

Analytical method for dodine in water

Reports: ECM: EPA MRID No. 50914404. Schoutsen, T.P., and I.F. Wierda. 2001. Validation of an Analytical Method for the Determination of Dodine Residues in Water. NOTOX Project No.: 335058. Report prepared by NOTOX B.V., 's-Hertogenbosch, The Netherlands, sponsored by Arysta LifeScience Benelux (formerly Chimac-Agriphar), Ougree, Belgium, and submitted by Arysta LifeScience North America LLC, Cary, North Carolina; 31 pages. Final report signed December 20, 2001 (pp. 3, 5); report submission dated July 16, 2019 (pp. 2-3).


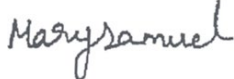
ILV: EPA MRID No. 50914403. Bacher, R. 2007. Dodine: Development and Validation of a Confirmatory Method for the Determination of Dodine in Surface Water. PTRL Europe Study No.: P 1271 G. Report prepared by PTRL Europe GmbH, Ulm, Germany, sponsored by Agriphar S.A., Ougree, Belgium, and submitted by Arysta LifeScience North America LLC, Cary, North Carolina; 29 pages. Final report issued September 21, 2007 (Original 1 of 2; p. 1); report submission dated July 16, 2019 (pp. 2-3).

Document No.: MRIDs 50914404 & 50914403

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with OECD Good Laboratory Practices (GLP) which are essentially in conformity with USEPA FIFRA (40 CFR Part 160), US FDA, and USEPA TSCA (40 CFR Part 792; p. 3 of MRID 50914404). 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. The GLP Certificate of the Test Facility was also included (p. 30).
ILV: The study was conducted in accordance with German GLP which are based on OECD GLP which are accepted by Regulatory Authorities throughout Europe, the USA (FDA and EPA, FIFRA (40 CFR Part 160)), and Japan (MHW, MAFF, and METI), as well as Council Directive 91/414/EEC Annex II (Part A, Section 4.2.; pp. 3, 6 of MRID 50914403). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-3, 5-6). A statement of the authenticity of the study report was included with the Quality Assurance statement. The GLP Certificate of the Test Facility was also included (p. 4).

Classification: This analytical method is classified as supplemental. The ILV was conducted to develop and validate a confirmatory method for dodine analysis in surface water. Although the ILV method was based on the ECM method, the reviewer considered the ILV to be an adapted, more rigorous ECM, as opposed to an ILV, of the ECM MRID 50914404, since there were multiple significant modifications to the sample processing procedure and analytical method, as well as establishment of a new method LOQ.

PC Code:	044301		
EFED Final Reviewer:	Taimei Harris, Ph.D., Chemist	Signature:	
		Date:	
	Lisa Muto, M.S., Environmental Scientist	Signature:	
CDM/CSS-Dynamac JV Reviewers:	Mary Samuel, M.S., Environmental Scientist	Date:	08/27/2020
		Signature:	
		Date:	08/27/2020

EPA Reviewer: Katrina White, Ph.D.

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac Joint Venture personnel. The CDM/CSS-Dynamac JV role does not include establishing Agency policies.

Executive Summary

This analytical method, NOTOX Project No. 335058, is designed for the quantitative determination of dodine in water using LC/MS/MS at the LOQ of 0.008 µg/L for surface water and at the LOQ of 0.1 µg/L for tap and ground water; however, only surface water was included in the internal validation. The surface water LOQ is less than the lowest toxicological level of concern in surface water matrices. The tap and ground water LOQ are less than the lowest toxicological level of concern in tap and ground water matrices. Although the ILV method (PTRL Europe Study No. P 1271 G) was based on the ECM method, the reviewer considered the ILV to be an adapted, more rigorous ECM, as opposed to an ILV, of the ECM MRID 50914404, since there were multiple significant modifications to the sample processing procedure and analytical method, as well as establishment of the method LOQ as 0.05 µg/L for surface water. Since the reported method LOQs of the ECM and ILV were not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQs of the ECM and ILV are the lowest levels of method validation (LLMVs) rather than LOQs.

