

# Empower Software Data Acquisition and Processing

*Theory Guide*

Waters

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# NOTICE

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# Table of Contents

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Preface .....	15
Chapter 1	
Data Acquisition .....	21
1.1 Overview .....	21
1.1.1 What Is Data Acquisition?.....	21
1.1.2 What Is Processing? .....	21
1.2 Integration Methods .....	22
1.2.1 Commonalities Between ApexTrack and Traditional Integration.....	22
1.3 Analog-to-Digital Conversion .....	22
1.3.1 Data Conversion .....	23
1.3.2 Data Transfer and Storage.....	23
1.4 Detection Sampling Rates .....	24
1.4.1 Determining the Optimum Sampling Rate .....	25
1.4.2 Displaying the Data Points.....	25
1.5 Effects of Data Acquisition Rate on Disk Space .....	25
1.6 Reference.....	26
Chapter 2	
ApexTrack Integration .....	27
2.1 Features and Capabilities .....	27
2.1.1 ApexTrack Features .....	27
2.1.2 How ApexTrack Performs Integration .....	28
2.1.3 Summary of Processing Method Parameters .....	28

2.1.4	Summary of Timed Events .....	31
2.1.5	Integration Peak Labels in ApexTrack .....	32
2.2	Apex Detection.....	33
2.2.1	Detecting Apices.....	34
2.2.2	Apex Detection Parameters .....	34
2.2.3	Obtaining the Second Derivative Plot .....	35
2.2.4	Detecting the Peak.....	36
2.2.5	Resolved Peaks and Shoulder Peaks.....	36
2.2.6	Round Peaks .....	37
2.2.7	Second Derivative Apex and Inflection Point Data in the Peaks Table .....	38
2.3	Baseline Location.....	39
2.3.1	How ApexTrack Determines the Slope Difference Threshold .....	39
2.3.2	How ApexTrack Locates the Baseline for an Isolated Peak .....	41
2.3.3	How ApexTrack Locates the Preliminary Baseline for a Cluster .....	42
2.3.4	How ApexTrack Determines the Final Cluster Baseline .....	43
2.3.5	Effect of Liftoff % and Touchdown % on Baseline Location.....	43
2.3.6	Effect of Changing Liftoff % and Touchdown % on Cluster Peaks .....	45
2.4	Determination of Peak Boundaries .....	46
2.4.1	Sequence of Operations .....	47

2.5	Computation of Integration Results.....	47
2.5.1	Peak Area .....	47
2.5.2	Peak Height .....	47
2.5.3	Retention Time .....	48
2.5.4	Retention Time and Height Values for a Manually Adjusted Peak .....	49
2.6	Peak Width Parameter .....	50
2.6.1	Peak Width in ApexTrack .....	50
2.6.2	Auto-Peak Width .....	51
2.6.3	Using Auto-Peak Width .....	52
2.6.4	Effect of Variation of Peak Width Parameter .....	52
2.7	Detection Threshold Parameter .....	53
2.7.1	Baseline Noise in ApexTrack.....	53
2.7.2	AutoThreshold .....	54
2.7.3	Using AutoThreshold .....	55
2.8	Peak Detection Events .....	56
2.8.1	Inhibit Integration Event .....	56
2.8.2	Detect Shoulders Event .....	57
2.8.3	Allow Negative Peaks Event .....	58
2.8.4	Set Events .....	60
2.9	Peak Integration Events .....	66
2.9.1	Valley-to-Valley Event .....	66
2.9.2	Gaussian Skim Event .....	70
2.9.3	Merge Peaks Event for GPC, GPCV, GPC-LS, and GPCV-LS.....	78
2.9.4	Set Liftoff % Event .....	79
2.9.5	Set Touchdown % Event.....	80

2.10 Incompatible Events for ApexTrack .....	80
2.11 When Timed Events Are Active .....	81
2.12 References.....	81

## Chapter 3

Traditional Integration .....	82
3.1 Peak Detection.....	82
3.1.1 Performing Data Bunching.....	83
3.1.2 Determining Peak Start .....	84
3.1.3 Determining Preliminary Peak Apex .....	85
3.1.4 Determining Peak End.....	85
3.1.5 Determining Peak Width and Threshold Values .....	86
3.1.6 Inhibiting Integration .....	90
3.2 Peak Integration .....	91
3.2.1 Determining Fused Peaks .....	91
3.2.2 Constructing the Baseline.....	93
3.2.3 Calculating Peak Retention Time, Height, and Area.....	95
3.2.4 Peak Rejection Criteria .....	97
3.3 Peak Detection Events.....	98
3.3.1 Allow Negative Peaks Event .....	98
3.3.2 Set Liftoff Event .....	100
3.3.3 Set Touchdown Event .....	100
3.3.4 Set Peak Width Event .....	101
3.4 Peak Integration Events .....	101
3.4.1 Integration Peak Labels .....	102
3.4.2 Force Baseline Events .....	103

3.4.3 Forward Horizontal Events.....	106
3.4.4 Reverse Horizontal Events .....	110
3.4.5 Valley-to-Valley Event .....	114
3.4.6 Force Drop Line Event.....	115
3.4.7 Force Peak Event.....	117
3.4.8 Skim Events.....	118
3.4.9 Set Minimum Height Event .....	123
3.4.10 Set Minimum Area Event .....	123
3.5 Incompatible Events.....	124
3.6 References.....	127

## Chapter 4

Peak Matching and Quantitation of Sample Components .....	128
4.1 Peak Matching .....	128
4.1.1 Calculating the Match Difference .....	129
4.1.2 Choosing the Optimal Peak Match .....	129
4.1.3 Shifting RT and RT Windows.....	130
4.2 Quantitation.....	132
4.2.1 Quantitation by Calibration .....	132
4.2.2 Quantitation Without Calibration .....	133
4.2.3 Quantitation Using Sample Weight and Dilution .....	133
4.2.4 Quantitation Using Injection Volume.....	135
4.2.5 Quantitation Using Responses Other than Peak Area and Height .....	136
4.2.6 External and Internal Standard Quantitation .....	137
4.2.7 External Standard Quantitation .....	137

4.2.8 Internal Standard Quantitation with Separate Standard and Unknown Samples .....	140
4.2.9 Internal Standard Quantitation Without Separate Standard and Unknown Samples (RF Internal Standard).....	145
4.3 Calibration Curve Fit Types .....	148
4.3.1 Single-Level Calibration Curve .....	149
4.3.2 Multilevel Calibration Matrix Operations .....	151
4.3.3 Multilevel Calibration Curves .....	154
4.3.4 Multilevel Forced-Through-Zero Calibration Curves .....	165
4.3.5 Weighting .....	166
4.3.6 Statistics .....	168
4.4 References.....	171
Appendix A	
Processing Codes .....	172
Appendix B	
Data Processing System Policies .....	202
B.1 Use v3.0X Style Peak Width and Threshold Determination .....	202
B.2 Use v2.XX Style Retention Time Calculations .....	204
B.3 Prompt User to Save Manual Changes Made in Review.....	204
B.4 Calculate % Deviation of Point from Curve .....	204



## Appendix C

Getting Started: Processing with ApexTrack.....	206
C.1 Starting Empower Software .....	206
C.2 Setting Up Peak Integration .....	207
C.3 ApexTrack Integration for the Empower Database .....	207
C.4 ApexTrack Integration in Projects .....	209
C.4.1 Enabling ApexTrack in a New Project .....	209
C.4.2 Enabling ApexTrack in an Existing Project .....	211
C.5 ApexTrack Integration in a Processing Method .....	212
C.5.1 Creating a New Processing Method with ApexTrack .....	212
C.5.2 Enabling ApexTrack in an Existing Processing Method .....	213
C.5.3 Changing the Integration Algorithm in a Processing Method.....	214
C.6 Summary of Integration Events .....	216
C.7 Peak Labels in Result .....	216
C.8 Manual Integration Guidelines .....	219
C.8.1 Adding or Deleting Peaks .....	219
C.8.2 Moving Peak Starts and Ends.....	219
C.8.3 Moving Vertical Drops .....	220
Index .....	221

# List of Figures

---

1-1	Data Acquisition Process.....	23
1-2	Acquired Data Points .....	24
2-1	ApexTrack Default Processing Method .....	29
2-2	ApexTrack Timed Events .....	31
2-3	Gaussian Peak and its Second Derivative .....	35
2-4	Baseline Resolved Peak, Valley Boundary, Shoulder Boundary, and Round Boundary .....	36
2-5	Fused Peaks with Valley (Left) and Fused Round Peaks (Right) .....	38
2-6	Setting Baseline Parameters .....	39
2-7	Inflection Point Baseline.....	40
2-8	Computing Slope Differences .....	40
2-9	Computing the Final Baseline.....	41
2-10	Example of Search for Baseline in a Tailed Peak .....	42
2-11	Preliminary Baselines in Cluster Peaks .....	42
2-12	Cluster Baselines.....	43
2-13	Touchdown of Largest Peak.....	44
2-14	Touchdown of 1/10 Peak.....	45
2-15	Touchdown of 1/100 Peak.....	45
2-16	Peak Width Definitions in a Gaussian Peak.....	51
2-17	Second Derivative Showing Baseline Noise .....	53
2-18	Example of Peak-to-Peak Baseline Noise .....	54
2-19	Automatic Measurement of Baseline Noise.....	55
2-20	Manual Measurement of Baseline Noise .....	55
2-21	Inhibit Integration Event .....	57
2-22	Detect Shoulders Event (Example).....	58
2-23	Example of Negative Peak Detection .....	59
2-24	Set Detection Threshold Event .....	61

2-25	Set Minimum Area Event .....	63
2-26	Set Minimum Height Event .....	65
2-27	No Valley-to-Valley Event in ApexTrack.....	67
2-28	With Valley-to-Valley Event in ApexTrack.....	67
2-29	Example of Valley-to-Valley Event in ApexTrack .....	69
2-30	Gaussian Skim Events (Examples) .....	71
2-31	Gaussian Skim Disabled on a Simple Chromatogram.....	73
2-32	Gaussian Skim Enabled on a Simple Chromatogram.....	74
2-33	Gaussian Skim Disabled on a Complex Chromatogram.....	74
2-34	Gaussian Skim Enabled on a Complex Chromatogram .....	75
2-35	Gaussian Skim with a Modified Launch Point.....	75
2-36	Gaussian Skim Boundary After Some Peaks Are Manually Deleted .....	76
2-37	Merge Peaks Event (GPC, GPCV, GPC-LS, and GPCV-LS) .....	78
2-38	Set Liftoff % and Set Touchdown % Events.....	79
3-1	Data Bunching Example .....	83
3-2	Determining Possible Peak Start .....	84
3-3	Determining the Preliminary Peak Apex .....	85
3-4	Determining Peak End .....	86
3-5	Inhibit Integration Event .....	90
3-6	Adjacent Peak Width Comparison .....	91
3-7	Determination of Resolved and Fused Peaks.....	93
3-8	Baseline Construction.....	94
3-9	Baseline Adjustment .....	95
3-10	Peak Retention Time and Peak Height Calculation .....	96
3-11	Peak Area Calculation .....	97
3-12	Allow Negative Peaks Event .....	99
3-13	Set Liftoff Event .....	100
3-14	Set Touchdown Event .....	101
3-15	Force Baseline by Time and Force Baseline by Peak Events.....	104
3-16	Force Baseline by Time Event with Baseline Averaging .....	105

3-17	Force Baseline and Allow Negative Peaks Events.....	106
3-18	Forward Horizontal by Time and Forward Horizontal by Peak Events .....	108
3-19	Forward Horizontal by Time Event with Baseline Averaging.....	109
3-20	Forward Horizontal by Time and Allow Negative Peaks Events .....	110
3-21	Reverse Horizontal by Time and Reverse Horizontal by Peak Events .....	112
3-22	Reverse Horizontal by Time Event with Baseline Averaging .....	113
3-23	Reverse Horizontal by Time and Allow Negative Peaks Events .....	114
3-24	Valley-to-Valley Event .....	115
3-25	Force Drop Line Event (with Only an Event Start Time) .....	116
3-26	Force Drop Line Event (Single Peak).....	116
3-27	Force Drop Line Event (Fused Peaks).....	117
3-28	Force Peak Event.....	117
3-29	Force Peak Event (Multiple Unresolved Peaks) .....	118
3-30	Height Ratio Test .....	119
3-31	Tangential Skim Events.....	120
3-32	Exponential Skim Events .....	122
3-33	Set Minimum Height Event .....	123
3-34	Set Minimum Area Event .....	124
4-1	External Standard Chromatogram.....	138
4-2	External Standard Component Calibration Curves (Single-Level, Concentration) .....	139
4-3	Classic Response Ratio Versus Amount (or Concentration) Ratio Plot .....	140
4-4	Multiplying Response Ratio by Internal Standard Amount (or Concentration) .....	141
4-5	Internal Standard Chromatogram .....	142
4-6	Internal Standard Component Calibration Curves (Single-Level, Amount) .....	144

4-7	RF Internal Standard Chromatogram .....	146
4-8	Single-Level Calibration Curve .....	149
4-9	Response Factor Calibration Curve.....	151
4-10	Point-to-Point Calibration Curve.....	155
4-11	Cubic Spline Calibration Curve.....	157
4-12	Linear Least-Squares Fit Calibration Curve.....	158
4-13	Quadratic Fit Calibration Curve .....	160
4-14	Cubic Fit Calibration Curve.....	162
4-15	Fourth-Order Fit Calibration Curve .....	163
4-16	Fifth-Order Fit Calibration Curve.....	164
C-1	Empower Login Dialog Box .....	206
C-2	New Project Policies Tab .....	208
C-3	Configuration Manager Message Box .....	209
C-4	Tablespace Page of New Project Wizard .....	210
C-5	General Tab of Project Properties Dialog Box .....	211
C-6	New Processing Method Dialog Box .....	212
C-7	Default ApexTrack Processing Method .....	213
C-8	Traditional Parameters in the Integration Tab .....	214
C-9	ApexTrack Parameters in the Integration Tab .....	215
C-10	Peak Labels for ApexTrack .....	218
C-11	Crossover Peaks for ApexTrack .....	218

# List of Tables

---

1-1	Effects of Sampling Rate and Run Time on Hard Disk Space .....	25
2-1	Integration Peak Labels on a Chromatogram .....	33
2-2	Valley-to-Valley Event Rules .....	68
3-1	Default Integration Peak Labels .....	94
3-2	Integration Peak Labels on a Chromatogram .....	102
3-3	Peak Labels for Tangentially Skimmed Peaks .....	121
3-4	Peak Labels for Exponentially Skimmed Peaks .....	123
3-5	Incompatible Events for Traditional Integration .....	124
4-1	Standard Peak Values, External Standard Calibration .....	138
4-2	Standard Peak Values, Internal Standard Calibration with Separate Standard and Unknown Samples .....	142
4-3	Standard Component Values, RF Internal Standard Calibration without Separate Standard and Unknown Samples .....	147
4-4	Unknown Component Values, RF Internal Standard Calibration without Separate Standard and Unknown Samples .....	148
4-5	Standard and Forced-Through-Zero Equation Format Comparison .....	165
4-6	Weighting Application Results .....	167
A-1	Processing Codes .....	173
C-1	ApexTrack Integration Peak Labels .....	217

# Preface

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The *Empower Software Data Acquisition and Processing Theory Guide* provides an overview of the Empower™ theories pertaining to data acquisition, peak detection and integration, and peak matching and quantitation of sample components used by the software to perform calculations.

