3M Environmental Laboratory

Final Report- Analytical Study

Single-Dose Intravenous Pharmacokinetic Study of T-6067 in Rabbits

In-Vivo Study Reference Number: HWI#6329-151

Study Number: AMDT-120694.1
Test Substance: L-13492 (T-6067)

Name and Address of Sponsor: 3M SCD Division
367 Grove Street
St. Paul, MN 55106

Name and Address of Testing Facility:
3M Environmental Technology & Services
935 Bush Avenue
St. Paul, MN 55106

Method Numbers and Revisions:

AMDT-M-1-0,	Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver
AMDT-M-2-0,	Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
AMDT-M-4-0,	Extraction of Fluorochemicals from Rabbit Liver
AMDT-M-5-0,	Analysis of Rabbit Liver Extract for Fluorochemicals Using Electrospray Mass Spectrometry
AMDT-M-8-0,	Analysis of Fluoride Using the Skalar Segmented Flow Analyzer with Ion Selective Electrode
AMDT-M-14-0,	Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Serum

Initiation Date: See attached protocol

Author: James D. Johnson

Approved By:

James D. Johnson
Study Director

11/28/95
Completion Date

1.0 SUMMARY

Tissue and serum samples were analyzed for total organic fluorine after single intravenous doses to individual rabbits in order to collect information in support of a dermal absorption study.

Serum samples were analyzed at 2, 4, 6, 8,12, 24, and 48 hours post dose. There is a rapid decrease in serum level of total organic fluorine with time. The biological half-life is on the order of 4 hours.

There is very little total organic fluorine present in liver or serum at 48 hours post intravenous dose of L-13492 (T-6067). The pharmacokinetic study suggests that interpretation of dermal absorption of L-13492 (to be done and reported in a separate study) will be more difficult. It is unlikely that the serum levels at 48 hours and the liver samples at 28 days will contain any detectable organic fluorine whatever the extent of dermal absorption.

2.0 INTRODUCTION

This study was designed to provide information as to whether L-13492 when administered as a single intravenous dose results in total organic fluoride increase in whole liver at 48 hours post dose or in serum at 2, 4, 8, 12, 24, or 48 hours. This information is to be acquired in order to interpret a subsequent dermal absorption study with the same compound (HWI#6329-152, AMDT-011095.1). The dermal absorption study will be reported separately.

3.0 TEST MATERIALS

3.1 Test, Control, and Reference Substances and Matrices

3.1.1 Analytical Reference Substance: FC-95, lot 161 or 171. They are equivalent.

3.1.2 Analytical Reference Matrix: Bovine liver and bovine serum

3.1.3 Analytical Control Substance: None

3.1.4 Analytical Control Matrix: Bovine liver and bovine serum

3.2 Source of Materials: 3M ICP/PCP Division for FC-95, bovine liver from grocery store, bovine serum from Sigma Chemical Company

3.3. Purity and Strength of Reference Substance: Responsibility of Sponsor.

3.4 Stability of Reference Substance: To be determined by Sponsor.

3.5 Storage Conditions for Test Materials: Room temperature for FC-95. For biological samples the storage is -20±10° C.

3.6 Disposition of Test and Control Substances: Biological tissues and fluids will be retained per GLP Regulation for the time period required for studies longer than 28 days. This study is in parallel with a 28 day absorption study, so all tissues will be retained.

4.0 EXPERIMENTAL - Overview

Serum and tissues from animals dosed as described (HWI#6329-151) were available for analysis by combustion with subsequent analysis by selective ion electrode or by electrospray mass spectrometry. The tissue and serum samples were analyzed for total organic fluorine to collect information in order to support a dermal absorption study. The extent of analysis will be at the discretion of the Study Director. The samples arise from a previous study (HWI#6329-151) in which single rabbits were dosed intravenously with L-13492 at doses ranging 4 to 24 mg/kg. (See attached protocol for dosing, tissue collection, etc.) In the final report from HWI on this study the dosing is from 4 to 24 mg/kg. In the original protocol, the dosing is to 160 mg/kg. A rabbit dosed at 40 mg/kg died 5 minutes after injection. No tissues were saved.

5.0 EXPERIMENTAL - METHODS

5.1 AMDT-M-1-0, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver

5.2 AMDT-M-2-0, Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer

5.3 AMDT-M-4-0, Extraction of Fluorochemicals from Rabbit Liver

5.4 AMDT-M-5-0, Analysis of Rabbit Liver Extract for Fluorochemicals Using Electrospray Mass Spectrometry

5.5 AMDT-M-8-0, Analysis of Fluoride Using the Skalar Segmented Flow Analyzer with Ion Selective Electrode

5.6 AMDT-M-14-0, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Serum

6.0 DATA ANALYSIS

The data are attached for total organic fluorine analysis of liver at 48 hours post dose. The total organic fluorine for whole liver for control animals, 4 mg/kg, 16 mg/kg, and 24 mg/kg intravenous doses of a 45% solution are 20 ug, 43 ug, 66 ug, and 54 ug. Each data point represents one animal. An animal dosed with 40 mg/kg died 5 minutes after injection.

The data are attached for serum concentrations of total organic fluorine at 2, 4, 6, 8, 12, 24, and 48 hours post dose. What is immediately apparent is that the concentrations decrease rapidly with time and are non-detectable at 48 hours. The biological half-life appears to be about 4 hours (see pages 84-86).

It appears likely that this salt of perfluorooctanoate is rapidly eliminated from female rabbits. It is not known if there is a sex difference in the elimination of the perfluorooctanoate in rabbits as is observed with rats.

Other data was collected using electrospray mass spectrometry and Skalar segmented flow analyzer with ion selective electrode (see appendices). This data, although supportive, in the opinion of the Study Director is not required to reach the conclusion stated here and therefore is not discussed in detail.

6.1 Circumstances that May Affect the Quality of the Data: The problem with this analysis is that there is not a good biological marker and thus the pharmacokinetic study does not provide a good avenue for the interpretation of a dermal absorption study

7.0 CONCLUSION

There is very little total organic fluorine present in liver at 48 hours post intravenous dose of L-13492. The pharmacokinetic study suggests that interpretation of dermal absorption of L-13492 (to be done and reported in a separate study) will be very difficult.

8.0 MAINTENANCE OF RAW DATA AND RECORDS

8.1 Raw Data and Data: Raw data, approved protocol, approved final report, appropriate specimens, and electronic data will be maintained in the AMDT archives.

9.0 APPENDICES

9.1 Protocol and Amendments

9.1.1 Protocol and Final Report: HWI#6329-151 "Single-Dose Intravenous Pharmacokinetic Study of T-6067 in Rabbits" (Protocol type TP8084.PK for dosing of animals, tissue collection, etc.)

9.1.2 Analytical protocol AMDT-120694.1

9.2 Signed Reports from Individual Scientists: None

9.3 Quality Assurance Unit Statement: See attached

9.4 Key Personnel Involved in the Study: See attached

9.5 Materials and Equipment: See methods

9.6 Solutions, Reagents, and Standards: See methods

9.7 Sample Preparation: See methods

9.8 Quality Control Practices: See methods

9.9 Test Methods: See Protocol AMDT-120694.1

9.10 Instrument Settings: See methods

9.11 Data: See attached.

9.11.1 Summary and raw data; ug F^- in whole liver as determined by thermal extraction followed by analysis using Orion ion analyzer.

9.11.2 Summary and raw data; ppm F^- in serum as determined by thermal extraction followed by analysis using Orion ion analyzer.

9.11.3 Summary and raw data; ppm F^- in serum as determined by thermal extraction followed by analysis using Skalar segmented flow analyzer with ion selective electrode.

9.11.4 Summary and raw data; analysis of liver extracts using electrospray mass spectrometry.

9.1.1 Protocol and Final Report: HWI#6329-151 "Single-Dose Intravenous Pharmacokinetic Study of T-6067 in Rabbits" (Protocol type TP8084.PK for dosing of animals, tissue collection, etc.)

HAZLETON
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Sponsor:

3M
St. Paul, Minnesota

FINAL REPORT

Study Title:

Single-Dose Intravenous Pharmacokinetic Study of T-6067 in Rabbits

Author:

Steven M. Glaza

Study Completion Date:

February 1, 1995

Performing Laboratory:

Hazleton Wisconsin, Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Project Identification:

HWI 6329-151

Phone 608 241-4471 Fax 608 241-7227
EXPRESS-MAIL DELIVERY 3301 KINSMAN BLVD MADISON, WI 53704

QUALITY ASSURANCE STATEMENT HWI 6329-151

This report has been reviewed by the Quality Assurance Unit of Hazleton Wisconsin, Inc., in accordance with the Food and Drug Administration (FDA) Good Laboratory Practice Regulations, 21 CFR 58.35 (b) (6) (7). The following inspections were conducted and findings reported to the Study Director and management. Written status reports of inspections and findings are issued to Hazleton management monthly according to standard operating procedures.

Inspection Dates From	To	Phase	Date Reported to Study Director	Date to Management
11/27/94	11/27/94	Protocol Review	11/27/94	12/10/94
12/09/94	12/09/94	Sample Collection	12/09/94	01/10/95
12/28/94	12/28/94	Protocol Amendment	12/28/94	01/10/95
01/17/95	01/19/95	Data/Report Review	01/19/95	02/10/95
01/30/95	01/30/95	Report Rereview	01/30/95	02/10/95

Cecilia M. Danner
Representative, Quality Assurance Unit

2/1/95
Date

STUDY IDENTIFICATION

Single-Dose Intravenous Pharmacokinetic Study of T-6067 in Rabbits

Test Material	T-6067
Sponsor	3M Toxicology Services 220-2E-02 3M Center St. Paul, MN 55144
Sponsor's Representative	John L. Butenhoff, PhD 3M Toxicology Services 220-2E-02 3M Center St. Paul, MN 55144 (612) 733-1962
Study Director	Steven M. Glaza Hazleton Wisconsin, Inc. P.O. Box 7545 Madison, WI 53707-7545 (608) 241-7292
Study Location	Hazleton Wisconsin, Inc. Building No. 3 3802 Packers Avenue Madison, WI 53704
Study Timetable	
Experimental Start Date	December 9, 1994
Experimental Termination Date	December 11, 1994

KEY PERSONNEL

Acute Toxicology

Steven M. Glaza
Study Director
Manager

Francis (Bud) W. McDonald
Study Coordinator

Patricia Padgham
In-life Supervisor

Rose M. Bridge
Report Supervisor

Quality Assurance

Sherry R. W. Petsel
Manager

Laboratory Animal Medicine

Cindy J. Cary, DVM
Diplomate, ACLAM
Supervisor

Anatomical Pathology

Thomas E. Palmer, PhD
Anatomical Pathologist

Jack Serfort/
Deborah L. Pirkel
Supervisors
Necropsy

Anne Mosher
Supervisor
Pathology Data

CONTENTS

SUMMARY

This study was done to assess the level of systemic exposure of T-6067 when administered by intravenous injection to rabbits.

Female Hra:(NZW)SPF rabbits were assigned at random to five groups (one/group). On Day 0, the animals received a single intravenous injection of the vehicle (sterile water for injection) or 4, 16, 24, or 40 mg of T-6067/kg of body weight at a dose volume of 0.5 mL/kg.

Clinical observations were conducted at approximately 0.5, 2, 4, 24, and 48 hours after intravenous injection. Body weights were determined just before test material administration (Day 0). A blood sample (approximately 4 mL) was collected from an auricular artery or marginal ear vein of the surviving animals at 2-, 4-, 6-, 8-, 12-, 24-hours post-injection. In addition, at the time of experimental termination (48-hours post-injection), approximately 20 mL of blood was obtained from each surviving animal. All samples were centrifuged, separated into serum and cellular fractions, and sent to the Sponsor. The one animal (40 mg/kg) found dead during the study was necropsied but tissues were not saved. Animals surviving to the end of the 48-hour post-injection period were anesthetized with sodium pentobarbital, bled via the posterior vena cava, and exsanguinated. An abbreviated gross necropsy examination was not done, however, tissues were collected. The whole liver, bile, and both kidneys from each animal surviving to termination were collected and sent frozen to the Sponsor after termination of the in-life phase.

The animals treated at 0, 4, 16, and 24 mg/kg appeared normal throughout the study. The animal treated at the 40 mg/kg dose level died within 5 minutes of dosing. At necropsy, the lungs in this animal were diffusely dark red. This change was attributed to acute death and postmortem change.

OBJECTIVE

The objective of this study was to assess the level of systemic exposure to the test material, T-6067, when administered as a single intravenous injection to rabbits.

REGULATORY COMPLIANCE

This study was conducted in accordance with the U.S. Food and Drug Administration's Good Laboratory Practice Regulations for Nonclinical Laboratory Studies, 21 CFR 58, with the exception that analysis of the test mixtures for concentration, homogeneity/solubility, and stability was not conducted. All procedures used in this study were in compliance with the Animal Welfare Act Regulations. In the opinion of the Sponsor and study director, the study did not unnecessarily duplicate any previous work.

TEST AND CONTROL MATERIALS

Identification

The test material was identified as T-6067 and described as a clear, colorless liquid. The control material was Sterile Water for Injection, USP (Abbott Laboratories, Lot No. 86-748-DM-02; Exp. March 1, 1996), and was described as a clear, colorless liquid.

Purity and Stability

The Sponsor assumes responsibility for test material purity and stability determinations (including under test conditions). A sample of the test material/vehicle mixtures for concentration, solubility, homogeneity, and stability analyses was not taken before administration as this was not requested by the Sponsor. The purity and stability of the USP grade control material were considered to be adequate for the purposes of this study.

Storage and Retention

The test material was stored at room temperature. The control material was stored refrigerated. Any unused test material was returned to the Sponsor after completion of all testing according to Hazleton Wisconsin (HWI) Standard Operating Procedure (SOP). Any remaining vehicle may be used for other testing and will not be discarded after issuance of the final report.

Safety Precautions

The test and control material handling procedures were according to HWI SOPs and policies.

TEST SYSTEM

Test Animal

Adult albino rabbits of the Hra:(NZW)SPF strain were received from HRP, Inc., Kalamazoo, Michigan on November 16, 1994 and maintained at the Hazleton Wisconsin facility at 3802 Packers Avenue, Madison, Wisconsin.

Housing

After receipt, the animals were acclimated for a period of at least 7 days. During acclimation and throughout the study, the animals were individually housed in screen-bottom stainless steel cages in temperature- and humidity-controlled quarters. Environmental controls for the animal room were set to maintain a temperature of 19° to 23°C, a relative humidity of 50% ±20%, and a 12-hour light/12-hour dark lighting cycle. In cases where variations from the required temperature and humidity conditions existed, they were documented and considered to have had no adverse effect on the study outcome. Animal husbandry and housing at HWI complied with standards outlined in the "Guide for the Care and Use of Laboratory Animals".[1]

Animal Diet

The animals were provided access to water *ad libitum* and a measured amount of Laboratory Rabbit Diet HF #5326, PMI Feeds, Inc. The feed is routinely analyzed by the manufacturer for nutritional components and environmental contaminants. Samples of the water are periodically analyzed by HWI. There were no known contaminants in the feed or water at levels that would have interfered with or affected the results of the study.

Selection of Test Animals

The animals were identified by animal number and corresponding ear tag and were selected at random based on health and body weight requirements.

Study Design

Female animals weighing from 2,511 to 2,781 g at initiation of treatment were placed into the following study groups:

Group	Treatment	Dose Level (mg T-6067/kg)	Dose Volume (mL/kg)	Number of Animals
1 (Control)	*	0	0.5	1
2	T-6067	4	0.5	1
3	T-6067	16	0.5	1
4	T-6067	40	0.5	1
5	T-6067	24	0.5	1

* Sterile Water for Injection, USP.

Justification for Species Selection

Historically, the New Zealand White albino rabbit has been the animal of choice because of the large amount of background information on this species.

PROCEDURES

Dose Preparation and Administration

The test material was diluted with Sterile Water for Injection to achieve a specific concentration for each dose level. An individual dose of each respective test solution or control was calculated for each animal based on its body weight on the day of treatment. The respective test solution was administered by intravenous injection into a marginal ear vein. The dose was given as a slow push (approximately 30 to 60 seconds in duration). The prepared test solutions were stored at room temperature until administered. After administration, any remaining test solutions were discarded.

Reason for Route of Administration

Intravenous injection is an acceptable route to assess systemic exposure.

Observations of Animals

Clinical observations were conducted at approximately 0.5, 2, 4, 24 and 48 hours after intravenous injection.

Body weights were determined just before test material administration (Day 0).

Sample Collection

Blood samples (approximately 4 mL) were collected from either ear via the catheterization of the auricular artery or from the marginal ear vein of all surviving animals at 2, 4, 6, 8, 12, and 24 hours post-injection. At the time of necropsy (approximately 48-hours post-injection) approximately 20 mL of blood was obtained from the posterior vena cava of each surviving animal. All samples were stored at room temperature until centrifuged and separated into serum and cellular fractions. The blood samples were then stored in a freezer set to maintain a temperature of -20°C ±10°C until shipped to the Sponsor.

Pathology

The animal found dead immediately after dosing (40 mg/kg) was subjected to an abbreviated gross necropsy examination and any abnormalities were recorded. No tissues were collected from this animal. At termination of the experimental phase, surviving animals were anesthetized with sodium pentobarbital and exsanguinated without necropsy however tissues were collected. The whole liver, bile, and both kidneys from each animal surviving to termination were collected and immediately placed on dry ice, then frozen by placing in a freezer set to maintain a temperature of -20°C ±10°C. After tissue/bile collection, the animals were discarded.

Shipment of Tissues

After completion of the in-life phase the blood samples, livers, bile, and kidneys were sent frozen (on dry ice) to the Sponsor (James D. Johnson, 3M E.E. & P.C., Bldg. 2-3E-09, 935 Bush Avenue, St. Paul, MN, 55106). The Sponsor is responsible for the retention and disposition of the samples. HWI does not accept any responsibility for the analysis of the samples collected in this study nor are these results presented in this report.

Statistical Analyses

No statistical analyses were required by the protocol.

Location of Raw Data, Records, and Final Report

The raw data, records, and an original signed copy of the final report will be retained in the archives of HWI in accordance with HWI SOP.

RESULTS

Body Weights

Individual body weights are in Table 1.

Clinical Observations

Individual clinical signs are in Table 2. The animals treated at 0, 4, 16, and 24 mg/kg appeared normal throughout the study. The animal treated at 40 mg/kg died within 5 minutes of dosing.

Pathology

The animals treated at 0, 4, 16, and 24 mg/kg survived to termination of the experimental phase and were not examined grossly when sacrificed. The animal treated at 40 mg/kg died on the day of treatment and was necropsied. The lungs in this animal were diffusely dark red. This change was attributed to acute death and postmortem change.

DISCUSSION

The level of systemic exposure of T-6067 was evaluated in female albino rabbits when administered as a single intravenous injection at levels of 0, 4, 16, 24, and 40 mg/kg. The animals treated at 0, 4, 16, and 24 mg/kg appeared normal throughout the study. Administration of this material at 40 mg/kg resulted in the death of the animal.

SIGNATURE

Steven M. Glaza
Study Director
Acute Toxicology

2-1-95
Date

REFERENCE

1. NIH Publication No. 86-23 (revised 1985).

HWI 6329-151

PATHOLOGY REPORT

There was one Group-4 female rabbit treated at 40 mg/kg that died on Day 0 and was necropsied.

At necropsy, the lungs in this animal were diffusely dark red. This change was attributed to acute death and postmortem change. The liver, bile, and both kidneys from this animal were inadvertently not collected as required by protocol. After necropsy, the animal was discarded.

Thomas E. Palmer, PhD
Pathologist

2-1-95
Date

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Table 1
Individual Body Weights (g)

Group	Dose Level (mg/kg)	Sex	Animal Number	Day 0
1	0	Female	F52762	2,781
2	4	Female	F52763	2,770
3	16	Female	F52768	2,735
4	40	Female	F52769	2,511
5	24	Female	F52770	2,778

Table 2
Individual Clinical Signs

Group	Dose Level (mg/kg)	Sex	Animal Number	Observation	Hour 0.5	2	4	24	48
1	0	Female	F52762	Appeared normal	✓	✓	✓	✓	✓
2	4	Female	F52763	Appeared normal	✓	✓	✓	✓	✓
3	16	Female	F52768	Appeared normal	✓	✓	✓	✓	✓
4	40	Female	F52769	Dead (≈5 minutes post-injection)	✓				
5	24	Female	F52770	Appeared normal	✓	✓	✓	✓	✓

✓ Indicates condition exists.
- Not applicable.

APPENDIX A

Protocol Deviation
Protocol TP8084.PK
Protocol Amendment No. 1

Protocol Deviation

Protocol	Actual Procedure
Page 8, 7. Experimental Design, E. Termination, (2) Scheduled Sacrifice, (a) Sample Collection. The whole liver and bile from each animal dying during the study, sacrifice in a moribund condition, or surviving to termination will be collected. Both kidneys from each animal will also be collected.	The liver, bile, and kidneys from the animal that died on test (Group 4, No. F52769) were inadvertently not collected at necropsy.

This deviation is not considered to have had an adverse effect on the outcome of the study.

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Sponsor:

3M
St. Paul, Minnesota

PROTOCOL TP8084.PK

Study Title:

Single-Dose Intravenous Pharmacokinetic Study
of T-6067 in Rabbits

Date:

December 6, 1994

Performing Laboratory:

Hazleton Wisconsin, Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Project Identification:

HWI 6329-151

STUDY IDENTIFICATION

Single-Dose Intravenous Pharmacokinetic Study
of T-6067 in Rabbits

HWI No.	6329-151
Test Material	T-6067
Sponsor	3M Toxicology Services 220-2E-02 3M Center St. Paul, MN 55144
Sponsor's Representative	John L. Butenhoff, PhD 3M Toxicology Services 220-2E-02 3M Center St. Paul, MN 55144 (612) 733-1962
Study Director	Steven M. Glaza Hazleton Wisconsin, Inc. P.O. Box 7545 Madison, WI 53707-7545 (608) 241-7292
Study Location	Hazleton Wisconsin, Inc. Building No. 3 3802 Packers Avenue Madison, WI 53704
Proposed Study Timetable	
Experimental Start Date	December 9, 1994
Experimental Termination Date	December 11, 1994
Draft Report Date	January 13, 1995

1. Study
Single-Dose Intravenous Pharmacokinetic Study in Rabbits

2. Purpose
To assess the level of systemic exposure when the test material is administered as a single intravenous injection to rabbits

3. Regulatory Compliance
This study will be conducted in accordance with the following Good Laboratory Practice Regulations/Standards/Guidelines with the exception that analysis of the test material mixtures for concentration, solubility, homogeneity, and stability will not be conducted:

[ ] Conduct as a Nonregulated Study
[X] 21 CFR 58 (FDA)
[ ] 40 CFR 160 (EPA-FIFRA)
[ ] 40 CFR 792 (EPA-TSCA)
[ ] C(81)30 (Final) (OECD)
[ ] 59 Nohsan No. 3850 (Japanese MAFF)
[ ] Notification No. 313 (Japanese MOHW)

All procedures in this protocol are in compliance with the Animal Welfare Act Regulations. In the opinion of the Sponsor and study director, the study does not unnecessarily duplicate any previous work.

4. Quality Assurance
The protocol, study conduct, and the final report will be audited by the Quality Assurance Unit in accordance with Hazleton Wisconsin (HWI) Standard Operating Procedures (SOPs) and policies.

5. Test Material

A. Identification
T-6067

B. Physical Description
(To be documented in the raw data)

C. Purity and Stability
The Sponsor assumes responsibility for purity and stability determinations (including under test conditions). Samples of test material/vehicle mixture(s) for concentration, solubility, homogeneity, and stability analyses will be taken before administration if requested by the Sponsor. These samples (if taken) will be sent to the Sponsor after experimental termination for possible analysis.

D. Storage
Room temperature

E. Reserve Samples
Reserve samples will not be required for this study.

F. Retention
Any unused test material will be returned to the Sponsor after completion of the in-life phase of the study.

G. Safety Precautions
As required by HWI SOPs and policies

6. Control Material

A. Identification
Sterile water for injection

B. Physical Description
Clear, colorless liquid

C. Purity and Stability
The purity and stability of this USP grade material is considered to be adequate for the purposes of this study.

