EPA Method 501.2 *(EPA 500-Series, November 1979)*
TITLE: Analysis Of Trihalomethanes In Drinking Water By Liquid/Liquid Extraction

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ANALYTE:	CAS #
Chloroform	67-66-3
Bromodichloromethane	75-27-4
Chlorodibromomethane	124-48-1
Bromoform	75-25-2
Trihalomethanes	
THM	

INSTRUMENTATION: GC

1.0 Scope

   1.1 This method (1,2) is applicable only to the determination of four trihalomethanes, i.e., chloroform, bromodichloromethane, chlorodibromomethane, and bromoform in finished drinking water, drinking water during intermediate stages of treatment, and the raw source water.

   1.2 For compounds other than the above-mentioned trihalomethanes, or for other sample sources, the analyst must demonstrate the usefulness of the method by collecting precision and accuracy data on actual samples as described in (3) and provide qualitative confirmation of results by Gas Chromatography/Mass Spectrometry (GC/MS) (4).

   1.3 Qualitative analyses using GC/MS or the purge and trap method (5) must be performed to characterize each raw source water if peaks appear as interferences in the raw source analysis.

   1.4 The method has been shown to be useful for the trihalomethanes over a concentration range from approximately 0.5 to 200 ug/L. Actual detection limits are highly dependent upon the characteristics of the gas chromatographic system used.

2.0 Summary

   2.1 Ten milliliters of sample are extracted one time with 2 mL of solvent. Three uL of the extract are then injected into a gas chromatograph equipped with a linearized electron capture detector for separation and analysis.

   2.2 The extraction and analysis time is 10 to 50 minutes per sample depending upon the analytical conditions chosen. (See Table 1 and Figures 1, 2, and 3.)

   2.3 Confirmatory evidence is obtained using dissimilar columns and temperature programming. When component concentrations are sufficiently high (>50 ug/L), halogen specific detectors may be employed for improved specificity.

   2.4 Unequivocal confirmatory analyses at high levels (>50 ug/L) can be

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performed using GC/MS in place of the electron capture detector. At levels below 50 ug/L, unequivocal confirmation can only be performed by the purge and trap technique using GC/MS (4,5).

2.5 Standards dosed into organic free water and the samples are extracted and analyzed in an identical manner in order to compensate for possible extraction losses.

2.6 The concentration of each trihalomethane is summed and reported as total trihalomethanes in ug/L.

3.0 Interferences

3.1 Impurities contained in the extracting solvent usually account for the majority of the analytical problems. Solvent blanks should be analyzed before a new bottle of solvent is used to extract samples. Indirect daily checks on the extracting solvent are obtained by monitoring the sample blanks (6.4.10). Whenever an interference is noted in the sample blank, the analyst should reanalyze the extracting solvent. The extraction solvent should be discarded whenever a high level (>10 ug/L) of interfering compounds are traced to it. Low level interferences generally can be removed by distillation or column chromatography (6); however, it is generally more economical to obtain a new source of solvent or select one of the approved alternative solvents listed in Section 5.1. Interference free solvent is defined as a solvent containing less than 0.4 ug/L individual trihalomethane interference. Protect interference-free solvents by storing in a non-laboratory area known to be free of organochlorine solvents. Subtracting blank values is not recommended.

3.2 Several instances of accidental sample contamination have been attributed to diffusion of volatile organics through the septum seal on the sample bottle during shipment and storage. The sample blank (6.4.10) is used to monitor for this problem.

