TRADE SECRET

Study Title

Hydrolytic Stability of H-25435 as a Function of pH

TEST GUIDELINES: OECD Guideline 111

AUTHOR: Bogdan Szostek, Ph.D.

STUDY COMPLETED ON: October 15, 2004

PERFORMING LABORATORY: E.I. du Pont de Nemours and Company
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LABORATORY PROJECT ID: DuPont-14965

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

WORK REQUEST NUMBER: 15028

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 compatible with the OECD Principles of Good Laboratory Practice (as revised 1997), ENV/MC/CHEM(98)17, OECD, Paris, 1998, and MAFF Japan Good Laboratory Practice Standards (11 NohSan Number 6283), except for the item documented below. The item listed does not impact the validity of the study.

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: _____ 15-OCT-2004
Bogdan Szostek, Ph.D. **Date**
Senior Research Chemist

Applicant / Sponsor: _____ _____
DuPont Representative Date

QUALITY ASSURANCE STATEMENT

Work Request Number: 15028
Study Code Number: 392

The conduct of this study has been subjected to periodic Quality Assurance inspections. The dates of inspection are indicated below.

Phase Audited	*Audit Dates*	*Dates Reported to Study Director and Management*
Conduct:	06 May 2004	07 May 2004
Report/Records:	09-12 Aug 2004	24 Aug 2004

Reported by: Donna M. Johnston 15 Oct 2004
Donna M. Johnston Date
Quality Assurance Auditor

CERTIFICATION

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

Approved by: _____ 15-OCT-2004
S. Mark Kennedy, Ph.D. Date
Manager

Issued by Study Director: _____ 15-OCT-2004
Bogdan Szostek, Ph.D. Date
Senior Research Chemist

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STUDY INFORMATION

Substance Tested: [ ]

Synonyms/Codes:
- [ ]
- H-25435
- [ ]

Haskell Number: 25435

Composition: [ ]
[ ]
[ ]
[ ]
[ ]

[ ]

Known Impurities: None identified

Physical Characteristics: Amber brown liquid

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

Study Initiated/Completed: May 4, 2004 / (see report cover page)

In-Life Initiated/Completed: May 6, 2004 / July 6, 2004

SUMMARY

The hydrolytic stability of H-25435 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. [ ] representing major chemical species present in H-25435 were monitored during the hydrolytic stability study. Measured concentrations and recoveries of individual [ ] reported as average concentrations and recoveries for Day 0 and Day 5 replicate samples, were used to evaluate the hydrolytic stability of H-25435. The average percent recoveries for the Day 0 and Day 5, pH 1.2 replicate samples were 76± 5.9% and 63 ± 28%, respectively. The average percent recoveries for the Day 0 and Day 5, pH 4.0 buffer replicate samples were 97 ± 3.0% and 95 ± 4.4%, respectively. The average percent recoveries for the Day 0 and Day 5, pH 7.0 replicate samples were 92± 1.1% and 90 ± 0.9%, respectively. The average percent recoveries for the Day 0 and Day 5, pH 9.0 replicate samples were 96± 0.6% and 95 ± 9.3%, respectively.

The H-25435 was demonstrated to be hydrolytically stable at pH 9.0, pH 7.0, and pH 4.0. The data obtained for hydrolytic stability of H-25435 at pH 1.2 show the difference of average recoveries for monitored [ ] between Day 0 and Day 5 samples is larger than 10 percent. However, a precipitate was observed after addition of the test substance to the pH 1.2 buffer. The precipitate was difficult to completely dissolve even after addition of acetonitrile and extended sonication, especially for the Day 5 samples. This lowered the observed recoveries, especially for the bis esters. Therefore, the observed change in the average recoveries between the Day 0 and Day 5 samples is most likely because of observed solubility problems rather than hydrolysis.

The measured concentration of 8-2 TBA did not indicate any hydrolytic degradation of H-25435 to 8-2 TBA for any of the investigated pHs. The observed concentrations of 8-2 TBA can be explained by the residual 8-2 TBA present in the test substance (H-25435). There was no significant increase of 8-2 TBA concentration observed between Day 0 and Day 5 samples for any of the investigated pHs.

INTRODUCTION

The purpose of the study was to investigate the hydrolytic stability of H-25435 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-25435 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.

MATERIALS AND METHODS

A. Materials

1. Test Substance

Name:	H-25435
Composition:	[ ] [ ] [ ] [ ] [ ] [ ]
Haskell number:	25435

2. Residual 8-2 TBA in H-25435

Residual 8-2 TBA content in the test substance (H-25435) was determined by LC/MS using the same analytical method as described in Analysis of 8-2 TBA in Methods. Five replicate samples of H-25435 (approximately 45 mg) were introduced to glass vials, weighed, 10 mL of THF (tetrahydrofurane) was added, vials crimped, and sonicated for 1.5 hours. A 0.1 mL aliquot of dissolved sample was transferred to a glass LC vial and 0.9 mL acetonitrile and internal standard (D-8-2 TBA) was added. Calibration standards were made in 10/90% THF/acetonitrile in the range from 100 to 10200 ng/mL 8-2 TBA.

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 3.73 g of KCl (purity 99.0%, Sigma) in 250 mL of water. The 0.2 M hydrochloric acid (HCl) was prepared by diluting 4.5 mL 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 [ ] 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 sodium hydroxide.

b. pH 4:

The 0.1 M solution of potassium hydrogen phthalate was prepared by dissolving 5.1 g of potassium hydrogen phthalate (purity 99+%, Sigma) in 250 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.

c. pH 7:

The 0.1 M solution of potassium phosphate was prepared by dissolving 3.4 g of potassium phosphate, monobasic (KH_2PO_4, purity 99.5%, Sigma) in 250 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 1.90 g of KCl (purity 99.0%, Sigma) and 1.55 g of H_3BO_3 (purity 99.5%, Sigma) in 250 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 hydrochloric acid.

Water used for buffer preparation was obtained from a Barnstead NANOpure Diamond water purification system. The water resistivity was > 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-25435 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-25435 was weighed into sixteen 10-mL autoclaved glass vials and 3 mL of appropriate buffer solution was added. The vials were crimp capped with the aluminum foil covering the part of the septa facing the inside of the vial. 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 1040 mg/L 8-2 TBA). The vials were crimp capped with the aluminum foil covering the part of the septa facing the inside of the vial. Three of the vials were used as the 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 H-25435 determination. The other four of the Day 0 or Day 5 vials, two blanks 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, designated for the H-25435 analysis. The vials were uncapped, the pH electrode was introduced to the vials and the pH reading was taken. These vials were further processed for H-25435 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

H-25435 is a mixture of [ ] The [ ]
present in H-25435 [ ] [

[

]

[

]

[

]

[

]

The test substance H-25435 has not been characterized to determine the actual contents of the chemical species that were monitored during the study. The concentrations of individual chemical species that were monitored during the study were represented as the total concentration of the H-25435.

Vials designated for H-25435 analysis were uncapped, 6 mL of acetonitrile was added, vials were recapped and vortexed for 30 minutes. The obtained extract was diluted 1:1000 with 2:1 acetonitrile:water before analysis. The blank samples were subjected to the same procedure, except the pH measurement.

The stock solution of H-25435 was made by dissolving 0.01 g of H-25435 in 10 mL of 2 mL isopropanol:8 mL water. Calibration standards for the H-25435 analysis were made by appropriate dilution of the stock. The calibration standards ranged from 1 to 10 mg/L of H-25435. Fresh calibration standards were prepared for each instance of analysis. Linear or quadratic regression of the peak area vs. standard concentration was used to establish the calibration curves.

