117.1
Dwell Time (msec)	200.0	
Expected Retention (min.)	5.18	
Declustering potential (DP)	65.0	
Collision energy (CE)	15.0	45.0
Collision cell exit potential	15.0	14.0

Figure 2. Instrument Conditions and Parameters (continued)

LC-MS/MS conditions for the Analysis of AB-1 Dimer in HPLC mode (Sandy Loam, German soil)

Agilent 1200 HPLC Conditions				
Column:	Acquity UPLC, BEH C_{18}: 1.7 µm × 2.1 mm × 50 mm[1]			
Temperature:	50 °C			
Gradient:	Time (min)	Flow (µL/min)	A (%)	B (%)
	0.00	500	70.0	30.0
	0.05	500	70.0	30.0
	0.90	500	20.0	80.0
	1.50	500	1.0	99.0
	2.45	500	1.0	99.0
	2.50	500	70.0	30.0
	3.00	500	70.0	30.0
Mobile Phase A:	1% formic acid in water			
Mobile Phase B:	0.1% formic acid in acetonitrile			
Injection Volume:	20.0 µL			

[1] LC gradient is operated in standard HPLC system (LC pressure approximately 4400 psi with the flow rate specified)

MS/MS Conditions		
Interface	AB SCIEX 5500 Triple Quad	
Polarity	Positive	
Curtain gas (CUR)	Nitrogen set at 30.0 (arbitrary units)	
Temperature (TEM)	500 °C	
Collision gas setting (CAD)	8.0	
GS1	40.0	
GS2	40.0	
Entrance potential (EP)	10.0	
Scan type	MRM	
MRM Conditions	**AB-1 Dimer**	
	Primary	**Secondary**
Q1 m/z	689.4	689.4
Q3 m/z	288.2	268.2
Dwell Time (msec)	200.0	
Expected Retention (min.)	2.80	
Declustering potential (DP)	100.0	
Collision energy (CE)	20.0	37.0
Collision cell exit potential	8.0	

1. INTRODUCTION

1.1 SCOPE OF THE METHOD

BAS 9210 I is a new insecticide that will be used for various crops (Citrus and fruiting vegetables) in the US, Canada and Europe. For registration of this insecticide and for establishing the DT50/90 values from field dissipation studies for these use patterns, a residue analytical method with a limit of quantitation of 0.01 mg/kg for the active ingredient and its metabolites in soil is developed.

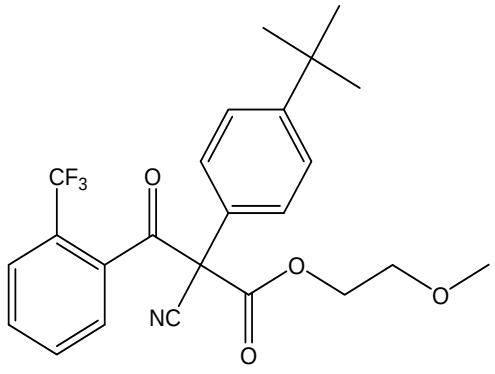
2. MATERIALS

Standard substances are stored in a freezer until use. Information on the characterization of these substances is available from BASF and is located at the Landwirtschaftliche Versuchsstation der BASF, Limburgerhof, Germany.

2.1 TEST AND REFERENCE SUBSTANCE

2.1.1 FORTIFICATION COMPOUND

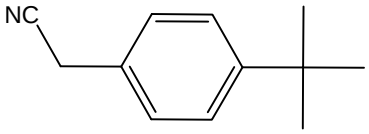
BASF Code Name:	BAS 9210 I (Cyflumetofen)
BASF Registry Number:	5465430
CAS Number:	400882-07-7
Molecular Formula:	$C_{24}H_{24}F_3NO_4$
Molecular Weight:	447.5
Structural Formula:	



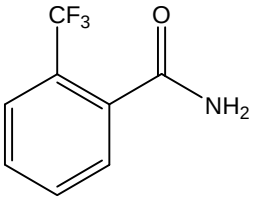
2. Materials (Continued)

BAS Code Name: A-2

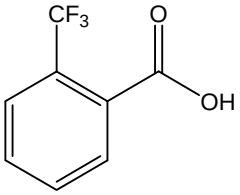
Chemical Name: (4-tert-butylphenyl)acetonitrile
BASF Registry Number: 133276
Molecular Formula: $C_{12}H_{15}N$
Molecular Weight: 173.3
Structural Formula:



BASF Code Name B-3
Chemical Name: 2-(trifluoromethyl)benzamide
BASF Registry Number: 4288294
CAS Number: 360-64-5
Molecular Formula: $C_8H_6F_3NO$
Molecular Weight: 189.13
Structural Formula:

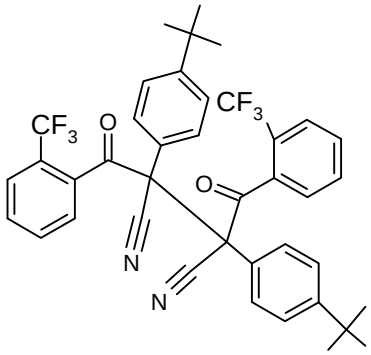


BASF Code Name B-1
Chemical Name: 2-(trifluoromethyl)benzoic acid
BASF Registry Number 104415
CAS Number: 433-97-6
Molecular Formula: $C_8H_5F_3O_2$
Molecular Weight: 190.12
Structural Formula:



2. Materials (Continued)

BASF Code Name	AB-1 Dimer
BASF Registry Number	5756389
Molecular Formula:	$C_{40}H_{34}F_6N_2O_2$
Molecular Weight:	688.7
Structural Formula:	



Reference Standard (used for calibration)

Same as fortification compound (section 2.1.1)

BASF has retained a reserve sample of these chemicals, and has documentation at the BASF Corporation, Research Triangle Park, North Carolina.

2. Materials (Continued)

2.2 EQUIPMENT—SUGGESTED SIZES/SUPPLIERS, MANUFACTURERS

Method Step	Equipment	Size, Description	Manufacturer/ Supplier	Catalog Number
2.4	Balance, Analytical	Model AT100	Mettler	
Various	Balance, Top Loading	Model PM 4800	Mettler	
Various	Bar, Magnetic Stirring	2 inch lengths	Various	
2.4	Bottle, Amber glass	Qorpak , 2 oz, 4 oz and 8 oz with Teflon®-lined screw cap	Qorpak	
3.2, 3.3	Centrifuge	Refrigerated Centrifuge Model CS-6KR	Beckmann	
3.2	Centrifuge Tubes (Teflon®)	50 mL	VWR	21009-477
3.2	Centrifuge Adapter	for 50 mL tubes	VWR	
Various	Wide neck glass bottle	250 mL	Kimble	13-756-393
Various	Cylinder, Graduated	Various sizes	Various	
Various	Flask, Erlenmeyer, 24/40	1000 mL	Various	
Various	Flask, Volumetric	100, 50, 25 ,10 and 5 mL	Various	
3.3	Liquid Handling System	Quadra96®, Model 320 or Quadra 3_{NS} ®	Tomtec Inc.	
Various	MicroMan pipettes	10-1000 µL	Gilson	M-25,M-50,M-250, M-1000
3.3	Multitube Vortexer	VX-2500	VWR	58816-116
Various	Pipet, Volumetric	0.5, 1-10, 25 mL	Various	

2. Materials (Continued)

Method Step	Equipment	Size, Description	Manufacturer/ Supplier	Catalog Number
Various	Pasteur Pipet, disposable	various size	VWR	
Various	Pipet tips	polypropylene	Matrix Inc.	196-205
3.3	Reagent reservoir	Dimpled polypropylene	Tomtec Inc.	
Various	Spatula		Various	
Various	Stopper, Teflon®	24/40	Various	
Various	Ultrasonic Bath	Model FS 7652H	Fisher Scientific	
Various	Vials, Amber Borosilicate	8 and 40 mL	VWR	224984 and 15900-018
3.2, 3.3	Reciprocal Shaker	HS 501 Digital Shaker	IKA	2527001
Various	Vortex mixer	Genie 2	Fisher Scientific Co	12-812
3.4	Filter Plate	AcroPrep, 0.45 um GHP	PALL	5054
3.4	Syringe	1mL	BD	0242421
3.4	Syringe Filter	0.45 um GHP	PALL	4556T
3.3	Well Plates (Storage block)	1.4 mL AlphaNumeric Tubes, 2.2 mL polypropylene with 1200 µL glass tubes	Matrix Inc., Hirschmann Laborgerate	4211, 4253, 924 05 96
3.3	Well Plate caps/seals	SepraSeal	Matrix Inc.	4463
3.7	LC-MS/MS	PE Sciex API 5000	PE Sciex	

NOTE: Other general laboratory glassware and equipment may be needed. Equipment with equivalent performance may be used, as required.

