**IDENTIFICATION AND CHARACTERIZATION OF
CONSERVATIVE ORGANIC TRACERS FOR USE AS
HYDROLOGIC TRACERS FOR THE YUCCA MOUNTAIN
SITE CHARACTERIZATION STUDY**

DOE/NV/10872--T2

DE92 014660

PROGRESS REPORT

JUNE 1 - DECEMBER 31, 1990

DOE Cooperative Agreement

No. DE-FC 08-90NV10872

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

Klaus J. Stetzenbach, Ph.D.
Environmental Research Center
University of Nevada-Las Vegas

MASTER

TRACER PROJECT PROGRESS REPORT

1 JUNE 1990 - 31 DECEMBER 1990

REVIEW OF PREVIOUS WORK

During the time period of October 1989 to May 1990 we purchased analytical instrumentation and developed the High Performance Liquid Chromatography (HPLC) analytical methods necessary for the stability tests. Tables 1 and 2 review the potential tracers and the HPLC method used during this phase. The stated detection limits were considered sufficient for this early phase of the study, and could be improved later if necessary. Several of the potential tracers (see notes in table 1) were eliminated from further study. The 3,5-Diiodosalicylic was eliminated because of poor sensitivity. Trans-3-fluorocinnamic acid, trans-2,4-difluorocinnamic acid, 2,6-difluorocinnamic acid and 3,4-difluorocinnamic acid could not be separated from other tracers to provide baseline resolution.

JUNE 1990 - DECEMBER 1990 WORK

Work during this period centered around two tasks. The first was the development of a Quality Assurance Project Plan (QAPjP) and Standard Operating Procedures (SOPs) for the technical equipment used during the batch studies. The second was the batch sorption and degradation studies for some of the candidate tracers. This second task is an ongoing task and will continue into the second and perhaps the third year of the study, depending on the number of tracers and their lack of degradation.

QUALITY ASSURANCE PROJECT PLAN

A draft copy of the QAPjP for the tracer project was transmitted to the Department of Energy, Yucca Mountain Project Office, on December 14, 1990. The Tracer Project QAPjP is based on quality assurance requirements of the Environmental Research Center (ERC) and the DOE. We are awaiting review comments from the DOE. A copy of the draft Tracer Project QAPjP is enclosed.

Standard Operating Procedures were written for the use of the HPLC with the ultraviolet absorption (UV) and fluorescence detectors, the autosampler for the HPLC, the computing integrator, the analytical balance and two statistical computer programs. Draft copies of the SOPs were transmitted to the DOE on December 14, 1990. Copies of the draft SOPs are enclosed.

INITIAL TRACER EVALUATIONS

COMPOUND NAME	DETECTION LIMIT*	VARIABILITY (AT DET.LIM.)
2-Fluoro- Cinnamic acid	100 ppb	8.35%
Trans 2,5-Difluoro- Cinnamic acid	100 ppb	7.05%
3,5-Difluoro- Cinnamic acid	100 ppb	3.75%
Alpha Fluoro- Cinnamic acid	100 ppb	5.18%
4-Fluoro- Cinnamic acid	100 ppb	5.78%
Trans 3-Fluoro- Cinnamic acid**	100 ppb	4.33%
Trans 2,4-Difluoro- Cinnamic acid**	100 ppb	9.52%
2,6-Difluoro- Cinnamic acid**	100 ppb	6.93%
3,4-Difluoro- Cinnamic acid**	100 ppb	13.32%
Salicylic acid***	10 ppb	3.77%
5-Fluoro- Salicylic acid	10 ppb	6.95%
3,5-Dichloro- Salicylic acid	25 ppb	4.35%
3,5-Diiodo- Salicylic acid****	1 ppm	6.24%

* See table 2 for analytical conditions.

** These compounds eluted so closely at all practical solvent mixtures, that they were removed from further consideration at this time.

*** The Salicylic acid was removed from further consideration at this time for two reasons: the Salicylic and 5-Fluorosalicylic acids co-eluted at all practical solvent mixtures, and it was thought that the Salicylic acid would decompose more rapidly than the other Salicylic acids; so the 5-Fluorosalicylic acid was chosen as the better candidate of the two.

**** 3,5-Diiodo Salicylic acid was removed from further consideration because of poor sensitivity.

TABLE 1

ANALYTICAL CONDITIONS

All analytical work was done with a 15cm, 5µm particle diameter octadecylsilane (C-18) bonded column.

Detection limits were evaluated under the following conditions:

Cinnamic Acids

Solvent Mixture: 50% Methanol and 50% 0.01M KH₂PO₄ @ pH = 3.0

Column Flow: 1.0 mL/minute

Sample Loop Volume: 10 µL

UV Absorption Detector Wavelength: 270 nm

Detector Range: 0.005

Salicylic Acids

Solvent Mixture: 65% Methanol and 35% 0.01M KH₂PO₄ @ pH = 3.0

Column Flow: 1.0 mL/minute

Sample Loop Volume: 10 µL

Fluorescence Detector Excitation Wavelength: 300 nm

Detector Cut-off filter: 380 nm

Detector Range: 50

TABLE 2

BATCH SORPTION AND DEGRADATION STUDIES

Batch sorption studies are static tests in which a potential tracer spiked water solution is placed in a bottle with aquifer material, 3 types of tuff for these experiments. The concentration of the tracer is monitored over regularly scheduled intervals. If the tracer sorbs or degrades, its concentration in the water is reduced or completely depleted. It is not possible to clearly distinguish between sorption and degradation with this test although, based on the decay curves, inferences can sometimes be made.

TUFF

The tuff used in these experiments was collected with the assistance of DOE geologists. On May 30, 1990, three types of tuff were collected from an outcropping in an area south of Yucca Mountain. These tuffs were named light, medium and dark according to their appearance. Each type of tuff was ground to a fine powder consistency approximately 40 mesh. On September 18, 1990, a second batch of tuff was collected from the same outcropping.

WATER

Two types of water are used in the batch experiments, distilled water from the ERC laboratory and J-13 well water. The first J-13 well water was collected on May 30, 1990, with additional collections on September 18 and December 6, 1990. By using both the distilled water and the J-13 well water we can derive additional information about possible chemical interferences in the J-13 well water.

EXPERIMENTAL RESULTS

BLANKS

Water and tuff blanks (no tracer) are set up with each batch that is tested. To date, none of the blanks has shown any peaks that could interfere with the analysis of the tracer.

LIGHT TUFF

The initial batch tests were set up with the light tuff collected on May 30, 1990. Upon addition of the tracer solution the pH increased to 11. This unusually high pH resulted in poor chromatographic peak shape and reduced the retention time by as much as 2 minutes over the standard solutions.

We attempted to wash the light tuff with distilled and J-13 water, but this only reduced the pH to approximately 9.5, which is still unsatisfactory and further study with this batch of light tuff was discontinued. No reason for this high pH has been determined.

Light tuff collected on September 18, 1990 does not produce unusual pH when the tracer is added, and studies with this tuff are currently underway.

5-FLUOROSALICYLIC ACID

Statistical analytical data for this batch experiment are shown in table 3. Plots of the experimental results are shown in figures 1 through 4.

The 5-fluorosalicilic acid exhibited some unusual behavior in both the dark and medium tuff

with J-13 well water and deionized water. In all cases the tracer is fairly close to the initial concentration for 2 to 3 days and then shows a rather sharp decline between days four and 14. However, after day 14 the tracer concentration remains constant for the remainder of the 93 days tested so far. The reduction is 43% for the medium tuff, J-13 water; 69% for the dark tuff, J-13 water and 71% for the duplicate; 48% for the medium tuff, DI water; and 48% for the dark tuff, DI water. Tracer decline is due to either bacterial degradation or chemical sorption. In this case, because of the levelling off, bacterial degradation is ruled out. The decline is most likely due to chemical sorption on specific, but limited number of sites in the tuff surface. Based on the data to date, this potential tracer will be studied further with the column experiments.

3,5-DICHLOROSALICYLIC ACID

Statistical analytical data for this batch experiment are shown in table 4. Plots of the experimental results are shown in figures 5 through 8.

This compound appears to be an excellent candidate for a tracer in tuff. All the data show good stability out to 60 days, and only the medium tuff/DI water samples shows significant reduction after 93 days (figure 7).

All of the samples show some unusual behavior at the start of the experiments. There is no explanation for this unusual reduction in concentration at this time. Further experiments will have to be conducted to verify this behavior.

This compound will be studied further with the column experiments.

CINNAMIC ACIDS

2-Fluorocinnamic acid, trans-2,5-difluorocinnamic acid and 3,5-difluorocinnamic acid have been tested in dark and medium tuff with DI water only (testing with J-13 water is in progress). Statistical analytical data for this batch experiment are shown in tables 5 through 7. Plots of the experimental results are shown in figures 9 through 14.

As can be seen from figures 9 - 14 these compounds appear not to be very stable. The cause of the reduction of the 2-fluorocinnamic acid and the trans-2,5-difluorocinnamic acid appears to be bacterial degradation. The rapid disappearance of 3,5-difluorocinnamic acid suggests strong sorption to the tuff.

MAJOR EQUIPMENT PURCHASES

During this reporting period a High Performance Liquid Chromatograph - Mass Spectrometer (HPLC-MS) and an autosampler were purchased. The autosampler is currently being used and the HPLC-MS is expected to be operational by February 1991.

SUMMARY AND CONCLUSIONS

Based on the data obtained to date, 2-fluorocinnamic acid, trans-2,5-difluorocinnamic acid and 3,5-difluorocinnamic acid are not stable enough to be useful as ground water tracers for the C-well experiments. Experiments are in progress with these compounds in J-13 water and light tuff. These experiments will be carried out to 90 days, but then no further work with these compounds is anticipated.

5-Fluorosalicylic acid appears to sorb on the tuffs used in the batch test. We will use this compound in the column tests to determine, if possible, a retardation factor to see if this might be a useful non-conservative tracer.

Based on the experimental results 3,5-dichlorosalicylic acid has the potential as a good ground water tracer. It must be studied further with the column experiments and the reason for the drop in concentration during the first 3 days must be determined.

FUTURE WORK (Jan 91- May 91)

Batch experiments are in progress with the above mentioned tracers in light tuff, 4-fluorocinnamic acid and alpha-fluoro-cinnamic acid with all three types of tuff. We will also start batch experiments with 2 pyridones, coumarin 343 and 2,7-dichloro-fluorescein. Once the HPLC-MS is operational, method development work will begin for trifluoroacetic acid, pentafluoropropionic acid and heptafluorobutyric acid.

5-FLUOROSALICYLIC ACID:

Dark and Medium Tuff (93 day analysis)

SAMPLE	VARIABILITY (ppb)	%VARIABILITY
50 ppb std.	2.43 ppb	4.87%
150 ppb std.	2.54 ppb	1.69%
250 ppb std.	2.97 ppb	1.19%
5-Fluorosal/ J-13 H ₂ O	2.05 ppb	0.93%
#2-1 (Med. Tuff, J-13 H ₂ O, 5-Fluorosal)	1.74 ppb	0.79%
#3-1 (Dk. Tuff, J-13 H ₂ O, 5-Fluorosal)	2.31 ppb	1.04%
#3-1D (Dk. Tuff, J-13 H ₂ O, 5-Fluorosal)	1.18 ppb	0.54%
5-Fluorosal/DI H ₂ O	1.89 ppb	1.30%
#5-1 (Med. Tuff, DI H ₂ O, 5-Fluorosal)	4.62 ppb	2.08%
#6-1 (Dk. Tuff, DI H ₂ O, 5-Fluorosal)	11.90 ppb	5.36%

5-FLUOROSALICYLIC ACID:

*Detection Limits: (Fluorescence) 10 ppb (U.V.) 10 ppm

*Wavelength: 300 nm with a 380 nm cut off filter

*Solvent Mix: 60% Methanol / 40% 0.05M KH₂PO₄ at pH=2.5*Soil Mix: 250 ml of Tracer solution to 250 grams of soil
Soil Blanks showed no interference compounds.

TABLE 3

3,5-DICHLOROSALICYLIC ACID:

Dark and Medium Tuff (93 day analysis)

SAMPLE	VARIABILITY (ppb)	%VARIABILITY
100 ppb std.	19.10 ppb	19.10%
300 ppb std.	11.03 ppb	3.68%
500 ppb std.	8.13 ppb	1.63%
3,5-Dichlorosal/ J-13 H ₂ O	6.88 ppb	1.69%
#2-2 (Med. Tuff, J-13 H ₂ O, 3,5-Dichlorosal)	15.99 ppb	3.93%
#3-2 (Dk. Tuff, J-13 H ₂ O, 3,5-Dichlorosal)	13.93 ppb	3.42%
3,5-Dichlorosal/ DI H ₂ O	7.12 ppb	1.78%
#5-2 (Med. Tuff, DI H ₂ O, 3,5-Dichlorosal)	6.47 ppb	1.62%
#6-2 (Dk. Tuff, DI H ₂ O, 3,5-Dichlorosal)	14.13 ppb	3.53%

3,5-DICHLOROSALICYLIC ACID:

*Detection Limits: (Fluorescence) 25 ppb (U.V.) 10 ppm

*Wavelength: 300 nm with a 380 nm cut off filter

*Solvent Mix: 60% Methanol / 40% 0.05M KH₂PO₄ at pH=2.5

*Soil Mix: 250 ml of Tracer solution to 250 grams of soil
Soil Blanks showed no interference compounds.

TABLE 4

2-FLUOROCINNAMIC ACID:

Dark and Medium Tuff (60 day analysis)

SAMPLE	VARIABILITY (ppb)	%VARIABILITY
200 ppb std.	2.84 ppb	1.42%
400 ppb std.	4.04 ppb	1.01%
600 ppb std.	5.28 ppb	0.88%
2-Fluoro cinn/ DI H ₂ O	6.56 ppb	1.35%
#5-6 (Med. Tuff, DI H ₂ O, 2-Fluoro cinn)	21.32 ppb	4.39%
#6-6 (Dk. Tuff, DI H ₂ O, 2-Fluoro cinn)	3.84 ppb	0.79%
#6-6D (Dk. Tuff, DI H ₂ O, 2-Fluoro cinn)	3.45 ppb	0.71%

2-FLUOROCINNAMIC ACID:

*Detection Limits: (U.V.) 25 ppb (Fluorescence) N/D

*Wavelength: 270 nm

*Solvent Mix: 60% Methanol / 40% 0.05M KH₂PO₄ at pH=2.5

*Soil Mix: 250 ml of Tracer solution to 250 grams of soil
Soil Blanks showed no interference compounds.

TABLE 5

TRANS 2,5-DIFLUOROCINNAMIC ACID:

Dark and Medium Tuff (60 day analysis)

SAMPLE	VARIABILITY (ppb)	%VARIABILITY
200 ppb std.	2.04 ppb	1.02%
400 ppb std.	7.88 ppb	1.97%
600 ppb std.	10.08 ppb	1.68%
t-2,5-Difluoro-cinn / DI H ₂ O	4.19 ppb	0.88%
#5-4 (Med. Tuff, DI H ₂ O, t-2,5-difluoro cinn)	5.57 ppb	1.17%
#5-4D (Med. Tuff, DI H ₂ O, t-2,5-difluoro cinn)	5.15 ppb	1.08%
#6-4 (Dk. Tuff, DI H ₂ O, t-2,5-difluoro cinn)	4.95 ppb	1.04%

TRANS 2,5-DIFLUOROCINNAMIC ACID:

*Detection Limits: (U.V.) 25 ppb (Fluorescence) N/D

*Wavelength: 270 nm

*Solvent Mix: 60% Methanol / 40% 0.05M KH₂PO₄ at pH=2.5*Soil Mix: 250 ml of Tracer solution to 250 grams of soil
Soil Blanks showed no interference compounds.

TABLE 6

3,5-DIFLUOROCINNAMIC ACID:

Dark and Medium Tuff (60 day analysis)

SAMPLE	VARIABILITY (ppb)	%VARIABILITY
200 ppb std.	2.64 ppb	1.32%
400 ppb std.	4.24 ppb	1.06%
600 ppb std.	5.70 ppb	0.95%
3,5-Difluoro- cinn / DI H ₂ O	7.56 ppb	1.53%
#5-3 (Med. Tuff, DI H ₂ O, 3,5 Difluoro cinn)	2.72 ppb	0.55%
#5-3D (Med. Tuff, DI H ₂ O, 3,5 Difluoro cinn)	31.62 ppb	6.39%
#6-3 (Dk. Tuff, DI H ₂ O, 3,5-Difluoro cinn)	33.50 ppb	6.78%

3,5-DIFLUOROCINNAMIC ACID:

*Detection Limits: (U.V.) 25 ppb (Fluorescence) N/D

*Wavelength: 270 nm

*Solvent Mix: 60% Methanol / 40% 0.05M KH₂PO₄ at pH=2.5

*Soil Mix: 250 ml of Tracer solution to 250 grams of soil
Soil Blanks showed no interference compounds.