Instrumentation and conditions:

HPLC Instrument: Model Agilent 1100, Agilent
MS Instrument: Quattro Micro, Waters

LC Parameters:
Column: Xterra MS C18, 30 mm x 2.1 mm, 2.5 µm
Mobile Phase: A- 0.15% (v/v) acetic acid/10 mM triethylamine in water
B- 0.15% (v/v) acetic acid/10 mM triethylamine in methanol
Gradient:

Time (min)	%B	Flow (mL/min)
0.0	50	0.25
2.0	100	0.25
8.0	100	0.25
8.1	100	0.50
10.1	100	0.50
10.2	50	0.25
15.0	50	0.25

Column Temperature: 30°C
Injection Volume: 10 µL

MS Parameters:
Ionization mode: Electrospray, negative ions
Capillary voltage: 3.2 kV
Cone voltage: 20 V
Source Temperature: 120°C
Desolvation Temperature: 450°C
Data Acquisition Function: **C6-P** mono ester ion: SIR of 443 m/z; 0-12 min
C8-P mono ester ion: SIR of 543 m/z; 0-12 min
C10-P mono ester ion: SIR of 643 m/z; 0-12 min
C6-P-IPA mixed ester ion: SIR of 485 m/z; 0-12 min
C8-P-IPA mixed ester ion: SIR of 585 m/z; 0-12 min
C10-P-IPA mixed ester ion: SIR of 685 m/z; 0-12 min
C6-P-C6 bis ester ion: SIR of 789 m/z; 0-12 min
C6-P-C8 bis ester ion: SIR of 889 m/z; 0-12 min
C8-P-C8/C6-P-C10 bis ester ion: SIR of 989 m/z; 0-12 min
C10-P-C10 bis ester ion: SIR of 1089 m/z; 0-12 min

4. Analysis of 8-2 TBA

Samples designated for 8-2 TBA analysis were processed by injecting 3 mL methyl-tertbutyl ether (MTBE) through the vial septum using a glass syringe. The pH 1.2 samples required neutralization before the extraction. Neutralization was done by injecting 0.27 mL of 1N sodium hydroxide to the sample vial before the MTBE addition. Neither Day 0 nor Day 5 samples were uncapped before MTBE injection to avoid any losses of 8-2 TBA. The vials containing injected MTBE were vortexed for 20 or 30 minutes on a multitube vortexer, centrifuged for 20 minutes at 4150 rpm at room temperature. Occasionally, the MTBE and aqueous layers would not separate even after centrifugation. In these cases, samples were be placed in a freezer at -20°C for overnight storage, removed from the freezer, allowed to come to room temperature and centrifuged for 20 minutes. Subsequently, the sample vials were uncapped and 0.1 mL of the

MTBE extract was transferred to a glass LC vial, 0.9 mL acetonitrile added, the extract was spiked with internal standard, crimped capped, and subjected to LC/MS analysis for 8-2 TBA.

The 1H, 1H, 2H, 2H-perfluorodecan-1-ol (97.6%, Oakwood Products, West Columbia, SC) was used as the analytical standard of 8-2 TBA. The 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 freshly for each calibration in 10/90% MTBE/acetonitrile. The calibration standards were made in the range from 100 to 10,000 µg/L 8-2 TBA. A constant level of internal standard was used: 515 µg/L D-8-2 TBA. The calibration curves were constructed using the ratio of the peak area for ions m/z monitored for 8-2 TBA and peak area for ions monitored for D-8-2 TBA and the concentrations of 8-2 TBA.

Instrumentation and conditions:

HPLC Instrument:	Model 2795, Waters
MS Instrument:	ZQ, Waters
LC Parameters:	
Column:	Xterra MS C18, 30 mm x 2.1 mm, 2.5 µm
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:	8-2 TBA: SIR of 463, 523; 0-6 min D- 8-2 TBA: SIR of 467, 528 m/z; 0-6 min

5. Calculations

The following calculations were used to determine the recoveries of individual chemical species monitored for H-25435 (Tables 1-4). For example, if 0.045 g of H-25435 was weighed into the vial and 3 mL of buffer was added to the vial the nominal concentration that was assigned to each of the chemical species was: (0.045 g *1000 mg/g) / 0.003 L = 15,000 mg/L. The nominal concentration used for recovery calculation was an average of four replicates. The % recovery was 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 1040 mg/L 8-2 TBA spike represents 4.5 µL x 1040 ng/µL= 4680 ng 8-2 TBA. This amount was extracted with 3 mL of MTBE. The expected nominal concentration of 8-2 TBA in the MTBE extract is: 4680 ng/3 mL=1560 ng/mL. The % recovery is calculated by dividing the measured concentration by the nominal concentration, times 100%.

The H-25435 contains residual 8-2 TBA. The expected, maximum concentration of 8-2 TBA derived from residual 8-2 TBA in the MTBE extract (3 mL), assuming complete extraction for a 45 mg sample of H-25435 is: 45 mg of H-25435 x (0.129%/100)/0.003 L= 19.4 mg/L (19,400 µg/L).

RESULTS AND DISCUSSION

Tables 1-4 present the results for measured concentrations and recoveries of individual [ ] measured for H-25435 and average concentrations and recoveries for Day 0 and Day 5 replicate samples for pH 1.2, 4.0, 7.0, 9.0, respectively. The average concentration and recoveries were calculated as an average of concentrations and recoveries of [ ] monitored for H-25435. The average percent recoveries for the Day 0 and Day 5, pH 1.2 replicate samples were 76± 5.9% and 63 ± 28%, respectively. The average percent recoveries for the Day 0 and Day 5, pH 4.0 buffer replicate samples were 97 ± 3.0% and 95 ± 4.4%, respectively. The average percent recoveries for the Day 0 and Day 5, pH 7.0 replicate samples were 92± 1.1% and 90 ± 0.9%, respectively. The average percent recoveries for the Day 0 and Day 5, pH 9.0 replicate samples were 96± 0.6% and 95 ± 9.3%, respectively. The difference of average recoveries between the Day 5 and Day 0 samples was less than 10% for pHs 4.0, pH 7.0, and pH 9.0 buffers. This indicates that the H-25435 is hydrolytically stable at these pHs ($t_{1/2}$ >one year). Similarly, the difference in recoveries [ ] for Day 0 and Day 5 samples at pH 4.0, pH 7.0, and pH 9.0 buffers were less than 10%, except the C10-P-C10 bis ester at pH 4.0 and pH 9.0 buffer. The average recoveries for Day 0 and Day 5 samples of the pH 1.2 buffer differ by more than 10%. However, in case of pH 1.2 buffer a precipitate was observed after addition of the test substance to the buffer. The precipitate was difficult to completely dissolve even after addition of acetonitrile and extended sonication, especially for the Day 5 samples. This lowered the observed recoveries, especially for the bis esters. Therefore, the observed change in the average recoveries between the Day 0 and Day 5 samples is most likely because of observed solubility problems rather than hydrolysis.

Figure 1 shows example calibration curves for [ ] monitored during the hydrolytic stability study. Figures 2-5 show example chromatograms obtained for the monitored [ ] for a pH 7.0, Day 0 blank sample, calibration standard containing 5.3 mg/L H-25435, pH 7.0, Day 0 test sample, and pH 7.0, Day 5 test sample.

The content of residual 8-2 TBA in H-25435 was measured using the procedure described in Section A.2. Based on the results of five replicates of the H-25435, the content of residual 8-2 TBA in H-25435 was calculated to be 0.129±0.0048%. Assuming that the buffer extracts all the residual 8-2 TBA from the test substance during the time of hydrolytic stability experiment, the expected concentration of 8-2 TBA in the test system would be 19.4 mg/L (19,400 µg/L). (see 5 Calculations). Tables 4-8 present the measured concentrations of 8-2 TBA for Day 0 and Day 5 replicate samples for pH 1.2, 4.0, 7.0, 9.0, respectively. The concentrations of 8-2 TBA measured for the Day 0 samples are slightly higher than the expected concentration of 8-2 TBA originating from residual 8-2 TBA. This may be due to different extraction methods used for determination of residual 8-2 TBA in the test substance and the method used for analysis of hydrolytic stability samples. The Day 5 concentrations of 8-2 TBA are comparable with these observed for Day 0 samples. The observed differences in concentration of 8-2 TBA between the Day 0 and Day 5 samples for pH 7.0, 9.0 buffers are in the range of error for the analytical method used (±15%). A decrease of 8-2 TBA concentration between Day 0 and Day 5 may be explained by adsorption of 8-2 TBA on the precipitate observed for this pH. The difference of 8-2 TBA concentrations between Day 0 and Day 5 for pH 4.0 samples is larger than can be

explained by the analytical method error (Table 6). However, the observed QC sample recoveries for Day 5 samples indicate higher than expected bias of the analytical method for this set of samples. Thus, the increase of 8-2 TBA concentration for Day 5 samples is most likely due to analytical method performance rather than the test substance hydrolysis.

Figure 6 shows a representative calibration curve obtained for 8-2 TBA. Figures 7-10 show chromatograms for 8-2 TBA calibration standard; Day 5, pH 7.0 blank sample; Day 5, pH 7.0 test sample; and Day 0, pH 7.0 test sample, respectively.

Table 9 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 with deviation not exceeding ± 0.1°C.

