Method Validation Study for the Determination of Residues of Methoxyfenozide and its A-ring Phenol Metabolite and B-ring Mono Acid Metabolite in Surface Water, Ground Water and Drinking Water by Liquid Chromatography with Tandem Mass Spectrometry

INTRODUCTION

Scope

This method is applicable for the quantitative determination of residues of methoxyfenozide and its A-ring phenol metabolite and B-ring mono acid metabolite in surface, ground and drinking water. The method was validated over the concentration range of 0.05-1.0 µg/L with a validated limit of quantitation of 0.05 µg/L. Common names, chemical names, and molecular formulas for the analytes are given in Table 1.

This study was conducted to fulfill data requirements outlined in the EPA Residue Chemistry Test Guidelines, OPPTS 850.7100 (*1*). The validation will also comply with the requirements of EU Council Regulation (EC) No. 1107/2009 with particular regard to Section 3 of SANCO/3029/99 rev.4 and Section 3 of SANCO/825/00 rev.8.1 as well as PMRA Regulatory Directive Dir98-02 (*2-4*). The validation was conducted following Dow AgroSciences SOP ECL-24 with exceptions noted in the protocol or by protocol amendment.

Method Principle

Residues of methoxyfenozide, its A-ring phenol metabolite and its B-ring mono acid metabolite are extracted from water samples by taking a 10.0-mL aliquot and placing in an 11-dram (45-mL) glass vial equipped with a PTFE-lined cap or a 50-mL polypropylene centrifuge tube equipped with a cap. The sample is acidified with a 1.0 N hydrochloric acid solution and purified and concentrated on a Strata -X polymeric sorbent SPE cartridge. The SPE eluate is evaporated to dryness. The sample is reconstituted with 1.0 mL of a 70% water/30% acetonitrile solution containing 0.1% formic acid. The sample is transferred to a 96-deep well plate along with the calibration standards, and these are analyzed for methoxyfenozide, the A-ring phenol metabolite and the B-ring mono acid metabolite by liquid chromatography coupled with positive-ion electrospray (ESI) tandem mass spectrometry (LC-MS/MS).

Test Substances/Reference Compounds/Analytical Standards

Test Substance	TSN	Percent Purity	Recertification Date	Reference
methoxyfenozide	TSN104129	99.9%	17-Oct-2014	FAPC 10-275236
A-ring phenol	TSN103265	99%	03-Sep-2013	FAPC 09-202030
B-ring mono acid	TSN029592-0001	99%	11-Jul-2012	FAPC 10-274917

The Certificates of Analysis for the test substances can be found in Figure 1-3. The above standards may be obtained free of charge from Test Substance Coordinator, Dow AgroSciences LLC, 9330 Zionsville Road, Building 304, Indianapolis, IN 46268-1054.

EXPERIMENTAL

Sample Origin, Numbering, Preparation, Storage, and Characterization

The test system was untreated control samples obtained from the Dow AgroSciences LLC Sample Management Group. All samples were tracked in the Dow AgroSciences LLC Regulatory Labs Information Management System (RLIMS) database. Unique sample numbers were assigned to the samples to track them during receipt, storage, and analysis. Complete source documentation was included in the study file.

Sample Group Number[a]	Water Type	pH	Hardness (mg equiv. $CaCO_3$/L)	Total Suspended Solids (ppm)	Alkalinity (mg $CaCO_3$/L)	Total Organic Carbon (ppm)	Dissolved Organic Carbon (ppm)
110356-003-0001[a]	Drinking Water (Tap)	8.6	4	8	292	3.1	2.6
110356-004-0001[b]	Ground Water (Bulk, Well)	8.2	330	6	183	3.4	2.2
110356-005-0001[c]	Surface Water (Pond)	8.0	135	8	80	8.0	6.9

[a]Sample originated as Control-585-0001.
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[a] Sample originated as Control-587-0001.

No sample preparation was required for the water samples prior to analysis. Samples were stored refrigerated at approximately 4°C after the time of sampling and during the course of the method validation study, except when they were removed for taking aliquots for sample analysis.

