DETERMINATION OF WATER SOLUBILITY OF TETRABROMOBISPHENOL A

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 439C-132

OPPTS 830.7860, Water Solubility (Generator Column Method)
and
OECD Guideline for the Testing of Chemicals, 105: Water Solubility

AUTHORS:

Jon A. MacGregor
Willard B. Nixon, Ph.D.

STUDY INITIATION DATE: March 11, 2002

STUDY COMPLETION DATE: August 26, 2002

Submitted to:

American Chemistry Council's
Brominated Flame Retardant Industry Panel
1300 Wilson Boulevard
Arlington, Virginia 22209

Wildlife International, Ltd.

8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8600

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: American Chemistry Council's Brominated Flame Retardant Industry Panel

TITLE: Determination of Water Solubility of Tetrabromobisphenol A

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 439C-132

STUDY COMPLETION: August 26, 2002

This study was conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Part 792, 17 August 1989 and OECD Principles of Good Laboratory Practice (OCDE/GD (92) 32, Environment Monograph No. 45).

STUDY DIRECTOR:

Jon A. MacGregor — DATE 8/26/02
Scientist

SPONSOR APPROVAL:

Sponsor — DATE

QUALITY ASSURANCE STATEMENT

This study was examined for compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Part 792, 17 August 1989 and OECD Principles of Good Laboratory Practice (OCDE/GD (92) 32, Environment Monograph No. 45). The dates of all inspections and audits and the dates that any findings were reported to the Study Director and Laboratory Management were as follows:

		DATE REPORTED TO:	
ACTIVITY:	DATE CONDUCTED:	STUDY DIRECTOR:	MANAGEMENT:
Matrix Fortification	March 14, 2002	March 14, 2002	March 18, 2002
Generator Column Preparation	March 19, 2002	March 19, 2002	March 26, 2002
Raw Data and Draft Report	July 24 & 29-31, 2002	July 31, 2002	August 1, 2002
Final Report	August 26, 2002	August 26, 2002	August 26, 2002

All inspections were study-based unless otherwise noted.

James H. Coleman
James H. Coleman
Quality Assurance Representative

8-26-02
DATE

REPORT APPROVAL

SPONSOR: American Chemistry Council's Brominated Flame Retardant Industry Panel

TITLE: Determination of Water Solubility of Tetrabromobisphenol A

WILDLIFE INTERNATIONAL, LTD. PROJECT NO.: 439C-132

This report was reviewed by the individuals involved in the conduct and management of the study, and was found to be an accurate reflection of the methods used, data collected and results of the study.

STUDY DIRECTOR:

Jon A. MacGregor — 8/26/02
Jon A. MacGregor
Scientist
DATE

MANAGEMENT:

Willard B. Nixon — 8/26/02
Willard B. Nixon, Ph.D.
Director of Chemistry
DATE

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SUMMARY

SPONSOR:	American Chemistry Council's Brominated Flame Retardant Industry Panel
SPONSOR'S REPRESENTATIVE:	Ms. Wendy Sherman
LOCATION OF STUDY, RAW DATA AND A COPY OF THE FINAL REPORT:	Wildlife International, Ltd. Easton, Maryland 21601

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER:	439C-132
TEST SUBSTANCE:	Tetrabromobisphenol A (TBBPA)
STUDY:	Determination of Water Solubility of Tetrabromobisphenol A
TEST DATES:	OECD Experimental Start – March 12, 2002 EPA Experimental Start – March 13, 2002 Experimental Termination – July 22, 2002

SUMMARY:	The water solubility of TBBPA was determined in pH 5.0, pH 7.0, pH 9.0 buffer solutions, and in non-buffered NANOpure® reagent water at a test temperature of 25.0 ± 0.1°C using the generator column method. The water solubility in pH 5.0 buffer was determined to be 0.148 mg TBBPA/L. The water solubility in pH 7.0 buffer was determined to be 1.26 mg TBBPA/L. The water solubility in pH 9.0 buffer was determined to be 2.34 mg TBBPA/L. The water solubility in non-buffered reagent water was determined to be 0.240 mg TBBPA/L.

INTRODUCTION

This study was conducted by Wildlife International, Ltd. for American Chemistry Council's Brominated Flame Retardant Industry Panel at the Wildlife International, Ltd. analytical chemistry facility in Easton, Maryland. Tests were performed using the generator column method. Aqueous solute samples were collected from separate generator column assemblies at three different buffered pHs, and in non-buffered reagent water, each at two different flow rates, and analyzed using high performance liquid chromatography with ultraviolet absorption detection (HPLC/UV). Generator columns for the pH 5.0, 7.0, and 9.0 buffer trials were prepared on March 13, 19, and 25, 2002, respectively. A generator column for the non-buffered reagent water determination was prepared on July 18, 2002. Aqueous solute samples were collected and analyzed between March 14 and July 19, 2002. Raw data generated by Wildlife International, Ltd. and a copy of the final report are filed under Project Number 439C-132 in archives located on the Wildlife International, Ltd. site.

OBJECTIVE

The objective of this study was to determine the water solubility of tetrabromobisphenol A (TBBPA) at 25 °C using the generator column method with water buffered at pH 5.0, 7.0 and 9.0 and non-buffered reagent water.

EXPERIMENTAL DESIGN

Separate generator columns were prepared for each determination during the definitive test. Each column was packed with Chromosorb W HP support material coated with the test substance. The column temperature was maintained at 25.0 ± 0.1 °C. The definitive generator column test consisted of generating aqueous solutions of the test substance at pH 5.0, 7.0, 9.0, and in non-buffered NANOpure® reagent water by pumping water through a generator column packed with solid support material coated with the test substance. The concentration of test substance in the saturated aqueous solutions eluted from the columns represented the water solubility at the test temperature for each determination.

MATERIALS AND METHODS

This study was conducted according to procedures outlined in the protocol, "Determination of Water Solubility of Tetrabromobisphenol A" (Appendix I). The protocol was based on procedures outlined in the U.S. EPA Product Properties Test Guidelines, OPPTS 830.7860 and the OECD

Guideline for the Testing of Chemicals, 105: Water Solubility. The generator column method was used to determine the water solubility of the test substance.

Test Substance

The test substance consisted of a composite of tetrabromobisphenol A (TBBPA) samples received from three manufacturers. The material's identity and date received from each of the manufacturers is given below:

Manufacturer	Lot/Batch	Date Received	Wildlife International Ltd. ID No.
Great Lakes Chemical Corp.	1008JE04B	August 16, 2001	5722
Albemarle Corp.	25115T-1	August 16, 2001	5721
Bromine Compounds Ltd.	010040	August 31, 2001	5733

An equal part (2000 g) of each of the manufacturer's TBBPA material was placed in a container and mixed on a shaker table for thirty minutes. The composite test substance was assigned the Wildlife International, Ltd. identification number 5754. Subsamples of the composite test substance were shipped to Albemarle Corporation on September 26, 2001 for characterization and homogeneity analyses (Appendix II). The analyses were performed on October12 and 25, 2001. The results of the analyses indicated the composite test substance was homogeneous and contained the following components:

Tetrabromobisphenol-A	99.17%
o,p'- Tetrabromobisphenol-A	0.05%
2,4,6-Tribromophenol	None Detected
Tribromobisphenol-A	0.79%

The composite test substance was stored under ambient conditions.

Reagent Water

The reagent water used in this study met the specifications for ASTM Type II water. The water was obtained from a well located on the Wildlife International, Ltd. site. The well water was pumped through a series of filters to remove microorganisms and particles greater than 0.45 µm. The water was further purified using a Culligan® Hi-Flo 1 Water Softener, a Culligan® S-Series Reverse Osmosis System, and a Barnstead NANOpure® ultrapure water system. The resistivity of the purified reagent water used for this study was at least 18.0 megohm-cm. The reagent water was used in the preparation of the pH 5.0, 7.0 and 9.0 aqueous buffer solutions and in the non-buffered determination.

Solvents

Acetone, Burdick and Jackson, was used to prepare a stock solution of the test substance to be used in coating the generator column support material with the test substance. Methanol (MeOH), Burdick and Jackson, was used to prepare stock solutions of the test substance for matrix fortifications (efficiency samples) and for the preparation of calibration standards. Acetonitrile (ACN), Burdick and Jackson, was used in the generator column aqueous solute sample processing/dilution procedure. All solvents used for this study were either suitable for HPLC and residue analysis or were certified reagents.

Preparation of Aqueous Buffer Solutions

pH 5.0- To 1000 mL of 0.1M potassium hydrogen phthalate added 452 mL of 0.1 M Sodium Hydroxide; adjusted to final volume of 2000 mL with reagent grade water. The final solution pH was verified to be 5.0 using a pH meter.

pH 7.0- To 1000 mL of 0.1 M potassium dihydrogen phosphate added 580 mL of 0.1 M Sodium Hydroxide; adjusted to final volume of 2000 mL with reagent grade water. The final solution pH was verified to be 7.0 using a pH meter.

pH 9.0- To 1000 mL of 0.1 M sodium borate (borax) added 276 mL of 0.1 M hydrochloric acid; adjusted to final volume of 2000 mL with reagent grade water. The final solution pH was verified to be 9.0 using a pH meter.

Preparation of Generator Column

The generator column was supplied by At-Mar Glass Co. (Kennett Square, PA). The glass column was ~20 cm long with an outside diameter (o.d.) of ~6 mm, and was joined to an enlarged

section with an o.d. of ~9 mm. The entire column was enclosed in a water jacket for temperature control. A diagram of the generator column is presented in Figure 1.

The inert support material used in the generator column was Chromosorb W HP (100 - 120 mesh), and was purchased from Supelco Inc.. A small plug of silanized glass wool was placed in the column just above the enlarged section. Next, an appropriate amount (~2-3 grams) of the support material was poured into a round bottom flask and was fortified with 20 mL of a ~1% (10.0 mg a.i./mL) acetone stock solution of the test substance. The acetone carrier solvent was removed by rotary evaporation at 35-40 °C. The coated support material was then poured into the generator column and another plug of glass wool was added to the top of the column to retain the packing material in the column. Gentle tapping and vibration were used to facilitate packing of the column. The column was connected to a constant temperature water bath and circulatory system (set to maintain a temperature of 25.0 ± 0.1 °C) and was allowed to thermally equilibrate for at least 2 hours.

The column was then back-flushed with the appropriate aqueous solution by applying gentle suction at the top of the column to remove any entrapped air. End fittings were attached to the column after it had been flushed. The column assembly was allowed to equilibrate overnight prior to collection of samples.

Apparatus Configuration

A Neslab Model IC-515 Constant Temperature Water Bath was used to maintain the test temperature (25.0 ± 0.1 °C) throughout the experiment. The constant temperature bath was filled with water. The temperature of the water bath was monitored using a N.I.S.T Traceable Digital Thermometer.

A submersible pump was placed in the constant temperature bath, and was used to pump a continuous stream of water through the jacket surrounding the generator column in order to maintain a constant temperature.

A 2-L Erlenmeyer flask was used as a reservoir for the appropriate aqueous buffered and un-buffered reagent water that was being pumped through the generator column. The flask was submerged in the constant temperature bath. The inlet line of a Waters Model 510B solvent delivery

system was placed in the 2-L flask, and the top of the flask covered with aluminum foil. The pump was used to control the flow rate of the aqueous solution through the generator column.

A diagram of the apparatus configuration is presented in Figure 2.

Aqueous Solute Sample Collection

Prior to the collection of aqueous solute samples from the generator column, the pump was set to deliver approximately 0.5 mL of aqueous buffered or non-buffered reagent water per minute overnight to equilibrate the system. Following the overnight equilibration period, the pump was set to deliver approximately 1.0 mL of aqueous solute per minute (first flow rate) through the generator column and allowed to equilibrate for at least 30 minutes. The generator column eluate was collected dropwise into 10-mL class A volumetric flasks containing 5.00 mL of the acetonitrile solvent. The aqueous solute was collected manually to the volume mark in each of the volumetric flasks. The volume of aqueous sample collected was 5.00 mL. Samples were collected at the 1.0 mL/minute flow rate until an equilibrium concentration was reached, as defined by five consecutive individual aqueous solute sample concentrations within 30% of each other. After fulfilling this criterion, a second trial was performed at approximately half the flow rate, 0.5 mL/minute. Aqueous eluate samples were collected until five consecutive individual aqueous eluate sample concentrations were within 30% of each other, and within 30% of the mean saturation concentration obtained with the first flow rate trial.

