AR226_3317

TRADE SECRET

Study Title

H-24616: Hydrolytic Stability of H-24616 as a Function of pH

TEST GUIDELINES: OECD Guideline 111

AUTHOR: Bogdan Szostek, Ph.D.

STUDY COMPLETED ON: June 9, 2004

PERFORMING LABORATORY: E.I. du Pont de Nemours and Company
Haskell Laboratory for Health and Environmental Sciences
Elkton Road, P.O. Box 50
Newark, Delaware 19714-0050

LABORATORY PROJECT ID: DuPont-13970

WORK REQUEST NUMBER: [redacted]

SERVICE CODE NUMBER: [redacted]

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. 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: _____ 09-JUN-2004
Bogdan Szostek, Ph.D. **Date**
Senior Research Chemist

Applicant / Sponsor: _____ _____
DuPont Representative Date

QUALITY ASSURANCE STATEMENT

Haskell Sample Number(s):

24616

Dates of Inspections:

Conduct: 29-Jan-2004

Records, Reports: 29-30-Mar-2004; 2-Apr-2004

Dates Findings Reported to:

Study Director: 29-Jan-2004; 7-Apr-2004

Management: 29-Jan-2004; 7-Apr-2004

Reported by: Matthew Frentzel 09-Jun-2004
Matthew Frentzel Date
Quality Assurance Auditor

CERTIFICATION

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

Approved by: _____ 09-JUN-2004
S. Mark Kennedy, Ph.D. Date
Manager

Issued by Study Director: _____ 09-JUN-2004
Bogdan Szostek, Ph.D. Date
Senior Research Chemist

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

STUDY INFORMATION

Substance Tested: [redacted]

Synonyms/Codes: • H-24616
[redacted]

Haskell Number: 24616

Composition: [redacted]

Known Impurities in Total Solids: [redacted]

Physical Characteristics: Opaque tan liquid

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

Study Initiated/Completed: December 3, 2003/ (see report cover page)

Experimental Start/Completion: December 22, 2003 / March 2, 2004

STUDY PERSONNEL

Study Director: Bogdan Szostek, Ph.D.
Analytical Associate: Keith B. Prickett, B.S.
Management: S. Mark Kennedy, Ph.D.

Report Preparation: Wanda F. Dinbokowitz
Management: Kim D. Birkmeyer, M.S.

SUMMARY

Hydrolytic stability of H-24616 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. Concentrations of fluorinated active ingredient of H-24616 were measured by ^{19}F NMR spectroscopy and percent recoveries were calculated for Day 0 and Day 5 replicate samples for pH 1.2, 4.0 phthalate, 4.0 citrate, 7.0, 9.0 buffers. The average percent recoveries for the Day 0 and Day 5, pH 1.2 replicate samples were 104± 3.6% and 86 ± 4.0%, respectively. The average percent recoveries for the Day 0 and Day 5, pH 4.0 phthalate buffer replicate samples were 93 ± 2.9% and 82 ± 6.9%, respectively. The average percent recoveries for the Day 0 and Day 5, pH 4.0 citrate buffer replicate samples were 92 ± 3.2% and 89 ± 3.1%, respectively. The average percent recoveries for the Day 0 and Day 5, pH 7.0 replicate samples were 97± 2.0% and 91 ± 1.8%, respectively. The average percent recoveries for the Day 0 and Day 5, pH 9.0 replicate samples were 98± 4.2% and 92 ± 0.81%, respectively. The fluorinated active ingredient of H-24616 was demonstrated to be hydrolytically stable at pH 9.0, pH 7.0, and pH 4.0 citrate buffer with $t_{1/2}$ >one year using the criterion of Day 0 and Day 5 fluorinated active ingredient recoveries differing by no more than 10 percent. The data obtained for hydrolytic stability of fluorinated active ingredient of H-24616 at pH 1.2 and pH 4.0-phthalate buffers is not conclusive. The difference of recoveries for fluorinated active ingredient between Day 0 and Day 5 samples was larger than 10 percent. The data indicates that a significant portion of fluorinated active ingredient stays dissolved after addition of acetonitrile to the samples that were exposed to test conditions in pH 1.2 and pH 4.0-phthalate buffer. Accurate quantitation of the dissolved fraction of fluorinated active ingredient was not achievable for acetonitrile extract. Therefore, accurate mass balance of fluorinated active ingredient could not be achieved. The ^{19}F NMR spectra of the acetonitrile extracts for these pHs did not indicate any changes of the fluorinated active ingredient material.

The measured concentration of [redacted] did not indicate any hydrolytic degradation of the fluorinated active ingredient material to [redacted] for pH 4.0 and pH 7.0 buffers. The observed concentrations of [redacted] can be explained by the residual [redacted] present in the test substance (H-24616). For pH 1.2 and 9.0 an increase of the [redacted] concentration beyond that accounted for by residual [redacted] present in the test substance was observed. It was estimated that this increase would represent hydrolysis of 0.06 and 0.03% the test substance for pH 1.2 and pH 9.0 buffers, respectively.

INTRODUCTION

The purpose of the study was to investigate the hydrolytic stability of H-24616 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 H-24616 as a function of pH", which was patterned after the OECD Guideline 111.[1] Additionally, the concentration of the [redacted] was monitored to investigate if [redacted] 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 pH 4.0, 7.0, 9.0 and at 37 ± 0.1°C for pH 1.2. If less than 10% test substance hydrolysis was 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 [redacted]

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. The test substance fluorinated active ingredient [redacted] is essentially not soluble in water. Therefore, the study was conducted with excess of undissolved fluorinated active ingredient and the amount of the test substance was sufficient to satisfy the sensitivity requirements of analytical method. The molar concentration of fluorinated active ingredient was estimated to be 0.002 M (in 5. Calculations).

MATERIALS AND METHODS

A. Materials

1. Test Substance

Name: H-24616

Composition: [redacted]

Haskell number: 24616

The test substance was characterized to determine % fluorine, % solids, pH, Mn, Mw, and polydispersity with the following results:

% Fluorine (w/w):	12.3%
% Solids (w/w)	24.3%
pH	6.3
Mn:	1500 amu
Mw:	1700 amu
polydispersity	1.13

2. Residual [redacted] in H-24616

Residual [redacted] content in the test substance (H-24616) was determined by LC/MS using the same analytical method as described in Analysis of [redacted] in Methods. Five replicate samples of H-24616 (approximately 45 mg) were introduced to glass vials, weighed, 10 mL of THF (tetrahydrofurane) was added, vials crimped, and vortexed for 10 minutes on a multitube vortexer. A 0.1 mL aliquot of dissolved sample was transferred to a glass LC vial and 0.9 mL acetonitrile and internal standard [redacted] was added. Calibration standards were made in 10/90% THF/acetonitrile in the range from 100 to 1020 ng/mL [redacted]

3. 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 3.78 g of KCl (purity 99.0%, Sigma) in 250 mL of water. The 0.2 M hydrochloric acid (HCl) was prepared by diluting 4.93 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:

Phthalate buffer: 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 4.0 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.

Citrate buffer: 0.1 M solution of potassium dihydrogen citrate was prepared by dissolving 11.5 g of potassium dihydrogen citrate (purity 99+%, Fluka) in 500 mL of water. The pH 4 buffer solution was prepared by placing 45 mL of a 0.1 M sodium hydroxide solution and 250 mL of a 0.1 M potassium dihydrogen citrate 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 1 M sodium hydroxide.

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 hydrochloric acid.

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 9.0 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 filter. The buffer solutions were stored at room temperature.

4. Equipment

pH Meter:	Orion Model 250 A
Water Bath:	Precision Reciprocal Shaking Water Bath, Model 50, Precision
Autoclave:	AMSCO Model 3023
Automatic Pipettes:	Research Pro 100, 1000, 5000; Eppendorff
Balance:	Mettler AE-100
Centrifuge:	Sorval Legend RT
Vortexer:	Multi-tube vortexer, VWR
Glass Vials:	10 mL borosilicate Serum Type Reaction vials (Supelco) with 20 mm Barrier Septa (Foil/Silicone), 0.1" thick (Supelco). The vials and septa were autoclaved at 121°C for 30 min before use.

B. Methods

1. Preliminary test

The preliminary test was performed on H-24616 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 pHs tested. Approximately 45 mg of the H-24616 was weighed into sixteen 10-mL autoclaved glass vials and 3 mL of appropriate buffer solution was added. The vials were crimp caped with the aluminum foil covered part of the septa facing inside the vial. Eight of the vials were processed for analysis as the Day 0 samples. 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 Al 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 [redacted] stock solution (4.5 µL of 1020 mg/L [redacted]. The vials were crimp capped with the aluminum foil covered part of the septa facing inside the vial. Three of the vials were used as the QC samples for [redacted] 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 [redacted] analysis on Day 5. Four of the Day 0 or four of the Day 5 vials and two blank vials were processed for the fluorinated active ingredient of H-24616 determination. The other four of the Day 0 or Day 5 vials, two blanks vials, and [redacted] QC vials were processed for the [redacted] determination.