TABLE 7

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Δ—Δ DCS-J13
▲—▲ DCS#2-2

DICHLOROSALICYLIC/MED TUFF/J-13 H₂O

Figure 6

Δ—Δ DCS-J13
▲—▲ DCS#3-2

DICHLOROSALICYLIC/DARK TUFF/J-13 H₂O

Figure 7

Δ—Δ DCS-DI
▲—▲ DCS#5-2

DICHLOROSALICYLIC/MED TUFF/DI H₂O

Figure 8

Δ—Δ DCS-DI
▲—▲ DCS#6-2

DICHLOROSALICYLIC/DARK TUFF/DI H₂O

Figure 9

O — O 2FC-DI
Δ — Δ 2FC#5-6

2-FLUORO CINNAMIC/MED TUFF/DI H₂O

Figure 10

○—○ 2FC-DI
△—△ 2FC#6-6
▲—▲ 2FC#6-6D

2-FLUORO CINNAMIC/DARK TUFF/DI H₂O

Figure 11

O—O t25DFC-DI
 Δ—Δ t25DFC#5-4
 ▲—▲ t25DFC#5-4D

t2,5 DIFLUOROCINNAMIC/MED TUFF/DI H₂O

Figure 12

○ —○ t25DFC-DI
△ —△ t25DFC#6-4

t2,5 DIFLUOROCINNAMIC/DARK TUFF/DI H₂O

Figure 13

○—○ 35DFC-DI
△—△ 35DFC#5-3
▲—▲ 35DFC#5-3D

3,5 DIFLUOROCINNAMIC/MED TUFF/DI H₂O

Figure 14

O—O 35DFC—DI
Δ—Δ 35DFC#6—3

3,5 DIFLUOROCINNAMIC/DARK TUFF/DI H₂O

UNIVERSITY OF NEVADA, LAS VEGAS
ENVIRONMENTAL RESEARCH CENTER

QUALITY ASSURANCE PROJECT PLAN

Identification and Characterization of
Conservative Organic Tracers for Use as Hydrologic Tracers
For the Yucca Mountain Site Characterization Study

DRAFT

Klaus J. Stetzenbach
Project Manager
Environmental Research Center
University of Nevada, Las Vegas
Las Vegas, Nevada 89154

Cooperative Agreement DE-FC08-90NV10872

Carl P. Gertz
Client Project Manager
U.S. Department of Energy
2753 S. Highland Dr.
Las Vegas, Nevada 89109

UNIVERSITY OF NEVADA, LAS VEGAS
ENVIRONMENTAL RESEARCH CENTER
QUALITY ASSURANCE PROJECT PLAN

Identification and Characterization of
Conservative Organic Tracers for Use as Hydrologic Tracers
For the Yucca Mountain Site Characterization Study

SIGNATURES OF APPROVAL

Ram Murthy, U.S. DOE QA Officer

Date

Carl P. Gertz, U.S. DOE Project Manager

Date

A.J. Cross-Smiecinski, ERC QA Officer

Date

Klaus J. Stetzenbach, ERC Coop Project Manager

Date

CONTENTS

QA Project Plan Locator Page.....	4
Section 1 Project Description.....	5
Section 2 Project Organization and Responsibilities.....	14
Section 3 Quality Assurance Program.....	20
Section 4 QA Objectives and Assessment Procedures.....	21
Section 5 Site Selection and Sampling Procedures.....	23
Section 6 Sample Custody.....	25
Section 7 Analytical Procedures.....	26
Section 8 Calibration Procedures and Requirements.....	27
Section 9 Preventive Maintenance Procedures and Schedules..	28
Section 10 Internal QC Checks.....	30
Section 11 Performance and Systems Audits.....	32
Section 12 Corrective Action.....	34
Section 13 Data Processing.....	35
Section 14 QA Reports to Management.....	38
Section 15 Computer Software.....	39
Section 16 Design Control.....	49
Section 17 Procurement Document Control.....	50
Section 18 Instructions, Plans, Procedure and Drawings.....	51
Section 19 Control of Purchased Items and Service.....	52
Section 20 Control of Processes.....	53
Section 21 Inspection.....	54
Section 22 Test Control.....	55
Section 23 Inspection, Test and Operating Status.....	56
Section 24 Control of Nonconforming Conditions.....	57
Section 25 ERC Organizational Chart.....	58
References.....	59
Appendix A.....	60

QA PROJECT PLAN LOCATOR PAGE

ERC QA Policy requires that the DOE QA requirements be supplemented to assure the production of quality data from a scientific investigation. Therefore, additional criteria have been addressed and intermingled with those requirements in this document. The purpose of the locator page is to allow the reader to locate the DOE QA requirements that are contained within this document.

<u>DOE QA Requirements</u>	<u>Section</u>
1. Organization	2, 25
2. Quality Assurance Program	3
3. Design Control	16
4. Procurement Document Control	17
5. Instructions, Plans, Procedures, and Drawings	18
6. Document Control	2
7. Control of Purchased Items and Services	19
8. Identification and Control of Samples and Data	2
9. Control of Processes	20
10. Inspection	21
11. Test Control	22
12. Control of Measuring and Test Equipment	8
13. Handling, Storage, and Shipping	2
14. Inspection, Test, and Operating Status	23
15. Control of Nonconforming Conditions	24
16. Corrective Action	12
17. Quality Assurance Records	2
18. Audits	11
19. Computer Software	15

SECTION 1.0

PROJECT DESCRIPTION

Ground water tracers are solutes dissolved in or carried by ground water to delineate flow pathways. Tracers provide information on direction and speed of water movement and that of contaminants that might be conveyed by the water. Tracers can also be used to measure effective porosity, hydraulic conductivity, dispersivity and solute distribution coefficients. Tracers can be naturally occurring compounds or elements or they can be completely foreign to the environment. The latter is generally preferred. For most applications tracers should be conservative, that is, move at the same rate as the water and not sorb to aquifer materials.

Tracers must have a number of properties to be functional. Regardless of the desired properties, the chemical and physical behavior of a tracer in ground water and the porous medium under study must be understood. Good estimates of tracer behavior can be obtained from laboratory studies. For the purposes of the Yucca Mountain C-well complex tracer study, the tracers should have the following properties:

- 1) Must be water soluble.
- 2) Should not sorb on the aquifer material (i.e., be conservative).
- 3) Should be chemically and biologically stable for the duration of the test.
- 4) Should be foreign to the Yucca Mountain environment.
- 5) Should have excellent analytical sensitivity.
- 6) Should be non-toxic.

The studies outlined in this proposal will address the above stated tracer properties with analytical method development, static sorption and degradation studies and column transport studies. Mutagenicity tests will be performed on promising candidates.

The tracers that will be used for these experiments are fluorinated organic acids and other organic compounds that have the chemical and biological stability necessary to be effective in the Yucca Mountain environment. Special emphasis will be placed on compounds that fluoresce or have very large ultraviolet absorption coefficients for very high analytical sensitivity.

The column tests (task 4) will be conducted relative to bromide. Bromide will also be used by other investigators as a standard tracer and will allow comparisons to be made of relative sorption rates between experiments.

1.1 STATEMENT OF PROBLEM

The purpose of this work is to identify and characterize candidate conservative organic tracers for use as hydrologic tracers for experiments to be conducted at the C-well complex.

General Outline of Tasks as received from the Department of Energy:

- 1) Identify candidate tracers for use as conservative tracers.
- 2) Conduct analytical chemical evaluation of promising candidate tracers by evaluating detection limits with respect to the Yucca Mountain environment.
- 3) Conduct batch tests on promising candidate tracers to evaluate suitability using a mass balance approach with consideration for time dependence.
- 4) Conduct column tests on promising candidate tracers with both crushed and intact core material and provide qualitative evaluations relative to bromide.
- 5) Conduct tests for degradation of candidate tracer compounds due to microbial action and chemical reactions under the environmental conditions anticipated at the C-well complex. Provide evidence for non-toxicity.
- 6) Prepare an appropriate level quality assurance program.
- 7) Report recommendations for field application of conservative organic tracers for hydrologic testing.

1.2 APPROACH

To successfully select a tracer or a suite of tracers for a specific application requires estimates of the length of time that tracers will reside in the subsurface and the volume of water that must be traced. These estimates must be provided by the USGS.

The identification of candidate conservative tracers (task 1) will be performed through a literature search and from previous experience with ground water tracers (1-5). Candidate tracers should meet as many of the criteria listed in the project description as possible. Under consideration at this time are fluorinated salicylic acids, pyridones and fluorescent whitening agents. All of these compounds fluoresce and can be detected at

extremely low levels. Another group of compounds that will be considered are the perfluorinated derivatives of acetic, propionic and butyric acids. These compounds are very inert and inexpensive, but are not as easily detected at low concentrations as other fluorinated acids. Other fluorinated organic acids with highly conjugated systems, that absorb strongly in the ultraviolet region will also be investigated.

It is anticipated that a significant portion of the tracer sample analysis during an actual test will be conducted in the field. For this reason the analytical procedures should be kept as simple as possible and be done with instrumentation rugged enough to be used in the field. An instrumental method that meets these requirements and is most convenient for analyzing water samples is High Performance Liquid Chromatography (HPLC) specifically, reversed phase HPLC. Reversed phase HPLC allows the tracer samples to be injected directly with no sample pretreatment other than filtration. The method can be used with fluorescence and ultraviolet absorption detectors and can be very sensitive with the appropriate tracers. For this reason, the major analytical efforts (task 2) will be directed towards the use of this method. The detection limits will be evaluated with fluids, either real or formulated to simulate the waters in the area of the C-well complex. Tracers that do not have suitable chromophores will be analyzed by HPLC-mass spectrometry (HPLC-MS). This technique will also be used for the identification of tracer degradation products.

The initial experiments to evaluate the suitability of the candidate tracers (task 3) will be batch stability tests that will test for both, but not differentiate between, chemical reactions including sorption) and biodegradation. The batch tests will be conducted with two water solutions and a number of water and soil mixtures. One of the water solutions will be distilled water for control purposes. The other water-only solution will be water obtained from the J-13 well. The water soil mixtures will be made up of crushed tuff from outcropping of tuff that will be in the tracer test horizon. The number and types of tuff will be determined with the aid of USDOE geologists. The concentration of the tracers used in these tests will be chosen for high analytical precision so that small reductions in concentration will be easy to recognize. The water and the tuff will be in a 1:1 weight ratio. Candidate tracers that show no significant reduction in concentration after 30 days will be used for further testing.

Since field tracer test chemicals and their degradation products may remain in the ground water for many years the laboratory tests will continue until the completion of the Yucca Mountain site characterization project to observe long term tracer stability and identify degradation products.

Column tests (task 4) will be conducted on all tracers that appear to be stable and conservative under conditions expected at the C-well complex. The column tests for this study will attempt to follow the procedures and methods used by Dr. Robert Bowman (1989). The columns will be constructed of plexiglas, about 30 cm in length and 5 cm in outside diameter, and will be packed with crushed core material obtained from the C-well corings or other suitable location from the Yucca Mountain area. The columns will be packed to a bulk density of about 1.5 grams per cubic centimeter. The exact bulk densities and core material mesh size will be determined later in discussions with the USGS and DOE. The columns will be fitted with stainless steel frits to keep the packing material in place. Solutions containing the tracer will be made of filtered water obtained from the C-wells. The concentration of tracer will be based upon results obtained from the batch studies. The linear flow rates through the column will be in the range of 30 cm per day, the actual flow rates will depend upon the pumps and the estimated linear velocity of the tracers in an actual test. A continuous UV or fluorescence monitor will be connected for real time analysis of the tracer solution as it comes off the column. The effluent from the continuous monitor will be directed to a fraction collector for later analysis by HPLC or other appropriate analytical instrumentation. Pulse injections of tracer, rather than a continuous infusion of traced solution, will be used as this method is more representative of the actual tracer experiments and is more convenient for repetitive (QA purposes) experiments. In addition to the tracer of interest, bromide will be added to every solution as a reference tracer. The concentration of tracer and the linear velocities of the solution will be varied to determine the effects of kinetics and to determine the capacity of the aquifer material.

Experiments with intact core will be performed in the manner similar to the one described above, but with the following qualifiers. The size of the column and therefore the size of the piece of core used will be dependent upon the hydraulic conductivity of the core. Since the hydraulic conductivity of tuff may be as low as 10^{-12} cm/sec the slice of core will have to be very thin to achieve a reasonable flow rate and such a thin slice may not very representative of the tuff on the C-well complex. The specific details of these experiments will be developed after consultation with the USGS and DOE.

Tracers that pass the batch test screening, task 3, will be tested more rigorously for chemical degradation, sorption and biodegradation. Tests will be conducted to determine the effects of temperature, oxygen, pH, microbial activity and surface area (task 5). These tests will be conducted in a batch mode and the

details of the chemical and biological degradation experiments will be worked out after sufficient data on the C-well core and water is made available.

The introduction of organic compounds into the subsurface at the proposed site of the Yucca Mountain Repository for use as tracers of groundwater movement may be affected by resident microorganisms. To assess the potential for degradation of the tracers by indigenous microbial populations, samples of the groundwater will be aseptically collected and amended with tracer. The concentration of tracer and the number of organisms per ml of water will be monitored over time. Control samples consisting of water without tracer and filtered water (to remove microorganisms) with and without tracer will also be monitored throughout the study. The test sampler will be incubated at in situ groundwater temperature under aerobic and/or anaerobic conditions.

The tracers will also be tested for mutagenicity using the Ames test. Ames testing uses specific strains of the bacterium *Salmonella typhimurium* selected for inability to grow without the addition of a particular amino acid. When exposed to a mutagen, however, the selected bacterium reverts to wild type and can grow in the absence of the amino acid.

A quality assurance program (task 6) will be developed under existing DOE and USGS guidelines. The program will include development of analytical protocols and set data quality objectives (DQOs). Included will be the number of standard and reference samples, duplicates and spike samples, relative standard deviation between replicate analysis, chain of custody and sample storage methods.

A final report (task 7) will be written that will cover the applicability as tracers of all compounds tested. Interim reports will be provided when significant findings for candidate tracers occur.

1.3 EXPERIMENTAL METHODS

1.3.1 Batch Tests (task 3)

Specific procedures covering the experimental methods are found in the Batch Test Task Quality Assurance Project Plan as well as the SOPs for the instruments used to analyze for the individual tracers.

1.3.2 Column Tests (task 4)

Specific procedures covering the experimental methods are found in the Column Test Task Quality Assurance Project Plan as well as the SOPs for the instruments used to analyze for the individual tracers.

1.3.3 Chemical and Biological Degradation Tests (task 5)

Specific procedures covering the experimental methods are found in the Chemical and Biological Degradation Test Task Quality Assurance Project Plan as well as the SOPs for the instruments used to analyze for the individual tracers.

1.3.4 Field Tests

If field tests are conducted with any of the tracers tested under this project, a Field Test Task Quality Assurance Project Plan will be written to cover the proposed work.

1.4 TIMELINE

An estimate of time required for each phase of the project and for the writing of the QA plan and final report is described in Table 1.

The schedule is approximate and may have to be modified depending upon the total number of potential tracers tested and the results of each testing Phase.

1.5 RESOURCE REQUIREMENTS

1.5.1 Personnel

See section 2.0.

1.5.2 Facilities

1.5.2.1 Laboratory facilities

All analytical chemistry work will be performed at the UNLV - ERC chemistry laboratory (with the possible exception of analysis of some samples from the field tests that will be done in the field). This laboratory is equipped with a variety of chemical instrumentation and equipment necessary to perform the analyses for the tracers.

1.5.2.2 Field Facilities

The location of field activities, if any, have as yet not been specified. Field activities will require climate controlled spaces with appropriate power to operate the analytical chemistry instrumentation and peripheral equipment.

1.5.3 Equipment and Instrumentation

Performing the batch and column tests will require routine laboratory equipment such as balances, volumetric glassware, pipettes etc. as well as sophisticated analytical chemistry instruments such as high performance liquid chromatographs (HPLC) with ultraviolet (UV) and fluorescence detectors and an HPLC-mass spectrometer (HPLC-MS).

Table 1

	90	91	92	93
	JJASOND	JFMAMJJASOND	JFMAMJJASOND	JFMAM
QA PLAN	XXXXXX			
BATCH TESTS	XXXXXXX	XXXXXXXXXXXXX	XXXXXX	
COLUMN TESTS		XXXXXXXXXXXXX	XXXXXXXXXXXXX	
TOXICITY TESTS		XXXX	XXXXXXXXXXXXX	
FIELD TESTS			XXXXXXXXXXXXX	XXX
FINAL REPORT				XXXXX

All of the times listed in table one are approximate. The actual time spent on each task will depend upon the total number of tracers that will be tested and the results of the tests. The tests will be sequential for individual tracers, and a tracer that does not pass the batch test will not be tested on the columns etc. The timing and length of the field tests will also depend upon availability and location of a suitable site.

SECTION 2.0

PROJECT ORGANIZATION AND RESPONSIBILITIES

2.1 KEY PERSONNEL (See Appendix A for qualifications.)

Project Manager/Principal Investigator:

Klaus Stetzenbach, ERC, Telephone: (702) 739-0840/3382

Investigators:

Tonya Dombrowski, ERC, Telephone: (702) 597-4120/739-3382

Tammy Jones, Graduate student, UNLV Department of Chemistry
U.S. Environmental Protection Agency, Environmental Monitoring
Systems Laboratory, P.O. Box 93478, Las Vegas, NV 89193-3478,
Telephone: (702) 798-2144, FTS 545-2144.

Kazamasu (Kaz) Lindley, ERC, Telephone: (702) 739-3382

Steve Ward, ERC, Telephone: (702) 597-4123/739-3382

Technicians:

Steve Butala, ERC, Telephone: (702) 597-4120/739-3382

Quality Assurance:

Amy Cross-Smiecinski, ERC, Telephone (702) 739-3742/739-3382

Kathy Lauckner, ERC, Telephone (702) 739-3142/739-3382

2.2 DIVISION OF RESPONSIBILITIES

Klaus Stetzenbach has the overall project technical lead and is also responsible for administrative and financial matters. The other investigators will be responsible for tasks as assigned by the project leader. A current (September 1990) ERC organizational chart is included in SECTION 25.

2.3 COMMUNICATIONS

Routes of communication basically follow the organizational scheme with project information flowing freely within ERC and formal communications moving through and between principal investigators and project managers. Formal external communication

travels from the project manager or his designee to the client or other outside entity.

2.4 DOCUMENT CONTROL

All QA plans, Standard Operating Procedures (SOPs), and other QA protocols developed for this project have the following identifying plate in the upper right hand corner of each page.

DOE Tracer Project
Revision No.
Date:
Page ____ of ____

"Revision number" is the numeric representation of the approved protocol revision. The original unrevised version is numbered 0. The "Date" indicates the date of the revision.

In all cases, employees possess only the most recent version of a protocol. A copy of each obsolete protocol is archived by the QA Officer for reference purposes.

Document control is applied to the following:

- * QA Program Plan
- * QA Project Plan
- * SOPs

The project manager Klaus Stetzenbach is responsible to see that personnel have current documents.

2.5 IDENTIFICATION OF SAMPLES

2.5.1 Collection

2.5.1.1 Collection of raw materials (tuff and water)

2.5.1.1.1 Tuff

Three types of tuff are used in the batch tests, light, medium and dark. These materials come from outcroppings of the formation in which the C-well tracer tests will be conducted. Determination of where these materials are collected are made by USDOE geologists. The actual collection is done by DOE and/or ERC research personnel.

The core material for the column tests comes from the Yucca Mountain Project (YMP) sample management facility. The origin of the core may be other than the C-wells, but will be from formations that are the same or similar to the ones in which the C-wells tracer tests will be conducted.

2.5.1.1.2 Water

Three types of water may be used for the batch and column tests. J-13 well water is obtained from the NTS in accordance with DOE and NTS procedures for collection. This water is then stored in the UNLV-ERC cold-room at 4°C in either glass or plastic containers.

Distilled water can be obtained from a variety of sources and should have a resistance greater than one megaohm.

Tap water will be water supplied by the Las Vegas Valley Water District, which has numerous sources and the chemistry of this water will vary accordingly.

2.5.1.2 Laboratory Samples

Samples from the batch and column tests will be collected according to good laboratory practice procedures as specified in the SOPs.

2.5.2 Identification

In order to insure that samples are identified and controlled in a manner consistent with their intended use, all sample labels shall include the following identification:

- Sample sequence number
- Date and time of collection
- Collection site

Initials of person collecting sample
Project name

All identification (labelling) methods shall be traceable to the appropriate documentation, such as laboratory notebooks and raw data.

All raw material samples are excluded from the labeling requirement until they are incorporated into an experiment.

2.5.3 Handling and Storage

All samples will be maintained in the laboratory and during transportation under conditions appropriate to the experimental and analytical conditions. J-13 water is stored in the UNLV-ERC cold-room at 4°C in either glass or plastic containers. Samples shall not be stored in an area that contains samples or reagents of high concentration which may cause contamination of the samples.

