

# **3M Environmental Laboratory**

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## **Final Report- Analytical Study**

### **Single-Dose Intravenous Pharmacokinetic Study of T-6054 in Rabbits**

**In-Vivo Study Reference Number:** HWI#6329-138

**Study Number:** AMDT-122094.2

**Test Substance:** FC-129 (T-6054)

**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

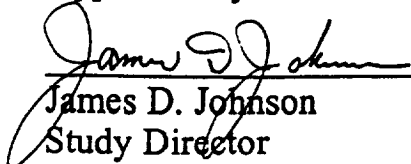
#### **Method Numbers and Revisions:**

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

**Initiation Date:** See attached protocol

**Author:** James D. Johnson

**Approved By:**

  
James D. Johnson  
Study Director

11/22/95  
Completion Date

## **1.0 SUMMARY**

Liver samples at 48 hours post intravenous dose of FC-129 (T-6054) in rabbits were analyzed for total organic fluorine and perfluorooctanesulfonate.

After an intravenous dose of FC-129 in rabbits, there is a detectable increase of total organic fluorine in liver at 48 hours post dose at doses ranging from 0.128 to 12.8 mg/kg. A substantial amount of this total organic fluorine is in the form of perfluorooctanesulfonate. This pharmacokinetic study shows that there is a convenient marker (perfluorooctanesulfonate) for assessing the extent of dermal absorption.

## **2.0 INTRODUCTION**

This study was performed in order to provide data for the assessment a subsequent dermal absorption study (HWI#6329-133). Knowing the disposition of an intravenous dose of FC-129, facilitates interpretation of a dermal absorption study.

Analysis of liver and serum samples from rabbits dosed intravenously with FC-129 for total organic fluoride (combustion analysis) and specific compounds (electrospray mass spectrometry) provide data as to whether fluorinated compounds are in the liver at 48 hours post dose and whether perfluorooctanesulfonate is present. Perfluorooctanesulfonate is a very good marker for a dermal study since previous work has shown it to be persistent in liver and serum in rabbits (biological half life >1 month).

## **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  $-20 \pm 10^{\circ}$  C.

**3.6 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 absorption study, so all tissues will be retained.

#### **4.0 EXPERIMENTAL - Overview**

Serum and tissues from animals dosed as described (HWI#6329-138), were available for analysis for total organic fluorine and fluorinated compounds. The samples were analyzed by combustion and/or electrospray mass spectrometry to the extent necessary to provide sufficient data for the interpretation of a second study on the extent of dermal absorption of FC-129 (HWI#6329-133).

#### **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

#### **6.0 DATA ANALYSIS**

The data are attached. For combustion analysis for total organic fluorine, the rabbits dosed with 0, 0.128, 0.64, 1.28, and 12.8 mg/kg had 18 (below the level of practical quantitation), 61, 118, 164, and 2239 ug/whole liver, respectively. The body weight

of the 12.8 mg/kg rabbit (F52752) was 2.7 kg. Thus, the total dose given this rabbit was 34.6 mg. The amount of total organic fluorine in this dose is 19 mg. The total organic fluorine in liver after the intravenous dose in rabbit F52752 represents 11.8% of the dose.

The data for an electrospray mass spectrometry analysis is attached. There is detectable perfluorooctanesulfonate in all treated rabbits. The high dose rabbit (F52752) had about 335 ug perfluorooctanesulfonate present in liver at 48 hours. If the 34.6 mg dose is expressed as FC-95, (that is, assuming 100% biotransformation) the dose is 31.8 mg. The 335 ug present in whole liver in this animal represents 1.05% of the dose. This represents a substantial amount of biotransformation of FC-129 to perfluorooctanesulfonate at 48 hours.

Other data was collected using 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 in a pharmacokinetic study reported separately (HWI#6329-159), there was a delay in perfluorooctanesulfonate concentration observed in serum that indicated an unexplained distribution pattern. The level dropped dramatically at 12 and 24 hours but returned to a maximum at 48 hours with a subsequent decay in serum levels that indicated a biological half-life of > 1 month. This delay in perfluorooctanesulfonate levels may be shifted to a later time when perfluorooctanesulfonate is resulting from biotransformation. There may actually be a higher level of perfluorooctanesulfonate in liver at a later time period and this study only went to 48 hours. This would indicate that perfluorooctanesulfonate is an even better marker for a dermal absorption study than would be indicated by this data.

## **7.0 CONCLUSION**

After an intravenous dose of FC-129 in rabbits, there is a detectable increase of total organic fluorine in liver at 48 hours at doses ranging from 0.64 to 12.8 mg/kg. A substantial amount of this total organic fluorine is in the form of perfluorooctanesulfonate. This pharmacokinetic study shows that there is a convenient marker for assessing the extent of dermal absorption.

## **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#6329-138 "Single-Dose Intravenous Pharmacokinetic Study of T-6054 in Rabbits" (Protocol type TP8084.PK for dosing of animals, tissue collection, etc.)

**9.1.2 Analytical protocol** AMDT-122094.2

**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-122094.2

**9.10 Instrument Settings:** See methods

**9.11 Data: See attached.**

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

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

**9.11.3 Summary and raw data; ug F<sup>-</sup> in whole liver as determined by thermal extraction followed by analysis using Skalar segmented flow analyzer with ion selective electrode.**

**9.1.1 Protocol and Final Report: HWI#6329-138 "Single-Dose Intravenous Pharmacokinetic Study of T-6054 in Rabbits" (Protocol type TP8084.PK for dosing of animals, tissue collection, etc.)**



**HAZLETON**  
W I S C O N S I N  
POST OFFICE BOX 7545  
MADISON, WI 53707-7545

a CORNING Company

Sponsor:

3M  
St. Paul, Minnesota



FINAL REPORT

Study Title:

Single-Dose Intravenous Pharmacokinetic  
Study of T-6054 in Rabbits

Author:

Steven M. Glaza

Study Completion Date:

February 24, 1995

Performing Laboratory:

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

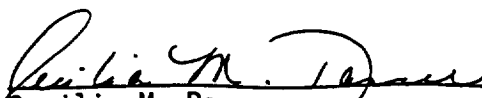
Laboratory Project Identification:

HWI 6329-138

## QUALITY ASSURANCE STATEMENT

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 |          | Phase              | Date                          | Date             |
|------------------|----------|--------------------|-------------------------------|------------------|
| From             | To       |                    | Reported to<br>Study Director | to<br>Management |
| 12/09/94         | 12/09/94 | Protocol Review    | 12/09/94                      | 01/10/95         |
| 12/19/94         | 12/19/94 | Animal Observation | 12/19/94                      | 01/10/95         |
| 02/02/95         | 02/02/95 | Data/Report Review | 02/02/95                      | 03/10/95         |

  
Cecilia M. Danner  
Representative, Quality Assurance Unit

2/24/95  
Date

STUDY IDENTIFICATION

Single-Dose Intravenous Pharmacokinetic  
Study of T-6054 in Rabbits

|                               |                                                                                                                                                                    |
|-------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Test Material                 | T-6054                                                                                                                                                             |
| Sponsor                       | 3M Toxicology Service Medical<br>Department<br>3M Center, Bldg. 220-2E-02<br>P.O. Box 33220<br>St. Paul, MN 55133-3220                                             |
| Sponsor's Representative      | John L. Butenhoff, PhD<br>3M Toxicology Service Medical<br>Department<br>3M Center, Bldg. 220-2E-02<br>P.O. Box 33220<br>St. Paul, MN 55133-3220<br>(612) 733-1962 |
| Study Director                | Steven M. Glaza<br>Hazleton Wisconsin, Inc.<br>P.O. Box 7545<br>Madison, WI 53707-7545<br>(608) 241-7292                                                           |
| Study Location                | Hazleton Wisconsin, Inc.<br>Building No. 3<br>3802 Packers Avenue<br>Madison, WI 53704                                                                             |
| Study Timetable               |                                                                                                                                                                    |
| Experimental Start Date       | December 17, 1994                                                                                                                                                  |
| Experimental Termination Date | December 19, 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. Petzel  
Manager

Laboratory Animal Medicine

Cindy J. Cary, DVM  
Diplomate, ACLAM  
Supervisor

Anatomical Pathology

Jack Serfort/  
Deborah L. Pirkel  
Supervisors  
Necropsy

Anne Mosher  
Supervisor  
Pathology Data

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#### SUMMARY

This study was done to assess the level of systemic exposure of T-6054 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 0.128, 0.64, 1.28, or 12.8 mg of T-6054/kg of body weight (Groups 1 through 5, respectively). The dose volume was 0.5 mL/kg for all groups.

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 animals at 2-, 4-, 6-, 8-, 12-, and 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 animal. All samples were centrifuged, separated into serum and cellular fractions, and sent to the Sponsor. Approximately 48 hours post-injection, the animals 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 were collected and sent frozen to the Sponsor after termination of the in-life phase.

All five animals appeared normal throughout the study.

## OBJECTIVE

The objective of this study was to assess the level of systemic exposure to the test material, T-6054, 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-6054 and described as an amber 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".<sup>1</sup>

### 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 2,702 to 2,891 g at initiation of treatment were placed into the following study groups:

| <u>Group</u> | <u>Treatment</u> | <u>Dose Level<br/>(mg T-6054/kg)</u> | <u>Dose Volume<br/>(mL/kg)</u> | <u>Number<br/>of Animals</u> |
|--------------|------------------|--------------------------------------|--------------------------------|------------------------------|
| 1 (Control)  | *                | 0                                    | 0.5                            | 1                            |
| 2            | T-6054           | 0.128                                | 0.5                            | 1                            |
| 3            | T-6054           | 0.64                                 | 0.5                            | 1                            |
| 4            | T-6054           | 1.28                                 | 0.5                            | 1                            |
| 5            | T-6054           | 12.8                                 | 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.

PROCEDURESDose 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 Collections

A blood sample (approximately 4 mL) was collected from either ear via the catheterization of the auricular artery or from the marginal ear vein of all 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 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  $-20^{\circ}\text{C} \pm 10^{\circ}\text{C}$  until shipped to the Sponsor.

### Pathology

At termination of the experimental phase (approximately 48-hours post-injection), animals were anesthetized with sodium pentobarbital, bled via the posterior vena cava, and exsanguinated. An abbreviated gross necropsy examination was not conducted, however, tissues were collected. The whole liver, bile, and both kidneys from each animal were collected and immediately placed on dry ice, then frozen by placing in a freezer set to maintain a temperature of  $-20^{\circ}\text{C} \pm 10^{\circ}\text{C}$ . 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 at initiation are in Table 1.

### Clinical Observations

Individual clinical signs are in Table 2. All five animals appeared normal throughout the study.

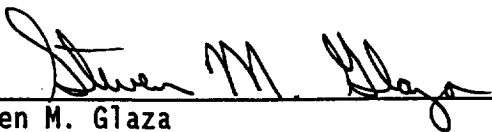
### Pathology

All animals survived to termination of the experimental phase and were not examined grossly when sacrificed.

## DISCUSSION

The level of systemic exposure of T-6054 was evaluated in female albino rabbits when administered as a single intravenous injection at levels of 0, 0.128, 0.64, 1.28, and 12.8 mg/kg. All animals appeared normal throughout the study following administration of this material.

## SIGNATURE

  
\_\_\_\_\_  
Steven M. Glaza  
Study Director  
Acute Toxicology

\_\_\_\_\_  
Date 2-24-95

## REFERENCE

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

Table 1  
Individual Body Weights (g)

| <u>Group</u> | <u>Dose<br/>Level<br/>(mg/kg)</u> | <u>Sex</u> | <u>Animal<br/>Number</u> | <u>Day 0</u> |
|--------------|-----------------------------------|------------|--------------------------|--------------|
| 1            | 0                                 | Female     | F52792                   | 2,784        |
| 2            | 0.128                             | Female     | F52793                   | 2,805        |
| 3            | 0.64                              | Female     | F52750                   | 2,891        |
| 4            | 1.28                              | Female     | F52751                   | 2,702        |
| 5            | 12.8                              | Female     | F52752                   | 2,783        |

Table 2  
Individual Clinical Signs

| <u>Group</u> | <u>Dose<br/>Level<br/>(mg/kg)</u> | <u>Sex</u> | <u>Animal<br/>Number</u> | <u>Observation</u> | <u>Hour</u> |          |          |           |           |
|--------------|-----------------------------------|------------|--------------------------|--------------------|-------------|----------|----------|-----------|-----------|
|              |                                   |            |                          |                    | <u>0.5</u>  | <u>2</u> | <u>4</u> | <u>24</u> | <u>48</u> |
| 1            | 0                                 | Female     | F52792                   | Appeared normal    | ✓           | ✓        | ✓        | ✓         | ✓         |
| 2            | 0.128                             | Female     | F52793                   | Appeared normal    | ✓           | ✓        | ✓        | ✓         | ✓         |
| 3            | 0.64                              | Female     | F52750                   | Appeared normal    | ✓           | ✓        | ✓        | ✓         | ✓         |
| 4            | 1.28                              | Female     | F52751                   | Appeared normal    | ✓           | ✓        | ✓        | ✓         | ✓         |
| 5            | 12.8                              | Female     | F52752                   | Appeared normal    | ✓           | ✓        | ✓        | ✓         | ✓         |

✓ Indicates condition exists.

