TRADE SECRET

Study Title

[ ]: Hydrolytic Stability of [ ] as a Function of pH

TEST GUIDELINES: OECD Guideline 111

AUTHOR: Bogdan Szostek, Ph.D.

STUDY COMPLETED ON: November 24, 2003

PERFORMING LABORATORY: E.I. du Pont de Nemours and Company
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LABORATORY PROJECT ID: DuPont-13202

WORK REQUEST NUMBER: 14643

SERVICE CODE NUMBER: 392

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with U.S. EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards, which are consistent with the OECD Principles of Good Laboratory Practice (as revised in 1997) published in ENV/MC/CHEM(98)17 and MAFF Japan Good Laboratory Practice Standards (59 NohSan Number 3850.

The test substance was characterized by the sponsor prior to the initiation of this study, except the percent content of 8-2 TBA. The analysis of percent content of 8-2 TBA in the test substance was completed after this study completion. Although the characterization was not performed under Good Laboratory Practice Standards, the characterization was done by an ISO 9000 certified laboratory, and the accuracy of the data is considered sufficient for the purposes of this study.

Applicant / Sponsor: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

Study Director: Bogdan Szostek _____ 24-NOV-2003
Bogdan Szostek, Ph.D. — Date
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Applicant / Sponsor: _____ _____
DuPont Representative — Date

QUALITY ASSURANCE STATEMENT

Haskell Sample Number(s):

25707

Dates of Inspections:

Protocol: July 25, 2003

Conduct: August 1, 2003

Records, Reports: September 30, 2003; October 1-3,6, 2003

Dates Findings Reported to:

Study Director: October 15, 2003; November 20, 2003

Management: October 15, 2003; November 20, 2003

Reported by: Wonda K. Kelly 24-Nov-2003

Wonda K. Kelly **Date**
Quality Assurance Auditor

CERTIFICATION

We, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

Approved by: _____ 24-NOV-2003
S. Mark Kennedy, Ph.D. Date
Manager, Analytical Chemistry

Issued by Study Director: _____ 24-NOV-2003
Bogdan Szostek, Ph.D. Date
Senior Research Chemist

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STUDY INFORMATION

Substance Tested: [ ]

Synonyms/Codes: • H-25707

Submitter's Notebook Number(s): E105186-11

Haskell Number: 25707

Purity: 100% Fluorinated polymer

Known Impurities: 0.016% 8-2 Telomer B Alcohol (1H,1H,2H,2H-perfluorodecan-1-ol, CAS# 678-39-7)

Physical Characteristics: Off-white solid

Stability: The test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.

Sponsor: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

Study Initiated/Completed: June 6, 2003 / (see report cover page)

Experimental Start/Completion: June 9, 2003 / September 15, 2003

STUDY PERSONNEL

Study Director: Bogdan Szostek, Ph.D.

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SUMMARY

The hydrolytic stability of [ ], a fluorotelomer-based acrylate copolymer, was tested in buffered aqueous solutions at pH 4.0, 7.0, 9.0 at 50°C and pH 1.2 at 37°C for five days. The [ ] was demonstrated to be hydrolytically stable under these test conditions. The stability was demonstrated by the comparison of the percent recoveries of [ ] for Day 0 and Day 5 samples and analysis of chromatographic peak shape for Day 5 samples. The average percent recoveries for the Day 0 and Day 5, pH 1.2 replicate samples are 101 ± 1.2% and 101 ± 1.0%, respectively. The average percent recoveries for the Day 0 and Day 5, pH 4.0 replicate samples are 100 ± 0.5% and 97 ± 2.1%, respectively. The average percent recoveries for the Day 0 and Day 5, pH 7.0 replicate samples are 101 ± 1.3% and 100 ± 0.8%, respectively. The average percent recoveries for the Day 0 and Day 5, pH 9.0 replicate samples are 100 ± 1.0% and 104 ± 2.5%, respectively. The difference in percent recoveries for Day 0 and Day 5 samples did not reach the critical level of 10% that would indicate the hydrolysis of the test substance for any of the investigated test conditions. The observed increase of 8-2 TBA concentration is approximately two orders of magnitude lower than the concentration of 8-2 TBA expected if 10% of the test substance hydrolyzed. In addition, the measured concentrations of 8-2 Telomer B Alcohol (8-2 TBA, $C8F17CH_2CH_2OH$) indicated an increase of the 8-2 TBA concentration between Day 0 and Day 5. The increase of the 8-2 TBA concentration can be accounted for by the presence of the residual 8-2 TBA raw material present in the test substance.

INTRODUCTION

The purpose of the study was to investigate the hydrolytic stability of [ ], a fluorotelomer-based acrylate copolymer, in pH 1.2, 4.0, 7.0, and 9.0 aqueous buffer solutions. The study was conducted according to the protocol: "Hydrolytic stability of [ ] as a function of pH", which was patterned after the OECD Guideline 111.[1] Additionally, the concentration of the 8-2 Telomer B Alcohol (8-2 TBA) was monitored to investigate if 8-2 TBA is released from the test substance during the test.

STUDY DESIGN

A preliminary test was performed on the test substance at 50 ± 0.1°C at each of the pH 4.0, 7.0, 9.0 and at 37 ± 0.1°C for pH 1.2. If less than 10% of the test substance hydrolysis is observed after 5 days, the test substance was considered hydrolytically stable and no additional testing was performed. In addition to the test substance determination during the preliminary test, the samples were analyzed for 8-2 TBA.

The study design complies with the following test guidelines: OECD Guideline 111[1] with the exception that the OECD Guideline 111 requires that the amount of the test substance used is the smaller of 0.01 M or half the saturation concentration in water. The test substance ([ ]) is insoluble in water and the analytical method does not allow detection of the test substance at trace levels. Therefore, the study was conducted with an excess of undissolved test substance.