Calculation of Standard Calibration Curve

Calculation of a standard curve begins with the injection of a series of calibration standards described in Appendix I and acquisition of peak areas for the following analyses:

methoxyfenozide	*m/z* Q1/Q3 369.1/313.2 (quantitative)
	m/z Q1/Q3 369.1/149.2 (confirmatory)
A-ring phenol	*m/z* Q1/Q3 355.0/299.2 (quantitative)
	m/z Q1/Q3 355.0/135.2 (confirmatory)
B-ring mono acid	*m/z* Q1/Q3 399.1/343.1 (quantitative)
	m/z Q1/Q3 399.1/149.2 (confirmatory)

In order to generate a standard curve, plot the analyte concentration on the abscissa (x-axis) and the respective peak area on the ordinate (y-axis) in Analyst. Using regression analysis, determine the equation for the curve with respect to the abscissa. Refer to

Figure 4-9 for example calibration plots and to Figures 10-15 for example calculations. Individual calibration data and set parameters can be found in Table 2-7.

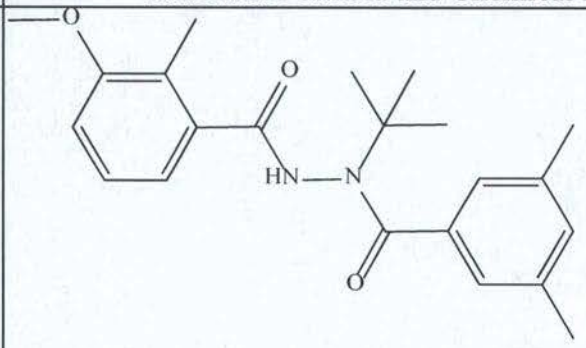
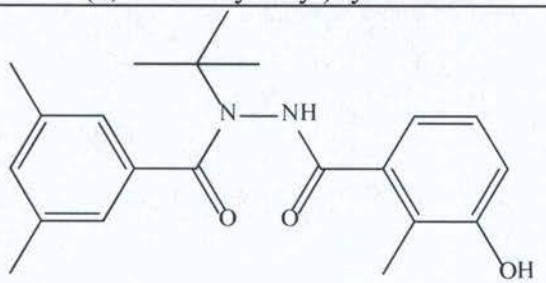
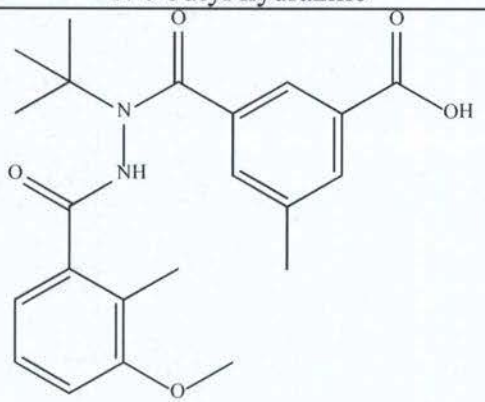
Full-Scan and Product-Ion Mass Spectra

A full scan and two product-ion mass spectra of methoxyfenozide, its A-ring phenol metabolite, and its B-ring mono acid metabolite are illustrated in Figures 16-18, respectively.

Statistical Treatment of Data

Statistical treatment of data included but was not limited to the calculation of regression equations, correlation coefficients (r) for describing the linearity of calibration curves, and means, standard deviations, and relative standard deviations of the results for the fortified recovery samples. Any potential outliers were evaluated by applying the Grubbs Test (*5*). Slight differences may be observed in the report data when verified by hand calculations due to rounding differences and/or use of significant figures in Analyst and the RLIMS system.

Table 1. Identities and Structures of Methoxyfenozide and its A-ring Phenol Metabolite and B-ring Mono Acid Metabolite

Common Name	Structural Formula and Chemical Name
Methoxyfenozide Molecular Formula: $C_{22}H_{28}N_2O_3$ CAS Number: 161050-58-4 Molecular Weight: 368.48	 3-methoxy-2-methylbenzoic acid 2-(3,5-dimethylbenzoyl)-2-(1,1-dimethylethyl)hydrazide
A-ring Phenol Metabolite Molecular Formula: $C_{21}H_{26}N_2O_3$ CAS Number: 252720-16-4 Molecular Weight: 354.45	 *N*'-(3-hydroxy-2-methylbenzoyl)-N-(3,5-dimethylbenzoyl)-*N*-*t*-butyl hydrazine
B-ring Mono Acid Metabolite Molecular Formula: $C_{22}H_{26}N_2O_5$ CAS Number: Unavailable Molecular Weight: 398.46	 (3-({1-*tert*-butyl-2-[(3-methoxy-2-methylphenol)carbonyl]hydrazinyl}-carbonyl)-5-methylbenzoic acid)