2. Materials (Continued)

2.3 Reagents and Chemicals—Suggested Sources

2.3.1 CHEMICALS

Chemical	Grade	Manufacturer/ Supplier	Catalog Number
Acetonitrile	High Purity	Fisher	A996-4
Formic Acid	98%	Fisher	MFX0440 6
Ammonium Hydroxide	28-30%	Mallinckrodt Chem	3256-14
HPLC Grade Water	High Purity	Fisher	W7-4

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

2.3.2 Solvent Mixtures and their Preparation

Solvent Mixtures	Method Step
Solution I: Acetonitrile-water, 60:40, v/v Add 600 mL of acetonitrile and 400 mL of water into a 1L Erlenmeyer flask and mix well to ensure complete homogeneous solution.	3.3
Solution II Acetonitrile with 0.1 % formic acid Add 1000 mL of acetonitrile and 1 mL of concentrated formic acid into a 1L Erlenmeyer flask and mix well to ensure complete homogeneous solution.	3.4
Solution III: Water with 0.1 % formic acid Add 1000 mL of water and 1 mL of concentrated formic acid into a 1L Erlenmeyer flask and mix well to ensure complete homogeneous solution.	3.4
Solution IV: 0.1% formic acid in Water:Acetonitrile, 75:25, v/v Add 250 mL of acetonitrile and 750 mL of water and 1 mL of concentrated formic acid into a 1L Erlenmeyer flask and mix well to ensure complete homogeneous solution.	3.4

2. Materials (Continued)

Solvent Mixtures	Method Step
Solution VI: Acetonitrile-Water, 85:15 v/v Add 850 mL of acetonitrile and 150 mL of water into a 1L Erlenmeyer flask and mix well to ensure complete homogeneous solution	3.2.3
Mobile Phase Solvent Mixtures: **LC-MS/MS Mobile Phase A: (Method A, E, G, H, I, L)** Water with 0.1 % formic acid (Same as **Solution III)** **LC-MS/MS Mobile Phase A: (Method B, C, D, F, J, K, M, N)** Water with 1 % formic acid Add 990 mL of water and 10 mL of concentrated formic acid into a 1L Erlenmeyer flask and mix well to ensure complete homogeneous solution. **LC-MS/MS Mobile Phase B (Method A and B):** Acetonitrile with 0.1 % formic acid (Same as **Solution II)**	3.7

2.4 STANDARD SOLUTIONS AND THEIR STORAGE STABILITY

2.4.1 STANDARD SOLUTION STORAGE STABILITY

Standard solutions are kept refrigerated. The storage stability of standard solutions made in acetonitrile and acetonitrile with 0.1% formic acid has been established during the course of the soil storage stability study (BASF Study 350786) for all analytes.

See the below table for stability information:

2. Materials (Continued)

2.4.1 STANDARD SOLUTION STORAGE STABILITY (CONT)

Analyte	Standard	Solvent	Duration (days)
BAS 9210 I	Stock	Acetonitrile	96
	Fortification	Acetonitrile with0.1% formic acid	35
	Calibration	Acetonitrile with 0.1% formic acid	35
B-1	Stock	Acetonitrile	96
	Fortification	Acetonitrile with0.1% formic acid	35
	Calibration	Acetonitrile with 0.1% formic acid	35
B-3	Stock	Acetonitrile	96
	Fortification	Acetonitrile with 0.1% formic acid	35
	Calibration	Acetonitrile with 0.1% formic acid	35
A-2	Stock	Acetonitrile	96
	Fortification	Acetonitrile with 0.1% formic acid	35
	Calibration	0.1% FA in Water:Acetonitrile, 75:25, v/v	35
AB-1 Dimer	Stock	Methanol	1 day
	Fortification	Methanol	1 day
	Calibration	Acetonitrile-water, 85:15 v/v	1 day

2.4.2 STANDARD SOLUTIONS

2.4.2.1 Stock solution of BAS 9210 I, B-1, B-3, A-2, (1 mg/mL) and AB-1 Dimer (0.1 mg/mL):

2.4.2.1a Stock solution of BAS 9210 I, B-1, B-3, A-2, (1 mg/mL)

Prepare a 1.0 mg/mL stock solution individually by weighing an appropriate amount of each analyte into a volumetric flask. Dissolve with acetonitrile as described below and dilute to mark

For example, to prepare a 10 mL of 1.0 mg/mL stock solution of BAS 9210 I in acetonitrile, weigh 10 mg BAS 9210 I into a 10 mL volumetric flask. Dissolve and dilute to mark with acetonitrile. Sonicate and vortex to ensure a complete homogeneous solution.

2.4.2.1b Stock solution of AB-1 dimer (0.1 mg/mL)

Prepare a **0.1** mg/mL stock solution of AB-1 Dimer by weighing an appropriate amount of the analyte into a volumetric flask. Dissolve with methanol as described below and dilute to mark.

NOTE: AB-1 Dimer only
The concentration of 0.1 mg/mL in methanol has been experimentally established to be optimal for stock solutions. A concentration higher than 0.1 mg/mL in methanol may result in precipitation and inaccurate results. AB-1 dimer is not readily soluble in many common organic solvents including acetonitrile, methanol , DMSO, DMF and in water.

2. Materials (Continued)

2.4.2.2.a Mix Standard Solution Preparation for Fortifications (BAS 9210 I, B-1, B-3, and A-2)

Prepare mixed standard solution for all analytes for fortification by combining stock solutions of each analyte (2.4.2.1) in a volumetric flask using the following scheme. Dilute to the mark with appropriate solvents as specified in the table below and vortex to ensure a complete homogeneous solution.

Analytes	Take Solution (µg/mL)	Volume (mL)	Dilute to a final volume (mL) Acetonitrile with 0.1% formic acid	Concentration of each analyte (µg/mL)
BAS 9210 I, B-1, B-3, A-2	1000	0.5 (each)	50	10
	10	5	50	1.0
	1	5	50	0.1

2.4.2.2.b Standard Solution Preparation for Fortifications (AB-1 Dimer)

For AB-1 Dimer, prepare standard solution for fortification by aliquoting stock solution (2.4.2.1) in a volumetric flask using the following scheme. Dilute to the mark with appropriate solvents as specified in the table below and vortex to ensure a complete homogeneous solution.

Analytes	Take Solution (µg/mL)	Volume (mL)	Dilute to a final volume (mL) (acetonitrile-water 85:15, v/v)	Concentration of each analyte (µg/mL)
AB-1 Dimer	100	0.5	5	10
	10	0.5	5	1.0
	1	0.5	5	0.1

2.4.2.3 Calibration Standard Solutions Preparation for LC-MS/MS Analysis

Prepare mixed calibration solution for LC-MS/MS analysis by using the solutions that were prepared in Section 2.4.2.2 in volumetric flasks using the following scheme in the table below. Dilute to the mark with appropriate solvents as specified and vortex to ensure a complete homogeneous solution.

2. Materials (Continued)

2.4.2.3a. Calibration Standard Solution Preparation of BAS 9210 I, B-1, and B-3:

Volume (mL) taken/ Solution used	Dilute to a final volume (mL) (Acetonitrile with 0.1% formic acid)	Concentration
5 mL of 0.1 μg/mL fortification solution	50	10 ng/mL
25 mL of 10 ng/mL calibration standard	50	5 ng/mL
10 mL of 5 ng/mL calibration standard	50	1 ng/mL
6.25 mL of 10 ng/mL calibration standard	100	0.625 ng/mL
25 mL of 1 ng/mL calibration standard	100	0.25 ng/mL
10 mL of 0.625 ng/mL calibration standard	50	0.125 ng/mL

2.4.2.3b Calibration Standard Solution Preparation of A-2

Volume (mL) taken/ Solution used	Dilute to a final volume (mL) (0.1% Formic acid in Water:Acetonitrile, 75:25, v/v)	Concentration
5 mL of 0.1 μg/mL fortification solution	50	10 ng/mL
25 mL of 10 ng/mL calibration standard	50	5 ng/mL
10 mL of 5 ng/mL calibration standard	50	1 ng/mL
6.25 mL of 10 ng/mL calibration standard	100	0.625 ng/mL
25 mL of 1 ng/mL calibration standard	100	0.25 ng/mL
10 mL of 0.625 ng/mL calibration standard	50	0.125 ng/mL

2. Materials (Continued)

2.4.2.3c Calibration Standard Preparation of AB-1 Dimer

Volume (mL) taken/ Solution used	Dilute to a final volume (mL) acetonitrile-water 85:15, v/v)	Concentration
0.5 mL of 0.1 μg/mL fortification solution	5	10 ng/mL
2 mL of 10 ng/mL calibration standard	4	5 ng/mL
1 mL of 5 ng/mL calibration standard	5	1 ng/mL
0.125 mL of 10 ng/mL calibration standard	2	0.625 ng/mL
1 mL of 1 ng/mL calibration standard	4	0.25 ng/mL
2 mL of 0.25 ng/mL calibration standard	4	0.125 ng/mL