You should understand the principles of chromatography and be familiar with acquiring, processing, and reporting data using Empower software.

**Note:** *Information on setting processing parameters and limits is included in the Empower Help.*

**Note:** *This guide uses the Empower Pro interface. If you do not have access to this interface, see your system administrator.*

## Organization

This guide contains the following:

[Chapter 1, Data Acquisition](#), describes the processes of analog-to-digital conversion and establishing correct sampling rates. These processes precede peak detection, integration, and data analysis.

[Chapter 2, ApexTrack Integration](#), contains theory on ApexTrack integration, including the peak detection processes, algorithms, and events; peak integration processes, events, and parameters; and restriction of certain event combinations.

[Chapter 3, Traditional Integration](#), describes the peak detection processes, algorithms, and events; peak integration processes, events, and parameters; and restriction of certain event combinations.

[Chapter 4, Peak Matching and Quantitation of Sample Components](#), describes the processes that Empower software uses to identify peaks and calculate specific component amounts.

[Appendix A, Processing Codes](#), lists the processing codes that appear when a problem is encountered during data processing.

[Appendix B, Data Processing System Policies](#), describes four system policies that control various aspects of data processing.

[Appendix C, Getting Started: Processing with ApexTrack](#), describes how to process data using the ApexTrack peak detection and integration processes, events, and parameters.

## Related Documentation

***Waters Licenses, Warranties, and Support:*** Provides software license and warranty information, describes training and extended support, and tells how Waters handles shipments, damages, claims, and returns.

### Online Documentation

***Empower Help:*** Describes all Empower windows, menus, menu selections, and dialog boxes for the base software and software options. Also includes reference information and procedures for performing all tasks required to use Empower software. Included as part of the Empower software.

***Empower Read Me File:*** Describes product features and enhancements, helpful tips, installation and/or configuration considerations, and changes since the previous version.

***Empower LIMS Help:*** Describes how to use the Empower LIMS Interface to export results and import worklists.

***Empower Toolkit Professional Help:*** Describes how to use the common-object-model, message-based protocol to communicate with the Empower software from a third-party application.

### Printed Documentation for Base Product

***Empower Software Getting Started Guide:*** Provides an introduction to the Empower software. Describes the basics of how to use Empower software to acquire data, develop a processing method, review results, and print a report. Also covers basic information for managing projects and configuring systems.

***Empower Software Data Acquisition and Processing Theory Guide:*** Provides theories pertaining to data acquisition, peak detection and integration, and quantitation of sample components.

***Empower System Installation and Configuration Guide:*** Describes Empower software installation, including the stand-alone Personal workstation, Workgroup configuration, and the Enterprise client/server system. Discusses how to configure the computer and chromatographic instruments as part of the Empower System. Also covers the installation, configuration, and use of acquisition servers such as the LAC/E<sup>32</sup> module, the busLAC/E™ card, and interface cards used to communicate with serial instruments.

***Empower System Upgrade and Configuration Guide:*** Describes how to add hardware and upgrade the Empower software using an import-and-export upgrade method.



**Empower Software System Administrator's Guide:** Describes how to administer the Empower Enterprise client/server system and Workgroup configuration.

**Empower Software Release Notes:** Contains last-minute information about the product. Also provides supplementary information about specific Empower software releases.

## Printed Documentation for Software Options

**Empower System Suitability Quick Reference Guide:** Describes the basics of the Empower System Suitability option and describes the equations used by the System Suitability software.

**Empower PDA Software Getting Started Guide:** Describes the basics of how to use the Empower PDA option to develop a PDA processing method and to review PDA results.

**Empower GC Software Getting Started Guide:** Describes how to use the Empower GC option to develop a GC processing method and to review GC results.

**Empower GPC Software Getting Started Guide:** Describes how to use the Empower GPC option to develop a GPC processing method and to review GPC results.

**Empower GPCV Software Getting Started Guide:** Describes how to use the Empower GPCV option to develop a GPCV processing method and to review GPCV results.

**Empower Light Scattering Software Getting Started Guide:** Describes how to use the Empower Light Scattering option to develop a light scattering processing method and to review light scattering results.

**Empower ZQ Mass Detector Software Getting Started Guide:** Describes installation, configuration, calibration, and tuning methods, as well as how to operate the ZQ Mass Detector with Empower software.

**Empower Chromatographic Pattern Matching Software Getting Started Guide:** Describes how to use the Chromatographic Pattern Matching option to develop a pattern matching processing method and to review pattern matching results.

**Empower Dissolution System Software Quick Start Guide:** Describes how to operate the Alliance<sup>®</sup> Dissolution System using Empower software.

**Empower Toolkit Programmer's Reference Guide:** Describes how to use the common-object-model, message-based protocol to communicate with Empower software from a third-party application.

**Waters Integrity System Getting Started Guide:** Describes features of the Waters Integrity® System and provides step-by-step tutorials that guide a user through the use of the Empower Mass Spectrometry (MS) option.

**Empower AutoArchive Software Installation and Configuration Guide:** Describes how to install and configure the Empower AutoArchive option.

## Documentation on the Web

Related product information and documentation can be found on the World Wide Web. Our address is <http://www.waters.com>.

## Related Adobe Acrobat Reader Documentation

For detailed information about using Adobe® Acrobat® Reader, see the *Adobe Acrobat Reader Online Guide*. This guide covers procedures such as viewing, navigating, and printing electronic documentation from Adobe Acrobat Reader.

## Printing This Electronic Document

Adobe Acrobat Reader lets you easily print pages, page ranges, or the entire document by selecting **File > Print**. For optimum print quantity, Waters recommends that you specify a PostScript® printer driver for your printer. Ideally, use a printer that supports 600 dpi print resolution.

## Documentation Conventions

The following conventions can be used in this guide:

Convention	Usage
Purple	Purple text indicates user action such as keys to press, menu selections, and commands. For example, “Click <b>Next</b> to go to the next page.”
<i>Italic</i>	Italic indicates information that you supply such as variables. It also indicates emphasis and document titles. For example, “Replace <i>file_name</i> with the actual name of your file.”
Courier	Courier indicates examples of source code and system output. For example, “The <code>SVRMGR&gt;</code> prompt appears.”
<b>Courier Bold</b>	Courier bold indicates characters that you type or keys you press in examples of source code. For example, “At the <code>LSNRCTL&gt;</code> prompt, enter <code>set password oracle</code> to access Oracle.”

Convention	Usage
<a href="#">Underlined Blue</a>	Indicates hypertext cross-references to a specific chapter, section, subsection, or sidehead. Clicking this topic using the hand symbol brings you to this topic within the document. Right-clicking and selecting <b>Go Back</b> from the shortcut menu returns you to the originating topic. For example, “The Equation for determining the optimum sampling rate can be found in <a href="#">Section 1.4, Detection Sampling Rates.</a> ”
Keys	The word <i>key</i> refers to a computer key on the keypad or keyboard. <i>Screen keys</i> refer to the keys on the instrument located immediately below the screen. For example, “The A/B screen key on the 2414 Detector displays the selected channel.”
...	Three periods indicate that more of the same type of item can optionally follow. For example, “You can store <i>filename1</i> , <i>filename2</i> , ... in each folder.”
>	A right arrow between menu options indicates you should choose each option in sequence. For example, “Select <b>File &gt; Exit</b> ” means you should select <b>File</b> from the menu bar, then select <b>Exit</b> from the File menu.

## Notes

Notes call out information that is helpful to the operator. For example:

**Note:** *Record your result before you proceed to the next step.*

## Attentions

Attentions provide information about preventing damage to the system or equipment. For example:



**Attention:** *To avoid damaging the detector flow cell, do not touch the flow cell window.*

## Cautions

Cautions provide information essential to the safety of the operator. For example:



**Caution:** To avoid burns, turn off the lamp at least 30 minutes before removing it for replacement or adjustment.



**Caution:** To avoid electrical shock and injury, unplug the power cord before performing maintenance procedures.



**Caution:** To avoid chemical or electrical hazards, observe safe laboratory practices when operating the system.

# Chapter 1

## Data Acquisition

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### 1.1 Overview

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#### 1.1.1 What Is Data Acquisition?

A *chromatogram* is a series of detector responses, sampled uniformly across a length of time. The elution of a compound results in a characteristic chromatographic peak profile.

*Integration* is the process of calculating an area that is bounded in part or in whole by a curved line. The goal of chromatographic peak integration is to obtain retention times, heights, and areas of these peaks.

Peak integration uses two key algorithms: one that detects peaks and one that determines their baselines. Once the peak apex and baseline are known, the retention time (RT), height, and area can be calculated.

#### 1.1.2 What Is Processing?

*Processing* is the manipulation of data to determine the identities and/or amounts of separated components. It most often involves integrating chromatographic peaks to calibrate standards and generate a calibration curve, and to quantitate the source components.

Processing methods define how Empower software detects, integrates, calibrates, and quantitates unprocessed, raw data from a 2D channel or a 2D-derived channel. The Processing Method wizard can help you create a processing method, or you can interactively develop the processing method in Review.

You start with unprocessed data acquired from a known standard (Channels). You can create a multipoint calibration curve by using a range of standard concentrations. Adding a processing method to a method set allows the software to process raw data while it is being acquired.

## 1.2 Integration Methods

---

Empower software offers two methods of peak detection and baseline determination:

- **ApexTrack integration** – Detects a peak at its apex using the second derivative of the chromatogram. Peak detection parameters are independent of baseline location parameters. The baseline of each peak is determined using the Liftoff% and Touchdown % parameters. A search algorithm starts from each peak's apex and works downward and outward to draw a baseline. Clusters are identified when the expanding baselines meet and fuse.

***Note:** To select the use of ApexTrack integration in a processing method, you must first enable system and project policies (see Section C.2, Setting Up Peak Integration).*

- **Traditional integration** – Compares a slope against a fixed threshold to identify the liftoff point of the peak (see [Chapter 3, Traditional Integration](#)). Peak touchdown is determined by algorithms that proceed down the chromatogram, finding features, valleys, apices, and so on, until a suitable slope threshold is met.

### 1.2.1 Commonalities Between ApexTrack and Traditional Integration

Although the algorithms that ApexTrack uses to detect peaks and to determine baselines differ from those used by Traditional processing, significant functionality is the same for both Traditional and ApexTrack integration.

- Auto-Peak Width and AutoThreshold are supported.
- Baselines start and end on real, sampled data points.
- Timed events such as Inhibit Integration, Set Peak Width, Set Detection Threshold, Set Minimum Height, and Set Minimum Area are supported.
- Peaks can be manually added or deleted.
- Peak start and stop markers can be manually changed.

## 1.3 Analog-to-Digital Conversion

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The detector analog output signal must be converted to a digital representation before Empower software can acquire and process data. This section describes the sequential processes of:

- Data conversion
- Data transfer and storage

### 1.3.1 Data Conversion

Analog-to-digital (A/D) conversion of detector data is performed in either of two ways (Figure 1-1):

- A detector controlled over the IEEE-488 bus (such as the Waters<sup>®</sup> 996/2996 or 2487 Detector) performs analog-to-digital conversion within the detector.
- A non-IEEE-488 detector transmits an analog output signal to a chromatographic interface (Waters busSAT/IN<sup>™</sup> module). The magnitude of this signal (in microvolts) corresponds to the amount of sample detected at a constant rate.

The voltage range over which the incoming analog signal can vary is  $-0.25$  to  $+2.25$  V. Each millivolt of signal represents 1,000 height counts (where 1 height count is equal to  $1 \mu\text{V}$ ). For example, with a detector set so that 1 AU is equal to 1 V, a 1 AU peak is equal to a peak height of 1,000,000 height counts (from the baseline at 0 V).

The busSAT/IN module converts analog signals to digital signals at a specified number of times per second (sampling rate).

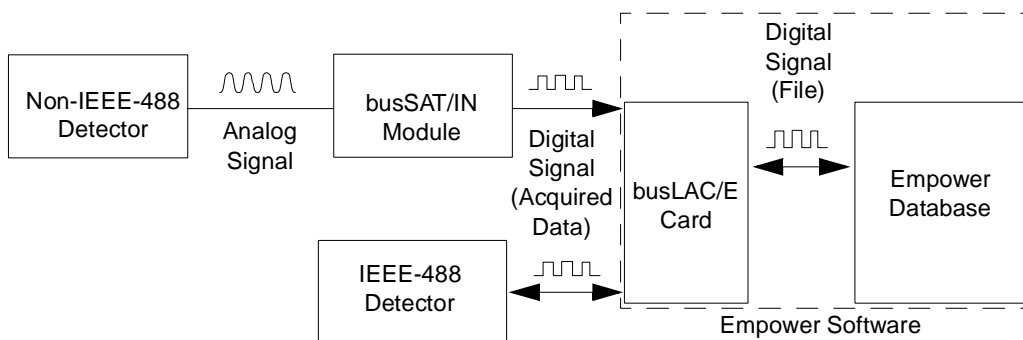


Figure 1-1 Data Acquisition Process

### 1.3.2 Data Transfer and Storage

When the software transfers and stores data:

1. The converted digital signal is transmitted to the busLAC/E<sup>™</sup> card, the Equinox serial card (both cards may be installed in an Empower workstation, LAC/E<sup>32</sup> Acquisition Server or acquisition client), or the computer's COM port.

**Note:** There are two different types of busLAC/E cards available: an ISA busLAC/E card (used in a computer's ISA card slot) and a PCI busLAC/E card (used in a computer's PCI card slot).

2. The collected data is transmitted from the busLAC/E card, the Equinox serial card, or the COM port to the computer's hard drive.

3. The digital voltage values are stored as acquired, unprocessed data. The stored digital values are the *raw data points* of the chromatogram. Raw data can be viewed in the Run Samples window as it is being acquired. The Sample Sets, Injections, and Channels views of the Project window represent the raw data in the current project.

[Figure 1-2](#) shows how acquired data points appear relative to analysis time for a typical analyte.