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Safety Precautions

Each analyst must be acquainted with the potential hazards of the equipment, reagents, products, solvents, and procedures used in this method before commencing laboratory work. Sources of information include operation manuals, material safety data sheets, literature, and other related data. Safety information should be obtained from the supplier. Disposal of waste materials, reagents, reactants, and solvents must be in compliance applicable governmental requirements.

Acetonitrile and methanol are flammable and should be used in well-ventilated areas away from ignition sources. Formic acid and hydrochloric acid are corrosive and can cause severe burns. It is imperative that proper eye and personal protection equipment be worn when handling these reagents.

Laboratory Equipment

Balance, analytical, Model AE100, Mettler-Toledo, Inc.

Balance, pan, Model BB2440, Mettler-Toledo, Inc.

Centrifuge, with rotor to accommodate 8-oz wide-mouth bottles, Model Sorvall Legend XFR, catalog number 75004539, Thermo Scientific

Evaporator, TurboVap LV, Zymark.

Pipet, positive-displacement, 10-100 µL capacity, Model F148504G, Gilson Inc.

Pipet, positive-displacement, 100-1000 µL capacity, Model F148506G, Gilson Inc.

Pipetter, adjustable, Eppendorf, 10-100 µL, catalog number 05-402-48, Brinkmann Instruments.

Pipetter, adjustable, Eppendorf, 50-1000 µL, catalog number 21-378-83, Brinkmann Instruments.

Pipetter, adjustable, Eppendorf, 1000-5000 µL, catalog number 22-46-134-6, , Brinkmann Instruments.

Shaker, variable speed reciprocating with box carrier, Model 6010, Eberbach Corporation.

Vacuum manifold, Model spe-12G, Mallinckrodt Baker, Inc.

Vortex mixer, Model Genie 2, catalog number 12-812, Fisher Scientific.

Chromatographic System

Column, analytical, Gemini 5µ C18 110A, 50.0 cm x 2.00 mm, 5-µm particle size, part number 00B-4435-B0, Phenomenex.

Guard column, SecurityGuard cartridge for Gemini C18 HPLC columns with 2.0 to 3.0 mm ID, part number AJ0-7596, Phenomenex.

Guard cartridge holder, part number KJ0-4282, Phenomenex.

Liquid chromatograph, Model 1100, Agilent Technologies.

Mass spectrometer, Model API 4000, AB/Sciex.

Mass spectrometer data system, Model Analyst 1.5.1, AB/Sciex.

Glassware and Materials

Bottle, 500-mL, media bottle, catalog number 06-423-3C, Fisher Scientific.

Bottle, 1.0-L, media bottle, catalog number 06-423-3D, Fisher Scientific.

Cartridges, SPE, Strata-X 33 µm polymeric sorbent, 60-mg, 3-mL, catalog number 8B-S100-UBJ, Phenomenex.

Collection plate, 96-deep well, 2-mL, catalog number 121-5203, International Sorbent Technology Ltd.

Collection plate sealing cap, catalog number 121-5205, Biotage.

Cylinder, graduated, 100-mL, catalog number 08-553B, Fisher Scientific.

Cylinder, graduated, 250-mL, catalog number 08-553F, Fisher Scientific.

Cylinder, graduated, 500-mL, catalog number 08-553C, Fisher Scientific.

Cylinder, graduated, 1000-mL, catalog number 08-553D, Fisher Scientific.

Cylinder, graduated mixing, 50-mL, catalog number 08-531C, Fisher Scientific.

Cylinder, graduated mixing, 2000-mL, catalog number 08-531H, Fisher Scientific.

Flask, volumetric, 50-mL, catalog number 10-209C, Fisher Scientific.

