

Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pesticide Name: Flonicamid

MRID #: 458548-14

Matrix: Soil

Analysis: LC/MS/MS

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STUDY TITLE

458548-14

**DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR THE
DETERMINATION OF IKI-220 RESIDUES IN SOIL-REPORT AMENDMENT**

NOTE: FLONICAMID (CAS #158062-67-0) is *N*-(cyanomethyl)-
4-(trifluoromethyl)-3-pyridinecarboximide. Other identifiers are
IKI-220 and F-1785.

DOCUMENT NO.

013323-1-1

DATA REQUIREMENT

Pesticide Assessment Guidelines
Series 860.1340

AUTHORS

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B. Dolan

DATE

March 28, 2002

PERFORMING LABORATORY

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STUDY SPONSOR

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JAPAN

SUBMITTED BY

ISK BIOSCIENCES CORPORATION
7470 Auburn Road, Suite A
Concord, Ohio 44077

1 of 97 pages in study with 3 front pages



**Electronic PDF
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For more info 305-5363 • Bob Schultz 308-8186

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS¹

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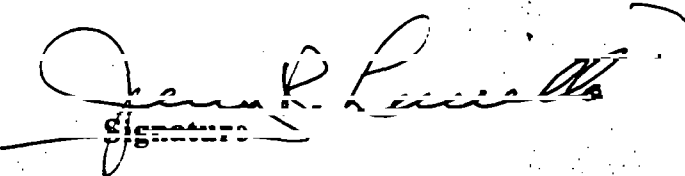
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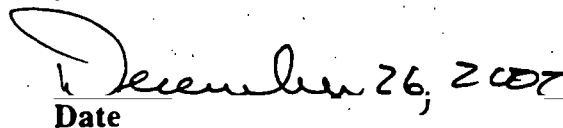
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Signature

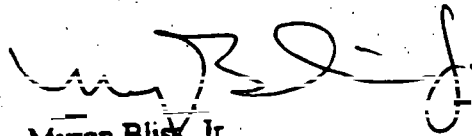

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COMPLIANCE STATEMENT

ISK Biosciences Corporation

This study was conducted and reported in compliance with Good Laboratory Practice Regulations set forth in Title 40, Part 160 of the Code of Federal Regulations of the United States of America.



Myron Bliss, Jr.
Manager, International Registrations
ISK BIOSCIENCES CORPORATION, APPLICANT

January 2, 2003
Date

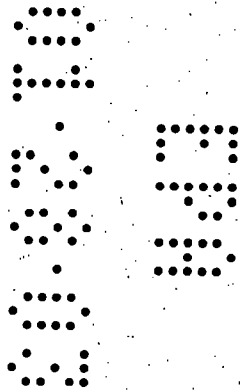
REFERENCE TO COMPLIANCE STATEMENT WITHIN THE REPORT

A GOOD LABORATORY PRACTICES compliance statement

with the signatures of

the STUDY DIRECTOR and the STUDY SPONSOR

is found on page 3 for the report





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REPORT AMENDMENT

Study Title:

Development and Validation of Analytical Method for the
Determination of IKI-220 Residues in Soil

Document Number: 013323-1-1

Ricerca Project Identification Number: 013323

Data Requirement:

OPPTS Series 850.7100

EU Guideline: SANCO / 825 / rev. 6 20/06/00.

Study Completed:

March 28, 2002

Authors:

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* Formerly Ricerca, LLC

STATEMENT OF NO DATA CONFIDENTIALITY CLAIM

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA § 10 (d) (1) (A), (B), or (C).

Company: Ishihara Sangyo Kaisha, Ltd.

Company Agent: S. Ogyu

Title: General Manager

Signature

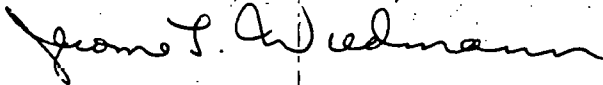
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Figure 1 shows a 4x4 grid of dots. The dots are arranged in a regular pattern, with some dots missing or faded, suggesting a sparse or disordered state. The grid is labeled with 'a' and 'b' axes.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The Report Amendment "Development and Validation of Analytical Method for the Determination of IKI-220 Residues in Soil," Ricerca Document Number 013323-1-1, did not generate new data. The data presented here was conducted and reported in compliance with the Good Laboratory Practice Standards set forth in Title 40, Part 160 of the Code of Federal Regulations of the United States of America in each of the four soil dissipation study reports (Ricerca Documents 12280-1, 12281-1, 12282-1 and 12283-1). This amendment contains all of the data reported in Report 013323-1 with changes to pages: A12 through A14 (addition of expiration dates of standards), A17 through A20 and A24 (additional text), and Appendix A (additional figures) as requested by the Sponsor; page A1 (Company name change). These changes had no effect on the integrity of the study.



Jerome L. Wiedmann, Study Director
Manager, Regulatory Studies
ISK Biosciences

12-20-02

Date



S. Ogyu, General Manager
Safety Science Laboratory
Central Research Institute, Ishihara Sangyo Kaisha, LTD.
Sponsor Representative

December 25, 2002

Date

Applicant

Date

Report Amendment:

**Development and Validation of Analytical Method for the
Determination of IKI-220 Residues in Soil**

Document Number: 013323-1-1

DISTRIBUTION

Original:

Ishihara Sangyo Kaisha, Ltd./S. Ogyu

Copies:

**Ricerca Archives (1)
Residue Analysis Files (2)
ISK Biosciences (3 - Unbound)**

APPROVALS

Study Title: Development and Validation of Analytical Method
for the Determination of IKI-220 Residues in Soil

Study Number: 013323

Document Number: 013323-1-1

Performing Laboratory: Department of Residue Analysis
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12-19-02

Date



Augustine Panthani, Director, Metabolism
Ricerca, LLC

12-19-02

Date



Jerome L. Wiedmann, Study Director
Manager, Regulatory Studies
ISK Biosciences

12-20-02

Date

QUALITY ASSURANCE STATEMENT

The Ricerca Quality Assurance Unit has performed inspections of the report amendment, "Development and Validation of Analytical Method for the Determination of IKI-220 Residues in Soil", Ricerca Document Number 013323-1-1, and reported findings to the Study Director and to Management on the dates listed below:

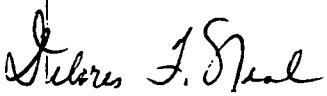
Dates of Inspection	Dates Reported to Ricerca Contact	Dates Reported to Management	Dates Reported to Sponsor Study Director/Management
3/04,05/02	3/5/02	3/27/02	3/27/02
			12/20/02
	Quality Assurance Auditor		Date

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ABSTRACT

This report amendment was prepared following comments generated from the Independent Laboratory Validation for IKI-220 and metabolites. The original text of Ricerca Report 13323-1 is included with some text changes.

An analytical method for the determination of residues of IKI-220 and its major soil metabolites, TFNA, TFNA-OH, TFNA-AM, TFNG and TFNG-AM, in soil was developed. Soil was extracted using an Accelerated Solvent Extractor (ASE). The extracts were then analyzed by LC-MS/MS using an 8-point calibration curve prepared from external standards. Residues of IKI-220 and TFNA-AM were determined using a turbo ion spray injector with the detector in the positive ion mode. Residues of TFNG, TFNA, TFNG-AM, TFNA-OH were determined also using turbo ion spray in the negative ion mode.

Soils from four different locations, California, North Carolina, North Dakota and Washington, were analyzed in sets consisting of a control and two fortifications at 5, 10, 25 or 50 ppb. These soils represented the major crop growing areas of the United States.

Mean IKI-220 and metabolites recoveries are shown below complete with standard deviations and relative standard deviations.

Soil Type	Mean Recovery % IKI-220	Mean Recovery % TFNG	Mean Recovery % TFNG-AM	Mean Recovery % TFNA	Mean Recovery % TFNA-AM	Mean Recovery % TFNA-OH
California	87.9 ± 8	87.8 ± 17	74.5 ± 11	99.4 ± 11	86.0 ± 8	96.4 ± 8
CA RSD*	10	19	15	10	9	8
North Carolina	92.8 ± 10	92.7 ± 11	84.1 ± 11	92.6 ± 11	92.4 ± 11	93.4 ± 11
NC RSD*	11	12	13	12	12	12
North Dakota	84.2 ± 12	87.1 ± 12	74.9 ± 16	90.1 ± 15	83.7 ± 11	89.1 ± 13
ND RSD*	14	13	21	17	13	15
Washington	85.3 ± 8	90.7 ± 11	78.3 ± 11	89.6 ± 12	88.8 ± 8	91.0 ± 11
WA RSD*	10	12	15	13	9	13

*RSD = relative standard deviation

The mean recoveries, standard deviations and relative standard deviations are in the acceptable ranges and indicate the method is precise and accurate. Mean recovery of IKI-220, TFNG, TFNA, TFNA-AM, and TFNA-OH from 5.0-ppb to 50-ppb fortifications exceeded 83% with relative standard deviations of less than 22%. Mean recoveries of TFNG-AM exceeded 74% with a standard deviation less than 21%, which is within EPA limits. These

data validate the method developed for the analysis of IKI-220 and its metabolites in the range of 5.0 to 50 ppb.

INTRODUCTION

A residue method for the determination of IKI-220, TFNG, TFNG-AM, TFNA, TFNA-AM and TFNA-OH in soil was developed and evaluated in this study. Method performance was discussed in terms of EU method parameters including accuracy, precision, limit of quantitation, specificity and linearity.

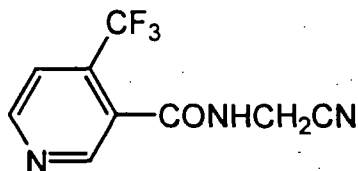
DOCUMENTS

This report summarizes the data acquired under Ricerca Protocols 12280-0 (Washington), 12281-0 (California), 12282-0 (North Dakota) and 12283-0 (North Carolina). These four studies were Residue Bareground Soil Dissipation Studies. The data generated in these studies is presented in this report as a validation of the soil method developed for the analysis of IKI-220 and its five soil metabolites.

TEST/REFERENCE SUBSTANCES

The structure, CAS registry number, and chemical name for IKI-220 and its metabolites are listed below.

- **IKI-220**



Chemical name: *N*-cyanomethyl-4-trifluoromethylnicotinamide

ISO name: Flonicamid (proposed)

CAS No.: 158062-67-0

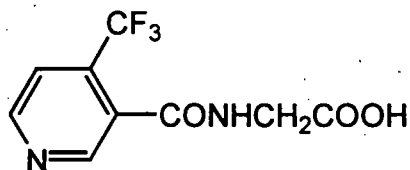
Molecular Wt.: 229.2

Lot Number: 9803

Purity: 99.7%

Expiration: November 11, 2003

• **TFNG**



Chemical names: *N*-(4-trifluoromethylnicotinoyl)glycine

CAS No.: 207502-65-6

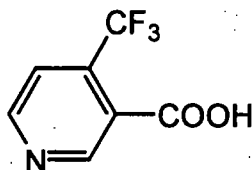
Molecular Wt.: 248.2

Lot Number: 0006

Purity: 99.4%

Expiration January 19, 2006

• **TFNA**



Chemical names: 4-trifluoromethylnicotinic acid

CAS No.: 158063-66-2

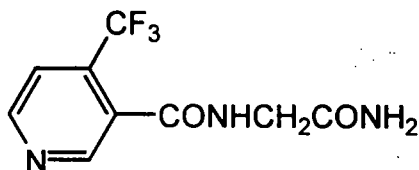
Molecular Wt.: 191.1

Lot Number: 0006

Purity: 99.4%

Expiration January 19, 2006

• **TFNG-AM**



Chemical names: *N*-(4-trifluoromethylnicotinoyl)glycinamide

CAS No.: 158062-96-5

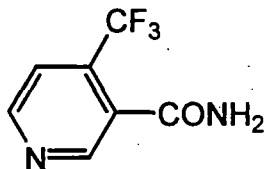
Molecular Wt.: 249.2

Lot Number: 0006

Purity: 99.5%

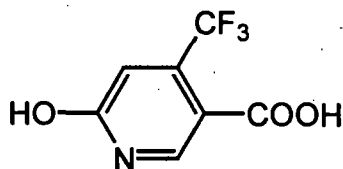
Expiration January 19, 2006

• **TFNA-AM**



Chemical names: 4-trifluoromethylnicotinamide
CAS No.: 158062-71-6
Molecular Wt.: 190.1
Lot Number: 0006
Purity: 100%
Expiration: January 19, 2006

• **TFNA-OH**



Chemical names: 6-hydroxy-4-trifluoromethylnicotinic acid
CAS No.: None
Molecular Wt.: 207.1
Lot Number: 0010
Purity: 100%
Expiration: January 19, 2006

OBJECTIVE

The objective of this study was to evaluate an analytical method for the determination of IKI-220, TFNG, TFNG-AM, TFNA, TFNA-AM, and TFNA-OH residues in soil. This report takes the data and explains how the method is valid based on method accuracy, precision (repeatability), limit of quantitation, specificity and linearity. The method demonstrated successful recovery of IKI-220 and its metabolites from 5.0 – 50.0 ppb-fortifications in four different soil types.

CONDUCT OF STUDY

SPONSOR

Ishihara Sangyo Kaisha, Ltd.
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Osaka, 550-0002
JAPAN

STUDY DIRECTOR

Jerome L. Wiedmann
Manager, Regulatory Studies
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Business Phone: (440) 357-4646

RETENTION OF DATA

This study was conducted by Ricerca LLC, 7528 Auburn Road, Concord, Ohio 44077, Department of Residue Analysis, in accordance with protocols mentioned in the document section of this report.

Raw data supporting this study and the final report are stored in the Ricerca, LLC Archives. The minimum storage period will be at least 10 years. Thereafter, no data will be withdrawn without the Sponsors consent.

MATERIALS AND METHODS

Personnel involved in these studies were:

J. L. Wiedmann	Study Director
P. S. Cassidy	Group Leader
B. Tan	Senior Research Spectrometrists
J. B. Formanik	Research Spectrometrists II
C. King	Research Chemist II
S. L. Amato	Research Spectrometrists I
B. Dolan	Associate Chemist
M. J. Lynch	Associate Chemist
J. Graham	Assistant Chemist

SAMPLE PROCUREMENT AND RECEIPT

Soil from the states of California, North Carolina, North Dakota and Washington was used to validate the method for IKI-220, TFNG, TFNG-AM, TFNA, TFNA-AM and TFNA-OH. The control samples used for concurrent fortifications were received from test sites at which IKI-220 bareground soil dissipation studies were being conducted. All control soils were received and stored frozen.

SOIL CHARACTERIZATION

Tables 1 to 4 contain results from soil characterization analyses for the 4 soils used in this method validation study. The percent organic matter of the 0-6" California soil is 1.5%; the pH is 7.1. The 0-6" soil from North Carolina had a percent organic matter of 0.8% and a pH of 6.3. North Dakota contained much more organic matter, 6.1%, with a pH of 6.6 as shown in Table 3. The percent organic matter of the 0-6" Washington soil was much lower containing only 0.3% organic matter and having a pH of 7.6.

SAMPLE IDENTIFICATION

At the time of receipt at Ricerca, samples were assigned a Ricerca identification code number. The soil sample numbers consisted of the project number, an integer, and a letter. The project numbers are 012281-California, 012282 - North Dakota, 012283 - North Carolina and 012280 - Washington. The integer identifies the time and plot for the sample. The letter (A-F) indicates the sample depth, where 'A' is for 0-6 inch (0-15cm), 'B' is for 6-12 inch (15-30cm), 'C' is for 12-18 inch (30-45cm) depths, etc. Lab codes were used to distinguish control and fortified samples, 1 = control, 2 = fortified at 5ppb and 3 = fortified at 50ppb. Examples of laboratory sample reference numbers are:

012281-035-1A: 0-6-inch, (0-15cm) control
012281-035-2A: 0-6-inch, (0-15cm) 5-ppb fortification
012281-035-3C: 12-18-inch, (30-45cm) 50-ppb fortification

The laboratory sample reference number and corresponding sample description were recorded on Operations and Data Collection Forms (flowsheets) and were used to identify sample residues in the report.

ANALYTICAL PROCEDURE

Summary

IKI-220, TFNG, TFNG-AM, TFNA, TFNA-AM, and TFNA-OH were extracted from soil using an Accelerated Solvent Extractor (ASE). Twenty grams of soil were extracted with a solution of water/methanol (70/30; v/v) at a temperature of 110°C and a pressure of 150 bar, over a seven-minute static cycle. The final volume was adjusted to 40 mL with 70/30 water/methanol if necessary. The extract was analyzed by LC-MS/MS using an 8-point calibration curve prepared from standards. IKI-220 and TFNA-AM are determined using turbo ion spray injector with the detector in the positive ion mode. TFNG, TFNA, TFNG-AM, TFNA-OH are determined also with turbo ion spray, but with the detector in the negative ion mode.

Preparation of Standards

Approximately 3 to 5 mg of each of the bulk standards are weighed into separate 25 mL Class A volumetric flasks. The solutions are brought to volume with acetonitrile (HPLC Grade) to make individual standards at 120 to 200 µg/mL.

A portion of each of these stock solutions is transferred to an appropriately sized Class A volumetric and diluted with 30 % methanol in water (HPLC Grade) to make a combined solution containing each of the six standards at 20 µg/mL (e.g. 10 mL of a 200 µg/mL individual standard diluted to 100 mL).

All lower concentration standards are prepared are prepared from the 20 µg/mL stock mixed standard by diluting with 30% Methanol in water. Calibration solutions should be in the range of 0.1 to 0.001 µg/mL with five standards in the range to evaluate linearity on the LC-MS/MS.

Standards prepared in this manner should be stored in a freezer and should be stable for four months. New standards should be compared against the old to evaluate stability over time.

Extraction

Reagents

Methanol (MeOH)	HPLC Grade, or equivalent
H ₂ O	HPLC Grade, or equivalent
Extraction Solvent: Water/Methanol (70/30). Combine and mix 700 mL HPLC grade water, 300 mL HPLC grade methanol.	

Equipment

Accelerated Solvent Extractor ASE 200 Dionex Corporation

Analytical Balance

Cell Filters – Dionex p/n 049458

Disposable pipettes

Eppendorf adjustable pipettors, 100 μ L or 1,000 μ L or equivalent

Hydromatrix, Varian Corp.

Mettler BB-2400 balance or equivalent

VWR Scientific Multi-Tube Vortexer or equivalent

ASE Extraction Solution and Sample Volume Adjustment Solution:

Water/Methanol (70/30). Combine and mix 700 mL HPLC grade water,
300 mL HPLC grade methanol.

Procedure

1. A 20 gram sample of soil homogenate is mixed with 4 grams of hydromatrix and is placed into a 33 mL ASE extraction cell.
2. The ASE extractor is programmed for a run cycle and the samples are extracted. The following are the ASE 200 programmed criteria:

Heat cycle set at default (6 min).

Static cycle set at 7 minutes at 110° C and 150 Bar (2176 psi).

Preheat cycle is set to 0 minutes.

Purge time is set at 300 second.

Cell volume is set at 35 to 50% in order to give a final extract volume of 40 mL.

3. Following extraction, the sample volume was adjusted to 40 mL and a portion of the extract was placed in an HPLC vial for LC-MS/MS quantitation.

Quantitation

Instruments Used

PE Sciex LC-MS/MS Model 3000

Typical parameters on a PE Sciex API 3000 (the parameters may be modified for best results):

IS (Ion Source)	-4500 (negative ion) 5000 (positive ion)
TEM (Temperature)	500 C
MR Pause	5.0 ms
NEB (Nebulizer Gas, N ₂)	12.0
CUR (Curtain Gas, N ₂)	14.0
CAD (Collision Activated Dissociation Gas-N ₂)	3.0

Reagents Used

Methanol – Optima, Fisher
Acetic Acid-ACS Grade, Fisher
Water – HPLC Grade, Fisher

HPLC Conditions—Positive Ion Mode

Column:	LUNA, Phenyl-hexyl 150 x 2 mm, 5 µm
Injection volume:	5 µL
Oven temperature:	Column at room temperature
Mobile phase:	A: 1% acetic acid in water B: methanol
Run time:	5 min
Flow rate (column):	0.3 mL/min
Retention times:	
IKI-220:	approx. 4.53 min.
TFNA-AMi:	approx. 3.94 min.

HPLC-Gradient¹

Time [min]	0	2	2.5	2.6	5
% A	90	50	50	90	stop
% B	10	50	50	10	stop

¹The gradient was modified, as necessary, to optimize the separation.

MS/MS Conditions - Positive Ion Mode

Ionization Mode: Turbo IonSpray® Positive Ion Mode

Scan Mode: Multiple Reaction Monitoring (MRM)

Precursor → Product Ion Pair

IKI-220 230.0 → 202.9

TFNA-AM 191.0 → 148.0

HPLC Conditions—Negative Ion Mode

Column: LUNA, Phenyl-hexyl 150 x 2 mm, 5 µm

Injection volume: 5 µL

Oven temperature: Column at room temperature

Mobile phase: A: 1% acetic acid in water
B: methanol

Run time: 8 min

Flow rate (column): 0.3 mL/min

Retention times:

TFNG: approx. 6.68 min.

TFNG-AM: approx. 5.52 min.

TFNA: approx. 7.29 min.

TFNA-OH: approx. 7.12 min.

HPLC-Gradient¹

Time [min]	0	6	6.1	8
% A	95	50	95	stop
% B	5	50	5	stop

¹The gradient was modified, as necessary, to optimize the separation.

MS/MS Conditions – Negative Ion Mode

Ionization Mode: Turbo IonSpray® Negative Ion Mode

Scan Mode: Multiple Reaction Monitoring (MRM)

Precursor → Product Ion Pair

TFNG-AM 246.3 → 99.0

TFNA-OH 206.1 → 162.0

TFNG 247.3 → 183.0

TFNA 190.3 → 145.8

METHOD MODIFICATION

The mobile phases and gradient profiles for the LC-MS/MS were altered from the conditions listed in protocol amendments 1 to each of the four protocols. The mobile phase was switched from an acetonitrile/water with 1% formic acid gradient to a methanol/water gradient with 1% acetic acid added as a pH modifier.

This method modification had no adverse affect on the study, as concurrent recoveries were run and validated with every sample set.

CALCULATION OF RESIDUES

IKI-220 and metabolite residues were quantitated using linear multi-point calibration curves generated from the injection of external standards. Detailed sample calculations are found in Appendix A.

RESULTS AND DISCUSSION

ACCURACY

Accuracy is defined as the nearness of the measured residue value to the true value. The overall IKI-220 mean recovery calculated from all fortifications is an indication of method accuracy. The table below summarizes recovery data for each soil type.

Soil Type	Fortification Levels		Mean Recovery
	(5 ppb)	(10, 25, 50 ppb)	
California	88.2	87.6	87.9
North Carolina	91.1	94.3	92.8
North Dakota	84.5	83.8	84.2
Washington	87.2	83.4	85.3

Recoveries of IKI-220 from individual fortified samples are shown in Tables 9-12, for California, North Carolina, North Dakota and Washington soil, respectively. Overall mean recoveries of IKI-220 for each soil type exceeded 70%. This indicates an acceptable degree of accuracy for the method. A few

individual recoveries were less than 70%, however the overall mean recovery for each soil type was still greater than 70% with these values.

PRECISION AND REPEATABILITY

The short-term precision afforded by an analytical method is given by the variability obtained in one laboratory with the same equipment on sets of replicate fortified samples. The relative standard deviation (RSD) calculated for a set of recoveries under these conditions is a measurement of repeatability. The range of recovery values is also an indicator of method precision. The table below lists the RSD of the mean recovery for each matrix and metabolites. Values are taken from Tables 5-8 which show individual sample recoveries for California, North Carolina, North Dakota and Washington soil, respectively.

Location	RSD of Mean IKI- 220 Recovery (%)	RSD of Mean TFNG Recovery (%)	RSD of Mean TFNG-AM Recovery (%)	RSD of Mean TFNA Recovery (%)	RSD of Mean TFNA-AM Recovery (%)	RSD of Mean TFNA-OH Recovery (%)
California	10	19	15	10	9	8
North Carolina	11	12	13	12	12	12
North Dakota	14	13	21	17	13	15
Washington	10	12	15	13	9	13

RSD values were less than 20% for each soil type, except for the RSD of TFNG-AM for North Dakota which was 21%. These RSD values indicate an acceptable level of method precision.

LIMIT OF QUANTITATION

The target method limit of quantitation (LOQ) or limit of determination (LDM) was 5.0 ppb. A practical assessment of method LOQ is given by measuring the accuracy and precision of recoveries obtained from replicate fortifications at the proposed LOQ. The mean recoveries and relative standard deviations (RSD) for concurrent recovery samples fortified at 5.0 ppb are listed in the table below.

Location	Mean / RSD IKI-220	Mean / RSD TFNG	Mean / RSD TFNG-AM	Mean / RSD TFNA	Mean / RSD TFNA-AM	Mean / RSD TFNA-OH
California	88/8	90/15	77/12	103/8	85/7	98/7
North Carolina	91/15	95/14	86/15	91/13	90/16	90/11
North Dakota	85/8	92/14	79/21	94/18	86/12	93/13
Washington	87/11	90/16	78/13	90/17	89/8	94/15

The mean recovery of ten 5.0 ppb soil fortifications for each of four soil types (9-5.0ppb fortifications for North Carolina) ranged from 85-91% for IKI-220, 90-96% for TFNG, 77-86% for TFNG-AM, 90-103% for TFNA, 85-90% for TFNA-AM and 89-98% for TFNA-OH, see Tables 5-8. The RSD of the 5.0 ppb soil fortifications in each soil type ranged from 8-15% for IKI-220, 13-16% for TFNG, 12-21% for TFNG-AM, 8-18% for TFNA, 7-16% for TFNA-AM and 7-13% for TFNG-OH, see Tables 5-8 (for a comprehensive listing of residues see Tables 9-12). These results support a method LOQ of 5.0 ppb for IKI-220 and its metabolites in soil.

RANGE

The upper range of analysis was 50.0 ppb. Assessment of this amount as the high point of the range was made by measuring the accuracy and precision of recoveries obtained from replicate fortifications at the proposed upper range. The mean recoveries and relative standard deviation (RSD) for each soil is listed in the table below, for the 50.0 ppb fortifications.

Location	Mean / RSD IKI-220	Mean / RSD TFNG	Mean / RSD TFNG-AM	Mean / RSD TFNA	Mean / RSD TFNA-AM	Mean / RSD TFNA-OH
California	88/12 ¹	86/23 ¹	72/17 ¹	96/13 ¹	87/11 ¹	95/10 ¹
North Carolina	94/8 ¹	90/10 ¹	82/10 ¹	94/11 ¹	95/8 ¹	98/11 ¹
North Dakota	84/18 ¹	83/11 ¹	72/22 ¹	87/15 ¹	82/14 ¹	86/17 ¹
Washington	83/8	92/9	79/17	89/8	88/10	88/9

1. Includes one 10 and one or two 25 ppb fortification. Otherwise, all fortifications made at 50 ppb.

The mean recovery of ten, 50.0 ppb soil fortifications for each of four soil types (8-50.0 ppb fortifications, 1-10.0 ppb and 1-25 ppb fortifications for North Carolina) ranged from 83-94% for IKI-220, 83-92% for TFNG, 72-82% for TFNG-AM, 87-94% for TFNA, 82-95% for TFNA-AM and 86-98% for TFNA-OH, see Tables 5-8. The RSD of the 50.0 ppb soil fortifications in each soil type ranged from 8-18% for IKI-220, 9-11% for TFNG, 10-22% for TFNG-AM, 8-15% for TFNA, 8-14% for TFNA-AM and 8-17% for TFNA-OH, see Tables 5-8 (for a comprehensive listing of residues see Tables 9-12). These results support an upper range of 50.0 ppb for quantitation of IKI-220 and its five metabolites in soil.

SPECIFICITY

Specificity by molecular ion for IKI-220 and its metabolites in the sample extracts was acceptable. Measurement was made by mass/mass detection, which is a highly discriminate analytical technique. Detection of peaks was based on mass and retention time.

LINEARITY

Linearity of calibration curves was very good in the concentration range 1 to 100 ng/mL IKI-220, TFNG, TFNG-AM, TFNA, TFNA-AM and TFNA-OH. The coefficient of determination (r^2) for all chromatographic runs was 0.995 or better. A first order linear fit with 1/X weighting was used for every calibration curve. See Appendix A for an example of typical detector linearity for IKI-220.

CAUTION/HINTS WITH THE METHOD

The analysis of TFNG-AM should be as rapid as possible. The analyte converts to TFNG over time on soil. This conversion was found in both ambient and frozen soil. Fortified concurrent recovery samples should be analyzed immediately (i.e. within six hours). However, it is noted that sample extracts and standards are stable when stored in a freezer. Otherwise there are no special issues with the method.

TIME TO ANALYZE A SET

Because of the automation of the ASE Extractor and with the use of an autosampler on the LC-MS/MS, samples can be efficiently processed. It is customary to analyze twenty samples or more in an eight-hour day.

CONCLUSIONS

The data shown in this report demonstrate that the analytical method for the determination of IKI-220 and its five major soil metabolites in soil provides adequate specificity, accuracy, precision and linearity. A method limit of quantitation of 5.0 and a range to 50 ppb was validated for IKI-220 and its metabolites in four different soil types.

Figure 1: Flow Diagram for IKI-220 Soil Method

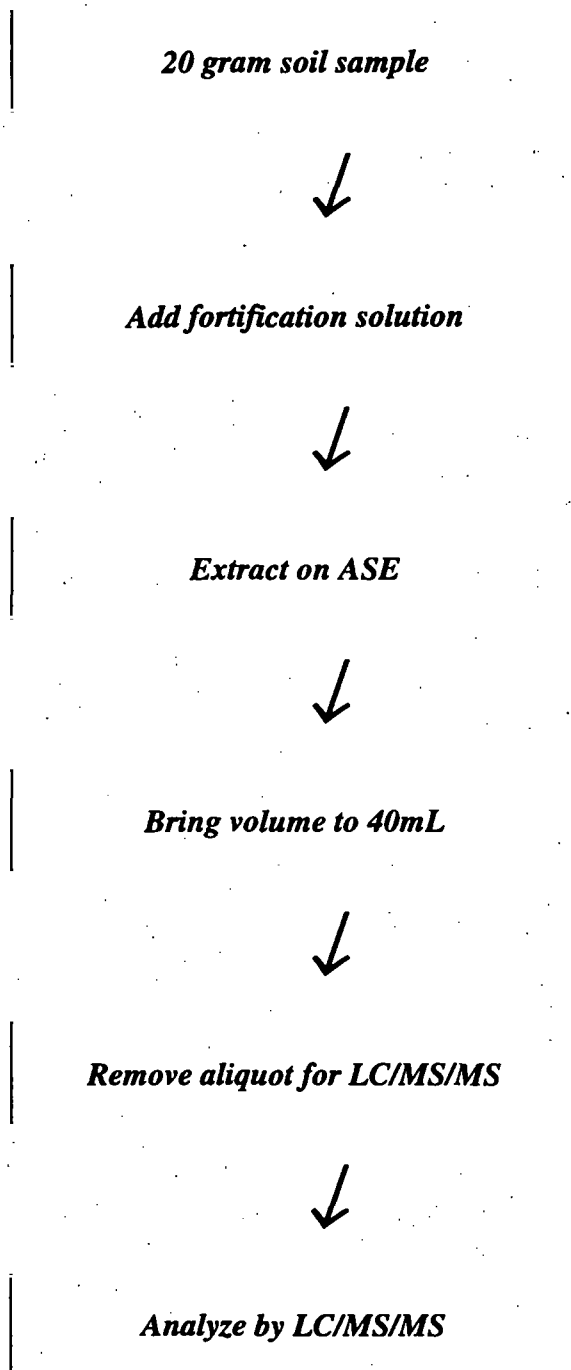


Table 1: Summary of Soil Characterization-California

Test Site: Excel Research Inc., Madera, California

Sample ID	Depth (Inches)	Depth (cm)	Texture	Sand (%)	Silt (%)	Clay (%)	OM (%)	pH	SP pH	CEC	%M 1/3 Bar (%)	%M 15 Bar (%)	Bulk Density (gm/cc)
00-1993	00-06	0-15	Sandy Loam	75	16	9	1.5	7.1	7.4	9.0	11.3	5.8	1.26
00-1994	06-12	15-30	Sandy Loam	73	18	9	0.8	7.1	7.0	6.4	10.2	5.4	1.35
00-1995	12-18	30-45	Sandy Loam	73	18	9	0.4	7.7	7.6	7.3	10.8	5.5	1.31
00-1996	18-24	45-60	Sandy Loam	71	18	11	0.4	8.0	7.9	8.0	12.1	6.1	1.27
00-1997	24-30	60-75	Sandy Loam	69	20	11	0.3	8.2	8.1	9.9	15.4	6.9	1.24
00-1998	30-36	75-90	Sandy Loam	67	24	9	0.3	8.4	8.2	10.0	16.4	7.3	1.21
00-1999	36-42	90-105	Sandy Loam	67	24	9	0.2	8.4	8.1	11.9	15.8	8.1	1.25
00-2000	42-48	105-120	Sandy Loam	65	24	11	0.6	8.1	8.0	13.1	17.9	9.5	1.19
00-2001	48-54	120-135	Sandy Loam	65	24	11	0.5	8.2	8.0	12.4	16.8	8.2	1.27
00-2002	54-60	135-150	Sandy Loam	69	22	9	0.2	8.3	8.1	13.9	17.5	8.4	1.27
00-2003	60-66	150-165	Sandy Loam	69	22	9	0.2	8.5	8.2	12.8	18.4	6.7	1.26
00-2004	66-72	165-180	Loam	41	38	21	0.3	8.5	8.3	14.8	21.4	8.1	1.21

OM: Organic Matter

SP pH: Saturation Paste pH

% M: % Moisture

CEC: Cation Exchange Capacity (meq/100 g)

Slope: 0 to 1%

Depth to Water Table: >6 ft.

