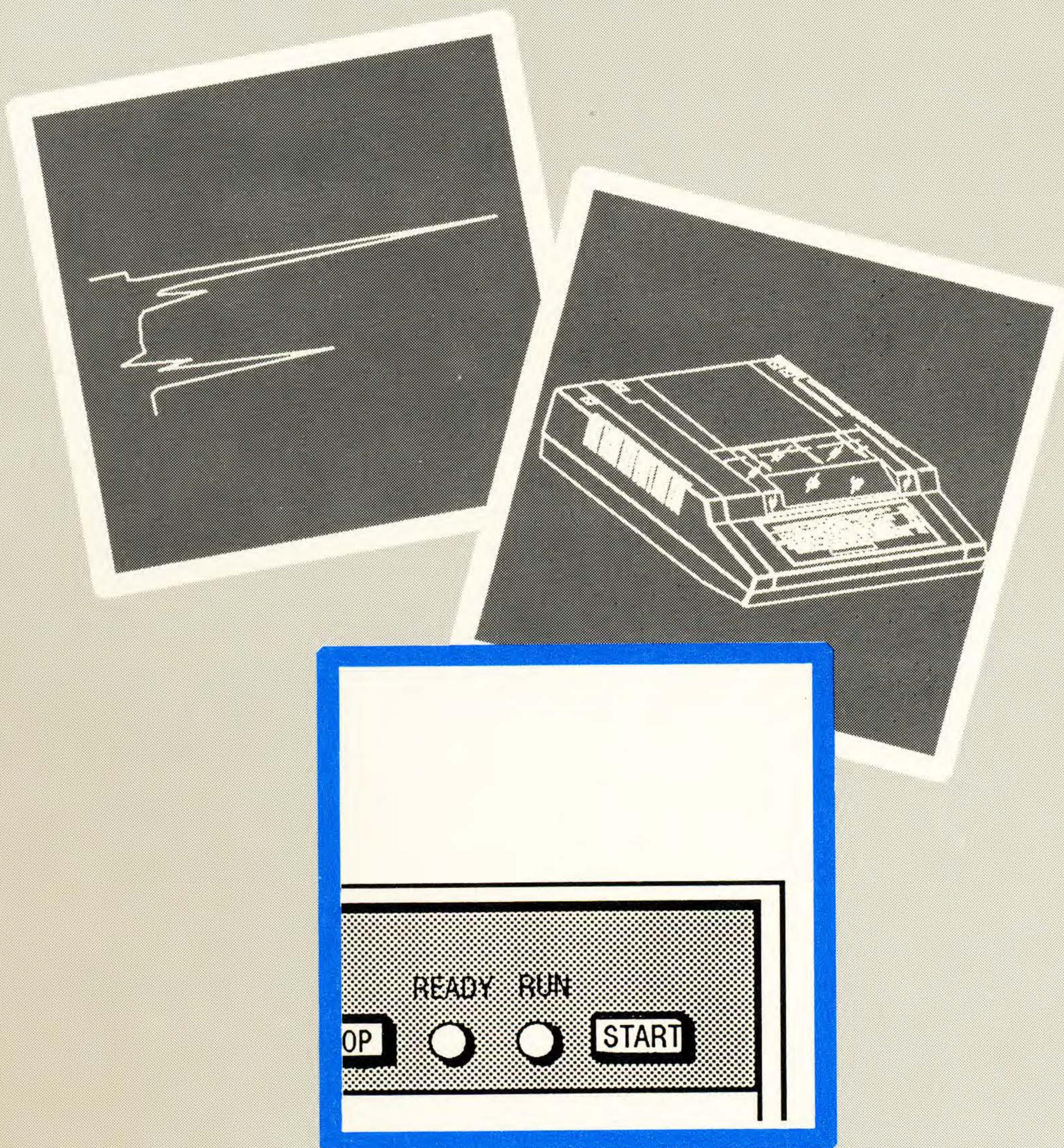


# HEWLETT-PACKARD



## HP 3396 Series III Integrator Tutorial

PLEASE FILL OUT BY USER AND MAIL.

**Tutorial**  
**HP 3396 Series II**  
**Part Number 03396-90235**

What is your major application of the product described in this manual?

---

How long have you been using this product?

Just received     1-3 months     3-6 months     6-12 months     Over 1 year

How often do you refer to this manual?

Daily     Weekly     Monthly     Seldom     Never

What was your level of integrator knowledge before you began using this manual?

None     Just beginning     Some experience     Experienced     Expert

Please indicate your agreement (or  
disagreement) with these statements:

	Strongly Agree	Agree	Neutral	Disagree	Strongly Disagree
• The manual contains the information I need.	<input type="checkbox"/>				
• The information is easy to find.	<input type="checkbox"/>				
• The information is accurate.	<input type="checkbox"/>				
• The instructions are easy to understand.	<input type="checkbox"/>				
• The manual contains enough examples.	<input type="checkbox"/>				
• The examples are appropriate and helpful.	<input type="checkbox"/>				
• The manual format and size are about right.	<input type="checkbox"/>				
• The illustrations are clear and helpful.	<input type="checkbox"/>				

Please feel free to write additional comments. Use additional sheets if you wish—the more detailed your comments, the more helpful they are to us.

---

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---

Name: \_\_\_\_\_ Date: \_\_\_\_\_

Thank you.

Title: \_\_\_\_\_

Company: \_\_\_\_\_

Address: \_\_\_\_\_

City/State/Zip: \_\_\_\_\_



June 1990

Phone: (\_\_\_\_\_) \_\_\_\_\_

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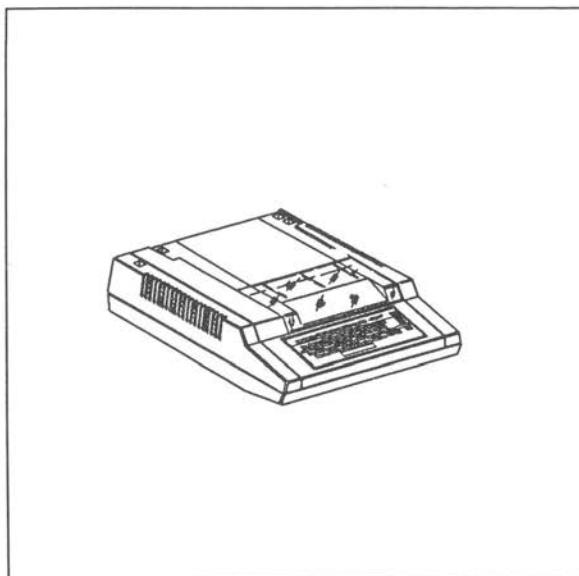
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# HP 3396 Series II Integrator

## Tutorial



# Printing History

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First edition—June 1990

Printed in USA

# Safety Information

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The HP 3396 Series II is an IEC (International Electrotechnical Commission) Safety Class 1 instrument. This unit has been designed and tested in accordance with recognized safety standards.

Whenever the safety protection of the HP 3396 Series II has been compromised, disconnect the unit from all power sources and secure the unit against unintended operation.

## WARNING

A WARNING CALLS ATTENTION TO A CONDITION OR POSSIBLE SITUATION THAT COULD CAUSE YOU OR OTHERS INJURY.

## CAUTION

A Caution calls attention to a condition or possible situation that could damage or destroy the product or your work.

## *Important User Information for In Vitro Diagnostic Applications*

This is a multipurpose product that may be used for qualitative or quantitative analyses in many applications. If used in conjunction with proven procedures (methodology) by a qualified operator, one of these applications may be in vitro diagnostic procedures.

General instrument performance characteristics and instructions are included in this manual. Specific in vitro diagnostic procedures and methodology remain the choice and the responsibility of the user and are not included.

# RFI Certification for the Federal Republic of Germany

---

## Manufacturer's Declaration

This is to certify that the equipment **HP 3396 Series II** is in accordance with the Radio Interference Requirements of Directive FTZ 1046/1984. The German Bundespost was notified that this equipment was put into circulation and the right to check the series for compliance with the requirements was granted.

## Herstellerbescheinigung

Hiermit wird bescheinigt, daß das Gerät/System

### **HP 3396 Series II**

in Übereinstimmung mit den Bestimmungen von Postverfügung 1046/84 funkentstört ist.

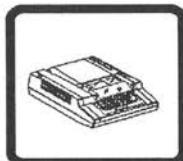
Der Deutschen Bundespost wurde das Inverkehrbringen dieses Gerätes/Systems angezeigt und die Berechtigung zur Überprüfung der Serie auf Einhaltung der Bestimmungen eingeräumt.

# Contents

---

## Chapter 1:

### Introduction



This chapter introduces the Tutorial Manual and describes the other manuals that arrived with your integrator. It also contains information for getting out of trouble.

## Chapter 2:

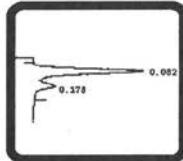
### Making Your First Run



This chapter teaches the basics of making a run. You can analyze the demo chromatogram using the default integrator conditions.

## Chapter 3:

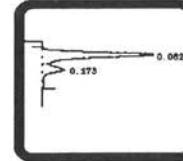
### Optimizing the Chromatogram



In this chapter you optimize the chromatogram by making several runs, changing the chart and integration parameters after each run.

