Test Material:	Methoxyfenozide
MRID:	49525703
Title:	Method Validation Study for the Determination 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
MRID:	49525702
Title:	Methoxyfenozide and its Metabolites - Independent Laboratory Validation of the Method for the Determination of Residues of Methoxyfenozide in Drinking, Surface and Ground Waters by LC-MS/MS
EPA PC Code:	121027
OCSPP Guideline:	850.6100

For CDM Smith

Primary Reviewer: Lisa Muto **Signature:** 

Date: 10/20/15

Secondary Reviewer: Kathleen Ferguson **Signature:**

Date: 10/20/15

QC/QA Manager: Joan Gaidos **Signature:** 

Date: 10/20/15

Analytical method for methoxyfenozide (RH-2485) and its transformation products, A-ring phenol metabolite of methoxyfenozide and B-ring mono acid metabolite of methoxyfenozide (RH-131154), in surface, ground and drinking water

Reports: ECM: EPA MRID No.: 49525703. Shackelford, D.D. and M.J. Walter. 2014. Method Validation Study for the Determination 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. Laboratory Study ID: 110356. Report prepared, sponsored and submitted by Regulatory Sciences and Government Affairs, Dow AgroSciences LLC, Indianapolis, Indiana; 100 pages. Final report issued April 25, 2014.
ILV: EPA MRID No. 49525702. Jones, S. 2012. Methoxyfenozide and its Metabolites - Independent Laboratory Validation of the Method for the Determination of Residues of Methoxyfenozide in Drinking, Surface and Ground Waters by LC-MS/MS. EAS Study No.: S11-04020. Dow AgroSciences Study Reference No.: 110757. Report prepared by Eurofins Agroscience Services (EAS) Ltd., Derbyshire, United Kingdom, and sponsored and submitted by Dow AgroSciences LLC, Indianapolis, Indiana; 148 pages. Final report issued September 21, 2012.

Document No.: MRIDs 49525703 & 49525702

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practices (GLP; p. 3 of MRID 49525703). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). A statement of the authenticity of the study report was included with the quality assurance statement (p. 4).
ILV: The study was conducted in accordance with USEPA and OECD GLP standards (1998), as well as the UK Department of Health (water characterization; p. 3; Appendix C, p. 127 of MRID 49525702). Signed and dated No Data Confidentiality, GLP, Quality Assurance and Authenticity statements were provided (pp. 2-4; Appendix C, p. 127). A statement of the authenticity of the study report was included with the quality assurance statement (p. 4).

Classification: This analytical method is classified as supplemental. In the ECM, no samples were prepared at 10×LOQ, and the number of samples was insufficient for all analyses at 2×LOQ. ILV representative chromatograms for control samples were not included for all analyte/matrices; ECM representative chromatograms were not included for the 2×LOQ samples. A reagent blank was not included in the ECM. Linearity coefficients (r^2) of methoxyfenozide were not always ≥ 0.995.

PC Code: 121027

Reviewer: Karen Milians

Signature:
Date: Digitally signed by KAREN MILIANS DN: c=US, o=U.S. Government, ou=USEPA, ou=Staff, cn=KAREN MILIANS, dnQualifier=0000048934 Date: 2016.11.15 13:47:43 -05'00'

All page numbers refer to those listed in the upper right-hand corner of the MRIDs.

Executive Summary

The analytical method, Method Validation No.110356, is designed for the quantitative determination of methoxyfenozide (RH-2485), A-ring phenol metabolite of methoxyfenozide and B-ring mono acid metabolite of methoxyfenozide (RH-131154) in drinking, ground and surface water matrices at the LOQ of 0.05 µg/L using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in water for all analytes. The ECM was validated by the ILV in the first trial for methoxyfenozide in ground water and A-ring phenol metabolite and B-ring mono acid metabolite in all three matrices. The ECM was validated by the ILV in the second trial for methoxyfenozide in drinking and surface water after the calibration standards were correctly prepared. In the ECM, no samples were prepared at 10×LOQ. ILV representative chromatograms for control samples were not included for all analyte/matrices.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/ yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Methoxy-fenozide	49525703	49525702		Water[1,2]	25/04/2014	Dow AgroSciences LLC	LC/MS/MS	0.05 µg/L
A-ring Phenol Metabolite								
B-ring Mono Acid Metabolite								

1 For the ECM, drinking (tap) water (110356-003-0001; pH 8.6, total organic carbon 3.1 ppm), ground (bulk, well) water (110356-004-0001; pH 8.2, total organic carbon 3.4 ppm), and surface (pond) water (110356-005-0001; pH 8.0, total organic carbon 8.0 ppm) were used (p. 13 of MRID 49525703).