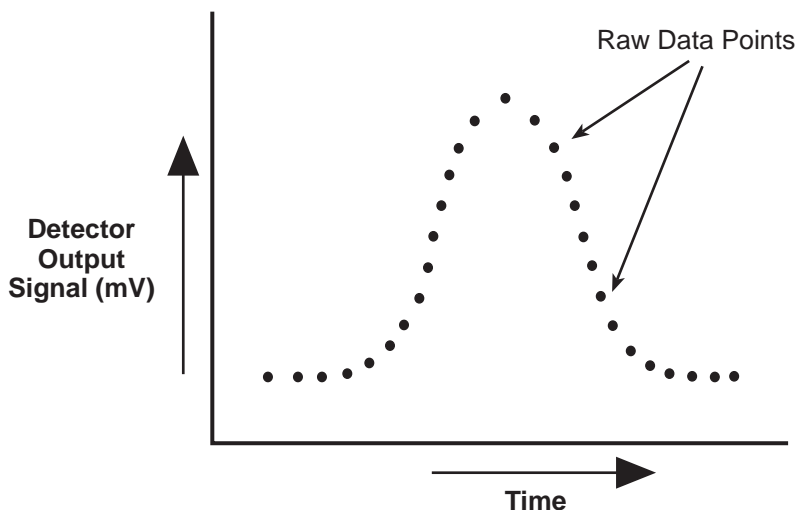


Figure 1-2 Acquired Data Points

## 1.4 Detection Sampling Rates

Empower software sets data collection frequency to the sampling rate you specify in the associated instrument tab of the Instrument Method Editor. The sampling rate needs to be high enough to provide a good representation of the chromatogram, but not so high that you are collecting more data than you need.

In liquid, gas, and ion chromatography, the best sampling rate produces a minimum of 15 data points from peak start to peak end for the narrowest peak of interest that is detected. The value of 15, as an optimum number of data points, is determined by typical signal-to-noise ratios and the frequency content of an exponentially modified Gaussian peak.

The amount of hard disk space required during data acquisition depends on the sampling rate and run time. (For additional information on the theory of data acquisition, see [Section 1.6, Reference.](#))



## 1.4.1 Determining the Optimum Sampling Rate

You can use the following equation to determine the optimum sampling rate:

$$SR = \frac{15}{W}$$

where:

$SR$  = Sampling rate (points/second)

15 = Optimum number of data points from peak start to peak end

$W$  = Measured width (in seconds) of the narrowest peak you want to detect

For example, with a measured peak width of 3 seconds, a sampling rate of 5 ensures data collection of 15 raw data points (where  $15/3 = 5$ ).

**Note:** If the number of data points across the narrowest peak of interest is less than 15, specify a faster sampling rate. Faster sampling rates produce more data points and require a greater amount of disk space for data storage (see [Section 1.5](#)). If the calculated sampling rate (as outlined above) is not available, select the next available higher rate.

## 1.4.2 Displaying the Data Points

The Peaks tab in Review displays the Start Time, End Time, and Points Across Peak for each integrated peak in the chromatogram. These are reportable fields that you can display in any report group.

## 1.5 Effects of Data Acquisition Rate on Disk Space

The amount of hard disk space required during data acquisition depends on the sampling rate and run time. [Table 1-1](#) illustrates the amount of hard disk space needed to store a single channel of data collected at different sampling rates and run times.

Table 1-1 Effects of Sampling Rate and Run Time on Hard Disk Space

Sampling Rate (points/sec)	Data Points Acquired per Minute Run Time	Kilobytes per Minute Run Time (1024 bytes)	Run Time (min)	Approx. Space Used (kilobytes)
1	60	0.23	10	2.3
5	300	1.17	10	12.0
20	1200	4.69	10	47.0

When you start data acquisition in Run Samples, the software determines the current amount of disk space available. If disk space is insufficient, the software warns you and

does not start acquisition. If space becomes limited during Run and Process or Run and Report modes, processing stops and acquisition continues until all remaining disk space is used.

## 1.6 Reference

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For further information on the theory of data acquisition, see:

Dyson, Norman, *Chromatographic Integration Methods*, The Royal Society of Chemistry, Thomas Graham House, Cambridge, 1990.

# Chapter 2

## ApexTrack Integration

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This chapter describes ApexTrack peak detection and integration theory. For step-by-step procedures, see [Appendix C, Getting Started: Processing with ApexTrack](#).

### 2.1 Features and Capabilities

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ApexTrack peak detection and integration by Empower software includes the following functions:

1. Automatically determines appropriate peak width and detection threshold values for the chromatogram unless already set in the processing method.
2. Detects peak apices in the chromatogram to determine the location of peaks and shoulders.
3. Integrates peaks to determine their retention times, areas, and heights.

The processing method defines the parameters that the software uses to detect and integrate the peaks within the raw data file (channel).

**Note:** *Functionality that is identical in Traditional processing is discussed in [“Commonalities Between ApexTrack and Traditional Integration” on page 22](#).*

#### 2.1.1 ApexTrack Features

Empower supports both Traditional integration and ApexTrack integration. The term “Traditional integration” refers to how data is processed (detecting peaks and locating baselines) by Millennium<sup>32</sup> software (see [Chapter 3, Traditional Integration](#)). ApexTrack processes data differently from Traditional integration:

- ApexTrack detects a peak at its apex rather than at its liftoff point. ApexTrack detects the apex by its curvature (second derivative). In contrast, Traditional integration detects a peak at its liftoff by its slope (first derivative).
- Because ApexTrack uses a curvature criterion, ApexTrack can reliably detect shouldered peaks.
- The ApexTrack algorithm finds baselines by starting at each peak’s apex, expanding a trial baseline downward and outward.
- ApexTrack determines the end points of peak and cluster baselines by internal slope comparisons. As a result, the location of the baseline is independent of detector drift, and ApexTrack can reliably integrate small peaks on sloped baselines.

Major features include:

- **Shoulder detection** – Detects shoulders and round peak pairs.
- **Gaussian skims** – Skims multiple peaks within a cluster with Gaussian profiles.
- **Negative peak detection and integration** – Integrates negative peaks and clusters containing negative (and positive) peaks. Supports shoulder detection and Gaussian skimming of negative peaks.

## 2.1.2 How ApexTrack Performs Integration

ApexTrack integration consists of three major processes:

1. **Detects peaks** – Detects a peak at its apex (using the second derivative of the chromatogram). Baseline slope does not affect peak detection. (ApexTrack uses a curvature threshold to detect the peak apex. Traditional integration detects a peak at its liftoff point.) (see [Section 2.2, Apex Detection](#)).
2. **Determines baselines** – Determines the baseline of each peak using the Liftoff % and Touchdown % parameters (see [Section 2.3, Baseline Location](#) and [Section 2.4, Determination of Peak Boundaries](#)).  
Peak detection and baseline determination are independent of each other.
3. **Calculates the peak area, height, and retention time (RT)** – Integrates peaks and determines height and retention time by the quadratic fit method (5-point or 3-point fit), or by the time of the second derivative apex, or the time of the highest point. (see [Section 2.5, Computation of Integration Results](#)).

## 2.1.3 Summary of Processing Method Parameters

[Figure 2-1](#) shows the default processing method for ApexTrack.

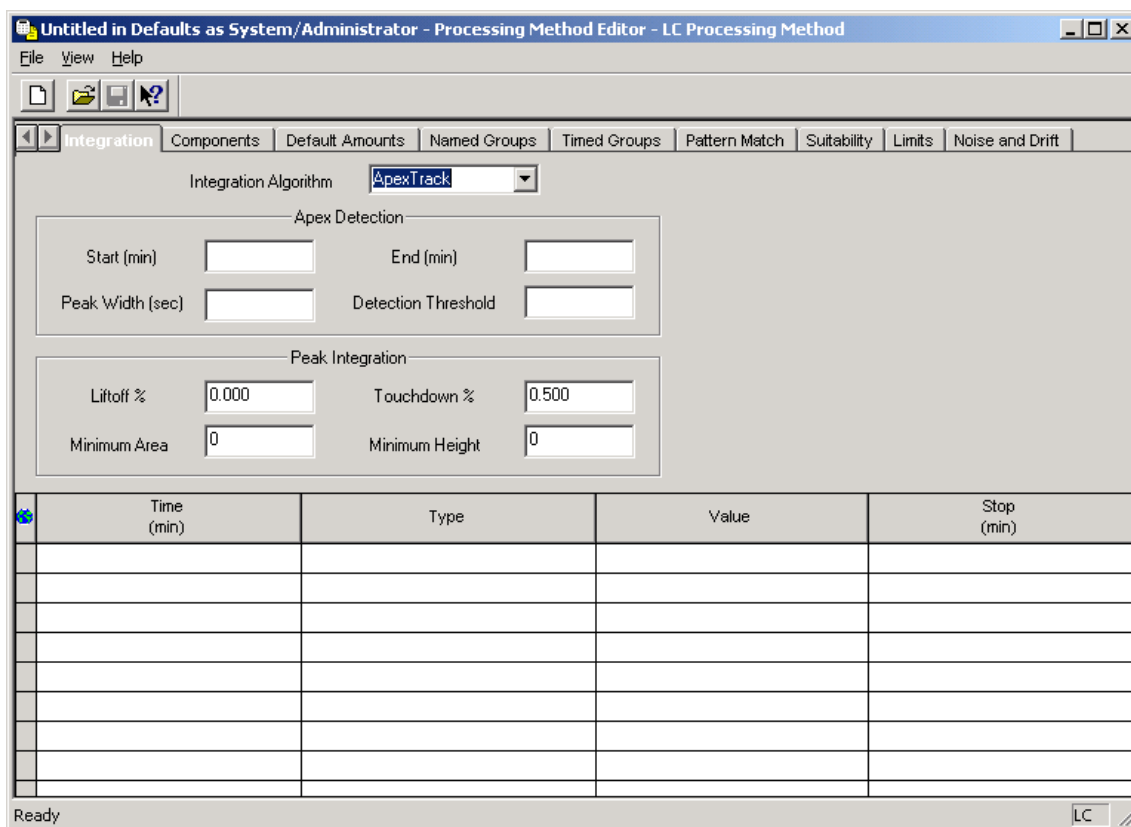


Figure 2-1 ApexTrack Default Processing Method

The processing parameters available in ApexTrack include the following:

- **Integration Algorithm** – Determines if ApexTrack or Traditional integration is used. The use of ApexTrack is enabled by the system policy and the project policy. Once enabled, you can switch a processing method between Traditional and ApexTrack (see Section C.4.2, Enabling ApexTrack in an Existing Project).
- **Start (min)** and **End (min)** – ApexTrack can only detect apices between the Start and End times. The effect of Start and End is similar to Inhibit Integration. You can enter these values manually or leave them blank (default). A blank entry in Start means start of data, and a blank entry in End means end of data. If you enter a value, the range is 0 to 655 min. If both Start and End values are not blank, Start must be less than End.

Two parameters control peak detection:

- **Peak Width (sec)** – The value for Peak Width is the width of a peak in seconds at 5% peak height. You can enter this value manually or leave it blank (default). The range is from 0.01 to 9999.9 seconds. If the field for the peak width is blank, the software applies the Auto-Peak Width algorithm to the region of the chromatogram between the Start and End times in the processing method. If an Inhibit Integration event occurs at the beginning and/or end of the chromatogram, the software applies the Auto-Peak Width algorithm to data between the first and last good data points outside the Inhibit Integration event. The peak width value controls only the smoothing of the data. The effect of smoothing is to set the minimum spacing that can occur between peaks. Reducing the peak width value generally increases the number of peaks that can potentially be detected (see [Section 2.6, Peak Width Parameter](#)).
- **Detection Threshold** – The value for Detection Threshold is the peak-to-peak baseline noise in response units scaled to the same units as peak height (microvolts). You can enter this value manually or leave it blank (default). The range is from 0.000 to 1.000e+090  $\mu\text{V}$ . If the field for the detection threshold is blank, the software applies the AutoThreshold algorithm to the region of the chromatogram between the Start and End times in the processing method. If an Inhibit Integration event occurs at the beginning and/or end of the chromatogram, the software applies the AutoThreshold algorithm to data between the first and last good data points outside the Inhibit Integration event. Reducing the detection threshold value increases the number of peaks that can be detected (see [Section 2.7, Detection Threshold Parameter](#)).

*Note: Generally, it is good practice to ensure that an Inhibit Integration event, and Start and End times occur within the baseline region of a chromatogram.*

Two parameters control baseline location:

- **Liftoff %** and **Touchdown %** – These parameters define the values ApexTrack uses when it determines the slope difference threshold to identify the start and end of peaks and peak clusters. The Liftoff % is used for peak start and the Touchdown % is used for peak end. The default value is 0.0 for Liftoff % and 0.5 for Touchdown %, and the values can range from 0 to 100%. Increasing a value raises the point on the peak at which liftoff or touchdown occurs (see [Section 2.3.1, How ApexTrack Determines the Slope Difference Threshold](#)).

*Note: The maximum value of Liftoff % and Touchdown % allowed in a GPC processing method is 5. The default value for Liftoff % and Touchdown % for GPC is 0.000.*

Two parameters reject peaks that fall below specified values:

- **Minimum Area** and **Minimum Height** – Reject peaks based on integration results.

## 2.1.4 Summary of Timed Events

ApexTrack peak detection and integration events are available in the Type column of the Integration table (Figure 2-2). ApexTrack events are available with all types of processing methods. The Merge Peaks event is also available with GPC processing methods.

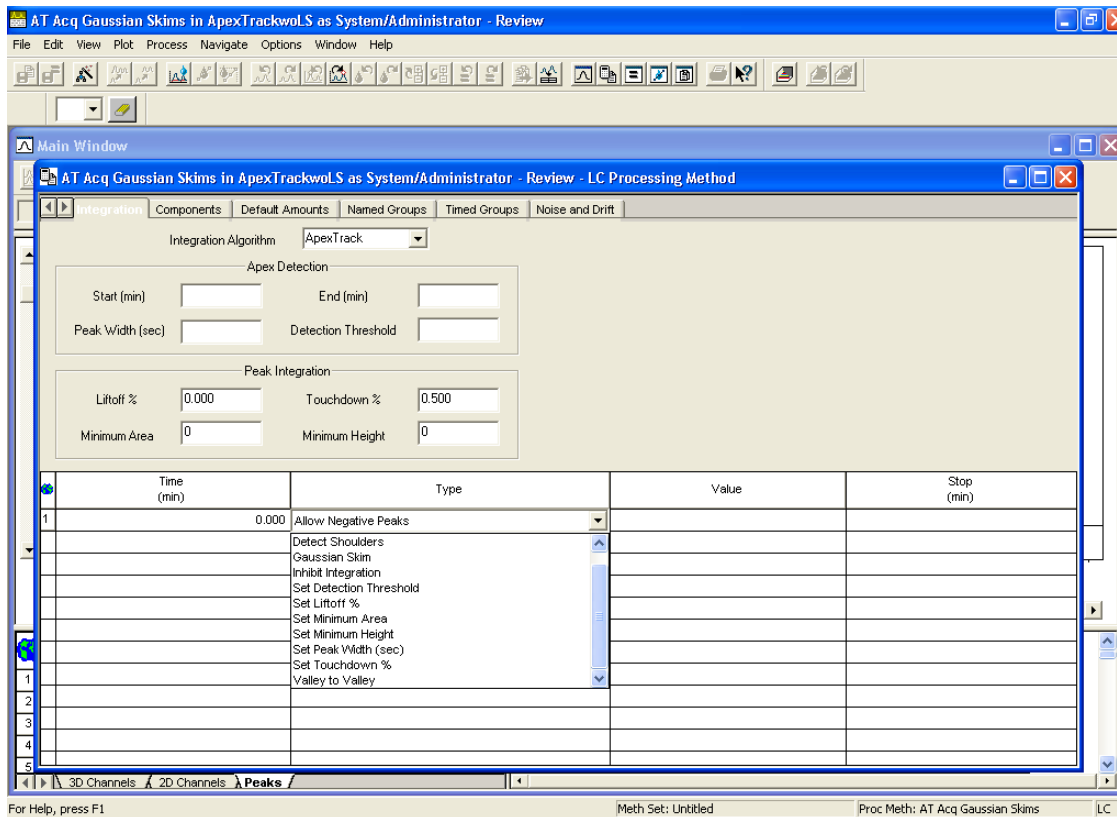


Figure 2-2 ApexTrack Timed Events

You can enable the following four ApexTrack timed events within the time range specified in the table entry:

- **Detect Shoulders** – Enables the detection of shoulder and round peaks. The peak boundary and the integration results reflect the detection of the shoulder and/or round peak (see [Section 2.8.2, Detect Shoulders Event](#)).
- **Valley-to-Valley** – Enables the replacement of cluster baselines with a separate baseline for each peak (see [Section 2.9.1, Valley-to-Valley Event](#)).
- **Gaussian Skim** – Enables the replacement of selected vertical drop lines with Gaussian skims (see [Section 2.9.2, Gaussian Skim Event](#)).
- **Allow Negative Peaks** – Enables negative peak detection (see [Section 2.8.3, Allow Negative Peaks Event](#)).

