Test Material:	Methoxyfenozide
MRID:	49201703
Title:	RH-2485 Analytical Method for Water
MRID:	49201702
Title:	Independent Laboratory Method Validation Trials of RH-2485 Analytical Method for Water
EPA PC Code:	121027
OCSPP Guideline:	850.6100

For CDM Smith

Primary Reviewer: Lisa Muto **Signature:** 

Date: 9/24/14

Secondary Reviewer: Lynne Binari **Signature:** 

Date: 9/24/14

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

Date: 9/24/14

Analytical method for methoxyfenozide in water

Reports: ECM: EPA MRID No.: 49201703. Stein, R. 1998. RH-2485 Analytical Method for Water. Rohm and Haas Technical Report No.: 34-98-25. Report prepared by Rohm and Haas Company, Spring House, Pennsylvania and Keystone Analytical Laboratories, Inc., North Wales, Pennsylvania; and sponsored and submitted by Rohm and Haas Company, Spring House, Pennsylvania; 46 pages including an un-paginated cover page. Final report issued May 5, 1998.
ILV: EPA MRID No. 49201702. Havel, J.A. and A. Xu. 1998. Independent Laboratory Method Validation Trials of RH-2485 Analytical Method for Water. KeyStone Analytical Laboratories Project No.: 980601. Protocol No.: 34P-98-35. TR No.: 34-98-102. Report prepared by KeyStone Analytical Laboratories, North Wales, Pennsylvania; and sponsored and submitted by Rohm and Haas Company, Spring House, Pennsylvania; 32 pages including page 2A. Final report issued June 26, 1998.

Document No.: MRIDs 49201703 & 49201702

Guideline: 850.6100

Statements: ECM: The study was conducted "in the spirit of" USEPA Good Laboratory Practice (GLP) standards (p. 3 of MRID 49201703). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-3). A statement of the authenticity of the study report was included as part of the Quality Assurance Statement.
ILV: The study was conducted in compliance with USEPA GLP standards (p. 3 of MRID 49201702). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2, 3). A statement of the authenticity of the study report was included as part of the Quality Assurance Statement.

Classification: This analytical method is classified as invalid. KeyStone Analytical Laboratories performed part of the ECM and all of the ILV. The ECM study report combined the results from two analyses; however, these two analyses could not be confirmed as equivalent. Neither of the two analyses of the ECM met all of the OCSPP guidelines independently due to insufficient number of samples, lack of raw data or lack of confirmation method. In the ECM and ILV chromatograms, resolution of the LOQ signal above the baseline was not satisfactory in the HPLC/UV analysis. The determinations of the LOQ and LOD were not shown to be based on scientifically acceptable procedures. The calibration curves provided by the ECM and ILV did not adequately bracket the expected concentrations of the fortified samples. A confirmation method was not employed by the ILV. The water matrices were not characterized in the ECM and ILV.

PC Code: 121027

Reviewer: Karen Milians, Chemist

Signature: Karen Milians Digitally signed by Karen Milians DN: cn=Karen Milians, o=EPA, ou, email=milians.karen@epa.gov, c=US Date: 2014.11.24 10:32:31 -05'00'

Date: 11/24/14

Page numbers of MRID 49201703 were written in the upper right-hand corner of the document. Page numbers of MRID 49201702 were written in the center of the bottom of the document.

Executive Summary

This analytical method, Rohm and Haas Technical Report No. 34-98-25, is designed for the quantitative determination of methoxyfenozide in untreated well water using HPLC/UV at the stated LOQ of 0.10 ppb. The LOQ is less than the lowest toxicological level of concern in water. The method could not be validated because KeyStone Analytical Laboratories performed part of the ECM and all of the ILV. The ECM study report combined the results from two analyses: TR 34-98-25 (termed the "current analysis") and TR 34-97-51 (dated July 30, 1997). These two analyses could not be confirmed as equivalent. Neither of the two analyses of the ECM met all of the OCSPP guidelines independently due to insufficient number of samples, lack of raw data or lack of confirmation method. The HPLC/UV analytical method which was validated by the ILV did not provide adequate resolution of the LOQ above the baseline and did not contain calibration curves which adequately bracketed the expected concentrations of the fortified samples. The HPLC/MS/MS confirmation method of the ECM was not included in the ILV. Additionally, the water matrices were not characterized in the ECM and ILV.

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						
Methoxyfenozide (RH-2485) (RH-112485)	49201703	49201702		water	05/05/1998	Rohm and Haas Company	HPLC/UV*	0.10 ppb

* The HPLC/MS/MS confirmation method of the ECM was not included in the ILV.