2.5.4 Control

At each sample process step, wet chemistry, analysis, etc., the information contained on the sample label will be used to identify associated data, extracts, etc.

2.5.5 Curation

After completion of the project, residual sample volume will be curated, until it is determined that no analyses need to be repeated. Samples will be curated for no longer than three years. Tracer samples may be discarded 60 days after chemical analysis or when analysis of the breakthrough curve is complete.

2.5.6 Records

A record of all samples, associated identification numbers, and circumstances of collection and handling are recorded in a laboratory notebook entitled DOE Tracer Project.

2.6 IDENTIFICATION AND CONTROL OF DATA

2.6.1 Generation

See section 13.

2.6.2 Identification

Data shall be labelled with the project name "DOE Tracer Project", name or initials of the data generator (e.g., the GC/MS analyst), the date, and the instrument or other source.

All data prepared from raw data shall possess the same label plus the name or initials of the subsequent data preparer, and the source of the data (e.g., log book number).

This information shall follow data in all of its forms, excluding formal reports, sufficiently to provide traceability among the data, analytical, wet preparation, and other components of the project data.

2.6.3 Handling and Storage

ERC QA Policy requires that all project data be stored in the Cooperative Agreement Files located in the ERC office or if too bulky, stored in the ERC's back storage room with a note of reference in the files. However, during data use, this will not be possible and the data will reside in the Environmental Chemistry Laboratory and in the offices of the various project personnel listed in Section 1.0.

Upon termination of each phase of the project, the data will be submitted to the QA officer and properly filed as described in the ERC QA Program Plan in the ERC Office project files or back storage room.

2.6.4 Transfer

The transfer of data from this project to other YMP researchers will be authorized by Dr. Stetzenbach on a case by case basis. Only photo copies or computer copies will be authorized for transfer.

2.6.5 Control

For the purpose of this project there are no controls for security purposes. The only other control is that Dr. Stetzenbach authorizes its release for proper intended uses.

2.6.6 Curation

All data produced and/or processed by the ERC for this project will be maintained in files as described in 2.6.3 for a minimum of three years from the termination of the project.

2.7 QA RECORDS

Records that furnish documentary evidence of quality and are prepared and maintained by ERC for this project are specified below. Records shall be distributed, handled, and controlled for a minimum of three years at ERC. Upon termination of the project or of a task, the following records will be submitted to the QA officer who will see that they are stored as described in 2.6.3.

Records shall be stored in offices/files or with personnel as indicated below for the duration of the project.

Cooperative Agreement File

QA Program Plan
QA Project Plan
Project Reports

QA Office Files

QA Program Plan
QA Project Plan
SOPs
Corrective Action Files
Audit Files

ERC Chemistry Laboratory DOE Tracer Project File

QA Program Plan
QA Project Plan
Project Reports
SOPs
Raw Data-hardcopy
Raw Data-magnetic
Audit Files
Corrective Action Files
Laboratory Notebooks

ERC Accounts Specialist Procurements

SOPs will also be available at each instrument (for which they will be written), so that operators will have them available while working on the DOE Tracer Project.

A completed record is a document that will either receive no more entries, or whose revision normally would consist of the reissue of the document and is signed and dated by the originator, and as applicable by other personnel authorized to approve the document.

SECTION 3.0

QUALITY ASSURANCE PROGRAM

The ERC QA Program for the YMP consists of ERC's QA Program Plan, this QA Project Plan, QA project plans of subcontractors, and all SOPs.

SECTION 4.0

QA OBJECTIVES AND ASSESSMENT PROCEDURES FOR MEASUREMENT DATA

4.1 OBJECTIVES

4.1.1 PRECISION

The precision or repeatability of the measurement process includes, but is not limited to the chemical nature of the sample, the sample matrix, sample concentration and the instrument(s) used to make the measurement. The precision that is obtained with each compound during this study will vary. The actual precision obtained will be one of many factors considered when evaluating the suitability of each potential tracer. The measurement precision of each compound will be evaluated throughout the proposed research program.

No corrective action will be taken based solely on the lack of precision or reproducibility. Corrective action i.e. repeating the analysis, will be done based on the investigators' experience with the instrument, tracer and the matrix of the sample.

4.1.2 ACCURACY

There are no traceable standards for the chemicals used for the batch and column experiments. Accuracy is therefore limited by the purity of the compound, the volumetric glassware and the calibration of the balance. Reference solutions (tracer solutions) in distilled water and J-13 water of each tracer are prepared for the batch tests. These solutions are analyzed with every sample batch. If the area counts vary more than 10% from the area counts obtained for the original analysis then a new solution of nearly identical concentration will be prepared and analyzed to determine if the original solution has degraded or the instrumental parameters have changed.

4.1.3 REPRESENTATIVENESS

Representativeness here concerns the conditions of the batch and column tests which are used to test the potential tracers. By using J-13 water, which is ground water from the general area of the C-well complex, and has been designated by DOE to be used for all studies involving Yucca Mountain ground water, and tuff from the formation of the planned tracer tests, the chemical environment of the C-well aquifer will be approximated.

4.1.4 COMPLETENESS

Completeness for the batch tests represents collecting data at scheduled intervals for a period of 60 days. If the tracer sorbs or is degraded before that time, data collection will stop for that potential tracer.

Completeness for the column tests collection of samples until 85% of the introduced mass is recovered for a tracer that does not sorb or is not degraded. Tracers that sorb or are degraded will have smaller percentages of recovery, and the experiment will be terminated, when in the estimation of the project leader, no further useful data will be obtained.

4.2 ROUTINE ASSESSMENT PROCEDURES

4.2.1 PRECISION

Precision will be calculated using a basic program called STDDEV (see Section 15)

4.2.2 ACCURACY

$$((A_0 - A)/A_0) \times 100$$

A_0 = original area counts
 A = current area counts

See section 4.1.2

4.2.3 REPRESENTATIVENESS

See Section 4.1.3

4.2.4 COMPLETENESS

See Section 4.1.4

SECTION 5.0

SITE SELECTION AND SAMPLING PROCEDURES

5.1 SAMPLING SITE SELECTION

5.1.1 TUFF

Three types of tuff are used in the batch tests, light, medium and dark. These materials come from outcroppings of the formation in which the C-well tracer tests will be conducted. Determination of where these materials are collected are made by USDOE geologists.

The core material for the column tests comes from the Yucca Mountain Project (YMP) sample management facility. The origin of the core may be other than the C-wells, but will be from formations that are the same or similar to the ones in which the C-wells tracer tests will be conducted.

5.1.2 J-13 WATER

J-13 well water is obtained from the NTS with the assistance of REECO personnel who are authorized to turn on the well. J-13 well water has been designated by DOE as the "standard" water to be used for experiments involving ground water.

5.2 SAMPLING SITE DESCRIPTION

5.2.1 TUFF

All tuff is obtained from the general vicinity of Yucca Mountain, Nevada, from outcroppings of the formations in which the C-well tracer tests will be conducted. The tuff core material may come from other locations on the NTS with similar formations.

5.3 SAMPLING PROCEDURES

5.3.1 TUFF

The tuff is collected by removing a manageable mass (with a sledge hammer or other suitable means) from larger sections. These material that is collected is then ground to a finer size to increase surface area and provide more uniform aliquots for individual experiments.

5.3.2 J-13 Water

J-13 water is collected from well J-13 at the Nevada Test Site. The well is purged prior to sample collection. The water is collected in an appropriate container, usually a clean 5 gallon plastic vessel.

5.3.3 Laboratory Samples

The description of how samples are collected from specific laboratory experiments is listed in the SOP used for that experiment.

SECTION 6.0

SAMPLE CUSTODY

All samples are stored in the ERC laboratory under conditions appropriate to the individual experiments and consistent with other laboratory functions. These samples are not considered to be sensitive or hazardous and no custody procedures are planned for this project.

SECTION 7.0

ANALYTICAL PROCEDURES

7.1 METHODS

High performance liquid chromatography (HPLC) and liquid chromatography - mass spectrometry (LC-MS) will be the primary instruments used to detect and quantify the tracers used in the tasks defined in this document. SOPs cover each specific procedure required to analyze tracers used in these tasks.

SECTION 8.0

CALIBRATION PROCEDURES AND FREQUENCIES

8.1 FREQUENCY

The frequency of calibration is done in accordance with manufacturers instructions (if specific calibration rates are listed in the instruments manual) and/or requirements set forth in the SOPs.

8.2 STANDARDS

The use of standards is covered in each SOP.

8.3 ACCEPTANCE CRITERIA

The instrument response is checked at a frequency covered in each SOP by analyzing one of the standards used to construct the initial calibration curve. The response of the standard must be within a range specified in the SOP for analyses to continue. The instrument is calibrated daily through analysis of a 3 or 4 point external standard curve. The correlation coefficient must be equal to or exceed the value specified in the SOP for that procedure.

SECTION 9.0

PREVENTIVE MAINTENANCE PROCEDURES AND SCHEDULES

9.1 SCHEDULE OF IMPORTANT PREVENTIVE MAINTENANCE TASKS

Measures have been established to insure that analytical balances, pH meters, instruments, and other measuring and test equipment used in activities that affect quality are controlled, calibrated, and adjusted properly at specified periods to maintain accuracy within necessary limits. SOPs have been composed where necessary for existing instrumentation, and will be written for new instrumentation as required.

9.1.1 ANALYTICAL BALANCE

Analytical balances are calibrated yearly by a contractor who has specific traceability to NIST. Further, analytical balances are calibrated with each use, with a weight from an S-Class set as described in the SOP. Each balance is labeled with the date of the next calibration and the contractor notifies ERC when the yearly calibration is approaching. All calibrations are recorded in the notebook or on the bench sheet that accompanies each balance.

Any deviation from calibration as determined routinely requires the user to label the balance as suspect and the laboratory director is immediately notified so that corrective action may be taken.

9.1.2 CHROMATOGRAPHIC INSTRUMENTATION

9.1.2.1 HPLC

The HPLCs are calibrated for flow rate at the factory. Any deviation from this factory calibration is accounted for by the routine use of standards as described in the HPLC SOP. Significant short term variability in flow rate will be detected by replicate analyses of samples and standards as outlined in the SOP, and by observation of trained operators.

9.1.2.2 FLUORESCENCE AND UV DETECTORS

The response of a detector depends upon many parameters, including certain settings on the detector and the power output of the lamp. The detector settings are covered by SOPs. Standards are used to compensate for long term variability in the lamp power

output. Short term power output variability will be detected by the use of replicate analyses.

9.1.2.3 MASS SPECTROMETER

The mass spectrometer will be calibrated according to manufacturers specifications and project SOPs.

9.2 CRITICAL SPARE PARTS

With most modern high technological instruments it is not feasible (financially) to keep all critical spare parts on hand. The spare parts that are kept on hand will depend upon instrument usage, cost of the part and availability from the supplier. Highly complex instrumentation such as the LC-MS will be purchased with service contracts contingent upon the availability of funds.

9.3 REFERENCE SERVICE CONTRACTS

A record of service contracts is available from the ERC Accounts Specialist.

SECTION 10.0

INTERNAL QC CHECKS

10.1 SAMPLES

10.1.1 Replicate Measurements

This refers to making multiple measurements on the same sample. This is covered in the SOPs for sample analysis.

10.1.2 Duplicates or Splits

Samples prepared in the same manner, with the same components but in separate containers. The use of duplicates is covered in the SOPs.

10.1.3 Collocated samples

Not Applicable.

10.2 SPIKED SAMPLES

10.2.1 Matrix spikes

Not Applicable.

10.2.2 Spiked blanks

Spiked blanks are distilled or J-13 water with tracer. Their use is covered in the SOPs.

10.2.3 Surrogates and internal standards

Not Applicable.

10.3 OTHERS

10.3.1 Standard Reference Materials

Not Applicable.

10.3.2 Blanks

10.3.2.1 Water Blanks

Distilled or J-13 water with no other components. Their use is covered in the SOPs.

10.3.2.2 Tuff Blanks

Distilled or J-13 water with tuff. Their use is covered in the SOPs.

10.3.3 Second column confirmation

Not Applicable.

10.3.4 Control charts

Control charts will be maintained for the balance.

10.3.5 Performance evaluation materials

Not Applicable.

10.3.6 Special project needs

None.

SECTION 11.0

PERFORMANCE AND SYSTEMS AUDITS

11.1 AUDIT SCHEDULES

At this time no audit schedules have been established. However, the general plan is that the ERC QA officer will perform systems audits of ERC projects yearly and provide materials for performance audits (if available) quarterly. Performance audit results will be discussed at systems audits.

All project activities are subject to planned and scheduled internal and external audits to assure that procedures and activities comply with the overall QA Program and to determine their effectiveness. Audits will provide an objective evaluation of quality-related practices, procedures, instructions, activities, and items, including the review of documents and records, to insure that the QA Program is effective and implemented properly.

The audits shall be performed in accordance with written procedures, using checklists, by the QA Officer and other appropriately trained personnel as necessary. Results of audits performed by the QA officer are to be reported to the principal investigator with copies to the director and the coop project manager. Results of audits performed by other ERC personnel are to be submitted to the principal investigator or director with copies to the director, coop project manager, and the QA officer. Corrective action shall be accomplished as addressed in Section 12.

All deficiencies, nonconformances, and potential quality problems identified during an audit are to be documented and monitored until verification of effective corrective action is made. That is, follow-up action, including verification of corrective action or re-audit of specific areas, shall be performed.

Management of the audited work group shall investigate adverse audit findings, and "give credit where credit is due" for good audit findings. In the case of adverse audit findings, the management shall determine root cause, schedule corrective action, including measures to prevent recurrence, and within 30 calendars days of receipt of the audit report, notify the QA Office and ERC management in writing of action taken or planned.

11.2 AUDIT REPORTING

The audit report shall be signed by the audit-team leader and issued within 7 calendar days. The report shall include the following information, as a minimum:

- * Scope of the audit.
- * Identification of the auditors.
- * Identification of persons contacted during audit activities.
- * Summary of audit results, including a statement of the effectiveness of the QA Program elements that were audited.
- * Description of each reported adverse audit finding in sufficient detail to enable corrective action to be taken by the audited work group.

11.3 AUDIT RECORDS

As a minimum, audit records shall include the following:

- * Identification of the entity or activities, or items audited and individuals contacted during the audits.
- * Description of any deficiencies, nonconformances, and potential quality problems identified.
- * Audit plans, audit reports, written replies, the record of completion of corrective action, and the close-out of the audit.
- * Records of personnel qualifications for auditors shall be established, updated annually, and maintained.

11.4 AUDIT PERSONNEL

11.4.1 Internal Audits

Internal audits will be performed by the ERC QA officer.

11.4.2 External Audits

External audits will be performed by the DOE QA officer or his designee and the ERC QA officer.

SECTION 12.0

CORRECTIVE ACTION

12.1 TRIGGER POINTS

Trigger points for corrective action are detailed in the SOPs for each procedure.

12.2 PERSONNEL

The ERC project manager shall be consulted if a situation arises that is not covered in the various QA plans or SOPs. If action must be taken before the project director can be consulted then the action shall be noted in the laboratory notebook and the project director shall be advised as soon as practical. Corrective actions shall be recorded in the laboratory notebooks for the QC indicators listed.

- * Exceeding calibration limits
- * Exceeding QC sample limits
- * Instrument/equipment failure
- * Laboratory notebook misuse

12.3 RESPONSE TO QA ACTIVITIES

Results of the following activities shall initiate a corrective action by project personnel which is documented by the project manager or principal investigator as described in the ERC QA Program Plan.

- * Performance audits
- * Systems audits
- * Interlab/Interfield Studies
- * Failure to adhere to or failure to document a deviation from the ERC QA Program Plan, this QAPjP, or a project SOP.

SECTION 13.0

DATA PROCESSING

13.1 Collection

13.1.1 Manual data collection

Procedures for the manual collection of data are written into the SOP for each instrument or procedure.

13.1.2 Computerized data collection

Procedures for the computerized collection of data are written into the SOP for each instrument or procedure.

13.2 Validation

The project staff shall review data as it is being generated and reported. Manual calculations will be spot-checked and initialled by the laboratory staff on a weekly basis and by the project director on a quarterly basis. Area counts of standards will be spot-checked by visual estimation by the laboratory staff on a weekly basis and on a quarterly basis by the project director. Laboratory notebooks will be spot-checked and initialled by the ERC QA officer on a monthly basis for errors and legibility. Transcribed data will be spot-checked by laboratory staff on a weekly basis.

13.2.1 Replicate analyses

In general, replicate analyses are used to determine the validity of the raw data. A minimum of two analyses are performed on each sample or standard. If the area counts agree within 5% then the data is accepted. If the difference between the replicate values is greater than 5% then a third analysis is performed.

The procedures are fully described in the SOP for each method, as well as exceptions to this general rule.

13.3 Outliers

13.3.1 Standards

The determination of an outlier for a standard will be made by analyzing the correlation data (minimum value for the correlation

coefficient is specified in the SOP). The outlier will first be reanalyzed, then, if necessary, prepared fresh. If this does not solve the problem the entire calibration curve will be rerun.

13.3.2 Samples

13.3.2.1 Replicate measurements

Outliers will be determined by comparing area counts for replicate measurements. If the two area counts do not agree within the percentage specified in the SOP then a third aliquot will be analyzed. All three values will be used to determine the mean unless one of the values can be rejected based on statistical information (see 13.3.2.3)

13.3.2.3 Statistical treatment of outliers

Outliers will be determined using any one of three methods listed in "Quality Assurance of Chemical Measurements" by John K. Taylor, Lewis Publishers, 1987, pages 33 - 36.

13.4 Data Reduction

Procedures for the reduction of data will be specified in the SOP for each instrument or procedure.

13.5 Reporting

The responsible individual is Klaus J. Stetzenbach, Ph.D., project director.

13.5.1 Flowchart of the data handling process

A flowchart of the data handling process is shown in figure 1.

Figure 1: DATA HANDLING PROCESS

SECTION 14.0

QA REPORTS TO MANAGEMENT

14.1 Report Types

Annual written reports will be prepared by the QA officer and the project manager and submitted to the ERC director, with copies sent to the DOE QA officer and the DOE project lead.

14.2 Reports Contents

Some of the topics that may be addressed in these reports are:

Summary of QA/QC programs, training, and accomplishments.

Results of technical systems and performance evaluation audits.

Significant QA/QC problems, recommended solutions, and results of corrective actions.

Data quality assessment in terms of precision, accuracy, representativeness, completeness and comparability.

Discussion of whether the QA objectives were met, and the resulting impact on technical conclusions.

Limitations on use of the measurement data, and discussion of the effects of such limitations.

14.2.1 Changes to QAPjP

This project plan will be reviewed and revised annually from the date of approval. The project manager will convene a meeting of project personnel and, with the aid of the QA staff, incorporate suggested changes into the document which in the text appear as italicized print. Items to be considered in the revision process are listed:

- * QC failures.
- * Documentation deficiencies.
- * GLP and traceability failures.
- * Added tasks.
- * Other agenda items.