3.3 This liquid/liquid extraction technique efficiently extracts a wide boiling range of non-polar organic compounds and, in addition, extracts the polar organic components of the sample with varying efficiencies. In order to perform the trihalomethane analysis as rapidly as possible with sensitivities in the low ug/L range, it is necessary to use the semi-specific electron capture detector and chromatographic columns which have relatively poor resolving power. Because of these concessions, the probability of experiencing chromatographic interferences is high. Trihalomethanes are primarily products of the chlorination process and generally do not a wear in the raw source water. The absence of peaks in the raw source water analysis with retention times similar to the trihalomethanes is generally adequate evidence of an interference-free finished drinking water analysis. Because of these possible interferences, in addition to each finished drinking water analysis, a representative raw source water (6.4.5) must be analyzed. When potential interferences are noted in the raw source water analysis, the alternate chromatographic columns must be used to reanalyze the sample

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set. If interferences are still noted, qualitative identifications should be
performed according to Sections 2.3 and 2.4. If the peaks are confirmed to
be other than trihalomethanes and add significantly to the total
trihalomethane value in the finished drinking water analysis, then the
sample set must be analyzed by the purge and trap method (5).

4.0 Apparatus

4.1 Extraction vessel - A 15 mL total volume glass vessel with a Teflon lined
screw-cap is required to efficiently extract the samples.

4.1.1 For samples that do not form emulsions 10 mL screw-cap flasks with a
Teflon faced septum (total volume is mL) are recommended. Flasks
and caps - Pierce - #13310 or equivalent Septa - Teflon silicone -
Pierce #12718 or equivalent.

4.1.2 For samples that form emulsions (turbid source water) 15 mL screw
cap centrifuge tubes with a Teflon cap liner are recommended.
Centrifuge tube - Corning 8062-15 or equivalent.

4.2 Sampling containers - 40 mL screw cap sealed with Teflon faced silicone
septa. Vials and caps - Pierce #13075 or equivalent. Septa - Pierce #12722
or equivalent.

4.3 Micro syringes - 10, 100 uL.

4.4 Micro syringe - 25 uL with a 2-inch by 0.006-inch needle - Hamilton 702N
or equivalent.

4.5 Syringes - 10 mL glass hypodermic with Luerlok tip (2 each).

4.6 Syringe valve - 2-way with luer ends (2 each) - Hamilton #86570-1FM1 or
equivalent.

4.7 Pipette - 2.0 mL transfer.

4.8 Glass stoppered volumetric flasks - 10 and 100 mL.

4.9 Gas chromatograph with linearized electron capture detector.
(Recommended option - temperature programmable. See Section 4.12).

4.10 Column A - 4 mm ID x 2m long glass packed with 3% SP-1000 on
Supelcoport (100/120 mesh) operated at 50 ˚C with 60 mL/min flow. (See
Figure 1 for a sample chromatogram and Table 1 for retention data).

4.11 Column B - 2 mm ID x 2m long glass packed with 10% squalane on
Chromosorb WAW (80/100 mesh) operated at 67 ˚C with 25 mL/min flow.
This column is recommended as the primary analytical column.
Trichloroethylene, a common raw source water contaminate, coelutes with
bromodichloromethane. (See Figure 2 for a sample chromatogram and
Table 1 for retention data.)

4.12 Column C - 2 mm ID x 3m long glass packed with 6% OV-11/4% SP-2100
on Supelcoport (100/120 mesh) temperature program 45 ˚C for 12 minutes,

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then program at 1-/minute to 70 ˚C with a 25 mL/min flow. (See Figure 3 for a sample chromatogram and Table I for retention data.)

4.13 Standard storage containers - 15 mL amber screw-cap septum bottles with Teflon faced silicone septa. Bottles and caps - Pierce #19830 or equivalent. Septa - Pierce #12716 or equivalent.

5.0 Reagents

5.1 Extraction solvent - (See 3.1). Recommended - Pentane(a) Alternative - hexane, methylcyclohexane or 2,2,4-trimethylpentane. (a) Pentane has been selected as the best solvent for this analysis because it elutes, on all of the columns, well before any of the trihalomethanes. High altitudes or laboratory temperatures in excess of 75-F may make the use of this solvent impractical. For these reasons, alternative solvents are acceptable; however, the analyst may experience baseline variances in the elution areas of the trihalomethanes due to coelution of these solvents. The degree of difficulty appears to be dependent upon the design and condition of the electron capture detector. Such problems should be insignificant when concentrations of the coeluting trihalomethane are in excess of 5 ug/L.

