METHOD 8331

TETRAZENE BY REVERSE PHASE
HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

1.0   SCOPE AND APPLICATION

    1.1   This method is intended for the analysis of tetrazene, an explosive residue, in soil and water. This method is limited to use by analysts experienced in handling and analyzing explosive materials. The following compounds can be determined by this method:

Compound	CAS No[a]
Tetrazene	31330-63-9

    [a] Chemical Abstracts Service Registry number

    1.2   Tetrazene degrades rapidly in water and methanol at room temperature. Special care must be taken to refrigerate or cool all solutions throughout the analytical process.

    1.3   Tetrazene, in its dry form, is extremely explosive. Caution must be taken during preparation of standards.

    1.4   The estimated quantitation limit (EQL) of Method 8331 for determining the concentration of tetrazene is approximately 7 µg/L in water and approximately 1 mg/kg in soil.

    1.5   This method is restricted to use by or under the supervision of analysts experienced in the use of HPLC, skilled in the interpretation of chromatograms, and experienced in handling explosive materials. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0   SUMMARY OF METHOD

    2.1   A 10 mL water sample is filtered, eluted on a C-18 column using ion pairing reverse phase HPLC, and quantitated at 280 nm.

    2.2   2 g of soil are extracted with 55:45 v/v methanol-water and 1-decanesulfonic acid on a platform shaker, filtered, and eluted on a C-18 column using ion pairing reverse phase HPLC, and quantitated at 280 nm.

3.0   INTERFERENCES

    3.1   No interferences are known. Tetrazene elutes early, however, and if a computing integrator is used for peak quantification, the baseline setting may have to be set to exclude baseline aberrations. Baseline setting is particularly important at low concentrations of analyte.

4.0   APPARATUS AND MATERIALS

    4.1   HPLC system

        4.1.1   HPLC - Pump capable of achieving 4000 psi.

        4.1.2   100 µL loop injector.

        4.1.3   Variable or fixed wavelength detector capable of reading 280 nm.

        4.1.4   C-18 reverse phase HPLC column, 25 cm x 4.6 mm (5 µm) (Supelco LC-18, or equivalent).

        4.1.5   Digital integrator - HP 3390A (or equivalent)

        4.1.6   Strip chart recorder.

    4.2   Other apparatus

        4.2.1   Platform orbital shaker.

        4.2.2   Analytical balance - ± 0.0001 g.

        4.2.3   Desiccator.

    4.3   Materials

        4.3.1   Injection syringe - 500 µL.

        4.3.2   Filters - 0.5 µm Millex-SR and 0.5 µm Millex-HV, disposable, or equivalent.

        4.3.3   Pipets - volumetric, glass, Class A.

        4.3.4   Scintillation vials - 20 mL, glass.

        4.3.5   Syringes - 10 mL.

        4.3.6   Volumetric flasks, Class A - 100 mL, 200 mL.

        4.3.7   Erlenmeyer flasks with ground glass stoppers - 125 mL.

4.4   Preparation

    4.4.1   Prepare all materials as described in Chapter 4 for volatile organics.

5.0   REAGENTS

    5.1   HPLC grade chemicals shall be used in all tests. It is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lowering the accuracy of the determination.

    5.2   General

        5.2.1   Methanol, CH_3OH - HPLC grade.

        5.2.2   Organic-free reagent water - All references to water in this method refer to organic-free reagent water, as defined in Chapter One.

        5.2.3   1-Decanesulfonic acid, sodium salt, $C_{10}H_{21}SO_3Na$ - HPLC grade.

        5.2.4   Acetic acid (glacial), CH_3COOH - reagent grade.

    5.3   Standard Solutions

        5.3.1   Tetrazene - Standard Analytical Reference Material.

        5.3.2   Stock standard solution - Dry tetrazene to constant weight in a vacuum desiccator in the dark. (Tetrazene is extremely explosive in the dry state. Do not dry more reagent than is necessary to prepare stock solutions.) Place about 0.0010 g (weighed to 0.0001 g) into a 100-ml volumetric flask and dilute to volume with methanol. Invert flask several times until tetrazene is dissolved. Store in freezer at -10°C. Stock solution is about 100 mg/L. Replace stock standard solution every week.

        5.3.3   Intermediate standard solutions

            5.3.3.1   Prepare a 4 mg/L standard by diluting the stock solution 1/25 v/v with methanol.

            5.3.3.2   Pipet 0.5, 1.0, 2.0, 5.0, 10.0, and 20.0 mL of the 4 mg/L standard solution into 6 separate 100 mL volumetric flasks, and make up to volume with methanol. Pipet 25.0 mL of the 4 mg/L standard solution into a 50 mL volumetric flask, and make up to volume with methanol. This results in intermediate standards of about 0.02, 0.04, 0.08, 0.2, 0.4, 0.8, 2 and 4 mg/L.