NOTE:
- Use amber bottles with Teflon®-lined screw caps as storage containers for all standard solutions.
- Suggested standard concentrations are listed here. A different concentration scheme may be used and additional standards may be prepared as needed.
- BAS 9210 I has shown to be unstable in aqueous conditions. The addition of formic acid has a stabilizing effect on BAS 9210 I. See potential problems.
- All standard solutions should be made in disposable glassware for AB-1 dimer. Certain contaminants can cause stability issues. See potential problems

2.4.2 EXTRACT STABILITY

The stability of analytes in both the extraction solvent and final solution for LC-MS/MS determination are established in the analytical phase of terrestrial field dissipation study (BASF Study 350785). The stability is shown below:

Analyte	Soil Extracts	Duration (days)
BAS 9210 I, B-1, and B-3	Extraction Solution: Acetonitrile-water, 70:30, v/v	9
	Final Solution for LC-MS/MS determination	9
A-2	Extraction Solution: Acetonitrile-water, 70:30, v/v	10
	Final Solution for LC-MS/MS determination	10
AB-1 Dimer	Extraction Solution: Acetonitrile-water, 70:30, v/v	1 day
	Final Solution for LC-MS/MS determination: Acetonitrile-water, 85:15, v/v	1 day

3. ANALYTICAL PROCEDURE

3.1 SAMPLE PREPARATION

Bulk soil samples received from the field are homogenized with dry ice using a Fitzmill. An aliquot of the homogenized soil samples are further homogenized in Retsch Ultra Centrifugal mill equipped with a 1.0 mm screen if necessary. The samples are stored frozen (<-5°C) before analysis.

3.2 PROCEDURE USING 5.0 G SAMPLE SIZE

3.2.1 PROCEDURE FOR THE ANALYSIS OF BAS 9210 I, B-1, B-3, A-2

3.2.1.1 Weighing and Fortification

Weigh a 5.0 g or to the nearest tenth of a gram aliquot of the soil sample into a 250 mL wide mouth glass jar.

For the fortification samples, add volumetrically an appropriate volume of standard solution to the respective control. For example, for a 0.01 ppm fortification sample, pipet 50 µL of the 1 µg/mL standard fortification solution (2.4.2.2) onto 5.0 g of control sample.

3.2.1.2 Extraction

Add 10 mL of acetonitrile volumetrically to the glass jar containing soil (Section 3.2.1.1). Firmly cap the vessel. Shake by hand to disperse the soil. Shake at 300 rpm for 30 minutes using a mechanical shaker.

Add 30 mL of **Solution I** (acetonitrile-water (60:40, v/v)) volumetrically to the centrifuge tube. Firmly cap and shake at 300 rpm for 30 minutes using a mechanical shaker. Allow the samples to settle for about 5 minutes and transfer about 20 mL of extract to Teflon centrifuge tube. Centrifuge samples at about 3500 rpm for 10 minutes in a swinging bucket centrifuge and then proceed to Step 3.4

3.2.2 PROCEDURE FOR THE ANALYSIS OF AB-1 DIMER

The procedure for the extraction of AB-1 Dimer follows the method for BAS 9210 I (Section 3.2.1). Proceed to Section 3.4.

3. Analytical Procedure (Continued)

3.3 PROCEDURE USING 0.1 G SAMPLE SIZE

3.3.1 PROCEDURE FOR THE ANALYSIS OF BAS 9210 I, B-1, B-3, A-2

3.3.1.1 Weighing and Fortification

Weigh a 100 mg ±10 mg aliquot of the soil sample into a 1.4 mL AlphaNumeric well plate tube (Matrix).

For the fortification samples, add volumetrically an appropriate volume of standard solution to the respective control sample by a micro pipet. For example, for a 0.01 ppm fortification sample, pipet 10 µL of the 0.1 µg/mL standard fortification solution (2.4.2.2) onto 100 mg of control sample.

3.3.1.2 Extraction

Add 0.2 mL of acetonitrile to the well tubes containing soil (Section 3.3.1.1) using a single or multi-channel automatic pipeter. Firmly cap the well tubes with Matrix SepraSeal cap. Vortex the capped well-plate tubes containing the soil samples upside down using Multitube vortexer at about 2400 rpm for 2 minutes to mix the solvent and the soil. Repeat vortexing the capped well plate tubes containing the soil samples upright position using Multi-tube vortexer at about 2400 rpm for 2 minutes. Shake the samples horizontally for 10 minutes on a reciprocal shaker for 10 minutes at 300 rpm. Centrifuge samples at about 2000 rpm for 5 minutes in a swinging bucket centrifuge. Detach the SepraSeal cap from the well-plate tubes containing the soil samples.

Add 0.6 mL of **Solution I** (acetonitrile-water (60:40, v/v)) to the well tubes containing soil with acetonitrile using a single or multi-channel automatic pipeter. Firmly cap the well tubes with Matrix SepraSeal cap. Vortex the capped well-plate tubes containing the soil samples upside down using Multitube vortexer at about 2400 rpm for 2 minutes to mix the solvent and the soil. Make sure the soil marc is completely dispersed. Repeat vortexing the capped well plate tubes containing the soil samples upright position using Multi-tube vortexer at about 2400 rpm for 2 minutes. Shake the samples horizontally for 10 minutes on a reciprocal shaker for 10 minutes at 300 rpm. Centrifuge samples at about 3500 rpm for 5 minutes in a swinging bucket centrifuge. Detach the SepraSeal cap from the well-plate tubes containing the soil samples. Proceed to Section 3.4.

NOTE:

- In case of some soil types, it may be necessary to increase the number of vortex cycles or to further agitate on a mechanical shaker for 5 minutes. The shaking step, if needed, should be performed in between vortex steps and document in the master sheet.
- The container type for the extraction of 0.1g can be important. Some container types can reduce recoveries. If using comparable labware for this method, test with procedurals before conducting analysis..

3. Analytical Procedure (Continued)

3.3.2 PROCEDURE FOR THE ANALYSIS OF AB-1 DIMER

The procedure for the extraction of AB-1 Dimer follows the method for BAS 9210 I (Section 3.3.1). Proceed to Section 3.4.3

3.4. EXTRACT PREPARATION FOR LC-MS/MS ANALYSIS

NOTE: Some soil types may require filtration. For analysis using 5 g sample size, syringe filter each sample through a 0.45 um GHP filter. For analysis using 0.1g sample size, load all samples onto a multi-well filter plate (0.45um GHP) and use vacuum to elute the samples into a 96 well plate for analysis. This is true for all analytes described below.

3.4.1 For analysis of BAS 9210 I, B-1, B-3, follow the steps below

a) For controls, and LOQ (0.01 ppm) fortifications samples, dilute the extract from Step 3.2.1.2 or 3.3.1.2 with **Solution II** (Acetonitrile with 0.1 % formic acid) 1:1, v/v. Transfer about 1 ml of sample extract into a HPLC vial (for 0.1g analysis, dilute the sample in the 96 well plate) and the samples are ready for injection. (see note above in section 3.4)

For samples with residues higher than LOQ should be diluted accordingly with **Solution II** (Acetonitrile with 0.1 % formic acid) to obtain quantitation within the calibration curves

3.4.2 For analysis of A-2, follow the steps below

a) For controls, and LOQ (0.01 ppm) fortifications samples, dilute the extract from Step 3.2.1.2 or 3.3.1.2 with **Solution III** (Water with 0.1 % formic acid) 1:1, v/v. Transfer about 1 ml of sample extract into a HPLC vial (for 0.1g analysis, dilute the sample in the 96 well plate) and the samples are ready for injection. (see note above in section 3.4)

For samples with residues higher than LOQ should be diluted accordingly with **Solution IV** (0.1% FA in water:acetonitrile, 75:25, v/v) to obtain quantitation within the calibration curves

3. Analytical Procedure (Continued)

3.4.3 For analysis of AB-1 Dimer follow the steps below:

Follow the same procedure as step 3.4.1.a, except dilute the extract with acetonitrile.

For samples with residues higher than LOQ should be diluted accordingly with **Solution VI** (water:acetonitrile 15:85 (v/v)) to obtain quantitation within the calibration curves

Flow charts of the analytical procedures are presented in **Figures 1 and 2.**

3.5 METHOD AUTOMATION

The method extraction and dilution procedures can be automated with the use of an automated liquid handling system. See Appendix A for examples of the automated liquid handling system programs. The instrument parameters (e.g. stage height, air gap, blow out, etc) that do not impact the data, may be changed, if needed. It is the responsibility of the analyst to ensure that the automated liquid handler delivers the correct volumes. Using automated liquid handling system, the analyst should print out the program that documents the parameters and keep this with the study file.

3.6 MOISTURE DETERMINATION

The moisture determination will be conducted for the treated samples with residue value above LOD.. Results of soil analysis are reported on a “dry weight” basis for residue determination. Therefore field treated soil sample weights must be corrected for moisture content by any method the laboratory customarily uses.

The procedural recoveries will not be corrected for moisture content of the sample.