Flask, volumetric, 100-mL, catalog number 10-209D, Fisher Scientific.

Flask, volumetric, 500-mL, catalog number 10-209G, Fisher Scientific.

Flask, volumetric, 1000-mL, catalog number 10-209H, Fisher Scientific.

Pipet, disposable serological, 25-mL, catalog number 56800-25210, Kimble/Kontes.

Pipet, 3.2-mL disposable transfer, catalog number 13-711-7, Fisher Scientific.

Pipet tip, positive-displacement, 50-µL capacity, catalog number CP50, Gilson Inc.

Pipet tip, positive-displacement, 100-µL capacity, catalog number CP100, Gilson Inc.

Pipet tip, positive-displacement, 1000-µL capacity, catalog number CP1000, Gilson Inc.

Pipet, volumetric, 0.5-mL, catalog number 13-650-2A, Fisher Scientific.

Pipet, volumetric, 1.0-mL, catalog number 13-650-2B, Fisher Scientific.

Pipet, volumetric, 2.0-mL, catalog number 13-650-2C, Fisher Scientific.

Pipet, volumetric, 5.0-mL, catalog number 13-650-2F, Fisher Scientific.

Tube, culture, 12-mL (16 x 100 mm), with screw cap, catalog number 99449-16, Corning Products.

Tube, round-bottle, polypropylene, graduated, 14-mL (17 x 100 mm), catalog number 352059, Becton Dickinson Labware, Franklin Lakes, NJ 07417.

Vial, 8-mL, with PTFE-lined screw cap, catalog number 60940A 8, Kimble/Kontes.

Reagents

Acetic acid, glacial, ACS plus grade, catalog number A38S-500, Fisher Scientific.

Acetonitrile, ChromaSolv for HPLC gradient grade, ≥99.9%, catalog number 439134-4L, Sigma-Aldrich.

Formic acid, Optima, LC/MS grade, catalog number A117-50, Fisher Scientific.

Hydrochloric acid, 1.0 N, certified concentration, catalog number SA48-1, Fisher Scientific.

Methanol, ChromaSolv for HPLC gradient grade, ≥99.9%, catalog number 34885-4L-R, Sigma Aldrich.

Nitrogen, refrigerated liquid, catalog number LQNI, BOC Gases.

Water, ChromaSolv for HPLC gradient grade, ≥99.9%, catalog number 270733-4L, Sigma Aldrich.

Prepared Solutions

acetonitrile + 0.1% formic acid (mobile phase B)

Pipet 2.0 mL of formic acid (96%) into a 2-L graduated mixing cylinder. Dilute to volume with HPLC grade acetonitrile. Cap the cylinder and invert several times to mix the solution prior to use.

50% methanol /50% water (v/v) (port #1 autosampler injection needle wash solution)

Using a 500-mL graduated cylinder, measure 500 mL of HPLC grade water and transfer this to a 1.0 L bottle. Using a 500-mL graduated cylinder, measure 500 mL of methanol and transfer this to the same 1.0 L bottle. Cap the bottle and mix well by shaking, and allow the solution to equilibrate to room temperature before use.

water + 0.1% formic acid (mobile phase A)

Pipet 2.0 mL of formic acid (96%) into a 2-L graduated mixing cylinder. Dilute to volume with HPLC grade water. Cap the cylinder and invert several times to mix the solution prior to use.

70% water/30% acetonitrile containing 0.1% acetic acid (v/v) (calibration standard diluent, original final sample diluent)

Using a 500-mL graduated cylinder, measure 500 mL of HPLC grade water and transfer to a 1000-mL volumetric flask. Using a 500-mL graduated cylinder, measure 300 mL of acetonitrile and transfer to the same 1000-mL volumetric flask. Pipet 1.0 mL of glacial acid into the same volumetric flask. Mix well by inverting the flask multiple times, and allow the solution to equilibrate to room temperature. Adjust to volume with HPLC grade water and invert the flask to mix well again before use.