D. Storage
Refrigerated

E. Reserve Samples
See Section, 5. E. Reserve Samples

F. Retention
Any remaining control material may be used for other testing and will not be discarded after issuance of the final report.

G. Safety Precautions
As required by HWI SOPs and policies

7. Experimental Design

A. Animals

(1) Species
Rabbit

(2) Strain/Source
Hra:(NZW)SPF/HRP, Inc.

(3) Age at Initiation
Adult

(4) Weight at Initiation
2.5 to 3.5 kg

(5) Number and Sex
5 females

(6) Identification
Individual numbered ear tag

(7) Husbandry

(a) Housing
Individually, in screen-bottom stainless steel cages (heavy gauge)

(b) Food
A measured amount of Laboratory Rabbit Diet HF #5326 (PMI Feeds, Inc.). The food is routinely analyzed by the manufacturer for nutritional components and environmental contaminants.

(c) Water
Ad libitum from an automatic system. Samples of the water are analyzed by HWI for total dissolved solids, hardness, and specified microbiological content and for selected elements, heavy metals, organophosphates, and chlorinated hydrocarbons.

(d) Contaminants
There are no known contaminants in the food or water that would interfere with this study.

(e) Environment
Environmental controls for the animal room will be set to maintain a temperature of 19°C to 23°C, a relative humidity of 50% ±20%, and a 12-hour light/12-hour dark cycle.

(f) Acclimation
At least 7 days

(8) Selection of Test Animals
Based on health and body weight according to HWI SOPs. An adequate number of extra animals will be purchased so that no animal in obviously poor health is placed on test.

(9) Justification for Species Selection
Historically, the New Zealand White albino rabbit has been the animal of choice because of the large amount of background information on this species.

B. Dose Administration

(1) Test Groups

Group	Dose Level (mg/kg)[a]	Number of Females
1	0 (Control)	1
2	4	1
3	16	1
4	40	1
5	160	1

a The dose volume will be 0.5 mL/kg of body weight.

C. Dosing Procedures

(1) Dosing Route
Intravenous injection into a marginal ear vein over approximately 30 to 60 seconds.

(2) Reason for Dosing Route
Intravenous injection is an acceptable route to assess systemic exposure.

(3) Dosing Duration
Single dose

(4) Dose Preparation
The test material will be diluted with sterile water for injection to achieve a specific concentration for each dose level. Individual doses will be calculated based on the animal's body weight taken just before test material administration. The prepared test mixtures will be stored at room temperature until administration.

D. Observation of Animals

(1) Clinical Observations
The animals will be observed for clinical signs of toxicity at approximately 0.5, 2.0, 4.0, 24, and 48 hours after treatment.

(2) Body Weights
Just before test material administration.

(3) Sample Collections

(a) Frequency
2, 4, 6, 8, 12, 24, and 48 hours post-injection

(b) Number of Animals
All

(c) Method of Collection
Blood samples (approximately 4 mL) will be collected from either ear via the catheterization of the auricular artery or from the marginal ear vein at 2, 4, 6, 8, 12, and 24 hours post-injection. Approximately 20 mL of blood (actual volume to be documented in the raw data) will be obtained from the posterior vena cava of each animal at the time of necropsy (48 hours post-injection). Approximately 20 mL of blood will be collected from moribund animals during the study, also, if possible. The samples will be stored at room temperature and then centrifuged, and the separate serum and cellular fractions stored in a freezer set to maintain a temperature of -20°C ±10°C. The separated serum and cellular fractions will be sent frozen to the Sponsor after experimental termination.

Samples will be shipped to:

James D. Johnson
3M E.E. & P.C.
Bldg. 2-3E-09
935 Bush Avenue
St. Paul, MN 55106

James D. Johnson or his alternate will be notified by telephone at (612) 778-5294 prior to the shipment of the samples.

E. Termination

(1) Unscheduled Sacrifices and Deaths
Any animal dying during the study or sacrificed in a moribund condition, will be subjected to an abbreviated gross necropsy examination and all abnormalities will be recorded. Animals in a moribund condition will be anesthetized with sodium pentobarbital (via injection in the marginal ear vein), bled via the vena cava, and exsanguinated.

(2) Scheduled Sacrifice
At approximately 48 hours post-injection, animals surviving to termination will be anesthetized with sodium pentobarbital (via injection in the marginal ear vein), bled via the vena cava, and exsanguinated. An abbreviated gross necropsy examination will not be done, however, tissues will be collected.

(a) Sample Collection
The whole liver and bile from each animal dying during the study, sacrificed in a moribund condition, or surviving to termination will be collected. Both kidneys from each animal will also be collected. The tissues will be placed on dry ice immediately after collection and then placed in a freezer set to maintain a temperature of -20°C ±10°C.

The tissues (liver, bile, kidneys) will be sent frozen on dry ice to the Sponsor after experimental termination. The samples will be shipped to the person listed in Section 7.D.(3).(c). The Sponsor is responsible for the retention and disposition of the samples.

F. Statistical Analyses
No statistical analyses are required.

8. Report
A final report including those items listed below will be submitted.

Description of the test and control materials
Description of the test system
Procedures
Dates of experimental initiation and termination
Description of any toxic effects
Gross pathology findings (if applicable)
Gross pathology report (if applicable and requested by the Study Director)

9. Location of Raw Data, Records, and Final Report
Original data, or copies thereof, will be available at HWI to facilitate auditing the study during its progress and before acceptance of the final report. When the final report is completed, all original paper data, including those item listed below will be retained in the archives of HWI according to HWI SOP.

Protocol and protocol amendments
Dose preparation records
In-life records
  Body weights
  Dose administration
  Observations
Sample collection records
Shipping records
Pathology Records
Study correspondence
Final report (original signed copy)

The following supporting records will be retained at HWI but will not be archived with the study data.

Animal receipt/acclimation records
Water analysis records
Animal room temperature and humidity records
Refrigerator and freezer temperature records
Instrument calibration and maintenance records

PROTOCOL APPROVAL

John L. Butenhoff, PhD
Sponsor's Representative
3M

12-12-94
Date

Steven M. Glaza
Study Director
Acute Toxicology
Hazleton Wisconsin, Inc.

12-6-94
Date

Representative
Quality Assurance Unit
Hazleton Wisconsin, Inc.

12/6/94
Date

(6329-151.protdskl)

HAZLETON
WISCONSIN
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MADISON, WI 53707-7545

a CORNING Company

PROTOCOL TP8084.PK

Single-Dose Intravenous Pharmacokinetic Study
of T-6067 in Rabbits

HWI 6329-151

Sponsor	Contractor
3M Toxicology Services 220-2E-02 3M Center St. Paul, MN 55144	Hazleton Wisconsin, Inc 3301 Kinsman Boulevard Madison, WI 53704
Sponsor's Representative	Study Director
John L. Butenhoff, PhD	Steven M. Glaza

Amendment No. 1

This amendment modifies the following portion of the protocol:

Effective December 9, 1994

1. Page 6, 7. Experimental Design; B. Dose Administration; (1) Test Groups. Due to the death of the Group 4 animal (40 mg/kg), modify the dose level for the Group 5 animal as indicated by the following underlined change:

Group	Dose Level (mg/kg)[a]	Number of Females
1	0 (Control)	1
2	4	1
3	16	1
4	40	1
5	24	1

a The dose volume will be 0.5 mL/kg of body weight

Amendment No. 1

PROTOCOL APPROVAL

John L. Butenhoff, PhD
Sponsor's Representative
3M

1-11-95
Date

Steven M. Glaza
Study Director
Acute Toxicology
Hazleton Wisconsin, Inc.

1-4-95
Date

Representative
Quality Assurance Unit
Hazleton Wisconsin, Inc.

1/4/95
Date

(6329-151.Am1.dsk2)

9.1.2 Analytical protocol AMDT-120694.1

3M Environmental Laboratory

Protocol - Analytical Study

Single-Dose Intravenous Pharmacokinetic Study of T-6067 in Rabbits

In-Vivo Study Reference Number: HWI#6329-151

Study Number: AMDT-120694.1
Test Substance: L-13492 (T-6067)

Name and Address of Sponsor: 3M SCD Division
367 Grove Street
St. Paul, MN 55106

Name and Address of Testing Facility:
3M Environmental Technology and Services
935 Bush Avenue
St. Paul, MN 55106

Proposed Initiation Date: July 25, 1995
Proposed Completion Date: August 25, 1995

Method Numbers and Revisions:

AMDT-M-1-0,	Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver
AMDT-M-2-0,	Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
AMDT-M-4-0,	Extraction of Fluorochemicals from Rabbit Liver
AMDT-M-5-0,	Analysis of Rabbit Liver Extract for Fluorochmicals Using Electrospray Mass Spectrometry
AMDT-M-8-0,	Analysis of Fluoride Using the Skalar Segmented Flow Analyzer with Ion Selective Electrode
AMDT-M-14-0,	Therman Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Serum

Author: James D. Johnson

Approved By:

James D. Johnson 11/16/95
James D. Johnson Date
Study Director

John L. Butenhoff 11/20/95
John Butenhoff, PhD Date
Sponsor Representative

1.0 PURPOSE

To assess the level of systemic exposure when the test material L-13492 (T-6067) is administered as a single intravenous injection to rabbits.

2.0 TEST MATERIALS

2.1 Test, Control, and Reference Substances and Matrices

2.1.1 Analytical Reference Substance: FC-95, lot 161 or 171. They are equivalent.

2.1.2 Analytical Reference Matrix: Bovine liver and bovine serum

2.1.3 Analytical Control Substance: None

2.1.4 Analytical Control Matrix: Bovine liver and bovine serum

2.2 Source of Materials: 3M ICP/PCP Division (2.1.1), grocery store (2.1.2, 2.1.4-liver), Sigma Chemical Company (2.1.2, 2.1.4-serum)

2.3 Number of Test and Control Samples: Liver and serum from 3 test animals and 1 control animal. One animal died after injection of a 40 mg/kg dose, and was not replaced. Other biological tissues (kidney, bile, cellular fraction) will be available for analysis if deemed appropriate by the Study Director.

2.4 Identification of Test and Control Samples: The samples are identified using the HWI animal identification number which consists of a letter and five digit number, plus the tissue identity and day identity (serum).

2.5 Purity and Strength of Reference Substance: To be determined by Sponsor.

2.6 Stability of Reference Substance: To be determined by Sponsor.

2.7 Storage Conditions for Test Materials: Room temperature (2.1.1), -20 ± 10°C (2.1.2, 2.1.4). Test and Control samples will be received according to AMDT-S-10-0.

2.8 Disposition of Specimens: Biological tissues and fluids will be retained per GLP Regulation for the time period required for studies longer than 28 days. This study is in parallel with a 28 day dermal absorption study so all tissues will be retained.

2.9 Safety Precautions: Refer to appropriate MSDS. Wear appropriate laboratory attire. Use caution when handling knives for cutting the samples.

3.0 EXPERIMENTAL - Overview

The tissues from animals dosed as described (HWI#6329-151), are available for analysis for fluorine compounds. At the discretion of the Study Director, a series of analytical tests can be performed. The screening for fluoride in liver via combustion (See Methods--next Section) is the appropriate analysis to present definitive data for fluorine in the liver.

4.0 EXPERIMENTAL - Methods

4.1 Liver and Serum screening methods: (attached)

4.1.1 AMDT-M-1-0, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver

4.1.2 AMDT-M-2-0, Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer

4.1.3 AMDT-M-4-0, Extraction of Fluorochemicals from Rabbit Liver

4.1.4 AMDT-M-5-0, Analysis of Rabbit Liver Extract for Fluorochmicals Using Electrospray Mass Spectrometry

4.1.5 AMDT-M-8-0, Analysis of Fluoride Using the Skalar Segmented Flow Analyzer with Ion Selective Electrode

4.1.6 AMDT-M-14-0, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Serum

5.0 DATA ANALYSIS

5.1 Data Reporting: Data will be reported as a concentration (weight/weight) of fluoride per tissue or fluid, or as FC-143 (electrospray mass spectrometry) per unit of tissue or fluid.

6.0 MAINTENANCE OF RAW DATA AND RECORDS

6.1 Raw Data and Records: Raw data, approved protocol, appropriate specimens, approved final report, and electronic data will be maintained in the AMDT archives.

7.0 REFERENCES

7.1 AMDT-S-10-0, Sample Tracking System

8.0 ATTACHMENTS

8.1 AMDT-M-1-0, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver

8.2 AMDT-M-2-0, Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer

8.3 AMDT-M-4-0, Extraction of Fluorochemicals from Rabbit Liver

8.4 AMDT-M-5-0, Analysis of Rabbit Liver Extract for Fluorochmicals Using Electrospray Mass Spectrometry

8.5 AMDT-M-8-0, Analysis of Fluoride Using the Skalar Segmented Flow Analyzer with Ion Selective Electrode

8.6 AMDT-M-14-0, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Serum

3M Environmental Laboratory

Method

Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer - Liver

Method Identification Number: AMDT-M-1 **Adoption Date:** 10-4-95

Revision Number: 0 **Revision Date:** None

Author: Rich Youngblom

Approved by:

Group Leader 10/3/95
Date

Quality Assurance 10-4-95
Date

Software: MS Word 5.1a

Affected Documents: AMDT-M-2 Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
AMDT-EP-3 Routine Maintenance of a Modified Dohrmann DX2000 Organic Halide Analyzer

1.0 SCOPE , APPLICABLE COMPOUNDS, AND MATRICES

1.1 Scope: This method is for the operation of a Dohrmann DX2000 when it is used to extract fluoride from various matrices. The fluoride is typically collected in TISAB solution for analysis with an ion selective electrode.
1.2 Applicable Compounds: Fluorochemicals or other fluorinated compounds.
1.3 Matrices: Biological tissues, particularly liver.

2.0 KEYWORDS

2.1 Fluoride, fluorine, extraction, pyrolysis, ionization, ion selective electrode, Dohrmann, halide, DX2000, fluorochemicals.

3.0 PRECAUTIONS

3.1 Glassware and exhaust gases can be extremely hot.
3.2 Glassware is fragile, broken glass may cause injuries.
3.3 Pressurized gases, proper compressed gas handling practices required.
3.4 Solvent based samples may flash, may need to allow them to dry down before starting run.
3.5 Potential biohazards due to the biological matrices. Use appropriate personal protective equipment.

4.0 SUPPLIES AND MATERIALS

4.1 Compressed Oxygen, Hydrocarbon free, regulated to 30 PSI.
4.2 Compressed Helium, High Purity Grade, regulated to 45 PSI.
4.3 Quartz glass sample boat with Teflon™ tubing, Dohrmann 890-097 or equivalent.
4.4 Quartz glass combustion tube, Reliance Glass G-9405-012 or equivalent.
4.5 Orion 940999 Total Ionic Strength Adjustment Buffer (TISAB II) or equivalent.
4.6 Sample collection vials, HDPE.
4.7 Milli-Q™ water
4.8 Polystyrene pipettes.
4.9 Activated Charcoal, E. Merck 2005 or equivalent.
4.10 Hamilton Syringe or equivalent.
4.11 Miscellaneous laboratory glassware

5.0 EQUIPMENT

5.1 Rosemount Dohrmann DX2000 Organic Halide Analyzer, modified for fluoride extraction.
5.2 IBM compatible 386 or 486 computer.
5.3 DX2000 software, version 1.00, modified for fluoride extraction.
5.4 Excel Spreadsheet, version 5.0 or greater

6.0 INTERFERENCES

6.1 Sample size is limited to approximately 150 mg, depending on sample moisture content. This may vary from matrix to matrix.

7.0 SAMPLE HANDLING

7.1 Samples are not to be handled with bare hands. Fluoride may leach from the skin to the sample. Use forceps or probe to transfer tissues.
7.2 Samples of liver are cut from frozen liver and placed in a tared and labeled weigh boat. Use a clean scalpel and cutting board. The cutting board and scalpel should be cleaned with water, methanol, or methanol-water solution after each liver is cut.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Calibration Standards

8.1.1 The standards required for each project will need to be appropriate for that individual project. Refer to protocol for that project.
8.1.2 Typically 50-500 ppm FC-95 in methanol standards are used.
8.1.3 For rabbit liver studies, use beef liver as the matrix. Cut a piece of frozen beef liver (100 - 150 mg) and weigh it in a labeled and tared weigh boat.

8.2 Calibration - Overview

The normal calibration is the fluoride curve (AMDT-M-2). However, if an optional spiked liver curve is required the procedure listed below is used.

8.2.1 A calibration curve for the DX2000 is generated by spiking samples with known standards and combusting them using the same methods and matrix type as the samples to be tested.
8.2.2 Typically, three replicates of each standard and five concentrations of standards will be spiked.
8.2.3 Standard curve will be plotted as Mass Spiked F (ug) on the x-axis and Standard Mass Recovered F (ug) on the y-axis. Generate a regression curve and calculate the equation for the line and the r^2 value.
8.2.4 Mass Spiked F (ug) = (Amount spiked in mL) x (Conc. of standard in ppm) x (0.6004)*
*FC-95 is 60.04% F therefore 0.6004 is the factor used to convert FC-95 to F
8.2.5 Standard Mass Recovered F (ug) = (TISAB volume in mL) x (Orion reading in ppm)

8.3 Calibration - Procedure

8.3.1 Start Up
8.3.1.1 Run 2 or more Clean Cycles when starting instrument each day. More clean cycles may be used if the previous samples contained high concentrations of fluoride.

8.3.2 Blanks
8.3.2.1 Prepare sample using the same methods and type of matrix as the test sample.
8.3.2.2 For rabbit studies, use beef liver as the matrix. Prepare at least 3 samples of beef liver (100 - 150 mg) for blanks.
8.3.2.3 Put sample in Dohrmann boat. Combust each sample as described in section 9.0 and analyze sample according to method AMDT-M-2 for the ion selective electrode analysis.

8.3.2.4 For rabbit studies, the meter reading for a blank sample should be 0.03 ppm or lower before proceeding with the calibration. Burn samples until this limit is reached, or until in the judgement of the operator the reading is stable with respect to historical readings (previous 48 hours).
8.3.2.5 For non-rabbit studies, the blank readings should reach a predetermined ion concentration before proceeding with the calibration.
8.3.2.6 It may be necessary to mix approximately 50 mg of charcoal with the sample to aid combustion.

8.3.3 Standard Curve
8.3.3.1 Weigh out at least 15 matrix samples (5 standards with 3 replicates each) in tared and labeled weigh boats. For rabbit studies, weigh 100-150 mg beef liver samples. Record weights in study data. Store the matrix samples on dry ice or ice packs to keep them frozen until used.
8.3.3.2 Place weighed beef liver sample in Dohrmann sample boat.
8.3.3.3 Start with the lowest standard concentration. Using a Hamilton syringe, eject a fixed quantity of the standard on or in the matrix. For rabbit studies, use 4 uL of standard and eject it on or in the beef liver.
8.3.3.4 At least 3 replicates should be used for the lowest standard concentration; more replicates may be used at the discretion of the analyst.
8.3.3.5 Combust the sample as described in section 9.3 and analyze according to AMDT-M-2.
8.3.3.6 Run all 15 standards. If one replicate is significantly different from the other two replicates, run another sample for that standard. Indicate in data that the new replicate replaces the old replicate and that the new replicate will be used to calculate the regression curve.
8.3.3.7 When all standards have been run, calculate the r^2. r^2 must be at least 0.95. If it is not at least 0.95, consult with supervisor.
8.3.3.8 A new standard curve should be run when the combustion tube or sample matrix is changed. New standard curve may also be run at the discretion of the analyst.

8.4 Storage Conditions for Standards

8.4.1 Storage requirements for standards are dependent on the individual standards used. Typically, standards are stored at room temperature in plastic screw top bottles.
8.4.2 New FC-95 standards should be prepared at least once a month.

9.0 PROCEDURES

9.1 Typical Operating Conditions:
9.1.1 Combustion tube temperature = 950°C.
9.1.2 Oxygen and Helium flow = 50 cc/minute.
9.1.3 Vaporization/Drying time = 240 seconds.
9.1.4 Bake time = 300 seconds.

9.2 Start Up Procedure:
9.2.1 If the program is not started, start the EOX program on the PC.
9.2.2 Open the SYSTEM SETUP window.
9.2.3 Put the furnace module and the cell in the READY mode.
9.2.4 Close the SYSTEM SETUP window.

9.2.5 When the oven has reached the READY temperature, run the CLEAN BOAT program found in the CELL CHECK menu.
9.2.6 See AMDT-EP-3 for details of the Dohrmann software.

9.3 Sample Extraction Procedure:
9.3.1 Open the SAMPLE HATCH and place the sample in the BOAT. It may be necessary to mix approximately 50 mg of charcoal with the sample to aid combustion. If this is done, charcoal should also be mixed in while establishing the baseline and when generating the standard curve.
9.3.2 Close SAMPLE HATCH.
9.3.3 Add appropriate volume of TISAB solution or 1:1 TISAB:Milli-Q™ water mixture to a labeled sample collection vial. Typically 0.6 mL to 15 mL are used. For rabbit studies, use 1.0 or 2.0 mL of 1:1 TISAB:Milli-Q™ water mixture.
9.3.4 Place the vial so that the tip of the COMBUSTION TUBE is in the TISAB at least 0.25 inches. Gases released during pyrolysis must bubble through the TISAB.
9.3.5 Run the EOX-SOLIDS program found in the RUN menu.
9.3.6 When the EOX program is finished, remove the collection vial from the combustion tube.
9.3.7 If undiluted TISAB was used to collect the sample, add an equal volume of Milli-Q™ water to the TISAB to make 1:1 TISAB:Milli-Q™.
9.3.8 Rinse the end of the combustion tube with Milli-Q™ water and wipe with a KIMWIPE to remove any TISAB remaining on the tube.
9.3.9 Open the sample hatch and remove any remaining ash from the boat. Ash can be removed with a cotton tipped applicator or vacuumed out. It may be necessary to scrap particles off the bottom with a spatula or other similar device. A drop of Milli-Q™ water may be added to the boat to aid in the Clean Cycle.
9.3.10 Close the hatch.
9.3.11 Run the CLEAN BOAT program.
9.3.12 Sample is ready for analysis by ion selective electrode (AMDT-M-2).

9.4 Sample Calculations

9.4.1 Use the standard curve to calculate the sample value.
9.4.2 Sample Mass Recovered F (ug) = (TISAB vol in mL) x $\frac{\text{(Orion reading in ppm - intercept)}}{\text{(Slope)}}$

10.0 VALIDATION

10.1 Quality Control
10.1.1 Daily Start Up Check Samples: Once the standard curve is established, each day of analysis is started by analyzing QC samples. The QC samples are to be the same as the lowest concentration spiked samples used to generate the standard curve. Each concentration must be done in triplicate unless the first two replicates are within 20% of the standard curve, then a third replicate is not necessary.

10.2 Precision and Accuracy: See method development analysis and sample analysis in Fluoride Notebooks 2,3, and 5. Precision and accuracy varies when analyzing samples of different matrices and different reference compounds.

10.3 Other Validation Parameters: NA

11.0 DATA ANALYSIS

11.1 Calculations

11.1.1 For the standard curve, use regression analysis in Excel, version 5.0 or greater.
11.1.2 To calculate the fluoride contraction in the sample, see method AMDT-M-2.

11.2 Analyzing the Data

11.2.1 r^2 must be at least 0.95 or greater. "Outliers" may be excluded if two of the three replicates are within 20% of each other and the outlier is greater than 200% of the average of those two or less than 50% of the average of those two. Any such outliers should be pointed out in the data and noted in the Final Report along with the reason it was considered an outlier.