An example of a moisture determination procedure is provided below:

The percent moisture is determined using a automated moisture determination equipment (Mettler Toledo HR83) using the formula below:

$$\text{Percent Moisture} = \frac{[\text{Wet Sample Weight (g)} - \text{Dry Sample Weight (g)}]}{\text{Wet Sample Weight (g)}} \times 100$$

$$\textbf{Residue in ppm (Dry residue)} = \frac{\textbf{Wet Sample Residue (ppm)}}{\textbf{(100 – “Percent Moisture”) / 100}}$$

3. Analytical Procedure (Continued)

3.7 INSTRUMENTATION: (SUGGESTED LC-MS/MS OPERATING CONDITION)

Method A: Used for the analysis of BAS 9210 I, B-1, and B-3

Instrument:	AB Sciex Instruments API 5000			
Inlet [HPLC System]:	ACQUITY UPLC System			
Software Version:	Analyst 1.4.2			
Column:	Acquity UPLC HSS T3 100 X 2.1 mm, 1.8µ			
Injection:	Typically 10 µL		Column Temperature: 50°C	
Mobile Phase:	A = Water with 0.1% formic acid B = Acetonitrile with 0.1% formic acid			
Gradient	Time (min.)	Flow rate: µL/min.	Composition	
			% A	%B
	0.0	600	90	10
	0.1	600	90	10
	0.4	600	60	40
	1.35	600	0	100
	2.25	600	0	100
	2.3	600	90	10
	2.6	600	90	10

3. Analytical Procedure (Continued)

	BAS 9210 I	B-1	B-3
Expected Retention Times	~1.94min	~1.26 min	~1.09 min
Transitions:* (m/z)	**448.2→ 173.0 (primary)** **448.2→ 145.1**	**189.1→ 68.8 (primary)** **189.1→ 144.9**	**190.0→ 130.0 (primary)** **190.0→ 102.0**
Ionization Mode	Positive (Switch at 1.87min)	Negative (Switch at 1.18 min)	Positive
	Turbo ion spray (500 °C)		

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

3. Analytical Procedures (Continued)

Method B: Used for the analysis of A-2

<table>
<tr><td>Instrument:</td><td colspan="4">AB Sciex Instruments API 5000</td></tr>
<tr><td>Inlet [HPLC System]:</td><td colspan="4">ACQUITY UPLC System</td></tr>
<tr><td>Software Version:</td><td colspan="4">Analyst 1.4.2</td></tr>
<tr><td>Column:</td><td colspan="4">BEH C18; 1.7 µm, 2.1 X 50 mm</td></tr>
<tr><td>Injection:</td><td colspan="2">Typically 40 µL or higher</td><td colspan="2">Column Temperature: 50°C</td></tr>
<tr><td>Mobile Phase:</td><td colspan="4">A = Water with 1% formic acid
B = Acetonitrile with 0.1% formic acid</td></tr>
<tr><td rowspan="9">Gradient</td><td rowspan="2">Time (min.)</td><td rowspan="2">Flow rate: µL/min.</td><td colspan="2">Composition</td></tr>
<tr><td>% A</td><td>%B</td></tr>
<tr><td>0.0</td><td>600</td><td>70</td><td>30</td></tr>
<tr><td>0.05</td><td>600</td><td>70</td><td>30</td></tr>
<tr><td>0.90</td><td>600</td><td>20</td><td>80</td></tr>
<tr><td>1.50</td><td>600</td><td>1</td><td>99</td></tr>
<tr><td>2.45</td><td>600</td><td>1</td><td>99</td></tr>
<tr><td>2.50</td><td>600</td><td>70</td><td>30</td></tr>
<tr><td>3.00</td><td>600</td><td>70</td><td>30</td></tr>
<tr><td></td><td colspan="4"></td></tr>
<tr><td>Expected Retention Times</td><td colspan="4">~ 1.14 min</td></tr>
<tr><td>Transitions (m/z):*</td><td colspan="4">174.1 → 147.1 (primary)
174.1 → 117.1</td></tr>
<tr><td>Ionization Mode:</td><td colspan="4">Turbo Ion Spray (450°C) Positive</td></tr>
</table>

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

3. Analytical Procedures (Continued)

Method C: Used for the analysis of AB-1 Dimer

Instrument:	AB Sciex Instruments API 5000 or API 5500			
Inlet [HPLC System]:	ACQUITY UPLC System			
Software Version:	Analyst 1.4.2			
Column:	BEH C18; 1.7 µm, 2.1 X 50 mm			
Injection:	Typically 10 µL or higher	Column Temperature: 50°C		
Mobile Phase:	A = Water with 1% formic acid B = Acetonitrile with 0.1% formic acid (Solution II)			
Gradient	Time (min.)	Flow rate: µL/min.	Composition	
			% A	%B
	0.0	600	70	30
	0.05	600	70	30
	0.90	600	20	80
	1.50	600	1	99
	2.45	600	1	99
	2.50	600	70	30
	3.00	600	70	30
	AB-1 Dimer			
Expected Retention Times	~ 1.78 min			
Transitions (m/z):*	689.4 → 288.2 **(primary)** 689.4 → 268.2			
Ionization Mode:	Turb Ion spray (500°C) Positive			

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

3. Analytical Procedures (Continued)

Method D: Used for the analysis of AB-1 Dimer in HPLC Mode (Alternate method)

<table>
<tr><td>Instrument:</td><td colspan="4">AB Sciex Instruments API 5000</td></tr>
<tr><td>Inlet [HPLC System]:</td><td colspan="4">ACQUITY UPLC System*</td></tr>
<tr><td>Software Version:</td><td colspan="4">Analyst 1.4.2</td></tr>
<tr><td>Column:</td><td colspan="4">BEH C18; 1.7 µm, 2.1 X 50 mm*</td></tr>
<tr><td>Injection:</td><td colspan="2">Typically 10 µL or higher</td><td colspan="2">Column Temperature: 50°C</td></tr>
<tr><td>Mobile Phase:</td><td colspan="4">A = Water with 1% formic acid
B = Acetonitrile with 0.1% formic acid (Solution II)</td></tr>
<tr><td rowspan="9">Gradient</td><td rowspan="2">Time (min.)</td><td rowspan="2">Flow rate: µL/min.</td><td colspan="2">Composition</td></tr>
<tr><td>% A</td><td>%B</td></tr>
<tr><td>0.0</td><td>500</td><td>70</td><td>30</td></tr>
<tr><td>0.05</td><td>500</td><td>70</td><td>30</td></tr>
<tr><td>0.90</td><td>500</td><td>20</td><td>80</td></tr>
<tr><td>1.50</td><td>500</td><td>1</td><td>99</td></tr>
<tr><td>2.45</td><td>500</td><td>1</td><td>99</td></tr>
<tr><td>2.50</td><td>500</td><td>70</td><td>30</td></tr>
<tr><td>3.00</td><td>500</td><td>70</td><td>30</td></tr>
<tr><td></td><td colspan="4"></td></tr>
<tr><td>Expected Retention Times</td><td colspan="4">~ 1.98 min</td></tr>
<tr><td>Transitions (m/z):**</td><td colspan="4">689.4 → 288.2 (primary)
689.4 → 268.2</td></tr>
<tr><td>Ionization Mode:</td><td colspan="4">Turbo ion spray (500°C) Positive</td></tr>
</table>

* LC gradient is operated in standard HPLC system (pressure around 4700 psi with the flow rate specified)

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

3. Analytical Procedures (Continued)

Method E: Used for the analysis of BAS 9210 I, B-1, and B-3 in HPLC Mode (Alternate method)

Instrument:	AB Sciex Instruments API 5000			
Inlet [HPLC System]:	ACQUITY UPLC System			
Software Version:	Analyst 1.4.2			
Column:	TOSOH Bioscience #18154 TSKgel Super-ODS 4.6 mm X 5 cm, 2u			
Injection:	Typically 10 μL		Column Temperature: 50°C	
Mobile Phase:	A = Water with 0.1% formic acid B = Acetonitrile with 0.1% formic acid			
Gradient	Time (min.)	Flow rate: μL/min.	Composition	
			% A	%B
	0.0	600	90	10
	0.1	600	90	10
	0.5	600	60	40
	1.35	600	0	100
	3.55	600	0	100
	3.60	600	90	10
	4.00	600	90	10

3. Analytical Procedure (Continued)

	BAS 9210 I	B-1**	B-3
Expected Retention Times	~2.4min	~1.83 min	~1.65 min
Transitions:* (m/z)	**448.2→ 173.0 (primary)** **448.2→ 145.1**	**189.1→ 68.8 (primary)** **189.1→ 144.9**	**190.0→ 130.0 (primary)** **190.0→ 102.0**
Ionization Mode	Positive (Switch at 2.20min)	Negative (Switch at 1.75 min)	Positive
	Turbo ion spray (500 °C)		

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

** This method is also used as the confirmatory method of B-1 (if necessary)

3. Analytical Procedure (Continued)

Method F: Used for the analysis of A-2 HPLC Mode (Alternate method)

