3M Environmental Laboratory

Final Report- Analytical Study

Single-Dose Dermal Absorption/Toxicity Study of T-6052 in Rabbits

In-Vivo Study Reference Number: HWI#6329-135

Study Number: AMDT-020795.1
Test Substance: FC-120 (T-6052)

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

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

Method Numbers and Revisions:

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

Initiation Date: See attached protocol

Author: James D. Johnson

Approved By:

James D. Johnson
Study Director

11/20/95
Completion Date

1.0 SUMMARY

Samples of liver at 28 days post dermal doses of FC-120 (T-6052) were analyzed by combustion for total organic fluorine. Even the highest dose of 1000 mg/kg (50 ug/kg) resulted in no organic fluorine at practical quantitation levels. There is a trace of some fluorine detectable if one uses just relative meter readings. Electrospray mass spectrometry was able to detect m/z=599 ion which is the perfluorodecanesulfonate anion.

The doses were too low to assess dermal absorption with this test method.

2.0 INTRODUCTION

The pharmacokinetic study for FC-120 was not successful. The highest dose was 50 ug/kg. Thus, in this study, if there was a substantial amount of organic fluorine present at 28 days it would indicate that a significant absorption of FC-120 had occurred. However, in the event of very little organic fluorine at 28 days, it would not be possible to make an assessment of dermal absorption. Liver samples and serum samples were available for analysis of total organic fluorine and perfluorodecanesulfonate anion. The samples were analyzed. Because the doses are quite low (high dose 50 ug/kg), it was not expected that fluorine would be detected.

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, bovine serum, rabbit serum

3.1.3 Analytical Control Substance: None

3.1.4 Analytical Control Matrix: Bovine liver, bovine serum and rabbit serum

3.2 Source of Materials: 3M ICP/PCP Division for FC-95, bovine liver from grocery store, bovine serum from Sigma Chemical Company, rabbit serum from AMDT-110394.1 (HWI#6329-123) control group animals.

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

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

3.5 Storage Conditions for Test Materials: Room temperature for FC-95. For biological samples the storage is -20 ± 10^{0} C.

3.6 Disposition Specimens: Biological tissues and fluids will be retained per GLP Regulation for the time period required for studies longer than 28 days.

4.0 EXPERIMENTAL - Overview

The tissues from animals dosed as described (HWI#6329-135), were available for analysis for fluorine compounds. At the discretion of the study director, a series of analytical tests could be performed. The screening for fluoride in liver via combustion was the most likely analysis to present definitive data for absorption if the pharmacokinetic test (IV administration) was positive for fluorine in the liver. Other available tests were electrospray mass spectroscopy. However, if the definitive results could be obtained with combustion analysis alone, only the liver samples would be analyzed and any other tests would be for confirmation.

5.0 EXPERIMENTAL - METHODS

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

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

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

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

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

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

6.0 DATA ANALYSIS

The data from combustion analysis are attached. There does not appear to be any total organic fluorine above the practical quantitation limit for any of the liver samples at 48 hours post intravenous dose for any of the dosing regimens. Even the 50 ug/kg dose is not above the practical quantitation limit. However, if one uses just the meter readings and compares those reading to the values for the controls, there is possibly a trace of fluorine being detected.

Electrospray mass spectrometry analysis is attached. The electrospray was able to detect perfluorodecanesulfonate anion in the 50 ug/kg dose livers. The amount present was not quantitated.

In view of the failure of the pharmacokinetic study (HWI#6329-134) to show a good marker for FC-120 at a dose of 50 ug/kg, it is not reasonable to make an assessment of the extent of dermal absorption from this study at the same dose level.

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 Have Affected the Quality of the Data: The problem with this analysis is that the pharmacokinetic study did not provide a good marker for dermal absorption because the dose level was too low. The dermal study is not at a higher dose.

7.0 CONCLUSION

This study does not provide a useful assessment of dermal absorption of FC-120. There is not a useful marker.

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#6329-135: "Single-Dose Dermal Absorption/Toxicity Study of T-6052 in Rabbits" (Protocol type TP3016.AB for dosing of animals, tissue collection, etc.)

9.1.2 Analytical protocol AMDT-020795.1

9.1.3 Amendment to Analytical Protocol AMDT-020795.1

9.2 Signed Reports from Individual Scientists: None

9.3 Quality Assurance Unit Statement: See attached

9.4 Key Personnel Involved in the Study: See attached

9.5 Materials and Equipment: See methods

9.6 Solutions, Reagents, and Standards: See methods

9.7 Sample Preparation: See methods

9.8 Quality Control Practices: See methods

9.9 Test Methods: See Protocol AMDT-020795.1

9.10 Instrument Settings: See methods

9.11 Data: See attached.

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

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

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

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

9.1.1 Protocol and Final Report: HWI#6329-135: "Single-Dose Dermal Absorption/Toxicity Study of T-6052 in Rabbits" (Protocol type TP3016.AB for dosing of animals, tissue collection, etc.)

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Sponsor:

3M Toxicology Service Medical Department
St. Paul, Minnesota

FINAL REPORT

Study Title:

Single-Dose Dermal Absorption/Toxicity Study of T-6052 in Rabbits

Author:

Steven M. Glaza

Study Completion Date:

June 27, 1995

Performing Laboratory:

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

Laboratory Project Identification:

HWI 6329-135

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HWI 6329-135

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 From	To	Phase	Date Reported to Study Director	Date to Management
12/21/94	12/21/94	Protocol Review	12/22/94	01/10/95
01/30/95	01/30/95	Protocol Amendment	01/30/95	02/10/95
02/02/95	02/02/95	Body Weight	02/02/95	03/10/95
03/29/95	03/29/95	Data/Report Review	03/29/95	04/10/95
03/29/95	03/29/95	Data Review	03/29/95	04/10/95
06/27/95	06/27/95	Report Rereview	06/27/95	07/10/95

Randy Lesty
Representative, Quality Assurance Unit

6.27.95
Date

STUDY IDENTIFICATION

Single-Dose Dermal Absorption/Toxicity
Study of T-6052 in Rabbits

Test Material	T-6052
Sponsor	3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220
Sponsor's Representative	John L. Butenhoff, PhD 3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220 (612) 733-1962
Study Director	Steven M. Glaza Hazleton Wisconsin, Inc. P.O. Box 7545 Madison, WI 53707-7545 (608) 241-7292
Study Location	Hazleton Wisconsin, Inc. Building No. 3 3802 Packers Avenue Madison, WI 53704
Study Timetable	
Study Initiation Date	December 30, 1994
Experimental (In-life) Start Date	January 5, 1995
In-life End Date	February 2, 1995
Experimental Termination Date	June 27, 1995
Study Completion Date	June 27, 1995

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

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Laboratory Animal Medicine

Cindy J. Cary, DVM
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Thomas E. Palmer, PhD
Anatomical Pathologist

Jack Serfort/
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Supervisors
Necropsy

Anne Mosher
Supervisor
Pathology Data

CONTENTS

SUMMARY

This study was done to assess the systemic absorption/toxicity and relative skin irritancy of T-6052 when applied to the skin of rabbits.

The study was conducted using three male and three female acclimated rabbits of the Hra:(NZW)SPF strain for each treatment group.

Group	Test Material	Dose Level (mg/kg)	Number of Animals Males	Females
1 (Control)	Distilled water	0[a]	3	3
2	T-6052	2	3	3
3	T-6052	200	3	3
4	T-6052	1,000	3	3

a Administered at a dose volume of 2.0 mL/kg.

The back of each rabbit was clipped free of hair and a single dose of the respective material at the indicated dose level was administered to the skin of the rabbits. The treatment sites remained intact. The area of application was covered with a gauze bandage secured with paper tape around all edges and overwrapped with Saran Wrap® and Elastoplast® tape to provide an occlusive dressing for a 24-hour exposure period.

Clinical observations were conducted predose and at approximately 1, 2.5, and 4 hours after test or control material administration. Additional clinical observations and twice a day mortality checks were conducted daily thereafter for 28 days. Body weights were determined on Day -8 for randomization purposes, before test or control material administration (Day 1), and at in-life termination (Day 29). The initial dermal irritation reading was made before test or control material administration (recorded as the Day 1 reading). Subsequent readings of dermal irritation were made approximately 30 minutes after bandage removal (Day 2) and on Days 4 and 8. Blood samples were collected from a marginal ear vein of the animals before in-life initiation (Day 1), approximately 24-hours postdose (Day 2), on Days 4, 8, 15, and 22. In addition, at the time of necropsy on Day 29, approximately 20 mL of blood was obtained from each animal. All samples were centrifuged and separated into serum and cellular fractions. All animals were euthanized at termination of the in-life phase and necropsied. The whole liver, bile, an approximate 1-cm x 1-cm section of the dermal application site from all animals, and both kidneys from one male and one female in each group were collected at necropsy and weighed (volume only determined for bile). The blood samples (serum and cellular fractions), livers, bile, dermal application sites, and kidneys were sent frozen to the Sponsor after termination of the in-life phase.

Application of T-6052 did not result in any test material-related changes in body weight gain or macroscopic findings at necropsy. All animals appeared clinically normal throughout the study. No dermal irritation was observed at the dermal scoring intervals as a result of the application of distilled water or T-6052 at any of the dose levels.

OBJECTIVE

The objective of this study was to assess the systemic toxicity/absorption and relative skin irritancy of a test material when applied to the skin of 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 material mixture prepared for the Group 2 animals for concentration, homogeneity/solubility, and stability was not conducted. All procedures used in this study are 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-6052 and described as a clear, colorless liquid. The control material was distilled water 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). Analysis of the test material mixture prepared for the Group 2 animals for concentration, homogeneity/solubility, and stability was not conducted or requested by the Sponsor. The purity and stability of the control material were considered to be adequate for the purposes of this study.

Storage and Retention

The test and control materials were stored at room temperature. A reserve sample of each test and control material was taken and will be retained in a freezer set to maintain a temperature of -20°C ±10° for 10 years in accordance with Hazleton Wisconsin (HWI) Standard Operating Procedure (SOP). Any unused test material was returned to the Sponsor after completion of the in-life phase according to HWI SOP. Any remaining control material is retained 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 procured from HRP, Inc., Denver, Pennsylvania on December 28, 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 these conditions existed, they were documented and considered to have had no adverse effect on the study outcome.

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 placed into study groups using a stratified body weight randomization program. The randomization body weights were determined on Day -8. The weight variation of the animals for each group of each sex selected for the study did not exceed ±2 standard deviations of the mean weight, and the mean body weights for each group of each sex were not statistically different at the 5% probability level. One female animal (No. F53409) was replaced in the study prior to treatment due to poor health. This animal was replaced with another female (No. F52982) which was treated in the same manner.

Study Design

Animals weighing from 2,052 to 2,471 g at initiation of treatment were placed into the following study groups:

Group	Test Material	Dose Level (mg/kg)	Number of Animals Males	Number of Animals Females
1 (Control)	Distilled water	0[a]	3	3
2	T-6052	2	3	3
3	T-6052	200	3	3
4	T-6052	1,000	3	3

a Administered at a dose volume of 2.0 mL/kg.

Justification for Species Selection

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

PROCEDURES

Preparation of Exposure Area

On the day before test material application, the back and, if necessary (to obtain unblemished skin), the flanks of each rabbit was clipped free of hair. The clipped area made up approximately 20% of the total body surface area. The intact skin of the test sites was inspected for interfering lesions, irritation, or defects that would preclude the use of any of the animals. The animals were clipped on Days 8 and 29 to aid in visualizing the application sites.

Dose Administration

All animals received a single administration of the respective test or control material. The day of treatment was designated as Day 1.

Group 1. An individual dose (2.0 mL/kg) was calculated and measured based on each animal's body weight on the day of treatment. The control material (distilled water) was applied evenly to the test site at a rate of approximately 0.04 mL/cm^2.

Groups 2, 3, and 4. For the Group 2 animals (2 mg/kg), the test material (T-6052) was mixed with distilled water to a concentration of 200 mg/mL and applied at a dose volume of 0.01 mL/kg. The mixture was stored at room temperature until administered. The test material was administered undiluted to the test sites of the Groups 3 and 4 animals (200 or 1,000 mg/kg, respectively) using the average bulk density of 0.98 g/mL to determine the dose volume for each dose level (0.20 and 1.02 mL/kg, respectively). An individual dose of the respective test material or test material mixture was calculated for each animal based on its body weight on the day of treatment. The area of exposure for the 2, 200, and 1,000 mg/kg dose levels was 4, 25, and 100 cm^2, respectively. The approximate rate of application ranged from 0.006 to 0.024 mL/cm^2.

Each area of application was covered with a 10-cm x 10-cm gauze bandage secured with paper tape around all edges and overwrapped with Saran Wrap® and Elastoplast® tape to provide an occlusive dressing. Collars were used to restrain the animals during the 24-hour exposure period.

Approximately 24 hours after test or control material application, the restraining collars and bandages were removed and any residual test material was removed with tap water and disposable paper towels.

Reason for Route of Administration

The dermal route is a potential route of exposure in humans.

Observations of Animals

Clinical observations were conducted predose and at approximately 1, 2.5, and 4 hours after test or control material administration. Additional clinical observations and twice a day mortality checks (morning and afternoon) were conducted daily thereafter for 28 days.

Body weights were determined for randomization purposes on Day -8, before test material administration (Day 1), and at in-life termination (Day 29).

The initial dermal irritation reading was made before test or control material administration according to the Draize[1] technique (recorded as the Day 1 reading). Subsequent readings of dermal irritation were made approximately 30 minutes after bandage removal (Day 2) and on Days 4 and 8.

Sample Collections

Blood samples (approximately 4 mL) were collected from a marginal ear vein of all animals before experimental initiation (Day 1). Subsequent collection of blood was conducted approximately 24-hours postdose (Day 2), and on Days 4, 8, 15, and 22. In addition, at the time of necropsy on Day 29, approximately 20 mL of blood was obtained from the posterior vena cava of each animal. All samples were centrifuged and separated into serum and cellular fractions. These samples were then stored in a freezer set to maintain a temperature of -20°C ±10°C until shipped to the Sponsor.

Pathology

At termination of the experimental phase (Day 29), animals were anesthetized with sodium pentobarbital, bled via the posterior vena cava, exsanguinated, and necropsied in random order. The sites of test and control material application were washed with lukewarm tap water before the necropsy procedure. All animals were subjected to an abbreviated gross necropsy examination and any abnormalities were recorded. The whole liver, bile, an approximate 1-cm x 1-cm section of the dermal application site from all animals, and both kidneys from the first male and female in each group were collected. The tissue samples were weighed (volume only determined for bile) and immediately placed on dry ice, then placed in a freezer set to maintain a temperature of -20°C ±10°C. After necropsy, the animals were discarded.

Shipment of Blood, Bile, and Tissues

After experimental termination, the blood samples (serum and cellular fractions), livers, bile, dermal application sites, 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), along with their corresponding weights or volumes. The Sponsor is responsible for the retention and disposition of the samples. HWI does not accept any responsibility for the analysis of the tissue 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 and mean body weights are in Table 1. All animals exhibited body weight gains from Day 1 to Day 29.

Clinical Observations

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

Dermal Irritation

Individual dermal irritation scores are in Table 3. The control material produced no dermal irritation. No dermal irritation was observed in the animals treated with T-6052 at any of the dose levels.

Pathology

Individual animal pathology comments are presented in Table 4. Individual animal tissue weights and bile volumes are in Table 5. There were no lesions observed in any of the animals.

Page 15 contains a pathology report by the study pathologist.

DISCUSSION

The acute systemic absorption/toxicity and relative skin irritancy of T-6052 were evaluated in male and female albino rabbits when administered as a single dermal application. Application of this material did not result in any dermal irritation or test material-related in-life clinical effects. There were no effects on body weight gain or macroscopic findings at necropsy.

SIGNATURE

Steven M. Glaza
Study Director
Acute Toxicology

6-27-95
Date

REFERENCE

1. Draize, J. H., "Acute Dermal Toxicity (Single Exposure)," In: *Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics - Dermal Toxicity*, Association of Food and Drug Officials of the U.S., pp. 54-56 (1959).

PATHOLOGY REPORT

There were six rabbits (three males and three females) each from four dose levels euthanized and necropsied at the termination of the study. The test material, dose level, day of death, and gross observations recorded for each animal are in the Individual Pathology Comments that follow this report.

At necropsy, there were no visible lesions in any of the animals. The liver, bile, an approximate 1-cm x 1-cm section of the dermal application site from all animals, and both kidneys from one male and one female in each group were collected. The tissue samples were weighed (volume only determined for bile), frozen, and sent to the Sponsor. After necropsy, the animals were discarded.

Thomas E. Palmer, PhD
Pathologist

6-27-95
Date

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Table 1
Individual and Mean Body Weights (g)

Male				Female			
Animal Number	Randomization Day -8	Day 1	Day 29	Animal Number	Randomization Day -8	Day 1	Day 29
Group 1 (Control) - Distilled Water (0 mg/kg)							
F52979	1,903	2,072	2,559	F52976	2,151	2,259	2,681
F52972	2,090	2,362	3,041	F52983	2,116	2,296	2,863
F52973	1,921	2,211	2,818	F52975	2,043	2,265	2,671
Mean	1,971	2,215	2,806		2,103	2,273	2,738
Group 2 - T-6052 (2 mg/kg)							
F52990	2,095	2,351	2,863	F52982[a]	1,885	2,145	2,681
F52997	2,031	2,205	2,928	F52994	2,022	2,261	2,914
F52986	2,034	2,332	2,816	F53410	2,220	2,471	2,875
Mean	2,053	2,296	2,869		2,042	2,292	2,823
Group 3 - T-6052 (200 mg/kg)							
F52996	2,190	2,302	2,897	F52989	2,097	2,316	2,874
F52992	1,889	2,052	2,729	F52993	1,993	2,234	2,651
F52984	1,950	2,257	2,993	F52977	2,049	2,323	2,687
Mean	2,010	2,204	2,873		2,046	2,291	2,737
Group 4 - T-6052 (1,000 mg/kg)							
F52980	1,936	2,184	2,637	F52995	2,131	2,249	2,644
F52978	2,181	2,384	3,063	F52987	2,140	2,274	2,817
F52991	2,108	2,351	3,142	F52988	2,188	2,423	3,015
Mean	2,075	2,306	2,947		2,153	2,315	2,825

a Animal No. F53409 was originally selected by the randomization program for use in the study but was replaced prior to treatment with No. F52982 due to poor health.

Table 2
Individual Clinical Signs

Sex	Animal Number	Observation	1-4 Hours (Day 1)	Day 2 through 29
		Group 1 (Control) - Distilled Water (0 mg/kg)		
Male	F52979	Appeared normal	✓	✓
	F52972	Appeared normal	✓	✓
	F52973	Appeared normal	✓	✓
Female	F52976	Appeared normal	✓	✓
	F52983	Appeared normal	✓	✓
	F52975	Appeared normal	✓	✓
		Group 2 - T-6052 (2 mg/kg)		
Male	F52990	Appeared normal	✓	✓
	F52997	Appeared normal	✓	✓
	F52986	Appeared normal	✓	✓
Female	F52982	Appeared normal	✓	✓
	F52994	Appeared normal	✓	✓
	F53410	Appeared normal	✓	✓

✓ Condition existed.

Table 2 (Continued)
Individual Clinical Signs

Sex	Animal Number	Observation	1-4 Hours (Day 1)	Day 2 through 29
		Group 3 - T-6052 (200 mg/kg)		
Male	F52996	Appeared normal	✓	✓
	F52992	Appeared normal	✓	✓
	F52984	Appeared normal	✓	✓
Female	F52989	Appeared normal	✓	✓
	F52993	Appeared normal	✓	✓
	F52977	Appeared normal	✓	✓
		Group 4 - T-6052 (1,000 mg/kg)		
Male	F52980	Appeared normal	✓	✓
	F52978	Appeared normal	✓	✓
	F52991	Appeared normal	✓	✓
Female	F52995	Appeared normal	✓	✓
	F52987	Appeared normal	✓	✓
	F52988	Appeared normal	✓	✓

✓ Condition existed.

Table 3
Individual Dermal Irritation Scores

Group 1 (Control) - Distilled Water (0 mg/kg)

Dermal Reaction	Males Study Day 1	2	4	8	Females Study Day 1	2	4	8
	Animal No. F52979				Animal No. F52976			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F52972				Animal No. F52983			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F52973				Animal No. F52975			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0

Table 3 (Continued)
Individual Dermal Irritation Scores

Group 2 - T-6052 (2 mg/kg)

Dermal Reaction	Males Study Day 1	2	4	8	Females Study Day 1	2	4	8
	Animal No. F52990				Animal No. F52982			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F52997				Animal No. F52994			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F52986				Animal No. F53410			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0

Table 3 (Continued)
Individual Dermal Irritation Scores

Group 3 - T-6052 (200 mg/kg)

Dermal Reaction	Males Study Day 1	2	4	8	Females Study Day 1	2	4	8
	Animal No. F52996				Animal No. F52989			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F52992				Animal No. F52993			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F52984				Animal No. F52977			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0

Table 3 (Continued)
Individual Dermal Irritation Scores

Group 4 - T-6052 (1,000 mg/kg)

Dermal Reaction	Males Study Day 1	2	4	8	Females Study Day 1	2	4	8
	Animal No. F52980				Animal No. F52995			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F52978				Animal No. F52987			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0
	Animal No. F52991				Animal No. F52988			
Erythema	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0
Atonia	0	0	0	0	0	0	0	0
Desquamation	0	0	0	0	0	0	0	0
Coriaceousness	0	0	0	0	0	0	0	0
Fissuring	0	0	0	0	0	0	0	0

Table 4
Individual Pathology Comments

Animal Number	Sex	Test Day Died	Test Day Sacrificed	Necropsy Observation
		Group 1 (Control) - Distilled Water (0 mg/kg)		
F52979	M	-	29	No visible lesions.
F52972	M	-	29	No visible lesions.
F52973	M	-	29	No visible lesions.
F52976	F	-	29	No visible lesions.
F52983	F	-	29	No visible lesions.
F52975	F	-	29	No visible lesions.
		Group 2 - T-6052 (2 mg/kg)		
F52990	M	-	29	No visible lesions.
F52997	M	-	29	No visible lesions.
F52986	M	-	29	No visible lesions.
F52982	F	-	29	No visible lesions.
F52994	F	-	29	No visible lesions.
F53410	F	-	29	No visible lesions.
		Group 3 - T-6052 (200 mg/kg)		
F52996	M	-	29	No visible lesions.
F52992	M	-	29	No visible lesions.
F52984	M	-	29	No visible lesions.
F52989	F	-	29	No visible lesions.
F52993	F	-	29	No visible lesions.
F52977	F	-	29	No visible lesions.

- Not applicable.

Table 4 (Continued)
Individual Pathology Comments

Animal Number	Sex	Test Day Died	Test Day Sacrificed	Necropsy Observation
		Group 4 - T-6052 (1,000 mg/kg)		
F52980	M	-	29	No visible lesions.
F52978	M	-	29	No visible lesions.
F52991	M	-	29	No visible lesions.
F52995	F	-	29	No visible lesions.
F52987	F	-	29	No visible lesions.
F52988	F	-	29	No visible lesions.

- Not applicable.

Table 5
Individual Animal Tissue Weights and Bile Volumes

Sex	Animal Number	Weight (g) Liver	Kidneys	Dermal Application Site	Bile Volume (mL)
		Group 1 (Control) - Distilled Water (0 mg/kg)			
Male	F52979	91.387	-	0.593	0.9
	F52972	83.176	16.196	0.358	1.15
	F52973	76.442	-	0.800	1.2
Female	F52976	61.443	-	0.445	0.8
	F52983	98.109	-	0.393	0.9
	F52975	87.984	14.617	0.608	0.4
		Group 2 - T-6052 (2 mg/kg)			
Male	F52990	81.033	-	0.281	0.4
	F52997	92.537	-	0.421	0.5
	F52986	83.745	16.924	0.632	1.1
Female	F52982	84.395	-	0.568	1.1
	F52994	83.650	15.618	0.432	1.4
	F53410	91.541	-	0.386	1.1
		Group 3 - T-6052 (200 mg/kg)			
Male	F52996	78.540	-	0.673	0.46
	F52992	80.845	-	0.511	1.31
	F52984	89.277	15.044	0.421	0.75
Female	F52989	98.425	16.883	0.837	0.7
	F52993	88.925	-	0.848	0.55
	F52977	72.840	-	0.526	0.9

- Not applicable.

Table 5 (Continued)
Individual Animal Tissue Weights and Bile Volumes

Sex	Animal Number	Weight (g)			Bile Volume (mL)
		Liver	Kidneys	Dermal Application Site	
		Group 4 - T-6052 (1,000 mg/kg)			
Male	F52980	83.049	15.630	0.896	1.0
	F52978	84.235	-	0.547	1.6
	F52991	88.738	-	0.439	0.9
Female	F52995	63.956	14.923	0.508	0.25
	F52987	82.019	-	0.287	1.35
	F52988	83.911	-	0.875	1.2

- Not applicable.

APPENDIX A

Protocol TP3016.AB
Protocol Amendment No. 1

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Sponsor:

3M Toxicology Service Medical Department
St. Paul, Minnesota

PROTOCOL TP3016.AB

Study Title:

Single-Dose Dermal Absorption/Toxicity Study of
T-6052 in Rabbits

Date:

December 30, 1994

Performing Laboratory:

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

Laboratory Project Identification:

HWI 6329-135

Phone 608 241 4471 Fax 608 241

STUDY IDENTIFICATION

Single-Dose Dermal Absorption/Toxicity Study of T-6052 in Rabbits

HWI No.	6329-135
Test Material	T-6052
Sponsor	3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220
Sponsor's Representative	John L. Butenhoff, PhD 3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220 (612) 733-1962
Study Director	Steven M. Glaza Hazleton Wisconsin, Inc. P.O. Box 7545 Madison, WI 53707-7545 (608) 241-7292
Study Location	Hazleton Wisconsin, Inc. Building No. 3 3802 Packers Avenue Madison, WI 53704
Proposed Study Timetable	
Experimental Start Date	January 5, 1995
Experimental Termination Date	February 2, 1995
Draft Report Date	March 16, 1995

1. Study
Single-Dose Dermal Absorption/Toxicity Study in Rabbits

2. Purpose
To assess the systemic absorption and toxicity and relative skin irritancy of a test material when applied to the skin of rabbits

3. Regulatory Compliance
This study will be conducted in accordance with the following Good Laboratory Practice Regulations/Standards/Guidelines:

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

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

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

5. Test Material

A. Identification
T-6052

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).

D. Storage
Room temperature

E. Reserve Samples
Reserve sample(s) of each batch/lot of test and control materials will be taken for this study.

The test and control material reserve samples will be stored at HWI in a freezer set to maintain a temperature of -20°C ±10°C for 10 years per HWI SOP. The Sponsor will be contacted after 10 years for disposition in accordance with the appropriate regulatory Good Laboratory Practices.

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
Distilled water

B. Physical Description
Clear, colorless liquid

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

D. Storage Conditions
Room temperature

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
2.0 to 3.0 kg

(5) Number and Sex
12 males and 12 females

(6) Identification
Individual numbered ear tag

(7) Husbandry

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

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

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

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

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

(f) Acclimation
At least 7 days

(8) Selection of Test Animals
Based on health and body weight according to HWI SOPs. An adequate number of extra animals will be purchased so that no animal in obviously poor health is placed on test. The animals will be placed into study groups using a stratified body weight randomization program within nine days of study initiation.

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

B. Dose Administration

(1) Test Groups

Group	Test Material	Dose Level (mg/kg)	Number of Animals Males	Number of Animals Females
1 (Control)	Distilled water	0*	3	3
2	T-6052	2**	3	3
3	T-6052	200	3	3
4	T-6052	1000	3	3

* To be administered at a dose volume of 2.0 mL/kg
** To be administered at a dose volume of .01 mL/kg

(2) Preparation of Exposure Area
On the day before test material application, the back and, if necessary (to obtain unblemished skin), the flanks of each rabbit will be clipped free of hair. The shaved area will constitute approximately 20% of the total body surface area. The treatment sites (intact skin) will be inspected for interfering lesions, irritation, or defects that would preclude the use of any of the animals. The animals will be clipped as needed throughout the study.

(3) Dose Administration
All animals will receive a single administration of the respective test or control material. The day of treatment will be designated as Day 1. The dose for each animal in Group 2 will be diluted with distilled water and applied at a dose volume of .01 mL/kg. The respective dose for each animal in Groups 3 and 4 will be applied undiluted. All doses in Groups 1-4 will be based on the animal's body weight just before administration and will be spread onto the area of exposure in a thin and uniform a layer. The area of application (Groups 1-4) will be covered with a 10-cm x 10-cm gauze bandage secured with paper tape around all edges and overwrapped with Saran Wrap® and Elastoplast® tape to provide an occlusive dressing. The rabbits will be collared during the 24-hour application period.

(4) Reason for Route of Administration
The dermal route is a potential route of exposure in humans.

(5) Removal of Test Material
Approximately 24 hours after test or control material application the bandages and collars will be removed and the residual test material will be removed using water or an appropriate solvent, if necessary.

C. Observation of Animals

(1) Clinical Observations
For clinical signs before test or control material administration and for clinical signs and mortality at approximately 1, 2.5, and 4 hours after test material administration (Day 1) and daily thereafter for clinical signs, and twice daily (a.m. and p.m.) for mortality for at least 28 days. Observations may be extended when directed by the study director.

(2) Reading of Dermal Irritation
Before test or control material administration the initial dermal irritation reading will be made and recorded as the Day 1 reading (Attachment 1). Additional dermal irritation readings will be made approximately 30 minutes after bandage removal (Day 2) and on Study Days 4 and 8. Individual dermal irritation records will be maintained for each animal.

(3) Body Weights
For randomization, before test or control material application (Day 1), on Day 29, and at unscheduled death (when survival exceeds 1 day)

(4) Sample Collections

(a) Frequency
Before initiation (Day 1), approximately 24 hours post-dose (Day 2), Days 4, 8, 15, 22, and at experimental termination (Day 29)

(b) Number of Animals
All

(c) Method of Collection
Blood samples (approximately 4 mL) will be collected from the marginal ear vein of either ear on Days 1, 2, 4, 8, 15, and 22. 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 sacrificed in a moribund condition or sacrificed at the time of necropsy (Day 29). 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 -20°C ±10°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 alternate will be notified by telephone at (612) 778-5294 prior to the shipment of the samples.

D. Pathology

(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 D. Pathology, (3) Sample Collection, will be collected. After necropsy, the animals will be discarded.

(2) Scheduled Sacrifice
At termination of the experimental phase (Day 29), surviving animals will be anesthetized with sodium pentobarbital (via injection in the marginal ear vein), bled via the vena cava, exsanguinated, and subjected to an abbreviated gross necropsy examination. The animals will be necropsied in random order and all abnormalities will be recorded.

(3) Sample Collection
The sites of test and control material application will be washed with lukewarm tap water prior to the necropsy procedure. The whole liver, bile, an approximate 1-cm x 1-cm section of the dermal application site from all animals, and both kidneys from the first male and female necopsied in each group will be collected and immediately placed in a freezer set to maintain a temperature of -20°C ±10°C. After necropsy, the animals will be discarded.

The tissues (liver, bile, dermal application site, 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.C.(4).(c). The Sponsor is responsible for the retention and disposition of the samples.

