DETERMINATION OF NTN 33893 IN WATER

1. Scope

   1.1 Applicable to water samples containing 0.1 ppb or higher concentrations of NTN 33893.

   1.2 Higher concentrations can be determined after diluting the sample extracts.

2. Principle

   2.1 Small amounts of NTN 33893 can be extracted from water with methylene chloride and determined using a high performance liquid chromatograph.

   2.2 Peak areas or peak heights of the sample and known standards are compared.

3. Reagents

   3.1 Acetonitrile, Burdick and Jackson "Distilled in Glass", or equivalent

   3.2 Methylene chloride, nanograde

   3.3 NTN 33893 analytical standard of known percentage purity (P)

   3.4 NTN 33893 standard solution, Weigh 0.01 ± 0.0001 g (Ws) of NTN 33893 analytical standard into a 100-mL volumetric flask. Dilute to volume with acetonitrile, stopper and mix thoroughly. This solution may be kept for one week only. Correct the amount weighed to obtain a 100% basis of NTN 33893 as follows:

$$\text{NTN 33893 (100\%)} = \frac{Ws \times P}{100}$$

   3.5 Sodium sulfate, anhydrous, ACS

   3.6 Water, HPLC grade.

4. Equipment

   4.1 Integration system, Hewlett-Packard LAS Model 3350 or equivalent.

   4.2 Liquid chromatograph, Shimadzu LC-6A or equivalent, equipped with a Zorbax ODS 4.6 mm × 25 cm column (DuPont P.N. 880952-702) ID # 715295 and a UV detector capable of measuring absorbances at 270 nm.

   4.3 Rotary vacuum evaporator, Buechi RE-11, or equivalent.

5. Procedure

5.1 Preparation of the standard solutions

1) Weigh 0.010 - 0.011 ± 0.0001 g NTN 33893 analytical standard (Book-ref. 88R11-19, purity 95.9%), into a 100-mL volumetric flask. Dilute to volume with acetonitrile and mix.

2) Pipet 1 mL of the standard solution from Step 1 into a 50-mL volumetric flask, dilute to volume with acetonitrile and mix. This is the 2-ppm standard solution.

3) Pipet 1 mL of the standard solution from Step 1 into a 100-mL volumetric flask. Dilute to volume with acetonitrile and mix. This is the 1-ppm standard solution.

4) Pipet 25 mL of the 1-ppm standard solution from Step 3 into a 50-mL volumetric flask. Dilute to volume with acetonitrile and mix. This is the 0.5-ppm standard solution.

5) Pipet 5 mL of the 1-ppm standard solution from Step 3 into a 50-mL volumetric flask. Dilute to volume with acetonitrile and mix. This is the 0.1-ppm standard solution.

6) Using glass syringes, filter the solutions from Step 2 through Step 5 with 0.45-μ Acrodisc filters into sample vials. These are the standard solutions for the analytical run.

5.2 Preparation of the samples

1) Mix the sample and measure 500-mL into a 1-L separatory funnel.

2) Extract three times by vigorously shaking for 1 minute each time with 75-mL portions of methylene chloride.

3) Drain the methylene chloride layer into a 500-mL boiling flask through a funnel containing 4 to 5 grams of anhydrous sodium sulfate.

4) Strip off the methylene chloride on a roto evaporator using a water bath at room temperature. Leave 1 to 2 mL of methylene chloride in the flask.

5) Transfer the methylene chloride from the boiling flask into a 1/2-oz. glass bottle using a disposable pipet.

6) Add 2 to 3 mL of methylene chloride to the boiling flask and swirl to rinse the inner wall of the flask, then transfer into the same bottle as in Step 5.

5.2 Preparation of the samples (continued)

7) Remove the methylene chloride from the glass vial using a stream of nitrogen gas.

8) Pipet 0.5 mL of acetonitrile into the glass bottle, then seal with a polyseal cap. Rotate to dissolve residue.

5 3 Spiking procedure for concurrent recoveries

1) Weigh 0.010 - 0.011 ± 0.0001 g of NTN 33893 analytical standard (Book-ref. 88R11-19, purity 95.9%), into a 100-mL volumetric flask. Dilute to volume with acetonitrile and mix.

2) Pipet 1 mL of standard solution from Step 1 into a 100 mL volumetric flask, dilute to volume with acetonitrile. Stopper and mix thoroughly.

3) Pipet 1 mL of standard solution from Step 2 into a 10 mL volumetric flask, dilute to volume with acetonitrile. Stopper and mix thoroughly.

4) Pipet 2.5 mL of spiking solution from Step 3 into a 500 mL volumetric flask, dilute to volume with HPLC water and mix to produce 0.5 ppb spike.

5) Concentrate and analyze the spiked samples with the method used for the samples.

5.4 Instrument

Set the following conditions on the instrument:

1.	Absorbance, AUFS	0.002
2.	Column Pressure, atm (approx)	220
3.	Column Temperature, °C	Ambient
4.	Flow, mL/min.	1.5
5.	Injection Volume, µL	20
6.	Lamp	UV
7.	Mobile phase acetonitrile/water	40:60
8.	Wavelength, nm	270

6. Calculation

6.1 Use least squares curve fitting to generate the "best" line which can be used to calculate the corresponding concentration for a given peak height or peak area.

6.2 Determine the concentration (Cppm) corresponding to each sample peak area from the standard curve.

6.3 Calculate the amount of NTN 33893 in the sample:
NTN 33893, µg/L = 1000xCppm X Dilution factor