2 For the ILV, untreated drinking water (pH 7.6, dissolved organic carbon 3.23 mg/L), untreated surface water (pH 7.7, dissolved organic carbon 6.67 mg/L) and untreated ground water (pH 7.5, dissolved organic carbon 154 µg/L) were characterized and used for validation (p. 14; Appendix B, pp. 116-118 of MRID 49525702).

I. Principle of the Method

Samples (10 mL) of water in vials equipped with PTFE-lined caps were fortified, as necessary, then acidified with 1.0 mL of 0.1N hydrochloric acid via vortex mixing (*ca*. 10 seconds; p. 12; Appendix 1, pp. 96-97 of MRID 49525703). The sample was purified using offline reversed-phase extraction (SPE) procedure (Strata-X polymeric sorbent reversed-phase SPE cartridge; 60-mg, 3-mL). The SPE column was pre-conditioned with methanol and water (3 mL each) with full vacuum (*ca*. -15 to -25 in Hg). The sample was applied to the column (*ca*. 1 mL/min rate); the eluate was discarded. The column was washed with 1.0 mL of water:methanol:formic acid (60:40:0.1, v:v:v). After drying the column with vacuum, the analytes were eluted with two 1.0-mL aliquots of acetonitrile (*ca*. 1 mL/min rate). The purified sample was evaporated to dryness under nitrogen on a TurboVap evaporator at *ca*. 40°C and reconstituted with 1.0 mL of water:acetonitrile containing 0.1% formic acid (70:30, v:v) via vortex mixing (*ca*. 10 seconds). The final solution was prepared in a 96-deep well plate and analyzed by liquid chromatography using positive-ion electrospray ionization (ESI) with tandem mass spectrometry.

Samples were analyzed for methoxyfenozide and its metabolites using an AB/Sciex API 4000 LC/MS/MS (p. 13; Appendix 1, pp. 92, 99-100 of MRID 49525703). The instrumental conditions consisted of a Gemini C18 110A column (2.00 x 50 mm, 5-µm; column temperature ambient, *ca*. 22°C), SecurityGuard cartridge for Gemini C18 HPLC column with 2.0 to 3.0 ID, a mobile phase gradient of (A) water containing 0.1% formic acid and (B) acetonitrile containing 0.1% formic acid [percent A:B (v:v) at 0.0-1.00 min. 70:30, 8.00 min. 10:90, 9.00-12.00 min. 70:30], MS/MS detection in positive turbo spray (MRM; temperature, 350°C), and injection volume 30 µL. Two parent-daughter ion transitions were monitored per analyte (quantification and confirmation, respectively): *m/z* 369.1 → 313.2 and *m/z* 369.1 → 149.2 for methoxyfenozide, *m/z* 355.0 → 299.2 and *m/z* 355.0 → 135.2 for A-ring phenol, and *m/z* 399.1 → 343.1 and *m/z* 399.1 → 149.2 for B-ring mono acid. Retention times were observed at *ca*. 5.1, 3.9, and 3.95 min. for methoxyfenozide, A-ring phenol and B-ring mono acid, respectively (Figures 23-30, pp. 82-89).

ILV

In the ILV, the ECM was performed exactly as written, except for the use of a driblock (40°C) instead of a TurboVap for evaporation and different LC/MS/MS conditions (pp. 15-16 of MRID 49525702). Samples were analyzed for methoxyfenozide and its metabolites using an Applied Biosystems API 4000 LC/MS/MS equipped with an Ascentis Express C18 column (2.1 x 50 mm, 2.7-µm; column temperature 40°C; pp. 17-19). The mobile phase gradient was (A) water containing 0.1% formic acid and (B) acetonitrile [percent A:B (v:v) at 0.01-1.00 min. 70:30, 8.00 min. 10:90, 9.00-12.00 min. 70:30] and MS/MS detection in positive turbo spray (MRM; temperature, 500°C), and injection volume 15 µL. These modifications of the LC/MS/MS conditions were minor changes and approved by the Study Director. Two parent-daughter ion transitions were monitored per analyte (quantification and confirmation, respectively): *m/z* 369.2 → 313.3 and *m/z* 369.2 → 149.2 for methoxyfenozide, *m/z* 355.1 → 299.2 and *m/z* 355.1 → 135.0 for A-ring phenol, and *m/z* 399.3 → 343.2 and *m/z* 399.3 → 149.0 for B-ring mono acid. Retention times were observed at *ca*. 4.5-4.7, 3.0-3.1 and 3.1-3.2 min. for methoxyfenozide, A-