E. 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
Tabulation of mortality data by sex and dose level
Description of any toxic effects/dermal irritation
Tabulation of mean body weights by sex and dose level
Gross pathology findings/gross pathology report

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
Anatomical pathology records
Sample collection records
Shipping records
Study correspondence
Final report (original signed copy)

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

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

PROTOCOL APPROVAL

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

1-5-95
Date

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

12-30-94
Date

Representative
Quality Assurance Unit
Hazleton Wisconsin, Inc.

12/30/94
Date

(6329-135.protdsk2)

Attachment 1

Scoring Scale for Acute Dermal Reactions

Erythema

0 - None
1 - Slight
2 - Moderate
3 - Severe

Edema

0 - None
1 - Slight (barely perceptible to well defined by definite raising)
2 - Moderate (raised approximately 1 mm)
3 - Severe (raised more than 1 mm)

Atonia

0 - None
1 - Slight (slight impairment of elasticity)
2 - Moderate (slow return to normal)
3 - Marked (no elasticity)

Desquamation

0 - None
1 - Slight (slight scaling)
2 - Moderate (scales and flakes)
3 - Marked (pronounced flaking with denuded areas)

Coriaceousness

0 - None
1 - Slight (decrease in pliability)
2 - Moderate (leathery texture)
3 - Marked (tough and brittle)

Fissuring

0 - None
1 - Slight (definite cracks in epidermis)
2 - Moderate (cracks in dermis)
3 - Marked (cracks with bleeding)

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PROTOCOL TP3016.AB

Single-Dose Dermal Absorption/Toxicity Study
of T-6052 in Rabbits

HWI 6329-135

Sponsor	Contractor
3M Toxicology Service Medical Department 3M Center, Bldg. 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220	Hazleton Wisconsin, Inc. 3301 Kinsman Boulevard Madison, WI 53704
Sponsor's Representative	Study Director
John L. Butenhoff, PhD	Steven M. Glaza

Amendment No. 1

This amendment modifies the following portions of the protocol:

Effective January 24, 1995

At the request of the Sponsor, the weights of tissues collected and the volume of bile collected will be documented in the raw data. These weights and volumes will be included with the sample shipment. Modify the following sections of the protocol to include these additions.

1. Page 9, 7. Experimental Design; D. Pathology; (3) Sample Collection. Modify the second sentence in the first and second paragraphs of this section with the following underlined additions:

   The whole liver, bile, an approximate 1-cm x 1-cm section of the dermal application site from all animals, and both kidneys from the first male and female necropsied in each group will be collected, weighed (volume only determined for bile), and immediately placed in a freezer set to maintain a temperature of -20°C ±10°C.

   The samples and their corresponding weights or volumes will be shipped to the person listed in Section 7.C.(4).(c).

2. Page 9, 8. Report. Add the following to this section:

   Individual animal tissue weights and bile volumes

Phone 608 241 4471 Fax 608 241 7227

Amendment No. 1

PROTOCOL AMENDMENT APPROVAL

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

2/15/95
Date

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

2-6-95
Date

Representative
Quality Assurance Unit
Hazleton Wisconsin, Inc.

2-7-95
Date

(6329-135.Am1.dsk2)

9.1.2 Analytical protocol AMDT-020795.1

3M Environmental Laboratory

Protocol - Analytical Study

Single-Dose Dermal Absorption/Toxicity Study of T-6052 in Rabbits

In-Vivo Study Reference Number: HWI#6329-135

Study Number: AMDT-020795.1
Test Substance: FC-120 (T-6052)

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

Author: James D. Johnson

Approved By:

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

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

1.0 PURPOSE

This study is designed to provide information as to whether FC-120 (T-6052) is dermally absorbed. The analytical aspect of this study is to determine fluorine-containing compounds in the liver and serum of rabbits. By comparison of the data obtained after dermal absorption with that obtained after intravenous injection, assessment of the extent of dermal absorption can be performed.

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, bovine serum and rabbit serum

2.1.3 Analytical Control Substance: None

2.1.4 Analytical Control Matrix: Bovine liver, bovine serum and rabbit 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 bovine serum), AMDT 110394.1 (Hwi#6329-123) control group animals (2.1.2, 2.1.4 rabbit serum)

2.3 Number of Test and Control Samples: Tissues and fluids from 18 test animals and 6 control animals. Tissues and fluids include liver, kidney, serum, cellular fraction, dermal application site and bile. Analysis of these tissues will be at the discretion of the Study Director.

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

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

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

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

2.8 Disposition of Specimens: Biological tissues and fluids will be retained per GLP Regulation for the time period required for studies longer than 28 days.

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

3.0 EXPERIMENTAL - Overview

The tissues from animals dosed as described (HWI#6329-135), 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. To confirm the identity of fluorine-containing compounds present in liver (if any at 28 days) and serum at various intervals, electrospray mass spectrometry may be selected as one of the analytical techniques employed. Not all of the tissues and fluid samples will be analyzed. When sufficient data has been collected to meet the objectives of the study in the opinion of the Study Director, analysis will cease.

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

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

5.0 DATA ANALYSIS

5.1 Data Reporting: Data will be reported as a concentration (weight/weight) of fluoride per tissue or fluid, or as FC-95 (electrospray mass spectrometry) per unit of tissue or fluid. Statistics used, at the discretion of the Study Director, may include regression analysis of serum concentrations with time and averages and standard deviations of concentrations for different dose groups. If necessary, simple statistical tests such as 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

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

3M Environmental Laboratory

Method

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

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

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

Author: Rich Youngblom

Approved by:

Group Leader 10/3/95
Date

Quality Assurance 10-4-95
Date

Software: MS Word 5.1a

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

1.0 SCOPE , APPLICABLE COMPOUNDS, AND MATRICES

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

2.0 KEYWORDS

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

3.0 PRECAUTIONS

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

4.0 SUPPLIES AND MATERIALS

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

5.0 EQUIPMENT

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

6.0 INTERFERENCES

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

7.0 SAMPLE HANDLING

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

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Calibration Standards

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

8.2 Calibration - Overview

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

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

8.3 Calibration - Procedure

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

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

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

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

8.4 Storage Conditions for Standards

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

9.0 PROCEDURES

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

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

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

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

9.4 Sample Calculations

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

10.0 VALIDATION

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

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

10.3 Other Validation Parameters: NA

11.0 DATA ANALYSIS

11.1 Calculations

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

11.2 Analyzing the Data

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

12.0 ATTACHMENTS

None

13.0 REFERENCES

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

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

3M Environmental Laboratory

Method

Fluoride Measurement by Means of an Orion EA940 Expandable Ion Analyzer

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

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

Author: Rich Youngblom

Approved By:

James D Johnson 10/3/95
Group Leader Date

Robert Van Buskirk 10-4-95
Quality Assurance Date

Software: MS Word 5.1a

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

1.0 SCOPE , APPLICABLE COMPOUNDS, AND MATRICES

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

1.2 APPLICABLE COMPOUNDS: Fluoride.

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

2.0 KEYWORDS

2.1 Fluoride, fluorine, ion selective electrode

3.0 PRECAUTIONS

3.1 No hazards identified with this method.

4.0 SUPPLIES AND MATERIALS

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

5.0 EQUIPMENT

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

6.0 INTERFERENCES

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

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

7.0 SAMPLE HANDLING

7.1 No special handling necessary.

8.0 CALIBRATION AND STANDARDIZATION

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

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

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

9.0 PROCEDURES

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

9.2 Calibration And Measurement, Using Orion 3E Software:

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

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

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

10.0 VALIDATION

10.1 Quality Control:

10.2 Precision and Accuracy

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

11.0 DATA ANALYSIS

11.1 Calculations None necessary.

11.2 Analyzing the Data None necessary.

12.0 ATTACHMENTS

None

13.0 REFERENCES

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

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

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 — Date: 10-31-95

Quality Assurance — Date: 10-31-95

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

1.0 SCOPE

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

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

1.3 **Matrices:** Rabbit Livers.

2.0 KEYWORDS

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

3.0 PRECAUTIONS

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

4.0 SUPPLIES AND MATERIALS

4.1 **Supplies**

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

4.2 **Reagents**

- 4.2.1 Aqueous Ammonium Acetate (Aldrich), approx. 250 ppm: Prepare a 2500 ppm aqueous solution of ammonium acetate by adding 250 mg ammonium acetate to a 100 mL volumetric flask and dilute to volume with Milli-Q water. Dilute this solution 1:10 for a 250 ppm solution.
- 4.2.2 Sodium carbonate/Sodium Bicarbonate Buffer (J.T. Baker), (Na_2CO_3/$NaHCO_3$) 0.25 M: Weigh 26.5 g of sodium carbonate (Na_2CO_3) and 21.0 g of sodium bicarbonate ($NaHCO_3$) into a 1 L volumetric flask and bring to volume with Milli-Q water.
- 4.2.3 Dilute acetonitrile solution, dilute acetonitrile 1:1 with Milli-Q water.
- 4.2.4 Ethyl Acetate
- 4.2.5 Methanol
- 4.2.6 Milli-Q water
- 4.2.7 1H,1H,2H,2H - perfluorooctanesulfonic acid (Aldrich)
- 4.2.8 FC-95 (3M Specialty Chemical Division)

5.0 EQUIPMENT

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

6.0 INTERFERENCES

6.1 There are no known interferences at this time.

7.0 SAMPLE HANDLING

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

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Internal Standards

8.1.1 Prepare an internal standard of approximately 12 ppm 1H,1H,2H,2H-perfluorooctanesulphonic acid to be added to each liver sample.
8.1.2 Weigh at least 0.1 g of 1H,1H,2H,2H-perfluorooctanesulphonic acid into a 100 mL volumetric flask. Record the actual weight.
8.1.3 Bring it up to volume with methanol, this is the stock standard.
8.1.4 To a 250 mL volumetric flask, add 3 mLs of the stock standard and bring to volume with Milli-Q water. Calculate the actual concentration of the standard.

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

8.2 Prepare FC-95 Anion Standards

8.2.1 Prepare FC-95 standards for the standard curve.
8.2.2 Weigh approximately 100 mg of FC-95 into a 100 mL volumetric flask. Record the actual weight.
8.2.3 Bring up to volume with dilute acetonitrile.
8.2.4 Dilute the solution with dilute acetonitrile 1:10 for a solution of approximately 100 ppm. Dilute this solution 1:10 with dilute acetonitrile for a solution of approx. 10 ppm.
8.2.5 Use the 10 ppm solution to make working standards with values close to 5.0 ppm, 1.0 ppm and 500 ppb.

8.3 Prepare Beef Liver Homogenate to Use for Standards

8.3.1 Weigh 40 g of Bovine liver into a 250 mL Nalgene bottle containing 200 mLs Milli-Q water. Grind to a homogenous solution.
8.3.2 Add 1 mL of the solution to a 15 mL centrifuge tube. Prepare a total of eight 1 mL aliquots of the solution in 15 mL centrifuge tubes. Be sure to re-suspend solution by shaking it between aliquots.

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

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

8.4 Calculate the actual value of the standards:

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

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

8.5 Calibration

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

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

8.6 Storage Conditions for Standards

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

8.7 Storage Conditions for Standards

8.7.1 Beef liver homogenates may be frozen after preparation.

9.0 PROCEDURES

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

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

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

9.4 Record the liver weight in the study note book.

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

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

10.0 VALIDATION

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

11.0 DATA ANALYSIS

11.1 **None**

12.0 ATTACHMENTS

12.1 Worksheet AMDT-M-4

13.0 REFERENCES

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

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

Worksheet AMDT-M-4

Study #	Sample Number set #	FC-95 approx 0.5 ppm actual ppm #W_____	FC-95 approx 1 ppm actual ppm #W_____	FC-95 approx. 5 ppm actual ppm #W_____	Date and Initials for Std.
-	Blank Liver	-	-	-	
-		100 uL	-	-	
-		200 uL	-	-	
-		300 uL	-	-	
-		400 uL	-	-	
-		-	500 uL	-	
-		-	-	200 uL	
-		-	-	300 uL	
[1]		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	

[1] study number where the original worksheet is located and place a copy.

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

3M Environmental Laboratory

Method

Analysis of Rabbit Liver Extract for Fluorochemicals using Electrospray Mass Spectroscopy

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

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

Author: Dave Christenson/Cynthia Weber

Approved By:

Group Leader 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 spectometer is performed. Samples and standards are NOT refrigerated.

8.4 Storage Conditions for Beef Liver Homogenates

8.4.1 Beef liver homogenates may be frozen after preparation.

9.0 PROCEDURE

9.1 Initial Set-up

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

9.1.2 Record backing pressure in the instrument log.

9.1.3 Fill the solvent cylinder with mobile phase.

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

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

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

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

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

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

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

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

9.2 Manual Injection

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

9.2.2 Turn the valve to "On".

9.2.3 Wait two minutes, and inject the next sample.

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

9.3 **Using the Autosampler**

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

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

9.3.3 Set-up the sampler:

9.3.3.1 Push the sample button

9.3.3.2 Set sample loop size = 100 uL

9.3.3.3 Set inject/sample = 2

9.3.3.4 Set Cycle time = 0

9.3.3.5 Name the file: Livers

9.3.3.6 Identify the tray used

9.3.3.7 Add the samples to Queue by pressing "Enter"

9.3.3.8 Press "Run" to start

10.0 VALIDATION

10.1 **Quality Control**

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

10.2 **Precision and Accuracy**

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

10.3 **Other Validation Parameters**

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

11.0 DATA ANALYSIS

11.1 Calculations

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

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

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

mg FC-95 anion in the total rabbit liver =

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

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

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

12.0 ATTACHMENTS

None

13.0 REFERENCES

13.1 AMDT-EP-17

14.0 REVISIONS

Revision Number	Reason for change	Revision Date

3M Environmental Laboratory

Method

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

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

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

Author: Deb Wright / Cynthia Weber

Approved By:

James D Johnson 10/5/95
Group Leader Date

Gale Kevin Burkett 9-27-95
Quality Assurance Date

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

1.0 SCOPE

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

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

2.0 KEYWORDS

2.1 Skalar, segmented flow, fluoride.

3.0 PRECAUTIONS

3.1 Follow standard laboratory safety practices.

4.0 SUPPLIES AND MATERIALS

4.1 Supplies

4.1.1 Sample cups, 4 mL plastic cups with caps

4.1.2 Autopipets, oxford or equivalent with plastic tips

4.1.3 Polypropylene volumetric flasks, 100 mL

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

4.1.5 Sample prefilters, Evergreen

4.2 Reagents

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

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

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

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

4.3 Standards

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

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

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

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

1.2 ppm	1.2	-
1.5 ppm	1.5	-

5.0 EQUIPMENT

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

6.0 INTERFERENCES

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

7.0 SAMPLE HANDLING

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

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Calibration Standards

8.1.1 Prepare calibration standards as in section **4.3.**

8.2 Calibration

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

8.3 Storage Conditions for Standards

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

9.0 PROCEDURE

9.1 Start Up Procedure

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

9.1.2 Put the fluoride electrodes in the electrode chamber.

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

9.1.4 Put the reagent-lines in the appropriate bottles.

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

9.1.6 Let the system stabilize for approximately 30 minutes.

9.2 Starting a Run

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

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

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

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

9.2.5 Set the baseline.

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

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

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

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

9.2.7 Press the spacebar to bring up the local menu.

9.2.8 Select START to start the analysis.

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

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

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

9.3 Loading and Printing the Data-File

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

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

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

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

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

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

9.4 Shutdown

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

9.4.2 Let the system rinse for approximately 30 minutes.

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

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

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

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

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

10.0 VALIDATION

10.1 Quality Control

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

10.2 Precision and Accuracy

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

10.3 Other Validation Parameters

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

11.0 DATA ANALYSIS

11.1 Calculations

11.1.1 The standard curve is plotted by the Skalar software.

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

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

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

11.4 Electronic Data

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

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

12.0 ATTACHMENTS

None

13.0 REFERENCES

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

13.2 Skalar Methods, #335, Skalar Methods Manual

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

14.0 REVISIONS

Revision Number	Reason for change	Revision Date

3M Environmental Laboratory

Method

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

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

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

Author: Rich Youngblom

Approved by:

James D Johnson
Group Leader 10/3/95
Date

Quality Assurance 9-27-95
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 fluids, particularly serum.

2.0 KEYWORDS

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

3.0 PRECAUTIONS

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

4.0 SUPPLIES AND MATERIALS

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

5.0 EQUIPMENT

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

6.0 INTERFERENCES

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

7.0 SAMPLE HANDLING

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

8.0 CALIBRATION AND STANDARDIZATION

8.1 Preparation of Calibration Standards

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

8.2 Calibration - Overview

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

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

8.3 Calibration - Procedure

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

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

8.3.3 Standard Curve

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

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

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

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

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

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

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

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

8.4 Storage Conditions for Standards

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

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

9.0 PROCEDURES

9.1 Typical Operating Conditions:

9.1.1 Combustion tube temperature = 950°C.

9.1.2 Oxygen and Helium flow = 50 cc/minute.

9.1.3 Vaporization/Drying time = 240 seconds.

9.1.4 Bake time = 300 seconds.

9.2 Start Up Procedure:

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

9.2.2 Open the SYSTEM SETUP window.

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

9.2.4 Close the SYSTEM SETUP window.

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

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

9.3 Sample Extraction Procedure:

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

9.3.2 Close SAMPLE HATCH.

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

9.4 Sample Calculations

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

10.0 VALIDATION

10.1 Quality Control

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

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

10.3 Other Validation Parameters: NA

11.0 DATA ANALYSIS

11.1 Calculations

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

11.2 Analyzing the Data

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

12.0 ATTACHMENTS

None

13.0 REFERENCES

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

14.0 REVISIONS

Revision Number	Reason for Change	Revision Date

9.1.3 Amendment to Analytical Protocol AMDT-020795.1

Attachment I

GLP Study

Protocol Amendment

Study Number: AMDT-020795.1

Study Title: Single-Dose Dermal Absorption /Toxicity Study of T-6052 in Rabbits

Study Director: James D. Johnson

Amendment Date: November 8, 1995 Amendment Number: 1

This amendment modifies the following portion of the protocol:

AMDT-M-14-0 specifies using bovine serum for the blanks in the thermal extraction. However, rabbit serum from the control animals (AMDT-110394.1) was used because the bovine serum blanks were higher than the samples.

Approved by:

James D. Johnson 11/20/95

Study Director 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 Dermal Absorption/Toxicity Study of T-6052 in Rabbits**

Study Number: AMDT-020795.1 Name of Auditor: Kari Rambo

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

Inspection Dates From	To	Phase	Date Inspection Reported to Management	Study Director
10/13/95	10/19/95	Final Report	10/19/95	10/19/95

Kari Rambo 10-19-95

QAU Auditor Date

9.4 Key Personnel Involved in the Study

3M Environmental Laboratory

Key Personnel

Thermal extraction followed by analysis using Orion ion analyzer:

Jim Johnson
Deb Wright
Rich Youngblom
Deann Plummer

Analysis of liver extracts using electrospray mass spectrometry:

Jim Johnson
Dave Christenson

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

Jim Johnson
Deb Wright
Rich Youngblom
Deann Plummer

Documentation and Reporting:

Jim Johnson
Rich Youngblom

Quality Assurance Unit:

Gale Van Buskirk
Cynthia Weber
Kari Rambo

9.11 Data

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

Summary of Combustion Data - Liver
AMDT-020795.1, HWI 6329-135
As Referenced in Final Report section 6.0 *DATA ANALYSIS*

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

	ug	Std. Dev.
Control Group	**16.7**	**± 5.9**
2.0 mg/kg dose (T6052)	**12.6**	**± 3.2**
200 mg/kg dose (T6052)	**18.3***	**± 4.2**
1000 mg/kg dose (T6052)	**24.1**	**± 4.2**

** Calculated as the mean of triplicate samples from each of three male and three female rabbits.
* One outlier omitted. Value of re-analyzed sample included in this report.

FC120 AB ID	% rcvry	Actual ppm F- in liver (W/W)	Average ppm F- in liver (W/W)	liver burned (grams)	Whole liver weight (grams)	Total F- in whole liver (ug)	Dosage (mg/kg)
Liver Blk-1		0.133		0.112			
Liver Blk-2		0.108		0.124			
Liver Spk-1	80%	1.10		0.110			
Liver Spk-2	90%	0.980		0.139			
Liver Spk-3	92%	1.06		0.132			
Liver Spk-4	94%	2.72		0.105			
Liver Spk-5	108%	3.24		0.101			
Liver Spk-6	94%	2.43		0.117			
Liver blank-3		0.393		0.119			
Liver blank-4		0.320		0.121			
F52972-1		0.360		0.107	82.5		
F52972-2		0.317	0.331	0.103	82.5	27.3	0.0
F52972-3		0.315		0.132	82.5		
F52973-1		0.240		0.148	75.8		
F52973-2		0.243	0.251	0.150	75.8	19.0	0.0
F52973-3		0.269		0.131	75.8		
F52979-1		0.214		0.119	90.6		
F52979-2		0.171	0.183	0.148	90.6	16.6	0.0
F52979-3		0.166		0.133	90.6		
Liver Blank-1		0.276		0.114			
Liver Blank-2		0.131		0.135			
Liver Spike-1	80%	0.864		0.141			
Liver Spike-2	89%	1.35		0.100			
Liver Spike-3	79%	0.863		0.138			
Liver Spike-4	77%	0.965		0.121			
Liver Blank-A		0.000		0.132			
Liver Spike-5	88%	1.02		0.130			
Liver Spike-6	90%	0.993		0.138			
Liver Spike-7	92%	1.04		0.134			
F52975-1		0.165		0.136	86.4		
F52975-2		0.135	0.138	0.135	86.4	12.0	0.0
F52975-3		0.116		0.139	86.4		
F52976-1		0.135		0.150	60.8		
F52976-2		0.125	0.185	0.141	60.8	11.2	0.0
F52976-3		0.294		0.127	60.8		
F52983-1		0.153		0.134	96.5		
F52983-2		0.151	0.145	0.116	96.5	14.0	0.0
F52983-3		0.132		0.128	96.5		
F52986-1		0.149		0.125	82.8		
F52986-2		0.147	0.147	0.108	82.8	12.2	2.0
F52986-3		0.145		0.135	82.8		
F52990-1		0.162		0.145	80.4		
F52990-2		0.196	0.184	0.120	80.4	14.8	2.0
F52990-3		0.195		0.107	80.4		
F52997-1		0.220		0.114	91.7		
F52997-2		0.243	0.195	0.116	91.7	17.9	2.0
F52997-3		0.123		0.150	91.7		

FC120 AB ID	% rcvry	Actual ppm F- in liver (W/W)	Average ppm F- in liver (W/W)	liver burned (grams)	Whole liver weight (grams)	Total F- in whole liver (ug)	Dosage (mg/kg)	
F52982-1		0.136		0.147	82.4			
F52982-2		0.153	0.134	0.105	82.4	11.0	2.0	
F52982-3		0.113		0.116	82.4			
F52994-1		0.121		0.130	82.9			
F52994-2		0.105	0.113	0.151	82.9	9.37	2.0	
F52994-3		0.113		0.148	82.9			
F53410-1		0.114		0.129	91.0			
F53410-2		0.130	0.115	0.119	91.0	10.5	2.0	
F53410-3		0.102		0.146	91.0			
Liver Blank-1		0.207		0.106				
Liver Blank-2		0.130		0.102				
Liver Spike-1	65%	0.911		0.108				
Liver Spike-2	65%	0.747		0.131				
Liver Spike-3	71%	0.751		0.144				
Liver Spike-4	85%	1.02		0.125				
Liver Spike-5	96%	1.13		0.129				
Liver Spike-6	90%	1.04		0.131				
Liver Spike-7	80%	0.946		0.128				
F52984-1		0.216		0.112	88.5			
F52984-2		0.224	0.200	0.104	88.5	17.7	200	
F52984-3		0.160		0.131	88.5			
F52992-1		0.173		0.121	80.6			
F52992-2		0.205	0.209	0.115	80.6	16.8	200	
F52992-3		0.247		0.136	80.6			
F52996-1		0.843		0.134	77.8			*Sample
F52996-2		0.272	1.06	0.115	77.8	82.5*	200	reanalyzed
F52996-3		2.07		0.142	77.8			below
F52977-1		0.207		0.140	72.0			
F52977-2		0.180	0.187	0.126	72.0	13.5	200	
F52977-3		0.173		0.128	72.0			
F52989-1		0.217		0.106	97.9			
F52989-2		0.249	0.219	0.134	97.9	21.5	200	
F52989-3		0.192		0.135	97.9			
F52993-1		0.174		0.141	88.3			
F52993-2		0.199	0.175	0.129	88.3	15.5	200	
F52993-3		0.153		0.147	88.3			
liver blank-1		0.312		0.148				
liver spike-1	72%	0.741		0.146				
liver spike-2	75%	0.928		0.123				
liver spike-3	67%	0.670		0.151				
liver spike-4	77%	0.826		0.141				
liver spike-5	77%	0.934		0.124				
liver spike-6	88%	1.27		0.105				
liver spike-7	152%	1.52		0.151				
liver spike-8	81%	0.829		0.148				
liver spike-9	88%	1.32		0.101				
liver spike-10	88%	1.12		0.118				

FC120 AB ID	% rcvry	Actual ppm F- in liver (W/W)	Average ppm F- in liver (W/W)	liver burned (grams)	Whole liver weight (grams)	Total F- in whole liver (ug)	Dosage (mg/kg)	
F52996-1		0.291		0.136	77.8			**Repeat
F52996-2		0.244	0.319	0.141	77.8	24.8**	200	of above
F52996-3		0.422		0.103	77.8			analysis
F52978-1		0.259		0.148	83.2			
F52978-2		0.224	0.236	0.141	83.2	19.6	1000	
F52978-3		0.225		0.143	83.2			
Blank liver 1		0.411		0.129				
Blank liver 2		4.45		0.146				
Blank liver 3		2.01		0.149				
Blank liver 4		0.340		0.137				
Blank liver 5		0.277		0.145				
Blank liver 6		0.404		0.114				
Liver Spike-1	130%	1.37		0.143				
Liver Spike-2	84%	1.09		0.117				
Liver Spike-3	87%	0.831		0.158				
Liver Spike-4	82%	0.955		0.130				
Liver Spike-5	77%	0.784		0.150				
F52980-1		0.289		0.133	82.0			
F52980-2		0.235	0.252	0.148	82.0	20.7	1000	
F52980-3		0.231		0.158	82.0			
F52991-1		0.330		0.134	86.9			
F52991-2		0.270	0.279	0.147	86.9	24.3	1000	
F52991-3		0.238		0.120	86.9			
F52987-1		0.204		0.122	81.2			
F52987-2		0.604	0.346	0.150	81.2	28.0	1000	
F52987-3		0.230		0.150	81.2			
F52988-1		0.249		0.118	83.2			
F52988-2		0.490	0.360	0.138	83.2	29.9	1000	
F52988-3		0.340		0.109	83.2			
F52995-1		0.453		0.147	63.1			
F52995-2		0.315	0.345	0.136	63.1	21.8	1000	
F52995-3		0.266		0.113	63.1			
liver blank-1		0.168		0.146				
liver spike-1	72%	0.862		0.126				
liver spike-2	81%	0.820		0.150				
liver spike-3	82%	0.847		0.146				
liver spike-4	77%	0.905		0.129				
liver spike-5	83%	2.03		0.123				
liver spike-6	84%	1.89		0.135				
liver spike-7	81%	1.75		0.141				
liver blank-2		0.266		0.131				

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

A-1
Containing pages
A-1 thru A-2
11/9/95
DLC

HWI # 6329-135

Study:	Single-Dose Dermal Absorption
Protocol Number:	TP3016.AB
Test Material:	T-6052 in Rabbits (FC-120)
Matrix:	Liver
R Squared Value:	Screening
Response Factor Amount:	N/A
Analyst:	DLC
Date:	4/6/95
Method:	
Instrument:	Fisons VG 2000 Electrospray MS
LABBASE File:	040695B

DLC 11-9-95

Rabbit liver extracts screened for M⁻ 599 ion only, no quantitation performed.

Group Dose	Sample #	Ion Count Area *	Extracted wt g	Dilution factor	Concentration µg/g ****	Total mass of liver g	Total amount of FC-95 per liver mg	% of FC-95
Group 1:								
0 mg/kg **	F52975	N.D.						
	F52976	re-extract						
	F52983	re-extract						
	F52972	N.D.						
	F52973	N.D.						
	F52979	N.D.						
Group 2:								
2 mg /kg ***	F52986	N.D.						
	F52990	N.D.						
	F52997	re-extract						
	F52982	N.D.						
	F52994	N.D.						
	F53410	N.D.						
Group 3:								
200 mg/kg ***	F52984	$						
	F52992	$						
	F52996	re-extract						
	F52977	$						
	F52989	N.D.						
	F52993	N.D.						
Group 4:								
1000 mg/kg ***	F52978	$						
	F52980	$						
	F52991	$						
	F52987	$						
	F52988	$						
	F52995	re-extract						

* SIR Monitoring of M⁻599 and 598.

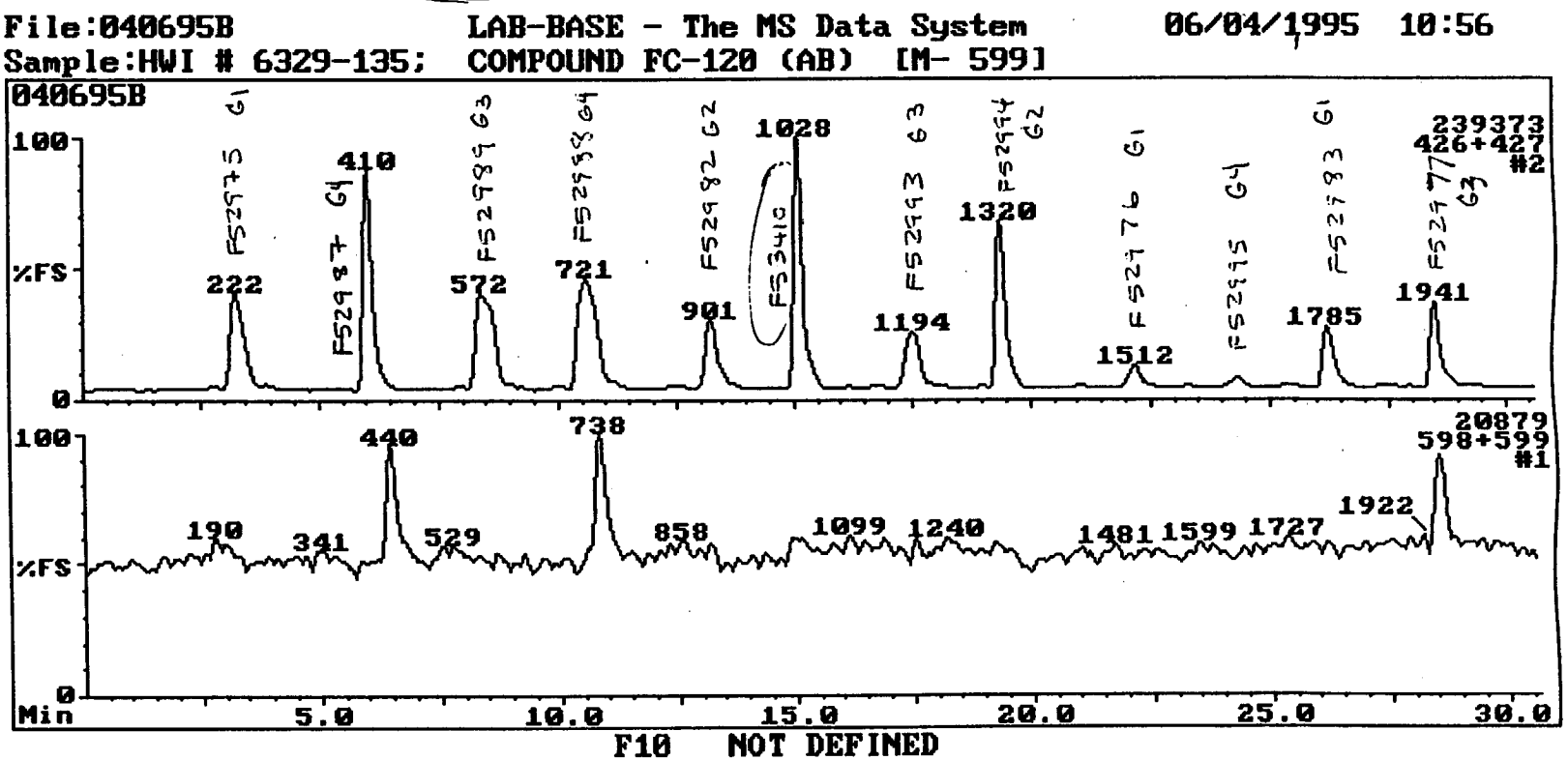
$ = Positive response for Ion monitored.