SECTION 15.0

COMPUTER SOFTWARE

15.1 General

The computer software development and control program required by the YMP and based on guidance specified in NUREG-0856, Final Technical Position on Documentation of Computer Codes for High-Level Waste Management, June 1983 is not applicable to ERC's participation in YMP. Therefore the requirements of this criterion are excluded in this QA Project Plan.

However it is the intent of the ERC to assure the quality and integrity of any software or programs that are modified or written by personnel. It is a requirement then that all software that is modified or program that is written, that may impact the quality of data or findings generated by the ERC, be validated as specified in the ERC QA Program Plan for Scientific Investigations.

15.2 MINITAB Statistical Software

MINITAB Statistical Software, Release 7.2, DOS Microcomputer Version. From: Minitab Inc., 3081 Enterprise Drive, State College, PA 16801-2756 USA.

15.3 Linear correlation program

The linear correlation program is used to construct calibration curves to determine the concentration of tracer in the sample solutions.

15.3.1 Curvefit batch file

The curvefit batch file allows the Curve program to be called up without having to execute the standard basic commands to load and run the program.

15.3.1.1 Listing of Curvefit batch file

```
echo off
cls
gwbasic \basic\curve
echo on
mode co80
```

15.3.2 Curve(fit) basic program

```
1000 REM curvefit program written by Ken R. Lauver
1010 REM variable definitions (types)
1020 DEFDBL A-H,K,L,M,O,P,R-Z:DEFINT I,J,N,Q:DIM
X(100,2),UNDO(100,2):I=1:J=1:N=0:SUM=0:UNDO=0:KEY OFF:COLOR
15,1,1:CLS:B$=""
1030 DEF FNCALCONC(X)=A+B*X
1040 REM concentration of standards input
1050 PRINT "Concentration (;I;)"
1060 INPUT Q$:IF Q$="" THEN 1160 ELSE X(I,2)=VAL(Q$)
1070 N=N+1
1080 REM response to standards input
1090 PRINT "Response (;I;) #";J;
1100 INPUT Q$
1110 REM if end of user input then calculate average response
entered for the current concentration
1120 IF Q$="" THEN IF J=1 THEN PRINT "You haven't entered a
response yet!":GOTO 1090 ELSE X(I,1)=SUM/(J-1):PRINT "Average
response (;I;) = ";X(I,1):J=1:SUM=0:I=I+1:PRINT:GOTO 1050
1130 SUM=SUM+VAL(Q$):J=J+1
1140 GOTO 1090
1150 REM set up screen to display data user has input
1160 CLS:PRINT
1170 PRINT "VALUES THAT WILL BE USED FOR THE CURVE"
1180 PRINT
1190 PRINT "PAIR #          CONCENTRATION          RESPONSE"
1200 FOR I=1 TO N
1210 PRINT USING "    ###          #####.#####
#####.#####";I,X(I,2),X(I,1)
1220 NEXT I:PRINT
1230 REM ask user if a typing error exists
1240 PRINT "Is this correct (":COLOR 1,15:PRINT "Y":COLOR
15,1:PRINT "/":COLOR 1,15:PRINT "N":COLOR 15,1:PRINT ")? "
1250 Q$=INKEY$:IF LEN(Q$)=0 THEN 1250
1260 IF Q$="y" OR Q$="Y" THEN 2150
1270 REM set up edit mode screen
1280 CLS
1290 PRINT "CORRECTION OPTIONS TO CHOOSE FROM:":PRINT
1300 COLOR 1,15:PRINT "D":COLOR 15,1:PRINT "elete data pair",,
1310 COLOR 1,15:PRINT "C":COLOR 15,1:PRINT "hange data pair":PRINT
1320 COLOR 1,15:PRINT "S":COLOR 15,1:PRINT "how data for curve",,
1330 COLOR 1,15:PRINT "U":COLOR 15,1:PRINT "ndo last at
c'hange":PRINT
1340 COLOR 1,15:PRINT "A":COLOR 15,1:PRINT "dd data to curve",,
1350 COLOR 1,15:PRINT "N":COLOR 15,1:PRINT "ew curve":PRINT
1360 PRINT "E":COLOR 1,15:PRINT "x":COLOR 15,1:PRINT "it from
correction routine and calculate correlation "
1370 PRINT:PRINT "OPTION?":PRINT
1380 Q$=INKEY$:IF LEN(Q$)=0 THEN 1380
1390 REM branch to appropriate routine
```

```
1400 Q=ASC(Q$)
1410 IF Q=65 OR Q=97 THEN 1490:REM add data pair
1420 IF Q=68 OR Q=100 THEN 1640:REM delete pair
1430 IF Q=67 OR Q=99 THEN 1790:REM change pair
1440 IF Q=78 OR Q=110 THEN RUN:REM new curve
1450 IF Q=83 OR Q=115 THEN 2050:REM show data for curve
1460 IF Q=85 OR Q=117 THEN 1970:REM undo last edit change
1470 IF Q=88 OR Q=120 THEN 2140:REM exit from editing
1480 GOTO 1380
1490 REM add data pair
1500 FOR I=1 TO N
1510 UNDO(I,1)=X(I,1):UNDO(I,2)=X(I,2)
1520 NEXT I
1530 UNDO=1:INITN=N:J=1:SUM=0
1540 PRINT "Concentration (";N+1;")";
1550 INPUT Q$
1560 IF Q$="" THEN 1620
1570 X(N+1,2)=VAL(Q$):N=N+1
1580 PRINT "Response (";I;") #";J;
1590 INPUT Q$
1600 IF Q$="" THEN IF J=1 THEN PRINT "You haven't entered a
response yet!":GOTO 1090 ELSE X(I,1)=SUM/(J-1):PRINT "Average
response (";I;") = ";X(I,1):GOTO 1620
1610 SUM=SUM+VAL(Q$):J=J+1:GOTO 1580
1620 PRINT:PRINT "Press any key to continue..."
1630 Q$=INKEY$:IF LEN(Q$)=0 THEN 1630 ELSE 1280
1640 REM delete pair
1650 FOR I=1 TO N
1660 UNDO(I,1)=X(I,1):UNDO(I,2)=X(I,2)
1670 NEXT I
1680 UNDO=1:INITN=N
1690 PRINT "Data pair to delete";:INPUT Q$
1700 IF Q$="" THEN 1760
1710 IF VAL(Q$)<1 OR VAL(Q$)>N THEN PRINT:PRINT "Out of range":GOTO
1690
1720 I=VAL(Q$)
1730 PRINT "Delete concentration (";I;") = ";X(I,2);" Response
(";I;") = ";X(I,1);
1740 INPUT I$
1750 IF LEFT$(I$,1)="y" OR LEFT$(I$,1)="Y" THEN FOR J=I+1 TO
N:X(J-1,1)=X(J,1):X(J-1,2)=X(J,2):NEXT J:N=N-1:PRINT "Data pairs
";N:GOTO 1770
1760 PRINT "No data deleted."
1770 PRINT "Press any key to continue..."
1780 Q$=INKEY$:IF LEN(Q$)=0 THEN 1780 ELSE 1280
1790 REM Change pair
1800 IF N=0 THEN PRINT "There isn't any data to change.":PRINT
:PRINT "Press any key to continue..."
1810 IF N=0 THEN Q$=INKEY$:IF LEN(Q$)=0 THEN 1810 ELSE 1280
```

```
1820 PRINT "Index pair to change";:INPUT Q$
1830 IF Q$="" THEN 1280
1840 IF VAL(Q$)<1 OR VAL(Q$)>N THEN PRINT:PRINT "Out of range":GOTO
1820
1850 I=VAL(Q$)
1860 FOR Q=1 TO N
1870 UNDO(Q,1)=X(Q,1):UNDO(Q,2)=X(Q,2)
1880 NEXT Q
1890 INITN=N:UNDO=1
1900 PRINT "Concentration (;I;) = ";X(I,2);" Response (;I;) =
";X(I,1)
1910 PRINT "New Concentration (;I;) = ";:INPUT X
1920 X(I,2)=X:J=1:SUM=0
1930 PRINT "New Response (;I;) #";J;
1940 INPUT Q$
1950 IF Q$="" THEN X(I,1)=SUM/(J-1):PRINT "Average Response (;I;)
= ";X(I,1):GOTO 1280
1960 SUM=SUM+VAL(Q$):J=J+1:GOTO 1930
1970 REM Undo last change
1980 IF UNDO<>1 THEN PRINT "You have not made any changes to the
data yet!":PRINT "Press any key to continue..."
1990 IF UNDO<>1 THEN Q$=INKEY$:IF LEN(Q$)=0 THEN 1990 ELSE 1280
2000 FOR Q=1 TO INITN
2010 X(Q,1)=UNDO(Q,1):X(Q,2)=UNDO(Q,2)
2020 NEXT Q
2030 N=INITN
2040 GOTO 1280
2050 REM Show curve data
2060 PRINT "VALUES THAT WILL BE USED FOR THE CURVE"
2070 PRINT,
2080 PRINT "PAIR #          CONCENTRATION          RESPONSE"
2090 FOR I=1 TO N
2100 PRINT USING "    ##          #####.#####
#####.#####";I,X(I,2),X(I,1)
2110 NEXT I:PRINT
2120 PRINT "Press any key to continue..."
2130 Q$=INKEY$:IF LEN(Q$)=0 THEN 2130 ELSE 1280
2140 REM Exit edit
2150 REM Regression calculation
2160 REM slope calculation
2170 B1=0#:FOR I=1 TO N:B1=B1+X(I,1)*X(I,2):NEXT I
2180 B21=0#:B22=0#:B2=0#
2190 FOR I=1 TO N:B21=B21+X(I,1):B22=B22+X(I,2):NEXT I
2200 B2=B21*B22/N
2210 B3=0#:FOR I=1 TO N:B3=B3+X(I,1)*X(I,1):NEXT I
2220 B4=0#:FOR I=1 TO N:B4=B4+X(I,1):NEXT I:B4=B4*B4/N
2230 B=(B1-B2)/(B3-B4)
2240 REM Calculate intercept
2250 A1=0#:FOR I=1 TO N:A1=A1+X(I,2):NEXT I:A1=A1/N
```

```

2260 A2=0#:FOR I=1 TO N:A2=A2+X(I,1):NEXT I:A2=A2/N
2270 A=A1-B*A2
2280 REM calculate correlation
2290 R1=0#:FOR I=1 TO N:R1=R1+X(I,2)*X(I,2):NEXT I
2300 R2=0#:FOR I=1 TO N:R2=R2+X(I,2):NEXT I:R2=R2*R2/N
2310 RSQ=((B1-B2)*(B1-B2))/((B3-B4)*(R1-R2))
2320 RSQ2=RSQ^.5
2330 REM display regression results to user to see if they are
satisfactory
2340 CLS
2350 PRINT USING "Correlation Coefficient" =
##.#####";RSQ2
2360 PRINT USING "Coefficient of Determination" =
##.#####";RSQ
2370 PRINT USING "Slope" =
#####.#####";B
2380 PRINT USING "Intercept" =
#####.#####";A
2390 PRINT
2400 PRINT " Standard Response
Calculated Percent "
2410 PRINT " Concentration
Concentration Deviation"
2420 PRINT
2430 FOR I=1 TO N
2440 IF X(I,2)=0 THEN PRINT USING "#####.###
#####.### Unable to
compute";X(I,2),X(I,1),FNCCALCONC(X(I,1)):GOTO 2460
2450 PRINT USING "#####.###
#####.###
# # # # # # # # # # # # # # # #
#####.#####";X(I,2),X(I,1),FNCCALCONC(X(I,1)),-(X(I,2)-FNCCALCONC(X(
I,1)))/X(I,2)*100
2460 NEXT I
2470 PRINT :PRINT "Is the correlation coefficient for this curve
satisfactory (":COLOR 1,15:PRINT "Y";:COLOR 15,1:PRINT "/":COLOR
1,15:PRINT "N";:COLOR 15,1:PRINT ")? "
2480 Q$=INKEY$:IF LEN(Q$)=0 THEN 2480
2490 IF Q$="y" OR Q$="Y" THEN 2570
2500 IF Q$<>"N" AND Q$<>"n" THEN BEEP:GOTO 2470
2510 PRINT:COLOR 1,15:PRINT "E";:COLOR 15,1:PRINT "dit or ":COLOR
1,15:PRINT "Q";:COLOR 15,1:PRINT "uit":REM NOT SATISFACTORY
2520 Q$=INKEY$:IF LEN(Q$)=0 THEN 2520
2530 IF Q$="E" OR Q$="e" THEN CLS:GOTO 1290
2540 IF Q$="Q" OR Q$="q" THEN CLOSE:SYSTEM
2550 BEEP:GOTO 2510
2560 REM output to a file or printer
2570 PRINT:PRINT "Output to ":COLOR 1,15:PRINT "P";:COLOR
15,1:PRINT "rinter or ":COLOR 1,15:PRINT "F";:COLOR 15,1:PRINT
"ile"

```

```

2580 Q$=INKEY$:IF LEN(Q$)=0 THEN 2580
2590 IF Q$="P" OR Q$="p" THEN F$="LPT1":FILE$=F$:GOTO 2620
2600 IF Q$="F" OR Q$="f" THEN PRINT:PRINT "Filename";:INPUT
F$:FILE$=F$:GOTO 2620
2610 BEEP:GOTO 2570
2620 PRINT:CLOSE:OPEN FILE$ FOR APPEND AS #1
2630 LINE INPUT "Title for output? ";T$
2640 PRINT:PRINT "Blank correction (";:COLOR 1,15:PRINT "Y";:COLOR
15,1:PRINT "/";:COLOR 1,15:PRINT "N";:COLOR 15,1:PRINT ")?";
2650 Q$=INKEY$:IF LEN(Q$)=0 THEN 2650
2660 IF Q$<>"Y" AND Q$<>"y" THEN BLANK=0:B$="":GOTO 2750
2670 J=1:BLANKSUM=0:BLANK=0
2680 PRINT:PRINT "Blank correction response (";J;")";:INPUT BLANK$
2690 IF BLANK$="" THEN BLANK=BLANKSUM/(J-1):PRINT:PRINT "Average
Blank correction response ";BLANK;:GOTO 2710
2700 BLANKSUM=BLANKSUM+VAL(BLANK$):J=J+1:GOTO 2680
2710 PRINT:PRINT:PRINT "Blank subtraction with value ";:COLOR
0,7:PRINT "b";:COLOR 7,0:PRINT "efore or ";:COLOR 0,7:PRINT
"a";:COLOR 7,0:PRINT "fter FITTING to the curve?"
2720 B$=INKEY$:IF LEN(B$)=0 THEN 2720
2730 IF B$="b" OR B$="B" OR B$="a" OR B$="A" THEN 2750
2740 BEEP:GOTO 2710
2750 PRINT:PRINT:PRINT "Dilution factor";:INPUT DILFACT
2760 PRINT:LINE INPUT "Conversion units for the dilution factor?
";U$
2770 REM output to the output device
2780 PAGE=1:LC=0
2790 PRINT #1,T$;" CURVE PAGE";PAGE:PRINT #1,
2800 PRINT #1,USING "Correlation = #####";RSQ
2810 PRINT #1,USING "Slope = #####";B
2820 PRINT #1,USING "Intercept = #####";A
2830 PRINT #1,
2840 PRINT #1, " Standard Response
Calculated Percent "
2850 PRINT #1, " Concentration
Concentration Deviation"
2860 PRINT #1,
2870 FOR I=1 TO N
2880 IF X(I,2)=0 THEN PRINT #1,USING "#####.###
#####.### Unable to
compute";X(I,2),X(I,1),FNCALCONC(X(I,1)):GOTO 2900
2890 PRINT #1,USING "#####.### #####.###
# # # # # # # # # # . # # #
####.#####";X(I,2),X(I,1),FNCALCONC(X(I,1)),-(X(I,2)-FNCALCONC(X(
I,1)))/X(I,2)*100
2900 NEXT I
2910 PRINT #1,:LC=LC+10+N
2920 IF BLANK=0 THEN PRINT #1,"Blank correction factor is
";BLANK:LC=LC+1:GOTO 2950

```

```
T      H      E      N      P      R      I      N      T
#1, "*****
*****":RUN
```

```
3160 IF ID$="new curve" OR ID$="NEW CURVE" THEN IF Q$="F" OR Q$="f"
THEN PRINT #1,CHR$(12); ELSE 3130
3170 IF ID$="new curve" OR ID$="NEW CURVE" THEN RUN
3180 IF ID$="QUIT" OR ID$="quit" THEN CLOSE:COLOR ,,0:SYSTEM
3190 IF ID$="edit" OR ID$="EDIT" THEN 1280
3200 REM
3210 IF ID$="HELP" OR ID$="help" THEN CLS:PRINT" H E L P   S C R E
E N":PRINT:PRINT "BAD ID   Lets you reenter a sample id.  SAMPLE
RESPONSE"
3220 IF ID$="HELP" OR ID$="help" THEN PRINT :PRINT "EDIT  Places
you in the edit mode.  COMMAND"
3230 IF ID$="HELP" OR ID$="help" THEN PRINT :PRINT "LAST BAD
Removes the last sample entered from the printer buffer.  COMMAND"
3240 IF ID$="HELP" OR ID$="help" THEN PRINT:PRINT "HELP  Displays
this useless information.  COMMAND"
3250 IF ID$="HELP" OR ID$="help" THEN PRINT:PRINT "NEW CURVE
Restarts the program.  COMMAND"
3260 IF ID$="HELP" OR ID$="help" THEN PRINT:PRINT "NEW DF  Change
the dilution factor and its units.  COMMAND"
3270 IF ID$="HELP" OR ID$="help" THEN PRINT:PRINT "QUIT  Leave the
program.  COMMAND":PRINT:GOTO 2990
3280 PRINT "Sample Response";
3290 INPUT SAMRES$
3300 IF SAMRES$="BAD ID" OR SAMRES$="bad id" THEN 2990
3310 IF SAMRES$="" THEN 3280
3320 SAMRES=VAL(SAMRES$)
3330 IF B$="" OR B$="B" OR B$="b" THEN
SAMCONC=FNCALCONC(SAMRES-BLANK)
3340 IF B$="A" OR B$="a" THEN
SAMCONC=FNCALCONC(SAMRES)-FNCALCONC(BLANK)
3350 USEFLAG=1:IDL$=ID$
3360 PRINT:GOTO 3010
3370 END
```

15.4 Standard deviation program

The standard deviation program is used to calculate the standard deviation for a specific method or analytical procedure.

15.4.1 Standard deviation program batch file

The "STDDEV" batch file allows the standard deviation program to be called up without having to execute the standard basic commands to load and run the program.

