Date Out: 6 APR 1994

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Chemical Code: 075003

ENVIRONMENTAL FATE AND GROUND WATER BRANCH

Review Action

To: W. Miller/S. Palamateer PM #16
Registration Division (H7505C)

From: Paul Mastradone, Section Chief
Chemistry Review Section 1
Environmental Fate & Ground Water Branch/EFED (H7507C)

Thru: Henry Jacoby, Chief
Environmental Fate & Ground Water Branch/EFED (H7507C)

4/7/94

Attached, please find the EFGWB review of...

Common Name:	Sodium Fluoroacetate	**Trade name:**	Compound 1080
Company Name:	Denver Wildlife Research Center		
ID #:	56228-22		
Purpose:	To review analytical method for determining Compound 1080 in coyote muscle tissue and in sheepskin and wool which pertains to environmental fate/ecological effects issues.		

Type Product:	Action Code:	EFGWB #(s):	Review Time:
Rodenticide	325	91-0334/0335	3 days

STATUS OF STUDIES IN THIS PACKAGE:
ADDRESSED IN THIS PACKAGE:

Guideline #	MRID	Status[1]

STATUS OF DATA REQUIREMENTS

Guideline #	Status[2]

[1]Study Status Codes: A=Acceptable U=Upgradeable C=Ancillary I=Invalid.
[2]Data Requirement Status Codes: S=Satisfied P=Partially satisfied N=Not satisfied R=Reserved W=Waived

1. CHEMICAL:

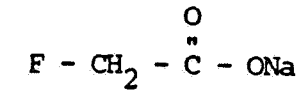
Chemical name: Sodium monofluroracetate

CAS no.: 62-74-8

Common name: Sodium fluoroacetate

Trade name: Compound 1080

Chemical structure:



Molecular formula: FCH_2CO_2Na

Molecular weight: 100.02

Physical/Chemical properties of active ingredient:

Physical characteristics: white power

Melting point: 35.2°C

Solubility: not given but highly soluble in water

Octanol/water partition coefficient: not available

Vapor Pressure: not available

2. TEST MATERIAL:

See individual DERs.

3. STUDY/ACTION TYPE:

To review analytical method for determining Compound 1080 in coyote muscle tissue and in sheepskin and wool as pertaining only to environmental fate/ ecological effects issues.

4. STUDY IDENTIFICATION:

Mishalanie, E.A. and Okuno, I. DETERMINATION OF COMPOUND 1080 IN TISSUE. Sponsored by U.S. Department of Agriculture, Animal and Plant Health Inspection Service, and Science and Technology, Washington, DC; Performed and Submitted by Denver Wildlife Research Center, Denver, CO under Laboratory Project ID Method No. 3A; Study complete 14 February 1990; Received by EPA 5 October 1990; MRID No. 41650201.

2

Mishalanie, E.A. and Kimball, B.A. DETERMINATION OF COMPOUND 1080 IN SHEEP-SKIN AND WOOL. Sponsored by U.S. Department of Agriculture, Animal and Plant Health Inspection Service, and Science and Technology, Washington, DC; Performed and Submitted by Denver Wildlife Research Center, Denver, CO under Laboratory Project ID Method No. 14A; Study complete 16 March 1990; Received by EPA 3 October 1990; MRID No. 41653401.

5. REVIEWED BY:

Gail Maske
Chemist, Review section #1
OPP/EFED/EFGWB

Signature: [signature]
Date: 6 APR 1994

6. APPROVED BY:

Paul Mastradone, Chief
Review section #1
OPP/EFED/EFGWB

Signature: Paul Mastradone
Date: 6 APR 1994

7. CONCLUSIONS:

These residue analytical methods were submitted for review by EEB due to environmental fate/ecological effects issues. This is a non-guideline review of a residue method.

Review of Submitted Data:

a. Analytical Method for Determination of Compound 1080 in Tissue (171-4); MRID No. 416502901

This residue analytical method for analyzing Compound 1080 in coyote muscle tissue is acceptable for analyzing Compound 1080 at 0.050 to 1.0 ppm concentrations.

Replicate fortified tissue samples with Compound 1080 at 50 ppb, 100 ppb, 400 ppb, and 1 ppm levels resulted in percent mean recoveries of 78.1%, 92.4%, 90.5% and 82.9%, respectively. From these data, a limit of quantitation in coyote muscle tissue of 50 ppb was reported. There are difficulties in obtaining repeatable calibration data below the 50 ppb level. In addition, a limit of detection in coyote muscle tissue of ≈25 ppb was reported due to the chromatographic interferences and difficulties in obtaining repeatable calibration data at the 25 ppb level.

The peak height verse concentration appear to be a linear function over the ranges of 2.5 to 10 ng/mL and 10 to 100 ng/mL. The r^2 factors ranged from 0.9940 to 0.9991. However, a 1:1 linear relationship was not indicated between peak height response and concentration (log/log data). the response vs concentration and the log response vs log concentration,

respectively. In addition, there was no chromatographic interferences reported for the control tissue samples.

b. Analytical Method for Determination of Compound 1080 in Sheepskin and Wool (171-4); MRID No. 41653401

This residue analytical method for analyzing Compound 1080 in blood stained sheepskin/wool tissue is acceptable for determining Compound 1080 at 5.00 to 100 mg/4" square tissue concentration.

For the 2.51 mg, 50.3 mg, 101 mg spiked concentration levels a mean reco very of 85.5 ± 2.2% , 80.0 ± 5.8%, and 76.7 ± 2.5% was reported, respectively. There did appear to be some decrease in the percent recoveries for the higher concentration levels.

A linear response was reported between the fluoroacetic acid/IS peak ratio and Compound 1080 concentration over the range tested. The fluoroacetic acid/IS was found to be directly proportional to the test material concentration. A r^2 of 0.9996 and 0.9998 were reported for the response vs concentration and the log response vs log concentration, respectively. In addition, there was no chromatographic interferences reported for the control tissue samples.

ENVIRONMENTAL FATE ASSESSMENT

Environmental fate data submitted up to this time was found not to be guideline studies and lacking significant data. Therefore, the studies were found of uncertain value and not reviewed in detail. Based on a review of the files, an environmental fate assessment can not be made at this time.

8. RECOMMENDATIONS:

The registrant should be informed of the following:

a. The analytical method for detection of sodium fluoroacetate in coyote muscle tissue and blood stained sheepskin/wool are acceptable.

b. The status of the Environmental Fate Data Requirements for the indoor use (impragnated collar/tag) is as follows:

Environmental Fate Data Requirement	Status of Data Requirement	MRID No.
Degradation Studies-Lab		
161-1 Hydrolysis	Not Fulfilled--See Attachment I	

9. BACKGROUND:

Sodium fluoroacetate, Compound 1080, is a rodenticide of which Tull Chemical Company, Oxford, AL is the sole U.S. producter. Compound 1080 is used in live stock protection collars and predator control. In 1972 the predacidal uses of Compound 1080 (primarily coyote control) was cancelled. However, in following a hearing in 1983, the Agency determined that significant new evidence warranted modifications of the 1972 cancellation of the livestock protection collars and single lethal dose baits uses. A registration for the livestock protection collar was issued in 1985 to the Department of Interior. In addition, the environmental fate data requirements were established and submission schedule. A Data-Call-in for terrestrial food use data requirements was issued in 1985, as well.

In 1986 the California Department of Food and Agriculture submitted data in support of registration of Compound 1080. The State of California package consisted of 14 studies (12 published and 2 unpublished) listed below:

Hydrolysis (161-1)

Goldman, P. 1965. The enzymatic cleavage of the carbon-fluorine bond in fluoroacetate. J.B.C. Vol 24 #8:3434-3438.

Goldman, P. and Milne, G.W.A. 1966. Carbon-fluroine bond cleavage. II. Studies on the mechanism of defluorination of fluoroacetate. J.B.C. 241 (23):5557-5559.

Harrison, B.L. et al. 1951. Deterioration of sodium monofluoroacetate in water and saline solutions (abstract). Fed. Proc. Pharm. Experi. Therapeutics 10: 306-307.

Photodegradation on soil (161-3)

Schroeder, P.M. et al. 1979, Ketron, Inc. Assessment of the environmental effects of predator and rodent control program in Wyoming using strychnine and 1080. Unpublished report. USDA (APHIS) contract #53-6395-81345.

Aerobic soil metabolism (162-1)

Walker, J.R.L. and Lien, B.C. 1978. Breakdown of 1080. Agri. Pests Destruction Council. (A.P.D.C.). Newsletter 5, 1-2.

David, W.A. and Gardiner, B.O. 1966. Persistence of fluoroacetate and fluoroacetamide in soil. Nature 209:1367-1368.