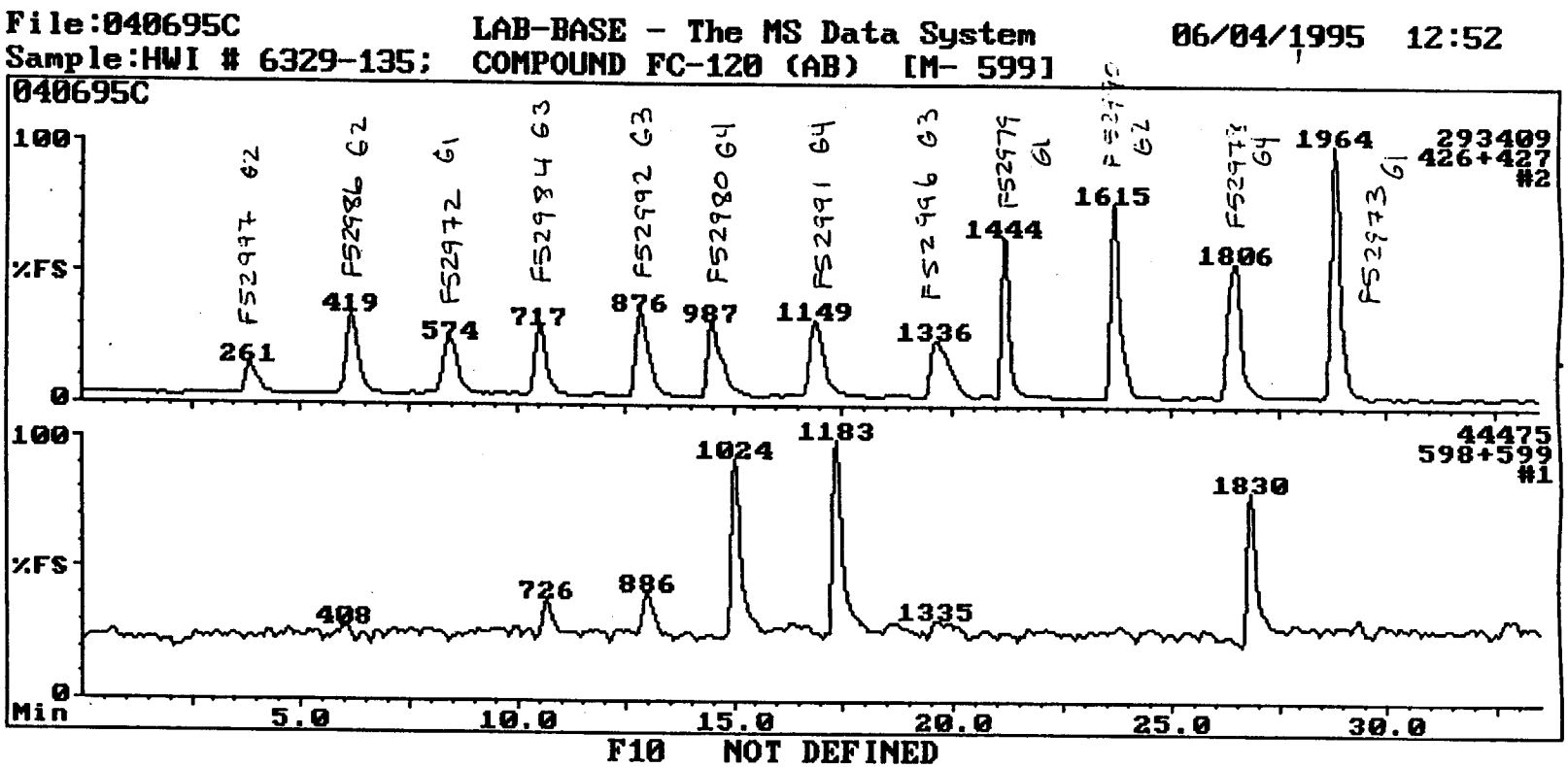
** Administered at a dose volume of 2.0 mL/kg.

*** Administered at a dose volume of 0.01 mL/kg.

****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.

Electrospray Chromatogram
from LABBase file: 040695B
Study # 6329-135
SIR: 599
negative ion
10-19-95 DLC

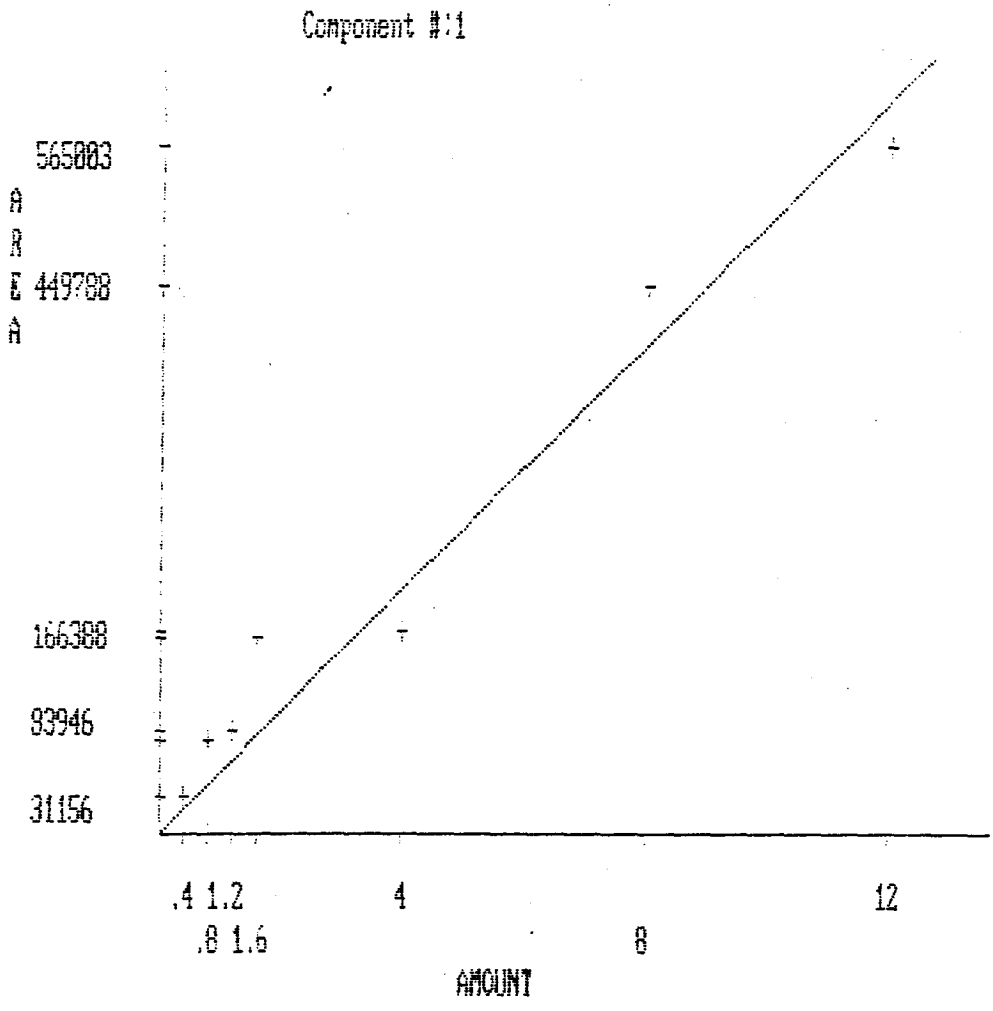



File:040695C
LAB-BASE - The MS Data System
06/04/1995 12:52
Sample:HWI # 6329-135; COMPOUND FC-120 (AB) [M- 599]
040695C
100
%FS
0
261
419
574
717
876
987
1149
1336
1444
1615
1806
1964
293409
426+427
#2
100
%FS
0
408
726
886
1024
1183
1335
1830
44475
598+599
#1
Min
5.0
10.0
15.0
20.0
25.0
30.0
F10 NOT DEFINED

LAB BASE
FILE 040395C
HWE # 6329-135
5/8/95
DLC

A-4

Method DLCLIV
Sample DLCLIV
Operator DLC
Run date 05-08-1995 11:39:38 Version: 8
Printed on 05-08-1995 AT 11:39:58
Straight Line Fit forced through Origin.



Component 1 =
EXTERNAL STANDARD CALIBRATION

LEVEL	AMOUNT	AREA
1	0.4000	31156
2	0.8000	77583
3	1.2000	83946
4	1.6000	160773
5	4.0000	166388
6	8.0000	449788
7	12.0000	565003

Y = SLOPE * X + INTERCEPT

Area = 5.0159E+04 * Amount + 0.0000E+00
Amount = 1.9937E-05 * Area + 0.0000E+00
R squared = 0.9467

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

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.

HWI 6329-135
AMDT 011095.1
Dohrmann Serum Analysis
Analysis Dates: 08/02/95 - 08/18/95

All serum samples were thermally extracted by a modified Dohrmann DX2000 Organic Halide Analyzer and collected in a 1:1 milli Q water and TISAB solution. The samples were measured on an Orion EA940 expandable ion analyzer. The Dohrmann was calibrated using 34ppm, 40ppm, 62ppm, 100ppm, 124ppm, 250ppm, and 500ppm FC-95 standards for serum curve 1. The same FC-95 standards were used to calibrate the Dohrmann with the exception of 250ppm and 500ppm for serum curve 2. The Orion was calibrated by direct measurement with no blank correction using 0.05ppm, 0.1ppm, 0.5ppm, 1.0ppm and 1.5ppm F^- standards. The slope, intercept, and correlation were recorded in the appropriate logbook.

A summary table is included, showing the ppm F^- in each sample (see page 2). An initial calibration curve with standard deviation, %RSD, R^2 value and equation of the line is on pages 8 - 11.

Pages 3 - 7 show the excel spreadsheet that was generated when the samples were being analyzed. The Dohrmann FC-95 initial calibration curve was not used to generate the data in the spreadsheet. Any Orion reading below 0.05 is below the Orion calibration and should be considered an estimate.

The FC-95 initial calibration curve 1 was spiked into bovine serum. Bovine serum was used as blanks and QC check samples on 08/02/95 and 08/03/95. However, due to problems with blanks having higher readings than the samples, serum curve 2 was analyzed using rabbit serum from study # 6329-123 rabbit # F52346. On days 08/15/95 - 08/18/95 rabbit serum from study # 6329-123 rabbits F52346, F52335, and F52332, were used for blanks and QC check samples

We were unable to located serum samples for Day 2 through Day 4. This study was discontinued after day 8 because nothing was found in the first day of sampling.

Dean K. Plummer

FC 120 AB

HWI 6329-135

Fluoride concentration in rabbit serum (ppm F-)

Group 1
Dosage: 0 mg/kg

Sample	Day 1	Day 8	Day 15	Day 22
F52972	0.533	0.311	0.553	0.582
F52973	0.498	0.178	0.859	0.712
F52979	0.551	0.490	0.612	0.561
F52975	0.517	0.436	0.674	0.524
F52976	0.479	0.323	0.501	0.472
F52983	0.506	0.286	0.503	0.677

Group 2
Dosage: 2 mg/kg

Sample	Day 1	Day 8	Day 15	Day 22
F52986	1.45	0.338	0.393	0.610
F52990	0.650	0.277	0.463	0.466
F52997	0.444	0.305	0.36	0.529
F52982	0.466	0.283	0.298	0.438
F52994	0.446	1.27	0.346	0.620
F53410	0.388	0.697	0.354	0.677

Group 3
Dosage: 200 mg/kg

Sample	Day 1	Day 8	Day 15	Day 22
F52984	0.594	0.694	0.271	0.620
F52992	0.550	0.526	0.281	0.636
F52996	0.568	0.845	0.257	0.712
F52977	0.602	1.10	0.274	0.685
F52989	0.642	1.57	0.641	0.814
F52993	0.642	0.612	0.777	0.580

Group 4
Dosage: 1000 mg/kg

Sample	Day 1	Day 8	Day 15	Day 22
F52978	0.762	0.449	0.777	0.449
F52980	1.01	0.566	0.807	0.516
F52991	1.07	0.709	0.752	0.581
F52987	0.886	0.630	0.700	0.335
F52988	0.549	0.478	0.668	0.249
F52995	0.491	0.513	0.57	0.275

STUDY # 6329-135 SERUM

Sample ID	Actual reading (ppm F-)	Sample Qty (mL or g)	TISAB final vol (mL)	mL FC95 spiked	Conc. FC95 solution (ppm)	% recovery (ug/ug)	Actual ppm F- in sample	Mass spiked (ug F-)	Mass recovered (ug F-)
BLANK	0.0613	0.1	2.0				1.23		0.123
BLANK	0.0396	0.1	2.0				0.792		0.0792
BLANK	0.0383	0.1	2.0				0.766		0.0766
BLANK	0.0412	0.1	2.0				0.824		0.0824
BLANK	0.0319	0.1	2.0				0.638		0.0638
62-PPM-1	0.0786	0.1	2.0	0.004	62	106%	1.57	0.149	0.157
62-PPM-2	0.0934	0.1	2.0	0.004	62	125%	1.87	0.149	0.187
250-PPM-1	0.304	0.1	2.0	0.004	250	101%	6.08	0.600	0.608
250-PPM-2	0.269	0.1	2.0	0.004	250	90%	5.38	0.600	0.538
BLANK	0.0903	0.1	2.0				1.81		0.181
BLANK	0.0527	0.1	2.0				1.05		0.105
BLANK	0.0326	0.1	2.0				0.652		0.0652
F52986-DAY1	0.0723	0.1	2.0				1.45		0.145
F52990-DAY1	0.0325	0.1	2.0				0.650		0.0650
F52997-DAY1	0.0222	0.1	2.0				0.444		0.0444
F52982-DAY1	0.0233	0.1	2.0				0.466		0.0466
F52994-DAY1	0.0223	0.1	2.0				0.446		0.0446
F53410-DAY1	0.0194	0.1	2.0				0.388		0.0388
62-PPM-1	0.0760	0.1	2.0	0.004	62	102%	1.52	0.149	0.152
250-PPM-1	0.246	0.1	2.0	0.004	250	82%	4.92	0.600	0.492
SERUM BLANK-1	0.207	0.1	2.0				4.13		0.413
SERUM BLANK-2	0.176	0.1	2.0				3.52		0.352
SERUM BLANK-3	0.107	0.1	2.0				2.15		0.215
SERUM BLANK-4	0.0842	0.1	2.0				1.68		0.168
SERUM BLANK-5	0.0745	0.1	2.0				1.49		0.149
SERUM BLANK-6	0.0856	0.1	2.0				1.71		0.171
SERUM BLANK-7	0.0755	0.1	2.0				1.51		0.151
SERUM BLANK-8	0.0691	0.1	2.0				1.38		0.138
SERUM BLANK-9	0.0615	0.1	2.0				1.23		0.123
SERUM BLANK-10	0.0657	0.1	2.0				1.31		0.131
SERUM BLANK-11	0.0592	0.1	2.0				1.18		0.118
SERUM BLANK-12	0.0672	0.1	2.0				1.34		0.134
SERUM BLANK-13	0.0703	0.1	2.0				1.41		0.141
SERUM BLANK-14	0.0674	0.1	2.0				1.35		0.135
SERUM BLANK-15	0.0655	0.1	2.0				1.31		0.131
SERUM BLANK-16	0.0507	0.1	2.0				1.01		0.101
SERUM BLANK-17	0.0434	0.1	2.0				0.868		0.0868
SERUM BLANK-18	0.0403	0.1	2.0				0.806		0.0806
SERUM BLANK-19	0.0398	0.1	2.0				0.796		0.0796
SERUM BLANK-20	0.0375	0.1	2.0				0.751		0.0751
62PPM-1	0.0969	0.1	2.0	0.004	62	130%	1.94	0.149	0.194
62PPM-2	0.0902	0.1	2.0	0.004	62	121%	1.80	0.149	0.180
62PPM-3	0.0869	0.1	2.0	0.004	62	117%	1.74	0.149	0.174
250PPM-1	0.262	0.1	2.0	0.004	250	87%	5.24	0.600	0.524
250PPM-2	0.270	0.1	2.0	0.004	250	90%	5.40	0.600	0.540
F52984DAY1	0.0297	0.1	2.0				0.594		0.0594

STUDY # 6329-135 SERUM

Sample ID	Actual reading (ppm F-)	Sample Qty (mL or g)	TISAB final vol (mL)	mL FC95 spiked	Conc. FC95 solution (ppm)	% recovery (ug/ug)	Actual ppm F- in sample	Mass spiked (ug F-)	Mass recovered (ug F-)
F52992DAY1	0.0275	0.1	2.0				0.550		0.0550
F52996DAY1	0.0284	0.1	2.0				0.568		0.0568
F52977DAY1	0.0301	0.1	2.0				0.602		0.0602
F52989DAY1	0.0321	0.1	2.0				0.642		0.0642
F52993DAY1	0.0321	0.1	2.0				0.642		0.0642
F52978DAY1	0.0381	0.1	2.0				0.762		0.0762
F52980DAY1	0.0507	0.1	2.0				1.01		0.101
F52991DAY1	0.0533	0.1	2.0				1.07		0.107
F52987DAY1	0.0443	0.1	2.0				0.886		0.0886
62PPM-1	0.0890	0.1	2.0	0.004	62	120%	1.78	0.149	0.178
250PPM-1	0.264	0.1	2.0	0.004	250	88%	5.28	0.600	0.528
8/15/95							0.803		0.0803
Blank Serum-1	0.0401	0.1	2.0				0.731		0.0731
Blank Serum-2	0.0365	0.1	2.0				0.558		0.0558
Blank Serum-3	0.0279	0.1	2.0				0.549		0.0549
F52988-day1	0.0275	0.1	2.0				0.491		0.0491
F52995-day1	0.0246	0.1	2.0				0.533		0.0533
F52972-day1	0.0266	0.1	2.0				0.498		0.0498
F52973-day1	0.0249	0.1	2.0				0.551		0.0551
F52979-day1	0.0276	0.1	2.0				0.517		0.0517
F52975-day1	0.0259	0.1	2.0				0.479		0.0479
F52976-day1	0.0240	0.1	2.0				0.506		0.0506
F52983-day1	0.0253	0.1	2.0				0.776	0.096	0.0776
40ppm-1	0.0388	0.1	2.0	0.004	40	81%	1.96	0.240	0.196
100ppm-1	0.0982	0.1	2.0	0.004	100	82%	0.403		0.0403
serum blank	0.0201	0.1	2.0				0.383		0.0383
serum blank	0.0192	0.1	2.0				0.294		0.0294
serum blank	0.0147	0.1	2.0				0.863	0.096	0.0863
spike 34-1	0.0431	0.1	2.0	0.004	40	90%	0.870	0.096	0.087
spike 34-2	0.0435	0.1	2.0	0.004	40	91%	1.86	0.240	0.186
spike 100-1	0.0928	0.1	2.0	0.004	100	77%	1.81	0.240	0.181
spike 100-2	0.0905	0.1	2.0	0.004	100	75%	1.97	0.240	0.197
spike 100-3	0.0984	0.1	2.0	0.004	100	82%	0.311		0.0311
F52972 DAY 8	0.0155	0.1	2.0				0.173		0.0173
F52973 DAY 8	0.00864	0.1	2.0				0.490		0.0490
F52979 DAY 8	0.0245	0.1	2.0				0.436		0.0436
F52975 DAY 8	0.0218	0.1	2.0				0.323		0.0323
F52976 DAY 8	0.0161	0.1	2.0				0.286		0.0286
F52983 DAY 8	0.0143	0.1	2.0				0.338		0.0338
F52986 DAY 8	0.0169	0.1	2.0				0.277		0.0277
F52990 DAY 8	0.0139	0.1	2.0				0.305		0.0305
F52997 DAY 8	0.0153	0.1	2.0				0.283		0.0283
F52982 DAY 8	0.0142	0.1	2.0				0.685	0.149	0.0685
SPIKE 62-1	0.0343	0.1	2.0	0.004	62	46%	1.71	0.149	0.171
SPIKE 62-2	0.0853	0.1	2.0	0.004	62	115%	2.11	0.298	0.211
SPIKE124-1	0.106	0.1	2.0	0.004	124	71%			

Sample ID	Actual reading (ppm F-)	Sample Qty (mL or g)	TISAB final vol (mL)	mL FC95 spiked	Conc. FC95 solution (ppm)	% recovery (ug/ug)	Actual ppm F- in sample	Mass spiked (ug F-)	Mass recovered (ug F-)
SPIKE124-2	0.134	0.1	2.0	0.004	124	90%	2.67	0.298	0.267
Blank	0.0722	0.1	2.0				1.44		0.144
F52994-day 8	0.0634	0.1	2.0				1.27		0.127
F53410 day 8	0.0348	0.1	2.0				0.697		0.0697
F52984 day8	0.0347	0.1	2.0				0.694		0.0694
F52992 day 8	0.0263	0.1	2.0				0.526		0.0526
F52996 day 8	0.0422	0.1	2.0				0.845		0.0845
F52977 day 8	0.0550	0.1	2.0				1.10		0.110
F52989 day 8	0.0407	0.1	2.0				0.814		0.0814
F52993 day 8	0.0306	0.1	2.0				0.612		0.0612
F52978 day 8	0.0272	0.1	2.0				0.544		0.0544
F52980 day 8	0.0283	0.1	2.0				0.566		0.0566
40ppm-1	0.0530	0.1	2.0	0.004	40	110%	1.06	0.096	0.106
124ppm-1	0.124	0.1	2.0	0.004	124	84%	2.49	0.298	0.249
Blank	0.0608	0.1	2.0				1.22		0.122
F52991 day 8	0.0354	0.1	2.0				0.709		0.0709
F52987 day 8	0.0315	0.1	2.0				0.630		0.0630
F52988 day 8	0.0239	0.1	2.0				0.478		0.0478
F52995 day 8	0.0257	0.1	2.0				0.513		0.0513
F52972 day 15	0.0277	0.1	2.0				0.553		0.0553
F52973 day 15	0.0430	0.1	2.0				0.859		0.0859
F52979 day 15	0.0306	0.1	2.0				0.612		0.0612
F52975 day 15	0.0337	0.1	2.0				0.674		0.0674
F52976 day 15	0.0250	0.1	2.0				0.501		0.0501
F52983 day 15	0.0251	0.1	2.0				0.503		0.0503
40 ppm-1	0.0452	0.1	2.0	0.004	40	94%	0.903	0.096	0.0903
124 ppm-1	0.123	0.1	2.0	0.004	124	83%	2.47	0.298	0.247
serum blank	0.0259	0.1	2.0				0.518		0.0518
serum blank	0.0199	0.1	2.0				0.398		0.0398
spike 40-1	0.0373	0.1	2.0	0.004	40	78%	0.745	0.096	0.0745
spike 40-2	0.0390	0.1	2.0	0.004	40	81%	0.781	0.096	0.0781
spike 40-3	0.0448	0.1	2.0	0.004	40	93%	0.896	0.096	0.0896
spike 124-1	0.122	0.1	2.0	0.004	124	82%	2.45	0.298	0.245
spike 124-2	0.134	0.1	2.0	0.004	124	90%	2.68	0.298	0.268
spike 124-3	0.116	0.1	2.0	0.004	124	78%	2.33	0.298	0.233
serum blank	0.0302	0.1	2.0				0.605		0.0605
F52986 DAY15	0.0196	0.1	2.0				0.393		0.0393
F52990 DAY15	0.0231	0.1	2.0				0.463		0.0463
F52997 DAY15	0.0180	0.1	2.0				0.360		0.0360
F52982 DAY15	0.0149	0.1	2.0				0.298		0.0298
F52994 DAY15	0.0173	0.1	2.0				0.346		0.0346
F53410 DAY15	0.0177	0.1	2.0				0.354		0.0354
F52984 DAY15	0.0135	0.1	2.0				0.271		0.0271
F52992 DAY15	0.0141	0.1	2.0				0.281		0.0281
F52996 DAY15	0.0129	0.1	2.0				0.257		0.0257
F52977 DAY15	0.0137	0.1	2.0				0.274		0.0274

Sample ID	Actual reading (ppm F-)	Sample Qty (mL or g)	TISAB final vol (mL)	mL FC95 spiked	Conc. FC95 solution (ppm)	% recovery (ug/ug)	Actual ppm F- in sample	Mass spiked (ug F-)	Mass recovered (ug F-)
SPIKE 40-1	0.0373	0.1	2.0	0.004	40	78%	0.746	0.096	0.0746
SPIKE 124-1	0.152	0.1	2.0	0.004	124	102%	3.04	0.298	0.304
serum blank	0.0465	0.1	2.0				0.931		0.0931
F52989 day15	0.0785	0.1	2.0				1.57		0.157
F52993 day 15	0.0389	0.1	2.0				0.777		0.0777
F52978 day 15	0.0389	0.1	2.0				0.777		0.0777
F52980 day 15	0.0404	0.1	2.0				0.807		0.0807
F52991 day 15	0.0376	0.1	2.0				0.752		0.0752
F52987 day 15	0.0350	0.1	2.0				0.700		0.0700
F52988 day 15	0.0334	0.1	2.0				0.668		0.0668
F52995 day 15	0.0285	0.1	2.0				0.570		0.0570
F52972 day 22	0.0291	0.1	2.0				0.582		0.0582
F52973 day 22	0.0356	0.1	2.0				0.712		0.0712
40ppm spike-1	0.0722	0.1	2.0	0.004	40	150%	1.44	0.096	0.144
40ppm spike-2	0.0460	0.1	2.0	0.004	40	96%	0.919	0.096	0.0919
124ppm spike-1	0.110	0.1	2.0	0.004	124	74%	2.20	0.298	0.220
F52979 day 22	0.0280	0.1	2.0				0.561		0.0561
F52975 day 22	0.0262	0.1	2.0				0.524		0.0524
F52976 day 22	0.0236	0.1	2.0				0.472		0.0472
F52983 day 22	0.0339	0.1	2.0				0.677		0.0677
F52986 day 22	0.0305	0.1	2.0				0.610		0.0610
F52990 day 22	0.0233	0.1	2.0				0.466		0.0466
F52997 day 22	0.0264	0.1	2.0				0.529		0.0529
F52982 day 22	0.0219	0.1	2.0				0.438		0.0438
F52994 day 22	0.0310	0.1	2.0				0.620		0.0620
F53410 day 22	0.0339	0.1	2.0				0.677		0.0677
40ppm spike-1	0.0645	0.1	2.0	0.004	40	134%	1.29	0.096	0.129
124 ppm spike -1	0.130	0.1	2.0	0.004	124	88%	2.61	0.298	0.261
62ppm spike-1	0.0898	0.1	2.0	0.004	62	121%	1.80	0.149	0.180
serum blank	0.0405	0.1	2.0				0.810		0.0810
F52984 day 22	0.0310	0.1	2.0				0.620		0.0620
F52992 day 22	0.0318	0.1	2.0				0.636		0.0636
F52996 day 22	0.0356	0.1	2.0				0.712		0.0712
F52977 day 22	0.0343	0.1	2.0				0.685		0.0685
F52989 day 22	0.0321	0.1	2.0				0.641		0.0641
F52993 day 22	0.0290	0.1	2.0				0.580		0.0580
40ppm spike-1	0.0616	0.1	2.0	0.004	40	128%	1.23	0.096	0.123
124 ppm spike -1	0.116	0.1	2.0	0.004	124	78%	2.32	0.298	0.232
serum blank	0.0221	0.1	2.0				0.442		0.0442
serum blank	0.0172	0.1	2.0				0.344		0.0344
spike 40-1	0.0235	0.1	2.0	0.004	40	49%	0.471	0.096	0.0471
spike 40-2	0.0333	0.1	2.0	0.004	40	69%	0.666	0.096	0.0666
spike 40-3	0.0343	0.1	2.0	0.004	40	71%	0.686	0.096	0.0686
spike 40-4	0.0371	0.1	2.0	0.004	40	77%	0.743	0.096	0.0743
spike 124-1	0.0562	0.1	2.0	0.004	124	38%	1.12	0.298	0.112
spike 124-2	0.0910	0.1	2.0	0.004	124	61%	1.82	0.298	0.182