Analytical Method

The analytical method involved a direct injection HPLC/UV analysis. Five milliliter volumes of acetonitrile (measured using a 5-mL class A volumetric pipet) were added to each 10-mL class A volumetric flask prior to sample collection. After collection, each flask was mixed well. For the pH 9.0 determination, the pH of the aqueous generator column samples was lowered to 7-8 using 1 drop of a 50% phosphoric acid solution/10 mL of solution. An aliquot of each of the final solutions was transferred to a labelled autosampler vial for analysis.

Concentrations of TBBPA in the samples were determined using a Hewlett-Packard Model 1090 High Performance Liquid Chromatograph equipped with a Hewlett-Packard 1100 variable wavelength detector (VWD) operated at 286nm. Chromatographic separations were achieved using a

YMC-Pack ODS-AM C-18 column (150 X 4.6 mm, 3 µm particle size). The instrument parameters are summarized in Table 1. An analytical method outline is provided in Figure 3.

Preparation of Quality Control Samples (Efficiency Samples)

Fortification stock solutions of TBBPA were prepared in methanol (MeOH) at concentrations of 10,000, 1000, 100, 10.0 and 1.00 mg TBBPA/L. One matrix blank and two matrix fortifications were prepared and analyzed along with the aqueous solute samples collected from the generator column for each solubility determination.

Calibration Curve and Quantitation

Calibration standards of TBBPA were prepared from the 100, 10.0 and 1.00 mg TBBPA/L stock solutions in acetonitrile: pH 5, 7, or 9 aqueous buffer solution (50:50, v/v) and in acetonitrile:NANOpure® water (50:50, v/v) for the non-buffered reagent water determination. For the analysis of the pH 5.0 aqueous solute samples, the calibration standards ranged from 0.0100 to 0.100 mg TBBPA/L. For the analysis of the pH 7.0 eluate samples, the calibration standards ranged from 0.100 to 1.00 mg TBBPA/L. For the analysis of the pH 9.0 aqueous solute samples, the calibration standards ranged from 0.500 to 5.00 mg TBBPA/L (Note: the pH 9.0 standards were lowered to pH 7-8 using 1 drop of a 50% phosphoric acid solution/10 mL of standard solution). For the analysis of the non-buffered reagent water aqueous solute samples, the calibration standards ranged from 0.0500 to 0.500 mg TBBPA/L. A set of calibration standards was analyzed before and after the set of samples, and a standard was injected a minimum of every five samples during the analytical run. A calibration curve was constructed from the linear regression equation using the respective concentration versus peak area responses of the calibration standards (Figures 4-7). Representative chromatograms of low and high-level calibration standards are shown in Figures 8 and 9, respectively. The concentration of TBBPA in the samples was determined by substituting the peak area responses of the samples into the applicable linear regression equation generated from the calibration curve as follows:

TBBPA in Sample (mg a.i./L) = [Peak Area – (Y-Intercept)/Slope] X Dilution Factor

$$\% \text{ Recovery} = \frac{\text{Measured TBBPA Concentration (mg a.i./L)}}{\text{Nominal TBBPA Concentration (mg a.i./L)}}$$

The method limit of quantitation (LOQ) for the pH 5.0 determination was set at 0.0200 mg TBBPA/L, calculated as the product of the lowest calibration standard (0.0100 mg TBBPA/L) and the dilution factor of the matrix blank sample (2).

The method limit of quantitation (LOQ) for the pH 7.0 determination was set at 0.200 mg TBBPA/L, calculated as the product of the lowest calibration standard (0. 100 mg TBBPA/L) and the dilution factor of the matrix blank sample (2).

The method limit of quantitation (LOQ) for the pH 9.0 determination was set at 1.00 mg TBBPA/L, calculated as the product of the lowest calibration standard (0.500 mg TBBPA/L) and the dilution factor of the matrix blank sample (2).

The method limit of quantitation (LOQ) for the non-buffered reagent water determination was set at 0.100 mg TBBPA/L, calculated as the product of the lowest calibration standard (0.0500 mg TBBPA/L) and the dilution factor of the matrix blank sample (2).

RESULTS AND DISCUSSION

Quality Control Samples

No interferences were observed at or above the LOQ's for the four matrix blank samples prepared and analyzed. Chromatograms of the pH 5, 7, 9 and the non-buffered reagent water matrix blanks are shown in Figures 10, 14, 18, and 22, respectively.

Along with the pH 5.0 generator column aqueous solute samples, extraction efficiency/QC samples were prepared at 0.0250 and 0.250 mg TBBPA/L in the aqueous pH 5.0 buffered reagent water. The percent recoveries of the 0.0250 and 0.250 mg TBBPA/L matrix fortifications were 100 and 63.6% of nominal concentrations, respectively. The 0.250 mg TBBPA/L sample recovery was low and a function of the test substance's limit of water solubility at pH 5.0. Representative chromatograms of the matrix fortification samples are shown in Figures 11 and 12, respectively.

Along with the pH 7.0 generator column eluate samples, extraction efficiency/QC samples were prepared at 0.500 and 1.50 mg TBBPA/L in the pH 7.0 buffered reagent water. The percent recoveries of the 0.500 and 1.50 mg TBBPA/L matrix fortifications were 101 and 100% of nominal concentrations, respectively. The mean recovery was calculated as 101±0.71% of nominal concentration. Representative chromatograms of the matrix fortification samples are shown in Figures 15 and 16, respectively.

Along with the pH 9.0 generator column eluate samples, extraction efficiency/QC samples were prepared at 1.50 and 2.50 mg TBBPA/L in the pH 9.0 buffered reagent water. The percent recoveries of the 1.50 and 2.50 mg TBBPA/L matrix fortifications were 100 and 101% of nominal concentrations, respectively. The mean recovery was calculated as 101±0.71% of nominal concentration. Representative chromatograms of the matrix fortification samples are shown in Figures 19 and 20, respectively.

Along with the non-buffered reagent water generator column aqueous solute samples, extraction efficiency/QC samples were prepared at 0.150 and 0.250 mg TBBPA/L in the aqueous non-buffered reagent water. The percent recoveries of the 0.150 and 0.250 mg TBBPA/L matrix fortifications were 98.9 and 99.2% of nominal concentrations, respectively. The mean recovery was calculated as 99.1±0.21% of nominal concentration. Representative chromatograms of the matrix fortification samples are shown in Figures 23 and 24, respectively.

Column Elution

The temperature of the water bath remained constant at 25.0 ± 0.1 °C throughout the experiment (Table 2).

The nominal flow rate of reagent water through the generator column was measured prior to the start of sample collection for each flow rate trial. At the pump setting of 1.0 mL/min. the flow rate was measured at ~1.0 mL/min. At the pump setting of 0.5 mL/min. the flow rate was measured at ~0.5 mL/min.

The results from the analyses of pH 5 eluate samples from the generator column are presented in Table 3. A representative chromatogram is shown in Figure 13. The measured concentration of TBBPA in the eluate samples collected at the first flow rate of 1.0 mL/minute ranged from 0.145 to 0.156 mg TBBPA/L. The measured concentration of TBBPA in the eluate samples collected at the second flow rate of 0.5 mL/minute ranged from 0.139 to 0.154 mg TBBPA/L.

The results from the analyses of pH 7 aqueous eluate samples from the generator column are presented in Table 4. A representative chromatogram is shown in Figure 17. The measured concentration of TBBPA in the aqueous eluate samples collected at the first flow rate of 1.0 mL/minute ranged from 1.26 to 1.28 mg TBBPA/L. The measured concentration of TBBPA in the eluate samples collected at the second flow rate of 0.5 mL/minute ranged from 1.25 to 1.26 mg TBBPA/L.

The results from the analyses of pH 9 eluate samples from the generator column are presented in Table 5. A representative chromatogram is shown in Figure 21. The measured concentration of TBBPA in the aqueous solute samples collected at the first flow rate of 1.0 mL/minute ranged from 2.33 to 2.49 mg TBBPA/L. The measured concentration of TBBPA in the aqueous solute samples collected at the second flow rate of 0.5 mL/minute ranged from 2.03 to 2.51 mg TBBPA/L.

The results from the analyses of non-buffered reagent water eluate samples from the generator column are presented in Table 6. A representative chromatogram is shown in Figure 25. The measured concentration of TBBPA in the aqueous solute samples collected at the first flow rate of 1.0 mL/minute ranged from 0.236 to 0.243 mg TBBPA/L. The measured concentration of TBBPA in the aqueous solute samples collected at the second flow rate of 0.5 mL/minute ranged from 0.240 to 0.242 mg TBBPA/L. The pH of the aqueous solute measured before the column was 6.71. The pH of the aqueous solute measured post generator column at the beginning and end of each flow rate determination was 6.83, 6.79, 7.23, and 7.12, respectively.

CONCLUSIONS

The mean aqueous TBBPA saturation concentrations for the pH 5.0 generator column samples collected at 1.0 and 0.5 mL/minute flow rates were determined to be 0.149 ± 0.0039 mg TBBPA/L and 0.146 ± 0.0057 mg TBBPA/L, respectively. The overall mean water solubility at pH 5.0 and 25.0 ± 0.1 °C was calculated to be 0.148 mg TBBPA/L

The mean aqueous TBBPA saturation concentrations for the pH 7.0 generator column samples collected at 1.0 and 0.5 mL/minute flow rates were determined to be 1.27 ± 0.0060 mg TBBPA/L and 1.26 ± 0.0055 mg TBBPA/L, respectively. The overall mean water solubility at pH 7.0 and 25.0 ± 0.1 °C was calculated to be 1.26 mg TBBPA/L

The mean aqueous TBBPA saturation concentrations for the pH 9.0 generator column samples collected at 1.0 and 0.5 mL/minute flow rates were determined to be 2.41 ● 0.0701 mg TBBPA/L and 2.27 ± 0.196 mg TBBPA/L, respectively. The overall mean water solubility at pH 9.0 and 25.0 ± 0.1 °C was calculated to be 2.34 mg TBBPA/L

The mean aqueous TBBPA saturation concentration for the non-buffered reagent water generator column samples collected at 1.0 and 0.5 mL/minute flow rates were determined to be 0.239 ± 0.0024 mg TBBPA/L and 0.241 ± 0.0008 mg TBBPA/L, respectively. The overall mean water solubility using non-buffered NANOpure® reagent water at 25.0 ± 0.1 °C was calculated to be 0.240 mg TBBPA/L.

REFERENCES

1. **U.S. Environmental Protection Agency.** 1996. Product Properties Test Guidelines, OPPTS 830.7560, Partition Coefficient (n-Octanol/Water), Generator Column Method. Washington, D.C.