Kelly, M 1965. Isolation of bacteria able to metabolize fluoroacetate or fluoroacetamide. Nature 208:809-810.

Lien, B.C. et al. 1979. Effect of sodium fluoroacetate (Compound 1080) on the soil microflora. Soil Biol. Biochem. 11:13-18.

Tonomura, K. et al. 1965 Defluorination of monofluoroacetate by bacteria. Part I. Isolation of bacteria and their activity of defluorination. Agri. Biol. Chem. 29 (4):124-128.7.

Walker, J.R.L. and Lien, B.C. 1981. Metabolism of fluoroacetate by soil Pseudomonas sp. and Fusarium Solani. Soil. Biol. Chem. 13:231-235.

Leaching, adsorption/desorption (163-1)

Corr, P.V. and Martire, P. 1971. Leaching by rain of sodium fluoroacetate (1080) from baits used for rabbit control. Austral. J. Exp. Agric. and Animal Husb. 11:278-281.

David, W.A. and Gardiner, B.O. 1966. Persistence of fluoroacetate and fluoroacetamide in soil. Nature 209:1367-1368.

Peters, J.A. 1974. Environmental Contamination, toxicology, and residues of Compound 1080 (sodium fluoroacetate) in relation to aerial control programmes in protection forests: A synopsis. Protection Forestry Division. Unpublished reports 6 pp. New Zealand Forest Service, Ranqiora.

Staple, E.L.J. 1968. The reduction of sodium monofluoroacetate (1080) content of carrot baits of various thickness by weathering. N. Z. Jour. Agric. Res., 11 (2):319-329.

See Attachment I for more detail. Anaerobic aquatic metabolism data was not submitted as requested in the Data-Call-In.

Since 1987 no environmental fate data has been submitted. A 1992 (PO;08/07/92) Phase IV review reflected the lack of environmental fate data, as well. The Luis report at the time of the Phase IV review lists only one use "impregnated collar/tag", which is considered an indoor use. The maximum application rate is 30.4 gm/animal and the number of collars/acre of pasture is limited ("do not use >20 collars/100 acres of pasture or >50 collars for 101 to 640 acres of pasture of >100 collars for 641 to 10,000 acres of pasture". In addition, the label has an endangered species restriction statement. The Luis report does not mention the grain bait for ground squirrel control use, although there are EFGWB actions which deal with the end-product (11085-E-1080 GROUND SQUIRREL BAIT 0.02) (PRD;05/04/88:ER;06/10/88). Therefore, assuming there was only one currently registered use (impragnated collar/tag), the hydrolysis data requirement was imposed as the sole data requirement.

10. DISCUSSION:

See individual DERs.

11: COMPLETION OF ONE-LINER:

See attached one-liner. Since no guideline environmental fate studies have been submitted, there is no environmental fate data encluded in the one-liner.

12: CBI APPENDIX:

N/A

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DATA EVALUATION RECORD

STUDY 1

CHEM 075003 SODIUM FLUOROACETATE § 171-4

STUDY ID 41650201

Mishalanie, E.A. and Okuno, I. DETERMINATION OF COMPOUND 1080 IN TISSUE. Sponsored by U.S. Department of Agriculture, Animal and Plant Health Inspection Service, and Science and Technology, Washington, DC; Performed and Submitted by Denver Wildlife Research Center, Denver, CO under Laboratory Project ID Method No. 3A; Study complete 14 February 1990; Received by EPA 5 October 1990.

DIRECT REVIEW TIME = 1.8 day

REVIEWED BY: G. Maske
TITLE: Chemist
ORG: EFGWB/EFED/OPP
TEL: 305-5245

SIGNATURE: [signature] 6 APR 1994

APPROVED BY: Paul Mastradone, Chief
Review section #1
OPP/EFED/EFGWB

Signature: Paul Mastradone

Date: 6 APR 1994

CONCLUSIONS:

This is a non-guideline review of a residue method for analyzing Compound 1080 in coyota muscle tissue. The analytical methodology is scientifically valid, and is acceptable for determining Compound 1080 at a concentration range of 0.050 to 1.0 ppm. There are two phases, extraction and analysis, to this analytical method.

Replicate fortified tissue samples with Compound 1080 at 50 ppb, 100 ppb, 400 ppb, and 1 ppm levels resulted in percent mean recoveries of 78.1%, 92.4%, 90.5% and 82.9%, respectively. From these data, a limit of quantitation in coyote muscle tissue of 50 ppb was reported. There are difficulties in obtaining repeatable calibration data below the 50 ppb level. In addition, a limit of detection in coyote muscle tissue of ≈25 ppb was reported due to the chromatographic interferences and difficulties in obtaining repeatable calibration data at the 25 ppb level.

The peak height verse concentration appear to be a linear function over the ranges of 2.5 to 10 ng/mL and 10 to 100 ng/mL. The r^2 factors ranged from 0.9940 to 0.9991. However, a 1:1 linear relationship was not indicated between peak height response and concentration (log/log data).

MATERIALS AND METHODS:

Reference Standards: Sodium monofluoroacetate

Stock Solution: 50 mg of sodium monofluoroacetate reference standard was dissolved in 10 mL of water which resulted in a concentration of 5000 µg/mL.

Intermediate Standard Solutions:

Solution A: 100 µL of the stock solution was transferred to a 10 mL flask and diluted to volume with 10% water/acetone. This resulted in a concentration of 50 µg/mL for solution A.

Solution B: 100 µL of the Solution A was transferred to a 10 mL flask and diluted to volume with 10% water/acetone. This resulted in a concentration of 0.5 µg/mL.

Solution C: 1.00 mL of Solution A was transferred to a 10 mL flask and diluted to volume with 10% water/acetone. This resulted in a concentration of 5 µg/mL.

Calibration Standards: Two sets of calibration standards were prepared by adding 100 µL of 10% triethanolamine/acetone, 5.00 mL of acetone, and 100 µL of 5% PFBB/isooctane to each culture tube containing the appropriate aliquot of intermediate standard and mixed.

set one: 2.5 to 10 ng/mL

Aliquot (µL)	Intermediate Standard	Concentration
25	B	2.39 ng/mL
35	B	3.34
50	B	4.76
75	B	7.10

set two: 10 to 100 ng/mL

Aliquot (µL)	Intermediate Standard	Concentration
10	C	9.60 ng/mL
20	C	19.2
40	C	38.2
60	C	57.0
80	C	75.8
100	C	94.3

Tissue Used: Coyote muscle tissue

METHODOLOGY:

One gram of tissue sample is transferred to a 25 mL tube, and 5 mL of 20% water/acetone solution was added and the contents mixed thoroughly. The sample was then sonicated for 10 minutes. After sonification the acetone phase was decanted. The extraction of the tissue sample was repeated twice using 5 mL of 20% water/acetone solution. The three extract phases were combine and the acetone phase evaporated. To the remaining aqueous extraction phase, 5 mL of hexane was added and the solution mixed. The hexane phase was decanted. The hexane wash was repeated one additional time. The aqueous extraction phase was extracted into ethyl acetate by adding 100 µL of 2.4 N HCl to the aqueous solution and mixing then adding 3 mL of ethyl acetate and shaking for 10 minutes. The ethyl acetate phase was transferred to a 12 mL tube and the ethyl acetate extraction repeated three additional times with 3 mL ethyl acetate. The ethyl acetate extraction phases were combined and 100 µL of triethanolamine/acetone added, mixed, and evaporated to dryness. Acetone (10 mL) was

added for reconstitution. Five milliliters of the reconstituted sample was transferred to another 12 mL tube. To the 5.00 mL acetone sample extract solution 100 µL of 10% triethanolamine/acetone and 100 µL of 5% PFBB/isooctane was added, mixed, and heated to 65°C for 30 minutes (See Sample Preparation Flow Chart, page 18 of 29). The extract sample solution was cooled and transferred into a GC sample vial. Repeated 1 µL samples were taken and injected into the GC to determine injection repeatability and chromatographic peak resolution. When system suitability has been demonstrated, the ten calibration standards and samples are injected. The peak height in each chromatogram is recorded.

The relative standard deviation of Compound 1080 is not greater than 3.5% for three consecutive injections of the sample solution from the 50 ppb fortified tissue samples. The resolution between the derivatized Compound 1080 chromatographic peak and the adjacent peak must be greater than 0.9.

Calibration standards from about 2.5 to 100 ng/mL were each injected into the GC. Two plots were constructed of peak response (Compound 1080) verse concentration. A linear regression was then performed on each of the two data sets and regression statistics calculated. In addition, control tissue samples were fortified with Compound 1080 at 50 ppb, 100 ppb, 400 ppb, and 1 ppm and analyzed using the above method and the percent recovery determine.