Table 2: Soil Characterization Data-North Carolina

Test Site: Research Agricultural Associates, Garner, North Carolina

Sample ID	Depth (Inches)	Depth (cm)	Texture	Sand (%)	Silt (%)	Clay (%)	OM (%)	pH	SP pH	CEC	%M 1/3 Bar (%)	%M 15 Bar (%)	Bulk Density (gm/cc)
00-1972	00-06	0-15	Loamy Sand	85	12	3	0.8	6.3	6.3	2.6	5.8	1.7	1.49
00-1973	06-12	15-30	Loamy Sand	79	14	7	0.3	6.2	6.1	2.2	7.4	2.4	1.53
00-1974	12-18	30-45	Sandy Clay Loam	63	14	23	0.4	6.0	6.0	5.1	12.8	7.6	1.28
00-1975	18-24	45-60	Sandy Clay Loam	59	14	27	0.3	5.8	5.9	5.9	15.4	10.1	1.24
00-1976	24-30	60-75	Sandy Clay Loam	59	14	27	0.2	5.6	5.8	5.8	15.9	10.8	1.21
00-1977	30-36	75-90	Sandy Clay Loam	55	12	33	0.2	5.4	5.5	7.1	20.2	13.2	1.19
00-1978	36-42	90-105	Sandy Loam	53	30	17	0.1	5.2	5.3	6.9	19.6	13.9	1.14
00-1979	42-48	105-120	Sandy Clay Loam	53	12	35	0.1	5.0	5.1	7.2	20.4	14.1	1.15
00-1980	48-54	120-135	Sandy Clay Loam	57	10	33	0.1	4.9	5.0	6.6	19.7	14.0	1.13
00-1981	54-60	135-150	Sandy Clay Loam	57	10	33	0.2	5.0	5.1	7.2	19.3	13.9	1.17
00-1982	60-66	150-165	Sandy Clay Loam	57	10	33	0.1	4.9	5.0	6.0	20.9	14.4	1.14
00-1983	66-72	165-180	Sandy Clay Loam	57	10	33	0.1	4.9	5.0	6.4	19.9	14.1	1.16

OM: Organic Matter

SP pH: Saturation Paste pH

%M: Percent moisture at different pressures (i.e. water holding capacity)

CEC: Cation Exchange Capacity (meq/100 g)

Slope: <1%

Depth to Water Table: Greater than 6 feet, seasonal high level

Table 3: Summary of Soil Characterization-North Dakota

Test Site: Agvise Agricultural Laboratory, Northwood, North Dakota

Sample ID	Depth (Inches)	Depth (cm)	Texture	Sand (%)	Silt (%)	Clay (%)	OM (%)	pH	SP pH	CEC	%M 1/3 Bar (%)	%M 15 Bar (%)	Bulk Density (gm/cc)
00-1865	00-06	0-15	Loam	27	47	26	6.1	6.6	6.6	26.9	40.3	24.0	1.11
00-1866	06-12	15-30	Loam	31	49	20	5.0	7.6	7.4	24.2	36.8	25.3	1.02
00-1867	12-18	30-45	Silt Loam	31	51	18	3.4	8.2	7.9	22.1	33.8	22.5	0.99
00-1868	18-24	45-60	Clay Loam	27	43	30	1.9	8.4	8.1	21.2	32.9	22.1	1.06
00-1869	24-30	60-75	Clay Loam	21	41	38	1.3	8.5	8.3	19.4	32.8	19.8	0.99
00-1870	30-36	75-90	Silty Clay Loam	19	43	38	1.0	8.5	8.3	17.3	32.3	20.4	1.00
00-1871	36-42	90-105	Silty Clay Loam	13	55	32	0.9	8.4	8.2	18.6	32.7	19.5	1.03
00-1872	42-48	105-120	Loam	25	49	26	0.5	8.3	8.1	19.3	33.4	17.6	1.03
00-1873	48-54	120-135	Loam	33	47	20	0.3	8.1	7.9	19.1	35.8	16.0	0.99
00-1874	54-60	135-150	Silt Loam	23	57	20	0.3	8.0	7.9	19.2	33.9	15.4	0.99
00-1875	60-66	150-165	Silt Loam	23	59	18	0.3	8.1	8.0	20.2	33.2	15.8	0.99
00-1876	66-72	165-180	Silt Loam	31	51	18	0.2	8.0	7.9	19.2	29.0	14.2	1.01

OM: Organic Matter

SP pH: Saturation Paste pH

%M: Percent moisture at different pressures (i.e. water holding capacity)

CEC: Cation Exchange Capacity (meq/100 g)

Slope: 0%

USDA Classification - Gardeia silt loam

Table 4: Summary of Soil Characterization-Washington

Test Site: Qualls Agricultural Laboratory, Ephrata, Washington

Sample ID	Depth (Inches)	Depth (cm)	Texture	Sand (%)	Silt (%)	Clay (%)	OM (%)	pH	SP pH	CEC	%M 1/3 Bar (%)	%M 15 Bar (%)	Bulk Density (gm/cc)
00-1903	00-06	0-15	Sand	92	8	0	0.3	7.6	7.8	7.6	5.4	3.7	1.46
00-1904	06-12	15-30	Sand	94	6	0	0.2	7.6	7.5	7.4	5.0	3.6	1.51
00-1905	12-18	30-45	Sand	90	10	0	0.2	7.7	7.6	8.4	6.1	4.2	1.48
00-1906	18-24	45-60	Sand	90	10	0	0.2	7.6	7.6	9.3	6.1	4.2	1.49
00-1907	24-30	60-75	Sand	90	8	2	0.2	7.7	7.6	9.0	6.1	4.0	1.48
00-1908	30-36	75-90	Sand	88	12	0	0.2	7.7	7.6	9.6	6.9	4.1	1.48
00-1909	36-42	90-105	Sand	88	10	2	0.2	7.7	7.6	9.0	6.8	3.8	1.49
00-1910	42-48	105-120	Sandy Loam	72	22	6	0.2	7.9	8.0	10.7	8.7	4.1	1.52
00-1911	48-54	120-135	Sandy Loam	58	38	4	0.2	8.2	8.1	13.9	16.3	5.5	1.36
00-1912	54-60	135-150	Sandy Loam	60	34	6	0.1	8.3	8.2	15.7	15.8	5.7	1.39
00-1913	60-66	150-165	Loamy Sand	74	24	2	0.1	8.6	8.4	15.3	10.5	4.5	1.52
00-1914	66-72	165-180	Loamy Sand	86	12	2	0.0	9.1	8.9	13.5	8.0	4.1	1.50

OM: Organic Matter

SP pH: Saturation Paste pH

% M: % Moisture

CEC: Cation Exchange Capacity (meq/100 g)

Slope: 0 to 1%

Depth to Water Table: Greater than 6 feet, seasonal high level

Table 5: Concurrent Recoveries of IKI-220, TFNG, TFNG-AM, TFNA, TFNA-AM and TFNA-OH from California Soil

Sample ID (12281-)	Fort (ppb)	IKI-220 Recovery %	TFNG Recovery %	TFNG-AM Recovery %	TFNA Recovery %	TFNA-AM Recovery %	TFNA-OH Recovery %
31-A-2	5	92.1	108.0	76.2	94.7	96.7	95.7
35-2-A	5	85.1	108.0	71.2	91.8	93.3	100.0
39-2-A	5	79.1	88.3	85.2	92.4	81.2	84.6
47-2-A	5	90.3	84.3	80.3	119.0	79.7	107.0
59-2-A	5	82.4	83.5	76.9	107.0	79.9	103.0
63-2-A	5	99.1	76.7	77.4	107.0	82.8	105.0
71-2-A	5	81.0	74.6	70.2	106.0	79.2	103.0
75-2-A	5	97.3	78.5	65.9	105.0	88.3	91.0
79-2-A	5	88.5	110.0	97.8	102.0	85.1	92.2
55-3-A	10	108.0	83.7	76.6	110.0	98.2	104.0
55-2-A	25	86.7	87.8	67.0	106.0	86.3	98.4
31-A-3	50	83.2	101.0	70.8	91.5	89.8	89.3
35-3-A	50	90.4	117.0	72.9	97.4	96.1	101.0
39-3-A	50	94.5	104.0	86.6	90.9	91.7	94.2
47-3-A	50	70.4	58.8	59.2	72.5	70.4	80.2
59-3-A	50	81.7	72.7	72.3	103.0	83.0	104.0
63-3-A	50	91.0	72.6	68.1	102.0	85.4	96.9
71-3-A	50	91.3	78.9	72.0	109.0	85.9	104.0
75-3-A	50	77.9	63.4	49.9	80.2	72.2	79.8
79-3-A	50	87.6	104.0	93.2	99.5	94.8	94.0
Mean Recovery	All	87.88	87.79	74.49	99.35	86	96.37
Std. Deviation	All	8.41	16.65	10.94	10.78	7.78	8.18
Relative Std. Dev.	All	9.63	19.00	14.62	10.25	9.02	8.49
Mean Recovery	5	88.16	89.97	76.81	103.09	85.25	97.99
Std. Deviation	5	6.68	13.65	9.51	8.28	6.01	7.08
Relative Std. Dev.	5	7.58	15.17	12.38	8.03	7.05	7.23
Mean Recovery	10, 25, 50	87.6	85.61	72.16	95.6	86.75	94.74
Std. Deviation	10, 25, 50	10.23	19.71	12.26	12.07	9.50	9.23
Relative Std. Dev.	10, 25, 50	11.70	23.00	16.99	12.60	10.95	9.70

Table 6: Concurrent Recoveries of IKI-220, TFNG, TFNG-AM, TFNA, TFNA-AM and TFNA-OH from North Carolina

Sample ID (12283-)	Fort (ppm)	IKI-220 Recovery %	TFNG Recovery %	TFNG-AM Recovery %	TFNA Recovery %	TFNA-AM Recovery %	TFNA-OH Recovery %
31-A-2	5	84.1	98.2	74.2	84.7	93.2	90.8
35-2-A	5	82.9	104	80.0	90.9	101	98.4
39-2-C	5	83.2	82.9	70.2	71.4	70.6	70.0
47-2-A	5	95.7	97.9	90.0	82.4	87.3	95.5
55-2-A	5	119	109	109	107	106	93.7
63-2-A	5	88.8	103	93.2	92.7	91.1	89.0
67-2-C	5	105	107	97.3	109	106	100
75-2-A	5	83.0	70.0	70.4	93.2	65.3	84.1
79-2-A	5	77.8	87.9	89.9	88.6	84.9	78.4
71-3-A	10	106	83.5	72.9	100	96.4	106
71-2-A	25	98.1	77.9	76.6	107	93.7	111
31-A-3	50	90.7	95.0	79.4	88.2	91.7	92.5
35-3-A	50	88.8	97.0	78.8	100	98.7	103
39-3-C	50	81.3	77.2	73.3	72.4	76.8	74.9
47-3-A	50	97.3	101	93.0	89.8	97.2	101
63-3-A	50	92.0	93.9	88.6	98.3	94.0	92.7
67-3-C	50	89.8	97.8	94.7	90.2	99.2	93.9
75-3-A	50	103	82.2	79.1	107	96.3	106
79-3-A	50	96.4	96.4	87.7	87.4	106	94.5
Mean Recovery	All	92.8	92.7	84.1	92.6	92.4	93.4
Std. Deviation	All	10.4	11.0	10.6	10.9	11.3	10.8
Relative Std. Dev.	All	11.2	11.9	12.7	11.8	12.3	11.5
Mean Recovery	5	91.1	95.5	86.0	91.1	89.5	88.9
Std. Deviation	5	13.3	12.9	13.3	11.7	14.4	9.8
Relative Std. Dev.	5	14.6	13.5	15.4	12.8	16.1	11.1
Mean Recovery	10,25,50	94.3	90.2	82.4	94.0	95.0	97.6
Std. Deviation	10,25,50	7.3	9.0	8.0	10.6	7.5	10.3
Relative Std. Dev.	10,25,50	7.7	10.0	9.7	11.2	7.9	10.5

Table 7: Concurrent Recoveries of IKI-220, TFNG, TFNG-AM, TFNA, TFNA-AM and TFNA-OH from North Dakota

Sample ID (12282-)	Fort (ppb)	IKI-220 Recovery %	TFNG Recovery %	TFNG-AM Recovery %	TFNA Recovery %	TFNA-AM Recovery %	TFNA-OH Recovery %
31-A-2	5	80.3	99.3	64.8	96.3	80.4	78.6
35-2-A	5	74.6	94.2	72.6	76.7	81.7	95.7
39-2-A	5	89.6	89.6	70.2	93.0	83.4	90.0
47-2-A	5	81.5	95.4	96.7	89.6	77.4	99.3
55-2-A	5	99.6	123	118	129	112	117
63-2-A	5	80.1	83.7	70.0	87.9	80.9	81.7
71-2-A	5	78.7	87.1	85.2	81.9	84.1	89.8
71-2-D	5	87.7	90.0	68.1	72.0	79.3	79.3
75-2-A	5	85.6	78.5	70.3	112	86.3	99.1
79-2-A	5	87.7	79.4	70.4	106	90.0	95.6
83-3-A	10	98.3	76.0	64.1	100	96.2	100
55-3-A	25	118	106	114	88.2	108	94.8
83-2-A	25	89.8	76.6	61.6	95.7	86.6	97.3
31-A-3	50	61.9	73.4	49.6	66.6	62.8	64.9
35-3-A	50	77.7	93.0	70.0	83.8	80.9	81.1
39-3-A	50	72.1	82.3	69.1	80.8	77.0	79.3
47-3-A	50	70.5	77.6	75.0	76.9	72.6	78.1
63-3-A	50	75.4	78.6	60.6	71.1	71.9	69.9
71-3-A	50	77.9	82.3	85.1	77.0	78.8	72.2
71-3-D	50	81.9	85.7	76.8	86.6	81.2	82.0
75-3-A	50	94.2	84.7	70.2	112	86.8	111
79-3-A	50	88.6	78.6	65.1	100	82.9	103
Mean Recovery	All	84.2	87.1	74.9	90.1	83.7	89.1
Std. Deviation	All	12	12	16	15	11	13
Relative Std. Dev.	All	14	13	21	17	13	15
Mean Recovery	5	84.5	92.0	78.6	94.4	85.6	92.6
Std. Deviation	5	7	13	17	17	10	12
Relative Std. Dev.	5	8	14	21	18	12	13
Mean Recovery	10,25,50	83.8	82.9	71.8	86.6	82.1	86.1
Std. Deviation	10,25,50	15	9	16	13	12	15
Relative Std. Dev.	10,25,50	18	11	22	15	14	17

Table 8: Concurrent Recoveries of IKI-220, TFNG, TFNG-AM, TFNA, TFNA-AM and TFNA-OH from Washington Soil

Sample ID (12280-)	Fortified (ppb)	IKI-220 Recovery %	TFNG Recovery %	TFNG-AM Recovery %	TFNA Recovery %	TFNA-AM Recovery %	TFNA-OH Recovery %
31-A-2	5	89.8	97.0	80.5	99.1	93.8	104.0
35-2-A	5	80.1	103.0	76.8	85.7	89.1	102.0
39-2-A	5	90.0	92.3	80.6	95.6	93.6	99.3
39-2-B	5	*	70.0	68.5	68.6	*	68.5
43-2-A	5	70.0	61.2	56.5	61.7	71.6	69.3
47-2-A	5	99.9	88.8	88.1	101.0	92.1	99.3
55-2-A	5	101.0	103.0	90.7	107.0	93.4	105.0
63-2-A	5	87.3	90.5	75.9	85.8	86.0	90.4
71-2-A	5	85.5	101.0	71.9	108.0	89.4	105.0
75-2-A	5	81.4	90.6	88.0	88.7	94.3	99.5
31-A-3	50	88.1	100.0	71.3	96.7	94.6	90.5
35-3-A	50	83.4	98.1	74.8	87.1	84.9	92.6
39-3-A	50	85.9	90.1	82.7	91.5	89.4	88.5
39-3-B	50	*	79.3	74.2	78.6	*	73.5
43-3-A	50	86.3	97.8	92.1	90.3	102.0	86.5
47-3-A	50	89.0	101.0	91.3	99.4	93.9	100.0
55-3-A	50	90.4	96.1	86.9	94.1	90.8	92.4
63-3-A	50	75.4	82.3	81.3	77.0	78.5	78.9
71-3-A	50	70.8	79.7	47.8	83.6	70.7	84.4
75-3-A	50	81.5	91.9	86.0	93.0	90.0	90.1
Mean Recovery	All	85.32	90.69	78.30	89.63	88.78	90.99
Std. Dev.	All	8.25	11.25	11.48	11.81	8.06	11.42
Relative Std. Dev.	All	9.67	12.40	14.66	13.18	9.07	12.55
Mean Recovery	5	87.22	89.74	77.75	90.12	89.26	94.23
Std. Dev.	5	9.68	13.92	10.39	15.44	7.18	14.00
Relative Std. Dev.	5	11.09	15.51	13.35	17.13	8.04	14.86
Mean Recovery	50	83.42	91.63	78.84	89.13	88.31	87.74
Std. Dev.	50	6.55	8.44	13.02	7.47	9.26	7.48
Relative Std. Dev.	50	7.85	9.21	16.5	8.38	10.49	8.53

*Negative ions were not analyzed for these samples.

Table 9: Comprehensive Data, California Control and Fortified Samples

Sample ID	Interval	Fort (ppb) ¹	Calculated Concentration(ng/mL) by Analyte					
			IKI-220	TFNG	TFNG-AM	TFNA	TFNA-AM	TFNA-OH
31-Reagent Blank	-1Day		N/A	N/A	N/A	N/A	N/A	N/A
31-1A	-1Day		N/A	N/A	N/A	N/A	N/A	N/A
31-2A	-1Day	5	2.30	2.70	1.91	2.37	2.42	2.39
31-3A	-1Day	50	20.8	25.2	17.70	22.9	22.42	22.3
35-Reagent Blank	0Day		N/A	N/A	N/A	N/A	N/A	N/A
35-1A	0Day		N/A	N/A	N/A	N/A	N/A	N/A
35-2A	0Day	5	2.13	2.70	1.78	2.29	2.33	2.51
35-3A	0Day	50	22.6	29.3	18.2	24.3	24.0	25.3
39-Reagent Blank	6Day		N/A	N/A	N/A	N/A	N/A	N/A
39-1A	6Day		N/A	N/A	N/A	N/A	N/A	N/A
39-2A	6Day	5	1.978	2.209	2.131	2.309	2.029	2.116
39-3A	6Day	50	23.62	25.99	21.65	22.73	22.92	23.55
47-Reagent Blank	0Day		N/A	N/A	N/A	N/A	N/A	N/A
47-1A	0Day		N/A	N/A	N/A	N/A	N/A	N/A
47-2A	0Day	5	2.257	2.108	2.007	2.976	1.992	2.685
47-3A	0Day	50	17.60	14.71	14.80	18.12	17.61	20.04

N/A=no residue present

¹ng/mL value must be multiplied x 2 to correct for volume and weight in residue data

Table 9 (cont.): Comprehensive Data, California Control and Fortified Samples

Sample ID	Interval	Fort (ppb) ¹	Calculated Concentration(ng/mL) by Analyte					
			IKI-220	TFNG	TFNG-AM	TFNA	TFNA-AM	TFNA-OH
55-Reagent Blank	1Day		N/A	N/A	N/A	N/A	N/A	N/A
55-1A	1Day		N/A	N/A	N/A	N/A	N/A	N/A
55-2A (25 ppb)	1Day	25	10.84	10.98	8.372	13.21	10.79	12.30
55-3A (10 ppb)	1Day	10	5.417	4.186	3.831	5.484	4.909	5.191
59-Reagent Blank	2Day		N/A	N/A	N/A	N/A	N/A	N/A
59-1A	2Day		N/A	N/A	N/A	N/A	N/A	N/A
59-2A	2Day	5	2.060	2.088	1.923	2.666	1.997	2.576
59-3A	2Day	50	20.43	18.16	18.08	25.72	20.74	26.09
63-Reagent Blank	4Day		N/A	N/A	N/A	N/A	N/A	N/A
63-1A	4Day		N/A	N/A	N/A	N/A	N/A	N/A
63-2A	4Day	5	2.479	1.918	1.935	2.681	2.070	2.624
63-3A	4Day	50	22.74	18.15	17.02	25.50	21.35	24.23
71-Reagent Blank	14Day		N/A	N/A	N/A	N/A	N/A	N/A
71-1A	14Day		N/A	N/A	N/A	N/A	N/A	N/A
71-2A	14Day	5	2.025	1.865	1.755	2.656	1.980	2.570
71-3A	14Day	50	22.81	19.73	18.00	27.26	21.47	25.91

N/A=no residue present

¹ng/mL value must be multiplied x 2 to correct for volume and weight in residue data

Table 9 (cont.): Comprehensive Data, California Control and Fortified Samples

Sample ID	Interval	Fort (ppb) ¹	Calculated Concentration(ng/mL) by Analyte					
			IKI-220	TFNG	TFNG-AM	TFNA	TFNA-AM	TFNA-OH
75-Reagent Blank	28Day		N/A	N/A	N/A	N/A	N/A	N/A
75-1A	28Day		N/A	N/A	N/A	N/A	N/A	N/A
75-2A	28Day	5	2.432	1.962	1.648	2.637	2.209	2.274
75-3A	28Day	50	19.47	15.85	12.47	20.05	18.05	19.96
79-Reagent Blank	60Day		N/A	N/A	N/A	N/A	N/A	N/A
79-1A	60Day		N/A	N/A	N/A	N/A	N/A	N/A
79-2A	60Day	5	2.21	2.755	2.445	2.549	2.13	2.306
79-3A	60Day	50	21.9	25.97	23.31	24.88	23.7	23.49

N/A=no residue present

¹ng/mL value must be multiplied x 2 to correct for volume and weight in residue data

Table 10: Comprehensive Data, North Carolina Control and Fortified Samples

Sample ID	Interval	Fort (ppb) ¹	Calculated Concentration(ng/mL) by Analyte					
			IKI-220	TFNG	TFNG-AM	TFNA	TFNA-AM	TFNA-OH
12283-								
31-Reagent Blank	-1Day		N/A	N/A	N/A	N/A	N/A	N/A
31-1A	-1Day		N/A	N/A	N/A	N/A	N/A	N/A
31-2A	-1Day	5	2.10	2.45	1.85	2.12	2.33	2.27
31-3A	-1Day	50	22.7	23.7	19.8	22.1	22.9	23.1
35-Reagent Blank	0Day App 1		N/A	N/A	N/A	N/A	N/A	N/A
35-1A	0Day App 1		N/A	N/A	N/A	N/A	N/A	N/A
35-2A	0Day App 1	5	2.07	2.61	2.00	2.27	2.53	2.46
35-3A	0Day App 1	50	22.2	24.3	19.7	25.0	24.7	25.8
39-Reagent Blank	6Day App 1		N/A	N/A	N/A	N/A	N/A	N/A
39-1C	6Day App 1		N/A	N/A	N/A	N/A	N/A	N/A
39-2C	6Day App 1	5	2.080	2.073	1.756	1.786	1.765	1.749
39-3C	6Day App 1	50	20.32	19.31	18.32	18.10	19.21	18.73
47-Reagent Blank	6 Day App 2		N/A	N/A	N/A	N/A	N/A	N/A
47-1A	6 Day App 2		N/A	N/A	N/A	N/A	N/A	N/A
47-2A	6 Day App 2	5	2.394	2.448	2.251	2.060	2.183	2.388
47-3A	6 Day App 2	50	24.33	25.17	23.25	22.46	24.30	25.16
55-Reagent Blank	1,2Day		N/A	N/A	N/A	N/A	N/A	N/A
55-1A	1,2Day		N/A	N/A	N/A	N/A	N/A	N/A
55-2A	1,2Day	5	2.976	2.726	2.728	2.678	2.638	2.755

N/A=no residue present

¹ng/mL value must be multiplied x 2 to correct for volume and weight in residue data

Table 10 (cont.): Comprehensive Data, North Carolina Control and Fortified Samples

Sample ID	Interval	Fort (ppb) ¹	Calculated Concentration(ng/mL) by Analyte					
12283-			IKI-220	TFNG	TFNG-AM	TFNA	TFNA-AM	TFNA-OH
63-Reagent Blank	4Day		N/A	N/A	N/A	N/A	N/A	N/A
63-1A	4Day		N/A	N/A	N/A	N/A	N/A	N/A
63-2A	4Day	5	2.219	2.577	2.331	2.318	2.278	2.224
63-3A	4Day	50	23.01	23.48	22.16	24.57	23.50	23.17
67-Reagent Blank	7Day		N/A	N/A	N/A	N/A	N/A	N/A
67-1C	7Day		N/A	N/A	N/A	N/A	N/A	N/A
67-2C	7Day	5	2.626	2.673	2.434	2.716	2.640	2.503
67-3C	7Day	50	22.46	24.45	23.67	22.54	24.80	23.47
71-Reagent Blank	14Day		N/A	N/A	N/A	N/A	N/A	N/A
71-1A	14Day		N/A	N/A	N/A	N/A	N/A	N/A
71-2A	14Day	25	12.27	9.740	9.579	13.37	11.71	13.89
71-3A	14Day	10	5.303	4.176	3.644	5.016	4.821	5.276
75-Reagent Blank	28Day		N/A	N/A	N/A	N/A	N/A	N/A
75-1A	28Day		N/A	N/A	N/A	N/A	N/A	N/A
75-2A	28Day	5	2.076	1.751	1.759	2.331	1.631	2.103
75-3A	28Day	50	25.70	20.55	19.78	26.70	24.07	26.48
79-Reagent Blank	60Day		N/A	N/A	N/A	N/A	N/A	N/A
79-1A	60Day		N/A	N/A	N/A	N/A	N/A	N/A
79-2A	60Day	5	1.95	2.198	2.249	2.216	2.12	1.959
79-3A	60Day	50	24.1	24.09	21.92	21.86	26.4	23.64

N/A=no residue present

¹ng/mL value must be multiplied x 2 to correct for volume and weight in residue data

Table 11: Comprehensive Data, North Dakota Control and Fortified Samples

Sample ID	Interval	Fort (ppb) ¹	Calculated Concentration(ng/mL) by Analyte					
			IKI-220	TFNG	TFNG-AM	TFNA	TFNA-AM	TFNA-OH
31-Reagent Blank	-11Day		N/A	N/A	N/A	N/A	N/A	N/A
31-1A	-11Day		N/A	N/A	N/A	N/A	N/A	N/A
31-2A	-11Day	5	2.01	2.48	1.62	2.41	2.01	1.96
31-3A	-11Day	50	15.5	18.4	12.4	16.6	15.7	16.2
35-Reagent Blank	0Day		N/A	N/A	N/A	N/A	N/A	N/A
35-1A	0Day		N/A	N/A	N/A	N/A	N/A	N/A
35-2A	0Day	5	1.87	2.36	1.81	1.92	2.04	2.39
35-3A	0Day	50	19.4	23.3	17.5	20.9	20.2	20.3
39-Reagent Blank	6Day App 1		N/A	N/A	N/A	N/A	N/A	N/A
39-1A	6Day App 1		N/A	N/A	N/A	N/A	N/A	N/A
39-2A	6Day App 1	5	2.241	2.240	1.756	2.324	2.086	2.249
39-3A	6Day App 1	50	18.03	20.57	17.26	20.21	19.26	19.84
47-Reagent Blank	6 Day App 2		N/A	N/A	N/A	N/A	N/A	N/A
47-1A	6 Day App 2		N/A	N/A	N/A	N/A	N/A	N/A
47-2A	6 Day App 2	5	2.04	2.384	2.418	2.241	1.93	2.482
47-3A	6 Day App 2	50	17.6	19.404	18.747	19.235	18.1	19.526

N/A= no residue present

¹ng/mL value must be multiplied x 2 to correct for volume and weight in residue data

Table 11 (cont.): Comprehensive Data, North Dakota Control and Fortified Samples

Sample ID	Interval	Fort (ppb) ¹	Calculated Concentration (ng/mL) by Analyte					
			IKI-220	TFNG	TFNG-AM	TFNA	TFNA-AM	TFNA-OH
55-Reagent Blank	1,2Day		N/A	N/A	N/A	N/A	N/A	N/A
55-1A	1,2Day		N/A	N/A	N/A	N/A	N/A	N/A
55-2A	1,2Day	5	2.489	3.068	2.940	3.230	2.810	2.914
55-3A	1,2Day	50	14.73	13.27	14.23	11.02	13.50	11.86
63-Reagent Blank	4,7Day		N/A	N/A	N/A	N/A	N/A	N/A
63-1A	4,7Day		N/A	N/A	N/A	N/A	N/A	N/A
63-2A	4,7Day	5	2.003	2.093	1.751	2.197	2.021	2.043
63-3A	4,7Day	50	18.85	19.65	15.15	17.78	17.97	17.48
71-Reagent Blank	14Day		N/A	N/A	N/A	N/A	N/A	N/A
71-1A	14Day		N/A	N/A	N/A	N/A	N/A	N/A
71-2A	14Day	5	1.97	2.176	2.130	2.049	2.10	2.244
71-3A	14Day	50	19.5	20.572	21.274	19.243	19.7	18.052
71D-Reagent Blank	14Day 0 Day App 2		N/A	N/A	N/A	N/A	N/A	N/A
71-1D	14Day 0 Day App 2		N/A	N/A	N/A	N/A	N/A	N/A
71-2D	14Day 0 Day App 2	5	2.194	2.251	1.702	1.801	1.982	1.983
71-3D	14Day 0 Day App 2	50	20.47	21.43	19.20	21.65	20.30	20.51

N/A=no residue present

¹ng/mL value must be multiplied x 2 to correct for volume and weight in residue data

Table 11 (cont.): Comprehensive Data, North Dakota Control and Fortified Samples

Sample ID	Interval	Fort (ppb)	Calculated Concentration(ng/mL) by Analyte					
			IKI-220	TFNG	TFNG-AM	TFNA	TFNA-AM	TFNA-OH
75-Reagent Blank	28Day		N/A	N/A	N/A	N/A	N/A	N/A
75-1A	28Day		N/A	N/A	N/A	N/A	N/A	N/A
75-2A	28Day	5	2.141	1.963	1.757	2.802	2.157	2.478
75-3A	28Day	50	23.55	21.18	17.54	28.10	21.71	27.63
79-Reagent Blank	60Day		N/A	N/A	N/A	N/A	N/A	N/A
79-1A	60Day		N/A	N/A	N/A	N/A	N/A	N/A
79-2A	60Day	5	2.192	1.985	1.760	2.657	2.251	2.390
79-3A	60Day	50	22.14	19.64	16.27	25.11	20.74	25.67
83-Reagent Blank	90Day		N/A	N/A	N/A	N/A	N/A	N/A
83-1A	90Day		N/A	N/A	N/A	N/A	N/A	N/A
83-2A	90Day	25	11.23	9.571	7.703	11.96	10.83	12.16
83-3A	90Day	10	4.914	3.798	3.205	5.007	4.811	5.016

N/A=no residue present

¹ng/mL value must be multiplied x 2 to correct for volume and weight in residue data

Table 12: Comprehensive Data, Washington Control and Fortified Samples

Sample ID	Interval	Fort (ppb)	Calculated Concentration(ng/mL) by Analyte ¹					
			IKI-220	TFNG	TFNG-AM	TFNA	TFNA-AM	TFNA-CH
31-Reagent Blank	-1Day		N/A	N/A	N/A	N/A	N/A	N/A
31-1A	-1Day		N/A	N/A	N/A	N/A	N/A	N/A
31-2A	-1Day	5	2.25	2.43	2.01	2.48	2.35	2.61
31-3A	-1Day	50	22.0	25.1	17.8	24.2	23.7	22.6
35-Reagent Blank	0Day		N/A	N/A	N/A	N/A	N/A	N/A
35-1A	0Day		N/A	N/A	N/A	N/A	N/A	N/A
35-2A	0Day	5	2.00	2.57	1.92	2.14	2.23	2.55
35-3A	0Day	50	20.8	24.5	18.7	21.8	21.2	23.1
39-Reagent Blank	6Day		N/A	N/A	N/A	N/A	N/A	N/A
39-1A	6Day		N/A	N/A	N/A	N/A	N/A	N/A
39-2A	6Day	5	2.25	2.31	2.01	2.39	2.34	2.48
39-3A	6Day	50	21.5	22.5	20.7	22.9	22.4	22.1
39-Reagent Blank	Verification		*	N/A	N/A	N/A	*	N/A
39-1B	Verification		*	N/A	N/A	N/A	*	N/A
39-2B	Verification	5	*	1.75	1.71	1.71	*	1.71
39-3B	Verification	50	*	19.8	18.6	19.7	*	18.4
43-Reagent Blank	0Day		N/A	N/A	N/A	N/A	N/A	N/A
43-1A	0Day		N/A	N/A	N/A	N/A	N/A	N/A
43-2A	0Day	5	1.76	1.53	1.41	1.54	1.79	1.73
43-3A	0Day	50	21.6	24.4	23.0	22.6	25.4	21.6

N/A=no residue present

¹ng/mL value must be multiplied x 2 to correct for volume and weight in residue data

*Negative ions were not run for these samples.