Table 10 summarizes results of the pH measurements for pH 1.2, 4.0, 7.0, and 9.0. The measured pH of Day 0 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-25435 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 H-25435 was demonstrated to be hydrolytically stable at pH 9.0, pH 7.0, and pH 4.0 with $t_{1/2}$ >one year. The data obtained for hydrolytic stability of H-25435 at pH 1.2 show the difference of average recoveries for monitored [ ] between Day 0 and Day 5 samples is larger than 10 percent. However, in case of pH 1.2 buffer a precipitate was observed after addition of the test substance to the buffer. The precipitate was difficult to completely dissolve even after addition of acetonitrile and extended sonication, especially for the Day 5 samples. This lowered the observed recoveries, especially for the [ ] Therefore, the observed change in the average recoveries between the Day 0 and Day 5 samples is most likely because of observed solubility problems rather than hydrolysis.

The measured concentration of 8-2 TBA did not indicate any hydrolytic degradation of H-25435 to 8-2 TBA for any of the investigated pHs. The observed concentrations of 8-2 TBA can be explained by the residual 8-2 TBA present in the test substance (H-25435). There was no significant increase of 8-2 TBA concentration observed between Day 0 and Day 5 samples for any of the investigated pHs.

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-25435, pH 1.2, 37°C, Days 0 (03-June-2004) and 5 (08-June-2004).

pH	Day	Rep	[ ] (mg/L)	[ ] (mg/L)	[ ] (mg/L)	[ ] (mg/L)	[ ] (mg/L)	[ ] (mg/L)	[ ] (mg/L)	[ ] (mg/L)	[ ] (mg/L)	[ ] (mg/L)	Average (mg/L)
1.2	0	1	17,800	17,200	14,200	16,900	16,500	14,200	15,700	8,670	2,970	1,270	12,500
1.2	0	2	16,400	16,400	13,400	15,600	15,000	12,800	12,800	5,810	1,630	648	11,000
1.2	0	3	17,100	17,100	13,900	16,800	15,700	13,500	14,700	7,420	2,570	1,130	12,000
1.2	0	4	18,300	18,200	14,500	16,700	16,500	14,400	15,100	7,740	2,620	1,300	12,500
Average			17,400	17,200	14,000	16,500	15,900	13,700	14,600	7,410	2,450	1,090	12,000
% RSD			4.8	4.3	3.4	3.7	4.5	5.3	8.6	16.1	23.4	27.7	5.9
% Recovery			**110**	**109**	**89**	**104**	**101**	**87**	**92**	**47**	**16**	**7.0**	**76**
1.2	5	1	14,700	14,900	14,200	10,400	10,400	11,000	10,000	7,980	6,890	3,230	10,400
1.2	5	2[1]	3,260	3,300	4,000	1,630	1,530	952	411	640	2,870	768	1,940
1.2	5	3	15,000	9,610	6,420	9,910	7,990	7,160	4,240	2,910	4,400	1,850	6,950
1.2	5	4	19,800	19,200	17,000	12,700	12,700	12,800	11,300	8,500	7,160	3,830	12,500
Average			16,500	14,600	12,500	11,000	10,400	10,300	8,510	6,460	6,150	2,970	9,950
% RSD			17.3	33	44	13.5	22.7	27.9	44	47.8	24.7	34.2	28.2
% Recovery			**109**	**97**	**83**	**73**	**69**	**68**	**56**	**43**	**41**	**20**	**66**

[1] Replicate excluded from calculations of Average and %RSD.

Table 2: Analytical results for aqueous stability of H-25435, pH 4.0, 50°C, Days 0 (13-May-2004) and 5 (18-May-2004).

pH	Day	Rep	[ ] (mg/L)	[ ] (mg/L)	[ ] (mg/L)	[ ] (mg/L)	[ ] (mg/L)	[ ] (mg/L)	[C6-P-C6] (mg/L)	[ ] (mg/L)	[ ] (mg/L)	[ ] (mg/L)	Average (mg/L)
4.0	0	1	15,300	14,600	14,900	14,900	15,200	15,300	15,100	15,200	14,400	10,600	14,600
4.0	0	2	16,100	15,300	15,700	15,600	15,600	15,800	15,500	15,400	15,600	11,300	15,200
4.0	0	3	16,200	16,700	16,400	16,000	15,700	16,300	16,200	16,100	15,900	11,300	15,700
4.0	0	4	16,600	16,500	15,400	15,800	15,800	15,400	15,800	15,500	15,400	10,800	15,300
Average			16,100	15,800	15,600	15,600	15,600	15,700	15,700	15,600	15,300	11,000	15,200
% RSD			3.4	6.3	4.0	3.1	1.7	2.9	3.0	2.5	4.2	3.2	3.0
% Recovery			**103**	**101**	**100**	**100**	**100**	**100**	**100**	**100**	**98**	**70**	**97**
4.0	5	1	15,500	15,300	14,300	15,100	14,700	15,000	15,000	15,000	14,300	8,760	14,300
4.0	5	2	15,400	16,600	15,300	14,900	14,600	15,100	15,000	15,100	14,800	8,800	14,600
4.0	5	3	14,400	15,500	14,400	13,800	13,400	13,600	14,100	14,400	13,800	8,220	13,600
4.0	5	4	16,200	16,700	15,600	15,300	15,100	15,200	15,400	15,900	15,300	9,890	15,100
Average			15,400	16,000	14,900	14,800	14,500	14,700	14,900	15,100	14,600	8,920	14,400
% RSD			4.8	4.5	4.3	4.5	5.1	5.1	3.7	4.1	4.4	7.8	4.4
% Recovery			**101**	**105**	**98**	**97**	**95**	**97**	**98**	**99**	**96**	**59**	**95**

Table 3: Analytical results for aqueous stability of H-25435, pH 7.0, 50°C, Days 0 (06-May-2004) and 5 (11-May-2004).

pH	Day	Rep	[ ] (mg/L)	[ ] (mg/L)	[ ] (mg/L)	[ ] (mg/L)	[ ] (mg/L)	[ ] (mg/L)	[ ] (mg/L)	[ ] (mg/L)	[ ] (mg/L)	[ ] (mg/L)	Average (mg/L)
7.0	0	1	15,800	15,700	15,500	15,400	15,400	14,800	15,600	15,800	15,000	9,890	14,900
7.0	0	2	15,700	15,900	15,300	15,000	15,200	15,100	15,500	14,900	14,700	9,720	14,700
7.0	0	3	15,300	16,500	15,300	15,400	15,300	15,300	15,400	14,800	14,300	9740	14,700
7.0	0	4	15,300	16,200	14,800	15,000	15,100	15,200	15,100	14,300	14,000	9570	14,500
Average			15,500	16,100	15,200	15,00	15,300	15,100	15,400	15,000	14,500	9,730	14,700
% RSD			1.7	2.2	2.0	1.5	0.8	1.4	1.4	4.2	3.0	1.3	1.1
% Recovery			**97**	**101**	**95**	**95**	**96**	**95**	**97**	**94**	**91**	**61**	**92**
7.0	5	1	15,000	14,300	13,400	14,500	14,300	14,500	14,600	14,400	13,500	9,330	13,800
7.0	5	2	14,400	14,900	13,600	14,500	14,300	15,100	14,300	14,500	14,000	9,410	13,900
7.0	5	3	15,300	15,500	15,000	14,600	14,300	14,000	14,700	14,600	13,800	9,580	14,100
7.0	5	4	14,700	15,400	14,100	14,600	14,400	14,700	14,300	14,500	13,800	9,660	14,000
Average			14,900	15,000	14,000	14,600	14,300	14,600	14,500	14,500	13,800	9,500	1,4000
% RSD			2.6	3.7	5.1	0.4	0.3	3.1	1.4	0.6	1.5	1.6	0.9
% Recovery			**96**	**96**	**90**	**94**	**92**	**94**	**93**	**93**	**89**	**61**	**90**

Table 4: Analytical results for aqueous stability of H-25435, pH 9.0, 50°C, Days 0 (13-May-2004) and 5 (18-May-2004).