## Chapter 4:

### Optimizing the Baseline



In chapter 4 you eliminate deficiencies in the default baseline by programming integration functions. The integrator reallocates the baseline, using your instructions, to create an optimized baseline.

## Chapter 5:

### Saving Your Method

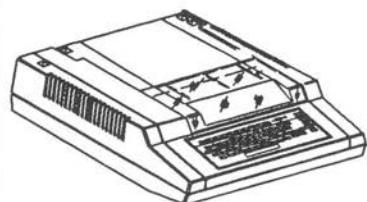


In this chapter you see how the integrator parameters can be saved in a "method" file. Review all the method entries you made in the course of this tutorial and save them in a method file for future recall.



# Introduction

---



## In this chapter....

- Introducing the Tutorial Manual ..... 1-2
- The Standard Manual Set ..... 1-3
- Manuals Included with Options and Accessories ..... 1-4
- Getting Out of Trouble ..... 1-5

---

## Introducing the Tutorial Manual

Welcome to the Hewlett-Packard 3396 Series II Integrator. If you are ready to use your integrator for the first time, you have come to the right place.

This Tutorial Manual introduces you to many of the major integrator functions by having you analyze a demo chromatogram supplied by Hewlett-Packard. You will edit several integrator parameters with the goal of optimizing your analysis. When you are finished, you will save the integrator parameters in a "method".

Once you start this manual, try to finish it in one sitting if possible; it should take no more than two hours.

- Before you start, disconnect any external devices from the integrator and verify that it is in good working order.
- Keep the *Quick Reference Card* handy. You will find it useful as a reference as you perform the tasks in this tutorial.

---

## The Standard Manual Set

The standard manual set comes with every HP 3396 Series II Integrator.

### **Installation, Maintenance, and Service Manual**

This is a three-part manual. It is the first manual you should open when your instrument arrives because it contains installation information. It also contains user maintenance, troubleshooting, and servicing information.

### **Tutorial**

This is the manual you are using now.

### **Operating Manual**

The Operating Manual contains procedures and facts needed during everyday operation.

### **Using Application Programs**

This manual, located in the Operating Manual binder, provides operating information for the application programs installed in your Series II integrator.

Using these programs, you can: schedule postrun programs, autoname data files, manage your files, reprocess data files, automate runs, plot peak calibration curves, and plot chromatographic baselines.

### **Networking Guide**

This manual describes how to use HP-IB, HP-IL, INET, and RS-232 devices with your Series II integrator. These devices include printers, disk drives, gas chromatographs, automatic samplers, etc.

### **Quick Reference Card**

This Quick Reference Card summarizes HP 3396 Series II operating tasks.

---

## **Manuals Included with Options and Accessories**

Depending on which options and/or accessories you ordered for your integrator, you may also have some of the following manuals.

### **HP Peak-96 Information Manager User's Guide**

This manual describes how to transfer files, manipulate and display data files, and remotely control your integrator using the HP Peak-96 Information Manager.

### **BASIC Language Manual Set**

The BASIC Quick Reference Guide is a handy and convenient reference for any BASIC programmer. It is small enough to keep by the integrator so it is ready when needed.

This BASIC User's Manual is intended for programmers new to the BASIC language or to those already familiar with BASIC but needing a brief review.

The BASIC Reference Manual is for the experienced BASIC programmer who must occasionally check the mnemonic or syntax of a BASIC command.

### **HP 5890 Dual Channel Operation**

This guide contains dual channel operating information for the HP 3396 Series II Integrator and HP 5890 GC combination.

### **Controlling the HP 1050 LC**

This guide contains operating information for controlling HP 1050 LC modules as a system under HP 3396 Series II method control.

### **HP EPROM Programmer Utility User's Guide**

This is an operating guide for users programming custom EPROMs for their HP 3396 Series II Integrator.

---

## Getting Out of Trouble

During the course of this tutorial, you may make a mistake. If you do, don't panic! You are bound to make a few mistakes when learning a new product. Some of the common problems that a new user may encounter are listed below.

### Stopping the Tutorial

To stop the demo chromatogram and end the tutorial, simply power the integrator Off and On. This resets the integrator to its default condition.

### Correcting a Typing Error

Correct a typing error in one of the following ways:

Use the [BKSP] key to erase the error and repeat your entry.

The printhead does not back up as a typewriter does. A block character is printed instead and the character to the left of the block is erased from integrator memory.

Press [ESC] to clear the entire entry and start again.

Press [BREAK] to cancel the entire command and start over.

### When You Hear a “Beep”

You will hear a “beep” when:

- You attempt to enter a command during plotting, integrating, or reintegrating. *Wait for the operation to end and try again.*
- You type faster than the integrator can accept input. *Try typing a little slower.*

### If You Get a “?” in Response to Your Entry

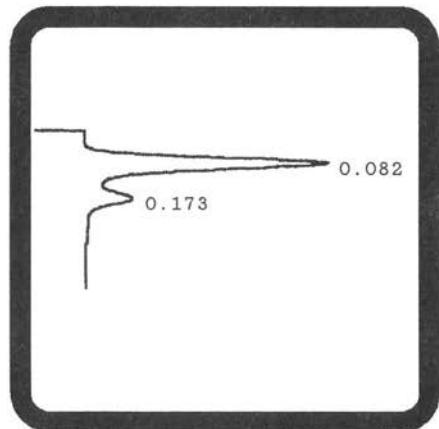
The numeric value is out of range. Enter a correct value after the “?”.

### If You Get an “Invalid System Command” Message

The command does not exist. Enter the correct command after the “?”.



# Making Your First Run



## In this chapter....

- Setting the Default Conditions ..... 2-2
- The Default Integrator Parameters ..... 2-3
- Integrator Parameters and the Method ..... 2-4
- Accessing the Demo Chromatogram ..... 2-5
- Making a Run ..... 2-7
- Reviewing the Run Data ..... 2-8

---

## Setting the Default Conditions

*Before starting this tutorial, disconnect any external devices from your integrator.*

When the integrator is switched on, it performs a number of internal checks called "power-on tests". When these tests are complete, the integrator's operating conditions are automatically set to their default values.

This process will erase whatever may currently be in the instrument memory. Be sure this is permissible before continuing.

To run the power-on tests:

1. Locate the instrument power switch. It is a white button on the rear panel, just below where the power cord plugs in.
2. Switch off the integrator. The indicator lights on the keyboard will turn off.
3. Wait about 10 seconds and switch on the integrator. The HP 3396 will perform its power-on tests.
4. When the tests are finished, the chart will look similar to this:

---

```
!"#$%&`()*+,-./0123456789: ;<=>?@ABCDEFGHIJKLMNPQRSTUVWXYZ[\]^_`abcde  
fghijklmnopqrstuvwxyz{|}-9: ;<=>?@ABCDEFGHIJKLMNPQRSTUVWXYZ[\]^_`abcde
```

---

```
Performing self test; unit will  
accept commands when KEYBD led is ON  
Model 3396B PLUS BASIC, Rev D.00.05 3/16/90  
Z80/HOST/INET/PP Rev = m/D/m/k w/ GENROM
```

\*

If your integrator fails to perform the power-on tests, put the tutorial aside and call your Hewlett-Packard service representative.

---

## The Default Integrator Parameters

When you first turn the integrator power on, the integrator parameters are automatically set to their default values.

To list the default parameters listed below, press [LIST] [METH] :

```
RUN PARAMETERS
ZERO      = 0
ATT 2^   = 0
CHT SP   = 1.0
AR REJ   = 0
THRSH    = 0
PK WD    = 0.04

TIMETABLE EVENTS
EMPTY

CALIBRATION
NO CALIB TBL

INTEGRATION PLOT TYPE ..... FILTERED

RUN DATA STORAGE
Store signal data ..... NO
Store processed peaks ..... NO

REPORT OPTIONS
Suppress local report ..... NO
HEIGHT% report ..... NO
Report uncalibrated peaks ... NO
Extended report ..... NO

POST-RUN LIST OPTIONS
Store post-run report ..... NO
External post-run report .... NO
List run parameters ..... NO
List timetable ..... NO
List calibration table ..... NO
List remote method ..... NO
Form-feed before report ..... NO
Form-feed after report ..... NO
Skip perforations in report . NO
Skip perforations in plot ... NO
```

The integrator parameters are stored in a file called a *method*.

---

## Integrator Parameters and the Method

When you edit any integrator parameter, you are editing a method.

A method is simply a file that contains all the parameters required by the integrator to perform an analysis—run parameters, data storage options, and report options.

A method will also contain:

- A calibration table when a calibrated report is specified.
- INET instrument set points when INET instruments are connected to the integrator.

### The Current Method

Throughout this tutorial you will be editing the current active method. The current active method is *the method currently controlling the analysis*.

The current active method has the following characteristics:

- There is always a method in memory—the current active method.
- You create a new method by modifying the current active method and saving it.
- When you start a run, all aspects of the analysis are controlled by the current active method.

Methods can be edited, stored, loaded, and listed.

---

## Accessing the Demo Chromatogram

To ensure a common starting point, you will have to set the default conditions.

The demonstration chromatogram is a set of data stored as part of the instrument diagnostics.