Table 1

Typical HPLC/UV Operational Parameters

INSTRUMENT:	Hewlett Packard Model 1090 High Performance Liquid Chromatograph (HPLC) equipped with a Hewlett-Packard Model 1100 Variable Wavelength Detector (VWD).
ANALYTICAL COLUMN:	YMC-Pack ODS-AM C-18 Column (150 x 4.6 mm, 3 µm particle size).
STOP TIME:	6.00 minutes
MOBILE PHASE:	80% Acetonitrile:20% NANOpure water: 0.1% Phosphoric Acid
FLOW RATE:	1.00 mL/minute
INJECTION VOLUME:	200 µL and 250 µL (pH 5.0 trial)
OVEN TEMPERATURE:	40 °C
TETRABROMOBISPHENOL A (TBBPA) PEAK RETENTION TIME:	~4.4 minutes
PRIMARY ANALYTICAL WAVELENGTH:	286 nm

Table 2

Water Bath Test Temperature Ranges

Generator Column Fraction (GCF)	Water Bath Temperature Ranges (°C)
GCF(pH-5)-1 to 10	25.0-25.1
GCF(pH-7)-1 to 10	25.0-25.1
GCF(pH-9)-11 to 15 and 17 to 21	25.0-25.0
GCF(NANO)-1 to 10	24.9-24.9

Table 3

Results for pH 5.0 Aqueous Buffer Solute Samples Collected from the Generator Column

Sample ID (439C-132-)	Peak Area	Sample Volume (mL)	Final Volume (mL)	Flow Rate (mL/min.)	Measured Concentration (mg TBBPA/L)[1]	Mean/S.D. Measured Concentration (mg TBBPA/L)[1]
GCF(pH5)-1	10.54829	5.00	10.0	1.0	0.149	0.149/
GCF(pH5)-2	10.29006	5.00	10.0	1.0	0.145	0.00389
GCF(pH5)-3	10.51674	5.00	10.0	1.0	0.148	
GCF(pH5)-4	10.62568	5.00	10.0	1.0	0.150	
GCF(pH5)-5	11.05683	5.00	10.0	1.0	0.156	
GCF(pH5)-6	10.41462	5.00	10.0	0.5	0.147	0.146/
GCF(pH5)-7	10.95357	5.00	10.0	0.5	0.154	0.00568
GCF(pH5)-8	10.37665	5.00	10.0	0.5	0.146	
GCF(pH5)-9	10.11951	5.00	10.0	0.5	0.143	
GCF(pH5)-10	9.85194	5.00	10.0	0.5	0.139	
						Overall: Mean=0.148 S.D.=0.00499 C.V.=3.37%

[1] All calculations performed using EXCEL 2000 in the full precision mode. Manual calculations may differ slightly.

Table 4

Results for pH 7.0 Aqueous Buffer Solute Samples Collected from the Generator Column

Sample ID (439C-132-)	Peak Area	Sample Volume (mL)	Final Volume (mL)	Flow Rate (mL/min.)	Measured Concentration (mg TBBPA/L)[1]	Mean/S.D. Measured Concentration (mg TBBPA/L)[1]
GCF(pH7)-1	73.56540	5.00	10.0	1.0	1.26	1.27/
GCF(pH7)-2	73.99676	5.00	10.0	1.0	1.27	0.00598
GCF(pH7)-3	74.31220	5.00	10.0	1.0	1.28	
GCF(pH7)-4	74.37923	5.00	10.0	1.0	1.28	
GCF(pH7)-5	73.76289	5.00	10.0	1.0	1.27	
GCF(pH7)-6	73.35008	5.00	10.0	0.5	1.26	1.26/
GCF(pH7)-7	73.44366	5.00	10.0	0.5	1.26	0.00545
GCF(pH7)-8	73.00670	5.00	10.0	0.5	1.25	
GCF(pH7)-9	72.95992	5.00	10.0	0.5	1.25	
GCF(pH7)-10	72.65605	5.00	10.0	0.5	1.25	
						Overall: Mean=1.26 S.D.=0.0116 C.V.=0.921%

[1] All calculations performed using EXCEL 2000 in the full precision mode. Manual calculations may differ slightly.

Table 5

Results for pH 9.0 Aqueous Buffer Solute Samples Collected from the Generator Column

Sample ID (439C-132-)	Peak Area	Sample Volume (mL)	Final Volume (mL)	Flow Rate (mL/min.)	Measured Concentration (mg TBBPA/L)[1]	Mean/S.D. Measured Concentration (mg TBBPA/L)[1]
GCF(pH9)-11	146.29611	5.00	10.0	1.0	2.46	2.41/
GCF(pH9)-12	138.57297	5.00	10.0	1.0	2.33	0.0701
GCF(pH9)-13	139.52513	5.00	10.0	1.0	2.35	
GCF(pH9)-14	148.04065	5.00	10.0	1.0	2.49	
GCF(pH9)-15	142.90840	5.00	10.0	1.0	2.40	
GCF(pH9)-17	149.44016	5.00	10.0	0.5	2.51	2.27/
GCF(pH9)-18	143.96487	5.00	10.0	0.5	2.42	0.196
GCF(pH9)-19	130.43855	5.00	10.0	0.5	2.19	
GCF(pH9)-20	121.14758	5.00	10.0	0.5	2.03	
GCF(pH9)-21	129.74464	5.00	10.0	0.5	2.18	
						Overall: Mean=2.34 S.D.=0.156 C.V.=6.67%

[1] All calculations performed using EXCEL 2000 in the full precision mode. Manual calculations may differ slightly.

Table 6

Results for Non-buffered Reagent Water Aqueous Solute Samples Collected from the Generator Column

Sample ID (439C-132-)	Peak Area	Sample Volume (mL)	Final Volume (mL)	Flow Rate (mL/min.)	Measured Concentration (mg TBBPA/L)[1]	Mean/S.D. Measured Concentration (mg TBBPA/L)[1]
GCF(NANO)-1	14.00432	5.00	10.0	1.0	0.239	0.239/
GCF(NANO)-2	14.19995	5.00	10.0	1.0	0.243	0.00241
GCF(NANO)-3	14.01078	5.00	10.0	1.0	0.240	
GCF(NANO)-4	14.01954	5.00	10.0	1.0	0.240	
GCF(NANO)-5	13.80115	5.00	10.0	1.0	0.236	
GCF(NANO)-6	14.10666	5.00	10.0	0.5	0.241	0.241/
GCF(NANO)-7	14.10619	5.00	10.0	0.5	0.241	0.00076
GCF(NANO)-8	14.14311	5.00	10.0	0.5	0.242	
GCF(NANO)-9	14.07202	5.00	10.0	0.5	0.241	
GCF(NANO)-10	14.02428	5.00	10.0	0.5	0.240	
						Overall: Mean=0.240 S.D.=0.00189 C.V.=0.786%

[1] All calculations performed using EXCEL 2000 in the full precision mode. Manual calculations may differ slightly.

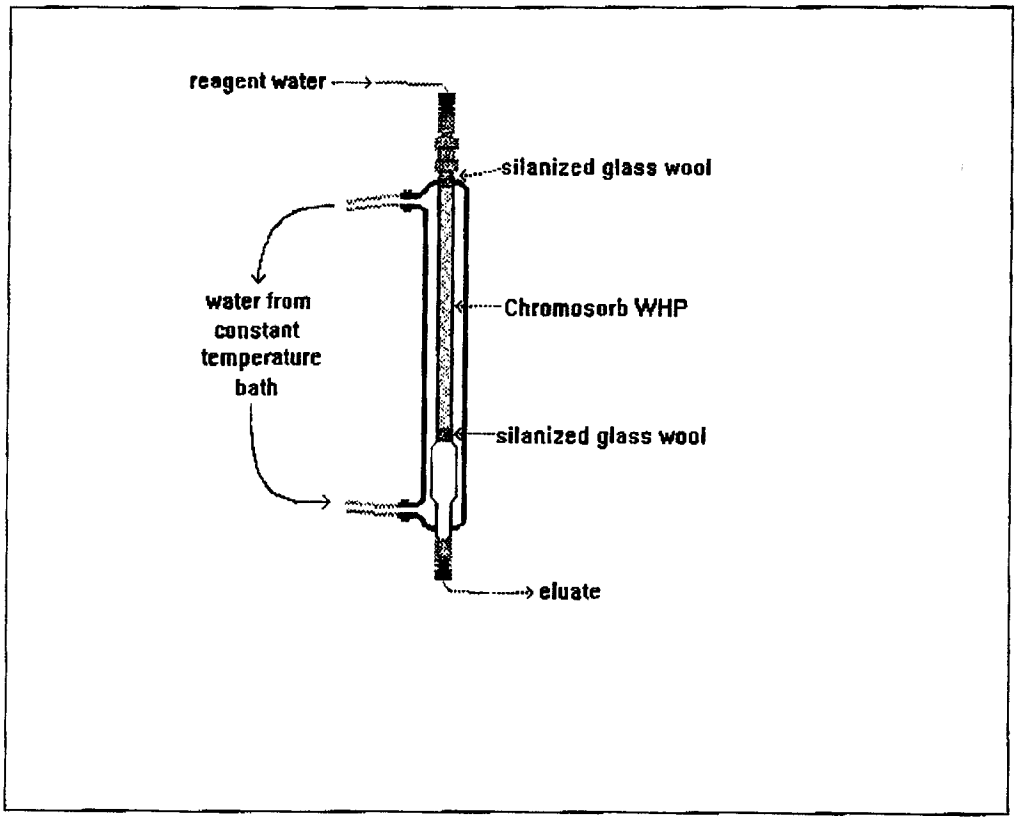

Figure 1. Diagram of generator column.

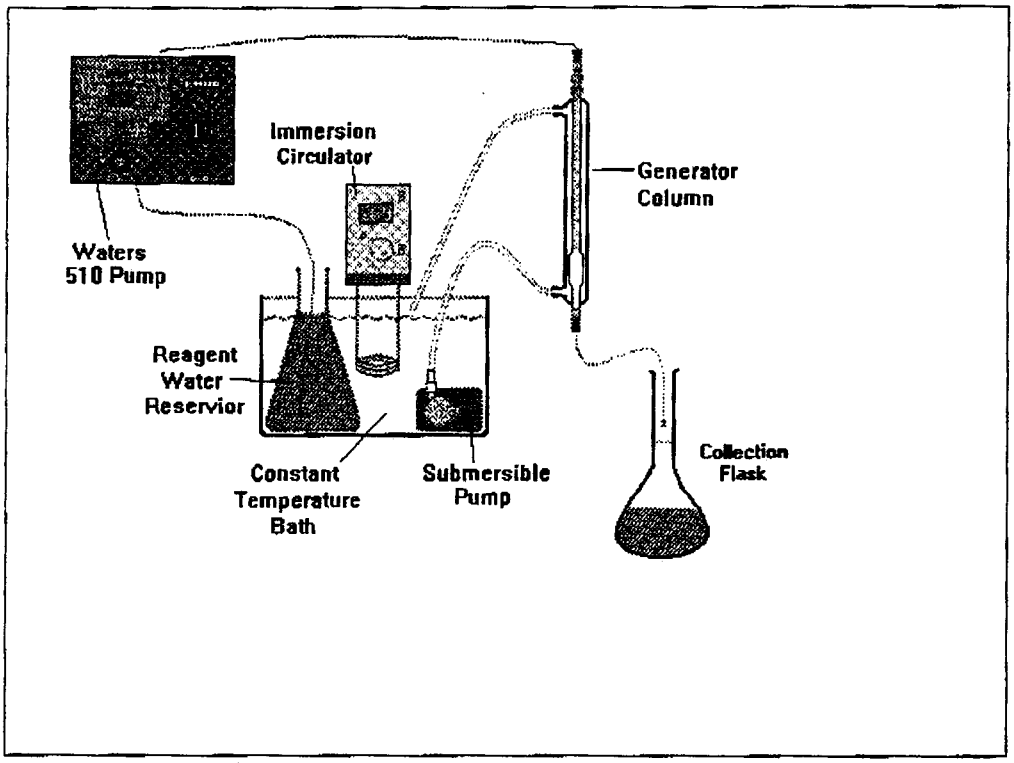

Figure 2. Diagram of apparatus configuration.

METHOD OUTLINE FOR THE DIRECT INJECTION ANALYSIS OF TETRABROMOBISPHENOL A (TBBPA) IN AQUEOUS SAMPLES FROM A WATER SOLUBILITY GENERATOR COLUMN STUDY (pH 5, 7 and 9, and non-buffered NANOpure® reagent water)

Prepare calibration standards in acetonitrile: pH 5, 7, 9 Buffer Solution or NANOpure® reagent water (50:50,v/v) using volumetric flasks and gas-tight syringes, STORE REFRIGERATED.

