US EPA - REGION 1

Technical Guidance for the Natural Attenuation Indicators: Methane, Ethane, and Ethene.

Based on Method: Analysis of Dissolved Methane, Ethane, and Ethene in Groundwater by a Standard Gas Chromatographic Technique, Don H. Kampbell and Steve A. Vandegrift, EPA, Ada, OK. J of Chrom, Vol 36, May 1998

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Disclaimer: The use or mention of trade names does not suggest or imply endorsement by the US EPA. This supplement to the reference method is intended for the analysis of Region 1 samples.

1. Introduction:

Analysis of dissolved methane, ethane, and ethene in conjunction with other natural attenuation parameters is used to determine whether or not natural attenuation is occurring. This document outlines principles to be used as guidance to generate data which are usable for the intended objectives.

2. Summary:

An inert gas is injected into the VOA or serum vial containing the water sample to create headspace. After equilibration, the headspace is analyzed for the target gases. The concentration of the gases in the water is calculated using Henry's Law. The concentration of the gas in the liquid is proportional to the partial pressure of the gas above the liquid.

3. Scope:

3.1 This method is for the analysis of groundwater samples for monitoring natural attenuation.

3.2 Target Compounds

Compound	Formula	Molecular Weight
Methane	CH_4	16
Ethane	C_2H_6	30
Ethene	C_2H_4	28

3.3 According to the Technical Protocol for Evaluating Natural Attenuation of Chlorinated Solvents in Ground Water[12.2], the laboratory needs to attain the following quantitation limits (QL).

Compound	Concentration (mg/L or ppm)
Methane	0.5
Ethane	0.1
Ethene	0.01

3.4 This method is amenable for the analysis of propane, propene, n-butane, and iso-butane if the quality control criteria are met.

3.5 If other gas analyses need to be included, e.g. carbon dioxide, it is recommended that separate vials are collected for the carbon dioxide analysis. The carbon dioxide samples must not be preserved, while samples for the methane, ethane and ethene are preserved by acidification.

4. Equipment and Reagents:

4.1 Gas Chromatograph (GC) equipped with a flame ionization detector (FID).

4.2 Column: Packed or capillary. Complete separation or baseline resolution must be achieved for all the target compounds. Typical columns include: Porapak Q, HayeSep, RT-UPLOT, or equivalent.

4.3 Apparatus needed for large volume injections: headspace analyzer, gas loop injector (>300 ul), or gas tight syringes.

4.4 Data System to calculate the concentration from area counts.

4.5 Collection Vials: VOA or serum vials, 40 - 125 mL, with a butyl rubber-Teflon faced septum, or equivalent. The Teflon-faced silicone septum are permeable to light hydrocarbon gases.[12.6]

4.6 Syringes: Gas tight or plastic in various sizes up to 60 mL with syringe valves.

4.7 Standards: Stock cylinder, NIST traceable gas standard, 1% or higher.

4.7.1 Gas Working Standards: Dilutions from the stock cylinder are prepared using syringes, a mass flow controller (MFC), or an absolute pressure gauge (Baratron). The absolute

pressure gauge or MFC is preferred over the syringe method.

4.8 Inert Gas: Helium or nitrogen, zero grade or better (less than 500 ppb THC).

4.9 Rotary or wrist shaker.

4.10 Containers for working standards: Tedlar® bags, Summa® or Silcosteel® canisters, or 1 - 2 L glass containers with a syringe valve.

5. Schedule:

5.1 A sampling batch consists of one or more Sample Delivery Groups (SDG). An SDG is:
- < Each Project Number of 20 field samples received, OR
- < Each 7 calendar day period during which the field samples are collected

5.2 Each SDG must include a set of quality control samples: field blank, field duplicates, laboratory reagent blank, matrix spike, and matrix spike duplicate.

5.4 Data deliverables must be submitted in one complete data package for a period set by the project manager, usually within 30 days of the sample receipt.