70% water/30% acetonitrile containing 0.1% formic acid (v/v) (calibration standard diluent, final sample diluent)

Using a 500-mL graduated cylinder, measure 500 mL of HPLC grade water and transfer to a 1000-mL volumetric flask. Using a 500-mL graduated cylinder, measure 300 mL of acetonitrile and transfer to the same 1000-mL volumetric flask. Pipet 1.0 mL of formic acid (96%) into the same volumetric flask. Mix well by inverting the flask multiple times, and allow the solution to equilibrate to room temperature. Adjust to volume with HPLC grade water and invert the flask to mix well again before use.

60% water/40% methanol containing 0.1% formic acid (v/v) (SPE wash solution)

Using a 250-mL graduated cylinder, measure 250 mL of HPLC grade water and transfer to a 500-mL volumetric flask. Using a 250-mL graduated cylinder, measure 200 mL of methanol and transfer to the same 1000-mL volumetric flask. Pipet 0.5 mL of formic acid (96%) into the same volumetric flask. Mix well by inverting the flask multiple times, and allow the solution to

equilibrate to room temperature. Adjust to volume with HPLC grade water and invert the flask to mix well again before use.

Preparation of Standard Stock and Fortification Solutions

1. Weigh 0.1000 g of methoxyfenozide, RH-2485 (RH-112485) and quantitatively transfer to a 100-mL volumetric flask with acetonitrile. Dilute to volume with acetonitrile to obtain a stock solution containing 1000 µg/mL of methoxyfenozide.

2. Weigh 0.1000 g of the A-ring phenol metabolite, (RH-117236) and quantitatively transfer to a 100-mL volumetric flask with acetonitrile. Dilute to volume with acetonitrile to obtain a stock solution containing 1000 µg/mL of the A-ring phenol metabolite.

3. Weigh 0.1000 g of the B-ring mono acid metabolite, (RH-131154) and quantitatively transfer to a 100-mL volumetric flask with acetonitrile. Add approximately 50 mL of acetonitrile to the flask plus 5 mL of HPLC grade water. Mix well and equilibrate to room temperature. Dilute to volume with acetonitrile to obtain a stock solution containing 1000 µg/mL of the B-ring mono acid metabolite.

4. Pipet 10.0 mL of each of the 1000-µg/mL standard solutions prepared in Step 1 through 3 above and quantitatively transfer each into the same 100-mL volumetric flask. Add approximately 60 mL of HPLC grade water and mix well. Equilibrate to room temperature and dilute to volume with HPLC grade water to obtain a mixed spiking solution containing 100.0 µg/mL of each analyte in a 70% water/30% acetonitrile solution.

5. Pipet 10.0 mL of the 100-µg/mL mixed standard solution prepared in Step 4 above into a 100-mL volumetric flask. Dilute to volume using a 70% water/30% acetonitrile solution containing 0.1% formic acid to obtain a 10.0-µg/mL mixed calibration stock solution.

6. Pipet 10.0 mL of the 10-µg/mL mixed standard solution prepared in Step 5 above into a 100-mL volumetric flask. Dilute to volume using a 70% water/30% acetonitrile solution containing 0.1% formic acid to obtain a 1.0-µg/mL mixed calibration stock solution.

7. Pipet 10.0 mL of the 1.0-µg/mL mixed standard solution prepared in Step 6 above into a 100-mL volumetric flask. Dilute to volume using a 70% water/30% acetonitrile solution containing 0.1% formic acid to obtain a 0.10-µg/mL mixed calibration stock solution.

8. Pipet 10.0 mL of the 0.10-µg/mL mixed standard solution prepared in Step 7 above into a 100-mL volumetric flask. Dilute to volume using a 70% water/30% acetonitrile solution containing 0.1% formic acid to obtain a 0.01-µg/mL mixed calibration stock solution.