MATERIALS AND METHODS

A. Materials

1. Test Substance

Name:	[ ]
Composition:	100% Fluorinated Polymer
CAS Number:	[ ]
Test Substance Chemistry:	[ ]
	[ ]
	[ ]
Haskell number:	25707

The test substance was characterized to determine the 8-2 TBA content, % fluorine, and polymer characterization parameters: Mn (number average), Mw (weight average), Mz (z average), and PDI (polydispersity index) with the following results:

% 8-2 TBA (w/w):	0.016%
% Fluorine (w/w):	[ ]
Mn:	[ ]
Mw:	[ ]
Mz:	[ ]
PDI:	[ ]

2. Preparation of buffer solutions

The following buffer solutions were prepared as described in the Annex to OECD guideline 111[(1)].

a. pH 1.2:

The 0.2 M potassium chloride (KCl) was prepared by dissolving 3.73 g of KCl (purity 99.0%, Sigma) in 250 mL of water. The 0.2 M hydrochloric acid (HCl) was prepared by diluting 1.83 g of hydrochloric acid (36.5-38%, Sigma) in 250 mL water. The pH 1.2 buffer solution was prepared by placing 125 mL of 0.2 M KCl and 161.3 mL of a 0.2 M HCl solution into a 500-mL flask and bringing the flask to volume with water. The pH of the buffer was adjusted to 1.2 with hydrochloric acid.

b. pH 4:

The 0.1 M solution of potassium hydrogen phthalate was prepared by dissolving 10.21 g of potassium hydrogen phthalate (purity 99+%, Sigma) in 500 mL of water. The pH 4 buffer solution was prepared by placing 2 mL of a 0.1 M sodium hydroxide solution and 250 mL of a 0.1 M potassium hydrogen phthalate solution into a 500-mL flask and bringing the flask to volume with water. The pH of the buffer was adjusted to 4.0 with hydrochloric acid.

c. pH 7:

The 0.1 M solution of potassium phosphate was prepared by dissolving 6.81 g of potassium phosphate, monobasic (KH_2PO_4, purity 99.5%, Sigma) in 500 mL of water. The pH 7 buffer solution was prepared by placing 148 mL of a 0.1 M sodium hydroxide solution and 250 mL of the 0.1 M potassium phosphate solution into a 500-mL flask and bringing the flask to volume with water. The pH of the buffer was adjusted to 7.0 with sodium hydroxide.

d. pH 9:

The 0.1 M boric acid (H_3BO_3) solution in 0.1 M potassium chloride (KCl) was prepared by dissolving 3.73 g of KCl (purity 99.0%, Sigma) and 3.09 g of H_3BO_3 (purity 99.5%, Sigma) in 500 mL of water. The pH 9 buffer solution was prepared by placing 250 mL of a 0.1 M H_3BO_3 solution in 0.1 M KCl and 106.5 mL of a 0.1 M sodium hydroxide solution into a 500-mL flask and bringing the flask to volume with water. The pH of the buffer was adjusted to 9.0 with sodium hydroxide.

Water used for buffer preparation was obtained from a Barnstead NANOpure® Diamond water purification system. The water resistivity is >18.2 megaohm•cm . All buffer solutions were

sterilized prior to use by filtering through Corning Sterilization Filter systems with 0.22 μm cellulose acetate filters. The buffer solutions were stored at room temperature.

3. Equipment

pH Meter:	Orion Model 250 A
Water Bath:	Water bath: Lab-Line Force Reciprocating Water Bath Shaker, Model 4682
Autoclave:	AMSCO Model 3023
Automatic Pipettes:	Research Pro 100, 1000, 5000; Eppendorff
Balance:	Mettler AE-100
Sonicator:	Branson Model 1210 or Branson Model 5200
Centrifuge:	Sorvall RT7
Vortexer:	Multi-tube vortexer, VWR
Glass Vials:	10 mL borosilicate crimp top glass vials (Supelco) with 20 mm T/butyl septa and aluminum foil. The vials, foil, and septa were autoclaved at 121°C for 30 min before use.

B. Methods

1. Preliminary test

The preliminary test was performed on [ ] at 50°C at each of the pH 4.0, 7.0, 9.0 and at 37°C for pH 1.2. The same sample preparation procedure and study design was followed for each of the pH levels tested. Approximately 100 mg of the [ ] was weighed into each of sixteen 10-mL autoclaved glass vials and 3 mL of appropriate buffer solution was added. The vials were crimp capped with the bottom of the septum covered with autoclaved aluminum foil. Eight of the vials were processed for analysis as the Day 0 samples. The remaining eight samples were wrapped with the aluminum foil, submerged in the water bath, and shaken at 100 rpm in the water bath for five days at the appropriate temperature. Eight vials containing only 3 mL of appropriate buffer were prepared to serve as the blanks. Four of them were used as the Day 0 blanks and the other four were wrapped in aluminum foil and placed in the water bath to be the Day 5 blanks. Additionally, six vials containing 3 mL of appropriate buffer were spiked with 8-2 TBA stock solution (4.5 μL of 1020 mg/L 8-2 TBA). The vials were crimp capped with the bottom of the septum covered with autoclaved aluminum foil. Three of the vials were used as the quality control (QC) samples for 8-2 TBA analysis on Day 0. The other three vials were wrapped in aluminum foil and placed in the water bath together with eight of the test substance vials. These served a role of QC samples for 8-2 TBA analysis on Day 5. Four of the Day 0 or four of the Day 5 vials and two blank vials were processed for the pH measurement and the [ ] determination. The other four of the Day 0 or Day 5 vials, two blank vials, and 8-2 TBA QC vials were processed for the 8-2 TBA determination.

2. pH measurement and temperature.

The pH was measured on Day 0 and Day 5 in the vials containing the buffer and the test substance that were designated for the [ ] analysis. The vials were uncapped, the pH electrode was introduced to the vials and the pH reading was recorded. These vials were further processed for [ ] analysis. The temperature of the water

bath was monitored daily. The water bath temperature readings from the automatic temperature control were checked and recorded in the study records daily.

3. Analysis of test substance

After the pH measurement, 0.3 mL of diluted [ ] fluorosurfactant (H-24678) was added to each vial, the vials were recapped, vortexed for ten minutes, and centrifuged for fifteen minutes at 3200 rpm. The addition of the [ ] fluorosurfactant is necessary for wetting of the [ ] and facilitates the pelleting of the material at the bottom of the vial after centrifugation. After centrifugation the vials were uncapped and the supernatant was discarded using a manual pipetter. The remaining solid material was dissolved by adding 10 mL of tetrahydrofuran (THF) and sonication for 30 minutes. Approximately, 1 mL of the THF solution was placed in a glass LC vial and subjected to LC-GPC analysis. The blank samples were subjected to the same procedure except the pH measurement.

The stock solution of the [ ] was made by dissolving 0.15 g of [ ] in 10 mL of THF. Thirty minutes of sonication were required to completely dissolve the test substance. Calibration standards for the [ ] analysis were made by appropriate dilution of the stock. The calibration standards ranged from 3000 to 15000 mg/L [ ]. Fresh calibration standards were prepared for each instance of analysis. Linear regression of the peak height vs. standard concentration was used to establish the calibration curves.