These four events are disabled by default. You can enable them in any combination and over any time range. However, these events cannot overlap themselves.

You can also enable the following events to modify the values already entered in the processing method:

- **Inhibit Integration** – Further delimits the time range within which peaks can be detected (see [Section 2.8.1, Inhibit Integration Event](#)).
- **Set Peak Width (sec)** and **Set Detection Threshold** – Modify the corresponding method values (see [Section 2.8.4, Set Events](#)).
- **Set Liftoff %** and **Set Touchdown %** – Modify the corresponding method values (see [Section 2.8.4, Set Events](#)).
- **Set Minimum Area** and **Set Minimum Height** – Modify the corresponding method values (see [Section 2.8.4, Set Events](#)).

The Merge Peaks event is also available for GPC type processing methods (see [Section 2.9.3, Merge Peaks Event for GPC, GPCV, GPC-LS, and GPCV-LS](#)).

All events require a Start time, the default is 0.000 minutes. For events that have a Stop (min) time, you can either leave it blank to indicate that this event is enabled until the end of the run, or you can enter a time in minutes. Both the Start and Stop times have a precision of three significant figures by default, and the valid range of each parameter is 0 to 655 minutes. The Start time must be less than the Stop time unless the Stop time is blank.

## 2.1.5 Integration Peak Labels in ApexTrack

Each identified peak in a chromatogram is given a two-letter label that describes the start and end boundary of that peak. The boundaries of a peak can be described by any pair of letters. These letters appear in the Int Type column of the Peaks tab in the Results and Main windows of Review.

If there are no integration events enabled, each peak starts or ends on the baseline (B) or in a valley (V) above the baseline. Each peak is labeled as follows:

- BB indicates a baseline-resolved peak.
- BV indicates a peak that starts a cluster.
- VB indicates a peak that ends a cluster.
- VV indicates a peak within a cluster.

ApexTrack integration supports four Int Type letters specific to ApexTrack ([Table 2-1](#)): Shoulder, Round, Gaussian Skim, and Crossover (see Section C.7, Peak Labels in Result).



Table 2-1 Integration Peak Labels on a Chromatogram

Name of Peak Start or End	Letter	Description
Baseline	B	The boundary of the peak is a baseline.
Valley	V	The boundary of the peak is a valley.
Shoulder <sup>a</sup>	S	The boundary of the peak is a shoulder.
Round <sup>a</sup>	R	The boundary of the peak is a round apex (see <a href="#">Section 2.2.6, Round Peaks</a> ).
Gaussian Skim <sup>a</sup>	G	The boundary of the peak is a Gaussian skim (see <a href="#">Section 2.9.2, Gaussian Skim Event</a> ).
Crossover <sup>a</sup>	X	The boundary of the peak occurs where the signal intersects the baseline. The peaks on either side of this point have opposite signs (one is positive, one is negative). See <a href="#">Section 2.8.3, Allow Negative Peaks Event</a> .

a. You must enable the appropriate timed event.

Capitalization of each letter indicates the following:

- **Uppercase letters** – ApexTrack performed the integration automatically.
- **Lowercase letters** – You performed manual integration.

For instance, a baseline label of Bb indicates that, while the peak start and peak end are both baseline-resolved, the peak start was automatically integrated by the software and you manually adjusted the peak end. See [Section C.7, Peak Labels in Result](#), for information on ApexTrack manual integration.

## 2.2 Apex Detection

When ApexTrack processing is invoked, the first process applied to the data is apex detection. The apex of a peak is the point of maximum curvature. Apex detection is based on measuring the *curvature* (the rate of change of the slope, or second derivative) of the peak. ApexTrack uses the curvature at the *peak apex* to detect peaks, and the algorithm associates a peak with each detected apex. After detecting peak apices, ApexTrack locates the baselines (see [Section 2.3, Baseline Location](#)).

**Note:** *In describing or plotting the curvature of a chromatogram, this guide adopts a negative curvature convention. The second derivative chromatogram measures the chromatogram's curvature at each point, and it is scaled (multiplied) by  $-1$  before plotting. With this convention, the apex of a positive peak has positive curvature, and the apex of a negative peak has negative curvature.*

## 2.2.1 Detecting Apices

ApexTrack software detects peaks as follows:

1. Obtains the peak width parameter.
2. Uses the peak width to obtain the second derivative smoothing filter.
3. Uses the second derivative filter to obtain the chromatogram's second derivative (curvature) plot.
4. Within the second derivative plot, locates the times of each maximum (for positive peaks) or minimum (for negative peaks, when Allow Negative Peaks is enabled), and records the values of the second derivative at each maximum (for positive peaks) or minimum (for negative peaks).
5. Obtains the detection threshold parameter.
6. Applies the second derivative threshold to the maximum (for positive peaks) or minimum (for negative peaks), and retains only the apices with curvatures above the threshold (for positive peaks) or below the threshold (for negative peaks).

## 2.2.2 Apex Detection Parameters

Two parameters control apex detection:

- Peak width
- Detection threshold

ApexTrack requires values for both parameters. You can manually enter peak width and detection threshold values into the processing method, or Auto-Peak Width and AutoThreshold can automatically determine the values. The operation of Auto-Peak Width is summarized in [Section 2.6, Peak Width Parameter](#). The operation of AutoThreshold is summarized in [Section 2.7, Detection Threshold Parameter](#).

### Auto-Peak Width

Auto-Peak Width is the automatic determination of the peak width. If the peak width is blank in the processing method, the software applies the Auto-Peak Width algorithm to the region of the chromatogram between the Start and End times in the processing method. If an Inhibit Integration event occurs at the beginning or end of the chromatogram, the software applies the Auto-Peak Width algorithm to data between the first and last good data points outside the Inhibit Integration event.

Auto-Peak Width measures the peak width in seconds at 5% height of the largest peak in the second derivative. This value is used in the integration of the chromatogram and is included in the integration result. You can enter this value into the processing method and save it for subsequent processing.

## AutoThreshold

AutoThreshold is the automatic determination of the threshold. If the detection threshold is blank in the processing method, the software applies the AutoThreshold algorithm to the region of the chromatogram between the Start and End times in the processing method. If an Inhibit Integration event occurs at the beginning or end of the chromatogram, the software applies the AutoThreshold algorithm to data between the first and last good data points outside the Inhibit Integration event.

AutoThreshold measures the peak-to-peak noise in the baseline segments between peaks. AutoThreshold reports its value in microvolts. ApexTrack uses this value in the integration of the chromatogram and includes it in the integration result. You can enter this value into the method and save it for subsequent processing.

**Note:** For additional information on peak detection theory, see [Section 2.12. References](#).

### 2.2.3 Obtaining the Second Derivative Plot

ApexTrack detects peaks by calculating the second derivative of the chromatogram. (Figure 2-3). The top plot shows an ideal Gaussian peak. The bottom plot shows its second derivative profile.

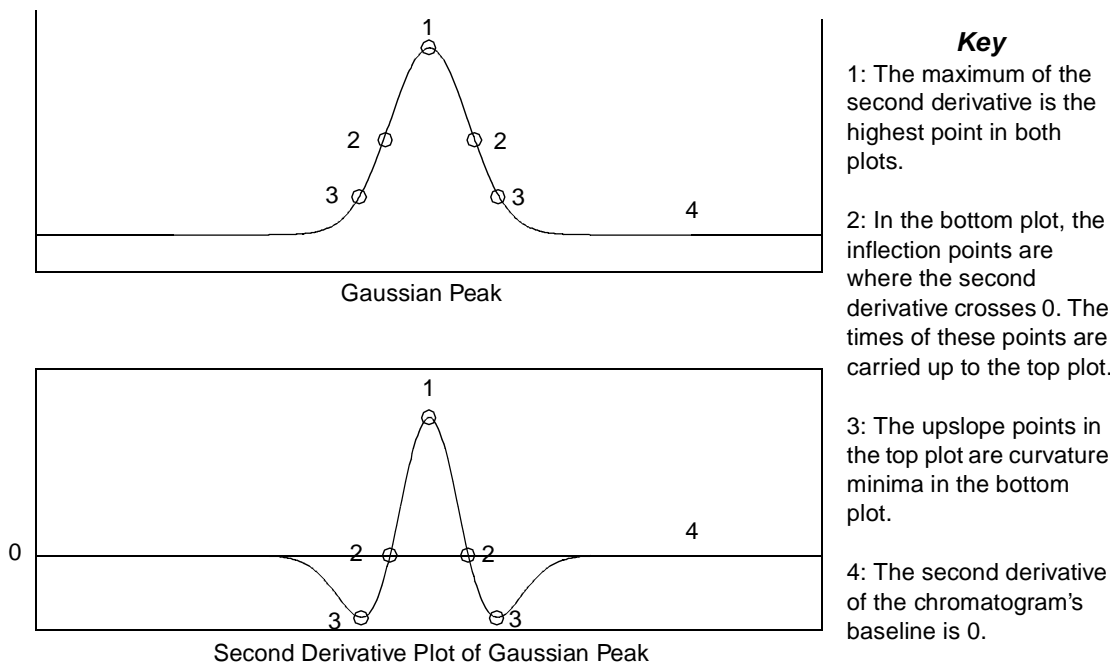


Figure 2-3 Gaussian Peak and its Second Derivative

**Note:** All second derivative plots in this guide are multiplied by  $-1$ , so the apex of a positive peak appears as a positive second derivative.

## 2.2.4 Detecting the Peak

A positive peak has a single maximum point of curvature. The time of that maximum identifies the peak's *apex* (Figure 2-3, 1).

Below the apex are the *inflection points* (Figure 2-3, 2), which straddle the apex and have zero curvature (pass through the zero line on the second derivative plot).

Continuing down the peak are the *upslope points* (Figure 2-3, 3), which have a minimum of curvature.

Finally, ApexTrack reaches the *baseline* (Figure 2-3, 4), which has zero curvature. Even if the baseline has significant drift, it still has zero curvature, because the curvature of a straight line is zero.

## 2.2.5 Resolved Peaks and Shoulder Peaks

Figure 2-4 shows the second derivative of a simulated chromatogram with an isolated peak on the left and three pairs of fused peaks. ApexTrack detects peaks by taking the second derivative of the chromatographic signal and locating the maxima. This process identifies all seven peaks, including the shoulder peak and round peaks.

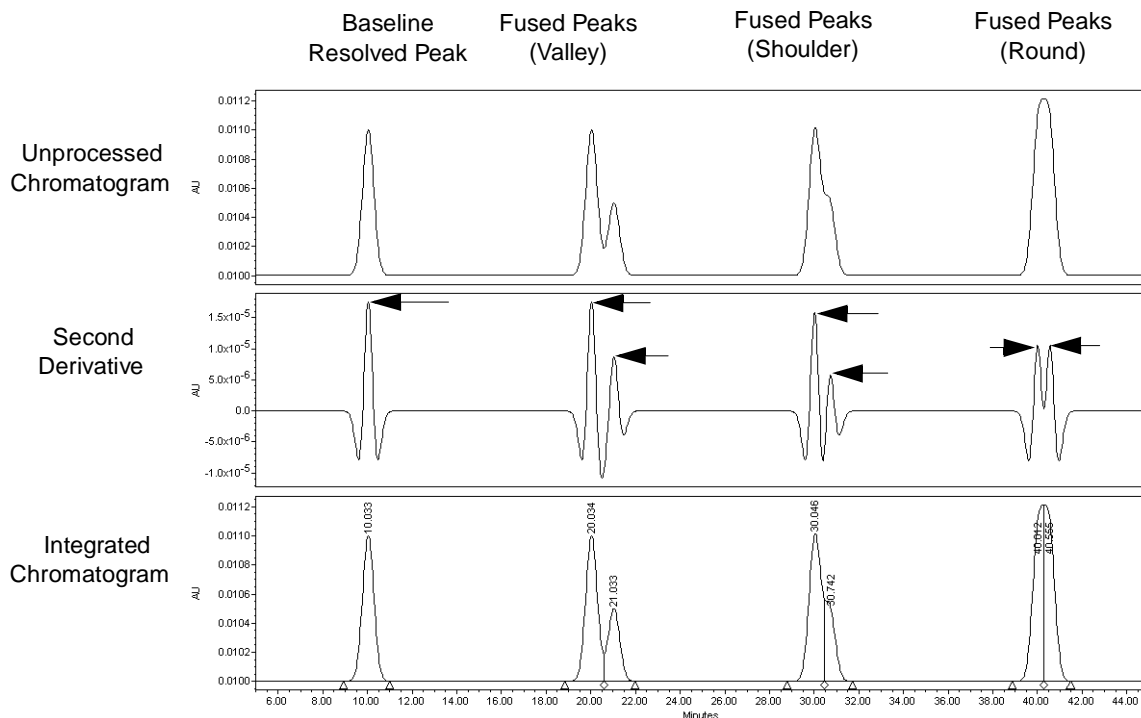


Figure 2-4 Baseline Resolved Peak, Valley Boundary, Shoulder Boundary, and Round Boundary

**Note:** All second derivative plots in this guide are multiplied by  $-1$ , so the apex of a positive peak appears as a positive second derivative.

The arrows in the second derivative plot point to the local maxima, referred to as the “second derivative apices.” All fused peaks are detected by their second derivative apex.

A valley drop line is added between fused peaks when there is a minimum in the chromatographic signal between the two second derivative apices.