To access the demo chromatogram:

1. Hold down the **[CTRL]** **[SHIFT]** and **[BREAK]** keys simultaneously until the instrument starts printing. If you release any of them too soon, the diagnostics will not start.
2. The integrator should display the following message:

SELF TEST: (Press (M) key for more help)

=>

If the self-test message does not appear, you probably released one of the keys too soon. Wait for the instrument to finish whatever it's doing and then try again.

3. Type M (for menu) to list the menu of integrator self-tests.

=> M

Press the keys for the tests you want to perform. If you select no tests, you will return to the system software. After you have selected the tests you want, press ENTER. The tests will run continuously unless an error halts them.

- (O) Clear all tests
  - (1) ROM crc and bank select test
  - (2) Quick RAM test
  - (3) Extended RAM test (10 min)
  - (4) 8051 ROM and RAM test
  - (5) 8051 interface test
  - (6) RS232 port test
  - (7) HP-IL port test
  - (8) HP-IL bus test
  - (9) Remote control and sample # input test
  - (B) A/D Noise test
  - (L) P/P test
  - (K) Keyboard test
  - (N) High Speed Printer Test
  - (A) Run all tests
  - (T) Enable Demo Chromatogram
- 
- (P) Print error messages
  - (S) Suppress error messages
  - (C) Continue testing if error occurs
  - (H) Halt testing if error occurs  
(press SPACE to continue)

=>

## Enabling the Demo Chromatogram

The demo chromatogram is item T in the menu listing.

1. Type T to enable the demo chromatogram.

The integrator prints the following message:

```
=> Demo Chromatogram Enabled
^BREAK
*
```

While running the demo chromatogram, the integrator cannot process an external analog signal.

2. To terminate this demonstration, turn the instrument off and back on again to reset the default conditions and ready it for normal operation.

Otherwise, continue to "Making a Run" on the next page.

## Making a Run

To initiate a run using the default method:

1. Press [START] to run the Demo chromatogram.
2. After the last peak (labeled 1.150) appears, stop the run by pressing [STOP]

```
* RUN # 1 JUN 1, 1990 00:07:11
START

STOP
RUN# 1 JUN 1, 1990 00:07:11
AREA%
    RT      AREA  TYPE    WIDTH      AREA%
.082    369702   BV    .039  28.85754
.173    79010    VB    .042  6.16722
.499    67789    PV    .040  5.29135
.574    246119   VB    .048  19.21111
.832    511335   BP    .053  39.91288
1.150    7173    VP    .072  .55990
TOTAL AREA=1281128
MUL FACTOR=1.0000E+00
```

## Reviewing the Run Data

The run data is displayed below:

```
* RUN # 1 JUN 1, 1990 00:07:11
START

STOP

RUN# 1 JUN 1, 1901 00:07:11

AREA%
  RT      AREA   TYPE   WIDTH     AREA%
.082    369702   BV    .039  28.85754
.173    79010    VB    .042  6.16722
.499    67789    PV    .040  5.29135
.574    246119   VB    .048  19.21111
.832    511335   BP    .053  39.91288
1.150    7173    VP    .072  .55990

TOTAL AREA=1281128
MUL FACTOR=1.0000E+00
```

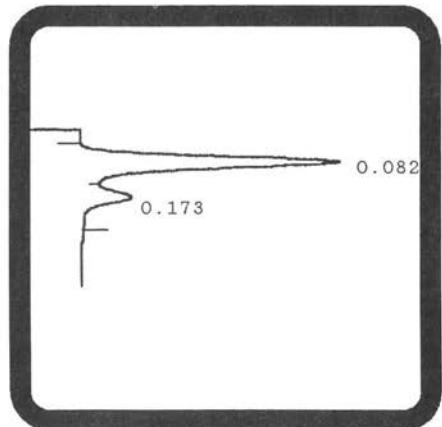
To review the run data:

- The first line identifies the run by supplying the run number, date, and time.
- The chromatogram appears next; in this case it looks terrible. The peaks are jammed together and they run off the right edge of the chart.
- The report of the analysis follows the chromatogram.

Until you have improved the chromatogram, you cannot accurately correlate much in the chromatogram with the report.

Your next step is *Optimizing the Chromatogram*.

# Optimizing the Chromatogram



## In this chapter...

- Optimizing Your Analysis ..... 3-2
- Setting the Chart Parameters ..... 3-3
- Setting a Timed Stop ..... 3-7
- Adding Integration Event Marks to the Chart ..... 3-9
- Evaluating the Integration ..... 3-12
- Changing the Integration Parameters ..... 3-14
- Evaluating the Improved Integration ..... 3-16

---

## Optimizing Your Analysis

To obtain the most reliable data, you should perform your analyses under the most favorable conditions by optimizing the analysis.

Each analysis has three components to be optimized:

- the chromatography
- the chromatogram
- the baseline

### The Chromatography

You optimize the chromatography by varying instrument temperatures, carrier gas flow, and the column selection. This is an important first step. A good analysis always starts with good chromatography.

In this tutorial you are using a demo chromatogram, so you can assume that the chromatography is good.

### The Chromatogram

Once the chromatography is under control, you take steps to optimize the chromatogram.

First, you adjust the chart parameters (attenuation and chart speed) to improve the appearance of the chromatogram. This is important when optimizing the integration where you must be able to see the integration marks clearly.

Next, you select the appropriate peak width and threshold parameters to control how the peaks are recognized and integrated.

### The Baseline

Finally, you optimize the baseline construction, using the integration functions. Your goal here is to get the best baseline fit for each peak in the chromatogram.

## Setting the Chart Parameters

The chart parameters are ATT 2<sup>^</sup> and CHT SP and ZERO.

### Run 1

In Run 1, the peaks run off scale and are too crowded to be seen properly. You have to adjust the ATT 2<sup>^</sup> and CHT SP values to improve the appearance of the chromatogram. The default integrator parameters are not suitable for the data supplied by the demo chromatogram.

```
* RUN # 1 JUN 1, 1990 00:07:11
START

STOP
RUN# 1 JUN 1, 1990 00:07:11
AREA%
    RT      AREA   TYPE   WIDTH      AREA%
.082    369702   BV    .039    28.85754
.173    79010    VB    .042    6.16722
.499    67789    PV    .040    5.29135
.574    246119   VB    .048    19.21111
.832    511335   BP    .053    39.91288
1.150    7173    VP    .072    .55990
TOTAL AREA=1281128
MUL FACTOR=1.0000E+00
```

1. To review the default integrator values, press [LIST] [LIST].

```
* LIST: LIST
```

```
PEAK CAPACITY: 1244
```

```
ZERO      = 0, -0.827
ATT 2^     = 0
CHT SP     = 1.0
AR REJ     = 0
THRSH     = 0
PK WD     = 0.04
```

## Attenuation (ATT 2<sup>^</sup>)

ATT 2<sup>^</sup> (attenuation) sets the scale for the chart width. Low numbers expand the chromatogram's width; large values compress it. The valid range is from -8 to 36.

Currently, the value is 0 and the peaks go off-scale; a larger value is needed to keep them on the paper.

2. Press [ATT 2↑] [5] [ENTER] to increase the attenuation to 5.

\* ATT 2<sup>^</sup> 5 @

Since each increase of 1 divides the peak heights by 2, going from 0 to 5 will scale them down by a factor of 32.

## Chart Speed (CHT SP)

CHT SP is the chart or paper speed. Low numbers cause peaks to be squeezed together into groups of narrow peaks. A faster chart speed spreads out the peaks.

The current setting of 1 cm/min is too slow. Try doubling it and then check the results.

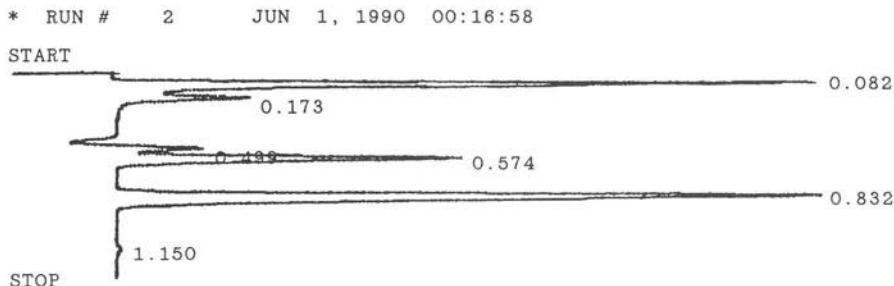
3. Press [CHT SP] [2] [ENTER] to double the chart speed.

\* CHT SP 2 @

Notice that each key press is printed on the chart so you can keep track of the changes. The [ENTER] key is acknowledged with an @ symbol.

## Run 2

1. Press [START] to begin a new run using new chart parameters.



RUN# 2 JUN 1, 1990 00:16:58

AREA%				
RT	AREA	TYPE	WIDTH	AREA%
.082	369702	BV	.039	28.85754
.173	79010	VB	.042	6.16722
.499	67789	PV	.040	5.29135
.574	246119	VB	.048	19.21111
.832	511335	BP	.053	39.91288
1.150	7173	VP	.072	.55990

TOTAL AREA=1281128  
MUL FACTOR=1.0000E+00

2. Use [STOP] to end the run after the peak at 1.150 is finished.

This chromatogram is much improved. Individual peaks are now separated and appear on scale.

You can make further improvements by increasing the chart speed and decreasing the attenuation. This will make some of the details at the base of the peaks become more apparent.