2. pH measurement and temperature

The pH was measured on Day 5 in the vials containing the buffer and the test substance, designated for the H-24616 analysis. Inadvertently, only pH of the buffers was measured on Day 0, not the pH of samples containing the test substance. Day 5 sample vials were uncapped, the pH electrode was introduced to the vials and the pH reading was taken. These vials were further processed for H-24616 analysis. The temperature of the water bath was monitored daily. The water bath has an automatic temperature control built in. The readings of the temperature control were checked and recorded daily.

3. Analysis of test substance

Vials designated for H-24616 analysis were uncapped, 6 mL of acetonitrile was added, vials were recapped and vortexed for 10 minutes. Addition of acetonitrile to the vial containing test substance suspended in a buffer solution causes the fluorinated active ingredient to precipitate. The vials were centrifuged for fifteen minutes at 3200 rpm, uncapped, and the liquid phase was removed from the vial. Ten milliliters of tetrahydrofurane (THF) was added to the vial, the vial was capped, and then sonicated for 30 minutes. Approximately 1 mL of the THF solution was placed in a glass LC vial, 0.1 mL of methyl-d_3-alcohol was added and subjected to ^{19}F-NMR analysis. The blank samples were subjected to the same procedure, without pH measurement.

The stock solution of H-24616 was made by dissolving 0.45 g of H-24616 in 10 mL of THF. Ten minutes of sonication was required to completely dissolve the test substance. Calibration standards for the H-24616 analysis were made by appropriate dilution of the stock. The calibration standards ranged from 200 to 2000 mg/L of fluorinated active ingredient of H-24616. Fresh stock solution and calibration standards were prepared for each instance of analysis. Linear regression of the peak area vs. standard concentration was used to establish the calibration curves.

Instrumentation and conditions:

NMR instrument:	Bruker AMX360 with a $^{19}F/^{1}H$ dual probe
Acquisition Parameters	Pulse Program: zg Time Domain Size: 16 k Number of Scans: 128 D1 Delay: 10 sec Dummy Scan: 2 Pulse Length: 8 µsec Receiver Gain: automated by the instrument Instrument Frequency: 338.8314566 MHz
Processing Parameters:	Size of Real Spectrum: 16 k Window Function: EM Window Size: 50 Hz
Peak Used for Quantitation	$-CF_3$ resonance at 32.5 ppm

4. Analysis of [redacted]

Samples designated for [redacted] analysis were processed by injecting 3 mL methyl-tert-butyl ether (MTBE) through the vial septum using a glass syringe. Neither Day 0 nor Day 5 samples were uncapped before MTBE injection to avoid any losses of [redacted] The vials containing injected MTBE were vortexed for 10 minutes on a multitube vortexer. Subsequently, the vials were uncapped and 0.1 mL of the MTBE extract was transferred to a glass LC vial, 0.9 mL acetonitrile added, spiked with internal standard, crimped capped, and subjected to LC/MS analysis for [redacted]

The [redacted] Oakwood Products, West Columbia, SC) was used as the analytical standard of [redacted] The [redacted] 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 10/90% MTBE/acetonitrile. The calibration standards were made in the range from 100 to 10000 µg/L [redacted] A constant level of internal standard was used: 520 µg/L [redacted] The calibration curves were contracted using the ratio of the peak area for ions m/z monitored for [redacted] and peak area for ions monitored for [redacted] and the concentrations of [redacted]

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	0.25

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:	[redacted] SIR of 463, 523; 0-6 min
	[redacted] SIR of 467, 528 m/z; 0-6 min

5. Calculations

The following calculations were used to determine the nominal concentrations of H-24616 fluorinated active ingredient (Tables 1-5). For example, if 0.045 g of H-24616 containing 22.6% fluorinated active ingredient was weighed into the vial and 3 mL of buffer was added to the vial the nominal concentration of fluorinated active ingredient is: (0.045 g* 0.226*1000 mg/g) / 0.003 L = 3390 mg/L of fluorinated active ingredient. The % recovery is calculated by dividing the measured concentration by the nominal concentration, times 100%.

The expected (nominal) concentration of [redacted] in the spiked samples was calculated as follows: the 4.5 µL of 1020 mg/L [redacted] spike represents 4.5 µL x 1020 ng/µL= 4590 ng [redacted] This amount was extracted with 3 mL of MTBE. The expected nominal concentration of [redacted] in the MTBE extract is: 4590 ng/3 mL=1530 ng/mL. The % recovery is calculated by dividing the measured concentration by the nominal concentration, times 100%.

The molar concentration of fluorinated active ingredient can be estimated using average molecular weight (Mn) of fluorinated active ingredient: 1500 g/mole. When 45 mg of H-24616 is used, it represents (0.045 g * 0.226)/1500 g/mole = 6.78 10^{-6} mole of fluorinated active ingredient. Taking into account that 3 mL of buffer is used this would represent the molar concentration: 6.78 10^{-6} mole/0.003 L = 0.0023 M. The expected concentration of [redacted] n

the MTBE extract if 10% of the H-24616 hydrolyzed to form [redacted] can be estimated in the following way. Ten percent of 45 mg of H-24616 represents 4.5 mg. Taking into account that the test substance contains 12.3% fluorine and assuming that all fluorine is present as [redacted] the hydrolysis of the 4.5 mg of the test substance would release: 4.5 mg x 0.123 /(324 mg F/mmole [redacted] 464.1 mg/mmole) = 0.79 mg [redacted] This amount of [redacted] would be contained in 3 mLs of MTBE extract. Therefore, expected concentration of [redacted] would be: 0.79 mg/0.003 L= 263 mg/L.

The H-24616 contains residual [redacted] The expected, maximum concentration of [redacted] derived from residual [redacted] n the MTBE extract (3 mL), assuming complete extraction for a 45 mg sample of H-24616 is: 45 mg of H-24616 x (0.019%/100)/0.003 L= 2.85 mg/L (2850 µg/L).

RESULTS AND DISCUSSION

Tables 1-5 present the results for measured concentrations of fluorinated active ingredient of H-24616 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 104± 3.6% and 86 ± 4.0%, respectively. The average percent recoveries for the Day 0 and Day 5, pH 4.0 phthalate buffer replicate samples are 93 ± 2.9% and 82 ± 6.9%, respectively. The average percent recoveries for the Day 0 and Day 5, pH 4.0 citrate buffer replicate samples are 92 ± 3.2% and 89 ± 3.1%, respectively. The average percent recoveries for the Day 0 and Day 5, pH 7.0 replicate samples are 97± 2.0% and 91 ± 1.8%, respectively. The average percent recoveries for the Day 0 and Day 5, pH 9.0 replicate samples are 98± 4.2% and 92 ± 0.81%, respectively. The difference of average recoveries between the Day 5 and Day 0 samples of the fluorinated active ingredient was less than 10% for pHs 4.0 citrate buffer, pH 7.0, and pH 9.0. This indicates that the fluorinated active ingredient of H-24616 is hydrolytically stable at these pHs ($t_{1/2}$ >one year).

The hydrolytic stability experiment for pH 4.0 was done with two different buffers: phthalate and citrate (Table 2 and Table 3). Two different buffers were used as initial experiments with phthalate buffer indicated that the buffer composition affects the solubility of fluorinated active ingredient in a buffer when subjected to prolonged exposure (5 days) at elevated temperature (50°C). Simply, the addition of acetonitrile to the vial containing the test substance would not completely precipitate the fluorinated active ingredient. The amount of the active ingredient recovered in the THF extract would be 30-50% (Table 2) for the phthalate buffer. In order to determine if the unaccounted portion of the fluorinated active ingredient was present in the acetonitrile extract, the NMR spectrum of the extracts was collected and a quantitation of the fluorinated active ingredient in the acetonitrile extract was attempted. Accurate quantitation of the fluorinated active ingredient in the acetonitrile extract (acetonitrile extract contains 6 mL acetonitrile and 3 mL of a buffer) was not possible because the calibration standards of fluorinated active ingredient in the acetonitrile extract could not be prepared using the THF stock of fluorinated active ingredient. The fluorinated active ingredient would precipitate if introduced to the acetonitrile extract using THF stock. In order to determine the sensitivity difference between the NMR measurements done for THF and acetonitrile extract matrix, a calibration standard of [redacted] in both solvents was made and measured by the NMR methods. This allowed the derivation of the sensitivity-difference factor that was applied to correct for the measurement of fluorinated active ingredient in the acetonitrile extract vs. the calibration standards made in THF. However, this procedure is considered to give only semi-quantitative results. Table 2 results indicate that significant portion of the fluorinated active ingredient is present in the acetonitrile extract for Day 5 samples. Comparison of the ^{19}F NMR spectra of Day 5 THF extract, Day 5 acetonitrile extract, and a calibration standard indicates that the fluorinated active ingredient present in the acetonitrile extract did not undergo a chemical change that would be visible by ^{19}F NMR (Figure 1). The average recovery of the fluorinated active ingredient for Day 5 samples is lower by 11% (Table 2) than the recovery for Day 0. However, taking into account the semi-quantitative nature of results for acetonitrile extracts, it is difficult to draw a conclusion about hydrolytic stability ($t_{1/2}$ >one year) of the fluorinated active ingredient as the difference may be coming from the inaccuracy of the results obtained for the

acetonitrile extracts. The observed solubility issues can be linked to buffer composition, not the buffer's pH for the pH 4.0 buffers. The solubility issue was not observed for citrate, pH 4.0 buffer (Table 3).