ring phenol and B-ring mono acid, respectively (Figures 20-21, pp. 52-53; Figures 26-27, pp. 58-59; Figures 32-33, pp. 64-65; Figures 38-39, pp. 70-71; Figures 41-42, pp. 73-74; Figures 47-48, pp. 79-80; Figures 53-58, pp. 85-90).

LOQ/LOD

The LOQ for all analytes was the same in the ECM and ILV at 0.05 µg/L (pp. 12, 16; Table 32, p. 54 of MRID 49525703; pp. 12, 24 of MRID 49525702). The LOD for all analytes was 0.015 µg/L in the ECM; the LOD was not reported in the ILV.

II. Recovery Findings

ECM (MRID 49525703): Mean recoveries and relative standard deviations (RSDs) were within guidelines for analysis of methoxyfenozide (RH-2485), A-ring phenol metabolite of methoxyfenozide and B-ring mono acid metabolite of methoxyfenozide (RH-131154) in drinking (tap), ground (well) and surface (pond) water matrices at fortification levels of 0.05 µg/L (LOQ), 0.10 µg/L (2×LOQ) and 1.00 µg/L (20×LOQ); however, the number of samples was insufficient for analyses at 0.10 µg/L (2×LOQ; n = 4; quantitative and confirmatory HPLC analyses; Tables 26-31, pp. 51-53). Performance data (recovery results) of the quantitative HPLC analysis and confirmatory HPLC analysis were comparable. The ECM calculations allowed for recovery data to be corrected for residues found in the control samples; however, residues were not quantified in any of the control samples (Tables 8-25, pp. 33-50; Figures 10-15, pp. 69-74). Recoveries from samples fortified at 0.015 µg/L (LOD) ranged (ions/matrices combined) from 71-91% for methoxyfenozide, 62-87% for A-ring phenol metabolite and 75-108% for B-ring mono acid metabolite (n = 2 for each matrix/analyte; DER Attachment 2). The water matrices were well characterized, and obtained from the Sample Management Group of Dow AgroSciences LLC (sources not further specified; p. 13). Drinking (tap) water (110356-003-0001; pH 8.6, total organic carbon 3.1 ppm), ground (bulk, well) water (110356-004-0001; pH 8.2, total organic carbon 3.4 ppm), and surface (pond) water (110356-005-0001; pH 8.0, total organic carbon 8.0 ppm) were used in the study.

ILV (MRID 49525702): Mean recoveries and RSDs were within guidelines for analysis of methoxyfenozide, A-ring phenol metabolite and B-ring mono acid metabolite in drinking (tap), ground (well) and surface (pond) water matrices at fortification levels of 0.05 µg/L (LOQ) and 0.50 µg/g (10×LOQ; quantitative and confirmatory HPLC analyses; Tables 5-10, pp. 29-31). Performance data (recovery results) of the quantitative HPLC analysis and confirmatory HPLC analysis were comparable. Untreated drinking water (pH 7.6, dissolved organic carbon 3.23 mg/L), untreated surface water (pH 7.7, dissolved organic carbon 6.67 mg/L) and untreated ground water (pH 7.5, dissolved organic carbon 154 µg/L) were characterized and used for validation (p. 14; Appendix B, pp. 116-118). The drinking water was obtained from a local water supply at EAS, Derbyshire, UK. The surface water was obtained from the River Trent by S. Jones. The ground water was purchased from a local supermarket (Evian ground water). The method was validated in the first trial for methoxyfenozide in ground water and A-ring phenol metabolite and B-ring mono acid metabolite in all three matrices (pp. 20-23; Tables 5-10, pp. 29-31). The method was validated in the second trial for methoxyfenozide in drinking and surface water after the calibration standards were correctly prepared.