6. Interference:

6.1 Methane is a very common contaminant and occurs naturally in the atmosphere. Automobile exhaust contains high levels of the target compounds so care must be exercised to prevent contamination in transport. Care must be also exercised in the sample analyses.

6.2 Moisture interferes with the low level analyses. Procedures to minimize the injection of moisture into the GC is advisable. A calcium sulfate moisture trap or cryogenics may be used.

7. Sample Collection:

7.1 Add 2 drops of 1:1 hydrochloric acid (HCl) or sulfuric acid (H_2SO_4) to the vial and add the sample by gently pouring the sample down the side of the vial without agitation.[12.7] Cap quickly cap so there are no bubbles and prepare 2 - 3 vials. Prepare 3 - 4 vials for a field duplicate site and 4 - 5 vials for a matrix spike and matrix spike

duplicate site. Check with the laboratory if additional vials are needed.

Note: Before adding the HCl to the sample vial, collect a test sample and test with pH paper to assure 2 drops are sufficient to decrease the pH to less than 2. Discard this test sample.

7.2 Place in a Ziploc® bag or equivalent. Record the following information on a sample label: facility, sample identification number, sample type (groundwater or surface), sample date and time, preservative, collector's initials. Ship the samples on ice at 4 ± 2 ^{0}C to the laboratory.

7.3 Complete the Chain of Custody Form.

7.4 Field records should include: location map, sample and sampling identification, sampling method, appearance and odor, weather conditions, water level prior to purging, total well depth, purge volume, water level after purging, well condition, sample depth, other field measurements and relevant information.

8. Storage and Holding Times:

8.1 Refrigerate at 4 ± 2 ^{0}C.

8.2 Holding time is 14 days from sampling collection.

9. Analytical Guidance:

9.1 Standards: At least 4 working standards that are evenly spaced are suggested. The low standard must be at or below the required quantitation limit (see section 3.3) to the highest concentration bracketing the samples at that site, possibly up to 40,000 ppmv (4 %) for methane and 10,000 ppmv (1 %) for ethane and ethene. For example, if the head space is 6 mL and water phase 54 mL at 25 ^{0}C, the ethene standard must be below 26 ppmv to achieve the required quantitation level of 0.01 mg/L, recommend 10 ppmv. The methane standards may be prepared separately because of the large differences observed in the methane concentrations in the environmental samples. The critical point is to bracket the concentration of the contaminants found in the samples.

9.2 Initial Calibration (IC): Using at least 4 working standards, create a linear (first order) regression of concentration (ppmv) versus area counts with a forced fit through the

origin.

9.3 Continuing Calibration Check (CCC): Using a mid-point standard, check initial calibration every 4 hours or 25 samples, whichever is more frequent, and at the end of the sample batch.

9.4 The sample must not be exposed to the atmosphere during the analysis. Air tight syringes must be used in transferring the sample.

9.5 General Steps: A known volume of 10% or more of headspace must be generated using nitrogen or helium at a constant temperature. The temperature is recorded at the beginning, every 4 hours, and at the end of the analyses. The displaced water can be measured in a syringe or by weight. The headspace is not pressurized. The sample is shaken on a rotary shaker at 1400 rpm for at least 5 minutes to establish an equilibrium. An aliquot of at least 300 uL of the headspace is injected into a gas chromatograph using a packed column or cryrogenic trapping on capillary columns. Smaller volumes are acceptable for capillary columns as long as the project DQOs are met. The injection volumes must be constant for the samples and standards except for samples exceeding the highest standard that require a smaller injection (see section 9.6).

9.6 Dilutions: If less than 10% of the original sample headspace was used in the sample analyses, a smaller injection from the same headspace can be made. Otherwise, a new sample is prepared at an appropriate dilution. For example, if the head space is 6 mL for a 60 mL serum vial and the sample injection is 300 ∶L (5 % of the headspace), a 50 - 100∶L of the headspace can be done for a 1:6 - 1:3 dilution. Dilutions are made to keep the area count response in the upper half of the calibration curve.

