INTRODUCTION

The purpose of this study was to conduct an independent laboratory validation for the determination of captan in surface water. The analysis of the reference/test substance was performed by gas chromatography with mass spectral detection based on the method, "Validation of a Method for the Determination of Captan in Freshwater for Support of Aquatic Field Dissipation Studies" (Bodle, Eric, Ph.D. and Zhang, Ling, Ph.D., Wildlife International, project number 234C-118, MANA study number R-35553, May 8, 2015).

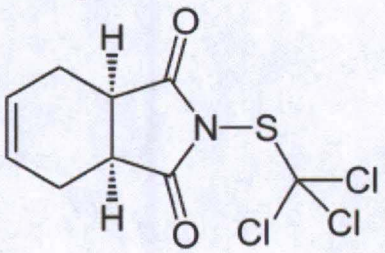
This study was designed to satisfy US EPA Guideline requirements described in US EPA Guidelines OCSPP 850.6100). The study was initiated on March 12, 2015. The experimental work was conducted from April 6, 2015 through April 8, 2015 at PTRL West, Hercules, CA 94547 under an approved protocol (Appendix A) according to the US EPA FIFRA Good Laboratory Practice Standards, 40 CFR §160.

PTRL-West is an independent organization in no way associated with Makhteshim Agan of North America, Inc. (d/b/a ADAMA).

The study director and performing chemists did not develop the analytical method, nor have they had any experience with the analytical method prior to this study. There was no communication with the originating laboratory (Wildlife International) regarding this methodology during the conduct of this study.

MATERIAL AND METHODS

Reference/Test Substance

Name:	Captan
IUPAC Name:	(3aR,7aS)-2-[(trichloromethyl)sulfanyl]-3a,4,7,7a-tetrahydro-1H-isoindole-1,3(2H)-dione
Purity:	99.5%
Supplier:	Chem Service
Lot no.:	3461500
Molecular formula:	$C_9H_8Cl_3NO_2S$
Molecular weight:	300.6 g/mole
Structure:	

Origin of Reference/Test Substance

The reference/test substance identified as Captan (lot no. 3461500) was provided by Chem Service and received at PTRL West on March 20, 2015. Upon receipt at PTRL West, the reference/test substance was given the PTRL inventory no. 2743W-001A;-001B. The reference/test substance was maintained at refrigerated when not in use. The Certificate of Analysis is provided in Appendix B.

Solvents/Reagents

Acetone, HPLC grade
Toluene, HPLC grade
Phosphoric acid (10% aqueous)

Equipment/Materials List

Laboratory balances
0.25 µm sieve
Weighing boats
Hamilton glass precision syringes
Volumetric flasks
Wrist-Action™ shaker
GC vials
Teflon tubes with caps
Amber glass bottles with Teflon® lined caps

Agilent 6890 Gas Chromatograph with Agilent 5973 Mass Selective Detector (GC/MSD) Chemstation Data System Software

Test Method

The analytical method for the analysis of captan was validated at PTRL West by Gas Chromatography with Mass Spectral Detection (GC/MS) and electron impact (EI) in SIM mode based on the analytical method (REFERENCE 1), provided by Wildlife International.

The water samples were spiked with known concentrations of captan. The samples were acidified with 10% aqueous phosphoric acid and extracted with toluene. The toluene extracts were analyzed by GC/MS. The percent recovery was determined using external standardization where a weighted linear curve for each transition (m/z 79, 149) was analyzed along with the samples.

Test System (Matrix)

Source and Characterization of Test System

Surface water was collected and characterized by Agvise Laboratories, Inc. (604 Highway 15 West, Northwood, North Dakota) under PTRL West test system repository study 2440W. Characterization of the surface water included pH, calcium, magnesium,

sodium, hardness, sodium adsorption ratio, conductivity, total dissolved solids, turbidity and alkalinity. Upon arrival at PTRL West, the water was assigned the inventory no. 2440W-102. The water sample was stored refrigerated (typically < 4°C) in the dark when not in use. The water characterization report, methods of characterization as well as collection documentation are presented in Appendix C.

Preparation of Captan Stock Solution

A stock solution containing captan was prepared by weighing an aliquot of the reference substance (10.19 mg) in a weighing boat and transferring into a 10 mL volumetric flask. The stock solution was dissolved and diluted to the mark with acetone. An additional volume of acetone (0.14 mL) was added into the flask to yield a nominal concentration of 1.0 mg/mL. The concentration of the stock solution was corrected for the purity of the reference substance (99.5%). The stock solution was transferred into an amber bottle and stored frozen (typically < -4°C) when not in use.