I. Principle of the Method

An Empore^{TM} 47-mm Extraction Disk (C-18) was pre-conditioned with 40 mL of 40% acetonitrile/water and then 40 mL Milli-Q water (pp. 10-11 of MRID 49201703). Samples (500 mL) of untreated well water were fortified, if necessary, then filtered through the Empore^{TM} 47-mm Extraction Disk (C-18). The loaded disk was rinsed with 30 mL of 15% acetonitrile/water, then residues were eluted with 40 mL of 40% acetonitrile/water under vacuum. The eluate was transferred to a 500-mL flask with a methanol rinsing. After 100 mL of n-propanol was added, the eluate was reduced to dryness using a rotovap (evaporation details were not reported). The residue was reconstituted in 2.5 mL of 52% acetonitrile/water. An aliquot was analyzed directly using HPLC/UV for quantification (TR 34-98-25 and TR 34-97-51 analyses) and HPLC/MS/MS for confirmation (TR 34-98-25 analysis only).

For quantification, samples were analyzed for methoxyfenozide (RH-2485) by HPLC (Supleco C-18, 25 cm x 4.6 mm ID column, 5 µm particle size; 45°C) using a mobile phase of acetonitrile:water (54:46, v:v; isocratic flow) with UV (240 nm) detection (pp. 8-9, 12 of MRID 49201703; Appendix 1, pp. 15-20; Appendix 1, pp. 22-25 of MRID 49201702). Injection volume was 50-150 µL. Methoxyfenozide was identified by its retention time, *ca*. 6.8 minutes.

For confirmation, samples were analyzed for methoxyfenozide by HPLC (Keystone BDS Hypersil C18, 50 mm x 3 mm column, 3 µm particle size; room temperature) using a mobile phase of acetonitrile:water (54:46, v:v) with 0.10% formic acid (isocratic flow) with MS/MS detection (turboionspray ionization, negative ion mode; pp. 9-10 of MRID 49201703). Injection volume was 25 µL. Methoxyfenozide was confirmed using one ion transition: *m/z* 367→149. The expected retention time was 4.12 minutes (Appendix 2, Figures 22-23, pp. 41-42 of MRID 49201703).

The ILV was performed exactly as above, except that only the HPLC/UV analysis was performed (pp. 8, 10 of MRID 49201702).

The limit of quantification (LOQ) for methoxyfenozide was the same in the ECM and ILV at 0.10 ppb (p. 13 of MRID 49201703; p. 11 of MRID 49201702). The limit of detection (LOD) was reported in the ECM as 0.03 ppb (p. 12 of MRID 49201703). The LOD was not reported in the ILV.

II. Recovery Findings

ECM (MRID 49201703): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of methoxyfenozide in untreated well water at fortification levels of 0.10 ppb (LOQ) and 1.00 ppb (10×LOQ; Tables 1-3, pp. 15-16). The study report used the results from two analyses: TR 34-98-25 and TR 34-97-51. TR 34-98-25 was termed the "current analysis" and included two samples at the LOQ and one sample at 10×LOQ, 0.25 ppb and 5.00 ppb. TR 34-97-51 (dated July 30, 1997; p. 14) included twelve samples at the LOQ, six samples at 10×LOQ and five samples at 0.25 ppb and 5.00 ppb. Methoxyfenozide was identified and quantified using HPLC/UV in both TR 34-98-25 and TR 34-97-51. A second analytical method, HPLC/MS/MS, was used for confirmation in TR 34-98-25 only; one ion transition was monitored. The untreated well water was not characterized (p. 10).

ILV (MRID 49201702): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of methoxyfenozide in untreated well water at fortification levels of 0.10 ppb (LOQ) and 1.0 ppb (10×LOQ; Table 2, p. 13). Analytes were identified and quantified using HPLC/UV. No confirmation method was used. The numbers of trials required to validated the method was not reported; however, it seemed to be one trial based on the terminology used on p. 11 ("this Independent Validation Trial"; pp. 6, 11).The untreated well water was not characterized (p. 8).