12.0 ATTACHMENTS

None

13.0 REFERENCES

13.1 Rosemount Dohrmann DX2000 Organic Halide Analyzer Operator's Manual (Manual 915-349, revision B, December 1993)
13.2 AMDT-M-2 Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
13.3 AMDT-EP-3 Routine Maintenance of a Modified Dohrmann DX2000 Organic Halide Analyzer

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

3M Environmental Laboratory

Method

Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer

Method Identification Number: AMDT-M-2 **Adoption Date:** 10-4-95

Revision Number: 0 **Revision Date:** None

Author: Rich Youngblom

Approved By:

James D. Johnson 10/3/95
Group Leader Date

Carl Van Buskirk 10-4-95
Quality Assurance Date

Software: MS Word 5.1a

Affected Documents: AMDT-M-1 Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer

1.0 SCOPE , APPLICABLE COMPOUNDS, AND MATRICES

1.1 SCOPE: This method is for the calibration and operation of an Orion EA940 Expandable Ion Analyzer.

1.2 APPLICABLE COMPOUNDS: Fluoride.

1.3 APPLICABLE MATRICES: Liquid samples in an appropriate buffer solution. Preferred pH of 6.0.

2.0 KEYWORDS

2.1 Fluoride, fluorine, ion selective electrode

3.0 PRECAUTIONS

3.1 No hazards identified with this method.

4.0 SUPPLIES AND MATERIALS

4.1 Orion 940999 Total Ionic Strength Adjustment Buffer II (TISABII) or equivalent.
4.2 Orion Model 900001 electrode filling solution (AgCl) or equivalent.
4.3 Orion 940907 100 ppm fluoride standard or equivalent.
4.4 Milli-Q™ water or equivalent.
4.5 Magnetic stir bars.
4.6 Lab tissues.
4.7 Sample collection vials.
4.8 Plastic 100 mL volumetric flasks.
4.9 Polystyrene pipettes.
4.10 Miscellaneous laboratory glassware.

5.0 EQUIPMENT

5.1 Orion Model EA940 Expandable Ion Analyzer or equivalent.
5.2 Orion Model 960900 Solid State Combination Fluoride electrode or equivalent.
5.3 Magnetic Stir Plate.
5.4 IBM compatible 386 or 486 computer (only needed if using Orion 3E software).
5.5 Orion RS232 interface cable (only needed if using Orion 3E software).
5.6 Microsoft Excel 5.0 (only needed if using Orion 3E software).

6.0 INTERFERENCES

6.1 It is recommended that the pH be at or near 6.0. A 1:1 mixture of TISAB and sample/Milli-Q™ water will generally bring sample to pH of 6.0.
6.2 Sample temperature may effect fluoride measurement. It is recommended that the sample be at room temperature as the standards were when the meter was calibrated.
6.3 The rate the samples are stirred at should be consistent with the rate the standards were stirred.

6.4 Air bubbles trapped under electrode can give erroneous readings. Make sure no air is trapped under electrode.

7.0 SAMPLE HANDLING

7.1 No special handling necessary.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Calibration Standards
8.1.1 Measure 50 mL of TISAB II into 5 100 mL plastic volumetric flasks.
8.1.2 Label the flasks as 0.05, 0.1, 0.5, 1.0, and 1.5 ppm F-, along with the date and your initials.
8.1.3 Pipette 0.05, 0.1, 0.5, 1.0, and 1.5 mL of 100 ppm fluoride standard into the appropriately labeled flasks.
8.1.4 Add approximately 30 mL of Milli-Q™ water to each flask.
8.1.5 Shake the flasks to mix the solutions.
8.1.6 Eliminate air bubbles from the flasks by tipping the flasks on their sides and rolling the air in the flasks over the air bubbles.
8.1.7 Bring the volume in the flasks up to the 100 mL mark with Milli-Q™ water.
8.1.8 Invert and shake the flasks for the final mixing.
8.1.9 Record standards in Standards Log Book.

8.2 Calibration
8.2.1 If necessary, remove tape from electrode filling hole.
8.2.2 Invert probe to wet top seal.
8.2.3 Eject a few drops of filling solution from bottom of electrode to wet lower seal.
8.2.4 Fill the electrode with filling solution.
8.2.5 The meter and the F- electrode are typically calibrated by direct measurement with no blank correction, using standards with concentrations of 0.05, 0.1, 0.5, 1.0, and 1.5 ppm F-, following the manufacturer's instructions.
8.2.6 Record the slope in the appropriate log book.
8.2.7 Clean the electrode by rinsing with Milli-Q™ water and wiping the sides down with lab tissues.

8.3 Storage Conditions for Standards
8.3.1 Calibration standards are stored at room temperature.

9.0 PROCEDURES

9.1 Calibration and Measurement, Standard method:
9.1.1 The sample to be measured needs to be mixed with TISAB using the proportions recommended by the TISAB manufacturer.
9.1.2 Place a stir bar in the sample and place the sample on the stir plate.
9.1.3 Allow the sample to mix for a few seconds before inserting the electrode. When the electrode is inserted, make sure there are no air bubbles trapped under the electrode.
9.1.4 The sample should be the same temperature as the calibration standards and stirred at the same rate as the calibration standards.
9.1.5 When the readings have stabilized, record the reading in the appropriate log book.

9.2 Calibration And Measurement, Using Orion 3E Software:

9.2.1 Calibration:
9.2.1.1 Follow steps 8.2.1 to 8.2.4.
9.2.1.2 Press Function Key #8 (F8).
9.2.1.3 The computer screen will ask you to confirm the number of standards to be used, concentration of the standards, and whether or not a blank is to be included in the calibration. Make any necessary changes to the information presented and click on CONTINUE.
9.2.1.4 Place the electrode in the first standard on the stir plate and click on CONTINUE.
9.2.1.5 Observe the readings on the graphic display on the computer. When the readings have stabilized, press ACCEPT READING.
9.2.1.6 Repeat step 9.2.1.4 and 9.2.1.5 for the remaining standards.
9.2.1.7 After the final standard, the computer will display the slope of the curve, as well as the intercept and correlation. Record the slope, intercept, and correlation in the appropriate log book and click on CONTINUE. The calibration data is automatically copied to C:\Orion\Data\Calib.txt.

9.2.2 Data Spreadsheet:
9.2.2.1 Select either NEW or OPEN from the FILE menu to open a new or existing spreadsheet to store data in.
9.2.2.2 Record the name of the spreadsheet used in the appropriate log book.

9.2.3 Fluoride Measurement:
9.2.3.1 Follow steps 9.2.1 through 9.2.4
9.2.3.2 Enter the name of the sample in the appropriate place on the screen.
9.2.3.3 Click on the NEW SAMPLE button
9.2.3.4 When the readings have stabilized, click on the RECORD button and write the result in the appropriate log book.

10.0 VALIDATION

10.1 Quality Control:

10.2 Precision and Accuracy

10.3 Other Validation Parameters According to Reference 13.2, the range of detection is 0.02 ppm fluoride up to a saturated solution of fluoride.

11.0 DATA ANALYSIS

11.1 Calculations None necessary.

11.2 Analyzing the Data None necessary.

12.0 ATTACHMENTS

None

13.0 REFERENCES

13.1 Orion Model EA940 Expandable Ion Analyzer Instruction Manual, Orion Research Incorporated, 1991.
13.2 Orion Model 960900 Solid State Combination Fluoride Electrode Instruction Manual, Orion Research Incorporated, 1991.

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

000050

3M Environmental Laboratory

Method

Extraction of Fluorochemicals from Rabbit Livers

SOP Identification Number: AMDT-M-4 **Adoption Date:** 10-21-95

Revision Number: 0 **Revision Date:** None

Author: Dave Christenson/Cynthia Weber

Approved By:

James W Johnson 10-31-95
Group Leader Date

Carl Von Bushek 10-31-95
Quality Assurance Date

Software: MS Word, 6.0
Affected Documents: M-5, Analysis of Rabbit Extract for Fluorochemicals Using Electrospray Mass Spectroscopy.

1.0 SCOPE

1.1 **Scope:** This method is for the extraction of fluorochemicals from rabbit livers. Ethyl acetate is used to extract fluorochemicals from the livers for analysis by electrospray mass spectroscopy.

1.2 **Applicable Compounds:** Fluorochemicals or other fluorinated compounds.

1.3 **Matrices:** Rabbit Livers.

2.0 KEYWORDS

2.1 Fluorochemicals, rabbit livers, electrospray mass spectrometer, fluorinated compounds, extraction.

3.0 PRECAUTIONS

3.1 Use gloves when handling the rabbit livers, they may contain pathogens.

4.0 SUPPLIES AND MATERIALS

4.1 Supplies

4.1.1 Syringe, capable of measuring 100 µL
4.1.2 Eppendorf type or disposable pipets
4.1.3 Gloves
4.1.4 Plastic grinding tubes
4.1.5 Plastic centrifuge tubes, 15 mL
4.1.6 Labels
4.1.7 Nitrogen
4.1.8 Timer
4.1.9 Filters, Titan nylon syringe filters, 0.2 µm.
4.1.10 Analytical pipets: glass volumetric pipets.
4.1.11 Disposable plastic 3 cc syringes.
4.1.12 Crimp cap autovials.

4.2 Reagents

4.2.1 Aqueous Ammonium Acetate (Aldrich), approx. 250 ppm: Prepare a 2500 ppm aqueous solution of ammonium acetate by adding 250 mg ammonium acetate to a 100 mL volumetric flask and dilute to volume with Milli-Q water. Dilute this solution 1:10 for a 250 ppm solution.

4.2.2 Sodium carbonate/Sodium Bicarbonate Buffer (J.T. Baker), (Na_2CO_3/$NaHCO_3$) 0.25 M: Weigh 26.5 g of sodium carbonate (Na_2CO_3) and 21.0 g of sodium bicarbonate ($NaHCO_3$) into a 1 L volumetric flask and bring to volume with Milli-Q water.

4.2.3 Dilute acetonitrile solution, dilute acetonitrile 1:1 with Milli-Q water.
4.2.4 Ethyl Acetate
4.2.5 Methanol
4.2.6 Milli-Q water
4.2.7 1H,1H,2H,2H - perfluorooctanesulfonic acid (Aldrich)
4.2.8 FC-95 (3M Specialty Chemical Division)

5.0 EQUIPMENT

5.1 Ultra-Turrax T25 Grinder for grinding liver samples.
5.2 Vortex mixer
5.3 Centrifuge
5.4 Shaker
5.5 Analytical Evaporator

6.0 INTERFERENCES

6.1 There are no known interferences at this time.

7.0 SAMPLE HANDLING

7.1 The rabbit livers are received frozen, and must be kept frozen until the extraction is performed.

8.0 CALIBRATION AND STANDARDIZATION

8.1 **Preparation of Internal Standards**

8.1.1 Prepare an internal standard of approximately 12 ppm 1H,1H,2H,2H-perfluorooctanesulphonic acid to be added to each liver sample.

8.1.2 Weigh at least 0.1 g of 1H,1H,2H,2H-perfluorooctanesulphonic acid into a 100 mL volumetric flask. Record the actual weight.

8.1.3 Bring it up to volume with methanol, this is the stock standard.

8.1.4 To a 250 mL volumetric flask, add 3 mLs of the stock standard and bring to volume with Milli-Q water. Calculate the actual concentration of the standard.

$$\frac{\text{actual mg perfluoroctane-sulphonic acid}}{0.1\ \text{L}} \times \frac{3\ \text{mL}}{250\ \text{mL}} = \text{actual concentration, ppm}$$

8.2 **Prepare FC-95 Anion Standards**

8.2.1 Prepare FC-95 standards for the standard curve.

8.2.2 Weigh approximately 100 mg of FC-95 into a 100 mL volumetric flask. Record the actual weight.

8.2.3 Bring up to volume with dilute acetonitrile.

8.2.4 Dilute the solution with dilute acetonitrile 1:10 for a solution of approximately 100 ppm. Dilute this solution 1:10 with dilute acetonitrile for a solution of approx. 10 ppm.

8.2.5 Use the 10 ppm solution to make working standards with values close to 5.0 ppm, 1.0 ppm and 500 ppb.

8.3 **Prepare Beef Liver Homogenate to Use for Standards**

8.3.1 Weigh 40 g of Bovine liver into a 250 mL Nalgene bottle containing 200 mLs Milli-Q water. Grind to a homogenous solution.

8.3.2 Add 1 mL of the solution to a 15 mL centrifuge tube. Prepare a total of eight 1 mL aliquots of the solution in 15 mL centrifuge tubes. Be sure to re-suspend solution by shaking it between aliquots.

8.3.3 Spike seven of the 1 mL aliquots with the following amounts of working standards in step **9.12** of the procedure. One 1 mL aliquot serves as the blank.

Working Standard (Approximate Conc.)	uL	Approximate final concentration of FC-95 in liver
-	-	Blank
500 ppb	100	0.292 ppm
500 ppb	200	0.584 ppm
500 ppb	300	0.877 ppm
500 ppb	400	1.168 ppm
1 ppm	500	2.924 ppm
5 ppm	200	5.848 ppm
5 ppm	300	8.772 ppm

8.4 Calculate the actual value of the standards:

$$\frac{\text{uL of standard} \times \text{concentration (in ppm)}}{\text{171 mg liver}^{*} / \text{1 ml homogenate}} = \text{final concentration (ppm) of FC -95 in liver}$$

*Average weight of bovine liver in solution as determined by weighing 1 mL homogenates of 40 mg liver in 200 mL of Milli-Q water. The amount of FC-95 is reported as equivalents of FC-95 potassium salt.

8.5 Calibration

8.5.1 Extract the spiked beef liver homogenate following 9.13 to 9.23 of this method. Use these standards to establish your curve on the mass spectrometer.

8.5.2 Alternatively, a standard curve may be generated using ratios of responses of the perfluorooctansulfonate anion and the internal standard anion versus concentration of the perfluorooctanesulfonate anion.

8.6 Storage Conditions for Standards

8.6.1 New standards are prepared with each analysis. Standards are stored in covered plastic centrifuge tubes until the analysis on the mass spectrometer is performed.

8.7 Storage Conditions for Standards

8.7.1 Beef liver homogenates may be frozen after preparation.

9.0 PROCEDURES

9.1 Obtain frozen liver samples. In spent tissue, note that the liver has not been packaged with other tissues.

9.2 Use a dissecting scalpel and cut off approximately 1 g of liver.

9.3 Weigh the sample directly into a tared plastic grinding tube.

9.4 Record the liver weight in the study note book.

9.5 Put a label on the vial with the study number, weight, rabbit ID, date and analyst initials.

9.6 Add 2.5 mLs water.
9.7 Grind the sample. Put the grinder probe in the sample and grind for about 2 minutes, until the sample is a homogeneous solution with no large chunks.
9.8 Rinse the probe off into the sample with 2.5 mLs water using a pipet.
9.9 Take the grinder apart and clean it with methanol after each sample. Follow AMDT-EP-22.
9.10 Cap the sample and vortex for 15 seconds.
9.11 Pipet 1 mL into a 15 mL centrifuge tube. Label the centrifuge tube with the identical information as the grinding tube. (See AMDT-M-4 Worksheet for documenting the remaining steps.)
9.12 Spike the beef liver homogenates with the appropriate amount of FC-95 standard as described in 8.3.
9.13 Spike the samples and beef liver homogenates with 100 uL of internal standard.
9.14 Add 1 mL of the sodium carbonate/sodium bicarbonate buffer and 1 mL ammonium acetate.
9.15 Using an analytical pipet, add 5 mL ethyl acetate.
9.16 Cap the sample and vortex 20 to 30 seconds.
9.17 Put them in the shaker for 20 min.
9.18 Centrifuge for 20 to 25 minutes, until the layers are well separated. Set the power on the centrifuge to 25.
9.19 Remove 4 mLs of the top organic layer to a fresh 15 mL centrifuge tube with a 5 mL graduated glass pipet. Transfer the label to the fresh tube.
9.20 Blow the sample down on the analytical evaporator to near dryness with nitrogen, approximately 30 to 40 minutes.
9.21 Bring the remaining sample up in 1 mL dilute acetonitrile with an analytical pipet.
9.22 Vortex 15 seconds.
9.23 Transfer the sample to a 3 mL syringe. Attach a 0.2 µm nylon mesh filter, and filter the sample into a fresh centrifuge tube or a autovial. Label the tube or vial with the study number and animal number.
9.24 Cap and hold for analysis by electrospray mass spectroscopy.
9.25 Complete AMDT-M-4 worksheet and attach to page of study notebook.

10.0 VALIDATION

10.1 **Quality Control** - not applicable
10.2 **Precision and Accuracy**- not applicable
10.3 **Other Validation Parameters**- not applicable

11.0 DATA ANALYSIS

11.1 None

12.0 ATTACHMENTS

12.1 Worksheet AMDT-M-4

13.0 REFERENCES

13.1 AMDT-EP-22 Routine Maintenance of Ultra-Turrax T-25

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

Worksheet AMDT-M-4

Study #	Sample Number set #	FC-95 approx 0.5 ppm actual ppm #W	FC-95 approx 1 ppm actual ppm #W	FC-95 approx. 5 ppm actual ppm #W	Date and Initials for Std.
-	Blank Liver	-	-	-	
-		100 uL	-	-	
-		200 uL	-	-	
-		300 uL	-	-	
-		400 uL	-	-	
-		-	500 uL	-	
-		-	-	200 uL	
-		-	-	300 uL	
[1]		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
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		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
[1] study number where the original worksheet is located and place a copy.					

Liver Extraction Process:	Date & Initials
Pipet 1 mL of Liver Solution	
Pipet 100 uL of 12 ppm Internal Standard	Std. #
Vortex 15 sec.	
Pipet 1 mL of 250 ppm Ammonium Acetate	Std. #
Pipet 1 mL of 0.25 Na_2CO_3/0.25M $NaHCO_3$ Buffer	
Pipet 5 mL of Ethyl Acetate	
Vortex 20-30 sec.	
Shake 20 min.	
Centrifuge 20-25 min.	
Remove a 4 mL aliquot of organic layer	
Blow down to near dryness (<0.25 mL) with N_2	
Add 1 mL of 1:1 Acetonitrile/H_2O	TN#
Vortex 15 sec.	
Filter using a 3cc B-D syringe with a 0.2um SRI filter into a 1.5 mL autosample vial.	

3M Environmental Laboratory

Method

Analysis of Rabbit Liver Extract for Fluorochemicals using Electrospray Mass Spectroscopy

SOP Identification Number: AMDT-M-5 **Adoption Date:** 6-6-95

Revision Number: 0 **Revision Date:** None

Author: Dave Christenson/Cynthia Weber

Approved By:

Group Leader — Date: 6/6/95

Quality Assurance — Date: 6/6/95

Software: MS Word, 6.0
Affected Documents: M-4, Extraction of Fluorochemicals from Rabbit Livers

1.0 SCOPE

1.1 **Scope:** This method is for the analysis of extracts of rabbit liver or other tissues or fluids for fluorochemicals using the electrospray mass spectrometer. The analysis is performed by single ion monitoring of FC-95 anion, M/Z= 499, the internal standard M/Z = 427, and other appropriate masses.

1.2 **Applicable Compounds:** Fluorochemicals or other fluorinated compounds.

1.3 **Matrices:** Rabbit Livers (samples), Beef Liver (standards), other tissues and fluids.

2.0 KEYWORDS

2.1 Fluorochemicals, fluorinated compounds, electrospray mass spectroscopy, mass spectrometer, rabbit livers.

3.0 PRECAUTIONS

3.1 Use caution with the voltage cable for the probe. When the voltage cable is plugged into the probe DO NOT TOUCH THE PROBE, there is risk of electrical shock.

3.2 Do not run the pump above it's capacity of 4000 psi. If pressure goes over 4000 psi stop and release pressure. The peak tubing may be plugged. Troubleshoot back to find the plug and replace the plugged tubing. See AMDT-EP-15

3.3 Do not run the pump to dryness.

4.0 SUPPLIES AND MATERIALS

4.1 **Supplies**

4.1.1 Nitrogen gas regulated to 140 psi.

4.1.2 Fluofix column or equivalent.

4.1.3 100 uL or 250 uL flat tip syringe for sample injection.

4.2 **Reagents**

4.2.1 Dilute acetonitrile mobile phase, dilute acetonitrile 1:1 with Milli-Q water.

4.2.2 Milli-Q water, all water used in this method should be Milli-Q water.

5.0 EQUIPMENT

5.1 VG Trio 2000 Electrospray Mass Spectrometer or equivalent.

5.2 ISCO Syringe Pump

5.3 Spectraphysics AS300 Autosampler

5.4 100 uL Assembly

5.5 Autovials or capped centrifuge tubes.

6.0 INTERFERENCES

6.1 There are no known interferences at this time.

7.0 SAMPLE HANDLING

7.1 Keep the extracted samples in capped 15 mL centrifuge tubes or in capped autovials until ready for analysis.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Calibration Standards

8.1.1 Seven beef liver standards and one blank beef liver are prepared during the extraction procedure. (See AMDT-M-4, section 8.0)

8.2 Calibration

8.2.1 Run the seven beef liver standards twice, starting with the lowest standard to obtain the standard curve.

8.2.2 Typically one standard is run after each 5 to 7 samples. Choose a standard in the same range of concentration as the samples.

8.3 Storage Conditions for Standards

8.3.1 Fresh standards are prepared with each analysis. Standards are stored in covered plastic centrifuge tubes until the analysis on the mass spectometer is performed. Samples and standards are NOT refrigerated.

8.4 Storage Conditions for Beef Liver Homogenates

8.4.1 Beef liver homogenates may be frozen after preparation.

9.0 PROCEDURE

9.1 Initial Set-up

9.1.1 Set software to "Operate on", Ion Mode ES$^-$.

9.1.2 Record backing pressure in the instrument log.

9.1.3 Fill the solvent cylinder with mobile phase.

9.1.4 Set the pump to "Run". Set the flow to 1000 uL/min. Observes droplets coming out of the tip of the probe. The pressure should be at 1700 to 1800 psi.

9.1.5 Check the fused silica at the end of the probe. Use an eye piece to check for chips. The tip should be flat with no jagged edges. If any chips are found cut off the tip of the silica with a column cutter and pull the silica through to the appropriate length.

9.1.6 Check your nitrogen supply. Turn on the nitrogen. There should be no nitrogen leaking around the tip of the probe. A fine mist should be coming out of the tip.

9.1.7 Carefully guide the probe into the opening. Insert it until it won't go any further. Connect the voltage cable to the probe.

9.1.8 Go to the "Editor" page, and set Ionization Mode to ES$^-$, and the appropriate masses to 427 and 499.

9.1.9 If it is not in single ion mode go to "Option" and set SIR.

9.1.10 Start Acquisition. Assign a file name, MO-DAY-YR + letter. Record it in the log book.

9.1.11 Run the beef liver samples first, running each standard twice at the beginning of the run.. Run a QC check by running one standard after every 5 to 7 samples.

9.2 Manual Injection

9.2.1 Draw 150 uL of sample into a syringe. Inject the sample into the rheodyne injection port. Inject slowly. Record the sample ID in the log book.

9.2.2 Turn the valve to "On".

9.2.3 Wait two minutes, and inject the next sample.

9.2.4 Record the scan number for each sample in the logbook.

9.3 Using the Autosampler

9.3.1 Set up sample tray A, B, or C.

9.3.2 Record the samples and their positions in the instrument log book. Up to 17 vials may be in each run.

9.3.3 Set-up the sampler:

- 9.3.3.1 Push the sample button
- 9.3.3.2 Set sample loop size = 100 uL
- 9.3.3.3 Set inject/sample = 2
- 9.3.3.4 Set Cycle time = 0
- 9.3.3.5 Name the file: Livers
- 9.3.3.6 Identify the tray used
- 9.3.3.7 Add the samples to Queue by pressing "Enter"
- 9.3.3.8 Press "Run" to start

10.0 VALIDATION

10.1 Quality Control

10.1.1 Run a standard every 5 to 7 samples. If a significant change(± 50%) in peak height occurs stop the run. Only the samples before the last acceptable standard will be used. The remaining samples will be reanalyzed.

10.2 Precision and Accuracy

10.2.1 See Method Validation Report number AMDT-M-5.0.V1

10.3 Other Validation Parameters

10.4 Refer to Method Validation Report Number AMDT-M-5.0.V1

11.0 DATA ANALYSIS

11.1 Calculations

11.2 Plot the standard curve, using the mean of the two values obtained for each standard.

11.2.1 Read peak heights or areas for the samples from the printout. Use linear regression to determine the sample concentrations.

11.2.2 Calculate the mg of FC-95 anion, or other fluorochemical in the total rabbit liver:

mg FC-95 anion in the total rabbit liver =

$$\frac{\text{mg FC-95 anion from std. curve}}{\text{gms of liver used for analysis}} \times \text{Total mass of liver, gms}$$

11.3 Make a results table and enter it in the study book.

11.4 Print a chromatogram for each sample, with the peaks labeled with the sample or standard ID. Write the study number on the printout, initial, date, and put it in the study folder. Staple all chromatograms together and number pages.

