

INTRODUCTION

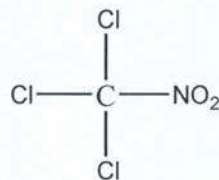
The purpose of this study was to conduct an independent laboratory validation (ILV) for the determination of chloropicrin in ground, surface and drinking water test systems. The quantitation analysis of chloropicrin was performed by Gas Chromatography with Electron Capture Detection (GC-ECD) and confirmatory analysis was performed by Gas Chromatography with Mass Spectrometric Detection (GC-MS), based on the method described in "Validation of Methodology for the Determination of Residues of Chloropicrin in Ground, Drinking and Surface Water", Huntingdon Life Sciences Ltd., Project Identity SXC 0007/072387. (Reference 1).

This study was designed to satisfy US EPA Guideline requirements described in OCSPP 850.6100. The study was initiated on June 16, 2016. The experimental work was conducted from June 28, 2016 through September 7, 2016 at EAG Laboratories-Hercules, 625-B Alfred Nobel Drive, Hercules, CA 94547 under the approved protocol (Appendix A) according to the US EPA FIFRA Good Laboratory Practice Standards, 40 CFR §160.

MATERIAL AND METHODS**Reference and Test Substances**

Common Name:	Chloropicrin
Chemical Name: (IUPAC):	Trichloronitromethane
CAS Registry No.:	76-06-2

Chemical Structure:



Molecular Formula:	CCl ₃ NO ₂
Molecular Mass:	164.4 grams/mole
Lot No.:	012-093A
Purity:	99.37%
Date of Expiry:	August 24, 2018
Storage Conditions:	Room Temperature

A copy of the Certificate of Analysis for the test/reference substance is provided in Appendix B.

Other Chemicals

HPLC grade acetone, and hexane were obtained from Burdick & Jackson.

Equipment List

Laboratory balances
Pipetman with plastic disposable tips
Vortex mixer
Centrifuge
Ultrasonic bath
Polypropylene bottles and tubes
Volumetric flask

Agilent 6890 Series Gas Chromatograph with Agilent 7683 Series Injector and Electron Capture Detector

Agilent 7890A Series Gas-Liquid Chromatograph with Agilent 7001B GC-MS/MS triple quad mass spectrometer

Test System

Source of Test System

The test systems that were used in this study were provided by EAG-Hercules and previously characterized by Agvise Laboratories. The ground water (2706W-032) was collected from Northwood, North Dakota, USA, the tap water (2706W-072) was collected from Hercules, California, USA and the surface water (2706W-069) was collected from Hercules, California, USA. The test systems were maintained under refrigerated conditions while not in use. The water characterization details are presented below and the characterization reports are provided in Appendix C.

Description	Ground	Tap	Surface
PTRL Identification (2706W-)	032	054/072	069
pH	7.3	7.5	7.8
Calcium (ppm)	147	8.5	295
Magnesium (ppm)	62	2.0	107
Sodium (ppm)	17	9.8	234
Hardness (mg equivalent CaCO ₃ /L)	627	30	1183
Conductivity (mmhos/cm)	1.00	0.10	2.44
Sodium Adsorption (SAR)	0.30	0.79	2.97
Total Dissolved Solids (ppm)	960	60	Not Determined
Turbidity (NTU)	1.04	0.83	Not Determined

Note: Tap water samples (2706W-054 and 2706W-072) both collected from PTRL West Laboratory at different times and are considered equivalent.

Test Method

The analytical method for the analysis of chloropicrin was independently validated at EAG Laboratories – Hercules by Gas Chromatography with Electron Capture Detection (GC-ECD) for chloropicrin and is described in “Validation of Methodology for the Determination of Residues of Chloropicrin in Ground, Drinking and Surface Water”, Huntingdon Life Sciences Ltd., Project Identity SXC 0007/072387. (Reference 1).

The water samples were spiked with known concentrations of chloropicrin and extracted with hexane. An aliquot of the final sample solution was injected onto the gas

chromatograph with electron capture detection (GC-ECD), for quantitation analysis and injected onto the gas chromatograph with mass spectrometric detection (GC-MS) for confirmation analysis of chloropicrin. The percent method recoveries were determined using external standardization where a linear curve for calibration standards was generated along with the samples.

Preparation of Stock Solution

A single stock solution was prepared for chloropicrin, by weighing an aliquot approximately 600 µL (Hamilton syringe) of the reference substance into a 4 mL amber vial with a Teflon lined septum screw cap containing approximately 3 mL of acetone. The vial was re-weighed and the mass of chloropicrin determined. The contents of the vial were chilled for approximately 20 minutes and then transferred to a 10 mL class A volumetric flask and brought to mark with acetone and then vortexed and inverted to mix. The concentration of the stock solution, corrected for purity was 99.08 mg/mL (100 mg/mL). The stock solution 100 mg/mL was stored in the freezer (typically < -10°C) when not in use.