The ECM and ILV each used one characterized surface water matrix. At least two sets of performance data should be submitted, and a maximum of three sample sets should be used to validate the ECM. Characterized tap (drinking) and ground water matrices were included in the ECM; however, these matrices were not fortified with dodine. The number of ILV trials required to validate the method was not reported. The ILV communications were not included in the study report, but the performing laboratories and personnel differed between the ECM and ILV.

The reproducibility of the method could not be determined at any fortification since only one set of performance data was submitted for each fortification. ILV data regarding precision and accuracy was acceptable, but only two samples were prepared for all ECM fortifications. All ILV and ECM data regarding linearity and specificity were satisfactory for dodine in the surface water matrices. The LOD was not reported in the ECM.

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						
Dodine	50914404 ¹	50914403 ²	Taimei Harris	Surface Water	20/12/2001	Arysta LifeScience North America LLC	LC/MS/MS	0.008 µg/L (ECM)
	50914404 ³	None submitted	Taimei Harris	Tap and Ground Water				0.05 µg/L (ILV)
								0.1 µg/L

1 In the ECM, the surface (river) water [pH 7.97, total hardness 14°dH (German degrees, medium-hard), dissolved organic carbon 7.0 mg/L] collected from the river Waal (Loenen, The Netherlands) was used in the study (p. 9 of MRID 50914404). The water characterization laboratory was not reported.

2 In the ILV, the surface (river) water (pH 7.6, total hardness 2.4 mmol/L corresponding to 13.2°dH, dissolved organic carbon 3.8 mg/L) was collected from Danube River near Bundesstrasse B30 in Ulm, Germany (p. 12 of MRID 50914403). The water characterization was performed by Institute Alpha in Ulm, Germany (non-GLP).

3 Tap (drinking) and ground (well) water matrices were included in the ECM; however, these matrices were not fortified with dodine (pp. 9, 17, 22; Figures 2-3, pp. 24-25 of MRID 50914404). Untreated control tap and ground water samples were only evaluated for specificity at the 0.008 µg/L LOQ level, but the ECM reported that the observed specificity equated to the fact that the method was applicable to tap and ground water matrices.

I. Principle of the Method

Water samples (99 mL) were fortified with 1.0 mL of the 0.8 or 8.0 µg/L fortification solutions, as necessary (sample total volume = 100 mL; pp. 10-14 of MRID 50914404). The samples were mixed with 10 mL of pH 7 phosphate buffer then applied to a Waters Oasis HLB C₁₈ solid phase extraction (SPE) cartridge (200 mg, 6 mL) preconditioned with 1 mL of methanol and 1 mL of water. The sample flask was rinsed with 3 mL Milli-Q water which was also added to the SPE column. After drawing the sample through, the cartridges were washed with 2 mL of wash solution 1 (21 mL acetonitrile + 9 mL methanol + 70 mL 2% ammonium hydroxide) then 2 mL of wash solution 2 (21 mL acetonitrile + 9 mL methanol + 70 mL 2% acetic acid). The analyte was eluted with 2.5 mL of elution solution (49 mL acetonitrile + 21 mL methanol + 30 mL 2% acetic acid). The keeper solution (1 mL; 100 µL glycerol in 99.9 mL methanol) was added then the sample was dried at 60°C under a stream of nitrogen. Methanol (*ca.* 1 mL) was added after 20-minute intervals until all the solvent was evaporated. The residue was reconstituted with 250 µL of end solution [acetonitrile:water (45:55, v:v) + 0.1% heptafluorbutyric acid] and analyzed by LC/MS/MS.