Instrument:	AB Sciex Instruments API 5000			
Inlet [HPLC System]:	ACQUITY UPLC System			
Software Version:	Analyst 1.4.2			
Column:	TSKgel Super-ODS 4.6 mm X 50 mm, 2μ [TOSOH Bioscience #18154]			
Injection:	Typically 40 μL or higher		Column Temperature: 50°C	
Mobile Phase:	A = Water with 1% formic acid B = Acetonitrile with 0.1% formic acid			
Gradient	Time (min.)	Flow rate: μL/min.	Composition	
			% A	%B
	0.0	600	70	30
	0.05	600	70	30
	0.90	600	20	80
	1.50	600	1	99
	2.45	600	1	99
	2.50	600	70	30
	3.00	600	70	30
Expected Retention Times	~ 2.03 min			
Transitions (m/z):*	174.1 → 147.1 **(primary)** 174.1 → 117.1			
Ionization Mode:	Turbo Ion Spray (450°C) Positive			

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

3. Analytical Procedure (Continued)

Method G: Used for the analysis of B-1 in HPLC Mode (Alternate method)

<table>
<tr><td>Instrument:</td><td colspan="4">AB Sciex Instruments API 5000</td></tr>
<tr><td>Inlet [HPLC System]:</td><td colspan="4">ACQUITY UPLC System*</td></tr>
<tr><td>Software Version:</td><td colspan="4">Analyst 1.4.2</td></tr>
<tr><td>Column:</td><td colspan="4">BEH C18; 1.7 µm, 2.1 X 50 mm*</td></tr>
<tr><td>Injection:</td><td colspan="2">Typically 10 µL or higher</td><td colspan="2">Column Temperature: 50°C</td></tr>
<tr><td>Mobile Phase:</td><td colspan="4">A = Water with 0.1% formic acid
B = Acetonitrile with 0.1% formic acid (Solution II)</td></tr>
<tr><td rowspan="9">Gradient</td><td rowspan="2">Time (min.)</td><td rowspan="2">Flow rate: µL/min.</td><td colspan="2">Composition</td></tr>
<tr><td>% A</td><td>%B</td></tr>
<tr><td>0.0</td><td>500</td><td>70</td><td>30</td></tr>
<tr><td>0.05</td><td>500</td><td>70</td><td>30</td></tr>
<tr><td>0.90</td><td>500</td><td>20</td><td>80</td></tr>
<tr><td>1.50</td><td>500</td><td>1</td><td>99</td></tr>
<tr><td>2.45</td><td>500</td><td>1</td><td>99</td></tr>
<tr><td>2.50</td><td>500</td><td>70</td><td>30</td></tr>
<tr><td>3.00</td><td>500</td><td>70</td><td>30</td></tr>
<tr><td></td><td colspan="4"></td></tr>
<tr><td>Expected Retention Times</td><td colspan="4">~ 0.8 min</td></tr>
<tr><td>Transitions (m/z):**</td><td colspan="4">189.1→ 68.8 (primary)
189.1→ 144.9</td></tr>
<tr><td>Ionization Mode:</td><td colspan="4">Turbo ion spray (500°C) Positive</td></tr>
</table>

* LC gradient is operated in standard HPLC system (pressure around 4700 psi with the flow rate specified)

**The primary ion listed will be used as default; any of these transitions could be used for quantitation in case interference is observed at the same retention time.

3. Analytical Procedures (Continued)

Method H: Used for the (analysis of BAS 9210 I, B-1, and B-3 (Independent Laboratory Method Validation)

Instrument:	AB Sciex Instruments API 5500			
Inlet [HPLC System]:	Agilent 1200SL HPLC System			
Software Version:	Analyst 1.5.1			
Column:	Acquity UPLC HSS T3 50 X 2.1 mm, 1.8µ			
Injection:	7 µL		Column Temperature: 60°C	
Mobile Phase:	A = Water with 0.1% formic acid B = Acetonitrile with 0.1% formic acid			
Gradient	Time (min.)	Flow rate (µL/min.)	Composition	
			% A	%B
	0.0	500	99	1
	0.1	500	99	1
	0.4	500	60	40
	1.35	500	0	100
	2.25	500	0	100
	2.3	500	99	1
	2.6	500	99	1

	BAS 9210 I	B-1	B-3
Expected Retention Times	~2.52 min	~2.00 min	~1.87 min
Transitions:* (m/z)	**448.2→ 173.0 (primary)** **448.2→ 145.1**	**189.1→ 69.0 (primary)** **189.1→ 145.1**	**190.0→ 130.0 (primary)** **190.0→ 102.0**
Ionization Mode	Positive (Switch at 2.44 min)	Negative (Switch at 1.93 min)	Positive
	Turbo ion spray (500 °C)		

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

3. Analytical Procedures (Continued)

Method I: Used for the analysis of B-1 (Alternative Chromatographic conditions used for Confirmatory purposes during Independent Laboratory Method Validation)

<table>
<tr><td>Instrument:</td><td colspan="4">AB Sciex Instruments API 5500</td></tr>
<tr><td>Inlet [HPLC System]:</td><td colspan="4">Agilent 1200SL HPLC System</td></tr>
<tr><td>Software Version:</td><td colspan="4">Analyst 1.5.1</td></tr>
<tr><td>Column:</td><td colspan="4">Acquity UPLC HSS T3 50 X 2.1 mm, 1.8µ</td></tr>
<tr><td>Injection:</td><td colspan="2">7 µL</td><td colspan="2">Column Temperature: 60°C</td></tr>
<tr><td>Mobile Phase:</td><td colspan="4">A = Water with 0.1% formic acid
B = Acetonitrile with 0.1% formic acid</td></tr>
<tr><td rowspan="9">Gradient</td><td rowspan="2">Time (min.)</td><td rowspan="2">Flow rate (µL/min.)</td><td colspan="2">Composition</td></tr>
<tr><td>% A</td><td>%B</td></tr>
<tr><td>0.0</td><td>500</td><td>99</td><td>1</td></tr>
<tr><td>0.1</td><td>500</td><td>99</td><td>1</td></tr>
<tr><td>0.4</td><td>500</td><td>60</td><td>40</td></tr>
<tr><td>1.35</td><td>500</td><td>0</td><td>100</td></tr>
<tr><td>2.25</td><td>500</td><td>0</td><td>100</td></tr>
<tr><td>2.3</td><td>500</td><td>99</td><td>1</td></tr>
<tr><td>2.6</td><td>500</td><td>99</td><td>1</td></tr>
<tr><td></td><td colspan="4">B-1</td></tr>
<tr><td>Expected Retention Times</td><td colspan="4">~2.00 min</td></tr>
<tr><td>Transitions:* (m/z)</td><td colspan="4">189.1→ 69.0 (primary)
189.1→ 145.1</td></tr>
<tr><td>Ionization Mode</td><td colspan="4">Negative</td></tr>
<tr><td></td><td colspan="4">Turbo ion spray (500 °C)</td></tr>
</table>

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

3. Analytical Procedures (Continued)

Method J: Used for the analysis of A-2
(Independent Laboratory Method Validation)

<table>
<tr><td>Instrument:</td><td colspan="4">AB Sciex Instruments API 5500</td></tr>
<tr><td>Inlet [HPLC System]:</td><td colspan="4">Agilent 1200SL HPLC System</td></tr>
<tr><td>Software Version:</td><td colspan="4">Analyst 1.5.1</td></tr>
<tr><td>Column:</td><td colspan="4">Acquity BEH C18; 1.7 µm, 2.1 X 50 mm</td></tr>
<tr><td>Injection:</td><td colspan="2">40 µL</td><td colspan="2">Column Temperature: 50°C</td></tr>
<tr><td>Mobile Phase:</td><td colspan="4">A = Water with 1% formic acid
B = Acetonitrile with 0.1% formic acid</td></tr>
<tr><td rowspan="9">Gradient</td><td rowspan="2">Time (min.)</td><td rowspan="2">Flow rate: µL/min.</td><td colspan="2">Composition</td></tr>
<tr><td>% A</td><td>%B</td></tr>
<tr><td>0.0</td><td>600</td><td>70</td><td>30</td></tr>
<tr><td>0.05</td><td>600</td><td>70</td><td>30</td></tr>
<tr><td>0.90</td><td>600</td><td>20</td><td>80</td></tr>
<tr><td>1.50</td><td>600</td><td>1</td><td>99</td></tr>
<tr><td>2.45</td><td>600</td><td>1</td><td>99</td></tr>
<tr><td>2.50</td><td>600</td><td>70</td><td>30</td></tr>
<tr><td>3.50</td><td>600</td><td>70</td><td>30</td></tr>
<tr><td></td><td colspan="4"></td></tr>
<tr><td>Expected Retention Times</td><td colspan="4">~ 1.74 min</td></tr>
<tr><td>Transitions (m/z):*</td><td colspan="4">174.1 → 147.1 (primary)
174.1 → 117.1</td></tr>
<tr><td>Ionization Mode:</td><td colspan="4">Turbo Ion Spray (400-500°C) Positive</td></tr>
</table>

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

3. Analytical Procedures (Continued)

Method K: Used for the analysis of AB-1 dimer (Independent Laboratory Method Validation)