15.4.1.1 Listing of the Standard Deviation program batch file

```
cls
gwbasic \basic\stddev
```


mode co80

15.4.2 STDDEV basic program

```
10 KEY OFF:COLOR 15,4,4:CLS
20 GOTO 80
30 PRINT
40 PRINT"*****"
50 PRINT"*****"
60 PRINT" "
70 PRINT" "
80 LOOP=1:REPSUM=0:SQDIF=0
90 TSUM=0
100 PRINT "DO YOU WANT A HARD COPY?      YES or NO";
110 INPUT Z$
120 IF Z$="QUIT" OR Z$="quit" THEN COLOR ,,0:SYSTEM
130 IF Z$="END" OR Z$="end" THEN 670
140 IF Z$="yes" OR Z$="YES" THEN PRINT "Do you want a name for the
hard copy?" ELSE 190
150 INPUT N$
160 LPRINT N$
170 LPRINT ""
180 LPRINT""
190 SUM=0:PRINT "Sample #";LOOP
200 IF Z$="yes" OR Z$="YES" THEN LPRINT "Sample #";LOOP
210 PRINT "Number of Replicates= ";
220 IF Z$="yes" OR Z$="YES" THEN LPRINT "Number of Replicates= ";
230 INPUT REP$
240 IF Z$="yes" OR Z$="YES" THEN LPRINT REP$
250 IF REP$="" THEN 460 ELSE REP=VAL(REP$):REPSUM=REPSUM+REP
260 IF REP$="QUIT" OR REP$="quit" THEN COLOR ,,0:SYSTEM
270 IF REP$="END" OR REP$="end" THEN 670
280 FOR I=1 TO REP
290 PRINT "Replicate #";I;
300 IF Z$="yes" OR Z$="YES" THEN LPRINT "Replicate #";I;
310 INPUT REPVAL
320 IF Z$="yes" OR Z$="YES" THEN LPRINT REPVAL
330 IF REPVAL=0 THEN 670
340 REP(I)=REPVAL
350 SUM=SUM+REPVAL
360 NEXT I
370 MEAN=SUM/REP
380 TSUM=TSUM+SUM
390 FOR I=1 TO REP
400 SQDIF=SQDIF+(MEAN-REP(I))^2
410 NEXT I
420 LOOP=LOOP+1
430 IF Z$="yes" OR Z$="YES" THEN LPRINT
440 PRINT
```

```
450 GOTO 190
460 PRINT ""
470 PRINT ""
480 PRINT"*****"
490 PRINT""
500 IF Z$="yes" OR Z$="YES" THEN LPRINT""
510 IF Z$="yes" OR Z$="YES" THEN LPRINT""
5 2 0   I F      Z $ = " y e s "      O R      Z $ = " Y E S "      T H E N
LPRINT"*****"
530 IF Z$="yes" OR Z$="YES" THEN LPRINT""
540 STDDEV=(SQDIF/(REPSUM-(LOOP-1)))^.5
550 PRINT "Std Dev=";STDDEV
560 IF Z$="yes" OR Z$="YES" THEN LPRINT "Std Dev=";STDDEV
570 PRINT "Degrees of freedom=";REPSUM-(LOOP-1)
580   I F      Z $ = " y e s "      O R      Z $ = " Y E S "      T H E N   L P R I N T      "Degrees   of
freedom=";REPSUM-(LOOP-1)
590 PRINT"Mean=";TSUM/REPSUM
600 IF Z$="yes" OR Z$="YES" THEN LPRINT "Mean=";TSUM/REPSUM
610 IF Z$="yes" OR Z$="YES" THEN LPRINT""
620 IF Z$="yes" OR Z$="YES" THEN LPRINT""
6 3 0   I F      Z $ = " y e s "      O R      Z $ = " Y E S "      T H E N
LPRINT"*****"
6 4 0   I F      Z $ = " y e s "      O R      Z $ = " Y E S "      T H E N
LPRINT"*****"
650 IF Z$="yes" OR Z$="YES" THEN LPRINT""
660 GOTO 30
670 KEY ON
680 END
```

SECTION 16.0

DESIGN CONTROL

All YMP projects require the development, review, and approval of planning documents prior to work initiation. Guidelines for the preparation of detailed technical procedures are given in Section 7.

SECTION 17.0

PROCUREMENT DOCUMENT CONTROL

Measures shall be established to insure that applicable regulatory requirements are included or referenced in the documents for procurement of material, equipment, and services used on the project. If necessary, UNLV procurement documents will require subcontractors to provide a QA program that is consistent with the pertinent provisions of this QA Project Plan.

SECTION 18.0

INSTRUCTIONS, PLANS, PROCEDURES, AND DRAWINGS

All laboratory work including preparation of standards, preparation of standards and solvents and sample analyses shall be recorded in the analysts notebook. In every case where findings and data are recorded the date and the investigator's initials and the date must be recorded with the observation. Records shall be in ink and deletions shall be made by a single line-through and initials/date of the change. The use of correction fluid is prohibited in notebooks and on data sheets. In cases where observations were recorded on extraneous paper, that paper should be permanently affixed to the notebook or sheet, rather than transferred, to avoid errors.

18.1 LABORATORY NOTEBOOKS

Experimental findings and details of this project are recorded in laboratory notebooks obtained from the UNLV Central Stores using the text Writing the Laboratory Notebook, available from ERC's QA officer. Specific laboratory notebooks pertaining to this project include:

- Instrument Maintenance Log
- Instrument Injection Log
- Sample Control
- Sample Processing

18.2 BENCH SHEETS

At this time there are no routine repetitive procedures requiring the use of bench data sheets.

18.3 PLANS AND DRAWINGS

At this time it is not planned that this project will use plans and drawings.

SECTION 19.0

CONTROL OF PURCHASED ITEMS AND SERVICES

GENERAL PURCHASING REQUIREMENTS

Purchases of material, equipment, and services conform to UNLV procurement documents (Title 4, Chapter 3).

SECTION 20.0

CONTROL OF PROCESSES

The activities associated with the ERC studies do not include processes that need to be controlled in the sense of this criterion.

SECTION 21.0

INSPECTION

The activities associated with the ERC studies do not require verification of conformance against standards or specified requirements, as specified in this criterion. Those activities requiring audits are described in this QAPjP.

SECTION 22.0

TEST CONTROL

The requirements of this section apply to engineered items and do not apply to scientific investigation activities. The requirements of this criterion are therefore excluded in this QA Project Plan.

SECTION 23.0

INSPECTION, TEST, AND OPERATING STATUS

The requirements of this section apply to engineered items and do not apply to scientific investigations. The requirements of this criterion are omitted from this QA Project Plan.

SECTION 24.0

CONTROL OF NONCONFORMING CONDITIONS

The requirements of this section apply to engineered items and do not apply to scientific investigations. The requirements of this criterion are omitted from this QA Project Plan.

SECTION 25.0 Environmental Research Center

Ellen M. Koivisto; Management Assistant II
Leslie E. Gorr; Word Processing Operator II
Carol L. Forsythe; Management Assistant II
Dorothy I. Adashefski; Administrative Aid

Patricia M. Baldwin; Grants & Contracts Manager
Jane A. Kappelman; Accounting Specialist
Ruby D. Bynum; Accounting Clerk II
Scott J. Lewandowski; Accounting Clerk II

Delbert S. Barth; Ph.D., Director
Edward J. Pozniomick; Ph.D., Sr. Scientist
Morton S. Bedinger; B.S., Geohydrologist

Technical Support

A. Smircinski; M.S., Director
Quality Assurance Officer
S. Burns; B.S.
Graphics Illustrator
K. Lauckner; M.F.E.
Quality Assurance Specialist
M. Schnell; B.A.
Technical Writer

Science Policy

G. Schweitzer; M.S., Director
Nuclear Engineer

Physical Sciences

K. Stetzenbach; Ph.D., Director
Environmental Chemist
S. Butala
Laboratory Technician
D. Diebel; Ph.D.
Physicist
I. Dombrowski; B.S.
Chemist
C. Fitzsimmons; M.S.
Environmental Scientist
R. Haas
Instrument Technician
V. Hodge; Ph.D.
Inorganic Chemist
R. Turner; M.S.
Electronics Engineer
R. Viswanathan; M.S.
Meteorologist
S. Ward; B.S.
Chemist

Biological Sciences

J. Santolucito; Ph.D., Director
Physiologist
D. Baepier; Ph.D., D.S.
Zoologist
M. Buttner; M.S.
Microbiologist
K. Lindley; M.S.
Chemist/Immunoassay
J. Meldrum; M.S.
Microbiologist
C. Palmer; Ph.D.
Soil Scientist
S. Paulsen; Ph.D.
Limnologist
L. Fifer; B.S.
Microbiology Lab Tech
W. Pratt; Ph.D.
Taxonomist
M. Saleh; Ph.D.
Biochemist
L. Stetzenbach; Ph.D.
Microbiologist
P. Vaux; Ph.D.
Limnologist

Environmental Statistics and Modeling

J. Flueck; Ph.D., Director
Senior Statistician
B. Conkling; Ph.D.
Soil Chemist
E. Furtaw, Jr.; B.E./P.E.
Environmental Engineer
D. Gonzales; B.S.
Systems Analyst
T. Lange; M.S.
Database Manager
M. Pandian; Ph.D.
Chemical Engineer
P. Phalke; M.S.
Agricultural Engineer
A. Singh; Ph.D.
Statistician
A. Sparks; B.S.
Applications Programmer
T. Starks; Ph.D.
Statistician
D. Weber; Ph.D.
Geophysicist

Earth Sciences

D. Trexler; M.S., Director
Geologist
P. Buchanan; B.S.
Geologist
T. Flynn; M.S.
Geologist
S. Wehrkamp
Management Assistant

Associates

D. Devitt; Ph.D.
Environmental Scientist
E. Hall; Ph.D.
Meteorologist
W. Jury; Ph.D.
Soil Physicist
D. Kreamer; Ph.D.
Hydrologist
G. Rupp; M.S./P.E.
Environmental Engineer

UNLV Students on Call

L. Hendricks
J. Mathews
S. MacMillan
M. Plyler
D. Poplin
E. Schmidt
R. Stockton
M. Wright

Anthropological Studies

L. Blair; M.S., Director
Research Specialist
S. Edwards; B.A.
Archaeologist
M. Kimball; M.A.
Archaeologist
J. Matthews; B.A.
Archaeologist
M. Roberson; B.A.
Archaeologist

Revised 11/90

REFERENCES

1. Stetzenbach, K. J., G. M. Thompson, and B. McCully. 1981. The Use of Fluorinated Organic Acids and Pseudo Halides as Hydrologic Tracers. Part I: Analytical Methods, Part II: Field Results. Rocky Mountain Groundwater Conference, Laramie, Wyoming.
2. Thompson, G. M.. 1982. Report to Rockwell Hanford Operations, Richland, Washington.
3. Walter, G. R., Bentley, H. W., Ward, J. J. and Gonzalez, D. D.. 1983. Field Characterization of Groundwater Transport Properties at the WIPP- SENM: Problems and Progress.
4. Davis, S. N., Campbell, D. J., Bentley, H. W. and Flynn, T. J.. 1985. Ground Water Tracers. National Water Well Association.
5. Thompson, G. M., and K. J. Stetzenbach. 1980. Assessment and Advances in Tracer Technology. A Topical Report to the Nuclear Regulatory Commission. Contract No. NRC-04-78-275.
6. Bowman, R. New Mexico Institute of Mining and Technology, Socorro, New Mexico, 1989 "Sorpton and Transport of Tritium and Anionic Tracers in Tuff."

APPENDIX A
PROJECT PERSONNEL QUALIFICATIONS

AUTOSAMPLER START-UP PROCEDURES

1.0 GENERAL INFORMATION

General instructions for the operation of the Spectra-Physics model SP8880/8875 Autosampler are given in the manual for the instrument. The instructions in this SOP are intended only for those individuals that have already familiarized themselves with the manual and have been instructed by the principal investigator, or other qualified personnel, in the use of this instrument.

All Autosampler functions can be controlled through the integrator keyboard when the Autosampler is connected through the Labnet software system. (Information on programming the Autosampler without the use of the Labnet software system can be found on page 3-5 of the Autosampler Operators manual.)

1.1 Autosampler Start-up

Ensure that the solvent reservoir is full (or contains enough solvent to complete the scheduled work). Methanol, or a solvent appropriate to the analysis (generally the strongest eluting solvent) should be used. This solvent is used to rinse the injection needle after each sample injection during a run.

1.1.1 Helium Purge

Helium purging is required to remove dissolved gasses from the solvent. Helium purging should be started approximately ten minutes prior to starting the autosampler.

The helium used as a purge gas is also used by the Autosampler to clean the remaining solvent from the syringe after each injection, and at the end of a run.

1.1.2 Autosampler power-up

Switch Autosampler to ON, and check that all other Labnet devices, (integrator, pump, etc.) are also ON, before powering up the computer. This allows the Labnet system to address all peripheral devices at initial start-up. As soon as power to the Autosampler is turned on, the tray turntable does a vial search to orient itself. Allow the Autosampler to run until the message

1.01 n.n (where n.n is the time from start-up)

appears in the LED display. The "Autosampler busy" light will go out shortly after the clock time appears.

1.1.3 Syringe check

Helium purging does not remove bubbles from the portion of solvent that is in the syringe barrel. If bubbles are apparent in the syringe barrel, the syringe must be flushed with solvent to remove them. This may be accomplished by pressing the "FLUSH" "8" "ENTER" keys. Wait for the system to flush itself. (Approximately eight minutes.) The bubbles in the syringe should be completely eliminated.

1.1.4 Vial preparation and loading

The Autosampler contains four vial trays which hold twenty vials each, as well as one position for a single "priority vial" (position 5.01, for more information see pages 4-1 and 4-2 of the Autosampler Operators Manual), giving a total capacity of eighty one vials.

1.1.4.1 Vial preparation

Two ml vials should be used with the current vial trays. These vials should NOT be filled above the straight sides of the vial, giving a sample volume of roughly 1.5 to 1.75ml. Two types of septa are available for use with the two ml vials: teflon, and teflon-faced silicone. When using the teflon-faced septa, be sure that the white, silicone side is visible through the vial cap. DO NOT overtighten the caps. Tighten only until the septum is snug. The teflon septa do not seal behind the needle after the septum is punctured. They should not be used with highly volatile or hazardous samples.

1.1.4.2 Tray loading

When loading filled vials, some record should be kept of sample placement as the Autosampler will only designate the sample injected by its vial position number, not the sample name.

If the calibration function is not being used, calibration standards should be placed at the beginning and end of each analyte sequence to give accurate initial calibration values, and final calibration checks. For analyses lasting longer than two hours, (not counting the calibration runs), additional calibration standards should be placed into the sampling sequence at two hour intervals.

For example: If ten samples were being run, each with three injections per vial, and a run time of ten minutes; a calibration standard should be placed in the tray after every four sample vials. Three runs at ten minutes each gives a thirty minute total run time for each sample. Four samples will then take two hours to analyze.

1.1.4.3 Tray placement

Each tray may be removed for easier loading. To replace the trays correctly, the tray must be placed on the carousel so that the small metal locating pin is properly seated in the opening in the tray. The pin marks the number one position of each tray so that the Autosampler can orient correctly throughout the run. Trays may be exchanged from position to position without problem, as long as they are seated correctly on the locator pin.

The door of the Autosampler must be shut completely for the Autosampler to orient to the correct vial position, and start or continue a run. Opening the Autosampler door during a run will not cause a fault or exit in the program, but the next injection in sequence will be delayed until the door is closed once more.

1.1.5 Creating/Editing Autosampler files

To set up a new Autosampler file, or edit an existing file, establish communication between the Labnet system and the Autosampler by using the command:

```
/1/ (Enter)  
DIALOG1 (Enter)
```

1.1.5.1 Solvent file entries

After the above commands have been made, the message:

```
SOLVENT FILE n  
ERASE FILE?
```

will appear, where n is the file number being used by the HPLC pump. The number of the solvent file being edited must be identical to the Autosampler file number. Typing N will give the following response:

TIME	%A	%B	%C	FLOW
------	----	----	----	------

(Pressing the ENTER key at this point will leave the current solvent parameters intact, and will skip this section of the dialogue.) To set up a solvent file or edit the existing file, enter the initial start time for the solvent run, usually 0.000, and press enter. If a solvent mix has been entered previously for this time, the values will be printed under the respective headings. New values can be entered by typing the time again and pressing enter. The printer head will stop at each of the solvent columns and allow values to be entered. Flow rate can also be entered or altered at this time.

It is very important that an end run time be entered, even

when using an isocratic solvent mix. This allows the pump to send an END OF RUN signal to the Autosampler so the next injection can occur. With an isocratic solvent file, an end run time may be specified simply by giving two time lines with the same solvent mix. For example:

TIME	%A	%B	%C	FLOW
0.00	0.00	40.0	60.0	1.00
5.00	0.00	40.0	60.0	1.00

This will send an END OF RUN signal to the Autosampler after five minutes, without changing the solvent mixture. Without the END OF RUN signal, the Autosampler will wait indefinitely to perform the next injection, as it requires an END OF RUN signal from all devices on line to start the next injection.

After all solvent values have been entered, depress the ENTER key once to give the message:

MAX. PRESS. LIMIT (nnnnPSI) = (nnnn equals some numeric value)

The desired pressure can be entered using the numeric keys followed by the ENTER key, or the current value can be accepted by pressing ENTER after the equal sign. (4500PSI is recommended, 6000PSI is the default value.) The message:

MIN. PRESS. LIMIT (nnnnPSI) = (nnnn equals some numeric value)

is then printed. The desired pressure can be entered using the numeric keys followed by the ENTER key, or the current value accepted by pressing ENTER after the equal sign. (50PSI is recommended, 0.00PSI is the default value.) The message:

VOLUME DELAY (nnnnML) = (where nnnn equals some numeric value)

is printed, and may be replaced with the desired value, or accepted as outlined above. (0.00ML is the default value.) Pressing ENTER once more gives:

END OF SOLVENT FILE DIALOG

1.1.5.2 Injector file entries

After the solvent file dialogue is complete, the following query is made:

INJECTOR FILE n ERASE? (Y OR N)

(where n is the file number.) Pressing N or ENTER at this point will yield the following response:

EQUILIBRATION TIME (nnnn) (where n is some time value)

Values can be accepted by pressing ENTER, or changed with the numeric keys. The equilibration time entered here is only in effect during the first injection of the run, and is active only the first time the file is used so that there is not a needless delay to following isocratic runs.

Pressing the ENTER key a second time gives:

GRADIENT DELAY (nnnn) (where n is some time value)

This option can help to compensate for pre column volume during gradient changes. (See section 4 of the Autosampler Operators Manual for further information.)

Pressing the ENTER key again gives the following line:

TIME	TIMED EVENTS
.00	

The timed event option allows the external activation of certain events at a specified time. An explanation for the use of this option can be found on page 5-20 of the Autosampler Operators Manual.

When ENTER is pressed after this query, the line,

END INJECTOR DIALOG

is printed, and the injector file is closed.

1.1.5.3 Autosampler file entries

After the injector dialogue ends, the message:

AUTOSAMPLER FILE ...PRESS <ENTER> TO SKIP TO NEXT QUERY
ERASE? (Y OR N)

is printed. Autosampler file parameters include all vial information; (ie vial number, injection volume, injection rate, etc.) and can be set through the dialogue outlined here, or through the command:

/1.AS/ DIALOG

If you do not want to alter or enter a parameter in this dialogue, simply press ENTER to move on to the next entry. Pressing ESCAPE exits from this dialogue.