A shoulder drop line is added between fused peaks when there is no minimum in the chromatographic signal between the two second derivative apices.

A round drop line is added between fused peaks where there is no minimum in the chromatographic signal between the two second derivative apices and the minimum in the second derivative plot (between the apices) is greater than zero.

## 2.2.6 Round Peaks

A pair of round peaks occurs when peaks of nearly equal height fuse at low (but not zero) resolution. When two peaks are not totally resolved, they could have a valley between them. At lower resolution, that boundary will be a shoulder (for two peaks of differing heights) or a round (for two peaks of similar height).

**Note:** When the Detect Shoulders timed event is enabled, both shoulder peaks and round pairs are detected. Shoulder boundaries are labeled by (S) and round boundaries are labeled by (R).

[Figure 2-5](#) shows two unresolved pairs of peaks illustrating the formation of a pair of round peaks. The pair on the left results in a valley boundary. The pair on the right results in a pair of round peaks. A pair of round peaks occurs when, at lower resolution, the valley disappears and the apex appears to be rounded or flattened. For valley (and shoulder) boundaries, each apex is straddled by a pair of inflection points (indicated by diamonds). The defining characteristic of the round boundary is that the two apices share the same single pair of inflection points.

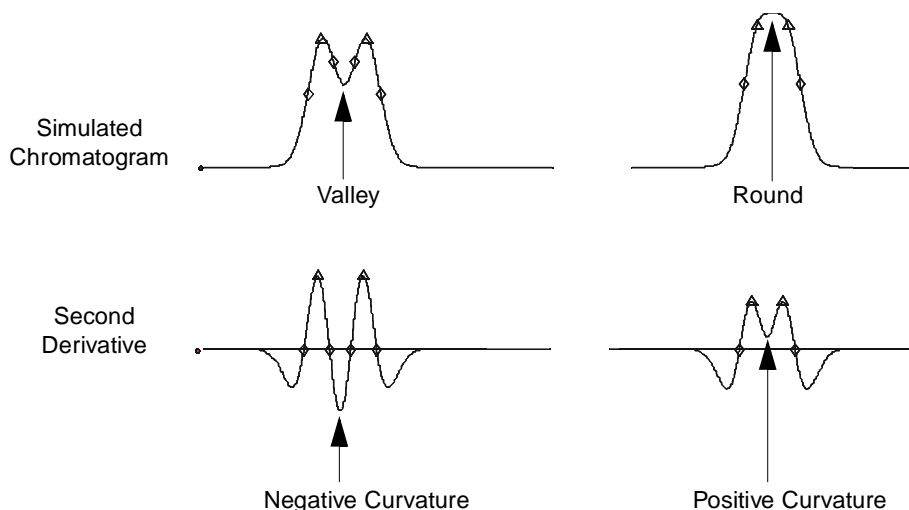


Figure 2-5 Fused Peaks with Valley (Left) and Fused Round Peaks (Right)

## 2.2.7 Second Derivative Apex and Inflection Point Data in the Peaks Table

### Second Derivative Apex

The time of the second derivative apex for each peak appears in the Peaks table in the column labeled 2nd Derivative Apex. This time is generally not the same as the peak retention time. If the time of the second derivative apex is used for the retention time, a peak code of I20 will be present. For tailed peaks, the second derivative apex time generally precedes the retention time.

For some timed events, the time of the second derivative apex is used to determine if timed event is enabled (see [Section 2.8, Peak Detection Events](#), and [Section 2.9, Peak Integration Events](#)).

The displayed value for the second derivative apex of a particular peak will not change with changes to the processing method unless the Peak Width parameter is changed. The only exception is if there are shoulder and/or round boundaries that have been removed from the peak because the Detect Shoulders event is not enabled. If a peak has “hidden” round or shoulder boundaries, the second derivative apex displayed for this peak may change with changes to the processing method.

### Inflection Points

Inflection points straddle the apex and have zero curvature (pass through the zero line on the second derivative plot). The time between a peak’s inflection points appears in the Peaks table in the column labeled Inflection Point Width (sec).

## 2.3 Baseline Location

After valid apices are found, ApexTrack determines the baselines associated with these apices. For positive peaks, the steps involved in baseline location are as follows:

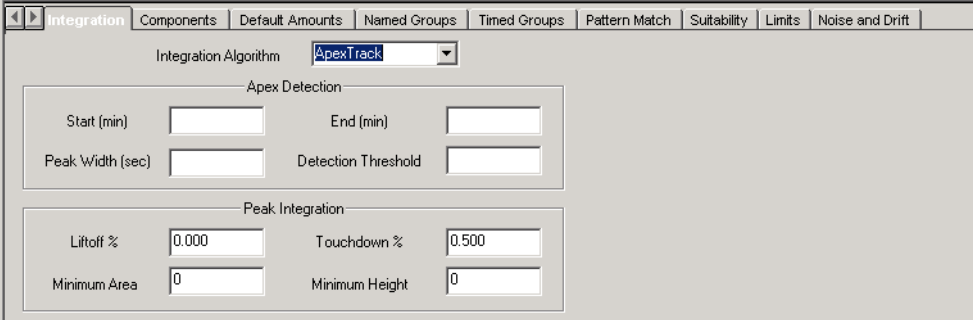
1. Initially draws a baseline between the inflection points of each peak.
2. Draws lines tangent to the inflection points.
3. Determines the slope differences between the inflection point baseline and a tangent line at each inflection point (upslope and downslope).
4. Determines the slope difference thresholds. The peak start slope difference threshold is defined as  $(\text{Liftoff \%} \times \text{slope difference}) / 100$ . The peak end slope difference threshold is defined as  $(\text{Touchdown \%} \times \text{slope difference}) / 100$ .  
A Liftoff % or Touchdown % of 100% is at the inflection point.  
A Liftoff % or Touchdown % of 0% merges with baseline noise.
5. Expands the baselines until the slope difference threshold criteria are met for peak start and peak end.

2

### 2.3.1 How ApexTrack Determines the Slope Difference Threshold

The processing method requires values for the following Peak Integration parameters (Figure 2-6):

- Liftoff % (default value is 0.000)
- Touchdown % (default value is 0.500, except GPC default value is 0.0)



The screenshot shows the 'integration' tab in the software interface. The 'Integration Algorithm' is set to 'ApexTrack'. Under the 'Apex Detection' section, there are input fields for 'Start (min)', 'End (min)', 'Peak Width (sec)', and 'Detection Threshold'. Under the 'Peak Integration' section, there are input fields for 'Liftoff %' (set to 0.000), 'Touchdown %' (set to 0.500), 'Minimum Area' (set to 0), and 'Minimum Height' (set to 0).

Figure 2-6 Setting Baseline Parameters

The algorithm then computes two slope difference thresholds for each peak, based on the Liftoff % and Touchdown %. ApexTrack calculates these slope difference thresholds as follows:

1. Identifies the inflection points that straddle a peak apex.

2. Draws a tangent at each inflection point and draws a baseline that connects the inflection points ([Figure 2-7](#)).

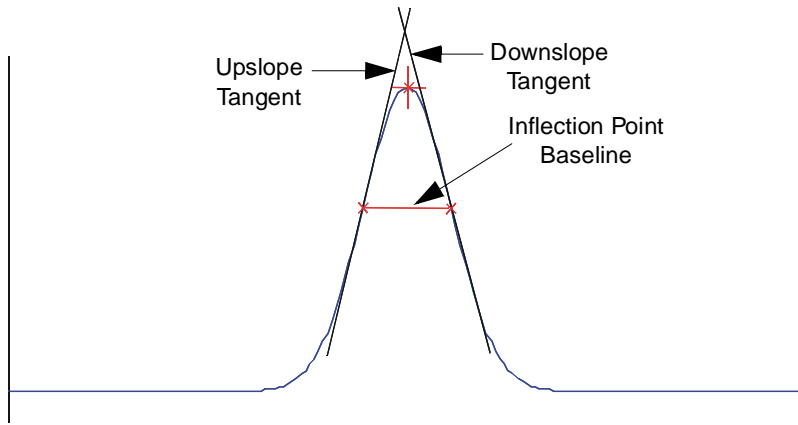


Figure 2-7 Inflection Point Baseline

3. Computes two slope differences,  $\Delta m_1$  and  $\Delta m_2$  ([Figure 2-8](#)):
- Between the slope of the tangent at the upslope inflection point and the inflection point baseline,  $\Delta m_1$ .
  - Between the tangent at the downslope inflection point and the inflection point baseline,  $\Delta m_2$ .

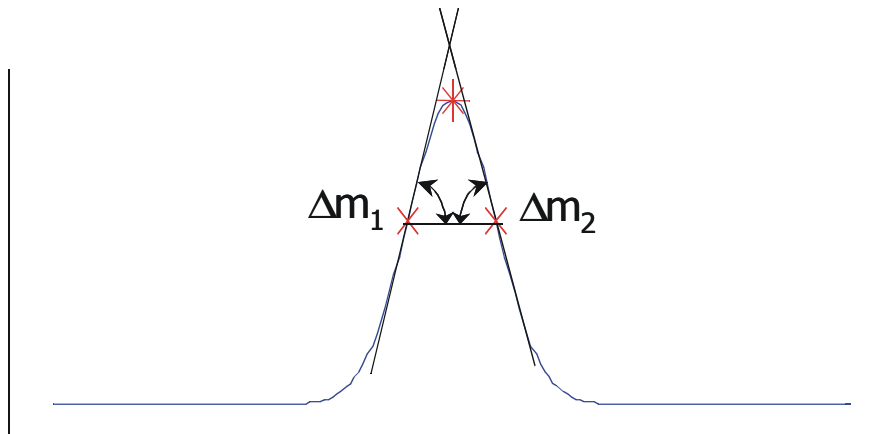


Figure 2-8 Computing Slope Differences



4. Computes two slope difference thresholds ( $T_{\text{start}}$  and  $T_{\text{end}}$ ) using Baseline % Thresholds from the method:
  - $T_{\text{start}} = (\Delta m_1 \times \text{Liftoff \%})/100$
  - $T_{\text{end}} = (\Delta m_2 \times \text{Touchdown \%})/100$

### 2.3.2 How ApexTrack Locates the Baseline for an Isolated Peak

For each peak, ApexTrack expands the baselines downward and outward until the slope difference threshold criteria are met ([Figure 2-9](#)). At each point in the expansion, the slope difference threshold criteria are tested.

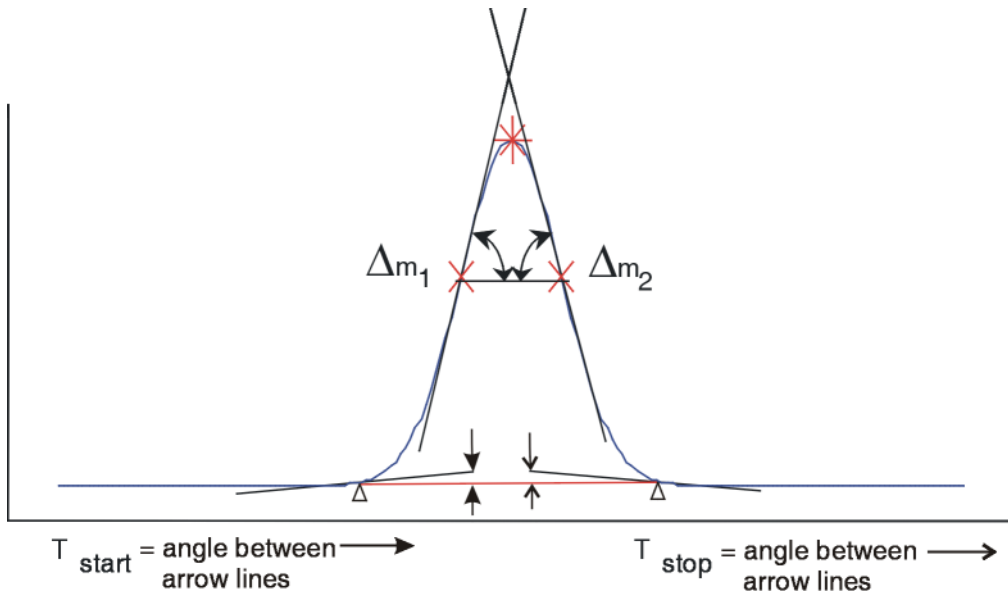


Figure 2-9 Computing the Final Baseline

[Figure 2-10](#) shows the simulation of a tailed peak, and uses it to illustrate how ApexTrack locates the baseline of a baseline-resolved peak. The initial baseline is the inflection point baseline. The baseline expands as it moves down the peak and the slope difference thresholds are tested. With each step, the ends of the baseline become more tangent to the peak. The expansion stops when the slope difference thresholds are met at both ends.

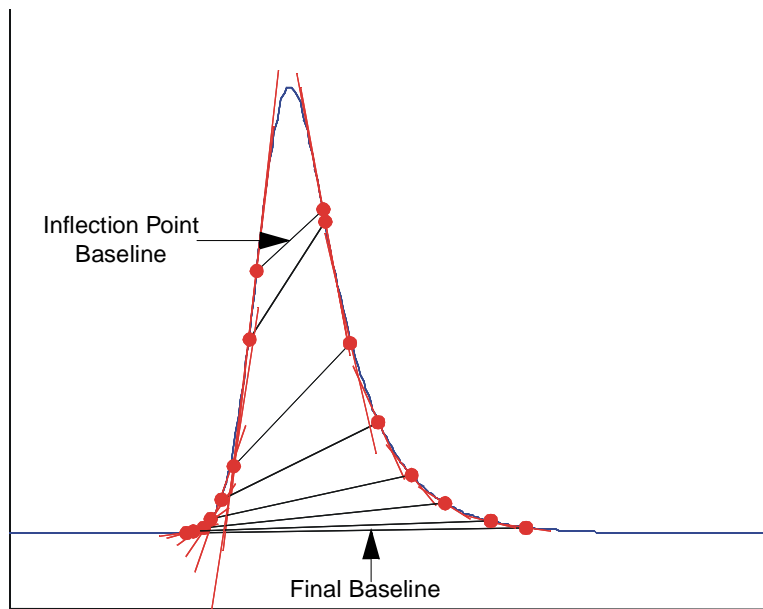


Figure 2-10 Example of Search for Baseline in a Tailed Peak

### 2.3.3 How ApexTrack Locates the Preliminary Baseline for a Cluster

1. ApexTrack expands each peak's baseline until its ends meet the slope difference threshold criteria. If peaks are not resolved, as the baselines are expanded they overlap. [Figure 2-11](#) shows the preliminary overlapped baselines for a two-peak cluster.