12.0 ATTACHMENTS

None

13.0 REFERENCES

13.1 AMDT-EP-17

14.0 REVISIONS

Revision Number	Reason for change	Revision Date

3M Environmental Laboratory

Method

Analysis of Fluoride Using the Skalar Segmented Flow Analyzer With Ion Selective Electrode

Method Identification Number: AMDT-M-8 **Adoption Date:** 10-5-95

Revision Number: 0 **Revision Date:** None

Author: Deb Wright / Cynthia Weber

Approved By:

James D Johnson 10/5/95
Group Leader Date

Gale Kevin Burbank 9-27-95
Quality Assurance Date

Software: IBM MS Word, 6.0
Affected Documents: AMDT-EP-26, Operation and Maintenance of the Skalar Segmented Flow Analyzer

000062

1.0 SCOPE

1.1 This method is for the analysis for fluoride, thermally extracted from samples using the Dohrmann DX2000 (AMDT-M-1), and collected in TISAB for analysis with an Ion Selective Electrode (ISE). The analysis is performed using the Skalar Segmented Flow Analyzer with ISE.

1.2 Samples can be tissues, serum, biological material, or other materials extracted on the Dohrmann.

2.0 KEYWORDS

2.1 Skalar, segmented flow, fluoride.

3.0 PRECAUTIONS

3.1 Follow standard laboratory safety practices.

4.0 SUPPLIES AND MATERIALS

4.1 Supplies

4.1.1 Sample cups, 4 mL plastic cups with caps

4.1.2 Autopipets, oxford or equivalent with plastic tips

4.1.3 Polypropylene volumetric flasks, 100 mL

4.1.4 Cartridge components, refer to the Skalar Methods for components and part numbers.

4.1.5 Sample prefilters, Evergreen

4.2 Reagents

4.2.1 Brij 35, 30% S.F.A.S. Detergent

4.2.2 TISAB II buffer solution: Purchase TISAB II from Orion. To 1 liter of TISAB II add 2.5 mL or 100 ppm fluoride solution and 1 mL Brij.

4.2.3 Sampler rinsing solution: Dilute TISAB II 1:1 with Milli-Q water.

4.2.4 Nitric acid solution for decontamination, 1 N (lab grade): Slowly add 64 mLs concentrated nitric acid (HNO_3) to 250 mLs of Milli-Q water. Bring the volume up to 1 L with Milli-Q water.

4.3 Standards

4.3.1 Stock solution, 100 ppm F: purchased from Orion.

4.3.2 Intermediate standard, 10 ppm: Dilute 10 mLs of stock solution to 100 mLs with Milli-Q water. Use polypropylene volumetric flasks.

4.3.3 Working standard: Make up the following working standards by adding the volumes of intermediate or stock standard indicated on the table, using oxford or pumpmate pipets, to 50 mLs of TISAB and diluting to 100 mLs with Milli-Q water.

Working Standard	mLs of Stock Standard	mLs of Intermediate Standard
0.015 ppm	-	0.15
0.03 ppm	-	0.3
0.06 ppm	-	0.6
0.09 ppm	-	0.9
0.12 ppm	-	1.2
0.15 ppm	-	1.5
0.3 ppm	0.3	-
0.6 ppm	0.6	-

1.2 ppm	1.2	-
1.5 ppm	1.5	-

5.0 EQUIPMENT

5.1 Skalar Segmented Flow Auto Analyzer Sans^{Plus} System equipped with ISE

6.0 INTERFERENCES

6.1 High concentrations of alkalinity, chloride, phosphate, sulfate or iron can cause interferences.

7.0 SAMPLE HANDLING

7.1 Samples should be stored in polyethylene bottles. Samples should be analyzed within 30 days.

8.0 CALIBRATION AND STANDARDIZATION

8.1 **Preparation of Calibration Standards**

8.1.1 Prepare calibration standards as in section 4.3.

8.2 **Calibration**

8.2.1 The standards are analyzed at the beginning of the run.

8.3 **Storage Conditions for Standards**

8.3.1 Standards are stored in capped polypropylene volumetric flasks. New standards are prepared at a minimum of every six months, or as necessary.

9.0 PROCEDURE

9.1 **Start Up Procedure**

9.1.1 Clamp down the pumpdecks, air bars and sampler-pump tubing.

9.1.2 Put the fluoride electrodes in the electrode chamber.

9.1.3 Turn on the power of the sampler, pumps, offset potentiometer and heating bath.

9.1.4 Put the reagent-lines in the appropriate bottles.

9.1.5 Turn on the interface, computer, display and printer. **Make sure you turn on the interface before the computer.**

9.1.6 Let the system stabilize for approximately 30 minutes.

9.2 **Starting a Run**

9.2.1 Create a sample table by selecting FILES, TABLE, and CREATE, type in the name of the file, and press ENTER.

9.2.2 Print the sample table, inserted in the system table by pushing ESC, PRINT, GROUP 1. This will print the entire run.

9.2.3 Dial the sampler settings to the appropriate number of samples, number of seconds for sample wash, and number of seconds for the sample.

9.2.4 Fill the sample tray with the standards, samples, washes and drifts. IW and FW/RUNOUT cups on the sampler do not need to be filled.

9.2.5 Set the baseline.

9.2.5.1 Select GRAPHICS, REAL TIME. If you cannot get real-time, you may be in the Data Handling Panel. Switch to the Analysis Panel by selecting CONTROL PANEL and pushing F7.

9.2.5.2 Use the small screwdriver for the offset potentiometer to set the base line. Adjust the baseline until it is approximately 3/4 inch from the bottom of the screen.

9.2.5.3 Check the highest standard and adjust the gain, if necessary, with the interface screw #3.

9.2.6 Go to CONTROL PANEL, and to analysis panel. Deselect the analysis that will not be run. (Select or deselect analysis by pressing ENTER.) Press Tab to return to the Analysis Panel.

9.2.7 Press the spacebar to bring up the local menu.

9.2.8 Select START to start the analysis.

9.2.9 Type your ID (initials), the sample table which you created under 9.2.1 (or press ENTER for choices), choose running with or without the system table and select START ANALYSIS.

9.2.10 After starting the software, start the sampler. Make sure that the sampler is set to the right number of samples and that the sample/wash/air times are OK.

9.2.11 Select GRAPHICS, REAL TIME to view the progress of the analysis.

9.3 Loading and Printing the Data-File

9.3.1 Go to CONTROL PANEL, press the spacebar to bring up the local menu and select LOAD. Select AUTOCALCULATION and enter the filename (or highlight the file to be printed and press ENTER).

9.3.2 To view the calibration curve, go to GRAPHICS, CALIBRATION CURVE.

9.3.3 To print the high level curve, push PRINT SCREEN.

9.3.4 To print the low level screen, push ESC to get out of graphics. Select SETTINGS. Change the max y value to approximately 900. Go to CAL CURVE and press ESC, and Enter. Press PRINT SCREEN.

9.3.5 Return to SETTINGS and change the max value back to 4095, go to EDIT, press ENTER and PRINT SCREEN to print sample peaks.

9.3.6 To print the results go to CONTROL PANEL, SPACEBAR, OUTPUT, OUTPUT. Select PRINTER for the Epson or PRN for the Laser.

9.4 Shutdown

9.4.1 Put all the reagent-lines in Milli-Q water.

9.4.2 Let the system rinse for approximately 30 minutes.

9.4.3 After the system has rinsed completely, turn off the sampler, pump and offset potentiometer. Turn off the heating bath on weekends. Leave liquid in the lines.

9.4.4 Take the electrode out and soak in 100 ppm F overnight.

9.4.5 Release the pump-decks, air bars and sampler pump-tubing.

9.4.6 Select FILES, press ALT F and select QUIT to exit the program.

9.4.7 On Friday, turn off the computer, display and interface for the weekend.

10.0 VALIDATION

10.1 Quality Control

10.1.1 Run a standard (mid to high concentration) every 10 samples. If a significant change in peak height occurs, only the samples before the last acceptable standard will be used. The remaining samples will be reanalyzed.

10.2 Precision and Accuracy

10.2.1 See Method Validation Report number AMDT-M-8.0.V1

10.3 Other Validation Parameters

10.4 Refer to Method Validation Report Number AMDT-M-8.0.V1

11.0 DATA ANALYSIS

11.1 Calculations

11.1.1 The standard curve is plotted by the Skalar software.

11.1.2 All calculations are done by the Skalar software. r^2 should be 0.995 or better.

11.2 Prepare spreadsheets to summarize data. Include sample volume, weights used etc.

11.3 Write the study number on the printouts, initial, date the printout, and bind together with all package documents and place in the study folder. Make a copy of the summary sheet and tape into the study notebook. Back up all data and spreadsheets onto study disk and backup disks.

11.4 Electronic Data

11.4.1 GLP studies: Electronic data is copied onto the Study floppy disk for each study, and also data is copied onto a floppy disk that is stored in the lab.

11.4.2 Other studies: All data is copied onto a floppy disk that is stored in the lab.

12.0 ATTACHMENTS

None

13.0 REFERENCES

13.1 AMDT-M-1, Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer-Liver

13.2 Skalar Methods, #335, Skalar Methods Manual

13.3 AMDT-EP-26, Operation and Maintenance of the Skalar Segmented Flow Analyzer

14.0 REVISIONS

Revision Number	Reason for change	Revision Date

3M Environmental Laboratory

Method

Thermal Extraction of Fluoride by Means of a Modified Dohrmann DX2000 Organic Halide Analyzer - Serum

Method Identification Number: AMDT-M-14 **Adoption Date:** 10-3-95

Revision Number: 0 **Revision Date:** None

Author: Rich Youngblom

Approved by:

James D Johnson 10/3/95
Group Leader Date

E.A. Van Buskirk 9-27-95
Quality Assurance Date

Software: MS Word 5.1a

Affected Documents: AMDT-M-2 Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
AMDT-EP-3 Routine Maintenance of a Modified Dohrmann DX2000 Organic Halide Analyzer

T.E. SP2 11/28/95

1.0 SCOPE , APPLICABLE COMPOUNDS, AND MATRICES

1.1 Scope: This method is for the operation of a Dohrmann DX2000 when it is used to extract fluoride from various matrices. The fluoride is typically collected in TISAB solution for analysis with an ion selective electrode.
1.2 Applicable Compounds: Fluorochemicals or other fluorinated compounds.
1.3 Matrices: Biological fluids, particularly serum.

2.0 KEYWORDS

2.1 Fluoride, fluorine, extraction, pyrolysis, ionization, ion selective electrode, Dohrmann, halide, DX2000, fluorochemicals.

3.0 PRECAUTIONS

3.1 Glassware and exhaust gases can be extremely hot.
3.2 Glassware is fragile, broken glass may cause injuries.
3.3 Pressurized gases, proper compressed gas handling practices required.
3.4 Solvent based samples may flash, may need to allow them to dry down before starting run.
3.5 Potential biohazards due to the biological matrices. Use appropriate personal protective equipment.

4.0 SUPPLIES AND MATERIALS

4.1 Compressed Oxygen, Hydrocarbon free, regulated to 30 PSI.
4.2 Compressed Helium, High Purity Grade, regulated to 45 PSI.
4.3 Quartz glass sample boat with Teflon™ tubing, Dohrmann 890-097 or equivalent.
4.4 Quartz glass combustion tube, Reliance Glass G-9405-012 or equivalent.
4.5 Orion 940999 Total Ionic Strength Adjustment Buffer (TISAB II) or equivalent.
4.6 Sample collection vials, HDPE.
4.7 Milli-Q™ water
4.8 Polystyrene pipettes.
4.9 Activated Charcoal, E. Merck 2005 or equivalent.
4.10 Hamilton Syringe or equivalent.
4.11 Miscellaneous laboratory glassware

5.0 EQUIPMENT

5.1 Rosemount Dohrmann DX2000 Organic Halide Analyzer, modified for fluoride extraction.
5.2 IBM compatible 386 or 486 computer.
5.3 DX2000 software, version 1.00, modified for fluoride extraction.
5.4 Excel Spreadsheet, version 5.0 or greater

6.0 INTERFERENCES

6.1 Sample size is limited to approximately 100 µl. This may vary from matrix to matrix.

7.0 SAMPLE HANDLING

7.1 Samples are to be handled with plastic pipettes. A new pipette is to be used for each sample.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Calibration Standards

8.1.1 The standards required for each project will need to be appropriate for that individual project. Refer to protocol for that project.
8.1.2 Typically 50-500 ppm FC-95 in methanol standards are used.
8.1.3 For rabbit serum studies, use beef serum as the matrix.

8.2 Calibration - Overview

The normal calibration is the fluoride curve (AMDT-M-2). However, if an optional spiked serum curve is required the procedure listed below is used.

8.2.1 A calibration curve for the DX2000 is generated by spiking samples with known standards and combusting them using the same methods and matrix type as the samples to be tested.
8.2.2 Typically, three replicates of each standard and five concentrations of standards will be spiked.
8.2.3 Standard curve will be plotted as Mass Spiked F (ug) on the x-axis and Standard Mass Recovered F (ug) on the y-axis. Generate a regression curve and calculate the equation for the line and the r^2 value.
8.2.4 Mass Spiked F (ug) = (Amount spiked in mL) x (Conc. of standard in ppm) x (0.6004)*
*FC-95 is 60.04% F therefore 0.6004 is the factor used to convert FC-95 to F
8.2.5 Standard Mass Recovered F (ug) = (TISAB volume in mL) x (Orion reading in ppm)

8.3 Calibration - Procedure

8.3.1 Start Up
8.3.1.1 Run 2 or more Clean Cycles when starting instrument each day. More clean cycles may be used if the previous samples contained high concentrations of fluoride.

8.3.2 Blanks
8.3.2.1 Prepare sample using the same methods and type of matrix as the test sample.
8.3.2.2 For rabbit studies, use beef serum as the matrix.
8.3.2.3 Put serum blank in Dohrmann boat. Combust sample as described in section 9.0 and analyze sample according to method AMDT-M-2 for the ion selective electrode analysis.
8.3.2.4 For rabbit studies, the meter reading for a blank sample should be 0.03 ppm or lower before proceeding with the calibration. Burn samples until this limit is reached, or until in the judgement of the operator the reading is stable with respect to historical readings (previous 48 hours).
8.3.2.5 For non-rabbit studies, the blank readings should reach a predetermined ion concentration before proceeding with the calibration.
8.3.2.6 It may be necessary to mix approximately 50 mg of charcoal with the sample to aid combustion.

8.3.3 Standard Curve

8.3.3.1 If beef serum is frozen, thaw at least enough to complete the standard curve analysis for the day (≈30 mL).

8.3.3.2 Pipette 100µL of beef serum into Dohrmann sample boat.

8.3.3.3 Start with the lowest standard concentration. Using a Hamilton syringe, eject a fixed quantity of the standard on or in the matrix. For rabbit studies, use 4 uL of standard and eject it on or in the beef serum.

8.3.3.4 At least 3 replicates should be used for the lowest standard concentration; more replicates may be used at the discretion of the analyst.

8.3.3.5 Combust the sample as described in section 9.3 and analyze according to AMDT-M-2.

8.3.3.6 Run all 15 standards. If one replicate is significantly different from the other two replicates, run another sample for that standard. Indicate in data that the new replicate replaces the old replicate and that the new replicate will be used to calculate the regression curve.

8.3.3.7 When all standards have been run, calculate the r^2. r^2 must be at least 0.95. If it is not at least 0.95, consult with supervisor.

8.3.3.8 A new standard curve should be run when the combustion tube or sample matrix is changed. New standard curve may also be run at the discretion of the analyst.

8.4 Storage Conditions for Standards

8.4.1 Storage requirements for standards are dependent on the individual standards used. Typically, standards are stored at room temperature in plastic screw top bottles.

8.4.2 New FC-95 standards should be prepared at least once a month.

9.0 PROCEDURES

9.1 Typical Operating Conditions:

9.1.1 Combustion tube temperature = 950°C.

9.1.2 Oxygen and Helium flow = 50 cc/minute.

9.1.3 Vaporization/Drying time = 240 seconds.

9.1.4 Bake time = 300 seconds.

9.2 Start Up Procedure:

9.2.1 If the program is not started, start the EOX program on the PC.

9.2.2 Open the SYSTEM SETUP window.

9.2.3 Put the furnace module and the cell in the READY mode.

9.2.4 Close the SYSTEM SETUP window.

9.2.5 When the oven has reached the READY temperature, run the CLEAN BOAT program found in the CELL CHECK menu.

9.2.6 See AMDT-EP-3 for details of the Dohrmann software.

9.3 Sample Extraction Procedure:

9.3.1 Open the SAMPLE HATCH and pipette 100µL of sample into the BOAT. It may be necessary to mix approximately 50 mg of charcoal with the sample to aid combustion. If this is done, charcoal should also be mixed in while establishing the baseline and when generating the standard curve.

9.3.2 Close SAMPLE HATCH.

9.3.3 Add appropriate volume of TISAB solution or 1:1 TISAB:Milli-Q™ water mixture to a labeled sample collection vial. Typically 0.6 mL to 15 mL are used. For rabbit studies, use 1.0 or 2.0 mL of 1:1 TISAB:Milli-Q™ water mixture.
9.3.4 Place the vial so that the tip of the COMBUSTION TUBE is in the TISAB at least 0.25 inches. Gases released during pyrolysis must bubble through the TISAB.
9.3.5 Run the EOX-WATER program found in the RUN menu.
9.3.6 When the EOX program is finished, remove the collection vial from the combustion tube.
9.3.7 If undiluted TISAB was used to collect the sample, add an equal volume of Milli-Q™ water to the TISAB to make 1:1 TISAB:Milli-Q™.
9.3.8 Rinse the end of the combustion tube with Milli-Q™ water and wipe with a KIMWIPE to remove any TISAB remaining on the tube.
9.3.9 Open the sample hatch and remove any remaining ash from the boat. Ash can be removed with a cotton tipped applicator and/or vacuumed out. It may be necessary to scrap particles off the bottom with a spatula or other similar device. A drop of Milli-Q™ water may be added to the boat to aid in the Clean Cycle.
9.3.10 Close the hatch.
9.3.11 Run the CLEAN BOAT program.
9.3.12 Sample is ready for analysis by ion selective electrode (AMDT-M-2).

9.4 Sample Calculations

9.4.1 Use the standard curve to calculate the sample value.
9.4.2 Sample Mass Recovered F (ug) = (TISAB vol in mL) x $\frac{\text{(Orion reading in ppm - intercept)}}{\text{(Slope)}}$

10.0 VALIDATION

10.1 Quality Control

10.1.1 Daily Start Up Check Samples: Once the standard curve is established, each day of analysis is started by analyzing QC samples. The QC samples are to be the same as the lowest concentration spiked samples used to generate the standard curve. Each concentration must be done in triplicate unless the first two replicates are within 20% of the standard curve, then a third replicate is not necessary.

10.2 Precision and Accuracy: See method development analysis and sample analysis in Fluoride Notebooks 2,3, and 5. Precision and accuracy varies when analyzing samples of different matrices and different reference compounds.

10.3 Other Validation Parameters: NA

11.0 DATA ANALYSIS

11.1 Calculations

11.1.1 For the standard curve, use regression analysis in Excel, version 5.0 or greater.
11.1.2 To calculate the fluoride contraction in the sample, see method AMDT-M-2.

11.2 Analyzing the Data

11.2.1 r^2 must be at least 0.95 or greater. "Outliers" may be excluded if two of the three replicates are within 20% of each other and the outlier is greater than 200% of the average of those two or less than 50% of the average of those two. Any such outliers should be pointed out in the data and noted in the Final Report along with the reason it was considered an outlier.

12.0 ATTACHMENTS

None

13.0 REFERENCES

13.1 Rosemount Dohrmann DX2000 Organic Halide Analyzer Operator's Manual (Manual 915-349, revision B, December 1993)
13.2 AMDT-M-2 Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer
13.3 AMDT-EP-3 Routine Maintenance of a Modified Dohrmann DX2000 Organic Halide Analyzer

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

9.3 Quality Assurance Unit Statement

Attachment D

GLP Study
Quality Assurance Statement

Completed by: QAU Auditor Original to: Study Director Copies to: QAU Files

Study Title: **Single-dose Intravenous Pharmacokinetic Study of T-6067 in Rabbits**

Study Number: AMDT-120694.1 Name of Auditor: Kari Rambo

This study has been inspected by the Quality Assurance Unit as indicated in the following table. The findings were reported to the study director and management.

Inspection Dates From	To	Phase	Date Inspection Reported to Management	Study Director
11-09-95	11-13-95	Final Report	11-13-95	11-13-95

Kari Rambo 11-13-95
QAU Auditor Date

000074

9.4 Key Personnel Involved in the Study

3M Environmental Laboratory

Key Personnel

Thermal extraction followed by analysis using Orion ion analyzer:

Jim Johnson
Deb Wright
Rich Youngblom
Deann Plummer

Analysis of liver extracts using electrospray mass spectrometry:

Jim Johnson
Dave Christenson

Thermal extraction followed by analysis using Skalar segmented flow analyzer with ion selective electrode:

Jim Johnson
Deb Wright
Rich Youngblom
Deann Plummer

Documentation and Reporting:

Jim Johnson
Rich Youngblom

Quality Assurance Unit:

Gale Van Buskirk
Cynthia Weber
Kari Rambo

9.11 Data

9.11.1 Summary and raw data; ug F^- in whole liver as determined by thermal extraction followed by analysis using Orion ion analyzer.

Summary of Combustion Data - Liver
AMDT-120694.1, HWI 6329-151
As Referenced in Final Report section 6.0 *DATA ANALYSIS*

Total ug Fluoride in Whole Liver
Mean per Dose Group

	ug
Control Group	19.7
4.0 mg/kg dose (T6067)	42.9
16 mg/kg dose (T6067)	66.4
24 mg/kg dose (T6067)	54.4

L13492 PK ID	% rcvry	Actual ppm F- in liver (W/W)	Average ppm F- in liver (W/W)	liver burned (grams)	Whole liver weight (grams)	Total F- in whole liver (µg)	Dosage (mg/kg)
liver blk 1		0.501		0.145			
liver blk 2		0.286		0.150			
liver blk 3		0.262		0.136			
liver spike 1	91%	1.08		0.127			
liver spike 2	94%	1.00		0.143			
liver spike 3	84%	0.985		0.129			
F52762 - 1		0.384		0.120	67.7		
F52762 - 2		0.245	0.290	0.136	67.7	19.7	0.0
F52762 - 3		0.242		0.142	67.7		
F52763 - 1		0.732		0.111	73.9		
F52763 - 2		0.483	0.580	0.146	73.9	42.9	4.0
F52763 - 3		0.524		0.133	73.9		
F52768-1		0.727		0.123	85.7		
F52768-2		0.794	0.775	0.133	85.7	66.4	16
F52768-3		0.803		0.115	85.7		
F52770-1		0.806		0.130	69.2		
F52770-2		0.775	0.786	0.134	69.2	54.4	24
F52770-3		0.776		0.142	69.2		
liver spike 4	96%	1.07		0.136			
liver spike 5	87%	1.19		0.111			
Liver blank		0.228		0.123			

9.11.2 Summary and raw data; ppm F^- in serum as determined by thermal extraction followed by analysis using Orion ion analyzer.

HWI 6329-151
AMDT 120694.1
Dohrmann Serum Analysis
Analysis Dates: 07/27/95 - 07/31/95

All serum samples were thermally extracted by a modified Dohrmann DX2000 Organic Halide Analyzer and collected in a 1:1 milli Q water and TISAB solution. The samples were measured on an Orion EA940 expandable ion analyzer. The Dohrmann was calibrated using 34ppm, 40ppm, 62ppm, 100ppm, 124ppm, 250ppm, and 500ppm FC-95 standards. The Orion was calibrated by direct measurement with no blank correction using 0.05ppm, 0.1ppm, 0.5ppm, 1.0ppm and 1.5ppm F^- standards. The slope, intercept, and correlation were recorded in the appropriate logbook.

A summary table is included, showing the ppm F^- in each sample (see page 2). The value of "ND" has been entered for any sample with an Orion reading of below 0.05. An initial calibration curve with standard deviation, %RSD, R^2 value and equation of the line is on pages 3 and 4.