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HWI 6329-138

APPENDIX A

Protocol TP8084.PK



a CORNING Company

Sponsor:

3M  
St. Paul, Minnesota

PROTOCOL TP8084.PK

Study Title:

Single-Dose Intravenous Pharmacokinetic Study  
of T-6054 in Rabbits

Date:

December 13, 1994

Performing Laboratory:

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

Laboratory Project Identification:

HWI 6329-138

STUDY IDENTIFICATION

Single-Dose Intravenous Pharmacokinetic Study  
of T-6054 in Rabbits

|                               |                                                                                                                                                                    |
|-------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| HWI No.                       | 6329-138                                                                                                                                                           |
| Test Material                 | T-6054                                                                                                                                                             |
| Sponsor                       | 3M Toxicology Service Medical<br>Department<br>3M Center, Bldg. 220-2E-02<br>P.O. Box 33220<br>St. Paul, MN 55133-3220                                             |
| Sponsor's Representative      | John L. Butenhoff, PhD<br>3M Toxicology Service Medical<br>Department<br>3M Center, Bldg. 220-2E-02<br>P.O. Box 33220<br>St. Paul, MN 55133-3220<br>(612) 733-1962 |
| Study Director                | Steven M. Glaza<br>Hazleton Wisconsin, Inc.<br>P.O. Box 7545<br>Madison, WI 53707-7545<br>(608) 241-7292                                                           |
| Study Location                | Hazleton Wisconsin, Inc.<br>Building No. 3<br>3802 Packers Avenue<br>Madison, WI 53704                                                                             |
| Proposed Study Timetable      |                                                                                                                                                                    |
| Experimental Start Date       | December 17, 1994                                                                                                                                                  |
| Experimental Termination Date | December 19, 1994                                                                                                                                                  |
| Draft Report Date             | January 23, 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
  - ☒ 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-6054
  - 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%  $\pm$ 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**

| <u>Group</u> | <u>Dose Level<br/>(mg/kg)<sup>a</sup></u> | <u>Number of<br/>Females</u> |
|--------------|-------------------------------------------|------------------------------|
| 1            | 0 (Control)                               | 1                            |
| 2            | 0.128                                     | 1                            |
| 3            | 0.64                                      | 1                            |
| 4            | 1.28                                      | 1                            |
| 5            | 12.8                                      | 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  $-20^{\circ}\text{C} \pm 10^{\circ}\text{C}$ . The separated serum and cellular fractions will be sent frozen on dry ice 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. Tissues, as described in section E. Termination, (3) Sample Collection, will be collected.

(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.

(3) 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  $-20^{\circ}\text{C} \pm 10^{\circ}\text{C}$ .

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*

John L. Butenhoff, PhD  
Sponsor's Representative  
3M Toxicology Service Medical Department

*12-15-94*

Date

*Steven M. Glaza*

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

*12-13-94*

Date

*Greg Thed*

Representative  
Quality Assurance Unit  
Hazleton Wisconsin, Inc.

*12-13-94*

Date

(6329-138.protdisk2)

### **9.1.2 Analytical protocol AMDT-122094.2**

# **3M Environmental Laboratory**

## **Protocol - Analytical Study**

### **Single-Dose Intravenous Pharmacokinetic Study of T-6054 in Rabbits**

**In-Vivo Study Reference Number:** HWI#6329-138

**Study Number:** AMDT-122094.2

**Test Substance:** FC-129 (T-6054)

**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

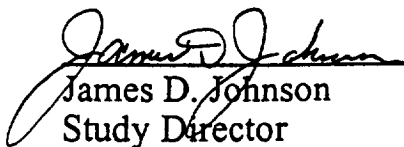
**Proposed Initiation Date:** July 25, 1995  
**Proposed Completion Date:** August 25, 1995

#### **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 Fluorochemicals Using  
Electrospray Mass Spectrometry  
AMDT-M-8-0, Analysis of Fluoride Using the Skalar Segmented Flow Analyzer  
with Ion Selective Electrode

**Author:** James D. Johnson

**Approved By:**

 10/30/95  
James D. Johnson Date  
Study Director

 10/30/95  
John Butenhoff, PhD Date  
Sponsor Representative

## **1.0 PURPOSE**

This study is performed in order to provide information necessary to assess the extent of dermal absorption of FC-129 (T-6054) in a subsequent dermal absorption study, HWI#6329-133.

The study is designed to provide information as to whether FC-129 and its metabolites are detectable in liver and other tissues, either as total organic fluorine or as specific compounds when the FC-129 is administered as an intravenous dose; and to ascertain whether perfluorooctanate will provide a marker for dermal absorption.

## **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 4 test animals and 1 control animal, 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),  $-20 \pm 10^{\circ}\text{C}$  (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 and serum from animals dosed as described (HWI#6329-138), 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. For confirmation of the presence of specific compounds in tissues and serum, electrospray mass spectrometry can be used.

### **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 Fluorochemicals Using Electrospray Mass Spectrometry**

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

## **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-95 (electrospray mass spectrometry) per unit of tissue or fluid. Statistics used, at the discretion of the Study Director, may include averages and standard deviations from different dose groups. If necessary, simple standard statistical tests such as the Student's t test may be applied to determine statistical difference.

## **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 Fluorochemicals Using Electrospray Mass Spectrometry

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

# 3M Environmental Laboratory

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## 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

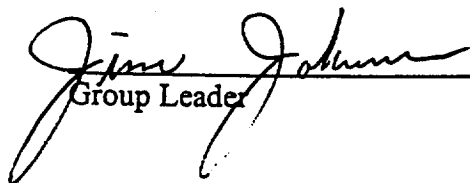
Revision Number: 0

Revision Date: None

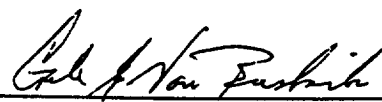
---

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**

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

## **3.0 PRECAUTIONS**

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**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**

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**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**

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**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**

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**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**

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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**

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### **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

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### 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  $\frac{(\text{Orion reading in ppm} - \text{intercept})}{(\text{Slope})}$

## 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

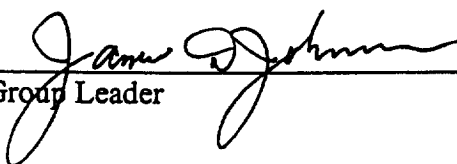
Revision Number: 0

Revision Date: None

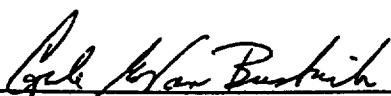
---

Author: Rich Youngblom

Approved By:

  
Group Leader

10/3/95  
Date

  
Quality Assurance

10-4-95  
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<sup>-</sup>, 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<sup>-</sup> 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<sup>-</sup>, 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**

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| <b><u>Revision<br/>Number</u></b> | <b><u>Reason for Change</u></b> | <b><u>Revision<br/>Date</u></b> |
|-----------------------------------|---------------------------------|---------------------------------|
|-----------------------------------|---------------------------------|---------------------------------|

# **3M Environmental Laboratory**

## **Method**

### **Extraction of Fluorochemicals from Rabbit Livers**

**SOP Identification Number: AMDT-M-4**

**Adoption Date: 10-31-95**

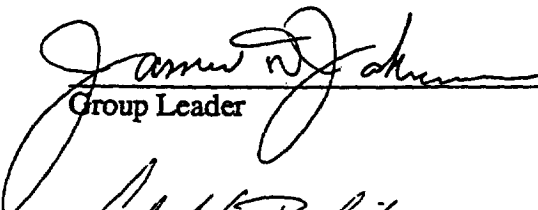
**Revision Number: 0**

**Revision Date: None**

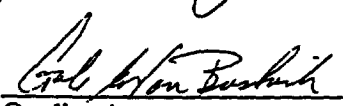
---

**Author: Dave Christenson/Cynthia Weber**

**Approved By:**

  
Group Leader

10-31-95  
Date

  
Quality Assurance

10-31-95  
Date

**Software: MS Word, 6.0**

**Affected Documents: M-5, Analysis of Rabbit Extract for Fluorochemicals Using Electrospray Mass Spectroscopy.**

## **1.0 SCOPE**

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- 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**

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

## **3.0 PRECAUTIONS**

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

## **4.0 SUPPLIES AND MATERIALS**

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### **4.1 Supplies**

- 4.1.1 Syringe, capable of measuring 100  $\mu$ L
- 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  $\mu$ m.
- 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), ( $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ ) 0.25 M: Weigh 26.5 g of sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) and 21.0 g of sodium bicarbonate ( $\text{NaHCO}_3$ ) 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**

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- 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.

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

### **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<br>(Approximate Conc.) | uL  | Approximate final<br>concentration of<br>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:

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

\*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 perfluorooctanesulfonate 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.

## 2.0 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  $\mu$ m 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**

[illegible]

# 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

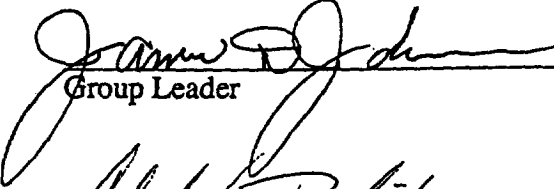
Revision Number: 0

Revision Date: None

---

Author: Dave Christenson/Cynthia Weber

Approved By:

  
Group Leader

6/6/95  
Date

  
Quality Assurance

6/6/95  
Date

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 spectrometer 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<sup>+</sup>.

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. Observe 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<sup>+</sup>, 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 ( $\pm 50\%$ ) 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

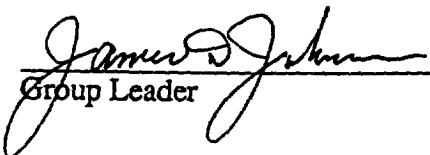
Revision Number: 0

Revision Date: None

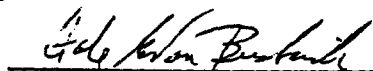
---

Author: Deb Wright / Cynthia Weber

Approved By:

  
Group Leader

10/5/95  
Date

  
Quality Assurance

1-27-95  
Date

Software: IBM MS Word, 6.0

Affected Documents: AMDT-EP-26, Operation and Maintenance of the Skalar Segmented Flow  
Analyzer

## **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 ( $\text{HNO}_3$ ) 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<sup>Plus</sup> 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**

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### **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

### **9.3 Quality Assurance Unit Statement**

Attachment D

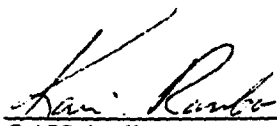
**GLP Study  
Quality Assurance Statement**

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

**Study Title: Single-dose Intravenous Pharmacokinetic Study of T-6054 in Rabbits**  
**Study Number: AMDT-122094.2**      **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 |          | Phase        | Date Inspection Reported to |                |
|------------------|----------|--------------|-----------------------------|----------------|
| From             | To       |              | Management                  | Study Director |
| 10/14/95         | 10/19/95 | Final Report | 10/19/95                    | 10/19/95       |

  
\_\_\_\_\_  
QAU Auditor      Date

## **9.4 Key Personnel Involved in the Study**

## **3M Environmental Laboratory**

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### **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<sup>-</sup> in whole liver as determined by thermal extraction followed by analysis using Orion ion analyzer.