Preparation of Calibration Standards

Prepare calibration standards by diluting the appropriate mixed calibration standard stock solutions (Steps 6-8) using a 70% water/30% acetonitrile solution containing 0.1% formic acid according to the following table:

Concentration of Stock Solution µg/mL	Aliquot of Stock Solution mL	Final Soln. Volume mL	Calibration Soln. Final Conc. ng/mL	Equivalent Sample Conc. [a] µg/L
1.0	5.0	100	50.0	5.00
1.0	3.5	100	35.0	3.50
1.0	2.0	100	20.0	2.00
1.0	1.0	100	10.0	1.00
0.10	5.0	100	5.0	0.500
0.10	1.0	100	1.0	0.100
0.01	5.0	100	0.50	0.050
0.01	1.5	100	0.15	0.015

[a] The equivalent sample concentration of methoxyfenozide, the A-ring phenol metabolite and the B-ring mono acid metabolite is based on taking a 10.0-mL initial aliquot of the water sample, concentrating and purifying it on an SPE cartridge, evaporating the eluate and reconstituting the sample to a final volume of 1.0 mL with using a 70% water/30% acetonitrile solution containing 0.1% formic acid.

Analytical Procedure

1. For preparing fortified samples containing methoxyfenozide, the A-ring phenol and the B-ring mono acid metabolites, add appropriate aliquots of the methoxyfenozide, the A-ring phenol and the B-ring mono acid metabolite solutions prepared in the "Preparation of Standard Solutions" section to the same 10.0 mL water sample (surface water, ground water and drinking water) to encompass the necessary concentrations range as described in the table below:

To spike methoxyfenozide, the A-ring phenol and the B-ring mono acid metabolite to 10 mL of water:			
Description	Spiking Volumes (µL)	Spiking Solutions (µg/mL)	Fortification Level (µg/L)
Control	---	---	---
LOD	15	0.01	0.015
LOQ	50	0.01	0.05
2× LOQ	100	0.01	0.1
20× LOQ	100	0.1	1

2. Add 1.0 mL of a 1.0N hydrochloric acid solution to each sample. Cap the sample vial with a PTFE-lined cap, pulse vortex mix for about 10 seconds.

3. Concentrate and purify samples using the following SPE procedure (each new lot of SPE cartridges should undergo profiling prior to use):

   a. Place a Strata -X polymeric sorbent reversed phase SPE cartridge (60-mg, 3-mL) on a vacuum manifold box.

   b. Condition the SPE cartridge with 3 mL of methanol followed by 3 mL of water, discarding the eluates. Apply full vacuum (approximately -15 to -25 inches of Hg) for about 10 seconds between solvent additions.

   c. Transfer the entire sample solution from Step 2 to the SPE cartridge. Pull the sample through the SPE cartridge at approximately 1 mL/min, discarding the eluate. Dry the plate under full vacuum for 10 seconds after sample has eluted.

   d. Wash the sample vial with a 1.0-mL aliquot of a 60% water/40% methanol/0.1% formic acid solution and transfer to the top of the SPE cartridge. Pull the solution through the SPE cartridge at approximately 1 mL/min, discarding the eluate. Dry the SPE cartridge under full vacuum for about 5 minutes.

   e. Elute the methoxyfenozide and its two metabolites from the SPE cartridge at approximately 1 mL/min with two 1.0-mL aliquots of acetonitrile, collecting the eluate in a 12-mL (16 x 100 mm) culture tube.

4. Evaporate the acetonitrile to dryness at approximately 40 °C using nitrogen (approximately 10 psi) on a TurboVap evaporator.

5. Reconstitute the samples using a positive displacement pipet to add 1.0 mL of a 70% water/30% acetonitrile solution containing 0.1% formic acid to the tube. Pulse vortex for about 10 seconds to mix well.

6. Transfer a portion of each sample that is ready for analysis to a 96-deep well plate.

7. Add approximately 1 mL of each of the calibration standards to the same plate and seal the plate. (Acceptable stopping point if sample is kept refrigerated.)

8. Analyze the calibration standards and samples by positive-ion ESI LC-MS/MS, injecting the calibration standards interspersed with the samples throughout the run.

9. If any sample net concentrations exceed 80% of the range of the standard calibration curve, re-analyze the sample after dilution using the 70% water/30% acetonitrile solution containing 0.1% formic acid. The concentration range of the calibration curve should at least cover from 30% of the LOQ to 20% above the highest sample concentration.

