

1.0 INTRODUCTION

1.1 Principle and Scope of the Method

JRFA Method AU-269R0 Attachment A was developed to determine residues of Aldicarb and its metabolites Aldicarb Sulfoxide and Aldicarb Sulfone in drinking, ground and surface water and independently validated by JRF America, Audubon, PA.

The independent lab validation was conducted using LOQ (0.1 µg/L) and 10×LOQ (1.0 µg/L) fortification levels for Aldicarb, Aldicarb Sulfoxide, and Aldicarb Sulfone in drinking, ground, and surface water. For each fortification level and matrix, five replicates were analyzed. Additionally, one method blank and two unfortified control samples were examined. The method LOD was set at 0.05 µg/L (50% of the LOQ) for each analyte. Three replicates of samples fortified at 0.03 µg/L were also analyzed to assess the method at 30% of the LOQ.

The residues of Aldicarb, Aldicarb Sulfoxide, and Aldicarb Sulfone were tested in 10 mL of drinking, ground, and surface water by vortex and centrifugation. The final determination was conducted using LC/MS/MS analysis in positive ion mode.

1.2 Specificity

Aldicarb, Aldicarb Sulfoxide, and Aldicarb Sulfone were identified and quantified as individual analytes.

2.0 MATERIALS AND METHODS

2.1 Test Systems

The following test systems were considered for this study:

Test System 1: Surface Water, Skippack Creek at Hoy Park, Collegeville, PA

Test System 2: Ground (Well) Water, residential well, West Chester, PA

Test System 3: Drinking Water, commercially available, Norristown, PA

Control drinking, ground, and surface water matrices were acquired by JRF America. The samples were logged in at JRF America and given unique laboratory identification numbers. The samples were stored refrigerated.

The characterization of the water used was performed by Agvise Laboratories and is given in the respective attached reports (Appendix B).

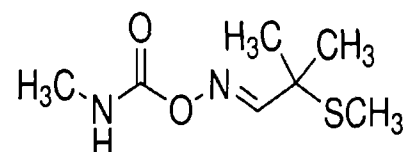
2.2 Test and Reference Substances

Three test/reference substances were evaluated: Aldicarb; Aldicarb Sulfoxide; and Aldicarb Sulfone. An archive sample of the three test/reference substances will be retained at JRF America. The following sample information and chemical/physical properties for the test/reference substances are:

2.2.1 Aldicarb

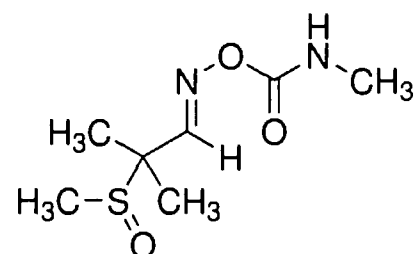
Chemical Name:	2-Methyl-2-(methylthio)propanal O-(N-methylcarbamoyl)oxime
CAS Registry No.:	116-06-3
Molecular Formula:	C ₇ H ₁₄ N ₂ O ₂ S
Molecular Weight:	190.26
Batch No.:	SZBC166XV
Re-Assay Date:	July 07, 2015
Purity:	99.9%
Recommended storage:	Ambient
Source:	Sigma-Aldrich

Chemical Structure:

**2.2.2 Aldicarb Sulfoxide**

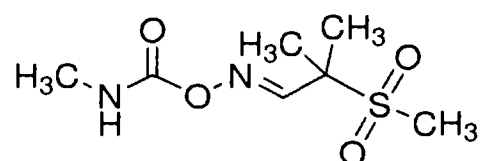
Chemical Name:	(1E)-2-Methyl-1-(((methylamino)carbonyl]oxy)imino)-2-(methylsulfinyl)propane
CAS Registry No.:	1646-87-3
Molecular Formula:	C ₇ H ₁₄ N ₂ O ₃ S
Molecular Weight:	206.26
Batch No.:	SZBD049XV
Re-Assay Date:	July 24, 2015
Purity:	99.2%
Recommended storage:	Refrigerated
Source:	Sigma-Aldrich

Chemical Structure:

**2.2.3 Aldicarb Sulfone**

Chemical Name:	2-methyl-2-(methylsulfonyl)-propionaldehyde-O-(methylcarbamoyl)oxime
CAS Registry No.:	1646-88-4
Molecular Formula:	C ₇ H ₁₄ N ₂ O ₄ S
Molecular Weight:	222.26
Batch No.:	SZBC313XV
Re-Assay Date:	7-Oct-15
Purity:	100%
Recommended storage:	Ambient
Source:	Sigma-Aldrich