PTRL West No.	Stock ID	Standard Name	Weight (mg)	Final volume (mL)	Purity (%)	Theoretical Conc. (mg/mL)
2269W-001	100 mg/mL	Chloropicrin	997.11	10.0	99.37	99.08

Preparation of Chloropicrin 1 mg/mL Stock Solution

The 100 mg/mL stock solution was further diluted to make a 1 mg/mL stock solution, by removing 1007 µL (volumetric pipette and 10 µL syringe) aliquot and diluting to mark with acetone into a 100 mL volumetric flask. The 1 mg/mL stock solution was vortexed and inverted to mix and then transferred into two separate 60 mL amber bottles. The stock solution 1 mg/mL was stored in the freezer (typically < -10°C) when not in use.

Preparation of Chloropicrin Intermediate Solution

A 10 µg/mL intermediate solution was prepared by measuring 0.1 mL (Hamilton syringe) of the 1 mg/mL stock solution into a 10 mL volumetric flask. The final solution was diluted to the mark with acetone, vortexed and inverted to mix and then transferred to a amber bottle for storage. The 10 µg/mL solution was stored in the freezer (typically < -10°C) when not in use.

Preparation of Chloropicrin Fortification Solutions

A 1000 ng/mL fortification solution was prepared by measuring 1.0 mL of the intermediate solution using a Hamilton syringe into a 10 mL volumetric flask. The final solution was diluted to the mark with acetone.

A 100 ng/mL fortification solution was prepared by measuring 1.0 mL of the 1000 ng/mL fortification solution using a Hamilton syringe into a 10 mL volumetric flask. The final solution was diluted to the mark with acetone.

The fortification solutions were mixed, transferred into amber bottles and stored in the freezer when not in use.

Preparation of Chloropicrin Calibration Solutions

Calibration standard solutions were prepared by mixing appropriate volumes of intermediate/calibrant solutions via Hamilton syringe with appropriate volumes of hexane into appropriately sized volumetric flasks as described below. The standard solutions ranged from 0.5 ng/mL to 100 ng/mL, representative preparations are shown below:

Standard ID	Theoretical Conc. (ng/mL)	Solution Used (ng/mL)	Volume of Solution (mL)	Final Volume (mL)
Cal A1	100	Intermediate	0.2	20
Cal A2	80	Intermediate	0.08	10
Cal A3	60	Intermediate	0.06	10
Cal A4	40	Intermediate	0.04	10
Cal A5	20	Cal A1	2.0	10
Cal A6	10	Cal A1	1.0	10
Cal A7	5	Cal A1	0.5	10
Cal A8	2	Cal A1	0.2	10
Cal A9	1	Cal A1	0.1	10
Cal A10	0.5	Cal A1	0.05	10

Fortification Procedure

Fortification of untreated water samples was conducted at the following two fortification levels as shown below using a 25 µL Hamilton syringe:

Fortification Level (ng/mL)	Fortification Solution
0.1	20 µL of 100 ng /mL in 20 g of water
1.0	20 µL of 1000 ng /mL in 20 g of water

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

Extraction Method for Chloropicrin in water

1. Measure and transfer 20 g of water into a 25 mL volumetric flask.
2. Fortify the samples as necessary.
3. Add 2 mL of hexane to each sample.
4. Partition each sample by vortexing and inversion for 1 minute.
5. Remove upper hexane layer with Pasteur pipette and transfer to amber vial for storage or HPLC vial for analysis.

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

Gas Chromatography with Electron Capture Detector Analytical Method (GC-ECD)

GC-ECD conditions (Chloropicrin Quantitation Analysis)

Instrument: Agilent 6890 Series Gas Chromatograph
Column: DB-17 (30 m x 530 µm x 1 µm film thickness)
Injector Temperature: 200 °C
Detector Temperature: 260 °C
Oven Program: 40 °C held for 2 minutes then ramped at 10 °C/min to 80 °C and then ramped at 30 °C/min to 200 °C
Carrier Gas: 7 mL/minute
Injection Volume: 1 µL
Retention Time: approximately 4.7 minutes

Gas Chromatography with Mass Spectrometric Detection Analytical Method (GC-MS)

GC-MS conditions (Chloropicrin Confirmation Analysis)

Instrument: Agilent 7890A Series Gas – Liquid Chromatograph
Ionisation mode: CI
Ion monitored: m/z 119
Column: DB-5ms (30 m x 0.25 mm x 0.25 µm film thickness)
Carrier Gas: Helium
Flow Rate: 1 mL/minute

Injector temperature: 200 °C

Oven Program: 40 °C held for 3 minutes then ramped at 10 °C/minute to 60 °C and then
ramped at 30 °C/minute to 200 °C

Injection Volume: 1 µL

Retention Time: Approximately 2.6 minutes

GC Analysis

Samples were analyzed in a set consisting of a solvent blank, reagent blank, two control extracts, five 0.1 ng/mL fortified controls, and five 1 ng/mL fortified controls interspersed between the calibrants. In addition, to ensure accuracy was maintained over the course of the analytical set, quality control (QC) calibrants were added to the sequence. The final QC calibrant response was within 20% when compared to its initial response. Calibrants and samples were analyzed in a single sequence of injections.

