INTRODUCTION

EAG Laboratories-Easton performed an independent laboratory validation (ILV) of a method for the determination of residues of Propargite in surface and drinking water. The protocol for this study titled "Independent Laboratory Validation of Methods for the Determination of Propargite in Surface and Drinking Water" is presented in Appendix I. The final report of the developing lab PTRL West-Hercules analytical method report entitled, "Development and Validation of a Method for the Determination of Propargite in Surface and Drinking Water" is presented in Appendix II.

This study was performed to satisfy regulatory requirements for independent laboratory validation of methods as set forth by the U.S. Environmental Protection Agency Series 860 - Residue Chemistry Test Guidelines, OCSPP 850.6100, *Environmental Chemistry Methods and Associated Independent Laboratory Validation* (1) and U.S. Environmental Protection Agency, 1996. Pesticide Regulation (PR) Notice 96-1: Notice to Manufacturers, Formulators, Producers and Registrants of Pesticides Products, *Tolerance Enforcement Methods - Independent Laboratory Validation By Petitioner* (2). The study was performed at the EAG Laboratories analytical chemistry facility in Easton, Maryland. The experimental portion of the study was conducted between March 01 and March 13, 2017. Raw data and a copy of the final report are archived at the EAG Laboratories-Easton site under project number 443C-128.

PURPOSE

This study was conducted to fulfill EPA requirements set forth in guideline OCSPP 850.6100 and PR Notice 96-1. This study provides validation data demonstrating that an independent researcher could reproduce the results of the analytical method with minimal contact with the method developers.

EXPERIMENTAL DESIGN

Surface and drinking water were fortified with Propargite at two concentrations and analyzed according to a method supplied by the Sponsor. The limit of quantitation (LOQ) for Propargite was set at 0.0100 µg/L. The higher concentration was ten-fold the LOQ, i.e., 0.100 µg/L. Reagent and matrix blanks (controls) were prepared and analyzed concurrently with the fortified samples to evaluate potential analytical interferences.

MATERIALS AND METHODS

Untreated Control Surface and Drinking Water - Origin

Surface water control matrix used for this study was obtained locally by EAG Laboratories-Easton from Tuckahoe Lake located in Tuckahoe State Park in Ridgely, MD. (Sample I.D. – WI-TL-302916). The water was collected on March 29, 2016 and was logged in and stored under refrigerated conditions at the testing facility upon receipt. The surface water was characterized internally and a summary report is presented in Appendix III.

Drinking (Well) water control matrix was collected on February 27, 2017 from a well at Easton Laboratories-Easton testing facility in Easton, MD. and had an assigned expiration date of March 13, 2017. The water was stored under refrigerated conditions in the dark following collection and when not

in use. The drinking water was characterized internally and the mean results for the 4 week period immediately preceding its use are summarized to Appendix IV.

Analytical Reference Substance

A reference substance of Propargite was received from Arysta on February 14, 2017 and was assigned the EAG Laboratories-Easton Identification Number 13555. The material was a liquid and was identified on the label and certificate of analysis as Propargite Standard; Lot# 2757-25-RRG; Purity 93.5%; CAS Number 2312-35-8; Expiration Date 10/31/2017. This reference substance was stored under refrigerated conditions. A certificate of analysis is presented in Appendix V.

The reference substance above was used to prepare primary analytical stocks and subsequently various secondary fortification stocks and calibration standards as discussed below.

Preparation of Primary Analytical Stock, Secondary Fortification Stocks and Calibration Standards

A primary stock solution of Propargite reference standard was prepared by weighing a 10.70 mg aliquot into a vial. The reference material was dissolved, transferred to a 10-mL volumetric flask, and adjusted to final volume using acetonitrile to yield a final nominal stock concentration of 1.00 mg/mL (corrected for purity).

Secondary fortification stocks of Propargite analyte for fortification of validations samples and preparation of calibration standards were prepared at 1.00, 0.100, and 0.0100 µg/mL in acetonitrile solvent as shown below:

Stock Conc. (µg/mL)	Aliquot (mL)	Final Volume (mL)	Fortification Stock Concentration (µg/mL)
1000	0.100	100	1.00
1.00	1.00	10.0	0.100
0.100	1.00	10.0	0.0100

All solutions were prepared using volumetric flasks and gas-tight syringes and were stored under freezer conditions when not in use.

