UNITED STATES
DEPARTMENT OF THE INTERIOR
GEOLOGICAL SURVEY

ANALYSIS OF PICRIC ACID IN WATER BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

OPEN-FILE REPORT 79-207

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Conversion Factors

For those readers who may prefer metric rather than inch-pound units, the conversion factors for terms used in this report are listed below.

Inch-pound	Multiply by:	Metric:
gal (gallons)	3.785	L (liters)
in (inches)	25.40	mm (millimeters)
in^2 (square inches)	645.2×10^2	mm^2 (square millimeters)
lb/in^2 (pounds/square inch)	6.897	kPa (kilopascal)

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ABSTRACT

Methods for the determination of picric acid (2,4,6, trinitrophenol) in water employing high-performance liquid chromatography are presented. Ion-pair chromatography under an isocratic condition is performed on a reverse-phase column. Picric acid can be determined directly in water at a lower detection limit of 10 micrograms per liter. By use of an extraction procedure, detection down to 0.1 microgram per liter can be realized.

INTRODUCTION

Picrates, derivatives of TNP 2,4,6-trinitrophenol, are used almost exclusively by the military as a secondary high explosive (Cook, 1971, p. 4). More commonly referred to as picric acid, TNP is suspected to be an environmental pollutant at sites of military-explosive manufacture, material fabrication, and munition demilitarization and disposal. TNP, a yellow crystalline substance that melts at 122°C, is one of the strongest of organic acids (pK_a 0.80), and has a solubility of 1.4 g in 100 g water at 25°C (Fieser and Fieser, 1961, p. 683-685).

A literature search revealed little information regarding the analysis of TNP in water and soils or sediment. A total of six methods was found, four using thin-layer chromatography, one using polarography, and one using HPLC (high-performance liquid chromatography). The HPLC method (Farey and Wilson, 1975) is well suited for the analysis of TNP in environmental samples. Midkiff (U. S. Bureau of Alcohol, Tobacco, and Firearms, written communication, 1977) suggested that TNP can be extracted from water using benzene because the compound is so readily soluble in this solvent. This is fortunate because the benzene extraction remains consistent with the method previously devised for the analysis of TNT and RDX explosives in water and soil-core material (Goerlitz and Law, 1975). Most important, however, HPLC permits direct analysis of an aqueous solution for TNP.

A new type of liquid chromatography, the so-called soap or ion-pair chromatography, has recently been introduced (Wittmer and others, 1975). This technique was adapted for use in reverse-phase HPLC for the determination of TNP in aqueous solutions and is the subject of this report.

METHOD

1. Summary of method

The explosive TNP or its salts, known as picrates, can be determined directly in water using this method. The sample is filtered and analyzed by HPLC using a reverse phase column and an ultraviolet detector. An extraction procedure is given for use if greater sensitivity is needed.

2. Application

This method is usable for the analysis of water or aqueous extracts of soil or sediment for TNP. The lower detection limit in water is 10 µg/L (micrograms per liter) of 10×10^{-9} g/mL (nanograms per milliliter). Use of a benzene extraction procedure permits detection down to 0.1 µg/L.

3. Interference

Any compounds having chemical and physical properties similar to TNP may cause interference. Interference is most likely during direct analysis of water or aqueous extracts. Compounds having nearly the same chromatographic capacity factor or retention volume and light-absorption chracteristics may interfere. TNP absorbs light radiation strongly in the 200-270 and 310-420 nm (nanometer) ranges, and most strongly at 365 nm. Owing to the very strong acidic property of TNP, any relatively alkaline surface will readily adsorb the compound, especially from benzene solution. Drying agents are usually alkaline and should be avoided. Glassware and boiling chips used in the solvent concentration procedure must be soaked in 6 M (molar) HCl (hydrochloric acid) prior to distilled-water rinse.

4. Apparatus

4.1 Centrifuge: Of bench-top type, accomodating 15-mL centrifuge tubes and having a variable speed to 3000 rpm (revolutions per minute).

4.2 Concentrating apparatus: A Kuderna-Danish concentrator, 500-mL capacity with a 1-ball Snyder column and both 4.00-mL graduated receiver tubes, and a 5.00-mL volumetric receiver flasks are used.

4.3 Chromatograph: A high-performance liquid chromatograph capable of operating to pressures of 4000 lb/in^2, having an injection port and a visible-ultraviolet detector capable of a wavelength setting of 365 nm. A Waters Associates[1/] ALC/GPC 204 liquid chromatograph equipped with the dual-channel detector, a model-6000A solvent-delivery system, and a model-660 solvent-flow programmer was used.

4.4 High-performance liquid chromatographic column: A stainless steel column 300-mm long with a 3.9-mm inside diameter, packed with reverse-phase material having a particle size of 10 µm (micrometer). A Waters Associates µBondapak C18, part number 27324, was used.