Table 2. Initial Validation Method Recoveries for Methoxyfenozide and Its Metabolites, A-ring Phenol and B-ring Mono Acid, in Tap, Ground and Surface Water[1,2]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Tap Water						
	Quantitation ion					
Methoxyfenozide	0.015 (LOD)	2	75, 91	--	--	--
	0.05 (LOQ)	6	89-98	95	3.5	3.7
	0.10	4	83-97	92	6.4	7.0
	1.00	6	68-99	88	13.6	15.5
A-ring Phenol Metabolite	0.015 (LOD)	2	83, 84	--	--	--
	0.05 (LOQ)	6	90-106	94	6.3	6.8
	0.10	4	86-99	93	5.5	5.9
	1.00	6	68-100	88	14.4	16.4
B-ring Mono Acid Metabolite	0.015 (LOD)	2	100	--	--	--
	0.05 (LOQ)	6	75-100	92	9.5	10.4
	0.10	4	82-102	93	8.6	9.3
	1.00	6	67-98	86	13.5	15.6
	Confirmation ion					
Methoxyfenozide	0.015 (LOD)	2	79, 89	--	--	--
	0.05 (LOQ)	6	91-98	94	2.9	3.1
	0.10	4	85-98	93	5.9	6.4
	1.00	6	69-101	88	13.6	15.4
A-ring Phenol Metabolite	0.015 (LOD)	2	79, 87	--	--	--
	0.05 (LOQ)	6	92-99	95	2.5	2.6
	0.10	4	83-100	94	8	8.5
	1.00	6	69-102	88	14.3	16.3
B-ring Mono Acid Metabolite	0.015 (LOD)	2	101	--	--	--
	0.05 (LOQ)	6	88-105	94	6.1	6.4
	0.10	4	78-97	89	9.7	10.9
	1.00	6	69-98	86	13.4	15.6
Ground (Well) Water						
	Quantitation ion					
Methoxyfenozide	0.015 (LOD)	2	71, 86	--	--	--
	0.05 (LOQ)	6	65-91	83	9.3	11.2
	0.10	4	83-99	92	7.5	8.1
	1.00	6	77-98	91	8.1	9.0
A-ring Phenol Metabolite	0.015 (LOD)	2	71, 80	--	--	--
	0.05 (LOQ)	6	64-94	82	10.2	12.5
	0.10	4	82-103	94	9.0	9.6
	1.00	6	78-99	91	8.4	9.2
B-ring Mono Acid Metabolite	0.015 (LOD)	2	101, 108	--	--	--
	0.05 (LOQ)	5	65-96	84	12.2	14.5
	0.10	4	85-102	92	7.9	8.6
	1.00	6	77-98	90	8.5	9.4
	Confirmation ion					

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Methoxyfenozide	0.015 (LOD)	2	73, 81	--	--	--
	0.05 (LOQ)	6	65-97	85	11.5	13.5
	0.10	4	85-95	91	4.4	4.8
	1.00	6	78-99	91	8.2	9.0
A-ring Phenol Metabolite	0.015 (LOD)	2	75, 76	--	--	--
	0.05 (LOQ)	6	67-92	83	9.7	11.7
	0.10	4	82-104	93	9.5	10.2
	1.00	6	77-100	92	9.0	9.8
B-ring Mono Acid Metabolite	0.015 (LOD)	2	85, 95	--	--	--
	0.05 (LOQ)	5	64-96	85	12.6	14.8
	0.10	4	77-102	90	10.4	11.5
	1.00	6	77-96	90	7.2	8.0
Surface (Pond) Water						
	Quantitation ion					
Methoxyfenozide	0.015 (LOD)	2	73	--	--	--
	0.05 (LOQ)	6	86-94	91	3.2	3.5
	0.10	4	89-95	93	2.8	3.0
	1.00	6	92-97	94	1.8	2.0
A-ring Phenol Metabolite	0.015 (LOD)	2	62, 73	--	--	--
	0.05 (LOQ)	6	84-94	90	3.5	3.9
	0.10	4	88-94	91	2.6	2.8
	1.00	6	90-97	94	2.6	2.7
B-ring Mono Acid Metabolite	0.015 (LOD)	2	91, 98	--	--	--
	0.05 (LOQ)	6	91-105	97	5.2	5.3
	0.10	4	87-95	90	3.7	4.1
	1.00	6	92-97	95	2.2	2.3
	Confirmation ion					
Methoxyfenozide	0.015 (LOD)	2	73, 84	--	--	--
	0.05 (LOQ)	6	85-98	91	4.6	5.0
	0.10	4	91-93	92	1	1.1
	1.00	6	93-98	96	2.2	2.3
A-ring Phenol Metabolite	0.015 (LOD)	2	66, 76	--	--	--
	0.05 (LOQ)	6	88-102	93	5.2	5.7
	0.10	4	88-95	93	3.1	3.3
	1.00	6	93-100	96	2.6	2.7
B-ring Mono Acid Metabolite	0.015 (LOD)	2	75, 106	--	--	--
	0.05 (LOQ)	6	89-104	96	5.9	6.1
	0.10	4	93-97	95	1.6	1.7
	1.00	6	94-97	95	0.9	1.0