DATA SUMMARY:

The peak height verse concentration appear to be a linear function over the ranges of 2.5 to 10 ng/mL and 10 to 100 ng/mL. The r^2 factors ranged from 0.9940 to 0.9991. However, a 1:1 linear relationship was not indicated between peak height response and concentration (log/log data).

Replicate fortified tissue samples with Compound 1080 at 50 ppb, 100 ppb, 400 ppb, and 1 ppm levels resulted in percent mean recoveries of 78.1%, 92.4%, 90.5% and 82.9%, respectively. From these data, a limit of quantitation in coyote muscle tissue of 50 ppb was reported. There are difficulties in obtaining repeatable calibration data below the 50 ppb level. In addition, a limit of detection in coyote muscle tissue of ≈25 ppb was reported due to the chromatographic interferences and difficulties in obtaining repeatable calibration data at the 25 ppb level.

COMMENTS:

1. GC data should be confirm by a confirmatory method such as MS to validate the data.

2. Purity of analytical standards and analytical solutions should be furnished. These were not included in the data.

DATA EVALUATION RECORD

STUDY 2

CHEM 075003 SODIUM FLUOROACETATE § 171-4

STUDY ID 41653401

Mishalanie, E.A. and Kimball, B.A. DETERMINATION OF COMPOUND 1080 IN SHEEPSKIN AND WOOL. Sponsored by U.S. Department of Agriculture, Animal and Plant Health Inspection Service, and Science and Technology, Washington, DC; Performed and Submitted by Denver Wildlife Research Center, Denver, CO under Laboratory Project ID Method No. 14A; Study complete 16 March 1990; Received by EPA 3 October 1990.

DIRECT REVIEW TIME = 1.8 day

REVIEWED BY: G. Maske
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SIGNATURE: 6 APR 1994

APPROVED BY: Paul Mastradone, Chief
Review section #1
OPP/EFED/EFGWB

Signature: Paul J Mastradone
Date: 6 APR 1994

CONCLUSIONS:

This is a non-guideline review of a residue method for analyzing Coumpound 1080 in blood stained sheepskin/wool tissue. The analytical methodology is scientifically valid, and is acceptable for determining Compound 1080 at a concentration range of 5.00 to 100 mg concentrations. There are two phases, extraction and analysis, to this analytical method.

For the 2.51 mg, 50.3 mg, 101 mg spiked concentration levels a mean recovery of 85.5 ± 2.2% , 80.0 ± 5.8%, and 76.7 ± 2.5% was reported, respectively. There did appear to be some decrease in the percent recoveries for the higher concentration levels.

A linear response was reported between the fluoroacetic acid/IS peak ratio and Compound 1080 concentration over the range tested. The fluoroacetic acid/IS was found to be directly proportional to the test material concentration. A r^2 of 0.9996 and 0.9998 were reported for the response vs concentration and the log response vs log concentration, respectively. In addition, there was no chromatographic interferences reported for the control tissue samples.

MATERIALS AND METHODS:

Reference Standards: Sodium monofluoroacetate

Stock Solution: 50 mg of sodium monofluoroacetate reference standard was dissolved in 10 mL of water which resulted in a concentration of 5000 µg/mL.

Internal (IS) Solution

200 mg of chloroacetic acid was transferred to a 10 mL flask partially filled with 1.0 N HCl, mixed, and diluted to volume with 1.0 N HCl.

Working Standard Solution:

200 µL of concentration standard solution was transferred to a 10 mL flask which contained 25 µL of IS solution/1.0 N HCl and then diluted to volume with 1.0 N HCl. This resulted in a concentration of 100 µg/mL.

Tissue Used: 4" by 4" Blood stained sheepskin/wool tissue

METHODOLOGY:

Two sets of Compound 1080 calibration standards were prepared ranging in concentration from 4.9 to 200 µg/mL. Each solution was analyzed by GC, and a plot was constructed of the fluoroacetic acid/IS chromatographic peak response ratio vs. Compound 1080 concentration.

After determining the fluoroacetic acid/I chromatographic and Compound 1080 peak response ratio, three representative sheepskin/wool tissue samples were fortified with LPC formulation that did not contain Compound 1080 for controls to determine if there were chromatographic interferences. These samples were analyzed in the same manner as the test samples. Replicate samples of control sheepskin/wool tissue were then fortified at concentrations of 2.51, 50.5, and 101 mg with Compound 1080, and dried prior to extraction.

Prior to extraction the test samples were cut into pieces that would ensure efficient extraction of Compound 1080. The test samples were extracted twice with 1.0 N HCl and the extracts combined. The combined extract was then passed through a SPE column and filtered through a nylon filter. For analyses 2 mL of the combined-cleaned extract was combined with 5 µL of internal IS solution, mixed, and a sample injected into the GC for analyses. After every five injections of test sample or standard, a single injection of 22N H_3PO_4 was inducted into the column to deactivate the injection port and column surfaces, and to desorb weak acids.

DATA SUMMARY:

A linear response was reported between the fluoroacetic acid/IS peak ratio and Compound 1080 concentration over the range tested. The fluoroacetic acid/IS was found to be directly proportional to the test material concentration. A r^2 of 0.9996 and 0.9998 were reported for the response vs concentration and the log response vs log concentration, respectively. In addition, there was no chromatographic interferences reported for the control tissue samples.

For the 2.51 mg, 50.3 mg, 101 mg spiked concentration levels a mean recovery of 85.5 ± 2.2% , 80.0 ± 5.8%, and 76.7 ± 2.5% was reported, respectively. Even though there was some decrease in the percent recoveries for the higher concentration levels, one preparation procedure was written.

COMMENTS:

1. The GC method should be confirmed using a confirmatory method such as MS to validate the data.

2. Purity of analytical standards and analytical solutions should be furnished. These were not included in the data.

XI. METHOD VALIDATION (CONTINUED)

Bias and Repeatability

Three replicate samples of control sheepskin/wool (4" x 4" pieces) were fortified with 2.51, 50.5, and 101 mg Compound 1080. The LPC formulation (10 mg 1080/mL) was used to deliver these quantities of 1080 onto the samples. The 1080-fortified samples were allowed to dry prior to extraction.

Spike Level and % Recovery

Sample #	2.51 mg	50.3 mg	101 mg
1	86.5	73.9	79.5
2	83.0	85.5	74.6
3	87.0	80.6	76.0
Mean	85.5	80.0	76.7
SD	2.2	5.8	2.5
RSD	2.6%	7.2%	3.3%

Since the recoveries at the three levels are comparable, the sample preparation procedure is written so that the extracts from each 4" x 4" sheepskin/wool piece are combined to produce one representative extract.

XII. REFERENCES

Taylor, J.K., Ed.,"Quality Assurance of Chemical Measurements," Lewis Publishers, Inc., Chelsea, Michigan 48118 (1987)

Analytical Chemistry Section Notebook RD1: pages 22 through 35.

SAMPLE PREPARATION FLOW CHART

N SHEEPSKIN/WOOL PIECES

↓ Extract each piece w/1 N HCl

↓ Centrifuge 10 mL of each extract

Discard sheepskin/wool ←

N SHEEPSKIN/WOOL EXTRACTS

↓ Remove equal volumes from each of the N extracts

COMBINED EXTRACT

↓ Condition SPE column

↓ Elute extract sample

Discard SPE column ←

COMBINED EXTRACT (CLEANED UP)

