

Reports: ECM: EPA MRID No.: 49687805. Zhang, L., E.S. Bodle. 2015. Validation for a method for the determination of captan in freshwater for support of aquatic field dissipation studies. Wildlife International Project No.: 234C-118. Mana Study No.: R-35353. Report prepared by Wildlife International, Evans Analytical Group, Easton, Maryland, sponsored and submitted by Makhteshim Agan of North America, Inc. (d/b/a ADAMA), Raleigh, North Carolina; 44 pages. Final report issued May 8, 2015; Report Amendment issued June 9, 2015.

ILV: EPA MRID No. 49687804. Arndt, T., L. Mannella. 2015. Independent Laboratory Validation of Method for Analysis of Captan in Surface Water. PTRL West Project No: 2743W. Sponsor Project No.: R-35996. Report prepared by PTRL West (a division of EAG, Inc.), Hercules, California, sponsored and submitted by Makhteshim Agan of North America, Inc. (d/b/a ADAMA), Raleigh, North Carolina; 56 pages. Final report issued June 3, 2015.

Document No.: MRIDs 49687805 & 49687804

Guideline: 850.6100

Statements: ECM: The study was conducted in compliance with USEPA FIFRA and OECD Good Laboratory Practice (GLP) standards, with the exception of the test and reference substance characterizations and storage stability at the testing facility (p. 3 of MRID 49687805). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). An Authenticity statement was not provided.

ILV: The study was conducted in compliance with USEPA GLP standards, with the exception of the sponsor-provided certification of captan standard (p. 3 of MRID 49687804). 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 in the QA statement.

Classification: This analytical method is classified as **supplemental**. The LOQ (100 µg/L) is not sensitive enough for the lowest toxicological level of concern (13.1 µg a.i./L) for aquatic organisms. The method used to calculate LOQ was not based on acceptable procedures, as defined by 40 CFR Part 136. Linearity was not satisfactory for the linear regression in the ECM. The ECM and ILV calibration curves did not adequately bracket the instrumental response at the LOQ. A reagent blank was not included in the ECM. An updated ECM including the confirmation ion which was validated by the ILV should have been provided.

Reviewer: Faruque Khan
Senior Scientist

Signature:
Date: 12-18-2015

Executive Summary

This analytical method, Mana Study No. R-35353, is designed for the quantitative determination of captan in surface water using GC/MS. The method is quantitative for captan at the stated LOQ of 0.100 mg/L. The LOQ is greater than the lowest toxicological level of concern 13.1 µg/L¹ (acute exposure to fish). The number of trials was not specified, but the reviewer assumed that the method was validated by the ILV after one trial. The ECM utilized lake surface water, while the ILV utilized creek surface water. Waters were sieved prior to processing and fortification. Captan was identified by one ion (*m/z* 79) in the ECM and by two ions (*m/z* 79 and *m/z* 149) in the ILV. Results were acceptable in the ECM and ILV; however, the calibration curves used for quantification were not fully optimized for accuracy. An updated ECM including the confirmation ion which was validated by the ILV could be recommended.

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						
Captan	49687805	49687804		Water ^{1,2}	08/05/2015 (Original) 09/06/2015 (Amendment 1)	Makhteshim Agan of North America, Inc. (d/b/a ADAMA)	GC/MS	0.100 mg/L

1 In the ECM, the surface water was collected from Tuckahoe Lake, Ridgely, Maryland (p. 11 of MRID 49687805). The water was characterized (pH 7.56; Appendix 4, p. 42).

2 In the ILV, the surface water (pH 7.0) was collected and fully characterized by Agvise Laboratories, Inc., Northwood, North Dakota (pp. 14-15; Appendix C, pp. 50-54 of MRID 49687804). The water was collected from Brandywine Creek, Chadds Ford, Pennsylvania.

I. Principle of the Method

Samples (unspecified volume) of freshwater were sieved (250 µm) and added to Teflon® centrifuge tubes (tubes pre-rinsed with toluene; pp. 12-13; Figure 1, p. 19 of MRID 49687805). For fortification recovery samples, an aliquot of the water equivalent to the fortification stock volume was removed, then the water samples were fortified using gas-tight syringes or equivalent. Samples were immediately acidified by adding *ca.* one drop of 10% phosphoric acid in water per 10 mL of sample. The samples were extracted with 20.0 mL of toluene (added via volumetric pipette) via mixing on a vortex mixer for *ca.* 5 minutes. Separate toluene layer and discard aqueous layer. Pool the toluene extracts of the matrix blank samples. Recovery samples were diluted, if necessary, using the pooled toluene extracts of the matrix blanks so that instrument response was in the range of the calibration curve. Calibration standards were prepared using the pooled matrix blank toluene extracts and volumetric pipettes, volumetric flasks or equivalent. Final extracts and standards were transferred to autosampler vials for analysis by GC/MS.