Data (uncorrected recovery results; Tables 8-25, pp. 33-50; Figures 10-15, pp. 69-74) were obtained from Tables 8-31, pp. 33-53 of MRID 49525703.

1 The water matrices were well characterized (p. 13). Drinking (tap) water (110356-003-0001; pH 8.6, total organic carbon 3.1 ppm), ground (bulk, well) water (110356-004-0001; pH 8.2, total organic carbon 3.4 ppm), and surface (pond) water (110356-005-0001; pH 8.0, total organic carbon 8.0 ppm) were used in the study.

2 Two parent-daughter ion transitions were monitored per analyte (quantification and confirmation, respectively): *m/z* 369.1 → 313.2 and *m/z* 369.1 → 149.2 for methoxyfenozide, *m/z* 355.0 → 299.2 and *m/z* 355.0 → 135.2 for

A-ring phenol, and *m/z* 399.1 → 343.1 and *m/z* 399.1 → 149.2 for B-ring mono acid (p. 13; Appendix 1, pp. 92, 99-100).

Table 3. Independent Validation Method Recoveries for Methoxyfenozide and Its Metabolites, A-ring Phenol and B-ring Mono Acid, in Tap, Ground and Surface Water[1,2]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Tap Water						
	Quantitation ion					
Methoxyfenozide	0.05 (LOQ)	5	85-94	89	4	4.0
	0.5	5	79-88	84	4	5.2
A-ring Phenol Metabolite	0.05 (LOQ)	5	85-92	88	3	3.8
	0.5	5	73-97	84	8	10.7
B-ring Mono Acid Metabolite	0.05 (LOQ)	5	82-88	85	2	2.6
	0.5	5	69-87	79	9	10.9
	Confirmation ion					
Methoxyfenozide	0.05 (LOQ)	5	82-92	87	4	4.6
	0.5	5	78-88	83	8	5.1
A-ring Phenol Metabolite	0.05 (LOQ)	5	90-96	93	3	2.8
	0.5	5	73-94	84	8	9.6
B-ring Mono Acid Metabolite	0.05 (LOQ)	5	83-95	88	5	5.2
	0.5	5	69-83	78	8	10.5
Ground Water						
	Quantitation ion					
Methoxyfenozide	0.05 (LOQ)	5	99-113	106	6	5.4
	0.5	5	92-102	96	4	3.9
A-ring Phenol Metabolite	0.05 (LOQ)	5	105-116	109	5	4.3
	0.5	5	96-107	101	4	3.9
B-ring Mono Acid Metabolite	0.05 (LOQ)	5	79-92	87	5	6.2
	0.5	5	81-89	86	3	3.6
	Confirmation ion					
Methoxyfenozide	0.05 (LOQ)	5	99-116	107	7	6.1
	0.5	5	90-102	95	5	4.9
A-ring Phenol Metabolite	0.05 (LOQ)	5	101-114	107	6	5.9
	0.5	5	94-103	98	3	3.5
B-ring Mono Acid Metabolite	0.05 (LOQ)	5	80-97	89	7	8.1
	0.5	5	82-92	88	4	4.2
Surface Water						
	Quantitation ion					
Methoxyfenozide	0.05 (LOQ)	5	84-91	87	3	3.2
	0.5	5	78-90	86	5	5.6
A-ring Phenol Metabolite	0.05 (LOQ)	5	80-88	85	3	3.8
	0.5	5	82-97	91	6	6.5
B-ring Mono Acid Metabolite	0.05 (LOQ)	5	82-92	88	3	4.1
	0.5	5	82-89	85	3	3.4
	Confirmation ion					
Methoxyfenozide	0.05 (LOQ)	5	86-92	89	2	2.7
	0.5	5	80-92	87	5	5.5
A-ring Phenol	0.05 (LOQ)	5	81-91	86	4	4.2