↓ Filter through nylon filter

↓ Remove 2.00 mL

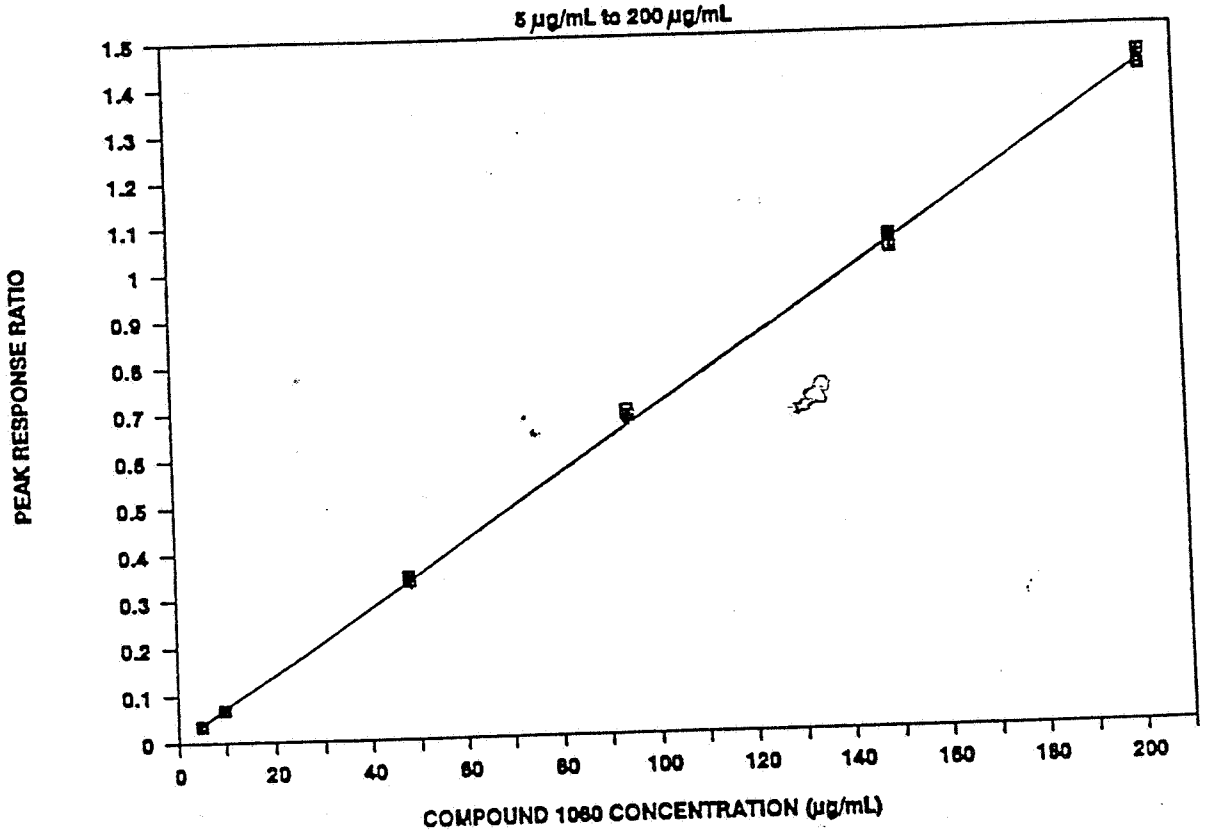
↓ Add 5 µL IS

SAMPLE FOR INJECTION INTO GC

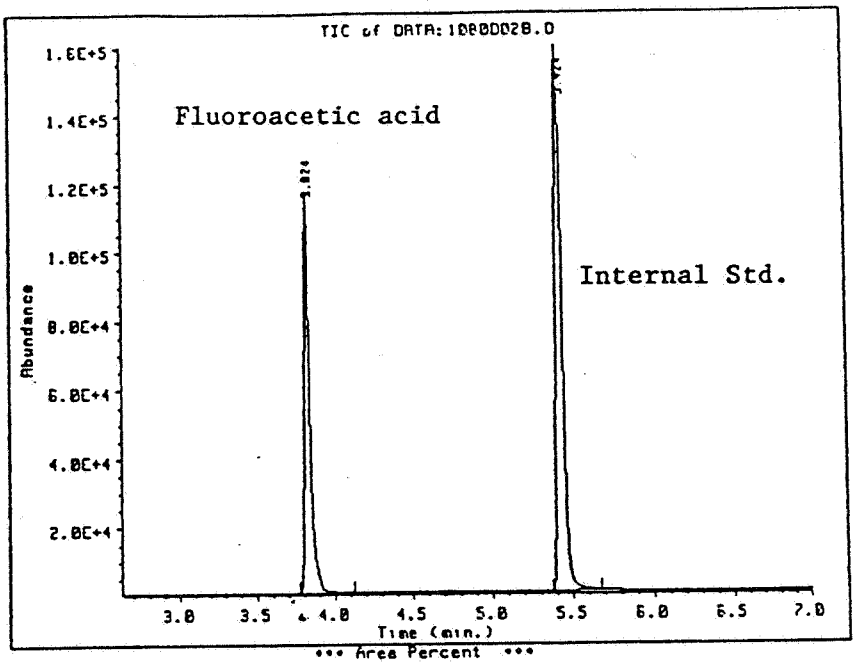
Developed By	Reviewed By	Approved By
Date 3-14-90	Date 3/14/90	Date 3-15-90

14

COMPOUND 1080 RESPONSE LINEARITY



Developed By	Reviewed By	Approved By
Date 3-14-90	Date 3/14/90	Date 3-15-90



Report by Signal

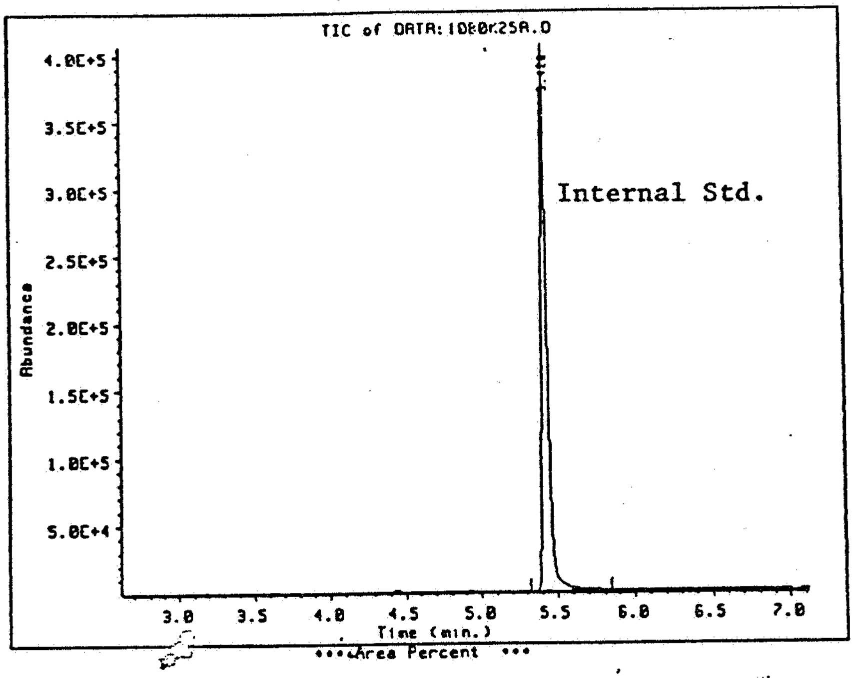
Operator: B. KIMBALL 21 Feb 90 3:53 pm
Method File Name : BKFULL1.M
Sample Info : 94.8 PPM 1080
Misc Info:
Integration File Name : DATA:10800028.I
Method Index : 4 Bottle Number : 2 Repetition Number: 2

Ret Time	Type	Total Ion Area	Height	Area %	Ratio %
3.824	PV	2785607.9075	113328.27477	41.965	72.31
5.424	PV	3852289.6867	159038.07657	58.035	100.00

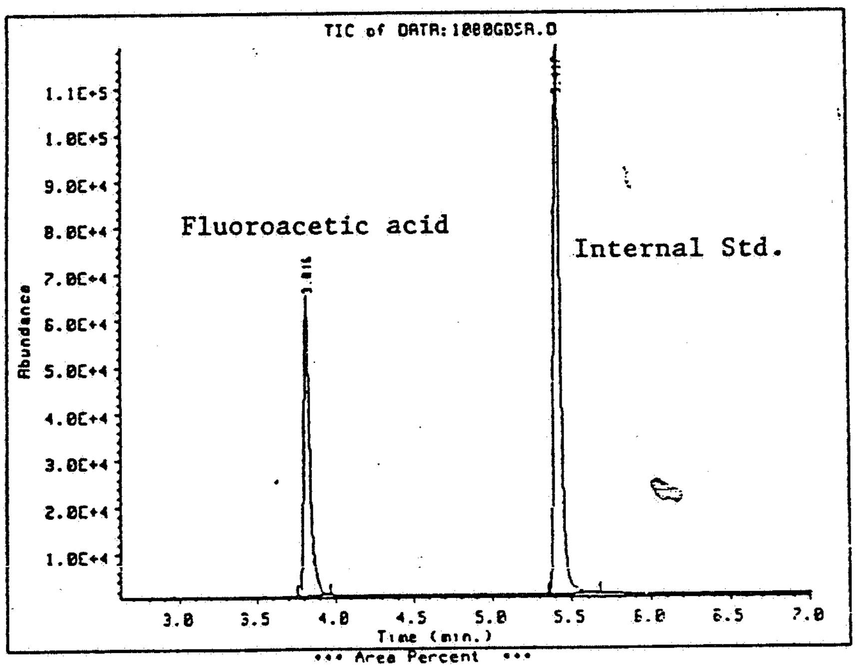
Chromatogram of a 94.8 µg/mL Compound 1080 working standard.