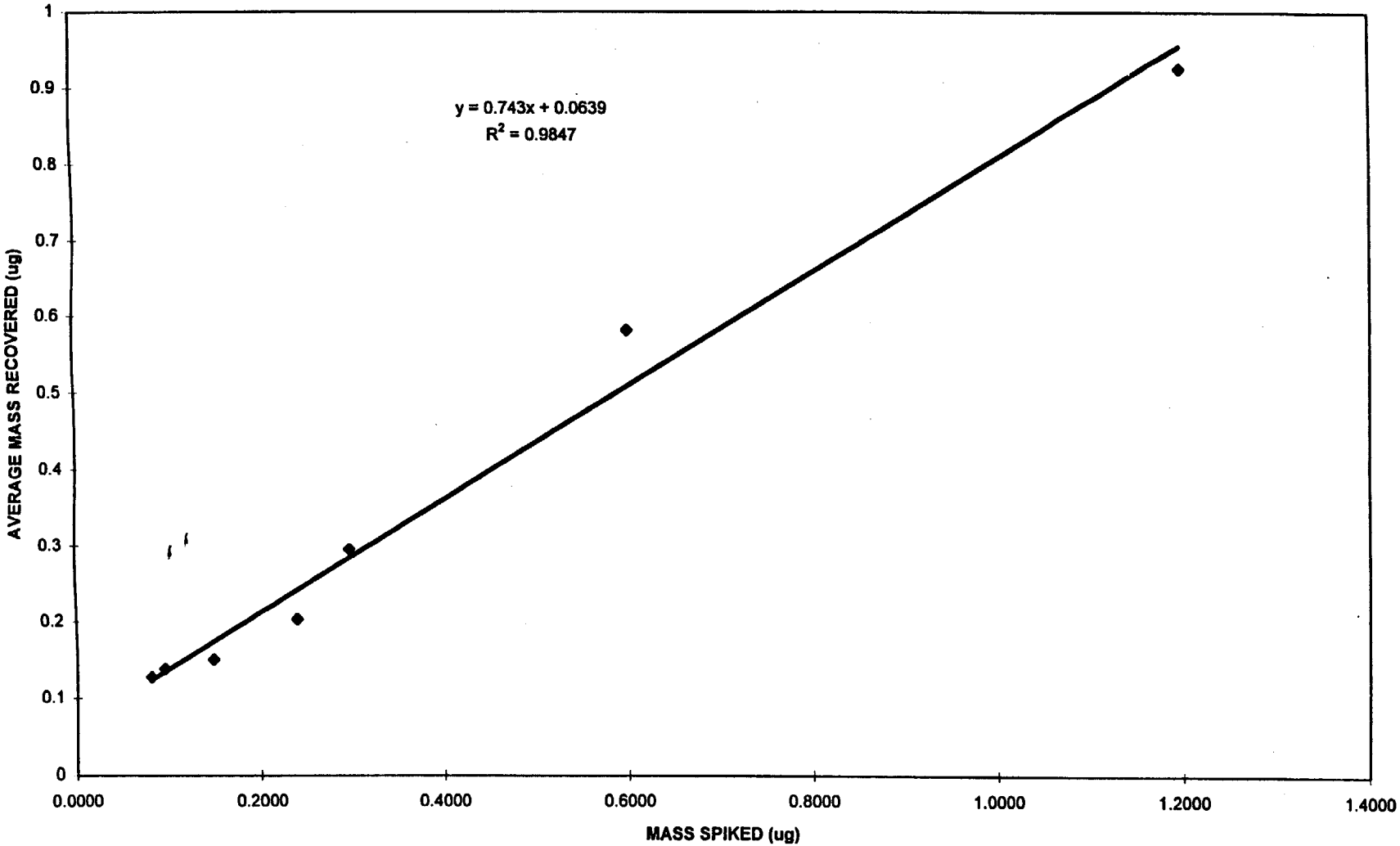
STUDY # 6329-135 SERUM

Sample ID	Actual reading (ppm F-)	Sample Qty (mL or g)	TISAB final vol (mL)	mL FC95 spiked	Conc. FC95 solution (ppm)	% recovery (ug/ug)	Actual ppm F- in sample	Mass spiked (ug F-)	Mass recovered (ug F-)
spike 124-3	0.205	0.1	2.0	0.004	124	138%	4.10	0.298	0.410
spike 124-4	0.0799	0.1	2.0	0.004	124	54%	1.60	0.298	0.160
SPIKE100-1	0.0659	0.1	2.0	0.004	100	55%	1.32	0.240	0.132
SPIKE100-2	0.0855	0.1	2.0	0.004	100	71%	1.71	0.240	0.171
SPIKE100-3	0.0851	0.1	2.0	0.004	100	71%	1.70	0.240	0.170
BLANK	0.0454	0.1	2.0				0.908		0.0908
BLANK	0.0222	0.1	2.0				0.444		0.0444
F52978 DAY22	0.0225	0.1	2.0				0.449		0.0449
F52980 DAY22	0.0258	0.1	2.0				0.516		0.0516
F52991 DAY22	0.0290	0.1	2.0				0.581		0.0581
F52987 DAY22	0.0168	0.1	2.0				0.335		0.0335
F52988 DAY22	0.0125	0.1	2.0				0.249		0.0249
F52995 DAY22	0.0138	0.1	2.0				0.275		0.0275
SPIKE 40-1	0.0337	0.1	2.0	0.004	40	70%	0.673	0.096	0.0673
SPIKE 100-1	0.0850	0.1	2.0	0.004	100	71%	1.70	0.240	0.170

NORMAN SERUM CURVE 1

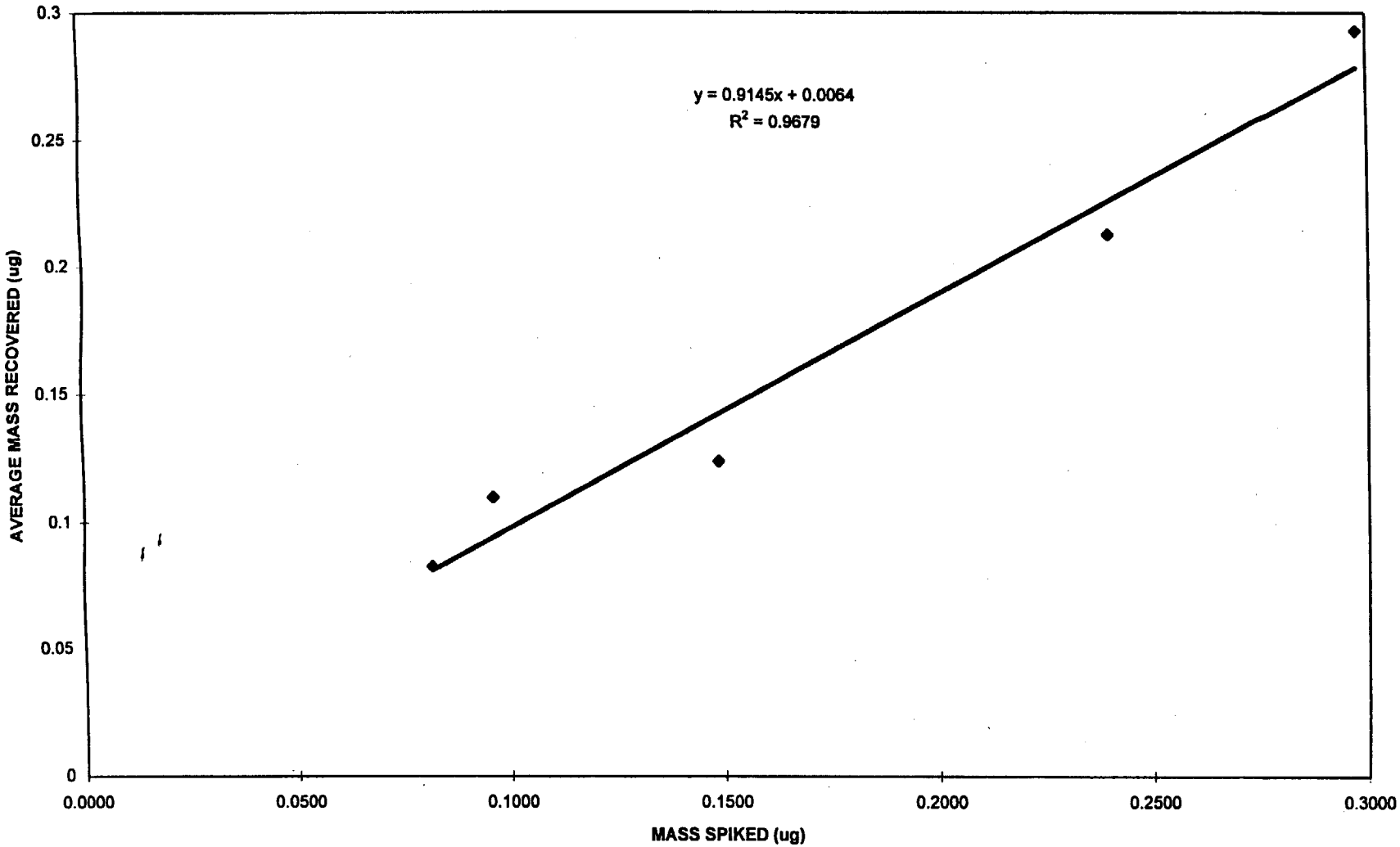
Sample ID	Actual reading (ppm F-)	Sample Qty (mL or g)	TISAB final vol (mL)	mL FC95 spiked	Conc. FC95 solution (ppm)	% recovery (ug/ug)	Actual ppm F- in sample	Mass spiked (ug F-)	Mass recovered (ug F-)		
4-ppm-1	0.07175	0.1	2.0	0.004	34	176%	1.4350	0.0817	0.1435	STDEV (34ppm):	0.0156
4-ppm-2	0.05614	0.1	2.0	0.004	34	138%	1.1228	0.0817	0.11228	%RSD:	12
4-ppm-3	0.06462	0.1	2.0	0.004	34	158%	1.2924	0.0817	0.12924	AVERAGE:	0.128
0-ppm-1	0.08668	0.1	2.0	0.004	40	180%	1.7336	0.0961	0.17336	STDEV (40ppm):	0.0241
0-ppm-2	0.06728	0.1	2.0	0.004	40	140%	1.3456	0.0961	0.13456	%RSD:	17
0-ppm-3	0.05939	0.1	2.0	0.004	40	124%	1.1878	0.0961	0.11878	AVERAGE:	0.139
0-ppm-4	0.06385	0.1	2.0	0.004	40	133%	1.2770	0.0961	0.1277		
2-ppm-1	0.07291	0.1	2.0	0.004	62	98%	1.4582	0.1489	0.14582	STDEV(62ppm):	0.00549
2-ppm-2	0.0753	0.1	2.0	0.004	62	101%	1.5060	0.1489	0.1506	%RSD:	3.6
2-ppm-3	0.07839	0.1	2.0	0.004	62	105%	1.5678	0.1489	0.15678	AVERAGE:	0.151
00-ppm-1	0.0902	0.1	2.0	0.004	100	75%	1.8040	0.2402	0.1804	STDEV (100ppm):	0.0224
00-ppm-2	0.1026	0.1	2.0	0.004	100	85%	2.0520	0.2402	0.2052	%RSD:	11
00-ppm-3	0.1126	0.1	2.0	0.004	100	94%	2.2520	0.2402	0.2252	AVERAGE:	0.204
24-ppm-1	0.1371	0.1	2.0	0.004	124	92%	2.7420	0.2978	0.2742	STDEV (124ppm):	0.0251
24-ppm-2	0.1451	0.1	2.0	0.004	124	97%	2.9020	0.2978	0.2902	%RSD:	8.5
24-ppm-3	0.1617	0.1	2.0	0.004	124	109%	3.2340	0.2978	0.3234	AVERAGE:	0.296
50-ppm-1	0.3217	0.1	2.0	0.004	250	107%	6.4340	0.6004	0.6434	STDEV (250ppm):	0.0821
50-ppm-2	0.2447	0.1	2.0	0.004	250	82%	4.8940	0.6004	0.4894	%RSD:	14
50-ppm-3	0.3078	0.1	2.0	0.004	250	103%	6.1560	0.6004	0.6156	AVERAGE:	0.583
00-ppm-1	0.4438	0.1	2.0	0.004	500	74%	8.8760	1.2008	0.8876	STDEV (500ppm):	0.0459
00-ppm-2	0.4584	0.1	2.0	0.004	500	76%	9.1680	1.2008	0.9168	%RSD:	5.0
00-ppm-3	0.4888	0.1	2.0	0.004	500	81%	9.7760	1.2008	0.9776	AVERAGE:	0.927

SERUM CURVE 1 NORMAN (07/25/95)



Sample ID	Actual reading (ppm F-)	Sample Qty (mL or g)	TISAB final vol (mL)	mL FC95 spiked	Conc. FC95 solution (ppm)	% recovery (ug/ug)	Actual ppm F- in sample	Mass spiked (ug F-)	Mass recovered (ug F-)		
SPIKE 34-1	0.0330	0.1	2.0	0.004	34	81%	0.660	0.0817	0.0660	STDEV (34ppm):	0.0152
SPIKE 34-2	0.0479	0.1	2.0	0.004	34	117%	0.957	0.0817	0.0957	%RSD:	18
SPIKE 34-3	0.0430	0.1	2.0	0.004	34	105%	0.861	0.0817	0.0861	AVERAGE:	0.0826
SPIKE 40-1	0.0682	0.1	2.0	0.004	40	142%	1.36	0.0961	0.136	STDEV (40ppm):	0.0234
SPIKE 40-2	0.0459	0.1	2.0	0.004	40	95%	0.917	0.0961	0.0917	%RSD:	21
SPIKE 40-3	0.0508	0.1	2.0	0.004	40	106%	1.02	0.0961	0.102	AVERAGE:	0.110
SPIKE 62PPM-1	0.0422	0.1	2.0	0.004	62	57%	0.845	0.149	0.0845	STDEV (62ppm):	0.0472
SPIKE 62PPM-2	0.0441	0.1	2.0	0.004	62	59%	0.882	0.149	0.0882	%RSD:	38
SPIKE 62PPM-3	0.0690	0.1	2.0	0.004	62	93%	1.38	0.149	0.138	AVERAGE:	0.124
SPIKE 62PPM-4	0.0922	0.1	2.0	0.004	62	124%	1.84	0.149	0.184		
SPIKE 100PPM-1	0.0962	0.1	2.0	0.004	100	80%	1.92	0.240	0.192	STDEV (100ppm):	0.0560
SPIKE 100PPM-2	0.159	0.1	2.0	0.004	100	132%	3.17	0.240	0.317	%RSD:	26
SPIKE 100PPM-3	0.0774	0.1	2.0	0.004	100	64%	1.55	0.240	0.155	AVERAGE:	0.213
SPIKE 100PPM-4	0.113	0.1	2.0	0.004	100	94%	2.25	0.240	0.225		
SPIKE 100PPM-5	0.0930	0.1	2.0	0.004	100	77%	1.86	0.240	0.186		
SPIKE 100PPM-6	0.100	0.1	2.0	0.004	100	83%	2.00	0.240	0.200		
SPIKE 124PPM-1	0.149	0.1	2.0	0.004	124	100%	2.97	0.298	0.297	STDEV (124ppm):	0.0108
SPIKE 124PPM-2	0.150	0.1	2.0	0.004	124	101%	3.00	0.298	0.300	%RSD:	3.7
SPIKE 124PPM-3	0.140	0.1	2.0	0.004	124	94%	2.80	0.298	0.280	AVERAGE:	0.293

SERUM CURVE 2 NORMAN (08/15/95)



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

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.

DDW '12694
Skalar Data

RE: 6329-135 SERUM SAMPLES
AMDT 20795.1
Date of Analysis: 8/16, 8/21, and 8/22/95
Analyst: DDW

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

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

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

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

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

Donald D Wright

DLW 7/26/95
AMDT 20795.1
HWI 6329-135
S Kalan DATA

SUMMARY OF 6329-135
SERUM SAMPLES
AMDT 020795.1

	Sample ID#	Fluoride in Sample (ppm) Day 1	Fluoride in Sample (ppm) Day 8	Fluoride in Sample (ppm) Day 15	Fluoride in Sample (ppm) Day22
	F52972	1.04	0.51	0.88	0.05
	F52973	0.98	ND	1.36	0.05
GROUP 1	F52979	1.02	0.78	1.00	0.04
Dose Level : 0	F52975	0.88	0.69	1.09	0.03
	F52976	0.84	0.52	0.82	0.03
	F52983	0.96	0.46	0.91	0.05
	F52986	1.78	0.54	0.25	0.04
	F52990	0.75	0.46	0.33	0.03
GROUP 2	F52997	0.47	0.61	0.23	0.04
Dose Level : 2 mg/kg	F52982	0.49	0.50	0.12	0.03
	F52994	0.56	1.85	0.33	0.04
	F53410	0.46	1.06	0.27	0.05
	F52984	0.81	1.05	0.14	0.05
	F52992	0.71	0.96	0.17	0.05
GROUP 3	F52996	0.62	1.29	0.18	0.05
Dose Level : 200 mg/kg	F52977	0.73	1.65	0.22	0.05
	F52989	0.71	1.26	2.07	0.04
	F52993	0.70	0.98	1.12	0.04
	F52978	0.81	0.82	1.17	0.03
	F52980	1.25	0.90	1.12	0.04
GROUP 4	F52991	1.31	1.22	1.06	0.03
Dose Level : 1000 mg/kg	F52987	1.03	1.09	0.94	0.02
	F52988	1.02	0.84	0.95	0.02
	F52995	0.92	0.86	0.77	0.03

1995-08-16 14:08 OutPut of : 950816A1
Operator : DDW
Date of the Analysis : 1995-08-16 08:58
Analysis File Name : C:\SKALAR\DATA\HWIDATA\SERUM\950816A1

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% Recovery	DI:TISAB final vol (mL)	Qty Sampl (mL)	Actual ppm F- in Sample	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery
1	Tracer	1.50	1.47	98%								
2	Drift	1.50	1.49	99%								
3	Wash		ND									
4	Standard 1	0.015	0.016	109%								
5	Standard 2	0.03	0.03	94%								
6	Standard 3	0.06	0.06	100%								
7	Standard 4	0.09	0.09	99%								
8	Standard 5	0.12	0.12	102%								
9	Standard 6	0.15	0.15	99%								
10	Standard 7	0.30	0.29	95%								
11	Standard 8	0.60	0.61	102%								
12	Standard 9	1.20	1.22	101%								
13	Standard 10	1.50	1.48	99%								
14	Drift	1.50	1.49	99%								
15	Wash		ND									
16	SPK 62-1		0.13		2.0	0.10	2.59	0.004	62.00	0.15	0.26	174%
17	SPK 250-1		0.35		2.0	0.10	6.92	0.004	250.0	0.60	0.69	115%
18	SPK 250-2		0.32		2.0	0.10	6.44	0.004	250.0	0.60	0.64	107%
19	BLANK		0.12		2.0	0.10	2.41					
20	BLANK		0.07		2.0	0.10	1.37					
21	BLANK		0.04		2.0	0.10	0.82					
22	F52986-1		0.09		2.0	0.10	1.78					
23	F52990-1		0.04		2.0	0.10	0.75					
24	F52997-1		0.02		2.0	0.10	0.47					
25	F52982-1		0.02		2.0	0.10	0.49					
26	Drift	1.50	1.48	98%								
27	Wash		ND									
28	F52994-1		0.03		2.0	0.10	0.56					
29	F53410-1		0.02		2.0	0.10	0.46					

QAU 9/14/95
AMDT 20795.1
HWI 6329-135
Skalar Data

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% Recovery	DI TISAB final vol (mL)	Qty Sampl (mL)	Actual ppm F- in Sample	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery
30	SPK 62-1		0.10		2.0	0.10	1.96	0.004	62.00	0.15	0.20	131%
31	SPK 250-1		0.29		2.0	0.10	5.88	0.004	250.0	0.60	0.59	98%
32	BLANK SERUM		0.06		2.0	0.10	1.25					
33	BLANK SERUM		0.05		2.0	0.10	1.04					
34	BLANK SERUM		0.06		2.0	0.10	1.13					
35	BLANK SERUM		0.05		2.0	0.10	0.97					
36	SPK 62-1		0.14		2.0	0.10	2.80	0.004	62.00	0.15	0.28	188%
37	SPK 62-2		0.11		2.0	0.10	2.29	0.004	62.00	0.15	0.23	154%
38	Drift	1.50	1.49	99%								
39	Wash		ND									
40	SPK 62-3		0.12		2.0	0.10	2.39	0.004	62.00	0.15	0.24	160%
41	SPK 250-1		0.25		2.0	0.10	5.08	0.004	250.0	0.60	0.51	85%
42	SPK 250-2		0.27		2.0	0.10	5.42	0.004	250.0	0.60	0.54	90%
43	F52984-1		0.04		2.0	0.10	0.81					
44	F52992-1		0.04		2.0	0.10	0.71					
45	F52996-1		0.03		2.0	0.10	0.62					
46	F52997-1		0.04		2.0	0.10	0.73					
47	F52984-1		0.04		2.0	0.10	0.71					
48	F52993-1		0.03		2.0	0.10	0.70					
49	F52978-1		0.04		2.0	0.10	0.81					
50	Drift	1.50	1.49	99%								
51	Wash		ND									
52	F52980-1		0.06		2.0	0.10	1.25					
53	F52991-1		0.07		2.0	0.10	1.31					
54	F52987-1		0.05		2.0	0.10	1.03					
55	SPK 62-1		0.11		2.0	0.10	2.10	0.004	62.00	0.15	0.21	141%
56	SPK 250-1		0.26		2.0	0.10	5.14	0.004	250.0	0.60	0.51	86%
57	Drift	1.50	1.50	100%								
58	Wash		ND									

1995-08-21 12:30 OutPut of : 950821A1
Operator : DDW
Date of the Analysis : 1995-08-21 07:55
Analysis File Name : C:\SKALAR\DATA\HWIDATA\SERUM\950821A1

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% Recovery	DI TISAB final vol (mL)	Qty Sampl (mL)	Actual ppm F- in Sample	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery
1	Tracer	1.50	1.47	98%								
2	Drift	1.50	1.48	99%								
3	Wash		ND									
4	Standard 1	0.015	0.014	96%								
5	Standard 2	0.03	0.03	99%								
6	Standard 3	0.06	0.06	104%								
7	Standard 4	0.09	0.09	99%								
8	Standard 5	0.12	0.12	99%								
9	Standard 6	0.15	0.15	101%								
10	Standard 7	0.30	0.28	93%								
11	Standard 8	0.60	0.62	103%								
12	Standard 9	1.20	1.22	102%								
13	Standard 10	1.50	1.47	98%								
14	Drift	1.50	1.54	103%								
15	Wash		ND									
16	SERUM BLK 1		0.08		2.0	0.10	1.53					
17	SERUM BLK 2		0.07		2.0	0.10	1.34					
18	SERUM BLK 3		0.06		2.0	0.10	1.12					
19	F52988-1		0.05		2.0	0.10	1.02					
20	F52995-1		0.05		2.0	0.10	0.92					
21	F52972-1		0.05		2.0	0.10	1.04					
22	F52973-1		0.05		2.0	0.10	0.98					
23	F52979-1		0.05		2.0	0.10	1.02					
24	F52975-1		0.04		2.0	0.10	0.88					
25	F52976-1		0.04		2.0	0.10	0.84					
26	Drift	1.50	1.53	102%								
27	Wash		ND									
28	F52983-1		0.05		2.0	0.10	0.96					
29	SPK 40-1		0.07		2.0	0.10	1.34	0.004	40.00	0.10	0.13	140%

QAW 12/6/95
AMDT 20795.1
HWI 6329-135
Skalar Data

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% Recovery	DI TISAB final vol (mL)	Qty Sample (mL)	Actual ppm F- in Sample	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery
30	SPK 100-1		0.14		2.0	0.10	2.83	0.004	100.0	0.24	0.28	118%
31	BLK-1		0.03		2.0	0.10	0.69					
32	BLK-2		0.03		2.0	0.10	0.64					
33	BLK-3		0.02		2.0	0.10	0.49					
34	SPK 40-1		0.06		2.0	0.10	1.29	0.004	40.00	0.10	0.13	134%
35	SPK 40-2		0.06		2.0	0.10	1.23	0.004	40.00	0.10	0.12	128%
36	SPK 100-1		0.13		2.0	0.10	2.50	0.004	100.0	0.24	0.25	104%
37	SPK 100-2		0.12		2.0	0.10	2.44	0.004	100.0	0.24	0.24	102%
38	Drift	1.50	1.54	103%								
39	Wash		ND									
40	SPK 100-3		0.14		2.0	0.10	2.70	0.004	100.0	0.24	0.27	113%
41	BLK		0.03		2.0	0.10	0.69					
42	F52972-8		0.03		2.0	0.10	0.51					
43	F52973-8		ND		2.0	0.10	ND					
44	F52979-8		0.04		2.0	0.10	0.78					
45	F52975-8		0.03		2.0	0.10	0.69					
46	F52976-8		0.03		2.0	0.10	0.52					
47	F52983-8		0.02		2.0	0.10	0.46					
48	F52986-8		0.03		2.0	0.10	0.54					
49	F52990-8		0.02		2.0	0.10	0.46					
50	Drift	1.50	1.53	102%								
51	Wash		ND									
52	F52997-8		0.03		2.0	0.10	0.61					
53	F52982-8		0.02		2.0	0.10	0.50					
54	SPK 62-1		0.05		2.0	0.10	1.10	0.004	62.00	0.15	0.11	74%
55	SPK 62-2		0.11		2.0	0.10	2.27	0.004	62.00	0.15	0.23	152%
56	SPK 124-1		0.14		2.0	0.10	2.75	0.004	124.0	0.30	0.27	92%
57	SPK 124-2		0.17		2.0	0.10	3.42	0.004	124.0	0.30	0.34	115%
58	BLK		0.10		2.0	0.10	2.07					
59	F52994-8		0.09		2.0	0.10	1.85					
60	F53410-8		0.05		2.0	0.10	1.06					
61	F52984-8		0.05		2.0	0.10	1.05					
62	Drift	1.50	1.55	103%								
63	Wash		ND									
64	F52992-8		0.05		2.0	0.10	0.96					
65	F52996-8		0.06		2.0	0.10	1.29					

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% Recovery	DI TISAB final vol (mL)	Qty Sampl (mL)	Actual ppm F- in Sample	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery
66	F52997-8		0.08		2.0	0.10	1.65					
67	F52989-8		0.06		2.0	0.10	1.26					
68	F52993-8		0.05		2.0	0.10	0.98					
69	F52978-8		0.04		2.0	0.10	0.82					
70	F52980-8		0.05		2.0	0.10	0.90					
71	SPK 40-1		0.08		2.0	0.10	1.51	0.004	40.00	0.10	0.15	157%
72	SPK 124-1		0.17		2.0	0.10	3.32	0.004	124.0	0.30	0.33	111%
73	BLK		0.09		2.0	0.10	1.79					
74	Drift	1.50	1.54	102%								
75	Wash		ND									
76	F52991-8		0.06		2.0	0.10	1.22					
77	F52987-8		0.05		2.0	0.10	1.09					
78	F52988-8		0.04		2.0	0.10	0.84					
79	F52995-8		0.04		2.0	0.10	0.86					
80	F52972-15		0.04		2.0	0.10	0.88					
81	F52973-15		0.07		2.0	0.10	1.36					
82	F52979-15		0.05		2.0	0.10	1.00					
83	F52975-15		0.05		2.0	0.10	1.09					
84	F52976-15		0.04		2.0	0.10	0.82					
85	F52983-15		0.05		2.0	0.10	0.91					
86	Drift	1.50	1.57	105%								
87	Wash		ND									
88	SPK 40-1		0.08		2.0	0.10	1.52	0.004	40.00	0.10	0.15	158%
89	SPK 124-1		0.17		2.0	0.10	3.30	0.004	124.0	0.30	0.33	111%
90	Drift	1.50	1.53	102%								
91	Wash		ND									

1995-08-21 16:58 OutPut of : 950821B1
Operator : DDW
Date of the Analysis : 1995-08-21 12:29
Analysis File Name : C:\SKALAR\DATA\HWIDATA\SERUM\950821B1

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% Recovery	DI:TISAB final vol (mL)	Qty Sampl (mL)	Actual ppm F- in Sample	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery
1	Tracer	1.50	1.49	99%								
2	Drift	1.50	1.48	99%								
3	Wash		ND									
4	Standard 1	0.015	0.016	105%								
5	Standard 2	0.03	0.03	95%								
6	Standard 3	0.06	0.06	102%								
7	Standard 4	0.09	0.09	101%								
8	Standard 5	0.12	0.12	98%								
9	Standard 6	0.15	0.15	100%								
10	Standard 7	0.30	0.28	93%								
11	Standard 8	0.60	0.62	103%								
12	Standard 9	1.20	1.22	102%								
13	Standard 10	1.50	1.47	98%								
14	Drift	1.50	1.44	96%								
15	Wash		ND									
16	SERUM BLK 1		0.03		2.0	0.10	0.63					
17	SERUM BLK 2		0.02		2.0	0.10	0.37					
18	SPK 40-1		0.04		2.0	0.10	0.81	0.004	40.00	0.10	0.08	85%
19	SPK 40-2		0.04		2.0	0.10	0.86	0.004	40.00	0.10	0.09	90%
20	SPK 40-3		0.05		2.0	0.10	1.03	0.004	40.00	0.10	0.10	107%
21	SPK 124-1		0.14		2.0	0.10	2.76	0.004	124.0	0.30	0.28	93%
22	SPK 124-2		0.14		2.0	0.10	2.88	0.004	124.0	0.30	0.29	97%
23	SPK 124-3		0.14		2.0	0.10	2.77	0.004	124.0	0.30	0.28	93%
24	BLK		0.03		2.0	0.10	0.65					
25	F52986-15		0.01		2.0	0.10	0.25					
26	Drift	1.50	1.41	94%								
27	Wash		ND									
28	F52990-15		0.02		2.0	0.10	0.33					
29	F52997-15		0.01		2.0	0.10	0.23					

DDW 1/26/95
AMDT 20795.1
HWI 6329-135
Skalar Data

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% Recovery	DI TISAB final vol (mL)	Qty Sampl (mL)	Actual ppm F- in Sample	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery
30	F52982-15		0.01		2.0	0.10	0.12					
31	F52994-15		0.02		2.0	0.10	0.33					
32	F53410-15		0.01		2.0	0.10	0.27					
33	F52984-15		0.01		2.0	0.10	0.14					
34	F52992-15		0.01		2.0	0.10	0.17					
35	F52996-15		0.01		2.0	0.10	0.18					
36	F52977-15		0.01		2.0	0.10	0.22					
37	SPK 40-1		0.04		2.0	0.10	0.86	0.004	40.00	0.10	0.09	90%
38	Drift	1.50	1.43	96%								
39	Wash		ND									
40	SPK 124-1		0.19		2.0	0.10	3.82					
41	BLK		0.07		2.0	0.10	1.43					
42	F52989-15		0.10		2.0	0.10	2.07					
43	F52993-15		0.06		2.0	0.10	1.12					
44	F52978-15		0.06		2.0	0.10	1.17					
45	F52980-15		0.06		2.0	0.10	1.12					
46	F52991-15		0.05		2.0	0.10	1.06					
47	F52987-15		0.05		2.0	0.10	0.94					
48	F52988-15		0.05		2.0	0.10	0.95					
49	F52995-15		0.04		2.0	0.10	0.77					
50	Drift	1.50	1.45	97%								
51	Wash		ND									
52	F52972-22		0.05		2.0	0.10	0.91					
53	F52973-22		0.05		2.0	0.10	1.02					
54	SPK 40-1		0.10		2.0	0.10	2.03	0.004	40.00	0.10	0.20	212%
55	SPK 40-2		0.07		2.0	0.10	1.31	0.004	40.00	0.10	0.13	136%
56	SPK 124-1		0.14		2.0	0.10	2.78	0.004	124.0	0.30	0.28	93%
57	BLK		0.06		2.0	0.10	1.19					
58	F52979-22		0.04		2.0	0.10	0.82					
59	F52975-22		0.03		2.0	0.10	0.68					
60	F52976-22		0.03		2.0	0.10	0.65					
61	F52983-22		0.05		2.0	0.10	0.91					
62	Drift	1.50	1.42	95%								
63	Wash		ND									
64	F52986-22		0.04		2.0	0.10	0.88					
65	F52990-22		0.03		2.0	0.10	0.69					

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% Recovery	DI TISAB final vol (mL)	Qty Sampl (mL)	Actual ppm F- in Sample	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery
66	F52997-22		0.04		2.0	0.10	0.73					
67	F52982-22		0.03		2.0	0.10	0.57					
68	F52994-22		0.04		2.0	0.10	0.89					
69	F53410-22		0.05		2.0	0.10	0.92					
70	SPK 40-1		0.09		2.0	0.10	1.79	0.004	40.00	0.10	0.18	186%
71	SPK 124-1		0.17		2.0	0.10	3.36	0.004	124.0	0.30	0.34	113%
72	SPK 62-1		0.12		2.0	0.10	2.42	0.004	62.00	0.15	0.24	163%
73	BLK		0.06		2.0	0.10	1.24					
74	Drift	1.50	1.47	98%								
75	Wash		ND									
76	F52984-22		0.05		2.0	0.10	0.94					
77	F52992-22		0.05		2.0	0.10	0.91					
78	F52996-22		0.05		2.0	0.10	0.96					
79	F52977-22		0.05		2.0	0.10	0.93					
80	F52989-22		0.04		2.0	0.10	0.88					
81	F52993-22		0.04		2.0	0.10	0.79					
82	SPK 40-1		0.09		2.0	0.10	1.71	0.004	40.00	0.10	0.17	178%
83	SPK 124-1		0.15		2.0	0.10	2.92	0.004	124.0	0.30	0.29	98%
84	Drift	1.50	1.46	97%								
85	Wash		ND									