pH	Day	Rep	[ ] (mg/L)	[ ] (mg/L)	[ ] (mg/L)	[ ] (mg/L)	[ ] (mg/L)	[ ] (mg/L)	[ ] (mg/L)	[ ] (mg/L)	[ ] (mg/L)	[ ] (mg/L)	Average (mg/L)
9.0	0	1	15,400	15,800	16,800	15,300	15,300	15,300	15,400	15,200	15,000	12,700	15,200
9.0	0	2	15,700	15,800	14,400	15,600	15,600	15,500	15,500	15,400	15,200	12,500	15,100
9.0	0	3	15,900	15,400	15,300	15,300	15,100	15,300	15,200	15,100	14,600	12,500	15,000
9.0	0	4	15,400	15,500	16,200	15,600	15,500	15,400	15,300	15,200	15,200	12,700	15,200
Average			15,600	15,600	15,700	15,500	15,400	15,400	15,400	15,200	15,000	12,600	15,100
% RSD			1.6	1.3	6.7	1.1	1.4	0.6	0.8	0.8	1.9	0.9	0.6
% Recovery			**99**	**99**	**100**	**98**	**98**	**98**	**98**	**96**	**95**	**80**	**96**
9.0	5	1	15,000	14,500	12,200	14,300	13,100	10,500	10,500	11,700	12,700	13,900	12,800
9.0	5	2	14,300	15,500	14,800	14,900	14,600	14,900	15,600	15,600	15,500	14,000	15,000
9.0	5	3	15,100	15,400	16,000	15,200	15,100	15,700	15,400	16,000	15,900	15,200	15,500
9.0	5	4	14,900	15,800	15,500	15,600	15,500	15,400	16,200	15,800	16,400	17,500	15,900
Average			14,800	15,300	14,600	15,000	14,600	14,100	14,400	14,800	15,100	15,200	14,800
% RSD			2.4	3.7	11.6	3.7	7.2	17.3	18.3	13.9	11.0	11.0	9.3
% Recovery			**95**	**98**	**94**	**96**	**94**	**91**	**93**	**95**	**97**	**98**	**95**

Table 5: Measured concentrations of 8-2 TBA for pH 1.2, 37°C, Days 0 (03-June-2004) and 5 (08-June-2004).

pH	Day	Rep	Nominal 8-2 TBA Concentration (µg/L)	Measured 8-2 TBA Concentration (µg/L)	% Recovery
1.2	0	1	-	25,300	-
1.2	0	2	-	25,700	-
1.2	0	3	-	25,600	-
1.2	0	4	-	24,800	-
			Average	25,400	
			Standard Deviation	404	
1.2	0	QC1	1560	1680	108
1.2	0	QC2	1560	1610	103
1.2	0	QC3	1560	1670	107
				Average	106
				Standard Deviation	2.7
1.2	5	1	-	16,800	-
1.2	5	2	-	24,900	-
1.2	5	3	-	14,800	-
1.2	5	4	-	22,700	-
			Average	19,800	
			Standard Deviation	4,780	
1.2	5	QC1	1560	1470	94
1.2	5	QC2	1560	1500	96
1.2	5	QC3	1560	1450	93
				Average	94
				Standard Deviation	1.5

Table 6: Measured concentrations of 8-2 TBA for pH 4.0, 50°C, Days 0 (13-May-2004) and 5 (18-May-2004).

pH	Day	Rep	Nominal 8-2 TBA Concentration (µg/L)	Measured 8-2 TBA Concentration (µg/L)	% Recovery
4.0	0	1	-	25,400	-
4.0	0	2	-	24,300	-
4.0	0	3	-	21,900	-
4.0	0	4	-	23,200	-
			Average	23,700	
			Standard Deviation	1,500	
4.0	0	QC1	1560	1680	108
4.0	0	QC2	1560	1660	106
4.0	0	QC3	1560	1620	104
				Average	106
				Standard Deviation	2.0
4.0	5	1	-	28,400	-
4.0	5	2	-	29,500	-
4.0	5	3	-	30,200	-
4.0	5	4	-	29,500	-
			Average	29,400	
			Standard Deviation	744	
4.0	5	QC1	1560	2010	129
4.0	5	QC2	1560	2030	130
4.0	5	QC3	1560	1630	104
				Average	121
				Standard Deviation	15

Table 7: Measured concentrations of 8-2 TBA for pH 7.0, 50°C, Days 0 (06-May-2004) and 5 (11-May-2004).

pH	Day	Rep	Nominal 8-2 TBA Concentration (µg/L)	Measured 8-2 TBA Concentration (µg/L)	% Recovery
7.0	0	1	-	22,300	-
7.0	0	2	-	22,300	-
7.0	0	3	-	22,800	-
7.0	0	4	-	23,900	-
		Average		22,800	
		Standard Deviation		750	
7.0	0	QC1	1560	1640	105
7.0	0	QC2	1560	1630	104
7.0	0	QC3	1560	1600	103
				Average	104
				Standard Deviation	1.0
7.0	5	1	-	24,000	-
7.0	5	2	-	24,300	-
7.0	5	3	-	24,200	-
7.0	5	4	-	12,300 [1]	-
		Average		24,200	
		Standard Deviation		150	
7.0	5	QC1	1560	1590	102
7.0	5	QC2	1560	1540	99
7.0	5	QC3	1560	1580	101
				Average	101
				Standard Deviation	1.5

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

Table 8: Measured concentrations of 8-2 TBA for pH 9.0, 50°C, Days 0 (13-May-2004) and 5 (18-May-2004).

pH	Day	Rep	Nominal 8-2 TBA Concentration (µg/L)	Measured 8-2 TBA Concentration (µg/L)	% Recovery
9.0	0	1	-	25,000	-
9.0	0	2	-	25,300	-
9.0	0	3	-	25,300	-
9.0	0	4	-	25,700	-
		Average		25,300	
		Standard Deviation		287	
9.0	0	QC1	1560	1590	102
9.0	0	QC2	1560	1640	105
9.0	0	QC3	1560	1680	108
				Average	105
				Standard Deviation	3.0
9.0	5	1	-	26,300	-
9.0	5	2	-	26,000	-
9.0	5	3	-	25,700	-
9.0	5	4	-	25,900	-
		Average		26,000	
		Standard Deviation		250	
9.0	5	QC1	1560	1550	99
9.0	5	QC2	1560	1620	104
9.0	5	QC3	1560	1510	97
				Average	100
				Standard Deviation	3.6

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

Time (Day)	pH	Date	Temperature (°C)
0	1.2	03-June-2004	37.0
1	1.2	04-June-2004	37.0
2	1.2	05-June-2004	37.0
3	1.2	06-June-2004	37.0
4	1.2	07-June-2004	37.0
5	1.2	08-June-2004	37.0
0	4.0	13-May-2004	50.0
1	4.0	14-May-2004	50.0
2	4.0	15-May-2004	50.0
3	4.0	16-May-2004	50.0
4	4.0	17-May-2004	50.0
5	4.0	18-May-2004	50.0
0	7.0	06-May-2004	50.0
1	7.0	07-May-2004	50.0
2	7.0	08-May-2004	-[1]
3	7.0	09-May-2004	-[1]
4	7.0	10-May-2004	50.0
5	7.0	11-May-2004	50.0
0	9.0	13-May-2004	50.0
1	9.0	14-May-2004	50.0
2	9.0	15-May-2004	50.0
3	9.0	16-May-2004	50.0
4	9.0	17-May-2004	50.0
5	9.0	18-May-2004	50.0

[1] Temperature was not recorded.

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

Day	Rep	Nominal pH	Measured pH
0	1	1.2	1.27
0	2	1.2	1.25
0	3	1.2	1.27
0	4	1.2	1.27
5	1	1.2	1.24
5	2	1.2	1.23
5	3	1.2	1.24
5	4	1.2	1.24
0	1	4.0	4.10
0	2	4.0	4.11
0	3	4.0	4.09
0	4	4.0	4.10
5	1	4.0	4.07
5	2	4.0	4.07
5	3	4.0	4.08
5	4	4.0	4.07
0	1	7.0	7.10
0	2	7.0	7.09
0	3	7.0	7.10
0	4	7.0	7.10
5	1	7.0	7.06
5	2	7.0	7.07
5	3	7.0	7.07
5	4	7.0	7.05
0	1	9.0	9.10
0	2	9.0	9.10
0	3	9.0	9.08
0	4	9.0	9.09
5	1	9.0	9.09
5	2	9.0	9.09
5	3	9.0	9.08
5	4	9.0	9.07

FIGURES

Figure 1: Example calibration curves obtained for monitored[ ].