3. Press [ATT 2↑] [4] [ENTER] to decrease the attenuation to 4.

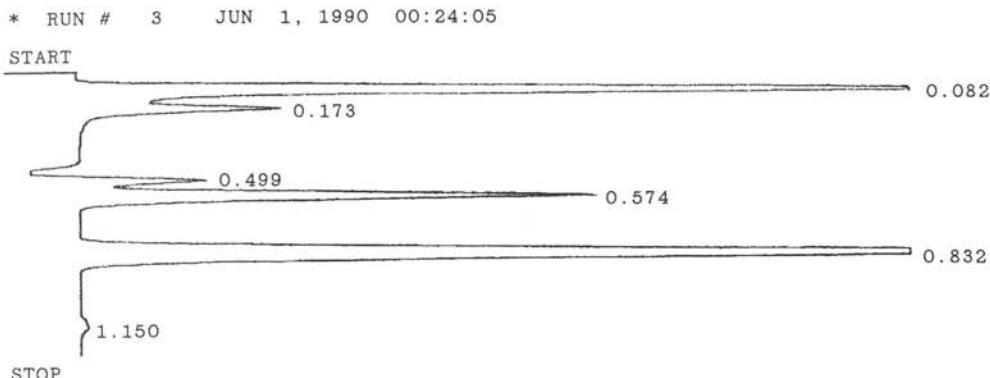
\* ATT 2^ 4 @

4. Press [CHT SP] [4] [ENTER] to double the chart speed.

\* CHT SP 4 @

## Run 3

1. Press [START] to begin a new run using the new chart parameters.
2. Press [STOP] to end the run after the peak at 1.150 is finished.



RUN# 3 JUN 1, 1990 00:24:05

### AREA%

RT	AREA	TYPE	WIDTH	AREA%
.082	369702	BV	.039	28.85754
.173	79010	VB	.042	6.16722
.499	67789	PV	.040	5.29135
.574	246119	VB	.048	19.21111
.832	511335	BP	.053	39.91288
1.150	7173	VP	.072	.55990

TOTAL AREA=1281128  
MUL FACTOR=1.0000E+00

Now compare the three reports that you've generated. The reports should be identical, since changes in ATT 2^ and CHT SP do not change the data; they just change the plot appearance. If you find differences between the reports, the most likely reason is that you ended a run too soon and missed the last peak.

What you have accomplished so far is to improve the appearance of the chromatogram to make the integration and baseline marks easier to observe. This will be important when you attempt to *optimize the baseline*.

---

## Setting a Timed Stop

So far, you've been using the [STOP] key to end the runs. This is satisfactory for single runs; however, it is usually more convenient to have the instrument stop the runs automatically.

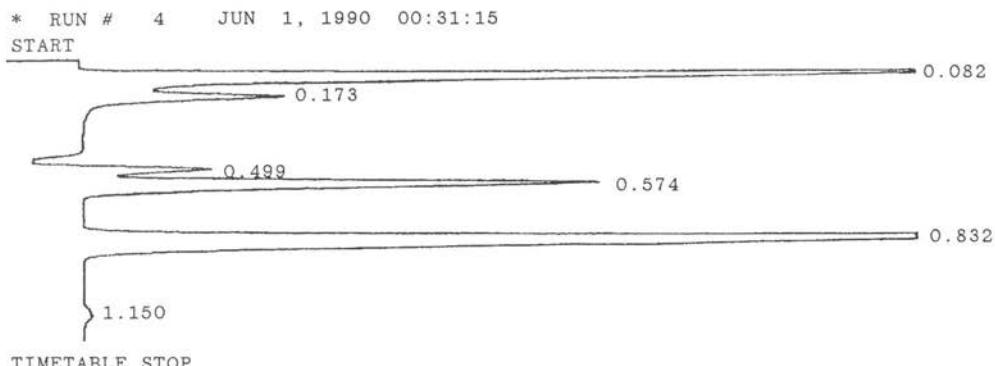
Note that the retention time of the last peak is 1.150 minutes. Stopping the run at 1.3 minutes should not interfere with that peak.

1. Press [TIME] [1] [.] [3] [STOP] to program the run to stop automatically 1.3 minutes after the run is started.

\* TIME 1.3 STOP @

## Run 4

1. Press [START] to begin a new run with a time programmed "Stop" command.



RUN# 4 JUN 1, 1990 00:31:15

### AREA%

RT	AREA	TYPE	WIDTH	AREA%
.082	369702	BV	.039	28.85754
.173	79010	VB	.042	6.16722
.499	67789	PV	.040	5.29135
.574	246119	VB	.048	19.21111
.832	511335	BP	.053	39.91288
1.150	7173	VP	.072	.55990

TOTAL AREA=1281128  
MUL FACTOR=1.0000E+00

2. This time the run ends automatically.

Note that the notation at the end of the chromatogram has changed from STOP to TIMETABLE STOP.

---

## Adding Integration Event Marks to the Chart

By adding integration event marks, you can see exactly how each peak was integrated. Then you can correct poor integrations by programming the appropriate integration function.

3. Press [TIME] [0] [INTG()] [8] [ENTER] to program integration function 8 to begin at the start of the run.

```
* TIME 0 INTG # 8 @
```

Programming integration function 8 will add integration event marks to the chart. A list of all the integration functions appears in section 4.

4. Press [LIST] [TIME] [ENTER] to verify that the timetable entry has been made.

```
0.000 INTG # = 8  
1.300 STOP
```

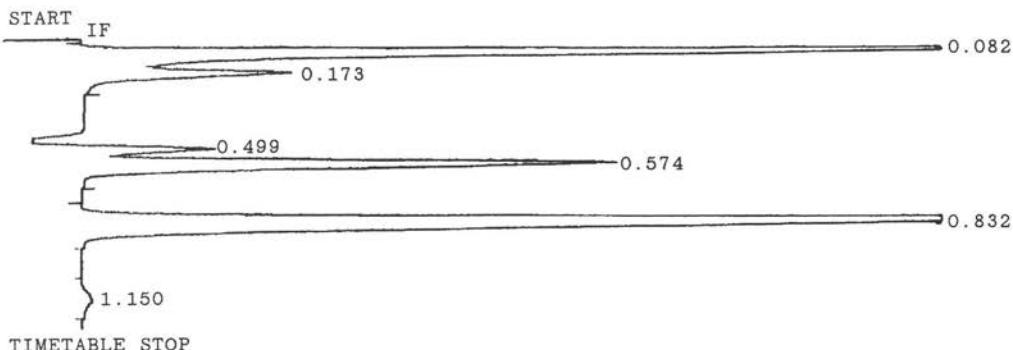
5. Press [LIST] [LIST] to list the current values for all the control parameters.

```
ZERO    = 0,0.002  
ATT 2^ = 4  
CHT SP = 4  
AR REJ = 0  
THRSH  = 0  
PK WD  = 0.04
```

## Run 5

1. Press [START] to make a run with integration event marks.

\* RUN # 5 JUN 1, 1990 00:54:45



RUN# 5 JUN 1, 1990 00:54:45

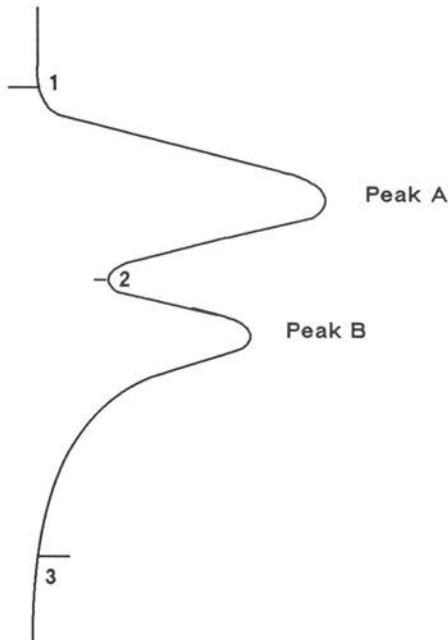
AREA%

RT	AREA	TYPE	WIDTH	AREA%
.082	369702	BV	.039	28.85754
.173	79010	VB	.042	6.16722
.499	67789	PV	.040	5.29135
.574	246119	VB	.048	19.21111
.832	511335	BP	.053	39.91288
1.150	7173	VP	.072	.55990

TOTAL AREA=1281128  
MUL FACTOR=1.0000E+00

## Integration Event Marks and Baseline Points

There are three kinds of integration event marks:



1. Long, to the left. This marks the start of a peak. It is often called a long down-tick. It is a baseline point.
2. Short, to the left. This marks the valley between two peaks that are not completely separated. It may be a baseline point.
3. Long, to the right. This marks the end of a peak. It is often called an up-tick. It is a baseline point.

When the baseline is drawn, it passes through all the baseline points. A baseline point is marked by a long down-tick or an up-tick.

---

## Evaluating the Integration

Notice the IF mark on the chart right at the beginning of the plot. This tells you that an integration function is executed at that time. The particular integrator function is not identified; you must consult the LIST TIME print-out for that.