5.2 Methyl alcohol - ACS Reagent Grade

5.3 Free and combined chlorine reducing agents - Sodium thiosulfate ACS Reagent Grade - sodium sulfite ACS Reagent Grade.

5.4 Activated carbon - Filtrasorb - 200, available from Calgon Corporation, Pittsburgh, PA, or equivalent.

5.5 Standards (b):

5.5.1 Bromoform 96% - available from Aldrich Chemical Company.

5.5.2 Bromodichloromethane 97% - available from Aldrich Chemical Company.

5.5.3 Chlorodibromomethane - available from Columbia Chemical, Incorporated, Columbia, S.C.

5.5.4 Chloroform 99% - available from Aldrich Chemical Company.

5.6 Organic-free water - Organic-free water is defined as water free of interference when employed in the procedure described herein.

5.6.1 Organic-free water is generated by passing tap water through a carbon filter bed containing carbon. Change the activated carbon whenever the concentration of any trihalomethane exceeds 0.4 ug/L.

5.6.2 A Millipore Super-Q Water System or its equivalent may be used to generate organic-free deionized water.

5.6.3 Organic-free water may also be prepared by boiling water for 15 minutes. Subsequently, while maintaining the temperature at 90 ˚C, bubble a contaminant free inert gas through the water at 100

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mL/minute for one hour. While still hot, transfer the water to a narrow mouth screw cap bottle with a Teflon seal.

5.6.4 Test organic free water each day it is used by analyzing it according to Section 7.

(b) As a precautionary measure, all standards must be checked for purity by boiling point determinations or GC/MS assays.

5.7 Standard Stock Solutions

5.7.1 Fill a 10.0 mL ground glass stoppered volumetric flask with approximately 9.8 mL of methyl alcohol.

5.7.2 Allow the flask to stand unstoppered about 10 minutes or until all alcohol wetted surfaces dry.

5.7.3 Weigh the unstoppered flask to the nearest 0.1 mg.

5.7.4 Using a 100 uL syringe, immediately add 2 to 3 drops of the reference standard to the flask, then reweigh. Be sure that the reference standard falls directly into the alcohol without contacting the neck of the flask.

5.7.5 Dilute to volume, stopper, then mix by inverting the flask several times.

5.7.6 Transfer the standard solution to a dated and labeled 15 mL screw-cap bottle with a Teflon cap liner. NOTE: Because of the toxicity of trihalomethanes, it is necessary to prepare primary dilutions in a hood. It is further recommended that a NIOSH/MESA- approved toxic gas respirator be used when the analyst handles high concentrations of such materials.

5.7.7 Calculate the concentration in micrograms per microliter from the net gain in weight.

5.7.8 Store the solution at 4 ˚C.

NOTE: All standard solutions prepared in methyl alcohol are stable up to 4 weeks when stored under these conditions. They should be discarded after that time has elapsed.

5.8 Aqueous Calibration Standard Precautions

5.8.1 In order to prepare accurate aqueous standard solutions, the following precautions must be observed:

a. Do not inject more than 20 uL of alcoholic standards into 100 mL of organic-free water.

b. Use a 25 uL Hamilton 702N microsyringe or equivalent. (Variations in needle geometry will adversely affect the ability to deliver reproducible volumes of methanolic standards into water.)

c. Rapidly inject the alcoholic standard into the expanded area of the

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filled volumetric flask. Remove the needle as fast as possible after injection.

d. Mix aqueous standards by inverting the flask three times only.

e. Discard the contents contained in the neck of the flask. Fill the sample syringe from the standard solution contained in the expanded area of the flask as directed in Section 7.0.

f. Never use pipets to dilute or transfer samples and aqueous standards.

g. Aqueous standards, when stored with a headspace, are not stable and should be discarded after one hour. Aqueous standards can be stored according to Sections 6.4.9 and 7.2.