Developed By	Reviewed By	Approved By
Bruce A. Kimball	J. Otis	Elizabeth A. Nicholine
Date 3-14-90	Date 3/14/90	Date 3-15-90

A



B



A) Chromatogram of an extract from a control sheepskin/wool sample.

B) Chromatogram of an extract from a control sheepskin/wool sample fortified with 50 mg of Compound 1080.

Developed By	Reviewed By	Approved By
Date	Date 3/14/90	Date 3-15-90

XI. METHOD VALIDATION

Calibration and Response Linearity

Ten calibration standards were prepared ranging in concentration from about 2.5 ng/mL to 100 ng/mL. Each solution was injected into the GC, and two plots were constructed of 1080 chromatographic peak response vs. concentration. A linear regression was performed on each of the two data sets. The x-y plots are attached, and the regression statistics are given below:

Set I: Response vs Concentration, 2.5 ng/mL to 10 ng/mL

r^2 = 0.9940

slope = 16.56 y-intercept = -11.20

Set II: Response vs Concentration, 10 ng/mL to 100 ng/mL

r^2 = 0.9983

slope = 19.55 y-intercept = -40.88

Set I: Log (Response) vs Log (Concentration), 2.5 ng/mL to 10 ng/mL

r^2 = 0.9986 slope = 1.177

Set II: Log (Response) vs Log (Concentration), 10 ng/mL to 100 ng/mL

r^2 = 0.9991 slope = 1.097

Result: Peak height response is a linear function of concentration over the ranges of interest. However, a 1:1 linear relationship does not exist between peak height response and concentration, as indicated by the slopes of the log/log data. Single point calibrations are not valid.

Selectivity

Representative control tissue samples (all components except the 1080) were subjected to the procedures in this method (control tissue - coyote muscle).

Result: A small chromatographic interference was observed at the 1080 retention time. This interference accounted for about 5% of the 1080 peak response that would be observed for a tissue sample containing 50 ppb 1080.

Chromatograms of calibration standards, reagent blanks, extracts from control tissues, and extracts from control tissues fortified with 1080 are attached.

Developed By	Reviewed By	Approved By
[signature]	[signature]	[signature]
Date 2/12/90	Date 2/13/90	Date 2-13-90

XI. METHOD VALIDATION (CONTINUED)

Method Bias and Reproducibility

Replicate samples of control tissue were fortified with Compound 1080 at the 50 ppb, 100 ppb, 400 ppb, and 1 ppm levels. The % recovery observed for each level is listed below.

	Spike Level and % Recovery			
Sample #	50 ppb	100 ppb	400 ppb	1 ppm
1	72.7	95.9	80.6	81.9
2	76.5	88.9	86.2	82.1
3	83.8	96.7	91.6	85.7
4	76.3	90.6	87.6	78.7
5	72.2	88.9	92.3	88.0
6	79.6	89.1	88.8	83.5
7	85.8	90.7	97.7	77.5
8		98.6	99.5	86.2
Mean	78.1	92.4	90.5	82.9
SD	5.2	4.0	6.2	3.7
RSD	6.7%	4.3%	6.8%	4.4%

Limit of Quantitation (LOQ) and Limit of Detection (LOD)

The LOQ for Compound 1080 in coyote muscle tissue is 50 ppb. Below this level, there are difficulties in obtaining repeatable calibration data.

The LOD for Compound 1080 in coyote muscle tissue is about 25 ppb. This limit was set because of chromatographic interferences and difficulties in obtaining repeatable calibration data at this concentration.

XII. REFERENCES

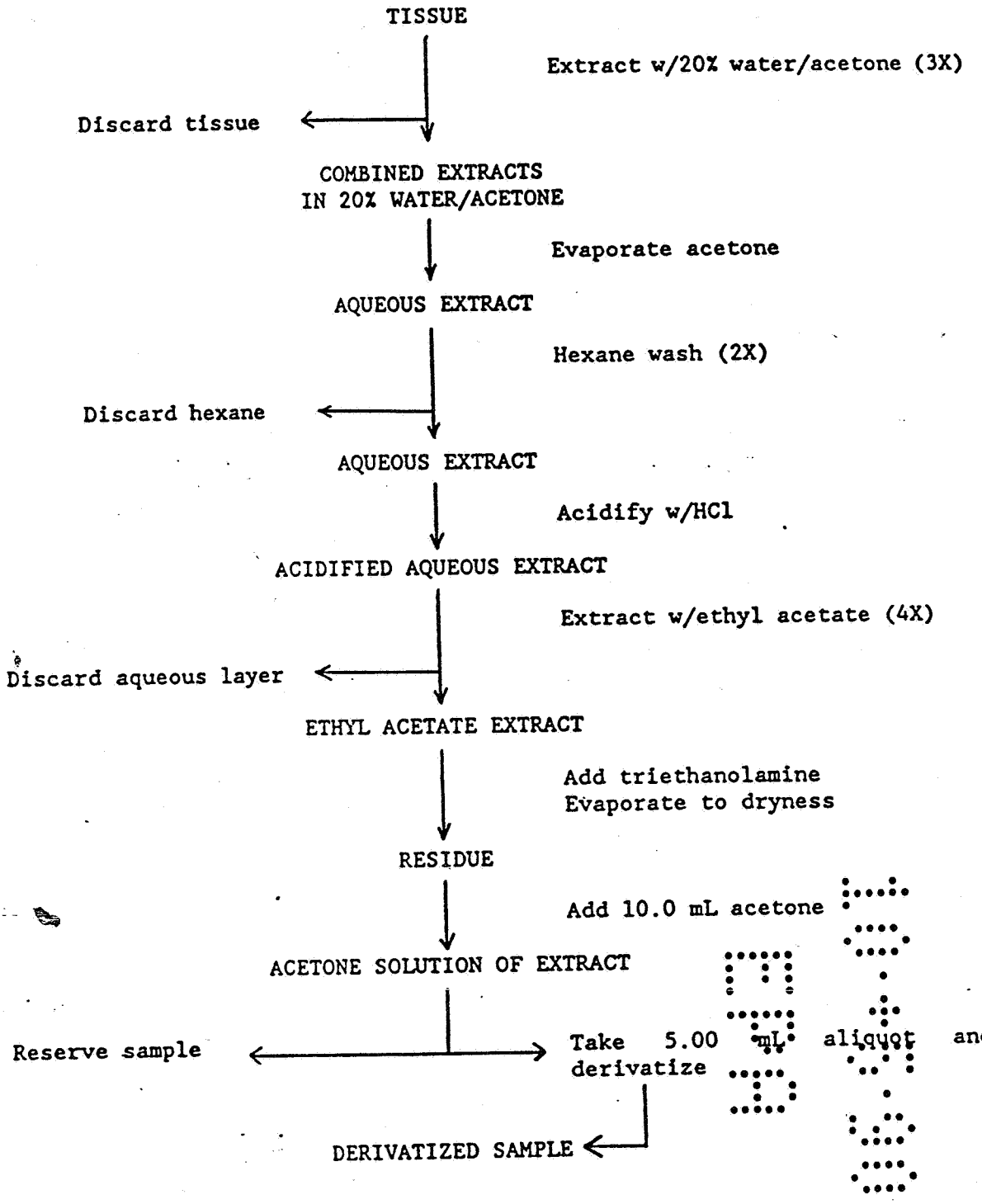
Okuno, I., Meeker, D.L., Felton, R.R. "Modified Gas-Liquid Chromatographic Method for Determination of Compound 1080 (Sodium Fluoroacetate)", J. Assoc. Off. Anal. Chem. 65, 1982, 1102-1105.

Taylor, J.K., Ed.,"Quality Assurance of Chemical Measurements," Lewis Publishers, Inc., Chelsea, Michigan 48118 (1987)

Analytical Chemistry Section Notebook IO-3R: pages 8 through 23.