10. Determine the suitability of the chromatographic system using the following criteria:

    a. Standard curve linearity: Determine that the correlation coefficient (r) equals or exceeds 0.995 for the least squares equation which describes the detector response as a function of standard curve concentration.

    b. Peak resolution: Visually determine that sufficient resolution has been achieved for each analyte relative to background interferences.

    c. Appearance of chromatograms: Visually determine that the chromatograms resemble those shown in Figures 19-30 with respect to peak response, baseline noise, and background interference. Visually determine that a minimum signal-to-noise ratio of 10:1 has been attained for the 0.50-ng/mL calibration standard (equivalent to 0.05 µg/L of methoxyfenozide and its A-ring phenol and B-ring mono acid metabolites in a water sample).

Supplemental Notes

1. Equipment, glassware, materials, reagents, and chemicals considered to be equivalent to those specified may be substituted with the understanding that their performance must be confirmed by appropriate tests. Common laboratory glassware and supplies are assumed to be readily available. Unless specified otherwise, class A volumetric glassware is used to prepare analytical standards, fortification solutions, and calibration standards.

2. Electronic pipets are used only for pipetting aqueous solutions. If they are used for pipetting non-aqueous solutions, the pipets should be calibrated following the manufacturer's instruction manual and Standard Operating Procedures (*10*).

3. Different volumes of solutions may be prepared when carrying out the analytical method as long as the same ratios are maintained.

4. The instrumental conditions may be modified to obtain optimal chromatographic separation and sensitivity.

5. Based on availability of material, weighing of the analytical standard can be modified and the subsequent solution preparation scheme adjusted.

6. Other types of regression models (either linear or non-linear) may be applied to give the best fit, correlation coefficient (r), for the data where this can be fully justified based on the detection system used.

Instrumental Conditions

Typical HPLC Operating Conditions

Instrumentation:	Agilent Model 1100 autosampler Agilent Model 1100 binary pump Agilent Model 1100 degasser
Column:	Gemini C18 110A 2.00 x 50 mm, 5.0-µm (SecurityGuard cartridge for Gemini C18 HPLC column with 2.0 to 3.0 mm ID)
Column Temperature:	ambient (approximately 22 °C)
Injection Volume:	30 µL
Injection Wash:	1) 700 µL of a 50% MeOH/50% water (no valve wash) 2) 2 x 700 µL of MeOH (no valve wash) 3) 2 x 700 µL of water (no valve wash)
Run Time:	approximately 12 minutes
Mobile Phase:	A –water containing 0.1% formic acid B –acetonitrile containing 0.1% formic acid
Mobile Phase Split:	approximately 250 µL/min split to source

Gradient:

Time (min)	Flow Rate (mL/min)	Solvent A (percent)	Solvent B (percent)
0:00	0.40	70	30
1:00	0.40	70	30
8:00	0.40	10	90
9:00	0.40	70	30
12:00	0.40	70	30

Flow Diverter

Flow to Waste	0.0 min → 1.0 min
Flow to Source	1.0 min → 8.0 min
Flow to Waste	8.0 min → end of run

Equilibration Time: 3.0 minutes

Typical Mass Spectrometry Operating Conditions

Instrumentation:	AB Sciex API 4000 LC-MS/MS System AB Sciex Analyst version 1.5.1 data System
Ionization Mode:	electrospray
Polarity:	positive
Scan Type:	MRM
Resolution:	Q1 – unit, Q3 – unit
Curtain Gas (CUR)	30
Collision Gas (CAD):	6
Ion Source Gas 1 (GS1)	50
Ion Source Gas 2 (GS2)	50
Temperature (TEM):	350 °C
Entrance Potential (EP):	10 volts
IonSpray Voltage (IS):	5000 volts
Acquisition Time Delay:	1.00 minutes
Period Duration:	7.00 minutes
Dwell Time:	150 ms

Analytes	Precursor Ion, Q1, *m/z*	Product Ion, Q3, *m/z*	Declustering Potential, V	Collision Energy, V	Cell Exit Potential, V
Methoxyfenozide (quantitation)	369.1	313.2	46	11	12
Methoxyfenozide (confirmation)	369.1	149.2	46	23	18
A-ring phenol (quantitation)	355.0	299.2	66	11	10
A-ring phenol (confirmation)	355.0	135.2	66	23	12
B-ring mono acid (quantitation)	399.1	343.1	76	11	12
B-ring mono acid (confirmation)	399.1	149.2	76	21	12