5.9 Calibration Standards

5.9.1 Prepare, from the standard stock solutions, a multicomponent secondary dilution mixture in methyl alcohol so that a 20 uL injection into 100 mL of organic-free water will generate a calibration standard which produces a response close (+/- 25%) to that of the unknown. (See 8.1).

5.9.2 Alternative Calibration Procedure

5.9.2.1 Construct a calibration curve for each trihalomethane containing a minimum of 3 different concentrations. Two of the concentrations must bracket each unknown.

5.9.3 Extract and analyze the aqueous calibration standards in the same manner as the unknowns.

5.9.4 Other calibration procedures (7) which require the delivery of less than 20 uL of methanolic standards to 10.0 mL volumes of water contained in the sample syringe are acceptable only if the methanolic standard is delivered by the solvent flush technique (8).

5.10 Quality Check Standard Mixture

5.10.1 Prepare, from the standard stock solutions, a secondary dilution mixture in methyl alcohol that contains 10.0 ng/uL of each compound. (See 5.7.6 and 5.7.8).

5.10.2 Daily, prepare and analyze a 2.0 ug/L aqueous dilution from this mixture by dosing 20.0 uL into 100 mL of organic-free water (See Section 8.1).

6.0 Sample Collection and Handling

6.1 The sample containers should have a total volume of at least 25 mL.

6.1.1 Narrow-mouth screw-cap bottles with the TFE fluorocarbon faced silicone septa cap liners are strongly recommended.

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6.2 Glassware Preparation

6.2.1 Wash all sample bottles, TFE seals, and extraction flasks in detergent. Rinse with tap water and finally with distilled water.

6.2.2 Allow the bottles and seals to air dry, then place in an 105 ˚C oven for 1 hour, then allow to cool in an area known to be free of organics.

NOTE: Do not heat the TFE seals for extended periods of time (> 1 hour) because the silicone layer slowly degrades at 105 ˚C.

6.2.3 When cool, seal the bottles using the TFE seals that will be used for sealing the samples.

6.3 Sample stabilization - A chemical reducing agent (Section 5.3) is added to all samples in order to arrest the formation of additional trihalomethanes after sample collection (7,9) and to eliminate the possibility of free chlorine reacting with impurities in the extraction solvent to form interfering organohalides. DO NOT ADD THE REDUCING AGENT TO SAMPLES AT COLLECTION TIME WHEN DATA FOR MAXIMUM TRIHALOMETHANE FORMATION IS DESIRED. If chemical stabilization is employed, then the reagent is also added to the blanks. The chemical agent (2.5 to 3 mg/40 mL) is added in crystalline form to the empty sample bottle just prior to shipping to the sampling site. If chemical stabilization is not employed at sampling time then the reducing agent is added just before extraction.

6.4 Sample Collection

6.4.1 Collect all samples in duplicate.

6.4.2 Fill the sample bottles in such a manner that no air bubbles pass through the sample as the bottle is filled.

6.4.3 Seal the bottle so that no air bubbles are entrapped in it.

6.4.4 Maintain the hermetic seal on the sample bottle until analysis.

6.4.5 The raw source water sample history should resemble the finished drinking water. The average retention time of the finished drinking water within the water plant should be taken into account when sampling the raw source water.

6.4.6 Sampling from a water tap.

6.4.6.1 Turn on the water and allow the system to flush until the temperature of the water has stabilized. Adjust the flow to about 500 mL/minute and collect duplicate samples from the flowing stream.

6.4.7 Sampling from an open body of water.

6.4.7.1 Fill a 1-quart wide-mouth bottle with sample from a representative area. Carefully fill duplicate sample bottles

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from the 1-quart bottle as in 6.4.

6.4.8 If a chemical reducing agent has been added to the sample bottles, fill with sample just to overflowing, seal the bottle, and shake vigorously for 1 minute.