Methods of Calculation

Quantitation

The quantitation of chloropicrin was conducted using peak area relative to the theoretical concentrations of the calibrants. The content of chloropicrin in samples was quantitated against a 1/x weighted linear curve of calibrants. An example for the quantitation of chloropicrin is presented below where:

$$\text{ng/mL analyte} = \frac{y - b}{m}$$

y = peak area

x = ng/mL compound injected

m = slope

b = intercept

Weighting of the calibration curve was applied to provide better curve fit at the lower concentration levels of chloropicrin.

The calculation of weighted curve equations (linear regression) and concentrations (ng/mL) present in samples and calibrants was conducted using Chromeleon® software.

The residue of the analyte in the sample is determined as follows:

$$\text{Residue (ng/L)} = \frac{\text{ng/mL analyte} \times \text{Dil. Factor} \times \text{Extract Volume (mL)}}{\text{Initial Volume (mL)}}$$

Initial Volume = 20 mL (volume of water sample assumed density of 1 g/mL)

The Percent Recovery of a fortified sample is determined as follows:

$$\frac{\text{Residue (ng/mL)} - \text{Average Residue of Controls (ng/mL)}}{\text{Fortification Level (ng/mL)}} \times 100$$

An example calculation for the recovery of chloropicrin in ground water fortified at 0.1 ng/mL (sample designated F1-A) is given in following:

Linear regression equation: $y = (2.16 \text{ e} +1) x + 1.22 \text{ e} +0$ ($r = 0.999$)

The calculated concentration in F1-A final extract (solving for x):

$$\text{Chloropicrin ng /mL} = \frac{2.03\text{e} +1 - 1.22 \text{ e} + 0}{2.16 \text{ e} +1} = 0.88 \text{ ng/mL}$$

Where: $2.03 \text{ e} +1$ is the peak area of chloropicrin for F1-A

$1.22 \text{ e} +0$ is the y-intercept from the linear regression equation

$2.16 \text{ e} + 1$ is the slope from the linear regression equation

The chloropicrin residue (ng/mL) for F1-A =

$$\frac{0.88 \text{ ng/mL} \times 2 \text{ mL (final volume)} \times 1 \text{ (Dil. Factor)}}{20 \text{ mL (initial volume)}}$$

$$= 0.088 \text{ ng/mL}$$

The percent recovery of fortified sample F1-A (chloropicrin):

$$\frac{\{[0.088 \text{ ng/mL} - 0.000 \text{ ng/mL (avg. control residue)]} \div 0.1 \text{ ng/mL (fort. level)}\} \times 100\%}{}$$

$$= 88\%$$

Note: values rounded for presentation and may differ slightly from reported values.

Calibration Range

The calibration curve, typically ranged from 0.5 ng/mL to 100 ng/mL for chloropicrin, was generated by Chromeleon® software for the water method validations.

Limit of Quantitation

The limit of quantitation (LOQ) was set at 0.1 ng/mL (ppb) for chloropicrin in water, which was defined as the lowest fortification level at which acceptable recovery data are obtained.

Limit of Detection

The LOD in ground water was calculated to be 0.004 ppb and 0.01 ppb for both drinking and surface water test systems for the quantitation analysis method of chloropicrin. Data for the LOD determinations is presented in Table 2, Table 3 and Table 4.

Time Required for Completion of a Sample Set

A sample set consisted of one solvent blank, one reagent blank, two controls (untreated water samples), and five fortified water samples (at each level i.e. LOQ and 10X LOQ). The time required for one sample set from preparation of standard solutions, initiation of extraction, until the completion of instrumental analysis and data evaluation is as follows:

- Preparation of standard solutions takes approximately 4 hours
- Sample preparation takes approximately 4 hours
- GC-ECD analysis and data processing (quantitation) takes approximately 2 hours, not including automated sample analysis
- GC-MS analysis and data processing (confirmation) takes approximately 2 hours, not including automated sample analysis

TOTAL = approximately 12 hours for one analyst to complete a set to satisfy the validation requirements.

Statistical Methods

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

Figure 1. Schematic Diagram for the Extraction of Chloropicrin from Water.