The difference of average recoveries between the Day 5 and Day 0 samples for pH 1.2 is more than 10 percent. However, similarly to pH 4.0-phthalate buffer, the solubility issues were encountered for the pH 1.2 buffer. Table 1 indicates that a significant amount of fluorinated active ingredient was detected in the acetonitrile extracts, but, for the reasons described above for the phthalate buffer, accurate quantitation of the fluorinated active ingredient in the acetonitrile extract was not possible. Therefore, this data was not conclusive about the hydrolytic stability of the fluorinated active ingredient at pH 1.2. However, comparison of the NMR spectra of Day 5 THF extract, Day 5 acetonitrile extract for pH 1.2 buffer, and a calibration standard indicated that the fluorinated active ingredient present in the acetonitrile extract did not undergo a chemical change that would be visible by ^{19}F NMR (Figure 2). Figure 3 presents the NMR spectra of a blank sample and the Day 0 THF extract for pH 1.2. Figures 4-6 present the comparison of NMR spectra obtained for Day 0 and Day 5 THF extracts for pH 4.0 citrate, pH 7.0, and pH 9.0 buffers, respectively.

The content of residual [redacted] in H-24616 was measured before the start of the hydrolytic study. Based on the results of five replicates of the H-24616, the content of residual [redacted] in H-24616 was calculated to be 0.019±0.0003%. Assuming that the buffer extracts all the residual [redacted] from the test substance during the time of hydrolytic stability experiment, the expected concentration of [redacted] in the test system would be 2.85 mg/L (see 5 Calculations). The hydrolysis of the fluorinated active ingredient of H-24616 would most likely result in the release of [redacted] If ten percent of H-24616 hydrolyzed releasing the [redacted] the expected concentration of [redacted] in the test system would be 263 mg/L (see 5 Calculations). Tables 6-10 present the measured concentrations of [redacted] for Day 0 and Day 5 replicate samples for pH 1.2, 4.0 phthalate, 4.0 citrate, 7.0, 9.0, respectively. The measured concentration of [redacted] for pH 1.2 (Table 6) indicates the increase of [redacted] concentration for Day 5 samples beyond the level that can be accounted for by the residual [redacted] present in the test substance. The concentration of [redacted] measured for the Day 0 samples is comparable with the expected concentration of [redacted] originating from residual [redacted] The Day 5 concentration of [redacted] is higher by approximately 1.5 mg/L more than the expected concentration originating from the residual [redacted] The observed increase of [redacted] concentration indicates the hydrolysis of the test substance to form [redacted] This increase represents only estimated hydrolysis of approximately 0.06% (1.5 mg/L / 2630 mg/L * 100%) of the test substance present in the test system. The acceptable recovery results for the quality control samples (QC) for Day 0 and Day 5 demonstrate the validity of the [redacted] measurement (Table 6). The measured concentrations of [redacted] for pH 4.0 citrate, pH 4.0 phthalate, pH 7.0 test system do not exceed significantly the expected concentration of [redacted] originating from the residual [redacted] (Tables 7-9). This indicates that there is no contribution of [redacted] from the hydrolysis of the test substance. The recovery results for QC samples are also in the acceptable range (Tables 7-9). The measured concentration of [redacted] for pH 9.0 (Table 10) indicate the increase of [redacted] concentration for Day 5 samples beyond the level that can be accounted for by the residual [redacted] present in the test substance. The increase is approximately 0.85 mg/L [redacted] beyond the concentration explainable by the residual [redacted] and is indicative of generation of [redacted] from hydrolysis of fluorinated active

ingredient at pH 9.0. This increase represents an estimated hydrolysis of approximately 0.03% (0.85 mg/L / 2630 mg/L * 100 %) of the test substance present in the test system.

Figure 7 shows a representative calibration curve obtained for [redacted] Figures 8-10 show chromatograms for the [redacted] calibration standard, Day 5, pH 4.0 test sample, and Day 0, pH 4.0 blank sample, respectively.

Table 11 summarizes the water bath temperature recording for pH 1.2, 4.0, 7.0, and 9.0. In all cases, the water bath temperature was maintained at the specified level ± 0.1°C.

Table 12 summarizes results of the pH measurements for pH 1.2, 4.0, 7.0, and 9.0. Inadvertently, only pH of buffers was measured on Day 0, not the pH of samples containing the test substance. The measured pH of Day 0 buffers and Day 5 replicate samples for all of the investigated pHs was at the specified nominal level with the deviation not exceeding ± 0.1.

CONCLUSIONS

The hydrolytic stability of H-24616 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 fluorinated active ingredient of H-24616 was demonstrated to be hydrolytically stable at pH 9.0, pH 7.0, and pH 4.0 citrate buffer with $t_{1/2}$ >one year. The data obtained for hydrolytic stability of the fluorinated active ingredient of H-24616 at pH 1.2 and pH 4.0-phthalate buffers is not conclusive. The difference of recoveries for fluorinated active ingredient between Day 0 and Day 5 samples was larger than 10 percent. The data indicates that a significant portion of fluorinated active ingredient stays dissolved after addition of acetonitrile to the samples that were exposed to test conditions in pH 1.2 and pH 4.0-phthalate buffer. Accurate quantitation of the dissolved fraction of fluorinated active ingredient was not achievable for acetonitrile extract. Therefore, accurate mass balance of fluorinated active ingredient could not be achieved. The ^{19}F NMR spectra of the acetonitrile extracts for these pHs did not indicate any changes to the fluorinated active ingredient material.

The measured concentration of [redacted] did not indicate any hydrolytic degradation of the fluorinated active ingredient material to [redacted] for pH 4.0 and pH 7.0 buffers. The observed concentrations of [redacted] can be explained by the residual [redacted] present in the test substance (H-24616). For pH 1.2 and 9.0 an increase of the [redacted] concentration beyond that which can be accounted for by residual [redacted] present in the test substance was observed. It was estimated that this increase would represent hydrolysis of 0.06 and 0.03% the test substance for pH 1.2 and pH 9.0 buffers, respectively.

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 H-24616, pH 1.2, 37°C, Days 0 (12-Feb-2004) and 5 (17-Feb-2004).

pH	Day	Rep	Fluorinated active ingredient of H-24616 Nominal Concentration[5] (mg/L)	Fluorinated active ingredient of H-24616 Measured Concentration (mg/L)	% Recovery
1.2	0	1	3320	3570	108
1.2	0	2	3280	3300	101
1.2	0	3	3330	3520	106
1.2	0	4	3330	3350	101
				Average	104
				Standard Deviation	3.6
1.2	5	1	3360	2660[1]+236[2]	86[4]
1.2	5	2	3320	2710[3]	82
1.2	5	3	3340	2790[1]+234[2]	91[4]
1.2	5	4	3260	2440[1]+255[2]	83[4]
				Average	86
				Standard Deviation	4.0

[1] recovered in the THF extract
[2] recovered in the acetonitrile extract
[3] sample of acetonitrile extract not saved
[4] represents total recovery from THF and acetonitrile extract
[5] represents nominal concentration of fluorinated active ingredient in the buffer

Table 2: Analytical results for aqueous stability of H-24616, pH 4.0 phthalate buffer, 50°C, Days 0 (5-Feb-2004) and 5 (10-Feb-2004).

pH	Day	Rep	Fluorinated active ingredient of H-24616 Nominal Concentration[4] (mg/L)	Fluorinated active ingredient of H-24616 Measured Concentration (mg/L)	% Recovery
4.0	0	1	3380	3100	92
4.0	0	2	3320	2970	89
4.0	0	3	3340	3180	95
4.0	0	4	3330	3170	95
				Average	93
				Standard Deviation	2.9
4.0	5	1	3420	1510[1]+940[2]	72[3]
4.0	5	2	3330	1320[1]+1540[2]	86[3]
4.0	5	3	3300	1090[1]+1670[2]	84[3]
4.0	5	4	3410	1340[1]+1590[2]	86[3]
				Average	82
				Standard Deviation	6.9