↓

For the preparation of concurrent matrix fortification samples (efficiency/QC samples), fortify appropriate aqueous solute solution matrix with stock solutions of TBBPA as necessary in class A volumetric flasks. Bring to volume with above matrix. Mix well. Volumetrically dilute solutions 1:1 with acetonitrile. Mix well. Dilute further, if necessary with acetonitrile : pH 5, 7 or 9 Buffer Solution or non-buffered NANOpure® reagent water (50:50,v/v) so that the final sample concentration falls within the calibration standard range. Mix well.

↓

For aqueous generator column definitive study samples, collect fractions of aqueous eluate from the generator column outlet directly into 10-mL class A volumetric flasks containing 5.00 mL (measured volumetrically) of acetonitrile solvent. Collect each fraction such that the final volume is 10.0 mL (Initial aqueous volume of 5.00 mL). Mix well. Volumetrically dilute solutions further, if necessary with acetonitrile : pH 5, 7 or 9 Buffer Solution or NANOpure® reagent water (50:50,v/v) so that the final sample concentration falls within the calibration standard range. Mix well.

↓

Transfer aliquots of final sample dilutions and appropriate calibration standards to autosampler vials for analysis by HPLC/UV.

Figure 3. Analytical method flow chart.

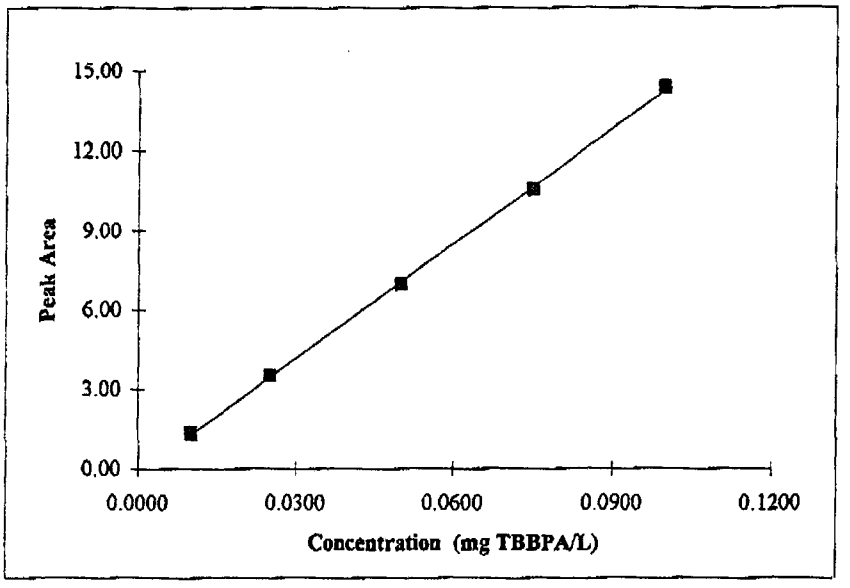

Figure 4. Representative calibration curve for pH 5.0 aqueous solute sample analyses (0.0100 to 0.100 mg TBBPA/L).
Intercept = -0.15757; Slope = 144.1109; $r^2 = 0.9997$.

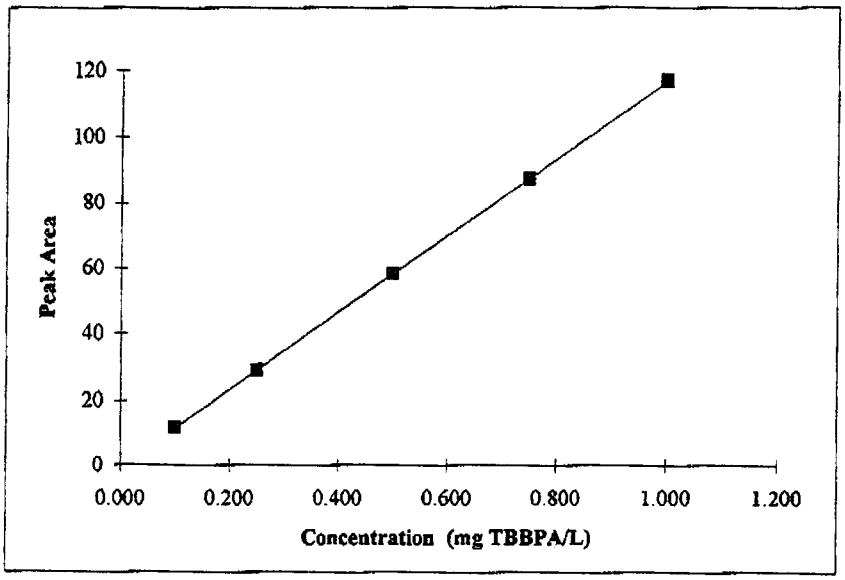

Figure 5. Representative calibration curve for pH 7.0 aqueous solute sample analyses (0.100 to 1.00 mg TBBPA/L).
Intercept = -0.16727; Slope = 116.6816; r^2 = 1.0000.

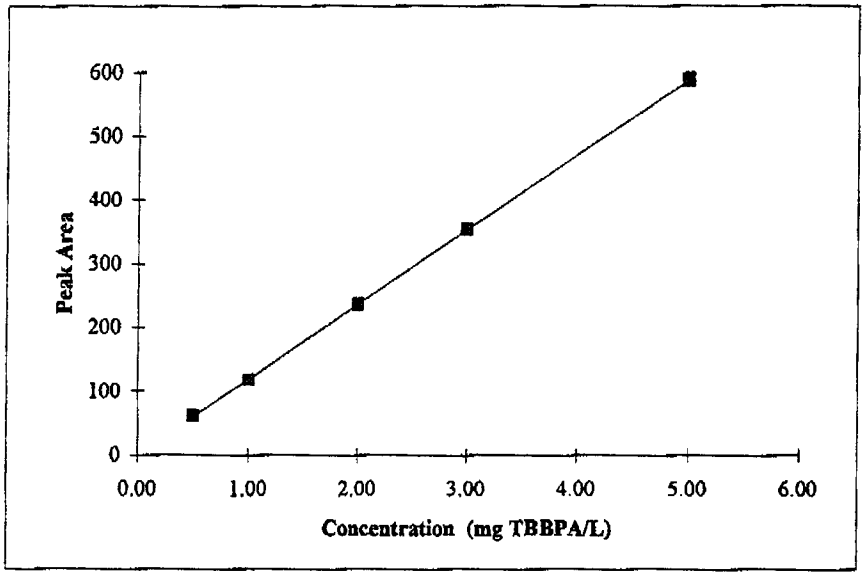

Figure 6. Representative calibration curve for pH 9.0 aqueous solute sample analyses (0.500 to 5.00 mg TBBPA/L).
Intercept = 1.66670; Slope = 117.5389; $r^2 = 1.0000$.

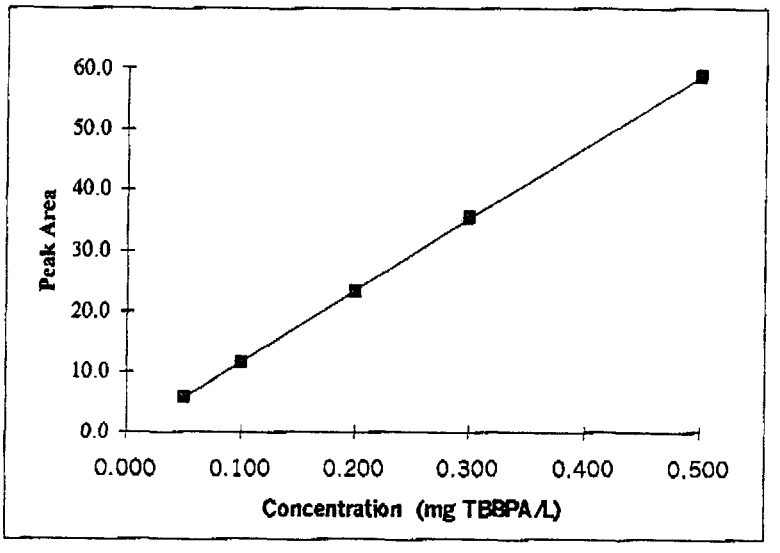

Figure 7. Representative calibration curve for non-buffered reagent water aqueous solute sample analyses(0.0500 to 0.500 mg TBBPA/L).
Intercept = -0.04566; Slope = 117.3616; r^2 = 1.0000.

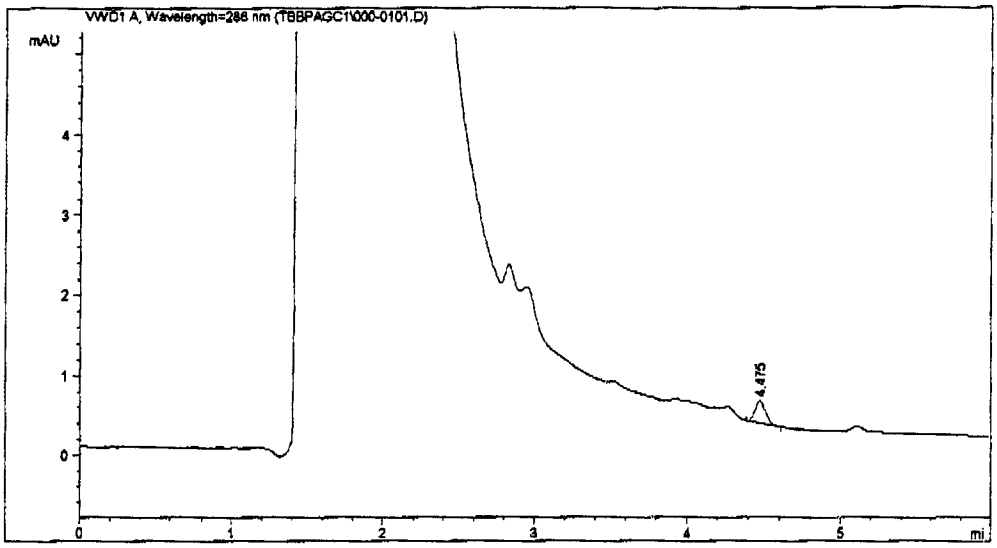

Figure 8. Representative chromatogram of a low-level calibration standard (0.0100 mg TBBPA/L).