**Summary of Combustion Data - Liver**  
**AMDT-122094.2, HWI 6329-138**  
**As Referenced in Final Report section 6.0 DATA ANALYSIS**

**Total ug Fluoride in Whole Liver**  
**Mean per Dose Group**

|                          | ug   |
|--------------------------|------|
| Control Group            | 18.0 |
| 0.128 mg/kg dose (T6054) | 60.5 |
| 0.64 mg/kg dose (T6054)  | 118  |
| 1.28 mg/kg dose (T6054)  | 164  |
| 12.8 mg/kg dose (T6054)  | 2239 |

| FC129 PK          |            | Actual                      | Average                     |                            | Whole                      | Total F-                  |                   |
|-------------------|------------|-----------------------------|-----------------------------|----------------------------|----------------------------|---------------------------|-------------------|
| ID                | %<br>rcvry | ppm F-<br>in liver<br>(W/W) | ppm F-<br>in liver<br>(W/W) | Liver<br>burned<br>(grams) | liver<br>weight<br>(grams) | in whole<br>liver<br>(µg) | Dosage<br>(mg/kg) |
| Liver Blank-1     |            | 1.11                        |                             | 0.145                      |                            |                           |                   |
| Liver Blank-2     |            | 0.371                       |                             | 0.137                      |                            |                           |                   |
| Liver Blank-3     |            | 0.253                       |                             | 0.128                      |                            |                           |                   |
| Liver Spike-1     | 97%        | 1.04                        |                             | 0.141                      |                            |                           |                   |
| Liver Spike-2     | 95%        | 0.860                       |                             | 0.168                      |                            |                           |                   |
| F52750-1          |            | 1.71                        |                             | 0.118                      |                            |                           |                   |
| F52750-2          |            | 1.53                        | 1.59                        | 0.121                      | 73.9                       | 118                       | 0.64              |
| F52750-3          |            | 1.54                        |                             | 0.130                      |                            |                           |                   |
| F52793-1          |            | 0.839                       |                             | 0.141                      |                            |                           |                   |
| F52793-2          |            | 0.840                       | 0.879                       | 0.125                      | 68.8                       | 60.5                      | 0.128             |
| F52793-3          |            | 0.959                       |                             | 0.123                      |                            |                           |                   |
| F52792-1          |            | 0.253                       |                             | 0.141                      |                            |                           |                   |
| F52792-2          |            | 0.234                       | 0.225                       | 0.129                      | 80.2                       | 18.0                      | 0.0               |
| F52792-3          |            | 0.187                       |                             | 0.148                      |                            |                           |                   |
| F52751-1          |            | 2.64                        |                             | 0.108                      |                            |                           |                   |
| F52751-2          |            | 2.34                        | 2.45                        | 0.133                      | 66.8                       | 164                       | 1.28              |
| F52751-3          |            | 2.37                        |                             | 0.133                      |                            |                           |                   |
| F52752-1          |            | 23.3                        |                             | 0.143                      |                            |                           |                   |
| F52752-2          |            | 24.5                        | 23.6                        | 0.142                      | 94.9                       | 2239                      | 12.8              |
| F52752-3          |            | 23.0                        |                             | 0.137                      |                            |                           |                   |
| Liver Blank-1     |            | 0.364                       |                             | 0.134                      |                            |                           |                   |
| Liver Blank-2     |            | 0.272                       |                             | 0.109                      |                            |                           |                   |
| Liver spike 63-1  | 97%        | 0.985                       |                             | 0.148                      |                            |                           |                   |
| Liver spike 63-2  | 96%        | 1.06                        |                             | 0.137                      |                            |                           |                   |
| Liver spike 126-1 | 95%        | 2.38                        |                             | 0.121                      |                            |                           |                   |
| Liver spike 126-2 | 82%        | 1.76                        |                             | 0.142                      |                            |                           |                   |
| Liver spike 126-3 | 83%        | 2.31                        |                             | 0.109                      |                            |                           |                   |

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

HWI# 6329-138

A-1  
Contains pages  
A-1 through A-  
11-2-95  
DLC

Study: Single-Dose Intravenous Pharmacokinetic  
Protocol Number: TP8084.PK  
Test Material: T-6054 in Rabbits (FC-129)  
Matrix: Liver  
R Squared Value: 0.9826  
Response Factor Amount: 1.92E00  
Analyst: DLC  
Date: 4/6/95  
Method: AMDT-M-4  
Instrument: Fisons VG 2000 Electrospray MS  
LABBASE File: 040695A

| Group<br>Dose            | Sample # | Ion Count<br>Ratio * | Extracted wt<br>g | Dilution<br>factor | Concentration<br>µg/g ** | Total mass<br>of liver<br>g | Total amount of<br>FC-95 per liver<br>mg |
|--------------------------|----------|----------------------|-------------------|--------------------|--------------------------|-----------------------------|------------------------------------------|
| Group 1:<br>0 mg/kg      | F52792   | 0.0245               | 1.2562            | 1                  | 0.0299                   | 80.1586                     | 0.002                                    |
| Group 2:<br>0.128 mg /kg | F52793   | 0.2346               | 1.3227            | 1                  | 0.2722                   | 68.8400                     | 0.019                                    |
| Group 3:<br>0.64 mg/kg   | F52750   | 0.4983               | 1.2397            | 1                  | 0.6169                   | 73.9156                     | 0.046                                    |
| Group 4:<br>1.28 mg/kg   | F52751   | 0.5016               | 1.2835            | 1                  | 0.5998                   | 66.7527                     | 0.040                                    |
| Group 5:<br>12.8 mg/kg   | F52752   | 2.7225               | 1.1852            | 1                  | 3.5254                   | 94.8862                     | 0.335                                    |

\* Ratio of M499 Ion Count/M427 Ion Count

\*\*The concentration was calculated by using the standard curve and multiplying the result by 4/5. The 4/5 factor is the result of a miscalculation in applying formula 8.4 in Method AMDT-M-4-0. 137 mg of liver was used in this calculation rather than 171 mg. The concentrations in the standard curve are therefore 5/4 larger than they should be. By multiplying the calculated concentration in the standard curve by 4/5, the correct result is obtained.

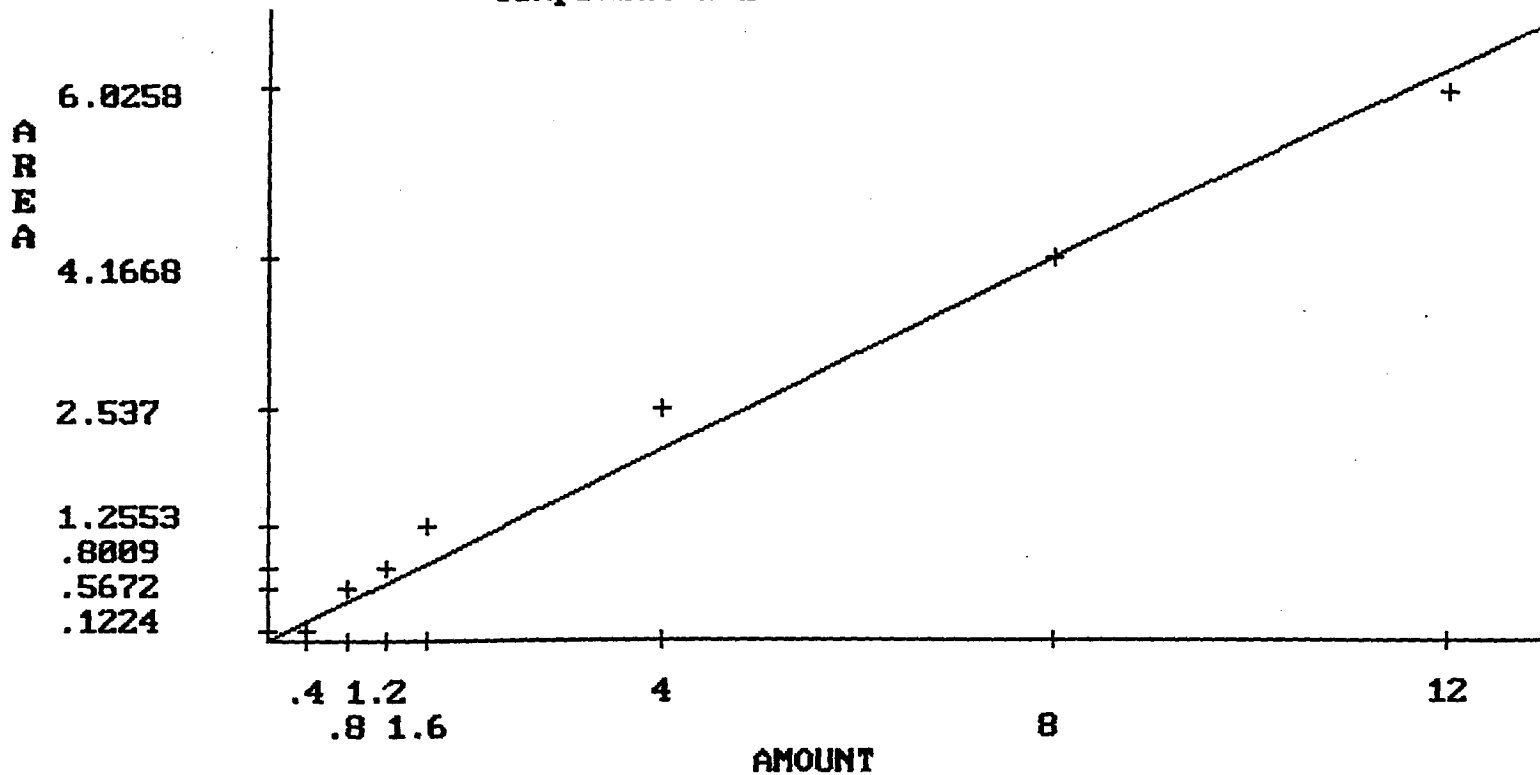
## HWI# 6329-138

| <u>Conc.</u> | <u>499 Ion Count</u> | <u>427 Ion Count</u> | <u>M=499 I.C./M=427 I.C.</u> |
|--------------|----------------------|----------------------|------------------------------|
| 0.4          | 9091                 | 74303                | 0.1224                       |
| 0.8          | 63720                | 112333               | 0.5672                       |
| 1.2          | 117051               | 146156               | 0.8009                       |
| 1.6          | 183223               | 145956               | 1.2553                       |
| 4            | 342495               | 135711               | 2.5237                       |
| 8            | 654597               | 157098               | 4.1668                       |
| 12           | 774077               | 128461               | 6.0258                       |

| <u>Sample #</u> | <u>499 Ion Count</u> | <u>427 Ion Count</u> | <u>M=499 I.C./M=427 I.C.</u> |
|-----------------|----------------------|----------------------|------------------------------|
| F52792          | 2399                 | 97837                | 0.0245                       |
| F52793          | 12826                | 54664                | 0.2346                       |
| F52750          | 41969                | 84226                | 0.4983                       |
| F52751          | 41908                | 83555                | 0.5016                       |
| F52752          | 256267               | 94129                | 2.7225                       |

Method C:DLCLIVE1  
 Sample  
 Operator  
 Run date 07-26-1995 16:12:19 version: 35  
 Printed on 07-26-1995 AT 16:12:32  
 Straight Line Fit forced through Origin.  
 -----

Component #:1



Component 1 =  
 EXTERNAL STANDARD CALIBRATION

| LEVEL | AMOUNT  | AREA |
|-------|---------|------|
| 1     | 0.4000  | 0    |
| 2     | 0.8000  | 1    |
| 3     | 1.2000  | 1    |
| 4     | 1.6000  | 1    |
| 5     | 4.0000  | 3    |
| 6     | 8.0000  | 4    |
| 7     | 12.0000 | 6    |

ratio

Area Ratio  $\frac{N=499 \text{ Area}}{N=427 \text{ Area}}$   
 10/16/95 DLG

Y = SLOPE \* X + INTERCEPT

Area = 5.2126E-01 \* Amount + 0.0000E+00  
 Amount = 1.9184E+00 \* Area + 0.0000E+00  
 R squared = 0.9826

Thurs 6 April 95

AWI# 6329- 131 (FC-135)

- 136 (FC-99)