<u>Peak R.T.</u>	<u>Where integration begins and ends</u>
0.082	Begins with a long down-tick. Ends at the valley between it and the second peak, which is marked with a short down-tick.
0.173	Begins at the valley between it and the previous one; ends at the up-tick mark.
0.499	This ends at the valley between it and the 0.574 peak, but where did it start? There is no long down-tick mark.
0.574	This is very similar to the 0.173 peak. It begins at a marked valley and ends at the up-tick.
0.832	Begins at the long down-tick, which is reasonable, but is followed by a valley marker instead of an up-tick. It doesn't look like a valley because there's no following peak visible.
1.150	Starts and ends at valley markers, but where are the valleys?

A word of warning: the signal makes a strong negative (downscale) move just before the 0.499 peak. What is this negative “object”? There are three major possibilities:

- *This is data from a gas chromatograph.* Since GC peaks are usually positive, the negative “object” is probably a signal disturbance caused by a valve operation or some other happening that is not related to the data. Thus you should probably ignore the “object”.
- *This is data from a liquid chromatograph.* LC data may go in either direction from the baseline, depending on the detector and sample. The “object” may be perfectly valid data, and you must be prepared to handle it.
- *This is data from some other instrument.* You must use your knowledge of that instrument to decide how to process the “object”.

The HP 3396 cannot know how to process everything that may happen; sometimes the decisions depend on information that is not part of the signal. In such cases, you must provide some assistance. For the moment, ignore the 0.499 peak and examine the other two questionable ones.

The problem for the 0.832 and 1.150 peaks is that you are integrating with a noisy signal. You can prove this to yourself by making a run with very low attenuation. This will expand the area near the baseline and show you how noisy this signal is. The integrator is much more sensitive than your eyes, and it is finding valley points between noise spikes and real peaks.

---

## Changing the Integration Parameters

So far, you have simply improved the chromatogram's appearance. The changes you've made have not affected the peak areas.

In this part of the tutorial, the objective is to find a combination of integration parameters that will find peaks where they exist and mark them with start and stop tick-marks.

- The start of a peak is marked by a long down-tick.
- The end of a peak is marked by an up-tick.
- If the signal does not reach the baseline between peaks, a short down-tick marks the end of one peak and the start of the next.

The two main integration control parameters are peak width and threshold. Peak width controls the peak finding process; threshold controls integrator sensitivity.

### Changing the Peak Width Value

PK WD (peak width) controls the ability to find peaks. Compare the present value (0.04) with the measured peak widths in the WIDTH column of the report.

2. PK WD should be *Greater than* WIDTH/4 and *Less than* 2\*WIDTH

In this case it is—for all peaks—so *the current value is satisfactory*.

### Changing the Threshold Value

THRSH (threshold) controls sensitivity. Increasing the threshold value makes the integrator less responsive to noise in the signal. The present value (0) is very low (the range is from -6 to +28).

3. Press [THRSH] [2] [ENTER] to increase the threshold to 2.

\* THRSH 2 @

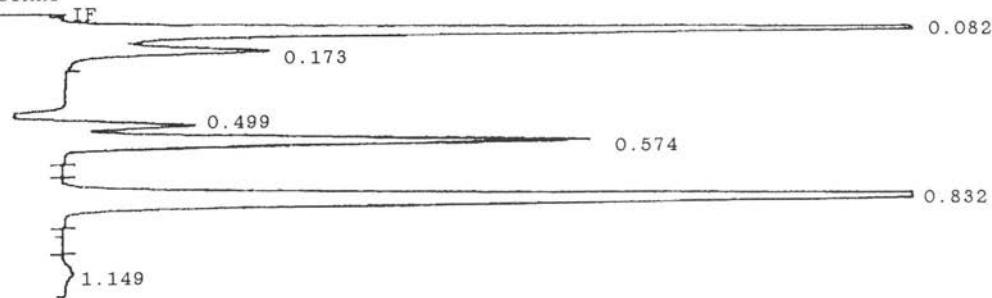
Since THRSH uses the same kind of scale as ATT 2<sup>^</sup>, that is a factor of 4 in sensitivity reduction.

## Run 6

Press [START] to make a run with an increased threshold value  
(from 0 to 2).

\* RUN # 6 JUN 1, 1990 01:08:45

START



TIMETABLE STOP

RUN# 6 JUN 1, 1990 01:08:45

AREA%

RT	AREA	TYPE	WIDTH	AREA%
.082	370294	BV	.039	28.89322
.173	79248	VB	.043	6.18355
.499	67827	PV	.040	5.29239
.574	246478	VB	.048	19.23213
.832	511110	PB	.053	39.88078
1.149	6638	BV	.070	.51795

TOTAL AREA=1281595  
MUL FACTOR=1.0000E+00

---

## Evaluating the Improved Integration

This integration has almost twice as many tick marks, showing that the data integration works better with this value of THRESH than with the original one.

- The first four peaks have the same tick marks as before.
- The 0.574 peak now ends with a definite end of peak mark rather than the noise-induced valley mark.
- The 0.832 peak also ends with an end mark.
- The last peak now begins with a start mark but still ends with a valley mark. Its retention time has changed from 1.150 to 1.149, something that can occur with very small peaks and a lot of noise.

You might want to experiment with other values for THRESH, but 2 is about optimal for this data. Lower values get back into a lot of valley marks, and higher values (starting at 4) reduce sensitivity so much that the last peak is not found.

## A Suggested Strategy for Setting the Integration Parameters

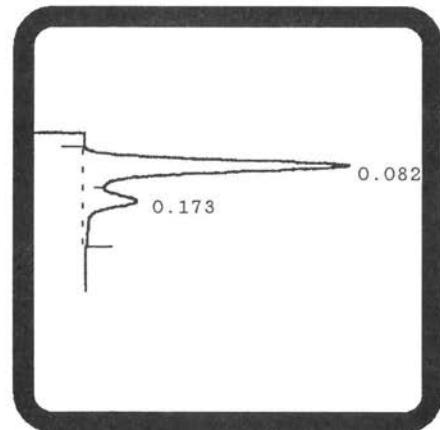
The strategy in selecting a certain peak width or threshold value is not to get the most or least possible number of tick marks.

Instead, you try to find a combination of values that produces the right kind of tick marks for each peak. Ideally, each peak begins with a long down-tick and ends with an up-tick. If peaks are merged, the valleys are marked with short down-ticks.

There will often be some excess marks, usually with more down-ticks than up-ticks. This happens when the instrument believes that a peak is starting (and writes a down-tick), but for some reason, the data that follows does not continue to look like a peak. Thus the down-tick is a false alarm, but it has already been printed and the integrator cannot back up and erase it. It could suppress the extra marks by reducing sensitivity, but this would almost certainly lose some real data.

Your next step is *Optimizing the Baseline*.

# Optimizing the Baseline



## In this chapter....

- Running the Baseline Program ..... 4-2
- Examining the Current Baseline ..... 4-4
- Using Integration Functions to Optimize the Baseline ... 4-7
- Archiving Run Data ..... 4-16

---

## Running the Baseline Program

In section 3 you improved the chromatogram by adjusting the main integration controls PK WD and THRSH.

In this section you will use the Baseline program to examine how the baseline is drawn and locate any peak or baseline situations that require special attention.

The Baseline program is a basic program that can be scheduled to execute after each run. When the Baseline program executes, it replots the chromatogram obtained during the analysis and draws in the baseline, showing exactly how the integration was performed.

### Storing Run Data

The Baseline program requires that you store the signal and processed peak files.

1. Press [OPO] [2] [ENTER] to select Option 2 and respond to the dialog as shown below.

```
RUN DATA STORAGE
Store signal data [Y/N*]: Y [ENTER]
Device [M*]: [ENTER]
Bunched or raw data [B/R*]: B [ENTER]
Store processed peaks [Y/N*]: Y [ENTER]
Device [M*]: [ENTER]
*
```

## Setting Up the Baseline Program

Assigning the Baseline program to Key 0 will make it execute after each run as a postrun program.

2. Type the command string **ASSIGN 0, E:BASELINE.BAS** and press **[ENTER]** to assign the Baseline program to Key 0.

```
*ASSIGN 0, E:BASELINE.BAS @
```

When each run completes, the Baseline program executes, replottedting the original chromatogram and drawing in the baseline according to the integration marks.

As you continue with this exercise, compare the baselines that you obtain with those in the illustrations.

## Examining the Current Baseline

Increase the chart speed to 12 to spread out the chromatogram, making the baseline easier to see.

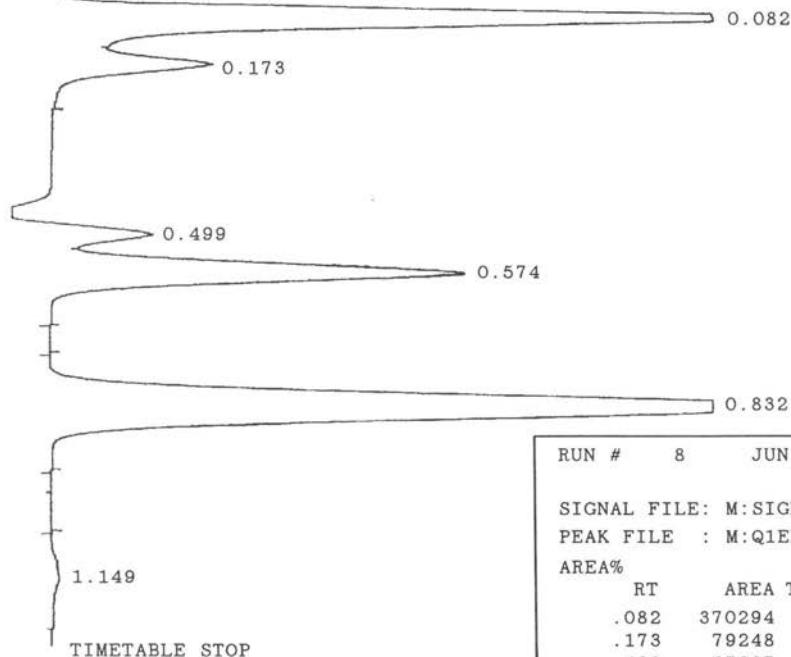
3. Press [CHT SP] [12] [ENTER] to increase the chart speed to 12.