Working calibration standards ranging in concentration from 0.0400 to 5.00 µg/L were prepared in acetonitrile from the secondary fortification stocks above as shown below:

Secondary Stock Concentration (ug/mL)	Aliquot (mL)	Final Volume (mL)	Working Calibration STD Conc. (µg/L)
0.100	0.500	10.0	5.00
0.100	0.200	10.0	2.00*
0.100	0.100	10.0	1.00
0.0100	0.500	10.0	0.500
0.0100	0.200	10.0	0.200
0.0100	0.100	10.0	0.100
0.00200*	0.200	10.0	0.0400

*Note: 2.00 µg/L working calibration standard level was used to prepare the low-level calibration standard as shown.

Calibration standard solutions were transferred to amber vials and stored under freezer conditions when not in use.

Analytical Method – Surface/Drinking Water

The analytical method for the extraction and analysis of Propargite in surface and drinking water employed a liquid-liquid partitioning procedure to extract and concentrate the samples. Final quantitation of samples was performed utilizing High Performance Liquid Chromatography with tandem mass spectrometric detection (HPLC/MS/MS).

Fortification of Recovery Samples

For each of the matrix validations, one reagent blank, two unfortified matrix blanks, five fortified control matrix samples at the LOQ, and five fortified control matrix samples at 10X the LOQ were prepared in ground and surface water as shown below:

Propargite Fortification Table

Nominal Concentration (µg/L)	Fortification Volume (mL)	Sample Volume (mL)	Fortification Stock Conc. (µg/mL)
0.0100 (LOQ)	0.100	100	0.100
0.100 (10X LOQ)	0.100	100	0.0100

All fortified samples were prepared with fortification solutions that were prepared compensating for the purity of the reference material. Therefore, residue fortification and recovery levels, expressed in µg/L, are equivalent to the expression as µg/L active ingredient (µg/L a.i.).

Extraction and Analysis of Propargite from Surface/Drinking Water

For analysis, 100-mL volumes of control surface/drinking water were measured into twelve individually labeled 250-mL separatory funnels, five of which were fortified with Propargite at the LOQ (0.0100 µg/L) and five at 10x the LOQ (0.100 µg/L) with secondary fortification stocks of the reference substance prepared as described above. A single reagent blank consisting HPLC grade reagent water, and

the two matrix blanks of unfortified control matrix were also prepared and carried through the methodology for each matrix. All samples were subsequently analyzed by methodology in Appendix II. Slight deviations in the LC/MS/MS source optimization parameters were utilized and were considered to be equivalent values related to inherent differences in instrumental performance and not a limitation of the methodology. Since specific details of the method are presented in Appendix II, a more general description is provided here.

Twenty-five milliliters (25 mL) of hexane extraction solvent and approximately 10 gram of sodium chloride were added to each separatory funnel. The funnels were shaken vigorously for approximately 1 minutes and the phases allowed to separate for approximately 10 minutes. The lower aqueous layers were collected into beakers. The remaining hexane phases were drained through a filter funnel containing approximately 5 g of sodium sulfate into 125-mL concentration flasks and the filter funnels were subsequently rinsed with approximately 5 mL of hexane. The aqueous layers were returned to the separatory funnels and the extraction procedure was repeated two additional times, combining the hexane phases in the concentration flasks. Each of the combined extracts was evaporated by rotary-evaporation at ~40°C (at approximately 230 mBar pressure) to near dryness. The extracts were reduced to complete dryness manually using a gentle stream of nitrogen. The final residues were reconstituted in 10.0 mL volumes of acetonitrile using ~ 1 minute of sonication to facilitate mixing. Approximate 500 µL aliquots of each final extract were centrifuged using a 0.45 µm microfilterfuge tube. The supernatants were transferred to auto-sampler vials for LC/MS/MS analysis.

The remainders of each sample acetonitrile extract were transferred to amber vials and stored under freezer conditions.

Quantitation of Propargite by LC/MS/MS

An Agilent Technologies Model 1200 Infinity Series High Performance Liquid Chromatograph connected to an AB Sciex API 5000 Mass Spectrometric Detector (LC/MS/MS) was used to analyze samples. A 10mM ammonium acetate methanol: water gradient was used.

Quantitation was performed using the response of the primary ion transitions for propargite. Confirmation analysis was performed using the response of the secondary confirmation ion transition as well. The ion transitions monitored are summarized below:

Analyte	Primary (Quantitation)	Secondary (Confirmation)
Propargite	368.1→231.1 amu	368.1→81.1 amu

Specific details of the LC/MS/MS instrumentation and operational parameters are presented in Table 1.