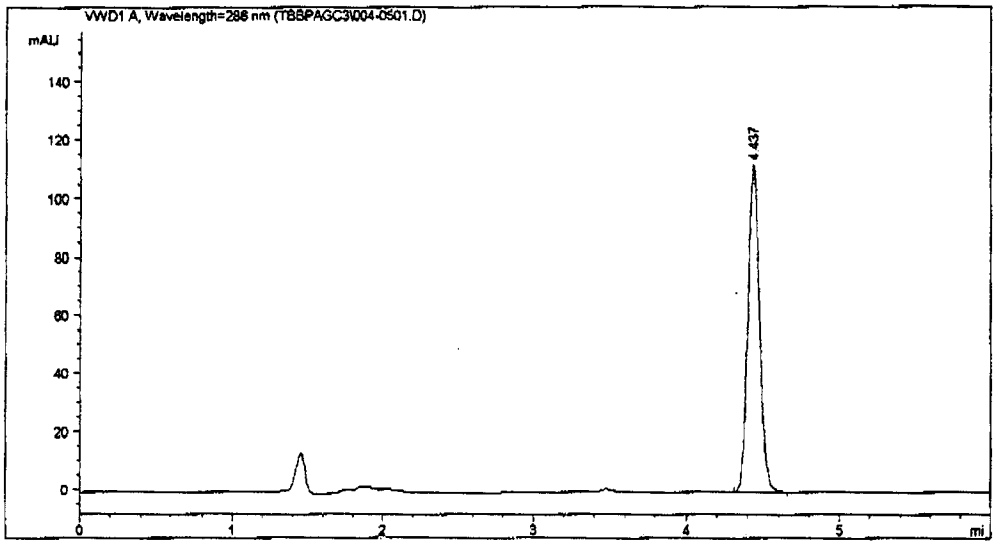

Figure 9. Representative chromatogram of a high-level calibration standard (5.00 mg TBBPA/L)

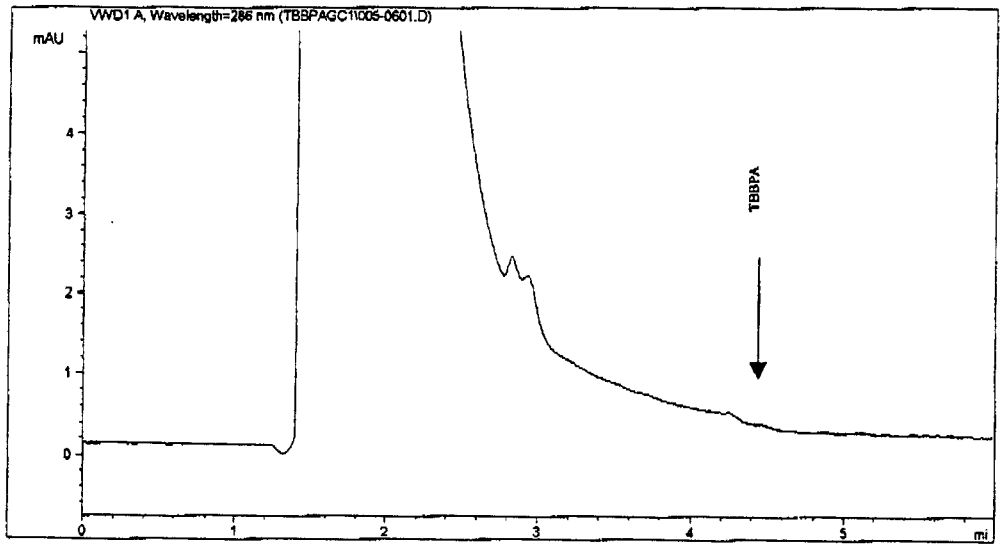

Figure 10. Representative chromatogram of the pH 5.0 aqueous buffer matrix blank sample (439C-132-MAB-1). The arrow indicates the approximate retention time of TBBPA peak.

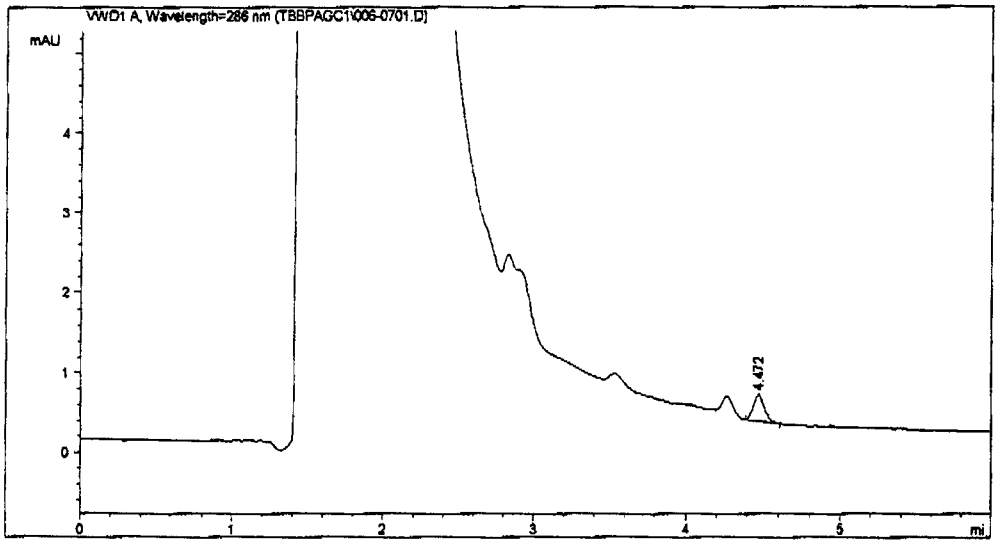

Figure 11. Representative chromatogram of the 0.0250 mg TBBPA/L pH 5.0 aqueous buffer matrix fortification sample (439C-132-MAS-1).

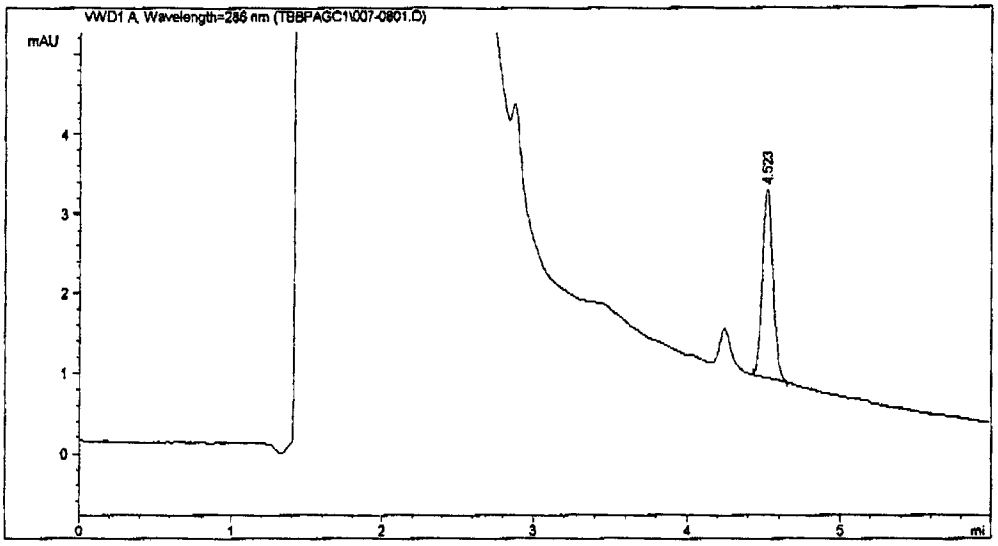

Figure 12. Representative chromatogram of the 0.250 mg TBBPA/L pH 5.0 aqueous buffer matrix fortification sample (439C-132-MAS-2).

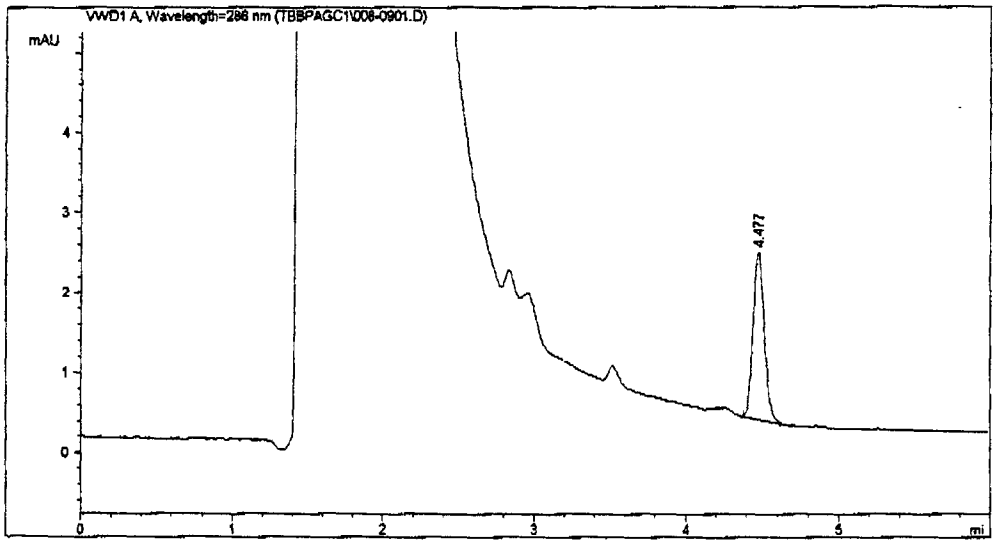

Figure 13. Representative chromatogram of a pH 5.0 aqueous solute sample collected from the generator column (439C-132-GCF(pH5)-1).

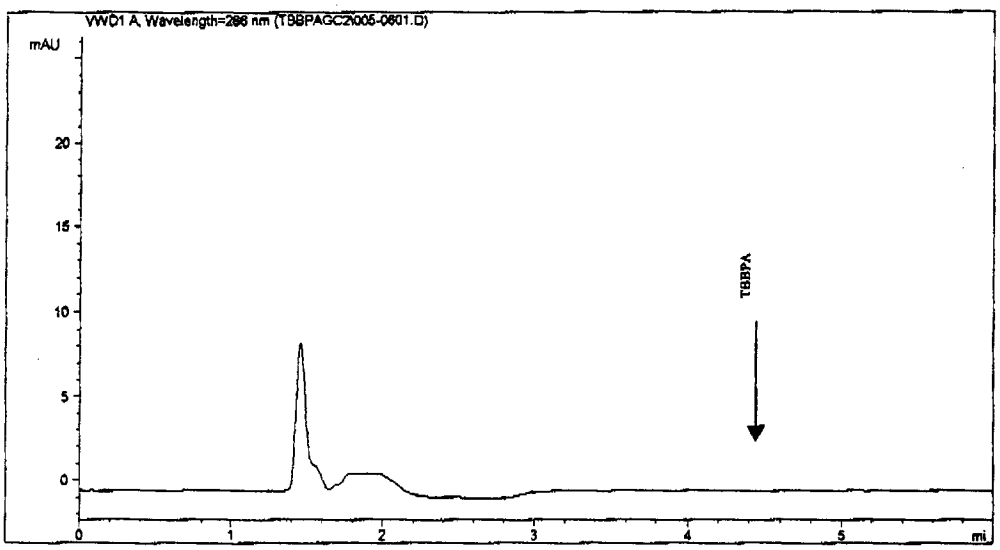

Figure 14. Representative chromatogram of the pH 7.0 aqueous buffer matrix blank sample (439C-132-MAB-2). The arrow indicates the approximate retention time of TBBPA peak.

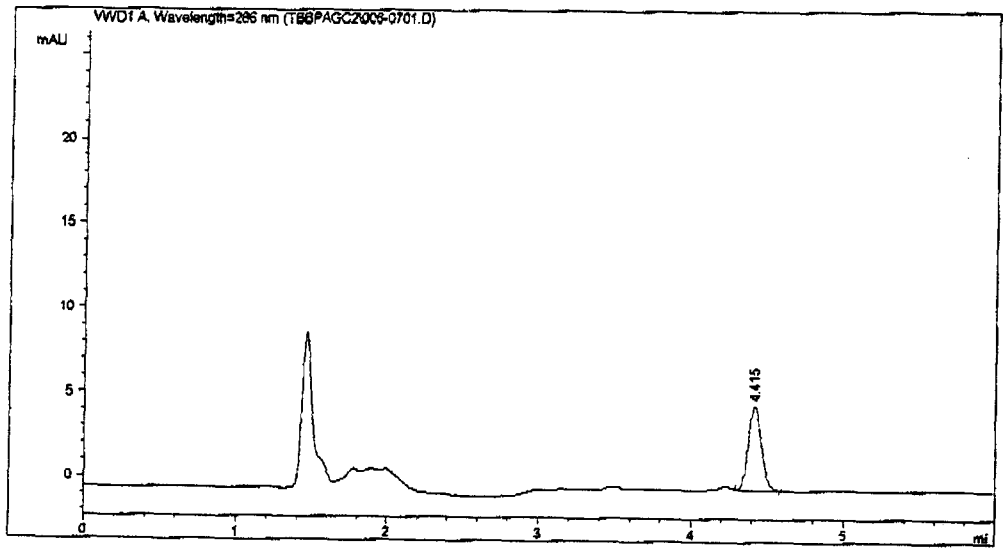

Figure 15. Representative chromatogram of the 0.500 mg TBBPA/L pH 7.0 aqueous buffer matrix fortification sample (439C-132-MAS-3).