[1] recovered in the THF extract
[2] recovered in the acetonitrile extract
[3] represents total recovery from THF and acetonitrile extract
[4] represents nominal concentration of fluorinated active ingredient in the buffer

Table 3: Analytical results for aqueous stability of H-24616, pH 4.0 citrate buffer, 50°C, Days 0 (5-Feb-2004) and 5 (10-Feb-2004).

pH	Day	Rep	Fluorinated active ingredient of H-24616 Nominal Concentration[5] (mg/L)	Fluorinated active ingredient of H-24616 Measured Concentration (mg/L)	% Recovery
4.0	0	1	3380	3080	91
4.0	0	2	3330	3170	95
4.0	0	3	3320	2920	88
4.0	0	4	3320	3130	94
				Average	92
				Standard Deviation	3.2
4.0	5	1	3350	2930	87
4.0	5	2	3320	834[1]+75[2]	27[3]
4.0	5	3	3330	2940	88
4.0	5	4	3360	3110	93
				Average	89[4]
				Standard Deviation	3.1[4]

[1] recovered in the THF extract
[2] recovered in the acetonitrile extract
[3] the vial most likely leaked during the experiment
[4] Day 5, replicate 2 excluded from calculation
[5] represents nominal concentration of fluorinated active ingredient in the buffer

Table 4: Analytical results for aqueous stability of H-24616, pH 7.0, 50°C, Days 0 (29-Jan-2004) and 5 (03-Feb-2004).

pH	Day	Rep	Fluorinated active ingredient of H-24616 Nominal Concentration (mg/L)[1]	Fluorinated active ingredient of H-24616 Measured Concentration (mg/L)	% Recovery
7.0	0	1	3420	3290	96
7.0	0	2	3320	3190	96
7.0	0	3	3360	3360	100
7.0	0	4	3360	3240	96
				Average	97
				Standard Deviation	2.0
7.0	5	1	3340	3060	92
7.0	5	2	3340	3100	93
7.0	5	3	3350	2990	89
7.0	5	4	3330	3000	90
				Average	91
				Standard Deviation	1.8

[1] represents nominal concentration of fluorinated active ingredient in the buffer

Table 5: Analytical results for aqueous stability of H-24616, pH 9.0, 50°C, Days 0 (29-Jan-2004) and 5 (03-Feb-2004).

pH	Day	Rep	Fluorinated active ingredient of H-24616 Nominal Concentration (mg/L)[1]	Fluorinated active ingredient of H-24616 Measured Concentration (mg/L)	% Recovery
9.0	0	1	3330	3180	95
9.0	0	2	3340	3180	95
9.0	0	3	3310	3440	104
9.0	0	4	3300	3240	98
				Average	98
				Standard Deviation	4.2
9.0	5	1	3340	3080	92
9.0	5	2	3330	3080	92
9.0	5	3	3330	3020	91
9.0	5	4	3330	3110	93
				Average	92
				Standard Deviation	0.81

[1] represents nominal concentration of fluorinated active ingredient in the buffer

Table 6: Measured concentrations of [redacted] for pH 1.2, 37°C, Days 0 (12-Feb-2004) and 5 (17-Feb-2004).

pH	Day	Rep	Nominal [redacted] Concentration (µg/L)	Measured [redacted] Concentration (µg/L)	% Recovery
1.2	0	1	-	2920	-
1.2	0	2	-	2950	-
1.2	0	3	-	2800	-
1.2	0	4	-	2950	-
			Average	2900	
			Standard Deviation	71	
1.2	0	QC1	1530	1580	103
1.2	0	QC2	1530	1580	103
1.2	0	QC3	1530	1580	103
				Average	103
				Standard Deviation	0.0
1.2	5	1	-	4010	-
1.2	5	2	-	4390	-
1.2	5	3	-	4060	-
1.2	5	4	-	4350	-
			Average	4200	
			Standard Deviation	200	
1.2	5	QC1	1530	1640	107
1.2	5	QC2	1530	1660	108
1.2	5	QC3	1530	1650	108
				Average	108
				Standard Deviation	0.6

Table 7: Measured concentrations of [redacted] for pH 4.0, 50°C, pH 4.0 phthalate buffer, 50°C, Days 0 (5-Feb-2004) and 5 (10-Feb-2004).

pH	Day	Rep	Nominal [redacted] Concentration (µg/L)	Measured [redacted] Concentration (µg/L)	% Recovery
4.0	0	1	-	2070	-
4.0	0	2	-	2210	-
4.0	0	3	-	2130	-
4.0	0	4	-	2070	-
			Average	2120	
			Standard Deviation	66	
4.0	0	QC1	1530	1570	103
4.0	0	QC2	1530	1600	105
4.0	0	QC3	1530	1580	103
				Average	104
				Standard Deviation	1.2
4.0	5	1	-	3400	-
4.0	5	2	-	3460	-
4.0	5	3	-	1710	-
4.0	5	4	-	1690	-
			Average	2570	
			Standard Deviation	1000	
4.0	5	QC1	1530	1650	108
4.0	5	QC2	1530	1430	93
4.0	5	QC3	1530	1680	110
				Average	104
				Standard Deviation	9.3

Table 8: Measured concentrations of [redacted] for pH 4.0, 50°C, pH 4.0 citrate buffer, 50°C, Days 0 (5-Feb-2004) and 5 (10-Feb-2004).

pH	Day	Rep	Nominal [redacted] Concentration (µg/L)	Measured [redacted] Concentration (µg/L)	% Recovery
4.0	0	1	-	2060	-
4.0	0	2	-	1990	-
4.0	0	3	-	2070	-
4.0	0	4	-	1600	-
			Average	1930	
			Standard Deviation	220	
4.0	0	QC1	1530	1740	114
4.0	0	QC2	1530	1600	105
4.0	0	QC3	1530	1560	102
				Average	107
				Standard Deviation	6.2
4.0	5	1	-	2720	-
4.0	5	2	-	3020	-
4.0	5	3	-	2960	-
4.0	5	4	-	2260	-
			Average	2740	
			Standard Deviation	350	
4.0	5	QC1	1530	1570	103
4.0	5	QC2	1530	1620	106
4.0	5	QC3	1530	1610	105
				Average	105
				Standard Deviation	1.5

Table 9: Measured concentrations of [redacted] for pH 7.0, 50°C, Days 0 (29-Jan-2004) and 5 (03-Feb-2004).

pH	Day	Rep	Nominal [redacted] Concentration (µg/L)	Measured [redacted] Concentration (µg/L)	% Recovery
7.0	0	1	-	2240	-
7.0	0	2	-	2280	-
7.0	0	3	-	2240	-
7.0	0	4	-	1450[1]	-
		Average		2250	
		Standard Deviation		23	
7.0	0	QC1	1530[2]	1660	108
7.0	0	QC2	1530	1660	108
7.0	0	QC3	1530	1670	109
				Average	108
				Standard Deviation	0.6
7.0	5	1	-	3180	-
7.0	5	2	-	3190	-
7.0	5	3	-	3180	-
7.0	5	4	-	3180	-
		Average		3180	
		Standard Deviation		5.0	
7.0	5	QC1	1530	1820	119
7.0	5	QC2	1530	1760	115
7.0	5	QC3	1530	1790	117
				Average	117
				Standard Deviation	2.0

[1] Not used in the calculation of the average and standard deviation.

Table 10: Measured concentrations of [redacted] for pH 9.0, 50°C, Days 0 (29-Jan-2004) and 5 (03-Feb-2003).

pH	Day	Rep	Nominal [redacted] Concentration (µg/L)	Measured [redacted] Concentration (µg/L)	% Recovery
9.0	0	1	-	2340	-
9.0	0	2	-	2340	-
9.0	0	3	-	2290	-
9.0	0	4	-	2360	-
		Average		2330	
		Standard Deviation		30	
9.0	0	QC1	1530	1660	108
9.0	0	QC2	1530	1680	110
9.0	0	QC3	1530	1520	99
				Average	106
				Standard Deviation	5.9
9.0	5	1	-	3680	-
9.0	5	2	-	3720	-
9.0	5	3	-	3690	-
9.0	5	4	-	3710	-
		Average		3700	
		Standard Deviation		18	
9.0	5	QC1	1530	1670	109
9.0	5	QC2	1530	1660	108
9.0	5	QC3	1530	1690	110
				Average	109
				Standard Deviation	1.0

Table 11: Daily temperature readings of the shaking water bath.