¹ <http://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/aquatic-life-benchmarks-pesticide-registration>

Samples were analyzed for captan by an Agilent Model 5890A GC system with an Agilent 5971A mass selective detector (p. 13; Table 1, p. 17 of MRID 49687805). The following instrumental conditions were employed: DB-5MS UI column (30 m x 250 μm i.d., 0.25 μm film thickness), injector temperature 250°C, helium carrier gas (0.7 mL/min.), temperature program 100°C (hold 1 min.) to 320°C (hold 1 min.; 15.0°C/min. rate), and selected ion monitoring (SIM) ionization mode. Injection volume was 2.00 μL (splitless). Captan was identified and quantified with one ion, m/z 79.00. The retention time for captan was *ca.* 11.7 minutes. No further confirmation was performed.

In the ILV, the extraction was performed exactly as above, except that the samples were extracted with toluene using a Wrist-ActionTM shaker, instead of a vortex mixer (pp. 14, 16-18, 21; Figure 1, p. 30 of MRID 49687804). Additionally, the sample volume was specified as 10 mL of water, and the remaining extracts were transferred to amber bottles and stored frozen (<-4°C). The analytical procedure was performed using an Agilent Model 6890 GC system with an Agilent 5973 mass selective detector. All GC/MS conditions were the same as the ECM, except the following: the column was reported as Agilent DB-5 (30 m x 250 μm i.d., 0.25 μm film thickness); helium carrier gas flow (1.1 mL/min.); and the MS conditions specified electron impact (EI) in SIM mode. Captan was identified and quantified with two ions, m/z 79 (quantification) and m/z 149 (confirmation). The retention time for captan was 10.2 minutes. The addition of a second ion for confirmation of the identification of captan was not considered a significant modification of the ECM since a confirmatory method is not usually required when LC/MS and GC/MS is the primary method.

The LOQ for captan was 0.100 mg/L in the ECM and ILV (pp. 8, 13 of MRID 49687805; pp. 10, 20, 22-23 of MRID 49687804). In the ECM, the instrumental LOD was reported as 0.00989 mg/L. The LOD was not reported in the ILV.

II. Recovery Findings

ECM (MRID 49687805): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD \leq 20%) for analysis of captan in surface water at fortification levels of 0.100 mg/L (LOQ) and 1.00 mg/L (10 \times LOQ; uncorrected recoveries; Table 2, p. 18). Captan was identified using one ion; no further confirmation was performed (Table 1, p. 17). The surface water matrix was collected from Tuckahoe Lake, Ridgely, Maryland (p. 11). The water was characterized (pH 7.56; Appendix 4, p. 42).

ILV (MRID 49687804): Mean recoveries and RSDs were within guideline requirements for analysis of captan in surface water at fortification levels of 0.1 mg/L (LOQ) and 1 mg/L (10 \times LOQ; uncorrected recoveries; Table I, p. 27; Appendix D, pp. 55-56). Captan was identified using two ions; recoveries of the quantification ion were higher than those of the confirmation ion at both fortification levels (pp. 10-11). The number of trials was not specified, but the reviewer assumed that the method was validated after one trial. The surface water (pH 7.0) was collected and fully characterized by Agvise Laboratories, Inc., Northwood, North Dakota (pp. 14-15; Appendix C, pp. 50-54). The water was collected from Brandywine Creek, Chadds Ford, Pennsylvania.

Table 2. Initial Validation Method Recoveries for Captan in Surface Water (Lake)^{1,2}

Analyte	Fortification Level (mg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Primary ion (<i>m/z</i> 79)						
Captan	0.100 (LOQ)	5	85.4-91.0	88.7	2.26	2.55
	1.00	5	87.6-98.7	92.6	4.15	4.48

Data (uncorrected recovery results; Table 2, p. 18) were obtained from Table 2, p. 18 of MRID 49687805.

1 The surface water was collected from Tuckahoe Lake, Ridgley, Maryland (p. 11). The water was characterized (pH 7.56; Appendix 4, p. 42).

2 Captan was identified and quantified with one ion, *m/z* 79.00 (Table 1, p. 17). No further confirmation was performed.