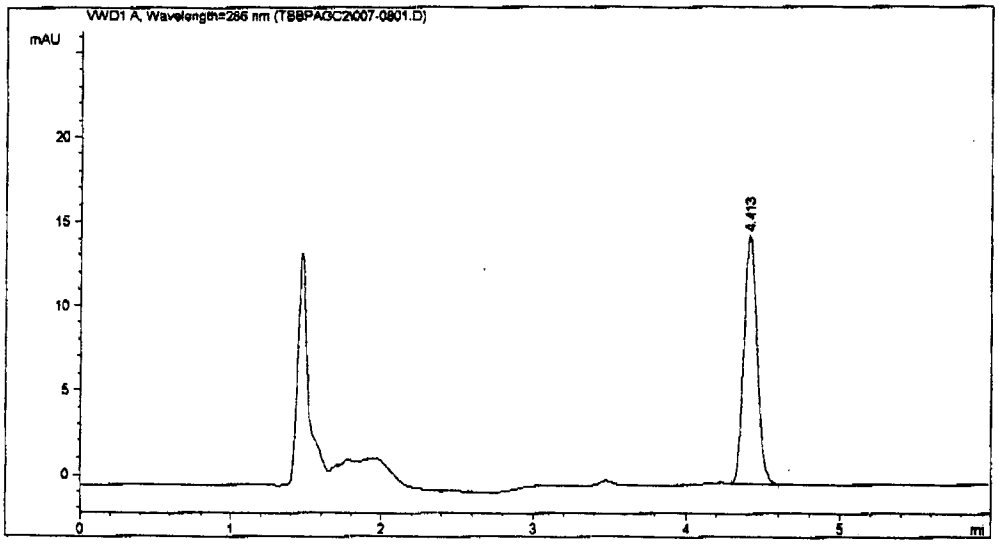

Figure 16. Representative chromatogram of the 1.50 mg TBBPA/L pH 7.0 aqueous buffer matrix fortification sample (439C-132-MAS-4).

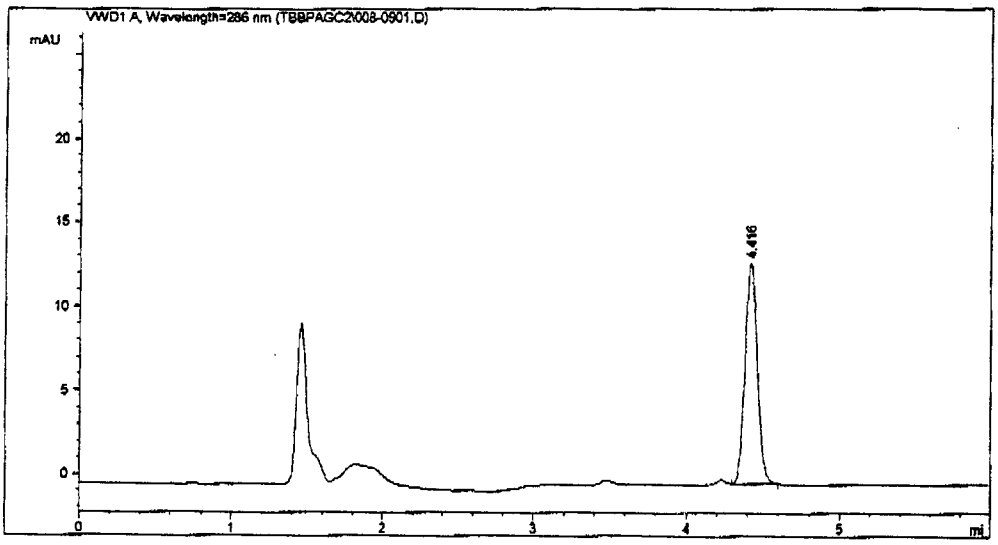

Figure 17. Representative chromatogram of a pH 7.0 aqueous solute sample collected from the generator column (439C-132-GCF(pH7)-1).

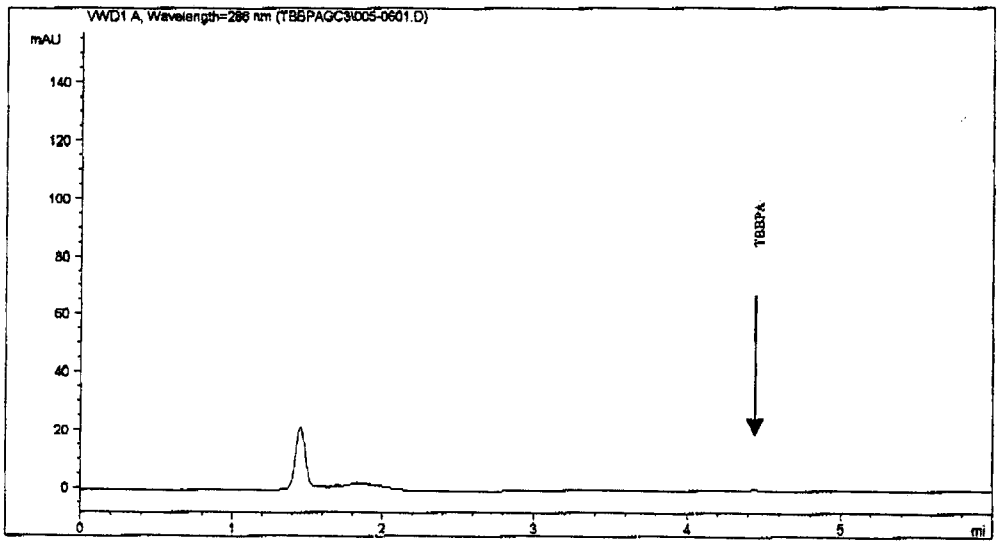

Figure 18. Representative chromatogram of the pH 9.0 aqueous buffer matrix blank sample (439C-132-MAB-3). The arrow indicates the approximate retention time of TBBPA peak.

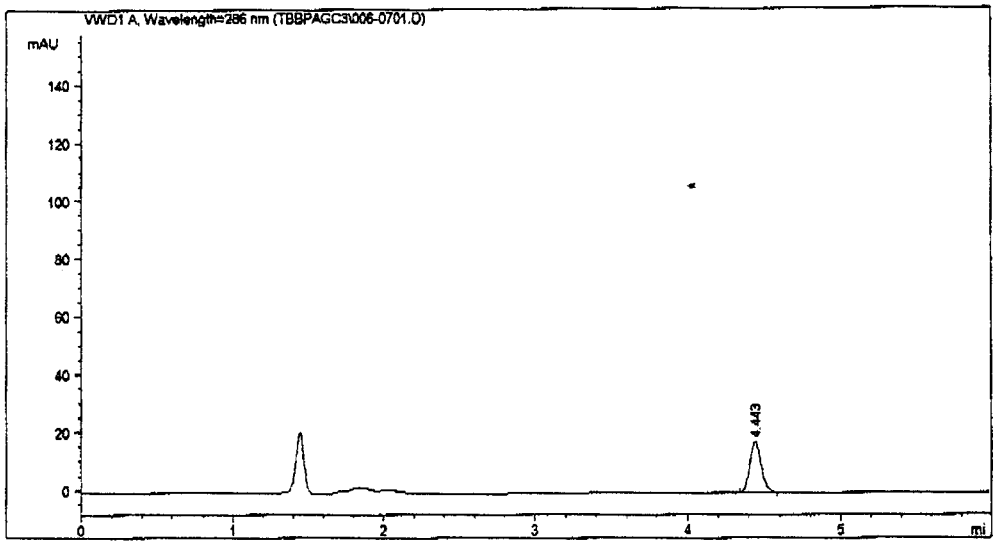

Figure 19. Representative chromatogram of the 1.50 mg TBBPA/L pH 9.0 aqueous buffer matrix fortification sample (439C-132-MAS-5).

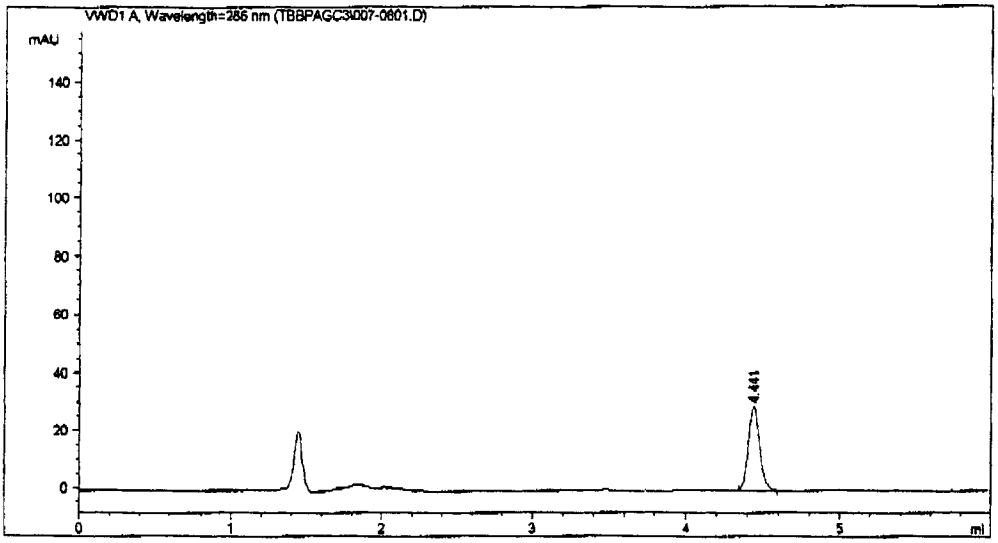

Figure 20. Representative chromatogram of the 2.50 mg TBBPA/L pH 9.0 aqueous buffer matrix fortification sample (439C-132-MAS-6).

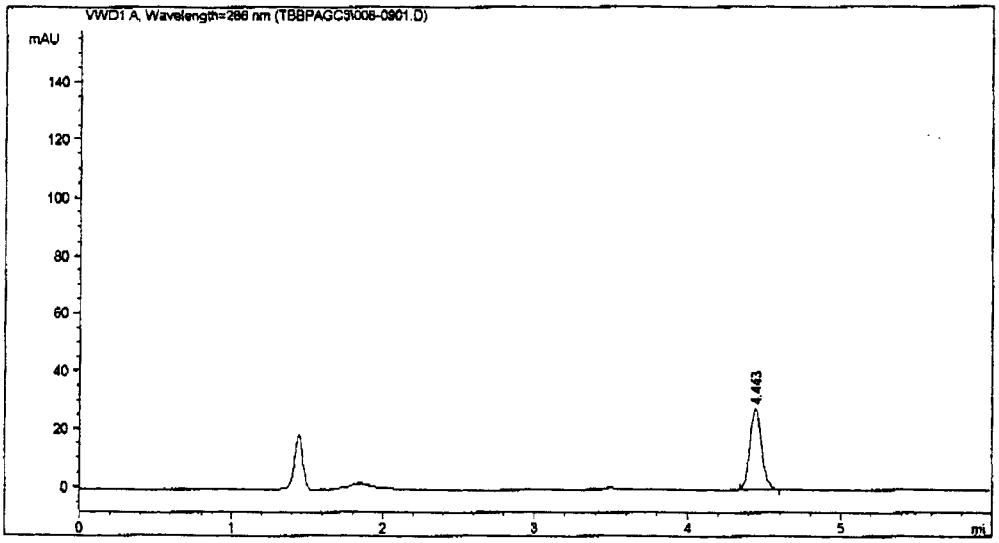

Figure 21. Representative chromatogram of a pH 9.0 aqueous solute sample collected from the generator column (439C-132-GCF(pH9)-11).