1995-08-22 09:05 OutPut of : 950822A1
Operator : DDW
Date of the Analysis : 1995-08-22 06:49
Analysis File Name : C:\SKALAR\DATA\HWIDATA\SERUM\950822A1

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% Recovery	DI:TISAB final vol (mL)	Qty Sampl (mL)	Actual ppm F- in Sample	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery
1	Tracer	1.50	1.46	97%								
2	Drift	1.50	1.48	98%								
3	Wash		ND									
4	Standard 1	0.015	0.016	104%								
5	Standard 2	0.03	0.03	94%								
6	Standard 3	0.06	0.06	104%								
7	Standard 4	0.09	0.09	101%								
8	Standard 5	0.12	0.12	97%								
9	Standard 6	0.15	0.15	101%								
10	Standard 7	0.30	0.28	92%								
11	Standard 8	0.60	0.62	104%								
12	Standard 9	1.20	1.23	102%								
13	Standard 10	1.50	1.47	98%								
14	Drift	1.50	1.54	102%								
15	Wash		ND									
16	SERUM BLK 1		0.05		2.0	0.10	0.94					
17	SERUM BLK 2		0.03		2.0	0.10	0.67					
18	SPK 40-1		0.04		2.0	0.10	0.86	0.004	40.00	0.10	0.09	90%
19	SPK 40-2		0.06		2.0	0.10	1.14	0.004	40.00	0.10	0.11	119%
20	SPK 40-3		0.06		2.0	0.10	1.15	0.004	40.00	0.10	0.12	120%
21	SPK 40-4		0.06		2.0	0.10	1.22	0.004	40.00	0.10	0.12	127%
22	SPK 124-1		0.08		2.0	0.10	1.66	0.004	124.0	0.30	0.17	56%
23	SPK 124-2		0.13		2.0	0.10	2.57	0.004	124.0	0.30	0.26	86%
24	SPK 124-3		0.28		2.0	0.10	5.56	0.004	124.0	0.30	0.56	187%
25	SPK 124-4		0.12		2.0	0.10	2.34	0.004	124.0	0.30	0.23	79%
26	Drift	1.50	1.53	102%								
27	Wash		ND									
28	SPK 100-1		0.11		2.0	0.10	2.12	0.004	100.0	0.24	0.21	88%
29	SPK 100-2		0.13		2.0	0.10	2.63	0.004	100.0	0.24	0.26	110%
30	SPK 100-3		0.13		2.0	0.10	2.56	0.004	100.0	0.24	0.26	106%

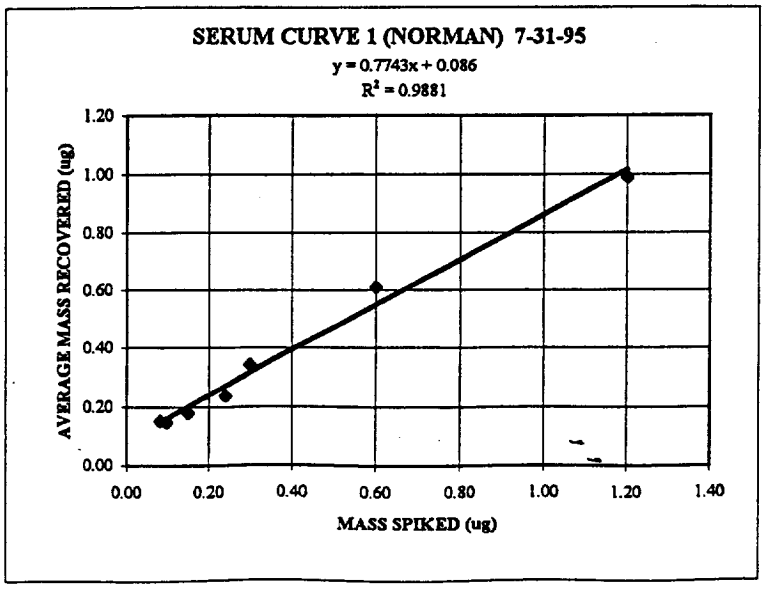
LDW 12/6/95
AMDT 20795.1
HWI 6329-135
Skalar Data

Sample #	Sample ID	Skalar Standard (ppm)	Skalar Result (ppm)	% Recovery	DI TISAB final vol (mL)	Qty Sampl (mL)	Actual ppm F- in Sample	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Mass Recovered (ug F-)	% Recovery
31	BLK		0.08		2.0	0.10	1.51					
32	BLK		0.04		2.0	0.10	0.78					
33	F52978-22		0.03		2.0	0.10	0.67					
34	F52980-22		0.04		2.0	0.10	0.85					
35	F52991-22		0.03		2.0	0.10	0.58					
36	F52987-22		0.02		2.0	0.10	0.50					
37	F52988-22		0.02		2.0	0.10	0.34					
38	Drift	1.50	1.53	102%								
39	Wash		ND									
40	F52995-22		0.03		2.0	0.10	0.54					
41	BLANK TISAB		ND									
42	SPK 100-1		0.11		2.0	0.10	2.12	0.004	100.0	0.24	0.21	88%
43	Drift	1.50	1.55	103%								
44	Wash		ND									

VOW '12014.J
AMDT 20795.J
HWI 6329-135
Skalar Data

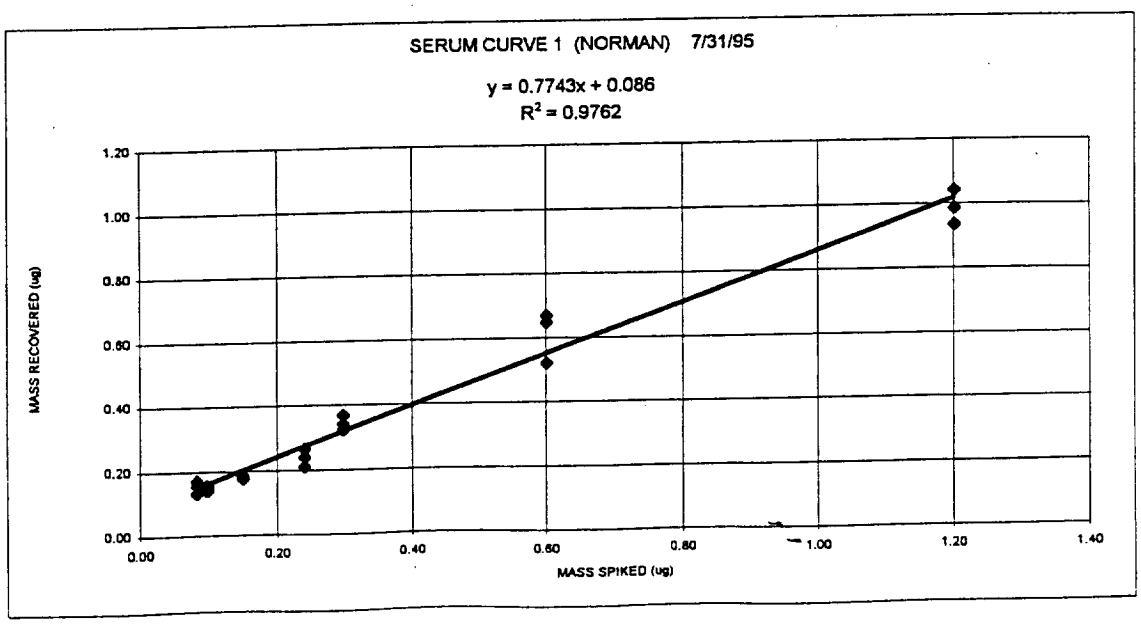
SERUM CURVE 1
7-31-95
NORMAN

Sample ID	Skalar Result (ppm)	DI:TISAB final vol (mL)	mL FC 95 Solution Spiked	Conc FC 95 Soln (ppm)	Mass Spiked (ug F-)	Average Mass Recovered (ug F-)	% Recovery
Spk 34-1	0.09	2.0	0.004	34.00			
Spk 34-2	0.07	2.0	0.004	34.00	0.08	0.15	188%
Spk 34-3	0.08	2.0	0.004	34.00			
Spk 40-1	0.08	2.0	0.004	40.00			
Spk 40-2	0.07	2.0	0.004	40.00	0.10	0.15	155%
Spk 40-3	0.07	2.0	0.004	40.00			
Spk 62-1	0.09	2.0	0.004	62.00			
Spk 62-2	0.09	2.0	0.004	62.00	0.15	0.18	121%
Spk 62-3	0.09	2.0	0.004	62.00			
Spk 100-1	0.11	2.0	0.004	100.0			
Spk 100-2	0.12	2.0	0.004	100.0	0.24	0.24	99%
Spk 100-3	0.13	2.0	0.004	100.0			
Spk 124-1	0.16	2.0	0.004	124.0			
Spk 124-2	0.17	2.0	0.004	124.0	0.30	0.34	115%
Spk 124-3	0.18	2.0	0.004	124.0			
Spk 250-1	0.33	2.0	0.004	250.0			
Spk 250-2	0.26	2.0	0.004	250.0	0.60	0.61	102%
Spk 250-3	0.32	2.0	0.004	250.0			
Spk 500-1	0.47	2.0	0.004	500.0			
Spk 500-2	0.49	2.0	0.004	500.0	1.20	0.99	82%
Spk 500-3	0.52	2.0	0.004	500.0			



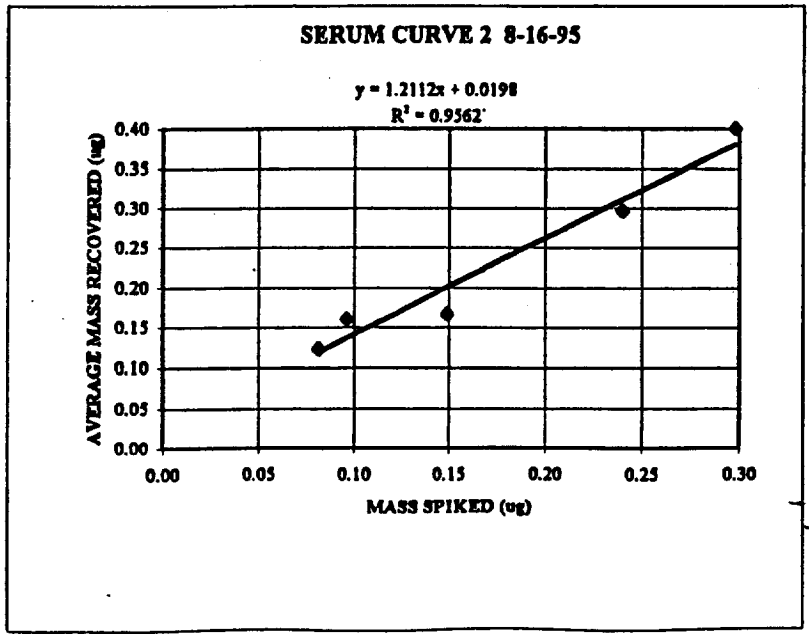
SERUM CURVE 1
7-31-95
NORMAN

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



SERUM CURVE 2
8-16-95
NORMAN

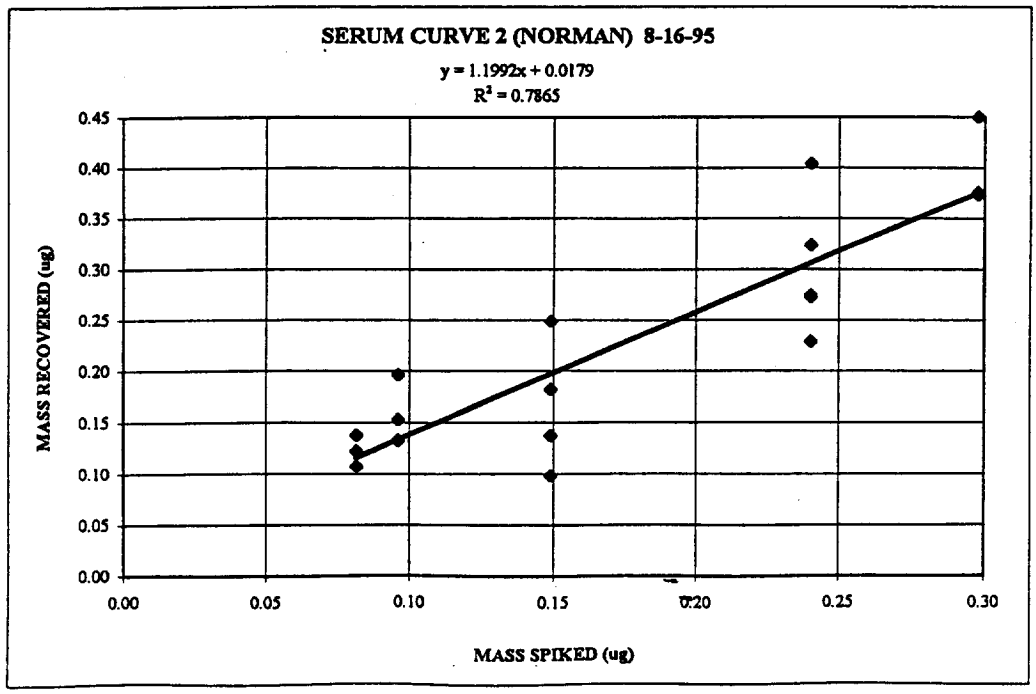
SPK 34-1	0.05	2.0	0.004	34.00			
SPK 34-2	0.07	2.0	0.004	34.00	0.08	0.12	150%
SPK 34-3	0.06	2.0	0.004	34.00			
SPK 40-1	0.10	2.0	0.004	40.00			
SPK 40-2	0.07	2.0	0.004	40.00	0.10	0.16	167%
SPK 40-3	0.08	2.0	0.004	40.00			
SPK 62-1	0.05	2.0	0.004	62.00			
SPK 62-2	0.07	2.0	0.004	62.00	0.15	0.17	112%
SPK 62-3	0.09	2.0	0.004	62.00			
SPK 62-4	0.12	2.0	0.004	62.00			
SPK 100-1	0.14	2.0	0.004	100.0			
SPK 100-2	0.20	2.0	0.004	100.0			
SPK 100-3	0.11	2.0	0.004	100.0	0.24	0.30	123%
SPK 100-4	0.16	2.0	0.004	100.0			
SPK 100-5	0.14	2.0	0.004	100.0			
SPK 100-6	0.14	2.0	0.004	100.0			
SPK 124-1	0.19	2.0	0.004	124.0			
SPK 124-2	0.23	2.0	0.004	124.0	0.30	0.40	134%
SPK 124-3	0.19	2.0	0.004	124.0			



Copy of Original
DAW
9/20/95

SERUM CURVE 2
8-16-95
NORMAN

Sample ID	Skelar Result (ppm)	DILUTED final vol (mL)	mL PC 95 Solution Spiked	Conc. PC 95 Soln (ppm)	Mass Spiked (ug PC)	Mass Recovered (ug PC)	% Recovery		
SPK 34-1	0.05	2.0	0.004	34.00	0.08	0.11	132%	STANDARD DEVIATION :	0.1837
SPK 34-2	0.07	2.0	0.004	34.00	0.08	0.14	169%	% RSD :	12.2150
SPK 34-3	0.06	2.0	0.004	34.00	0.08	0.12	150%		
SPK 40-1	0.10	2.0	0.004	40.00	0.10	0.20	204%	STANDARD DEVIATION :	0.3354
SPK 40-2	0.07	2.0	0.004	40.00	0.10	0.13	139%	% RSD :	20.0349
SPK 40-3	0.08	2.0	0.004	40.00	0.10	0.15	159%		
SPK 62-1	0.05	2.0	0.004	62.00	0.15	0.10	66%	STANDARD DEVIATION :	0.4333
SPK 62-2	0.07	2.0	0.004	62.00	0.15	0.14	92%	% RSD :	38.7472
SPK 62-3	0.09	2.0	0.004	62.00	0.15	0.18	122%		
SPK 62-4	0.12	2.0	0.004	62.00	0.15	0.25	167%		
SPK 100-1	0.14	2.0	0.004	100.0	0.24	0.27	114%	STANDARD DEVIATION :	0.2535
SPK 100-2	0.20	2.0	0.004	100.0	0.24	0.40	168%	% RSD :	20.5563
SPK 100-3	0.11	2.0	0.004	100.0	0.24	0.23	95%		
SPK 100-4	0.16	2.0	0.004	100.0	0.24	0.32	135%		
SPK 100-5	0.14	2.0	0.004	100.0	0.24	0.27	114%		
SPK 100-6	0.14	2.0	0.004	100.0	0.24	0.27	114%		
SPK 124-1	0.19	2.0	0.004	124.0	0.30	0.38	126%	STANDARD DEVIATION :	0.1454
SPK 124-2	0.23	2.0	0.004	124.0	0.30	0.45	151%	% RSD :	10.8282
SPK 124-3	0.19	2.0	0.004	124.0	0.30	0.37	126%		



QAU 8/25/95
AMDT 20795.1
HWI 6329-135
Serum Curve 1-Non

1995-08-16 14:10 OutPut of : 950816A1

Software : version 6.1 c1990,93

Operator : DDW

Date of the Analysis : 1995-08-16 08:58

Analysis File Name : C:\SKALAR\DATA\HWIDATA\SERUM\950816A1

Fluoride 1.5
Calibration order = Inverse Logarithm

Slope : s = #.#####

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

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

a2 = -0.00000
a1 = 0.00065
a0 = -1.15706

Fluoride L
Calibration order = 2

Correlation : r = 0.99717

$\text{Result} = a2 * x^2 + a1 * x + a0$

a2 = 0.00000
a1 = 0.00018
a0 = 0.00912

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

1995-08-16 14:10 OutPut of : 950816A1

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

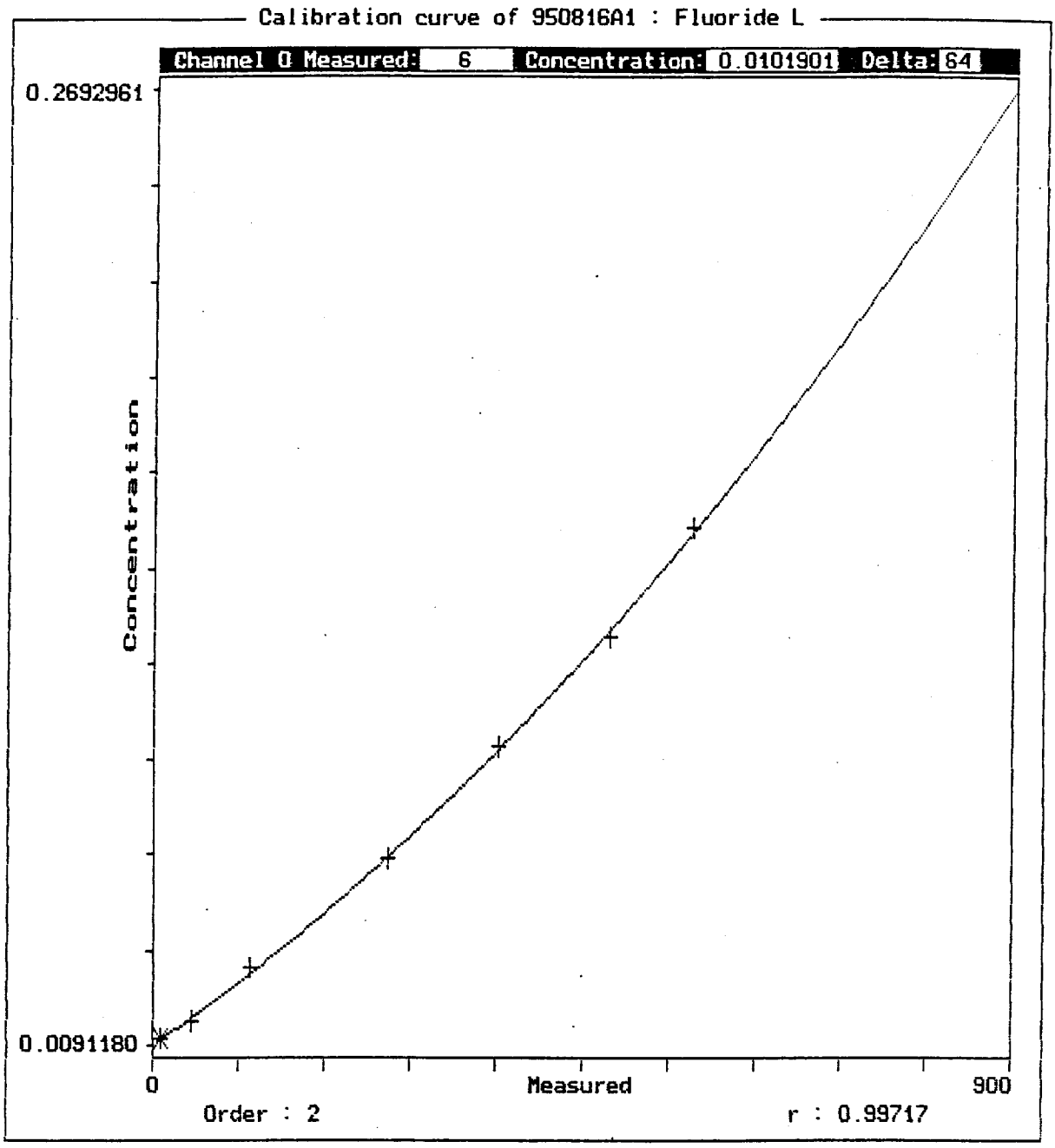
```
Path number    :  0
Signal type    : Debubbled
Decolor        : No
system Number  : 0
diLute         : No
Resample       : No
dil Threshold  : 4095
diG output     : 0
Window event   : Off
```

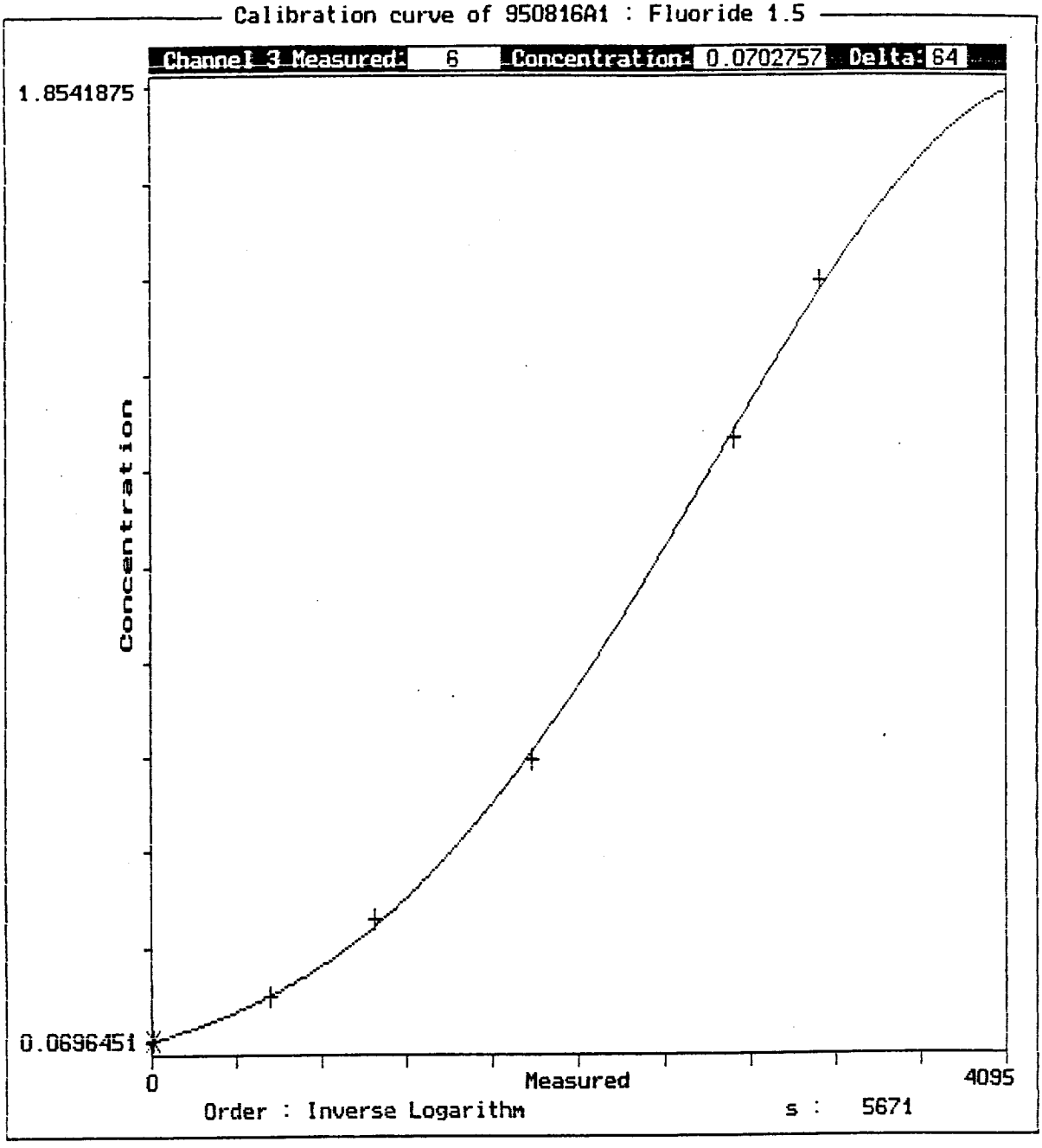
1995-08-16 14:10 OutPut of : 950816A1

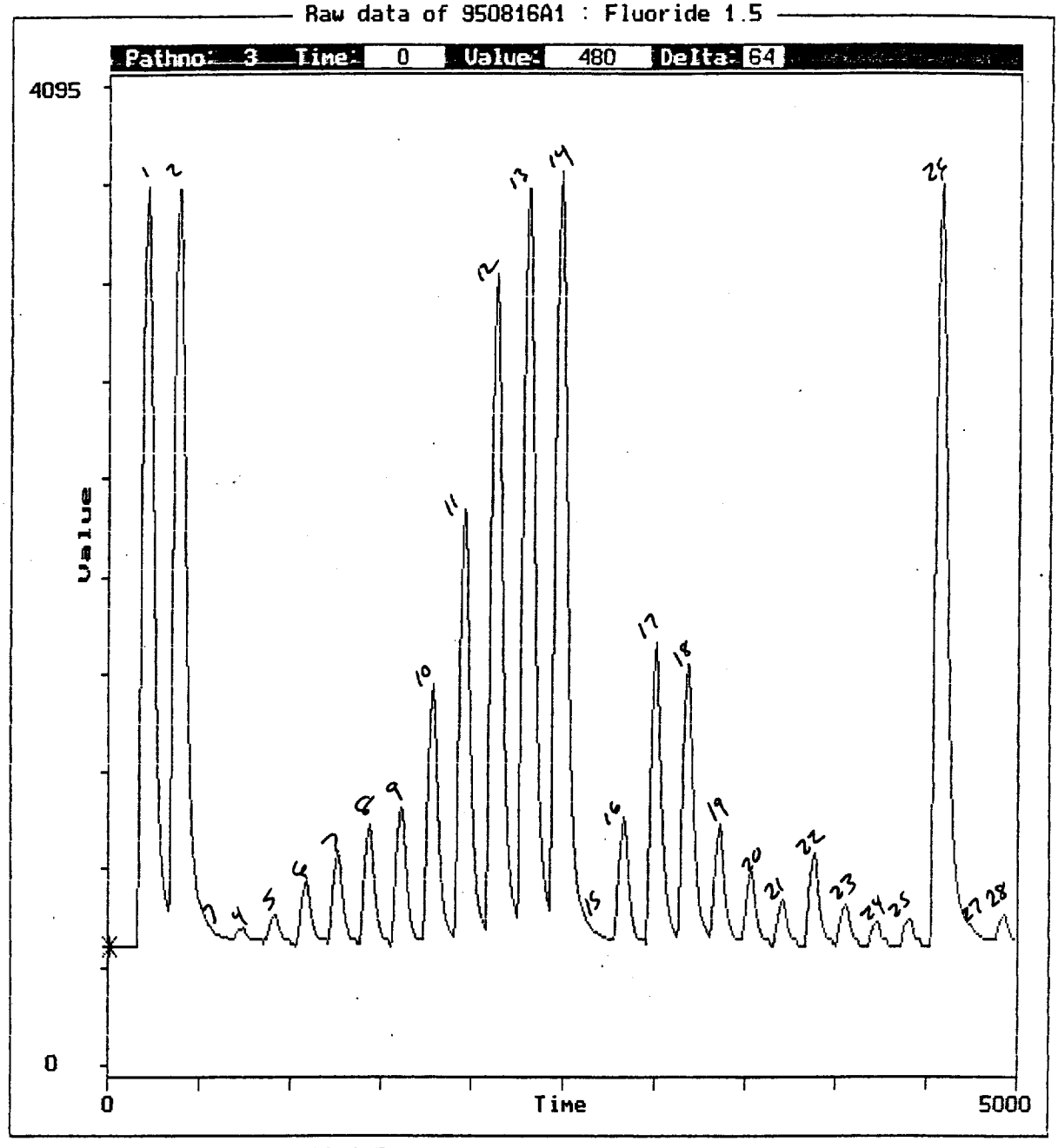
```
s1   sTandard :    0.015
s2   sTandard :    0.030
s3   sTandard :    0.060
s4   sTandard :    0.090
s5   sTandard :    0.120
s6   sTandard :    0.150
s7   sTandard : Ignore
s8   sTandard : Ignore
s9   sTandard : Ignore
s10  sTandard : Ignore
Order : 2
Dimension : PPM
start Value     : 500  DU
trigger 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     : #.####
```