Preparation of Captan Intermediate Solutions

Intermediate solutions were prepared in 10 mL volumetric flasks by volumetrically measuring aliquots of captan stock solution (1.0 mg/mL) and in serial dilution as follows:

Solution used	Aliquot soln (mL)	Final volume (mL)	Theoretical conc. (µg/mL) [1]	Sample ID
stock	1	10	100	100 µg/mL
100 µg/mL	1	10	10	10 µg/mL
10 µg/mL	1	10	1	1 µg/mL

[1] Theoretical conc. (µg/mL) = [theoretical conc. soln used x aliquot (mL)] ÷ final volume (mL)

Final solutions were diluted to the mark with acetone. Intermediate solutions were mixed, transferred into amber bottles and stored frozen (typically < -4°C) when not in use.

Preparation of Captan Solvent Based Calibrants

Solvent based calibrant was prepared at only one concentration as follows: an aliquot (0.05 mL) of the 10 µg/mL Captan intermediate solution was transferred and diluted in a 5 mL volumetric flask with toluene to yield a nominal concentration of 0.10 µg/mL.

Preparation of Captan Matrix Based Calibrants

Five calibrants were prepared in matrix (untreated samples) by volumetrically measuring an appropriate volume of the captan intermediate solutions (1 µg/mL and 10 µg/mL) into separate 5 mL volumetric flasks and diluting to the mark with untreated samples. Two untreated samples (matrix blanks) were previously combined for the preparation of the matrix based calibrants. Calibrants were stored refrigerated (typically < 10°C) when not in use.

Aliquot soln (mL)	Solution used	Final volume (mL)	Theoretical conc. (µg/mL)[2]
0.100	1 µg/mL	5	0.02
0.250	1 µg/mL	5	0.05
0.050	10 µg/mL	5	0.10
0.075	10 µg/mL	5	0.15
0.100	10 µg/mL	5	0.20

[2] Theoretical conc stds (µg/mL) = [theoretical conc sol used (µg/mL) x aliquot (mL)] ÷ final volume (mL)

Preparation of Captan Fortification Solutions

Fortification solutions were captan intermediate solutions 10 µg/mL (for LOQ) and 100 µg/mL (for 10X LOQ) described in the previous section above.

Fortification Procedure

Fortification of untreated water samples was conducted at two fortification levels as shown below:

Fortification Level (mg/L)	Captan
0.1	0.1 mL of 10 µg/mL in 10 mL water
1.0	0.1 mL of 100 µg/mL in 10 mL water

Fortification was conducted to determine the percent recovery within the method validation. This procedure was performed in quintuplicate during method validation at each fortification level.

Extraction Method

1. Sieve surface water through a 250 µm sieve.

2. Measure 10 mL aliquots of water in Teflon tubes (previously rinsed with toluene). Note, 0.1 mL of test system water removed prior to spiking with fortification solution (0.1 mL) in step 3.

3. Fortify as necessary using Hamilton glass precision syringes.

4. Acidify samples with one drop of 10% aqueous phosphoric acid.

5. Extract samples with 20 mL toluene.

6. Place samples on a Wrist-Action™ shaker for 5 minutes and let aqueous and organic phases to separate.

7. Transfer organic phase of all samples into glass bottles.

8. Dilute organic sample extracts with combined controls (untreated samples) if necessary so as to fit into the range of the calibration curve

9. Analyze organic phase by GC/MS.

10. Transfer remaining extracts (organic phase) into amber bottles and store in freezer (typically < -4°C) when not in use. Discard aqueous phase of all samples.

A schematic diagram of the extraction method is presented in Figure 1.

Gas Chromatography with Mass Spectrometry Analytical Method (GC/MS)

GC conditions

Column: Agilent DB-5, 30 m x 0.25 mm x 0.25 µm
Injection Volume: 2 µL
Injector temp: 250°C
Splitless mode
Splitless time: 2 min
GC liner: Single Goose Neck
Temp program:

- Initial conditions: 100°C hold for 1 minute
- Ramp: 15°C/minute to 320°C hold for 1 minute

Initial flow (He): 1.1 mL/min
Pressure: 11.7 psi

Run time: 16.67 minutes

Approximate retention time:

- Captan: 10.2 min

MS conditions

Electron Impact mode (EI)
Transfer line temp: 280°C
MS quad temp: 150°C
MS source temp: 230°C
Acquisition mode: Selected Ion Monitoring (SIM): m/z 79 (quantitation ion) and 149 (confirmation ion)
Data acquisition: from 5 min to 14 min

Note: the full scan (m/z 50 – 450 amu) for captan is provided in Figure 2.