[1/]The use of brand names in this report is for identification purposes only and does not imply endorsement by the U. S. Geological Survey.

4.5 Erlenmeyer flasks: 250-mL plain-lip erlenmeyer flasks.

4.6 Filtration apparatus: All-glass filter apparatus for vacuum filtering, using 47-mm-diameter membrane filters with a funnel volume of 300 mL and a flask volume of 1 L. Millipore apparatus, part no. XX 15 047 00, was used. A Swinny-syringe filtration apparatus consisting of a 10-mL glass syringe and a 25-mm-diameter filter holder having standard Luer fittings. Millipore apparatus, part no. XX 30 025 00, was used.

4.7 Filter membrane: A membrane filter inert to acetonitrile, having a pore diameter of 0.45 μm or less, for vacuum filtration. Selas Flotronics silver-membrane filter, catalog no. FM-47.45, was used. For syringe filtration, Millipore part no. FGLP 025 00 was used.

4.8 Integrating equipment: A compensating polar planimeter readable to the nearest 0.1 mm^2 is acceptable. Other instruments or methods of integration demonstrating equal or greater accuracy can be used. A Spectra-Physics System 1, with calculation accessory, was used in this study.

4.9 Microbalance: A Cahn Gram Electrobalance or equivalent.

4.10 Microliter syringes: Microsyringes having capacities of 10 $\pm$ 0.1 and 100 $\pm$ 1.0 μL, with a needle size compatible with the liquid chromatograph injection port.

4.11 Recorder: A strip-chart recorder having a chart speed of 13 mm/min, a 1-sec pen speed, and a 10.0-mv (millivolt) span.

4.12 Steambath: Steambath or thermostated, fluidized sandbath. A Tecam fluidized sandbath at 100°C was used.

4.13 Separatory funnels: Squibb form, 1- or 2-L capacity. No lubricant is used on the stopcocks.

4.14 Volumetric glassware: Class A volumetric flasks in 5-, 10-, and 25-mL sizes, and graduated 15-mL centrifuge tubes having glass stoppers.

5. Reagents

5.1 Acetonitrile, of quality suitable for high-pressure liquid chromatography.

5.2 Benzene, pesticide-analysis quality, distilled in glass.

5.3 Hexane, pesticide-analysis quality, distilled in glass.

5.4 HCl (hydrochloric acid), ACS reagent grade.

5.5 Na_2HPO_4 (Sodium phosphate), dibasic, anhydrous, ACS reagent grade.

5.6 NaOH (Sodium hydroxide), ACS reagent grade.

5.7 Tetrabutylammonium phosphate solution: Ion pairing solution. Waters Associates PIC reagent A, part no. 85101, was used.

5.8 TNP (2,4,6-Trinitrophenol): Reference or analytical grade can be obtained from chemical specialty suppliers or from military sources. TNP as supplied usually contains 10-15 percent water and its purity must be determined by titration.

5.9 Water reagent, distilled, obtained from a high-purity tin-lined still. The feed water is passed through an activated carbon filter. The distillate is collected in a tin-silver storage tank, and the stored water is irradiated by ultraviolet light. A gravity delivery system is used and no plastic material other than Teflon is in contact with the distilled water. For HPLC, the water is filtered through a 0.45-μm silver-membrane filter.

6. Procedure

6.1 Calibration: Each chromatographic system must be calibrated using a TNP reference standard at a concentration ten times greater than the lower detection limit, under operating conditions to be used for analysis. A 100-μL injection of solution containing 100 μg/L TNP should give a response approximately three-quarters of full scale on the strip-chart recorder, at a solvent flow rate of 1.5 mL/min. TNP elutes at 220 sec, which is equivalent to a capacity factor of 1.3 for the system.

The elution solvent or mobile phase is prepared on each day of use by mixing 500 mL of acetonitrile, 500 mL of reagent water and a vial of ion pairing solution. The resulting 0.005 M tetrabutylammonium phosphate solution, buffered at pH 7.5, is filtered through a 0.45 μm membrane filter before use.

6.2 Sampling: Samples are collected according to recommended practice for organic analysis (see, for example, Goerlitz and Brown, 1972, p. 2-3) to ensure their quality. Special care is required to avoid contamination; see Section 3 of this report, Interference. The sample is cooled in ice, or is refrigerated, immediately after collection. Shipment is made by the most expeditious means to avoid delays in transit to the laboratory. No preservative is used, and the sample is analyzed without delay.

6.3 Direct aqueous prcoedure: Adjust the pH of the sample to pH 7 $\pm$ 0.5 using 0.1 M HCl or 0.1 M NaOH as needed.

6.3.1 Centrifuge 15 mL of sample at 2000 rev/min for 5 min.

6.3.2 Prepare the Swinny-syringe filtration unit and clean by filtering 2 mL of acetonitrile and then 5 mL of reagent water.