<table>
<tr><td>Instrument:</td><td colspan="4">AB Sciex Instruments API 5500</td></tr>
<tr><td>Inlet [HPLC System]:</td><td colspan="4">Agilent 1200SL HPLC System</td></tr>
<tr><td>Software Version:</td><td colspan="4">Analyst 1.5.1</td></tr>
<tr><td>Column:</td><td colspan="4">Aquity BEH C18; 1.7 µm, 2.1 X 50 mm</td></tr>
<tr><td>Injection:</td><td colspan="2">20 µL</td><td colspan="2">Column Temperature: 50°C</td></tr>
<tr><td>Mobile Phase:</td><td colspan="4">A = Water with 1% formic acid
B = Acetonitrile with 0.1% formic acid</td></tr>
<tr><td rowspan="9">Gradient</td><td rowspan="2">Time (min.)</td><td rowspan="2">Flow rate: µL/min.</td><td colspan="2">Composition</td></tr>
<tr><td>% A</td><td>%B</td></tr>
<tr><td>0.0</td><td>500</td><td>70</td><td>30</td></tr>
<tr><td>0.05</td><td>500</td><td>70</td><td>30</td></tr>
<tr><td>0.90</td><td>500</td><td>20</td><td>80</td></tr>
<tr><td>1.50</td><td>500</td><td>1</td><td>99</td></tr>
<tr><td>2.45</td><td>500</td><td>1</td><td>99</td></tr>
<tr><td>2.50</td><td>500</td><td>70</td><td>30</td></tr>
<tr><td>3.50</td><td>500</td><td>70</td><td>30</td></tr>
<tr><td></td><td colspan="4">AB-1 Dimer</td></tr>
<tr><td>Expected Retention Times</td><td colspan="4">~ 2.80 min</td></tr>
<tr><td>Transitions (m/z):*</td><td colspan="4">689.4 → 288.2 (primary)
689.4 → 268.2</td></tr>
<tr><td>Ionization Mode:</td><td colspan="4">Turbo Ion Spray (500°C) Positive</td></tr>
</table>

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

3. Analytical Procedures (Continued)

Method L: Used for the analysis of BAS 9210 I, B-1, and B-3 in HPLC mode (Soil Method Validation)

Instrument:	AB Sciex Instruments API 5500			
Inlet [HPLC System]:	Agilent 1200SL HPLC System			
Software Version:	Analyst 1.5.1			
Column:	Gemini C18 4.6 X 100 mm, 3.0 μm			
Injection:	20 μL		Column Temperature: 50°C	
Mobile Phase:	A = Water with 0.1% formic acid B = Acetonitrile with 0.1% formic acid			
Gradient	Time (min.)	Flow rate (μL/min.)	Composition	
			% A	%B
	0.0	800	95.0	5.0
	0.1	800	95.0	5.0
	0.6	800	60.0	40.0
	2.8	800	25.0	75.0
	7.0	800	0.0	100.0
	7.5	800	0.0	100.0
	7.6	800	95.0	5.0
	10.6	800	95.0	5.0

	BAS 9210 I	B-1	B-3
Expected Retention Times	~6.40 min	~4.10 min	~3.35 min
Transitions:* (m/z)	**448.2→ 173.0 (primary)** **448.2→ 145.1**	**189.1→ 69.0 (primary)** **189.1→ 145.1**	**190.0→ 130.0 (primary)** **190.0→ 102.0**
Ionization Mode	Positive (Switch at 4.5 min)	Negative (Switch at 3.5 min)	Positive
	Turbo ion spray (500 °C)		

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

3. Analytical Procedures (Continued)

Method M: Used for the analysis of A-2 in HPLC mode (Soil Method Validation)

Instrument:	AB Sciex Instruments API 5500			
Inlet [HPLC System]:	Agilent 1200SL HPLC System			
Software Version:	Analyst 1.5.1			
Column:	Gemini C18 4.6 X 100 mm, 3.0 μm			
Injection:	40 μL		Column Temperature: 50°C	
Mobile Phase:	A = Water with 1% formic acid B = Acetonitrile with 0.1% formic acid			
Gradient	Time (min.)	Flow rate: μL/min.	Composition	
			% A	%B
	0.0	800	70	30
	0.1	800	70	30
	3.0	800	20	80
	4.0	800	1	99
	5.0	800	1	99
	5.1	800	70	30
	8.0	800	70	30
	A-2			
Expected Retention Times	~ 5.18 min			
Transitions (m/z):*	174.1 → 147.1 **(primary)** 174.1 → 117.1			
Ionization Mode:	Turbo Ion Spray (400°C) Positive			

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

3. Analytical Procedures (Continued)

Method N: Used for the analysis of AB-1 dimer in HPLC mode (Soil Method Validation)

<table>
<tr><td>Instrument:</td><td colspan="4">AB Sciex Instruments API 5500</td></tr>
<tr><td>Inlet [HPLC System]:</td><td colspan="4">Agilent 1200SL HPLC System</td></tr>
<tr><td>Software Version:</td><td colspan="4">Analyst 1.5.1</td></tr>
<tr><td>Column:</td><td colspan="4">Aquity BEH C18; 1.7 µm, 2.1 X 50 mm*</td></tr>
<tr><td>Injection:</td><td colspan="2">20 µL</td><td colspan="2">Column Temperature: 50°C</td></tr>
<tr><td>Mobile Phase:</td><td colspan="4">A = Water with 1% formic acid
B = Acetonitrile with 0.1% formic acid</td></tr>
<tr><td rowspan="9">Gradient</td><td rowspan="2">Time (min.)</td><td rowspan="2">Flow rate: µL/min.</td><td colspan="2">Composition</td></tr>
<tr><td>% A</td><td>%B</td></tr>
<tr><td>0.0</td><td>500</td><td>70</td><td>30</td></tr>
<tr><td>0.05</td><td>500</td><td>70</td><td>30</td></tr>
<tr><td>0.90</td><td>500</td><td>20</td><td>80</td></tr>
<tr><td>1.50</td><td>500</td><td>1</td><td>99</td></tr>
<tr><td>2.45</td><td>500</td><td>1</td><td>99</td></tr>
<tr><td>2.50</td><td>500</td><td>70</td><td>30</td></tr>
<tr><td>3.00</td><td>500</td><td>70</td><td>30</td></tr>
<tr><td></td><td colspan="4">AB-1 Dimer</td></tr>
<tr><td>Expected Retention Times</td><td colspan="4">~ 3.22 min</td></tr>
<tr><td>Transitions (m/z):**</td><td colspan="4">689.4 → 288.2 (primary)
689.4 → 268.2</td></tr>
<tr><td>Ionization Mode:</td><td colspan="4">Turbo Ion Spray (500°C) Positive</td></tr>
</table>

* LC gradient is operated in standard HPLC system (LC pressure approximately 4400 psi with the flow rate specified)

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

NOTE:

1. The LC-MS/MS instrument and equipment listed was used for method development. Other equivalent hardware may be used. The use of a guard column is optional.
2. The recommended instrument parameters and chromatographic systems were found to be optimal for the instrument used. The exact values used must be optimized for each instrument.
3. Two transitions are provided and either transition maybe used for quantitation depending on instrument response and matrix interference.
4. Although ACQUITY UPLC System is capable of running at ultra high pressure (up to 15,000 psi) with the column that has a particle size of 1.7 µm, this system operates within standard LC parameters (e.g. standard HPLC column, at ~ 4700 psi) with lower flow rate
5. In case a new chromatographic method for AB-1 dimer is used, care should be taken that the degradation product peak is separated from the AB-1 dimer peak
6. Due to instability of the AB-I dimer solution, it recommended not exceed instrument analysis time beyond 5 to 6 hours

3.8 CALIBRATION PROCEDURES

Calculation of results is based on peak area measurements using a calibration curve. The calibration curve is obtained by direct injection of BAS 9210 I, B-1, and B-3 mix standards for LC-MS/MS in the range of 10 ng/mL to 0.125 ng/mL. The calibration curve is obtained by direct injection of AB-1 Dimer standards for LC-MS/MS in the range of 10 ng/mL to 0.125 ng/mL. The calibration curve is obtained by direct injection of A-2 standards for LC-MS/MS in the range of 10 ng/mL to 0.125 ng/mL. In a given injection run, the same injection volume is used for all samples and standards.

Calibration curves are prepared by plotting the peak area versus the weight using a linear least squares working curve in the form of $y = bx + c$.

Establish the stability of the detection response by injecting several concentrations of standards. For analysis, alternate samples and standards. For each injection set, the set should begin and end with standard injections, and each standard level should be injected at least in duplicate.

Note: It is advisable to “stabilize” on column retention time of the analytes before injecting the first sample of an analytical series.

3.9 LIMIT OF QUANTITATION AND LIMIT OF DETECTION

The limit of quantitation is defined as the lowest fortification level successfully tested. The limit of quantitation is 0.01 ppm each for all analytes. The limit of detection was estimated at 20% of the limit of quantitation, equivalent to 0.002 ppm each for all analytes. Therefore at the LOQ of 0.01 ppm the LOD is 0.002 ppm. The lowest standard for each analyte in the calibration curve has good detectability (signal to noise ratio greater than 3:1).

