

INTRODUCTION

The purpose of this study was to verify the performance of methodology for the analysis of Captan in freshwater to be used in support of aquatic field dissipation studies. Validation samples were analyzed based upon procedures developed by Wildlife International. The study was performed based on U.S. Environmental Protection Agency Series 850 – Ecological Effects Test Guidelines, OCSPP 850.6100, *Environmental Chemistry Methods and Associated Independent Laboratory Validation* (1) and SANCO/3029/99 rev.4 dated 11/07/00, *Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (Part A, Section 4 and Annex III (part A, Section 5) of Directive 91/414 (2))*. The analysis of the samples were performed using gas chromatography (GC) with Mass Selective Detection (MS). Freshwater validation samples were prepared and analyzed on February 23, 2015. All raw data generated by Wildlife International and the original final report are filed under Project Number 234C-118 in archives located on the Wildlife International site.

OBJECTIVE

This study was conducted according to the protocol “Validation of a Method for the Determination of Captan in Freshwater for Support of Aquatic Field Dissipation Studies” (Appendix 1).

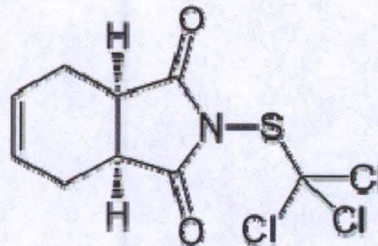
MATERIALS AND METHODS

Freshwater was fortified with Captan at two different concentrations and analyzed according to the method developed by Wildlife International. Matrix blanks (controls) were analyzed concurrently to evaluate possible analytical interferences. A calibration curve was generated from analyses of standard solutions of the test substance that were analyzed with the series of matrix fortification samples.

Test Substance and Analytical Standard

The test substance, Captan Tec., was received from ADAMA on December 1, 2014 and was assigned Wildlife International Identification Number 12013 and stored under ambient conditions. This test substance was used to prepare method validation samples for this study. The test substance chemical structure and additional information are shown below:

Structure:



Name: Captan Tec

Supplier: ADAMA Makhteshim

Batch#: 94138620

CAS Number: 133-06-2

Molecular Formula: C₉H₈Cl₃NO₂S

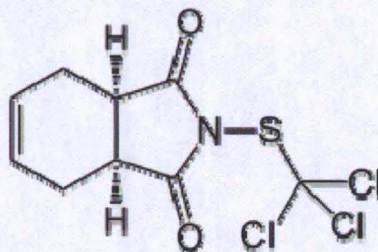
Molecular Weight: 300.6 grams/mole

Purity: 94.8%

The Captan test substance was stored under ambient conditions at the testing facility. A Certificate of Analysis for the Captan test substance is presented in Appendix 2.

The analytical standard was received from ChemService on November 14, 2014 and was assigned Wildlife International Identification Number 11999. This analytical standard was used to prepare method calibration standards for this study. The analytical standard chemical structure and additional information are shown below:

Structure:



Name: Captan

Supplier: ChemService

Lot#: 3324700

CAS Number: 133-06-2

Molecular Formula: C₉H₈Cl₃NO₂S

Molecular Weight: 300.6 grams/mole

Purity: 99.5%

The Captan analytical standard was stored under ambient conditions at the testing facility. A Certificate of Analysis for the Captan analytical standard is presented in Appendix 3.

Reagents and Solvents

All solvents and reagents used in this study were HPLC-grade or equivalent.

Equipment

Laboratory Balances

Beakers

Teflon Centrifuge Tubes

Class A Volumetric Flasks

Class A Volumetric Pipettes

Pasteur pipettes

Assorted Hamilton Gas-tight Syringes

Vortex Mixer

Sieve - 250 μm

Autosampler Vials with Crimp Caps

Agilent Model 5890A Gas Chromatography with Agilent Model 5791A Mass Selective Detector (GC/MSD)

Alternative equipment may be substituted as long it is considered equivalent in function and generates successful method outcome.

Test System

The freshwater used to prepare the method validation samples was obtained locally from Tuckahoe Lake, located in Ridgley, MD. The water was collected from the surface of the lake and was characterized at Wildlife International by measuring the specific conductance, hardness, alkalinity and pH. The results of this characterization are summarized in Appendix 4.

Stocks and Standards Preparation

A stock solution of Captan test substance was prepared by weighing 0.100 grams of the test substance on an analytical balance. The test substance was transferred to a 100-mL volumetric flask and the contents were brought to volume using acetone. The primary stock (1.00 mg/mL) was diluted in acetone to prepare 0.100 and 0.0100 mg/mL stock solutions. The 0.100 and 0.0100 mg/mL stock solutions were used to prepare the matrix fortification samples.