            5.3.3.3   Cool immediately on preparation in refrigerator or ice bath.

5.3.4   Working standard solutions

        5.3.4.1   Inject 4 mL of each of the intermediate standard solutions into 6.0 mL of water. This results in concentrations of about 0.008, 0.016, 0.032, 0.08, 0.16, 0.3, 0.8 and 1.6 mg/L.

        5.3.4.2   Cool immediately on preparation in refrigerator or ice bath.

    5.5   QC spike concentrate solution

    5.5.1   Dry tetrazene to constant weight in a vacuum desiccator in the dark. (Tetrazene is extremely explosive in the dry state. Do not dry any more than necessary to prepare standards.) Place about 0.0011 g (weighed to 0.0001 g) into a 200-ml volumetric flask and dilute to volume with methanol. Invert flask several times until tetrazene is dissolved. Store in freezer at -10°C. QC spike concentrate solution is about 55 mg/L. Replace stock standard solution every week.

    5.5.2   Prepare spiking solutions, at concentrations appropriate to the concentration range of the samples being analyzed, by diluting the QC spike concentrate solution with methanol. Cool on preparation in refrigerator or ice bath.

    5.6   Eluent

    5.6.1   To make about 1 liter of eluent, add 2.44 g of 1-decanesulfonic acid, sodium salt to 400/600 v/v methanol/water, and add 2.0 mL of glacial acetic acid.

6.0   SAMPLE COLLECTION, PRESERVATION, AND HANDLING

    6.1   See the introductory material to this Chapter, Organic Analytes, Section 4.1.

    6.2   Samples must be collected and stored in glass containers. Follow conventional sampling procedures.

    6.3   Samples must be kept below 4°C from the time of collection through analysis.

7.0   PROCEDURE

    7.1   Sample Preparation

    7.1.1   Filtration of Water Samples

        7.1.1.1   Place a 10 mL portion of each water sample in a syringe and filter through a 0.5 μm Millex-HV filter unit. Discard first 5 mL of filtrate, and retain 5 mL for analysis.

7.1.2   Extraction and Filtration of Soil Samples

7.1.2.1   Determination of sample % dry weight - In certain cases, sample results are desired based on dry-weight basis. When such data is desired, a portion of sample for this determination should be weighed out at the same time as the portion used for analytical determination.

WARNING: The drying oven should be contained in a hood or vented. Significant laboratory contamination may result from a heavily contaminated hazardous waste sample.

7.1.2.1.1   Immediately after weighing the sample for extraction, weigh 5-10 g of the sample into a tared crucible. Determine the % dry weight of the sample by drying overnight at 105°C. Allow to cool in a desiccator before weighing:

$$\% \text{ dry weight} = \frac{\text{g of dry sample} \times 100}{\text{g of sample}}$$

7.1.2.2   Weigh 2 g soil subsamples into 125 mL Erlenmeyer flasks with ground glass stoppers.

7.1.2.3   Add 50 mL of 55/45 v/v methanol-water with 1-decanesulfonic acid, sodium salt added to make a 0.1 M solution.

7.1.2.4   Vortex for 15 seconds.

7.1.2.5   Shake for 5 hr at 2000 rpm on platform shaker.

7.1.2.6   Place a 10 mL portion of each soil sample extract in a syringe and filter through a 0.5 μm Millex-SR filter unit. Discard first 5 mL of filtrate, and retain 5 mL for analysis.

7.2   Sample Analysis

7.2.1   Analyze the samples using the chromatographic conditions given in Section 7.2.1.1. Under these conditions, the retention time of tetrazene is 2.8 min. A sample chromatogram, including other compounds likely to be present in samples containing tetrazene, is shown in Figure 1.

7.2.1.1   Chromatographic Conditions

Solvent: 0.01 M 1-decanesulfonic acid, in acidic
                   methanol/water (Section 5.5)
Flow rate: 1.5 mL/min
Injection volume: 100 μL
UV Detector: 280 nm

7.3   Calibration of HPLC

    7.3.1   Initial Calibration - Analyze the working standards (Section 5.3.4), starting with the 0.008 mg/L standards and ending with the 0.30 mg/L standard. If the percent relative standard deviation (%RSD) of the mean response factor (RF) for each analyte does not exceed 20%, the system is calibrated and the analysis of samples may proceed. If the %RSD for any analyte exceeds 20%, recheck the system and/or recalibrate with freshly prepared calibration solutions.