Instrumentation and conditions:

LC System:	Agilent HP 1100 with Agilent Refractive Index Detector (RID)
Column:	Styragel HR 4, 7.8 mm x 300 mm, Waters
LC Solvent:	Tetrahydrofuran (THF)
Flow Rate:	1 mL/min
Column Temperature:	40 °C
RI Detector Temperature:	35 °C
Data Collection:	0-15 min
Injection Volume:	50 µL

4. Analysis of 8-2 TBA

Samples designated for 8-2 TBA analysis were processed by injecting 6 mL acetonitrile through the vial septum using a glass syringe. Neither Day 0 nor Day 5 samples were uncapped before acetonitrile injection to avoid any losses of 8-2 TBA. The vials containing injected acetonitrile were vortexed for 15 minutes on a multitube vortexer. Subsequently, the vials were uncapped and 1 mL of the extract was transferred to a glass LC vial, spiked with internal standard, crimp capped, and subjected to LC/MS analysis for 8-2 TBA.

1H, 1H, 2H, 2H-perfluorodecan-1-ol (97.6%, Oakwood Products, West Columbia, SC) was used as the analytical standard of 8-2 TBA. 1D, 1D, 2D, 2D, 3-^{13}C-heptadecafluoro decanol (M+5) (abbreviated as D-8-2 TBA) was used as the internal standard. Stock solutions (1000 mg/L) of the analytical standard and the internal standard were prepared in methanol and were stored refrigerated. The calibration standards were prepared fresh for each calibration in 50/50%

water/acetonitrile. The calibration standards were made in the range from 25 to 2550 µg/L 8-2 TBA. A constant concentration of internal standard was used: 520 µg/L D-C8-2A. The calibration curves were contracted using the ratio of the peak area for ions m/z monitored for 8-2 TBA and peak area for ions monitored for D-8-TBA and the concentrations of 8-2 TBA.

Instrumentation and conditions:

HPLC Instrument:	Model 2795, Waters
MS Instrument:	ZQ, Micromass
LC Parameters:	
Column:	Luna 3u C18 (2), 20 mm x 2mm
Mobile Phase:	A– water B - methanol

Gradient:

Time (min	%A	%B	Flow (mL/min)
0	30	70	0.25
1	30	70	0.25
2	0	100	0.25
6	0	100	0.25
6.1	30	70	0.25
8	30	70	0.15

Column Temperature:	30°C
Injection Volume:	20 µL
Column Switch:	2 and 6 min
MS Parameters:	
Ionization mode:	Electrospray, negative ions
Capillary voltage:	2.5 kV
Cone voltage:	10 V
Source Temperature:	120°C
Desolvation Temperature:	300°C
Data Acquisition Function:	8-2 TBA: SIR* of 463, 509, 523; 0-6 min D- 8-2 TBA: SIR of 468, 514, 528 m/z; 0-6 min

* These ions represent the deprotonated molecular ion of 8-2 TBA (m/z 463), formate adduct of 8-2 TBA (m/z 509), and acetate adduct of 8-2 TBA (m/z 523). The formate and acetate adduct are formed because of residual presence of acetate and/or formate in the system originating from other methods that are run on the system and utilize these components in the mobile phase.

5. Calculations

The following calculations were used to determine the nominal concentrations of [ ] (Tables 1-4). For example, if 0.1009 g of [ ] was weighed into the vial and subsequently dissolved in 10 mL of THF during sample processing, this would represent the nominal concentration of 100.9 mg/0.01 L=10,090 mg/L. The percent recovery is calculated by dividing the measured concentration by the nominal concentration, times 100%.

The expected (nominal) concentration of 8-2 TBA in the spiked samples was calculated as follows: the 4.5 µL of 1020 mg/L 8-2 TBA spike represents 4.5 µL x 1020 ng/µL= 4590 ng 8-2 TBA. Total volume of extract is 9 mL: 3 mL of buffer and 6 mL of acetonitrile. The expected

nominal concentration of 8-2 TBA in the extract is: 4590 ng/9 mL=510 ng/mL. The percent recovery is calculated by dividing the measured concentration by the nominal concentration, times 100%.

The expected concentration of 8-2 TBA in the acetonitrile extract if 10% of the [ ] hydrolyzed can be estimated in the following way. Ten percent of 100 mg of [ ] represents 10 mg. Taking into account that the test substance contain [ ] fluorine and assuming that all fluorine is present in the form of bound 8-2 TBA, the hydrolysis of the 10 mg of the test substance would release: [ ] [ ]5.56 mg 8-2 TBA. This amount of 8-2 TBA would be contained in 9 mLs of extract. Therefore, expected concentration of 8-2 TBA would be: 5.56 mg/0.009 L= 618 mg/L.

The [ ] contains residual raw material, 8-2 TBA. The expected, maximum concentration of 8-2 TBA derived from the 8-2 TBA present in the extract, assuming complete extraction, is: 100 mg of [ ] x (0.016%/100)/0.009 L= 1.78 mg/L (1780 µg/L).

RESULTS AND DISCUSSION

Tables 1-4 present the results for measured concentrations of [ ] and percent recoveries obtained for Day 0 and Day 5 replicate samples for pH 1.2, 4.0, 7.0, 9.0, respectively. The average percent recoveries for the Day 0 and Day 5, pH 1.2 replicate samples are 101 ± 1.2% and 101 ± 1.0%, respectively. The average percent recoveries for the Day 0 and Day 5, pH 4.0 replicate samples are 100 ± 0.5% and 97 ± 2.1%, respectively. The average percent recoveries for the Day 0 and Day 5, pH 7.0 replicate samples are 101 ± 1.3% and 100 ± 0.8%, respectively. The average percent recoveries for the Day 0 and Day 5, pH 9.0 replicate samples are 100 ± 1.0% and 104 ± 2.5%, respectively. The relative difference between the Day 5 and Day 0 concentrations of the test substance was less than 10% for any of the investigated pHs. This indicates that the [ ] is hydrolytically stable at investigated pHs. Figures 1 to 4 compare the LC chromatograms of four replicates of Day 5 samples with the chromatograms of two calibration standards for pH 1.2, 4.0, 7.0, 9.0, respectively. The shape of the chromatographic peak, eluting from 7 to 11 minutes, is indicative of the molecular weight distribution of the [ ]. The hydrolysis of [ ] would result in change of the observed peak shape. Comparison of the peak shape of the freshly made calibration standards of [ ] and the Day 5 samples indicates that no evident change of the peak shape for Day 5 samples was observed for any of the investigated pHs. This further supports the conclusion of [ ] hydrolytic stability. Figure 5 presents the LC chromatograms of the Day 5 blank samples for pH 1.2, 4.0, 7.0, and 9.0.