A stock solution of Captan analytical standard was prepared by weighing 0.01005 grams (weight corrected for purity of 99.5%) of the standard on an analytical balance. The analytical standard was transferred to a 100-mL volumetric flask and the contents were brought to volume using acetone. The primary stock (0.100 mg a.i./mL) was diluted in acetone to prepare 0.0100 and 0.00100 mg a.i./mL stock solutions. The 0.0100 and 0.00100 mg a.i./mL stock solutions were used to prepare the calibration standards with the pooled matrix blank toluene extract phase. The following shows the dilution scheme for the calibration standards:

Stock Concentration (mg a.i./mL)	Aliquot (mL)	Final Volume (mL)	Stock Concentration (mg a.i./L)
0.00100	0.100	5.00	0.0200
0.00100	0.250	5.00	0.0500
0.0100	0.0500	5.00	0.100
0.0100	0.0750	5.00	0.150
0.0100	0.200	5.00	0.200

Analytical Method

The method used for the analysis of Captan in surface water was fortified at two different concentrations in quintuplet (0.100 and 1.00 mg/L) and analyzed based on methodology developed by Wildlife International. Four matrix blanks were prepared for analysis to evaluate potential analytical method interferences.

The method validation samples were fortified with the appropriate test substance stock solutions and were acidified using one drop of 10% phosphoric acid in water to every 10 mL of sample. Samples were extracted with toluene. The toluene extract phases from the matrix blank samples were pooled to be used to prepare matrix-matched calibration standards. Sample extracts were diluted with the pooled matrix

blank toluene extract phase, as necessary. Extracts and standards were transferred to autosampler vials and submitted for GC/MS analysis.

Concentrations of Captan in water samples were determined using an Agilent Model 5890A gas chromatograph, equipped with an Agilent Model 5971A mass selective detector operated in selected ion monitoring (SIM) mode. Chromatographic separations were achieved using a DB-5MS UI column (30 m x 250 µm, 0.25 µm film thickness). The operational parameters are summarized in Table 1. A detailed analytical method outline is provided in Figure 1.

Method Limit of Quantitation (LOQ)

The limit of quantitation (LOQ) for this freshwater method verification was set at 0.100 mg/L as defined in the protocol, the lowest level fortified and analyzed during the verification set (Note: this method validation was conducted at levels well above the actual limit of quantitation for this method). Reagent blank and matrix blank samples were further screened to confirm any potential interference to be < 30% of the fortified LOQ level. The theoretical LOQ was 0.0422 mg/L, calculated as the product of the lowest calibration standard (0.0200 mg a.i./L) and the dilution factor of the matrix blank samples (2.0), divided by the purity of the test substance (94.8%). The actual LOQ was determined to be 0.0659 mg/L, calculated as the product of the lowest calibration standard / (average signal to noise ratio) x 10 x the dilution factor of the matrix blank samples (2.0) / purity of the test substance (94.8%) as shown below:

$$0.0200 \text{ mg a.i./L} \div 6.4 \times 10 \times 2.0 \div 0.948 = 0.0659 \text{ mg/L}$$

Limit of Detection (LOD)

The instrumental limit of detection (LOD) was determined to be 0.00989 mg/L, calculated as the product of the lowest calibration standard / (average signal to noise ratio) x 3 x the dilution factor of the standard (1.0) / purity of the test substance (94.8%) as shown below:

$$0.0200 \text{ mg a.i./L} \div 6.4 \times 3 \times 1.0 \div 0.948 = 0.00989 \text{ mg/L}$$

Table 1

Typical Gas Chromatograph (GC) Operational Parameters

INSTRUMENT:	Agilent Model 5890A gas chromatograph (GC)
DETECTOR:	Agilent Model 5971A mass selective detector (MSD)
ANALYTICAL COLUMN:	DB-5MS UI (30 m x 250 μ m, 0.25 μ m film thickness)
INJECTOR TEMPERATURE:	250°C
RUN TIME:	14 minutes
OVEN:	Initial temperature: 100°C Initial hold time: 1.00 minute Rate: 15.0°C/minute Final temperature: 320°C Final hold time: 1.00 minute
INJECTION VOLUME:	2.00 μ L (splitless)
CARRIER GAS:	Helium
INITIAL FLOW:	0.7 mL/min
INITIAL PRESSURE:	12 psi
ACQUISITION MODE:	Selected ion monitoring (SIM)
IONS MONITORED:	79.00 m/z
APPROXIMATE RETENTION TIME:	11.7 minutes

METHOD OUTLINE FOR THE PROCESSING OF CAPTAN IN FRESHWATER

1. Sieve freshwater through a 250 μm sieve.
2. Rinse all Teflon® centrifuge tubes with toluene.
3. Add freshwater using a volumetric pipette to Teflon® centrifuge tubes. Remove an aliquot of freshwater equivalent to the fortification stock volume. Prepare recovery samples by fortifying freshwater with the appropriate test substance stock solutions using gas-tight syringes or equivalent. Unfortified freshwater will serve as the matrix blank.
4. Immediately acidify samples by adding approximately one drop of 10% phosphoric acid in water to every 10 mL of sample.
5. Using a volumetric pipette, add 20.0 mL of toluene to each sample. Cap and mix on a vortex mixer for approximately five minutes.
6. Pool the toluene extract phase of matrix blank samples to be used to prepare standards and dilute samples. Extra matrix blank samples may be extracted as needed.
7. Discard the aqueous phase and dilute samples with the pooled matrix blank toluene extract phase, as necessary, into the range of calibration curve using volumetric pipettes, volumetric flasks, or equivalent.
8. Prepare calibration standards with the pooled matrix blank toluene extract phase using volumetric flasks and gas-tight syringes, or equivalent.
9. Transfer each extract and standard to an autosampler vial and submit samples for GC/MS analysis.

Figure 1. Analytical method outline for the analysis of Captan in freshwater.