Samples were analyzed using an Agilent 1100 HPLC series and Waters 510 HPLC pump (pump 2) coupled to an Applied Biosystems Sciex API 3000 MS (pp. 11, 14 of MRID 50914404). The following LC conditions were used: Xterra MS C₈ (2.1 mm x 50 mm column; 3.5 µm particle

size; column temperature 20°C), mobile phase of acetonitrile:water (45:55, v:v) + 0.1% heptafluorbutyric acid, flow 0.3 mL/min., and injection volume of 10 µL. The following MS/MS conditions were used: positive mode (source temperature 500°C), Turbo Ion Spray interface, and multiple reaction monitoring (MRM). Dodine was identified using one ion pair transition: m/z 228.2→186.2. Expected retention time was 3.5 minutes.

The independent laboratory performed the ECM as written, with the following modifications: 1) wash solution 1 = 21 mL acetonitrile + 9 mL methanol + 70 mL 0.5% aqueous ammonia solution; 2) 10 mL versus 2.5 mL of elution solution was used due to low recoveries; 3) no keeper solution was used prior to evaporation; 4) the sample was evaporated to *ca.* 3 mL instead of dryness; 5) the reduced sample was reconstituted to a final volume of 5.0 mL using methanol:water (2:8, v:v); 6) significant analytical instrumentation, parameter, and equipment modifications; and 7) the method LOQ was 0.05 µg/L not 0.008 µg/L (pp. 13-16 of MRID 50914403). The same SPE cartridges were used. An Agilent 1200 HPLC system coupled to an Applied Biosystems MDS Sciex API 4000 Triple Quadrupole MS was used. All LC and MS parameters were very different from those of the ECM. The following LC conditions were used: Thermo Aquasil C₈ (3.0 mm x 150 mm column; 3.0 µm particle size; column temperature 20°C), Phenomenex C18 pre-column (4 mm x 3 mm), mobile phase of A) 1% formic acid in water and B) 0.1% formic acid in methanol [percent A:B; 0.0 min. 80:20, 5.0-14.0 min. 0:100, 14.1-18.0 min. 80:20], flow not reported, and injection volume of 20 µL. The following MS/MS conditions were used: positive mode (source temperature 550°C), Turbo Ion Spray interface, and multiple reaction monitoring (MRM). Dodine was identified using two ion pair transitions (quantitation and confirmation, respectively): m/z 228→187 and m/z 228→60; quantitation ion transition was the same as that of the ECM. Expected retention time was *ca.* 8.9 minutes.

The Limit of Quantification (LOQ) for dodine in surface water was reported as 0.008 µg/L in the ECM and 0.05 µg/L in the ILV (pp. 6, 9, 15, 20, 22 of MRID 50914404; pp. 7, 11, 19-20 of MRID 50914403). The Limit of Detection (LOD) for dodine in surface water was reported as 0.005 µg/L in the ILV. The LOD was not reported in the ECM. Since the LOQs were not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQs are the lowest levels of method validation (LLMV) rather than LOQs. The LOQ for tap and ground water was reported in the ECM as 0.1 µg/L; however, no validation was performed in these two matrices.

II. Recovery Findings

ECM (MRID 50914404): Recoveries were acceptable (77.2-88.6%) for analysis of dodine in one surface water matrix at the LOQ (0.008 µg/L) and 10×LOQ (0.08 µg/L); however, only two samples were prepared at each fortification so means and relative standard deviations (RSDs) could not be calculated (Tables 9-11, pp. 19-20). The study report recovery data was presented as two analytical batches. Each analytical batch was analyzed in triplicate. Each analytical batch was considered to be one sample where the mean of the triplicate analyses was considered to be the batch recovery. One ion transition was quantified; a confirmatory method is not usually required when LC/MS or GC/MS is used as the primary method to generate study data. Surface (river) water [pH 7.97, total hardness 14°dH (German degrees, medium-hard), dissolved organic carbon 7.0 mg/L] collected from the river Waal (Loenen, The Netherlands) was used in the study (p. 9). The surface water characterization laboratory was not reported. Tap and ground water matrices were included in the ECM; however, these matrices were not fortified with dodine (pp. 9, 17, 22). Untreated control tap and ground water samples were only evaluated for specificity at the 0.008 µg/L LOQ level, but the ECM reported that the observed specificity equated to the fact that the method was applicable to tap and ground water matrices.