Time (Day)	pH	Date	Temperature (°C)
0	1.2	12-Feb-2004	37.0
1	1.2	13-Feb-2004	37.0
2	1.2	14-Feb-2004	37.0
3	1.2	15-Feb-2004	37.0
4	1.2	16-Feb-2004	37.0
5	1.2	17-Feb-2004	37.0
0	4.0	05-Feb-2004	50.0
1	4.0	06-Feb-2004	50.0
2	4.0	07-Feb-2004	50.0
3	4.0	08-Feb-2004	50.0
4	4.0	09-Feb-2004	50.0
5	4.0	10-Feb-2004	50.0
0	7.0	29-Jan-2004	50.0
1	7.0	30-Jan-2004	50.0
2	7.0	31-Jan-2004	50.0
3	7.0	01-Feb-2004	50.0
4	7.0	02-Feb-2004	50.0
5	7.0	03-Feb-2004	50.0
0	9.0	29-Jan-2004	50.0
1	9.0	30-Jan-2004	50.0
2	9.0	31-Jan-2004	50.0
3	9.0	01-Feb-2004	50.0
4	9.0	02-Feb-2004	50.0
5	9.0	03-Feb-2004	50.0

Table 12: pH measurements for Day 0 and Day 5.

Day	Rep	Nominal pH	Measured pH
0	1, 2, 3, 4	1.2	-[1]
5	1	1.2	1.30
5	2	1.2	1.29
5	3	1.2	1.28
5	4	1.2	1.30
0	1, 2, 3, 4	4.0[2]	-[3]
5	1	4.0[2]	4.08
5	2	4.0[2]	4.08
5	3	4.0[2]	4.08
5	4	4.0[2]	4.09
0	1, 2, 3, 4	4.0[4]	-[5]
5	1	4.0[4]	4.06
5	2	4.0[4]	4.05
5	3	4.0[4]	4.06
5	4	4.0[4]	4.06
0	1, 2, 3, 4	7.0	-[6]
5	1	7.0	7.08
5	2	7.0	7.09
5	3	7.0	7.08
5	4	7.0	7.08
0	1, 2, 3, 4	9.0	-[7]
5	1	9.0	9.05
5	2	9.0	9.05
5	3	9.0	9.05
5	4	9.0	9.06

[1] pH not measured for replicate samples; measured buffer pH was 1.20
[2] pH 4.0 phthalate buffer
[3] pH not measured for replicate samples; measured buffer pH was 3.99
[4] pH 4.0 citrate buffer
[5] pH not measured replicate samples; measured buffer pH was 4.01
[6] pH not measured for replicate samples; measured buffer pH was 6.99
[7] pH not measured for replicate samples; measured buffer pH was 9.00

FIGURES

Figure 1: Example ^{19}F NMR spectra of 1050 mg/L fluorinated active ingredient calibration standard (A), fluorinated active ingredient recovered in THF extract (B), and acetonitrile extract (C) for Day 5, pH 4.0 phthalate buffer.

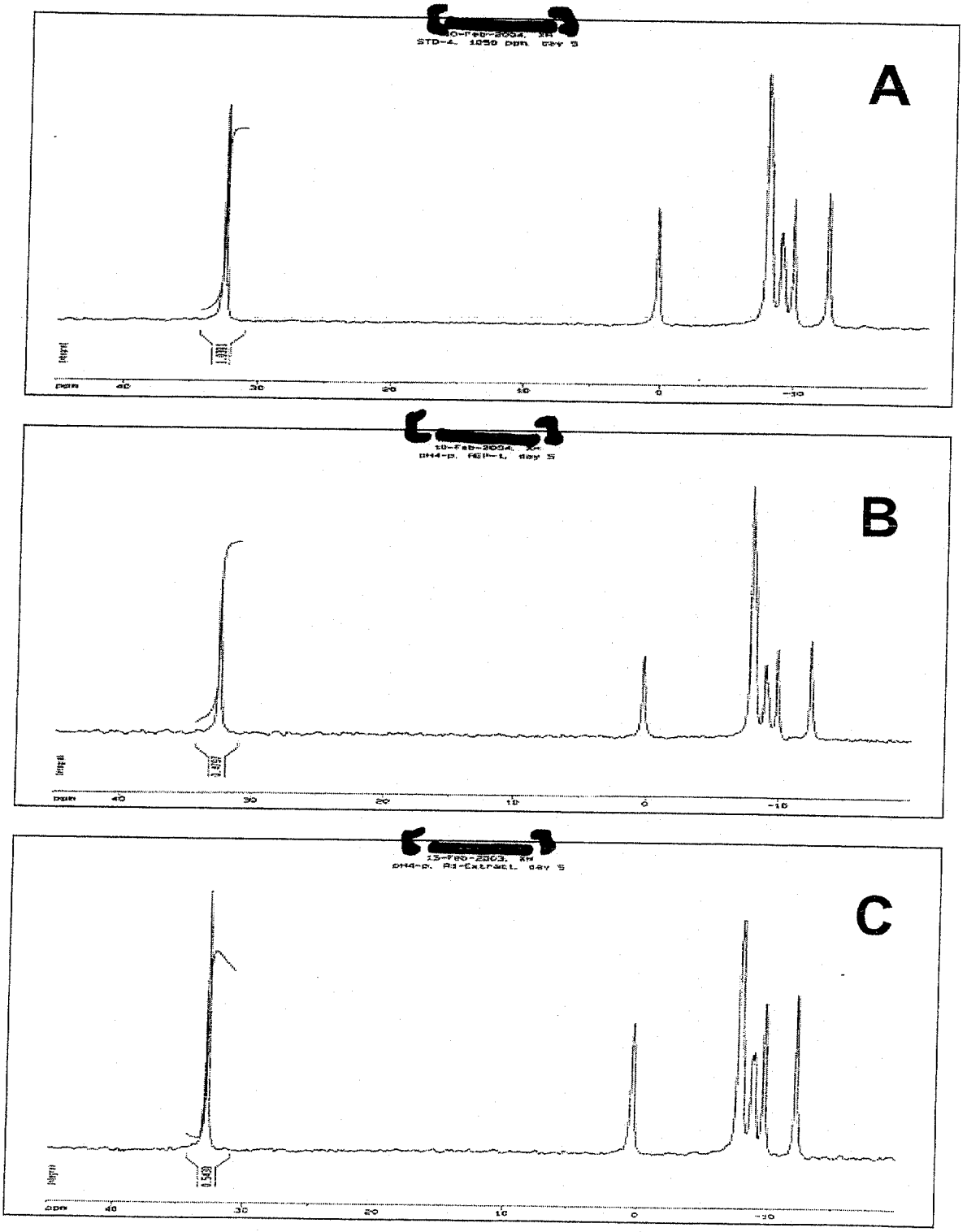

Figure 2: Example ^{19}F NMR spectra of 210 mg/L fluorinated active ingredient calibration standard (A), fluorinated active ingredient recovered in THF extract (B), and acetonitrile extract (C) for Day 5, pH 1.2 buffer.