CHT SP 12 @

4. Press [START] to begin your examination of the default baseline.

\* RUN # 8 JUN 1, 1990 01:17:53

START  
IF



Closing Signal File M: SIGNAL.BNC  
Storing processed peaks to M: Q1EB8423.PRO

RUN # 8 JUN 1, 1990 01:17:53

SIGNAL FILE: M: SIGNAL.BNC  
PEAK FILE : M: Q1EB8423.PRO

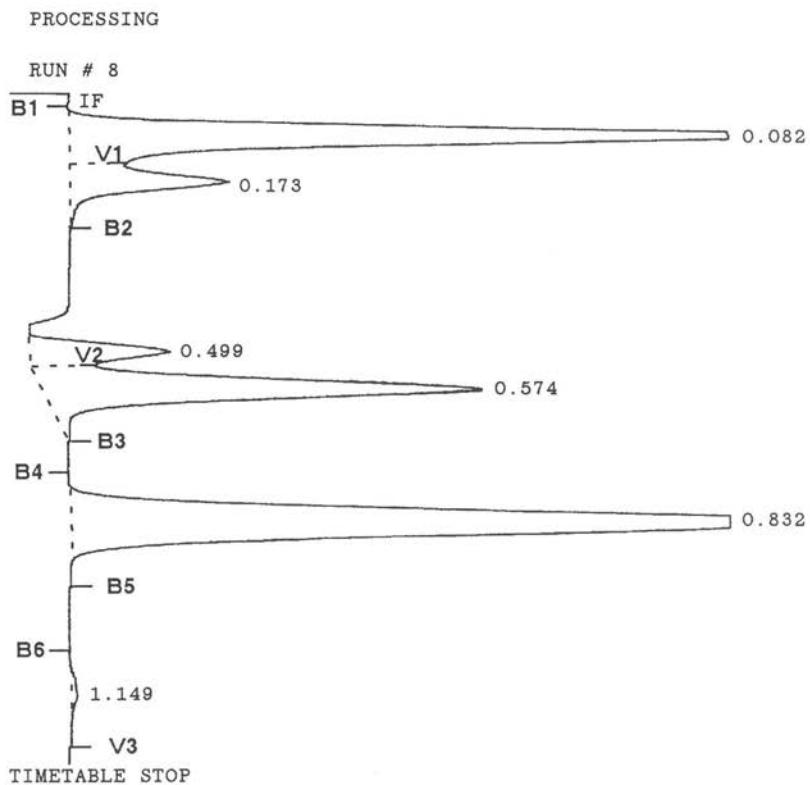
AREA%

RT	AREA	TYPE	WIDTH	AREA%
.082	370294	BV	.039	28.89322
.173	79248	VB	.043	6.18355
.499	67827	PV	.040	5.29239
.574	246478	VB	.048	19.23213
.832	511110	PB	.053	39.88078
1.149	6638	BV	.070	.51795

TOTAL AREA=1281595  
MUL FACTOR=1.0000E+00

## Examining the Baseline Construction

First note that the replotted chromatogram has fewer integration event marks than the original run. This is because any false start-of-peak integration marks that appear on the original chromatogram are not saved in the processed peak file. When the Baseline program replots the chromatogram, it includes only those integration event marks stored in the processed peak file.



For illustration purposes, marks have been added to the chromatograms to indicate baseline points. The baseline points are labeled with the letter **B** and a number to reference them. Valley points are labeled with the letter **V**.

The baseline starts at the first data point in the chromatogram and then goes from baseline point to baseline point (in this instance from B1 to B6) unless some special situation arises.

There is a baseline penetration between points B2 and B3. This section of the baseline is allocated as a rubber band line anchored at the penetration point and B3. However, because the negative-going peak is clipped, the baseline cannot be drawn as allocated. It has to be drawn horizontally while inside the clipped region.

The baseline is drawn normally once it is outside the clipped region. *Negative peaks should be inverted or clamped to make the baseline allocation obvious. This is especially true when using the Baseline program.*

At the end of the run, the last baseline point is B6. The line is drawn vertically down from that point until the run ends and passes below point V3. How do you know? If the line went through V3, it would be another baseline point, not a valley point. If it went above it, V3 would become a penetration point and lose the V label.

The table below shows the peak types that appear in the printed report and the baseline constructions they represent.

<b>Peak</b>	<b>Type</b>	<b>Baseline Construction</b>
0.082	<b>BV</b>	Starts at a baseline point. It ends at a dropline from a valley point.
0.173	<b>VB</b>	Begins at a valley dropline and ends at a baseline point.
0.499	<b>PV</b>	Begins at a penetration point and ends at a dropline from a valley point.
0.574	<b>VB</b>	Begins at a valley dropline and ends at a baseline point.
0.832	<b>PB</b>	Begins at a penetration point and ends at a dropline from a valley. The dropline is too short to be visible.

Obviously, the third and fourth peaks are measured from a very odd baseline—but what is the “correct” baseline? That depends on the type of data and, to some extent, on the information you want to extract. In this case it’s quite clear that you must optimize the baseline construction so that the baseline penetration doesn’t cause mismeasurement of the 0.499 and 0.574 peaks.

In the runs that follow, only the replotted chromatogram and baseline (per the Baseline program) are shown. The report for each run is superimposed for your reference.

---

## Using the Integration Functions to Optimize the Baseline

The tools for correcting or customizing the baseline construction are the integration functions, all controlled by the [INTG( )] key. These functions must be time programmed.

### The 15 Integration Functions

<u>Function No.</u>	<u>Resulting Action</u>
0	Set baseline now
1	Set baseline at next valley
2	Set baseline at all valleys
3	Tangent skim from next peak
4	Turn off tangent skim
5	Draw horizontal baseline
6	Measure and update threshold
7	Turn off retention time labeling
8	Turn on start/stop marks
9	Turn off integration
10	Increment threshold
11	Invert negative peaks
12	Clamp negative peaks
13	Show functions 11 and 12
14	Start peak sum window

## Choosing the Integration Functions

When a baseline penetration goes well below the expected baseline, use integration function 11 (invert negative peaks) and integration function 12 (clamp negative peaks). The penetration may actually be a peak you want to quantify.

<u>Function No.</u>	<u>Resulting Action</u>
11	Sets a baseline level and inverts any signal changes that go below that level. This turns negative peaks into positive peaks before integration.
12	“Clamps” the signal at the baseline level; any signal that goes below baseline is ignored.
13	Does not change the signal treatment, it just causes the chart to display the result of functions 11 or 12 if they are used. The inversion or clamping occurs regardless of whether or not you make it visible with function 13.

## Consider the Source

Before you program the integration functions, consider the data source.

You may be collecting any one of the following types of data:

- Gas Chromatographic Data
- Liquid Chromatographic Data
- Data from Some Other Source

Each source of chromatographic data has different characteristics which, in turn, require different treatment using the integration functions.

## Analyzing Gas Chromatographic Data

Assume that you are analyzing gas chromatographic data. All the peaks should be positive. The baseline penetration is almost certainly a baseline upset. To prevent inaccurate areas for peaks nearby, it should be ignored. Use function 12 (clamp negative peaks) to do this and function 13 to display IF (for integrator function) on the chart to see when it occurs.

Function 12 must be programmed to start before the baseline goes negative and after the end of the preceding (0.173) peak. An event time of 0.38 minute seems reasonable.