9.7 Calculations:

Calculate the total concentration of the analyte in the original sample using equations outlined in section 9.7.2. Determine the analyte concentration (ppmv) in the sample's headspace from the area response of the analyte using the multi-point initial calibration (IC) described in section 9.2.

9.7.1 Blank subtraction is not allowed.

9.7.2 Equations for calculating the concentration of the analyte in the original sample::

Eq. 1: Density factor of analyte in headspace = $\frac{\text{MW (g/mole)} * 273\ (^0\text{K})}{22.4\ \text{(L/mole)} * \text{Temp}\ (^0\text{K})}$

MW = Molecular weight
Temp (^{0}K) = Method temperature used

Eq. 2: Concentration in headspace (mg/L) = $\frac{\text{Conc (ppmv)} * \text{Density factor} * \text{Vol}_h}{\text{Vol}_w * 1000}$

Vol_h = Volume of the headspace
Vol_w = Volume of the water phase

Eq. 3: Concentration in water (mg/L) = $\frac{\text{Conc (ppmv)} * 55.5 * \text{MW}}{\text{Henry's Constant} * 1000}$

1 L of water = 55.5 g-mole

Eq. 4: Final concentration in the original water is the sum of both phases.

Total Sample Concentration (mg/L) = Conc in headspace (#2) + Conc in water (#3)

9.8 Quantitation Limits (QL): The reporting limit of the sample (mg/L) is the calculation of the lowest gas standard used in the initial calibration.

10. Quality Control Requirements:

10.1 Initial Calibration (IC): The initial calibration must be performed and the acceptance criteria must be achieved before samples are analyzed. The acceptance criteria is a regression coefficient (r^2) greater than 0.995 and the lowest gas standard must have a signal/noise ratio greater than 5.

10.2 Continuing Calibration Check (CCC): The validity of the initial calibration is checked every 4 hours or 25 samples, whichever is more frequent, and at the end of the sample batch with the continuing calibration check. The acceptance criterion is less than 20% difference from the true value.

10.3 Laboratory Reagent Blank (LRB): An aliquot of reagent water or other blank matrix

that is handled exactly as a sample including exposure to all glassware, equipment, environmental conditions, and solvents that are used with other samples. The LRB is used to determine if the method analytes or interferences are present in the laboratory environment, the reagents, or the apparatus. The LRB criteria must be met before field samples are analyzed. A LRB is prepared and analyzed with each SDG and the acceptance criteria are:

methane < 0.1 mg/L
ethane < 0.02 mg/L
ethene < 0.01 mg/L

10.4 Field or Trip Blank (FB): An aliquot of reagent water or other blank matrix that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, storage, preservation, and all analytical procedures. The purpose of the FB is to determine if method analytes or other interferences are present in the field environment. A FB is prepared and analyzed with each SDG and the acceptance criteria are:

methane < 0.1 mg/L
ethane < 0.02 mg/L
ethene < 0.01 mg/L

10.5 Field Duplicates (FD1 and FD2): Two separate samples collected at the same time and location under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with the sample collection, preservation, and storage, as well as with laboratory procedures. The acceptance criteria for field duplicates is less than 30% RPD.

10.6 Matrix Spike (MS): Create the head space in a separate sample as outlined in section 9.5. Keeping the vial inverted, inject an aliquot (100 - 500 uL) of the highest standard or stock cylinder containing methane, ethane, and ethene, into the environmental sample. The MS is analyzed exactly like a field sample to determine if the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the MS corrected for background concentrations. The percent recovery will be based on the sample concentration (mg/L) as reported for the sample. The MS is prepared and analyzed with each SDG and the acceptance criteria recovery is between

80 - 120 %. The matrix spike calculations are attached to this document to calculate the theoretical headspace concentration (ppmv). See attachment C for the calculations.

$$\% \text{ Recovery} = \frac{\text{MS Concentration (mg/L) - Sample Concentration (mg/L)}}{\text{Theoretical Concentration (mg/L)}} * 100$$

Another approach is to calculate the percent recovery based on the headspace concentration, ppmv. These calculations are also given in attachment C.