Table 2. Initial Validation Method Recoveries for Methoxyfenozide in Untreated Well Water[1,2]

Analyte	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
TR 34-98-25 & TR 34-97-51 Analyses (HPLC/UV Results)						
Methoxyfenozide (RH-2485)	0.10 (LOQ)	14	94.0-151	113	18.3	16.2
	0.25	6	94.8-121	106	9.2	8.7
	1.00	7	93.0-118	108	7.7	7.1
	5.00	6	94.6-106	99	5.2	5.3
TR 34-98-25 Analysis (HPLC/UV Results)						
Methoxyfenozide (RH-2485)	0.10 (LOQ)	2	96.0, 100	--[3]	--	--
	0.25	1	94.8	--	--	--
	1.00	1	93.0	--	--	--
	5.00	1	94.6	--	--	--
TR 34-97-51 Analysis (HPLC/UV Results)						
Methoxyfenozide (RH-2485)	0.10 (LOQ)	12	94.0-151	116	18.6	16.1
	0.25	5	98.7-121	108	8.4	7.8
	1.00	6	106-118	110	4.6	4.2
	5.00	5	94.7-106	100	5.3	5.4
TR 34-98-25 Analysis (HPLC/MS/MS Results)						
Methoxyfenozide (RH-2485)	0.10 (LOQ)	2	96.9, 111	--	--	--
	0.25	1	107	--	--	--
	1.00	1	94.0	--	--	--
	5.00	1	84.4	--	--	--

Data (**corrected** recovery results) were obtained from Tables 1-3, pp. 15-16 of MRID 49201703.

1 Untreated well water was not characterized (p. 10).

2 Mean recoveries, standard deviations and relative standard deviations were reviewer-calculated from the data in the study report since the study author only reported means and SDs for all fortification levels together (see DER Attachment 2).

3 Mean recoveries, standard deviations and relative standard deviations were not applicable.

Table 3. Independent Validation Method Recoveries for Methoxyfenozide in Well Water[1]

Analyte	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)[2]
TR 34-98-102 Analysis (HPLC/UV Results)						
Methoxyfenozide (RH-2485)	0.10 (LOQ)	5	86.5-97.2	92.1	3.80	4.2
	1.0	5	103.2-104.4	103.6	0.50	0.5

Data (uncorrected recovery results) were obtained from Tables 1-2, pp. 12-13 of MRID 49201702.

1 Untreated well water was not characterized (p. 8).

2 Relative standard deviations were reviewer-calculated from the data in the study report since the study author only reported means and SDs (see DER Attachment 2).

III. Method Characteristics

The limit of quantification (LOQ) for methoxyfenozide was the same in the ECM and ILV at 0.10 ppb (pp. 7, 12-13 of MRID 49201703; p. 11 of MRID 49201702). In the ECM, the LOQ was applied to the HPLC/UV and HPLC/MS/MS analyses. The LOQ was established by the fortification and analysis of samples at that level. No calculations were reported. In the ECM, the limit of detection (LOD) was defined as 30% of the LOQ which corresponded to 0.03 ppb. No calculations were reported. The LOD was not reported in the ILV. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

Table 4. Method Characteristics for Methoxyfenozide in Untreated Well Water

	Methoxyfenozide	
	HPLC/UV[1,2]	HPLC/MS/MS[3]
Limit of Quantitation (LOQ)	0.10 ppb	
Limit of Detection (LOD)	0.03 ppb[4]	
Linearity (calibration curve r^2 and concentration range)[5]	ECM: r^2 = 1.0000 (0.01-1.00 µg/mL)[6] ILV: r^2 = 0.995256 (0.01-0.50 µg/mL)	r^2 = 0.9996 (0.01-1.00 µg/mL)[6]
Repeatable	Yes	Yes[7]
Reproducible	No[8]	No[8,9]
Specific	No[10,11]	No[11]

Data were obtained from p. 12; Appendix 1, Figure 7, p. 25; Appendix 2, Figure 20, p. 39 of MRID 49201703; and Appendix 1, p. 21 of MRID 49201702.

1 For the ECM, the HPLC/UV analysis data was a compilation of two separate analyses: TR 34-98-25, which included two samples at the LOQ and one sample each at 10×LOQ, 0.25 ppb and 5.00 ppb, and TR 34-97-51 (dated July 30, 1997; p. 14), which included twelve samples at the LOQ, six samples at 10×LOQ and five samples each at 0.25 ppb and 5.00 ppb. The reviewer has separated the data from these analyses in Table 2 above. The reviewer assumed that the calibration data for the linearity assessment was part of TR 34-98-25.

2 For the ILV, five samples were analyzed by HPLC/UV at the LOQ and 10×LOQ.

3 Only the ECM performed HPLC/MS/MS analysis, as a confirmation method. Only samples in the TR 34-98-25 analysis were included in the data set.

4 The LOD was only reported in the ECM.

5 The linearity of the ECM and ILV calibration curves were validated by the reviewer using the data from the chromatograms. For the ECM, the reviewer graphed peak area versus concentration to generate calibration curves for the HPLC/UV (r^2 = 0.9999, 0.01-1.00 µg/mL) and HPLC/MS/MS (r^2 = 0.9993, 0.01-1.00 µg/mL) analyses (see Reviewer Comment #5; Appendix 1, Figures 1-6, pp. 19-24 and Appendix 2, Figures 14-19, pp. 33-38 of MRID 49201703). For the ILV, the reviewer graphed peak height versus concentration to generate a calibration curve for the HPLC/UV (r^2 = 1, 0.01-0.50 µg/mL) analyses (Appendix 1, pp. 15-20 of MRID 49201702).