Additional evidence supporting the hydrolytic stability of [ ] can be derived from the measured concentration of 8-2 TBA. The hydrolysis of [ ] would result in the release of 8-2 TBA and the increase of 8-2 TBA concentration in the test system. Based on the charcterization data, the test substance contains 0.016% of residual 8-2 TBA. Assuming that the buffer extracts all the 8-2 TBA during the time of hydrolytic stability experiment, the expected concentration of 8-2 TBA in the processed extract (3 mL buffer and 6mL acetonitrile) would be 1.78 mg/L (see B.5 Calculations). Similarly, the expected concentration of 8-2 TBA in the extract, if 10% of [ ] was hydrolyzed, is 618 mg/L (see B.5 Calculations). Tables 5-8 present the measured concentrations of 8-2 TBA in the extracts for Day 0 and Day 5 replicate samples for pH 1.2, 4.0, 7.0, 9.0, respectively. The data shows a significant increase of 8-2 TBA concentration in the Day 5 samples, when the average concentration of 8-2 TBA for Day 0 and Day 5 samples are compared. However, the concentrations reach only some of the expected level of fully extracted 8-2 TBA (1780 µg/L) and these are approximately two orders of magnitude lower than the concentrations expected when 10% of the test substance would hydrolyze. The increase of the 8-2 TBA concentration between the Day 0 and Day 5 samples is simply a result of extraction of the 8-2 TBA from [ ] during the hydrolytic stability experiment at elevated temperature (50°C or 37°C). The percent recovery results for 8-2 TBA (Tables 5-8) support the validity of the 8-2 TBA measurement in the Day 0 and Day 5 samples for all investigated pHs. The Day 5 sample percent recovery results demonstrate that no significant loss of 8-2 TBA occurred from the test system during the hydrolytic stability experiment. Figure 6 shows representative calibration curve obtained for 8-2

TBA. Figures 7-9 show chromatograms for 8-2 TBA calibration standard, Day 0, pH 4.0 test sample, and Day 0, pH 4.0 blank sample, respectively.

Table 9 summarizes the water bath temperature data for pH 1.2, 4.0, 7.0, and 9.0. In all cases, the water bath temperature was maintained at the specified level with deviation not exceeding ± 0.1°C.

Table 10 summarizes results of the pH measurements for Day 0 and Day 5 samples for pH 1.2, 4.0, 7.0, and 9.0. The measured pH for Day 0 and Day 5 replicate samples for all of the investigated pHs was at the specified nominal level with the deviations not exceeding ± 0.1.

CONCLUSIONS

The hydrolytic stability of [ ], a fluorotelomer-based acrylate copolymer, was tested in buffered aqueous solutions at pH 4.0, 7.0, 9.0 at 50°C and pH 1.2 at 37°C for five days. The [ ] was demonstrated to be hydrolytically stable under these test conditions. The stability of the [ ] was demonstrated by the comparison of the percent recoveries of [ ] for Day 0 and Day 5 samples and analysis of chromatographic peak shape for Day 5 samples. The difference in percent recoveries for Day 0 and Day 5 samples did not reach the critical level of 10% that would indicate the hydrolysis of the test substance for any of the investigated test conditions. In addition, the measured concentrations of 8-2 TBA indicated an increase of the 8-2 TBA concentration between Day 0 and Day 5. The increase of the 8-2 TBA concentration can be accounted for by the presence of the residual 8-2 TBA in the test substance. The increase of 8-2 TBA concentration is approximately two orders of magnitude lower than the concentration of 8-2 TBA expected if 10% of the test substance hydrolyzed.

RECORDS AND SAMPLE STORAGE

Specimens (if applicable), raw data, and the final report will be retained at Haskell Laboratory, Newark, Delaware, or at Iron Mountain Records Management, Wilmington, Delaware.

REFERENCES

1. Organisation for Economic Cooperation and Development (OECD). May 12, 1981. OECD Guidelines for Testing of Chemicals, Hydrolysis as a Function of pH, OECD Guideline No. 111.

TABLES

Table 1: Analytical results for aqueous stability of [ ], pH 1.2, 37°C, Days 0 (10-Sep-2003) and 5 (15-Sep-2003).

pH	Day	Rep	[ ] Nominal Concentration (mg/L)	[ ] Measured Concentration (mg/L)	% Recovery
1.2	0	1	10100	10100	100
1.2	0	2	10100	10300	102
1.2	0	3	10000	10200	102
1.2	0	4	10200	10200	100
				Average	101
				Standard Deviation	1.2
1.2	5	1	10200	10100	99
1.2	5	2	10000	10100	101
1.2	5	3	10200	10300	101
1.2	5	4	10300	10400	101
				Average	101
				Standard Deviation	1.0

Table 2: Analytical results for aqueous stability of [ ], pH 4.0, 50°C, Days 0 (28-Aug-2003) and 5 (02-Sep-2003).

pH	Day	Rep	[ ] Nominal Concentration (mg/L)	[ ] Measured Concentration (mg/L)	% Recovery
4.0	0	1	10100	9960	99
4.0	0	2	10100	10100	100
4.0	0	3	10000	10000	100
4.0	0	4	10200	10200	100
				Average	100
				Standard Deviation	0.5
4.0	5	1	10200	9660	95
4.0	5	2	10100	9810	97
4.0	5	3	10100	10100	100
4.0	5	4	10100	9810	97
				Average	97
				Standard Deviation	2.1

Table 3: Analytical results for aqueous stability of [ ], pH 7.0, 50°C, Days 0 (28-Aug-2003) and 5 (02-Sep-2003).

pH	Day	Rep	[ ] Nominal Concentration (mg/L)	[ ] Measured Concentration (mg/L)	% Recovery
7.0	0	1	10200	10100	99
7.0	0	2	10100	10200	101
7.0	0	3	10100	10300	102
7.0	0	4	10000	10100	101
				Average	101
				Standard Deviation	1.3
7.0	5	1	10200	10200	100
7.0	5	2	10200	10200	100
7.0	5	3	10100	10200	101
7.0	5	4	10000	9910	99
				Average	100
				Standard Deviation	0.8

Table 4: Analytical results for aqueous stability of [ ], pH 9.0, 50°C, Days 0 (03-Sep-2003) and 5 (08-Sep-2003).