Pages 5 and 6 show the excel spreadsheet that was generated when the samples were being analyzed. Pages 7 and 8 show the same spreadsheet with "ND" inserted where the Orion reading is below 0.05.

Dawn K. Plummer

Charts on pages 3, 4 & 5 were added to report on 11/28/95. Pages were renumbered to reflect changes DP² 11/28/95

T.E. DP² 11/28/95

L13492 PK

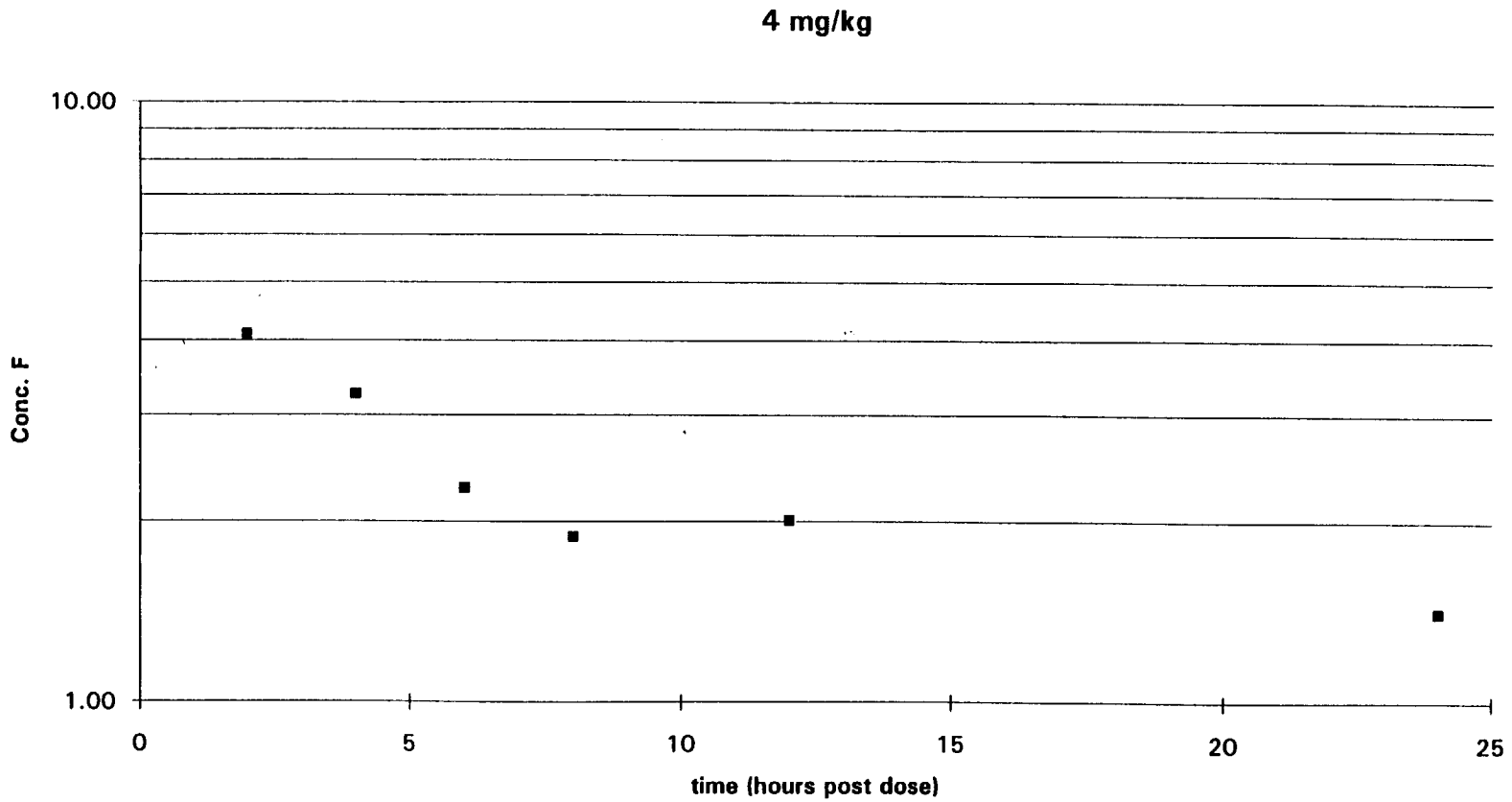
HWI 6329-151

Fluoride concentration in rabbit serum (ppm F-)

	Sample	2.0 hour	4.0 hour	6.0 hour	8.0 hour	12.0 hour	24.0 hour	48.0 hour
Dosage: 0mg/kg	F52762	1.25	5.84	ND	3.56	2.98	1.22	ND
Dosage: 4 mg/kg	F52763	4.09	3.25	2.27	1.89	2.01	1.41	ND
Dosage: 16 mg/kg	F52768	14.9	8.05	4.39	2.87	2.72	ND	ND
Dosage: ~~160~~ 24 mg/kg	F52770	41.0	19.4	12.7	8.08	4.40	ND	ND

SPZ 11/9/95

PSY
11-13-95



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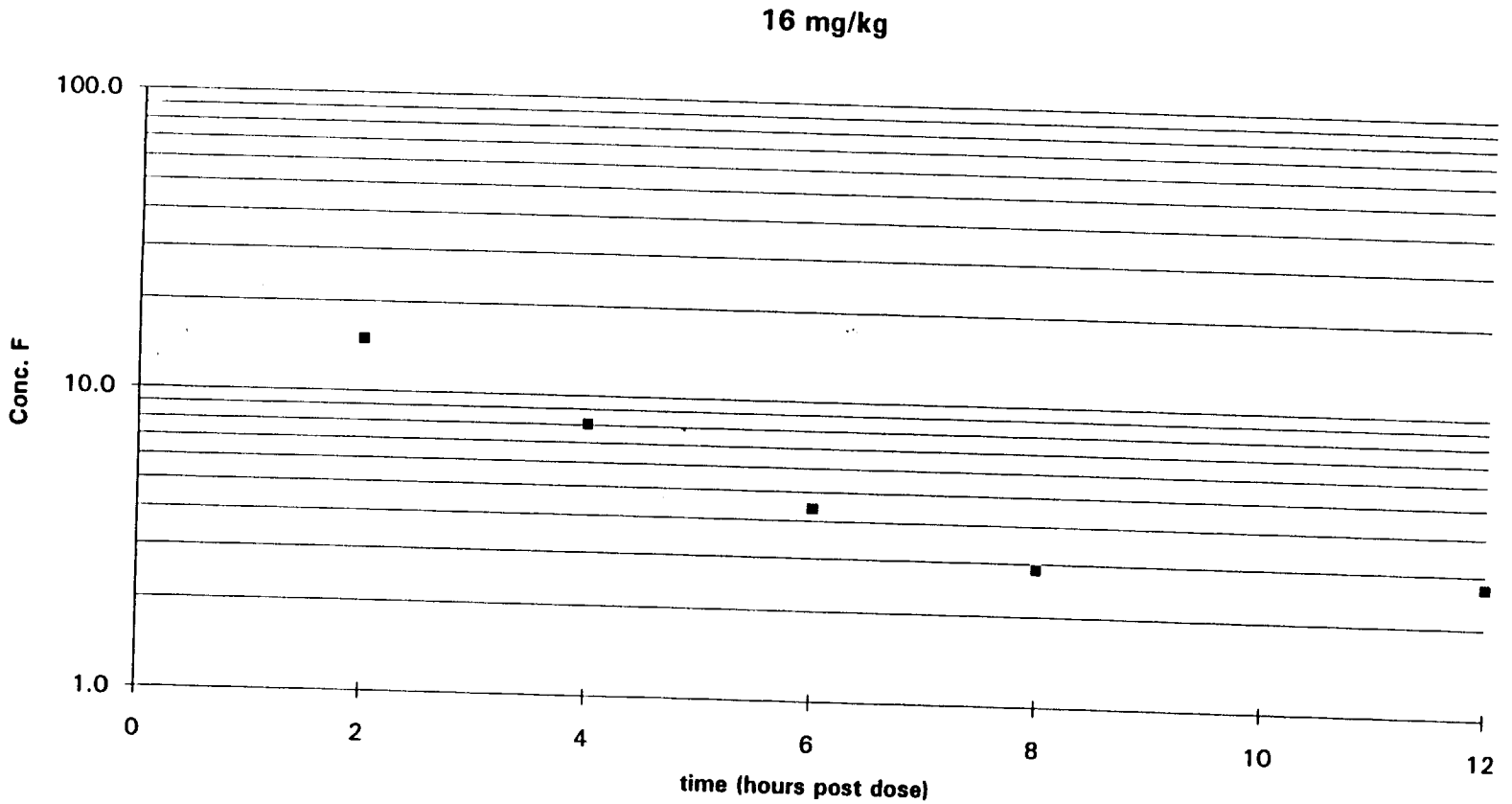

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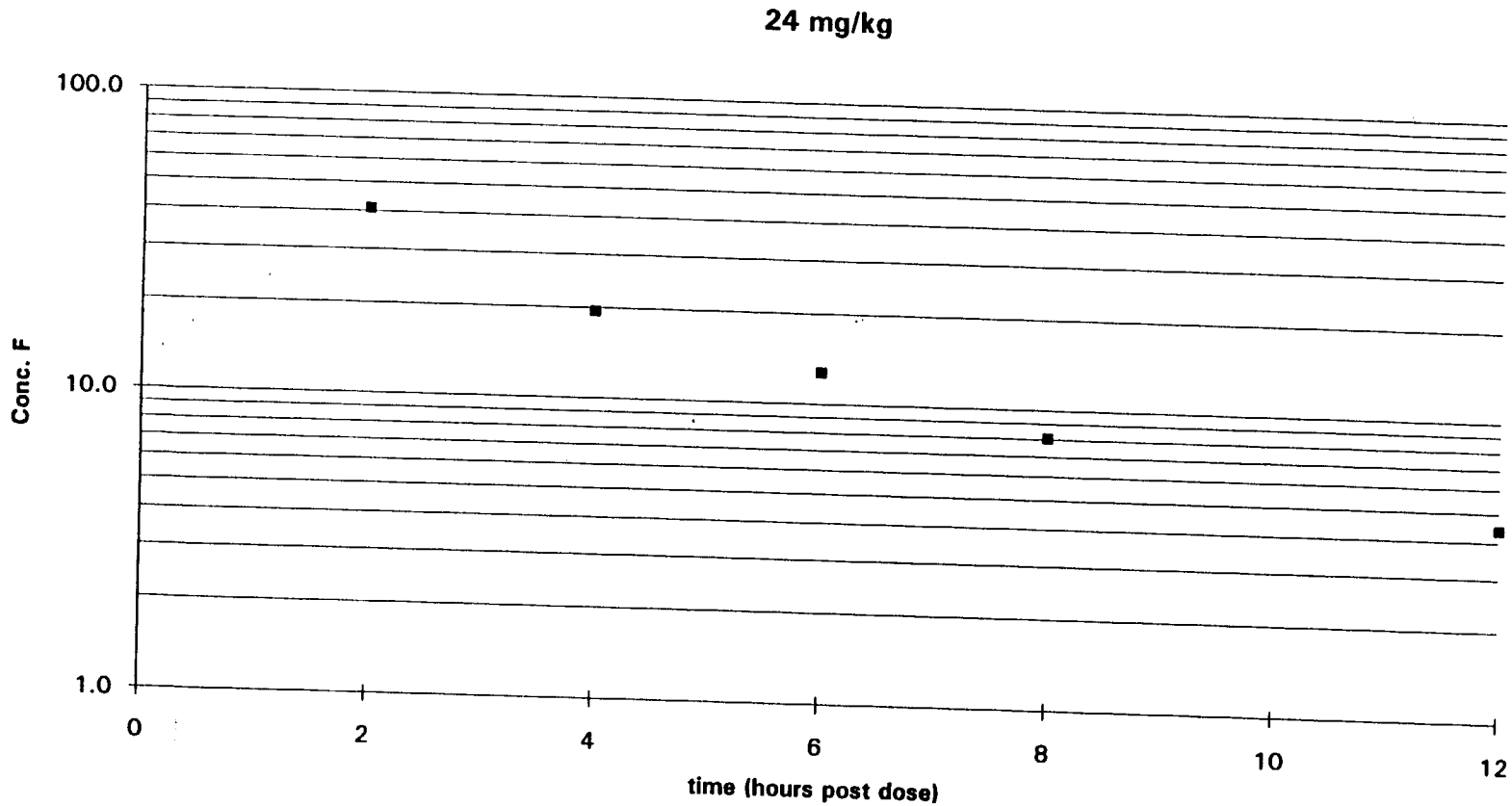
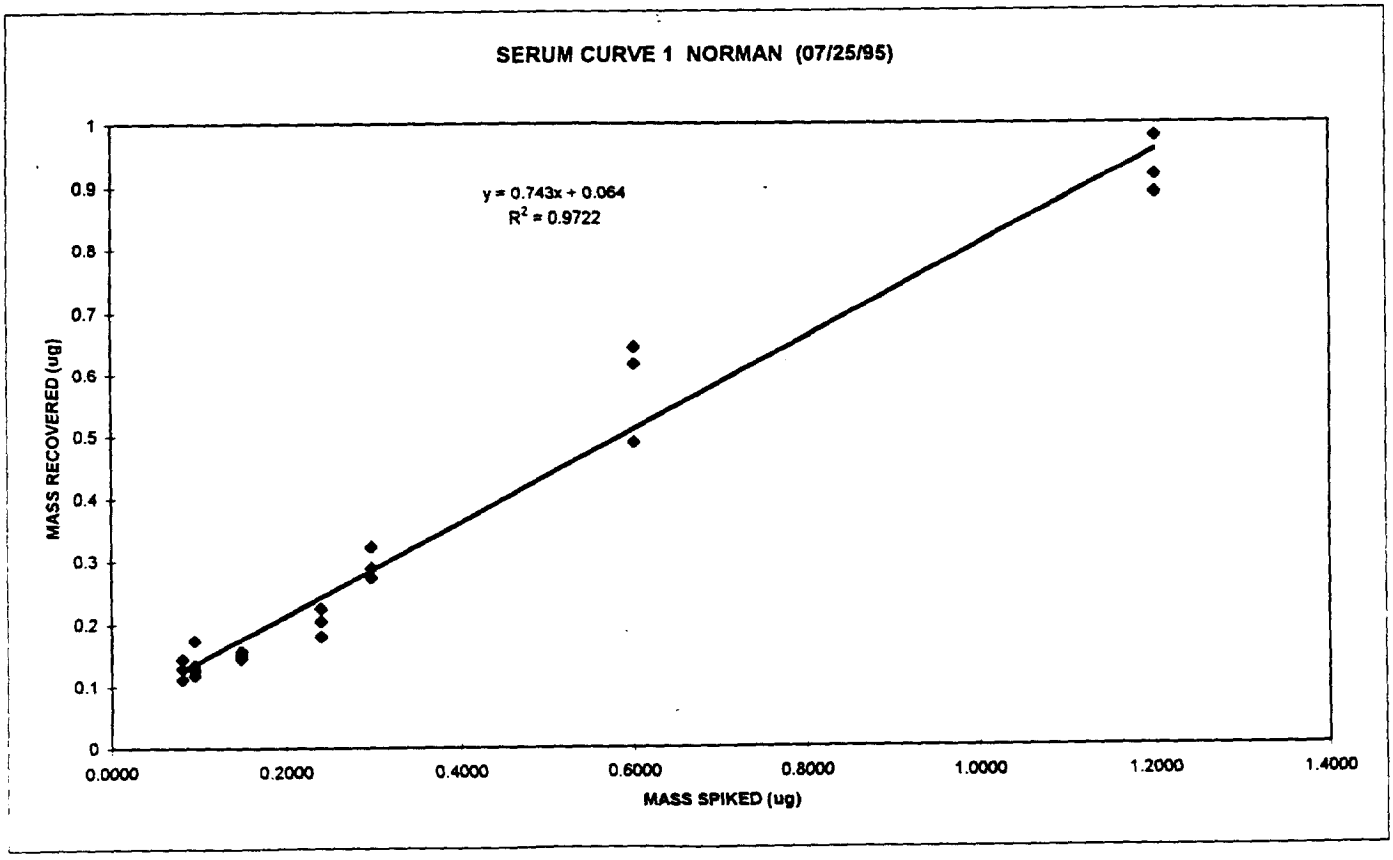

24 mg/kg
100.0
10.0
1.0
Conc. F
0
2
4
6
8
10
12
time (hours post dose)

NORMAN SERUM CURVE 1

Sample ID	Actual reading (ppm F-)	Sample Qty (mL or g)	TISAB final vol (mL)	mL FC95 spiked	Conc. FC95 solution (ppm)	% recovery (ug/ug)	Actual ppm F- in sample	Mass spiked (ug F-)	Mass recovered (ug F-)		
34-ppm-1	0.07175	0.1	2.0	0.004	34	176%	1.4350	0.0817	0.1435	STDEV:	0.015629
34-ppm-2	0.05614	0.1	2.0	0.004	34	138%	1.1228	0.0817	0.11228	%RSD:	12
34-ppm-3	0.06462	0.1	2.0	0.004	34	158%	1.2924	0.0817	0.12924		
40-ppm-1	0.08668	0.1	2.0	0.004	40	180%	1.7336	0.0961	0.17336	STDEV:	0.024057
40-ppm-2	0.06728	0.1	2.0	0.004	40	140%	1.3456	0.0961	0.13456	%RSD:	17
40-ppm-3	0.05939	0.1	2.0	0.004	40	124%	1.1878	0.0961	0.11878		
40-ppm-4	0.06385	0.1	2.0	0.004	40	133%	1.2770	0.0961	0.1277		
62-ppm-1	0.07291	0.1	2.0	0.004	62	98%	1.4582	0.1489	0.14582	STDEV:	0.005495
62-ppm-2	0.0753	0.1	2.0	0.004	62	101%	1.5060	0.1489	0.1506	%RSD:	3.6
62-ppm-3	0.07839	0.1	2.0	0.004	62	105%	1.5678	0.1489	0.15678		
100-ppm-1	0.0902	0.1	2.0	0.004	100	75%	1.8040	0.2402	0.1804	STDEV:	0.022443
100-ppm-2	0.1026	0.1	2.0	0.004	100	85%	2.0520	0.2402	0.2052	%RSD:	11
100-ppm-3	0.1126	0.1	2.0	0.004	100	94%	2.2520	0.2402	0.2252		
124-ppm-1	0.1371	0.1	2.0	0.004	124	92%	2.7420	0.2978	0.2742	STDEV:	0.025096
124-ppm-2	0.1451	0.1	2.0	0.004	124	97%	2.9020	0.2978	0.2902	%RSD:	8.5
124-ppm-3	0.1617	0.1	2.0	0.004	124	109%	3.2340	0.2978	0.3234		
250-ppm-1	0.3217	0.1	2.0	0.004	250	107%	6.4340	0.6004	0.6434	STDEV:	0.082072
250-ppm-2	0.2447	0.1	2.0	0.004	250	82%	4.8940	0.6004	0.4894	%RSD:	14
250-ppm-3	0.3078	0.1	2.0	0.004	250	103%	6.1560	0.6004	0.6156		
500-ppm-1	0.4438	0.1	2.0	0.004	500	74%	8.8760	1.2008	0.8876	STDEV:	0.045915
500-ppm-2	0.4584	0.1	2.0	0.004	500	76%	9.1680	1.2008	0.9168	%RSD:	5.0
500-ppm-3	0.4888	0.1	2.0	0.004	500	81%	9.7760	1.2008	0.9776		



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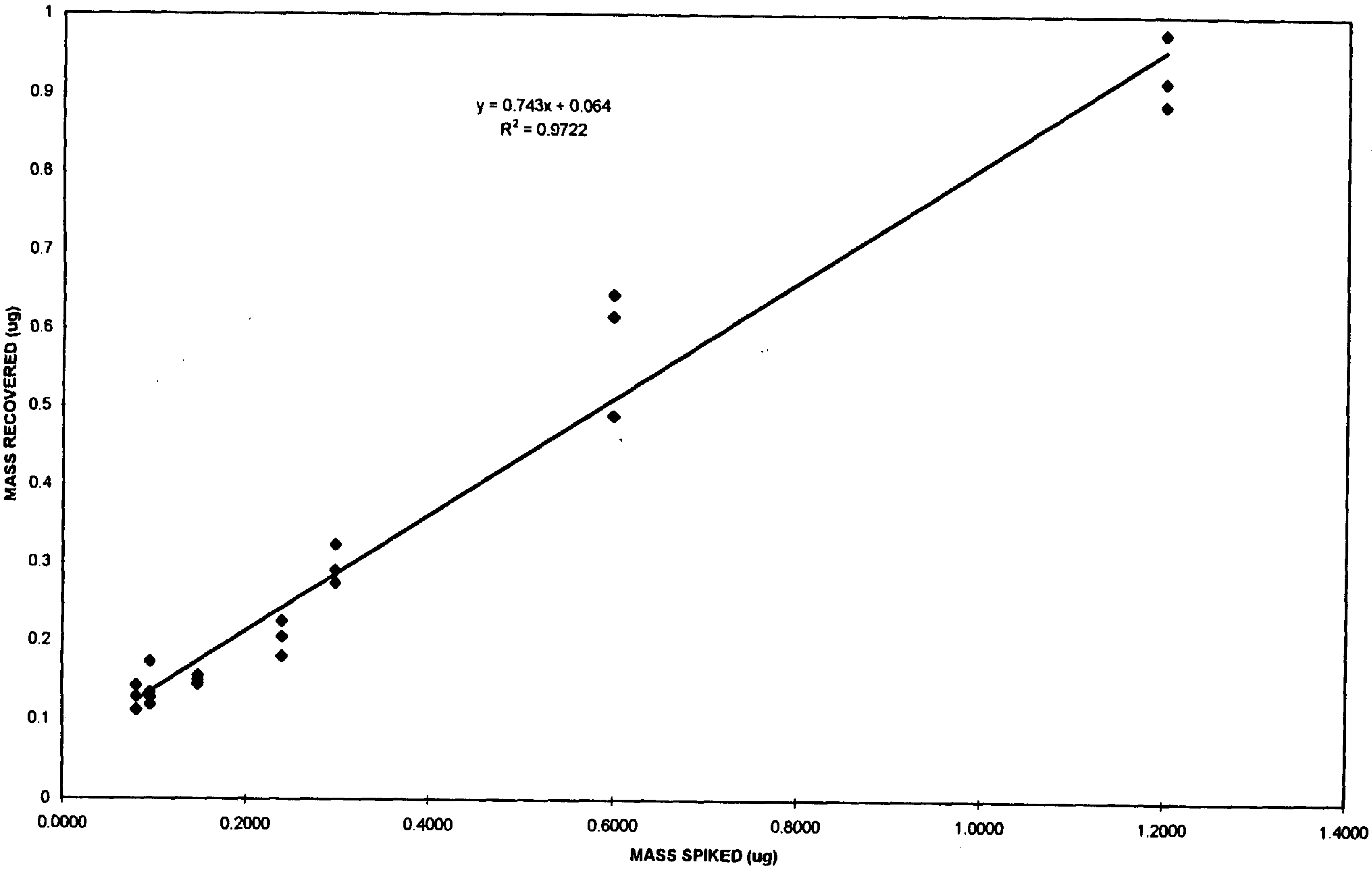
T.E 8/2 11/28/95 B

SERUM CURVE 1 NORMAN (07/25/95)