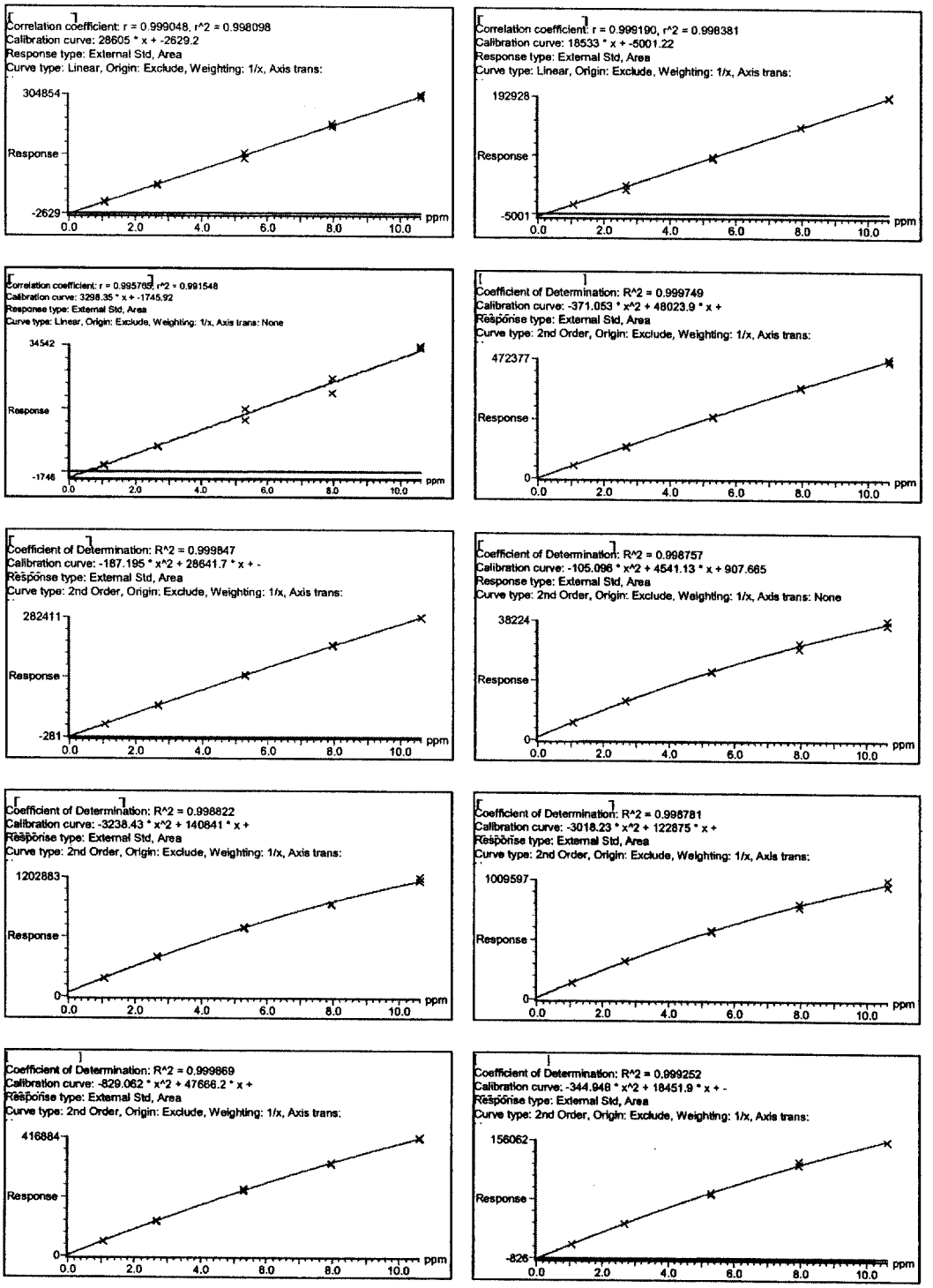

Figure 2: Example chromatograms of monitored [ ]obtained for a blank sample for pH 7.0 buffer on Day 0.

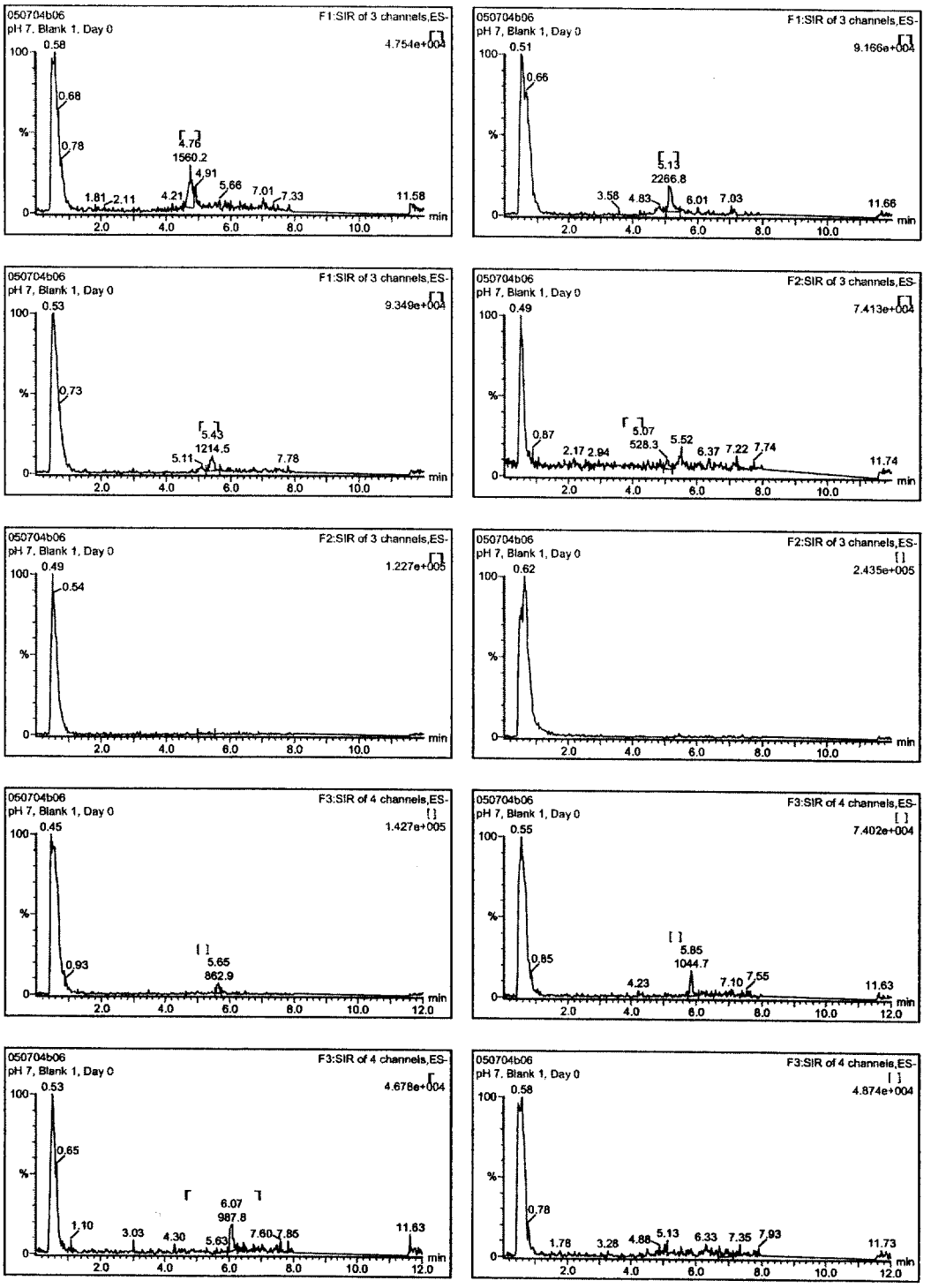

Figure 3: Example chromatograms of monitored [ ] obtained for a calibration standard containing 5.3 mg/L H-25435.