6 The concentration range which was reported in the titles of the Figures of the ECM ("mg/mL") seemed to be an error because the ECM method dictated the concentration range of 0.010 µg/mL to 1.0 µg/mL (pp. 11-12).

7 n = 2 for the LOQ level; n = 1 for the 10×LOQ level.

8 The ECM and ILV were not performed by two separate, independent laboratories. KeyStone Analytical Laboratories performed part of the ECM and all of the ILV (pp. 1, 14 of MRID 49201703; and p. 1 of MRID 49201702).

9 No ILV performed using HPLC/MS/MS analysis.

10 No confirmation method was used for the HPLC/UV analysis of the ILV. The analyte peak was not distinct from the background/baseline at the LOQ.

11 The calibration curves provided by the ECM and ILV did not adequately bracket the expected concentrations of the fortified samples.

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IV. Method Deficiencies and Reviewer's Comments

1. The reviewer determined that the ECM and ILV were not entirely performed by two separate laboratories. The ECM report was prepared by Rohm and Haas Company, Spring House, Pennsylvania and **Keystone Analytical Laboratories, Inc.,** North Wales, Pennsylvania, and the ILV report was prepared by **KeyStone Analytical Laboratories**, North Wales, Pennsylvania (p. 1 of MRIDs 49201703 & 49201702). Aside from the slight difference in titling, the KeyStone laboratory of the ECM was the same as that of the ILV, including the Unit # and address of the laboratory. Additionally, the ECM study personnel from KeyStone Laboratories, Inc., Allen Xu (reported on p. 14 of MRID 49201703), was one of the ILV study authors (p. 1 of MRID 49201702). OCSPP guidelines specify that the laboratory which performs the ILV must be independent from that which performed the ECM.

2. The ECM study report used the results from two analyses: TR 34-98-25 (termed the "current analysis") and TR 34-97-51 (dated July 30, 1997; p. 14; Tables 1-3, pp. 15-16). The reviewer compiled Table 2 of the DER showing the results of these two studies together and separated. All of the results met OCSPP guidelines regarding acceptable recoveries and fortifications at the LOQ and 10×LOQ; however, the data set for TR 34-98-25 contained only one or two samples for each fortification level. OCSPP guidelines specify a minimum of five samples to be fortified at LOQ and 10×LOQ. The data set for TR 34-97-51 lacked a confirmation method (see Reviewer Comment #7), and no raw data or calibration curve from this analysis were provided. The data set from each analysis did not meet all of the OCSPP guidelines. When the two analyses were combined, OCSPP guidelines were met, but these two analyses could not be confirmed as equivalent (see Reviewer Comment #3 for more discussion).

3. The method of quantification of the analyte differed between the ECM and ILV, and possibly differed within the ECM "combined" data. Both the ECM and ILV calculations specify that the quantification of the analyte concentration was based on the standard curve (p. 12 of MRID 49201703; p. 9 of MRID 49201702). In the ECM, the provided calibration curves were part of the TR 34-98-25 analysis (labeled as such) and were graphed, contrary to preference, as peak area versus concentration (p. 11; Table 1, p. 15; Appendix 1, Figure 7, p. 25; Appendix 2, Figure 20, p. 39 of MRID 49201703). The study report dictated that "standards and samples are preferably quantitated by peak height, although area may be used" (p. 11 of MRID 49201703). The reviewer believed that the quantification method employed for the TR 34-98-25 analysis could have differed from that employed for the TR 34-97-51 analysis. This indicated a possible discrepancy in the method of quantification of the samples of each analysis, so combining the data from these two analyses may not be appropriate. The ILV calibration curve, and quantification of fortified samples, was based on peak height versus concentration; therefore, the quantification method of the ILV differed from what was reported in the ECM (Appendix 1, p. 21 of MRID 49201702). See Reviewer Comments #6 & #10 for more discussion of the calibration curves.

4. The chromatograms of the LOQ were not distinctive from the baseline in the HPLC/UV analysis of the ECM and ILV (Appendix 1, Figures 9-10, pp. 27-28 of MRID 49201703; and Appendix 1, p. 24 of MRID 49201702). Interferences in the ILV chromatogram greatly obscured the LOQ peak, although the ILV study authors used height for quantification. More distinction was observed in the HPLC/MS/MS chromatogram; however, this method

was not validated or employed by the ILV (Appendix 2, Figures 22-23, pp. 41-42 of MRID 49201703).