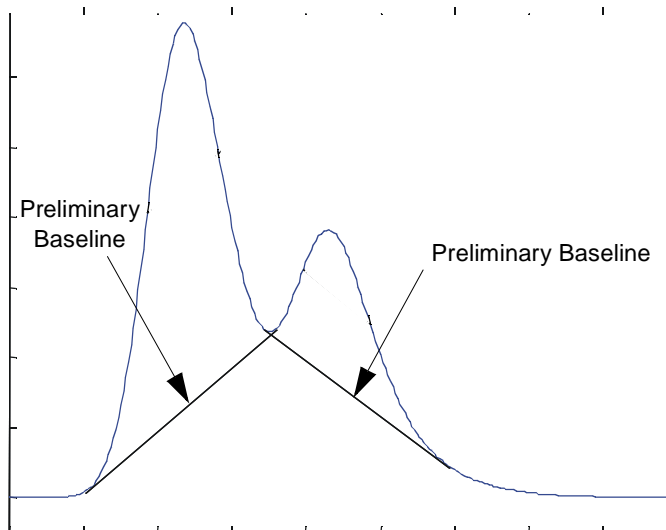


Figure 2-11 Preliminary Baselines in Cluster Peaks

2. Identifies the valleys by the overlap of the expanded baselines.
3. Replaces the two overlapped preliminary baselines with one fused baseline that starts at the beginning of the first preliminary baseline and ends at the last preliminary baseline on the cluster ([Figure 2-12](#)).

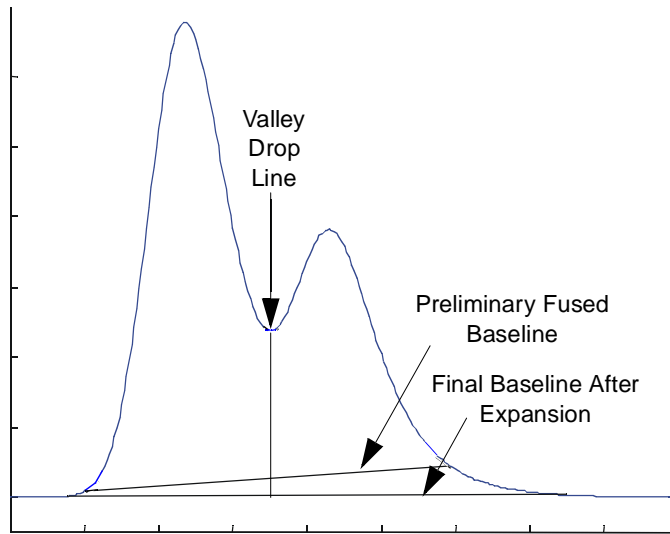


Figure 2-12 Cluster Baselines

### 2.3.4 How ApexTrack Determines the Final Cluster Baseline

After a baseline is fused, the slope difference thresholds are tested at the beginning and end of the cluster baseline ([Figure 2-12](#)). If the slope difference thresholds have not been met, ApexTrack:

1. Expands the cluster baseline as before.
2. Stops the expansion when the slope difference thresholds are met:
  - Slope difference threshold  $T_{\text{start}} = (\Delta m_1 \times \text{Liftoff \%})/100$
  - Slope difference threshold  $T_{\text{end}} = (\Delta m_2 \times \text{Touchdown \%})/100$
3. Positions valley drop lines at the point of minimum height above the final baseline.

### 2.3.5 Effect of Liftoff % and Touchdown % on Baseline Location

The location of the baseline is controlled by Liftoff % and Touchdown %. If both are set to 0%, the resulting baselines are tangent to the detector baseline. If both are set to 1%, then the slope difference thresholds are 1% of the inflection points' slope differences ( $\Delta m_1$  and  $\Delta m_2$ ). The resulting baselines terminate at about 1% of the peak's height. If both are set to 100%, the baseline used for each peak is its inflection point baseline.

Because ApexTrack uses a percentage to calculate the slope difference threshold, the threshold computed for a series of peaks is proportional to peak height. Thus, big peaks have big slope difference thresholds and small peaks have small slope difference thresholds, which allows a single method to successfully integrate peaks of varying sizes using the same Liftoff % and Touchdown % values.

[Figure 2-13](#) shows an example of peaks with height ratios of 1, 1/10, and 1/100, using the default values Liftoff % = 0 and Touchdown % = 0.5.

- Liftoff is the same for each peak.
- Touchdown is the same for each peak.
- Touchdown is well positioned for each peak.

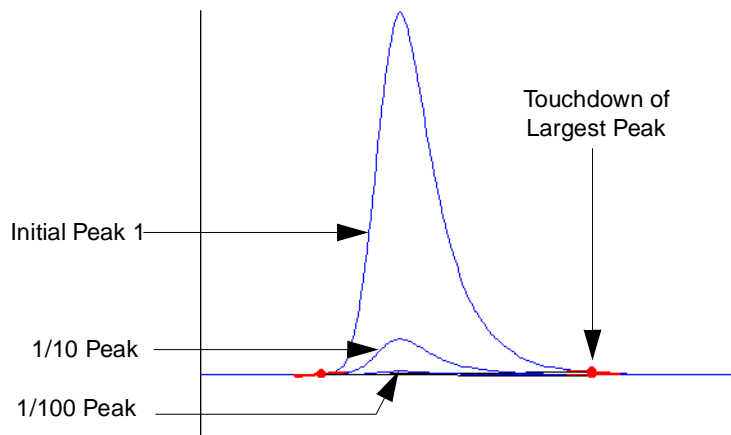


Figure 2-13 Touchdown of Largest Peak

Zooming in to focus on the middle (1/10) peak, touchdown is well positioned, although the slopes are different ([Figure 2-14](#)). The end point for the middle peak occurs at the same relative point to the peak tail.

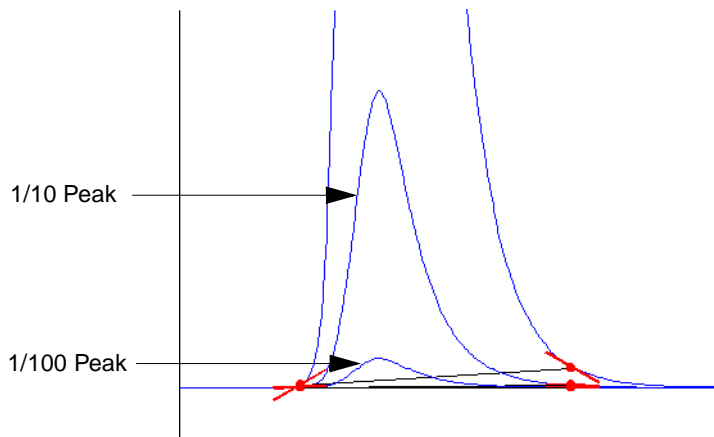


Figure 2-14 Touchdown of 1/10 Peak

Zooming in to focus on the smallest (1/100) peak, touchdown is well positioned, although the slopes are different (Figure 2-15). The end point for the third peak occurs at the same relative point to the peak tail. The slope difference threshold for the largest peak is 100 times that of the smallest peak.

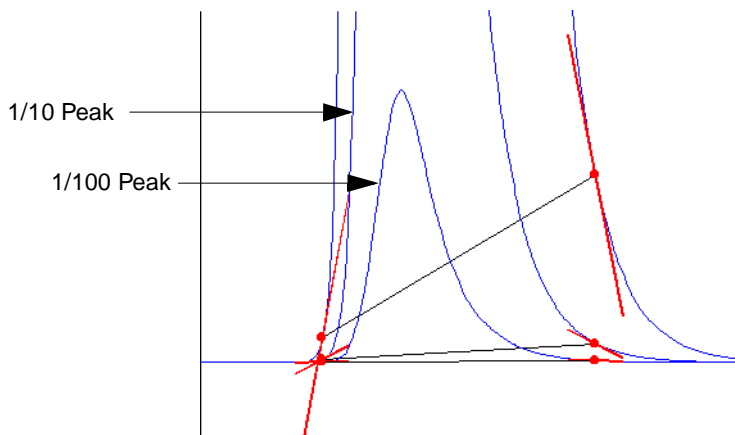


Figure 2-15 Touchdown of 1/100 Peak

### 2.3.6 Effect of Changing Ltoff % and Touchdown % on Cluster Peaks

Changing the Ltoff % and Touchdown % changes the baseline location, and may cause the time of a valley drop line to change. This is because the drop line is set at the lowest point relative to the current baseline.

Changing Lutoff % and Touchdown % can cause shoulder drop lines to become valley drop lines and vice versa. The determination of whether a drop line is a shoulder or valley depends upon the slope of the baseline. If there is a minimum between the two adjoining peak apices with respect to the current baseline, the drop line is a Valley boundary (V). If there is no minimum, the drop line is a Shoulder boundary (S). Shoulder drop lines can become valley drop lines (and vice versa) when changes in Lutoff % and Touchdown % change the slope of the baseline.

If Detect Shoulders is not enabled, peaks can be added or deleted as you change Lutoff % and Touchdown %. The determination of whether a peak is a shoulder is made using the current baseline. If Detect Shoulders is not enabled, a shoulder peak will not appear. However, if you change Lutoff % and Touchdown %, the slope of the baseline changes. The boundary of that peak may appear as a valley with respect to the new baseline and the peak will appear. In general, the disappearance of a shoulder boundary has the effect of combining the two adjoining peaks into one.

## 2.4 Determination of Peak Boundaries

---

After detecting the apices and locating the baselines, ApexTrack identifies the start and stop of each peak. The default boundaries are baseline and valley. If a peak is baseline resolved, the start and stop are the baseline's end points, and are labeled by a B. If a peak is in a cluster, the boundaries between peaks are vertical drop lines placed at valley points, and are labeled by a V (see [Section 2.1.5, Integration Peak Labels in ApexTrack](#)).

Timed events enable the following additional types of boundaries:

**Detect Shoulders** – In regions where shoulder detection is enabled, the boundaries for shoulder and round peaks are vertical drops, labeled by S and R respectively (see [Section 2.8.2, Detect Shoulders Event](#)).

**Gaussian Skim** – In regions where Gaussian skimming is enabled, a Gaussian profile replaces vertical drop lines and the new peak boundary is labeled by a G (see [Section 2.9.2, Gaussian Skim Event](#)).

**Allow Negative Peaks** – If a peak cluster contains only negative peaks and Allow Negative Peaks is enabled, peak start and end boundaries are labeled by B and V. If Detect Shoulders or Gaussian Skim is enabled in these regions, the S, R, and G boundaries are also allowed (see [Section 2.8.3, Allow Negative Peaks Event](#)).

**Crossover** – If a cluster contains positive and negative peaks, the chromatographic signal will intersect the baseline between these adjoining peaks. In this case, the boundary is a crossing point and is labeled by an X (see [Section 2.1.5, Integration Peak Labels in ApexTrack](#)).

## 2.4.1 Sequence of Operations

The determination of peaks, baselines, and boundaries involves three processes (apex detection, baseline location, and boundary determination) with the processing of four timed events (Allow Negative Peaks, Detect Shoulders, Valley-to-Valley, and Gaussian Skim). The order of operation of these processes is as follows:

1. Detect apices.
2. Process Allow Negative Peak events.
3. Locate baselines.
4. Locate peak boundaries.
5. Process Valley-to-Valley events.
6. Process Detect Shoulders events.
7. Process Gaussian Skim events.

## 2.5 Computation of Integration Results

---

Once the apices are detected, the baselines are placed, and the boundaries are identified, ApexTrack obtains the integration results for each peak. Peak area, retention time, and height are all computed using the baseline-corrected signal.

**Note:** *ApexTrack integration uses the baseline-corrected signal to determine retention time. In contrast, Traditional integration uses the uncorrected signal to determine retention time. For relatively flat baselines, the retention time values will match or will differ by amounts that are not significant. For peaks on highly sloped baselines, the ApexTrack calculation, based on the baseline-corrected signal, is more accurate.*

If one or both of a peak's boundaries is a Gaussian skim, then a portion of the peak profile and/or baseline is replaced by the skim, prior to baseline correction. For example, if a peak generates a skim, the skim profile replaces the responses for the larger (parent) peak between the start and stop time of the skim. This same profile becomes the baseline of the adjoining, smaller (child) peak that is being skimmed.

### 2.5.1 Peak Area

Peak area is obtained by Simpson's rule. The contribution to peak area from each adjoining pair of sample points is the average of the baseline-corrected responses at those sample points, multiplied by the sample period (the time between the adjoining sample points).

### 2.5.2 Peak Height

Peak height is the value of the baseline-corrected response at the retention time.

## 2.5.3 Retention Time

### Obtaining the Peak Retention Time

ApexTrack determines the retention time and height in one of four ways, depending on the peak boundaries and the properties of the peak shape. For a given peak, the retention time and height can be obtained from:

- A 5-point fit of a quadratic curve to the points at the peak apex.
- 3-point fit of a quadratic curve to the points at the peak apex.
- The time of the second derivative apex.
- The time of the highest point.

**Note:** Under most conditions, the 5-point quadratic fit is used to determine a peak's height and retention time and no processing code is reported.

**Note:** The 3-point fit and the time of the second derivative are unique to ApexTrack and are not implemented in Traditional integration.

**Note:** In ApexTrack, the 3-point and 5-point fit is to the baseline-corrected signal. In Traditional, only the 5-point fit is implemented, and that fit is to the uncorrected signal.

### Rules that Determine Which Retention Time Method is Used

For each peak, a hierarchy of tests is carried out to determine the retention time method. The tests and the order in which these tests are done is as follows:

1. The retention time of the second derivative apex is used:
  - If either boundary of the peak is a round (R).
  - If the highest point on the baseline-corrected signal is at a peak boundary. In general this ensures that the retention time of a shoulder peak is obtained from the second derivative apex.

A processing code I20 is included in the integration result of the peak whenever the retention time and height reported in the result are calculated at the second derivative apex.

If the retention time of the second derivative apex cannot be used because it falls outside the peak boundary, the retention time of the highest point is used instead. A processing code of I23 is included in the integration result, signifying that the attempt at using the second derivative apex retention time failed.

2. If the peak does not fit the criteria for the first test, the 3-point fit is used:
  - If there are fewer than four sample points within the inflection point width of the peak.

A processing code I19 is included in the integration result of the peak whenever the retention time and height reported in the result are calculated from a 3-point fit.



If the retention time falls outside the 3 points used for the fit, the second derivative apex value is used, and a processing code of I22 is included in the integration result to indicate that a 3-point fit was attempted but failed. A processing code I20 is included in the integration result of the peak whenever the retention time and height reported in the result are calculated at the second derivative apex.

If the retention time of the second derivative apex falls outside the peak boundary, the retention time of the highest point is used instead. A processing code of I23 is included in the integration result, signifying that the attempt at using the second derivative retention time failed.

3. If the peak does not fit the criteria for either test, the 5-point fit is used.
  - No processing code is reported.

The 5-point fit may fail if:

- The first or last point of the 5 points used for the fit, lies outside the start and stop times of the peak.
- The retention time from the fit falls outside the 5 points used for the fit.

A processing code of I21 is included in the integration result signifying that a 5-point fit was attempted, but failed. In either case a 3-point fit is then attempted. A processing code I19 is included in the integration result of the peak whenever the retention time and height reported in the result are calculated from a 3-point fit.