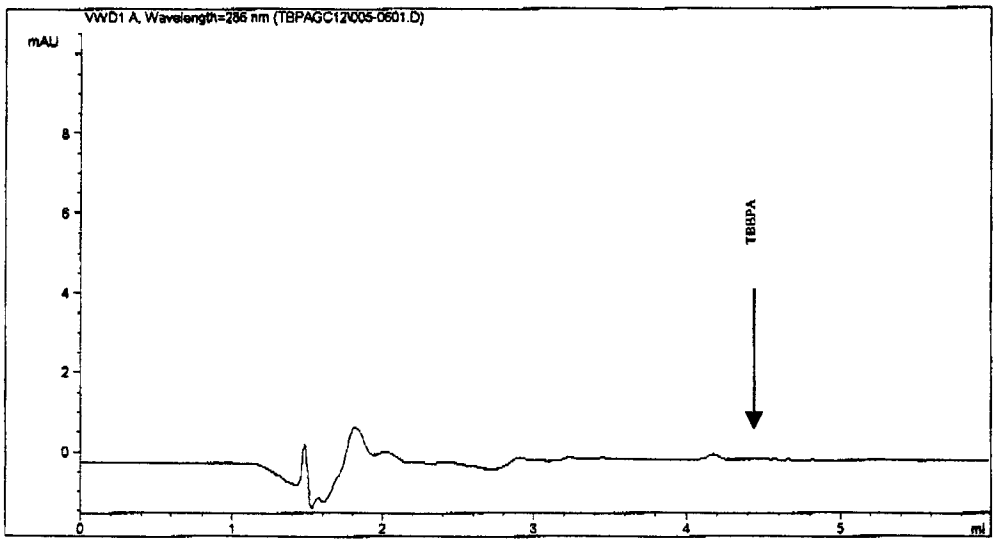

Figure 22. Representative chromatogram of the non-buffered reagent water matrix blank sample (439C-132-MAB-4). The arrow indicates the approximate retention time of TBBPA peak.

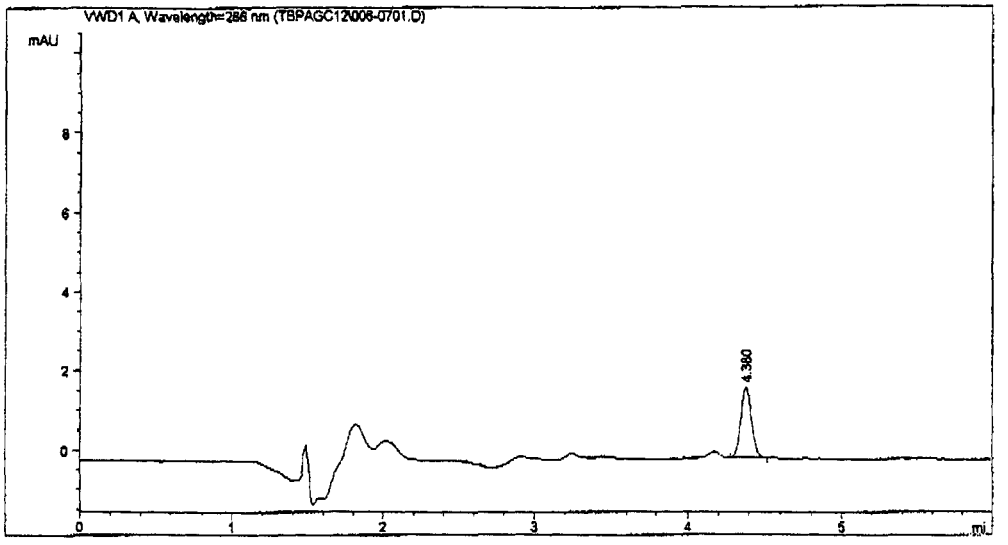

Figure 23. Representative chromatogram of the 0.150 mg TBBPA/L non-buffered reagent water matrix fortification sample (439C-132-MAS-7).

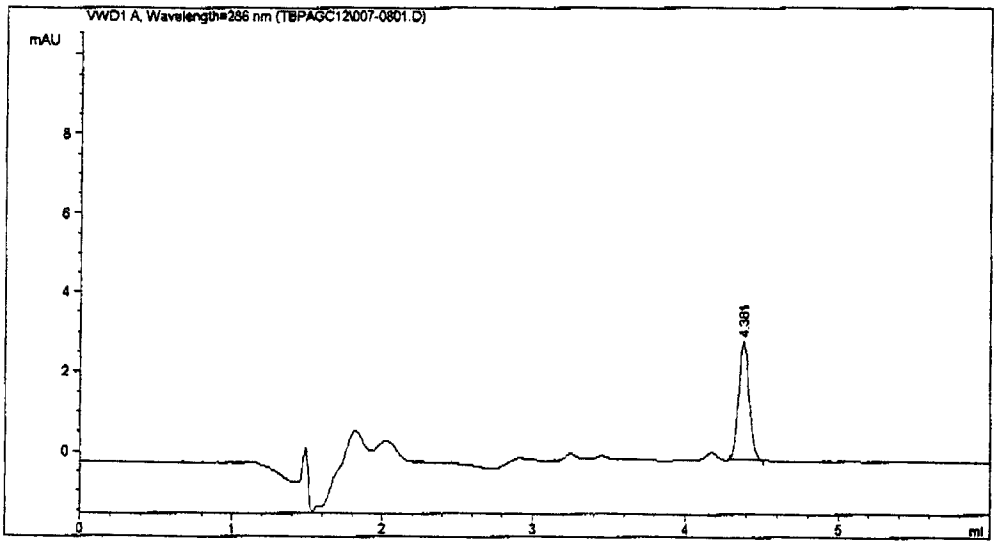

Figure 24. Representative chromatogram of the 0.250 mg TBBPA/L non-buffered reagent water matrix fortification sample (439C-132-MAS-8).

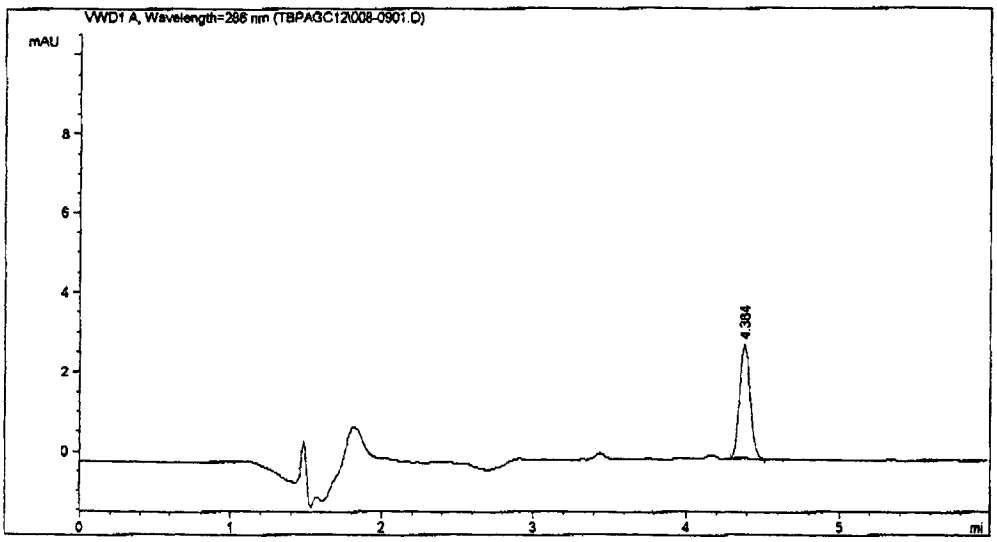

Figure 25. Representative chromatogram of a non-buffered reagent water aqueous solute sample collected from the generator column (439C-132-GCF(NANO)-1).

APPENDIX I

Protocol and Protocol Amendment

PROTOCOL

DETERMINATION OF WATER SOLUBILITY OF TETRABROMOBISPHENOL A

U.S. EPA Product Properties Test Guidelines

OPPTS 830.7860, Water Solubility (Generator Column Method)

and

OECD Guideline for the Testing of Chemicals, 105: Water Solubility

Submitted to

American Chemistry Council's
Brominated Flame Retardant Industry Panel
1300 Wilson Boulevard
Arlington, Virginia 22209

Wildlife International, Ltd.

8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8600

February 5, 2002

Wildlife International, Ltd.

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DETERMINATION OF WATER SOLUBILITY OF TETRABROMOBISPHENOL A

SPONSOR:	American Chemistry Council's Brominated Flame Retardant Industry Panel 1300 Wilson Boulevard Arlington, Virginia 22209
SPONSOR'S REPRESENTATIVE:	Ms. Wendy Sherman
TESTING FACILITY:	Wildlife International Ltd. 8598 Commerce Drive Easton, Maryland 21601
STUDY DIRECTOR:	Jon MacGregor, ~~Chemist~~ Study Director Wildlife International, Ltd.
LABORATORY MANAGEMENT:	Willard B. Nixon, Ph.D. Director of Analytical Chemistry

FOR LABORATORY USE ONLY

Proposed Dates:

Experimental Start Date: 3-11-02	Experimental Termination Date: 5-11-02
Project No.: 439C-132	Initiation Date: 3-11-02
Test Substance No.: 5284D 5754	Receipt Date: 3-30-01 3-29-01

PROTOCOL APPROVAL

Jon A. MacGregor STUDY DIRECTOR	3-11-02 DATE
Willard B. Nixon LABORATORY MANAGEMENT	3/11/02 DATE
Wendy K. Sherman SPONSOR'S REPRESENTATIVE	2/21/02 DATE

Wildlife International, Ltd.

INTRODUCTION

Wildlife International Ltd. will determine the water solubility of the test substance, Tetrabromobisphenol A. The study will be conducted at the Wildlife International Ltd. analytical chemistry facility in Easton, Maryland. The study will be performed following procedures in the U.S. EPA Product Properties Test Guidelines, OPPTS 830.7860, *Water Solubility (Generator Column Method)* (1). Additional guidance presented in the OECD Guideline for Testing of Chemicals, 105: *Water Solubility* (2) may be used. Raw data for all work performed at Wildlife International Ltd. and a copy of the final report will be filed by project number in archives located on the Wildlife International Ltd. site or at an alternative location to be specified in the final report.

OBJECTIVE

The objective of this study is to experimentally determine the water solubility of Tetrabromobisphenol A at 25°C using the generator column method at pH 5.0, 7.0 and 9.0.

EXPERIMENTAL DESIGN

The generator column method is generally applicable to liquid or solid test substances that are stable in water and are soluble in the range from 1 µg/L to 5000 mg/L. For test substances with an aqueous solubility less than 1 µg/L, the solubility will be reported as <1 µg/L without further quantitation. The definitive generator column test consists of generating aqueous solutions of the test substance at pH 5.0, 7.0 and 9.0 by pumping water through a generator column packed with a solid support coated with the test substance. The concentration of test substance in the saturated aqueous solutions eluting from the column represents the water solubility at the test temperature and pH.

MATERIALS AND METHODS

Test Substance

The test substance consisted of a composite of TBBPA samples received from three manufacturers. The material's identity and date received from each of the manufacturers is given below:

Manufacturer	Lot/Batch	Date Received	Wildlife International Ltd. Identification Number
Great Lakes Chemical Corporation	1008JE04B	August 16, 2001	5722
Albemarle Corporation	25115T-1	August 16, 2001	5721
Bromine Compounds, Ltd.	010040	August 31, 2001	5733

Wildlife International, Ltd.

The composite test substance was assigned Wildlife International Ltd. identification number 5754 and was stored under ambient conditions.

The Sponsor is responsible for all information related to the test substance and agrees to accept any unused test substance and/or test substance containers remaining at the end of the study.

The attached form **IDENTIFICATION OF TEST SUBSTANCE BY SPONSOR** (Appendix I) is to be used to provide available information. The Sponsor is responsible for information on the purity and composition of the test substance, as well as all information related to the safe handling of the test substance. The Sponsor also agrees to accept any unused test substance or test substance containers remaining at the end of the study.

Reagents

Water that meets or exceeds ASTM Type II standards (ASTM D 1193-91) will be used. Other solvents and reagents will be ACS reagent or HPLC grade or better. The solid support material for the column elution method will be Chromosorb WHP (100 - 120 mesh) chromatographic support material.

Preparation of Buffer Solutions

Buffer solutions will be prepared based on recommendations of OPPTS 830.7860 Guideline (1). Equivalent reagents and approximate volumes may be used to achieve the desired pH.