Table 12 (cont.): Comprehensive Data, Washington Control and Fortified Samples

Sample ID	Interval	Fort (ppb) ²	Calculated Concentration(ng/mL) by Analyte ¹					
			IKI-220	TFNG	TFNG-AM	TFNA	TFNA-AM	TFNA-OH
47-Reagent Blank	6Day		N/A	N/A	N/A	N/A	N/A	N/A
47-1A	6Day		N/A	N/A	N/A	N/A	N/A	N/A
47-2A	6Day	5	2.5	2.22	2.20	2.53	2.30	2.48
47-3A	6Day	50	22.3	25.2	22.8	24.8	23.5	25.0
55-Reagent Blank	1Day		N/A	N/A	N/A	N/A	N/A	N/A
55-1A	1Day		N/A	N/A	N/A	N/A	N/A	N/A
55-2A	1Day	5	2.52	2.58	2.27	2.68	2.34	2.63
55-3A	1Day	50	22.6	24.0	21.7	23.5	22.7	23.1
63-Reagent Blank	4Day		N/A	N/A	N/A	N/A	N/A	N/A
63-1A	4Day		N/A	N/A	N/A	N/A	N/A	N/A
63-2A	4Day	5	2.18	2.26	1.90	2.15	2.15	2.26
63-3A	4Day	50	18.8	20.6	20.3	19.2	19.6	19.7
71-Reagent Blank	14Day		N/A	N/A	N/A	N/A	N/A	N/A
71-1A	14Day		N/A	N/A	N/A	N/A	N/A	N/A
71-2A	14Day	5	2.138	2.537	1.798	2.690	2.235	2.613
71-3A	14Day	50	17.69	19.94	11.94	20.91	17.67	21.09
75-Reagent Blank	28Day		N/A	N/A	N/A	N/A	N/A	N/A
75-1A	28Day		N/A	N/A	N/A	N/A	N/A	N/A
75-2A	28Day	5	2.03	2.27	2.20	2.22	2.34	2.49
75-3A	28Day	50	20.4	23.00	21.5	23.3	22.4	22.5

N/A=no residue present

¹ng/mL value must be multiplied x 2 to correct for volume and weight in residue data

²0-6 inches = 0-15 cm

APPENDIX A

Representative Chromatograms and Sample Calculations

Note that only the chromatograms and sample calculations for the North Carolina location (12283) were included in this document. Chromatograms and sample calculations for the other three locations are similar to these documents and therefore are not included.

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Explanation of Chromatograms

Figures A-1 through A-8 show a representative set of chromatograms including control, concurrent recovery samples, as well as actual sample assays from two different time points.

Residue Sample Calculations

Residues were calculated by plotting the peak areas for the eight standards run within the sample set to obtain a best-fit linear external standard calibration plot. Peak areas of the samples analyzed with the calibration samples, were calculated using the slope and intercept of the line to generate a result in ng/mL based on the injected sample. These calculated amounts were put in a Summary Sheet. This amount was adjusted for sample volume and weight to generate a ppb result. The calculation is shown below:

$$\text{Gross residue ppm} = \frac{(D - I)}{S} * \frac{V}{W}$$

Where: S = Slope (μV-sec/μg/mL)
D = Detector Response, (Peak Area, μV-sec)
I = Intercept (μV -sec)
V = Volume for HPLC or Multiplier (mL)
W = Sample Aliquot Weight (g)

For sample 012282-55-2-A (Figure A-4) the IKI-220 residue was calculated as follows:

$$\begin{aligned} \text{Gross Residue ppm} &= \frac{6774.4 - 79.6}{2250} * \frac{40}{20} \\ &= 5.95 \text{ ppb} \end{aligned}$$

$$\% \text{ Recovery} = \frac{(\text{ppm found} - \text{ppm control}) * 100}{\text{ppm added}}$$

$$\% \text{ Recovery} = \frac{(5.95 - 0) * 100}{5} = 119 \%$$

Calibration and quantitation of the metabolites TFNG, TFNG-AM, TFNA, TFNA-OH and TFNA-AM were calculated in the same manner as IKI-220.

Moisture-Correction Sample Calculations

Treated sample residues were corrected for moisture as shown below.

$$\text{corrected residue ppm} = \frac{(\text{uncorrected residue ppm})}{(100 - \% \text{moisture})} \times 100$$

An example of moisture-correction is shown below for treated sample 012282-45-1A, application 2, day 0, 0-6-inch, plot A. The uncorrected residue value taken from the comprehensive data table is 37.2 ppb, the moisture is 21.3%. Substitute the values in the above equation to give:

$$\text{corrected residue ppm} = \frac{(37.2 \text{ ppb})}{(100 - 21.3\%)} \times 100$$

$$\text{corrected residue ppm} = \frac{(37.2 \text{ ppm})}{(0.787)}$$

$$\text{corrected dry residue} = 47.3 \text{ ppb}$$

Figure A - 1: Calibration Curve for IKI-220

E062801C.rdb (IKI-220): "Linear" Regression ("1/x" weighting): $y = 2.25e+003 x + 79.6$ ($r = 0.9995$)

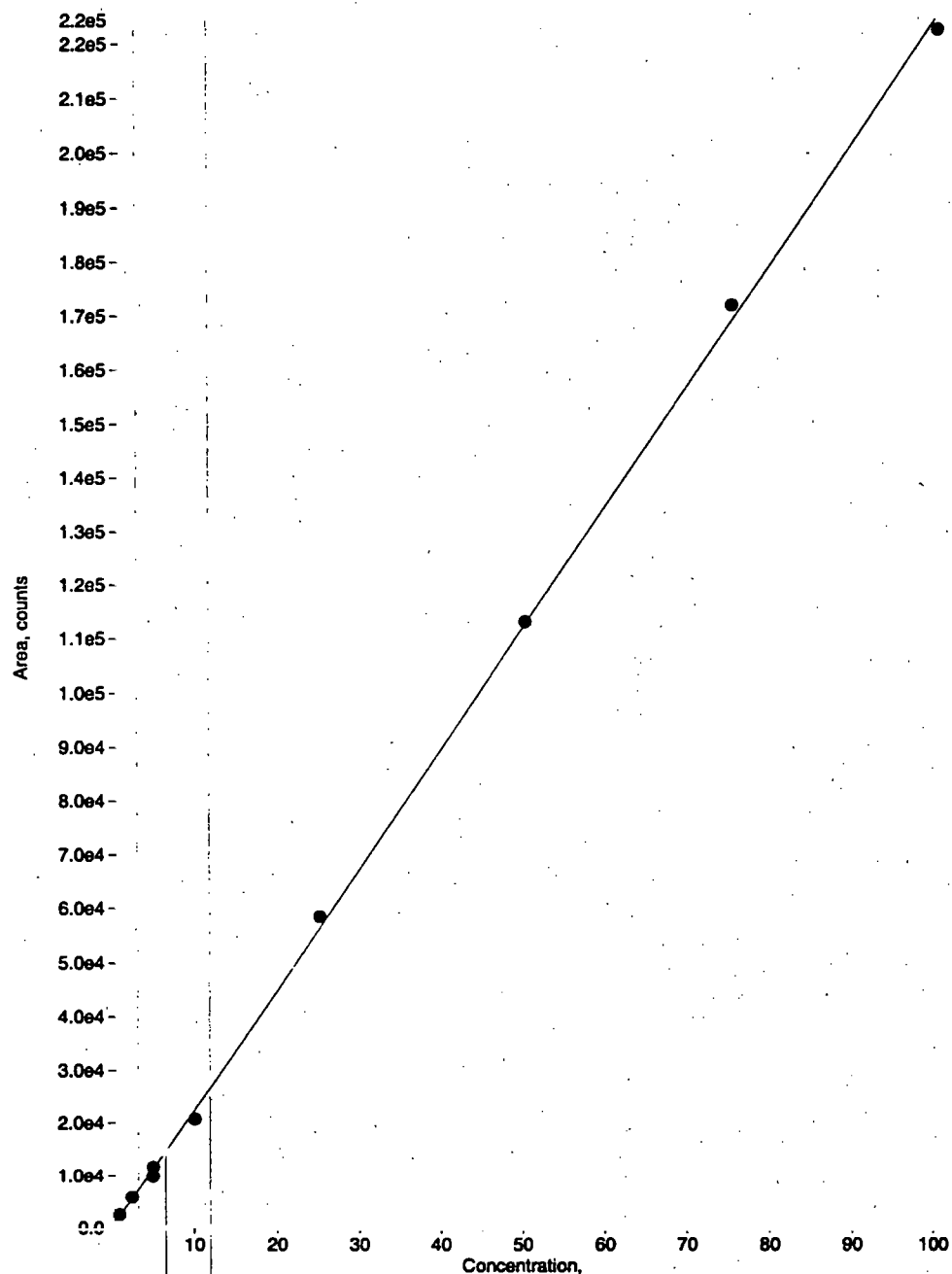


Figure A - 2: Calibration Curve for TFNA-AM

E062801C.rdb (TFNA-AM): Linear Regression (1/x weighting): $y = 1.55e+004 \cdot x + 1.41e+003$ ($r = 0.9999$)

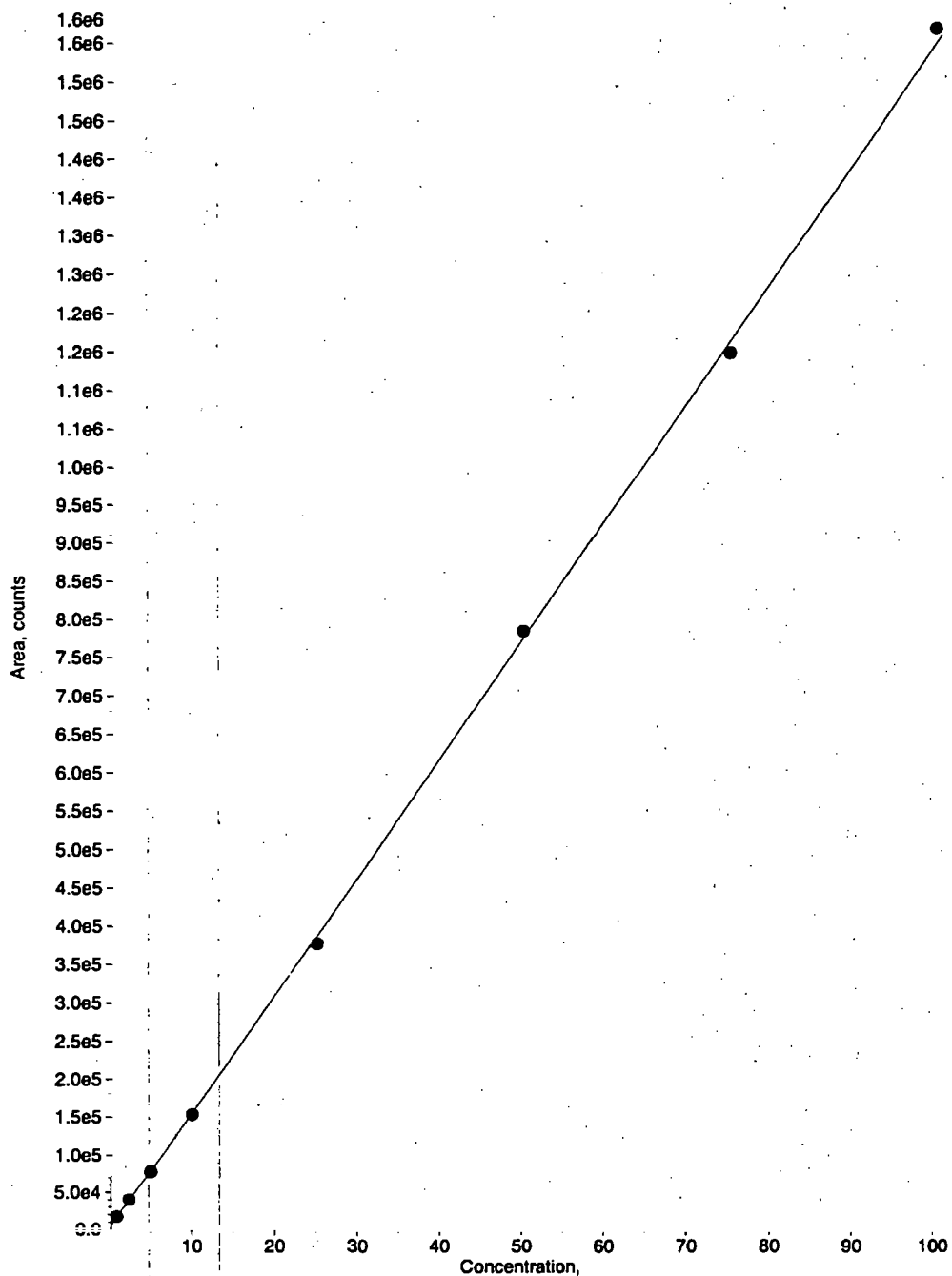


Figure A - 3: Calibration Curve for TFNG

E062801B.rdb (TF-VG): Linear Regression (*1/x weighting): $y = 2.09e+003 x + .193$ ($r = 0.9998$)

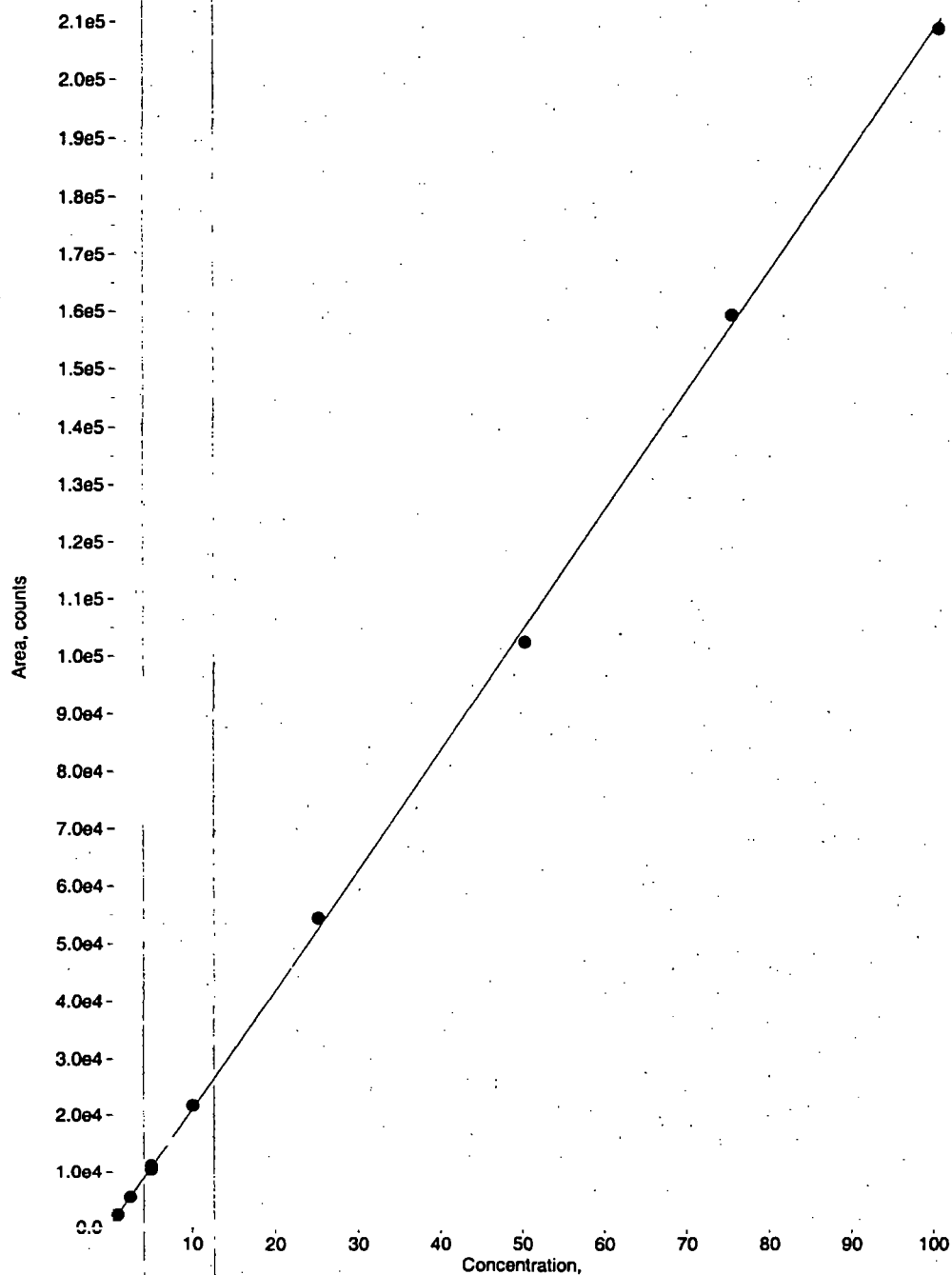


Figure A - 4: Calibration Curve for TFNG-AM

E062801B.rdb (TFNG-AM): Linear Regression ("1/x" weighting) $y = 2.42e+003 x + 433$ ($r = 0.9974$)

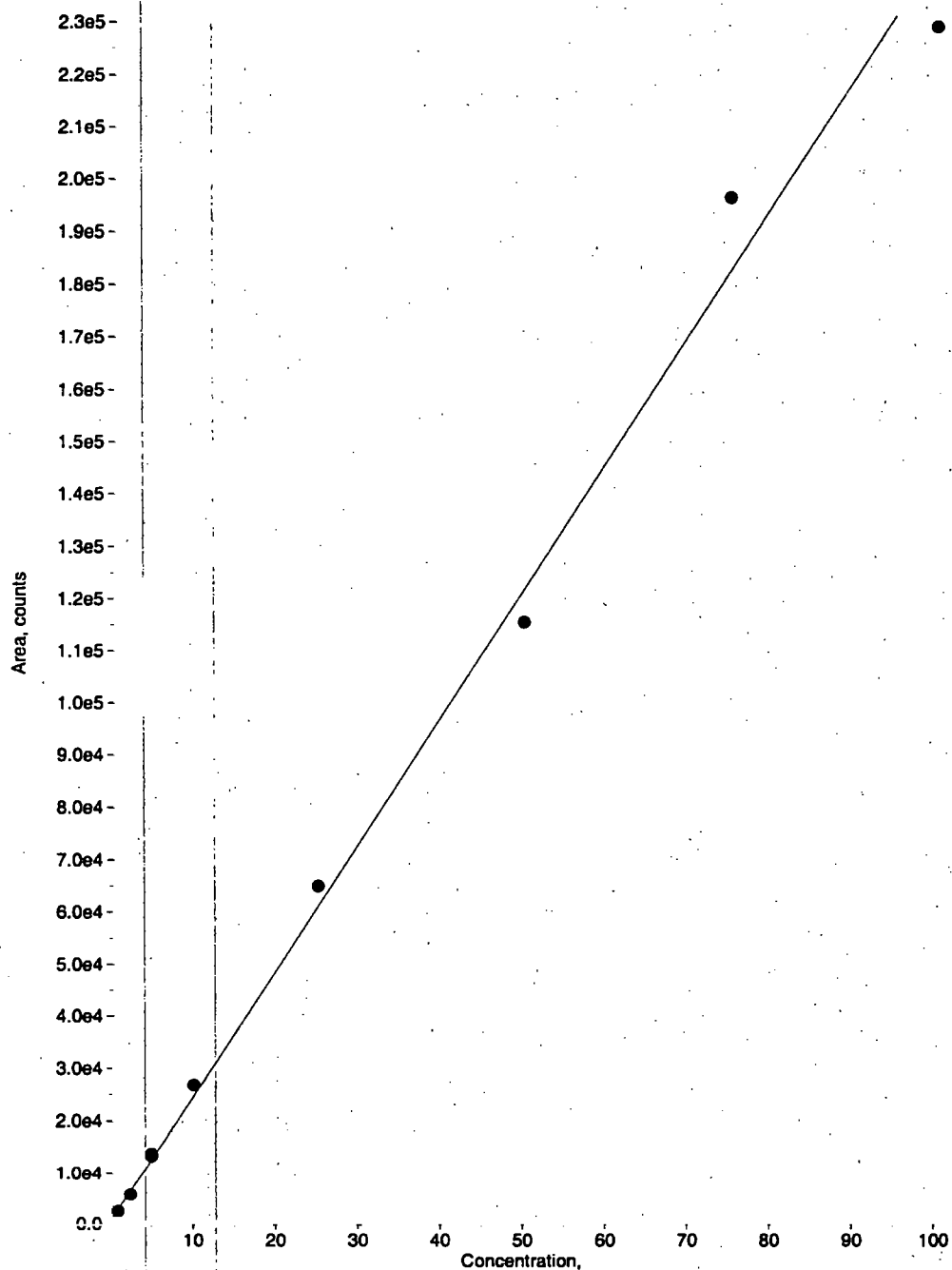


Figure A - 5: Calibration Curve for TFNA

E062801B.rdb (TFNA): Linear Regression (1/x weighting): $y = 1.92e+004 \cdot x + 70$ ($r = 0.9998$)

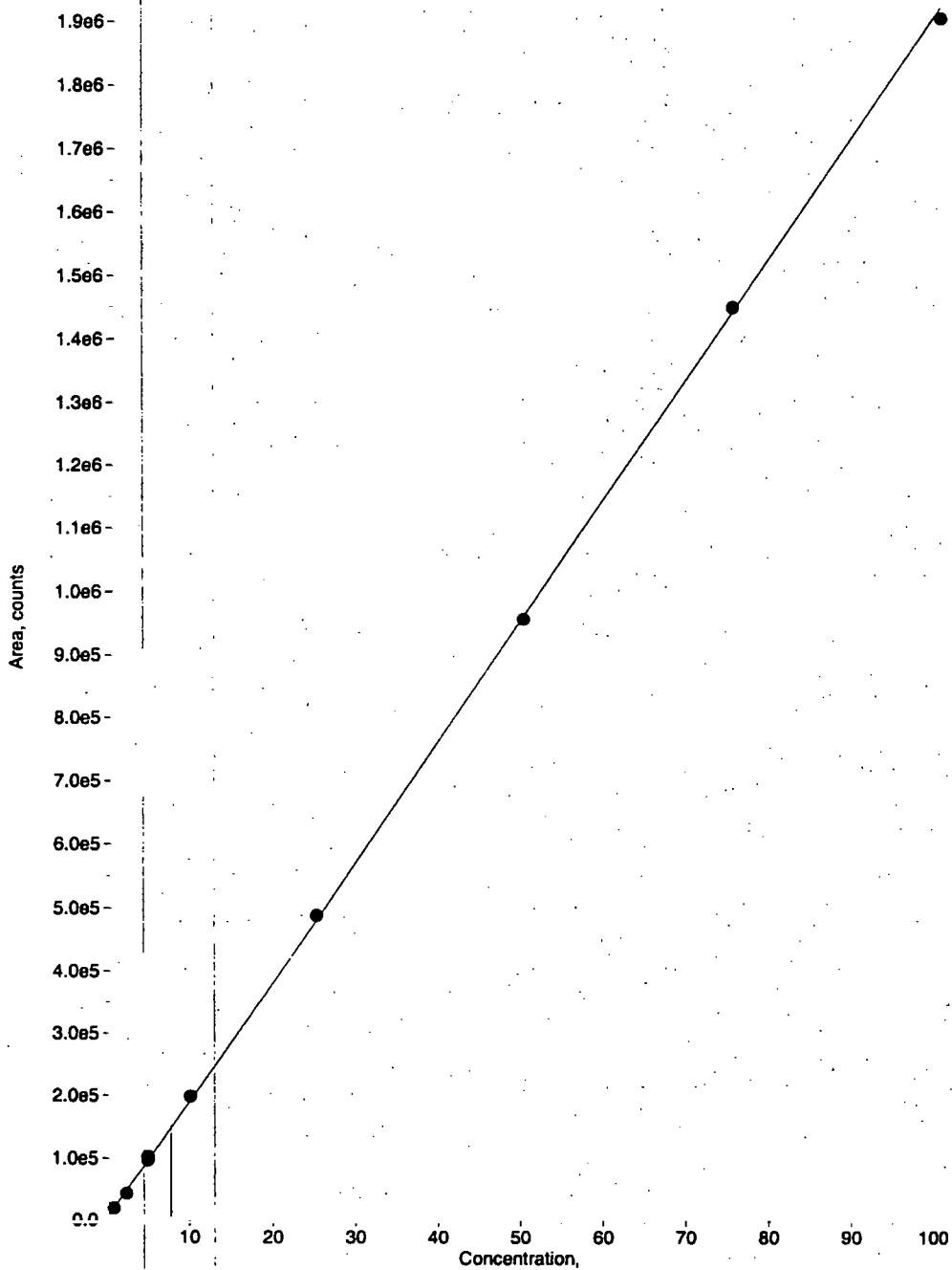


Figure A - 6: Calibration Curve for TFNA-OH

E062801B.rdb (TFNA-OH): Linear Regression (1/x weighting): $y = 1.33e+004 x + 142$ ($r = 0.9999$)

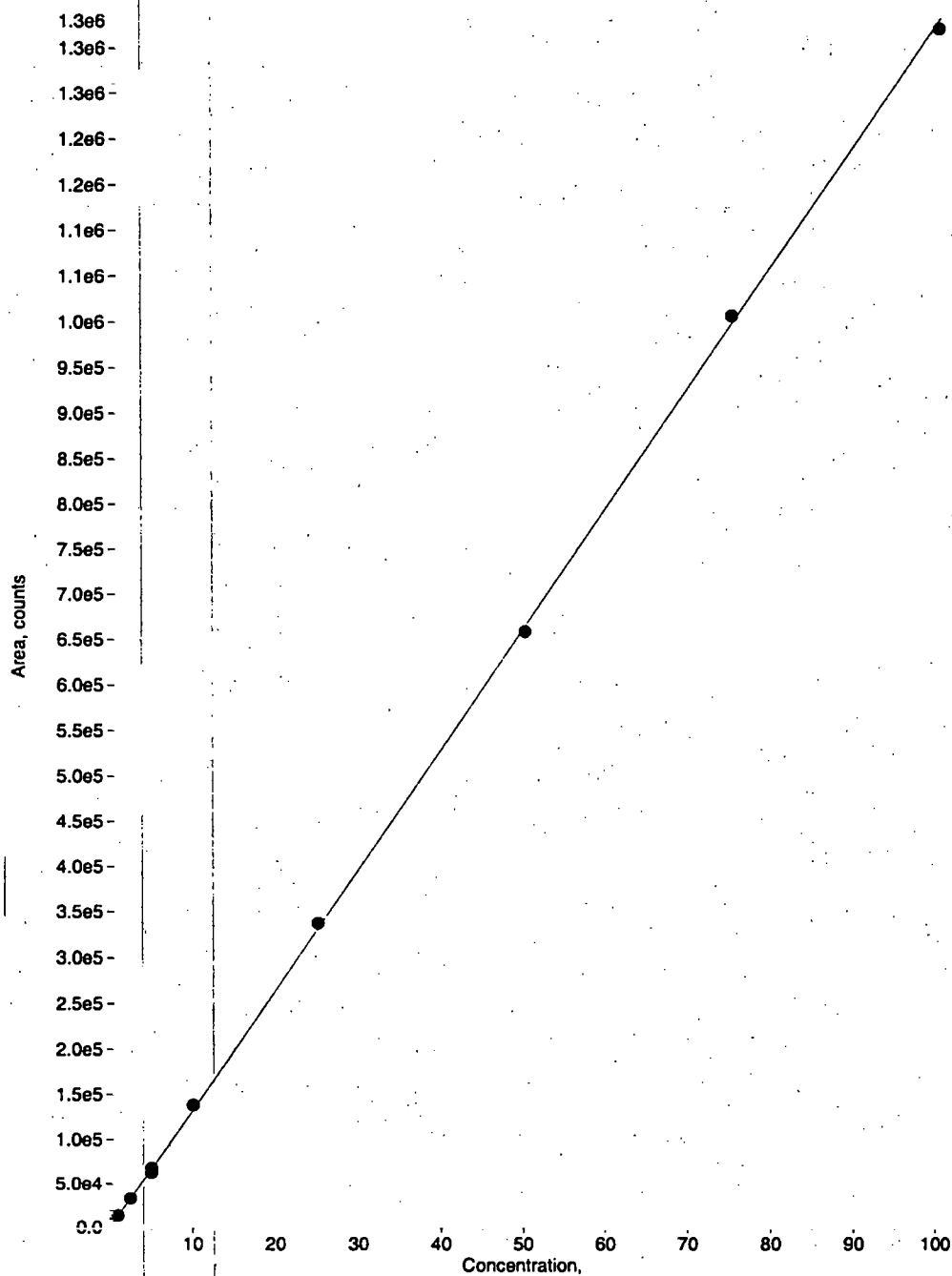


Figure A - 7: Example Analyst Version: 1.1A Results Summary for Analysis of Samples, IKI-220

Analyte Peak Name	File Name	Sample Name	Sample Type	Accuracy (%)	Analyte Concentration	Calculated Concentration	Peak Area (counts)
1 IKI-220	E062801CIE062801C001.wif	1 ng/mL Std	Standard	110	1.000	1.104	2562.2
2 IKI-220	E062801CIE062801C002.wif	2.5 ng/mL Std	Standard	104	2.500	2.588	5900.6
3 IKI-220	E062801CIE062801C003.wif	50 ng/mL Std	Standard	100	50.000	50.14	112875.6
4 IKI-220	E062801CIE062801C004.wif	100 ng/mL Std	Standard	98.8	100.000	98.80	222328.3
5 IKI-220	E062801CIE062801C005.wif	Mobile Phase	Unknown	N/A	N/A	Too small	0.0
6 IKI-220	E062801CIE062801C006.wif	12283 NC 55	Unknown	N/A	N/A	Too small	0.0
7 IKI-220	E062801CIE062801C007.wif	12283 55 1-A	Unknown	N/A	N/A	Too small	0.0
8 IKI-220	E062801CIE062801C008.wif	12283 55 2-A 5 ppb	Quality C	119	2.500	2.976	6774.4
9 IKI-220	E062801CIE062801C009.wif	12283 55 B	Unknown	N/A	N/A	Too small	0.0
10 IKI-220	E062801CIE062801C010.wif	5 ng/mL	Standard	103	5.000	5.150	11664.1
11 IKI-220	E062801CIE062801C011.wif	12283 55 C	Unknown	N/A	N/A	Too small	0.0
12 IKI-220	E062801CIE062801C012.wif	12283 56 A	Unknown	N/A	N/A	10.31	23267.4
13 IKI-220	E062801CIE062801C013.wif	12283 56 B	Unknown	N/A	N/A	0.4297	1046.1
14 IKI-220	E062801CIE062801C014.wif	12283 56 C	Unknown	N/A	N/A	Too small	0.0
15 IKI-220	E062801CIE062801C015.wif	12283 57 A	Unknown	N/A	N/A	10.95	24704.9
16 IKI-220	E062801CIE062801C016.wif	75 ng/mL	Standard	102	75.000	76.28	171657.4
17 IKI-220	E062801CIE062801C017.wif	12283 57 B	Unknown	N/A	N/A	Too small	0.0
18 IKI-220	E062801CIE062801C018.wif	12283 57 C	Unknown	N/A	N/A	Too small	0.0
19 IKI-220	E062801CIE062801C019.wif	12283 58 A	Unknown	N/A	N/A	5.220	11821.7
20 IKI-220	E062801CIE062801C020.wif	12283 58 B	Unknown	N/A	N/A	Too small	0.0
21 IKI-220	E062801CIE062801C021.wif	12283 58 C	Unknown	N/A	N/A	Too small	0.0
22 IKI-220	E062801CIE062801C022.wif	25 ng/mL Std	Standard	104	25.000	25.92	58377.6
23 IKI-220	E062801CIE062801C023.wif	12283 60 A	Unknown	N/A	N/A	1.309	3024.3
24 IKI-220	E062801CIE062801C024.wif	12283 60 B	Unknown	N/A	N/A	Too small	0.0
25 IKI-220	E062801CIE062801C025.wif	12283 60 C	Unknown	N/A	N/A	Too small	0.0
26 IKI-220	E062801CIE062801C026.wif	12283 61 A	Unknown	N/A	N/A	2.214	5060.5
27 IKI-220	E062801CIE062801C027.wif	12283 61 B	Unknown	N/A	N/A	Too small	0.0
28 IKI-220	E062801CIE062801C028.wif	10 ng/mL	Standard	91.8	10.000	9.178	20725.2
29 IKI-220	E062801CIE062801C029.wif	12283 61 C	Unknown	N/A	N/A	Too small	0.0
30 IKI-220	E062801CIE062801C030.wif	12283 62 A	Unknown	N/A	N/A	1.273	2944.0
31 IKI-220	E062801CIE062801C031.wif	12283 62 B	Unknown	N/A	N/A	Too small	0.0
32 IKI-220	E062801CIE062801C032.wif	12283 62 C	Unknown	N/A	N/A	Too small	0.0
33 IKI-220	E062801CIE062801C033.wif	5 ng/mL	Standard	96.9	5.000	4.345	9852.9

Figure A - 8: Calibration Standard, 1 ng/mL

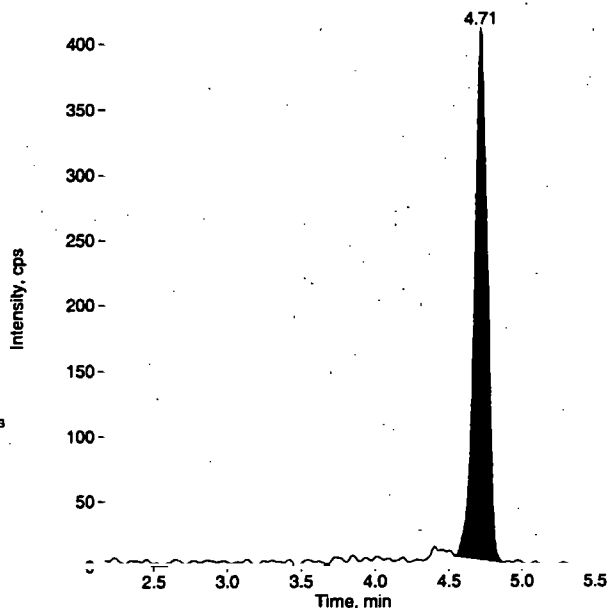
Sample Name: "1 ng/mL Std" Sample ID: " " File: "E062801C001.w" Peak Name: "IKI-220" Mass(es): "230.0/203.0 amu"

Comment: " " Annotation: " "

Sample Index: 1
Sample Type: Standard
Concentration: 1.000
Acq. Date: 06/28/01
Acq. Time: 08:56:54 PM

Modified: No
Bunching Factor: 2
Noise Threshold: 0.73
Area Threshold: 3.65
Num. Smoother: 5
RT Window: 30.0 sec
Expected RT: 4.71 min
Sep. Width: 0.20
Sep. Height: 0.01
Exp. Peak Ratio: 5.00
Exp. Adj. Ratio: 4.00
Exp. Val. Ratio: 1.00
Use Relative RT: No