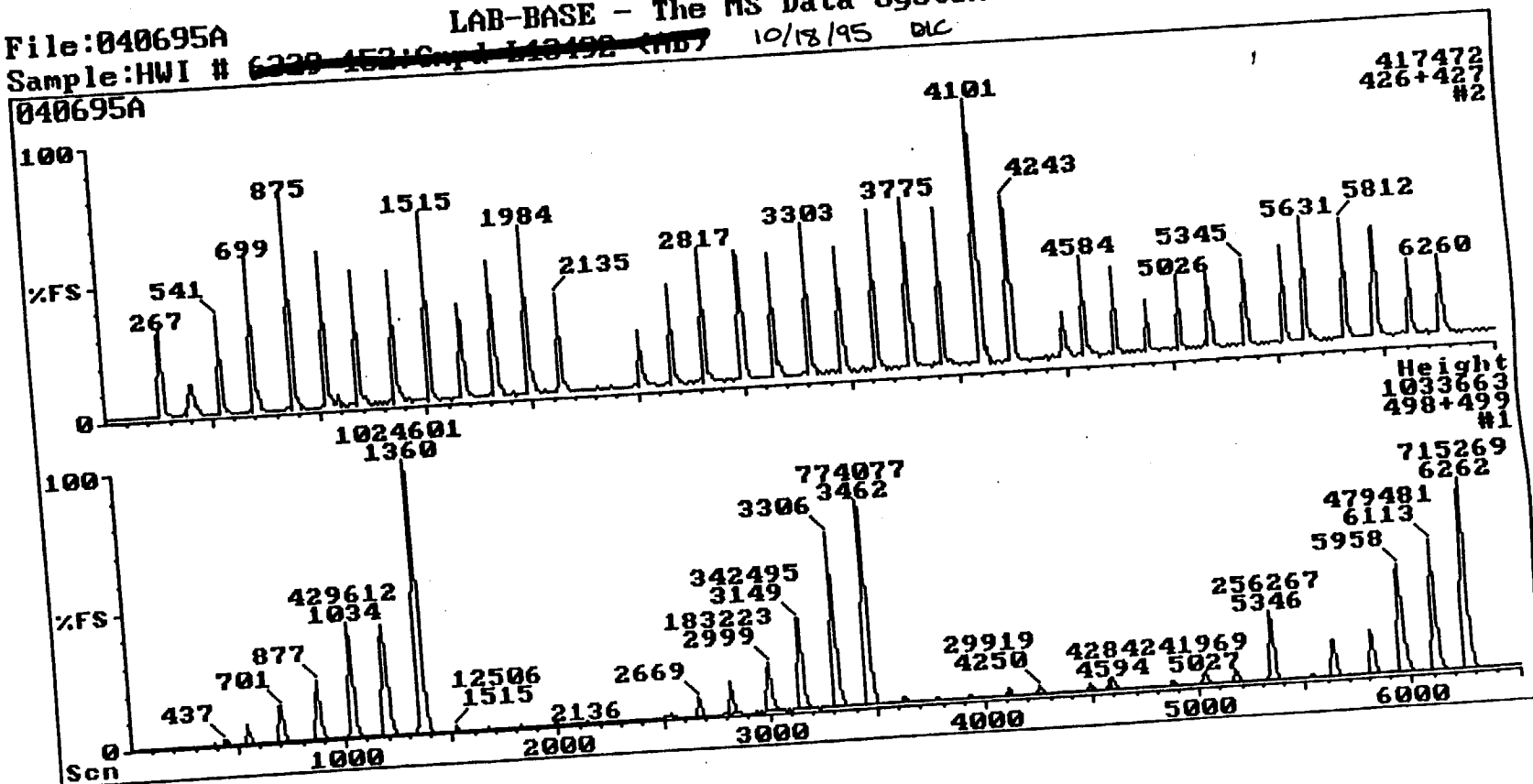
6329-138 (FC-129)

06/04/1995 08:14

**File:040695A**

File:040695A  
Sample:HWI # ~~6229 152:G1:1143492 (ind)~~ 10/18/95

040695A



A-5

#HWI #

6329-138 (FC-129)

File:040695A

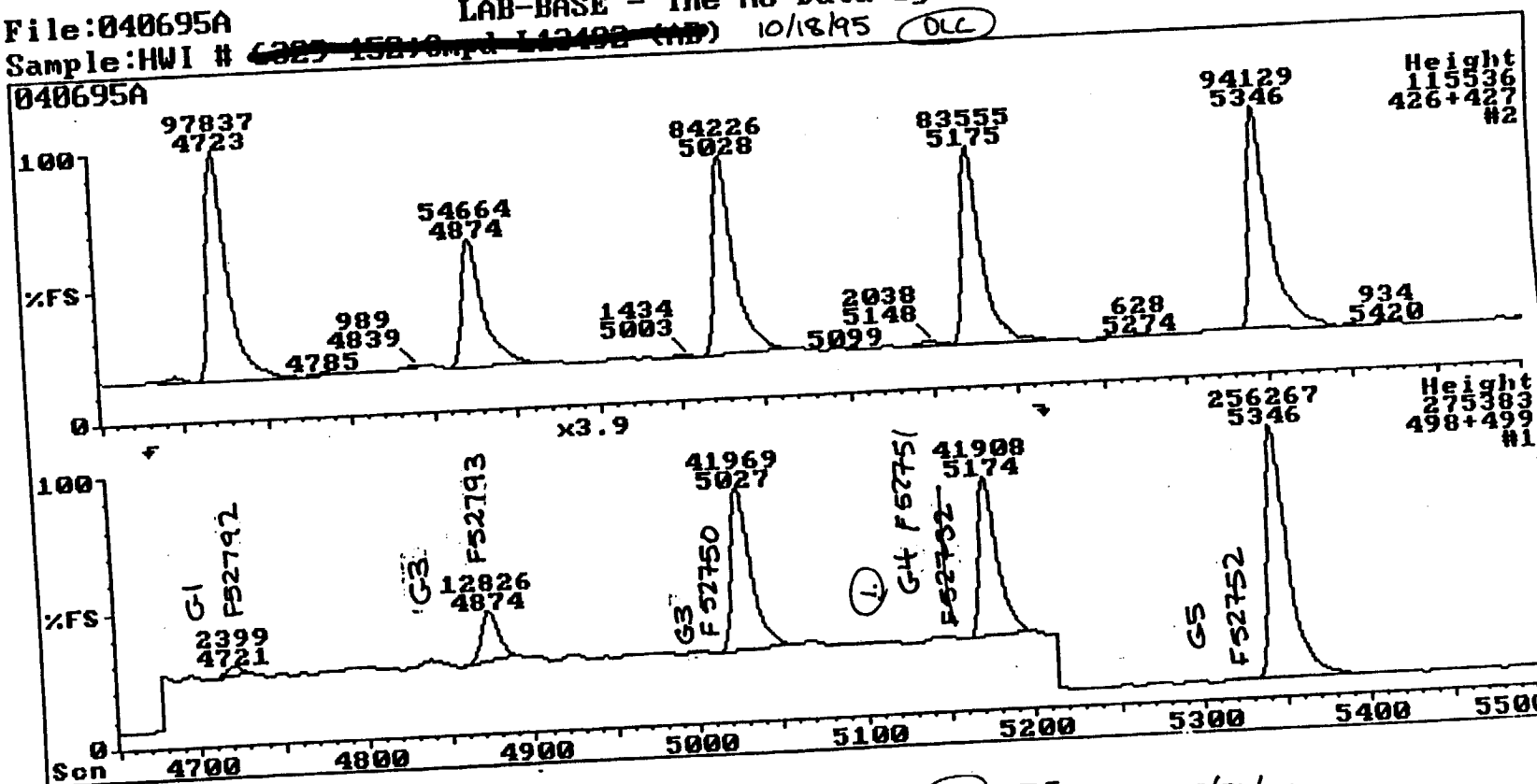
LAB-BASE - The MS Data System

06/04/1995 08:14

Sample:HWI #

~~6329-15210mpd L42492 (AD)~~ 10/18/95

DLC



(1.) T.E. DLC 7/31/95  
See Electrospray Log Book

A-6

BEEF LIVER Standards  
10/18/95  
DLC

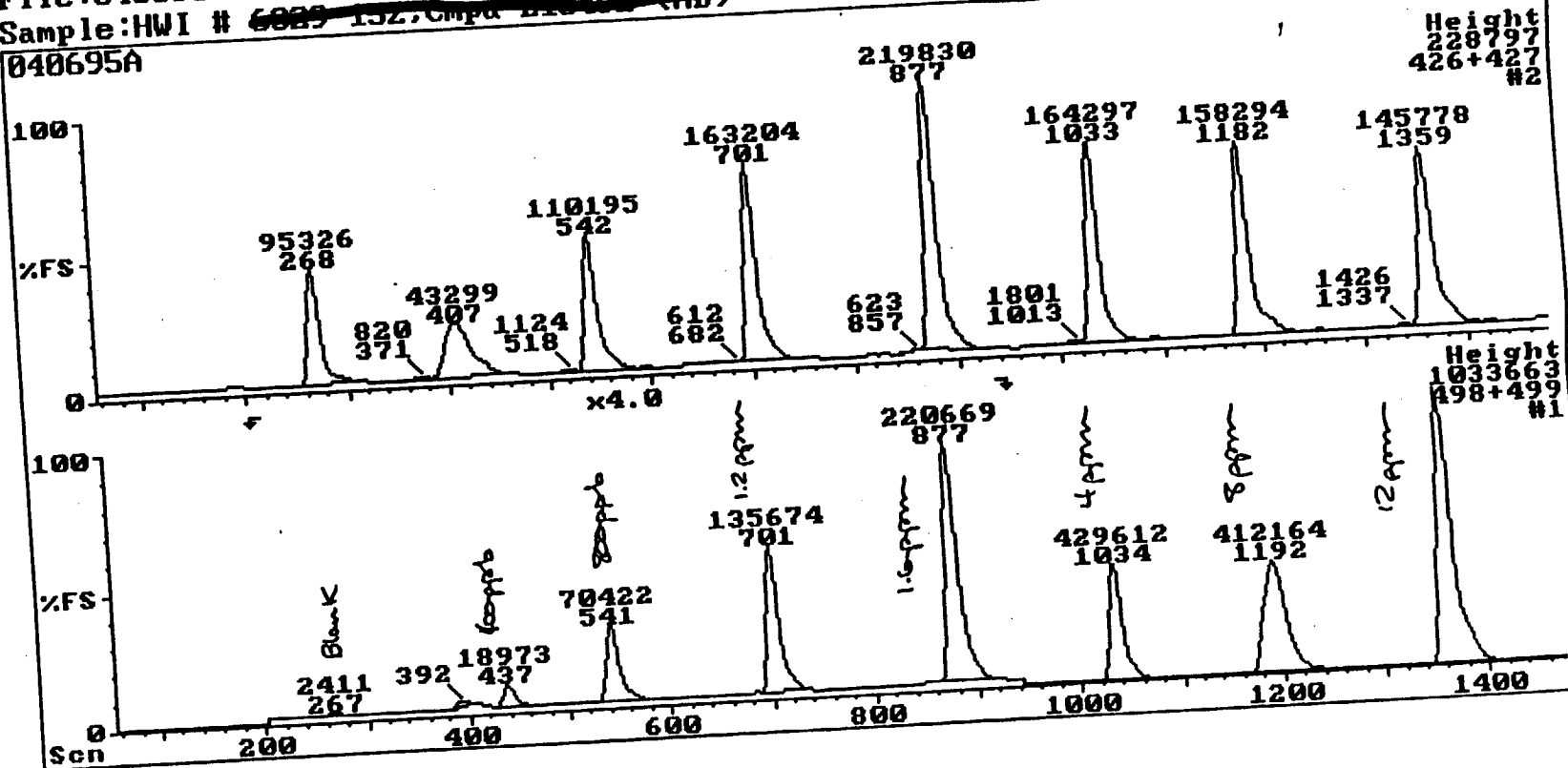
06/04/1995 08:14

LAB-BASE - The MS Data System  
10/18/95 DLC

File:040695A

Sample:HWI # ~~6089~~ 152.Cmpd L10402 (HW)