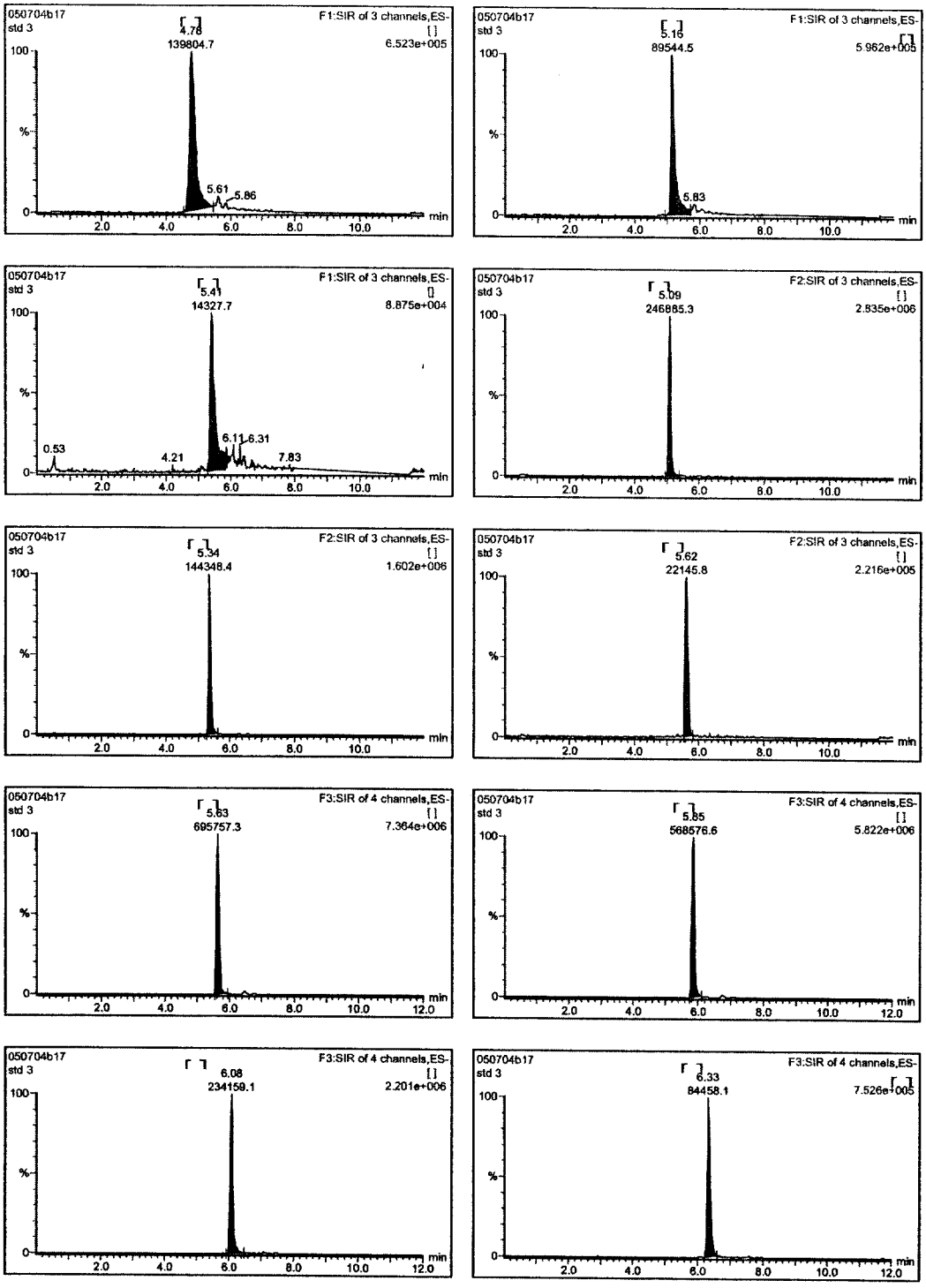

Figure 4: Example chromatograms of monitored [ ]obtained for Day 0, pH 7.0 hydrolytic stability sample.

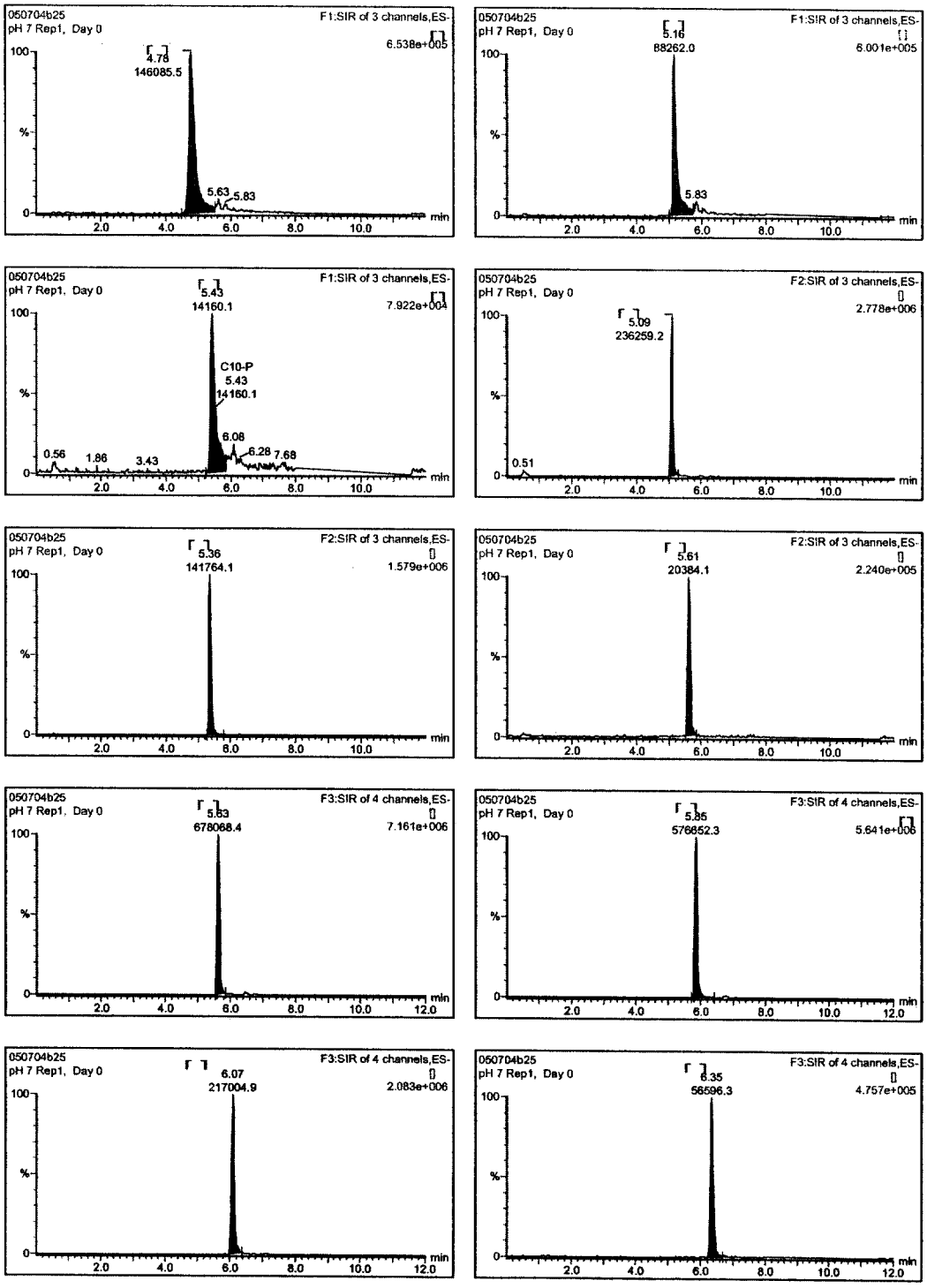

Figure 5: Example chromatograms of monitored [ ]obtained for Day 5, pH 7.0 hydrolytic stability sample.