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Metabolite	0.5	5	82-94	89	5	5.7
B-ring Mono Acid Metabolite	0.05 (LOQ)	5	81-90	85	4	4.9
	0.5	5	78-88	82	4	4.7

Data (uncorrected results; Appendix D, p. 129) were obtained from Tables 5-10, pp. 29-31 of MRID 49525702 and DER Attachment 2 (s.d. at LOQ and 10×LOQ).

1 Untreated drinking water (pH 7.6, dissolved organic carbon 3.23 mg/L), untreated surface water (pH 7.7, dissolved organic carbon 6.67 mg/L) and untreated ground water (pH 7.5, dissolved organic carbon 154 µg/L) were characterized and used for validation (p. 14; Appendix B, pp. 116-118).

2 Two parent-daughter ion transitions were monitored per analyte (quantification and confirmation, respectively): *m/z* 369.2 → 313.3 and *m/z* 369.2 → 149.2 for methoxyfenozide, *m/z* 355.1 → 299.2 and *m/z* 355.1 → 135.0 for A-ring phenol, and *m/z* 399.3 → 343.2 and *m/z* 399.3 → 149.0 for B-ring mono acid (pp. 17--19).

III. Method Characteristics

In the ECM and ILV, the LOQ value for methoxyfenozide and its metabolites, A-ring phenol and B-ring mono acid, was established at The LOQ for all analytes was the same in the ECM and ILV at 0.05 µg/L (pp. 12, 16, 19; Table 32, p. 54 of MRID 49525703; pp. 12, 24 of MRID 49525702). The LOD for all analytes was 0.015 µg/L in the ECM. The LOD was not reported in the ILV. Following the method of Keith, L. H., *et al*. (see section **V. References** below), the LOD and LOQ for determination of methoxyfenozide and its metabolites in water/sediment were calculated in the ECM using the standard deviation from the 0.05 µg/L recovery results. The LOD was calculated as three times the standard deviation (3*s*), and the LOQ was calculated as ten times the standard deviation (10*s*) of the recovery results. The calculated values support the LOQ and LOD established for the study and are presented in **Table 4** below.

Table 4. Method Characteristics

			Methoxyfenozide	A-ring phenol metabolite	B-ring mono acid metabolite
Limit of Quantitation (LOQ)	Established		0.05 µg/L		
	Calculated (ECM)		0.0144-0.0575 µg/L	0.0125-0.0509 µg/L	0.0260-0.0629 µg/L
Limit of Detection (LOD)	Established		0.015 µg/L		
	Calculated (ECM)		0.00432-0.0173 µg/L	0.00375-0.0153 µg/L	0.00780-0.0189 µg/L
Linearity (Least squares calibration curve r and concentration range)	ECM[1]		r^2 = 0.99986 (Q) r^2 = 0.99979 (C)	r^2 = 0.99944 (Q) r^2 = 0.99988 (C)	r^2 = 0.99979 (Q) r^2 = 0.99985 (C)
			0.10-75 ng/mL		
	ILV[2]		r^2 = **0.9926**-0.9996 (Q) r^2 = **0.9888**-0.9996 (C)	r^2 = 0.9990-0.9994 (Q) r^2 = 0.9992-0.9996 (C)	r^2 = 0.9988 (Q) r^2 = 0.9986 (C)
			0.00015-0.01 µg/mL		
Repeatable	ECM[3]		Yes at LOQ and 20×LOQ. Yes at 2×LOQ, but n = **4**. No samples were prepared at 10×LOQ.		
	ILV[4]		Yes at LOQ and 10×LOQ (n = 5).		
Reproducible			Yes at the LOQ and 10×LOQ.		
Specific	ECM		Yes, no interferences were observed in the matrix control.		
	ILV	Tap	Yes, no interferences were observed in the matrix control.		Yes, no interferences were observed in the matrix control; however, baseline noise interfered with peak integration at the LOQ.
		Surface	Yes, no interferences were observed in the matrix control.	Matrix control chromatogram not provided.	Matrix control chromatogram not provided. Baseline noise interfered with peak integration at the LOQ.
		Ground	Yes, only minor residues (<10% of the LOQ) in the matrix control.		