040695A



A-1

Beef Liver Standards  
10/18/95  
DLC

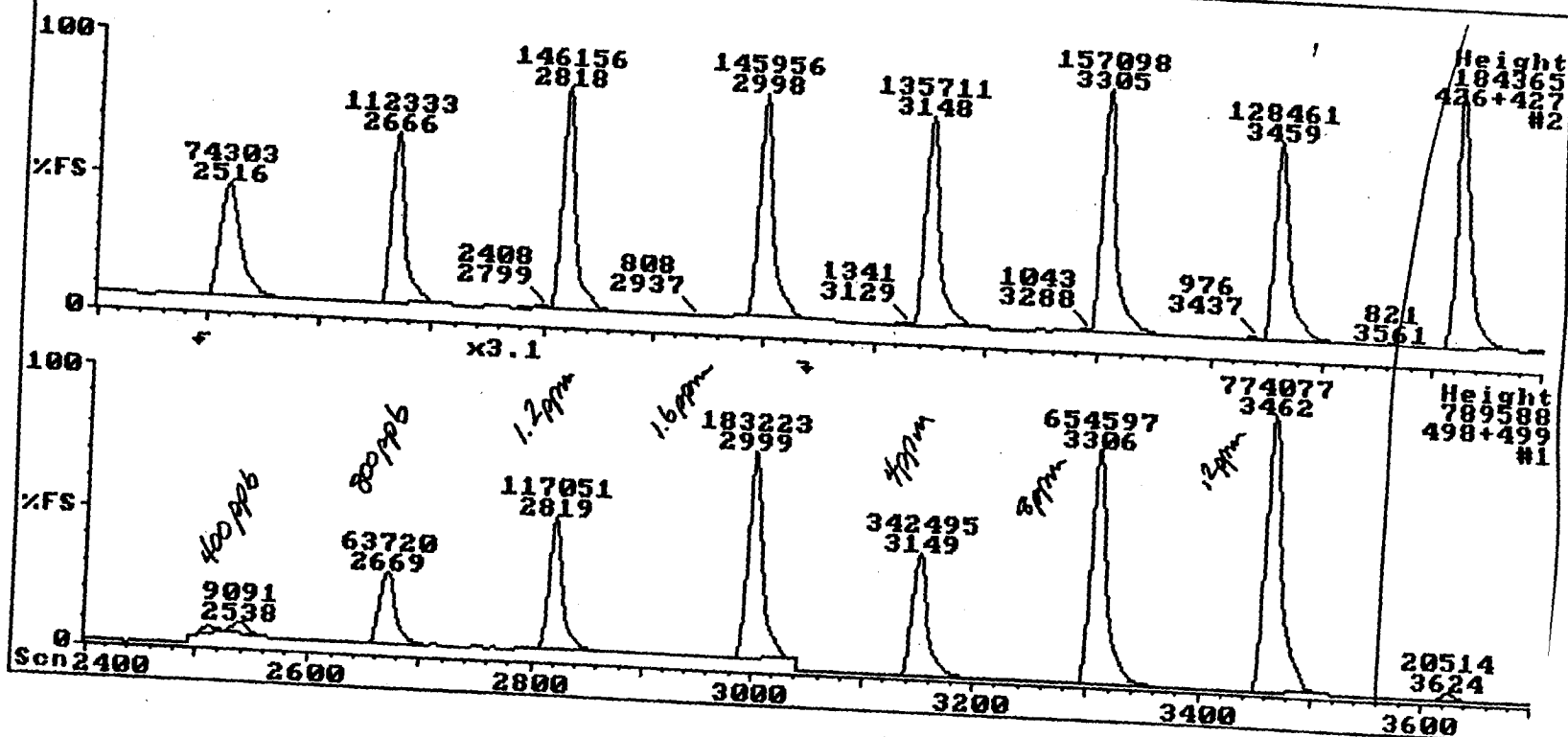
File:040695A

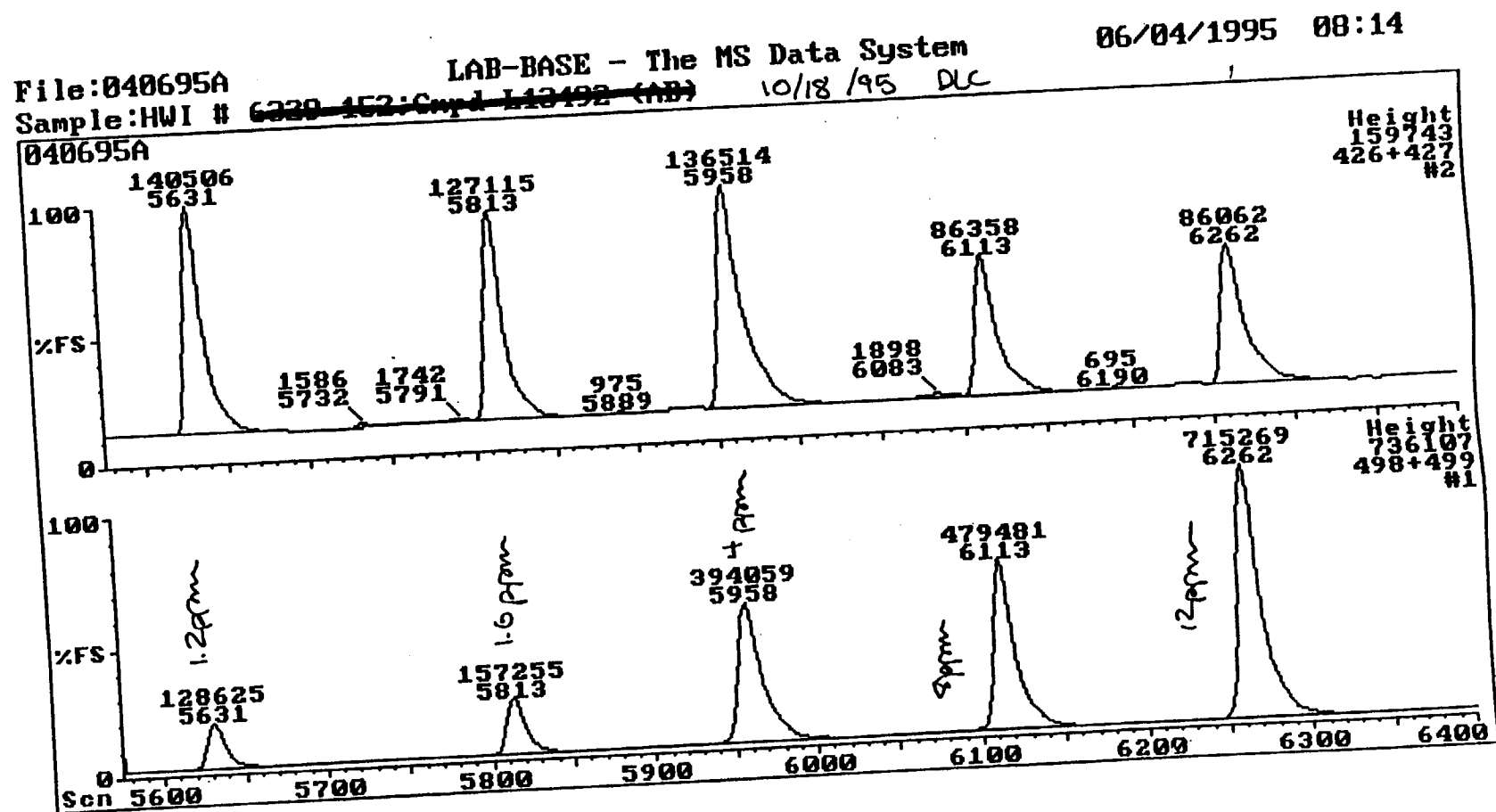
Sample:HLI # ~~6329~~ 152: Compd L15152 (HD)  
040695A

LAB-BASE - The MS Data System

10/18/95 DLC

06/04/1995 08:14





**9.11.3 Summary and raw data; ug F<sup>-</sup> in whole liver as determined by thermal extraction followed by analysis using Skalar segmented flow analyzer with ion selective electrode.**

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.

RE: 6329-138 LIVER SAMPLES  
AMDT 122094.2  
Date of Analysis: 4-20-95  
Analyst: DDW

The samples are burned in the Dohrman at 950 C using between 0.1 and 0.2 grams of the liver. The gas is collected in 1.0 mL of 1:1 TISAB/Milli-Q water then an additional 1 mL of 1:1 TISAB/Milli-Q is added to allow for sufficient volume for Skalar analysis. 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.

Diana Wright

**SUMMARY OF 6329-138  
LIVER SAMPLES  
AMDT 122094.2**

|                                            | Sample ID | Skalar Result (ppm) | DI TISAB final vol (ml) | Sample Weight (grams) | Actual ppm F- in Sample | Average Actual ppm F- in Sample | Total Tissue Wt (grams) | Total F- per tissue (ug) | Average Total F- per tissue (ug) |
|--------------------------------------------|-----------|---------------------|-------------------------|-----------------------|-------------------------|---------------------------------|-------------------------|--------------------------|----------------------------------|
| <b>GROUP 1</b><br>Dose Level : 0           | F52792-1  | 0.02                | 2.0                     | 0.1409                | 0.27                    |                                 | 80.1586                 | 22                       |                                  |
|                                            | F52792-2  | ND                  | 2.0                     | 0.1287                | ND                      | ND                              | 80.1586                 | ND                       | ND                               |
|                                            | F52792-3  | ND                  | 2.0                     | 0.1481                | ND                      |                                 | 80.1586                 | ND                       |                                  |
| <b>GROUP 2</b><br>Dose Level : 0.128 mg/kg | F52793-1  | 0.07                | 2.0                     | 0.1410                | 1.00                    |                                 | 68.8400                 | 69                       |                                  |
|                                            | F52793-2  | 0.06                | 2.0                     | 0.1253                | 0.99                    | 1.00                            | 68.8400                 | 68                       | 69                               |
|                                            | F52793-3  | 0.06                | 2.0                     | 0.1229                | 1.01                    |                                 | 68.8400                 | 70                       |                                  |
| <b>GROUP 3</b><br>Dose Level : 0.64 mg/kg  | F52750-1  | 0.11                | 2.0                     | 0.1176                | 1.95                    |                                 | 73.9156                 | 144                      |                                  |
|                                            | F52750-2  | 0.11                | 2.0                     | 0.1206                | 1.85                    | 1.84                            | 73.9156                 | 137                      | 136                              |
|                                            | F52750-3  | 0.11                | 2.0                     | 0.1303                | 1.73                    |                                 | 73.9156                 | 128                      |                                  |
| <b>GROUP 4</b><br>Dose Level : 1.28 mg/kg  | F52751-1  | 0.16                | 2.0                     | 0.1078                | 3.02                    |                                 | 66.7527                 | 202                      |                                  |
|                                            | F52751-2  | 0.17                | 2.0                     | 0.1332                | 2.58                    | 2.74                            | 66.7527                 | 172                      | 183                              |
|                                            | F52751-3  | 0.17                | 2.0                     | 0.1328                | 2.62                    |                                 | 66.7527                 | 175                      |                                  |
| <b>GROUP 5</b><br>Dose Level : 12.8 mg/kg  | F52752-1  | 0.42                | 2.0                     | 0.1429                | 5.91                    |                                 | 94.8862                 | 560                      |                                  |
|                                            | F52752-2  | 0.76                | 2.0                     | 0.1419                | 10.67                   | 9.05                            | 94.8862                 | 1012                     | 859                              |
|                                            | F52752-3  | 0.73                | 2.0                     | 0.1370                | 10.58                   |                                 | 94.8862                 | 1004                     |                                  |

## 138LIVER.XLS

95-07-06 15:57 OutPut of : 950420A1  
 Operator : DDW  
 Date of the Analysis : 1995-04-20 07:12  
 Analysis File Name : C:\SKALAR\DATA\HWIDATA\LIVERS\950420A1