pH	Day	Rep	[ ] Nominal Concentration (mg/L)	[ ] Measured Concentration (mg/L)	% Recovery
9.0	0	1	10100	9950	99
9.0	0	2	10000	10100	101
9.0	0	3	10000	10000	100
9.0	0	4	10100	10000	99
				Average	100
				Standard Deviation	1.0
9.0	5	1	10000	10200	102
9.0	5	2	10100	10300	102
9.0	5	3	10000	10500	105
9.0	5	4	10300	11000	107
				Average	104
				Standard Deviation	2.5

Table 5: Measured concentrations of 8-2 TBA for pH 1.2, 37°C, Days 0 (10-Sep-2003) and 5 (15-Sep-2003).

pH	Day	Rep [a]	Nominal 8-2 TBA Concentration (µg/L)	Measured 8-2 TBA Concentration (µg/L) [b]	% Recovery
1.2	0	1	-	727	-
1.2	0	2	-	774	-
1.2	0	3	-	762	-
1.2	0	4	-	895	-
			Average	790	
			Standard Deviation	73	
1.2	0	QC1	510	595	117
1.2	0	QC2	510	594	116
1.2	0	QC3	510	633	124
				Average	119
				Standard Deviation	4.4
1.2	5	1	-	493	-
1.2	5	2	-	688	-
1.2	5	3	-	658	-
1.2	5	4	-	490	-
			Average	582	
			Standard Deviation	110	
1.2	5	QC1	510	440	86
1.2	5	QC2	510	471	92
1.2	5	QC3	510	476	93
				Average	90
				Standard Deviation	3.8

[a] QC1, QC2 and QC3 represent three 8-2 TBA recovery samples for each test day.
[b] Concentrations of 8-2 TBA in the extract (3 mL buffer and 6 mL acetonitrile).

Table 6: Measured concentrations of 8-2 TBA for pH 4.0, 50°C, Days 0 (28-Aug-2003) and 5 (02-Sep-2003).

pH	Day	Rep [a]	Nominal 8-2 TBA Concentration (µg/L)	Measured 8-2 TBA Concentration (µg/L) [b]	% Recovery
4.0	0	1	-	778	-
4.0	0	2	-	699	-
4.0	0	3	-	795	-
4.0	0	4	-	740	-
			Average	753	
			Standard Deviation	43	
4.0	0	QC1	510	495	97
4.0	0	QC2	510	508	100
4.0	0	QC3	510	487	95
				Average	97
				Standard Deviation	2.5
4.0	5	1	-	1560	-
4.0	5	2	-	1680	-
4.0	5	3	-	1680	-
4.0	5	4	-	1430	-
			Average	1590	
			Standard Deviation	120	
4.0	5	QC1	510	464	91
4.0	5	QC2	510	471	92
4.0	5	QC3	510	447	88
				Average	90
				Standard Deviation	2.1

[a] QC1, QC2 and QC3 represent three 8-2 TBA recovery samples for each test day.
[b] Concentrations of 8-2 TBA in the extract (3 mL buffer and 6 mL acetonitrile).

Table 7: Measured concentrations of 8-2 TBA for pH 7.0, 50°C, Days 0 (28-Aug-2003) and 5 (02-Sep-2003).

pH	Day	Rep [a]	Nominal 8-2 TBA Concentration (µg/L)	Measured 8-2 TBA Concentration (µg/L) [b]	% Recovery
7.0	0	1	-	796	-
7.0	0	2	-	875	-
7.0	0	3	-	841	-
7.0	0	4	-	801	-
			Average	828	
			Standard Deviation	37	
7.0	0	QC1	510	519	102
7.0	0	QC2	510	494	97
7.0	0	QC3	510	499	98
				Average	99
				Standard Deviation	2.7
7.0	5	1	-	1670	-
7.0	5	2	-	1300	-
7.0	5	3	-	1800	-
7.0	5	4	-	1380	-
			Average	1540	
			Standard Deviation	240	
7.0	5	QC1	510	565	111
7.0	5	QC2	510	411	81
7.0	5	QC3	510	462	91
				Average	94
				Standard Deviation	15

[a] QC1, QC2 and QC3 represent three 8-2 TBA recovery samples for each test day.
[b] Concentrations of 8-2 TBA in the extract (3 mL buffer and 6 mL acetonitrile).

Table 8: Measured concentrations of 8-2 TBA for pH 9.0, 50°C, Days 0 (03-Sep-2003) and 5 (08-Sep-2003).

pH	Day	Rep [a]	Nominal 8-2 TBA Concentration (µg/L)	Measured 8-2 TBA Concentration (µg/L) [b]	% Recovery
9.0	0	1	-	796	-
9.0	0	2	-	519	-
9.0	0	3	-	864	-
9.0	0	4	-	704	-
			Average	721	
			Standard Deviation	150	
9.0	0	QC1	510	483	95
9.0	0	QC2	510	492	96
9.0	0	QC3	510	-	-
				Average	96
				Standard Deviation	0.7
9.0	5	1	-	800	-
9.0	5	2	-	757	-
9.0	5	3	-	868	-
9.0	5	4	-	1010	-
			Average	859	
			Standard Deviation	110	
9.0	5	QC1	510	437	86
9.0	5	QC2	510	472	93
9.0	5	QC3	510	417	82
				Average	87
				Standard Deviation	5.6

[a] QC1, QC2 and QC3 represent three 8-2 TBA recovery samples for each test day.
[b] Concentrations of 8-2 TBA in the extract (3 mL buffer and 6 mL acetonitrile).

Table 9: Daily temperature readings of the shaking water bath.

Time (Day)	pH	Date	Temperature (°C)
0	1.2	10-Sep-2003	37.1
1	1.2	11-Sep-2003	37.0
2	1.2	12-Sep-2003	37.0
3	1.2	13-Sep-2003	37.0
4	1.2	14-Sep-2003	37.1
5	1.2	15-Sep-2003	37.0
0	4.0	28-Aug-2003	50.0
1	4.0	29-Aug-2003	50.1
2	4.0	30-Aug-2003	50.0
3	4.0	31-Aug-2003	49.9
4	4.0	01-Sep-2003	50.0
5	4.0	02-Sep-2003	50.0
0	7.0	28-Aug-2003	50.0
1	7.0	29-Aug-2003	50.1
2	7.0	30-Aug-2003	50.0
3	7.0	31-Aug-2003	49.9
4	7.0	01-Sep-2003	50.0
5	7.0	02-Sep-2003	50.0
0	9.0	03-Sep-2003	50.0
1	9.0	04-Sep-2003	50.0
2	9.0	05-Sep-2003	49.9
3	9.0	06-Sep-2003	50.0
4	9.0	07-Sep-2003	50.0
5	9.0	08-Sep-2003	50.0

Table 10: pH measurements for Day 0 and Day 5.