ILV (MRID 50914403): Mean recoveries and relative standard deviations (RSDs) met requirements (mean 70-120%; RSD ≤20%) for analysis of dodine in one water matrix at the LOQ (0.05 µg/L) and 10×LOQ (0.5 µg/L; Table 1, p. 21). Two ion transitions were quantified; recovery results of the quantitative and confirmatory ion transitions were comparable. RSDs at the LOQ were high (17-18%). The surface (river) water (pH 7.6, total hardness 2.4 mmol/L corresponding to 13.2°dH, dissolved organic carbon 3.8 mg/L) was collected from Danube River near Bundesstrasse B30 in Ulm, Germany (p. 12). The water characterization was performed by Institute Alpha in Ulm, Germany (non-GLP). The method was validated by the with multiple significant modifications to the sample processing procedure and analytical method, as well as establishment of the method LOQ as 0.05 µg/L not 0.008 µg/L (see Reviewer's Comment #2; pp. 13-16). The number of ILV trials required to validate the method was not reported. The ILV method was based on the ECM method; however, the ILV was conducted to develop and validate a confirmatory method for dodine analysis in surface water (pp. 15, 19). The ILV is considered to be an adapted ECM, as opposed to an ILV, of the ECM MRID 50914404 (see Reviewer's Comment #1).

Table 2. Initial Validation Method Recoveries for Dodine in Water^{1,2}

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Surface (River) Water						
Dodine	0.008 (LOQ)	2 ³	80.3, 88.6	--	--	--
	0.08	2 ³	77.2, 78.8	--	--	--

Data (uncorrected recovery results; pp. 15-16) were obtained from Tables 10-11, pp. 19-20 of MRID 50914404. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ. The reported LOQ of the ECM differed from that of the ILV.

1 Dodine was identified using one ion pair transition: m/z 228.2→186.2.

2 The surface (river) water [pH 7.97, total hardness 14°dH (German degrees, medium-hard), dissolved organic carbon 7.0 mg/L] collected from the river Waal (Loenen, The Netherlands) was used in the study (p. 9). The surface water characterization laboratory was not reported. Drinking and ground water matrices were reported, but these matrices were not fortified for validation recovery.

3 The study report recovery data was presented as two analytical batches (Tables 9-11, pp. 19-20). Each analytical batch was analyzed in triplicate. Each analytical batch was considered to be one sample where the mean of the triplicate analyses was considered to be the batch recovery. Means and relative standard deviations (RSDs) could not be calculated since $n = 2$.

Table 3. Independent Validation Method Recoveries for Dodine in Water^{1,2}

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) ³	Relative Standard Deviation (%)
Surface (River) Water						
Quantitation ion transition						
Dodine	0.05 (LOQ)	5	67-97	80	14	18
	0.5	5	66-88	80	9	11
Confirmation ion transition						
Dodine	0.05 (LOQ)	5	68-97	80	14	17
	0.5	5	69-90	82	8	10

Data (uncorrected recovery results; pp. 17-18) were obtained from Table 1, p. 21 of MRID 50914403 and DER Attachment 2. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ. The reported LOQ of the ECM differed from that of the ILV.

1 Dodine was identified using two ion pair transitions (quantitation and confirmation, respectively): m/z 228→187 and m/z 228→60. The quantitation ion transition was the same as that of the ECM.

2 The surface (river) water (pH 7.6, total hardness 2.4 mmol/L corresponding to 13.2°dH, dissolved organic carbon 3.8 mg/L) was collected from Danube River near Bundesstrasse B30 in Ulm, Germany (p. 12). The water characterization was performed by Institute Alpha in Ulm, Germany (non-GLP).

3 Standard deviations were not reported in the study but were reviewer-calculated based on recovery results (DER Attachment 2). Rules of significant figures were followed.