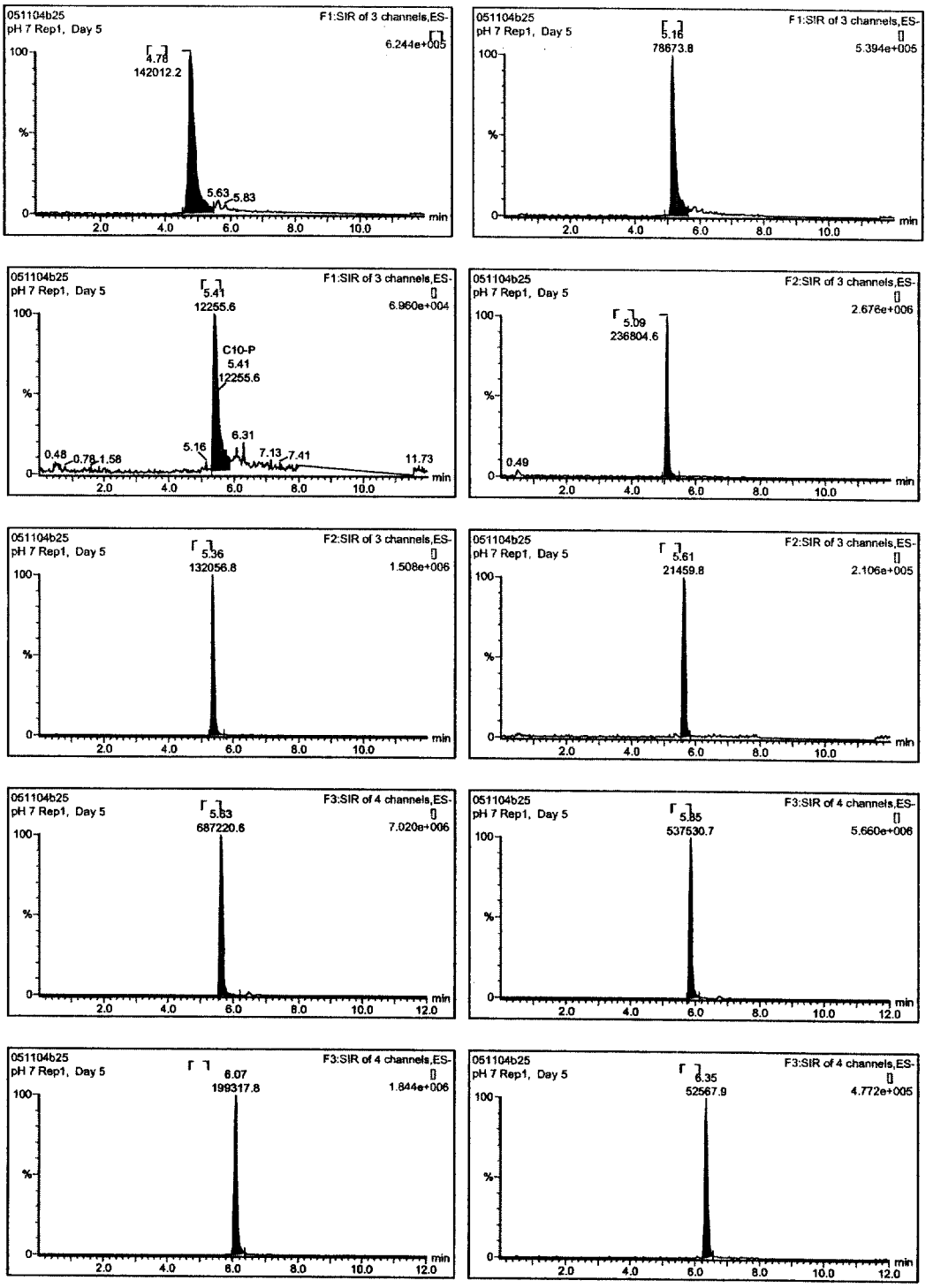

Figure 6: Representative calibration curve for 8-2 TBA.

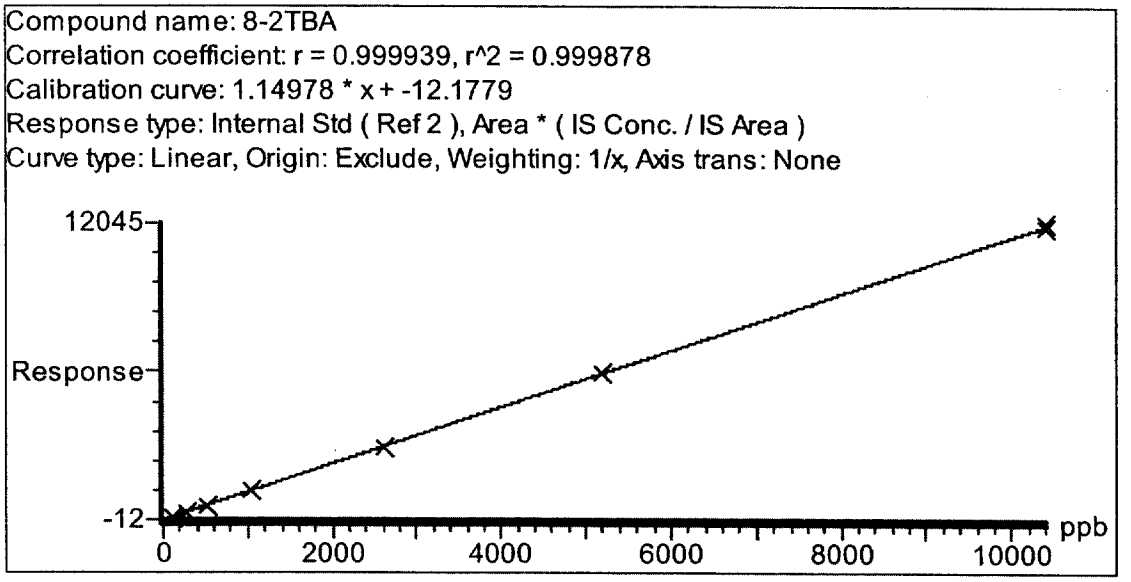

Figure 7: Representative chromatograms of 104 µg/L 8-2 TBA calibration standard and 515 µg/L D-8-2 TBA internal standard.

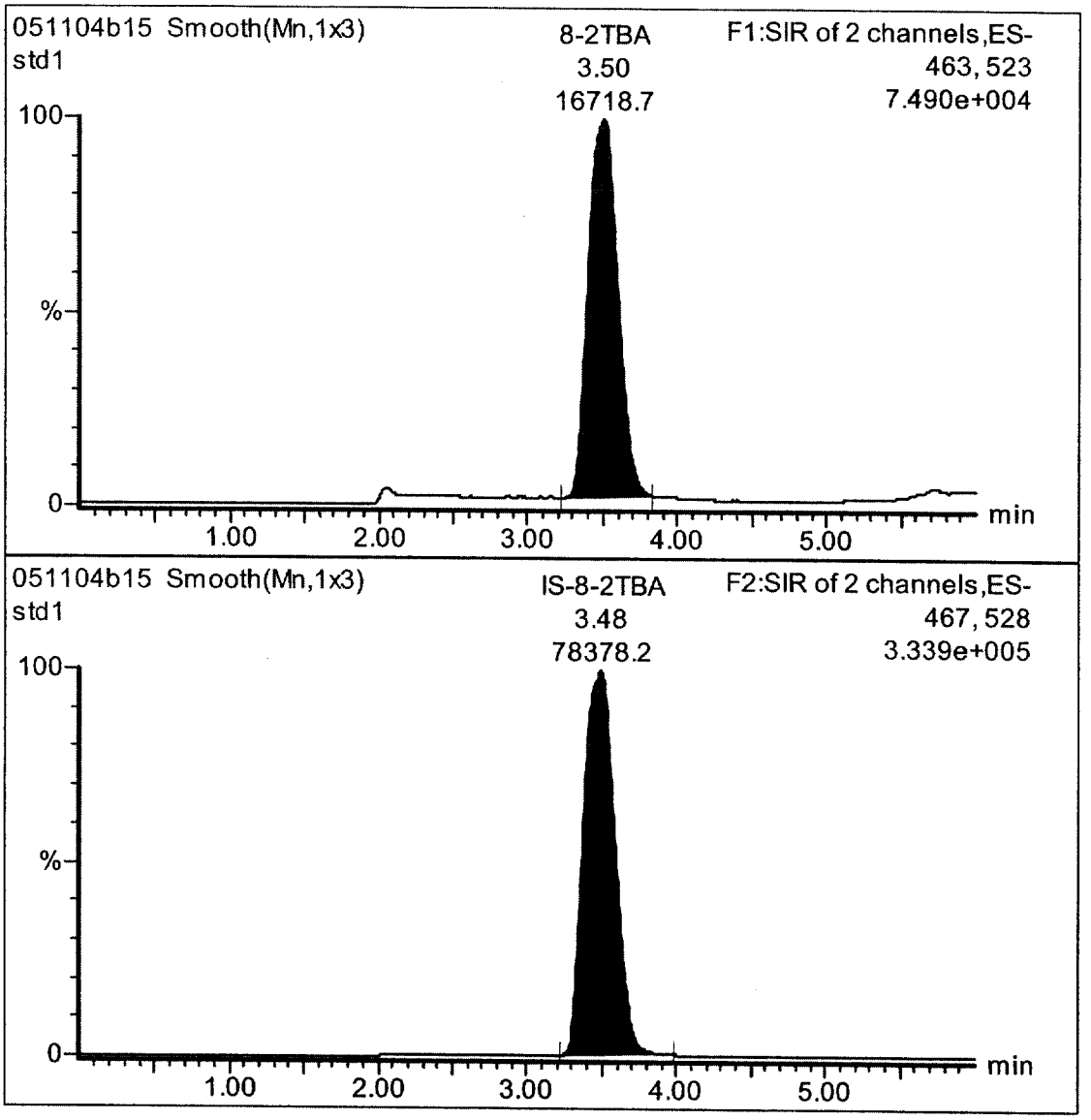

Figure 8: Representative chromatograms for Day 5, pH 7.0 buffer blank sample.