A NO answer to the ERASE? question will yield the following table:

VIAL	INJ VOL	INJ/VIAL	CAL INT	CAL VIAL	FILE
------	---------	----------	---------	----------	------

After the headings are printed, the print head stops in the VIAL column. Enter the vial number of the first sample vial in the following manner: the first number is that of the TRAY, followed by a period, and then by the position number of the vial. For example: the fourth vial in tray number three would be designated 3.04, while the first vial in tray one would be 1.01. Specify the first vial in the run in this manner.

Enter the injection volume of the sample loop at the prompt. The Autosampler automatically overfills the sample loop using the equation:

$$V_i = 1.5 \times V_s + 50\mu\text{L}$$

(V_i = Volume Injected)
(V_s = Volume Specified)

Thus a 10 μL injection volume will actually use 65 μL of sample. The default value for this parameter is 20 μL , and will be used if no other value is specified.

Enter the number of injections per vial (max. of 10) at the INJ/VIAL prompt.

The CAL INT prompt defines the number of samples to be run between calibrations, and may be left blank if the integrator is not figuring calibration values, and other calibration procedures are being used. (See section 1.1.4.2.)

The CAL VIAL prompt defines the calibration vial and points to the next standard to be run. This also may be left blank if the integrator is not figuring calibration values. (More information about these two options can be found on pages 5-14 through 5-19 of the Autosampler Operators Manual.)

The entry at the FILE prompt must be the same file number as specified in the previous dialogues with the pump, solvent file, and etc.

These parameters do not need to be entered for each vial separately, as the entered values "carry down" until a new value is entered. Thus if all the vials in the run were to have the same injection volume, number of injections per vial, and calibration sequence, the parameters would only have to be entered for the first vial, and would be used by the Autosampler on all succeeding vials. For example, if the following sequence were entered:

VIAL	INJ VOL	INJ/VIAL	CAL INT	CAL VIAL	FILE
1.01	10	3			1
1.17	10	1			1
1.20	10	3			1

2.05 10 1 1

vials one through sixteen on tray one would each be injected three times; vials seventeen through nineteen on tray one would be injected once each; vial twenty on tray one, and vials one through four on tray two would be injected three times; with vial five on tray two being injected once.

After all entries in this table are complete, press ENTER, and the query:

LAST VIAL? (0)

will be printed. Specify the last vial in the run using the numbering scheme explained above, and press ENTER. The Autosampler then responds:

SYRINGE RATE? (2)

This parameter controls the rate at which solvent is drawn into and expelled from the syringe. The default value of two is adequate in most cases, but a lower value may be required for more viscous samples.

When ENTER is pressed after this query, the Autosampler terminates this section of the dialogue with the message:

END A/S DIALOG

1.1.5.4 Detector parameter entries

Having completed the previous dialogue sections, the Autosampler now requires the entry of the detector parameters. There are two detectors currently in use with the Spectra-physics HPLC system; the UV detector is addressed by the Labnet system as Channel A, the Fluorescence detector is addressed as Channel B. Dialogue with the UV detector starts immediately after the END A/S DIALOGUE command with the message:

FI= 1 FE= 1 MN= 0
PRESS 'ENTER' TO SKIP ENTRY
CAPTURE DATA? [Y/N]

Since both detectors are assumed by the Autosampler to be on line, it is important that the appropriate responses be made to all queries for both detectors. (See the SP4400 Integrator Users Manual Section 3, for an explanation of all detector dialogue queries.)

For the detector being used for the analysis, a Y response must be entered here. If a detector is NOT being used for the

current analysis, a N response must be entered. This will prevent the collection of data from the unused detector.

After a response has been entered, the following query is printed:

CAPTURE REPORT? [Y/N]

Once again, for the detector being used for the analysis, a Y response must be entered here. If a detector is NOT being used for the current analysis, a N response must be entered to prevent the collection of a report from the unused detector.

After a response has been entered, the Autosampler responds:

ENABLE BASELINE DRAWING? [Y/N]

For a more thorough understanding of the actual baseline used for peak integration, a Y response should be entered for the detector being used in the analysis. A N response should be given for the unused detector.

If a N response has been entered for the above queries, the file name under which the data will be collected and stored is displayed in the line:

FILE NAME (xxxxxxxxxxxxxxxx) ="

This name can contain up to fifteen characters, and has currently been designated as U.V. DETECTOR, and FLUORESCENCE DET, for the respective detector file names. Pressing ENTER after this query will leave the existing file name in place. Alterations can be made by typing the desired file name in through the keyboard, and pressing ENTER.

If a Y response was entered for the CAPTURE DATA? query, the disk file name, (the name under which the file will be stored to, and retrieved from the disk) is displayed in the line:

DISK FILE? (xxxxxxx)

This file name can contain up to eight characters, and will be automatically incremented at the end of each run if the last characters are numeric. (Alphabetical characters at the end of the file name will also be incremented automatically, but will cycle every twenty six characters, and therefore should not be used for runs containing more than twenty six samples.) The file names currently being used for the U.V., and Fluorescence detectors are DOEN###, and DOEA### respectively.

After a response is entered for the for the DISK FILE? query, the Autosampler then requests the number by which all devices on line will be accessed by the Labnet system, using the question:

LNET # (n)

For the ERC Spectra-physics HPLC system, the number one (1) must always be entered in response to this query. If any other number is entered, the system will not function.

The path-name through which the generated data and reports will be stored is then listed in the line:

PATHNAME? (xxxxxxxxxxxxxxxxxx)

This may contain up to fifteen characters. The current pathname may be accepted by pressing ENTER, or changed using the keyboard. Currently the pathname /LABNET/TEMP is being used for data collection and storage.

If a Y response was entered for the CAPTURE DATA? query, the disk file name, (the name under which the file will be stored to, and retrieved from the disk) is now displayed in the line:

FILE NAME (xxxxxxxxxxxxxxxxxx) ="

This name can contain up to fifteen characters, and has currently been designated as U.V. DETECTOR, and FLUORESCENCE DET, for the respective detector file names. Pressing ENTER after this query will leave the existing file name in place. Alterations can be made by typing the desired file name in through the keyboard, and pressing ENTER.

The following table is then printed:

TIME	FUNCTION VALUE
TT=	(indicates time value)

It is important that an end run (ER) time be listed for BOTH detectors even when one of them is not being used. If no end run time is listed for the unused detector, the Autosampler will wait indefinitely to start the next injection as it must receive an END OF RUN signal from all devices on line before proceeding with the next injection. It is convenient if the same end run (ER) time is listed for both detectors. The Autosampler will then receive the END OF RUN signal from both simultaneously, and no delay will be caused in the initiation of the next injection.

To enter an end run time, simply type in the numeric minute value (ie typing in 5.0 at the TT= prompt would designate five minutes), then press ENTER. The print head moves to the right and prints TF= (this indicates Timed Function). Type ER (for End Run) then press ENTER. The print head moves once more to the right and prints TV= (for Timed Value, which indicates whether you are turning the function ON or OFF at the designated time). Type in 1 which will activate the end run function at 5.0 minutes run time. Press ENTER twice to exit the TIME table dialogue. (For more

information on entering timed functions through this dialogue, please see the SP4400 Integrator Users Manual, section 8.)

After exiting from the TIME table dialogue, the message :

METHOD NUMBER [0,1,2,5] (n) MN=

is printed, where n is one of the numbers listed. These numbers designate different integration/calibration methods used by the integrator. For analyses not using the integrator for calibration, the method number (MN) selected should be 0. (For more information on these methods, please see the SP4400 Integrator Users Manual, pages 3-7 to 3-21.)

1.2 STARTING A RUN

When the sample vials are loaded, and all parameters have been correctly entered through the DIALOG1 command; the run parameters can be checked using the command:

PRMETHOD <ENTER>

This gives a printout of all the run parameters and conditions, as well as current solvent file and detector conditions, and may be helpful in pin-pointing possible errors or problem areas before the run is started. It should be noted that once a run has been started, the integrator keyboard is locked to all non-Autosampler commands. Therefore, parameters such as chart speed, attenuation, peak threshold and etc., must be set prior to starting the run, and are best done prior to starting the Autosampler dialogue sequence.

To start to Autosampler run type:

/1.AS/ START <ENTER>

The "Autosampler busy" light should come on, and the valve should switch to the FILL position. When the sample is injected, the integrator should start a new run file, listing the injection number, injection volume, tray number, equilibration time, gradient delay, channel designation, disk file name, date, and time of injection.

STOPPING A RUN

The command:

/1.AS/ STOP

can be used to stop a run before it is finished if necessary. The power OFF of the integrator, pump, computer, or autosampler will

also stop the run.

The Autosampler will automatically stop at the end of a run, and the timed events file of the Autosampler can be used to shut down the other devices on line (please see page 5-20 of the Autosampler Operators Manual). The clean-up file of the SP8800 HPLC pump can also be used in conjunction with the Autosampler (please see the SP8800/8810 LC Pump Operators Manual, page 4-7 for more information.)

2.0 AUTOSAMPLER SHUT-DOWN

When the Autosampler has completed the run, and all data has been collected from the final injection, the power switch can be pushed to the OFF position. Sample vials may be removed and cleaned, or new vials loaded at this time.

2.0.1 Helium shut-down

Turn off the toggle switch on the Autosampler lower front panel. If the LC pump has also been turned off, close the regulator valve on the Helium tank.

STANDARD DEVIATION PROGRAM

1.0 General information

This program is used to calculate the standard deviation for a method. The standard deviation, for the purposes of this study, is used to determine significant differences between samples and reference materials and evaluate the precision of the analytical methods.

2.0 Starting the program

The program can be executed by typing:
STDDEV <ENTER>

The user is then asked if a hard copy is desired and, if yes, what to name it. Then, the number of replicates and their values are entered for each sample. After the last value is entered, <ENTER> is depressed twice and the calculated standard deviation, the number of degrees of freedom (N) and the mean* for all entered values is shown on the screen.

At this point, the program is ready for more input if desired. If you wish to end the program answer no to the hard copy question and then type quit.

If an incorrect value is entered, then the program must be restarted. This is done by pressing <ENTER> until you are asked "Number of replicates?" by the program. Then type "end" and depress the F2 key to restart.

* The mean is provides useful information only when all the replicate values are from the same sample or batch.

3.0 BATCH FILE

A simple batch file, called "STDDEV", is used to execute the standard deviation program from any directory in the computer.

3.1 Listing of the STDDEV batch file

```
cls
gwbasic \basic\stddev
mode co80
```

4.0 LISTING OF THE STDDEV BASIC PROGRAM

```
10 KEY OFF:COLOR 15,4,4:CLS
20 GOTO 80
30 PRINT
40 PRINT"*****"
50 PRINT"*****"
60 PRINT " "
70 PRINT " "
80 LOOP=1:REPSUM=0:SQDIF=0
90 TSUM=0
100 PRINT "DO YOU WANT A HARD COPY?      YES or NO";
110 INPUT Z$
120 IF Z$="QUIT" OR Z$="quit" THEN COLOR ,,0:SYSTEM
130 IF Z$="END" OR Z$="end" THEN 670
140 IF Z$="yes" OR Z$="YES" THEN PRINT "Do you want a name for the
hard copy?" ELSE 190
150 INPUT N$
160 LPRINT N$
170 LPRINT ""
180 LPRINT""
190 SUM=0:PRINT "Sample #";LOOP
200 IF Z$="yes" OR Z$="YES" THEN LPRINT "Sample #";LOOP
210 PRINT "Number of Replicates= ";
220 IF Z$="yes" OR Z$="YES" THEN LPRINT "Number of Replicates= ";
230 INPUT REP$
240 IF Z$="yes" OR Z$="YES" THEN LPRINT REP$
250 IF REP$="" THEN 460 ELSE REP=VAL(REP$):REPSUM=REPSUM+REP
260 IF REP$="QUIT" OR REP$="quit" THEN COLOR ,,0:SYSTEM
270 IF REP$="END" OR REP$="erd" THEN 670
280 FOR I=1 TO REP
290 PRINT "Replicate #";I;
300 IF Z$="yes" OR Z$="YES" THEN LPRINT "Replicate #";I;
310 INPUT REPVAL
320 IF Z$="yes" OR Z$="YES" THEN LPRINT REPVAL
330 IF REPVAL=0 THEN 670
340 REP(I)=REPVAL
350 SUM=SUM+REPVAL
360 NEXT I
370 MEAN=SUM/REP
380 TSUM=TSUM+SUM
390 FOR I=1 TO REP
400 SQDIF=SQDIF+(MEAN-REP(I))^2
410 NEXT I
420 LOOP=LOOP+1
430 IF Z$="yes" OR Z$="YES" THEN LPRINT
440 PRINT
450 GOTO 190
460 PRINT ""
```

```
470 PRINT ""
480 PRINT"*****"
490 PRINT""
500 IF Z$="yes" OR Z$="YES" THEN LPRINT""
510 IF Z$="yes" OR Z$="YES" THEN LPRINT""
520 IF Z$="yes" OR Z$="YES" THEN LPRINT"*****"
530 IF Z$="yes" OR Z$="YES" THEN LPRINT""
540 STDDEV=(SQDIF/(REPSUM-(LOOP-1)))^.5
550 PRINT "Std Dev=";STDDEV
560 IF Z$="yes" OR Z$="YES" THEN LPRINT "Std Dev=";STDDEV
570 PRINT "Degrees of freedom=";REPSUM-(LOOP-1)
580 IF Z$="yes" OR Z$="YES" THEN LPRINT "Degrees of
freedom=";REPSUM-(LOOP-1)
590 PRINT"Mean=";TSUM/REPSUM
600 IF Z$="yes" OR Z$="YES" THEN LPRINT "Mean=";TSUM/REPSUM
610 IF Z$="yes" OR Z$="YES" THEN LPRINT""
620 IF Z$="yes" OR Z$="YES" THEN LPRINT""
630 IF Z$="yes" OR Z$="YES" THEN
LPRINT"*****"
640 IF Z$="yes" OR Z$="YES" THEN
LPRINT"*****"
650 IF Z$="yes" OR Z$="YES" THEN LPRINT""
660 GOTO 30
670 KEY ON
680 END
```

INTEGRATOR PROCEDURES

1.0 GENERAL INFORMATION

General instructions for the operation of the Spectra-Physics model SP4400 Chromjet Integrator are given in the manual for the instrument. The instructions in this SOP are intended only for those individuals that have already familiarized themselves with the manual and have been instructed by the principal investigator, or other qualified personnel, in the use of this instrument.

When connected by the Labnet software system, all system parameters (ie. integrator functions, data storage, detector files and timed events, and the Autosampler functions) can be controlled through the integrator keyboard except for the actual detector settings, and data retrieval from the computer hard drive.

1.1 SYSTEM CONFIGURATION

System configuration should only need to be done in the event of a prolonged power failure, or when a change in configuration is necessary due to addition or removal of peripheral equipment (ie. detectors).

Information on configuring the system is located in the SP4400 Integrator Users Guide, starting on page 1-10. Currently the system is configured to include two detectors, (the UV detector as Channel A, and the Fluorescence detector as Channel B; both have Unit #1), the Epson computer, the SP4400 Integrator, and the Autosampler.

1.2 KEYBOARD PARAMETERS/SETTINGS

In depth information on all keyboard functions can be found in the SP4400 Integrator Users Guide, Section 3. For information on keys not covered here, please see the Users Guide.

Most of the keyboard functions are locked and cannot be changed when the autosampler is running, so all settings should be checked prior to starting the autosampler run.

1.2.1 LCD Status Key

When this key (LCD STATUS) is pressed, the display will show the status state which is active at the current time, as well as the channel, file number, attenuation, run time, level, and memory

usage. This display is continuously updated.

1.2.2 Inject/End Keys

These keys (INJ/END A and INJ/END B) can be used to start a run for the channel designated, by pressing the key when the display indicates a "ready" status. Pressing the key during a run will end the run. Pressing the SHIFT key and the INJ/END key simultaneously during a run will abort the run. The data and report from an aborted run will not be saved.

1.2.3 Attenuation Key

The ATTEN key can be used at any time during a run or between runs to check or set the attenuation. When this key is pressed, a list of values for the attenuation parameter will be displayed on the LCD, (the list will also be printed out if the integrator is not in the middle of a run), and the system is set to receive a numerical value. Press ENTER after typing in the desired setting.

1.2.4 Channel Switch/Lock Key

Pressing the A<->B key switches the keyboard assignment between channels. The new channel assignment is shown on both the LCD display, and the printout. The default setting is channel A. Pressing SHIFT and this key simultaneously locks the current channel setting. Pressing CTRL and this key simultaneously will unlock this function.

1.2.5 Chart Speed/Time Key

This key (CHART SPEED) is used to check and set chart speed at any time during a run or between runs. A listing of all possible settings for the chart speed is displayed when the key is pressed, with the current value shown in parentheses. To select a new chart speed, type in the desired value from the list, and press ENTER.

When this key is pressed together with the SHIFT key, the current time of day is displayed.

1.2.6 Dialog Key

This key (DIALOG) initiates dialogue for file setup and edit. For more information see section 2.0 of this S.O.P., and the discussion of Dialog in the SP4400 Integrator Users Manual, Section 3.

1.2.7 Integration Inhibit Key

Pressing this key (INTEG INHIB) inhibits integration of the

detector signal on the active channel. Pressing this key once turns this function ON, pressing it a second time during the run will turn the function OFF. The detector signal continues to be plotted when this function is active, but affected peaks will not be integrated or assigned retention times, and will therefore not appear in the report. When the Integration Inhibit function is active, "INT INH" will be displayed in the lower right corner of the LCD.

1.2.8 Peak Threshold Key

The PT EVAL key evaluates the detector signal when pressed, and sets the peak threshold value accordingly to avoid integrating baseline noise as peaks. The lowest value the system will accept is 12. The values will be higher if the signal is noisy. The peak threshold can be set manually by typing PT = nnn, where nnn is the desired numerical value. The peak threshold evaluation function should NOT be used during a run.

1.2.9 Plot Auto Key

The PLOT AUTO function, when active, allows plotting to start with each use of an INJ/END key, or upon externally started injections when an autosampler is used. Each time the PLOT AUTO key is pressed, the setting switches between PLOT "A" AUTO and PLOT "B" AUTO.

1.2.10 Plot On/Plot Off Key

The PLOT ON function key is used to plot the baseline on the active channel. Pressing SHIFT and PLOT ON together stops the plot, and turns the plotter off.

1.2.11 Print File Key

Pressing this key (PRINT FILE) prints a list of current file parameters in the edit file. It is a good practice to print out the existing file before making any changes.

1.2.12 Paper Key

Pressing the PAPER key advances the paper on the printer as long as the key is held down. To set the page length correctly in the printer, hold down the CTRL and the K key simultaneously until the perforation is exactly at the top of the paper guide. Then press the CTRL and the T key. The printer will automatically align the paper correctly for the next run, and will bypass perforations in succeeding runs.