6.4.9 Sealing practice for septum seal screw cap bottles.

6.4.9.1 Open the bottle and fill to overflowing. Place on a level surface. Position the TFE side of the septum seal upon the convex sample meniscus and seal the bottle by screwing the cap on tightly.

6.4.9.2 Invert the sample and lightly tap the cap on a solid surface. The absence of entrapped air indicates a successful seal. If bubbles are present, open the bottle, add a few additional drops of sample, then reseal bottle as above.

6.4.10 Sample Blanks

6.4.10.1 Prepare blanks in duplicate at the laboratory by filling and sealing sample bottles with organic-free water just prior to shipping the sample bottles to the sampling site.

6.4.10.2 If the sample is to be stabilized, add an identical amount of reducing agent to the blanks.

6.4.10.3 Ship the blanks to and from the sampling site along with the sample bottles from the 1-quart bottle as in 6.4.

6.4.10.4 Store the blanks and the samples, collected at a given site (sample set), together in a protected area known to be free from contamination. A sample set is defined as the samples collected at a given site (a water treatment plant, duplicate raw sample water, duplicate finished water and the duplicate sample blanks comprise the sample set).

6.5 When samples are collected and stored under these conditions, a measurable loss of trihalomethanes has been detected over extended periods of time (7). It is recommended that the samples be analyzed within 14 days of collection.

7.0 Extraction and Analysis

7.1 Remove the plungers from two 10-mL syringes and attach a closed syringe valve to each.

7.2 Open the sample bottle (c) (or standard) and carefully pour the sample into one of the syringe barrels until it overflows. Replace the plunger and compress the sample. Open the syringe valve and vent any residue air while adjusting the sample volume to 10 mL. Close the valve.

7.3 Fill the second syringe in an identical manner from the same bottle. This

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syringe is reserved for a replicate analysis (and 8.4).

(C) If for any reason the chemical reducing agent has not been added to sample, then it must be added just prior to analyses at the rate of 3 mg/40 mL or by adding 1 mg directly to the sample in the extracion.

7.4 Pipette 2.0 mL of extraction solvent into a clean extraction flask.

7.5 Carefully inject the contents of the syringe into the extraction flask.

7.6 Seal with a Teflon faced septum.

7.7 Shake vigorously for 1 minute.

7.8 Let stand until the phases separate (approx. 60 seconds).

7.8.1 If the phases do not separate on standing then centrifugation can be used to facilitate separation.

7.9 Analyze the sample by injecting 3.0 uL (solvent flush technique, (8)) of the upper (organic) phase into the gas chromatograph.

8.0 Analytical Quality Control

8.1 A 2 ug/L quality check standard (See 5.10) should be extracted and analyzed each day before any samples are analyzed. Instrument status checks and lower limit of detection estimations based upon response factor calculations at 5 times the noise level are obtained from these data. In addition, the data obtained from the quality check standard can be used to estimate the concentration of the unknowns. From this information the appropriate standards can be determined.

8.2 Analyze the sample blank and the raw source water to monitor for potential interferences as described in Sections 3.1, 3.2, and 3.3.

8.3 Spiked samples

8.3.1 For those laboratories analyzing more than 10 samples a day, each 10th sample analyzed should be a laboratory-generated spike which closely duplicates the average finished drinking water in trihalomethane composition and concentration. Prepare the spiked sample in organic-free water as described in S.9.

8.3.2 In those laboratories analyzing less than 10 samples daily, each time the analysis is performed, analyze at least one laboratory generated spike sample which closely duplicates the average finished drinking water in trihalomethane composition and concentration. Prepare the spiked sample in organic-free water as described in Section 5.9.

8.3.3 Maintain an up-to-date log on the accuracy and precision data collected in Sections 8.3 and 8.4. If results are significantly different than those cited in Section 10.1, the analyst should check out the entire analysis scheme to determine why the laboratory's precision and accuracy limits are greater.