10.7 Matrix Spike Duplicate (MSD): A laboratory duplicate of the MS and analyzed with the MS. Analysis of the MS and MSD represent a measure of the precision associated with the laboratory, field procedures, and matrix effects. The acceptance criteria are recoveries between 80 - 120 % and agreement between the two spikes within 25 % RPD.

$$\%RPD = \frac{\text{(MS Concentration (mg/L)- MSD Concentration (mg/L))}}{\text{((MS Concentration (mg/L) + MSD Concentration (mg/L)/2)}} * 100$$

10.8 Laboratory Fortified Blank (LFB): Identical to MS except an aliquot of reagent water or other blank matrix is used instead of the field sample. Optional, if a matrix spike and matrix spike duplicate were prepare and analyzed. This is for situations in which the laboratory does not have enough sample to do a matrix spike. The acceptance criteria recovery is between 80 - 120 %.

10.9 Secondary Standard: A standard from another vendor used to check the primary working standard. This is optional because it is difficult to obtain another source. The acceptance criteria is within 25 % agreement.

10.10 Method Detection Limit (MDL): Minimum concentration of a substance that can be reported with a 99% confidence that the concentration is greater than zero. A minimum of seven replicates of a low standard are analyzed and the MDL value is calculated, [12.4]. The MDL is preformed annually and the acceptance criteria are:

methane < 0.05 mg/L
ethane < 0.005 mg/L
ethene < 0.005 mg/L

10.11 Response Factors (RF): The response for a FID is the current resulting from the ions produced in the flame from the analyte. This is usually measured by area counts or peak heights. The response factor is the response divide by the concentration.

$$RF = \frac{\text{Working Standard's Area Counts}}{\text{Working Standard's Concentration (ppmv)}}$$

Since the FID response is directly proportional to the carbon number, a visual check on the peak heights, areas, or a comparison of the absolute response factors is recommended for gross errors. The ethane and ethene will have similar RFs which are twice the methane RF. The recommended criteria is C2's is within 25% agreement and methane is within 25% of half the average C2's RF.

10.12 Data must be flagged with a qualifier if a quality control sample fails the acceptance criteria. This must be explained in the narrative.

11. Data Deliverables:

11.1 The deliverables must be complete and accurate to support the reported data.

11.2 A narrative must be provided. The narrative must include a paragraph which describes the analytical method performed by the laboratory, a description of the QC performed with the acceptance criteria, equipment used, any deviations, problems encountered and their resolution. This narrative must also include a table of the laboratory sample numbers and corresponding field sample numbers, if they are different. Additionally, the vendor of the stock standard, concentration documentation, and a description of how the working calibration standards were prepared should be noted.

11.3 An example of the calculations for the methane, ethane, and ethene concentrations using Henry's Law Constant, Attachment B, must be included.

11.4 The data package must contain the following information:

- Chain of Custody Form
- A description of the laboratory's preparation of the working standards and spike solutions in the data deliverable narrative.
- Instrument Sequence Log
- Initial Calibration (area counts, response factors, retention times, date, time, and instrument ID) with the linear regression coefficient

- Continuing Calibration Check Standards (area counts, retention times, date, time, and instrument ID) with the percent differences
- Laboratory blank results and sample data results including the concentration in the water (mg/L) and the raw data in each SDG. The raw data must include area counts, retention times, and concentration (ppmv) with the chromatograms
- Matrix Spikes (contents, spiked amount, and concentration) with recoveries
- Matrix Spike Duplicate recovery results (contents, spiked amount, and concentration) and relative percent differences
- Copies of the laboratory bench notes, sample preparation logs, or logbook (condition of the sample when received).