5. The determinations of LOQ and LOD were not based on scientifically acceptable procedures. In the ECM, the LOQ was applied to the HPLC/UV and HPLC/MS/MS analyses (pp. 7, 12-13 of MRID 49201703; p. 11 of MRID 49201702). The LOQ was established by the fortification and analysis of samples at that level. In the ECM, the limit of detection (LOD) was defined as 30% of the LOQ which corresponded to 0.03 ppb. The LOD was not reported in the ILV. No calculations related to background values were provided.

   Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples. Additionally, the lowest toxicological level of concern in water was not reported. An LOQ above toxicological levels of concern results in an unacceptable method classification.

6. The calibration curves provided by the ECM and ILV did not adequately bracket the expected concentrations of the fortified samples. In the ECM, the area and height of the lowest calibration standard (0.01 "mg/mL") were similar to that of the LOQ (0.10 ppb; Appendix 1, Figure 1, p. 19; Appendix 1, Figures 9-10, pp. 27-28; and Appendix 2, Figure 14, p. 33; Appendix 2, Figures 22-23, pp. 41-42 of MRID 49201703). Likewise in the ILV, the height of the lowest calibration standard (0.01 µg/mL) was similar to that of the LOQ (0.01 ppb; Appendix 1, pp. 15, 24 of MRID 49201702). That the calibration standards did not bracket the fortified samples may have resulted in the wide range of recovery values at the LOQ (94-151% for HPLC/UV and 96.9-111% for HPLC/MS/MS; Table 1, p. 15; Table 3, p. 16 of MRID 49201703; and Table 2, p. 13 of MRID 49201702).

7. In the ECM study, confirmation of the analytes was performed with HPLC/MS/MS in the TR 34-98-25 analysis; however, an insufficient number of samples was included in the TR 34-98-25 analysis (Tables 1-3, pp. 15-16 of MRID 49201703). So, in the portion of the study which contained a sufficient number of samples (TR 34-97-51), no confirmation method was used, only the primary HPLC/UV method. In the ILV, only HPLC/UV analysis was employed (pp. 9-10 of MRID 49201702). The protocol which the ILV was following, which was provided by the Sponsor, was Protocol Number 34P-98-35 (Appendix 2, pp. 26-31 of MRID 49201702). This protocol specified for the water samples to be analyzed by method TR 34-98-25 (p. 28), but did not specify the analytical instrument or analysis to be employed. So, the reviewer could not determine if the protocol specified HPLC/UV and HPLC/MS/MS analysis or just HPLC/UV analysis.

8. In the ECM, no raw data from the TR 34-97-51 analysis (dated July 30, 1997; p. 14) was provided. Reagent blank samples were not included for the TR 34-98-25 analysis.

9. The water matrices were not characterized in the ECM and ILV (p. 10 of MRID 49201703; p. 8 of MRID 49201702).

10. For the ECM and ILV, the individual peak area/height count data used to generate the provided multi-point standard curves were not reported (Appendix 1, Figure 7, p. 25; Appendix 2, Figure 20, p. 39 of MRID 49201703; and Appendix 1, p. 21 of MRID 49201702). The reviewer generated five-point curves using peak area/height count data from

provided chromatograms of calibration standards (see Table 4 above; DER Attachment 2). For the ECM, the reviewer graphed peak area versus concentration, as opposed to peak height versus concentration, in order to validate the calibration curves which were provided by the ECM; however, the reviewer noted that the study report dictated that quantification by peak height was preferable (p. 11 of MRID 49201703). In the ILV, the calibration curve was generated by graphing peak height versus concentration (Appendix 1, p. 21 of MRID 49201702).

11. The ECM residue calculations specify correcting recovery results for any residues detected in the matrix control samples (p. 12 of MRID 49201703). Also, recoveries in the data tables were listed as "Corrected Recovery" (Tables 1-4, pp. 15-16 of MRID 49201703). However, residues were not corrected in the ILV, based on the calculations which were provided (pp. 9-10 of MRID 49201702).

12. In the ECM report, the cover page indicated another MRID # for the study report: 44617824 (Submitted Date, 07/17/1998; un-paginated cover page of MRID 49201703).

13. The ILV study authors reported that "no communication took place between KeyStone and Rohm & Haas" during the trial (p. 11 of MRID 49201702).

14. It was reported in the ILV that a set of twelve samples could be completed in 6 hours or less (p. 6 of MRID 49201702).

V. References

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 (p. 7 of MRID 49201703)

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