1. Press [TIME] [.38] [INTGO] [12] [ENTER] to clamp the baseline penetration.

```
* TIME .38 INTG # 12 @
```

2. Press [TIME] [0] [INTGO] [13] [ENTER] to display IF on the chart to see when an integrator function occurs.

```
* TIME 0 INTG # 13 @
```

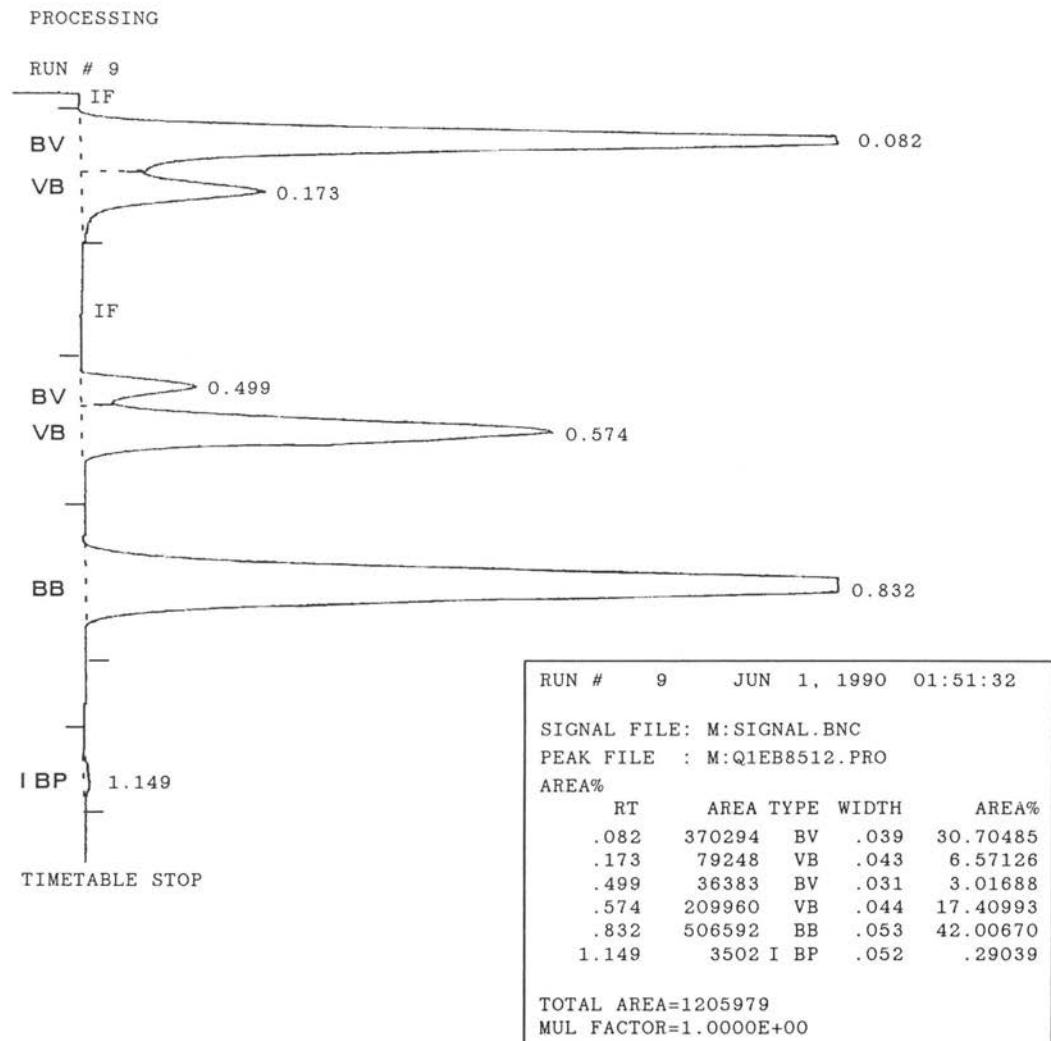
Function 13 can be activated for the entire run; it takes no action until function 12 occurs.

3. Press **LIST**] [TIME] [ENTER] to display the list of your timetable entries.

```
* LIST: TIME @  
0.000 INTG # = 8  
0.000 INTG # = 13  
0.380 INTG # = 12  
1.300 STOP
```

**4.** Press [START] to make another run.

The baseline is drawn in and the peak area allocations are indicated with droplines.



This baseline looks much better. Note the IF mark where the clamping started. The baseline penetration has vanished and the third and fourth peaks now have reasonable baselines.

However, look over the chromatogram and report of run 9 (not its replot with baseline).

- There are not as many tick marks as in run 8, and some of them have changed type.
- The areas reported for the last two peaks have decreased. The 1.149 peak does not have a tick mark showing when it ends; its type in the report is now I BP, incomplete baseline point to penetration point.

IF 12, the clamp is the cause. The clamping function occurs at 0.38 minute and the baseline remains unchanged for the remainder of the run. The true baseline drifts very slightly, but the clamp prevents the software from compensating for the drift.

The solution to this drift dilemma is simple; make another timetable entry to release the clamp as soon as the baseline penetration has passed. The time selected must be after the end of the baseline penetration but before the retention time of the next peak. An event time of 0.48 minute seems reasonable here.

5. Press [TIME] [.48] [INTG0] [-] [12] [ENTER] to release the clamp.

```
* TIME .48 INTG # - 12 @
```

6. Press [LIST] [TIME] [ENTER] to display the list of your timetable entries.

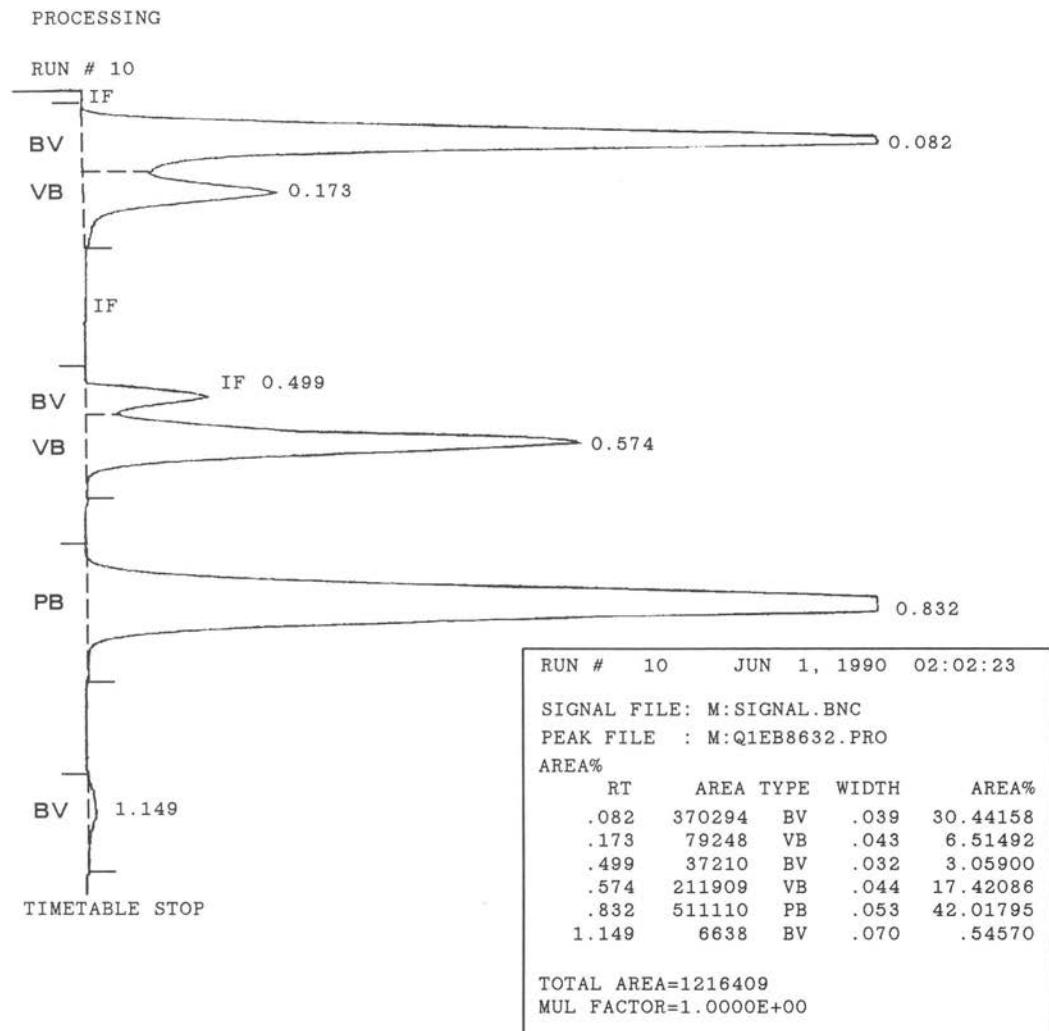
```
* LIST: TIME @
```

```
0.000 INTG # = 8
0.000 INTG # = 13
0.380 INTG # = 12
0.480 INTG # = -12
1.300 STOP
```

In the next run the baseline penetration will be inverted and we can tell if it is a chromatographic peak.

7. Press [START] to begin run 10.

The baseline and droplines indicate the peak area allocations.



Notice that the last two peaks now have the areas they had before we experimented with the clamp. This means that the drift compensation is working again and that the clamp has been effectively canceled. The IF mark on the leading edge of the 0.499 peak shows that the time chosen for the event was appropriate.

## Analyzing Liquid Chromatographic Data

With some HPLC sample and detector combinations, negative peaks are perfectly valid data. The baseline penetration does not look much like a peak, but we'll treat it as such for this experiment.

To do this, you must replace the function 12 entries, which clamp negative peaks, with function 11 entries, which will invert them.