GC/MS Analysis

Samples were analyzed interspersed between the calibrants. Calibrants and samples were analyzed in single injections. Toluene was analyzed as the solvent blank. Some standard solutions were reanalyzed as check standards (quality control standards) to ensure good chromatography and consistent instrument performance. The stability of the signal was monitored by comparing the response (captan peak area) of a quality control standard injection with that of a comparable standard from the linear curve within the sequence.

Methods of Calculation

Preparation of Stock Standard Solutions

$$\text{Volume of solvent (mL)} = \frac{(W) \times 1000\,\mu g/mg \times (P)}{(FC)}$$

where W = Milligrams of neat standard
P = Chemical purity of neat standard
FC = Final Concentration (µg/mL)

Quantitation

Separation of captan was achieved by GC. The detection was by GC/MS with EI in SIM mode. The target analyte was identified by the coincidence of its retention time with its reference substance and MS characteristics. The quantitation of captan was determined by measuring peak area relative to the concentration of the calibrants. The content of captan in samples was quantitated against a 1/x weighted linear curve ($y = mx + b$) of each transition ion from captan calibrants where:

y = peak area
x = µg/mL (mg/L) analyte
m = slope
b = intercept

The calculation of weighted curve equation (linear regression) and concentration (mg/L) present in samples and calibrants was conducted using Excel software. The amount of captan was determined for the quantitation ion at m/z 79 and for the confirmation ion at m/z 149.

Recoveries from fortified samples were determined by calculating the found concentration and dividing by the relevant fortification level.

Residue in water

Detected conc (mg/L captan) = [calculated conc (mg/L) x dilution factor x extraction volume] ÷ sample volume

Where:

Dilution factor = (final volume for analysis) ÷ (aliquot extract)
Sample volume (mL) = 10
Extraction volume (mL) = 20

Example: Fortified sample F2A (m/z 79)
Aliquot extract = 0.1 mL
Final volume for analysis = 1 mL
Calculated conc (mg/L) = 0.0469

Dilution factor = (0.1 mL ÷ 1 mL) = 10
mg/L captan = (0.0469 x 10 x 20) ÷ 10 = 0.938

Percent recovery of captan in water

$$\% \text{ Recovery} = \frac{\text{mg/L detected - mg/L Control}}{\text{Fortification Level (mg/L)}} \times 100$$

Example: Fortified sample F2A (m/z 79)

$$\% \text{ Captan} = \frac{0.938 \text{ mg/L}}{1 \text{ mg/L}} \times 100 = 94\%$$

No captan residue was detected in the controls.

Transcriptions (spreadsheets) of the raw data to support calculations for this study are presented in Appendix D.

Limit of Quantitation

The limit of quantitation was assigned as the lowest fortification level of captan validated by the analytical method. The LOQ for captan in water was 0.1 mg/L.

Time Required for Completion of a Sample Set

A sample set consisted of fourteen samples which was comprised of five fortified water samples at each level (LOQ and 10X LOQ), and four controls (untreated water samples). Time required per sample set from initiation of extraction until the completion of instrumental analysis and data evaluation by one analyst is as follows:

- Sample preparation (fortification and dilution) takes approximately 8 hours
- GC/MS analysis and data processing (two transition ions) take approximately 5 hours

TOTAL = approximately 13 hours for one analyst to complete a sample set. This does not include preparation of calibrants and fortification solutions.

Modification of the Analytical Method Validation

The analytical method was run as written in Wildlife report (reference 1) with minor modifications:

1. Samples were placed on a Wrist-Action™ shaker after the addition of toluene for extraction. In the original method, samples were vortexed to mix.

2. An additional transition ion (m/z 149) was monitored as confirmation ion. The original method reported one ion (m/z 79) only.

No impact on the study was observed. Acceptable recoveries within the 70% - 120% range were achieved at each fortification level. In addition, results for m/z 149 confirmation ion were reported in the present study.

Statistical Methods

Means, standard deviation, relative standard deviation, and 1/x linear regression were the only statistical methods employed in this study.

Communication Pertaining to Independent Laboratory Validation

Communication between the Study Director and the Sponsor Representative was limited to e-mail correspondence as follows:

April 8 2015: a protocol amendment was sent to the Sponsor in regards of solvent based and matrix based calibrants preparation, linearity, quantitation, and matrix effect assessment.

Figure 1. Schematic Diagram of the Analytical Method.