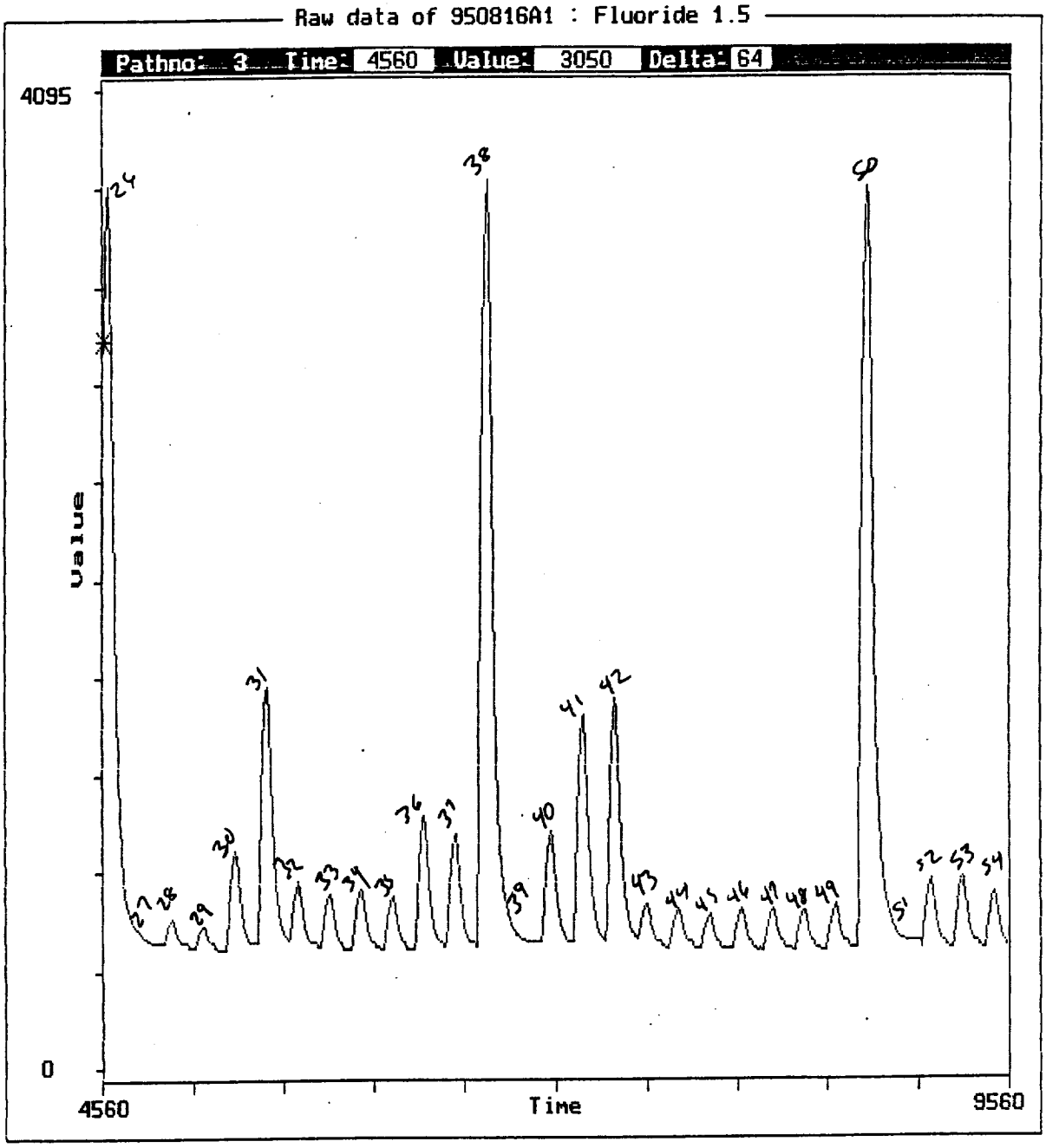
			Fluoride 1.5			Fluoride L		
			PPM			*PPM*		
Pos	Typ	Ident	Ch	Result F	Time	Ch	Result F	Time
wt	iw	Initial Wash	3	0.070	65	4	0.0091	0
1	t	Tracer	3	1.472	207	4	1.8335	0
2	d	Drift	3	1.486	383	4	1.8558	0
3	w	Wash	3	0.070	619	4	0.0091	0
4	s1	Standard 1	3	0.074	729	4	0.0164	0
5	s2	Standard 2	3	0.081	907	4	0.0281	0
6	s3	Standard 3	3	0.100	1081	4	0.0601	0
7	s4	Standard 4	3	0.117	1258	4	0.0894	0
8	s5	Standard 5	3	0.137	1434	4	0.1221	0
9	s6	Standard 6	3	0.154	1608	4	0.1488	0
10	s7	Standard 7	3	0.285	1784	4	0.3400	0
11	s8	Standard 8	3	0.614	1958	4	0.7483	0
12	s9	Standard 9	3	1.217	2133	4	1.4687	0
13	s10	Standard 10	3	1.479	2308	4	1.8442	0
14	d	Drift	3	1.490	2484	4	1.8617	0
15	w	Wash	3	0.070	2726	4	0.0091	0
16	u	SPK 62-1	3	0.142	2830	4	0.1296	0
17	u	SPK 250-1	3	0.346	3011	4	0.4211	0
18	u	SPK 250-2	3	0.322	3187	4	0.3892	0
19	u	BLANK	3	0.136	3361	4	0.1206	0
20	u	BLANK	3	0.105	3536	4	0.0686	0
21	u	BLANK	3	0.088	3710	4	0.0410	0
22	u	F52986-1	3	0.117	3886	4	0.0889	0
23	u	F52990-1	3	0.086	4062	4	0.0377	0
24	u	F52997-1	3	0.078	4236	4	0.0234	0
25	u	F52982-1	3	0.079	4410	4	0.0247	0
26	d	Drift	3	1.477	4586	4	1.8413	0
27	w	Wash	3	0.070	4825	4	0.0091	0
28	u	F52994-1	3	0.081	4934	4	0.0281	0
29	u	F53410-1	3	0.078	5110	4	0.0228	0
30	u	SPK 62-1	3	0.122	5288	4	0.0978	0
31	u	SPK 250-1	3	0.294	5462	4	0.3525	0
32	u	BLANK SERUM	3	0.101	5635	4	0.0625	0
33	u	BLANK SERUM	3	0.095	5810	4	0.0519	0
34	u	BLANK SERUM	3	0.098	5986	4	0.0566	0
35	u	BLANK SERUM	3	0.093	6161	4	0.0485	0
36	u	SPK 62-1	3	0.148	6335	4	0.1400	0
37	u	SPK 62-2	3	0.132	6510	4	0.1145	0
38	d	Drift	3	1.485	6684	4	1.8539	0
39	w	Wash	3	0.070	6911	4	0.0091	0
40	u	SPK 62-3	3	0.136	7034	4	0.1194	0
41	u	SPK 250-1	3	0.254	7210	4	0.2971	0
42	u	SPK 250-2	3	0.271	7382	4	0.3208	0
43	u	F52984-1	3	0.088	7557	4	0.0407	0
44	u	F52992-1	3	0.085	7734	4	0.0356	0
45	u	F52996-1	3	0.082	7910	4	0.0310	0
46	u	F52997-1	3	0.086	8086	4	0.0364	0
47	u	F52984-1	3	0.085	8258	4	0.0356	0
48	u	F52993-1	3	0.085	8434	4	0.0348	0
49	u	F52978-1	3	0.088	8608	4	0.0407	0
50	d	Drift	3	1.488	8785	4	1.8587	0
51	w	Wash	3	0.070	9004	4	0.0091	0
52	u	F52980-1	3	0.101	9131	4	0.0625	0
53	u	F52991-1	3	0.103	9311	4	0.0654	0

			Fluoride 1.5			Fluoride L		
			PPM			*PPM*		
Pos	Typ	Ident	Ch	Result	F Time	Ch	Result	F Time
54	u	F52987-1	3	0.095	9486	4	0.0517	0
55	u	SPK 62-1	3	0.127	9662	4	0.1051	0
56	u	SPK 250-1	3	0.257	9838	4	0.3017	0
57	d	Drift	3	1.503	10012	4	1.8831	0
58	w	Wash	3	0.070	10247	4	0.0091	0
wt	rw	RunOut Wash	3	0.070	10487	4	0.0091	0

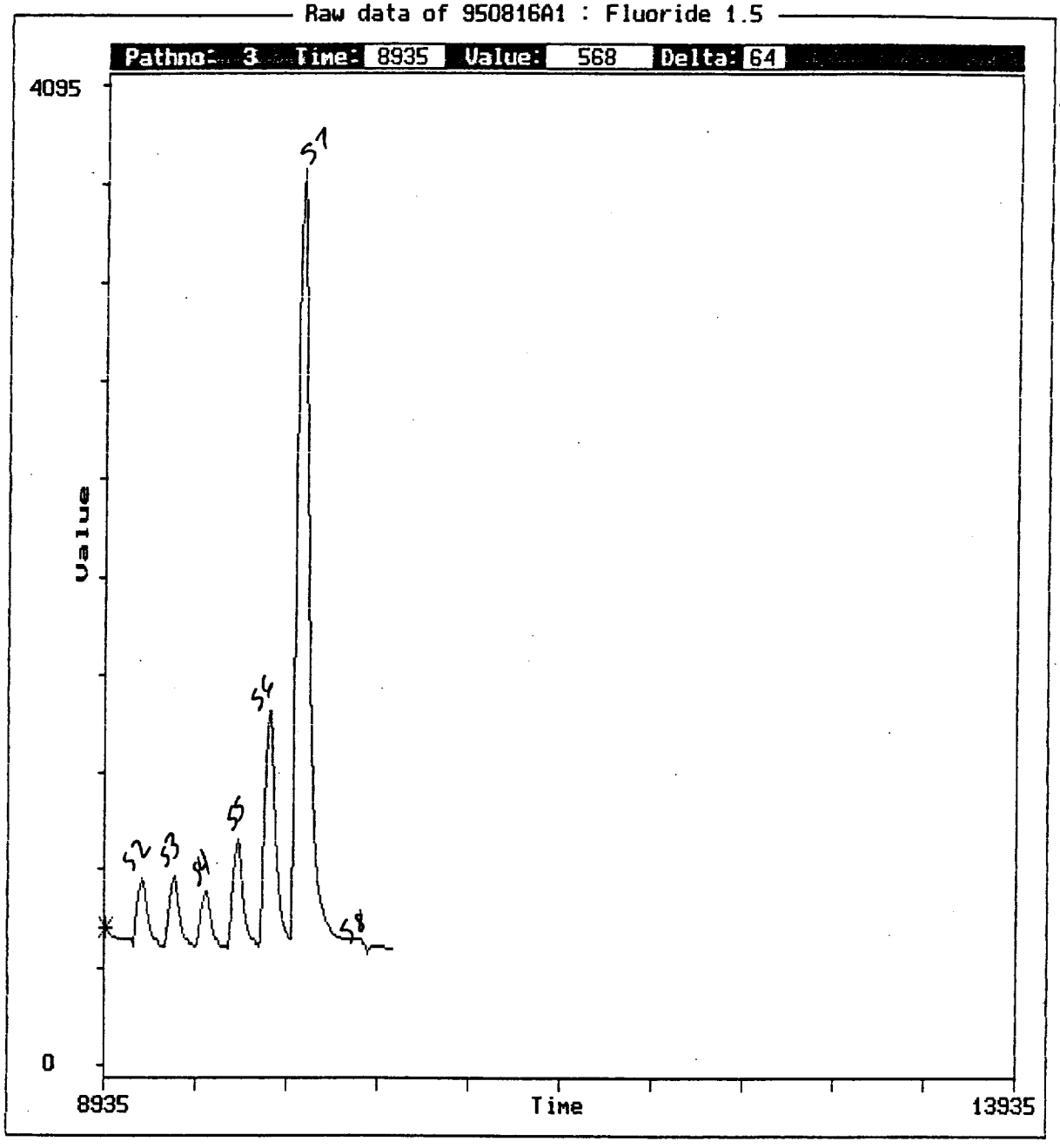

Calibration curve of 950816A1 : Fluoride L
Channel 0 Measured: 6
Concentration: 0.0101901
Delta: 64
0.2692961
0.0091180
Concentration
0
Measured
900
Order : 2
r : 0.99717


Calibration curve of 950816A1 : Fluoride 1.5
Channel 3 Measured: 6
Concentration: 0.0702757
Delta: 64
1.8541875
0.0696451
Concentration
0
Measured
4095
Order : Inverse Logarithm
s : 5671


Raw data of 950816A1 : Fluoride 1.5
Pathno: 3 Time: 0 Value: 480 Delta: 64
4095
0
Value
0
Time
5000
Esc=Exit ¦ F1=Help ¦ Crtl-P=Edit peaks ¦



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



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

DDW 8/25/95
AMDT 20795
HW= 6329-1
Serum Curve 2-

1995-03-21 12:30 OutPut of : 950821A1

Software : version 6.1 c1990,93

Operator : DDW

Date of the Analysis : 1995-08-21 07:55

Analysis File Name : C:\SKALAR\DATA\HWIDATA\SERUM\950821A1

Fluoride 1.5
Calibration order = Inverse Logarithm

Slope : s = #.#####

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

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

a2 = -0.00000
a1 = 0.00071
a0 = -1.20061

Fluoride L
Calibration order = 2

Correlation : r = 0.99953

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

a2 = -0.00000
a1 = 0.00025
a0 = 0.00771

Sampler
Type : SA1000
Number : 1
Sample Time : 50 sec.
Wash Time : 120 sec.
Air Time : 1 sec.
Take up : Single
sPecial : None
needle Height : 70 mm.

Diluter
needle Height : 80 mm
dilution Factor : 10
dilution Volume : 2.5 ml.
Resample : 1
Dilution runs : 1

User file : . TXT
Reproces : No

1995-08-21 12:30 OutPut of : 950821A1

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

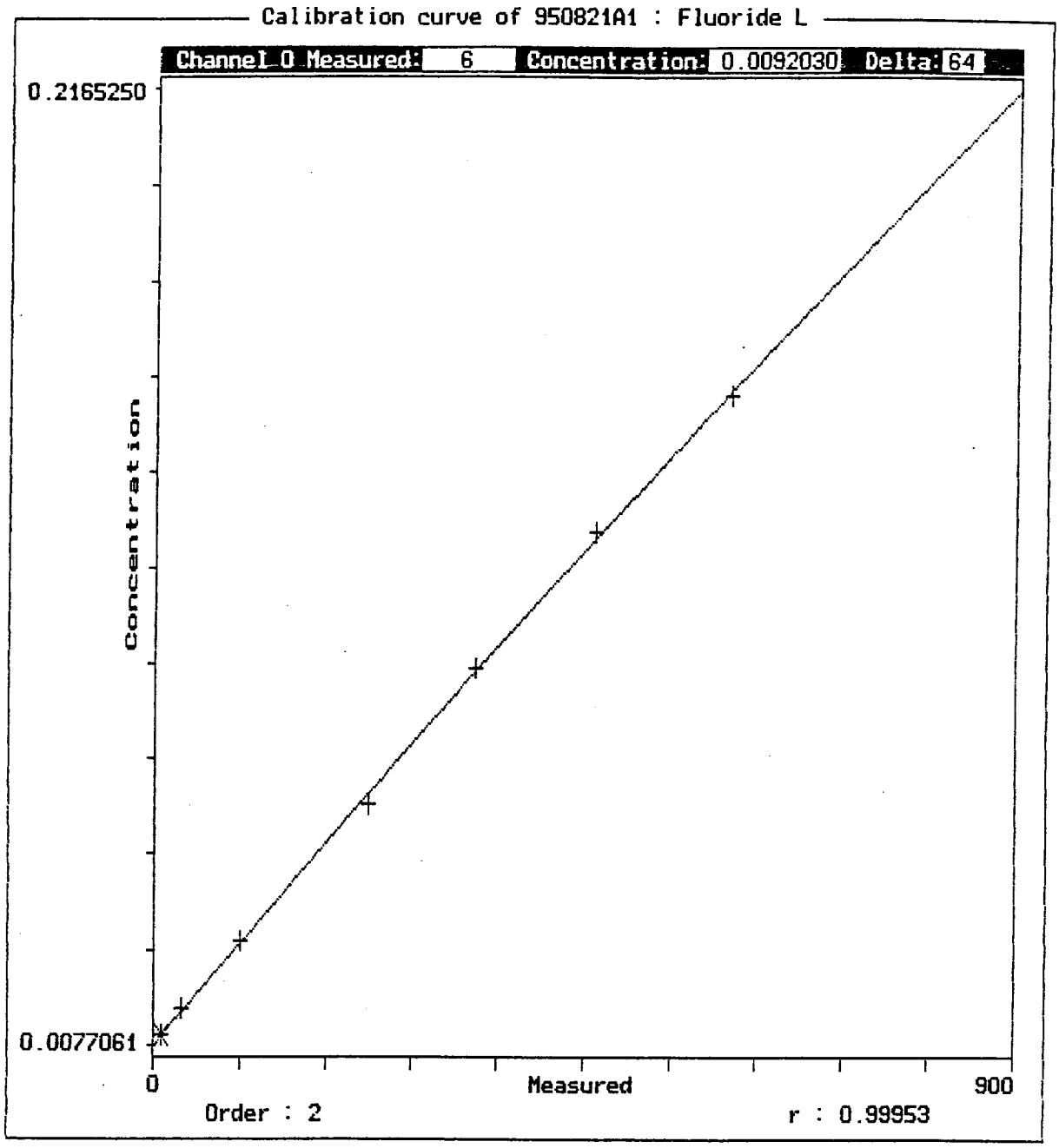
Path number : 0
Signal type : Debubbled
Decolor : No
system Number : 0
diLute : No
Resample : No
dil Threshold : 4095
diG output : 0
Window event : Off