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Sample ID	Actual reading (ppm F-)	Sample Qty (mL or g)	TISAB final vol (mL)	mL FC95 spiked	Conc. FC95 solution (ppm)	% recovery (ug/ug)	Actual ppm F- in sample	Mass spiked (ug F-)	Mass recovered (ug F-)
BLANK-1	0.0507	0.1	2.0				1.01		0.101
BLANK-2	0.0355	0.1	2.0				0.710		0.0710
BLANK-3	0.0344	0.1	2.0				0.689		0.0689
BLANK-4	0.0308	0.1	2.0				0.617		0.0617
BLANK-5	0.0353	0.1	2.0				0.707		0.0707
BLANK-6	0.0290	0.1	2.0				0.581		0.0581
62-PPM-1	0.0856	0.1	2.0	0.004	62	115%	1.71	0.15	0.171
62-PPM-2	0.110	0.1	2.0	0.004	62	148%	2.20	0.15	0.220
62-PPM-3	0.0835	0.1	2.0	0.004	62	112%	1.67	0.15	0.167
62-PPM-4	0.0778	0.1	2.0	0.004	62	104%	1.56	0.15	0.156
62-PPM-5	0.0746	0.1	2.0	0.004	62	100%	1.49	0.15	0.149
62-PPM-6	0.0718	0.1	2.0	0.004	62	96%	1.44	0.15	0.144
250-PPM-1	0.190	0.1	2.0	0.004	250	63%	3.80	0.60	0.380
250-PPM-2	0.213	0.1	2.0	0.004	250	71%	4.27	0.60	0.427
250-PPM-3	0.223	0.1	2.0	0.004	250	74%	4.47	0.60	0.447
F52762-2.0HR	0.0626	0.1	2.0				1.25		0.125
F52763-2.0HR	0.205	0.1	2.0				4.09		0.409
F52768-2.0HR	0.746	0.1	2.0				14.9		1.49
F52770-2.0HR	2.05	0.1	2.0				41.0		4.10
F52762-4.0HR	0.292	0.1	2.0				5.84		0.584
F52763-4.0HR	0.163	0.1	2.0				3.25		0.325
F52768-4.0HR	0.402	0.1	2.0				8.05		0.805
F52770-4.0HR	0.971	0.1	2.0				19.4		1.94
62-PPM-1	0.189	0.1	2.0	0.004	62	254%	3.78	0.15	0.378
62-PPM-2	0.135	0.1	2.0	0.004	62	181%	2.70	0.15	0.270
250-PPM-1	0.221	0.1	2.0	0.004	250	74%	4.42	0.60	0.442
250-PPM-2	0.246	0.1	2.0	0.004	250	82%	4.92	0.60	0.492
62-PPM-3	0.130	0.1	2.0	0.004	62	174%	2.60	0.15	0.260
SERUM BLANK	0.0458	0.1	2.0				0.916		0.0916
SERUM BLANK	0.0352	0.1	2.0				0.703		0.0703
SERUM BLANK	0.0340	0.1	2.0				0.680		0.0680
SERUM BLANK	0.0358	0.1	2.0				0.715		0.0715
SERUM BLANK	0.0350	0.1	2.0				0.700		0.0700
SPIKE 62-1	0.0628	0.1	2.0	0.004	62	84%	1.26	0.15	0.126
SPIKE 62-2	0.0689	0.1	2.0	0.004	62	92%	1.38	0.15	0.138
SPIKE 62-3	0.0715	0.1	2.0	0.004	62	96%	1.43	0.15	0.143
SPIKE 250-1	0.155	0.1	2.0	0.004	250	52%	3.10	0.60	0.310
SPIKE 250-2	0.261	0.1	2.0	0.004	250	87%	5.22	0.60	0.522
SPIKE 250-3	0.217	0.1	2.0	0.004	250	72%	4.34	0.60	0.434
SERUM BLANK	0.0936	0.1	2.0				1.87		0.187
SERUM BLANK	0.0289	0.1	2.0				0.577		0.0577
F52762-6HR	0.0222	0.1	2.0				0.445		0.0445
F52763-6HR	0.114	0.1	2.0				2.27		0.227
F52768-6HR	0.220	0.1	2.0				4.39		0.439
F52770-6HR	0.636	0.1	2.0				12.7		1.27
F52762-8HR	0.178	0.1	2.0				3.56		0.356
F52763-8HR	0.094	0.1	2.0				1.89		0.189
F52768-8HR	0.144	0.1	2.0				2.87		0.287

Sample ID	Actual reading (ppm F-)	Sample Qty (mL or g)	TISAB final vol (mL)	mL FC95 spiked	Conc. FC95 solution (ppm)	% recovery (ug/ug)	Actual ppm F- in sample	Mass spiked (ug F-)	Mass recovered (ug F-)
F52770-8HR	0.404	0.1	2.0				8.08		0.808
F52762-12HR	0.149	0.1	2.0				2.98		0.298
F52763-12HR	0.101	0.1	2.0				2.01		0.201
SPIKE 62-1	0.0920	0.1	2.0	0.004	62	124%	1.84	0.15	0.184
SPIKE 250-1	0.187	0.1	2.0	0.004	250	62%	3.74	0.60	0.374
SPIKE 250-2	0.225	0.1	2.0	0.004	250	75%	4.51	0.60	0.451
F52768-12HR	0.136	0.1	2.0				2.72		0.272
F52770-12HR	0.220	0.1	2.0				4.40		0.440
F52762-24HR	0.0609	0.1	2.0				1.22		0.122
F52763-24HR	0.0704	0.1	2.0				1.41		0.141
F52768-24HR	0.0442	0.1	2.0				0.883		0.0883
F52770-24HR	0.0387	0.1	2.0				0.775		0.0775
F52762-48HR	0.0282	0.1	2.0				0.564		0.0564
F52763-48HR	0.0264	0.1	2.0				0.527		0.0527
F52768-48HR	0.0260	0.1	2.0				0.520		0.0520
F52770-48HR	0.0357	0.1	2.0				0.715		0.0715
BLANK-1	0.0830	0.1	2.0				1.66		0.166
BLANK-2	0.0314	0.1	2.0				0.627		0.0627
SPIKE 62-1	0.0635	0.1	2.0	0.004	62	85%	1.27	0.15	0.127
SPIKE 62-2	0.0644	0.1	2.0	0.004	62	86%	1.29	0.15	0.129
SPIKE250-1	0.157	0.1	2.0	0.004	250	52%	3.15	0.60	0.315
SPIKE250-2	0.239	0.1	2.0	0.004	250	80%	4.78	0.60	0.478
SPIKE250-3	0.240	0.1	2.0	0.004	250	80%	4.80	0.60	0.480

T.E. 98 11/28/95 98

Sample ID	Actual reading (ppm F-)	Sample Qty (mL or g)	TISAB final vol (mL)	mL FC95 spiked	Conc. FC95 solution (ppm)	% recovery (ug/ug)	Actual ppm F- in sample	Mass spiked (ug F-)	Mass recovered (ug F-)
BLANK-1	0.0507	0.1	2.0				1.01		0.101
BLANK-2	0.0355	0.1	2.0				ND		ND
BLANK-3	0.0344	0.1	2.0				ND		ND
BLANK-4	0.0308	0.1	2.0				ND		ND
BLANK-5	0.0353	0.1	2.0				ND		ND
BLANK-6	0.0290	0.1	2.0				ND		ND
62-PPM-1	0.0856	0.1	2.0	0.004	62	115%	1.71	0.15	0.171
62-PPM-2	0.110	0.1	2.0	0.004	62	148%	2.20	0.15	0.220
62-PPM-3	0.0835	0.1	2.0	0.004	62	112%	1.67	0.15	0.167
62-PPM-4	0.0778	0.1	2.0	0.004	62	104%	1.56	0.15	0.156
62-PPM-5	0.0746	0.1	2.0	0.004	62	100%	1.49	0.15	0.149
62-PPM-6	0.0718	0.1	2.0	0.004	62	96%	1.44	0.15	0.144
250-PPM-1	0.190	0.1	2.0	0.004	250	63%	3.80	0.60	0.380
250-PPM-2	0.213	0.1	2.0	0.004	250	71%	4.27	0.60	0.427
250-PPM-3	0.223	0.1	2.0	0.004	250	74%	4.47	0.60	0.447
F52762-2.0HR	0.0626	0.1	2.0				1.25		0.125
F52763-2.0HR	0.205	0.1	2.0				4.09		0.409
F52768-2.0HR	0.746	0.1	2.0				14.9		1.49
F52770-2.0HR	2.05	0.1	2.0				41.0		4.10
F52762-4.0HR	0.292	0.1	2.0				5.84		0.584
F52763-4.0HR	0.163	0.1	2.0				3.25		0.325
F52768-4.0HR	0.402	0.1	2.0				8.05		0.805
F52770-4.0HR	0.971	0.1	2.0				19.4		1.94
62-PPM-1	0.189	0.1	2.0	0.004	62	254%	3.78	0.15	0.378
62-PPM-2	0.135	0.1	2.0	0.004	62	181%	2.70	0.15	0.270
250-PPM-1	0.221	0.1	2.0	0.004	250	74%	4.42	0.60	0.442
250-PPM-2	0.246	0.1	2.0	0.004	250	82%	4.92	0.60	0.492
62-PPM-3	0.130	0.1	2.0	0.004	62	174%	2.60	0.15	0.260
SERUM BLANK	0.0458	0.1	2.0				ND		ND
SERUM BLANK	0.0352	0.1	2.0				ND		ND
SERUM BLANK	0.0340	0.1	2.0				ND		ND
SERUM BLANK	0.0358	0.1	2.0				ND		ND
SERUM BLANK	0.0350	0.1	2.0				ND		ND
SPIKE 62-1	0.0628	0.1	2.0	0.004	62	84%	1.26	0.15	0.126
SPIKE 62-2	0.0689	0.1	2.0	0.004	62	92%	1.38	0.15	0.138
SPIKE 62-3	0.0715	0.1	2.0	0.004	62	96%	1.43	0.15	0.143
SPIKE 250-1	0.155	0.1	2.0	0.004	250	52%	3.10	0.60	0.310
SPIKE 250-2	0.261	0.1	2.0	0.004	250	87%	5.22	0.60	0.522
SPIKE 250-3	0.217	0.1	2.0	0.004	250	72%	4.34	0.60	0.434
SERUM BLANK	0.0936	0.1	2.0				1.87		0.187
SERUM BLANK	0.0289	0.1	2.0				ND		ND
F52762-6HR	0.0222	0.1	2.0				ND		ND
F52763-6HR	0.114	0.1	2.0				2.27		0.227
F52768-6HR	0.220	0.1	2.0				4.39		0.439
F52770-6HR	0.636	0.1	2.0				12.7		1.27
F52762-8HR	0.178	0.1	2.0				3.56		0.356
F52763-8HR	0.094	0.1	2.0				1.89		0.189
F52768-8HR	0.144	0.1	2.0				2.87		0.287

10 F

F.E. Sp² 11/28/95

Sample ID	Actual reading (ppm F-)	Sample Qty (mL or g)	TISAB final vol (mL)	mL FC95 spiked	Conc. FC95 solution (ppm)	% recovery (ug/ug)	Actual ppm F- in sample	Mass spiked (ug F-)	Mass recovered (ug F-)
F52770-8HR	0.404	0.1	2.0				8.08		0.808
F52762-12HR	0.149	0.1	2.0				2.98		0.298
F52763-12HR	0.101	0.1	2.0				2.01		0.201
SPIKE 62-1	0.0920	0.1	2.0	0.004	62	124%	1.84	0.15	0.184
SPIKE 250-1	0.187	0.1	2.0	0.004	250	62%	3.74	0.60	0.374
SPIKE 250-2	0.225	0.1	2.0	0.004	250	75%	4.51	0.60	0.451
F52768-12HR	0.136	0.1	2.0				2.72		0.272
F52770-12HR	0.220	0.1	2.0				4.40		0.440
F52762-24HR	0.0609	0.1	2.0				1.22		0.122
F52763-24HR	0.0704	0.1	2.0				1.41		0.141
F52768-24HR	0.0442	0.1	2.0				ND		ND
F52770-24HR	0.0387	0.1	2.0				ND		ND
F52762-48HR	0.0282	0.1	2.0				ND		ND
F52763-48HR	0.0264	0.1	2.0				ND		ND
F52768-48HR	0.0260	0.1	2.0				ND		ND
F52770-48HR	0.0357	0.1	2.0				ND		ND
BLANK-1	0.0830	0.1	2.0				1.66		0.166
BLANK-2	0.0314	0.1	2.0				ND		ND
SPIKE 62-1	0.0635	0.1	2.0	0.004	62	85%	1.27	0.15	0.127
SPIKE 62-2	0.0644	0.1	2.0	0.004	62	86%	1.29	0.15	0.129
SPIKE250-1	0.157	0.1	2.0	0.004	250	52%	3.15	0.60	0.315
SPIKE250-2	0.239	0.1	2.0	0.004	250	80%	4.78	0.60	0.478
SPIKE250-3	0.240	0.1	2.0	0.004	250	80%	4.80	0.60	0.480

T.G. 11/28/95 11 8

9.11.3 Summary and raw data; ppm F^- in serum as determined by thermal extraction followed by analysis using Skalar segmented flow analyzer with ion selective electrode.

DDW 8/2/95

RE: 6329-151 SERUM SAMPLES
AMDT 120694.1
Date of Analysis: 8/2/95
Analyst: DDW

The samples are burned in the Dohrman at 950 C using 0.10 mL of the serum. The gas is collected in 2.0 mL of 1:1 TISAB/Milli-Q water. The samples are then analyzed on a Skalar Segmented Flow Analyzer using the Ion Specific Electrode (ISE) Method.

TISAB buffer is added to each sample as it proceeds through the system. The sample then goes through a heated mixing coil before the potential between the ion selective electrode and the reference electrode is measured. The signal is amplified and related to the fluoride concentration.

The instrument was calibrated in the ranges of 0.015 - 0.15 ppm and 0.15 - 1.50 ppm fluoride. The standard curve for the high range was plotted using the inverse logarithm option. The standard curve for the low range is linear. All standards and samples were then calculated by the Skalar software using these curves. All results below 0.0001 ppm appear on the raw data as #.####.

A quality control standard was analyzed every 10 samples to check for accuracy and drift.

Raw data is taken from the appropriate calibrated range of the Skalar printout and summarized on an Excel spreadsheet. The final results are adjusted for the collection volume and any subsequent dilutions.

Debra D Wright

8/9/17

aaw 9/2/95
Stolen Data

SUMMARY OF
6329-151
SERUM SAMPLES
AMDT 120694.1

	Sample ID	Fluoride in Sample (ppm) 2 hr	Fluoride in Sample (ppm) 4 hr	Fluoride in Sample (ppm) 6 hr	Fluoride in Sample (ppm) 8 hr	Fluoride in Sample (ppm) 12 hr	Fluoride in Sample (ppm) 24 hr	Fluoride in Sample (ppm) 48 hr
Dose Level : 0	**F52762**	1.56	6.18	ND	3.96	3.44	1.43	0.55
Dose Level : 4 mg/kg	**F52763**	4.10	3.44	2.49	2.24	2.51	1.60	0.46
Dose Level : 16 mg/kg	**F52768**	15.6	8.08	4.72	3.36	2.94	0.96	0.57
Dose Level : ~~160~~ 24 mg/kg	**F52770**	32.9	20.2	14.8	9.26	4.62	0.80	0.82

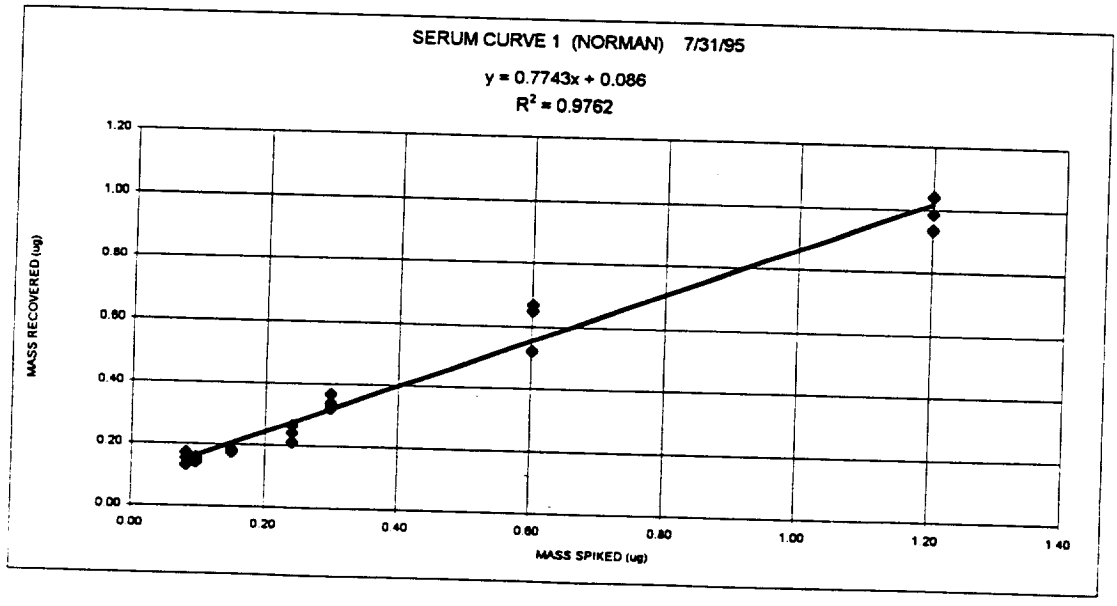
* 11/9/95 aaw
Dose levels in protocol were changed after analysis aaw 11/9/95

000095

DOW 1/2/95
Skalar Data

SERUM CURVE 1
7-31-95
NORMAN

Sample ID	Skalar Result (ppm)	DI:TISAB final vol (mL)	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery		
Spk 34-1	0.09	2.0	0.004	34.00	0.08	0.17	211%	STANDARD DEVIATION :	0.2450
Spk 34-2	0.07	2.0	0.004	34.00	0.08	0.13	163%	% RSD :	12.9998
Spk 34-3	0.08	2.0	0.004	34.00	0.08	0.16	191%		
Spk 40-1	0.08	2.0	0.004	40.00	0.10	0.16	164%	STANDARD DEVIATION :	0.0826
Spk 40-2	0.07	2.0	0.004	40.00	0.10	0.14	147%	% RSD :	5.3307
Spk 40-3	0.07	2.0	0.004	40.00	0.10	0.15	154%		
Spk 62-1	0.09	2.0	0.004	62.00	0.15	0.18	120%	STANDARD DEVIATION :	0.0263
Spk 62-2	0.09	2.0	0.004	62.00	0.15	0.18	119%	% RSD :	2.1670
Spk 62-3	0.09	2.0	0.004	62.00	0.15	0.18	124%		
Spk 100-1	0.11	2.0	0.004	100.0	0.24	0.21	88%	STANDARD DEVIATION :	0.1138
Spk 100-2	0.12	2.0	0.004	100.0	0.24	0.24	100%	% RSD :	11.4530
Spk 100-3	0.13	2.0	0.004	100.0	0.24	0.27	110%		
Spk 124-1	0.16	2.0	0.004	124.0	0.30	0.32	108%	STANDARD DEVIATION :	0.0778
Spk 124-2	0.17	2.0	0.004	124.0	0.30	0.34	114%	% RSD :	6.7516
Spk 124-3	0.18	2.0	0.004	124.0	0.30	0.37	124%		
Spk 250-1	0.33	2.0	0.004	250.0	0.60	0.67	111%	STANDARD DEVIATION :	0.1318
Spk 250-2	0.26	2.0	0.004	250.0	0.60	0.52	87%	% RSD :	12.9196
Spk 250-3	0.32	2.0	0.004	250.0	0.60	0.65	108%		
Spk 500-1	0.47	2.0	0.004	500.0	1.20	0.94	78%	STANDARD DEVIATION :	0.0442
Spk 500-2	0.49	2.0	0.004	500.0	1.20	0.99	82%	% RSD :	5.3672
Spk 500-3	0.52	2.0	0.004	500.0	1.20	1.04	87%		



000096

1995-08-02 13:20 OutPut of : 950802B1
Operator : DDW
Date of the Analysis : 1995-08-02 08:38
Analysis File Name : C:\SKALAR\DATA\HWIDATA\SERUM\950802B1

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% Recovery	DI:TISAB final vol (mL)	Qty Sampl (mL or grams)	Actual ppm F- in Sample	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery
1	Tracer	1.50	1.48	98%								
2	Drift	1.50	1.48	99%								
3	Wash		ND									
4	Standard 1	0.015	0.017	111%								
5	Standard 2	0.03	0.03	92%								
6	Standard 3	0.06	0.06	101%								
7	Standard 4	0.09	0.09	101%								
8	Standard 5	0.12	0.12	99%								
9	Standard 6	0.15	0.15	100%								
10	Standard 7	0.30	0.28	93%								
11	Standard 8	0.60	0.62	103%								
12	Standard 9	1.20	1.23	102%								
13	Standard 10	1.50	1.47	98%								
14	Drift	1.50	1.49	99%								
15	Wash		ND									
16	SERUM BLK 1		0.07		2.0	0.10	1.39					
17	SERUM BLK 2		0.05		2.0	0.10	0.97					
18	SERUM BLK 3		0.05		2.0	0.10	0.96					
19	SERUM BLK 4		0.04		2.0	0.10	0.80					
20	SERUM BLK 5		0.05		2.0	0.10	0.96					
21	SERUM BLK 6		0.04		2.0	0.10	0.78					
22	SPK 62-1		0.11		2.0	0.10	2.17	0.004	62.00	0.15	0.22	146%
23	SPK 62-2		0.12		2.0	0.10	2.45	0.004	62.00	0.15	0.25	165%
24	SPK 62-3		0.10		2.0	0.10	1.94	0.004	62.00	0.15	0.19	131%
25	SPK 62-4		0.09		2.0	0.10	1.89	0.004	62.00	0.15	0.19	127%
26	Drift	1.50	1.47	98%								
27	Wash		ND									
28	SPK 62-5		0.09		2.0	0.10	1.84	0.004	62.00	0.15	0.18	124%
29	SPK 62-6		0.09		2.0	0.10	1.74	0.004	62.00	0.15	0.17	117%
30	SPK 250-1		0.20		2.0	0.10	3.96	0.004	250.00	0.60	0.40	66%

000097

4

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% Recovery	DI TISAB final vol. (mL)	Qty Sampl (mL or grams)	Actual ppm F- in Sample	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F)	Mass Recovered (ug F)	% Recovery
31	SPK 250-2		0.22		2.0	0.10	4.40	0.004	250.00	0.60	0.44	73%
32	SPK 250-3		0.23		2.0	0.10	4.54	0.004	250.00	0.60	0.45	76%
33	F52762-2		0.08		2.0	0.10	1.56					
34	F52763-2		0.21		2.0	0.10	4.10					
35	F52768-2		0.78		2.0	0.10	15.58					
36	F52770-2		1.65		2.0	0.10	32.94					
37	F52762-4		0.31		2.0	0.10	6.18					
38	Drift	1.50	1.47	98%								
39	Wash		ND									
40	F52763-4		0.17		2.0	0.10	3.44					
41	F52768-4		0.40		2.0	0.10	8.08					
42	F52770-4		1.01		2.0	0.10	20.18					
43	SPK 62-1		0.26		2.0	0.10	5.18	0.004	62.00	0.15	0.52	348%
44	SPK 62-2		0.15		2.0	0.10	2.97	0.004	62.00	0.15	0.30	199%
45	SPK 250-1		0.23		2.0	0.10	4.52	0.004	250.00	0.60	0.45	75%
46	SPK 250-2		0.25		2.0	0.10	5.08	0.004	250.00	0.60	0.51	85%
47	SPK 62-3		0.15		2.0	0.10	2.93	0.004	62.00	0.15	0.29	197%
48	SERUM BLK 1		0.06		2.0	0.10	1.20					
49	SERUM BLK 2		0.04		2.0	0.10	0.90					
50	Drift	1.50	1.42	95%								
51	Wash		ND									
52	SERUM BLK 3		0.05		2.0	0.10	0.92					
53	SERUM BLK 4		0.04		2.0	0.10	0.88					
54	SERUM BLK 5		0.04		2.0	0.10	0.80					
55	SPK 62-1		0.07		2.0	0.10	1.47	0.004	62.00	0.15	0.15	99%
56	SPK 62-2		0.08		2.0	0.10	1.62	0.004	62.00	0.15	0.16	109%
57	SPK 62-3		0.08		2.0	0.10	1.65	0.004	62.00	0.15	0.17	111%
58	SPK 250-1		0.17		2.0	0.10	3.38	0.004	250.00	0.60	0.34	56%
59	SPK 250-2		0.28		2.0	0.10	5.64	0.004	250.00	0.60	0.56	94%
60	SPK 250-3		0.23		2.0	0.10	4.62	0.004	250.00	0.60	0.46	77%
61	BLK 1		0.11		2.0	0.10	2.15					
62	Drift	1.50	1.38	92%								
63	Wash		ND									
64	BLK 2		0.04		2.0	0.10	0.74					
65	BLK 3		0.02		2.0	0.10	0.47					
66	F52762-6		ND		2.0	0.10	ND					