- pH 5.0—To 250 mL of 0.1M potassium hydrogen phthalate add 113 mL of 0.1M sodium hydroxide; adjust the final volume to 500 mL with reagent grade water.
- pH 7.0—To 250 mL of 0.1M potassium dihydrogen phosphate add 145 mL of 0.1M sodium hydroxide; adjust the final volume to 500 mL with reagent grade water.
- pH 9.0—To 250 mL of 0.075M borax add 69 mL of 0.1M HCl; adjust the final volume to 500 mL with reagent grade water.

Analytical Method(s)

Tetrabromobisphenol A will be analyzed by high performance liquid chromatography (HPLC) with ultraviolet absorption detection (UV) or gas chromatography (GC). The analytical method(s)

Wildlife International, Ltd.

used for quantitation of the test substance in water will be based upon procedures provided by the Sponsor or procedures developed by Wildlife International Ltd.

Preparation of the Generator Column

The generator column consists of a water-jacketed glass column approximately 6-mm outside diameter (OD), approximately 20 cm in length, joined to a 9-mm OD section, approximately 5 cm in length, which in turn is connected to another section of 6-mm OD tubing, approximately 3 cm in length. The inlet and outlet are fitted with adapters for connection to chemically-inert tubing.

If the test substance is a liquid, a weighed amount may be added directly to the carrier support material or mixed with a volatile solvent before addition. If the test substance is a solid, a solution containing approximately 1% of the test substance in a volatile solvent will be added to the carrier support material. For test substances added to the support material with solvent, the flask will be placed on a rotary evaporator and the solvent evaporated to evenly coat the carrier material with the test substance. The loaded carrier material will be allowed to soak for approximately 2 hours in water. The generator column will then be prepared as follows. A plug of silanized glass wool will be inserted into one end of a water-jacketed glass column. The slurry of loaded carrier material in water will be added to the column, followed by another plug of silanized glass wool to retain the carrier material. The column will be allowed to equilibrate for 2 hours at the test temperature. A separate column will be prepared for each of the three pH's investigated. The generator columns will be labeled with the project number and a unique column code.

Test Procedure

Aqueous buffer solutions for eluting the test material will be pumped through the water-jacketed generator column, maintained at approximately 25 ± 0.1°C, at a flow rate of approximately 0.5 mL/min. Temperatures of the water flowing through the jacket will be measured daily or more frequently during column elution. Water for column elution will be maintained at 25 ± 0.1°C. Water will be drawn through the column initially (back flushed) to remove entrapped air. Water will then be pumped through the column for a minimum of 15 minutes at a rate of approximately 0.5 mL/min to equilibrate the system. Following equilibration, water will be pumped through the column at a rate of approximately 1.0 mL/min. The eluate from the generator column will be directed to a collection vessel. A collection vessel will be used for collection of eluate fractions when HPLC analysis by

Wildlife International, Ltd.

direct injection is not suitable or a pre-concentration step is required. The apparatus will be allowed to run until five consecutive samples differ by less than ±30%. The test will then be repeated at approximately one-half the previous flow rate until two consecutive runs give the same solubility (within 30%). The volume of each aqueous sample will be dictated by the amount of test substance required for quantitation.

If a concentration step is necessary prior to analysis, the initial column eluats during column equilibration will be collected in a waste container. A collection vessel containing extracting solvent may be used to collect each water sample. The water volume may be determined either by weight differences of the extraction vessel before and after water collection or measurement of the volume following extraction. Samples will be identified with the project number and a unique sample identification code.

Calculations

The concentration of the test substance in each sample will be expressed in milligrams per liter (mg/L) or micrograms per liter (µg/L) as appropriate for the concentration. The average solubility and standard deviation will be calculated for at least five samples from each run for each pH.

Sample and Test Substance Retention

Upon completion of testing, portions of the test substance used as part of this study will be disposed of in accordance with federal, state and local regulations. Test substance containers and any unused portion of the test substance remaining at the end of the study will be returned to the Sponsor.

RECORDS TO BE MAINTAINED

Records to be maintained for data generated by Wildlife International Ltd. will include, but not be limited to:

1. A copy of the signed protocol.
2. Identification and characterization of the test substance, if provided by the Sponsor.
3. Dates of initiation and completion of the study.
4. Dates of experimental start and termination.
5. Storage conditions of the test substance.

Wildlife International, Ltd.

6. Test substance use log.
7. Concentration calculations and records of solution preparation.
8. Instrument operating conditions and chromatograms, if applicable.
9. Statistical calculations.
10. Test conditions.
11. A copy of the final report.

FINAL REPORT

Wildlife International Ltd. will prepare a final report of the results of the study. The report will include, but not be limited to the following, when applicable

1. Name and address of the facility performing the study.
2. Dates upon which the study was initiated and completed.
3. A statement of compliance signed by the Study Director addressing any exceptions to Good Laboratory Practice Standards.
4. Purpose and procedure, as stated in the approved protocol, including all amendments and deviations to the protocol.
5. A copy of the protocol and protocol amendments.
6. The test substance identification, including name, chemical abstract number or code number, purity, composition, empirical formula, molecular formula, manufacturer's lot/batch number, dissociation in water, method of analysis, and any other information provided by the Sponsor.
7. Description of the test method or reference to the method used along with any modifications made.
8. Water bath temperatures measured during column elution.
9. The individual concentrations and measured flow rates for each sample.
10. The means and standard deviation for at least five samples from the saturation plateau of each run.
11. The average concentration from two successive, acceptable runs.
12. Description of any problems experienced and how they were resolved.
13. A statement prepared by the Quality Assurance Unit listing the dates that study inspections and audits were made and findings reported to the Study Director and Management.

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CHANGING OF PROTOCOL

Planned changes to the protocol will be in the form of written amendments signed by the Study Director and approved by the Sponsor. Amendments will be considered as part of the protocol and will be attached to the final protocol. Any other changes will be in the form of written deviations filed with the raw data. All changes to the protocol will be indicated in the final report.

GOOD LABORATORY PRACTICES

This study will be conducted according to the Good Laboratory Practices described in OECD (OCDE/GD (92) 32, Environment Monograph No. 45) and EPA (40 CFR Part 792). Each study conducted by Wildlife International, Ltd. is routinely examined by the Wildlife International, Ltd. Quality Assurance Unit for compliance with Good Laboratory Practices, Standard Operating Procedures and the specified protocol. A statement of compliance with Good Laboratory Practices will be prepared for all portions of the study conducted by Wildlife International Ltd. The Sponsor will be responsible for compliance with Good Laboratory Practices for procedures performed by other laboratories.

Wildlife International, Ltd.

REFERENCES

1. **U.S. EPA Product Properties Test Guidelines.** 1998. OPPTS 830.7860. *Water Solubility (Generator Column Method).*

2. **Organization for Economic Cooperation and Development.** 1995. Guideline for Testing of Chemicals, 105: *Water Solubility.*

Wildlife International, Ltd.

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APPENDIX I

IDENTIFICATION OF TEST SUBSTANCE BY SPONSOR

To be Completed by Sponsor

I. Test Substance Identity (name to be used in the report): Tetrabromobisphenol-A

Reference Standard (if applicable): N/A

Test Substance Sample Code or Batch Number: Wildlife International, Ltd. No.5754

Test Substance Purity (% Active Ingredient): 99.17% Expiration Date: _____

II. Test Substance Characterization

Have the identity, strength, purity and composition or other characteristics which appropriately define the test substance and reference standard been determined prior to its use in this study in accordance with GLP Standards? X Yes __ No

III. Test Substance Storage Conditions

Please indicate the recommended storage conditions at Wildlife International, Ltd..

Ambient temperature; protect from light and moisture

Has the stability of the test substance under these storage conditions been determined in accordance with GLP Standards? X Yes __ No

Other pertinent stability information:

N/A

IV. Toxicity Information:

Mammalian: Rat LD50 > 5 g/kg Mouse LD50: > 10 g/kg

Aquatic:	Invertebrate Toxicity (EC/LC50)	Fish Toxicity (LC50)
	N/A	N/A

Other Toxicity Information (including findings of chronic and subchronic tests):

V. Classification of the Compound:

_____Insecticide _____Herbicide _____Fungicide

_____Microbial Agent _____Economic Poison

Other: Halogenated flame retardant

Project Number 439C-132

Wildlife International, Ltd.

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: Determination of Water Solubility of Tetrabromobisphenol A

PROTOCOL NO.: 439/020502/WATSSOL/SUB439 **AMENDMENT NO.:** 1

SPONSOR: American Chemistry Council's Brominated Flame Retardant Industry Panel

PROJECT NUMBER: 439C-132

EFFECTIVE DATE: July 17, 2002

AMENDMENT: Page 3, Experimental Design: Add an additional definitive generator column water solubility determination trial at 25°C using NANOpure® (ASTM Type II equivalent) reagent grade water. Additionally, the pH of the column eluate will be measured and recorded at the start and end of each flow rate and the pH of the NANOpure water feed to the column will also be measured and recorded.

REASON: The experimental scope was increased at the request of the Sponsor to determine the effect of a non-buffered high purity water eluent on the measured solubility of the Tetrabromobisphenol A test substance using the generator column method.

STUDY DIRECTOR	DATE 7/18/02
LABORATORY MANAGEMENT	DATE 7/22/02
SPONSOR'S REPRESENTATIVE	DATE

Project Number 439C-132

Wildlife International, Ltd.

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: Determination of Water Solubility of Tetrabromobisphenol A

PROTOCOL NO.: 439/020502/WATSSOL/SUB439 **AMENDMENT NO.:** 1

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STUDY DIRECTOR	DATE
LABORATORY MANAGEMENT	DATE
Wendy K. Sherman SPONSOR'S REPRESENTATIVE	July 19, 2002 DATE

APPENDIX II

Certificate of Analysis

CONCLUSIONS AND TEST ARTICLE ANALYTICAL DATA. 1.

CHEMICAL NAME: Tetrabromobisphenol-A
C.A.S. No.: 79-94-7
MOLECULAR FORMULA: $C_{15}H_{12}Br_4O_2$
PHYSICAL FORM: White Powder
CHEMICAL STRUCTURE:

ANALYSIS	RESULTS		ANALYSIS DATES	ANALYST
FT-IR	The sample FT-IR spectrum matched that of the reference spectrum. All spectra are on file with the original data.		10/12/01	W. T. Cobb
HPLC				
Sample	Purity (area% TBBPA)	Average	Difference (%) from average			
middle right	99.14	99.17	<5%	10/25/01	J. S. Arroyave
middle left	99.18	99.17	<5%	10/25/01	J. S. Arroyave
bottom right	99.09	99.17	<5%	10/25/01	J. S. Arroyave
top right	99.19	99.17	<5%	10/25/01	J. S. Arroyave
top left	99.16	99.17	<5%	10/25/01	J. S. Arroyave
bottom left	99.23	99.17	<5%	10/25/01	J. S. Arroyave

CONCLUSION: Based on these analytical data, the test article identity was confirmed as tetrabromobisphenol-A. The composite sample was shown to be homogeneous with a purity of 99.17%.

Conclusions and Test Article Data. 2.

Characterization of Test Article by HPLC (Area%)

	TBBPA	Tribromophenol	TriBPA	opp'-TBBPA
Middle Right	99.14	Not detected	0.82	0.04
Middle Left	99.18	Not detected	0.76	0.06
Bottom Right	99.09	Not detected	0.84	0.06
Top Right	99.19	Not detected	0.78	0.04
Top Left	99.16	Not detected	0.8	0.04
Bottom Left	99.23	Not detected	0.72	0.04

APPENDIX III

Personnel Involved in the Study

The following key Wildlife International, Ltd. personnel were involved in the conduct or management of this study:

1. Willard B. Nixon, Ph.D., Director of Chemistry
2. Timothy Z. Kendall, M.S., Supervisor
3. Jon A. MacGregor, Scientist