Chemical Structure:



2.3 Equipment

Equipment	Size, Description	Manufacturer	Catalog No.
Analytical Balance	AT 200	Mettler Toledo	----
Flasks, Volumetric	Various sizes	Various	----
Cylinder, graduated	Various sizes	Various	----
Pipettes	Various volumes	Eppendorf	----
Centrifuge	Allegra 6	Beckman Coulter	----
Bottle, Amber glass	Qorpak , 2 oz and 4 oz with Teflon®-lined screw cap	VWR Scientific Products Boston Round, Amber	----
Plastic Centrifuge Tubes	15 mL	VWR	525-0400
Mixer	BenchMark, vortex	BenchMark Scientific, Inc.	13112194
Ultrasonic bath	Model FS 7652H	Fisher Scientific	----
HPLC vials	2 mL	Agilent Technologies	5182-0716
HPLC vial caps	PTFE/red silicone septa	Agilent Technologies	5182-0717
Liquid Chromatographic System	Waters Acquity UPLC	Waters	----
Mass Spectrometer	AB Sciex API 4000 QTrap	AB Sciex	----
HPLC Column	Nomura Develosil RP aqueous, C30, 150 x 2 mm, 5µm	Nomura	

2.4 Reagents

2.4.1 Chemicals

Chemical	Grade	Manufacturer/Supplier	Catalog No.
Water	LC/MS	Sigma-Aldrich	39253-4x4L-R
Ammonium acetate	LC/MS	Supelco/Sigma-Aldrich	14267-25G
Methanol	LC/MS	Fluka	34966-4L
Acetonitrile	LC/MS	EMD	AX0156-1

2.4.2 Solutions and Solvent Mixtures

Description	Code	Composition
Solvent system 1	S1	50/50 LC/MS grade Water/Methanol 250 mL of Water and 250 mL of Methanol in 500 mL graduated cylinder.
HPLC mobile phase A	LC1	5mM Ammonium acetate in LC/MS Water Add 0.385 g of Ammonium acetate into 1 L volumetric flask. Add LC/MS Water to line. Mix well to ensure complete homogeneous solution.
HPLC mobile phase B	LC2	100% LC/MS Grade Methanol

2.4.3 Standard Solutions

Standard solutions were stored refrigerated in amber glass bottles with screw caps.

Preparation of Stock Solutions

A 0.1 mg/mL stock solution was prepared for each individual analyte by weighing an appropriate amount of each analyte into a 100 mL volumetric flask and diluting to volume in acetonitrile.

Analyte ID	Purity	Weight (mg) ^a	Final Volume (mL)	Concentration (mg/mL)
Aldicarb	99.9%	11.6	100	0.116
Aldicarb Sulfoxide	99.2%	10.0	100	0.100
Aldicarb Sulfone	100%	10.2	100	0.102

^a Corrected for purity

Preparation of Mixed Analyte Solutions

A mixed analyte solution was prepared by dilution of the intermediate stock dilution solutions with methanol/water (50:50, v/v) in a 50 mL volumetric flask.

Analyte	Concentration (µg/mL)	Aliquot Volume (mL)	Final Volume (mL)	Final Concentration (µg/mL)
Aldicarb	116	0.431	50	1.0
Aldicarb Sulfoxide	100	0.500		
Aldicarb Sulfone	102	0.4901		

Preparation of Fortification Solutions

Fortification solutions were prepared by serial dilution of the mixed analyte solution with methanol/water (50:50, v/v) in 10 mL volumetric flasks and diluting to volume.

Analyte	Concentration (µg/mL)	Aliquot Volume (mL)	Final Volume (mL)	Final Concentration (µg/mL)
Mixed	1.0	5	50	0.1
Mixed	0.1	1	10	0.01

Preparation of Calibration Standard Solutions

Standard solutions for calibration were prepared by dilution of the fortification solutions with methanol/water (50:50, v/v) in 10 mL volumetric flasks, or HPLC vial for the 0.03 ng/mL (30% LOQ) standard.