4. CALCULATION OF RESULTS

4.1 PRINCIPLE

Calculation of results is based on area measurements.

For the procedural recoveries, the sample weight will be considered 5 g in the final calculation of residues [μg/g (ppm)]. The method requires that the sample weight to be 5 ± 0.1 g for fortification samples. The recovery is the percentage of the fortified amount (μg or ng), which is recovered through the method and the weights cancels out, as shown in the equation (Sec 4.2)

4.2 EQUATIONS

The recoveries and residues of all analytes in μg/g (ppm) are calculated with the following formulas:

Residue in ppm = ng found per injection/ mg injected

Percent recovery (%) = $\frac{\text{Residue (μg/g) for [fortified sample - control sample]}}{\text{Amount (μg/g) fortified}} \times 100$

ng found per injection = $\frac{\text{Amount of analyte calculated from calibration curve}}{1000}$

Standard curve: pg = $\frac{\text{Peak Area - intercept}}{\text{slope}}$

mg injected = $\frac{\text{Sample weight (5 g) extracted X μL injected X Dilution factor (F1)}}{\text{Final extraction volume (40 mL)}}$

5. TIME REQUIREMENT FOR ANALYSIS

The time required for a set of 13 samples (2 fortified, 1 control and 10 unknown 5 g. samples) is approximately 8 person-hours, provided that no special problems arise, such as matrix interference.
The time required for a set of 26 samples (2 fortified, 1 control and 23 unknown 0.1 g. samples) is approximately 4 person-hours, provided that no special problems arise, such as matrix interference.

6. CONFIRMATORY TECHNIQUES

The method of determination is LC-MS/MS, which is a highly selective and self-confirmatory detection technique. However, two ions were monitored during analysis for peak confirmation.

7. POTENTIAL PROBLEMS

- The glassware used for the method should be thoroughly rinsed with acetonitrile to prevent contamination.
- It is important to keep the stock solution of BAS 9210 I sealed properly. Condensation can form inside the bottle when the stock solution is moved in and out of the refrigerator. The analyte, BAS 9210 I is unstable in water (pH 7 and higher). It is recommended to add 0.1% formic acid to increase the stability in aqueous solution.
- It is recommended that the samples containing the residues of BAS 9210 I should not be thawed and re-frozen for analysis at any time.
- The stability of the stock/fortification solutions of AB-1 Dimer in methanol and in the calibration standards in acetonitrile:water, 85:15, v/v is around 1 day when refrigerated. It is recommended to use standard solutions out of the refrigerator and keep it cold during its use
- Stability of AB-1 Dimer can also be affected by contaminants in glassware. It was seen that using disposable glassware decreases this risk.. Therefore it is recommended to pre wash the glassware with the solvent to be used prior to their usage.
- Due to the micro procedure in this method (small sample amounts and volumes) and poor solubility in water it is important to avoid contamination. Use disposable items as much as possible.
- Any non-disposable item used in the method should be rinsed with methanol followed by acetone prior to its use. In case of an automated liquid handling system (e.g Quadra 96), cleaning the system, tip shucking station and the reservoirs with acetone prior to its use is essential to avoid contamination.
- It is highly recommended to perform instrument check routinely during LC-MS/MS analysis for standard peak enhancement or suppression. The instrument check sample is basically prepared by adding known amount of standard to the control matrix at the limit of quantitation (0.01 ppm level). It is recommended to clean the LC-MS thoroughly, if peak enhancement or suppression has been observed. Some of the cleaning procedure includes exhaustive cleaning of the hardware, such as skimmer, fused silica for sample introduction, and several gradient systems to wash the column.
- It was noticed during method development that large dilutions (1 to 100 or greater) of BAS 9210 I can produce erratic results. For best results add the dilution solvent first and then add the aliquot when making dilutions. Also keep the dilution scheme to 1 to 50 or less.

8. SAFETY AND HEALTH CONSIDERATIONS

All procedures involving organic solvents should be performed in a well-ventilated hood. Personal protective equipment (gloves, lab coats and safety glasses) should be worn while performing this method. Read all label statements and precautions.

FIGURE 1: FLOW DIAGRAM OF ANALYTICAL METHOD NO. D1002 (5.0 OR 0.1 G SAMPLE SIZE) FOR BAS 9210 I, B-1, B-3, A-2

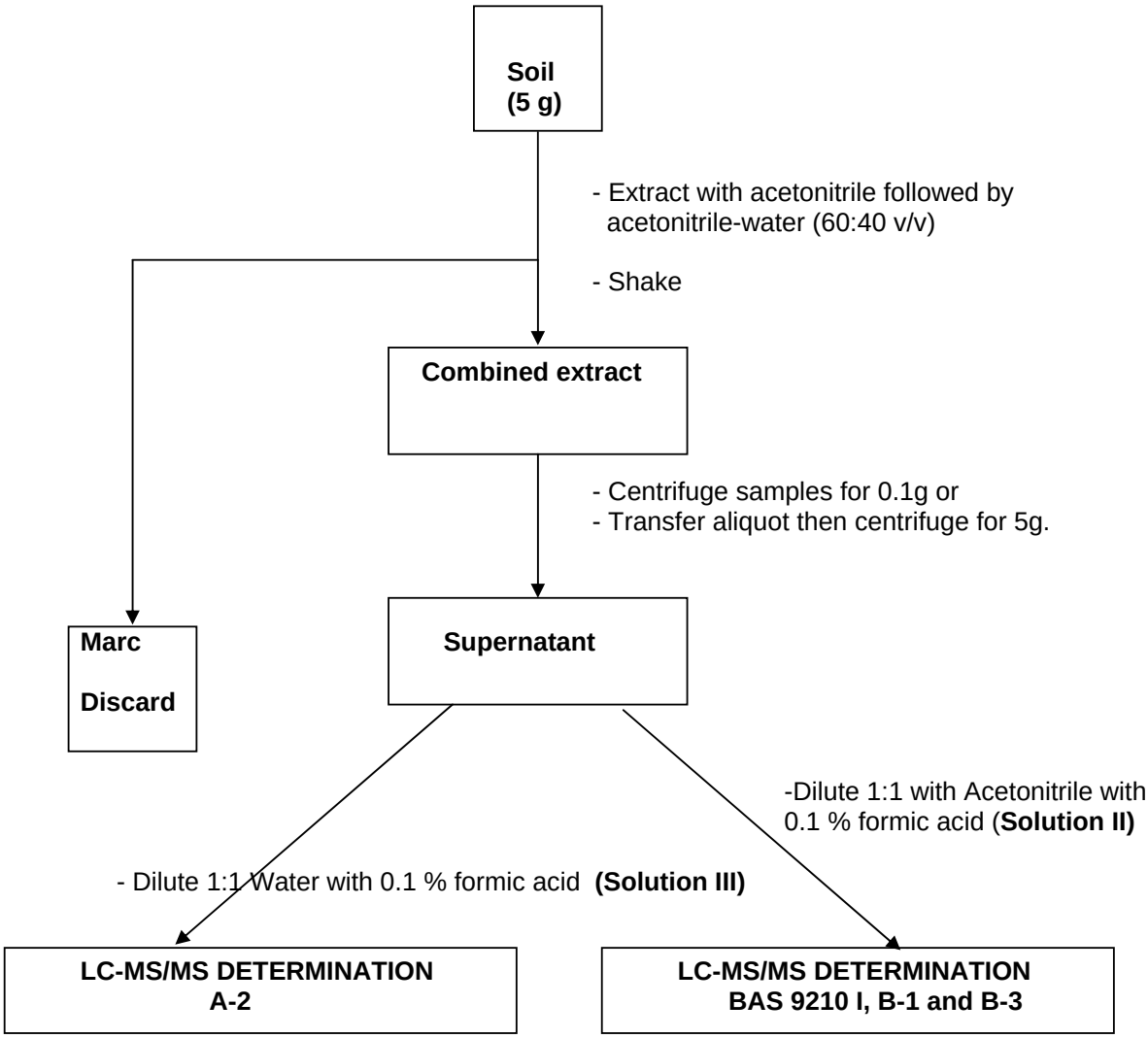
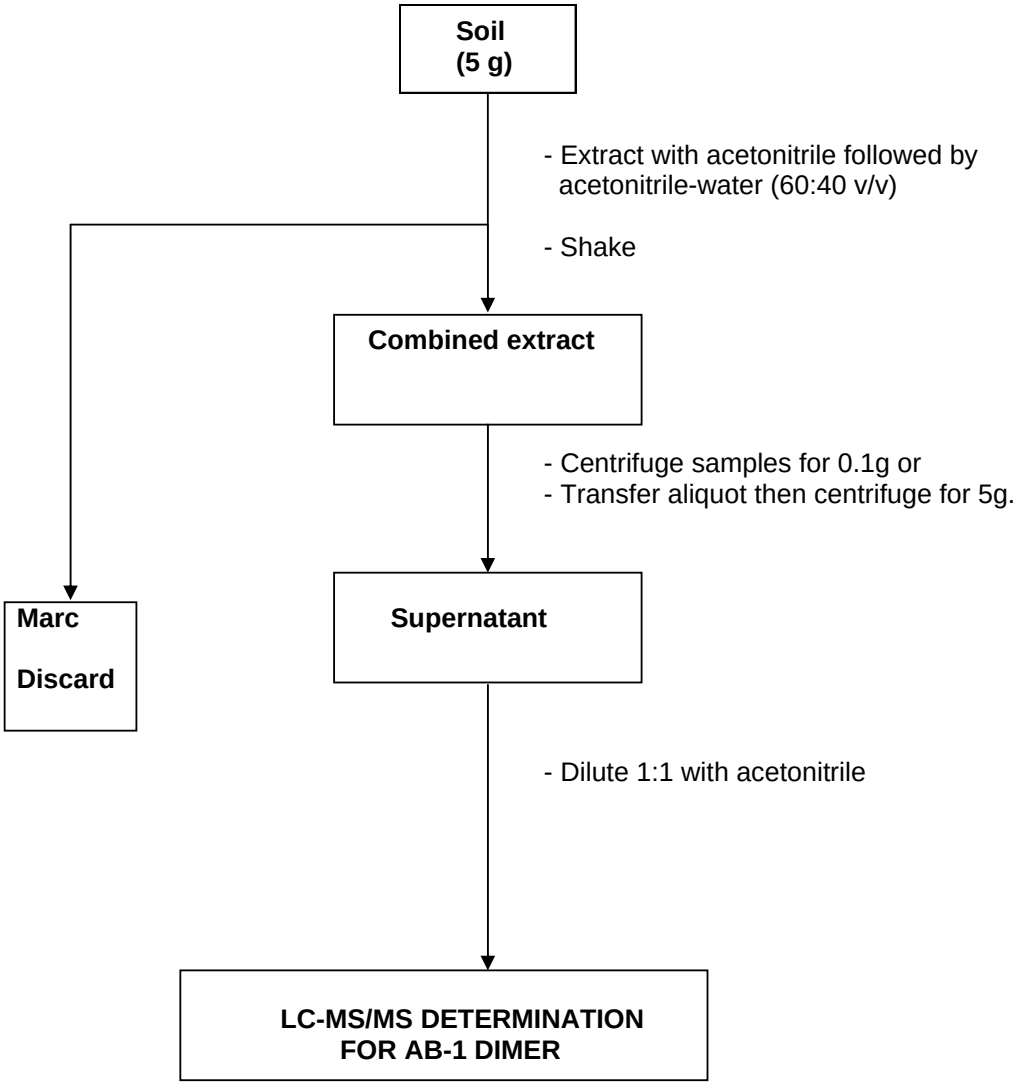