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% Recovery	DI TISAB final vol (mL)	Qty Sampl (mL or grams)	Actual ppm F- in Sample	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery
67	F52763-6		0.12		2.0	0.10	2.49					
68	F52768-6		0.24		2.0	0.10	4.72					
69	F52770-6		0.74		2.0	0.10	14.80					
70	F52762-8		0.20		2.0	0.10	3.96					
71	F52763-8		0.11		2.0	0.10	2.24					
72	F52768-8		0.17		2.0	0.10	3.36					
73	F52770-8		0.46		2.0	0.10	9.26					
74	Drift	1.50	1.43	95%								
75	Wash		ND									
76	F52762-12		0.17		2.0	0.10	3.44					
77	F52763-12		0.13		2.0	0.10	2.51					
78	SPK 62-1		0.12		2.0	0.10	2.48	0.004	62.00	0.15	0.25	166%
79	SPK 250-1		0.21		2.0	0.10	4.18	0.004	250.00	0.60	0.42	70%
80	SPK 250-2		0.24		2.0	0.10	4.80	0.004	250.00	0.60	0.48	80%
81	F52768-12		0.15		2.0	0.10	2.94					
82	F52770-12		0.23		2.0	0.10	4.62					
83	F52762-24		0.07		2.0	0.10	1.43					
84	F52763-24		0.08		2.0	0.10	1.60					
85	F52768-24		0.05		2.0	0.10	0.96					
86	Drift	1.50	1.39	93%								
87	Wash		ND									
88	F52770-24		0.04		2.0	0.10	0.80					
89	F52762-48		0.03		2.0	0.10	0.55					
90	F52763-48		0.02		2.0	0.10	0.46					
91	F52768-48		0.03		2.0	0.10	0.57					
92	F52770-48		0.04		2.0	0.10	0.82					
93	Drift	1.50	1.43	95%								
94	Wash		ND									

660099

DDW 9/2/95
AMDT 120694.1
HWI 6329-151
Serum

1995-08-02 13:20 OutPut of : 950802B1

Software : version 6.1 c1990,93

Operator : DDW

Date of the Analysis : 1995-08-02 08:38

Analysis File Name : C:\SKALAR\DATA\HWIDATA\SERUM\950802B1

Fluoride 1.5
Calibration order = Inverse Logarithm

Slope : s = 1.11111

$$\text{Result} = 10^{\left[\frac{x - c1}{s}\right]}$$

x = corrected value of the sample
c1 = corrected value of the concentration 1
s = Slope of the electrode

a2 = -0.00000
a1 = 0.00070
a0 = -1.23789

Fluoride L
Calibration order = 2

Correlation : r = 0.99908

Result = a2 * x^2 + a1 * x + a0

a2 = -0.00000
a1 = 0.00025
a0 = -0.00103

Sampler	Type	: SA1000
	Number	: 1
	Sample Time	: 50 sec.
	Wash Time	: 120 sec.
	Air Time	: 1 sec.
	Take up	: Single
	sPecial	: None
	needle Height	: 70 mm.
Diluter	needle Height	: 80 mm
	dilution Factor	: 10
	dilution Volume	: 2.5 ml.
	Resample	: 1
	Dilution runs	: 1
	User file	: . TXT
	Reproces	: No

1995-08-02 13:20 OutPut of : 95080281

Fluoride 1.5 Path number : 3
Signal type : Debubbled
Decolor : Yes
system Number : 0
diLute : No
Resample : No
dil Threshold : 4095
diG output : 0
Window event : Off

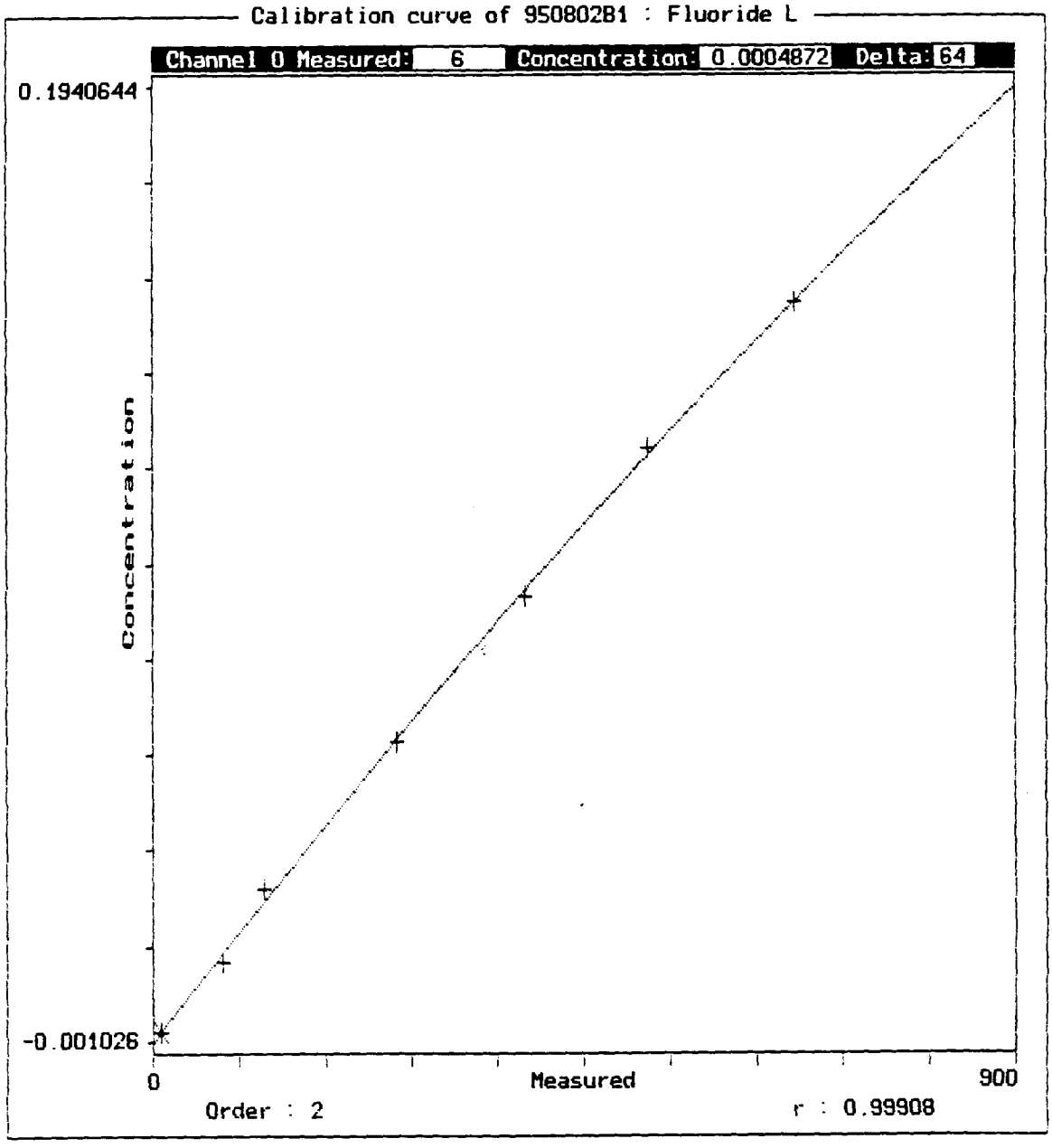
s1 sTandard : Ignore
s2 sTandard : Ignore
s3 sTandard : Ignore
s4 sTandard : Ignore
s5 sTandard : Ignore
s6 sTandard : 0.150
s7 sTandard : 0.300
s8 sTandard : 0.600
s9 sTandard : 1.200
s10 sTandard : 1.500
Order : Inverse Logarithm
Dimension : PPM
start Value : 500 DU
trigger Limit : 1800 Sec
Peak shape : Pointed
stArt ignore : 60 Sec
eNd ignore : 120 Sec
Measure window : 75 %
Filter : No
Regeneration : No
formUla :
output : ##.###

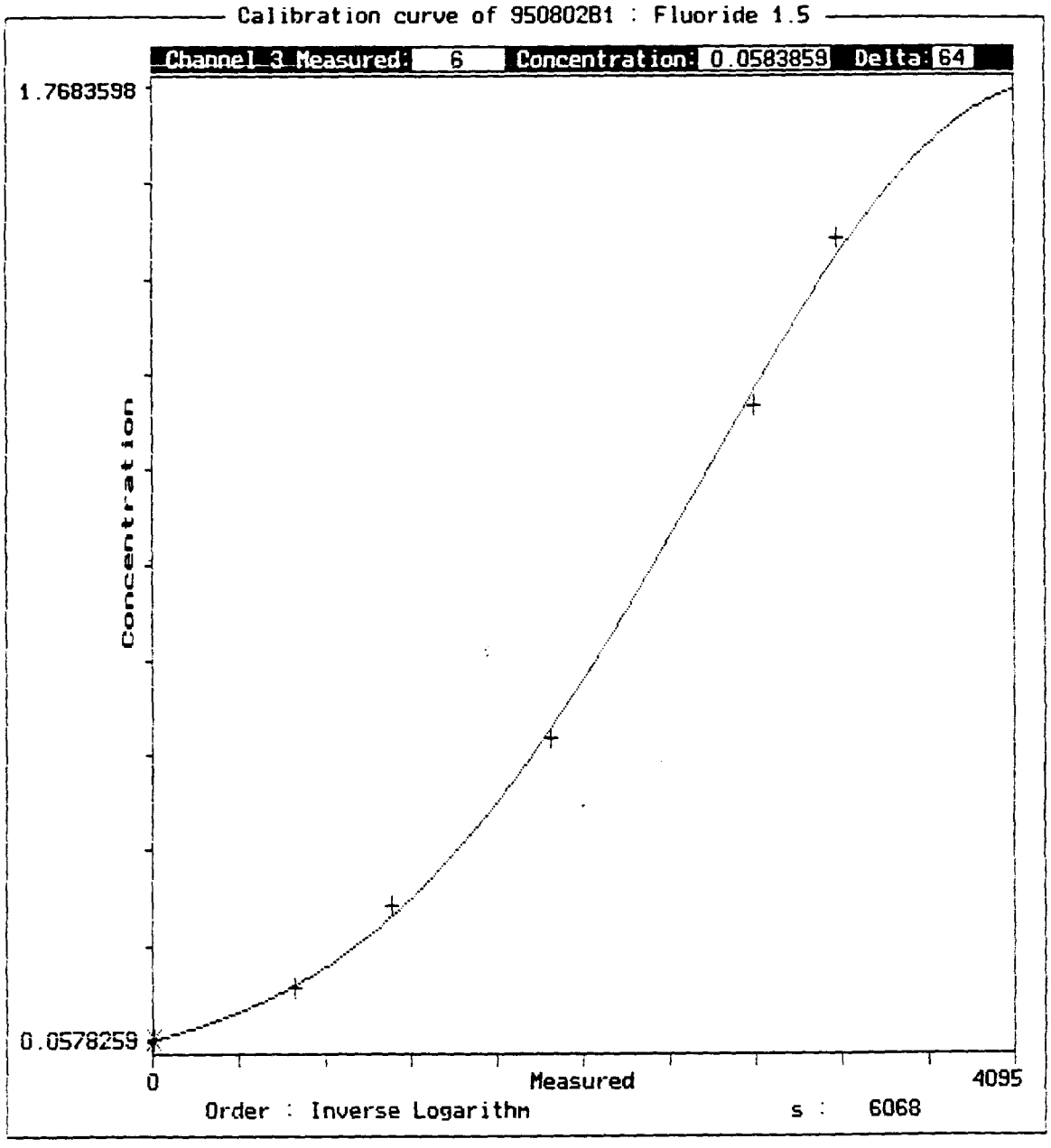
Fluoride L Path number : 0
Signal type : Debubbled
Decolor : No
system Number : 0
diLute : No
Resample : No
dil Threshold : 4095
diG output : 0
Window event : Off

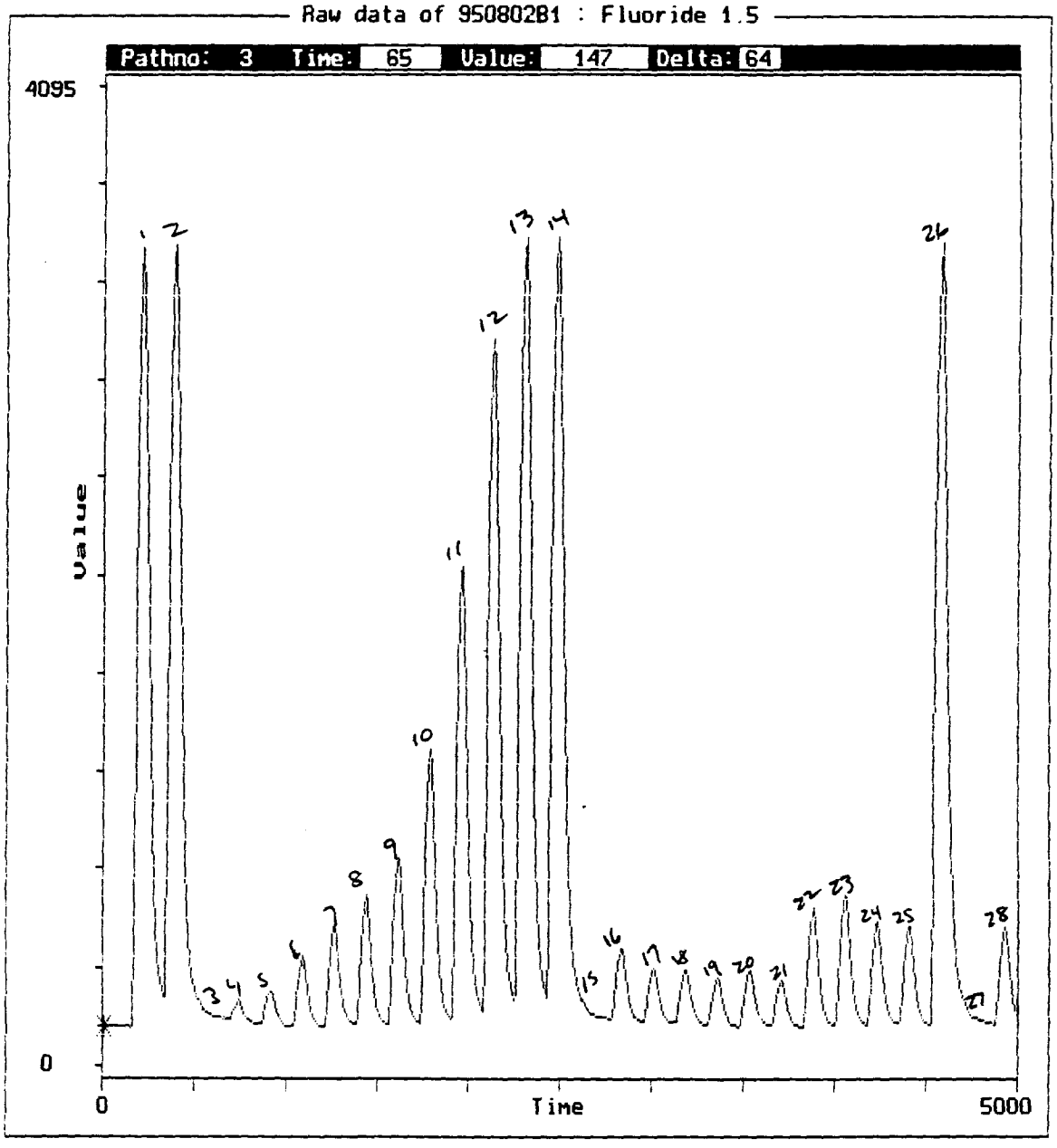
s1 sTandard : 0.015
s2 sTandard : 0.030
s3 sTandard : 0.060
s4 sTandard : 0.090
s5 sTandard : 0.120
s6 sTandard : 0.150
s7 sTandard : Ignore
s8 sTandard : Ignore
s9 sTandard : Ignore
s10 sTandard : Ignore
Order : 2
Dimension : PPM
start Value : 500 DU
trigger Limit : 1800 Sec
Peak shape : Pointed
stArt ignore : 60 Sec
eNd ignore : 120 Sec
Measure window : 75 %
Filter : No
Regeneration : No
formUla : c4:=c3
output : #.####

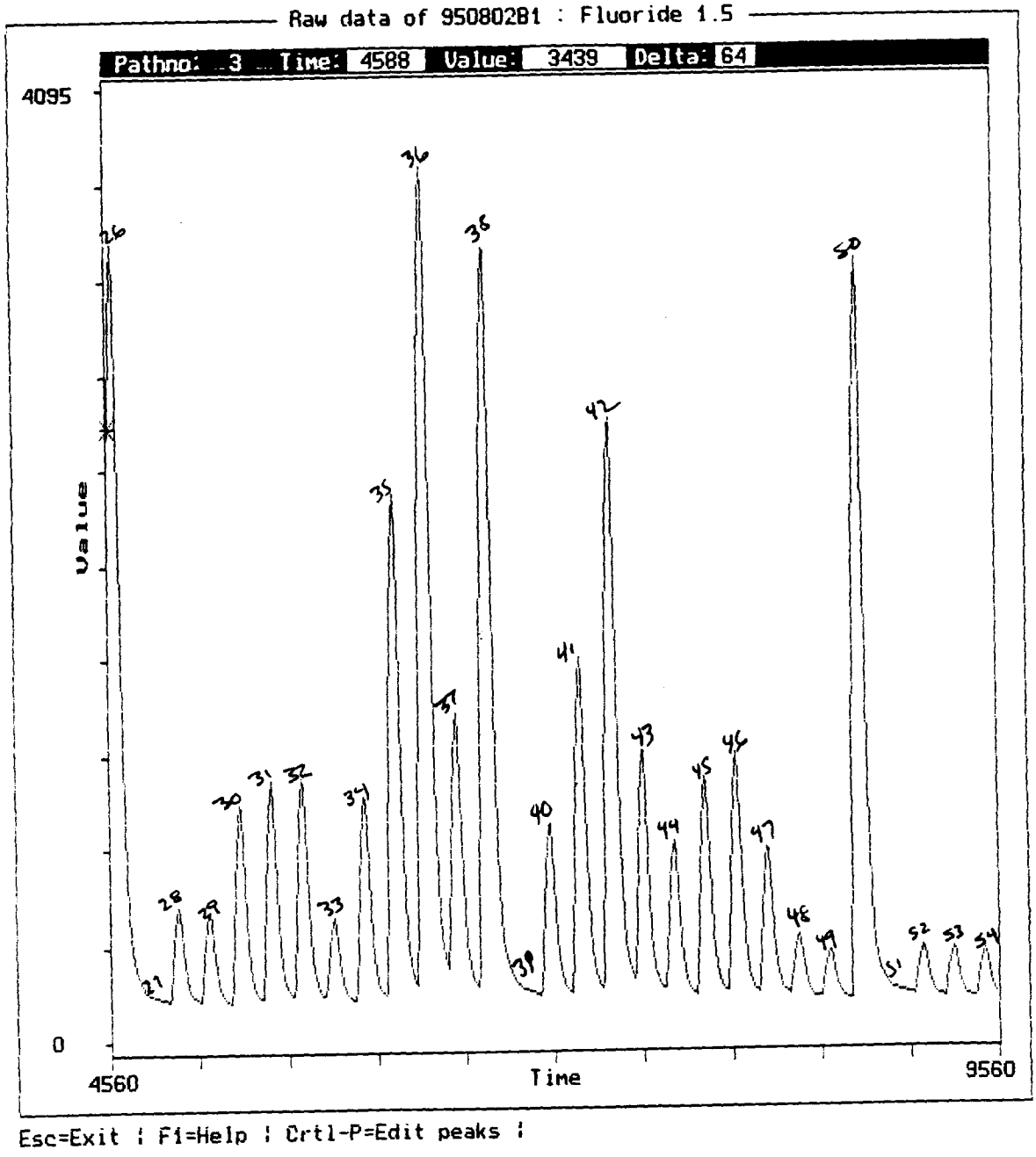
			Fluoride 1.5			Fluoride L		
			PPM			PPM		
Pos	Typ	Ident	Ch	Result	F Time	Ch	Result	F Time
wt	iv	Initial Wash	3	0.058	65	4	#.####	0
1	t	Tracer	3	1.475	209	4	0.3996	0
2	d	Drift	3	1.481	385	4	0.3995	0
3	w	Wash	3	0.058	624	4	#.####	0
4	s1	Standard 1	3	0.065	735	4	0.0167	0
5	s2	Standard 2	3	0.069	911	4	0.0275	0
6	s3	Standard 3	3	0.086	1085	4	0.0606	0
7	s4	Standard 4	3	0.105	1262	4	0.0912	0
8	s5	Standard 5	3	0.126	1436	4	0.1187	0
9	s6	Standard 6	3	0.156	1612	4	0.1503	0
10	s7	Standard 7	3	0.280	1786	4	0.2336	0
11	s8	Standard 8	3	0.618	1962	4	0.3348	0
12	s9	Standard 9	3	1.228	2136	4	0.3960	0
13	s10	Standard 10	3	1.469	2310	4	0.3997	0
14	d	Drift	3	1.489	2486	4	0.3994	0
15	w	Wash	3	0.058	2700	4	#.####	0
16	u	SERUM BLK 1	3	0.091	2836	4	0.0696	0
17	u	SERUM BLK 2	3	0.080	3011	4	0.0486	0
18	u	SERUM BLK 3	3	0.079	3187	4	0.0481	0
19	u	SERUM BLK 4	3	0.075	3363	4	0.0400	0
20	u	SERUM BLK 5	3	0.079	3533	4	0.0479	0
21	u	SERUM BLK 6	3	0.075	3711	4	0.0388	0
22	u	SPK 62-1	3	0.118	3887	4	0.1087	0
23	u	SPK 62-2	3	0.129	4063	4	0.1225	0
24	u	SPK 62-3	3	0.109	4237	4	0.0972	0
25	u	SPK 62-4	3	0.107	4414	4	0.0945	0
26	d	Drift	3	1.474	4588	4	0.3996	0
27	w	Wash	3	0.058	4829	4	#.####	0
28	u	SPK 62-5	3	0.106	4939	4	0.0921	0
29	u	SPK 62-6	3	0.102	5113	4	0.0870	0
30	u	SPK 250-1	3	0.198	5287	4	0.1849	0
31	u	SPK 250-2	3	0.220	5465	4	0.2000	0
32	u	SPK 250-3	3	0.227	5639	4	0.2048	0
33	u	P52762-2	3	0.096	5810	4	0.0782	0
34	u	P52763-2	3	0.205	5989	4	0.1902	0
35	u	P52768-2	3	0.779	6165	4	0.3598	0
36	u	P52770-2	3	1.647	6339	4	0.3919	0
37	u	P52762-4	3	0.309	6515	4	0.2471	0
38	d	Drift	3	1.474	6689	4	0.3996	0
39	w	Wash	3	0.058	6918	4	#.####	0
40	u	P52763-4	3	0.172	7039	4	0.1647	0
41	u	P52768-4	3	0.404	7214	4	0.2828	0
42	u	P52770-4	3	1.009	7389	4	0.3833	0
43	u	SPK 62-1	3	0.259	7567	4	0.2232	0
44	u	SPK 62-2	3	0.154	7740	4	0.1483	0
45	u	SPK 250-1	3	0.226	7914	4	0.2041	0
46	u	SPK 250-2	3	0.254	8090	4	0.2203	0
47	u	SPK 62-3	3	0.152	8266	4	0.1467	0
48	u	SERUM BLK 1	3	0.086	8439	4	0.0599	0
49	u	SERUM BLK 2	3	0.078	8616	4	0.0448	0
50	d	Drift	3	1.418	8790	4	0.3999	0
51	w	Wash	3	0.058	9016	4	#.####	0
52	u	SERUM BLK 3	3	0.078	9139	4	0.0462	0
53	u	SERUM BLK 4	3	0.077	9315	4	0.0441	0