If the retention time from the 3-point fit falls outside the 3 points used for the fit, then a processing code of I22 is included in the integration result signifying that a 3-point fit was attempted, but failed. In this case the second derivative value is attempted. A processing code I20 is included in the integration result of the peak whenever the retention time and height reported in the result are calculated at the second derivative apex.

If the retention time of the second derivative apex falls outside the peak boundary, the retention time of the highest point is used instead. A processing code of I23 is included in the integration result, signifying that the second derivative retention time was attempted, but failed.

## 2.5.4 Retention Time and Height Values for a Manually Adjusted Peak

Baseline location and peak boundaries are needed to determine a peak's retention time, height, and area. When a manually determined baseline location and peak boundaries coincides with the automatically determined values, the manually determined retention time, height, and area values will be identical to the automatically determined values.

**Note:** *If retention time and height are computed using the 2<sup>nd</sup> derivative apex, an I20 integration event is reported. If an I20 peak is manually adjusted, the second derivative value is unavailable so the software uses a different set of rules to determine how to calculate retention time and height. As a result the retention time and height will be determined by a 5-point, 3-point fit, or by time of the highest point.*

If Detect Shoulders is enabled, an I20 event can occur if a peak is determined to be a shoulder or round. If Detect Shoulders is not enabled, an I20 event can occur for a narrow, low level peak.

## 2.6 Peak Width Parameter

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### 2.6.1 Peak Width in ApexTrack

Different separations can produce peaks whose widths vary over a wide range, from seconds to minutes. ApexTrack requires the peak width as input into the processing method. The peak width sets the widths of the digital filters, which are used internally to obtain the smoothed, first and second derivative chromatograms. In ApexTrack, the role of the peak width is solely to determine these filter widths.

**Note:** *The number of points in the filtered chromatograms is the same as in the original chromatogram. Unlike Traditional Integration, data points are not bunched in ApexTrack. ApexTrack processing uses either the original or the filtered chromatograms.*

The width of a filter determines how much smoothing is included. Wider filters produce increased smoothing in the smoothed, first or second derivative chromatogram. Smoothing removes high frequency components (noise), leaving frequencies that correspond to chromatographic features (peaks).

Different conventions can be employed to express the width of a chromatographic peak. The ApexTrack processing method expects as input the width of a peak measured at 5% of its height, expressed in seconds ([Figure 2-16](#)).

**Note:** *The most universal measure of the width of a distribution is its standard deviation (SD). For a Gaussian peak, the chromatographic peak width is defined as  $4.0 \times SD$ .*

In ApexTrack, you can measure the peak width at 5% height by visual inspection and enter this value in the method, or you can use Auto-Peak Width to determine the peak width automatically.

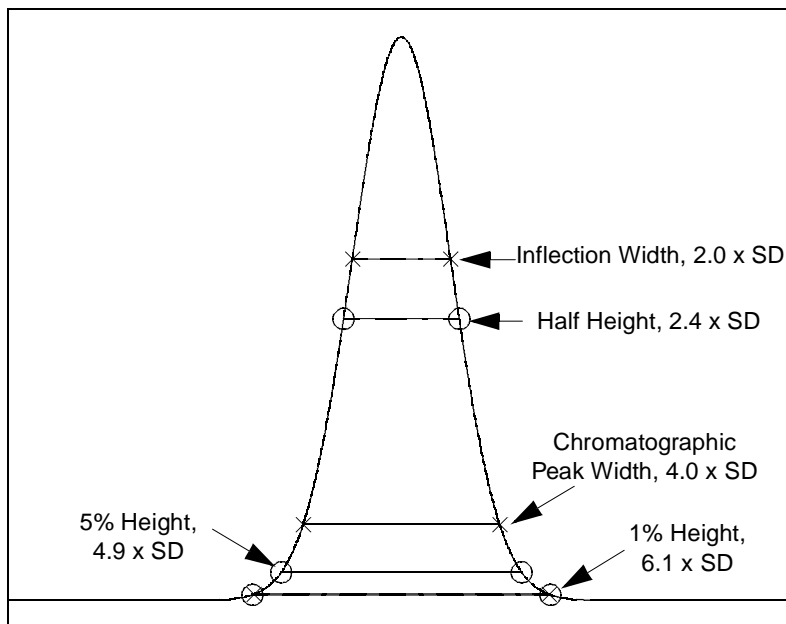



Figure 2-16 Peak Width Definitions in a Gaussian Peak

## 2.6.2 Auto-Peak Width

Auto-Peak Width automatically measures the peak width. A region of a chromatogram containing one or more peaks is required. Auto-Peak Width selects the peak having the largest magnitude second derivative in that region and determines its peak width. The width of that peak is determined by accurately measuring the time between the inflection points. This time is multiplied by a factor of  $4.89549/2$ , which gives the width at 5% for a Gaussian peak.

You can select the region that is used to calculate Auto-Peak Width two ways:


- Zoom in on a region of the chromatogram, and click  (Set Processing Method Peak Width). The peak width is entered into the processing method. When you process the data, ApexTrack reports and uses this value.
- Leave the Peak Width field in the processing method blank. When you process the data, Auto-Peak Width uses the regions between the Start and End times, and the region between any Inhibit Integration events at the start and end of the chromatogram, to determine the peak width. ApexTrack reports and uses this value.

### 2.6.3 Using Auto-Peak Width

If the Peak Width field is blank, Auto-Peak Width uses the data between Start and End, and the region between any Inhibit Integration events at the start and end of the chromatogram, to determine the peak width. Choose the Start and End times to exclude injection artifacts and artifacts associated with the return-to-initial conditions. For example, if the void volume is included, ApexTrack might select an injection artifact as the highest peak, which generally gives a width that is too small.

Even if Start and End times are properly chosen, Auto-Peak Width can give inaccurate results under the following circumstances:

- If the largest peak is saturated, the peak width value can be too large.
- If the largest peak is coeluting with another peak, the peak width value can be too large.
- If the largest peak is noisy, Auto-Peak Width can measure the width of a noise artifact and produce a width that is too small.

To address these problems, zoom in on a region of the chromatogram that contains a valid peak with a valid width, then click  (Set Processing Method Peak Width). The peak width is entered into the processing method. When you process the data with this method, ApexTrack reports and uses this value. Generally, the peak width obtained from a reference separation is relevant for subsequent separations.

When you enable the Inhibit Integration event (see [Section 2.8.1, Inhibit Integration Event](#)), Auto-Peak Width uses the data between the first and last good data points outside the Inhibit Integration event. For example, if you enter an Inhibit Integration start time at 0 minutes and end time at 1 minute, then another Inhibit Integration event starting at 5 minutes to end, Auto-Peak Width is calculated using the first good data point after 1 minute and the last good data point before 5 minutes.

### 2.6.4 Effect of Variation of Peak Width Parameter

On baseline-resolved peaks, the variation of width about the Auto-Peak Width value by a factor of up to 1.5 should have little effect on peak detection or baseline placement.

For complex chromatograms with coeluted peaks that span a range of peak heights, changing the width by a factor of 1.5 (and redetermining the threshold with AutoThreshold) can change which low-level peaks are detected.

Increasing the width by about a factor of 2 (and redetermining the threshold with AutoThreshold) should result in detection of peaks near the baseline that might otherwise be missed. However, this level of increase also reduces the number of detected shoulders.

## 2.7 Detection Threshold Parameter

### 2.7.1 Baseline Noise in ApexTrack

Two sources of noise can add fluctuations to the chromatographic signal:

- The irreducible statistical fluctuations inherent in any detection process.
- The detector's response to the solvent stream.

To distinguish chromatogram peaks from noise peaks, ApexTrack requires a detection threshold as input into the processing method. The software interprets this value as the baseline's peak-to-peak noise, expressed in microvolts.

In ApexTrack, the baseline noise in the second derivative chromatogram is relevant in distinguishing peaks from baseline artifacts. [Figure 2-17](#) is the second derivative of a chromatogram with two chromatographic peaks and the baseline noise.

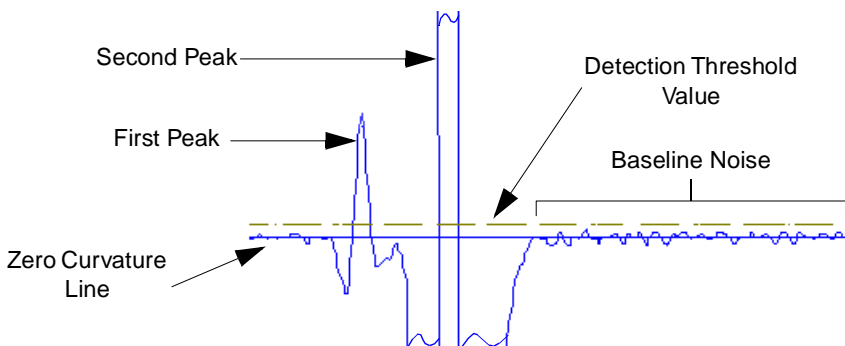


Figure 2-17 Second Derivative Showing Baseline Noise

The noise in the baseline of the second derivative chromatogram is proportional to the noise in the baseline of the original chromatogram. Thus, the software can obtain a second derivative threshold from the peak-to-peak noise in the original chromatogram's baseline.

Internally, the threshold entered into the processing method is converted to a value of curvature (microvolts/sec/sec). This converted value is applied to the second derivative chromatogram. Only peaks whose second derivative rises above the curvature threshold are accepted as valid detections of peak apices. A properly chosen threshold rejects all artifacts due to detector noise and accepts only valid peaks.

In ApexTrack, you can measure the baseline's peak-to-peak noise by visual inspection and enter this value in the method ([Figure 2-18](#)), or you can use AutoThreshold to measure the peak-to-peak noise automatically.

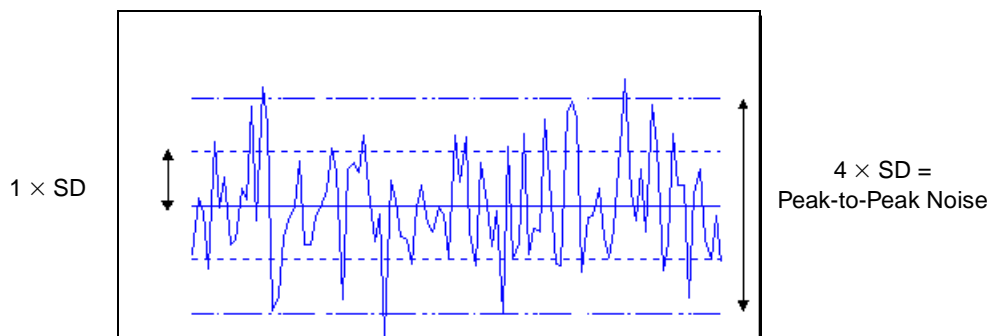



Figure 2-18 Example of Peak-to-Peak Baseline Noise

## 2.7.2 AutoThreshold

AutoThreshold automatically determines the baseline noise amplitude. AutoThreshold requires a region of a chromatogram and a value for peak width as input. AutoThreshold identifies the regions within the chromatogram that are free of peaks, and estimates the peak-to-peak noise in those regions. The selected region might contain one or more peaks, or no peaks. For an accurate measurement, the selected region must contain a segment of the chromatogram that is free of peaks and is at least one peak width wide.

As with Auto-Peak Width, you can select the region that is input to AutoThreshold in two ways:

- Zoom in on a region of the chromatogram, and click  (Set Processing Method Threshold). This button is active only if a peak width is entered into the processing method. The Threshold is entered into the processing method. When you process the data, ApexTrack reports and uses this value.
- Leave the Threshold field in the processing method blank. When you process the data, AutoThreshold uses the regions between the Start and End times and between Inhibit Integration events at the beginning and end, to determine the detection threshold. ApexTrack reports and uses this value.

AutoThreshold determines the threshold by examining the noise regions in the second derivative chromatogram. It converts the noise in the second derivative chromatogram to the equivalent peak-to-peak noise threshold that would be seen in the original baseline, and reports this value.

[Figure 2-19](#) shows the integration of a sample chromatogram whose baseline is shown in [Figure 2-20](#). AutoThreshold reports the threshold as 23.00  $\mu\text{V}$ .

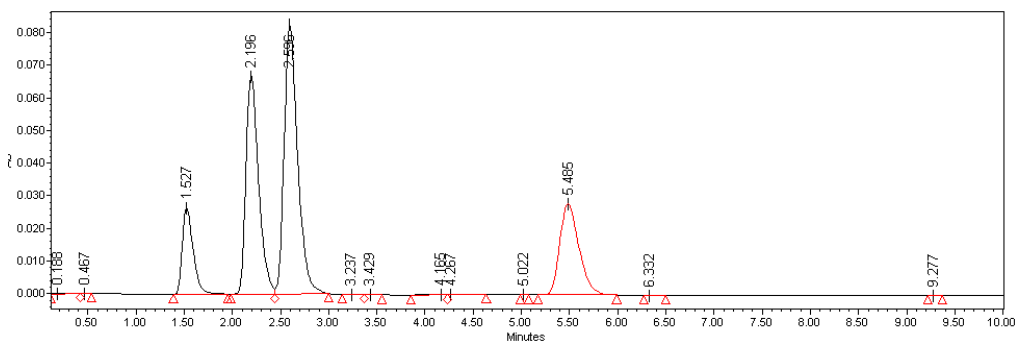


Figure 2-19 Automatic Measurement of Baseline Noise

You can also manually zoom in on a baseline and estimate the peak-to-peak noise visually ([Figure 2-20](#)). A straight line with a positive or negative slope indicates drift, and you can estimate the peak-to-peak height. The magnitude of peak-to-peak noise in this example is approximately 25  $\mu\text{V}$ . You can enter this value into the ApexTrack method.

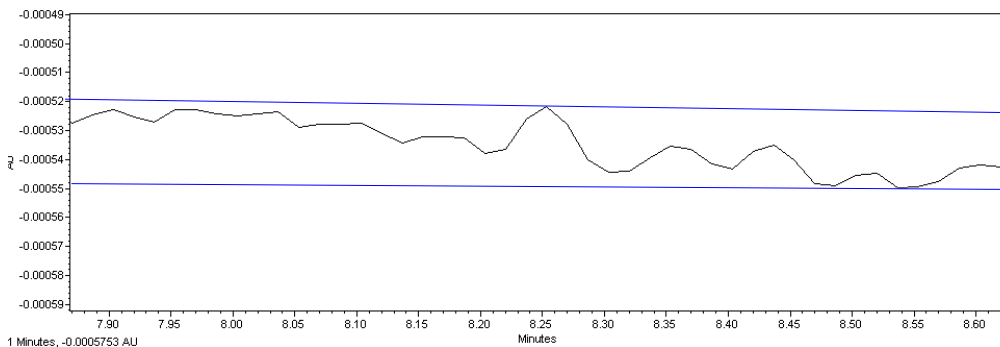


Figure 2-20 Manual Measurement of Baseline Noise

### 2.7.3 Using AutoThreshold

If the Detection Threshold field is blank, AutoThreshold uses the data between Start and End times and between Inhibit Integration events to determine the detection threshold. Choose the Start and End times to exclude regions that can have baseline noise that differs from the noise in the separation region.