Int. Type: Base To Base
Retention Time: 4.72 min
Area: 2562.2 counts
Height: 409.2 cps
Start Time: 4.55 min
End Time: 4.87 min



Sample Name: "1 ng/mL Std" Sample ID: " " File: "E062801C001.w" Peak Name: "TFNA-AM" Mass(es): "191.0/148.0 amu"

Comment: " " Annotation: " "

Sample Index: 1
Sample Type: Standard
Concentration: 1.000
Acq. Date: 06/28/01
Acq. Time: 08:56:54 PM

Modified: Yes
Bunching Factor: 1
Noise Threshold: 6.05
Area Threshold: 30.25
Num. Smoother: 5
RT Window: 30.0 sec
Expected RT: 4.11 min
Sep. Width: 0.20
Sep. Height: 0.01
Exp. Peak Ratio: 5.00
Exp. Adj. Ratio: 4.00
Exp. Val. Ratio: 1.00
Use Relative RT: No

Int. Type: Base To Base
Retention Time: 4.11 min
Area: 17260.0 counts
Height: 3067.9 cps
Start Time: 3.96 min
End Time: 4.29 min

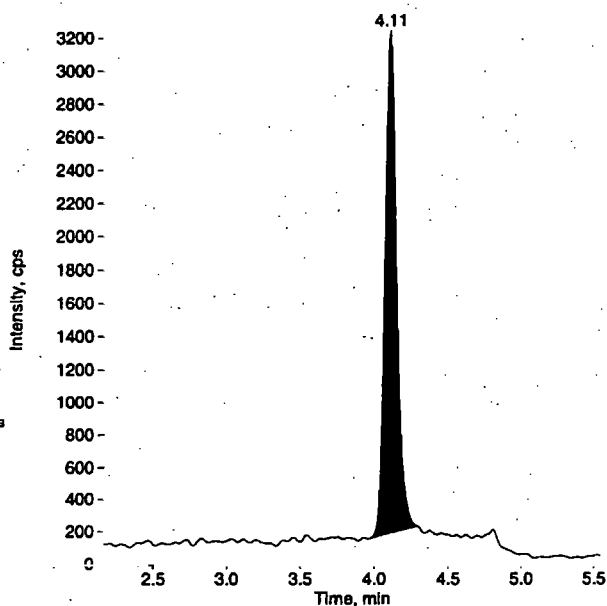


Figure A - 8 (Contd.): Calibration Standard, 1 ng/mL

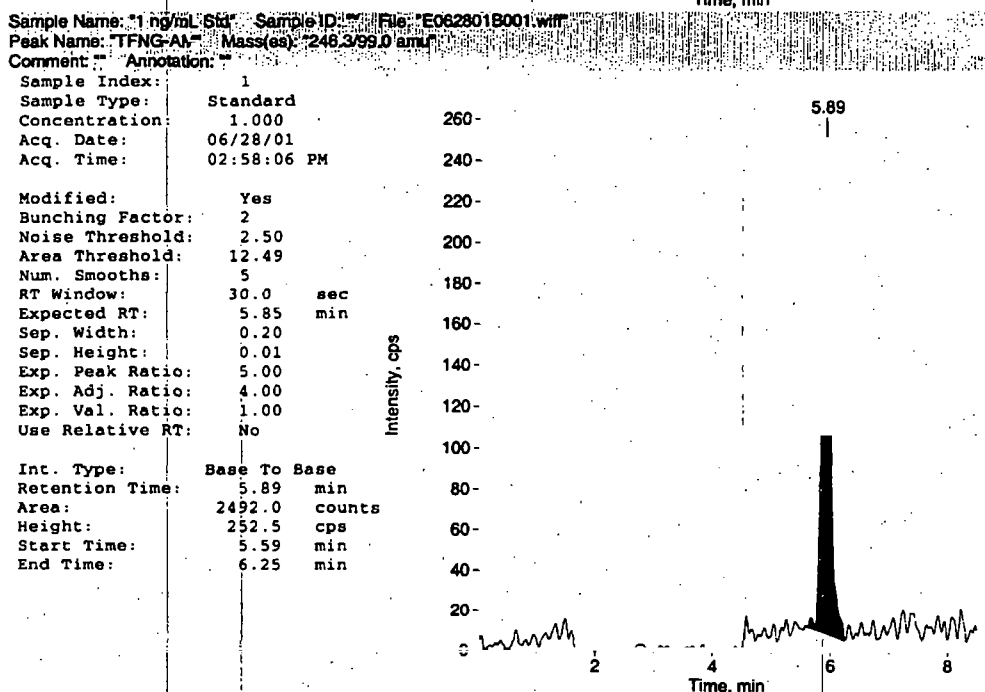
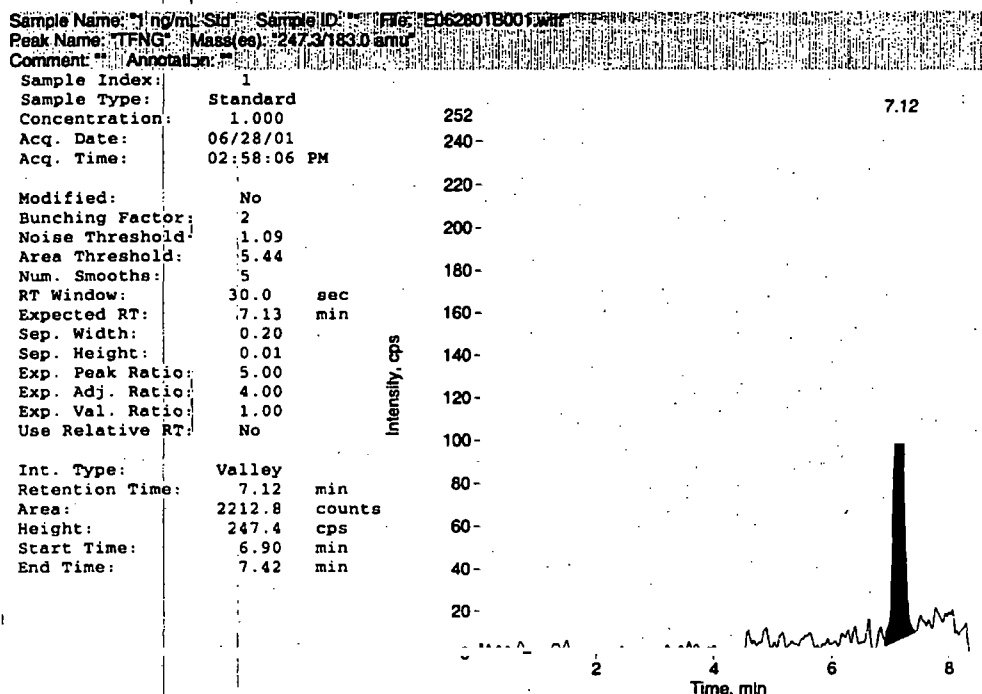


Figure A - 8 (Contd.): Calibration Standard, 1 ng/mL

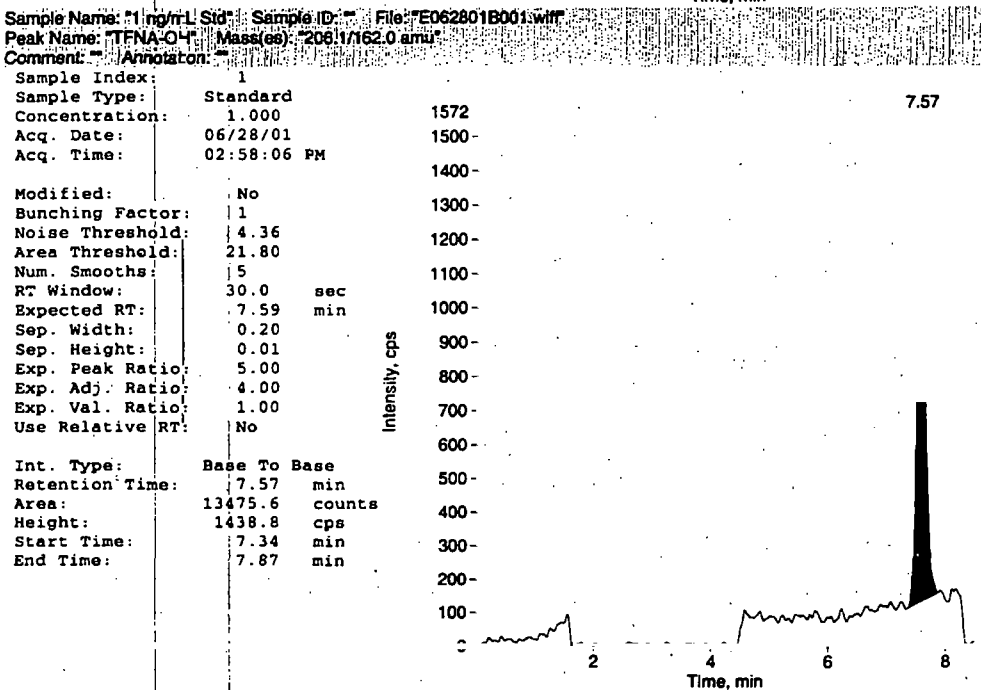
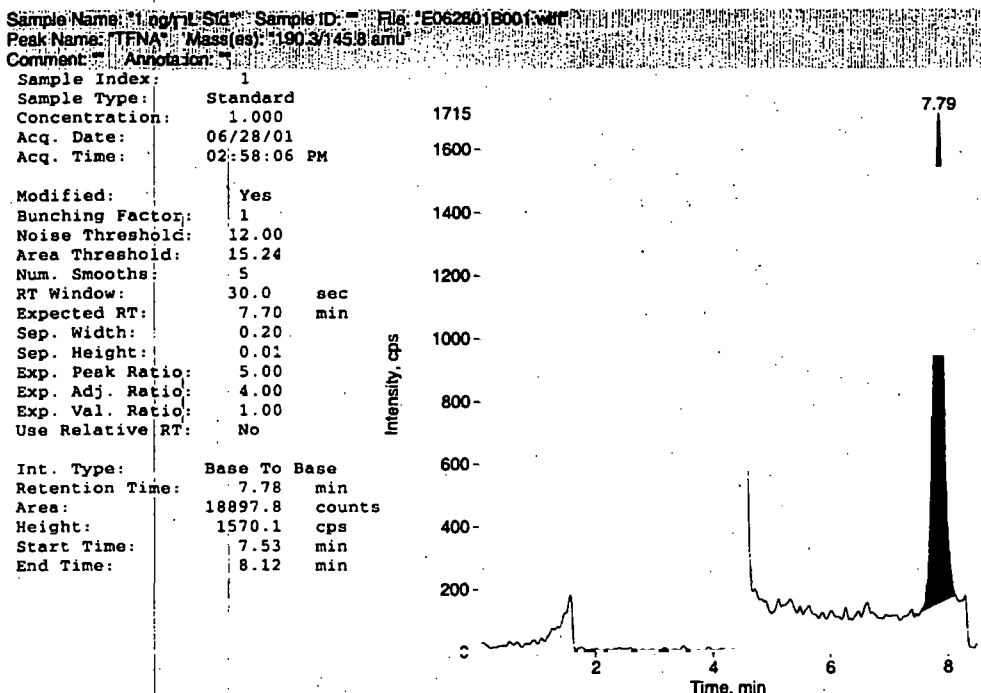


Figure A - 9: Calibration Standard, 2.5 ng/mL

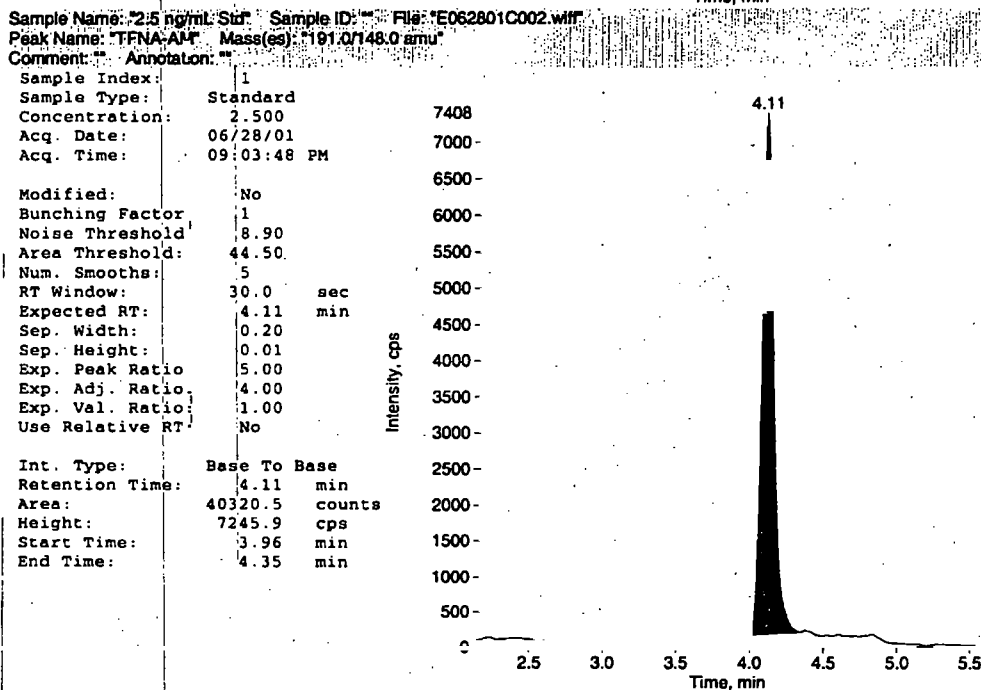
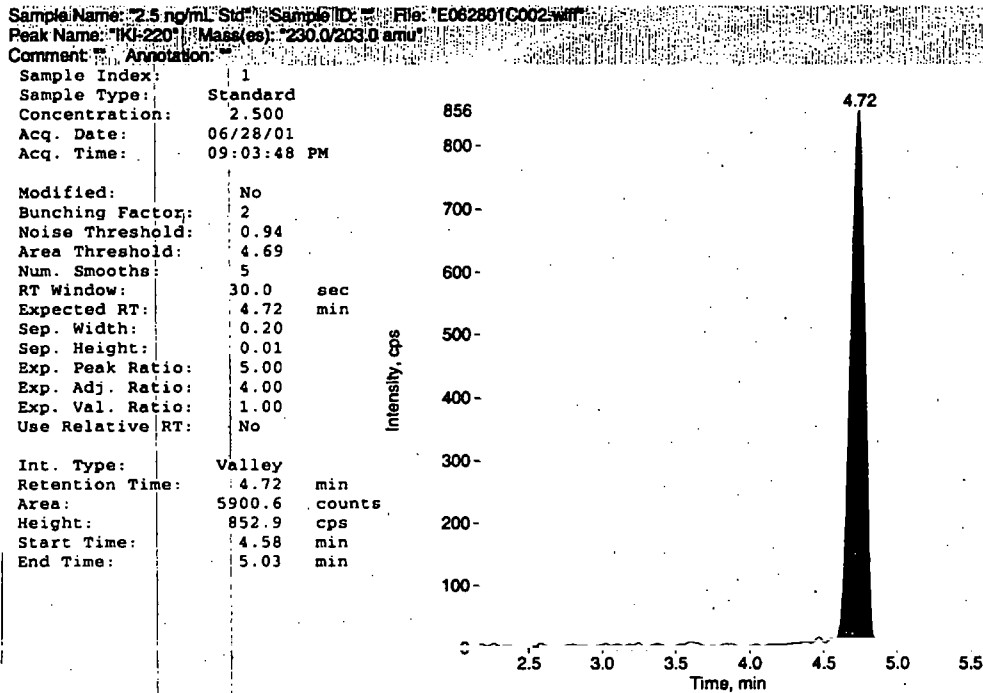


Figure A - 9 (Contd.): Calibration Standard, 2.5 ng/mL

Sample Name: "2.5 ng/mL Std" Sample ID: "" File: "E062801B002.wiff"
Peak Name: "TFNG" Mass(es): "247.3/183.0 amu"

Comment: "" Annotation: ""

Sample Index:	1	
Sample Type:	Standard	
Concentration:	2.500	550-
Acq. Date:	06/28/01	
Acq. Time:	03:08:47 PM	500-
Modified:	No	
Bunching Factor:	2	450-
Noise Threshold:	1.09	
Area Threshold:	5.44	400-
Num. Smoother:	5	
RT Window:	30.0 sec	
Expected RT:	7.13 min	350-
Sep. Width:	0.20	
Sep. Height:	0.01	300-
Exp. Peak Ratio:	5.00	
Exp. Adj. Ratio:	4.00	250-
Exp. Val. Ratio:	1.00	
Use Relative RT:	No	200-
Int. Type:	Valley	
Retention Time:	7.11 min	
Area:	5378.9 counts	150-
Height:	553.3 cps	
Start Time:	6.89 min	100-
End Time:	7.52 min	50-

Time, min

Sample Name: "2.5 ng/mL Std" Sample ID: "" File: "E062801B002.wiff"

Peak Name: "TFNG-AM" Mass(es): "246.3/99.0 amu"

Comment: "" Annotation: ""

Sample Index:	1	
Sample Type:	Standard	
Concentration:	2.500	600-
Acq. Date:	06/28/01	
Acq. Time:	03:08:47 PM	550-
Modified:	Yes	
Bunching Factor:	2	500-
Noise Threshold:	2.50	
Area Threshold:	12.49	450-
Num. Smoother:	5	
RT Window:	30.0 sec	
Expected RT:	5.85 min	400-
Sep. Width:	0.20	
Sep. Height:	0.01	350-
Exp. Peak Ratio:	5.00	
Exp. Adj. Ratio:	4.00	300-
Exp. Val. Ratio:	1.00	
Use Relative RT:	No	250-
Int. Type:	Base To Base	
Retention Time:	5.86 min	200-
Area:	5782.2 counts	
Height:	604.1 cps	150-
Start Time:	5.59 min	
End Time:	6.19 min	100-

Time, min

Figure A - 9 (Contd.): Calibration Standard, 2.5 ng/mL

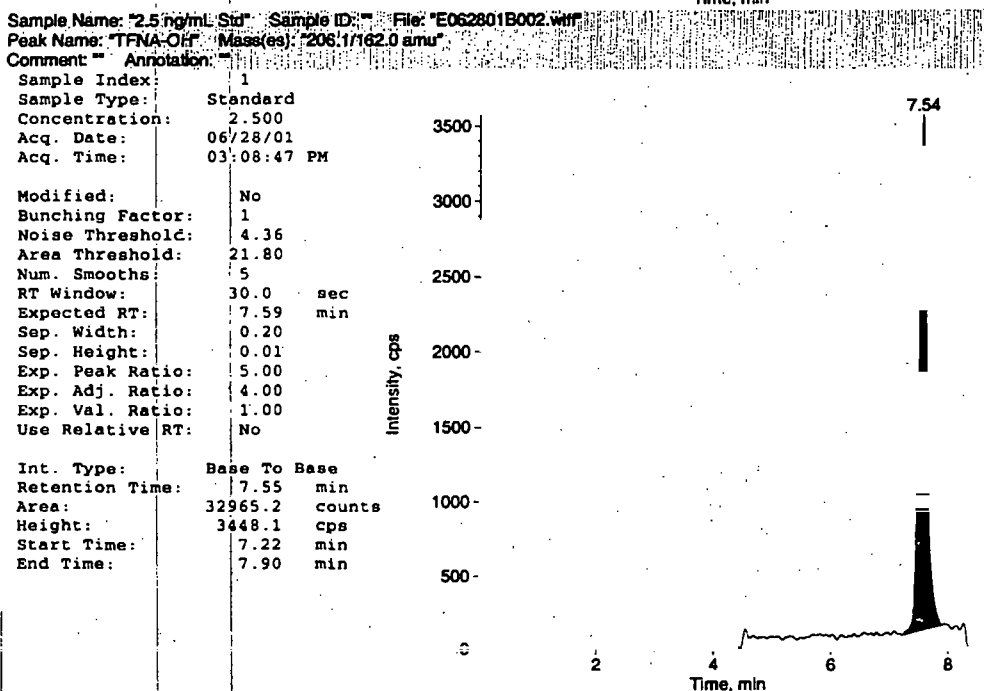
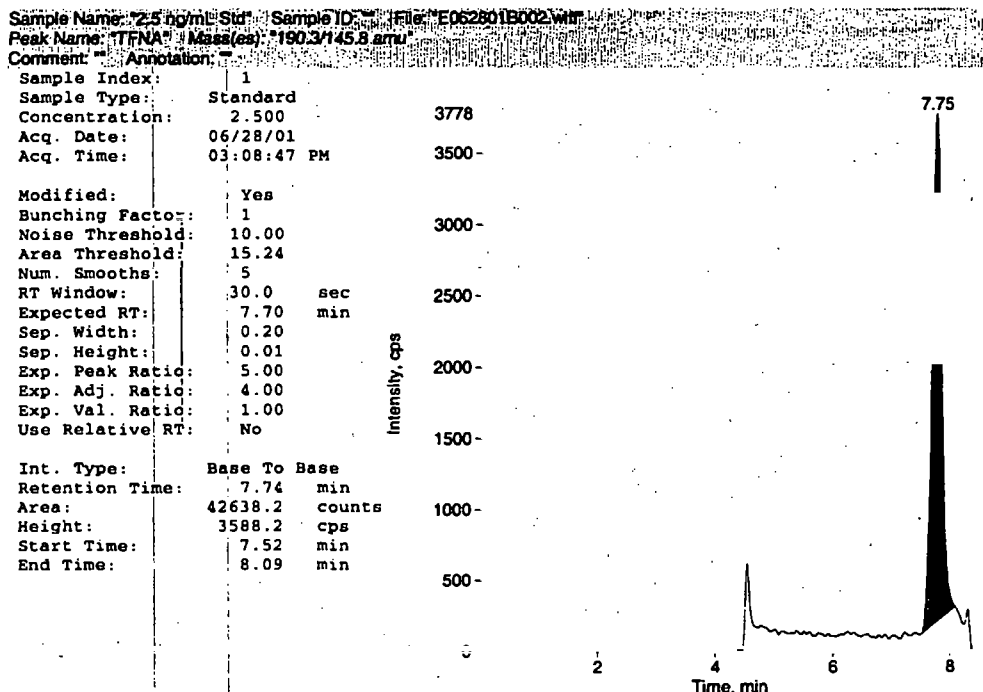


Figure A - 10: Calibration Standard, 5 ng/mL

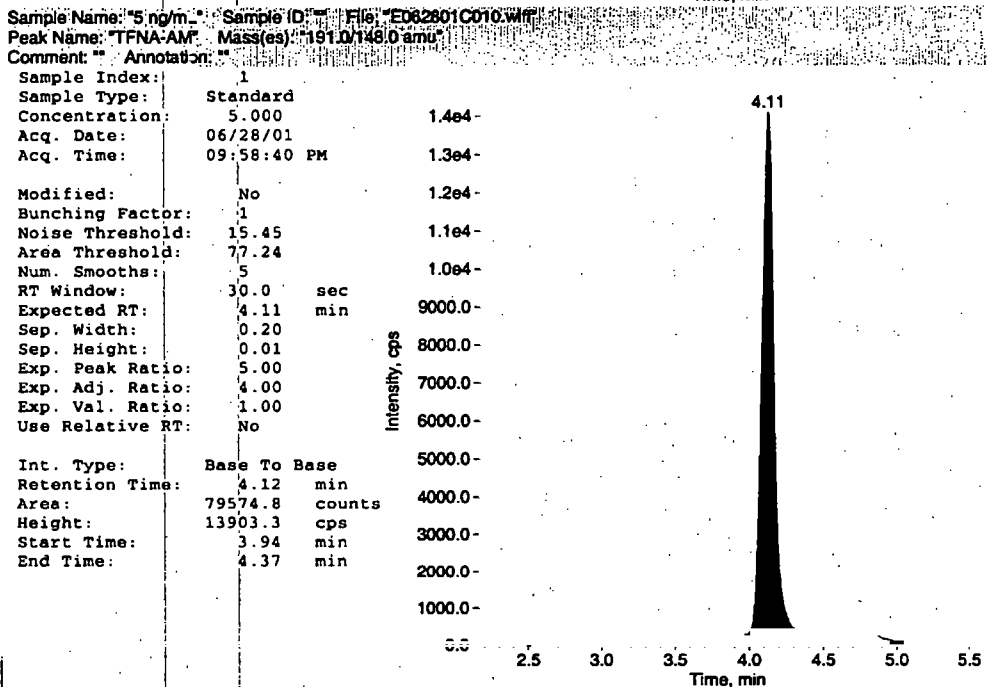
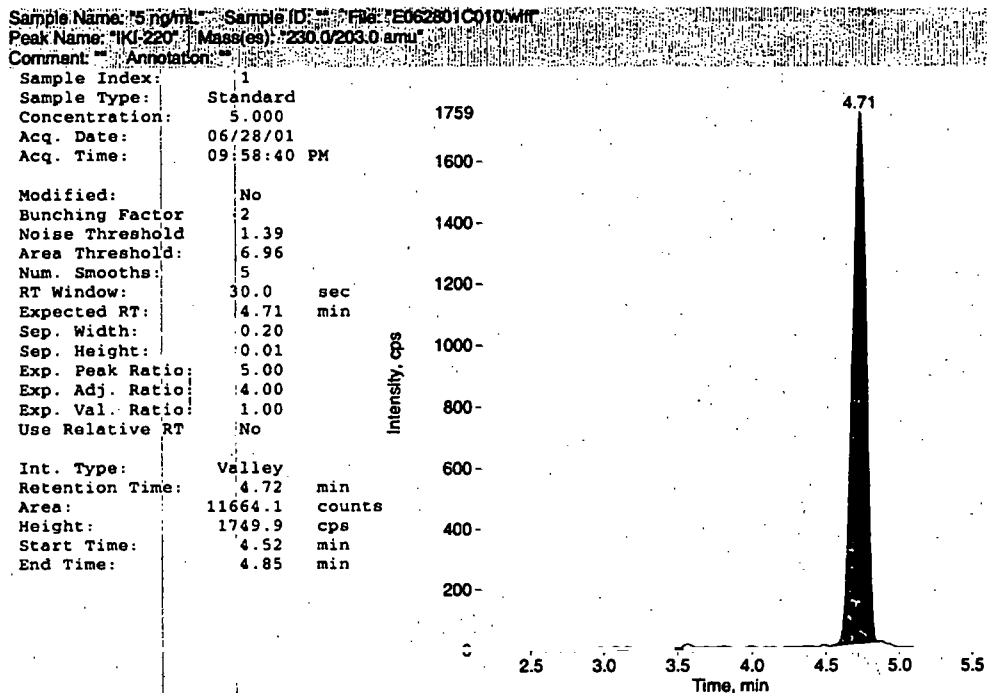


Figure A - 10 (Contd.): Calibration Standard, 5 ng/mL

Sample Name: "5 ng/mL" Sample ID: " " File: "E062801B010.w" File

Peak Name: "TFNG" Mass(es): "247.3/183.0 amu"

Comment: " " Annotation: " "

Sample Index:	1	
Sample Type:	Standard	
Concentration:	5.000	1178
Acq. Date:	06/28/01	
Acq. Time:	04:41:14 PM	1100-
Modified:	No	1000-
Bunching Factor:	2	
Noise Threshold:	1.09	900-
Area Threshold:	5.44	
Num. Smoother:	5	800-
RT Window:	30.0 sec	
Expected RT:	7.13 min	700-
Sep. Width:	0.20	
Sep. Height:	0.01	600-
Exp. Peak Ratio:	5.00	
Exp. Adj. Ratio:	4.00	500-
Exp. Val. Ratio:	1.00	
Use Relative RT:	No	400-
Int. Type:	Base To Base	
Retention Time:	7.07 min	300-
Area:	10330.3 counts	
Height:	1178.2 cps	200-
Start Time:	6.88 min	
End Time:	7.50 min	100-

Intensity, cps

Time, min

Sample Name: "5 ng/mL" Sample ID: " " File: "E062801B010.w" File

Peak Name: "TFNG-AM" Mass(es): "248.3/99.0 amu"

Comment: " " Annotation: " "

Sample Index:	1	
Sample Type:	Standard	
Concentration:	5.000	1400-
Acq. Date:	06/28/01	
Acq. Time:	04:41:14 PM	1300-
Modified:	No	1200-
Bunching Factor:	2	
Noise Threshold:	2.50	1100-
Area Threshold:	12.49	
Num. Smoother:	5	1000-
RT Window:	30.0 sec	
Expected RT:	5.85 min	900-
Sep. Width:	0.20	
Sep. Height:	0.01	800-
Exp. Peak Ratio:	5.00	
Exp. Adj. Ratio:	4.00	700-
Exp. Val. Ratio:	1.00	
Use Relative RT:	No	600-
Int. Type:	Base To Base	500-
Retention Time:	5.82 min	400-
Area:	13324.9 counts	
Height:	1436.7 cps	300-
Start Time:	5.52 min	
End Time:	6.44 min	200-

Intensity, cps

Time, min

Figure A - 10 (Contd.): Calibration Standard, 5 ng/mL

Sample Name: "5 ng/mL" Sample ID: " " File: "E062801B010.wiff"

Peak Name: "TFNA" Mass(es): "190.3/145.8 amu"

Comment: " " Annotation: " "

Sample Index: 1
Sample Type: Standard
Concentration: 5.000
Acq. Date: 06/28/01
Acq. Time: 04:41:14 PM
Modified: No
Bunching Factor: 1
Noise Threshold: 3.05
Area Threshold: 15.24
Num. Smoother: 5
RT Window: 30.0 sec
Expected RT: 7.70 min
Sep. Width: 0.20
Sep. Height: 0.01
Exp. Peak Ratio: 5.00
Exp. Adj. Ratio: 4.00
Exp. Val. Ratio: 1.00
Use Relative RT: No

Int. Type: Base To Base
Retention Time: 7.67 min
Area: 96998.9 counts
Height: 7728.2 cps
Start Time: 7.33 min
End Time: 8.36 min

Intensity, cps



Sample Name: "5 ng/mL" Sample ID: " " File: "E062801B010.wiff"

Peak Name: "TFNA-OH" Mass(es): "206.1/162.0 amu"

Comment: " " Annotation: " "

Sample Index: 1
Sample Type: Standard
Concentration: 5.000
Acq. Date: 06/28/01
Acq. Time: 04:41:14 PM
Modified: No
Bunching Factor: 1
Noise Threshold: 4.36
Area Threshold: 21.80
Num. Smoother: 5
RT Window: 30.0 sec
Expected RT: 7.59 min
Sep. Width: 0.20
Sep. Height: 0.01
Exp. Peak Ratio: 5.00
Exp. Adj. Ratio: 4.00
Exp. Val. Ratio: 1.00
Use Relative RT: No

Int. Type: Base To Base
Retention Time: 7.50 min
Area: 62288.8 counts
Height: 6857.5 cps
Start Time: 7.30 min
End Time: 7.80 min

Intensity, cps



Figure A - 11: Calibration Standard, 10 ng/mL

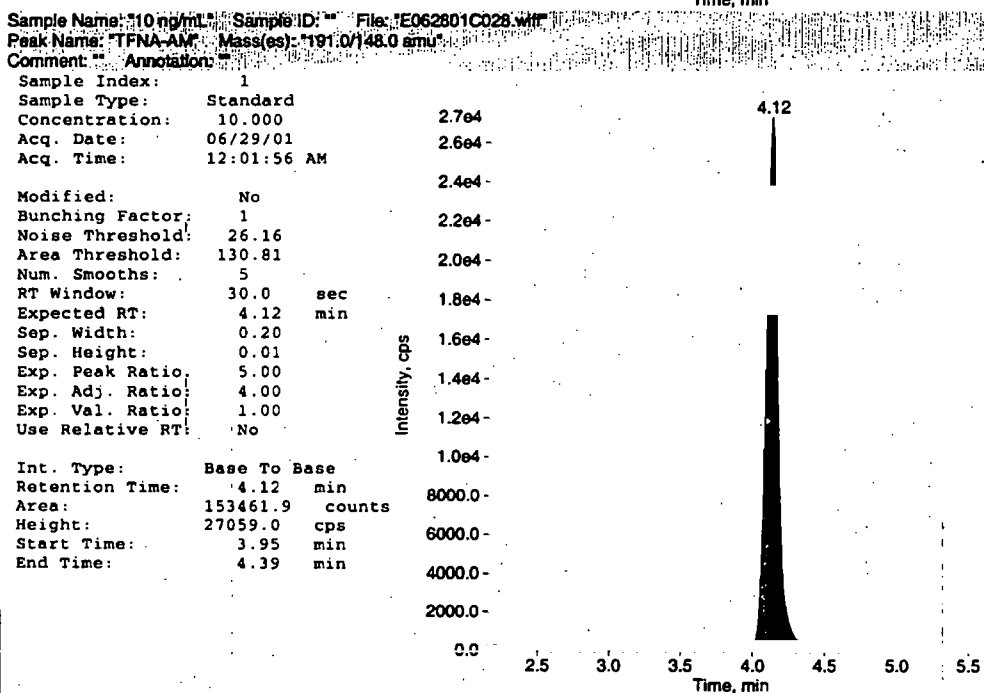
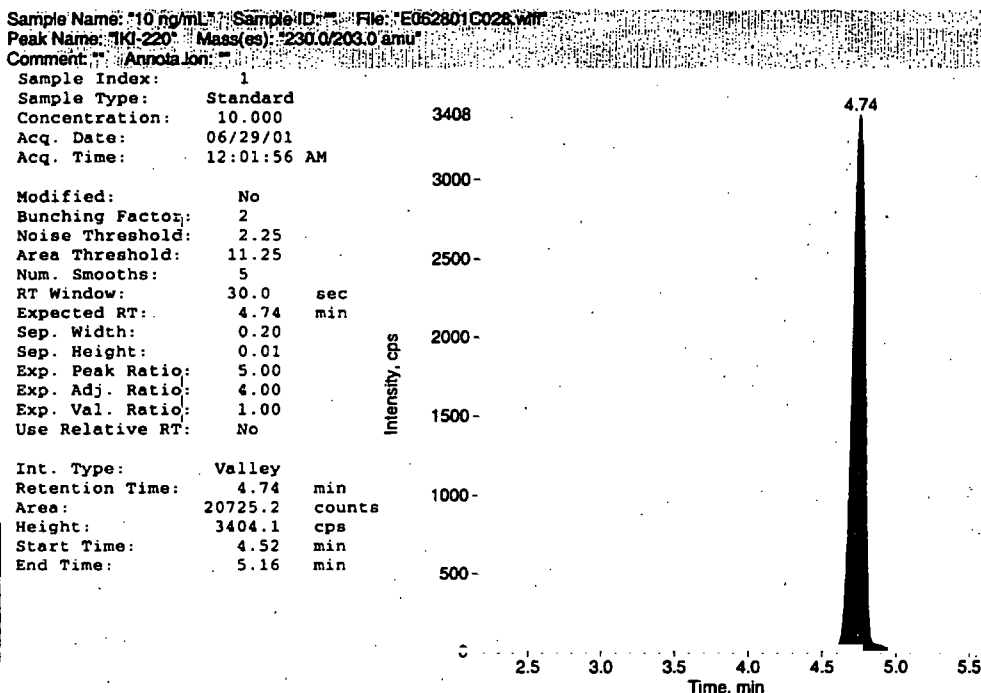