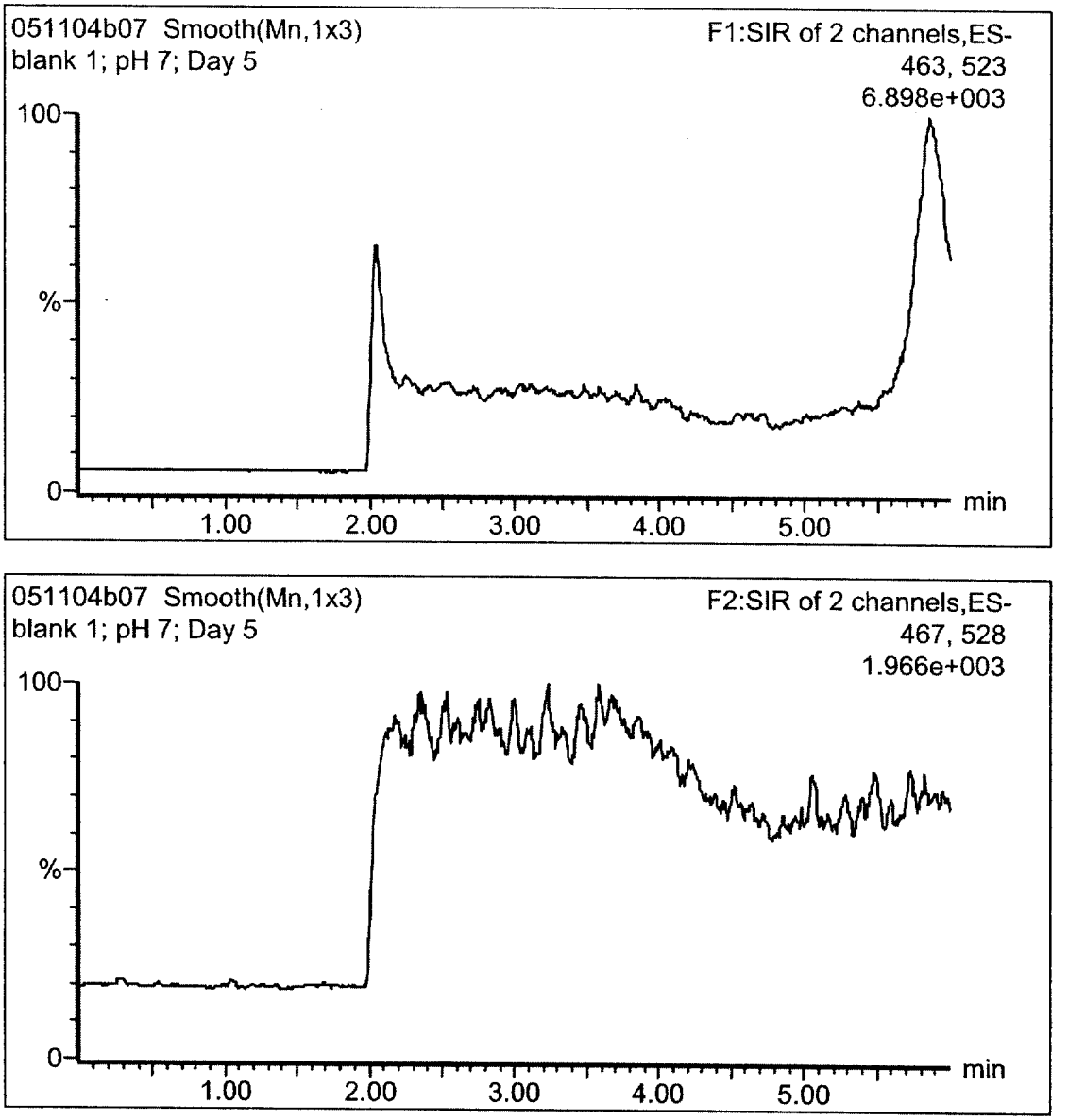

Figure 9: Representative chromatograms for Day 5, pH 7.0 hydrolytic stability sample and 515 µg/L D-8-2 TBA internal standard.

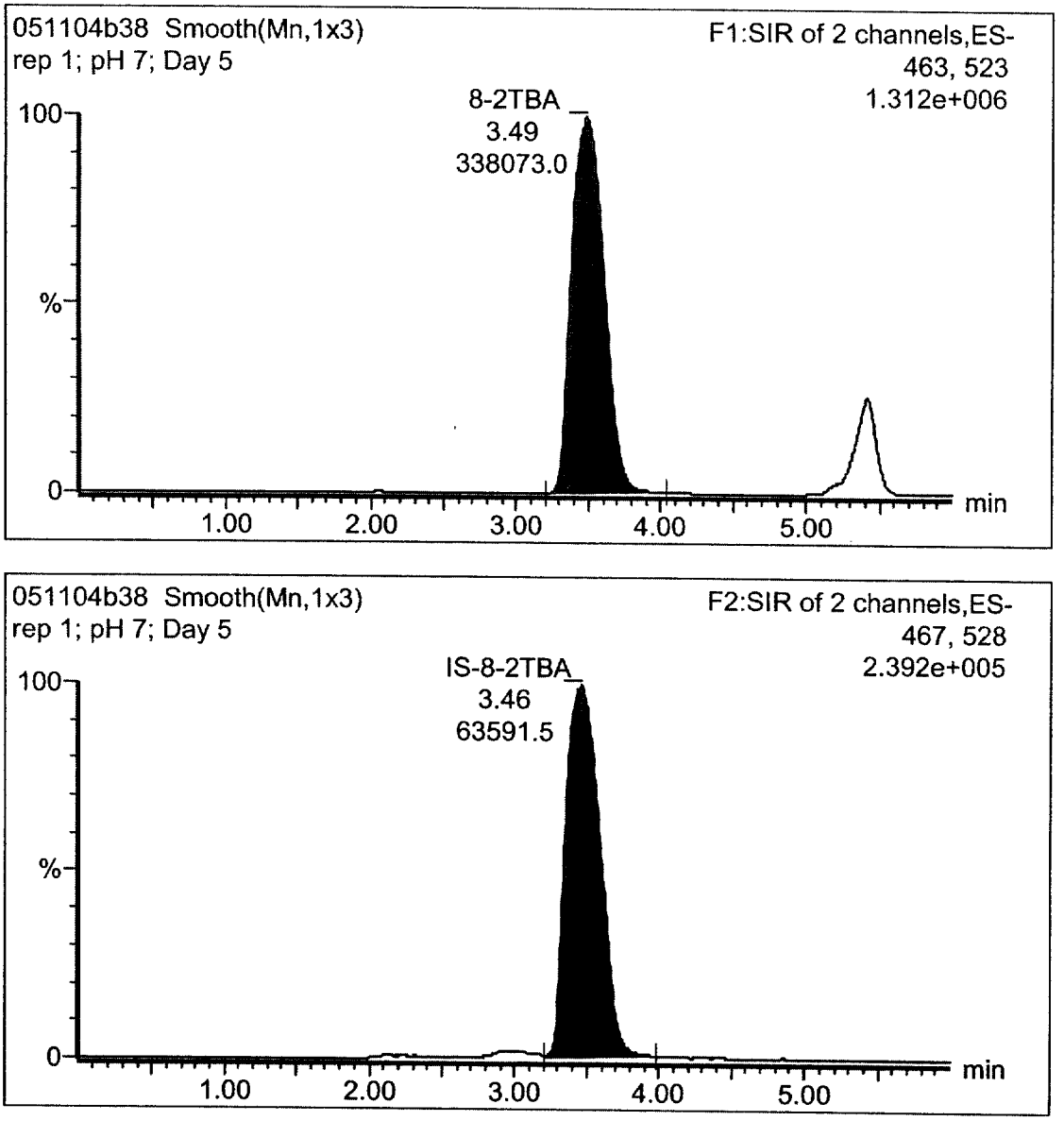

Figure 10: Representative chromatograms for Day 0, pH 7.0 hydrolytic stability sample and 515 µg/L D-8-2 TBA internal standard.