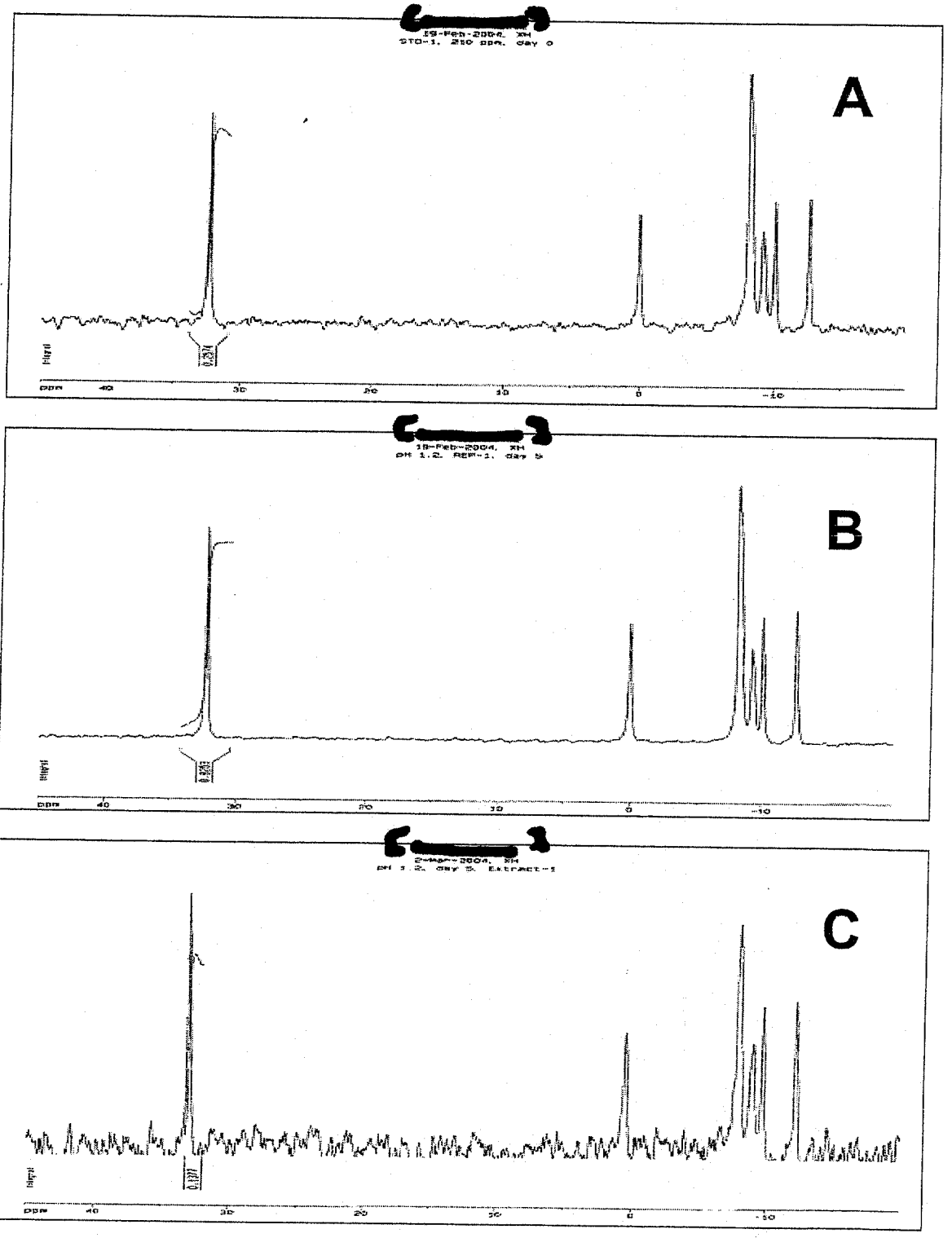

Figure 3: Example ^{19}F NMR spectra of a blank control sample for Day 5, pH 1.2 buffer (A) and the fluorinated active ingredient recovered in THF extract (B) sample for Day 0, pH 1.2 buffer (B).

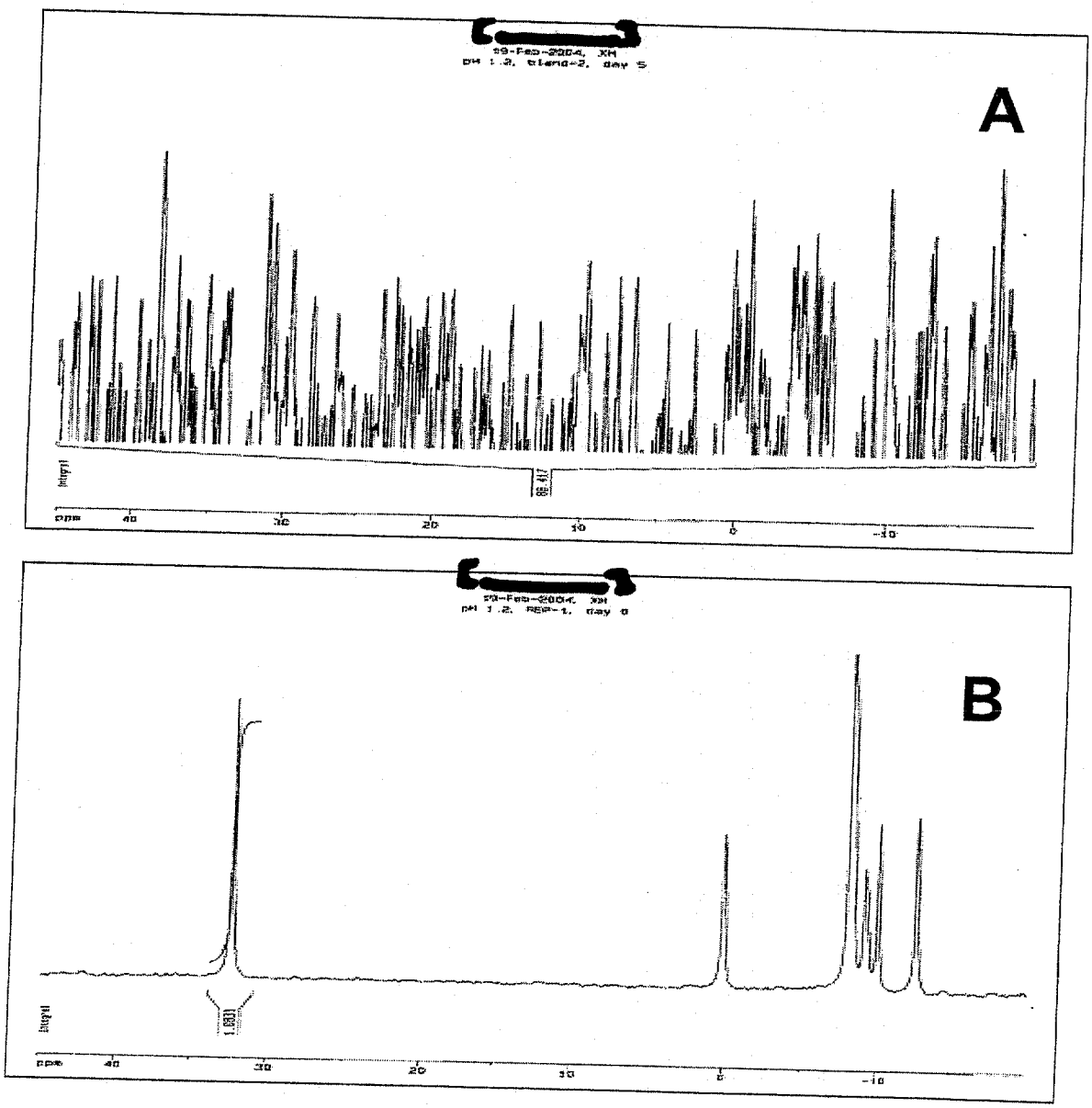

Figure 4: Example ^{19}F NMR spectra of the fluorinated active ingredient recovered in THF extract for Day 0, pH 4.0 citrate buffer (A) and Day 5, pH 4.0 citrate buffer (B).

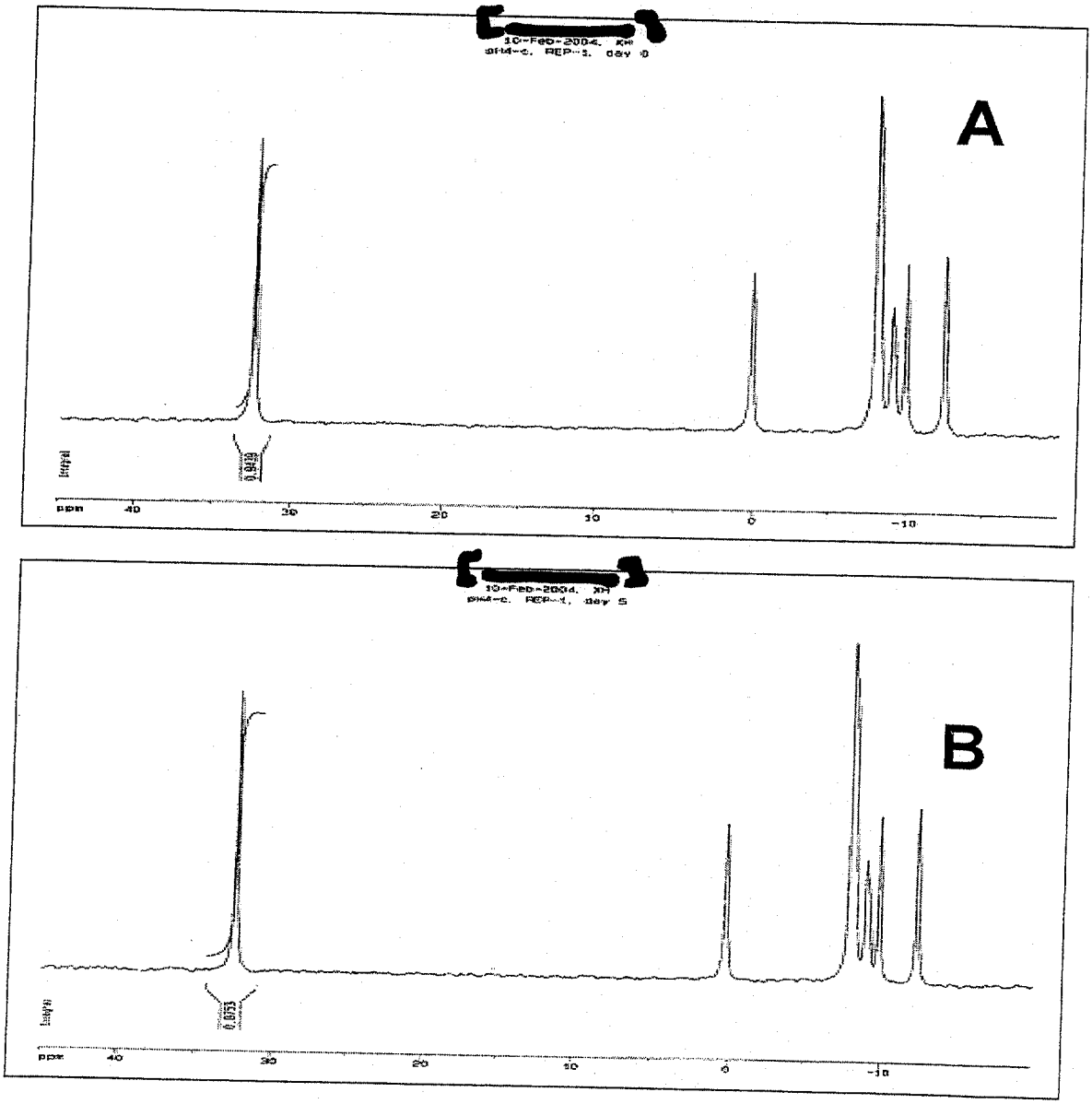

Figure 5: Example ^{19}F NMR spectra of the fluorinated active ingredient recovered in THF extract for Day 0, pH 7.0 buffer (A) and Day 5, pH 7.0 buffer (B).