FIGURE 2: FLOW DIAGRAM OF ANALYTICAL METHOD NO. D1002 (5.0 G OR 0.1G SAMPLE SIZE) FOR AB-1 DIMER



APPENDIX A.

EXAMPLE QUADRA METHOD

The examples for the programming of the automated liquid handling equipment for the performance of the method are presented only as a guide. The user manual should be consulted for definitions of terminology and directions for operation. Other programs can be used that are proven to be equivalent. The individual steps should be modified, as needed, to ensure that the proper volumes are taken.

Soil Extraction

Protocol Name: 9210 Soil Extraction Final

Model : Quadra 3 - 96 Tip 450µL

Shuttle Layout

```
 +--------------------------------
 | Pos. 1: Dilution Solvent
 |        96-Dimpled Bottom Res. with Baffles-1.9" Tall  on Stacker Nest
 | Pos. 2: 96-Dimpled Bottom Res. with Baffles-1.9" Tall  on Stacker Nest
 | Pos. 3: Plastic Tips on Tip Jig - 450 µL Plastic Tips
 | Pos. 4: Weighed Samples
 |        96-Dimpled Bottom Res. with Baffles-1.9" Tall  on Stacker Nest
 | Pos. 5: ACN/Water
 |        96-Dimpled Bottom Res. with Baffles-1.9" Tall  on Stacker Nest
 | Pos. 6: ACN
 |        96-Dimpled Bottom Res. with Baffles-1.9" Tall  on Stacker Nest
 +--------------------------------
```

(1) Load Tips from Pos. 3
(2) Mix 400.0 µL @1300, 5 times, at ACN on 6, 10.0 µL Air Gap
(3) Aspirate 200. µL l @1300 from ACN on 6, 10.0 µL Air Gap
(4) Aspirate 10.0 µL @0 from ACN on 6
(5) Dispense 210. µL @825 to Weighed Samples on 4, 10.0 µL Blowout @825
(6) Pause Program at Pos. 2
(7) Aspirate 10.0 µL @625 from ACN/Water on 5
(8) Aspirate 300. µL @1250 from ACN/Water on 5, 10.0 µL l Air Gap
(9) Aspirate 10.0 µL @625 from ACN/Water on 5
(10) Dispense 310.0 µL l @825 to Weighed Samples on 4, 10.0 µL Blowout @825
(11) Aspirate 10.0 µL @625 from ACN/Water on 5
(12) Aspirate 300.0 µL @1250 from ACN/Water on 5, 10.0 µL Air Gap
(13) Aspirate 10.0 µL @625 from ACN/Water on 5
(14) Dispense 310.0 µL @825 to Weighed Samples on 4, 10.0 µL Blowout @825
(15) Pause Program at Pos. 2
(16) Shuck Tips to Pos. 3

Soil Dilution

Protocol Name: 9210 Soil Dilution Step Final

Model : Quadra 3 - 96 Tip 450 µL
Shuttle Layout
+----------
| Pos. 1: Dilution Solvent
| 96-Dimpled Bottom Res. with Baffles-1.9" Tall on Stacker Nest
| Pos. 2: Extraction Sample
| 96-Dimpled Bottom Res. with Baffles-1.9" Tall on Stacker Nest
| Pos. 3: Plastic Tips on Tip Jig - 450 µL Plastic Tips
| Pos. 4: Weighed Samples
| Alpha Matrix Tubes on Stacker Nest
| Pos. 5: Final Sample
| 96-Dimpled Bottom Res. with Baffles-1.9" Tall on Stacker Nest
| Pos. 6: ACN
| 96-Dimpled Bottom Res. with Baffles-1.9" Tall on Stacker Nest
+----------
(1) Load Tips from Pos. 3
(2) Mix 400.0 µL @1340, 3 times, at Dilution Solvent on 1
(3) Aspirate 200.0 µL @1335 from Dilution Solvent on 1, 50.0 µL Air Gap
(4) Aspirate 10.0 µL l @0 from Dilution Solvent on 1
(5) Empty Sample @750 to Final Sample on 5
(6) Aspirate 200.0 ul @1290 from Extraction Sample on 2, 50.0 µL Air Gap
(7) Aspirate 10.0 µL @0 from Extraction Sample on 2
(8) Empty Sample @750 to Final Sample on 5
(9) Timed Dispense for 2 cycles @650 to Final Sample on 5
(10) Mix 400.0 ul @890, 5 times, at Final Sample on 5
(11) Empty Sample @725 to Final Sample on 5
(12) Shuck Tips to Pos. 3
(13) Pause Program
(14) Load Tips from Pos. 3
(15) Mix 400.0 µL @1340, 3 times, at Dilution Solvent on 1
(16) Aspirate 200.0 µL l @1340 from Dilution Solvent on 1, 50.0 µL Air Gap
(17) Aspirate 10.0 µL @0 from Dilution Solvent on 1
(18) Empty Sample @750 to Final Sample on 5
(19) Aspirate 200.0 µL @1250 from Extraction Sample on 2, 50.0 µL Air Gap
(20) Aspirate 10.0 µL @0 from Extraction Sample on 2
(21) Empty Sample @750 to Final Sample on 5
(22) Timed Dispense for 2 cycles @750 to Final Sample on 5
(23) Mix 400.0 µL @1000, 5 times, at Final Sample on 5
(24) Empty Sample @750 to Final Sample on 5
(25) Shuck Tips to Pos. 3

Appendix E. Recommendations/ Modifications from the Independent Laboratory Validation

The method D1002 was validated in the first trial and met the criteria for a successful validation of the method in an independent laboratory. Acceptable average recoveries were obtained for a representative soil sample. The modification to the method is described below.

Method Modifications

Method modifications include the following:

The suggested LC-MS/MS conditions for the B-1 secondary ion transition (m/z 189.1 → 145.1) were not adequate for quantitation when injected with BAS 9210 I and B-3. The B-1 metabolite, which is detected in negative ionization mode, elutes between two positive ionization periods and switching between polarities caused distorted peak shape in the secondary ion transition (m/z 189.1 → 145.1). Therefore, a separate chromatographic method should be used for the analysis of B-1 for confirmatory purposes. These LC-MS/MS parameters are in Section 3.7, Method I of the technical procedure, which is presented Appendix D.