| Sample # | Sample ID   | Skalar Standard (ppm) | Skalar Result (ppm) | % Recovery | DIETISAB final vol (ml) | Qty Sample (ml or grams) | Actual ppm F- in Sample | Total Tissue Wt (grams) | Total F- per flame (ug) | ml PC 95 Solution Spiked | Conc PC 95 Soln (ppm) | Mass Spiked (ug F-) | Mass Recovered (ug F-) | % Recovery |
|----------|-------------|-----------------------|---------------------|------------|-------------------------|--------------------------|-------------------------|-------------------------|-------------------------|--------------------------|-----------------------|---------------------|------------------------|------------|
| 1        | Tracer      | 1.50                  | 1.46                | 97%        |                         |                          |                         |                         |                         |                          |                       |                     |                        |            |
| 2        | Drift       | 1.50                  | 1.47                | 98%        |                         |                          |                         |                         |                         |                          |                       |                     |                        |            |
| 3        | Wash        |                       | ND                  |            |                         |                          |                         |                         |                         |                          |                       |                     |                        |            |
| 4        | Standard 1  | 0.015                 | 0.015               | 98%        |                         |                          |                         |                         |                         |                          |                       |                     |                        |            |
| 5        | Standard 2  | 0.03                  | 0.03                | 101%       |                         |                          |                         |                         |                         |                          |                       |                     |                        |            |
| 6        | Standard 3  | 0.06                  | 0.06                | 101%       |                         |                          |                         |                         |                         |                          |                       |                     |                        |            |
| 7        | Standard 4  | 0.09                  | 0.09                | 100%       |                         |                          |                         |                         |                         |                          |                       |                     |                        |            |
| 8        | Standard 5  | 0.12                  | 0.12                | 99%        |                         |                          |                         |                         |                         |                          |                       |                     |                        |            |
| 9        | Standard 6  | 0.15                  | 0.15                | 100%       |                         |                          |                         |                         |                         |                          |                       |                     |                        |            |
| 10       | Standard 7  | 0.30                  | 0.28                | 94%        |                         |                          |                         |                         |                         |                          |                       |                     |                        |            |
| 11       | Standard 8  | 0.60                  | 0.61                | 102%       |                         |                          |                         |                         |                         |                          |                       |                     |                        |            |
| 12       | Standard 9  | 1.20                  | 1.23                | 103%       |                         |                          |                         |                         |                         |                          |                       |                     |                        |            |
| 13       | Standard 10 | 1.50                  | 1.47                | 98%        |                         |                          |                         |                         |                         |                          |                       |                     |                        |            |
| 14       | Drift       | 1.50                  | 1.48                | 99%        |                         |                          |                         |                         |                         |                          |                       |                     |                        |            |
| 15       | Wash        |                       | ND                  |            |                         |                          |                         |                         |                         |                          |                       |                     |                        |            |
| 16       | BLK-1       |                       | 0.10                |            | 2.0                     | 0.1445                   | 1.36                    |                         |                         |                          |                       |                     |                        |            |
| 17       | BLK-2       |                       | 0.03                |            | 2.0                     | 0.1365                   | 0.48                    |                         |                         | 0.004                    | 63.00                 | 0.15                | 0.18                   | 118%       |
| 18       | SPK-1       |                       | 0.09                |            | 2.0                     | 0.1410                   | 1.26                    |                         |                         | 0.004                    | 63.00                 | 0.15                | 0.17                   | 113%       |
| 19       | SPK-2       |                       | 0.09                |            | 2.0                     | 0.1677                   | 1.02                    |                         |                         |                          |                       |                     |                        |            |
| 20       | 50-1        |                       | 0.11                |            | 2.0                     | 0.1176                   | 1.95                    | 73.9156                 | 144.06                  |                          |                       |                     |                        |            |
| 21       | 50-2        |                       | 0.11                |            | 2.0                     | 0.1206                   | 1.85                    | 73.9156                 | 136.68                  |                          |                       |                     |                        |            |
| 22       | 50-3        |                       | 0.11                |            | 2.0                     | 0.1303                   | 1.73                    | 73.9156                 | 128.20                  |                          |                       |                     |                        |            |
| 23       | 93-1        |                       | 0.07                |            | 2.0                     | 0.1410                   | 1.00                    | 68.8400                 | 68.55                   |                          |                       |                     |                        |            |
| 24       | 93-2        |                       | 0.06                |            | 2.0                     | 0.1253                   | 0.99                    | 68.8400                 | 68.46                   |                          |                       |                     |                        |            |
| 25       | 93-3        |                       | 0.06                |            | 2.0                     | 0.1229                   | 1.01                    | 68.8400                 | 69.57                   |                          |                       |                     |                        |            |
| 26       | Drift       | 1.50                  | 1.47                | 98%        |                         |                          |                         |                         |                         |                          |                       |                     |                        |            |
| 27       | Wash        |                       | ND                  |            |                         |                          |                         |                         |                         |                          |                       |                     |                        |            |
| 28       | 92-1        |                       | 0.02                |            | 2.0                     | 0.1409                   | 0.27                    | 80.1586                 | 21.73                   |                          |                       |                     |                        |            |
| 29       | 92-2        |                       | ND                  |            | 2.0                     | 0.1287                   | ND                      | 80.1586                 | ND                      |                          |                       |                     |                        |            |
| 30       | 92-3        |                       | ND                  |            | 2.0                     | 0.1481                   | ND                      | 80.1586                 | ND                      |                          |                       |                     |                        |            |
| 31       | 51-1        |                       | 0.16                |            | 2.0                     | 0.1078                   | 3.02                    | 66.7527                 | 201.87                  |                          |                       |                     |                        |            |

new 171143  
 And T 122094.2  
 HWI 6329-138  
 Liver Samples  
 Skalar Data

## 138LIVER.XLS

| Sample # | Sample ID | Skalar Standard (ppm) | Skalar Result (ppm) | % Recovery | DI TISAB final vol (ml.) | Qty Sample (ml. or gram) | Actual ppm P- in Sample | Total Tissue Wt (grams) | Total F- per tissue (ug) | ml. PC 95 Solution Spiked | Conc. PC 95 Soln (ppm) | Mass Spiked (ug F-) | Mass Recovered (ug F-) | % Recovery |
|----------|-----------|-----------------------|---------------------|------------|--------------------------|--------------------------|-------------------------|-------------------------|--------------------------|---------------------------|------------------------|---------------------|------------------------|------------|
| 32       | 51-2      |                       | 0.17                |            | 2.0                      | 0.1332                   | 2.58                    | 66.7527                 | 172.39                   |                           |                        |                     |                        |            |
| 33       | 51-3      |                       | 0.17                |            | 2.0                      | 0.1328                   | 2.62                    | 66.7527                 | 174.92                   |                           |                        |                     |                        |            |
| 34       | 52-1      |                       | 0.42                |            | 2.0                      | 0.1429                   | 5.91                    | 94.8862                 | 560.42                   |                           |                        |                     |                        |            |
| 35       | 52-2      |                       | 0.76                |            | 2.0                      | 0.1419                   | 10.67                   | 94.8862                 | 1012.39                  |                           |                        |                     |                        |            |
| 36       | 52-3      |                       | 0.73                |            | 2.0                      | 0.1370                   | 10.58                   | 94.8862                 | 1004.27                  |                           |                        |                     |                        |            |
| 37       | BLK-1     |                       | 0.04                |            | 2.0                      | 0.1334                   | 0.55                    |                         |                          |                           |                        |                     |                        |            |
| 38       | Drift     | 1.50                  | 1.51                | 100%       |                          |                          |                         |                         |                          |                           |                        |                     |                        |            |
| 39       | Wash      |                       | ND                  |            |                          |                          |                         |                         |                          |                           |                        |                     |                        |            |
| 40       | BLK-2     |                       | 0.02                |            | 2.0                      | 0.1092                   | 0.44                    |                         |                          | 0.004                     | 63.00                  | 0.15                | 0.18                   | 119%       |
| 41       | SPK-63-1  |                       | 0.09                |            | 2.0                      | 0.1484                   | 1.21                    |                         |                          | 0.004                     | 63.00                  | 0.15                | 0.18                   | 119%       |
| 42       | SPK-63-2  |                       | 0.09                |            | 2.0                      | 0.1370                   | 1.32                    |                         |                          | 0.004                     | 126.00                 | 0.30                | 0.29                   | 96%        |
| 43       | SPK 126-1 |                       | 0.15                |            | 2.0                      | 0.1212                   | 2.40                    |                         |                          | 0.004                     | 126.00                 | 0.30                | 0.28                   | 91%        |
| 44       | SPK 126-2 |                       | 0.14                |            | 2.0                      | 0.1439                   | 1.91                    |                         |                          | 0.004                     | 126.00                 | 0.30                | 0.27                   | 91%        |
| 45       | SPK 126-3 |                       | 0.14                |            | 2.0                      | 0.1085                   | 2.53                    |                         |                          |                           |                        |                     |                        |            |
| 46       | Drift     | 1.50                  | 1.50                | 100%       |                          |                          |                         |                         |                          |                           |                        |                     |                        |            |
| 47       | Wash      |                       | ND                  |            |                          |                          |                         |                         |                          |                           |                        |                     |                        |            |

OutPut of : 950420A1

Software : version 6.1 c1990.93

Operator : DDW

Date of the Analysis : 1995-04-20 07:12

Analysis File Name : C:\SKALAR\DATA\HWIDATA\LIVERS\950420A1

QOW 7/17/95  
AMDT 122094.2  
HWI 6329-138  
Liner Samples

Fluoride 1.5

Calibration order = Inverse Logarithm

**Slope** :  $S = \#.####$

Result =  $10 \left[ \frac{x - c_1}{s} \right]$

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

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

**a2 = -0.00000**

```
a1 = 0.00065
```

```
a0 = -1.24984
```

Fluoride L

Calibration order = 2

Correlation :  $r = 0.99991$

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

```
a2 = 0.00000
```

```
a1 = 0.00020
```

```
a0 = 0.00010
```

```

Sampler          Type          : SA1000
                  Number        : 1
                  Sample Time   : 50 sec.
                  Wash Time     : 120 sec.
                  Air Time      : 1 sec.
                  Take up       : Single
                  sSpecial      : 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
```