Figure A -11 (Contd.): Calibration Standard, 10 ng/mL

Sample Name: "10 ng/mL" Sample ID: " " File: "E062801B028.wif"

Peak Name: "TFNG" Mass(es): "247.3/183.0 amu"

Comment: " " Annotation: " "

Sample Index:	1	
Sample Type:	Standard	
Concentration:	10.000	2400-
Acq. Date:	06/28/01	
Acq. Time:	07:47:31 PM	2200-
Modified:	No	
Bunching Factor:	2	2000-
Noise Threshold:	1.09	
Area Threshold:	5.44	1800-
Num. Smooths:	5	
RT Window:	30.0 sec	1600-
Expected RT:	7.13 min	
Sep. Width:	0.20	1400-
Sep. Height:	0.01	
Exp. Peak Ratio:	5.00	1200-
Exp. Adj. Ratio:	4.00	
Exp. Val. Ratio:	1.00	1000-
Use Relative RT:	No	
Int. Type:	Valley	
Retention Time:	7.07 min	800-
Area:	21698.7 counts	
Height:	2413.2 cps	600-
Start Time:	6.79 min	
End Time:	7.59 min	400-
		200-
		0

Time, min

Sample Name: "10 ng/mL" Sample ID: " " File: "E062801B028.wif"

Peak Name: "TFNG-AM" Mass(es): "248.3/99.0 amu"

Comment: " " Annotation: " "

Sample Index:	1	
Sample Type:	Standard	
Concentration:	10.000	2800-
Acq. Date:	06/28/01	
Acq. Time:	07:47:31 PM	2600-
Modified:	No	
Bunching Factor:	2	2400-
Noise Threshold:	2.50	
Area Threshold:	12.49	2200-
Num. Smooths:	5	
RT Window:	30.0 sec	2000-
Expected RT:	5.85 min	1800-
Sep. Width:	0.20	
Sep. Height:	0.01	1600-
Exp. Peak Ratio:	5.00	
Exp. Adj. Ratio:	4.00	1400-
Exp. Val. Ratio:	1.00	
Use Relative RT:	No	1200-
Int. Type:	Base To Base	
Retention Time:	5.81 min	1000-
Area:	26767.6 counts	
Height:	2877.2 cps	800-
Start Time:	5.59 min	
End Time:	6.27 min	400-
		200-
		0

Time, min

Figure A -11 (Contd.): Calibration Standard, 10 ng/mL

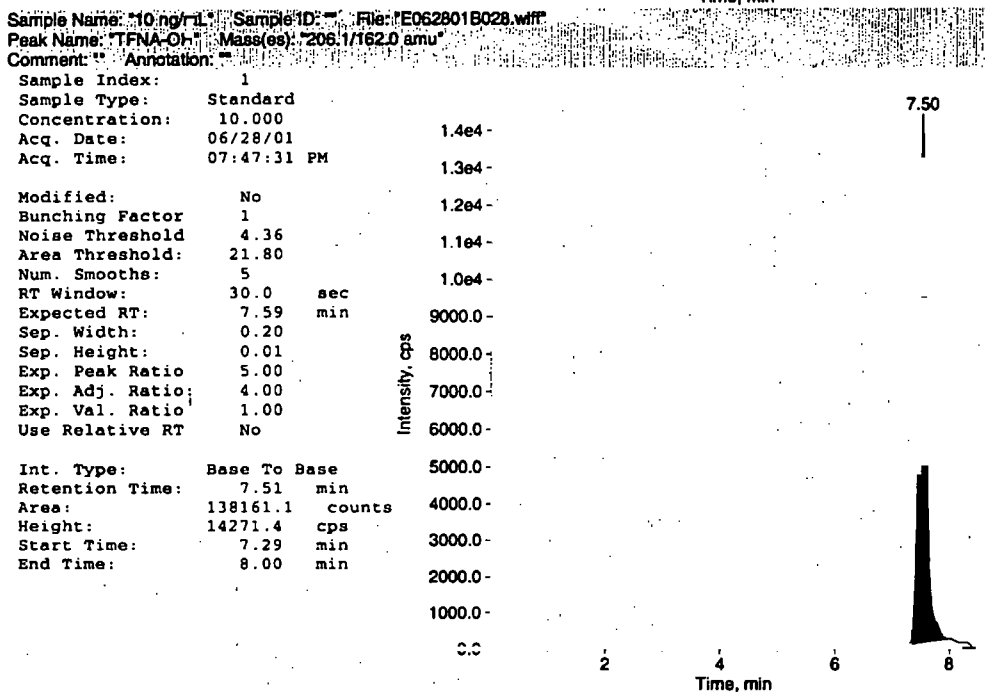
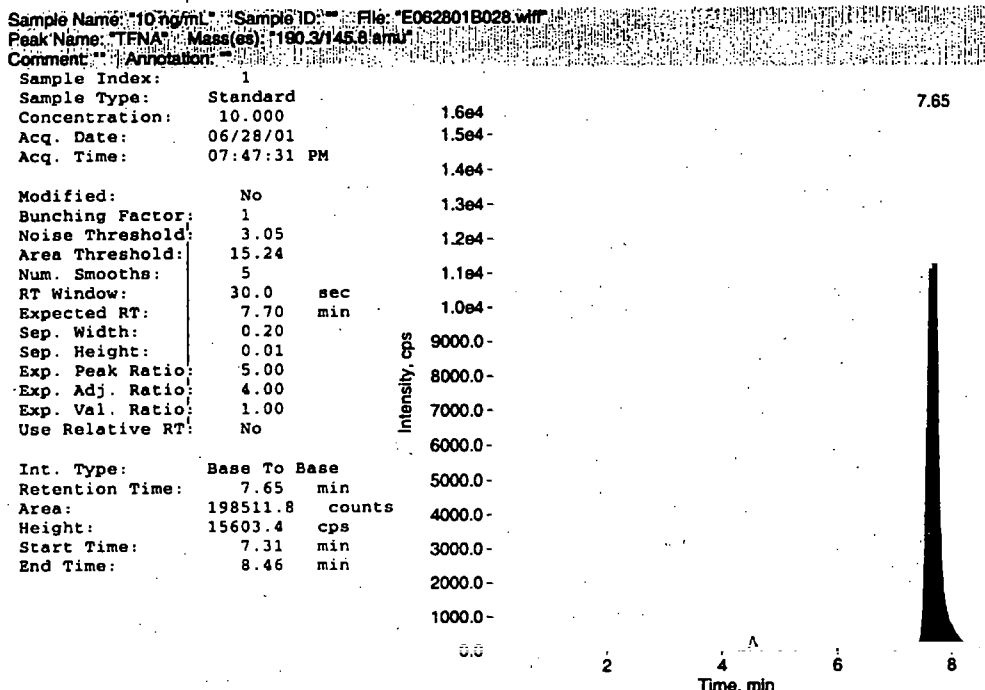


Figure A - 12: Calibration Standard, 25 ng/mL

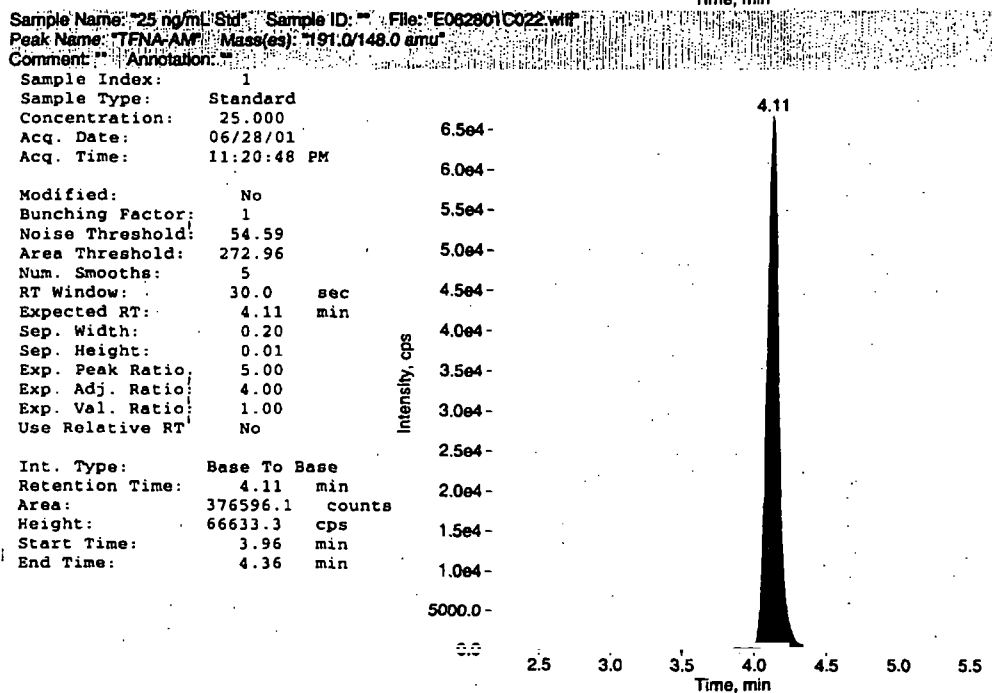
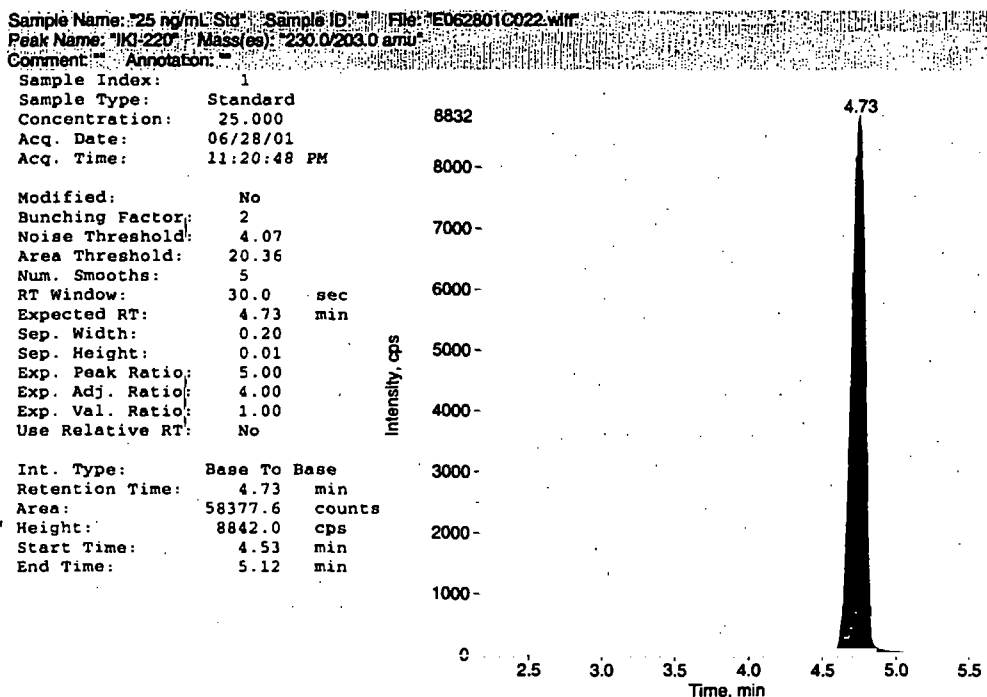


Figure A - 12 (Contd.): Calibration Standard, 25 ng/mL

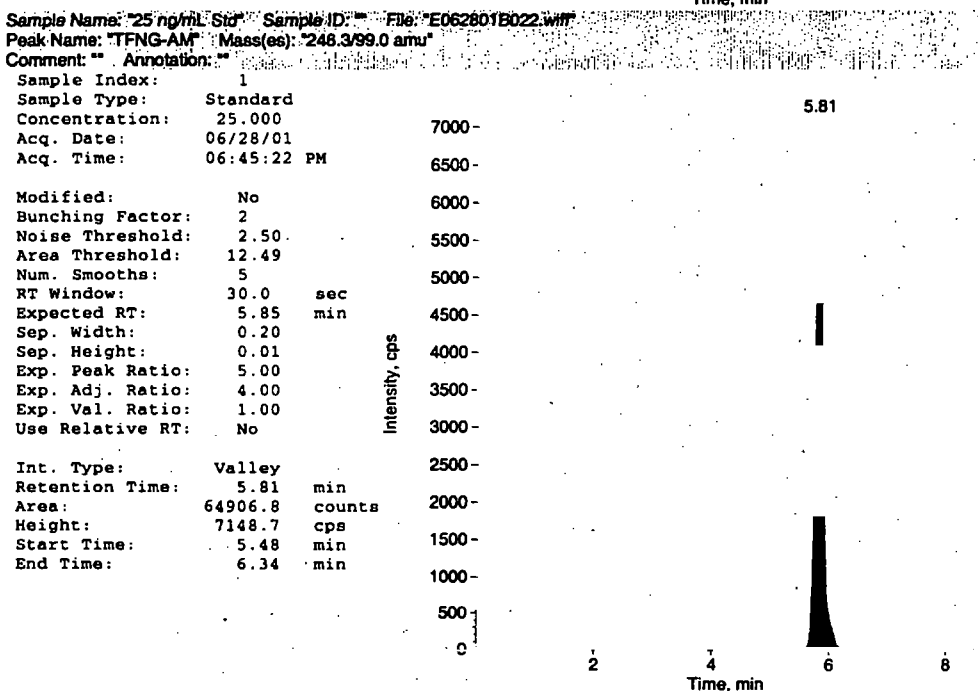
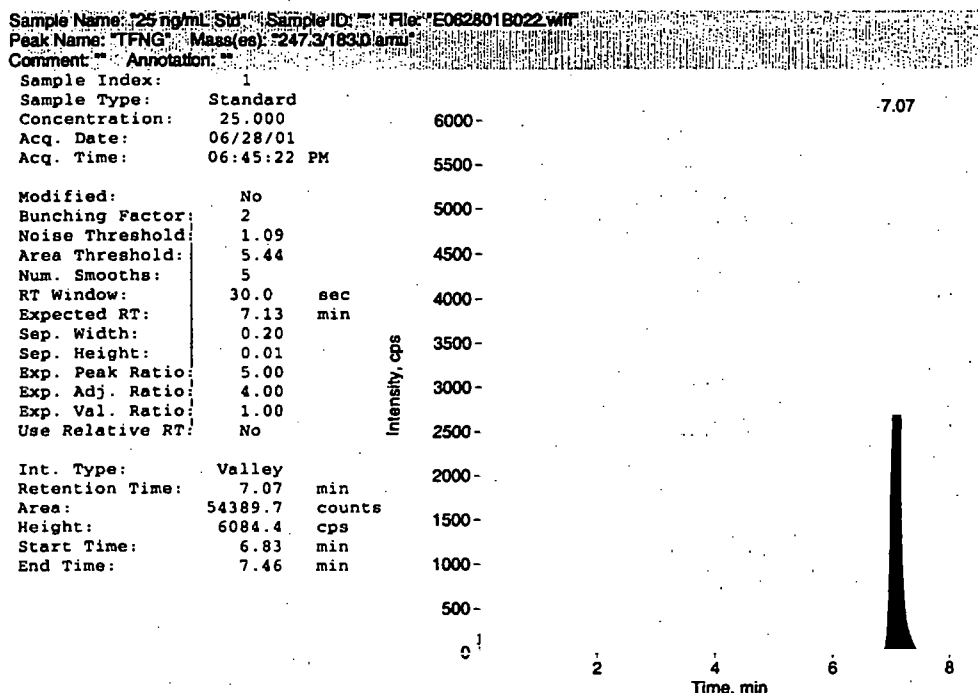


Figure A - 12 (Contd.): Calibration Standard, 25 ng/mL

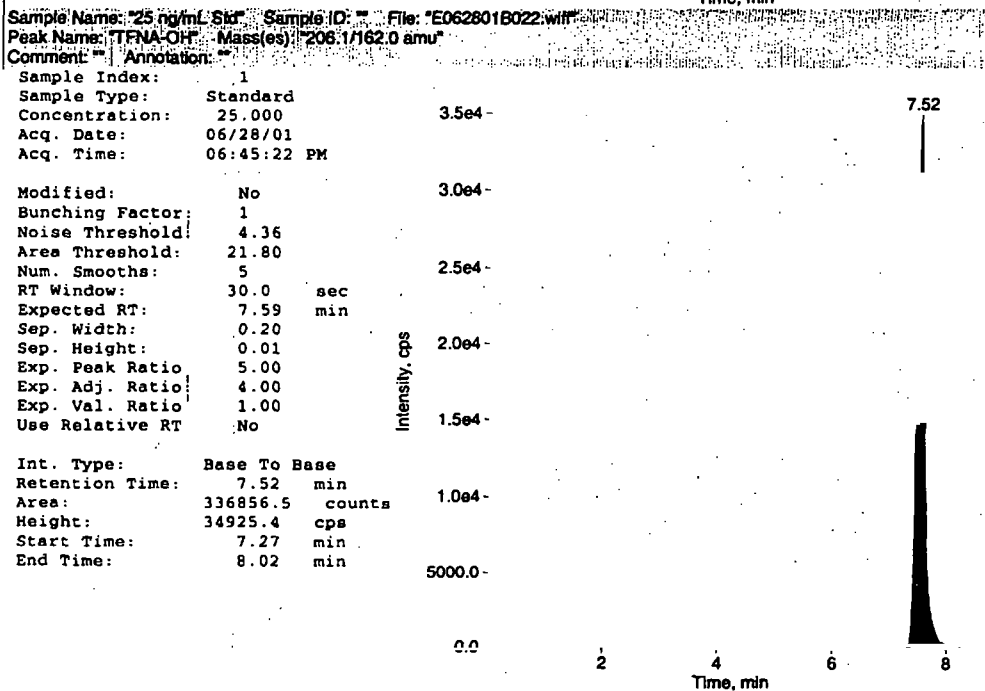
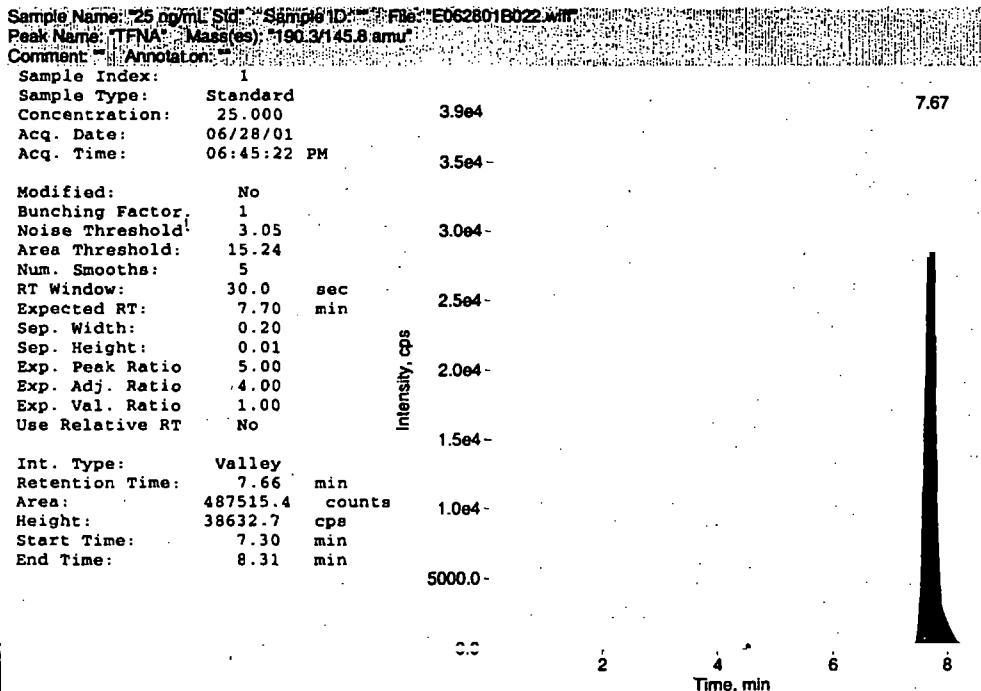


Figure A - 13: Calibration Standard, 50 ng/mL

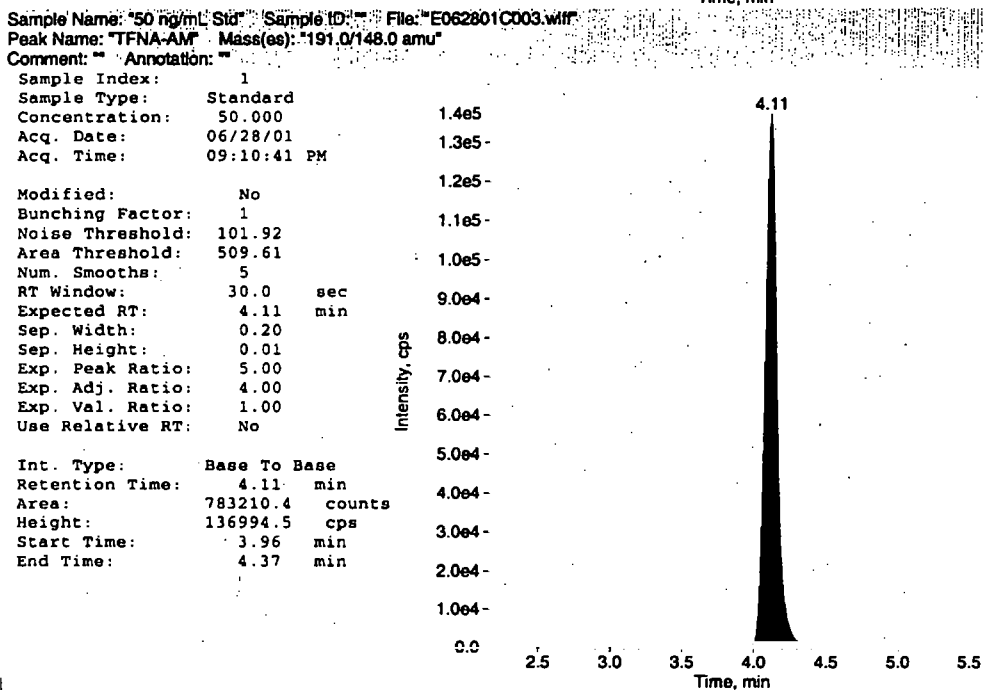
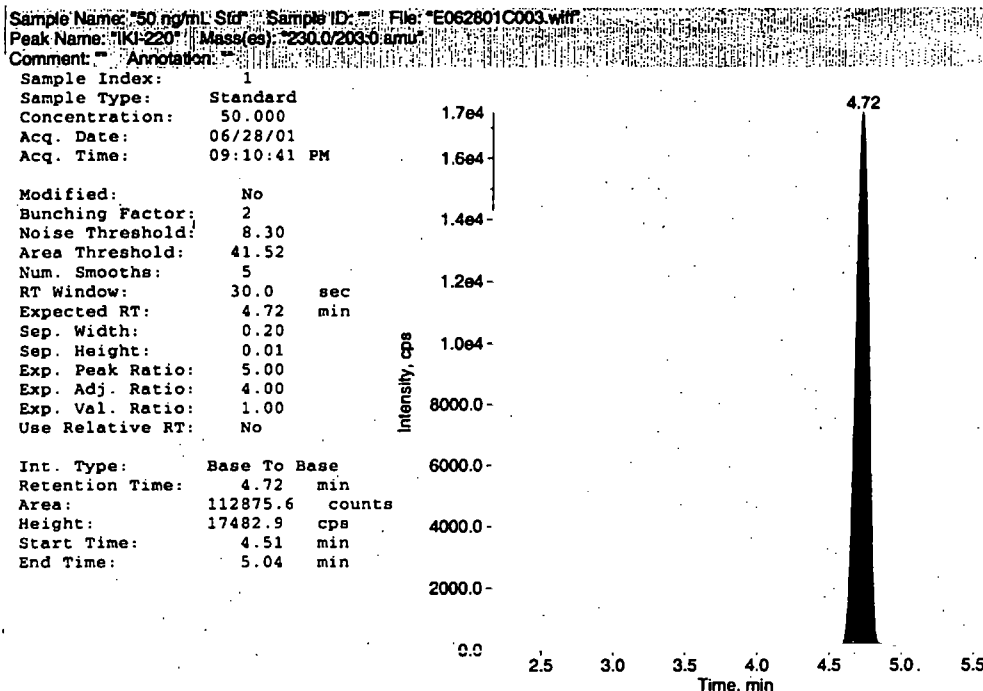


Figure A - 13 (Contd.): Calibration Standard, 50 ng/mL

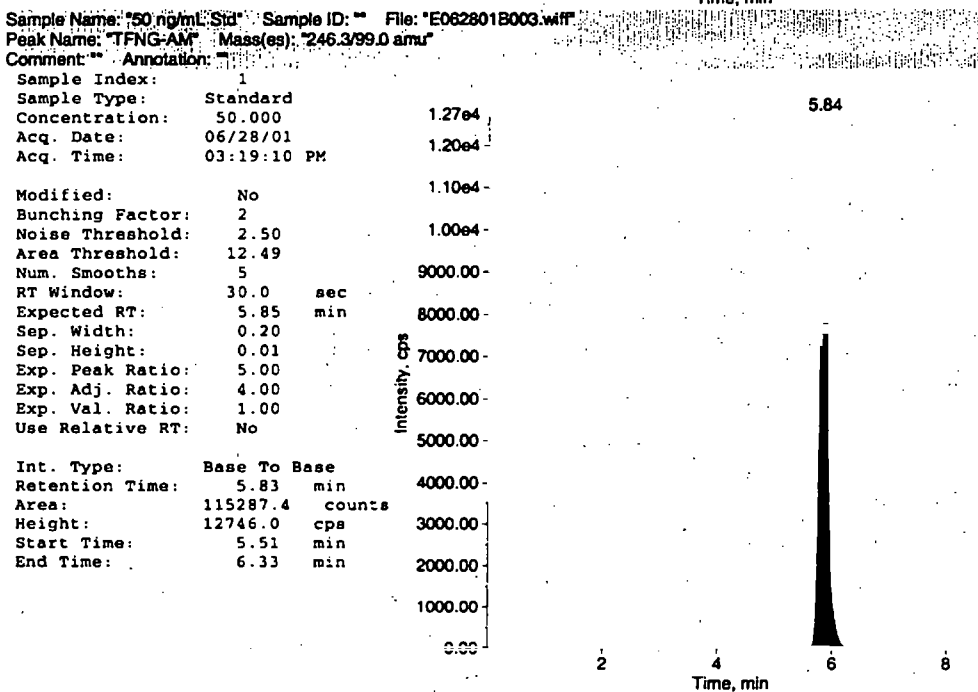
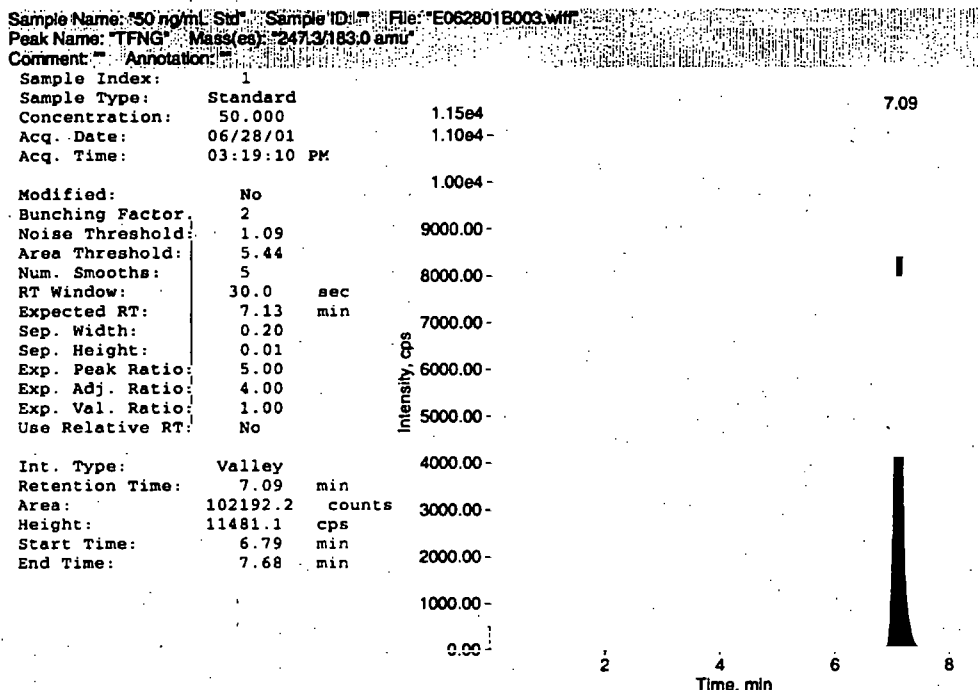


Figure A - 13 (Contd.): Calibration Standard, 50 ng/mL

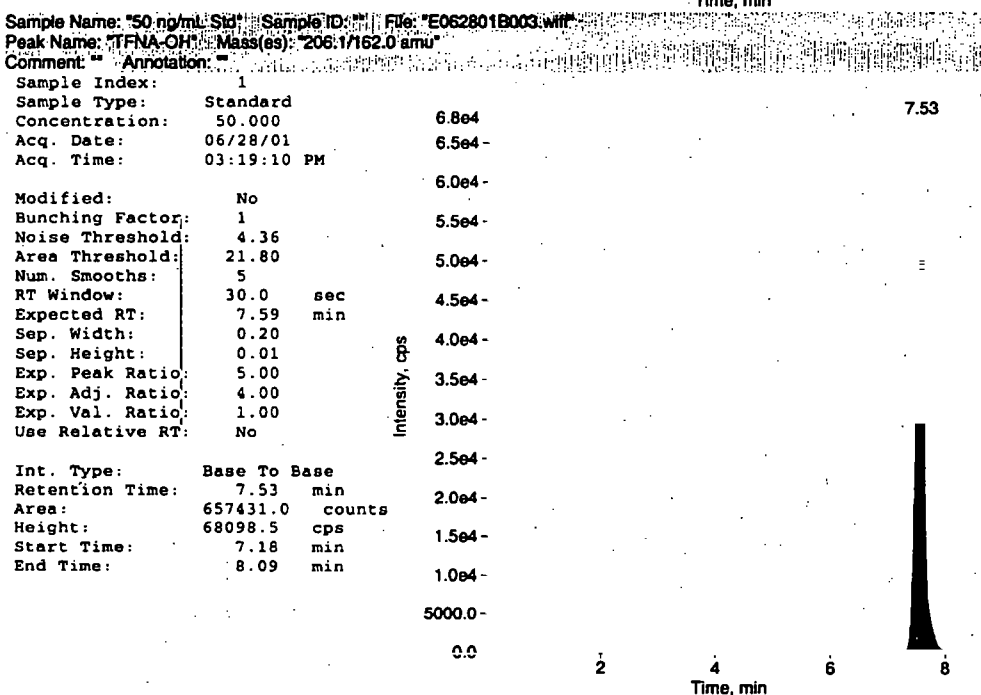
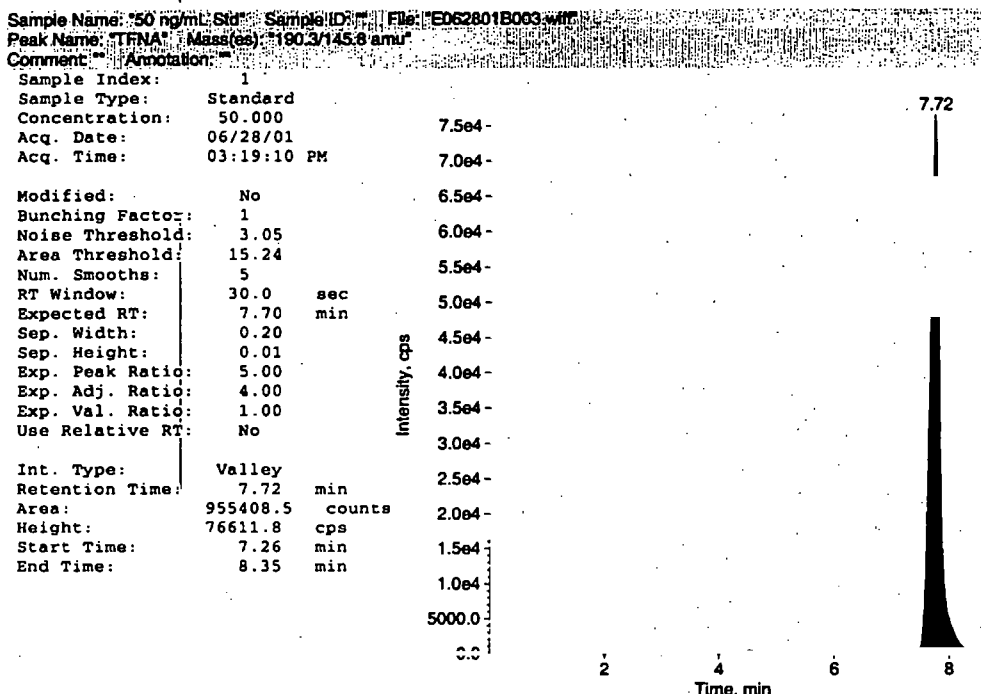