Table 3. Independent Validation Method Recoveries for Captan in Surface Water (Creek)^{1,2}

Analyte	Fortification Level (mg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Primary ion (<i>m/z</i> 79)						
Captan	0.100 (LOQ)	5	84-103	95	7	7
	1.00	5	82-94	88	5	6
Secondary ion (<i>m/z</i> 149)						
Captan	0.100 (LOQ)	5	77-100	89	9	10
	1.00	5	79-83	81	1	1

Data (uncorrected recovery results; p. 20; Appendix D, pp. 55-56) were obtained from Table I, p. 27 of MRID 49687804.

1 The surface water (pH 7.0) was collected and fully characterized by Agvise Laboratories, Inc., Northwood, North Dakota (pp. 14-15; Appendix C, pp. 50-54). The water was collected from Brandywine Creek, Chadds Ford, Pennsylvania.

2 Captan was identified and quantified with two ions, *m/z* 79 (quantification) and *m/z* 149 (confirmation; p. 18).

III. Method Characteristics

The method LOQ for captan was 0.100 mg/L in the ECM and ILV (pp. 8, 13 of MRID 49687805; pp. 10, 20, 22-23 of MRID 49687804). In the ECM, the LOQ was defined as the lowest fortification level fortified and analyzed during the verification set. Other criteria included the confirmation that interferences in the reagent blank and matrix blank were <30% of the LOQ. The theoretical LOQ was calculated to be 0.0422 mg/L in the ECM; the actual LOQ was calculated to be 0.0659 mg/L. Both values supported the method LOQ. The calculations for each were as follows:

Theoretical LOQ = (lowest calibration standard) × (dilution factor of the matrix blank samples) ÷ (purity of test substance);

Actual LOQ = (lowest calibration standard) ÷ (average signal to noise ratio) × 10 × (dilution factor of the matrix blank samples) ÷ (purity of test substance).

In the ECM, the instrumental LOD was reported as 0.00989 mg/L. The calculation was as follows:

Instrumental LOD = (lowest calibration standard) ÷ (average signal to noise ratio) × 3 × (dilution factor of the matrix blank samples) ÷ (purity of test substance).

In the ILV, the LOQ was defined as the lowest fortification level yielding acceptable recovery. The LOD was mentioned in the ILV, but no value was reported. No calculations for the LOQ or LOD were reported in the ILV.

Table 4. Method Characteristics

		Captan
Limit of Quantitation (LOQ)		0.100 mg/L
Limit of Detection (LOD)	ECM	0.00989 mg/L (instrumental)
	ILV	Not reported
Linearity (calibration curve r^2 and concentration range)	ECM	$r^2 = 0.993$ (m/z 79)
	ILV	$r^2 = 0.9992$ (m/z 79) $r^2 = 0.9964$ (m/z 149)
	Concentration range	0.02 to 0.2 $\mu\text{g/mL}$ (solvent-based calibrants)
Repeatable	ECM ¹	Yes at LOQ and 10 \times LOQ (m/z 79). No confirmation ion was monitored. ²
	ILV ³	Yes at LOQ and 10 \times LOQ (m/z 79 and m/z 149).
Reproducible		Yes at LOQ and 10 \times LOQ.
Specific	ECM	Only chromatograms for m/z 79 provided. Matrix interferences were not observed.
	ILV	Chromatograms for m/z 79 and m/z 149 provided. Matrix interferences were not observed. Multiple non-interfering peaks were observed in the confirmation ion chromatograms (peak heights were >3xs the peak height of the analyte).

Data were obtained from pp. 8, 12-13; Table 2, p. 18; Figure 2, p. 20; Figures 5-7, pp. 23-25 of MRID 49687805; pp. 10-11, 20, 22-23; Tables I-II, pp. 27-28; Figures 4-9, pp. 34-39; Appendix 4, pp. 55-56 of MRID 49687804.

1 The surface water was collected from Tuckahoe Lake, Ridgley, Maryland (p. 11 of MRID 49687805). The water was characterized (pH 7.56; Appendix 4, p. 42).

2 A confirmatory method is not usually required when LC/MS and GC/MS is the primary method.

3 The surface water (pH 7.0) was collected and fully characterized by Agvise Laboratories, Inc., Northwood, North Dakota (pp. 14-15; Appendix C, pp. 50-54 of MRID 49687804). The water was collected from Brandywine Creek, Chadds Ford, Pennsylvania.