Example Calculations

For each analyte, a regression equation was derived from the chromatographic peak area responses of the analytes determined in calibration standard solutions versus the respective nominal concentrations of the standards. Standard curves were generated by plotting this function with analyte concentration (µg/L) on the abscissa and the respective analyte peak area response on the ordinate. The applied regression was weighted 1/x with respect to concentration and expressed as a linear regression as follows:

$$y = mx + b$$

Where: Y = peak area
m = slope
b = Y-intercept
x = analyte concentration

Concentrations of analytes in the samples (quantitation and confirmation analyses) were determined by substituting peak area responses of the samples into the re-arranged weighted (1/x) regression equation as follows:

$$\text{Analyte Concentration} = \frac{\text{Peak area - (Y-intercept)}}{\text{Slope}}$$

Using the data from the surface water method validation sample 443C-128-SWVMAS-1, 0.0100 µg/L shown below, the analytical result and percent recovery was calculated as follows using the software algorithms of Analyst version 1.6 of the AB Sciex API 5000 mass spectrometer system in full precision mode. Note: manual calculations shown here may differ slightly than reported.

Where:

Peak area = 21184
Y-intercept = 1554.14
Slope = 193298

The concentration of Propargite at instrument was determined by substituting the resulting analyte peak area response into the above equation. Using the values above, the concentration in the final sample solution was calculated as:

$$\text{Concentration at instrument (µg/L): } = \frac{21184 - (1554.14)}{193298}$$

Concentration at instrument (µg/L): = 0.10155

The residue concentration (µg/L) for Proparite in the fortified water recovery sample was determined as the product of the at instrument solution concentration determined above and the overall dilution factor as follows:

$$\text{Concentration in µg/L} = \text{Propargite Concentration at Instrument x } \frac{\text{(Final Volume)}}{\text{(Initial Volume)}}$$

Where: Initial Volume= 100 mL
Final Volume = 10.0 mL

Using the nominal concentration (µg/L) from above, the concentration of Propargite in water sample was calculated as follows:

Concentration in sample (µg/L) = 0.10155 x 0.100

Concentration in sample (µg/L) = 0.0102

The percent recovery was determined by dividing the concentration of the analyte recovered in the fortified sample – average control residue (µg/L), if measured, by the nominal concentration added as shown below:

$$\text{Recovery (\%)} = \frac{\text{µg/L Found - average control residue.}}{\text{µg/L Added}} \text{ x } 100$$

For the above 0.0100 µg/L fortified sample, the percent recovery of Propargite was calculated as:

$$\text{Recovery (\%)} = \frac{\text{0.0102 µg/L - 0.00 µg/L Found}}{\text{0.0100 µg/L Added}} \text{ x } 100$$

Recovery (%) = 102%

The same calculation procedure was applied for the confirmation analyses of Propargite for this study as well.

Statistical Treatment of Data

Mean recoveries for Propargite for each fortification level were calculated by dividing the sum of the percent recoveries by the total number of fortified samples. The standard deviation and relative standard deviation (coefficient of variation) for the recoveries were also determined and reported for both quantitation and confirmation analyses.

Table 1. LC/MS/MS Instrumentation and Operational Parameters

Instrumentation	Agilent Technologies Model 1200 Infinity Series High Performance Liquid Chromatograph with a AB Sciex API 5000 Mass Spectrometric Detector (LC/MS/MS) and Turbo-V Ion Spray Source, operated in the positive, multiple reaction monitoring (MRM) mode.
Analytical Column	PHENOMENEX Gemini® 3 µm C18 110A (50 mm x 2.0 mm)
Guard Column	None
Mobile Phases	A2: 10mM Ammonium Acetate in HPLC-grade water B2: 10mM Ammonium Acetate in Methanol Gradient Elution Program: Time (min) / %A2 / %B2 / Flow Rate (µL/min) / Temp (°C) 0.00 / 70.0 / 30.0 / 200 / 55.0 1.00 / 5.00 / 95.0 / 200 / 55.0 5.00 / 5.00 / 95.0 / 200 / 55.0 5.10 / 70.0 / 30.0 / 200 / 55.0 8.00 / 70.0 / 30.0 / 200 / 55.0
Diverter Valve (Valco)	Time (min) / Position 0.0 / A 4.0 / B 5.9 / A
Injection Volume	12 µL
Total Run Time	8.00 minutes
Parameters	Period 1-Experiment 1: Scan Type/Polarity: MRM/Positive: GS1 = 40, GS2 = 40, CUR = 30, CAD = 12, IS = 5500, TEM = 300, DP = 61, EP = 10 Quantitation: (368.1/231.1 amu), CE = 13, CXP = 10 Confirmation: (368.1/81.1 amu), CE = 37, CXP = 10 Retention Time: Approximately 5.0 minutes

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Figure 1. Schematic of Extraction Procedure