12. **Analytical References:**

12.1 Reference Method: Analysis of Dissolved Methane, Ethane, and Ethylene in Ground Water by a Standard Gas Chromatographic Technique, Don Kampbell and Steve Vandegrift, J of Chrom, Vol 36, May 1998

12.2 Technical Protocol for Evaluating Natural Attenuation of Chlorinated Solvents in Ground Water, EPA/600/R-98/128, September, 1998

12.3 Rapid Analysis of Natural Attenuation Indicators by Gas Chromatography, Tim Slagle and Frank Allen, EPA, presentation at EPA meeting, Annapolis, MD, May, 2001

12.4 Method detection limit: 40 CFR, part 136, Appendix B

12.5 Henry's Law Constant: Perry, J.H., Chemical Engineer's Handbook

12.6 Per conversation with Steve Vandegrift, 09/28/01

12.7 Sample Preparation and Calculations for Dissolved Gas Analysis in Water Samples using a GC Headspace Equilibration Technique, EPA, Ada, OK, RSKSOP-175.

Acknowledgment:

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Attachment A:
Quality Control Table:

Parameter	**Frequency**	**Acceptance Criteria**	**Corrective Action**
Initial Calibration (IC)	Prior to the analysis of any field samples	Linear regression coefficient, $r^2 > 0.995$	Repeat, prepare new standards
Lowest Standard	Every IC	Signal/Noise > 5	Repeat, bake GC, prepare new standard
Continuous Calibration Check (CCC)	Every 4 hrs, 25 samples or less, whichever is more frequent, and at the end of each sample batch	Difference from the calculated value < 20% D	Repeat, recalibrate, prepare new standards
Laboratory Reagent Blank (LRB)	Every SDG	methane < 0.1 mg/L ethane < 0.02 mg/L ethene < 0.01 mg/L	Repeat with fresh water.
Field or Trip Blank (FB)	Every field sampling event	methane < 0.1 mg/L ethane < 0.02 mg/L ethene < 0.01 mg/L	Contact project manager
Field Duplicates (FD1 and FD2)	Every field sampling event	Agreement within 30 % RPD	Contact project manager
Matrix Spike (MS)	Every field sampling event or 10% of the samples; whichever is greater	Recoveries between 80 - 120 %	Repeat MS, check the calculations, dilute and inject the spike std into the GC
Matrix Spike Duplicate (MSD)	Every field sampling event or 10% of the samples; whichever is greater	Recoveries between 80 - 120 % Agreement within 25 % RPD	Repeat MSD, check the calculations, dilute and inject the spike std into the GC

Parameter	**Frequency**	**Acceptance Criteria**	**Corrective Action**
Laboratory Fortified Blank (LFB)	Optional, when insufficient sample volume for the MS	Recoveries between 80 - 120 %	Repeat MS, check the calculations, dilute and inject the spike std into the GC
Secondary Source	Optional	Agreement within 25 % of accepted value	Prepare new stds
Method Detection Limit	Annually	methane < 0.05 mg/L ethane < 0.005 mg/L ethene < 0.005 mg/L	Repeat, use a lower standard

Attachment B:

Henry's Law Constants

Temperature (^{0}C)	**Methane**	**Ethane**	**Ethene**
0	22,400	12,600	5,520
5	25,900	15,500	6,530
10	29,700	18,900	7,680
15	33,700	22,600	8,950
20	37,600	26,300	10,200
25	41,300	30,200	11,400
30	44,900	34,200	12,700
35	48,600	38,300	NA
40	52,000	42,300	NA

Attachment C:

Matrix Spike Calculations by Sample Concentration (mg/L):

1. Calculate the Amount of the Spike (S):

S(ug) = Density of Gas (ug/uL) * Volume of Gas (uL)

$$S(ug) = \left[\frac{MW * 273^{\circ} K}{22.4 * Temp^{\circ} K}\right] * \left[\frac{Std(ppmv) * Vol_{inj}(mL)}{1000}\right]$$

$$S(ug) = \frac{273\ ^{0}K * MW * Std\ (ppmv) * Vol_{inj}\ (mL)}{22{,}400 * Temp\ ^{0}K}$$

2. Calculate the Theoretical Matrix Spike Concentration in the Sample:

$$\text{MS Concentration (mg/L)} = \frac{S(ug)}{Vol_{w}(mL)}$$

Example: Using a method used in EPA, Region 4 by Frank Allen.