2.0 DETECTOR PARAMETER ENTRIES

There are two detectors currently in use with the Spectra-physics HPLC system; the UV detector is addressed by the Labnet system as Channel A, the Fluorescence detector is addressed as Channel B. (See the Autosampler SOP section 1.1.5.4 for information on setting the detector file parameters when using the Autosampler dialogue.)

Dialogue with the UV detector (channel A), can be initiated by pressing the DIALOGUE key while the LCD status shows Channel A in the upper left corner. Dialogue with the Fluorescence detector (channel B), can be initiated the same way when Channel B shows in the upper left hand corner of the LCD display. The dialogue begins with the message:

```
FI= 1      FE= 1      MN= 0  
PRESS 'ENTER' TO SKIP ENTRY  
CAPTURE DATA?      [Y/N]
```

For the detector being used for the analysis, a Y response must be entered here. If a N response is entered, no data from the run will be collected.

After a response has been entered, the following query is printed:

```
CAPTURE REPORT?      [Y/N]
```

Once again, for the detector being used for the analysis, a Y response must be entered here. If a N response is given, the report will not be stored on the hard drive.

After a response has been entered, the Integrator responds:

```
ENABLE BASELINE DRAWING? [Y/N]
```

For a more thorough understanding of the actual baseline used for peak integration, a Y response should be entered for the detector being used in the analysis. This will cause the integrator to draw in the baseline used in the peak integration, and will allow interferences or incorrect integration to be more easily spotted and corrected.

If a N response has been entered for the above queries, the file name under which the data will be collected and stored is displayed in the line:

```
FILE NAME (xxxxxxxxxxxxxxxx) ="
```


This name can contain up to fifteen characters, and has currently been designated as U.V. DETECTOR, and FLUORESCENCE DET, for the respective detector file names. Pressing ENTER after this query will leave the existing file name in place. Alterations can be made by typing the desired file name in through the keyboard, and pressing ENTER.

If a Y response was entered for the CAPTURE DATA? query, the disk file name, (the name under which the file will be stored to, and retrieved from the disk) is displayed in the line:

DISK FILE? (xxxxxxx)

This file name can contain up to eight characters, and will be automatically incremented at the end of each run if the last characters are numeric. (Alphabetical characters at the end of the file name will also be incremented automatically, but will cycle every twenty six characters, and therefore should not be used for runs containing more than twenty six samples.) The file names currently being used for the U.V., and Fluorescence detectors are DOEN###, and DOEA### (where ### represents some number) respectively.

After a response is entered for the DISK FILE? query, the Integrator then requests the number by which all devices on line will be accessed by the Labnet system, using the question:

LNET # (n)

For the ERC Spectra-physics HPLC system, the number one (1) must always be entered in response to this query. If any other number is entered, the system will not function.

The path-name through which the generated data and reports will be stored is then listed in the line:

PATHNAME? (xxxxxxxxxxxxxxxxx)

This may contain up to fifteen characters. The current pathname may be accepted by pressing ENTER, or changed using the keyboard. Currently the pathname /LABNET/TEMP is being used for data collection and storage.

If a Y response was entered for the CAPTURE DATA? query, the disk file name, (the name under which the file will be stored to, and retrieved from the disk) is now displayed in the line:

FILE NAME (xxxxxxxxxxxxxxxxx) ="

This name can contain up to fifteen characters, and has currently been designated as U.V. DETECTOR, and FLUORESCENCE DET, for the respective detector file names. Pressing ENTER after this query will leave the existing file name in place. Alterations can be

made by typing the desired file name in through the keyboard, and pressing ENTER.

The following table is then printed:

TIME	FUNCTION	VALUE
TT=	TF=	TV=

For more information on these functions, see the SP4400 Integrator users Guide, pages 3-5 to 3-7, also Section 8, and Appendix A. TT= indicates the time the function is to take place during the run. The recommended minimum time is 0.02 min, this is also the recommended minimum time between functions. TF= indicates the Timed Function to be used. TV= Timed Value, which indicates whether you are turning the function ON or OFF at the designated time. Typing in 1 will activate the end run function at the specified run time.

After exiting from the TIME table dialogue, the message :

METHOD NUMBER [0,1,2,5] (n) MN=

is printed, where n is one of the numbers listed. These numbers designate different integration/calibration methods used by the integrator. For analyses not using the integrator for calibration, the method number (MN) selected should be 0. (For more information on these methods, please see the SP4400 Integrator Users Manual, pages 3-7 to 3-21.)

3.0 FILE AND REPORT STORAGE/RETRIEVAL

The SP4400 Chromjet Integrator is linked to the Epson Equity 1+ computer so that all data and reports generated during the course of a run are stored on the hard drive under a unique pathname, (currently LABNET/TEMP), and file names specific to each channel and detector. (Currently the file names DOEN### for channel A (the U.V. detector) and DOEA### for channel B (the Fluorescence detector), where ### represents some number, are being used.)

Files thus stored can be retrieved through the DISPLAY option on the Labnet software system. Once they are retrieved using DISPLAY, they can be viewed, parameter changes made, and then rewritten to the hard drive. If parameter changes have been made, an option is given where the altered chromatogram can be stored to the hard drive under a different file name, thus preserving the original data. Chromatograms can also be printed from these files using the Labnet software.

All files generated by the integrator are stored on the hard

drive, then downloaded to 5 1/4" floppy disks. They are then stored to the hard drive of another computer, downloaded to 3 1/2" floppy disks, and the hard drive data copied to a backup tape periodically. Currently, the 5 1/4" disks, the 3 1/2" disks, and the tape backup of the electronic data are stored for future reference. All hard copy printouts of relevant data are also stored.

HPLC STANDARD OPERATING PROCEDURES

1 GENERAL INFORMATION

General instructions for the operation of the Spectra-Physics model 8800 HPLC are given in the manual for the instrument. The instructions in this SOP are intended only for those individuals that have already familiarized themselves with the manual and have been instructed by the principal investigator, or other qualified personnel, in the use of this instrument.

1.1 HPLC Start-up

Ensure that the solvent reservoirs are full (or contain enough solvent to complete the scheduled work). A variety of solvents and numerous compositions of solvents may be used in this analytical procedure. The choice of solvents will depend upon the analyte, interferences and the type of column used for the separation. A log is kept in appendix A that lists columns, solvent composition, retention times etc. Appendix A is not meant to be comprehensive or all inclusive, but rather as a guide for mobile phases that have and have not been suitable. When working with new columns and/or matrices it may be necessary to experiment with solvents and solvent composition until a suitable combination is found.

1.1.1 Helium Purge

Helium purging is required to remove dissolved gasses from the solvents. If the dissolved gasses are not completely removed, bubbles will form in the proportioning valve and can be seen in the plastic tubing connecting the proportioning valve and the pump inlet valve. The bubbles cause erratic pump behavior and affect the flow rate, and may significantly impact measurement precision. Helium purging should be started approximately ten minutes prior to starting the pump.

1.1.2 Solvent tubing purge

Helium purging does not remove gasses from the portion of solvent that is in the tubing from the uptake filter to the pump inlet valve. This portion of solvent must be purged from the system using the pump with the column bypass valve open. Each of the solvents used (up to 3) must be purged. It is possible to purge 2 or 3 solvent tubings simultaneously, however it is more efficient to purge them separately. The solvent that is stored in the column (usually methanol or acetonitrile) should be purged last.

1.1.3 Programmed solvent start

In order to increase the lifetime of the HPLC columns they are stored in non-aqueous solvents such as methanol or acetonitrile after each use. To keep the column from being shocked with a new solvent, or to prevent buffers from precipitating when they contact the non-aqueous solvent, a programmed start is used.

1.1.3.1

After purging is complete and the column bypass valve is closed, program a file to start at 100% of the column storage solvent with a 5 to 10 minute gradient to arrive at the desired solvent composition. Make sure that this start file holds the desired composition until another analytical file can be programmed and initiated. The column will require 10 to 20 column volumes or more of the new solvent composition to come to equilibrium.

1.1.4 DETECTOR START-UP PROCEDURE

Detector (lamps) can be turned on and allowed to warm up during initial HPLC start-up (sec 1.1). The Excitation wavelength setting and column-detector connections should be checked at this time. Specific detector settings for each tracer can be found in the appendices of this SOP. Avoid unnecessary movement of the wavelength adjustment dial, as exact replacement of a previous setting is difficult, and even small changes in the wavelength setting may cause noticeable differences in the signal output.

After solvent mixture has been allowed to equilibrate (section 1.1.3.1) and pressure readings have stabilized, the detector can be zeroed and baseline readings allowed to stabilize. Detectors can be re-zeroed after the baseline has stabilized, if necessary.

1.1.4.1 UV Detector

On the UV detector, the settings that will vary from compound to compound are the wavelength, the range and the time constant or rise time. These settings should be checked and changed or reset if necessary.

1.1.4.2 Fluorescence Detector

The excitation wavelength, emission filter, range, response setting and the lamp frequency may vary depending on sample and solvent composition. These settings should be checked and changed or reset if necessary. Excitation filters should be checked periodically and cleaned if necessary.

1.1.5 Replenishing Solvents

If each workday is started with full solvent reservoirs it is highly unlikely that they will have to be refilled during the day. However, should the need arise, stop the pumps, refill the reservoirs and wait 10 minutes to allow the solvents to degas. Start pumps, wait for stable baseline, then run standard and continue work.

2.0 HPLC shut-down procedures

The reversed phase HPLC columns should be stored in methanol or acetonitrile when not in use or overnight. This will reduce dissolution of the silica and the bonded phase and eliminate bacterial growth. If the column is to be stored for an extended period of time it should be labeled as to the storage solvent.

2.1 Programmed shut-down

To keep the column from being shocked with the storage solvent, and to prevent buffers from precipitating when they contact the non-aqueous solvent, a programmed shut-down is used. First, any buffered solvent is replaced by distilled water and 5 to 10 column volumes of the new mixture or pure distilled water is pumped through the column. Then, a gradient that changes about 20% per minute is used to switch the column to the storage solvent. Approximately 10 to 20 column volumes of the storage solvent should be pumped through the column before shutting off the pumps.

2.2 Pump shut-down

Push stop button on SP-8800.

2.3 Helium shut-down

Turn of toggle switch on SP-8800. Close regulator valve on the Helium tank.

3.0 Column clean-up procedures

From time to time it may be necessary to clean up the HPLC columns to elute compounds that do not migrate or migrate very slowly with the analytical solvent mixture. The frequency with which this must be done will depend upon the sample matrix and possibly the purity of the solvents of the mobile phase. The indications are a drifting baseline and/or extraneous peaks or large broad interfering peaks in the chromatogram. Since most ground water is free of organic compounds, this will not happen

very often and the daily shut-down procedures will be sufficient to clean the column.

3.1 Clean-up steps

Follow HPLC shut-down procedures (section 2.1) except that at least 20 column volumes of the non-aqueous solvent should be pumped through the column. Also, monitor the detector baseline (using the integrator numeric values or the detector numeric readout) and continue pumping until the baseline is stable. Once a stable baseline has been obtained, program the solvent composition back to the analytical mobile phase at a rate similar to that used during the start-up procedures.

4.0 Preparation of standards

4.1 Preparation of standard stock solution

Aliquots of the standard material will be weighed on a balance to the nearest 0.1 milligram (mg) in accordance with the SOP for the balance. This weighed amount of standard shall then be diluted in a volumetric flask with an appropriate solvent.

The amount that is weighed and the volume of solvent will depend upon the final dilution range for the standard.

The stock solution shall be dated with the date of preparation and stored in a refrigerator.

4.2 Preparation of standard solutions

The standard stock solution will be prepared from dilutions of the standard stock solution.

NOTE:

UNLESS OTHERWISE SPECIFIED AT LEAST TWO (REPLICATE)
ANALYSES WILL BE PERFORMED ON EVERY SAMPLE AND STANDARD

4.0 Replicate measurements

At least two analyses (measurements) will be made on each sample and standard. If the two area counts do not agree within the percentage specified in appendix --- of this SOP then a third aliquot will be analyzed. All three values will be used to determine the mean unless one of the values can be rejected based on statistical information (see 13.3.2.3 of the QA Project Plan).

6.0 Calibration procedures

Calibration curves will be constructed using 2 or 3 standard solutions of different concentrations and a blank solution. The concentration ranges of the standards should be higher than the highest expected concentration and lower than the lowest expected concentration. Since it is often not possible to predict these high and low values, it shall be acceptable to have either of these concentrations be outside the range of the standards but not by more than one order of magnitude. If this situation occurs, additional standards must be analyzed. Ranges of standards will be based on known or expected experimental concentrations.

6.1 Calibration results

Results will be calculated using the "Curve" program (Curve.SOP). The minimum acceptable correlation coefficient is listed in appendix --- of this SOP.

**STANDARD OPERATING PROCEDURES
FOR THE
SARTORIUS 2432 ANALYTICAL BALANCE**

1.0 Sartorius Analytical Balance

Standard procedures for this balance will be covered in two sections, actual balance operation, and calibration check procedures.

2.0 BALANCE OPERATION

2.1 CHECK ZERO

Set all controls to zero. Turn release lever (left side, behind weight dial) down to the "0" position. If scale does not stop on zero, adjust with the zero control knob. (right side, behind micrometer control.)

2.2 WEIGHING AN UNKNOWN

Place object on pan. Close balance doors and turn release lever to "1/2" position. Dial in weights, starting with the largest series. When too much weight is added, the scale moves below zero; go back one step (if 30 is too much, go back to 20). Then use the next lower series.

2.3 READING THE RESULT

When all the weights have been used, turn the release lever down to the "1" position. When the scale stops, use the micrometer knob to superimpose the pointer on the next lower division. Read the total weight from left to right observing the decimal point.

2.4 MAKING ADJUSTMENTS

NOTE: Make all adjustments smoothly

NOTE: NEVER DIAL WEIGHTS IN THE FULL RELEASE POSITION

Stop momentarily in the full arrest, ("0" position) before moving the release lever to full release, ("1" position). If pan swings too much, arrest once more.

3.0 CALIBRATION CHECK PROCEDURES

Everyone using the balance must observe the following procedures.

3.1 LOGBOOK ENTRIES

All log book entries must be dated and initialed or signed by the person making the entry. A brief description of the substance weighed should be entered on the appropriate line (example: Potassium Phosphate crystals).

3.2 STANDARD WEIGHTS

The two (2) gram weight from the PERMAS Standard weight set should be weighed as a calibration check before and after each sample.

3.3 WEIGHING PROCEDURE

When an object or substance is to be weighed, first weigh the standard two (2) gram weight and record this value in the appropriate column in the log book. Re-zero the balance, then weigh the sample, according to the directions outlined above. Enter this value, along with a brief description of the sample, into the log book. Re-zero the balance and then re-weigh the two (2) gram weight. Record this value in the log book. Sign or initial the log book below your entries, and check to be sure that the date is recorded correctly. Re-set all weight values to zero, and place the release lever in the arrested or "0" position before leaving the balance.

CURVEFIT PROGRAM

1.0 General Information

The curve program is used to construct a calibration curve from which unknown concentrations of analyte can be measured.

The signal that is detected from a chromatographic column appears as a gaussian peak, and the area under this peak is directly proportional to the concentration. The calibration curve is constructed using samples of known concentration and the concentration of the unknown samples is interpolated from this curve.

The data are obtained using an integrator that electronically integrates the area under the peak and then prints the results as an "area count" for each sample. The area count data is entered in the curve program, which calculates a correlation coefficient and a slope value. The correlation coefficient, for the purpose of this project, is an estimate of how close the line is to being a straight line (a straight line has a correlation coefficient of 1). The slope value is used to interpolate the concentrations of the unknown samples.

2.0 Instructions on the use of the curve program

2.1 Starting the program

The program may be started from any directory by typing:
Curvefit <ENTER>

The program then directs the user to enter:
Concentration 1 (for standard #1)

then

enter all replicate response values for
standard #1..

After the last response for #1, press <ENTER> and repeat for Concentration #2, then 3 etc. until all entries for the standards have been made.

After the last entry has been made press <ENTER> twice and follow the instruction displayed on the screen.

The results can be sent directly to the printer or stored in a file for printing at a later time. The stored files can be called up and printed with Word Perfect 5.1 or Word Star 2000. (Word Perfect requires that the columns be aligned before printing. For proper printing with Word Star, the file should be block inserted into an empty but formatted file.)

Note: If an incorrect sample identification is entered the line can be deleted by entering "bad id" for the response.

3.0 Curvefit batch file

The curvefit batch file is a convenient way to execute the curve program from any directory in the computer.

3.1 Listing of the curvefit batch file

```
echo off
cls
gwbasic \basic\curve
echo on
mode co80
```

4.0 Curve basic program

The curve program is a basic program that can be executed on a personal computer that has any of numerous versions of basic.