Developed By	Reviewed By	Approved By
L. Okuno	Bruce A. Kimball	Elizabeth A. Middleton
Date 2/12/90	Date 2/13/90	Date 2-13-90

SAMPLE PREPARATION FLOW CHART



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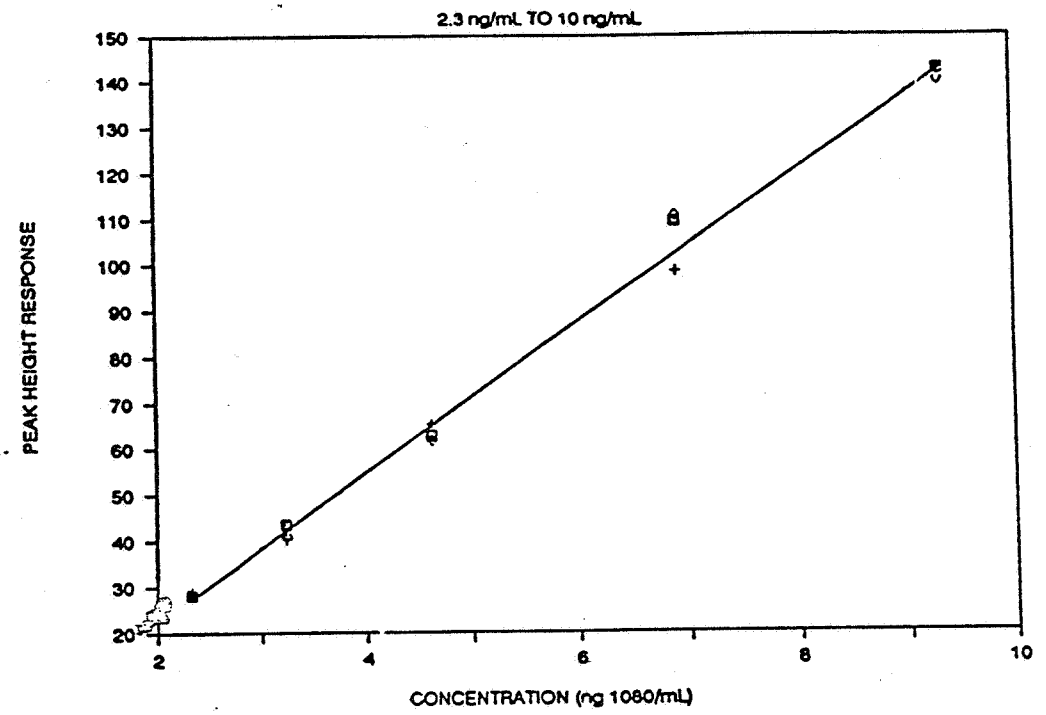
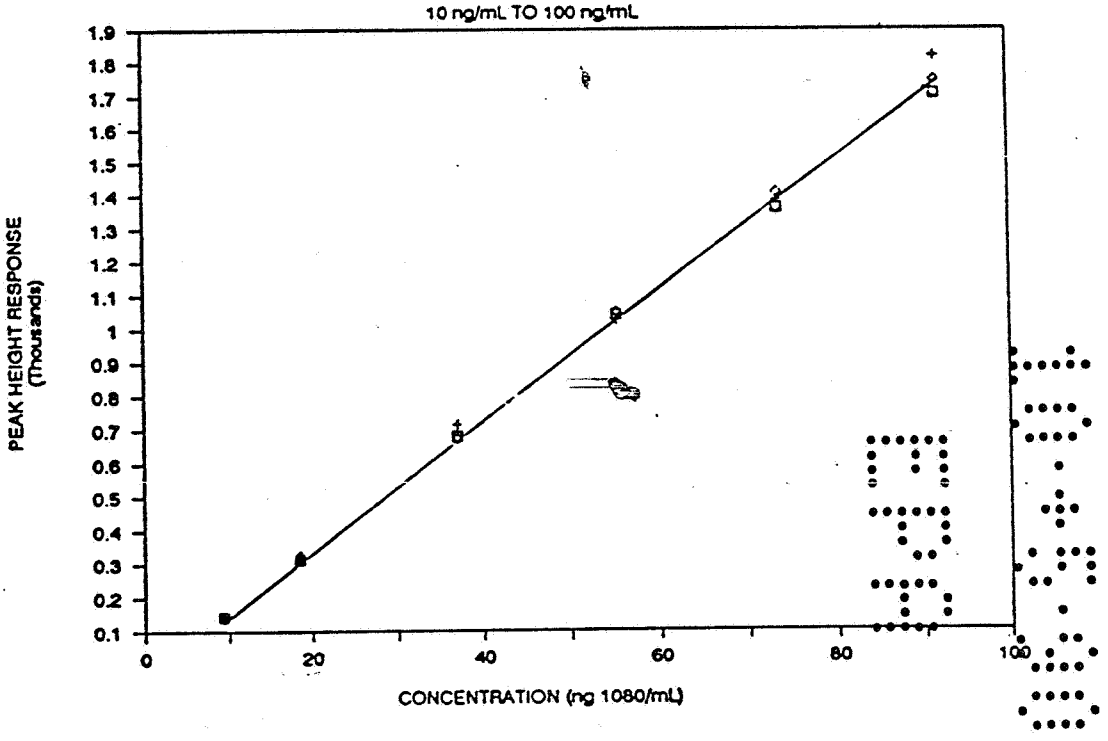
COMPOUND 1080 RESPONSE LINEARITY





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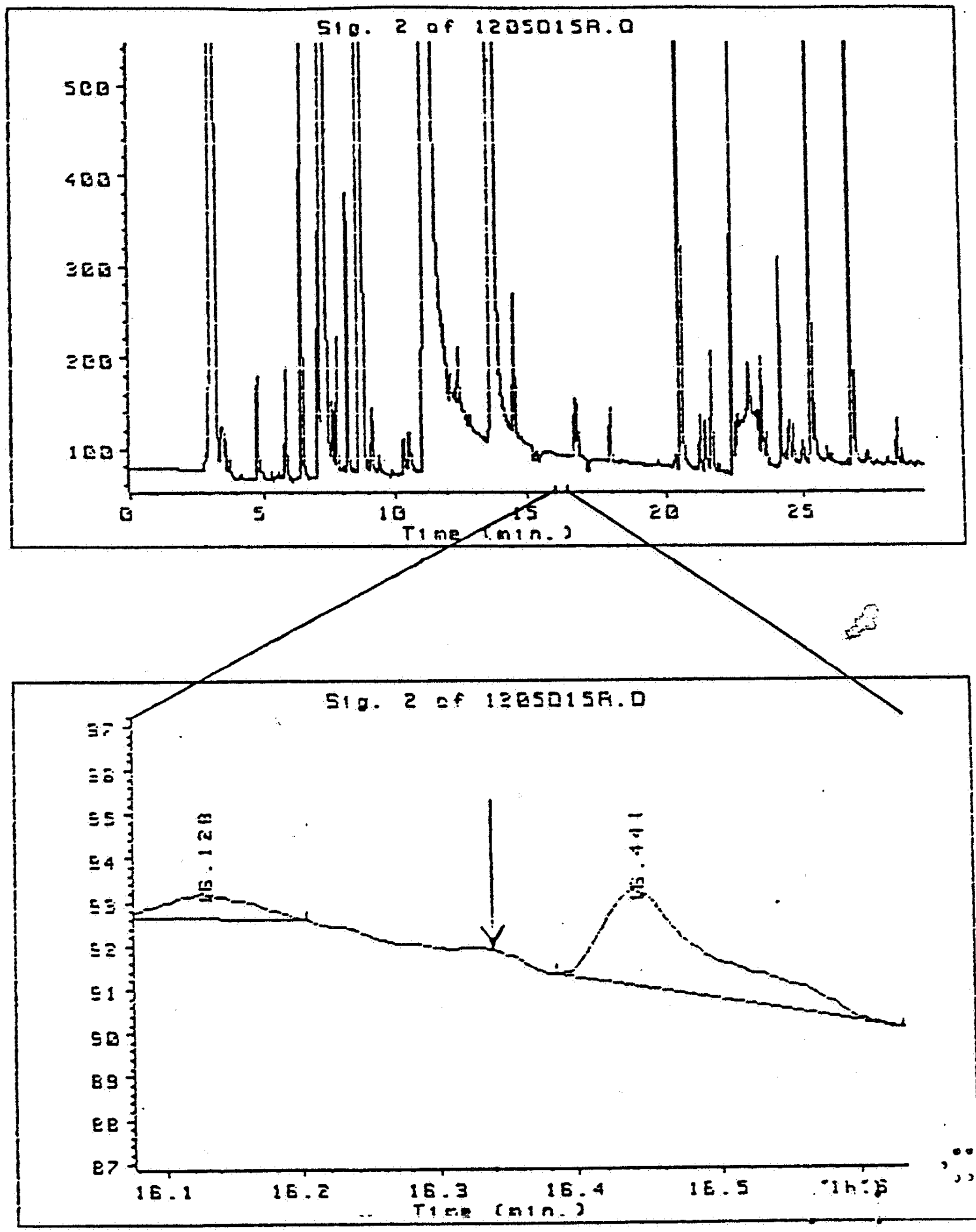

Chromatogram of a reagent blank. All sample preparation procedures were performed. The retention time for derivatized 1080 is indicated by an arrow.