6.3.3 Filter the supernatant, discarding the first 2 mL to aid in rinsing. Collect 5.0 mL of filtrate in a clean graduated centrifuge tube.

6.3.4 Add 0.10 mL of ion pairing reagent to the sample and mix thoroughly.

6.3.5 Analyze the sample by HPLC under conditions used for calibration. Note: the comparison standard is prepared as above, adding the 0.10 mL of reagent to have an equivalent volume.

6.4 Solvent extraction procedure: Determine the weight of the water sample to three significant figures and pour the water into a 1- or 2-L separatory funnel.

6.4.1 To each liter of water add 25 mL of concentrated HCl. Add more acid, if necessary, to bring the pH of the resulting solution to less than 1.

6.4.2 Add 50 mL of benzene to the sample in the separatory funnel, and stopper and shake vigorously for 1 full minute, venting the pressure frequently. Allow the contents to separate for at least 10 min and draw off the aqueous layer into the original container. Collect the benzene layer in a 250-mL erlenmeyer flask. Do not use drying agent.

6.4.3 Repeat the extraction of the water sample three more times in the same manner, using 50 mL of benzene each time. Combine the extracts in the 250-mL erlenmeyer flask.

6.4.4 Transfer the extract to a 500-mL Kuderna-Danish apparatus fitted with a 4.0-mL receiver tube. Add a small boiling chip to prevent bumping, and remove most the benzene by heating on a steambath or fluidized sandbath at 100-125°C. (CAUTION: the evaporation of benzene must be conducted in a well-ventilated fume hood.) When the ball in the Snyder column stops bouncing, remove the apparatus from the heat and allow it to cool. Further reduce the volume of solvent to 0.5-1.0 mL by directing a stream of nitrogen on the surface of the liquid, while gently warming with a heat lamp.

6.4.5 To a 5.0-mL volumetric receiver flask, add 0.2 M aqueous solution of phosphate buffer until the miniscus just enters the lower constriction of the flask. Transfer the extract concentrate to the volumetric receiver, using a dropping pipet. Stopper and shake for 1 min, mixing the contents thoroughly. Allow layers to separate for 10 min.

6.4.6 Using a dropping pipet, remove the benzene layer.

6.4.7 Add 1.0 mL hexane to the flask, shake for 1 min, and allow to separate. Remove the hexane layer and repeat this step again.

6.4.8 Add 0.1 mL of concentrated HCl and bring the contents of the receiver to volume with additional 0.2 M phosphate buffer solution.

6.4.9 Add 0.10 mL ion-pairing reagent and filter the aqueous solution through a cleaned membrane filter using the Swinny-syringe unit.

6.4.10 Analyze the sample by HPLC under conditions used for calibration.

7. Calculations

Each chromatographic system must be calibrated with standards. The response, or absorbance, of the detector is displayed as an analog tracing on the strip-chart recorder, and the area under the curve is proportional to the amount of material passing through. The time elapsed from the introductior of samples to the differential curve maximum is designated as the retention time for a particular component.

7.1 Qualitative analysis: Direct comparison of the retention time of the sample component to that of an authentic standard of TNP is the method used for qualitative identification.

7.2 Quantitative analysis: Measurement of the chromatogram peak area by use of a planimeter or by any other method of equal or greater accuracy is acceptable. If a planimeter is used, the average of at least two measurements is taken.

7.2.1 Calculation for direct aqueous procedure: The concentration of TNP in water can be calculated using the following equation:

$$\text{Concentration of TNP } (\mu g/L) = \frac{A}{m} \cdot \frac{1000}{Vi},$$

where

A = area of component response,

m = slope (calibration factor), unit area per μg, and

Vi = mL of sample injected.

7.2.2 Calculation for extraction procedure: The concentration of TNP in the water sample can be calculated using the following equation:

$$\text{Concentration of TNP } (\mu g/L) = \frac{A}{m} \cdot \frac{5}{Vi} \cdot \frac{1000}{VS},$$

where

A = area of component response,

m = slope (calibration factor), unit area per μg,

Vi = mL of solution injected, and

Vs = volume of sample, in mL (computed from sample mass).

8. Report

For the direct analysis procedure, the TNP found is reported as follows: At concentration less than 10 μg/L, report to one significant figure. At concentrations of 10 μg/L or greater, report to two significant figures. For the extraction procedure, report as follows: At concentration less than 1.0 μg/L, report to nearest tenth; at concentrations of 1.0 μg/L or greater, report to two significant figures.

9. Precision

Precision of the direct procedure at 100 μg/L is ± 5 percent. Tests performed using the extraction procedure on distilled water fortified with 10 μg/L TNP gave 75 ± 15 percent recovery.

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