4.1 Listing of the curve program

```
1000 REM curvefit program written by Ken R. Lauver
1010 REM variable definitions (types)
1020 DEFDBL A-H,K,L,M,O,P,R-Z:DEFINT I,J,N,Q:DIM
X(100,2),UNDO(100,2):I=1:J=1:N=0:SUM=0:UNDO=0:KEY OFF:COLOR
15,1,1:CLS:B$=""
1030 DEF FNCONC(X)=A+B*X
1040 REM concentration of standards input
1050 PRINT "Concentration (;I;)";
1060 INPUT Q$:IF Q$="" THEN 1160 ELSE X(I,2)=VAL(Q$)
1070 N=N+1
1080 REM response to standards input
1090 PRINT "Response (;I;) #";J;
1100 INPUT Q$
1110 REM if end of user input then calculate average response
entered for the current concentration
1120 IF Q$="" THEN IF J=1 THEN PRINT "You haven't entered a
response yet!":GOTO 1090 ELSE X(I,1)=SUM/(J-1):PRINT "Average
response (;I;) = ";X(I,1):J=1:SUM=0:I=I+1:PRINT:GOTO 1050
1130 SUM=SUM+VAL(Q$):J=J+1
1140 GOTO 1090
1150 REM set up screen to display data user has input
1160 CLS:PRINT
1170 PRINT "VALUES THAT WILL BE USED FOR THE CURVE"
```

```
1180 PRINT
1190 PRINT "PAIR #          CONCENTRATION          RESPONSE"
1200 FOR I=1 TO N
1210 PRINT USING "    ###          #####.#####"
#####.#####";I,X(I,2),X(I,1)
1220 NEXT I:PRINT
1230 REM ask user if a typing error exists
1240 PRINT "Is this correct (";:COLOR 1,15:PRINT "Y";:COLOR
15,1:PRINT "/";:COLOR 1,15:PRINT "N";:COLOR 15,1:PRINT ")??"
1250 Q$=INKEY$:IF LEN(Q$)=0 THEN 1250
1260 IF Q$="y" OR Q$="Y" THEN 2150
1270 REM set up edit mode screen
1280 CLS
1290 PRINT "CORRECTION OPTIONS TO CHOOSE FROM:":PRINT
1300 COLOR 1,15:PRINT "D";:COLOR 15,1:PRINT "elete data pair",,
1310 COLOR 1,15:PRINT "C";:COLOR 15,1:PRINT "hange data pair":PRINT
1320 COLOR 1,15:PRINT "S";:COLOR 15,1:PRINT "how data for curve",,
1330 COLOR 1,15:PRINT "U";:COLOR 15,1:PRINT "ndo last edit
change":PRINT
1340 COLOR 1,15:PRINT "A";:COLOR 15,1:PRINT "dd data to curve",,
1350 COLOR 1,15:PRINT "N";:COLOR 15,1:PRINT "ew curve":PRINT
1360 PRINT "E";:COLOR 1,15:PRINT "x";:COLOR 15,1:PRINT "it from
correction routine and calculate correlation "
1370 PRINT:PRINT "OPTION?":PRINT
1380 Q$=INKEY$:IF LEN(Q$)=0 THEN 1380
1390 REM branch to appropriate routine
1400 Q=ASC(Q$)
1410 IF Q=65 OR Q=97 THEN 1490:REM add data pair
1420 IF Q=68 OR Q=100 THEN 1640:REM delete pair
1430 IF Q=67 OR Q=99 THEN 1790:REM change pair
1440 IF Q=78 OR Q=110 THEN RUN:REM new curve
1450 IF Q=83 OR Q=115 THEN 2050:REM show data for curve
1460 IF Q=85 OR Q=117 THEN 1970:REM undo last edit change
1470 IF Q=88 OR Q=120 THEN 2140:REM exit from editing
1480 GOTO 1380
1490 REM add data pair
1500 FOR I=1 TO N
1510 UNDO(I,1)=X(I,1):UNDO(I,2)=X(I,2)
1520 NEXT I
1530 UNDO=1:INITN=N:J=1:SUM=0
1540 PRINT "Concentration (";N+1;")";
1550 INPUT Q$
1560 IF Q$="" THEN 1620
1570 X(N+1,2)=VAL(Q$):N=N+1
1580 PRINT "Response (";I;") #";J;
1590 INPUT Q$
1600 IF Q$="" THEN IF J=1 THEN PRINT "You haven't entered a
response yet!":GOTO 1090 ELSE X(I,1)=SUM/(J-1):PRINT "Average
response (";I;") = ";X(I,1):GOTO 1620
```

```
1610 SUM=SUM+VAL(Q$):J=J+1:GOTO 1580
1620 PRINT:PRINT "Press any key to continue..."
1630 Q$=INKEY$:IF LEN(Q$)=0 THEN 1630 ELSE 1280
1640 REM delete pair
1650 FOR I=1 TO N
1660 UNDO(I,1)=X(I,1):UNDO(I,2)=X(I,2)
1670 NEXT I
1680 UNDO=1:INITN=N
1690 PRINT "Data pair to delete";:INPUT Q$
1700 IF Q$="" THEN 1760
1710 IF VAL(Q$)<1 OR VAL(Q$)>N THEN PRINT:PRINT "Out of range":GOTO
1690
1720 I=VAL(Q$)
1730 PRINT "Delete concentration (";I;") = ";X(I,2);" Response
(";I;") = ";X(I,1);
1740 INPUT I$
1750 IF LEFT$(I$,1)="y" OR LEFT$(I$,1)="Y" THEN FOR J=I+1 TO
N:X(J-1,1)=X(J,1):X(J-1,2)=X(J,2):NEXT J:N=N-1:PRINT "Data pairs
";N:GOTO 1770
1760 PRINT "No data deleted."
1770 PRINT "Press any key to continue..."
1780 Q$=INKEY$:IF LEN(Q$)=0 THEN 1780 ELSE 1280
1790 REM Change pair
1800 IF N=0 THEN PRINT "There isn't any data to change.":PRINT
:PRINT "Press any key to continue..."
1810 IF N=0 THEN Q$=INKEY$:IF LEN(Q$)=0 THEN 1810 ELSE 1280
1820 PRINT "Index pair to change";:INPUT Q$
1830 IF Q$="" THEN 1280
1840 IF VAL(Q$)<1 OR VAL(Q$)>N THEN PRINT:PRINT "Out of range":GOTO
1820
1850 I=VAL(Q$)
1860 FOR Q=1 TO N
1870 UNDO(Q,1)=X(Q,1):UNDO(Q,2)=X(Q,2)
1880 NEXT Q
1890 INITN=N:UNDO=1
1900 PRINT "Concentration (";I;") = ";X(I,2);" Response (";I;") =
";X(I,1)
1910 PRINT "New Concentration (";I;") = ";:INPUT X
1920 X(I,2)=X:J=1:SUM=0
1930 PRINT "New Response (";I;") #";J;
1940 INPUT Q$
1950 IF Q$="" THEN X(I,1)=SUM/(J-1):PRINT "Average Response (";I;")
= ";X(I,1):GOTO 1280
1960 SUM=SUM+VAL(Q$):J=J+1:GOTO 1930
1970 REM Undo last change
1980 IF UNDO<>1 THEN PRINT "You have not made any changes to the
data yet!":PRINT "Press any key to continue..."
1990 IF UNDO<>1 THEN Q$=INKEY$:IF LEN(Q$)=0 THEN 1990 ELSE 1280
2000 FOR Q=1 TO INITN
```

```

2010 X(Q,1)=UNDO(Q,1):X(Q,2)=UNDO(Q,2)
2020 NEXT Q
2030 N=INITN
2040 GOTO 1280
2050 REM Show curve data
2060 PRINT "VALUES THAT WILL BE USED FOR THE CURVE"
2070 PRINT
2080 PRINT "PAIR #          CONCENTRATION          RESPONSE"
2090 FOR I=1 TO N
2100 PRINT USING "    ##          #####.#####
#####.#####";I,X(I,2),X(I,1)
2110 NEXT I:PRINT
2120 PRINT "Press any key to continue..."
2130 Q$=INKEY$:IF LEN(Q$)=0 THEN 2130 ELSE 1280
2140 REM Exit edit
2150 REM Regression calculation
2160 REM slope calculation
2170 B1=0#:FOR I=1 TO N:B1=B1+X(I,1)*X(I,2):NEXT I
2180 B21=0#:B22=0#:B2=0#
2190 FOR I=1 TO N:B21=B21+X(I,1):B22=B22+X(I,2):NEXT I
2200 B2=B21*B22/N
2210 B3=0#:FOR I=1 TO N:B3=B3+X(I,1)*X(I,1):NEXT I
2220 B4=0#:FOR I=1 TO N:B4=B4+X(I,1):NEXT I:B4=B4*B4/N
2230 B=(B1-B2)/(B3-B4)
2240 REM Calculate intercept
2250 A1=0#:FOR I=1 TO N:A1=A1+X(I,2):NEXT I:A1=A1/N
2260 A2=0#:FOR I=1 TO N:A2=A2+X(I,1):NEXT I:A2=A2/N
2270 A=A1-B*A2
2280 REM calculate correlation
2290 R1=0#:FOR I=1 TO N:R1=R1+X(I,2)*X(I,2):NEXT I
2300 R2=0#:FOR I=1 TO N:R2=R2+X(I,2):NEXT I:R2=R2*R2/N
2310 RSQ=((B1-B2)*(B1-B2))/((B3-B4)*(R1-R2))
2320 RSQ2=RSQ^.5
2330 REM display regression results to user to see if they are
satisfactory
2340 CLS
2350 PRINT USING "Correlation Coefficient          ="
#####.#####";RSQ2
2360 PRINT USING "Coefficient of Determination    ="
#####.#####";RSQ
2370 PRINT USING "Slope                                ="
#####.#####";B
2380 PRINT USING "Intercept                                ="
#####.#####";A
2390 PRINT
2400 PRINT          "      Standard          Response
Calculated          Percent      "
2410 PRINT          "      Concentration
Concentration          Deviation"

```

```
2420 PRINT
2430 FOR I=1 TO N
2440 IF X(I,2)=0 THEN PRINT USING "#####.###
#####.###          Unable to
compute";X(I,2),X(I,1),FNCALCONC(X(I,1)):GOTO 2460
2450 PRINT USING "#####.###          #####.###
#####.###";X(I,2),X(I,1),FNCALCONC(X(I,1)),-(X(I,2)-FNCALCONC(X(
I,1)))/X(I,2)*100
2460 NEXT I
2470 PRINT:PRINT "Is the correlation coefficient for this curve
satisfactory (";:COLOR 1,15:PRINT "Y";:COLOR 15,1:PRINT "/";:COLOR
1,15:PRINT "N";:COLOR 15,1:PRINT ")?";
2480 Q$=INKEY$:IF LEN(Q$)=0 THEN 2480
2490 IF Q$="y" OR Q$="Y" THEN 2570
2500 IF Q$<>"N" AND Q$<>"n" THEN BEEP:GOTO 2470
2510 PRINT:COLOR 1,15:PRINT "E";:COLOR 15,1:PRINT "dit or ";:COLOR
1,15:PRINT "Q";:COLOR 15,1:PRINT "uit":REM NOT SATISFACTORY
2520 Q$=INKEY$:IF LEN(Q$)=0 THEN 2520
2530 IF Q$="E" OR Q$="e" THEN CLS:GOTO 1290
2540 IF Q$="Q" OR Q$="q" THEN CLOSE:SYSTEM
2550 BEEP:GOTO 2510
2560 REM output to a file or printer
2570 PRINT:PRINT "Output to ";:COLOR 1,15:PRINT "P";:COLOR
15,1:PRINT "rinter or ";:COLOR 1,15:PRINT "F";:COLOR 15,1:PRINT
"ile"
2580 Q$=INKEY$:IF LEN(Q$)=0 THEN 2580
2590 IF Q$="P" OR Q$="p" THEN F$="LPT1":FILE$=F$:GOTO 2620
2600 IF Q$="F" OR Q$="f" THEN PRINT:PRINT "Filename";:INPUT
F$:FILE$=F$:GOTO 2620
2610 BEEP:GOTO 2570
2620 PRINT:CLOSE:OPEN FILE$ FOR APPEND AS #1
2630 LINE INPUT "Title for output? ";T$
2640 PRINT:PRINT "Blank correction (";:COLOR 1,15:PRINT "Y";:COLOR
15,1:PRINT "/";:COLOR 1,15:PRINT "N";:COLOR 15,1:PRINT ")?";
2650 Q$=INKEY$:IF LEN(Q$)=0 THEN 2650
2660 IF Q$<>"Y" AND Q$<>"y" THEN BLANK=0:B$="":GOTO 2750
2670 J=1:BLANKSUM=0:BLANK=0
2680 PRINT:PRINT "Blank correction response (";J;")";:INPUT BLANK$
2690 IF BLANK$="" THEN BLANK=BLANKSUM/(J-1):PRINT:PRINT "Average
Blank correction response ";BLANK;:GOTO 2710
2700 BLANKSUM=BLANKSUM+VAL(BLANK$):J=J+1:GOTO 2680
2710 PRINT:PRINT:PRINT "Blank subtraction with value ";:COLOR
0,7:PRINT "b";:COLOR 7,0:PRINT "efore or ";:COLOR 0,7:PRINT
"a";:COLOR 7,0:PRINT "fter FITTING to the curve?"
2720 B$=INKEY$:IF LEN(B$)=0 THEN 2720
2730 IF B$="b" OR B$="B" OR B$="a" OR B$="A" THEN 2750
2740 BEEP:GOTO 2710
2750 PRINT:PRINT:PRINT "Dilution factor";:INPUT DILFACT
```



```

2760 PRINT:LINE INPUT "Conversion units for the dilution factor?
";U$
2770 REM output to the output device
2780 PAGE=1:LC=0
2790 PRINT #1,T$;" CURVE          PAGE";PAGE:PRINT #1,
2800 PRINT #1,USING "Correlation =   ##.#####";RSQ
2810 PRINT #1,USING "Slope          = #####.#####";B
2820 PRINT #1,USING "Intercept     = #####.#####";A
2830 PRINT #1,
2840 PRINT #1,          "    Standard          Response
Calculated            Percent    "
2850 PRINT #1,          " Concentration
Concentration          Deviation"
2860 PRINT #1,
2870 FOR I=1 TO N
2880 IF X(I,2)=0 THEN PRINT #1,USING "#####.###
#####.###          #####.###          Unable to
compute";X(I,2),X(I,1),FNCALCONC(X(I,1)):GOTO 2900
2890 PRINT #1,USING "#####.###          #####.###
# # # # # # # # # # # # # # # # # # #
#####.#####";X(I,2),X(I,1),FNCALCONC(X(I,1)),-(X(I,2)-FNCALCONC(X(
I,1)))/X(I,2)*100
2900 NEXT I
2910 PRINT #1,:LC=LC+10+N
2920 IF BLANK=0 THEN PRINT #1,"Blank correction factor is
";BLANK:LC=LC+1:GOTO 2950
2930 IF B$="b" OR B$="B" THEN PRINT #1,"Blank correction factor is
";BLANK;" with subtraction performed":PRINT #1,"before fitting
response to the curve":LC=LC+2
2940 IF B$="a" OR B$="A" THEN PRINT #1,"Blank correction factor is
";BLANK;" with subtraction performed":PRINT #1,"after fitting
response to the curve":LC=LC+2
2950 PRINT #1,"Dilution factor is ";DILFACT;" with conversion units
of ";U$;"":PRINT #1,
2960 PRINT #1,          "    Sample          Sample
Calculated            Times DF"
2970 PRINT #1,          "Identification          Response
Concentration"
2980 PRINT #1,:LC=LC+5
2990 USEFLAG=0
3000 REM user inputs data one at a time to be fitted to the curve
3010 PRINT:LINE INPUT "Sample Identification or Command:";ID$
3020 IF LC>=60 THEN LC=2:PAGE=PAGE+1:PRINT #1,CHR$(12);T$;" CURVE
PAGE";PAGE
3030 IF ID$="" THEN 3010
3040 REM user has several commands to use which affect the running
of the program. These include help,new dilution factor,new
curve,edit,& quit.
3050 IF ID$<>"LAST BAD" AND ID$<>"last bad" AND USEFLAG<>0 THEN

```

```
PRINT #1,USING "\          \          #####.###  
# # # # # # # # # # # # # # # # # # #  
#####.###";IDL$,SAMRES,SAMCONC,SAMCONC*DILFACT:LC=LC+1  
3060 IF ID$="LAST BAD" OR ID$="last bad" THEN 3010  
3070 IF ID$="new df" OR ID$="NEW DF" THEN PRINT:PRINT "Dilution  
factor";:INPUT DILFACT  
3080 IF ID$="new df" OR ID$="NEW DF" THEN PRINT:PRINT "Are the  
units still ";U$;"("::COLOR 1,15:PRINT "Y";:COLOR 15,1:PRINT  
"/";:COLOR 1,15:PRINT "N";:COLOR 15,1:PRINT ")?"  
3090 IF ID$="new df" OR ID$="NEW DF" THEN Q$=INKEY$:IF LEN(Q$)=0  
THEN 3090  
3100 IF ID$="new df" OR ID$="NEW DF" THEN IF Q$<>"Y" AND Q$<>"y"  
THEN LINE INPUT "Conversion units for the dilution factor? ";U$  
3110 IF ID$="new df" OR ID$="NEW DF" THEN PRINT #1,:PRINT #1,"New  
dilution factor is ";DILFACT;" with conversion units of  
";U$;".":PRINT #1,:LC=LC+3  
3120 IF ID$="NEW DF" OR ID$="new df" THEN 2990  
3130 IF ID$="NEW CURVE" OR ID$="new curve" THEN PRINT:COLOR  
1,15:PRINT "C";:COLOR 15,1:PRINT "ontinue printing on this page or  
";:COLOR 1,15:PRINT "F";:COLOR 15,1:PRINT "orm feed to next page?"  
3140 IF ID$="NEW CURVE" OR ID$="new curve" THEN Q$=INKEY$:IF  
LEN(Q$)=0 THEN 3140  
3150 IF ID$="new curve" OR ID$="NEW CURVE" THEN IF Q$="C" OR Q$="c"  
T H E N P R I N T  
#1,"*****  
*****":RUN  
3160 IF ID$="new curve" OR ID$="NEW CURVE" THEN IF Q$="F" OR Q$="f"  
THEN PRINT #1,CHR$(12); ELSE 3130  
3170 IF ID$="new curve" OR ID$="NEW CURVE" THEN RUN  
3180 IF ID$="QUIT" OR ID$="quit" THEN CLOSE:COLOR ,,0:SYSTEM  
3190 IF ID$="edit" OR ID$="EDIT" THEN 1280  
3200 REM  
3210 IF ID$="HELP" OR ID$="help" THEN CLS:PRINT" H E L P S C R E  
E N":PRINT:PRINT "BAD ID Lets you reenter a sample id. SAMPLE  
RESPONSE"  
3220 IF ID$="HELP" OR ID$="help" THEN PRINT :PRINT "EDIT Places  
you in the edit mode. COMMAND"  
3230 IF ID$="HELP" OR ID$="help" THEN PRINT :PRINT "LAST BAD  
Removes the last sample entered from the printer buffer. COMMAND"  
3240 IF ID$="HELP" OR ID$="help" THEN PRINT:PRINT "HELP Displays  
this useless information. COMMAND"  
3250 IF ID$="HELP" OR ID$="help" THEN PRINT:PRINT "NEW CURVE  
Restarts the program. COMMAND"  
3260 IF ID$="HELP" OR ID$="help" THEN PRINT:PRINT "NEW DF Change  
the dilution factor and its units. COMMAND"  
3270 IF ID$="HELP" OR ID$="help" THEN PRINT:PRINT "QUIT Leave the  
program. COMMAND":PRINT:GOTO 2990  
3280 PRINT "Sample Response";  
3290 INPUT SAMRES$
```

```

3300 IF SAMRES$="BAD ID" OR SAMRES$="bad id" THEN 2990
3310 IF SAMRES$="" THEN 3280
3320 SAMRES=VAL(SAMRES$)
3330 IF B$="" OR B$="B" OR B$="b" THEN
SAMCONC=FNCALCONC(SAMRES-BLANK)
3340 IF B$="A" OR B$="a" THEN
SAMCONC=FNCALCONC(SAMRES)-FNCALCONC(BLANK)
3350 USEFLAG=1:IDL$=ID$
3360 PRINT:GOTO 3010
3370 END

```

5.0 Test sample print out

TEST CURVE PAGE 1

Correlation = 0.9999721496
Slope = 1.0237374840
Intercept = 0.1224305187

Standard Concentration	Response	Calculated Concentration	Percent Deviation
10.000	9.500	9.848	-1.52063
50.000	48.750	50.030	0.05927
0.000	0.000	0.122	Unable to compute

Blank correction factor is 0
Dilution factor is 1 with conversion units of ppm.

Sample Identification	Sample Response	Calculated Concentration	Times DF
test 1	25.300	26.023	26.023
test 1d	25.700	26.432	26.432
test 2	37.600	38.615	38.615
test 2d	35.500	36.465	36.465

END

**DATE
FILMED**

7 / 17 / 92