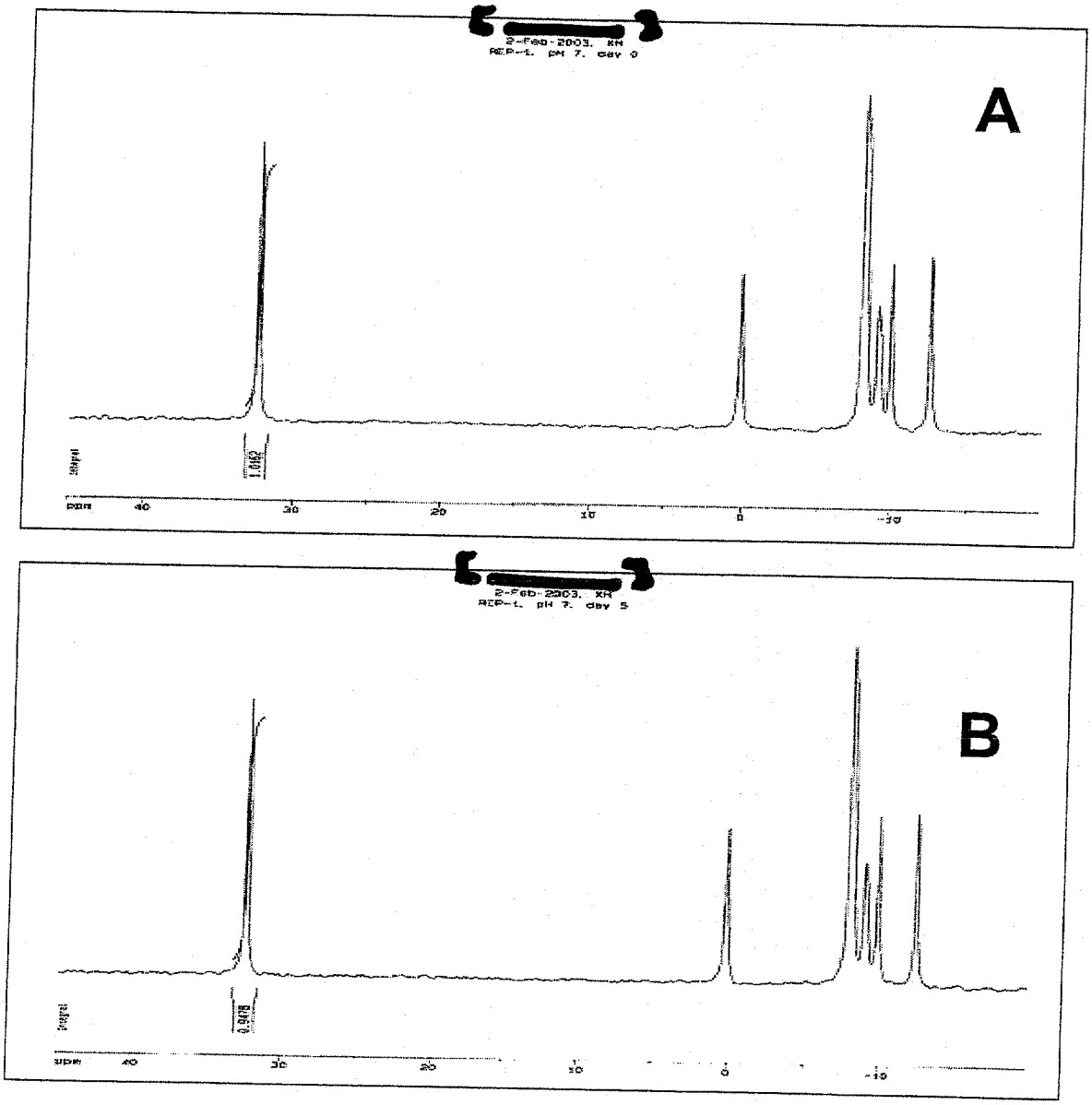

Figure 6: Example ^{19}F NMR spectra of the fluorinated active ingredient recovered in THF extract for Day 0, pH 9.0 buffer (A) and Day 5, pH 9.0 buffer (B).

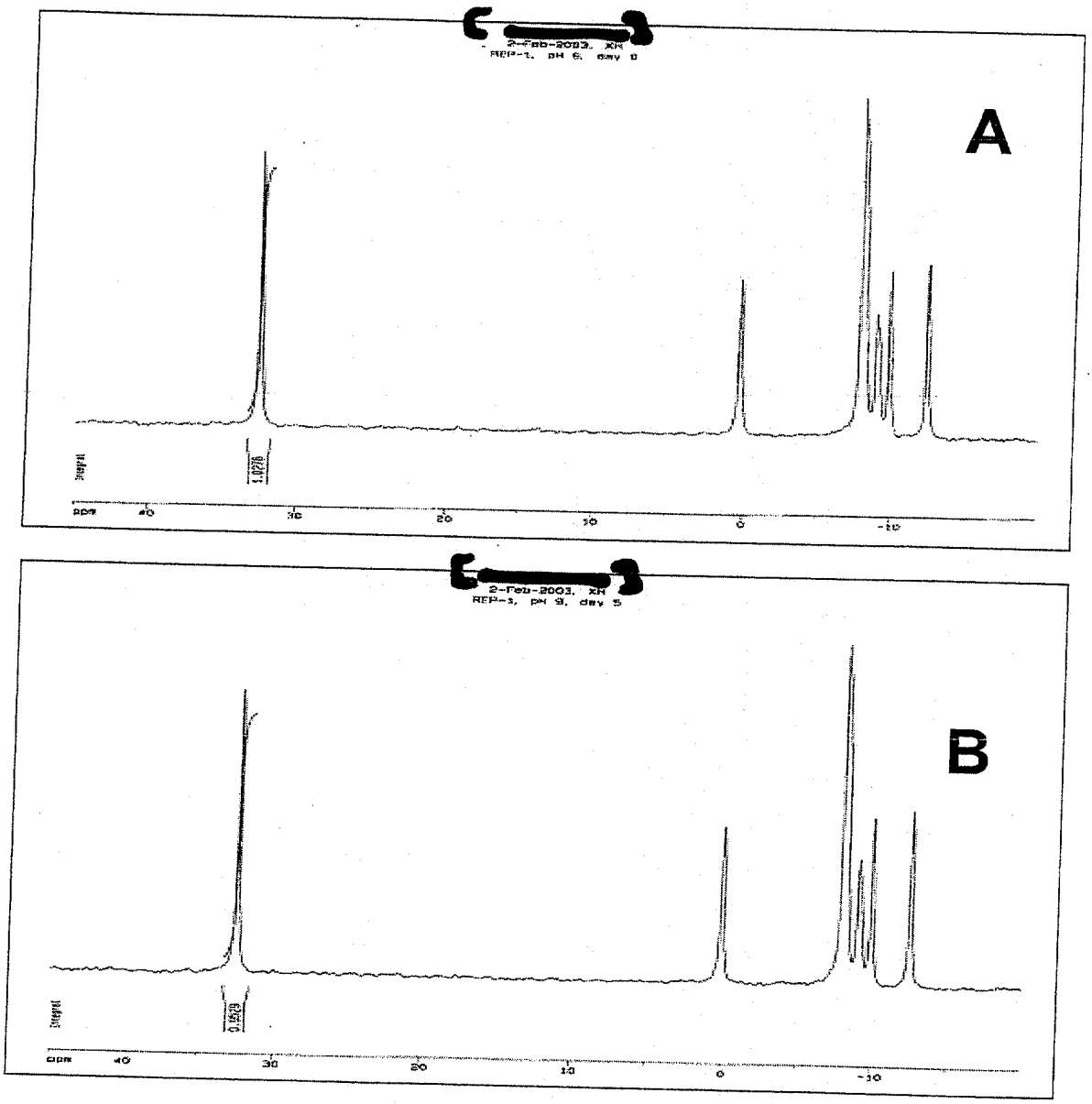

Figure 7: Representative calibration curve for [redacted]