Data were obtained from pp. 12, 16, 19; Tables 8-32, pp. 33-54; Figures 4-9, pp. 63-68; Figures 19-30, pp. 78-89 of MRID 49525703; pp. 12, 24; Tables 5-10, pp. 29-31; Figures 1-12, pp. 33-44; Figures 16-58, pp. 48-90 of MRID 49525702. Q = Quantitative HPLC analysis; C = Confirmatory HPLC analysis.

1 ECM standard curves were weighted 1/x. ECM r^2 values are reviewer-generated for all analytes from reported r values of 0.9997206-0.9999347 (Q) and 0.9998778-0.9999269 (C; calculated from data in Figures 4-9, pp. 63-68 of MRID 49525703; see DER Attachment 2).

2 ILV standard curves were weighted 1/x. ILV r^2 values are reviewer-generated for all analytes from reported r values of 0.9963-0.9997 (Q) and 0.9944-0.9998 (C; calculated from data in Figures 1-12, pp. 33-43 of MRID 49525702; see DER Attachment 2).

3 For the ECM, drinking (tap) water (110356-003-0001; pH 8.6, total organic carbon 3.1 ppm), ground (bulk, well) water (110356-004-0001; pH 8.2, total organic carbon 3.4 ppm), and surface (pond) water (110356-005-0001; pH 8.0, total organic carbon 8.0 ppm) were used (p. 13 of MRID 49525703).

4 For the ILV, untreated drinking water (pH 7.6, dissolved organic carbon 3.23 mg/L), untreated surface water (pH 7.7, dissolved organic carbon 6.67 mg/L) and untreated ground water (pH 7.5, dissolved organic carbon 154 µg/L) were characterized and used for validation (p. 14; Appendix B, pp. 116-118 of MRID 49525702).

Linearity is satisfactory when $r^2 \geq 0.995$.

IV. Method Deficiencies and Reviewer's Comments

1. In the ECM, no samples were prepared at 10×LOQ. Also, the number of samples was insufficient for all analyses at 2×LOQ (n = 4; Tables 8-31, pp. 33-53 of MRID 49525703). OSCPP guidelines recommend a minimum of five samples spiked at each fortification level (*i.e.*, minimally, the LOQ and 10× LOQ) for each analyte.

2. In the ILV, control matrix chromatograms were not provided for the following analyses: A-ring phenol metabolite with surface water; and B-ring mono acid metabolite with surface and ground water (Figures 16-58, pp. 48-90 of MRID 49525702). In the ECM, representative chromatograms for spiked samples at 2× LOQ were not included (Figures 4-9, pp. 63-68; Figures 19-30, pp. 78-89 of MRID 49525703). OCSPP guidelines recommend that chromatograms are provided for all matrices which were included in the validation.

   In the ECM, a reagent blank was not included (Tables 8-25, pp. 33-50 of MRID 49525703).

3. The linearity coefficient (r^2) of methoxyfenozide was not always ≥ 0.995 (Figures 1-2, pp. 33-34 of MRID 49525702 and DER Attachment 2). The reviewer-calculated r^2 values were 0.9926 (Q) and 0.9888 (C) for the drinking water set.

4. In the ILV, minor residues were observed in the control samples of methoxyfenozide with ground water (results table and chromatogram) and A-ring phenol metabolite with ground water (chromatogram only; Tables 5-10, pp. 29-31; Figures 1-12, pp. 33-44; Figures 16-58, pp. 48-90 of MRID 49525702). In the results table, the residues in the methoxyfenozide ground water control were only quantified as "DR = detectable residues less than 10% of the LOQ found in one sample" (Table 5, p. 29). Residues in the A-ring phenol metabolite ground water control were observed in the chromatogram (Figure 46, p. 78) and were not identified by the study author due to insignificance. Additionally, the reviewer noted that the baseline noise interfered with peak integration of B-ring mono acid metabolite at the LOQ (Figure 53, p. 85; Figure 55, p. 87; Figure 57, p. 89). The baseline was faint in these chromatograms, but the irregular baseline and peak base was able to be seen.