Developed By	Reviewed By	Approved By
[signature]	[signature]	Elizabeth A. Mishalanie
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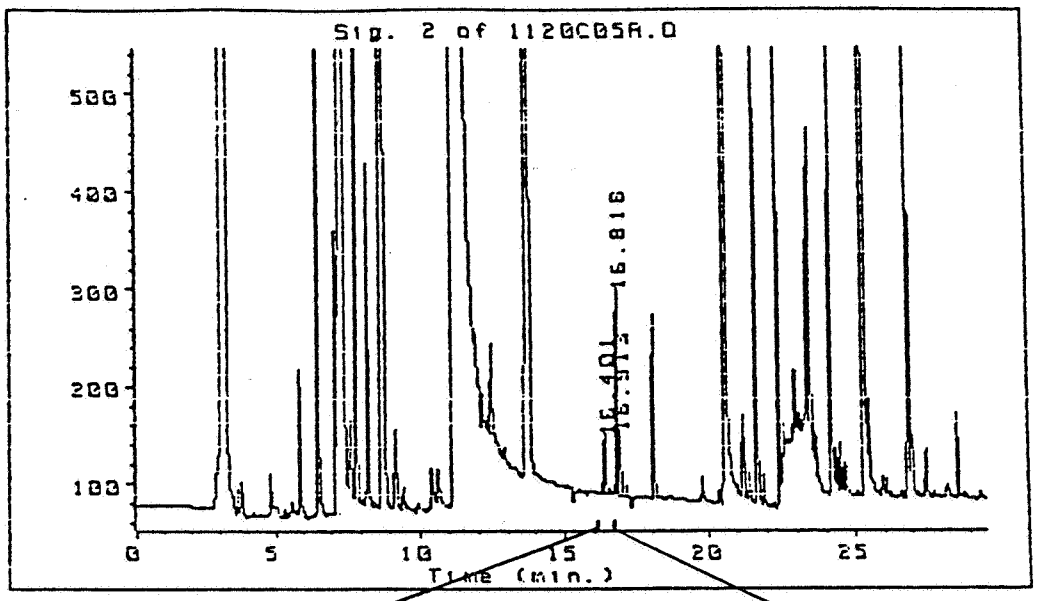

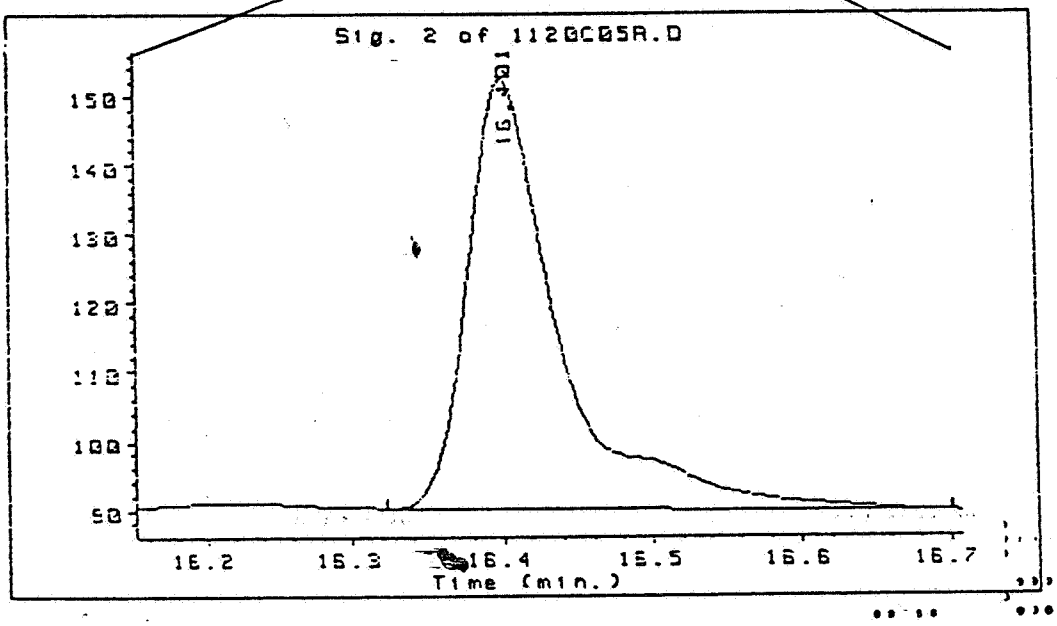

Chromatogram of a 4.62 ng/mL calibration standard.

Developed By	Reviewed By	Approved By
L. Ahrens	Bruce A. Kilbride	Elizabeth A. Michaelis
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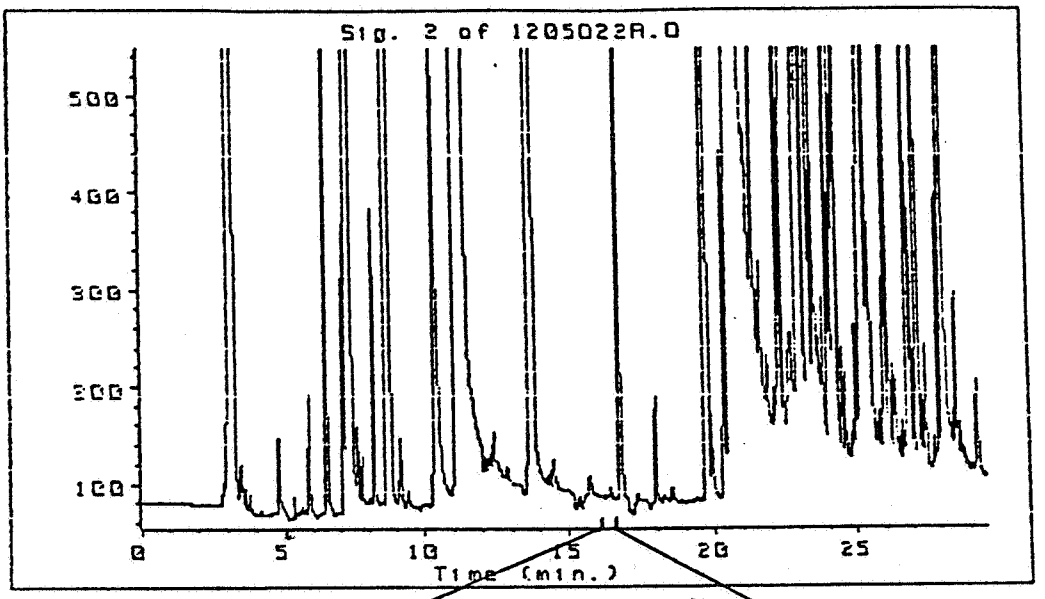

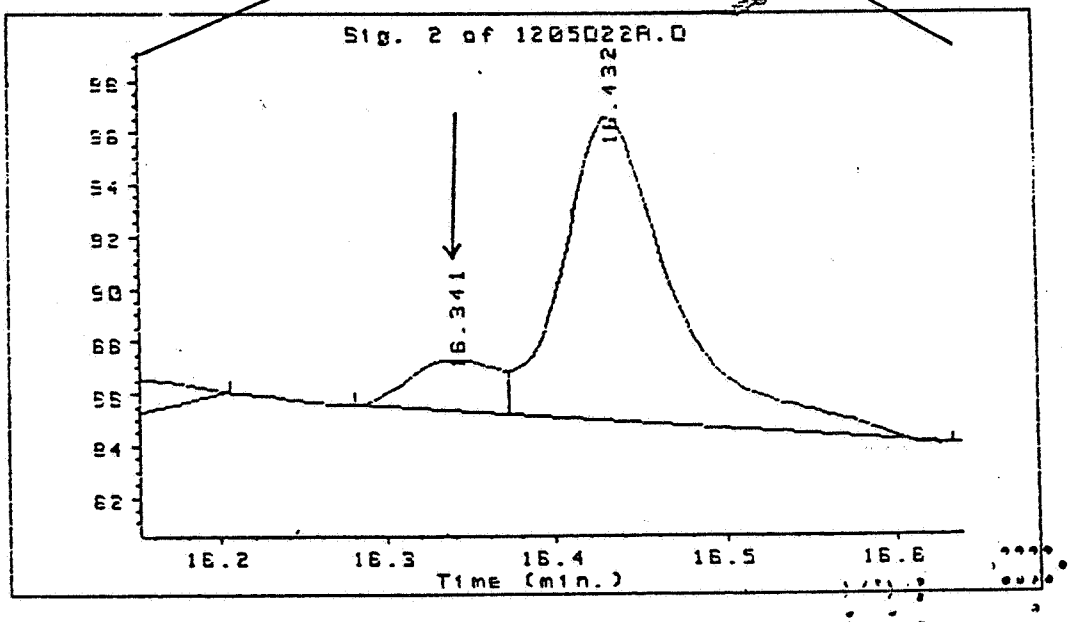

Chromatogram of an extract from a coyote muscle control sample. The retention time for derivatized 1080 is indicated by an arrow. The interference observed at this retention time accounts for about 5% of the 1080 peak height response that would be observed for a tissue sample containing 50 ppb 1080.