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8.4 Randomly select and analyze 10% of all samples in duplicate.

8.5 Analyze all samples in duplicate which appear to deviate more than 30% from any established norm.

8.6 Quarterly, spike an EMSL-Cincinnati trihalomethane quality control sample into organic-free water and analyze.

8.6.1 The results of the EMSL trihalomethane quality control sample should agree within 20% of the true value for each trihalomethane. If they do not, the analyst must check each step in the standard generation procedure to solve the problem.

8.7 It is important that the analyst be aware of the linear response characteristics of the electron capture system that is utilized. Calibration curves should be generated and rechecked quarterly for each trihalomethane over the concentration range encountered in the samples in order to confirm the linear response range of the system Quantitative data cannot be calculated from non-linear responses. Whenever non-linear responses are noted, the analyst must dilute the sample for reanalysis.

8.8 Maintain a record of the retention times for each trihalomethane using data gathered from spiked samples and standards.

8.8.1 Daily calculate the average retention time for each trihalomethane and the variance encountered for the analyses.

8.8.2 If individual trihalomethane retention time varies by more than 10% over an eight hour period or does not fall within l0% of an established norm, the system is "out of control." the source of retention data variation must be corrected before acceptable data can be generated.

9.0 Calculations

9.1 Locate each trihalomethane in the sample chromatogram by comparing the retention time of the suspect peak to the data gathered in 8.8.1. The retention time of the suspect peak must fall within the limits established in 8.8.1 for a single column identification.

9.2 Calculate the concentration of each trihalomethane by comparing the peak heights or peak areas of the samples to those of the standards. Round off the data to the nearest ug/L or two significant figures.

```
                        sample peak height
Concentration (ug/L) = ------------------- * std conc.(ug/L)
                        standard peak height
```

9.3 Calculate the total trihalomethane concentration (TTHM) by summing the 4 individual trihalomethane concentrations in ug/L:

```
TTHM (ug/L) = (conc. CHCl3)+(conc. CHBrCl2) +(conc.
CHBr2Cl)+(conc.CHBr3)
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9.4 Calculate the limit of detection (LOD) for each trihalomethane not detected
using the following criteria:

```
                 (A * ATT)
    LOD (ug/L) = --------- * (2 ug/L)
                 (B * ATT)
where

    B = peak height (mm) of 2 ug/L quality check standard
    A = 5 times the noise level in mm at the exact retention
        time of the trihalomethane or the base line
        displacement in mm from theoretical zero at the exact
        retention time for the trihalomethane.
  ATT = attenuation factor.
```

9.5 Report the results obtained from the lower limit of detection estimates along
with the data for the samples.

10.0 Precision and Accuracy

10.1 Single lab precision and accuracy. The data in Table II were generated by
spiking organic-free water with trihalomethanes as described in 5.9. The
mixtures were analyzed by the analyst as true unknowns.

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TABLE I. Retention Times for Trihalomethanes

Trihalomethane	Retention Time, Minutes		
	Column A	Column B	Column C
Chloroform	1.0	1.3	4.9
Bromodichloromethane	1.5	2.5(d)	11.0
Chlorodibromomethane (dibromochloromethane)	2.6	5.6	23.1
Bromoform	5.5	10.9	39.4

(d) On this column, trichloroethylene, a common raw source water contaminate, coelutes with bromodichloromethane.

Table II. Single Laboratory Accuracy and Precision

Compound	Dose Level ug/L	No. of Samples	Mean ug/L	Precision Relative Standard Deviation (%)	Accuracy Percent Recovery
CHCl3	9.1	5	10	11	110
CHCl3	69	3	73	5.3	106
CHBrCl2	1.2	5	1.3	9.8	108
CHBrCl2	12	2	15	1.4	125
CHBr2Cl	2.7	5	2.0	17	74
CHBr2Cl	17	3	16	9.9	94
CHBr3	2.9	5	2.2	10	76
CHBr3	14	3	16	12	114