5. The toxicological level of concern was not reported for the analytes in water. A LOQ above toxicological levels of concern results in an unacceptable method classification.

6. The ILV study author reported that the first trial was unsuccessful for methoxyfenozide in drinking, surface and ground water (pp. 20-23 of MRID 49525702). A problem in the calibration standard preparation was suspected. The drinking and surface water batches were re-extracted for methoxyfenozide for a successful second trial. The extracts of the ground water batch with methoxyfenozide were re-injected successfully using the new calibration standards. Likewise, the extracts of all water batches with A-ring phenol metabolite and B-ring mono acid metabolite were re-injected successfully. The re-injections were considered to be part of the first trial results.

7. The reviewer noted that the titling of the confirmation chromatograms of the analytes contained "qualifier(IS)" versus "qualifier" in the quantification chromatograms (Figures 16-58, pp. 48-90 of MRID 49525702). The reviewer noted that the "IS" did not refer to an internal standard.

8. The ILV reported that communications between the ILV and the sponsor were unnecessary (p. 23 of MRID 49525702).

9. In the ECM, the calibration standards (water:acetonitrile containing 0.1% formic or acetic acid, 70:30, v:v) were stable for at least 136-141 days when protected from light under ambient conditions (pp. 16-17; Tables 33-37, pp. 55-58 of MRID 49525703). The sample extracts from water and sediment were stable for up to 7 days under refrigeration storage (*ca*. 4°C).

   In the ECM, matrix effects were also studied (p. 18; Tables 38-39, p. 59 of MRID 49525702). Matrix effects were insignificant (±16%) for all matrices.

10. It was reported for the ILV that the analytical procedure for one set of 15 samples required approximately 7.5 person hours for preparation (p. 23 of MRID 49525702). The LC/MS/MS was conducted overnight unattended. The interpretation of data required approximately 3 hours. The overall time to complete a set of samples was 1.5 calendar days.

V. References

Keith, L. H.; Crummett, W.; Deegan, J., Jr.; Libby, R. A.; Taylor, J. K.; Wentler, G. *Anal. Chem.* 1983, *55*, 2210-2218 (p. 19 of MRID 49525703).

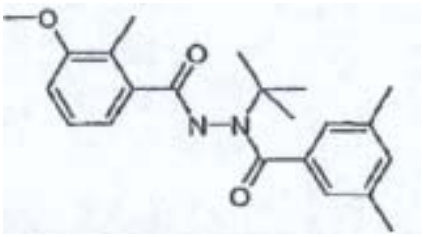
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

Attachment 1: Chemical Names and Structures

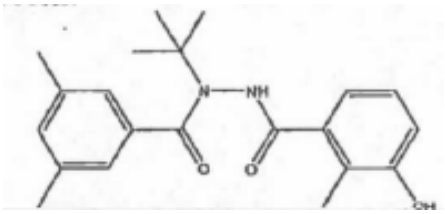
Methoxyfenozide (RH-2485; RH-112485)

IUPAC Name: Not reported
CAS Name: 3-Methoxy-2-methylbenzoic acid 2-(3,5-dimethylbenzoyl)-2-(1,1-dimethylethyl)hydrazide
CAS Number: 161050-58-4
SMILES String: Not found



A-ring Phenol Metabolite of RH-2485

IUPAC Name: Not reported
CAS Name: N'-(3-hydroxy-2-methylbenzoyl)-N-(3,5-dimethylbenzoyl)-N-t-butyl hydrazine
CAS Number: 252720-16-4
SMILES String: Not found



B-ring Mono Acid Metabolite of Methoxyfenozide (RH-131154)

IUPAC Name: Not reported
CAS Name: (3-({1-tert-butyl-2-[(3-methoxy-2-methylphenol)carbonyl]hydrazinyl}-carbonyl)-5-methylbenzoic acid)
CAS Number: Not found
SMILES String: Not found