    7.3.2   Continuing Calibration - On a daily basis, inject 250 μL of stock standard into 20 mL water. Keep solution in refrigerator until analysis. Analyze in triplicate (by overfilling loop) at the beginning of the day, singly after each five samples, and singly after the last sample of the day. Compare response factors from the mean peak area or peak height obtained over the day with the response factor at initial calibration. If these values do not agree within 10%, recalibrate.

8.0   QUALITY CONTROL

    8.1   Refer to Chapter One for specific quality control procedures.

    8.2   Prior to preparation of stock solutions, methanol should be analyzed to determine possible interferences with the tetrazene peak. If the methanol shows contamination, a different batch of methanol should be used.

    8.3   Method Blanks

    8.3.1   Method blanks for the analysis of water samples should be organic-free reagent water carried through all sample storage and handling procedures.

    8.3.2   Method blanks for the analysis of soil samples should be uncontaminated soil carried through all sample storage, extraction, and handling procedures.

9.0   METHOD PERFORMANCE

    9.1   Method 8331 was tested in a laboratory over a period of four days. Spiked organic-free reagent water and standard soil were analyzed in duplicate each day for four days. The HPLC was calibrated daily according to the procedures given in Section 7.1. Method performance data are presented in Tables 1 and 2.

10.0   REFERENCES

1.   Walsh, M.E., and T.F. Jenkins, "Analytical Method for Determining Tetrazene in Water," U.S. Army Corps of Engineers, Cold Regions Research & Engineering Laboratory, Special Report 87-25, 1987.

2.   Walsh, M.E., and T.F. Jenkins, "Analytical Method for Determining Tetrazene in Soil," U.S. Army Corps of Engineers, Cold Regions Research & Engineering Laboratory, Special Report 88-15, 1988.

11.0 SAFETY

    11.1 Standard precautionary measures used for handling other organic compounds should be sufficient for safe handling of the analytes targeted by Method 8331.

FIGURE 1
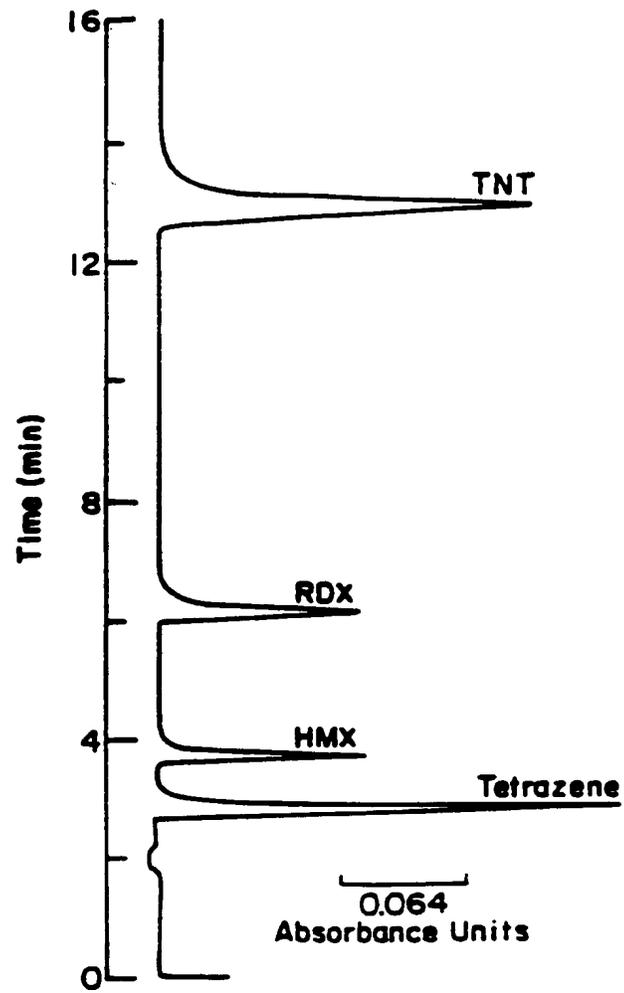
TABLE 1.
METHOD PERFORMANCE, WATER MATRIX