Analyte	Concentration (ng/mL)	Aliquot Volume (mL)	Final Volume (mL)	Final Concentration (ng/mL)
Mixed	100	0.1	10	1.0
	1.0	5	10	0.5
	0.5	5	10	0.25
	0.25	4	10	0.1
	0.1	5	10	0.05
	0.05	0.9	1.5	0.03

3.0 Analytical Procedure

3.1 Weighing and Fortification

Sixteen samples were prepared for each matrix (surface, ground, and drinking water): One method blank, two control samples, three treated at 30% of the limit of quantitation (LOQ), five samples treated at the LOQ, and five treated at 10×LOQ. For each, 10 mL of water was measured into a 15 mL centrifuge tube and spiked with the appropriate fortification solution.

The following scheme was used:

Sample Type	Sample Volume	Concentration of Spiking Solution [µg/mL]	Volume of Spiking Solution [µL]	Level of Fortification [µg/L]
Control	10 mL	-	-	0.00
Fortification (30% LOQ)	10 mL	0.01	30	0.03
Fortification (LOQ)	10 mL	0.01	100	0.1
Fortification (10× LOQ)	10 mL	0.1	100	1.0

3.2 Extraction of the Sample Material

The samples were mixed using a vortex for 1 min. and centrifuged for 5 min. at 3,000 rpm prior to dilution (if needed).

3.3 Preparation for Measurement

For Control and LOQ levels, samples were ready for injection. The 10×LOQ samples were diluted 10 times with methanol/water (50:50, v/v). The final concentration of each sample, as prepared, is presented below:

Sample Type	Aldicarb Concentration (ng/mL)	Aldicarb Sulfoxide Concentration (ng/mL)	Aldicarb Sulfone Concentration (ng/mL)
Control	0.0	0.0	0.0
Fortification (30% LOQ)	0.03	0.03	0.03
Fortification (LOQ)	0.1	0.1	0.1
Fortification (10×LOQ)	1.0	1.0	1.0

4.0 Instrumentation and Conditions

UPLC/MS/MS Conditions for the Analysis of Aldicarb, Aldicarb Sulfoxide, and Aldicarb Sulfone

Waters Acquity UPLC Conditions			
Analytical Column	Nomura Develosil RPaqueous-3, C30, 150 x 2 mm, 5µm		
Column Temperature	40 °C		
Flow Rate	0.45 mL/min		
Gradient:	Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
	Initial	95	5
	1.0	90	10
	1.5	75	25
	3.00	0	100
	6.00	95	5
	8.00	95	5
	8.01	95	5
Mobile Phase A:	5 mM Ammonium acetate in Water		
Mobile Phase B:	100% Methanol		
Injection Volume (µL):	10		

MS/MS Conditions	
Interface	AB SCIEX 4000 Qtrap® with Turbo Ion Spray®
Polarity	Positive
Ionization	Electrospray (ESI)
Curtain gas (CUR)	30
Temperature (TEM)	450 °C
Ion transfer voltage (IS)	5500
Collision gas setting (CAD)	10
GS1	40
GS2	45
Scan type	MRM

Analyte	Transitions (m/z)		polarity	Approximate Retention Time (min.)
	Primary	Secondary		
Aldicarb	208 → 88.6	208 → 115.7	Positive	3.45
Aldicarb Sulfoxide	207 → 88.5	207 → 68.5		2.72
Aldicarb Sulfone	240 → 75.5	240 → 86.0		2.82

4.1 Calibration Procedures

Calculation of results is based on peak area measurements using a calibration curve. The calibration curve is obtained by injection of the calibration standards containing known amounts of Aldicarb and its metabolites at appropriate concentrations for each analyte (0.03 to 1.0 ng/mL). Typical calibration curves are presented for Aldicarb and its metabolites in Figure 11.1 through Figure 11.3. Representative calibration standard chromatograms are presented in Figure 11.4 through Figure 11.21. Representative blank, untreated control, and fortified control sample chromatograms are presented in Figure 11.22 through Figure 11.75.