Figure A - 14: Calibration Standard, 75 ng/mL

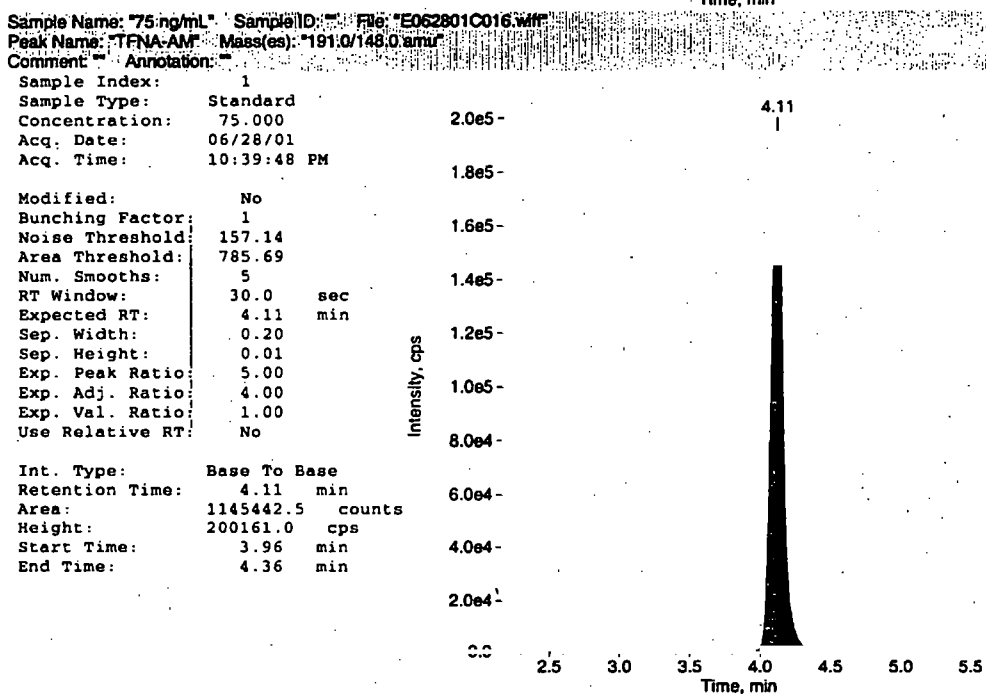
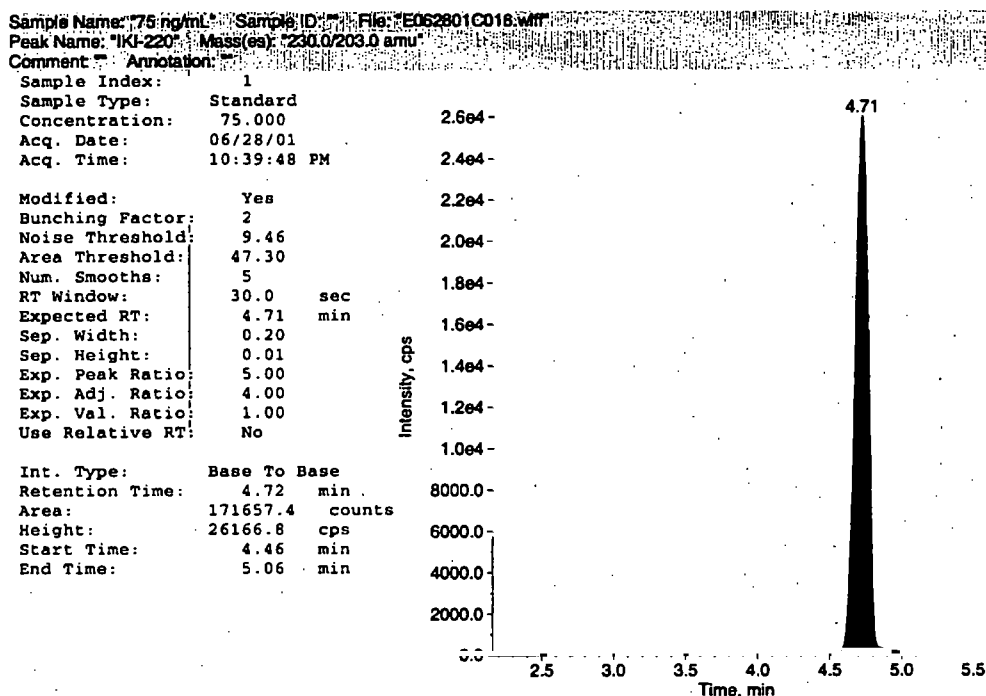


Figure A - 14 (Cont): Calibration Standard, 75 ng/mL

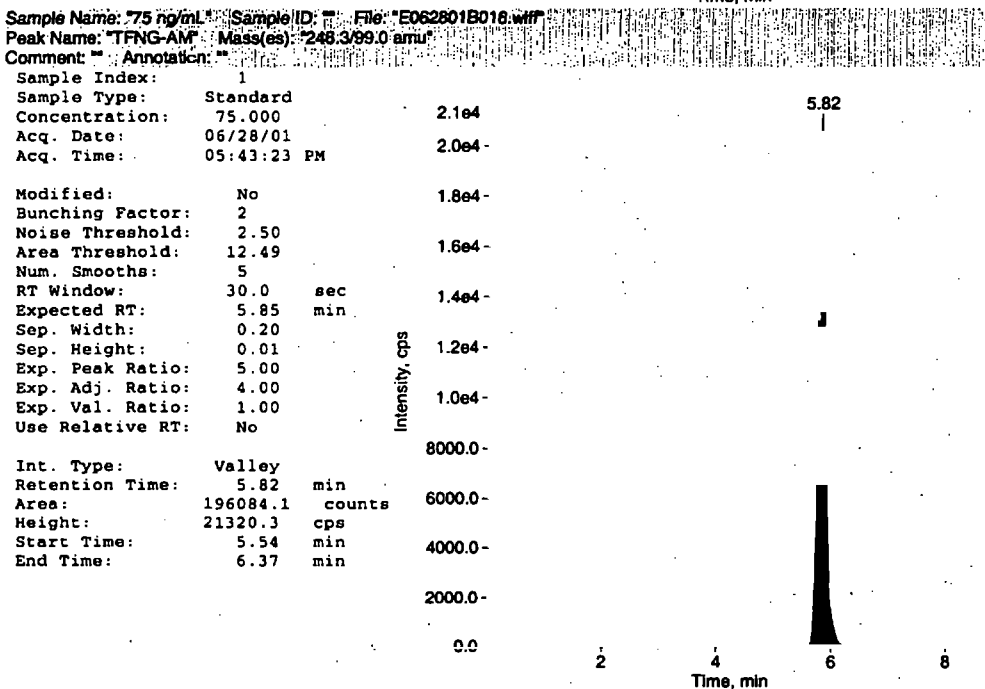
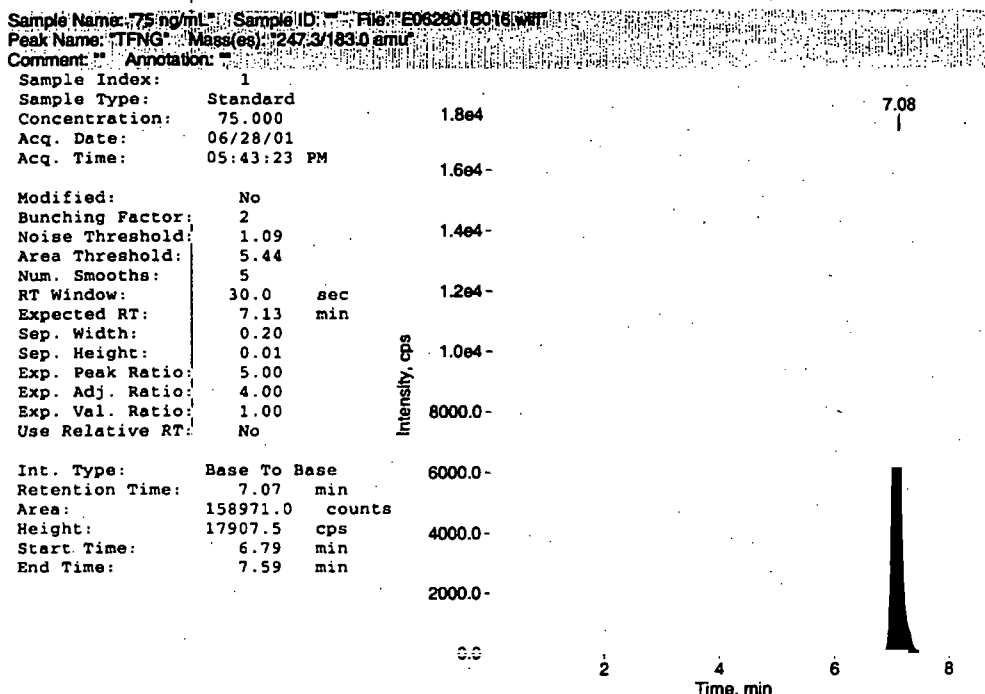


Figure A - 14 (Cont): Calibration Standard, 75 ng/mL

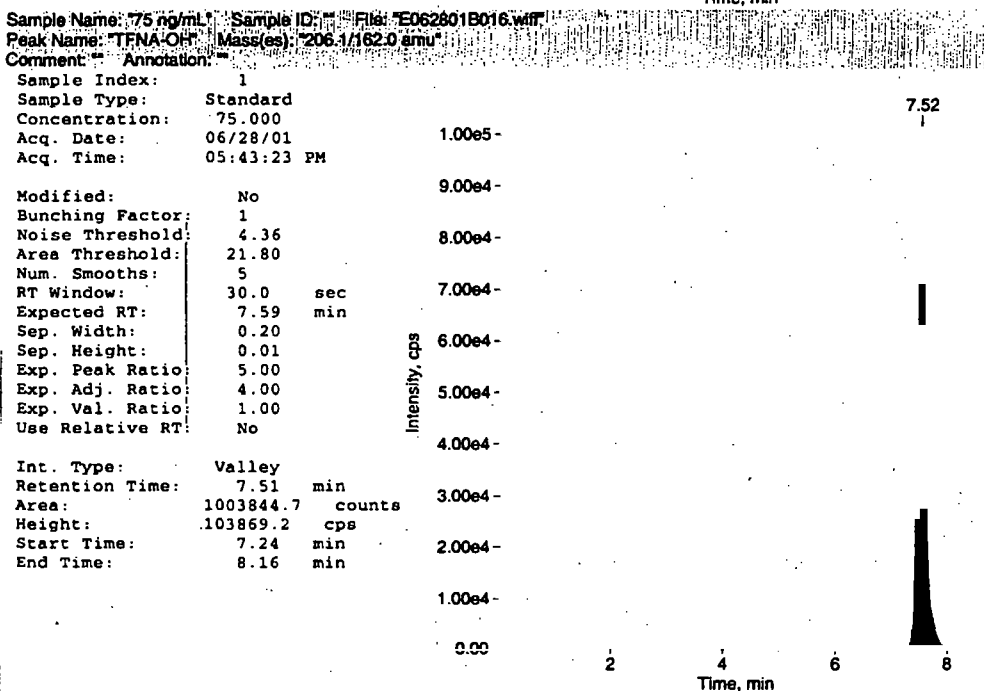
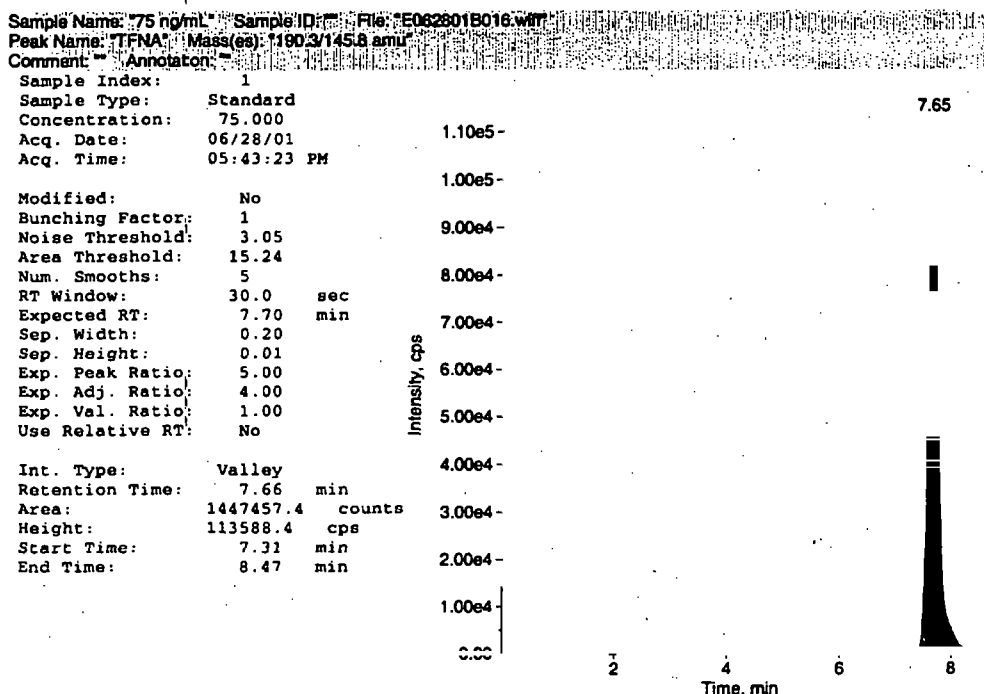


Figure A - 15: Calibration Standard, 100 ng/mL

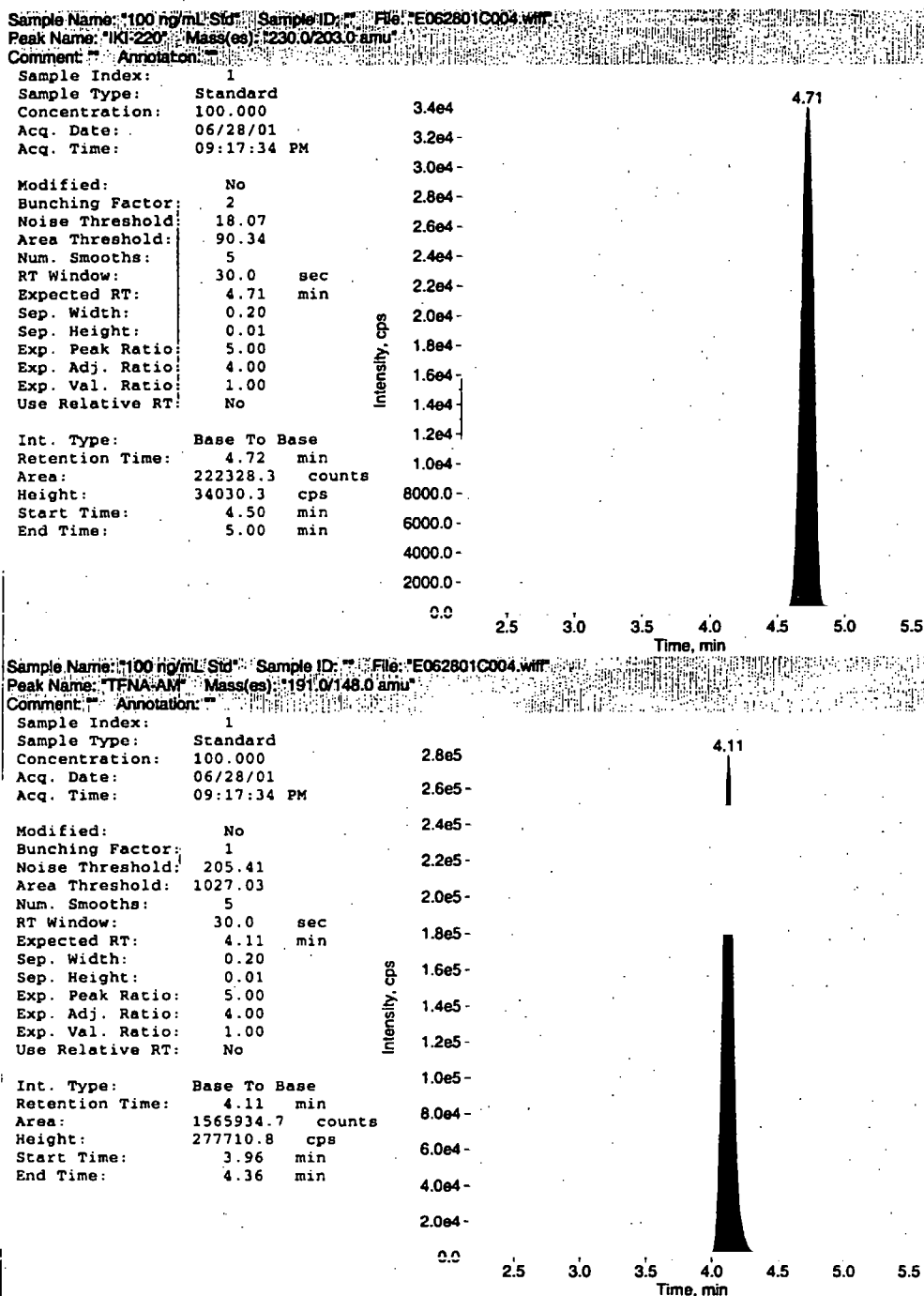


Figure A - 15 (Cont): Calibration Standard, 100 ng/mL

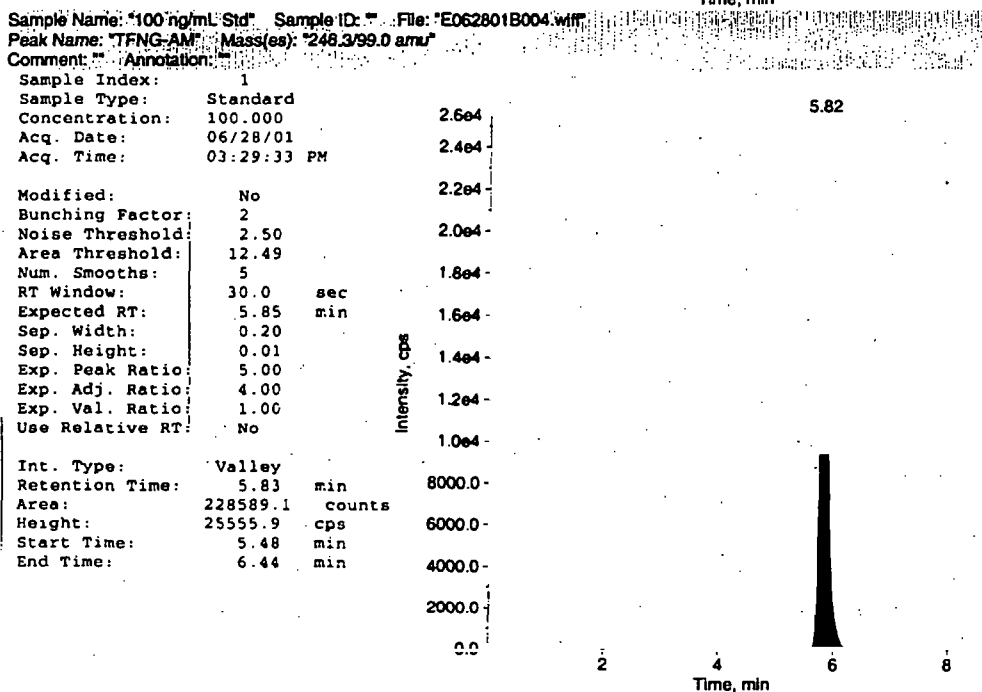
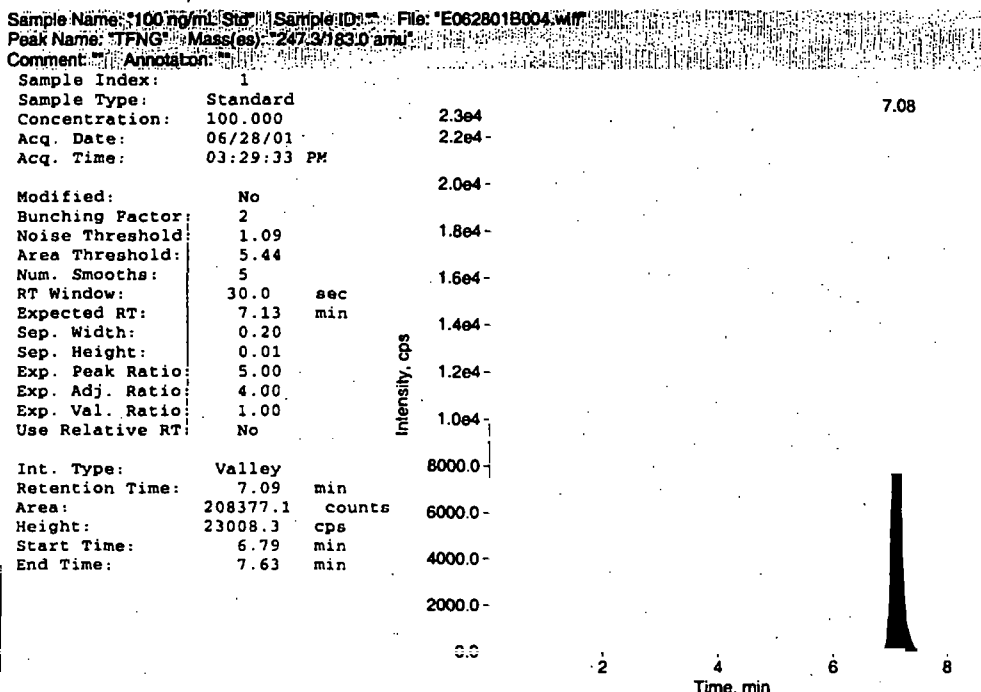


Figure A - 15 (Cont): Calibration Standard, 100 ng/mL

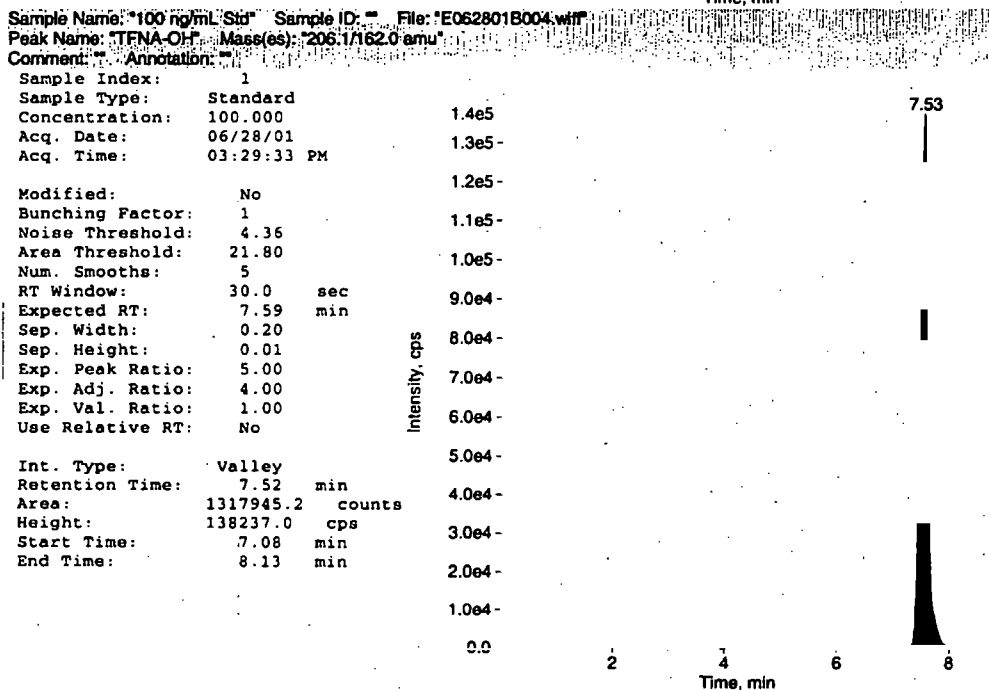
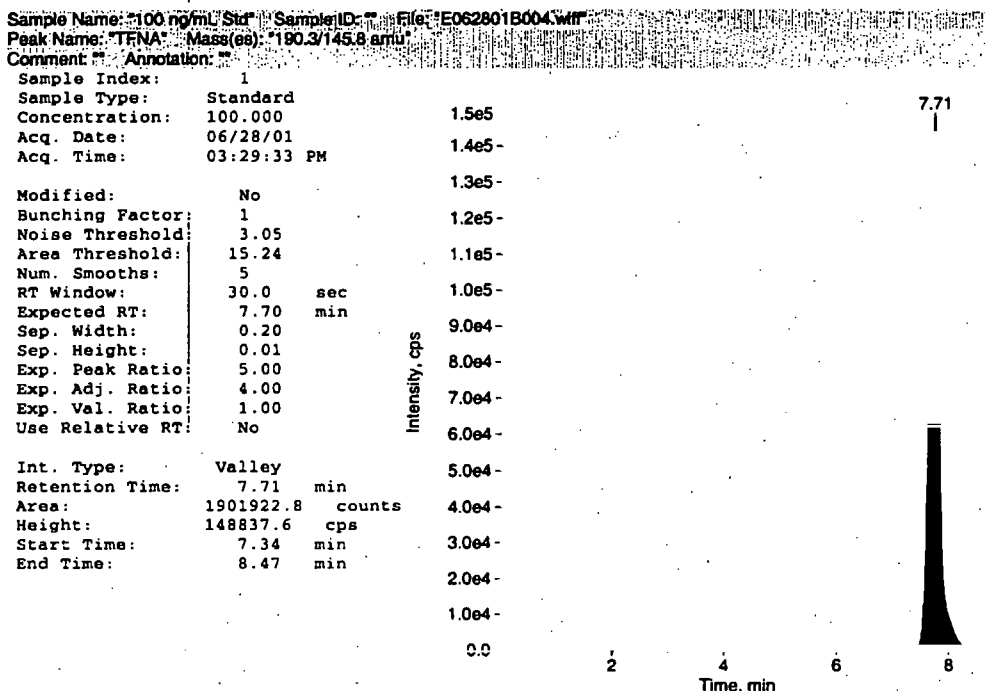


Figure A - 16: Control Sample, 12283-55-1-A, Day 1, 0-6"

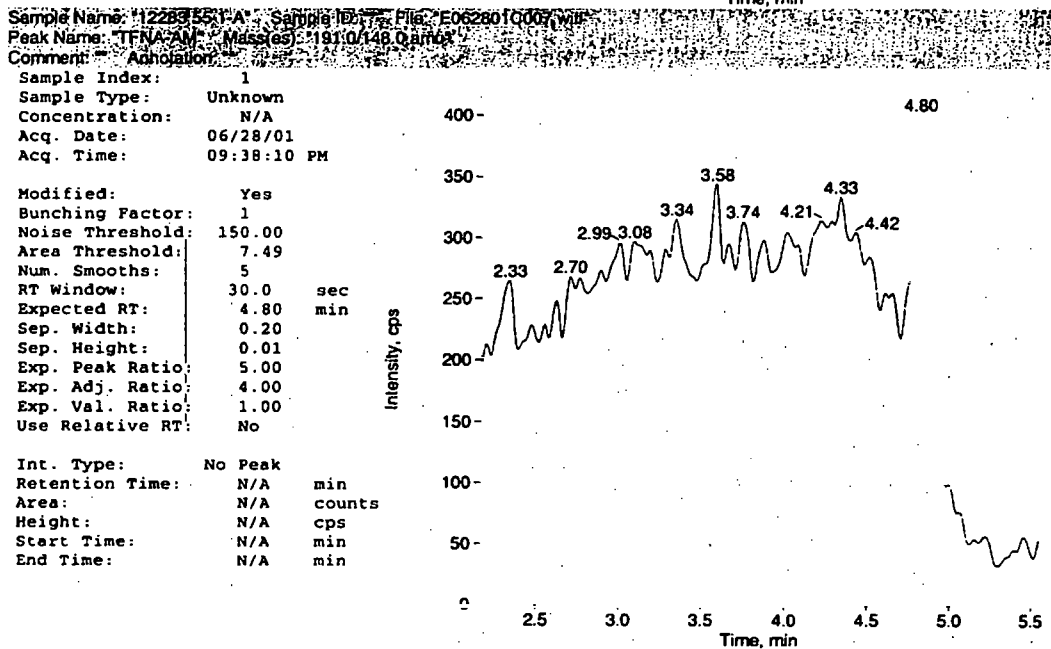
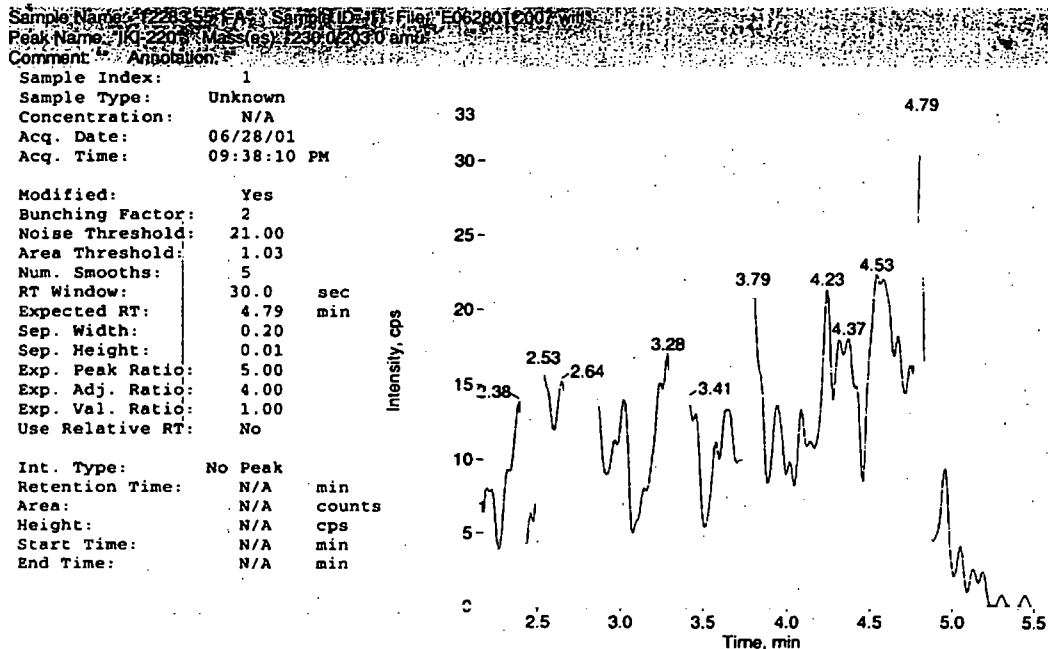


Figure A - 16 (Contd.): Control Sample, 12283-55-1-A, Day 1, 0-6"

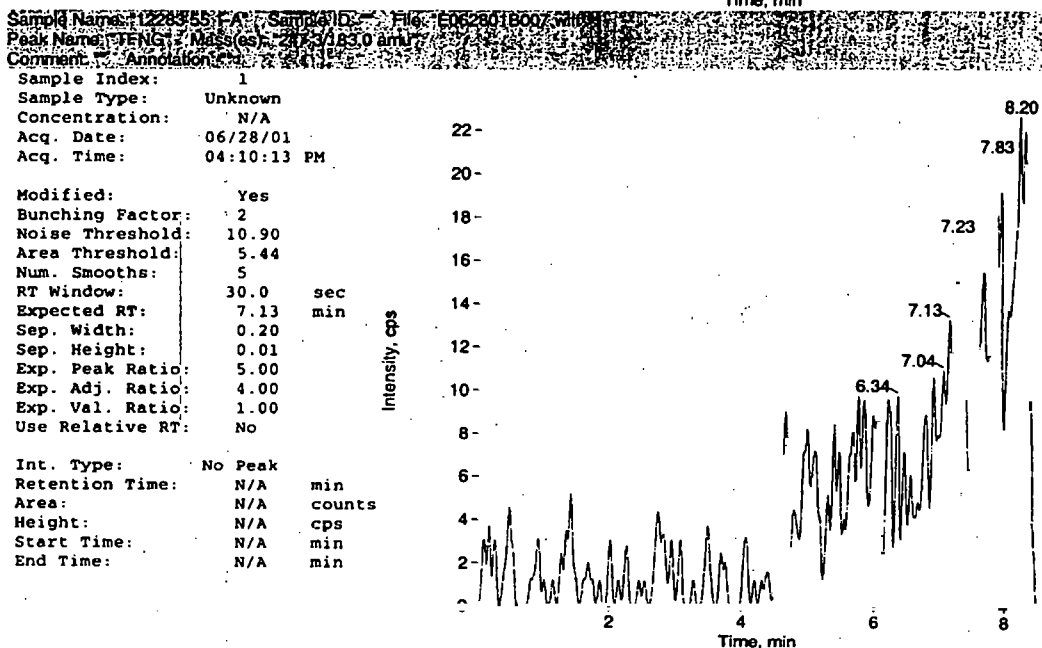
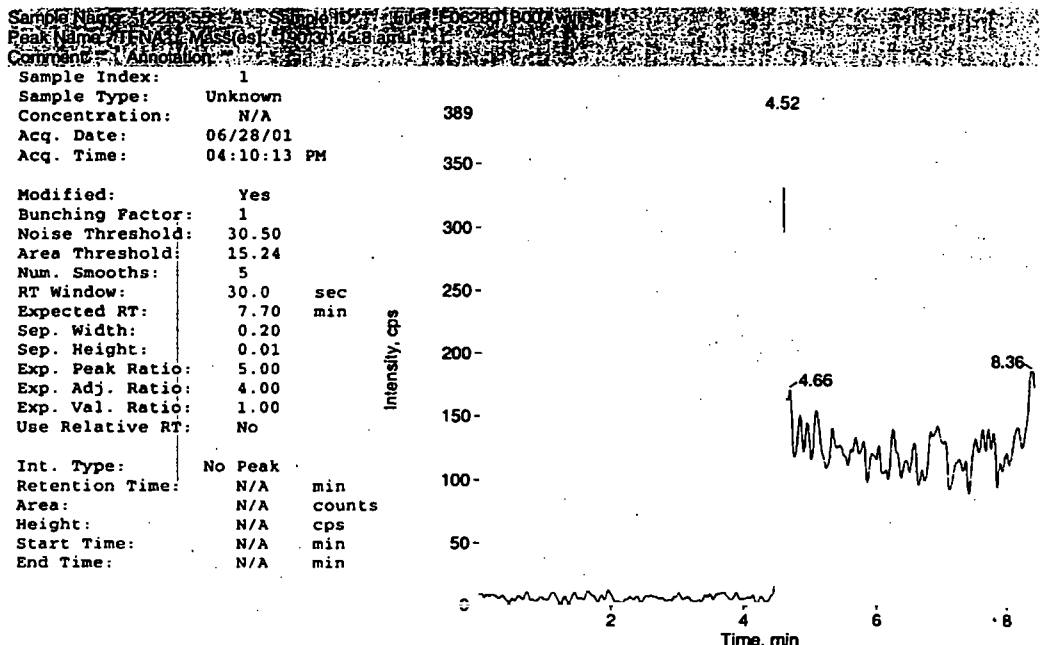