Spike Conc. (µg/L)	Replicate	Day 1	Day 2	Day 3	Average Day 4	% Recovery
				Avg % Recovery		
0.00	Replicate 1	0.0	0.0	0.0	0.0	
	% Recovery	NA	NA	NA	NA	NA
	Replicate 2	0.0	0.0	0.0	0.0	
	% Recovery	NA	NA	NA	NA	NA
7.25	Replicate 1	8.9	7.8	7.4	9.4	
	% Recovery	122	108	102	130	116
	Replicate 2	6.6	9.9	8.5	6.7	
	% Recovery	91	137	117	92	109
14.5	Replicate 1	14.6	14.6	13.8	14.6	
	% Recovery	101	101	95	101	99
	Replicate 2	14.8	14.1	14.1	15.2	
	% Recovery	102	97	98	105	100
29	Replicate 1	31.8	30.0	30.8	28.7	
	% Recovery	110	103	106	99	105
	Replicate 2	29.5	29.7	30.4	30.7	
	% Recovery	102	102	105	106	104
72.5	Replicate 1	71.1	73.6	75.7	73.9	
	% Recovery	98	102	104	102	101
	Replicate 2	71.2	71.3	70.7	71.6	
	% Recovery	98	98	98	99	98
145	Replicate 1	140.6	143.8	144.7	142.1	
	% Recovery	97	99	100	98	98
	Replicate 2	138.5	140.8	140.9	136.9	
	% Recovery	96	97	97	94	96
290	Replicate 1	289.4	288.5	291.0	289.8	
	% Recovery	100	99	100	100	100
	Replicate 2	282.0	284.2	281.9	282.5	
	% Recovery	97	98	97	97	97
725	Replicate 1	737.6	707.2	714.3	722.0	
	% Recovery	102	98	99	100	99
	Replicate 2	700.2	695.8	714.2	716.3	
	% Recovery	97	96	99	99	97
OVERALL						102

TABLE 2
METHOD PERFORMANCE, SOIL MATRIX

| Spike Conc. (µg/L) | Replicate | Avg % Recovery | | | | |
		Day 1	Day 2	Day 3	Average Day 4	% Recovery
0.00	Replicate 1	0.0	0.0	0.0	0.0	
	% Recovery	NA	NA	NA	NA	NA
	Replicate 2	0.0	0.0	0.0	0.0	
	% Recovery	NA	NA	NA	NA	NA
1.28	Replicate 1	0.6	0.9	0.6	1.0	
	% Recovery	49	73	48	74	61
	Replicate 2	1.2	0.7	0.8	0.7	
	% Recovery	92	56	63	56	67
2.56	Replicate 1	1.4	1.5	1.6	1.6	
	% Recovery	56	58	61	61	59
	Replicate 2	1.5	2.0	1.4	1.3	
	% Recovery	59	79	56	50	61
5.12	Replicate 1	2.9	3.0	2.9	2.9	
	% Recovery	57	58	56	56	57
	Replicate 2	3.0	3.0	3.5	3.1	
	% Recovery	58	59	69	60	61
12.8	Replicate 1	7.8	7.6	7.8	8.1	
	% Recovery	61	59	61	63	61
	Replicate 2	8.0	8.4	7.7	8.2	
	% Recovery	62	66	60	64	63
25.6	Replicate 1	17.2	16.7	17.4	17.3	
	% Recovery	67	65	68	68	67
	Replicate 2	16.7	16.8	17.6	17.2	
	% Recovery	65	66	69	67	67
OVERALL						62

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                    ┌─────────────┐
                    │    Start    │
                    └──────┬──────┘
                           │
                           ▼
          ┌──────────────────────────┐
          │   7.1.1 Filter 10 mL     │          ┌──────────────────────┐
          │   water sample; discard  │          │  7.2 Analyze samples │
          │   first 5 mL; analyze    │─────────▶│  using chromatographic│
          │   last 5.                │          │     conditions in    │
          └────────────┬─────────────┘          │    Section 7.2.1.1.  │
                       │                         └──────────┬───────────┘
                       ▼                                    │
          ┌──────────────────────────┐                     ▼
          │   7.1.2.1 Determine      │          ┌──────────────────────┐
          │   % dry weight.          │          │ 7.3.1 Initial        │
          └────────────┬─────────────┘          │ calibration: Analyze │◀───┐
                       │                         │   working standards  │    │
                       ▼                         │   (Section 5.3.3).   │    │
          ┌──────────────────────────┐          └──────────┬───────────┘    │
          │  7.1.2.2 - 7.1.2.5       │                     │                │
          │  Extract 2 g soil        │                     ▼                │
          │  with 50 mL solvent.     │                  ◇ 7.3.1 is % RSD ◇  │
          └────────────┬─────────────┘            of mean RF  >20%?   Yes   │
                       │                                                    │
                       ▼                                 │  No              │
          ┌──────────────────────────┐                  ▼                  │
          │  7.1.2.6 Filter 10 mL    │          ┌──────────────────────┐   │
          │  extract; discard 5 mL;  │          │   7.3.2 Continuing    │   │
          │  analyze last 5 mL.      │          │   Calibration.        │   │
          └──────────────────────────┘          └──────────┬───────────┘   │
                                                            │               │
                                                            ▼               │
                                                   ┌─────────────┐          │
                                                   │    Stop     │          │
                                                   └─────────────┘          │
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7.3.1 is % RSD of mean RF >20%? — Yes → **7.3.1 Recheck system/recalibrate with new calibration solution.**