III. Method Characteristics

The LOQ for dodine in surface water was reported as 0.008 µg/L in the ECM and 0.05 µg/L in the ILV (pp. 6, 9, 15, 20, 22 of MRID 50914404; pp. 7, 11, 19-20 of MRID 50914403). In the ECM, the LOQ was justified by the acceptability of the recovery results. No justification of the LOQ was reported in the ILV. No calculations to support the LOQ were reported in the ECM or ILV. The LOD for dodine in surface water was reported as 0.005 µg/L in the ILV. The LOD was not reported in the ECM. The LOD in the ILV was based on the level of matrix interferences in extracts of untreated blank control specimens ($\leq 10\%$ of the LOQ). No calculations were reported for the LOQ or LOD in the ECM or ILV.

Since the LOQs were not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQs are the lowest levels of method validation (LLMV) rather than LOQs.

The LOQ for tap and ground water was reported in the ECM as 0.1 µg/L; however, no validation was performed in these two matrices (pp. 6, 9, 22 of MRID 50914404).

Table 4. Method Characteristics – Surface Water

		Dodine
Limit of Quantitation (LOQ)*	ECM	0.008 µg/L ¹
	ILV	0.05 µg/L
Limit of Detection (LOD)	ECM	Not reported
	ILV	0.005 µg/L (10% of the LOQ)
Linearity (calibration curve r and concentration range) ²	ECM	r = 0.9986532006
		0.005-0.1 µg/L
	ILV	r = 0.9992 (Q & C)
		0.10-20 ng/mL
Repeatable	ECM ^{3,4}	Yes for 0.008 µg/L and 0.08 µg/L in one characterized surface water matrix, but n = 2 . ⁵
	ILV ^{6,7}	Yes for 0.05 µg/L and 0.5 µg/L in one characterized surface water matrix
Reproducible		Could not be determined. Only one set of performance data submitted for each fortification.
Specific	ECM	Yes, no matrix interferences were observed.
	ILV	Yes, matrix interferences were <i>ca.</i> 10% of the LOQ (based on peak height). ⁸

Data were obtained from pp. 6, 9, 15, 20, 22 (LOQ/LOD); Tables 9-11, pp. 19-20 (recovery data); Figure 7, p. 29 (calibration curves); Figures 1-6, pp. 23-28 (chromatograms) of MRID 50914404; pp. 7, 11, 19-20 (LOQ/LOD); Table 1, p. 21 (recovery data); Figure 1, p. 22 (calibration curves); Figures 2-7, pp. 23-28 (chromatograms) of MRID 50914403; DER Attachment 2. Q = quantitative ion transition; C = confirmatory ion transition.

* Since the LOQs were not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQs are the lowest levels of method validation (LLMVs) rather than LOQs. The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV.

1 The reported LOQ of the ECM differed from that of the ILV.

2 Solvent-based calibrations were used in the ECM while matrix-matched calibrations were used in the ILV (p. 13 of MRID 50914404; pp. 14, 19 of MRID 50914403).

3 In the ECM, the surface (river) water [pH 7.97, total hardness 14°dH (German degrees, medium-hard), dissolved organic carbon 7.0 mg/L] collected from the river Waal (Loenen, The Netherlands) was used in the study (p. 9 of MRID 50914404). The surface water characterization laboratory was not reported.

4 Tap and ground water matrices were included in the ECM; however, these matrices were not fortified with dodine (pp. 9, 17, 22; Figures 2-3, pp. 24-25 of MRID 50914404). Untreated control tap and ground water samples were only evaluated for specificity at the 0.008 µg/L LOQ level, but the ECM reported that the observed specificity equated to the fact that the method was applicable to tap and ground water matrices.

5 The study report recovery data was presented as two analytical batches (Tables 9-11, pp. 19-20 of MRID 50914404). Each analytical batch was analyzed in triplicate. Each analytical batch was considered to be one sample where the mean of the triplicate analyses was considered to be the batch recovery.