Figure A - 16 (Contd.): Control Sample, 12283-55-1-A, Day 1, 0-6"

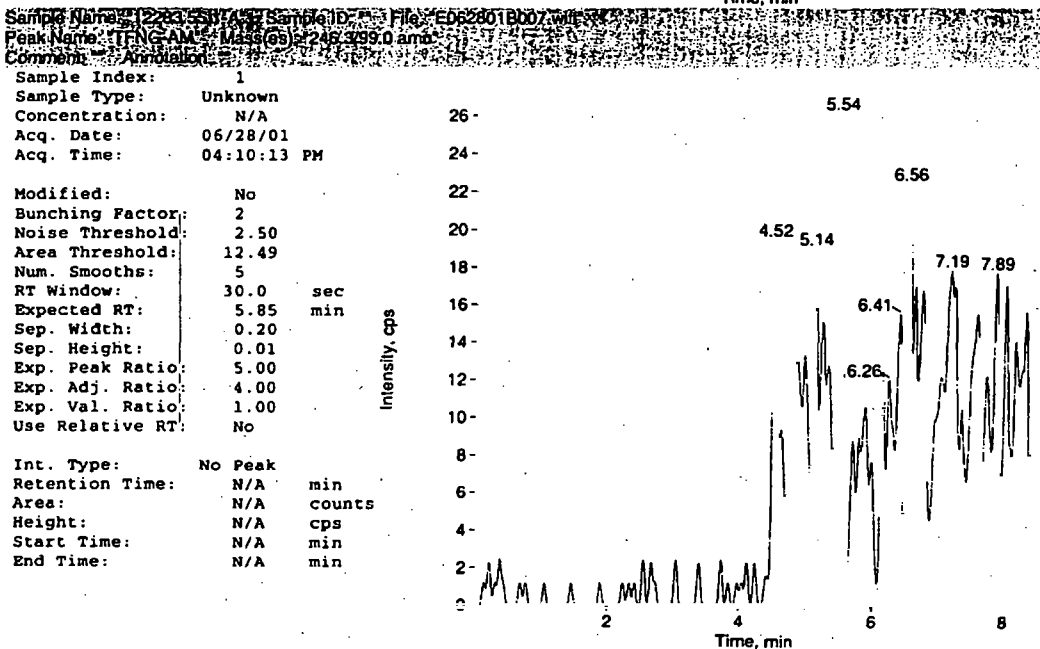
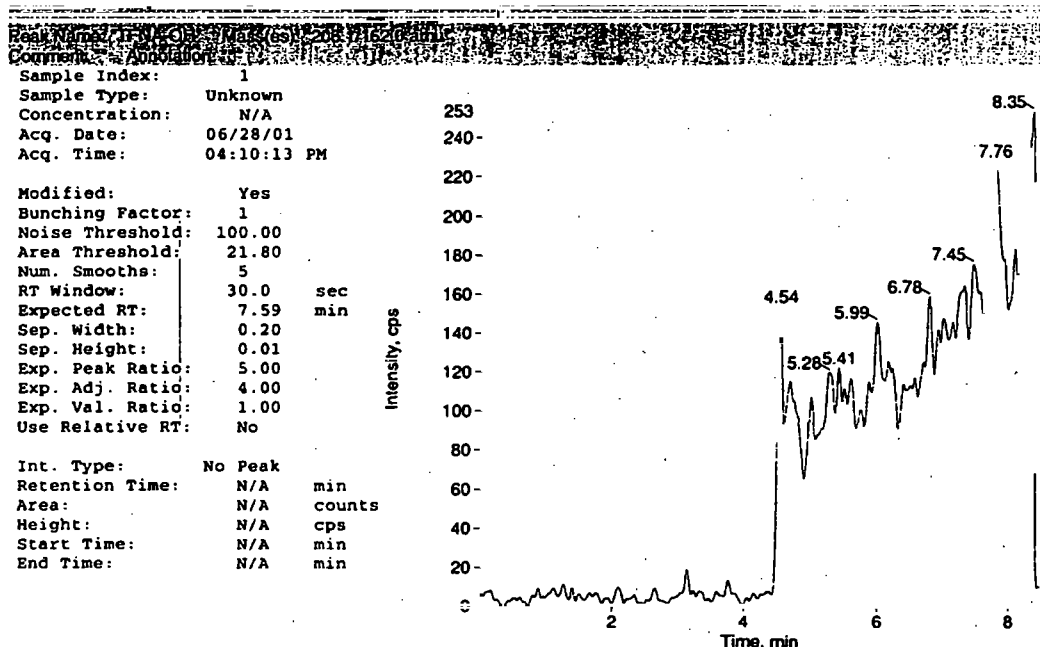


Figure A - 17: Control Sample fortified at 5 ppb, 12283-55-2-A, Day 1, 0-6"

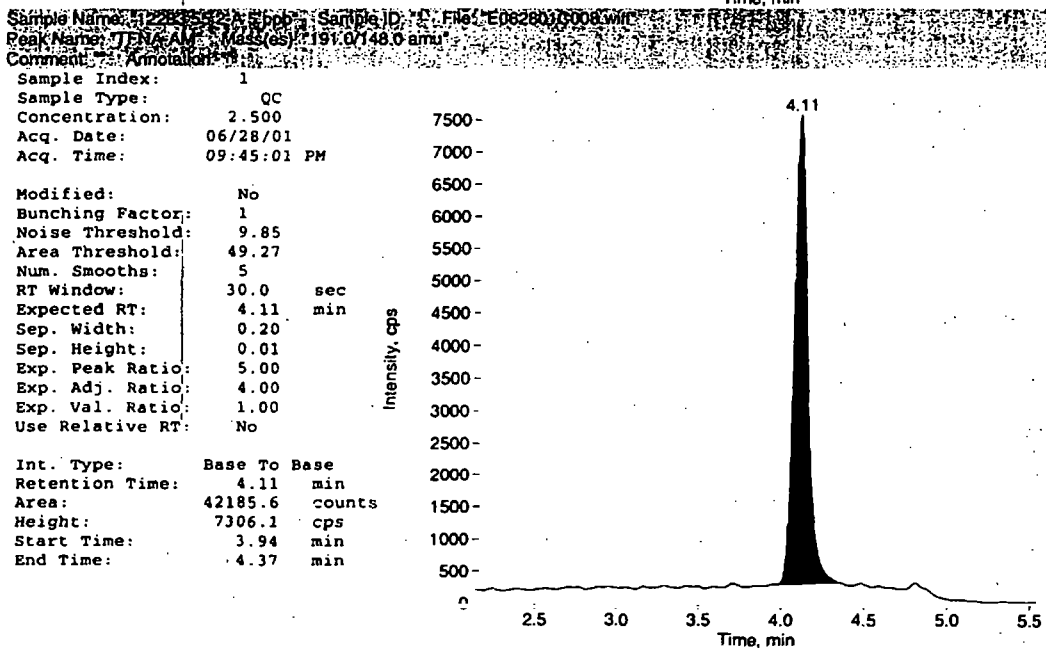
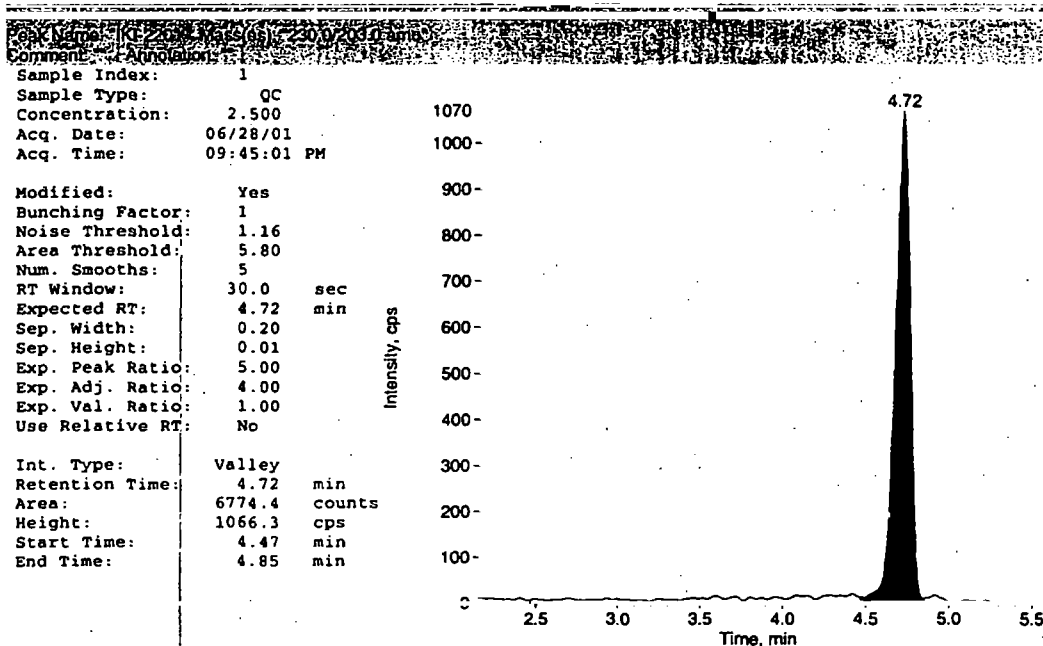


Figure A - 17 (Contd.): Control Sample fortified at 5 ppb, 12283-55-2-A, Day 1, 0-6"

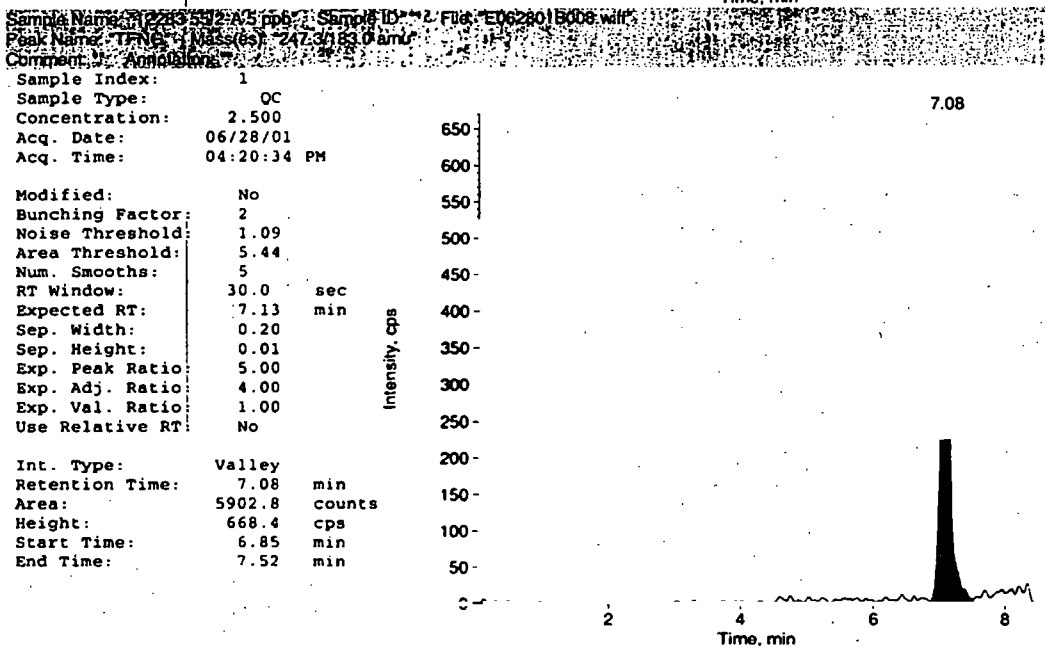
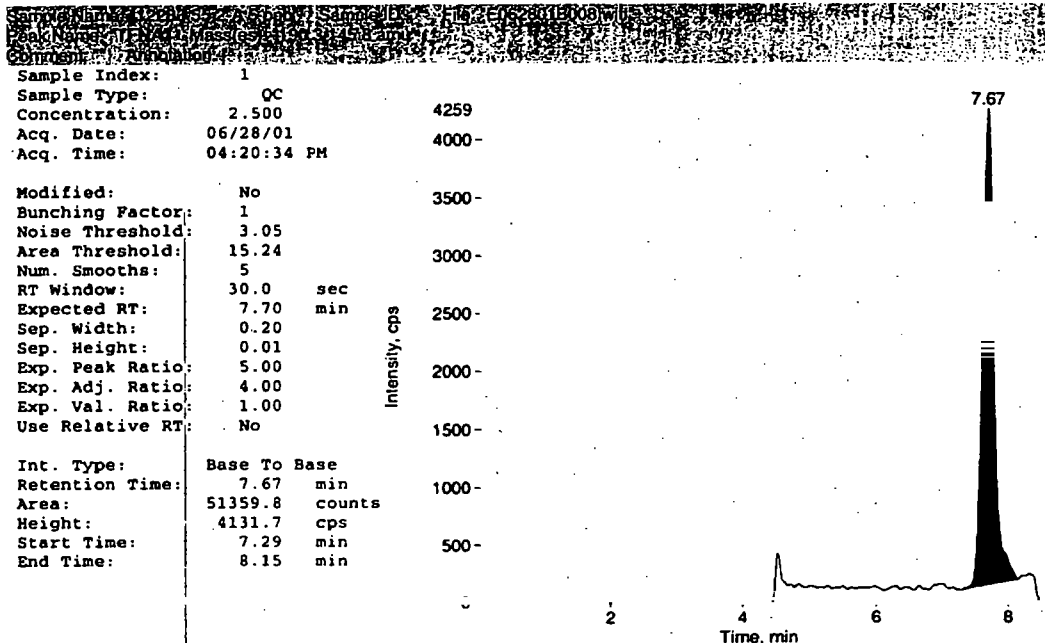


Figure A - 17 (Contd.): Control Sample fortified at 5 ppb, 12283-55-2-A, Day 1, 0-6"

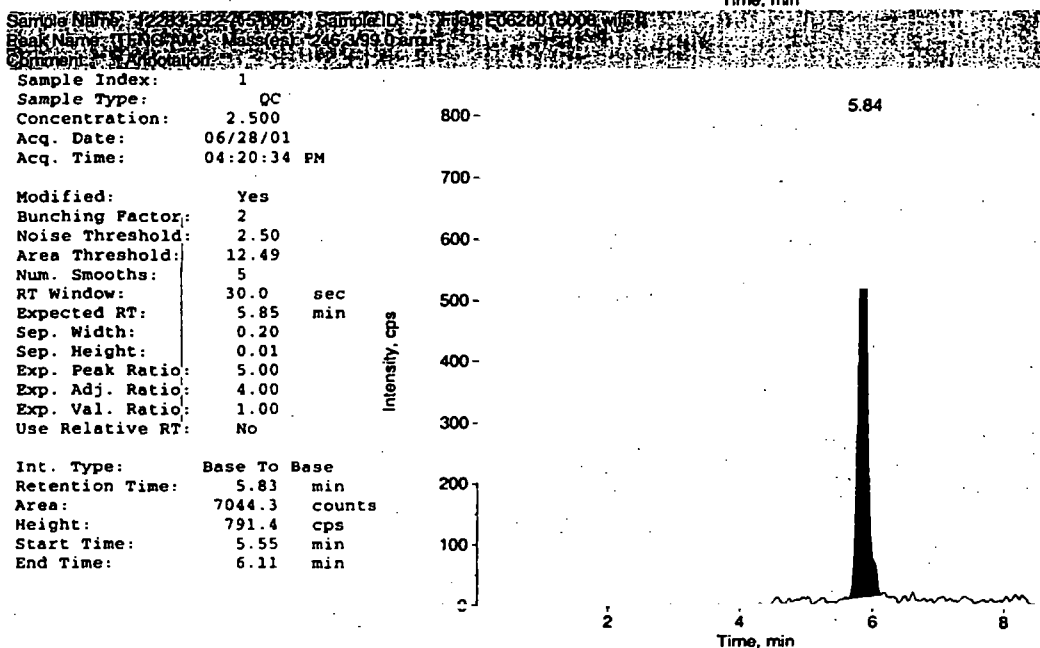
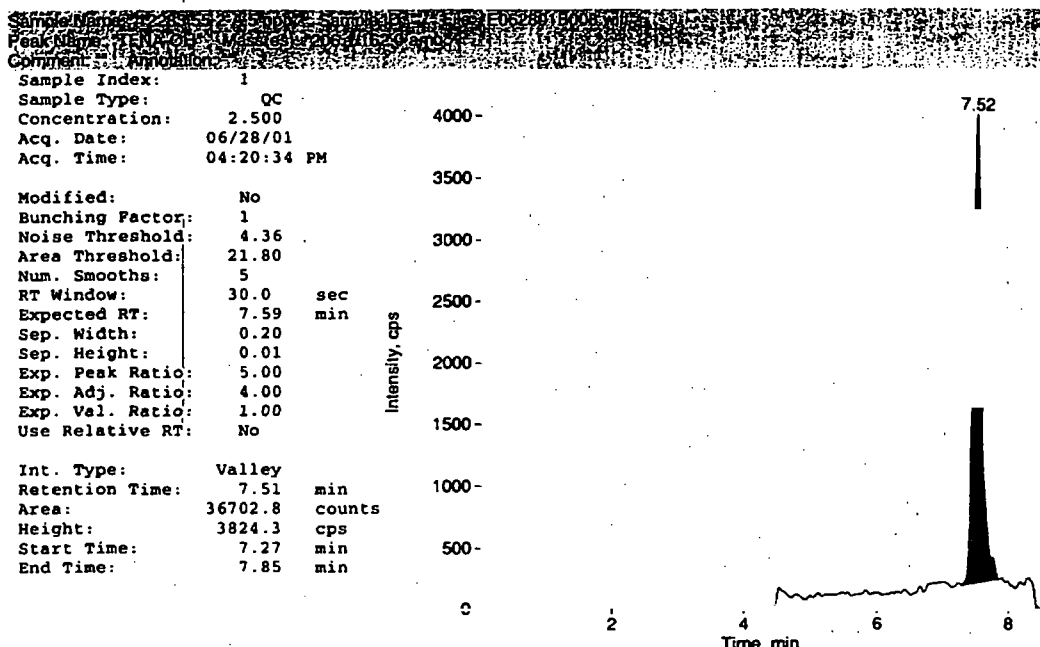


Figure A - 18: Concurrent Recovery Sample No. 12283-47- 3-A at 50 ppb

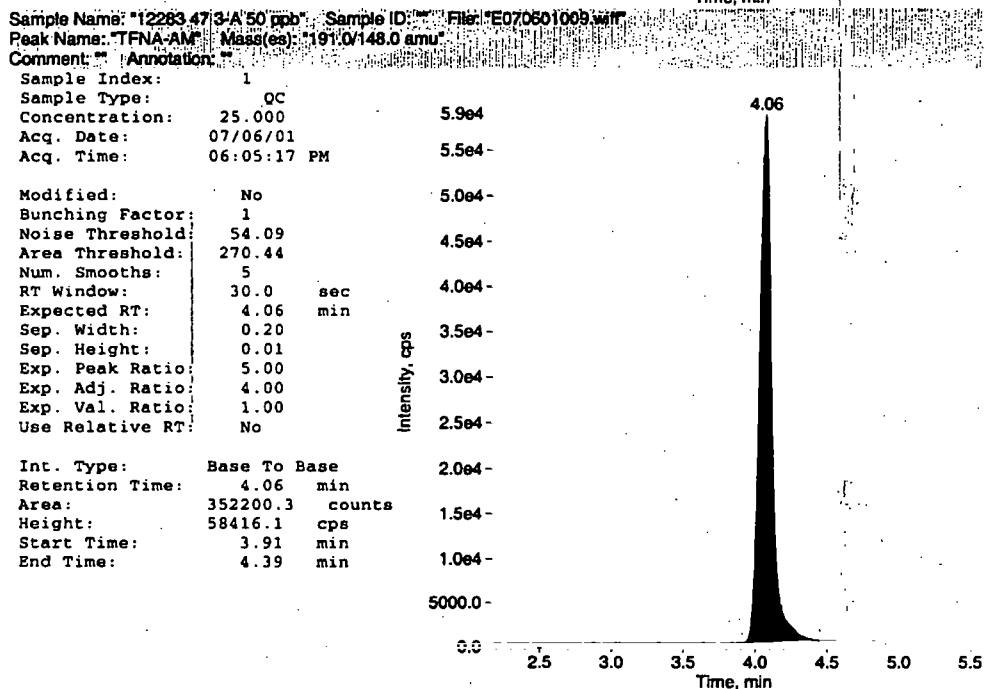
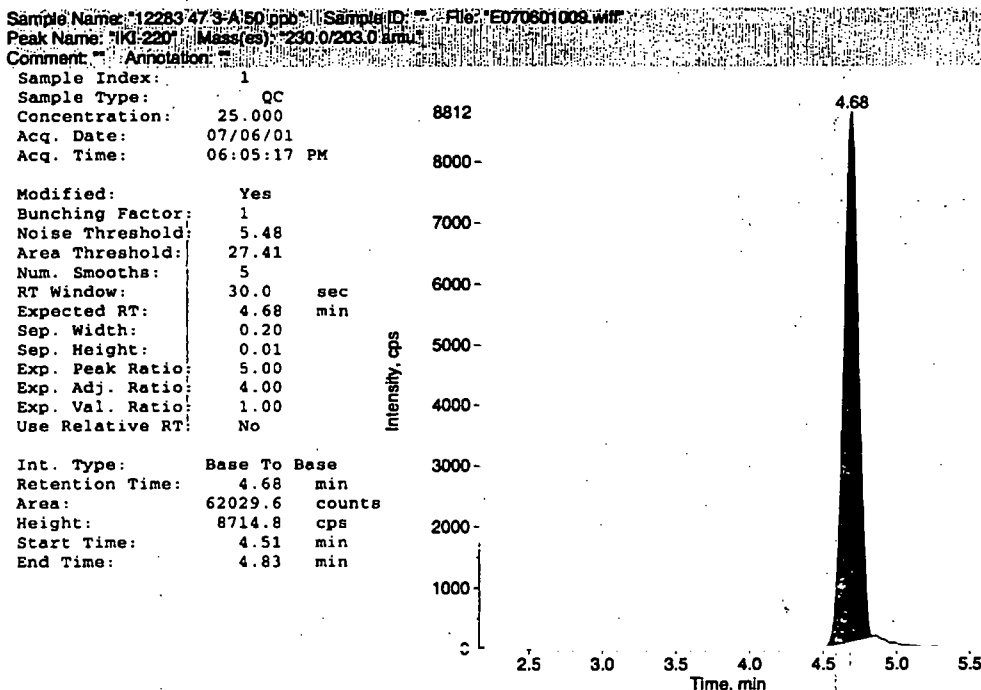


Figure A - 18 (Contd.): Concurrent Recovery Sample No. 12283-47- 3-A at 50 ppb

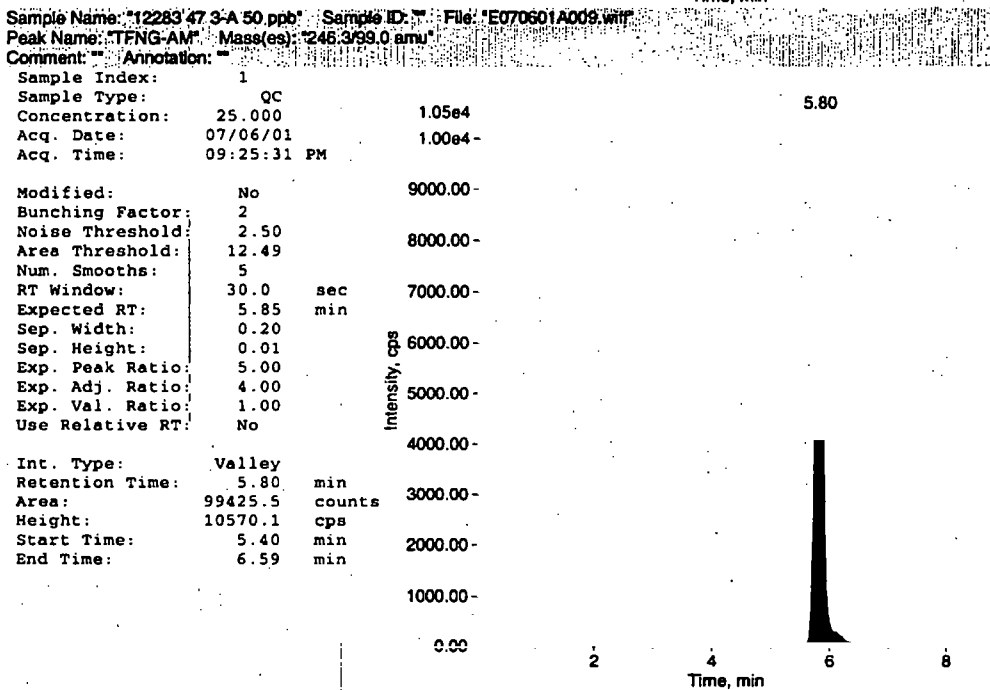
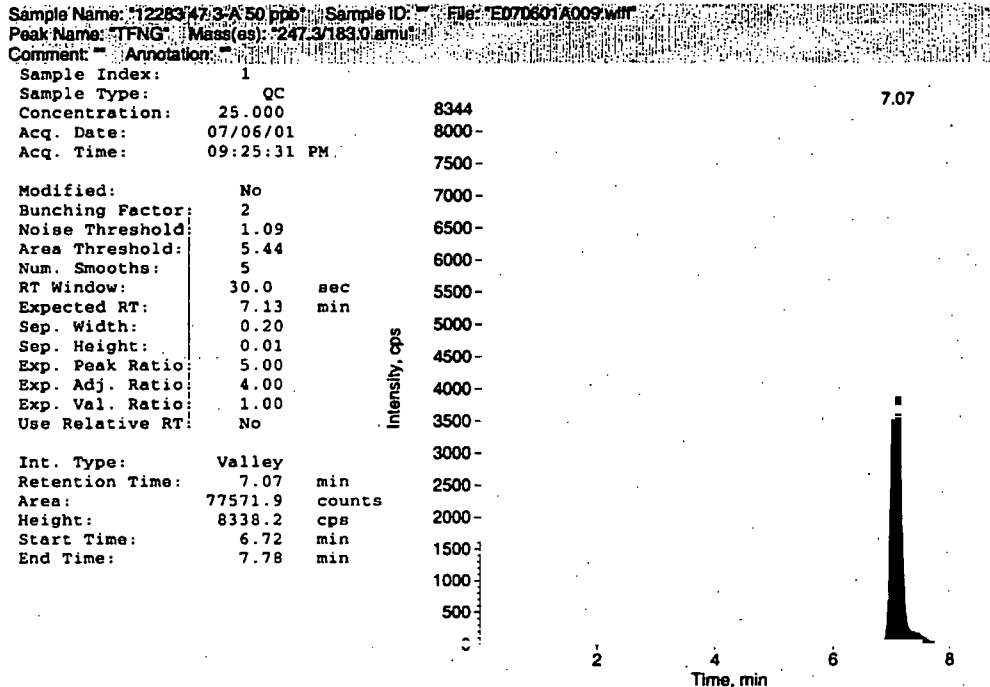


Figure A - 18 (Contd.): Concurrent Recovery Sample No. 12283-47- 3-A at 50 ppb

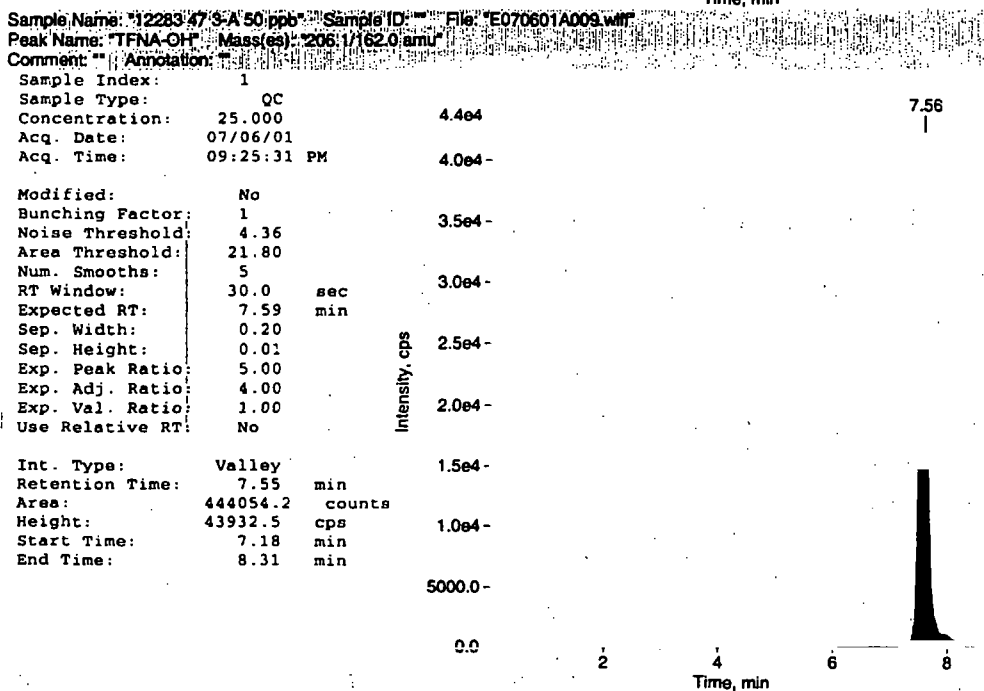
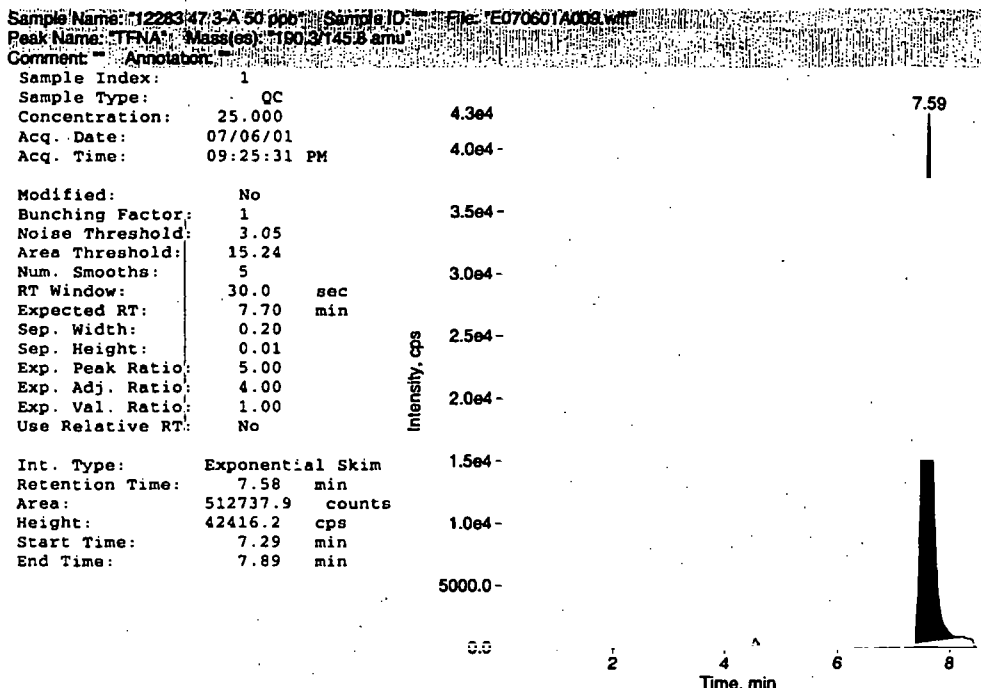


Figure A - 19: Treated Sample, 12283-56-A, Day 1, 0-6"