Vol_h = Headspace Volume = 30 mL
Vol_w = Water Phase Volume = 30 mL
Spike 400 ：L of 10,000 ppmv (stock) of methane at 25 ^{0}C
Analytical Temperature = 25 ^{0}C
Observed Sample Concentration = 50.7 ：g/L
Observed Matrix Concentration = 131.7 ：g/L

1. Calculate the Amount of the Spike (S):

$$S(ug) = \frac{273\ ^{0}K * 16 * 10{,}000ppmv * 0.4\ mL}{22{,}400 * 298\ ^{0}K}$$

S(ug) = 2.616 ：g

2. Calculate the Theoretical Matrix Spike Concentration in the Sample:

MS Concentration (mg/L) = 2.616 ：g

30 mL

MS Concentration (mg/L) = 0.0872 mg/L = 87.2 ：g/L

3. Calculate the Recovery for the Matrix Spike:

MS Recovery = 131.7 ：g/L - 50.7 ：g/L = 93%
87.2 ：g/L

Matrix Spike Calculations by Sample Headspace (ppmv):

1. Calculate the Amount of the Spike (S):

S(ug) = Density of Gas (ug/uL) * Volume of Gas (uL)

$$S(ug) = \left[\frac{MW * 273^{\circ} K}{22.4 * Temp^{\circ} K}\right] * \left[\frac{Std(ppmv) * Vol_{inj}(mL)}{1000}\right]$$

S(ug) = 273 * MW * Std (ppmv) * Vol Inj (mL)
22,400 * Temp K

2. Calculate the Theoretical Headspace Concentration of the Spike Compound in the Sample (C):

Total Concentration = Concentration in Headspace + Concentration in Water of Analyte (mg/L)

$$\frac{S(ug)}{Vol_w(mL)} = \left[\frac{C(ppmv) * Densityfactor * Vol_h(mL)}{Vol_w(mL) * 1000}\right] + \left[\frac{C(ppmv) * 55.5 * MW}{Henry'sConstant * 1000}\right]$$

Solve for C (ppmv).

Example: Using a method used in EPA, Region 4 by Frank Allen.

Vol_h = Headspace Volume = 30 mL

Vol_w = Water Phase Volume = 30 mL
Spike 400 ∶L of 10,000 ppmv (stock) of methane at 25 ^{0}C
Analytical Temperature = 25 ^{0}C
Observed Sample's Headspace Concentration = 75 ppmv.
Observed MS Headspace Concentration = 195 ppmv

1. Calculate the Amount of the Methane Spike (S):

$$S(ug) = \left[\frac{16*273K}{22.4*298K}\right] * \left[\frac{10{,}000\,ppmv*0.4mL}{1000}\right]$$

S(ug) = 2.62 ug of methane

2. Calculate the Theoretical Headspace Concentration (C):

$$\frac{2.62ug}{(30mL)} = \left[\frac{C(ppmv)*0.654*(30mL)}{(30mL)*1000}\right] + \left[\frac{C(ppmv)*55.5*16}{41{,}300*1000}\right]$$

Solve for C.

C(ppmv) = 129.2 ppmv in the headspace

3. Calculated Recovery:

$$\%\ Recovery = \frac{\text{MS Concentration (ppmv) - Sample Concentration (ppmv)}}{\text{Theretical Concentration (ppmv)}} * 100$$

$$\%\ Recovery = \frac{195\ ppmv - 75\ ppmv}{129.2\ ppmv} * 100 = 93\ \%\ MS\ Recovery$$