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		Elizabeth A. Nicholaisen
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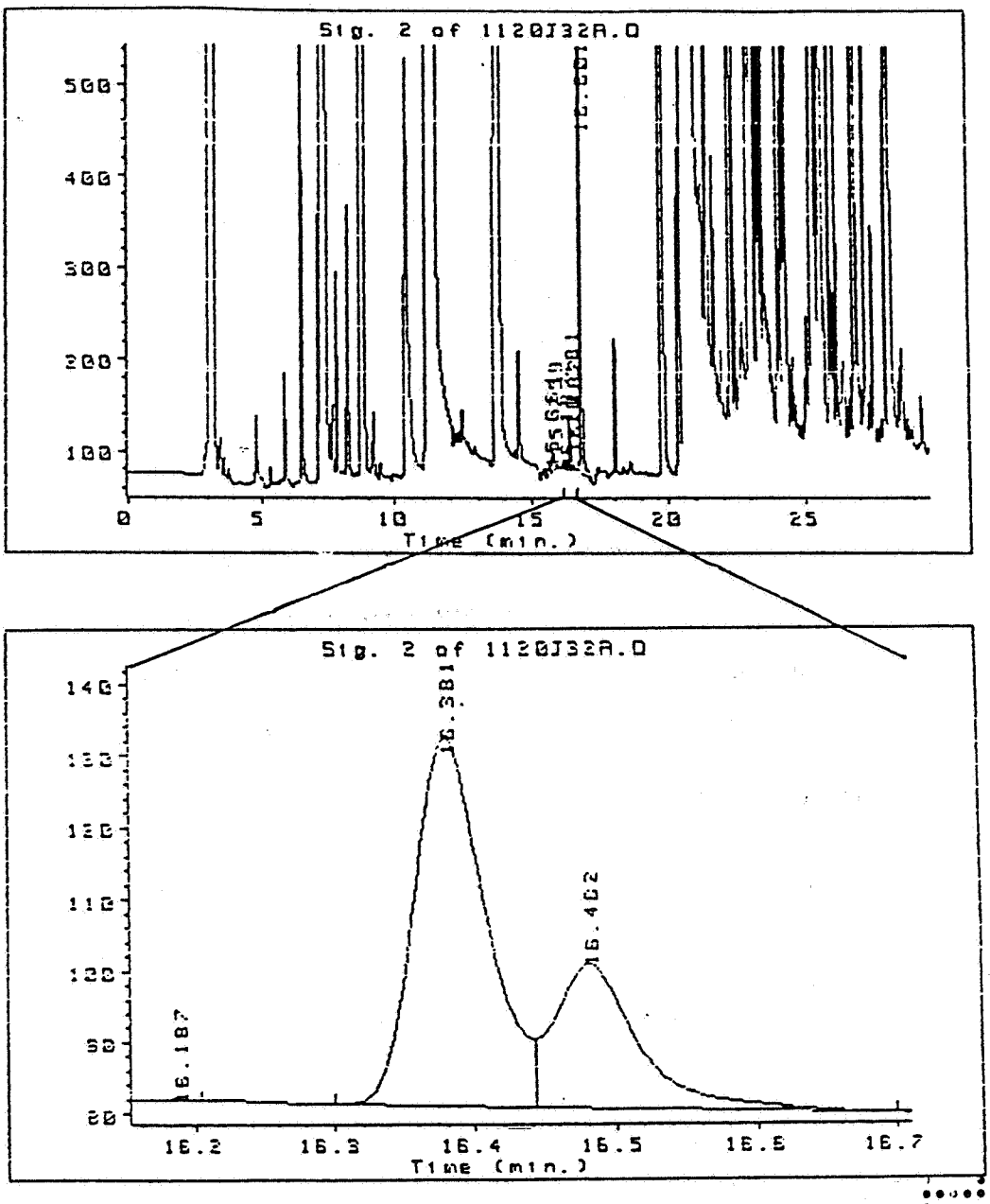
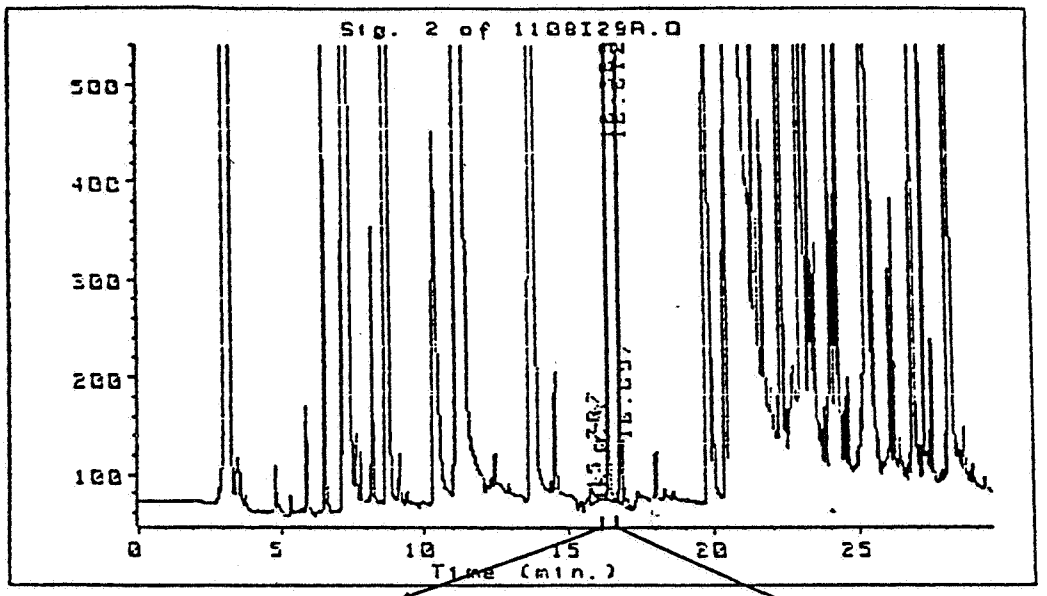
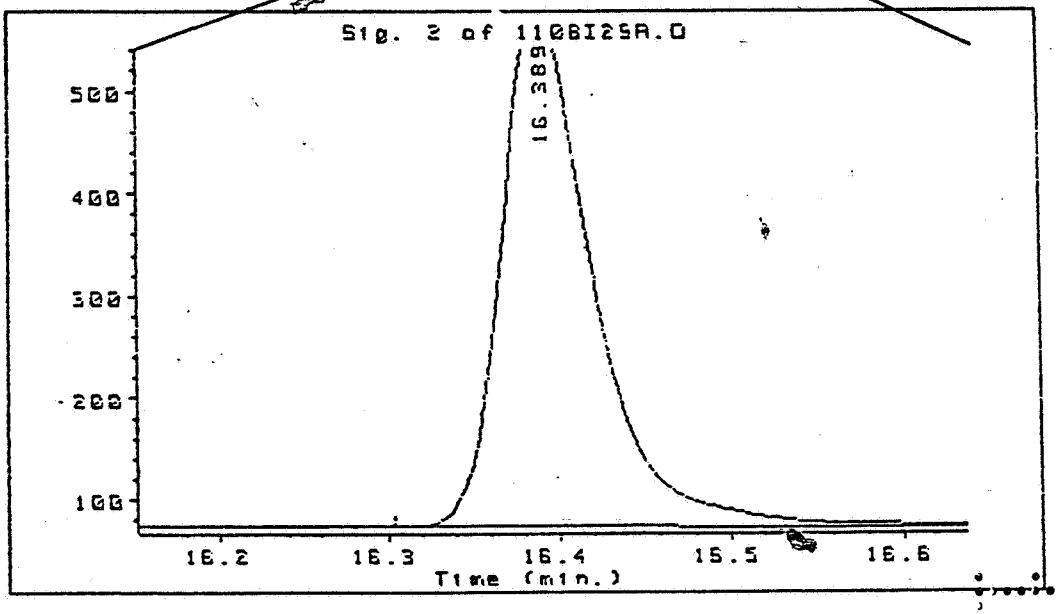


Chromatogram of an extract from a coyote muscle control sample fortified at the 50 ppb Compound 1080 level.

Developed By	Reviewed By	Approved By
L Ofsus	Brian A. Mitchell	Elizabeth A. Michalenes
Date 2/12/90	Date 2/13/90	Date 2-13-90





Chromatogram of an extract from a coyote muscle control sample fortified at the 400 ppb Compound 1080 level.

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