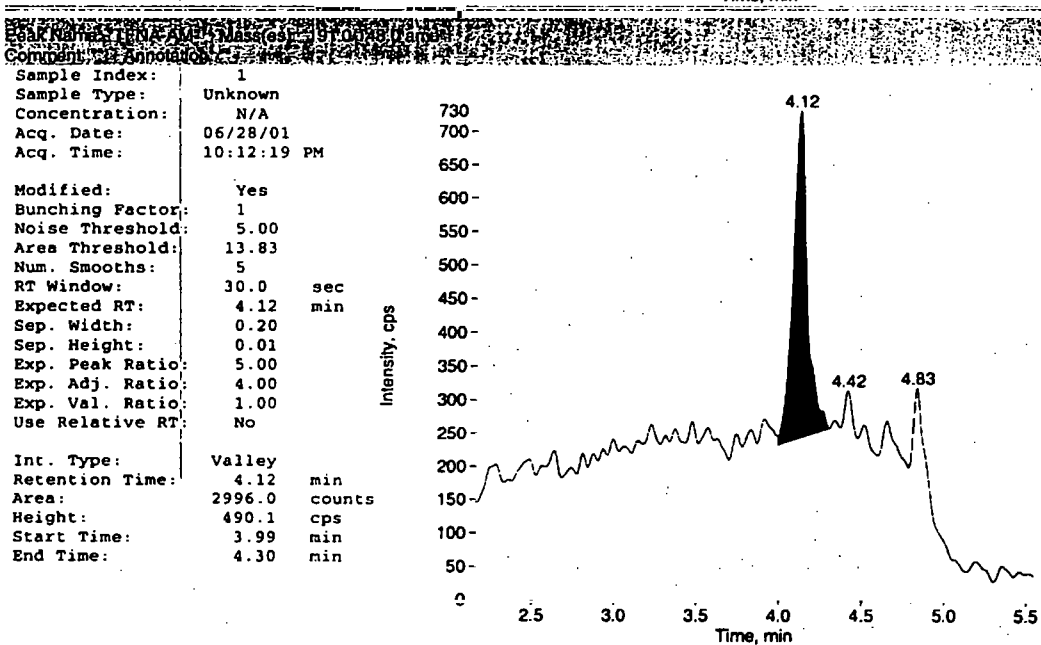
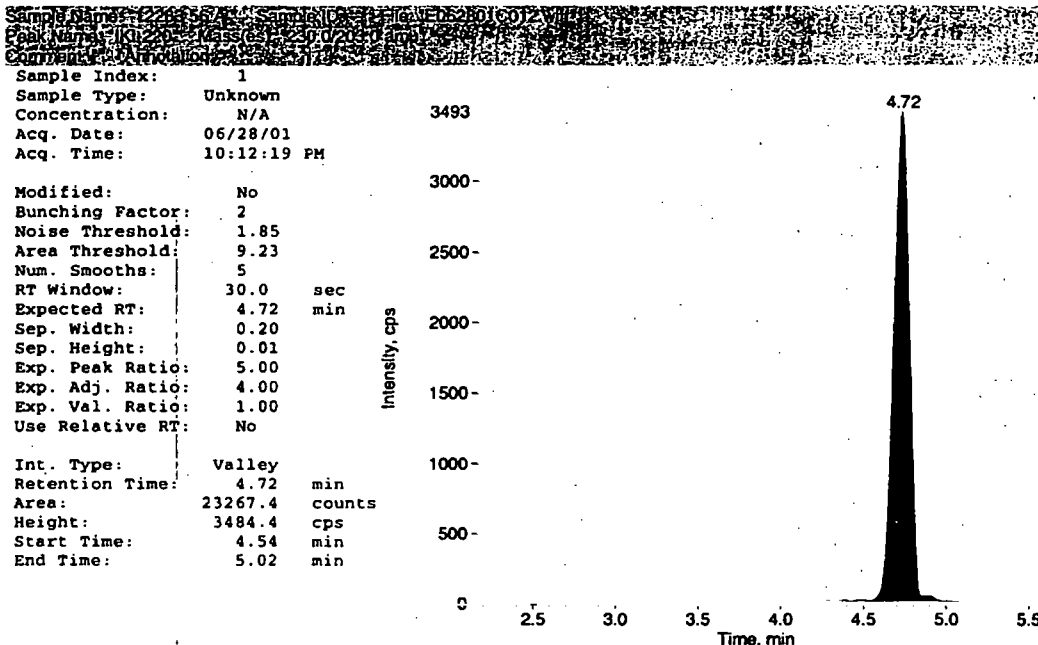


Figure A - 19 (Contd.): Treated Sample, 12283-56-A, Day 1, 0-6"

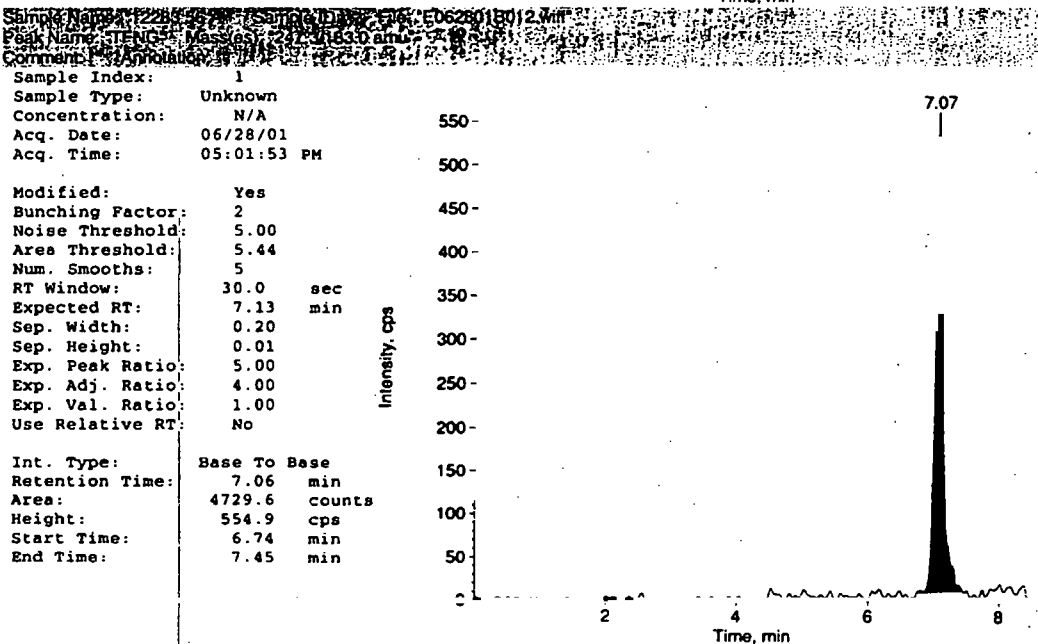
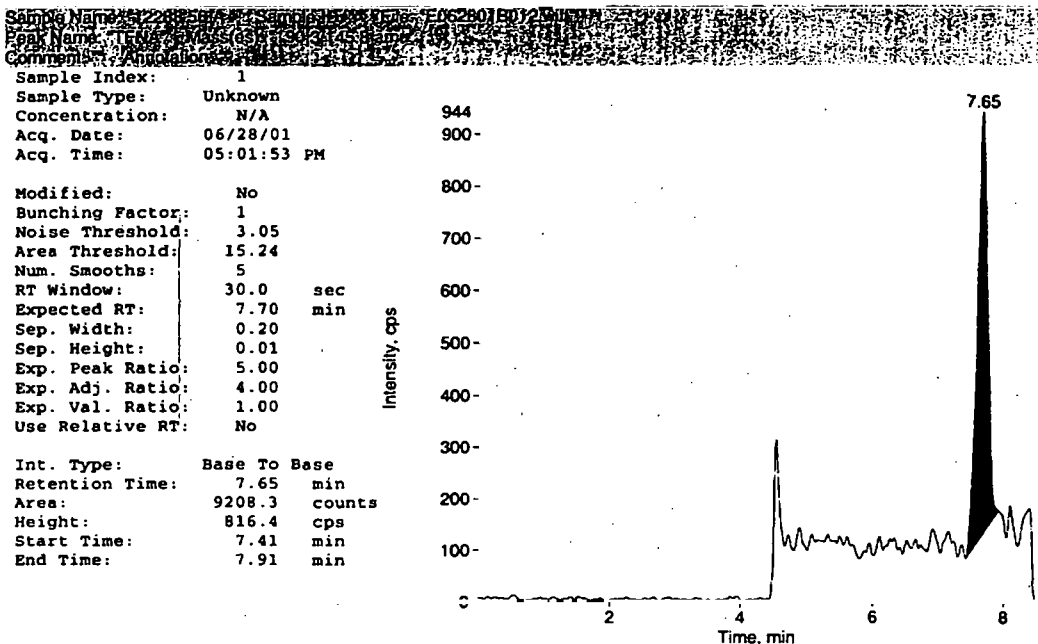


Figure A - 19 (Contd.): Treated Sample, 12283-56-A, Day 1, 0-6"

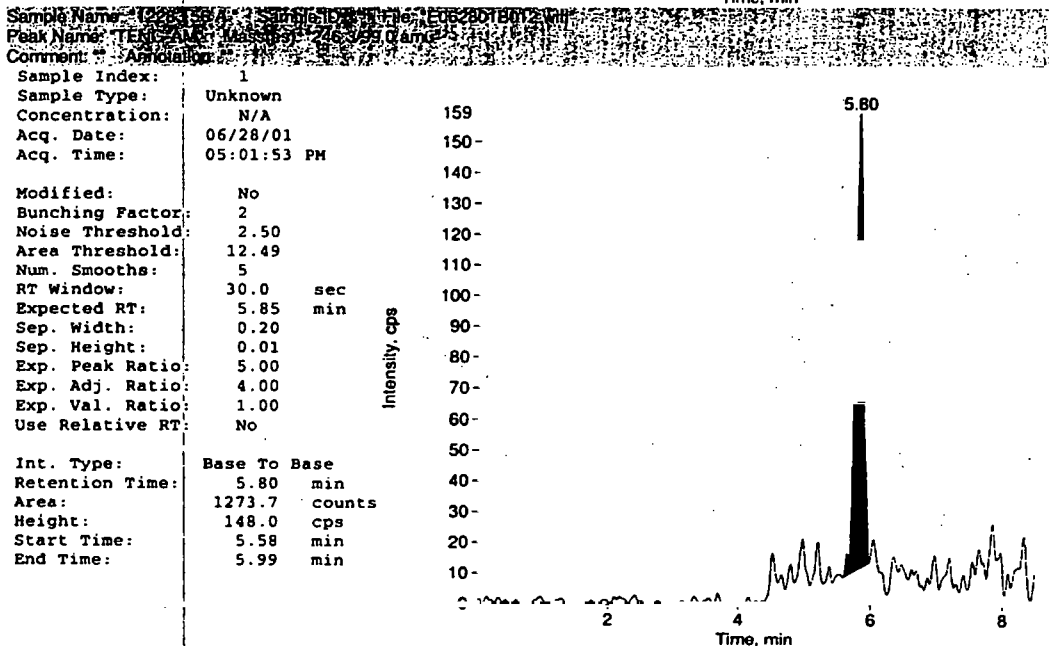
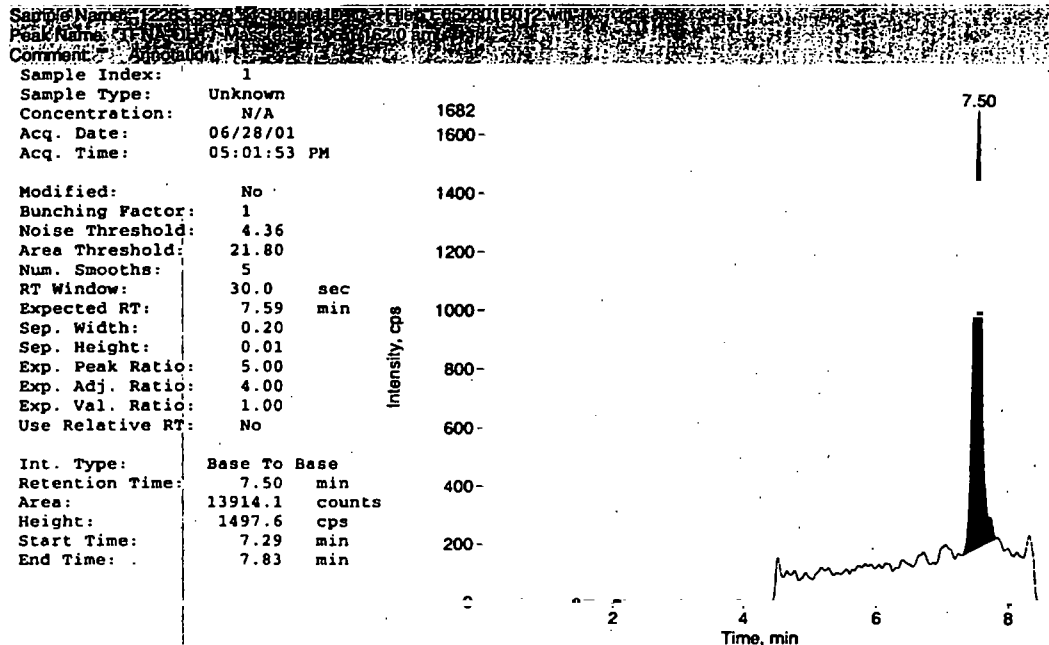


Figure A - 20: Treated Sample, 12283-56-B, Day 1, 6-12"

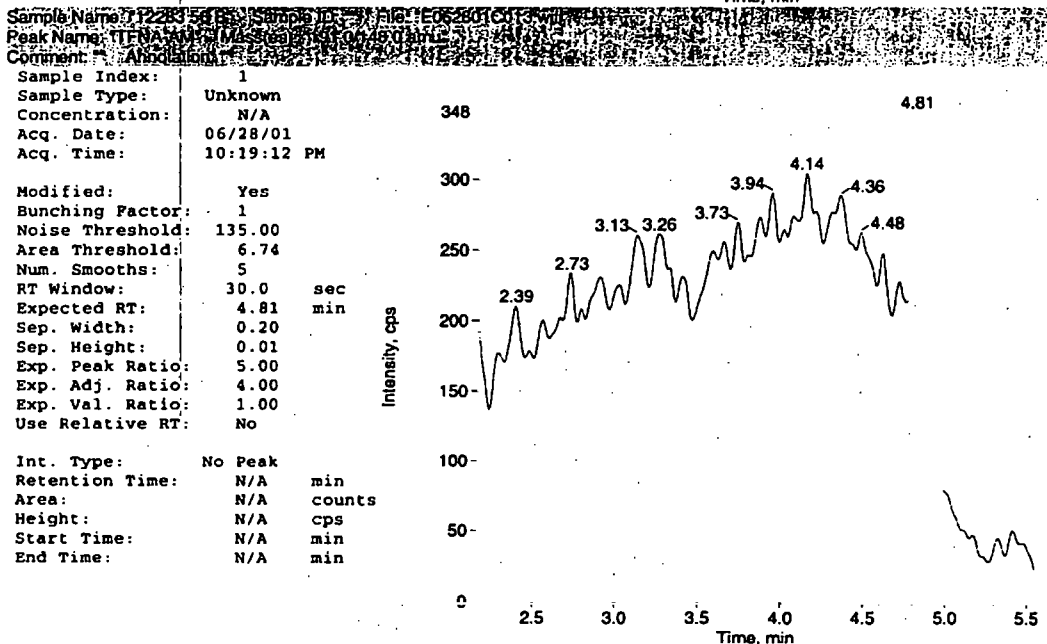
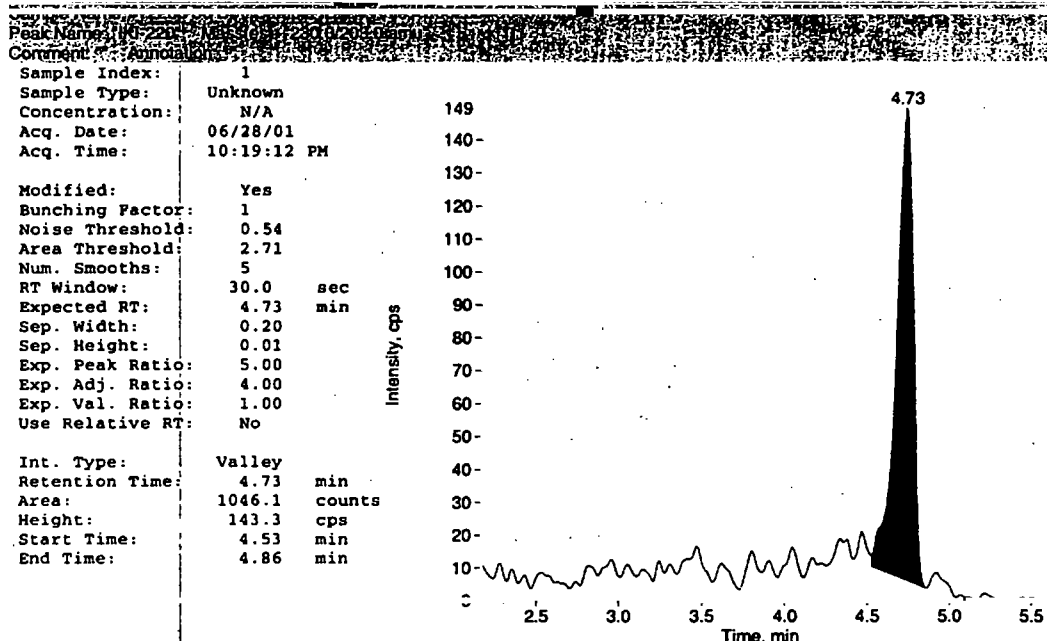


Figure A -20 (Contd.): Treated Sample, 12283-56-B, Day 1, 6-12"

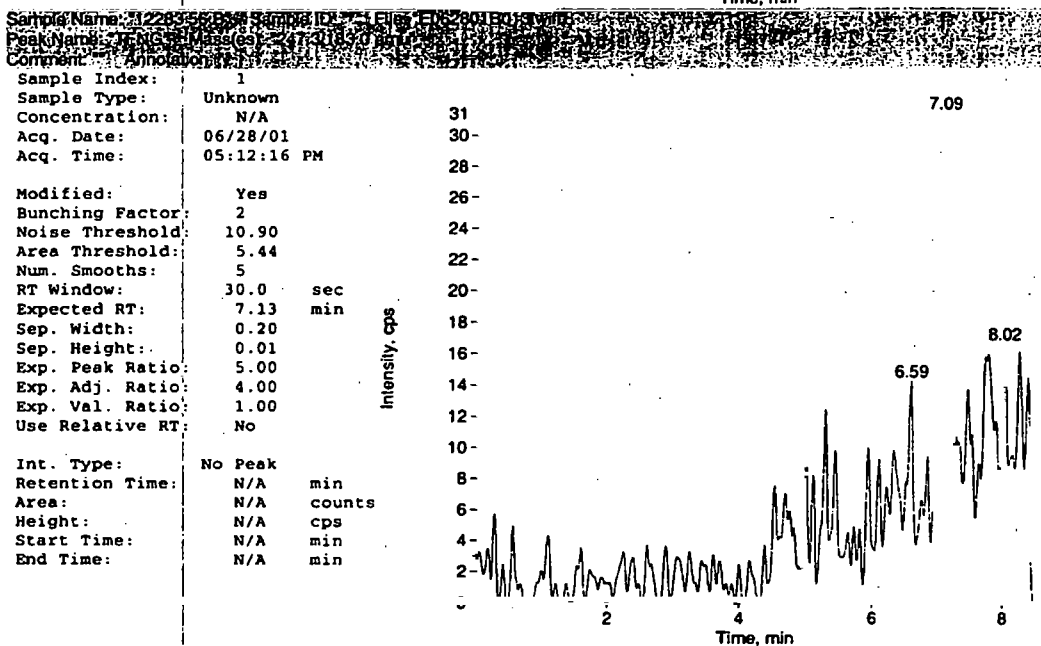
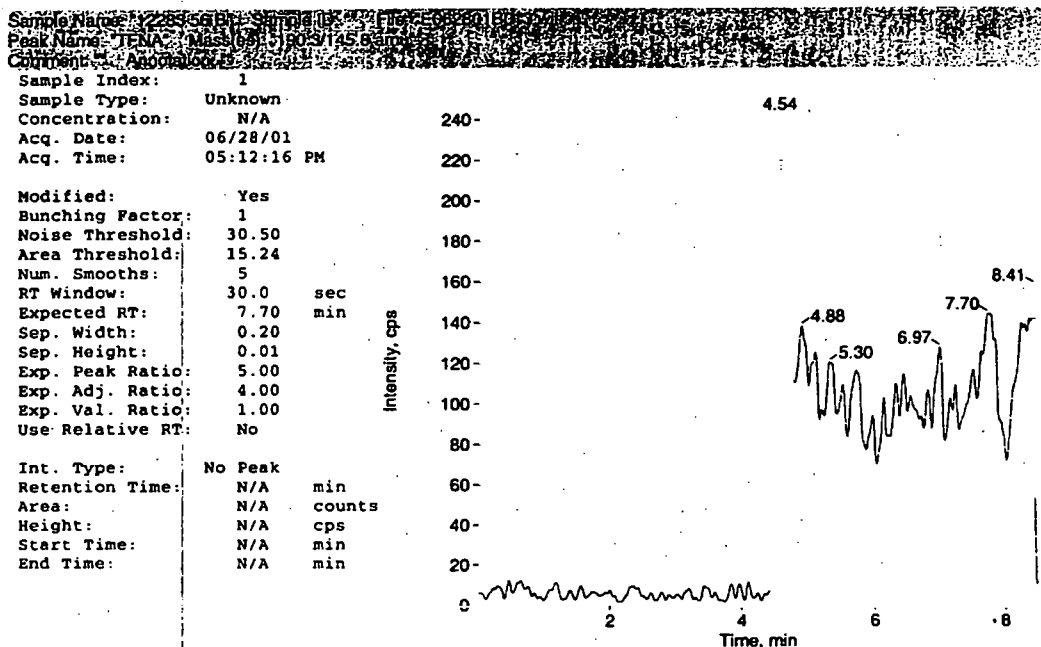


Figure A-20 (Contd.): Treated Sample, 12283-56-B, Day 1, 6-12"

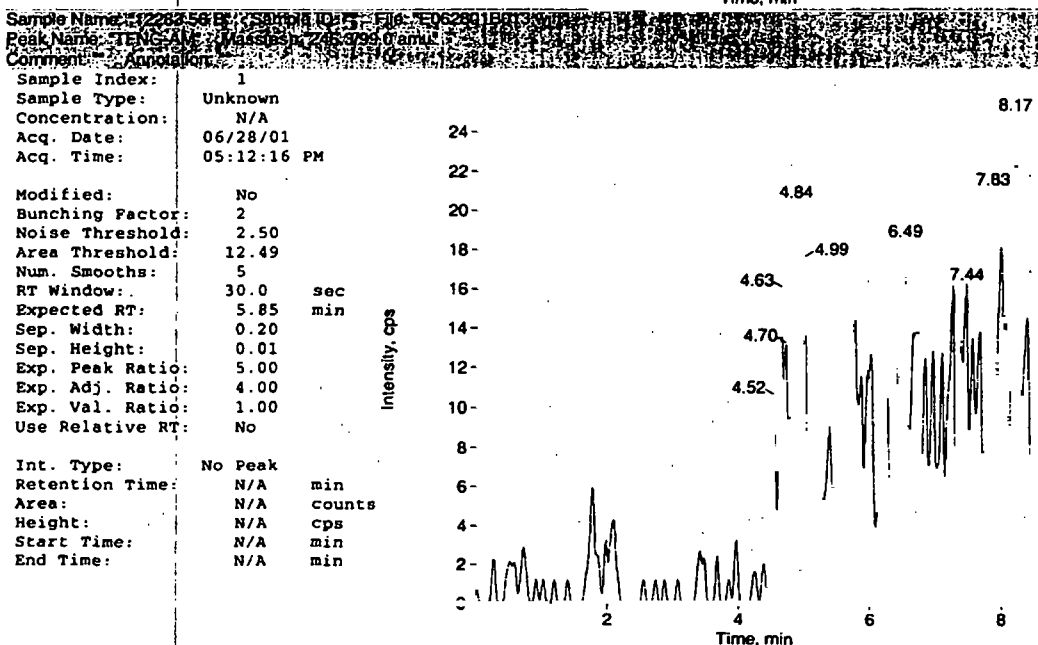
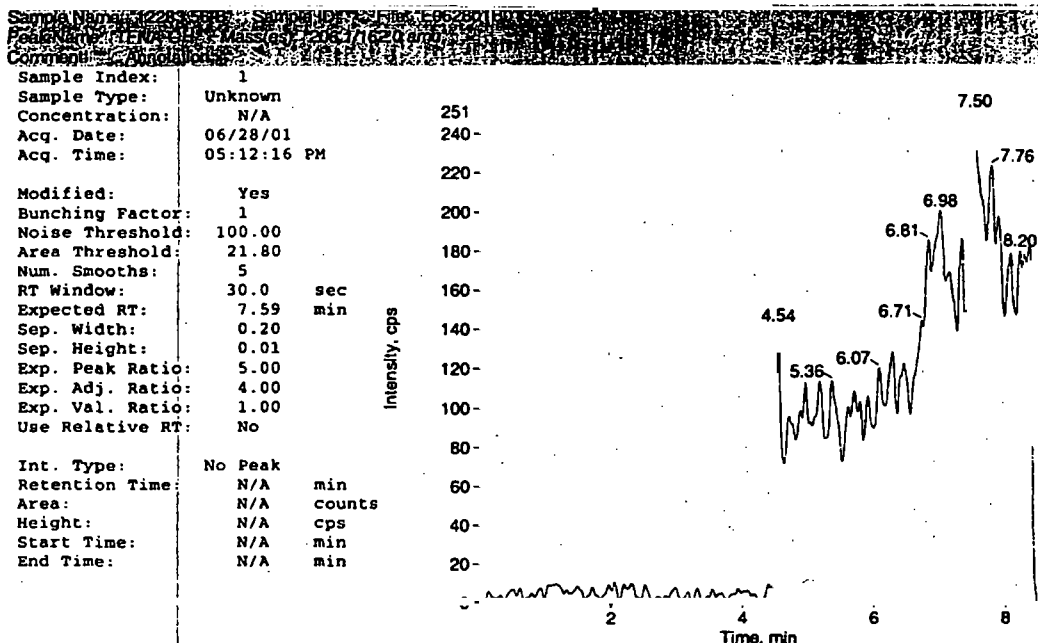


Figure A - 21: Treated Sample, 12283-56-C, Day 1, 12-18"

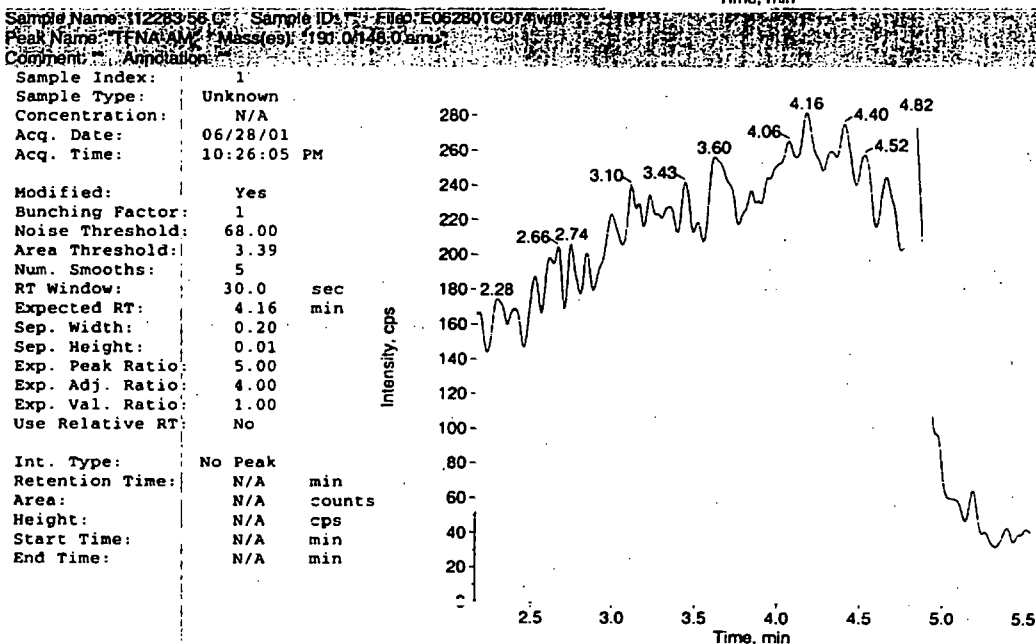
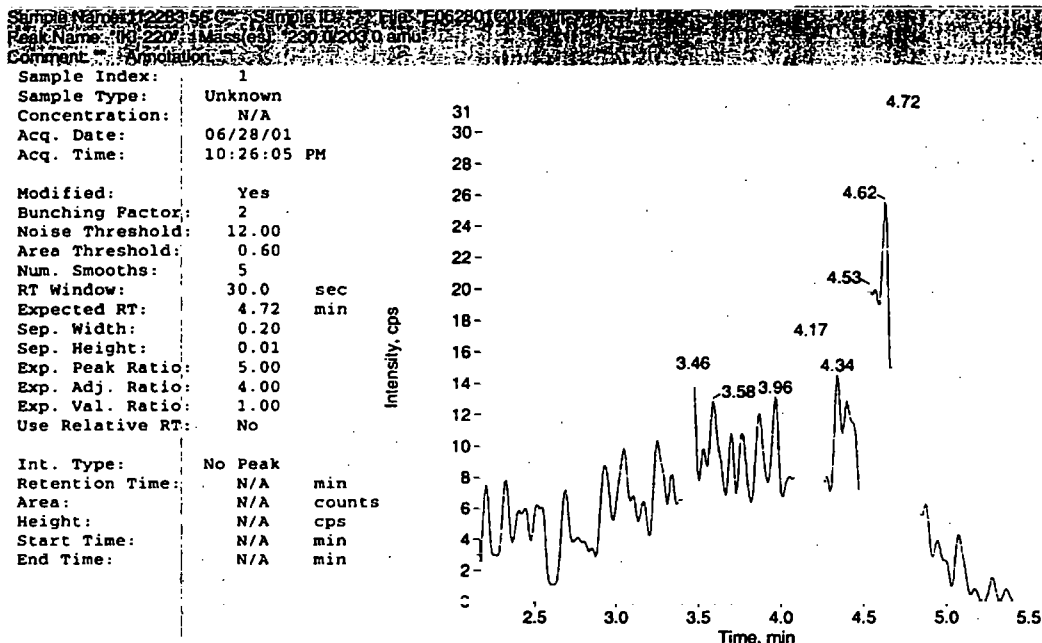


Figure A - 21 (Contd.): Treated Sample, 12283-56-C, Day 1, 12-18"

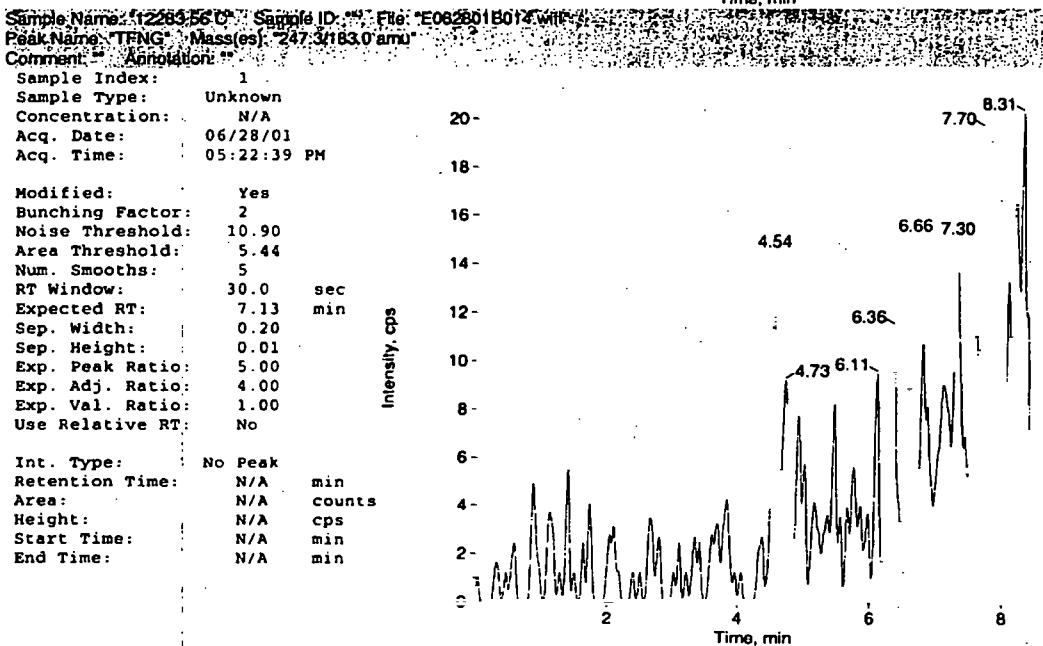
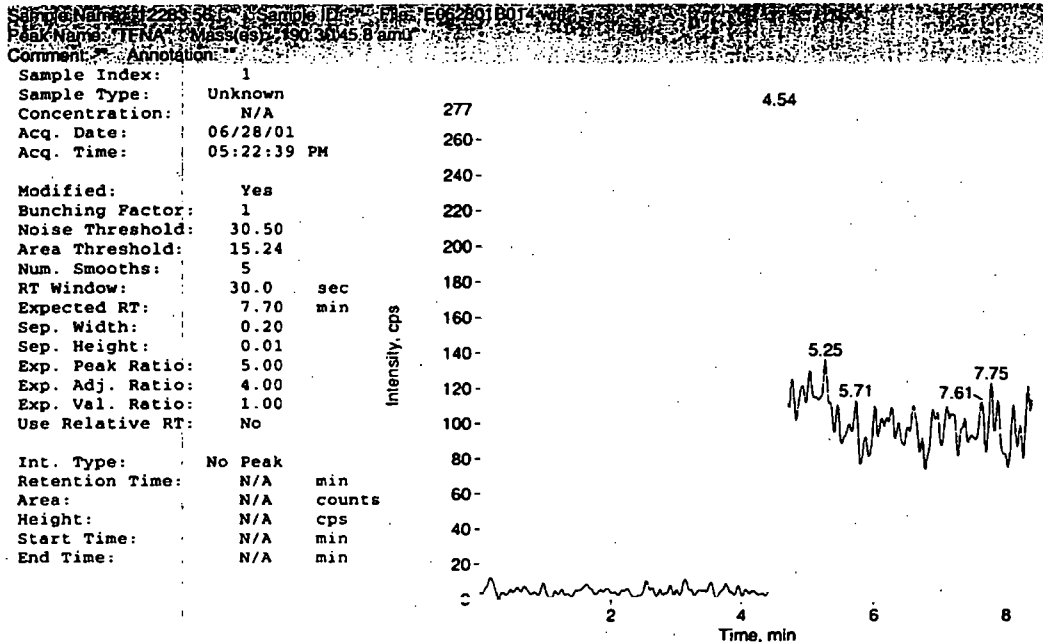


Figure A - 21 (Contd.): Treated Sample, 12283-56-C, Day 1, 12-18"