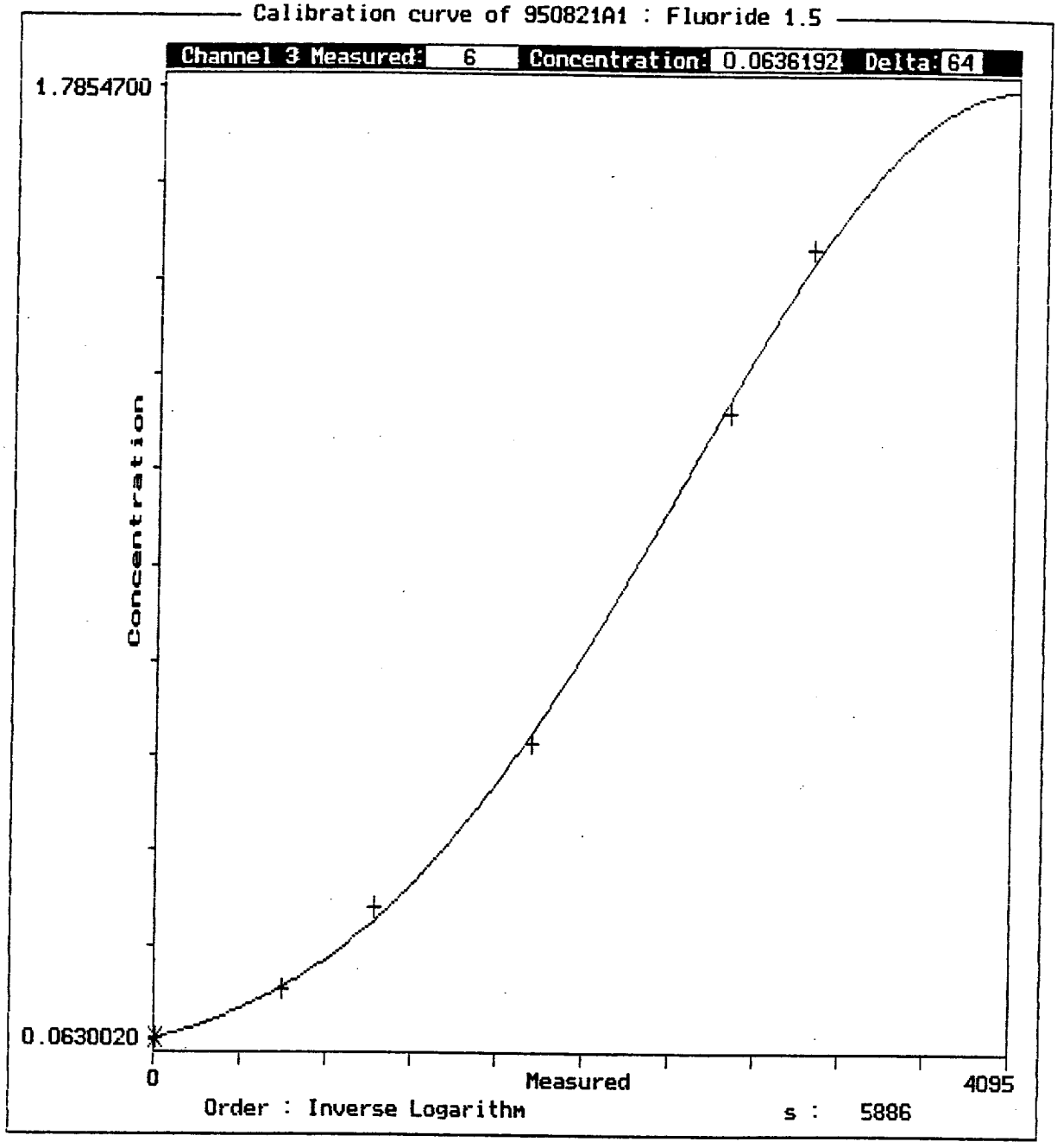
1995-08-21 12:30 OutPut of : 950821A1

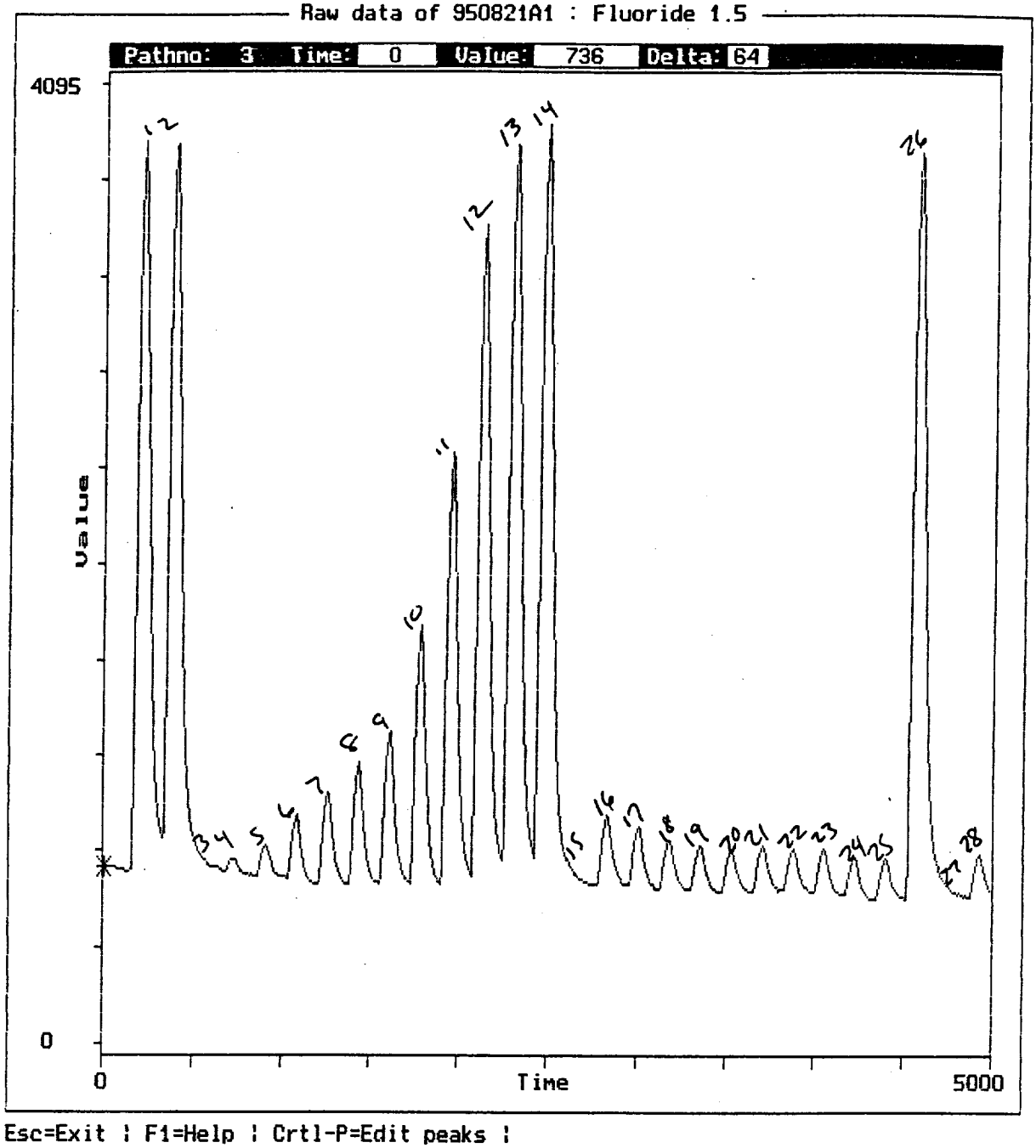
```
s1   sTandard :    0.015
s2   sTandard :    0.030
s3   sTandard :    0.060
s4   sTandard :    0.090
s5   sTandard :    0.120
s6   sTandard :    0.150
s7   sTandard : Ignore
s8   sTandard : Ignore
s9   sTandard : Ignore
s10  sTandard : Ignore
Order : 2
Dimension : PPM
start Value     : 500  DU
trigger 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	0.063	65	4	0.0077	0
1	t	Tracer	3	1.468	208	4	0.5962	0
2	d	Drift	3	1.482	382	4	0.5992	0
3	w	Wash	3	0.063	570	4	0.0077	0
4	s1	Standard 1	3	0.066	728	4	0.0144	0
5	s2	Standard 2	3	0.073	903	4	0.0298	0
6	s3	Standard 3	3	0.090	1083	4	0.0624	0
7	s4	Standard 4	3	0.106	1259	4	0.0891	0
8	s5	Standard 5	3	0.128	1435	4	0.1184	0
9	s6	Standard 6	3	0.156	1609	4	0.1509	0
10	s7	Standard 7	3	0.279	1783	4	0.2480	0
11	s8	Standard 8	3	0.619	1958	4	0.3935	0
12	s9	Standard 9	3	1.224	2133	4	0.5438	0
13	s10	Standard 10	3	1.471	2309	4	0.5968	0
14	d	Drift	3	1.539	2483	4	0.6121	0
15	w	Wash	3	0.063	2723	4	0.0077	0
16	u	SERUM BLK 1	3	0.098	2832	4	0.0765	0
17	u	SERUM BLK 2	3	0.092	3012	4	0.0672	0
18	u	SERUM BLK 3	3	0.086	3184	4	0.0559	0
19	u	F52988-1	3	0.083	3360	4	0.0510	0
20	u	F52995-1	3	0.081	3534	4	0.0462	0
21	u	F52972-1	3	0.084	3710	4	0.0518	0
22	u	F52973-1	3	0.082	3885	4	0.0488	0
23	u	F52979-1	3	0.083	4060	4	0.0508	0
24	u	F52975-1	3	0.080	4234	4	0.0440	0
25	u	F52976-1	3	0.079	4410	4	0.0420	0
26	d	Drift	3	1.529	4583	4	0.6099	0
27	w	Wash	3	0.063	4810	4	0.0077	0
28	u	F52983-1	3	0.082	4933	4	0.0481	0
29	u	SPK 40-1	3	0.092	5111	4	0.0672	0
30	u	SPK 100-1	3	0.147	5285	4	0.1414	0
31	u	BLK-1	3	0.075	5460	4	0.0347	0
32	u	BLK-2	3	0.074	5632	4	0.0322	0
33	u	BLK-3	3	0.070	5810	4	0.0246	0
34	u	SPK 40-1	3	0.091	5988	4	0.0643	0
35	u	SPK 40-2	3	0.089	6162	4	0.0617	0
36	u	SPK 100-1	3	0.133	6336	4	0.1251	0
37	u	SPK 100-2	3	0.131	6512	4	0.1221	0
38	d	Drift	3	1.540	6684	4	0.6123	0
39	w	Wash	3	0.063	6902	4	0.0077	0
40	u	SPK 100-3	3	0.142	7037	4	0.1352	0
41	u	BLK	3	0.075	7207	4	0.0347	0
42	u	F52972-8	3	0.071	7384	4	0.0256	0
43	u	F52973-8	3	0.064	7558	4	0.0090	0
44	u	F52979-8	3	0.077	7736	4	0.0388	0
45	u	F52975-8	3	0.075	7909	4	0.0347	0
46	u	F52976-8	3	0.071	8085	4	0.0261	0
47	u	F52983-8	3	0.070	8257	4	0.0231	0
48	u	F52986-8	3	0.071	8437	4	0.0271	0
49	u	F52990-8	3	0.070	8609	4	0.0231	0
50	d	Drift	3	1.525	8785	4	0.6090	0
51	w	Wash	3	0.063	9012	4	0.0077	0
52	u	F52997-8	3	0.073	9136	4	0.0303	0
53	u	F52982-8	3	0.070	9310	4	0.0248	0

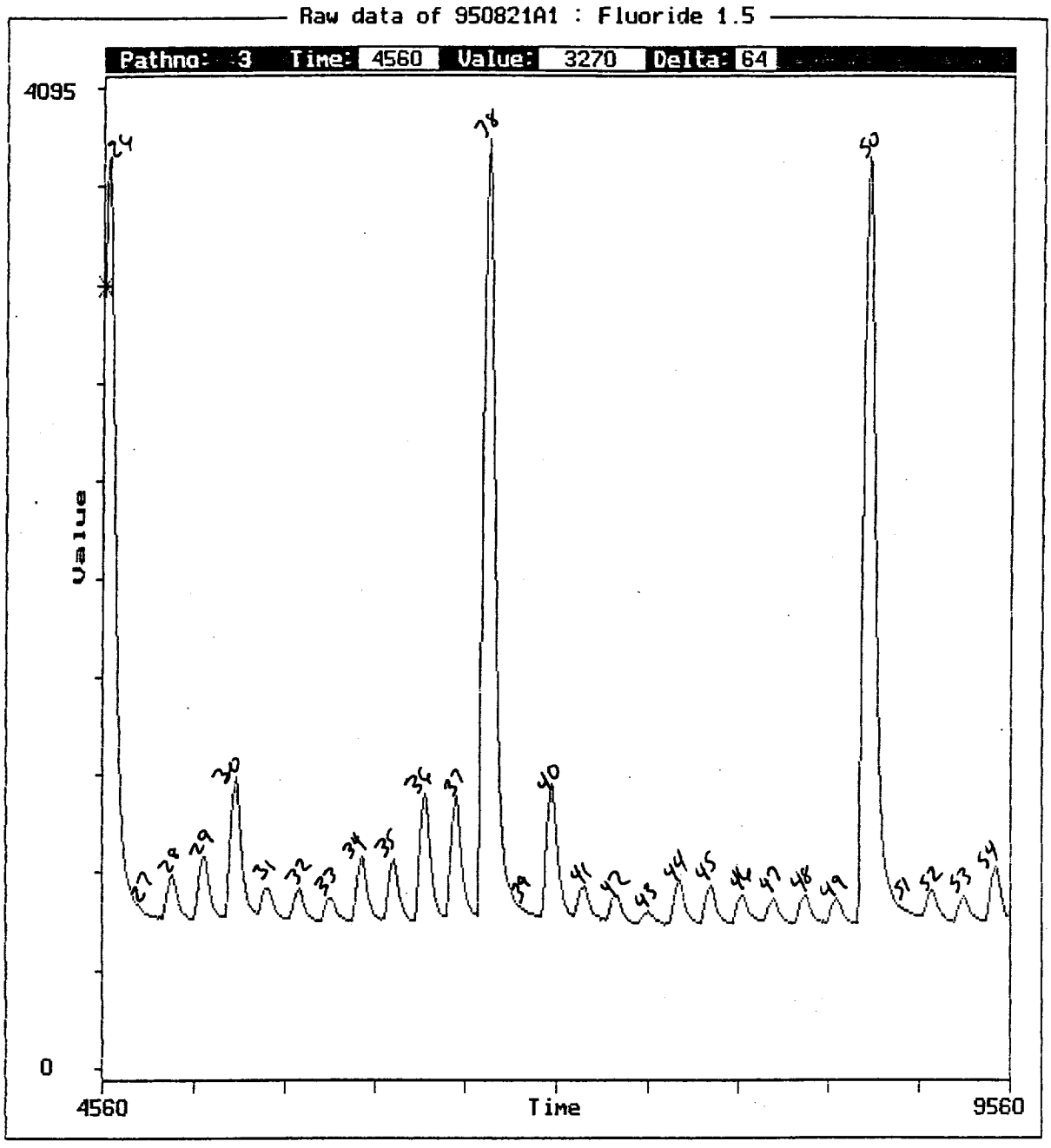
			Fluoride 1.5			Fluoride L		
			PPM			*PPM*		
Pos	Typ	Ident	Ch	Result	F Time	Ch	Result	F Time
54	u	SPK 62-1	3	0.085	9488	4	0.0549	0
55	u	SPK 62-2	3	0.124	9664	4	0.1135	0
56	u	SPK 124-1	3	0.144	9838	4	0.1373	0
57	u	SPK 124-2	3	0.171	10014	4	0.1659	0
58	u	BLK	3	0.116	10189	4	0.1037	0
59	u	F52994-8	3	0.109	10365	4	0.0927	0
60	u	F53410-8	3	0.085	10539	4	0.0532	0
61	u	F52984-8	3	0.084	10715	4	0.0527	0
62	d	Drift	3	1.552	10888	4	0.6153	0
63	w	Wash	3	0.063	11124	4	0.0077	0
64	u	F52992-8	3	0.082	11239	4	0.0479	0
65	u	F52996-8	3	0.091	11415	4	0.0646	0
66	u	F52997-8	3	0.102	11589	4	0.0823	0
67	u	F52989-8	3	0.090	11766	4	0.0629	0
68	u	F52993-8	3	0.082	11938	4	0.0488	0
69	u	F52978-8	3	0.078	12108	4	0.0410	0
70	u	F52980-8	3	0.080	12290	4	0.0452	0
71	u	SPK 40-1	3	0.098	12466	4	0.0756	0
72	u	SPK 124-1	3	0.166	12642	4	0.1606	0
73	u	BLK	3	0.106	12816	4	0.0894	0
74	d	Drift	3	1.536	12990	4	0.6115	0
75	w	Wash	3	0.063	13232	4	0.0077	0
76	u	F52991-8	3	0.089	13341	4	0.0612	0
77	u	F52987-8	3	0.085	13517	4	0.0544	0
78	u	F52988-8	3	0.079	13683	4	0.0420	0
79	u	F52995-8	3	0.079	13867	4	0.0432	0
80	u	F52972-15	3	0.080	14042	4	0.0442	0
81	u	F52973-15	3	0.093	14218	4	0.0679	0
82	u	F52979-15	3	0.083	14392	4	0.0501	0
83	u	F52975-15	3	0.085	14566	4	0.0547	0
84	u	F52976-15	3	0.078	14740	4	0.0410	0
85	u	F52983-15	3	0.080	14916	4	0.0454	0
86	d	Drift	3	1.574	15092	4	0.6204	0
87	w	Wash	3	0.063	15334	4	0.0077	0
88	u	SPK 40-1	3	0.098	15441	4	0.0761	0
89	u	SPK 124-1	3	0.165	15619	4	0.1601	0
90	d	Drift	3	1.534	15792	4	0.6110	0
91	w	Wash	3	0.063	16032	4	0.0077	0
wt	rw	RunOut Wash	3	0.063	16267	4	0.0077	0


Calibration curve of 950821A1 : Fluoride L
Channel 0 Measured: 6
Concentration: 0.0092030
Delta: 64
0.2165250
0.0077061
Concentration
0
Measured
900
Order : 2
r : 0.99953

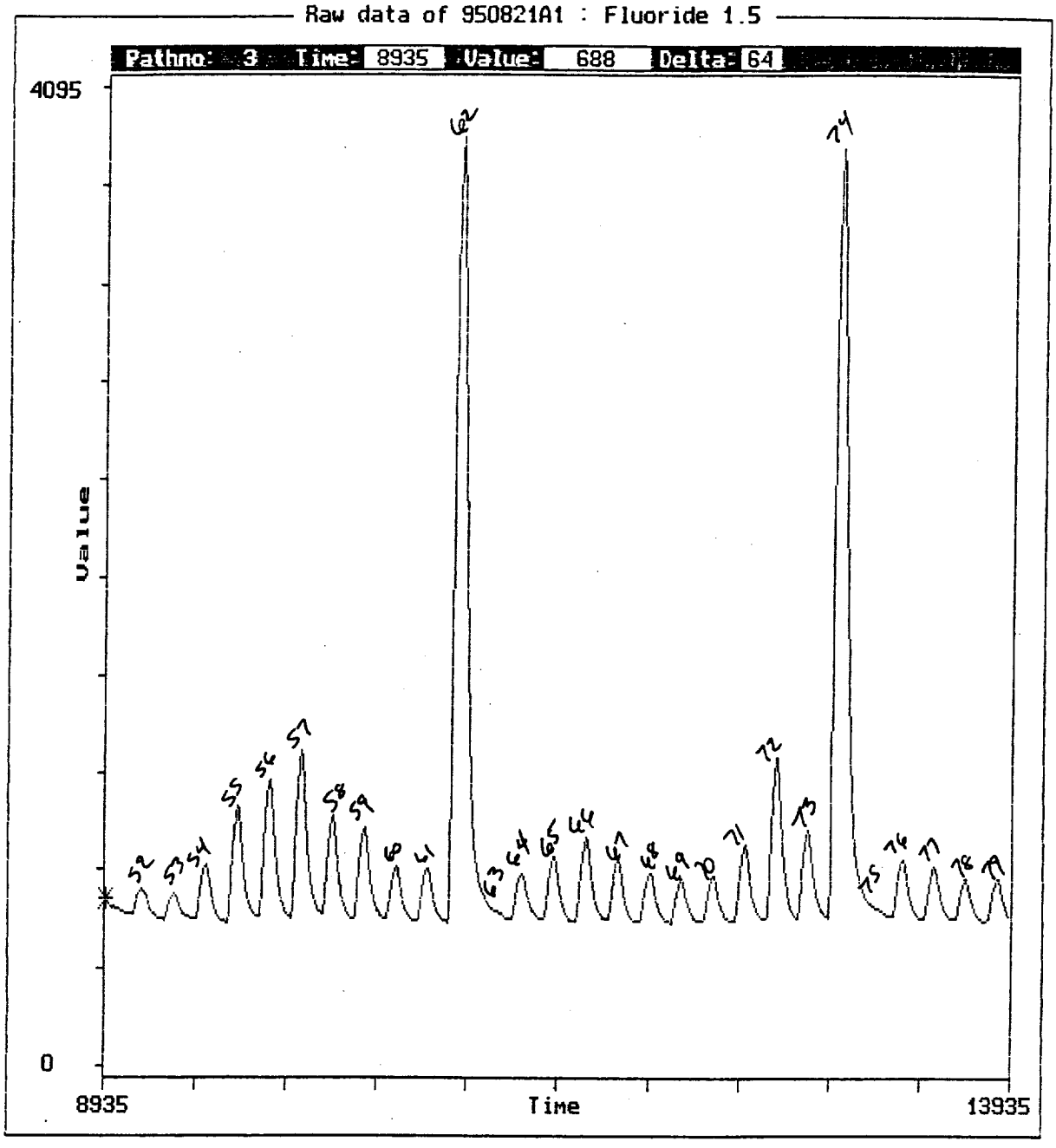

Calibration curve of 950821A1 : Fluoride 1.5
Channel 3 Measured: 6
Concentration: 0.0636192
Delta: 64
1.7854700
0.0630020
Concentration
0
Measured
4095
Order : Inverse Logarithm
s : 5886

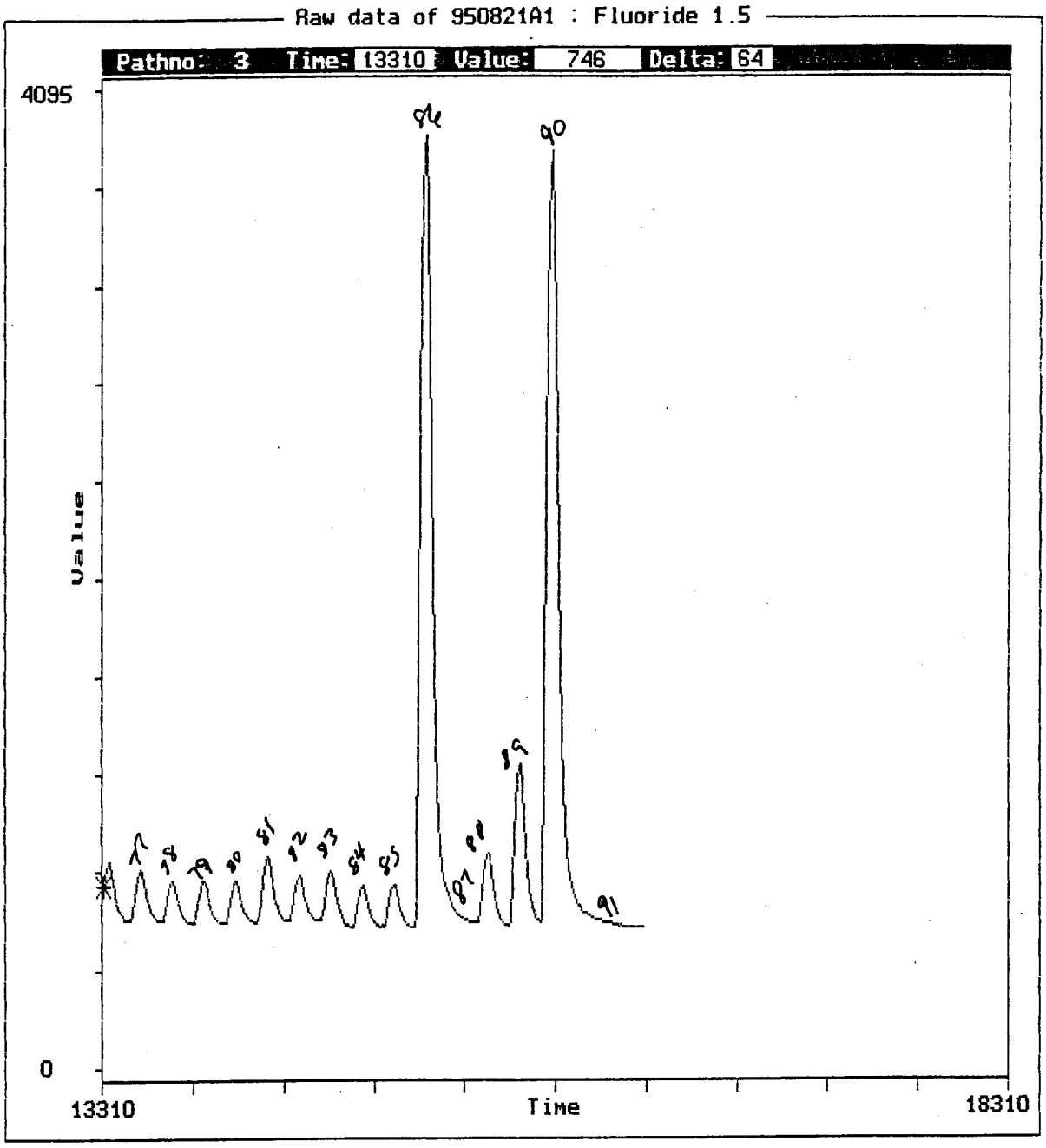


Esc=Exit ¦ F1=Help ¦ Crtl-P=Edit peaks ¦



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


Raw data of 950821A1 : Fluoride 1.5
Pathno: 3 Time: 8935 Value: 688 Delta: 64
4095
0
Value
8935
Time
13935
52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79
Esc=Exit ¦ F1=Help ¦ Crtl-P=Edit peaks ¦



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

DDW 8/25/95
AMDT 20795.1
HWI 6329-135
Serum Curve 2-Nb

1995-08-21 16:58 OutPut of : 950821B1

Software : version 6.1 c1990,93

Operator : DDW

Date of the Analysis : 1995-08-21 12:29

Analysis File Name : C:\SKALAR\DATA\HWIDATA\SERUM\950821B1

Fluoride 1.5
Calibration order = Inverse Logarithm

Slope : s = #.#####

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

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

a2 = -0.00000
a1 = 0.00068
a0 = -1.19997

Fluoride L
Calibration order = 2

Correlation : r = 0.99960

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

a2 = -0.00000
a1 = 0.00025
a0 = 0.00307

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

1995-08-21 16:58 OutPut of : 950821B1

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

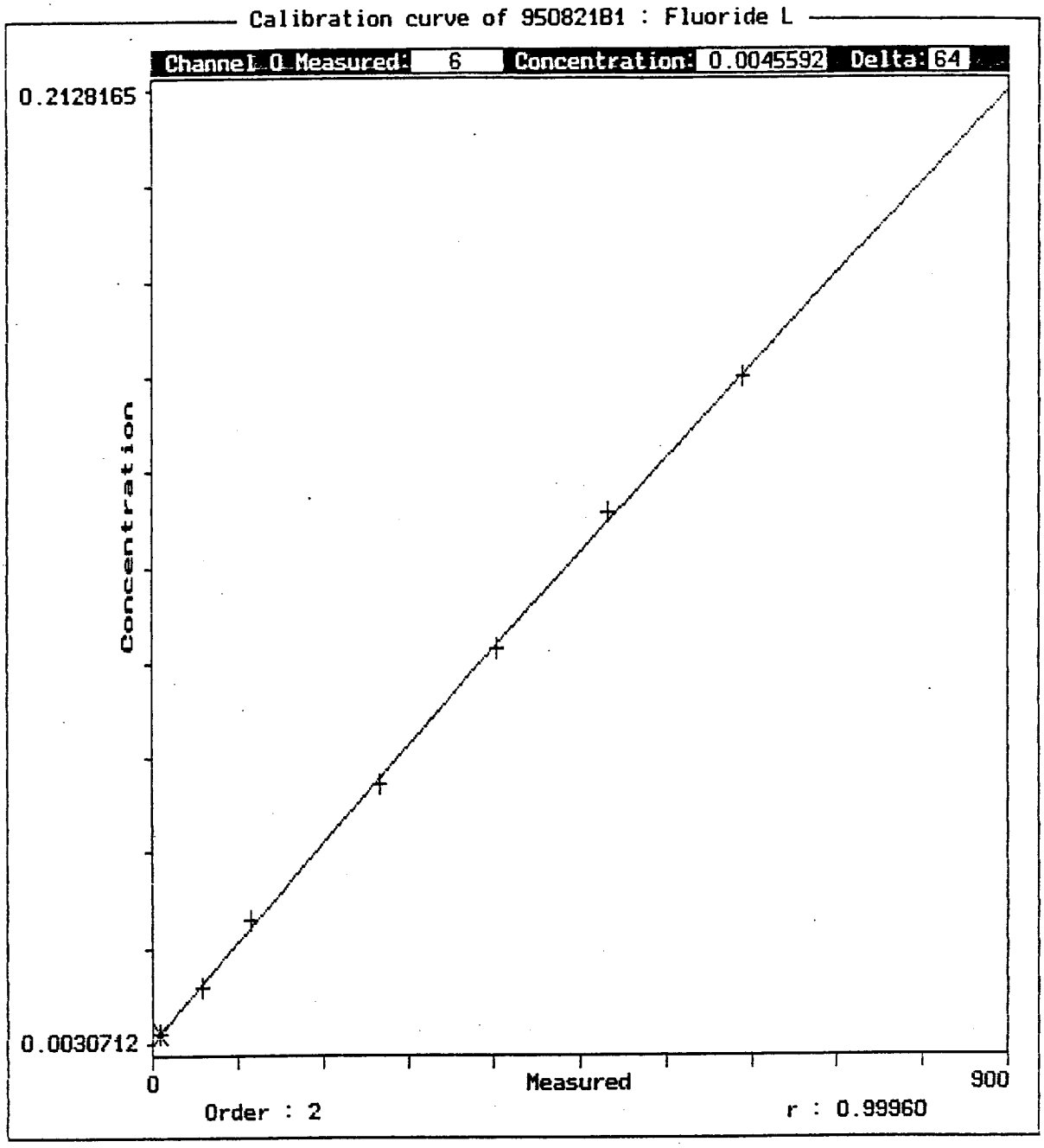
```
Path number   :  0
Signal type   : Debubbled
Decolor       : No
system Number : 0
diLute        : No
Resample      : No
dil Threshold : 4095
diG output    : 0
Window event  : Off
```

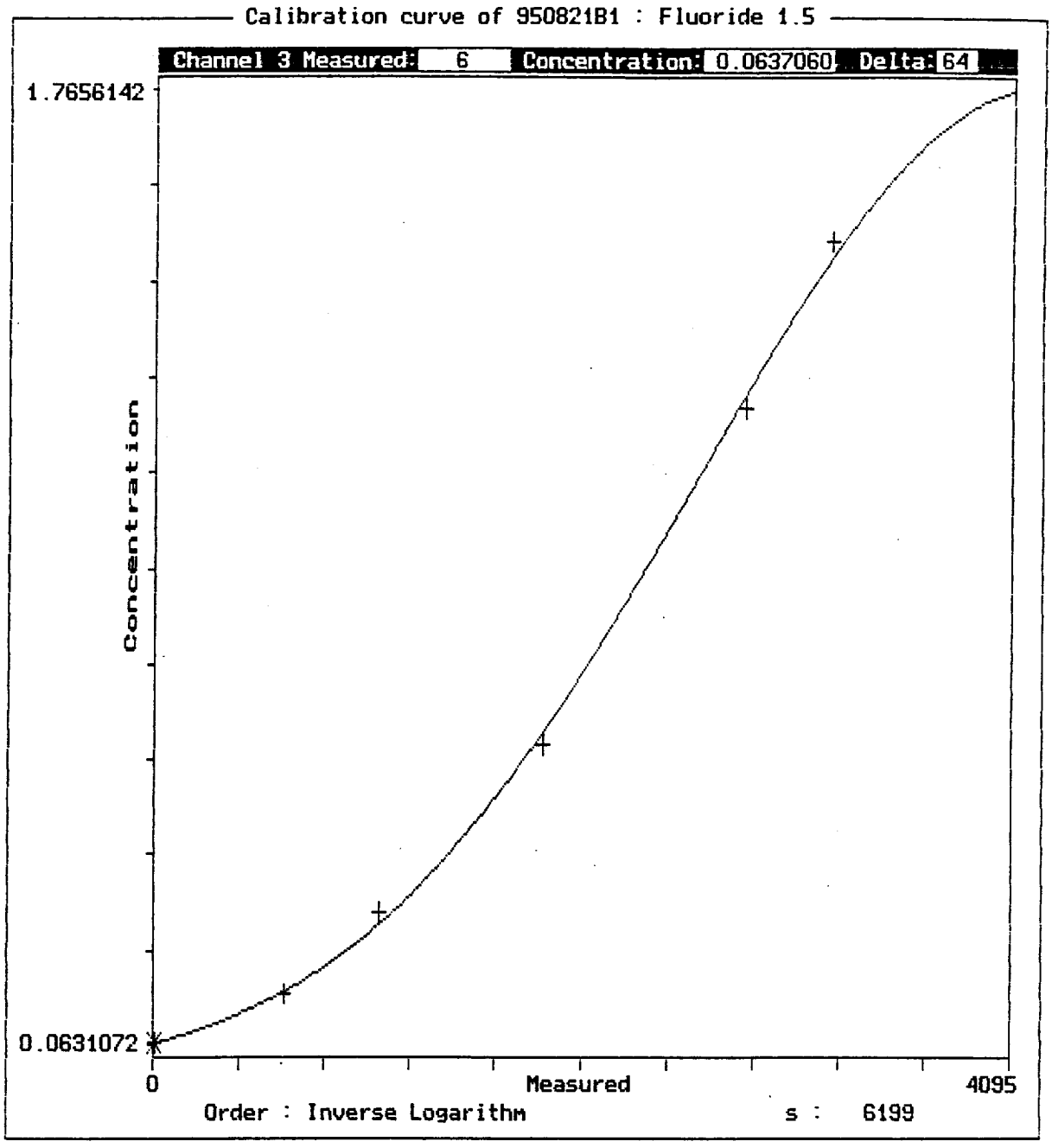
1995-08-21 16:58 OutPut of : 950821B1

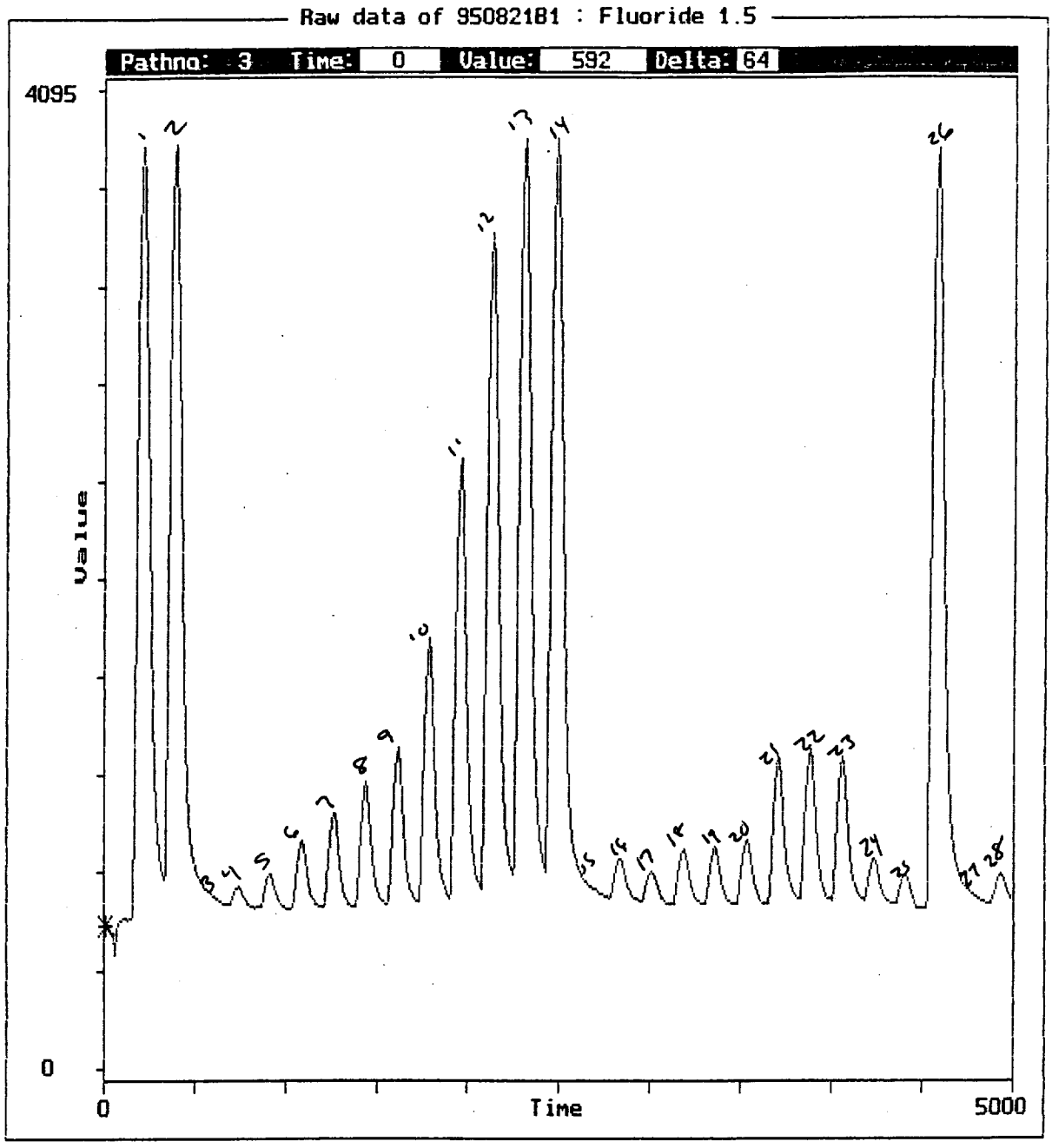
```
s1   sTandard :    0.015
s2   sTandard :    0.030
s3   sTandard :    0.060
s4   sTandard :    0.090
s5   sTandard :    0.120
s6   sTandard :    0.150
s7   sTandard : Ignore
s8   sTandard : Ignore
s9   sTandard : Ignore
s10  sTandard : Ignore
Order : 2
Dimension : PPM
start Value     : 500  DU
trigger 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	0.063	65	4	0.0031	0
1	t	Tracer	3	1.488	208	4	0.6348	0
2	d	Drift	3	1.478	384	4	0.6322	0
3	w	Wash	3	0.063	625	4	0.0031	0
4	s1	Standard 1	3	0.068	733	4	0.0157	0
5	s2	Standard 2	3	0.074	911	4	0.0284	0
6	s3	Standard 3	3	0.091	1085	4	0.0612	0
7	s4	Standard 4	3	0.109	1261	4	0.0909	0
8	s5	Standard 5	3	0.129	1435	4	0.1181	0
9	s6	Standard 6	3	0.156	1611	4	0.1507	0
10	s7	Standard 7	3	0.279	1787	4	0.2517	0
11	s8	Standard 8	3	0.620	1960	4	0.4063	0
12	s9	Standard 9	3	1.224	2135	4	0.5703	0
13	s10	Standard 10	3	1.472	2311	4	0.6308	0
14	d	Drift	3	1.435	2485	4	0.6213	0
15	w	Wash	3	0.063	2662	4	0.0031	0
16	u	SERUM BLK 1	3	0.075	2837	4	0.0314	0
17	u	SERUM BLK 2	3	0.070	3013	4	0.0184	0
18	u	SPK 40-1	3	0.080	3186	4	0.0406	0
19	u	SPK 40-2	3	0.081	3362	4	0.0431	0
20	u	SPK 40-3	3	0.086	3536	4	0.0515	0
21	u	SPK 124-1	3	0.145	3714	4	0.1381	0
22	u	SPK 124-2	3	0.150	3888	4	0.1441	0
23	u	SPK 124-3	3	0.145	4062	4	0.1386	0
24	u	BLK	3	0.076	4238	4	0.0326	0
25	u	F52986-15	3	0.067	4411	4	0.0127	0
26	d	Drift	3	1.408	4586	4	0.6146	0
27	w	Wash	3	0.063	4732	4	0.0031	0
28	u	F52990-15	3	0.069	4938	4	0.0164	0
29	u	F52997-15	3	0.067	5109	4	0.0115	0
30	u	F52982-15	3	0.064	5285	4	0.0058	0
31	u	F52994-15	3	0.069	5459	4	0.0167	0
32	u	F53410-15	3	0.068	5635	4	0.0137	0
33	u	F52984-15	3	0.065	5811	4	0.0070	0
34	u	F52992-15	3	0.065	5987	4	0.0083	0
35	u	F52996-15	3	0.066	6163	4	0.0090	0
36	u	F52977-15	3	0.066	6334	4	0.0110	0
37	u	SPK 40-1	3	0.081	6513	4	0.0431	0
38	d	Drift	3	1.434	6686	4	0.6211	0
39	w	Wash	3	0.063	6924	4	0.0031	0
40	u	SPK 124-1	3	0.191	7036	4	0.1851	0
41	u	BLK	3	0.097	7212	4	0.0715	0
42	u	F52989-15	3	0.118	7386	4	0.1036	0
43	u	F52993-15	3	0.088	7559	4	0.0561	0
44	u	F52978-15	3	0.089	7736	4	0.0585	0
45	u	F52980-15	3	0.088	7911	4	0.0559	0
46	u	F52991-15	3	0.086	8083	4	0.0530	0
47	u	F52987-15	3	0.083	8263	4	0.0469	0
48	u	F52988-15	3	0.083	8437	4	0.0474	0
49	u	F52995-15	3	0.079	8613	4	0.0385	0
50	d	Drift	3	1.454	8787	4	0.6263	0
51	w	Wash	3	0.063	9024	4	0.0031	0
52	u	F52972-22	3	0.083	9137	4	0.0457	0
53	u	F52973-22	3	0.085	9313	4	0.0511	0

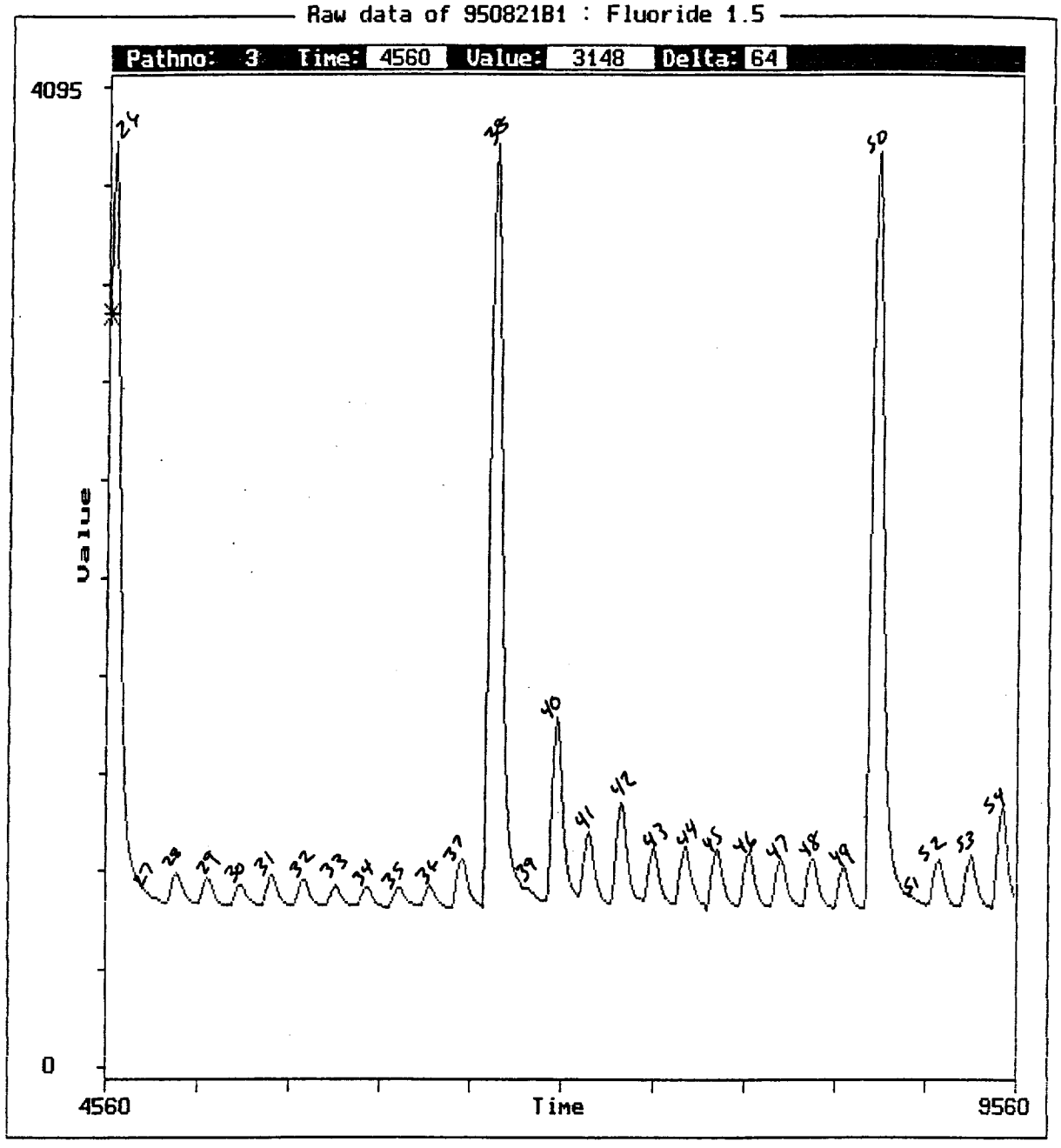
			Fluoride 1.5			Fluoride L		
			PPM			*PPM*		
Pos	Typ	Ident	Ch	Result	F Time	Ch	Result	F Time
54	u	SPK 40-1	3	0.117	9489	4	0.1017	0
55	u	SPK 40-2	3	0.093	9662	4	0.0655	0
56	u	SPK 124-1	3	0.146	9838	4	0.1391	0
57	u	BLK	3	0.090	10010	4	0.0593	0
58	u	F52979-22	3	0.080	10186	4	0.0411	0
59	u	F52975-22	3	0.077	10365	4	0.0341	0
60	u	F52976-22	3	0.076	10537	4	0.0324	0
61	u	F52983-22	3	0.082	10713	4	0.0453	0
62	d	Drift	3	1.419	10886	4	0.6175	0
63	w	Wash	3	0.063	11120	4	0.0031	0
64	u	F52986-22	3	0.082	11237	4	0.0438	0
65	u	F52990-22	3	0.077	11409	4	0.0343	0
66	u	F52997-22	3	0.078	11583	4	0.0363	0
67	u	F52982-22	3	0.074	11762	4	0.0284	0
68	u	F52994-22	3	0.082	11936	4	0.0445	0
69	u	F53410-22	3	0.083	12114	4	0.0460	0
70	u	SPK 40-1	3	0.108	12288	4	0.0893	0
71	u	SPK 124-1	3	0.168	12462	4	0.1627	0
72	u	SPK 62-1	3	0.131	12638	4	0.1211	0
73	u	BLK	3	0.091	12812	4	0.0621	0
74	d	Drift	3	1.469	12985	4	0.6301	0
75	w	Wash	3	0.063	13227	4	0.0031	0
76	u	F52984-22	3	0.083	13337	4	0.0469	0
77	u	F52992-22	3	0.082	13511	4	0.0455	0
78	u	F52996-22	3	0.084	13685	4	0.0479	0
79	u	F52977-22	3	0.083	13858	4	0.0467	0
80	u	F52989-22	3	0.082	14035	4	0.0440	0
81	u	F52993-22	3	0.079	14211	4	0.0397	0
82	u	SPK 40-1	3	0.106	14379	4	0.0857	0
83	u	SPK 124-1	3	0.152	14559	4	0.1459	0
84	d	Drift	3	1.461	14735	4	0.6280	0
85	w	Wash	3	0.063	14976	4	0.0031	0
wt	rw	RunOut Wash	3	0.063	15210	4	0.0031	0