Day	Rep	Nominal pH	Measured pH
0	1	1.2	1.25
0	2	1.2	1.25
0	3	1.2	1.26
0	4	1.2	1.25
5	1	1.2	1.29
5	2	1.2	1.30
5	3	1.2	1.28
5	4	1.2	1.29
0	1	4.0	4.10
0	2	4.0	4.10
0	3	4.0	4.10
0	4	4.0	4.10
5	1	4.0	4.10
5	2	4.0	4.10
5	3	4.0	4.10
5	4	4.0	4.10
0	1	7.0	7.05
0	2	7.0	7.06
0	3	7.0	7.05
0	4	7.0	7.05
5	1	7.0	7.10
5	2	7.0	7.10
5	3	7.0	7.10
5	4	7.0	7.10
0	1	9.0	9.05
0	2	9.0	9.06
0	3	9.0	9.05
0	4	9.0	9.05
5	1	9.0	9.10
5	2	9.0	9.08
5	3	9.0	9.09
5	4	9.0	9.08

FIGURES

Figure 1: LC chromatograms of four Day 5, pH 1.2 replicate samples (A), 7500 mg/L (B), and 15000 mg/L (C) calibration standards of [ ].

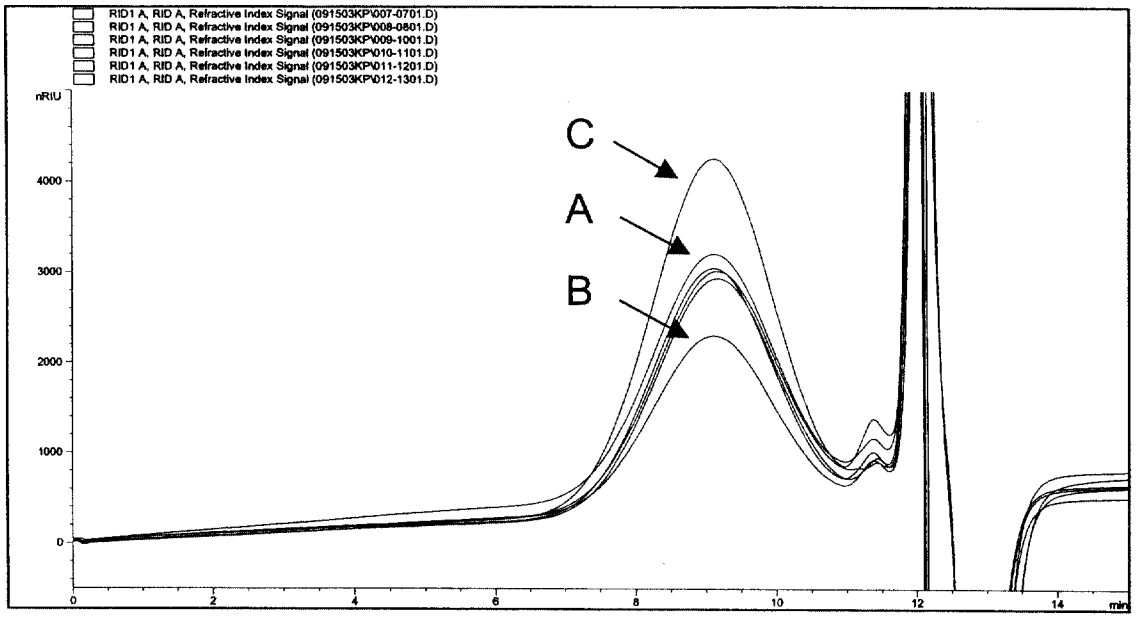

Figure 2: LC chromatograms of four Day 5, pH 4.0 replicate samples (A), 7500 mg/L (B), and 15000 mg/L (C) calibration standards of [ ].

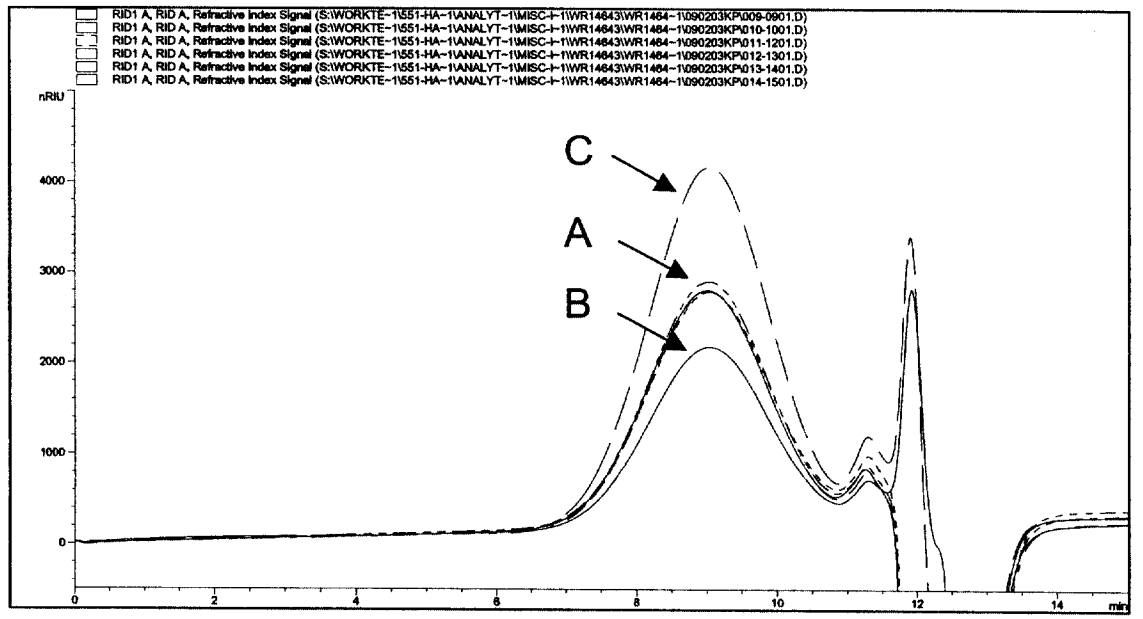

Figure 3: LC chromatograms of four Day 5, pH 7.0 replicate samples (A), 7500 mg/L (B), and 15000 mg/L (C) calibration standards of [ ].