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

IV. Method Deficiencies and Reviewer's Comments

- The ECM study authors supported their determinations of the LOQ and LOD with calculations; however, these determinations of the LOQ and LOD were not based on scientifically acceptable procedures, as defined by 40 CFR Part 136 (pp. 8, 13 of MRID 49687805). The theoretical LOQ equaled the product of the lowest calibration standard and dilution factor of the matrix blank samples, divided by the purity of test substance. The actual LOQ equaled the product of the lowest calibration standard, which was divided by the average signal to noise ratio, and 10 and the dilution factor of the matrix blank samples, divided by the purity of test substance. The reported LOQ was higher than both the theoretical and actual LOQs. In the ECM, the instrumental LOD was calculated as the lowest calibration standard, divided by the average signal to noise ratio, multiplied by the product of 3 and the dilution factor of the matrix blank samples, divided by the purity of test substance. In the ILV, the LOQ was reported from the ECM, and the LOD was not reported.

It is preferred that detection limits are not be based on the arbitrarily selected lowest concentration in the spiked samples.

2. In the ECM, linearity was not satisfactory for the linear regression of the quantification ion of captan (see above). Linearity is satisfactory when $r^2 \geq 0.995$. Linear regressions were matrix-matched. A confirmation ion was not monitored in the ECM.
3. The calibration curves did not adequately bracket the instrumental response at the LOQ. In the ILV, the two lowest standards, 0.02 and 0.05 mg/L generated instrument responses (peak areas) of 3398 (Q) and 517 (C) and 10176 (Q) and 1467 (C), respectively (Figure 3, pp. 32-33 of MRID 49687804). The LOQ peak area was 8596 (Q) and 1116 (C; Figure 8, p. 38). In the ECM, the bracketing of the LOQ by the calibration standards could not be evaluated since the individual peak response data was not provided of the calibration curves or the fortified samples and representative chromatograms were only provided for the highest and lowest calibration standard. For accuracy of calculations at the LOQ based on the calibration curve, at least two calibration standards should generate instrumental responses below and above the instrument response at the LOQ.
4. A reagent blank was not included in the ECM.
5. No confirmation method was included in the ECM. A confirmation ion was not monitored, and a second confirmatory analytical method was not presented. A confirmatory method is not usually required when LC/MS and GC/MS is the primary method.

In the ILV, captan was identified and quantified with two ions, m/z 79 (quantification) and m/z 149 (confirmation; pp. 17-18, 21 of MRID 49687804). The addition of a second ion for confirmation of the identification of captan was not considered a significant modification of the ECM since a confirmatory method is not usually required when LC/MS and GC/MS is the primary method; however, an updated ECM including the confirmation ion which was validated by the ILV should have been provided.

6. The ILV study authors noted that recoveries of the quantification ion were higher than those of the confirmation ion at both fortification levels (pp. 10-11 of MRID 49687804). No reason was proposed to explain this observation. The smaller peak response of the confirmation ion versus the quantification ion was noted in the ILV representative chromatograms (Figures 8-9, pp. 38-39). The GC/MS full scan of captan was included in the ILV (Figure 2, p. 31)
7. The ECM reported the reason for the amendment of the original study report: to correct the Sponsor Study number from R-35553 to R-35353 (Appendix 6, p. 44 of MRID 49687805).
8. The ILV study author reported that communications between the independent laboratory and the originating laboratory, Wildlife International, were not necessary (p. 25 of MRID 49687804).
9. In the ILV, no significant matrix effects were found (p. 23; Table III, p. 29 of MRID 49687804). Matrix effects were determined to be 9-13% for both ions monitored. Non-matrix matched standards were used in the ECM and ILV (p. 12 of MRID 49687805; pp. 15-16; Figures 5-6, pp. 35-36 of MRID 49687804).

10. It was reported in the ILV that the method could be performed within 24 hours or 3 eight-hour working days (p. 25 of MRID 49687805).

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.

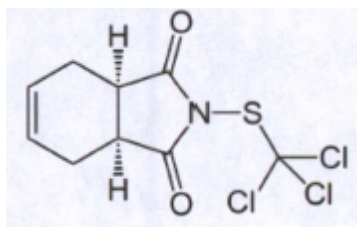
Attachment 1: Chemical Names and Structures**Captan (Captan Tec)**

IUPAC Name: (3aR,7aS)-2-[(trichloromethyl)sulfanyl]-3a,4,7,7a-tetrahydro-1H-isoindole-1,3(2H)-dione

CAS Name: Not reported

CAS Number: 133-06-2

SMILES String: Not found



Information related to Primary Review

For CDM Smith

Primary Reviewer: Lisa Muto

Signature: 

Date: 11/10/15

Secondary Reviewer: Kathleen Ferguson

Signature:

Date: 11/10/15

QC/QA Manager: Joan Gaidos

Signature: 

Date: 11/10/15