Calibration curve of 950821B1 : Fluoride L
Channel 0 Measured: 6
Concentration: 0.0045592
Delta: 64
0.2128165
0.0030712
Concentration
0
Measured
900
Order : 2
r : 0.99960

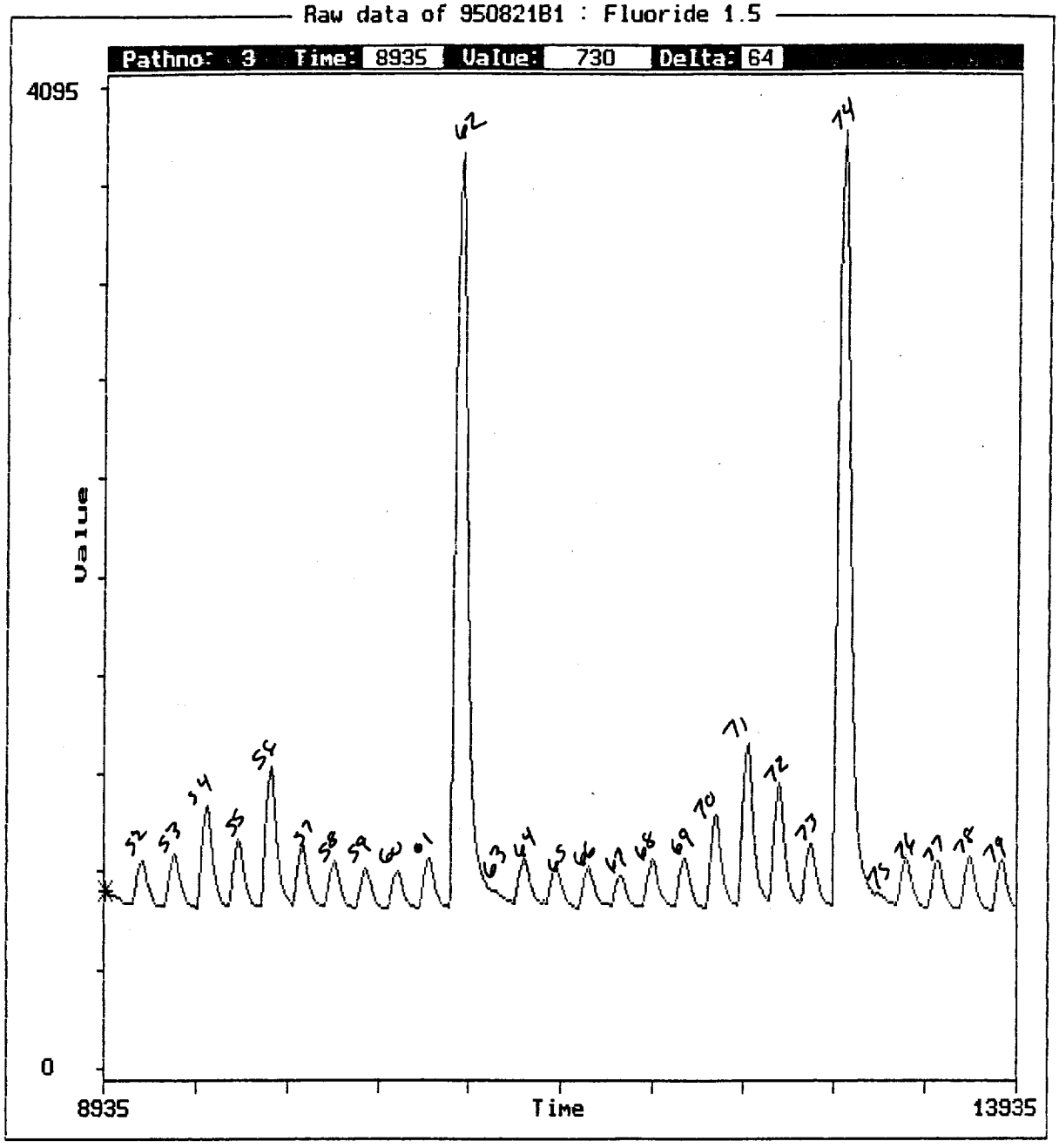

Calibration curve of 950821B1 : Fluoride 1.5
Channel 3 Measured: 6
Concentration: 0.0637060
Delta: 64
1.7656142
0.0631072
Concentration
0
Measured
4095
Order : Inverse Logarithm
s : 6199



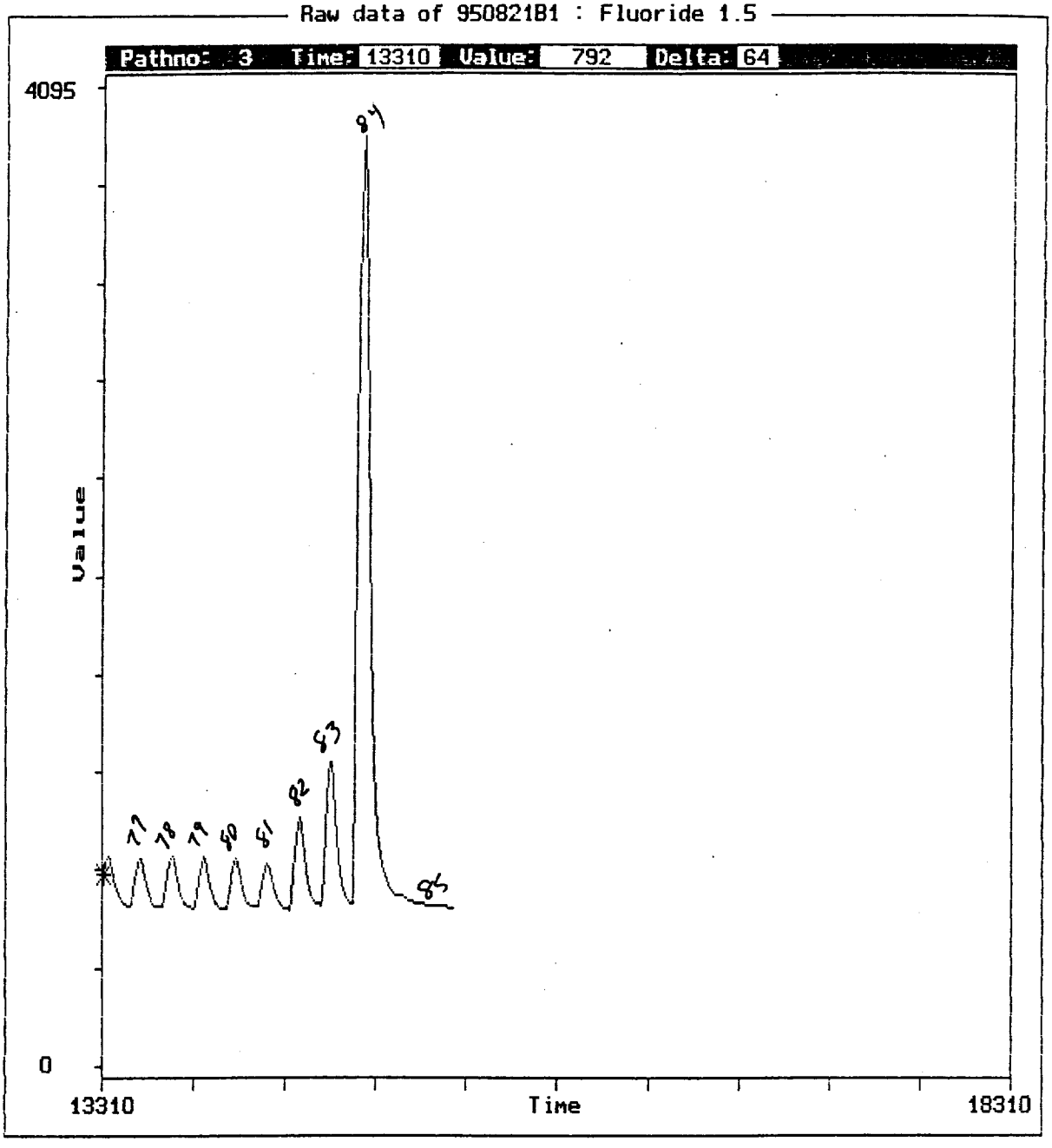
Esc=Exit ¦ F1=Help ¦ Crtl-P=Edit peaks ¦



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



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



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

Printout of Sample Table : 6329135D Inserted in System Table of : group 1

0	iw	Initial Wash	1	1.0000
1	t	Tracer	1	1.000
2	d	Drift	1	1.000
3	w	Wash	1	1.000
4	s1	Standard 1	1	1.000
5	s2	Standard 2	1	1.000
6	s3	Standard 3	1	1.000
7	s4	Standard 4	1	1.000
8	s5	Standard 5	1	1.000
9	s6	Standard 6	1	1.000
10	s7	Standard 7	1	1.000
11	s8	Standard 8	1	1.000

DDW 8/25/95 (D) 6 pp
MBT 20795.1
HWI 6329-135
Serum Cmmn 2 hr

1995-08-22 09:05 OutPut of : 950822A1

Software : version 6.1 c1990,93

Operator : DDW

Date of the Analysis : 1995-08-22 06:49

Analysis File Name : C:\SKALAR\DATA\HWIDATA\SERUM\950822A1

Fluoride 1.5
Calibration order = Inverse Logarithm

Slope : s = #.#####

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

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

a2 = -0.00000
a1 = 0.00075
a0 = -1.18734

Fluoride L
Calibration order = 2

Correlation : r = 0.99917

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

a2 = -0.00000
a1 = 0.00027
a0 = 0.00577

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

1995-08-22 09:05 OutPut of : 950822A1

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

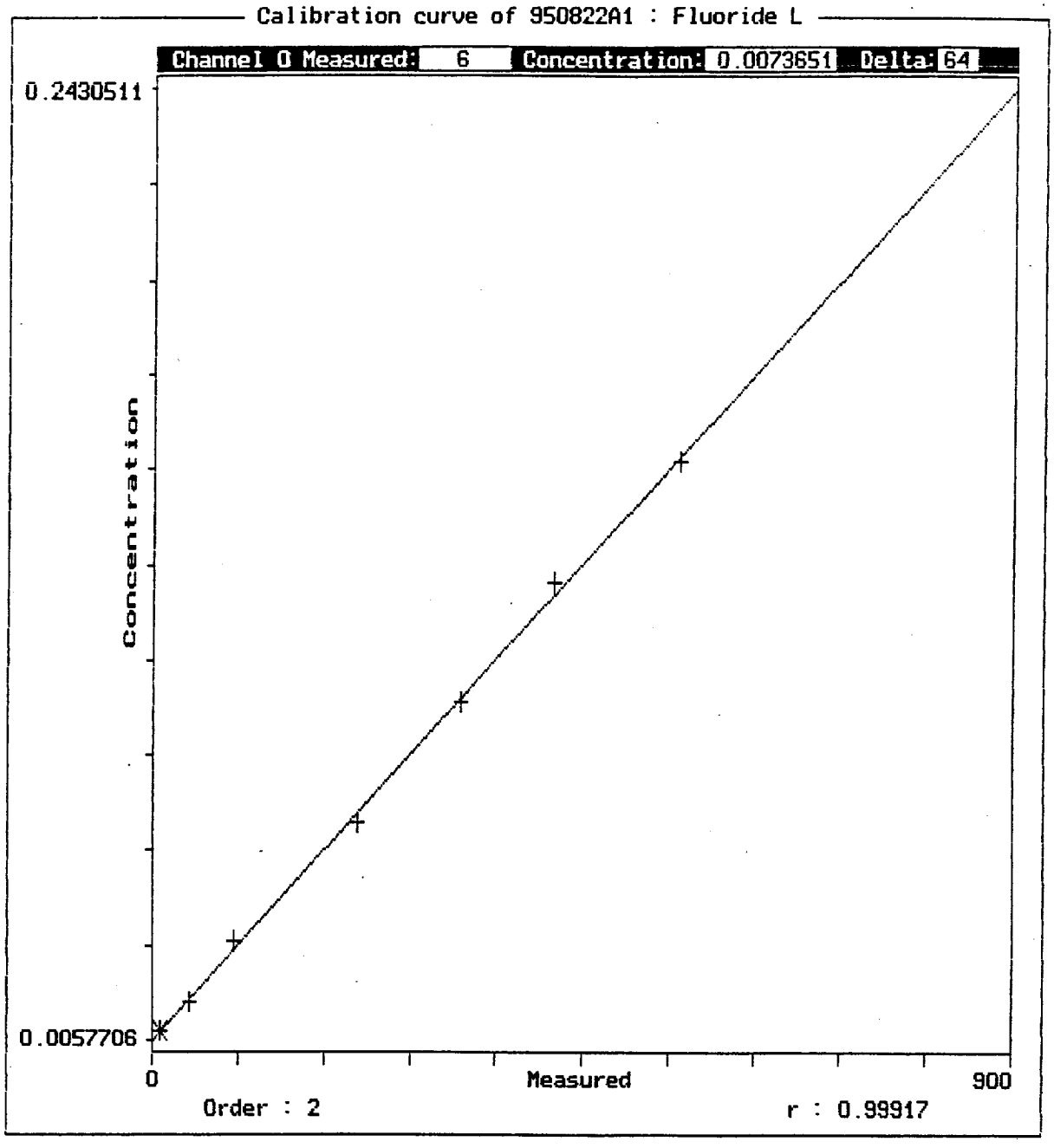
Path number : 0
Signal type : Debubbled
Decolor : No
system Number : 0
diLute : No
Resample : No
dil Threshold : 4095
diG output : 0
Window event : Off

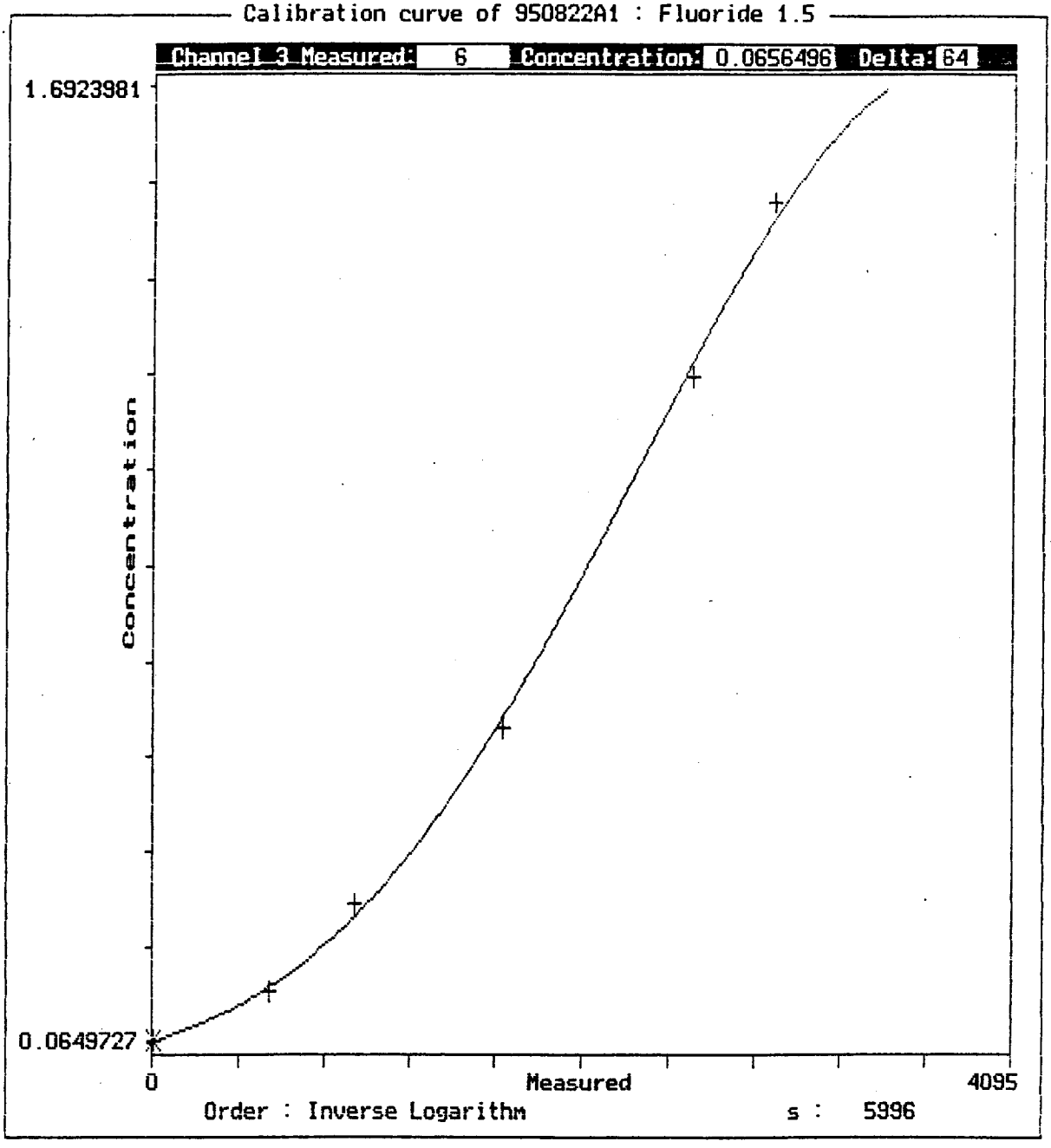
1995-08-22 09:05 OutPut of : 950822A1

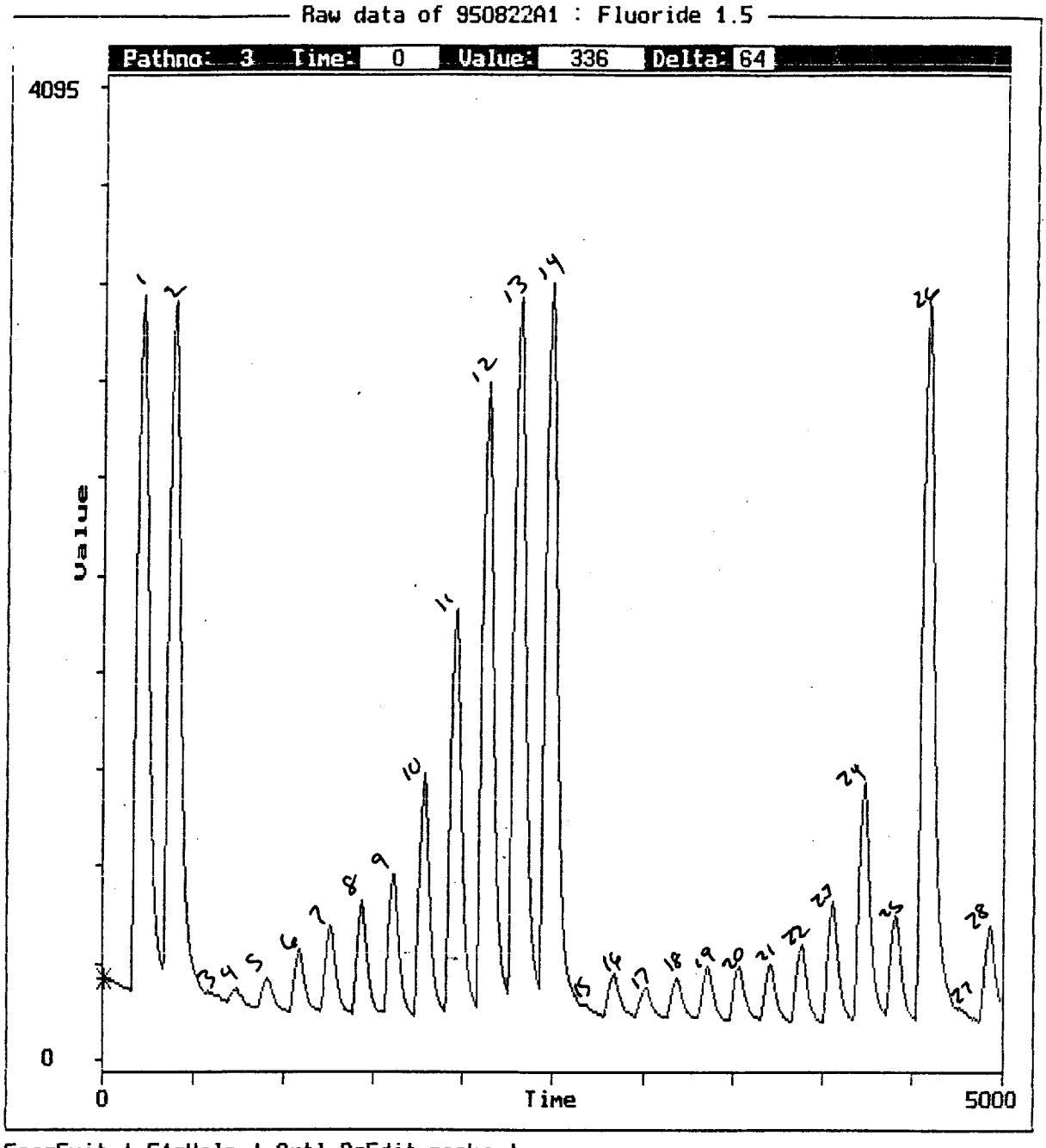
```
s1   sTandard :    0.015
s2   sTandard :    0.030
s3   sTandard :    0.060
s4   sTandard :    0.090
s5   sTandard :    0.120
s6   sTandard :    0.150
s7   sTandard : Ignore
s8   sTandard : Ignore
s9   sTandard : Ignore
s10  sTandard : Ignore
Order : 2
Dimension : PPM
start Value     : 500  DU
trigger 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	0.065	65	4	0.0058	0
1	t	Tracer	3	1.461	208	4	0.7660	0
2	d	Drift	3	1.475	383	4	0.7723	0
3	w	Wash	3	0.065	616	4	0.0058	0
4	s1	Standard 1	3	0.069	729	4	0.0156	0
5	s2	Standard 2	3	0.075	907	4	0.0281	0
6	s3	Standard 3	3	0.093	1083	4	0.0623	0
7	s4	Standard 4	3	0.111	1259	4	0.0911	0
8	s5	Standard 5	3	0.129	1433	4	0.1167	0
9	s6	Standard 6	3	0.157	1609	4	0.1512	0
10	s7	Standard 7	3	0.277	1785	4	0.2587	0
11	s8	Standard 8	3	0.621	1959	4	0.4443	0
12	s9	Standard 9	3	1.227	2134	4	0.6732	0
13	s10	Standard 10	3	1.469	2309	4	0.7697	0
14	d	Drift	3	1.536	2484	4	0.8009	0
15	w	Wash	3	0.065	2722	4	0.0058	0
16	u	SERUM BLK 1	3	0.085	2837	4	0.0472	0
17	u	SERUM BLK 2	3	0.078	3011	4	0.0334	0
18	u	SPK 40-1	3	0.083	3187	4	0.0432	0
19	u	SPK 40-2	3	0.090	3361	4	0.0570	0
20	u	SPK 40-3	3	0.090	3533	4	0.0575	0
21	u	SPK 40-4	3	0.092	3710	4	0.0609	0
22	u	SPK 124-1	3	0.105	3886	4	0.0829	0
23	u	SPK 124-2	3	0.138	4059	4	0.1283	0
24	u	SPK 124-3	3	0.278	4236	4	0.2595	0
25	u	SPK 124-4	3	0.129	4412	4	0.1172	0
26	d	Drift	3	1.533	4584	4	0.7994	0
27	w	Wash	3	0.065	4825	4	0.0058	0
28	u	SPK 100-1	3	0.121	4936	4	0.1059	0
29	u	SPK 100-2	3	0.141	5112	4	0.1317	0
30	u	SPK 100-3	3	0.137	5286	4	0.1278	0
31	u	BLK	3	0.101	5460	4	0.0755	0
32	u	BLK	3	0.080	5635	4	0.0390	0
33	u	F52978-22	3	0.078	5811	4	0.0334	0
34	u	F52980-22	3	0.082	5987	4	0.0424	0
35	u	F52991-22	3	0.076	6151	4	0.0291	0
36	u	F52987-22	3	0.073	6335	4	0.0249	0
37	u	F52988-22	3	0.070	6511	4	0.0169	0
38	d	Drift	3	1.532	6685	4	0.7989	0
39	w	Wash	3	0.065	6927	4	0.0058	0
40	u	F52995-22	3	0.074	7037	4	0.0268	0
41	u	~~SPK 40-1~~	3	~~0.062 A~~	~~7210~~	4	~~# ####~~	~~0~~
42	u	SPK 100-1	3	0.121	7386	4	0.1059	0
43	d	Drift	3	1.550	7561	4	0.8082	0
44	w	Wash	3	0.065	7803	4	0.0058	0
wt	rw	RunOut Wash	3	0.065	8036	4	0.0058	0

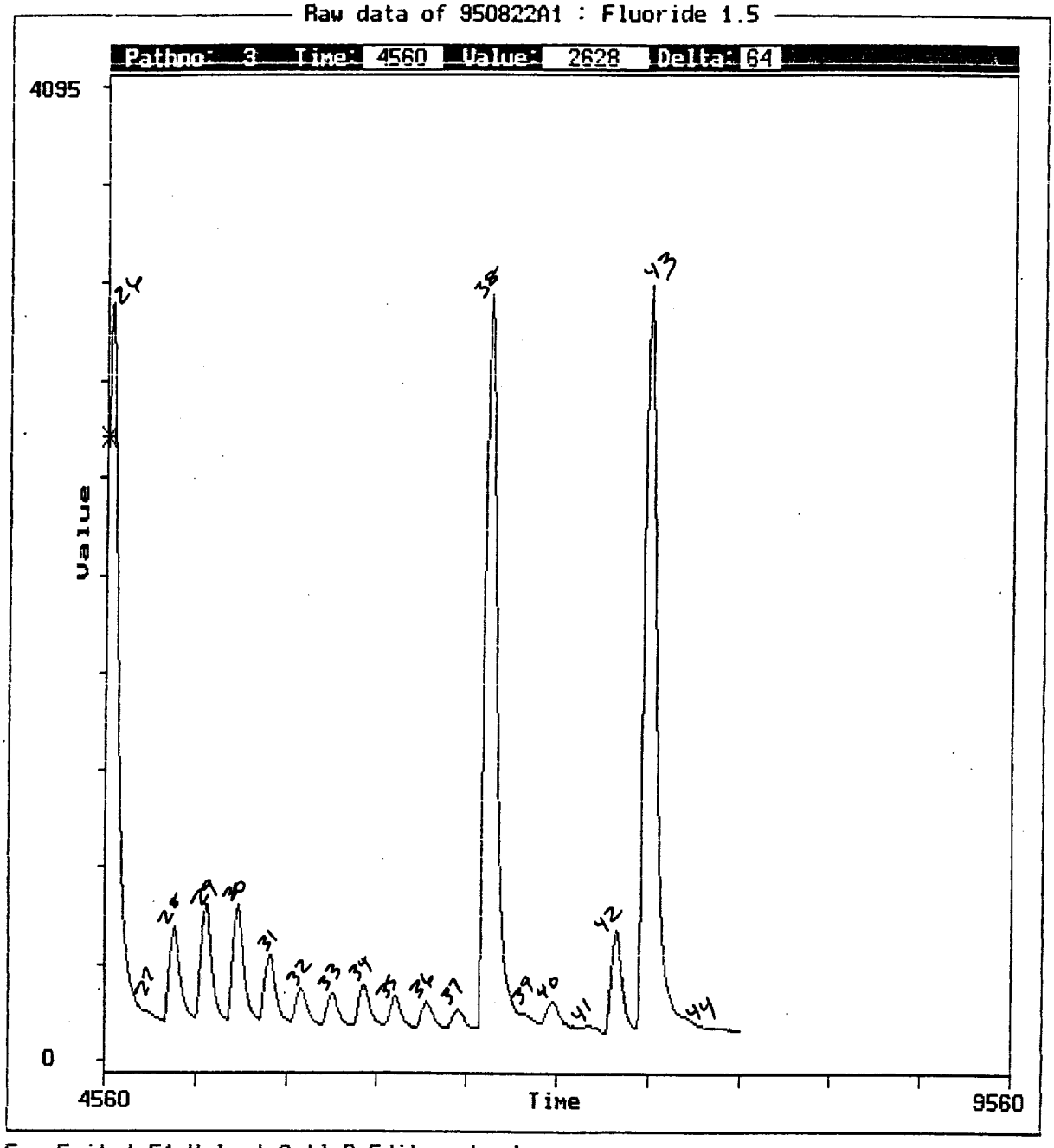
Blank TISAB - sample sp 8/22/95 QAJ


Calibration curve of 950822A1 : Fluoride L
Channel 0 Measured: 6
Concentration: 0.0073651
Delta: 64
0.2430511
0.0057706
Concentration
0
Measured
900
Order : 2
r : 0.99917


Calibration curve of 950822A1 : Fluoride 1.5
Channel 3 Measured: 6 Concentration: 0.0656496 Delta: 64
1.6923981
0.0649727
Concentration
0
Measured
4095
Order : Inverse Logarithm
s : 5996



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


Raw data of 950822A1 : Fluoride 1.5
Pathno: 3 Time: 4560 Value: 2628 Delta: 64
4095
0
Value
4560
Time
9560
24
38
43
26
29
30
31
32
33
34
35
36
37
39
40
41
42
44
Esc=Exit ¦ F1=Help ¦ Crtl-P=Edit peaks ¦