6 In the ILV, the surface (river) water (pH 7.6, total hardness 2.4 mmol/L corresponding to 13.2°dH, dissolved organic carbon 3.8 mg/L) was collected from Danube River near Bundesstrasse B30 in Ulm, Germany (p. 12 of MRID 50914403). The water characterization was performed by Institute Alpha in Ulm, Germany (non-GLP).

7 The ILV validated the method with significant modifications to the sample processing procedure and analytical method, as well as establishment of the method LOQ as 0.05 µg/L not 0.008 µg/L (pp. 13-16 of MRID 50914403). The number of ILV trials required to validate the method was not reported. The ILV method was based on the ECM method; however, the ILV was conducted to develop and validate a confirmatory method for dodine analysis in surface water (pp. 15, 19). The reviewer considered the ILV to be an adapted ECM, as opposed to an ILV, of the ECM MRID 50914404.

8 Based on Figures 6-7, pp. 27-28 of MRID 50914403. No peak was integrated by the study authors; however, a small peak was observed in the surface water blank control at the retention time of dodine (RT = 8.85 min.). The reviewer estimated the matrix interference based on interpreted peak heights.

IV. Method Deficiencies and Reviewer's Comments

1. Only surface water matrices were included in the ECM and ILV validations.
2. The submitted ILV MRID 50914403 was conducted to develop and validate a confirmatory method for dodine analysis in surface water (pp. 15, 19 of MRID 50914403). Although the ILV method was based on the ECM method, the ILV is considered to be an adapted ECM, as opposed to an ILV, of the ECM MRID 50914404, since there were multiple significant modifications to the sample processing procedure and analytical method, as well as establishment of the method LOQ as 0.05 µg/L not 0.008 µg/L, as in the ECM (pp. 13-16).
3. The ILV made the following significant modifications to the ECM (NOTOX Project No. 335058): 1) wash solution 1 = 21 mL acetonitrile + 9 mL methanol + 70 mL 0.5% aqueous ammonia solution; 2) 10 mL versus 2.5 mL of elution solution was used due to low recoveries; 3) no keeper solution was used prior to evaporation; 4) the sample was evaporated to *ca.* 3 mL instead of dryness; 5) the reduced sample was reconstituted to a final volume of 5.0 mL using methanol:water (2:8, v:v); 6) significant analytical instrumentation, parameter, and equipment modifications; and 7) the method LOQ was 0.05 µg/L not 0.008 µg/L (pp. 13-16 of MRID 50914403). The significant analytical instrumentation, parameter, and equipment modifications included the following: 1) addition of pre-column; 2) the use of a two-solvent mobile phase (versus one-solvent mobile phase); 3) a mobile (versus isocratic) phase gradient; 4) injection volume change; 5) MS temperature change; and 6) addition of confirmatory ion transition for quantification and identification. Dodine had a later retention time in the ILV which would minimize interference from possible polar contaminants. The use of acetonitrile:water (45:55, v:v) + 0.1% heptafluorobutyric acid as an end solution and HPLC mobile phase was eliminated by the ILV. The reviewer noted that the same SPE cartridges were used. Overall, the ILV method (PTRL Europe Study No. P 1271 G) was more rigorous version of the ECM method (NOTOX Project No. 335058), which had been updated with multiple significant modifications.
4. Since the reported method LOQs of the ECM and ILV were not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQs of the ECM and ILV are the lowest levels of method validation (LLMV) rather than LOQs (pp. 6, 9, 15, 20, 22 of MRID 50914404; pp. 7, 11, 19-20 of MRID 50914403). The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV.
5. The reproducibility of the method could not be determined at any fortification since only one set of performance data was submitted for each fortification. OCSPP guidelines state that two sets of performance data should be submitted, one for the initial or other internal validation and one for the ILV.
6. An insufficient number of samples (n = 2) were prepared for the ECM surface water matrix at the LOQ (0.008 µg/L) and 10×LOQ (0.08 µg/L). OCSPP guidelines state that a minimum of five spiked replicates were analyzed at each concentration (*i.e.*, minimally,

the LOQ and 10×LOQ) for each analyte. The study report recovery data was presented as two analytical batches (Tables 9-11, pp. 19-20 of MRID 50914404). Each analytical batch was analyzed in triplicate. Each analytical batch was considered to be one sample where the mean of the triplicate analyses was considered to be the batch recovery. Means and relative standard deviations (RSDs) could not be calculated since $n = 2$.