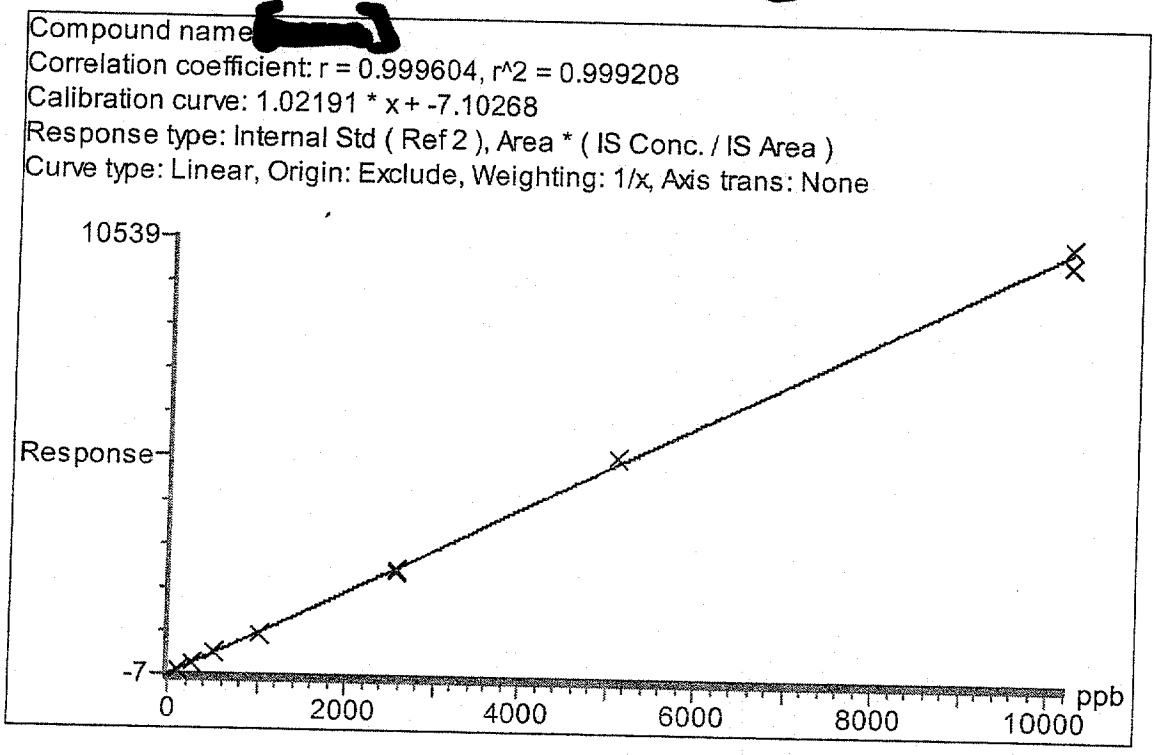

Figure 8: Representative chromatograms of 102 µg/L [redacted] **calibration standard and 520 µg/L** [redacted] **internal standard.**

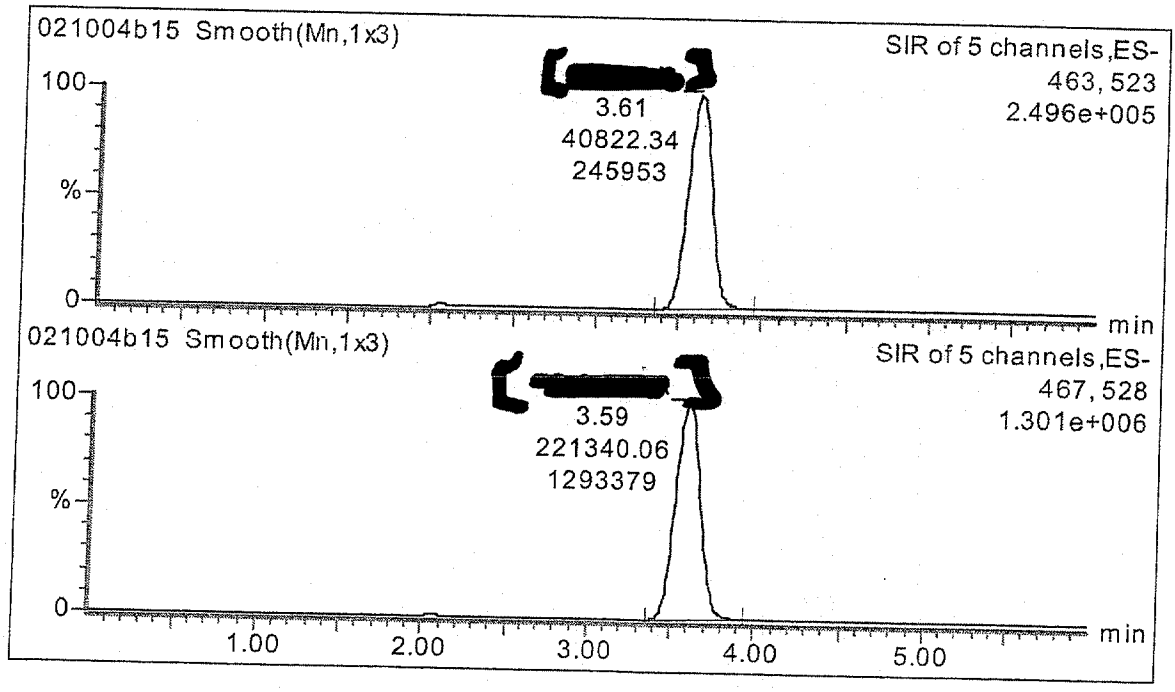

Figure 9: Representative chromatograms for Day 5, pH 4.0 citrate buffer hydrolytic stability sample and 520 µg/L [redacted] internal standard.

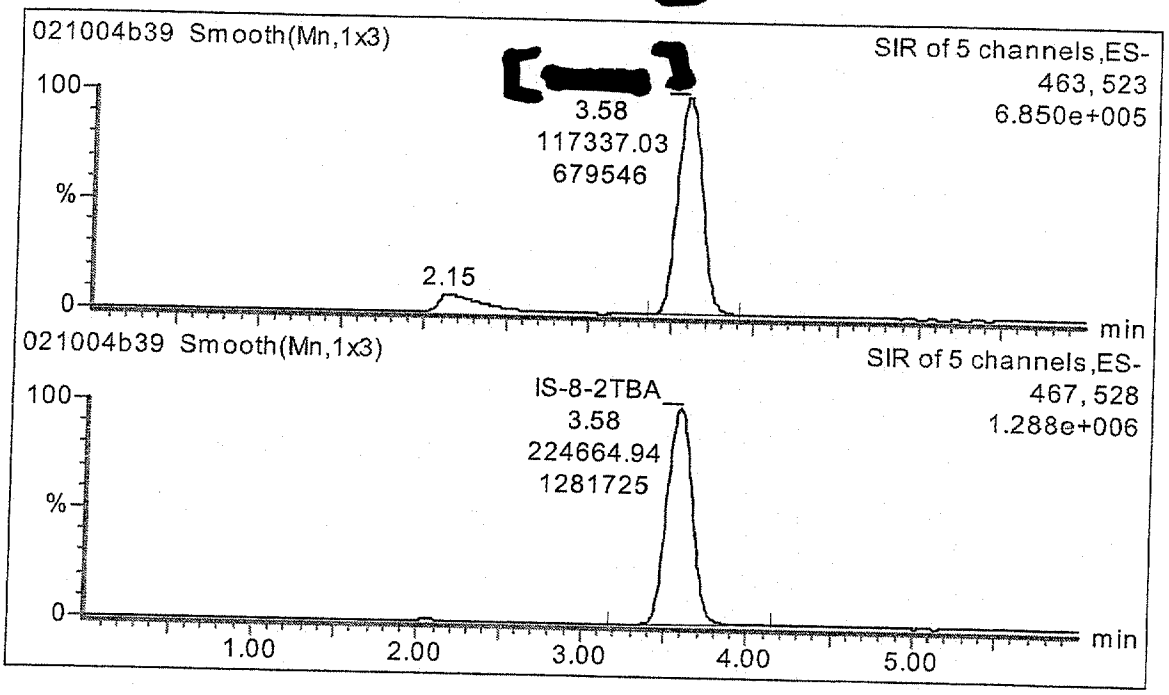

Figure 10: Representative chromatograms for Day 5, pH 4.0 citrate buffer blank sample and 520 µg/L [redacted] internal standard.