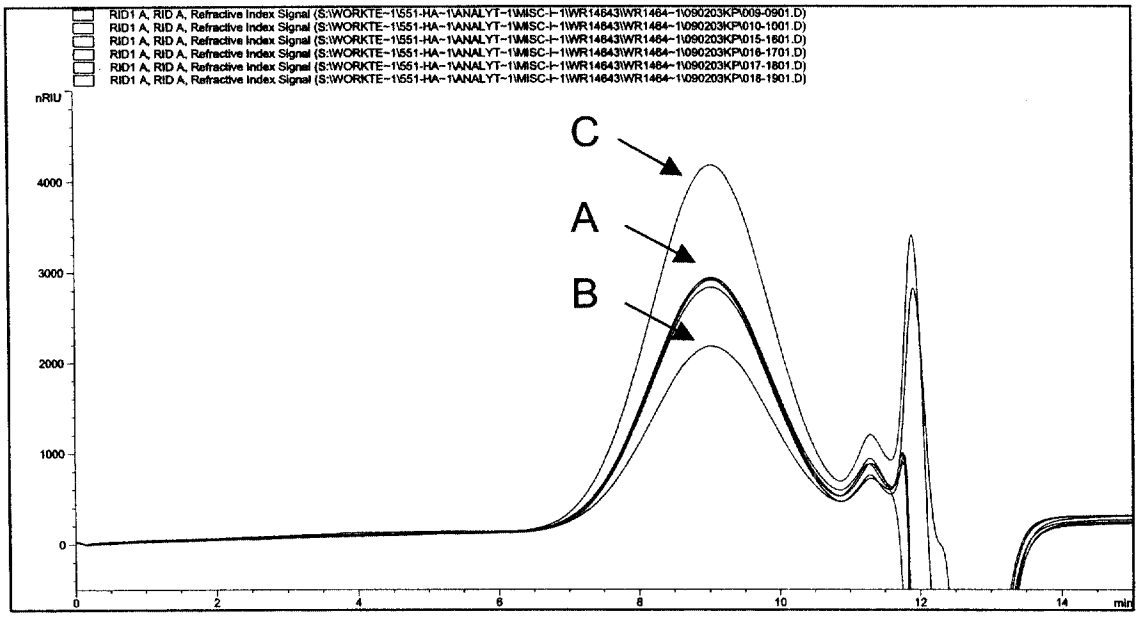

Figure 4: LC chromatograms of four Day 5, pH 9.0 replicate samples (A), 7500 mg/L (B), and 15000 mg/L (C) calibration standards of [ ].

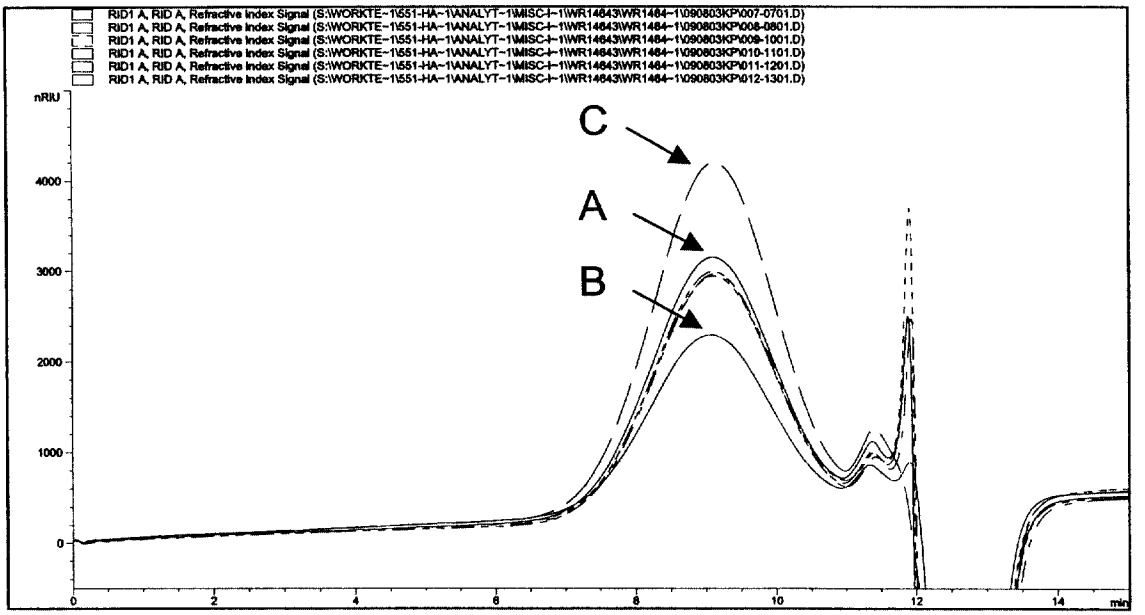

Figure 5: Representative LC chromatograms of blank samples for Day 5, pH 1.2, 4.0, 7.0, and 9.0.

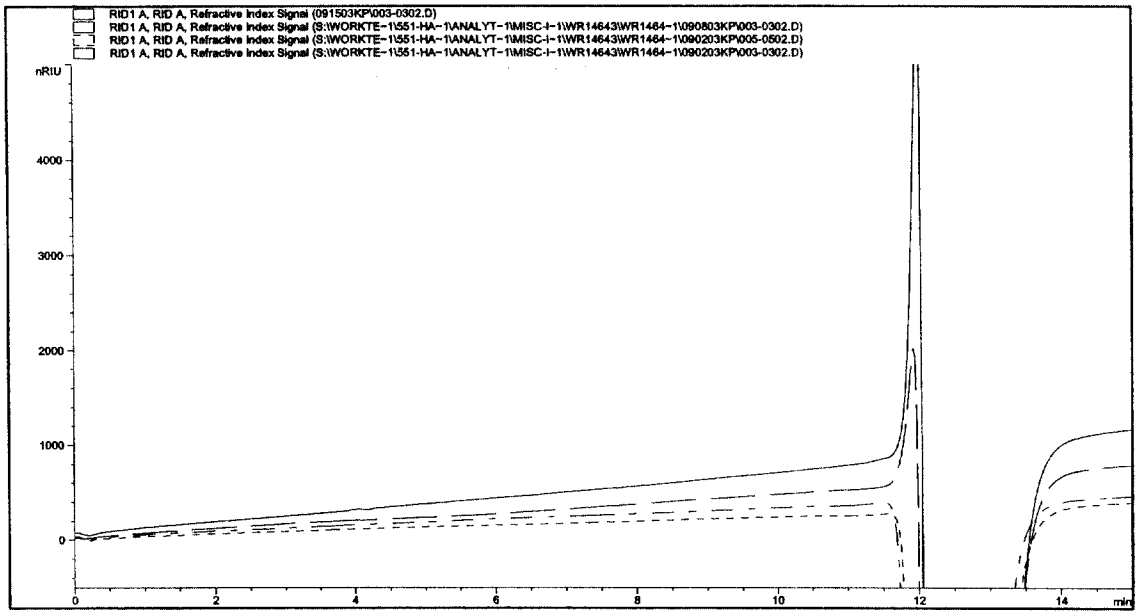

Figure 6: Representative calibration curve for 8-2 TBA.