To invert the baseline penetration instead of clamping it:

1. Press **[DEL] [TIME] [.38] [ENTER]** to delete the function 12 entry at .38 minute.

```
* DELETE TIME .38 @
```

2. Press **[DEL] [TIME] [.48] [ENTER]** to delete the function 12 entry at .48 minute.

```
* DELETE TIME .48 @
```

3. Press **[TIME] [.38] [INTG0] [11] [ENTER]** to invert the negative peak.

```
* TIME .38 INTG # 11 @
```

4. Press **[TIME] [.48] [INTG0] [-] [11] [ENTER]** to turn off function 11.

```
* TIME .48 INTG # - 11 @
```

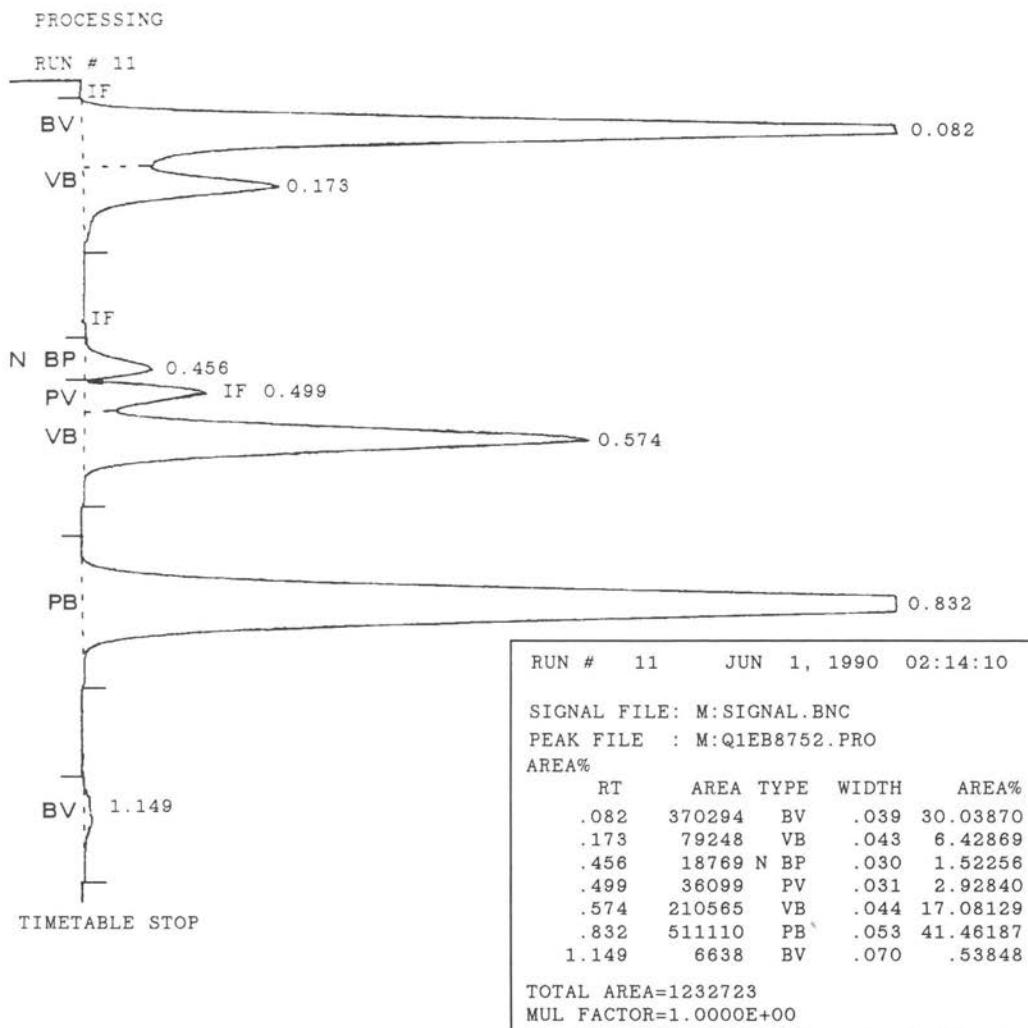
If you left function 11 on, all peaks after .38 minute would be inverted.

5. Press **[LIST] [TIME] [ENTER]** to display the list of your timetable entries.

```
0.000 INTG # = 8
0.000 INTG # = 13
0.380 INTG # = 11
0.480 INTG # = -11
1.300 STOP
```

6. Press [START] to begin run 11.

The baseline and droplines indicate the peak area allocations.



The 0.456 peak contains a letter N in the peak type column to show that this is a negative peak inverted for integration. It is a very well-shaped peak. The distortion apparent in the earlier runs was due to "clipping" when it reached the mechanical limit of the plotter.

## Analyzing Data from Other Sources

Make your first run without using the integration functions. This will probably do a fair-to-good job of integrating your peaks. Then use the integration functions where necessary to optimize your integration.

The Operating Manual contains a complete discussion of integration and the integration functions.

**Note:** Do not disable the default chromatogram or change any method parameters at this time. You will be using them in the next section.

---

## Archiving Run Data

When you store run data in the Memory Disk (M:), it is saved in a file named SIGNAL. An appended extension identifies the type of data stored in the file.

- M: SIGNAL . RAW      Raw data is unprocessed integrator signal input.
- M: SIGNAL . BNC      Bunched signal data is raw data that has been processed by the peak width filter. It contains much less “noise” than raw data and can be stored in much less memory. Unless your application requires raw data, store run data in bunched files. Bunched data requires less memory and takes less time to reintegrate than raw data.

### Archiving Files by Renaming Them

Unfortunately, the SIGNAL file is overwritten with new data after each run, destroying the results of the previous analysis. To prevent run data from being overwritten, you can archive the Signal file by renaming it after each run.

```
* RENAME M:SIGNAL.BNC M:RUN11.BNC
```

### Using a Disk Drive

You can archive your run data automatically by using a disk drive. With a disk drive, the integrator automatically assigns a unique name to the Signal Data file for each run. The unique eight-character name is based on the integrator’s clock time.

The first character is always Q, for example:

- A:QABCDEFG1.RAW      Raw signal data
- A:QABCDEFG1.BNC      Bunched signal data

## Automatically Renaming Result Files

You can run the AUTONAME program to assign unique and meaningful names automatically to the Signal Data, Processed Peak, and Report files. This prevents the files from being overwritten and archives them automatically without requiring a disk drive.

See section 3 in the *Using Application Programs* manual for more information about renaming result files.

## Processed Peak Files

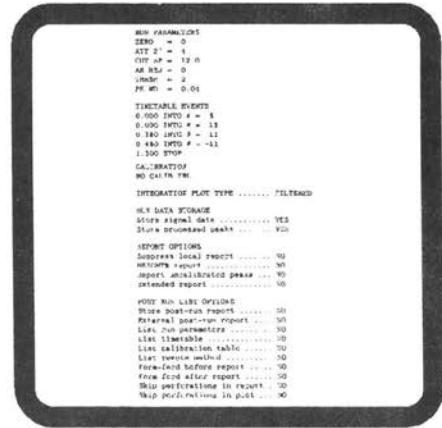
Processed Peak files are not overwritten after each run. Each Processed Peak file is automatically assigned a unique name based on the HP 3396 clock time.

The first character is always Q, for example:

- A:QABCDEF1.PRO      Processed peak file
- A:QABCDEF1.PRA      Processed peak file with bunched data
- A:QABCDEF1.RPT      Report file



# Saving Your Method



## In this chapter....

- Listing Your Method ..... 5-2
- Saving Your Method for Future Recall ..... 5-3
- Ending the Tutorial Lesson ..... 5-4

---

## Listing Your Method

1. To list the method you have created by following this tutorial, enter the following key commands [**LIST**] [**METH**][**ENTER**].

```
RUN PARAMETERS
ZERO      = 0
ATT 2^    = 4
CHT SP    = 12.0
AR REJ    = 0
THRSH    = 2
PK WD    = 0.04

TIMETABLE EVENTS
0.000 INTG # = 8
0.000 INTG # = 13
0.380 INTG # = 11
0.480 INTG # = -11
1.300 STOP

CALIBRATION
NO CALIB TBL

INTEGRATION PLOT TYPE ..... FILTERED

RUN DATA STORAGE
Store signal data ..... YES
Store processed peaks ..... YES

REPORT OPTIONS
Suppress local report ..... NO
HEIGHT% report ..... NO
Report uncalibrated peaks ... NO
Extended report ..... NO

POST-RUN LIST OPTIONS
Store post-run report ..... NO
External post-run report .... NO
List run parameters ..... NO
List timetable ..... NO
List calibration table ..... NO
List remote method ..... NO
Form-feed before report ..... NO
Form-feed after report ..... NO
Skip perforations in report . NO
Skip perforations in plot ... NO
```

---

## Saving Your Method for Future Recall

You may wish to save the current method:

- After you have created or edited a method.
  - Before you switch off the integrator (clearing the memory and erasing the current method).
  - Before you load a new method (overwriting the current method).
2. To save your method, the current method, with the name DEMO, enter the following keystrokes:

[STORE] [METH] M:DEMO.MET [ENTER]

---

## Ending the Tutorial Lesson

When you are finished with the tutorial, you should return the integrator to its normal operation.

To return the instrument to normal (nondiagnostic) operation:

1. Hold down the **[CTRL]** **[SHIFT]** and **[BREAK]** keys simultaneously until the instrument starts printing. If you release any of them too soon, the diagnostics will not start.
2. The integrator should display the following message:

```
SELF TEST: (Press (M) key for more help)
```

```
=>
```

If the self-test message does not appear, you probably released one of the keys too soon. Wait for the instrument to finish whatever it's doing and then try again.

3. Press **0** (zero) to return the integrator to its normal operation.

```
=> 0  *
^BREAK
*
```

**Note:** You can also return the instrument to normal operation by turning it off and back on again. The power switch is on the rear panel, just below where the power cord connects. Turning the power off clears the integrator memory, including the current method.

## Congratulations!

You have completed the tutorial. Take a break!



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PACKARD