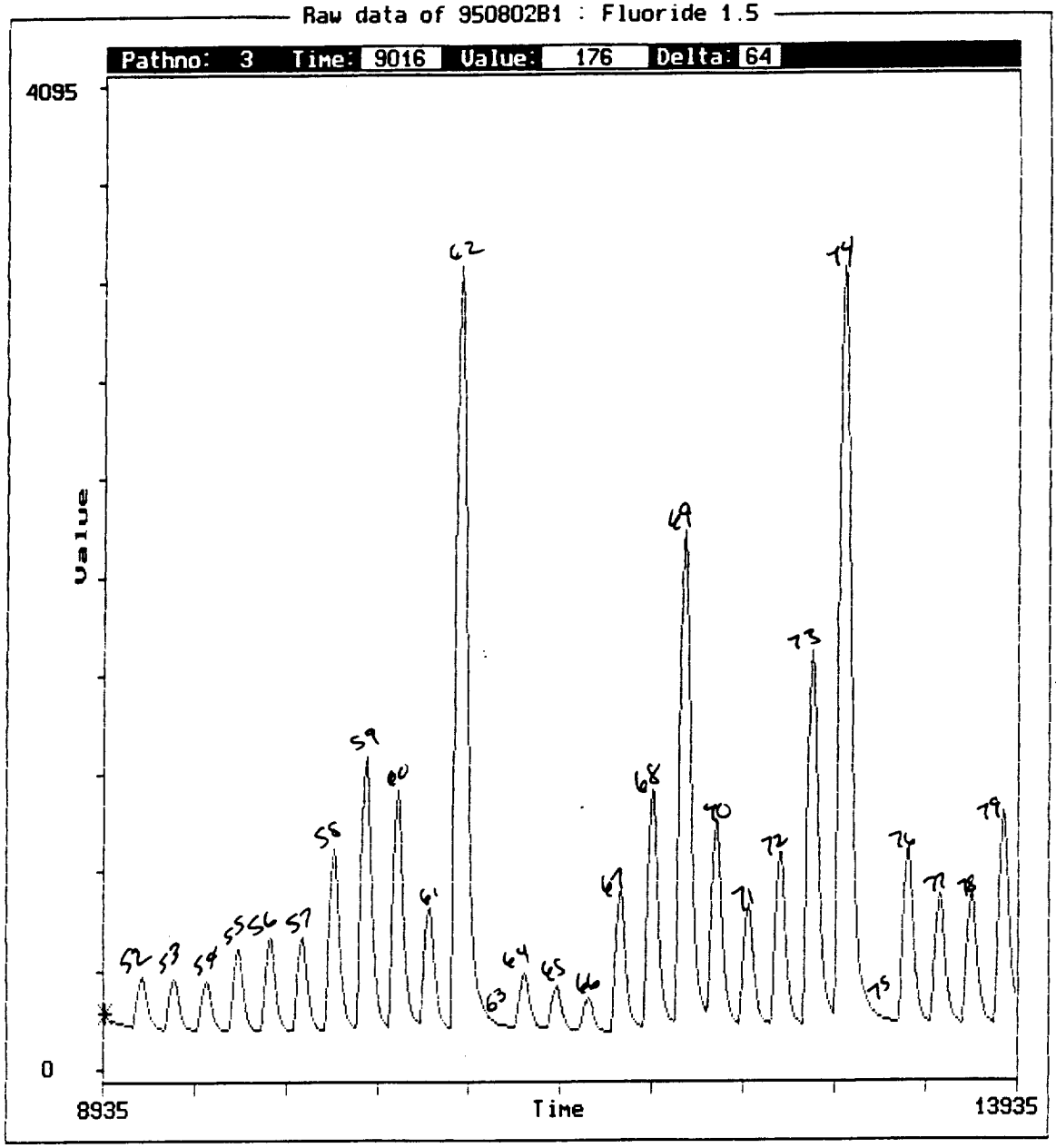
			Fluoride 1.5			Fluoride L		
			PPM			PPM		
Pos	Typ	Ident	Ch	Result	F Time	Ch	Result	F Time
54	u	SERUM BLK 5	3	0.075	9491	4	0.0400	0
55	u	SPK 62-1	3	0.093	9665	4	0.0734	0
56	u	SPK 62-2	3	0.098	9841	4	0.0809	0
57	u	SPK 62-3	3	0.099	10016	4	0.0825	0
58	u	SPK 250-1	3	0.169	10191	4	0.1621	0
59	u	SPK 250-2	3	0.282	10366	4	0.2347	0
60	u	SPK 250-3	3	0.231	10539	4	0.2071	0
61	u	BLK 1	3	0.117	10715	4	0.1074	0
62	d	Drift	3	1.382	10890	4	0.3997	0
63	w	Wash	3	0.058	11011	4	#.####	0
64	u	BLK 2	3	0.074	11242	4	0.0369	0
65	u	BLK 3	3	0.068	11414	4	0.0236	0
66	u	P52762-6	3	0.063	11588	4	0.0122	0
67	u	P52763-6	3	0.131	11766	4	0.1244	0
68	u	P52768-6	3	0.236	11940	4	0.2099	0
69	u	P52770-6	3	0.740	12116	4	0.3546	0
70	u	P52762-8	3	0.198	12292	4	0.1849	0
71	u	P52763-8	3	0.121	12466	4	0.1121	0
72	u	P52768-8	3	0.168	12640	4	0.1614	0
73	u	P52770-8	3	0.463	12817	4	0.3001	0
74	d	Drift	3	1.428	12990	4	0.3999	0
75	w	Wash	3	0.058	13230	4	#.####	0
76	u	P52762-12	3	0.172	13341	4	0.1647	0
77	u	P52763-12	3	0.132	13517	4	0.1256	0
78	u	SPK 62-1	3	0.130	13691	4	0.1238	0
79	u	SPK 250-1	3	0.209	13867	4	0.1924	0
80	u	SPK 250-2	3	0.240	14043	4	0.2121	0
81	u	P52768-12	3	0.152	14217	4	0.1469	0
82	u	P52770-12	3	0.231	14391	4	0.2072	0
83	u	P52762-24	3	0.092	14561	4	0.0714	0
84	u	P52763-24	3	0.098	14743	4	0.0802	0
85	u	P52768-24	3	0.079	14918	4	0.0479	0
86	d	Drift	3	1.388	15091	4	0.3998	0
87	w	Wash	3	0.058	15223	4	#.####	0
88	u	P52770-24	3	0.075	15444	4	0.0400	0
89	u	P52762-48	3	0.070	15618	4	0.0277	0
90	u	P52763-48	3	0.067	15790	4	0.0231	0
91	u	P52768-48	3	0.070	15964	4	0.0285	0
92	u	P52770-48	3	0.076	16142	4	0.0412	0
93	d	Drift	3	1.432	16318	4	0.3999	0
94	w	Wash	3	0.058	16545	4	#.####	0
wt	rw	RunOut Wash	3	0.058	16793	4	#.####	0


Calibration curve of 950802B1 : Fluoride L
Channel 0 Measured: 6
Concentration: 0.0004872
Delta: 64
0.1940644
-0.001026
Concentration
0
Measured
900
Order : 2
r : 0.99908


Calibration curve of 950802B1 : Fluoride 1.5
Channel 3 Measured: 6
Concentration: 0.0583859
Delta: 64
1.7683598
0.0578259
Concentration
0
Measured
4095
Order : Inverse Logarithm
s : 6068

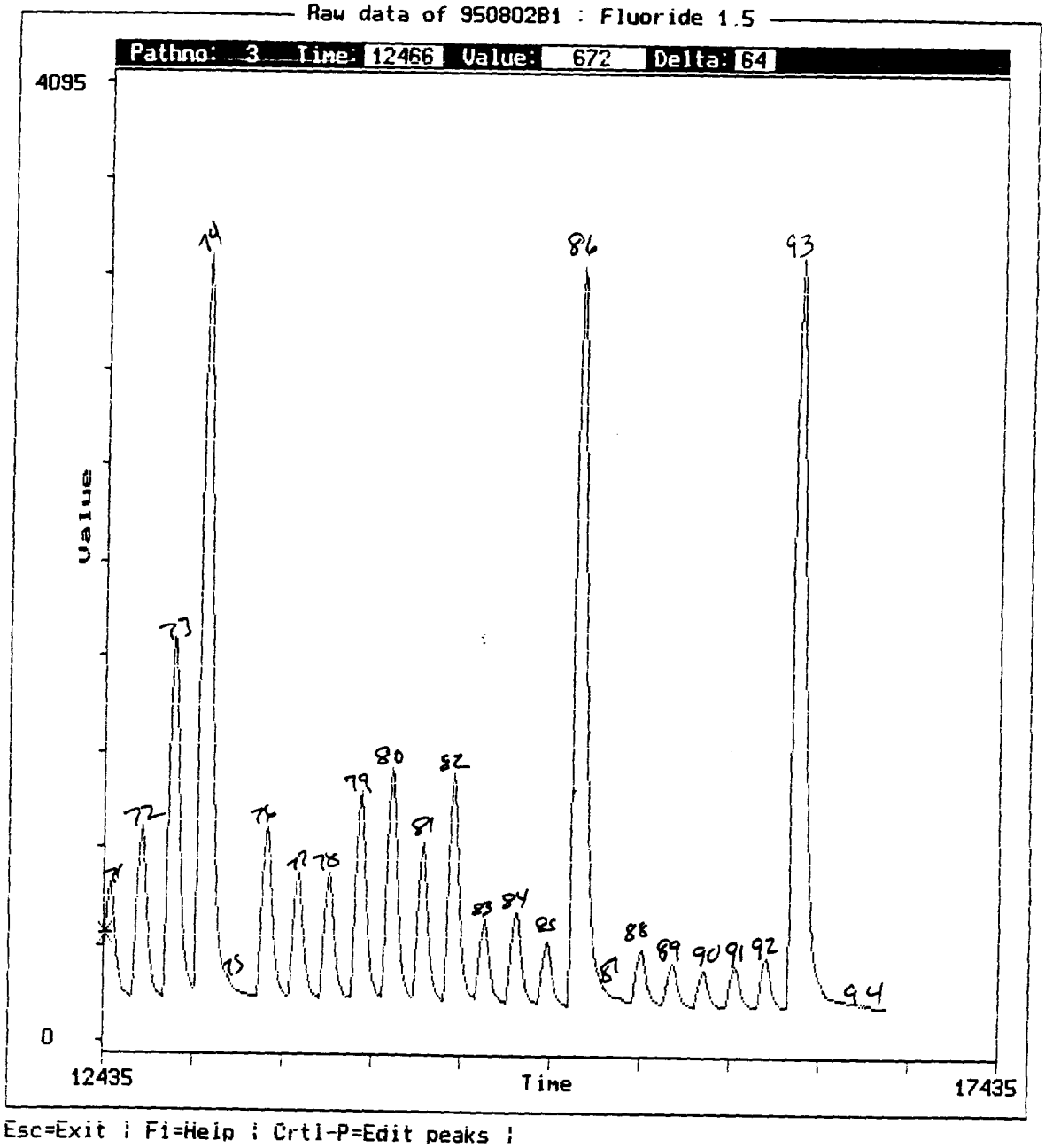

Raw data of 950802B1 : Fluoride 1.5
Pathno: 3 Time: 65 Value: 147 Delta: 64
4095
0
Value
0
Time
5000
Esc=Exit ¦ F1=Help ¦ Crtl-P=Edit peaks ¦


Raw data of 950802B1 : Fluoride 1.5
Pathno: 3 Time: 4588 Value: 3439 Delta: 64
4095
Value
0
4560
Time
9560
Esc=Exit ¦ F1=Help ¦ Crtl-P=Edit peaks ¦



Esc=Exit ¦ F1=Help ¦ Crtl-P=Edit peaks ¦

000109


Raw data of 950802B1 : Fluoride 1.5
Pathno: 3 Time: 12466 Value: 672 Delta: 64
4095
0
Value
12435
Time
17435
Esc=Exit ¦ F1=Help ¦ Ctrl-P=Edit peaks ¦

9.11.4 Summary and raw data; analysis of liver extracts using electrospray mass spectrometry.

This data, although supportive, in the opinion of the Study Director is not required to reach the conclusion stated in Final Report Section 6.0, and therefore is not discussed in detail.

contains pages
A-1 through A-6
DLC
11-10-95

NO DATA SUMMARY TABLE
FOR THIS STUDY.

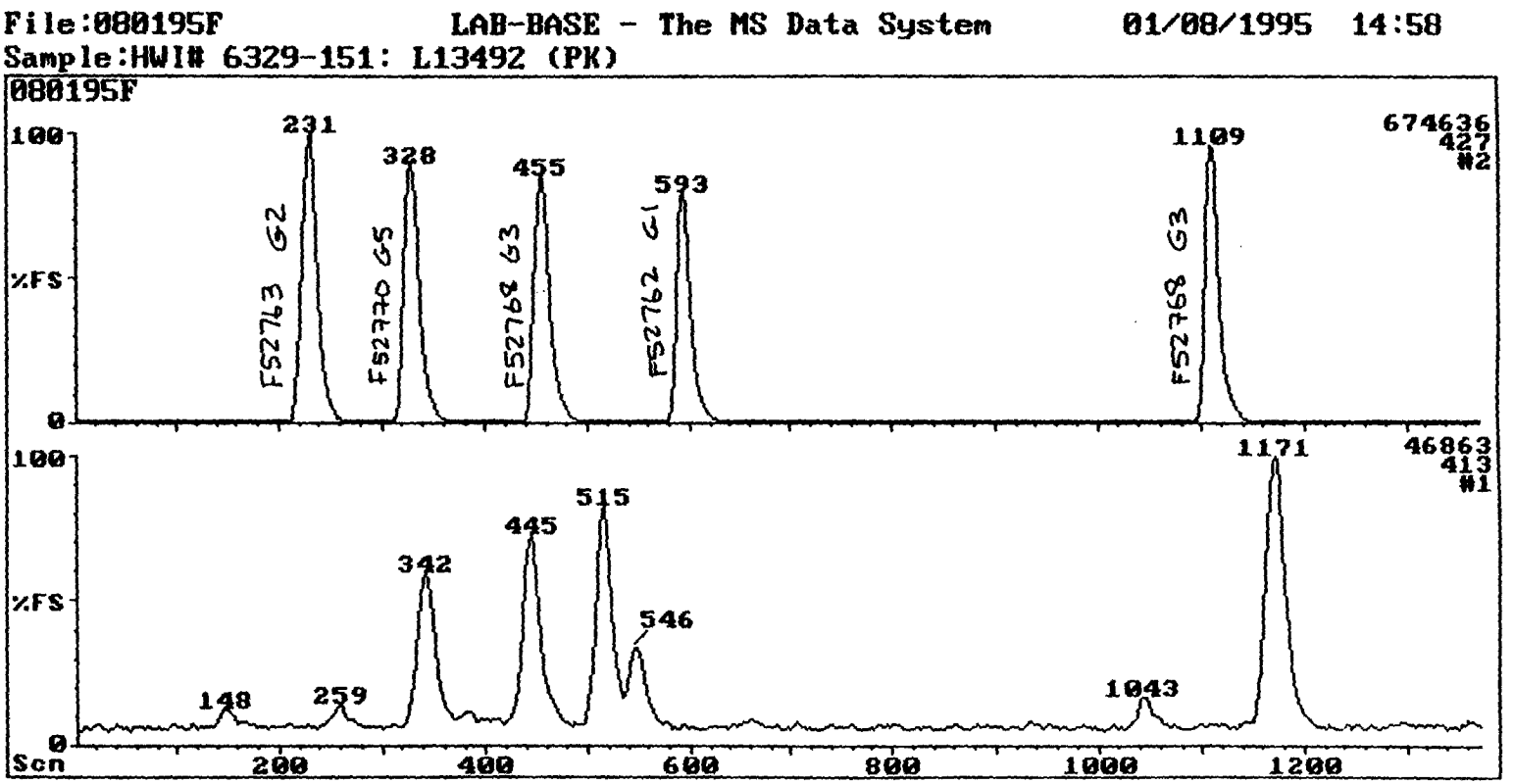
Samples were simply screened for M-413 ion only and not quantitated.

11-10-95 DLC

A-1

000112

SCREENING



HWI# 6329-151 Cmpd L13492 (PK)

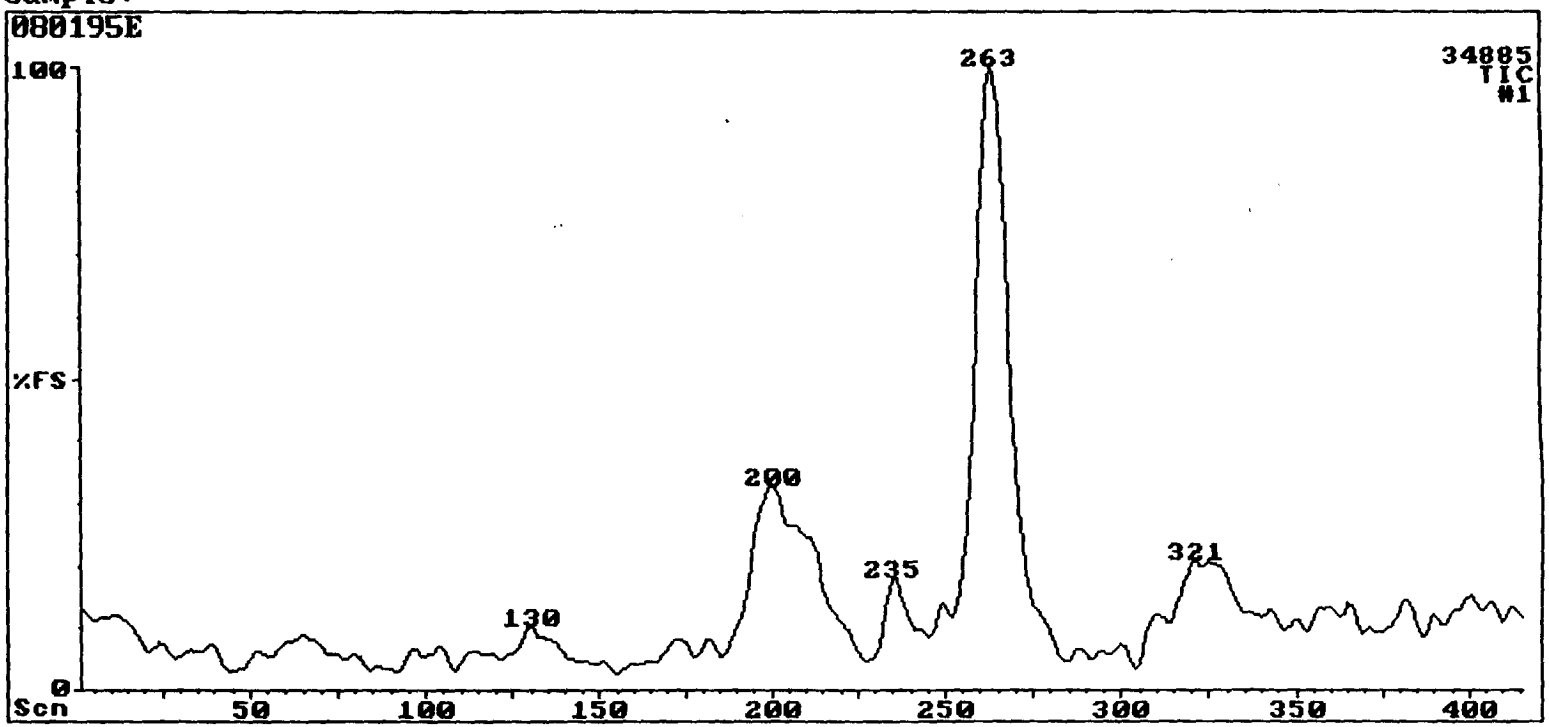
F52768

MODE : SCN

ES -

File:080195E LAB-BASE - The MS Data System 01/08/1995 14:22

Sample:



A-3

File:080195E
Sample:
LAB-BASE - The MS Data System
01/08/1995 14:22
080195E'202 (5.387) COMBINE:(196 to 206)-(((91 to 110)+(229 to 243))*1.000)
100
435.00
2032
%FS
471.00
436.00
472.00
473.00
356.00
402.00
396.00
453.00
474.00
491.00
515.00
533.00
578.00
582.00
552.00
583.00
648.00
0
M/z
350
400
450
500
550
600
650

A-4

000115

File:080195E
Sample:

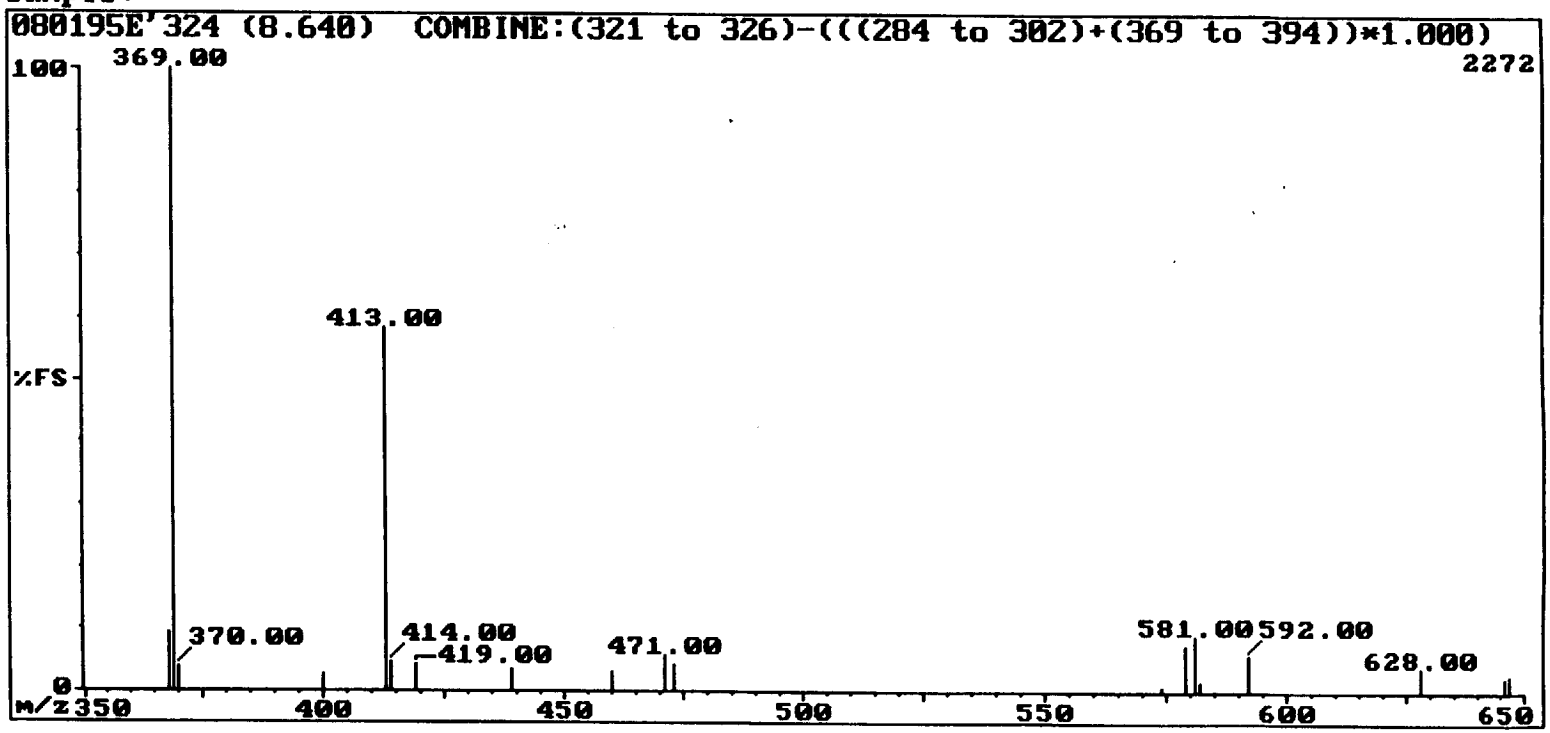
LAB-BASE - The MS Data System 01/08/1995 14:22

080195E'262 (6.987) COMBINE:(262 to 266)-(((108 to 155)+(288 to 308))*1.000)

A-5

000116

File:080195E LAB-BASE - The MS Data System 01/08/1995 14:22
Sample:



A-4

AWI # 6329-151 L13492 (PK)

000117