4.2 Calculation of Residues and Recoveries

Calculations of results were based on peak area measurements. Analyst[®] 1.5.1 software created the standard curve based on linear regression analysis using 1/x weighting. The recoveries of the analytes were calculated relative to the linearity curve generated with each set.

$$\text{I. Concentration (ng/mL)} = \frac{\text{Response} - \text{Intercept}}{\text{Slope}} = C_A$$

The recoveries of spiked compounds are calculated according to equation II:

$$\text{II. Recovery \%} = \frac{\text{Conc. of Fortified Sample (ng/mL)} - \text{Conc. of Control (ng/mL)}}{\text{Conc. of Sample as Prepared}} \times 100$$

Example Calculation: Aldicarb (208 → 88.6), Surface Water Trial 1, 10×LOQ #1 fortified at 1 ppm, Analytical Batch: SURFACE_TRIAL_1_10202014.wiff

The following values were used in this calculation:

Response of fortified sample	3679.32
Response of control sample	0.000
Slope:	35400
Intercept:	-83.7

$$\text{I. Concentration (ng/mL)} = \frac{3679.32 + 83.7}{35400} = 0.1063 \text{ ng/mL}$$

$$\text{II. Recovery \%} = \frac{0.1063 \text{ ng/mL}}{0.1 \text{ ng/mL}} = 106\%$$

5.2 Summary of Method

Type of method: LC/MS/MS

Test systems: Surface (Creek) Water
Ground (Well) Water
Drinking (Bottled) Water

Analytes and selected mass transitions (*m/z*):

<u>Analyte</u>	<u>Primary</u>	<u>Secondary</u>
Aldicarb	208 → 88.6	208 → 115.7
Aldicarb Sulfoxide	207 → 88.5	207 → 68.5
Aldicarb Sulfone	240 → 75.5	240 → 86.0

Analytical procedure: 10 mL of test water was fortified with Aldicarb and its metabolites Aldicarb Sulfoxide and Aldicarb Sulfone. The final determination was conducted using LC/MS/MS electrospray ionization in positive ion mode.

Confirmatory technique: For every compound the quantitation is possible at two different transitions. Due to the high selectivity and specificity of LC/MS/MS an additional confirmatory technique was not necessary.

Limit of detection (LOD): 0.05 µg/L for Aldicarb, Aldicarb Sulfoxide, and Aldicarb Sulfone.

Limit of quantification (LOQ): 0.1 µg/L for Aldicarb metabolites, corresponding to a concentration of 0.1 ng/mL in the final extract.

Levels of fortification: 0.1, and 1.0 µg/L for Aldicarb and metabolites. 0.03 µg/L level was also analyzed to assess the method at 30% of the LOQ.

Time required: The analysis of 16 samples per matrix requires about 8 hours of work including the calculation of the results and reporting of all raw data under GLP.

7.0 CONCLUSIONS/RECOMMENDATIONS FROM ILV

The ILV was completed successfully on the first trial for Aldicarb, Aldicarb Sulfoxide, and Aldicarb Sulfone in drinking, ground, and surface water. All average recoveries (70-110%) and relative standard deviations ($\leq 20\%$) were within the acceptable range.

The following modifications to JRFA Analytical method AU-269R0 are recommended:

1. Change "Preparation of Fortification Solutions" table in Section 2.4.3 to correspond to the preceding text. The heading in column four of the table should read "Dilute with 50:50, *methanol:water* to a final volume of (mL)".
2. Change "Instrumental Analysis" table in Section 4.2. The mobile phase composition at the 3.00 min. time point of the gradient should read "0" in the "Phase A" column in the Parameter section.

8.0 PROTOCOL AMENDMENTS AND DEVIATIONS

This study was conducted according to the study protocol approved on October 08, 2014. One protocol deviation was documented for the study and is presented in Appendix D. Records of all communication with the Study Monitor are contained in the raw data package and a summary can be found in Appendix E. The protocol deviation is summarized below.

1. No peaks were detected for 0.03 ppb (30% LOQ) standard for the confirmatory transition for Aldicarb Sulfoxide in the ground and surface water matrices. Deviation has no impact on study, since calibration curve covers 50% LOQ (0.05 ppb) as target LOD, for the aforementioned transitions, analyte and matrices.

9.0 REFERENCES

1. OCSP 850.6100, Ecological Effects Test Guidelines, U.S. Environmental Protection Agency. U.S. Government Printing Office: Washington, DC, 2012; EPA-712-C-001.
2. SANCO/825/00 rev. 8.1 (16 November 2010), Guidance Document on Pesticide Residue Analytical Methods.
3. SANCO/3029/99 rev. 4 (11 July 2000), Guidance for Generating and Reporting Methods of Analysis in Support of Pre-Registration Data Requirements for Annex II.
4. C. Bianca "Analytical Method for Analysis of Aldicarb, Aldicarb Sulfone and Aldicarb Sulfoxide in Three Water Types: Surface Water; Ground Water; and Drinking Water"; JRF America Method Number AU-269R0; JRF America: Audubon, PA, October 3, 2014.