7. The number of ILV trials required to validate the method was not reported.
8. ILV communications were not reported, summarized, or provided in the study report. The communications should be included in the study report to assess whether the ILV was performed independently from the ECM. The performing laboratories and personnel differed between the ECM and ILV (pp. 1, 5 of MRID 50914404; pp. 1, 6 of MRID 50914403).
9. Tap (drinking) and ground (well) water matrices were included in the ECM; however, these matrices were not fortified with dodine (pp. 9, 17, 22; Figures 2-3, pp. 24-25 of MRID 50914404). Untreated control tap and ground water samples were only evaluated for specificity at the 0.008 µg/L LOQ level. No matrix interferences were observed in the matrix blanks of the tap and ground water matrices. The ECM reported that the observed specificity equated to the fact that the method was applicable to tap and ground water matrices. The LOQ for tap and ground water was reported in the ECM as 0.1 µg/L; however, no validation was performed in these two matrices (pp. 6, 9, 22).
10. No reagent blank was included in the ECM or ILV (p. 16 of MRID 50914404; p. 11 of MRID 50914403).
11. In the ECM, the final extracts in the surface water matrix were determined to be stable for up to 27 hours at 4°C (pp. 21-22 of MRID 50914404). The stock solution was determined to be stable for up to 18 days at ≤10°C.

The reviewer noted that the stability of dodine in end solution was reportedly assessed in the ECM by adding the dodine stock solution to the end solution [acetonitrile:water (45:55, v:v) + 0.1% heptafluorbutyric acid] at a nominal concentration of 0.05 µg/L (pp. 13, 21; Table 14, p. 21 of MRID 50914404). The reviewer noted that this concentration was equivalent to the ILV LOQ.

12. The reviewer noted that ILV RSDs at the LOQ were high (17-18%; Table 1, p. 21 of MRID 50914403).
13. The reviewer noted the following typographical error in the ILV GLP Compliance Statement: Japan (MHW, MAFF, and **MITI**) instead of Japan (MHW, MAFF, and **METI**) (p. 3 of MRID 50914403).
14. The ILV Certificate of Analysis for dodine was faint and difficult to read (HPLC purity 95.06 ± 0.52%; Appendix 1, p. 29 of MRID 50914403).

15. The determinations of the LOD and LOQ in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 6, 9, 15, 20, 22 of MRID 50914404; pp. 7, 11, 19-20 of MRID 50914403). In the ECM, the LOQ was justified by the acceptability of the recovery results. No justification of the LOQ was reported in the ILV. No calculations to support the LOQ were reported in the ECM or ILV. The LOD in the ILV was based on the level of matrix interferences in extracts of untreated blank control specimens ($\leq 10\%$ of the LOQ). No calculations were reported for the LOQ or LOD in the ECM or ILV. Detection Limit should not be based on the arbitrarily selected lowest concentration in the spiked samples.

Since the LOQs were not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQs are the lowest levels of method validation (LLMVs) rather than LOQs.

16. In the ECM, no significant matrix effects were observed ($< 20\%$; p. 20 of MRID 50914404). Solvent-based calibrations were used in the ECM while matrix-matched calibrations were used in the ILV (p. 13 of MRID 50914404; pp. 14, 19 of MRID 50914403).

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, and Revision 2; 1994 and 2016.

Attachment 1: Chemical Names and Structures**Dodine****IUPAC Name:** 1-Dodecylguanidinium acetate**CAS Name:** Not reported**CAS Number:** 2439-10-3 (acetate)

112-65-2 (free base)

SMILES String: Not found