1995-04-20 09:36

OutPut of : 950420A1

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

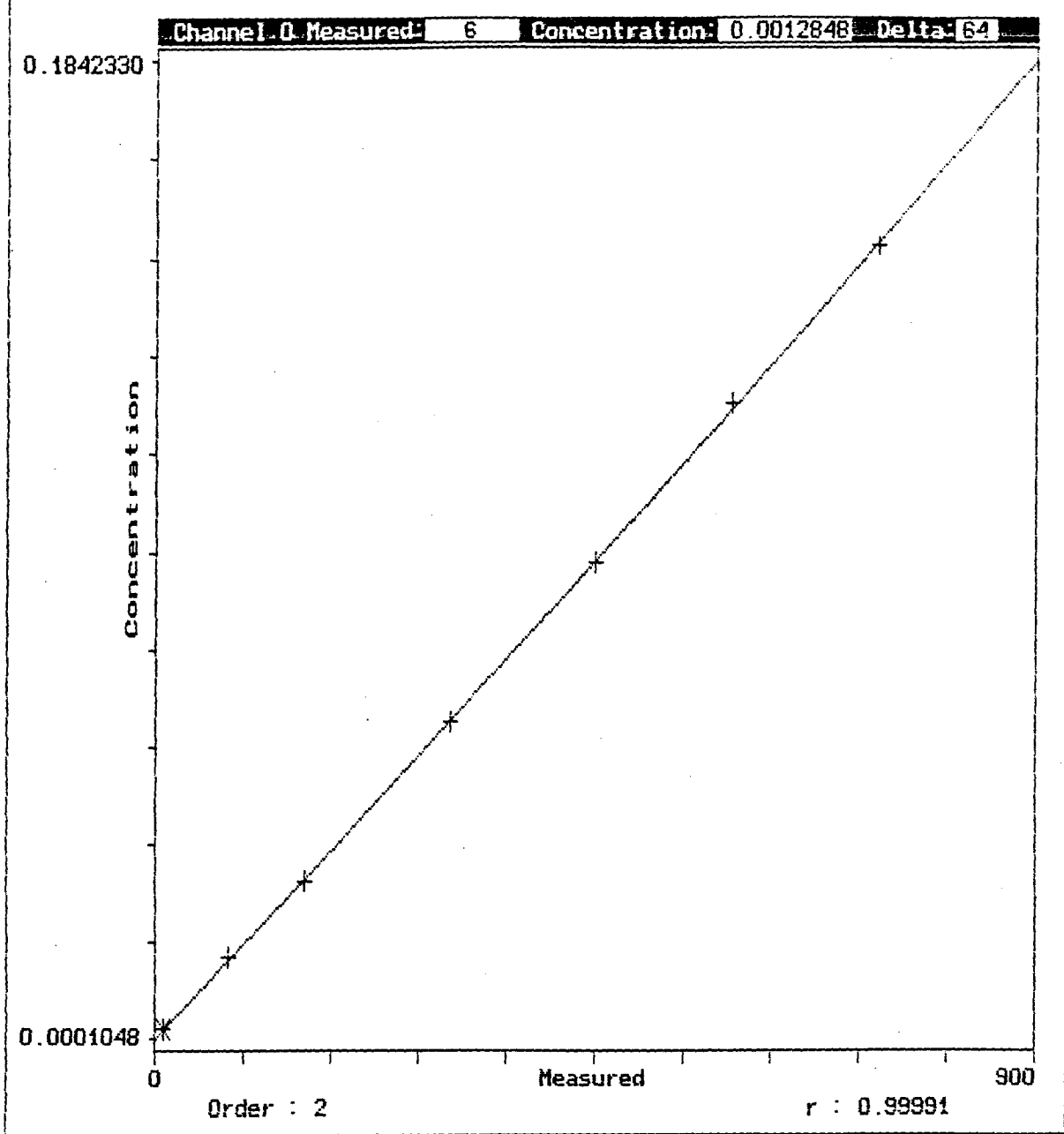
1995-04-20 09:36

OutPut of : 950420A1

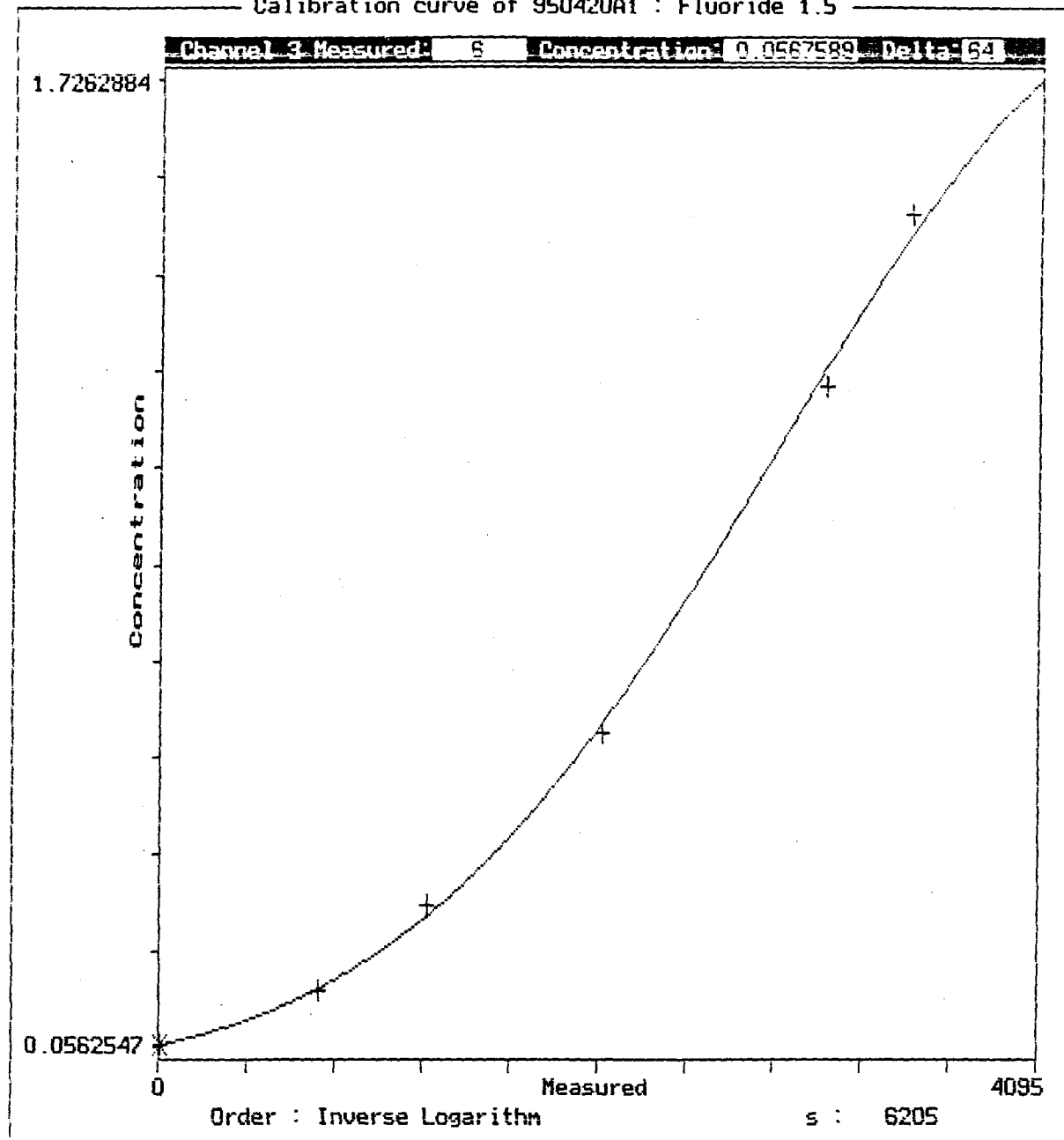
```
s1 sTandard : 0.015
s2 sTandard : 0.030
s3 sTandard : 0.060
s4 sTandard : 0.090
s5 sTandard : 0.120
s6 sTandard : 0.150
s7 sTandard : Ignore
s8 sTandard : Ignore
s9 sTandard : Ignore
s10 sTandard : Ignore
Order : 2
Dimension : PPM
start Value : 500 DU
trigger Limit : 1800 Sec
Peak shape : Pointed
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  | iw  | Initial Wash | 3            | 0.056  | 65     | 4          | 0.0001 | 0      |
| 1   | t   | Tracer       | 3            | 1.457  | 210    | 4          | 0.7942 | 0      |
| 2   | d   | Drift        | 3            | 1.470  | 384    | 4          | 0.8006 | 0      |
| 3   | w   | Wash         | 3            | 0.056  | 626    | 4          | 0.0001 | 0      |
| 4   | s1  | Standard 1   | 3            | 0.063  | 733    | 4          | 0.0147 | 0      |
| 5   | s2  | Standard 2   | 3            | 0.070  | 907    | 4          | 0.0302 | 0      |
| 6   | s3  | Standard 3   | 3            | 0.087  | 1085   | 4          | 0.0603 | 0      |
| 7   | s4  | Standard 4   | 3            | 0.106  | 1261   | 4          | 0.0904 | 0      |
| 8   | s5  | Standard 5   | 3            | 0.128  | 1436   | 4          | 0.1190 | 0      |
| 9   | s6  | Standard 6   | 3            | 0.155  | 1611   | 4          | 0.1505 | 0      |
| 10  | s7  | Standard 7   | 3            | 0.282  | 1785   | 4          | 0.2602 | 0      |
| 11  | s8  | Standard 8   | 3            | 0.614  | 1959   | 4          | 0.4429 | 0      |
| 12  | s9  | Standard 9   | 3            | 1.232  | 2135   | 4          | 0.6965 | 0      |
| 13  | s10 | Standard 10  | 3            | 1.467  | 2311   | 4          | 0.7988 | 0      |
| 14  | d   | Drift        | 3            | 1.483  | 2485   | 4          | 0.8066 | 0      |
| 15  | w   | Wash         | 3            | 0.056  | 2727   | 4          | 0.0001 | 0      |
| 16  | u   | BLK-1        | 3            | 0.112  | 2836   | 4          | 0.0986 | 0      |
| 17  | u   | BLK-2        | 3            | 0.071  | 3011   | 4          | 0.0326 | 0      |
| 18  | u   | SPK-1        | 3            | 0.105  | 3187   | 4          | 0.0889 | 0      |
| 19  | u   | SPK-2        | 3            | 0.103  | 3363   | 4          | 0.0857 | 0      |
| 20  | u   | 50-1         | 3            | 0.124  | 3538   | 4          | 0.1146 | 0      |
| 21  | u   | 50-2         | 3            | 0.122  | 3714   | 4          | 0.1115 | 0      |
| 22  | u   | 50-3         | 3            | 0.123  | 3886   | 4          | 0.1130 | 0      |
| 23  | u   | 93-1         | 3            | 0.093  | 4060   | 4          | 0.0702 | 0      |
| 24  | u   | 93-2         | 3            | 0.088  | 4238   | 4          | 0.0623 | 0      |