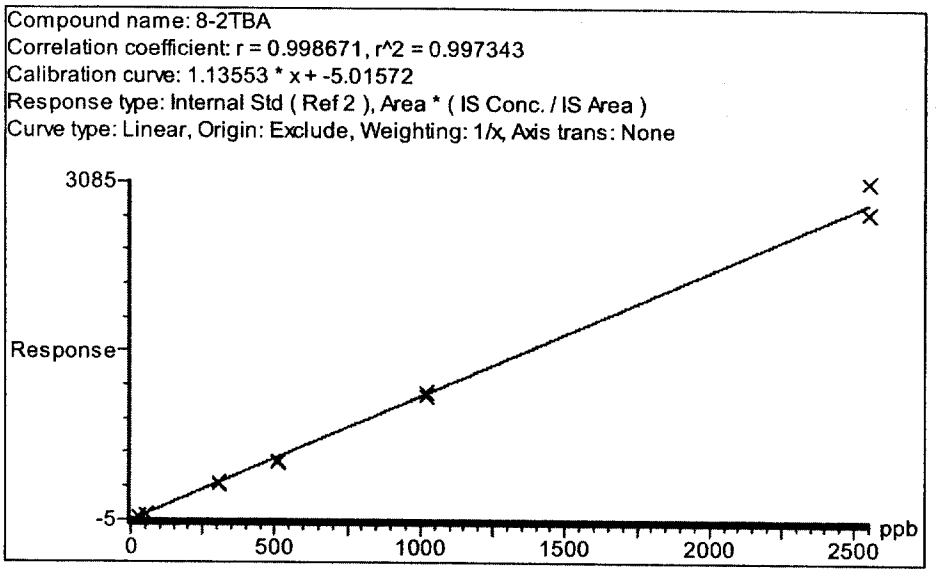

Figure 7: Representative chromatograms of 25.5 µg/L 8-2 TBA calibration standard and 520 µg/L D-8-2 TBA internal standard.

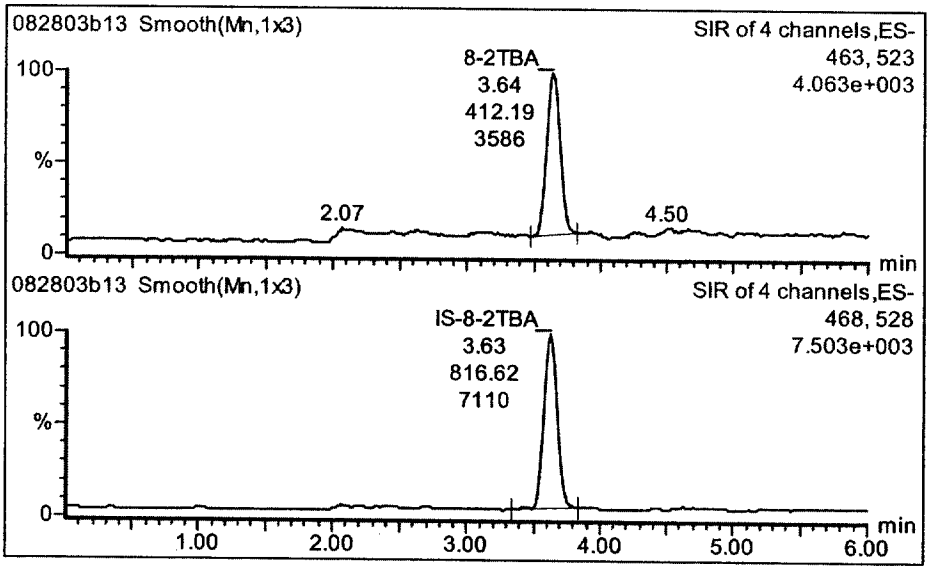

Figure 8: Representative chromatograms for Day 0, pH 4.0 hydrolytic stability sample and 520 µg/L D-8-2 TBA internal standard.

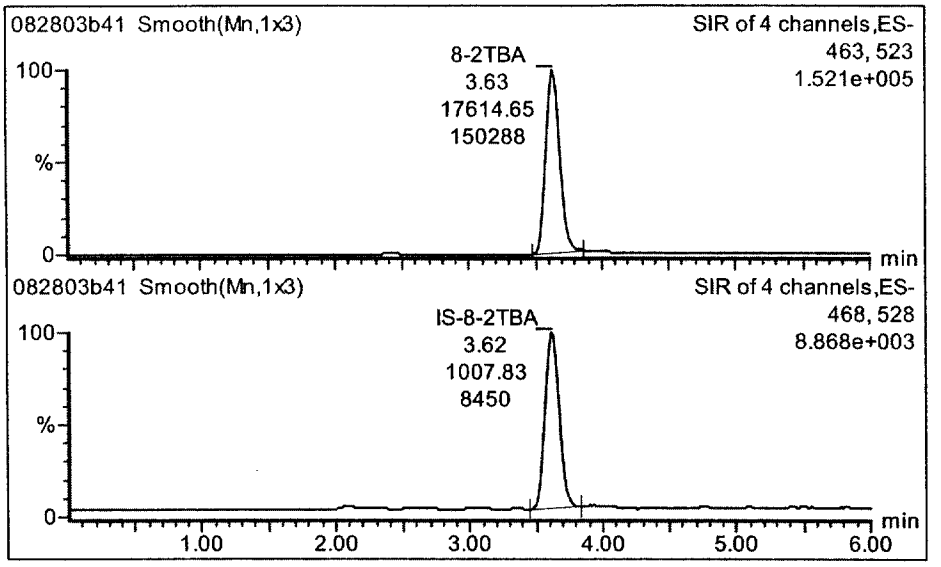

Figure 9: Representative chromatograms for Day 0, pH 4.0 blank sample.