| 25  | u   | 93-3         | 3            | 0.088  | 4414   | 4          | 0.0621 | 0      |
| 26  | d   | Drift        | 3            | 1.467  | 4588   | 4          | 0.7988 | 0      |
| 27  | w   | Wash         | 3            | 0.056  | 4740   | 4          | 0.0001 | 0      |
| 28  | u   | 92-1         | 3            | 0.065  | 4936   | 4          | 0.0191 | 0      |
| 29  | u   | 92-2         | 3            | 0.063  | 5111   | 4          | 0.0143 | 0      |
| 30  | u   | 92-3         | 3            | 0.061  | 5285   | 4          | 0.0117 | 0      |
| 31  | u   | 51-1         | 3            | 0.163  | 5463   | 4          | 0.1586 | 0      |
| 32  | u   | 51-2         | 3            | 0.172  | 5637   | 4          | 0.1686 | 0      |
| 33  | u   | 51-3         | 3            | 0.174  | 5813   | 4          | 0.1705 | 0      |
| 34  | u   | 52-1         | 3            | 0.422  | 5989   | 4          | 0.3468 | 0      |
| 35  | u   | 52-2         | 3            | 0.757  | 6163   | 4          | 0.5053 | 0      |
| 36  | u   | 52-3         | 3            | 0.725  | 6339   | 4          | 0.4918 | 0      |
| 37  | u   | BLK-1        | 3            | 0.074  | 6515   | 4          | 0.0368 | 0      |
| 38  | d   | Drift        | 3            | 1.507  | 6689   | 4          | 0.8183 | 0      |
| 39  | w   | Wash         | 3            | 0.056  | 6930   | 4          | 0.0001 | 0      |
| 40  | u   | BLK-2        | 3            | 0.067  | 7034   | 4          | 0.0238 | 0      |
| 41  | u   | SPK-63-1     | 3            | 0.106  | 7214   | 4          | 0.0898 | 0      |
| 42  | u   | SPK-63-2     | 3            | 0.106  | 7390   | 4          | 0.0902 | 0      |
| 43  | u   | SPK 126-1    | 3            | 0.151  | 7566   | 4          | 0.1456 | 0      |
| 44  | u   | SPK 126-2    | 3            | 0.143  | 7740   | 4          | 0.1375 | 0      |
| 45  | u   | SPK 126-3    | 3            | 0.143  | 7914   | 4          | 0.1371 | 0      |
| 46  | d   | Drift        | 3            | 1.495  | 8090   | 4          | 0.8123 | 0      |
| 47  | w   | Wash         | 3            | 0.056  | 8324   | 4          | 0.0001 | 0      |
| wt  | rw  | RunOut Wash  | 3            | 0.056  | 8565   | 4          | 0.0001 | 0      |

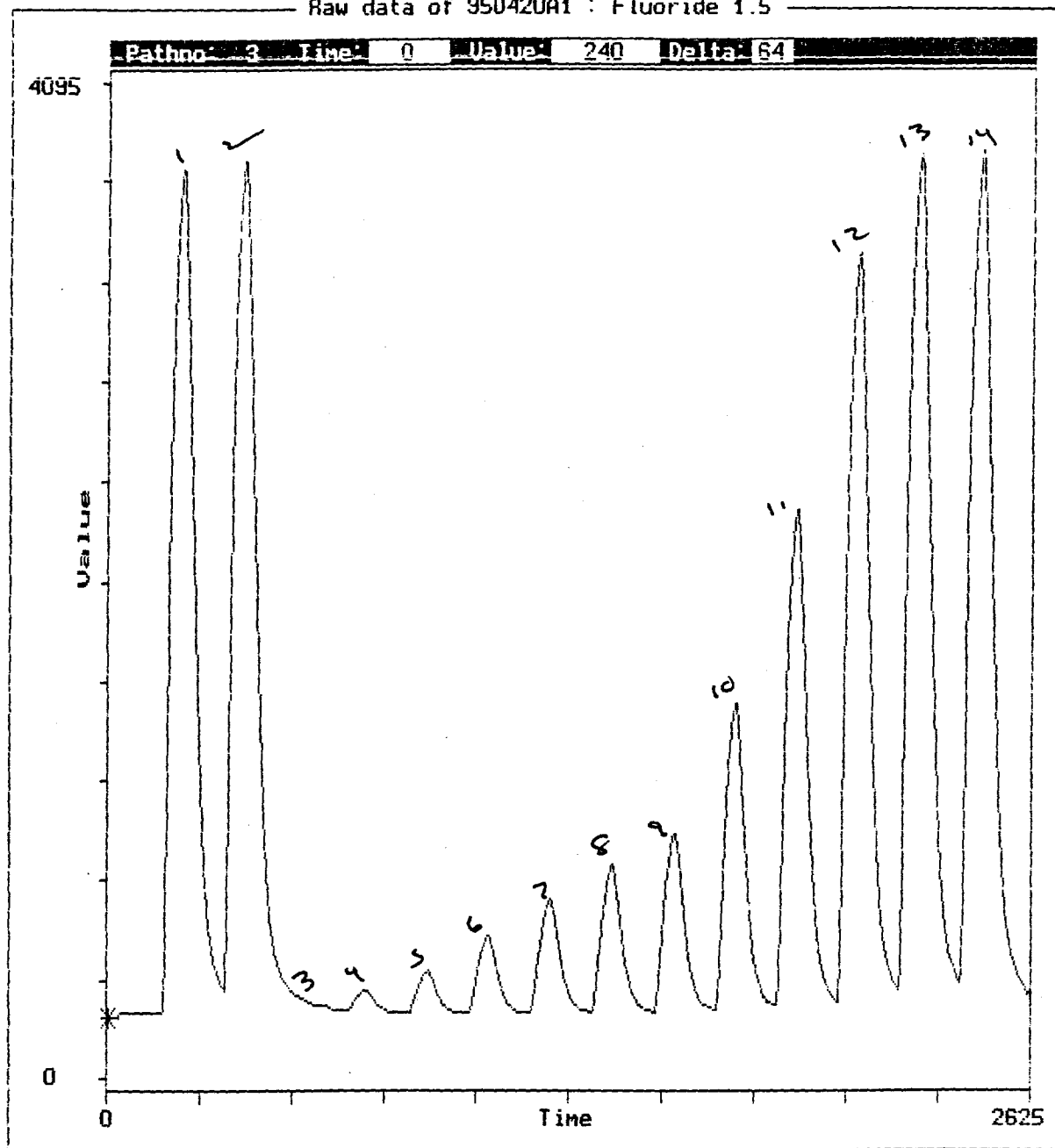
Calibration curve of 950420A1 : Fluoride L



Calibration curve of 950420A1 : Fluoride 1.5

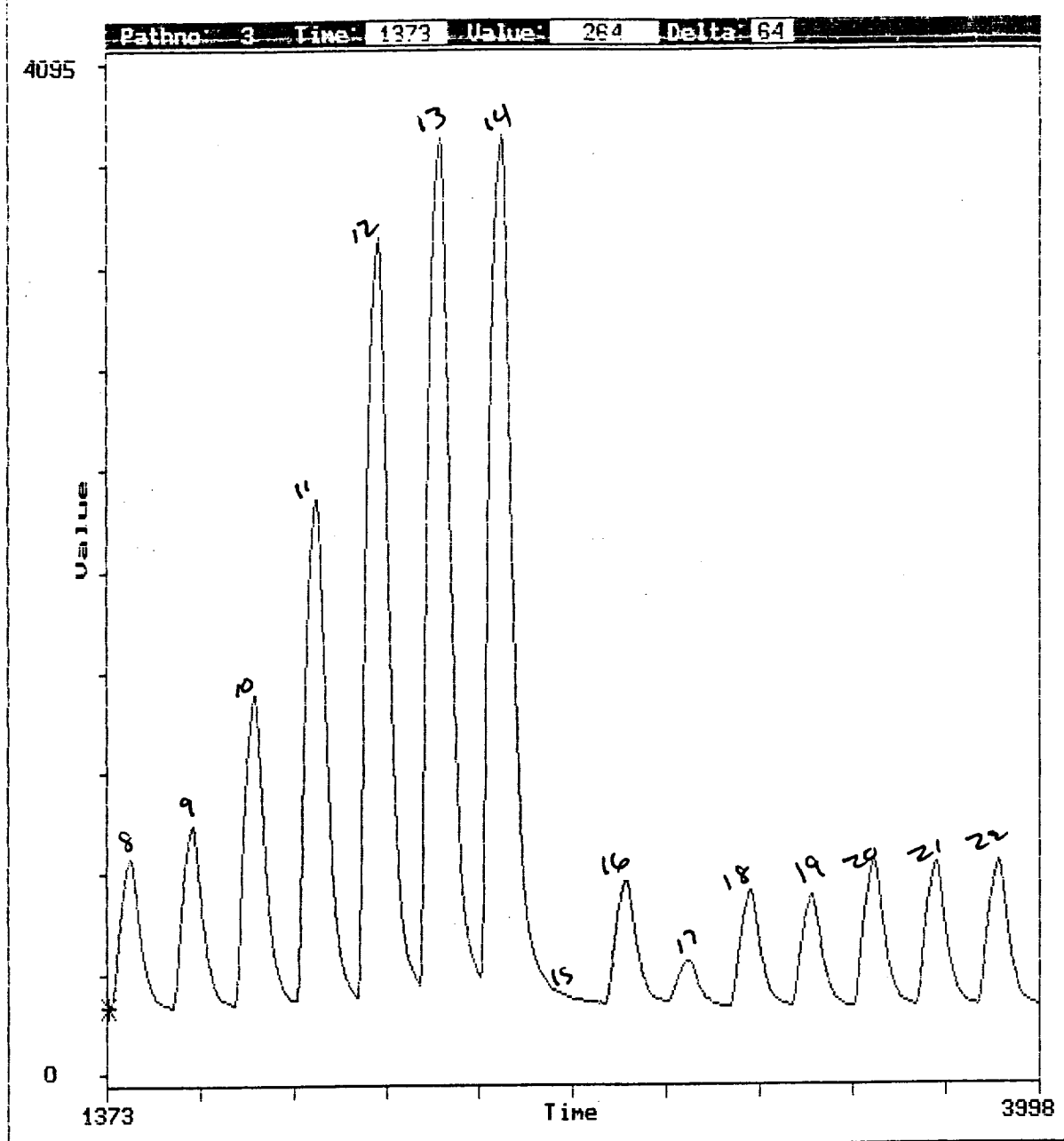


Raw data of 950420A1 : Fluoride 1.5



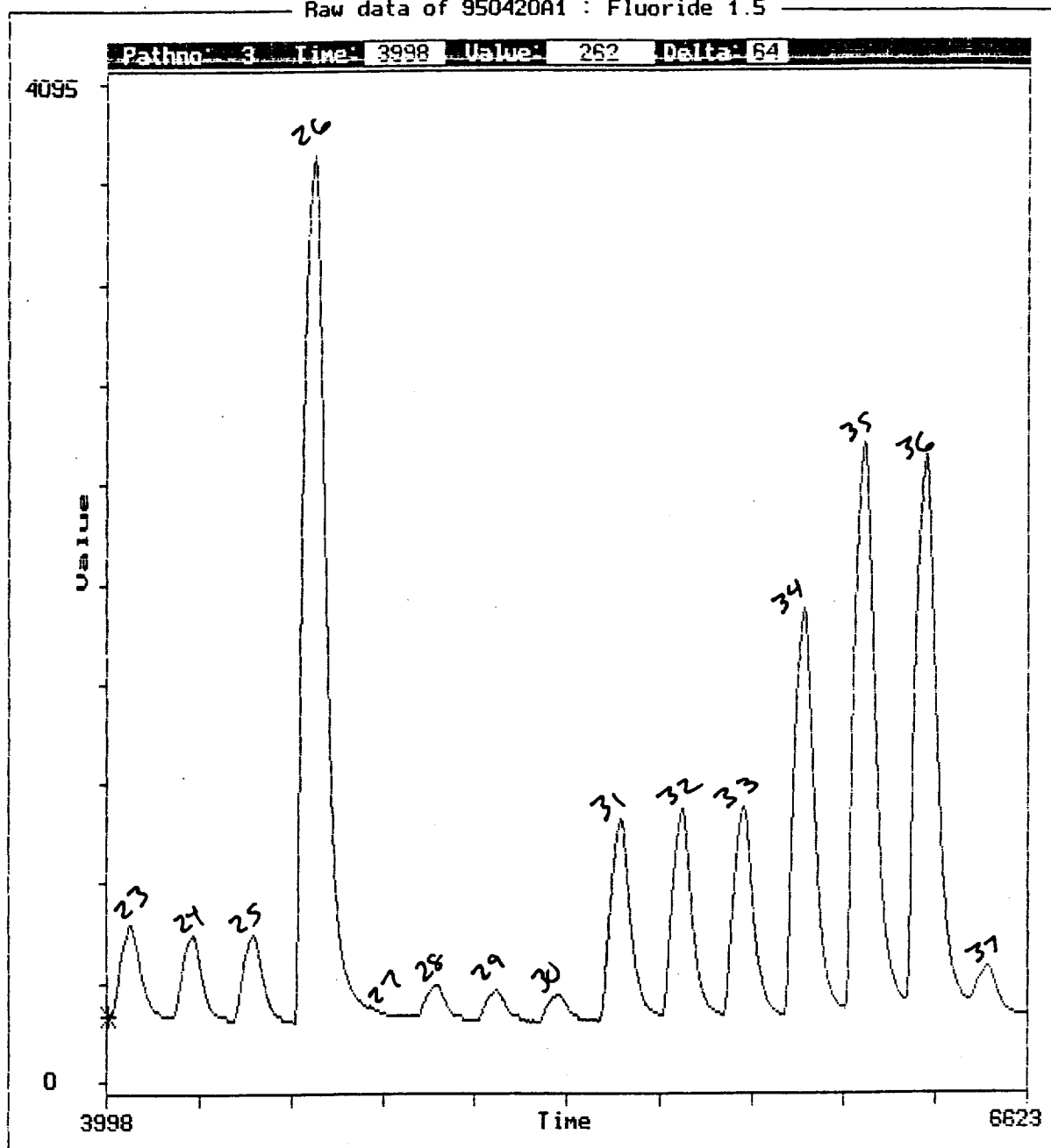
Esc=Exit : F1=Help : Ctrl-P=Edit peaks :

Raw data of 950420A1 : Fluoride 1.5



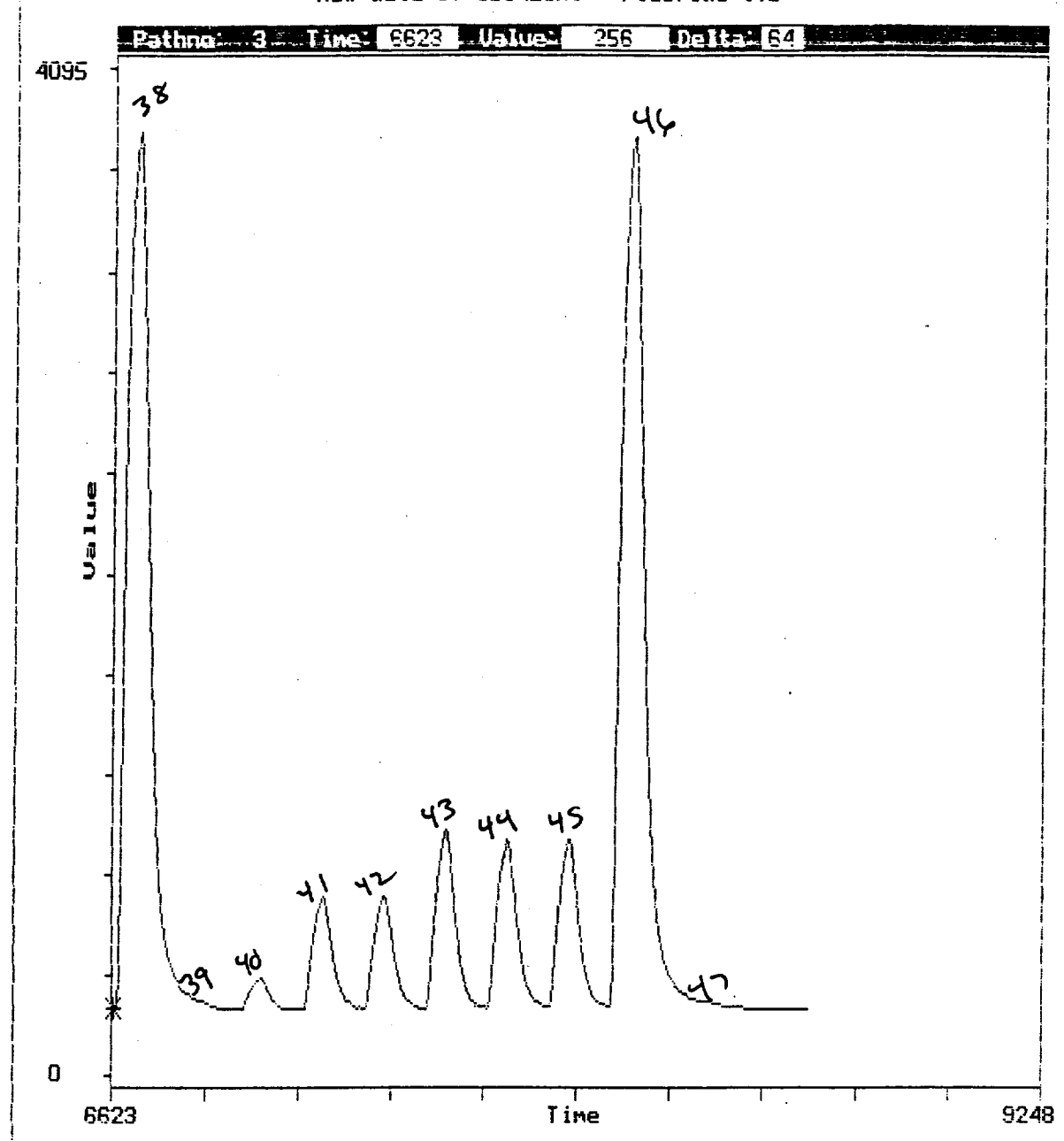
Esc=Exit : F1=Help : Ctrl-P=Edit peaks :

Raw data of 950420A1 : Fluoride 1.5



Esc=Exit : F1=Help : Ctrl-P=Edit peaks :

Raw data of 950420A1 : Fluoride 1.5



Esc=Exit : F1=Help : Ctrl-P=Edit peaks :