When you enable the Inhibit Integration event at the beginning and/or end of the chromatogram (see [Section 2.8.1, Inhibit Integration Event](#)), AutoThreshold uses the data between the first and last good data points outside the Inhibit Integration event. For example, if you enter an Inhibit Integration start time at 0 minute and end time at 1 minute, then another Inhibit Integration event starting at 5 minutes to end, AutoThreshold is calculated using the first good data point after 1 minute and the last good data point before 5 minutes.

Even if the processing method parameters are properly chosen, there is a circumstance under which AutoThreshold can give inaccurate results. In a chromatogram containing many components, there may be no region that is free of peaks. In this case, AutoThreshold may give a value that is too high, so that valid peaks are not detected. To address this problem, zoom in on the baseline region and set the Detection Threshold to the height of the smallest peak. It can be useful to temporarily set the Detection Threshold to 0.0, so you can see all the peaks that previously were not detected.

## 2.8 Peak Detection Events

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In ApexTrack, timed events can modify the detection of peaks. You select ApexTrack peak detection events from the Type column of the Integration table ([Figure 2-2 on page 31](#)).

**Note:** [Section 2.9 on page 66](#) lists ApexTrack events that affect peak integration.

**Note:** [Section 2.10 on page 80](#) lists events that cannot overlap.

ApexTrack events that affect peak detection are as follows:

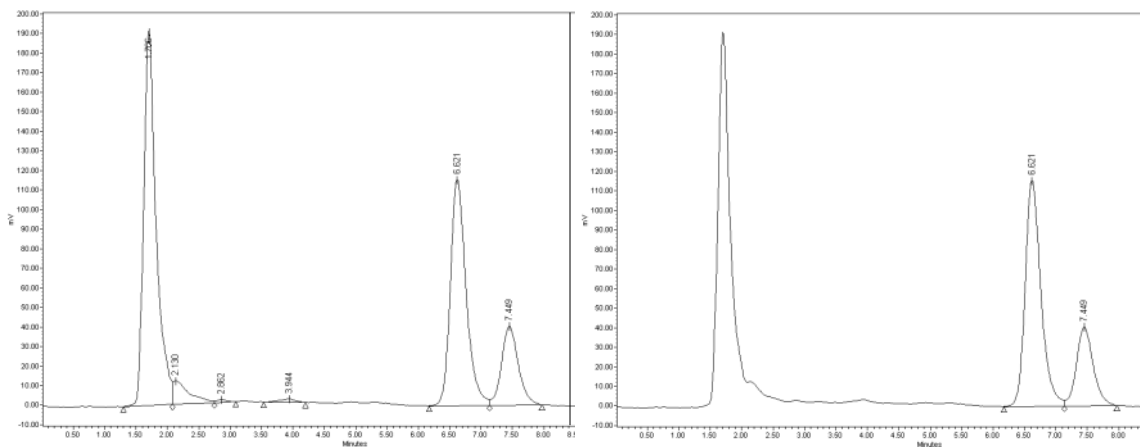
- [Section 2.8.1, Inhibit Integration Event](#)
- [Section 2.8.2, Detect Shoulders Event](#)
- [Section 2.8.3, Allow Negative Peaks Event](#)
- [Section 2.8.4, Set Events](#), as follows:
  - Set Peak Width (sec)
  - Set Detection Threshold
  - Set Minimum Area
  - Set Minimum Height

### 2.8.1 Inhibit Integration Event

The Inhibit Integration event inhibits apex detection. Thus, during the Inhibit Integration event, ApexTrack does not detect any peak whose second derivative apex falls during the event time, whether positive or negative ([Figure 2-21](#)). If this event overlaps a peak, it can affect the detection of the overlapped peak. If the peak is within a cluster, it can affect the location of the baseline in the cluster.

**Note:** *It is good practice to ensure that an Inhibit Integration event, and Start and End times occur within the baseline region of a chromatogram.*





Inhibit Integration Event is Not Enabled

Inhibit Integration Event Enabled  
from 0 to 5 Minutes

Figure 2-21 Inhibit Integration Event

This event requires a Start time, which is 0.000 minutes by default. The Stop time is optional and is blank (which means the end of the chromatogram) by default. The event does not have a value. This event can overlap all other events without conflict, except a Valley-to-Valley event or another Inhibit Integration event.

Auto-Peak Width and AutoThreshold use the data between the first and last data points inside the Start and End time and any Inhibit Integration events at the start and end of the run. For example, if you enter an Inhibit Integration start time at 0 minutes and end time at 1 minute, then another Inhibit Integration event starting at 5 minutes to end, Auto-Peak Width and AutoThreshold are calculated using the first data point after 1 minute and the last data point before 5 minutes.

## 2.8.2 Detect Shoulders Event

**Note:** It is good practice to enable the Detect Shoulders event in regions where the Gaussian Skim event is enabled. You must enable Detect Shoulders in order for shoulder peaks to be skimmed off adjoining larger peaks.

The Detect Shoulders event detects shoulder peaks and pairs of round peaks (see [Section 2.2.5, Resolved Peaks and Shoulder Peaks](#), and [Section 2.2.6, Round Peaks](#)). Shoulders and rounds occur when two adjoining peaks elute at a low enough resolution that no valley appears between them. If resolution is sufficient, ApexTrack detects each peak even when it is discernible only by its curvature ([Figure 2-22](#)).

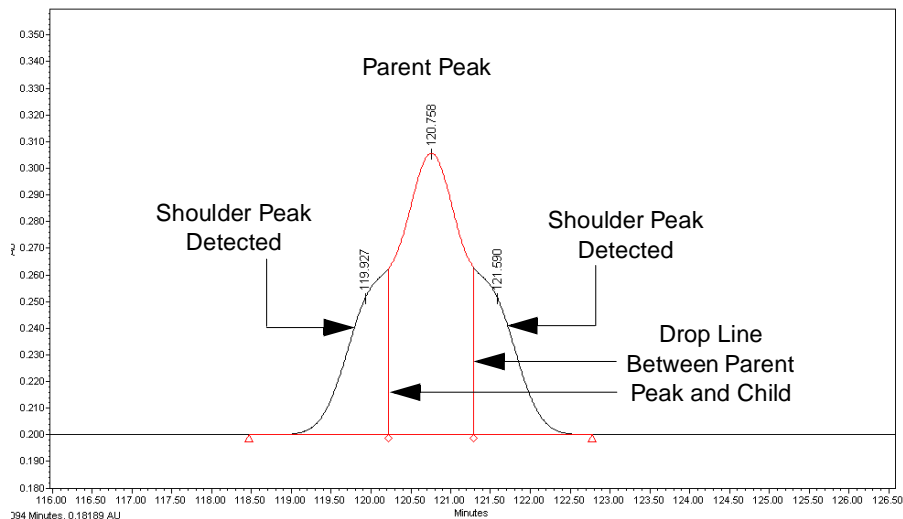


Figure 2-22 Detect Shoulders Event (Example)

The Detect Shoulders event has a Stop time, but does not have a value. This event cannot overlap the Merge Peaks event or another Detect Shoulders event, but it can overlap all other events.

This event works as follows:

- The ApexTrack algorithm detects positive peaks as local maxima of curvature using the second derivative of the chromatogram. Therefore, shoulder and round peaks are detected together with the other peaks.
- If you add a Detect Shoulders integration event, ApexTrack retains all shoulder and round vertical drop lines within the time period when the event is active.
- Shoulder drop lines are labeled with an S, and round drop lines are labeled with an R.
- If you do not add this event, a separate algorithm determines the apices that are shoulders or rounds, then folds them into the adjoining peak.
- When a shoulder or round peak is rejected because its area and/or height are too small, it is removed from the list of peaks.
- If you enable Allow Negative Peaks, negative shoulders and rounds within a negative peak are retained.

### 2.8.3 Allow Negative Peaks Event

ApexTrack detects negative peaks as apices that have curvatures whose sign is opposite that of positive peaks. Negative peaks can be isolated or part of a cluster ([Figure 2-23](#)). In a cluster, adjoining peaks can have the same or opposite signs (for height). You must activate negative peak detection by entering an Allow Negative Peaks timed event.

**Note:** This event affects peaks whose second derivative apices occur during the time of the event.

**Note:** The location of the crossing point is marked by an "x" in the chromatogram.

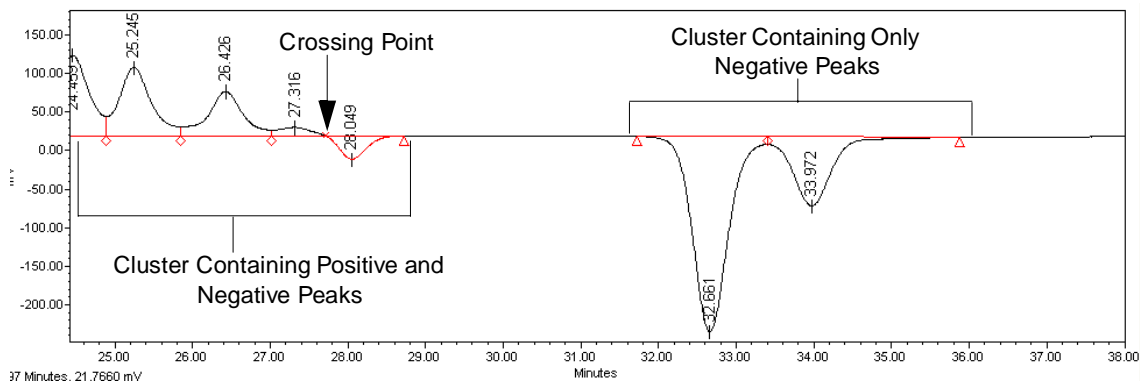


Figure 2-23 Example of Negative Peak Detection

**Note:** To ensure the best results, use this timed event sparingly. This event requires a period of stable, clean baseline (approximately the width of one peak) both before and after the peak or peak cluster. Always place the event Start time and/or End time in an area of the baseline with no peaks.

The Allow Negative Peaks event can have a Stop time (in minutes), but does not have a value. This event can overlap all other events without conflict, except another Allow Negative Peaks event.

Auto-Peak Width and AutoThreshold are compatible with negative peaks. The input to both operations can include negative peaks. Auto-Peak Width uses the largest peak to determine the value for the peak width parameter, regardless of the sign of the peak. AutoThreshold rejects both negative and positive peaks in identifying regions of baseline to use for threshold determination.

This event works as follows:

- If you enable the Allow Negative Peaks event, ApexTrack detects the apices of both positive and negative peaks.
- If you do not enable the Allow Negative Peaks event, ApexTrack detects only the apices of positive peaks.
- If clusters contain both positive and negative peaks and you do not enable the Allow Negative Peaks event, ApexTrack often detects the negative peaks as valleys between positive peaks.
- The Detect Shoulders and Gaussian Skim events are compatible with Allow Negative Peaks. If these events are enabled, shoulders and rounds of negative peaks are detected and vertical boundaries can be replaced by skim baselines.

## 2.8.4 Set Events

Set events cause the specified integration parameter to be changed to the event value for the remainder of the run or until another Set event of the same type occurs. All Set events require a time and a value. Set events do not allow a Stop time. All Set events can overlap in time with all events without conflict (including the same Set event).

You can enable the following Set events in ApexTrack processing:

- Set Peak Width (sec)
- Set Detection Threshold
- Set Minimum Area
- Set Minimum Height

**Note:** The *Set Liftoff%* and *Set Touchdown%* events are discussed in [Section 2.9, Peak Integration Events](#).

### Set Peak Width (sec) Event

This event changes the peak width that is used to calculate the width of smoothing filters used to obtain the first and second derivative chromatograms. This event takes place immediately by causing a new set of filters to be used, starting from the sample point corresponding to the event time.

**Note:** The *Set Peak Width (sec)* event overrides the global *Peak Width (sec)* parameter for the remainder of the run or until another *Set Peak Width* event is encountered. To return to the global value, you must enable another *Set Peak Width* event specifying that value.

This event requires a Start time, which is 0.000 minutes by default. A Stop time is not allowed. The event requires a value, which is blank by default, and allowed values are from 0.01 to 9999.99 seconds.

### Set Detection Threshold Event

The Set Detection Threshold event redefines the detection threshold required to include a peak apex in the peak list ([Figure 2-24](#)). A higher value excludes smaller peaks. This event affects peaks whose second derivative apices occur during the time of the event.

**Note:** The *Set Detection Threshold* event overrides the global *Detection Threshold* parameter for the remainder of the run or until another *Set Detection Threshold* event is encountered. To return to the global value, you must enable another *Set Detection Threshold* event specifying that value.

This event requires a Start time, which is 0.000 minutes by default. A Stop time is not allowed. The event requires a value, which is blank by default, and allowed values are from 0.0 to 1.000e+090. This event is applied to the time of the peak's second derivative apex.

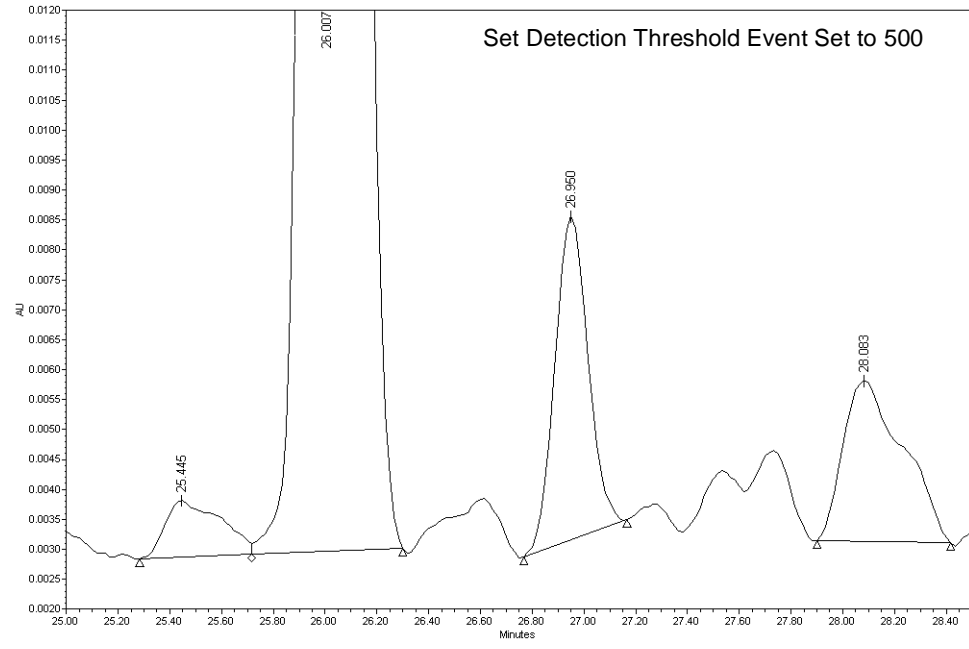
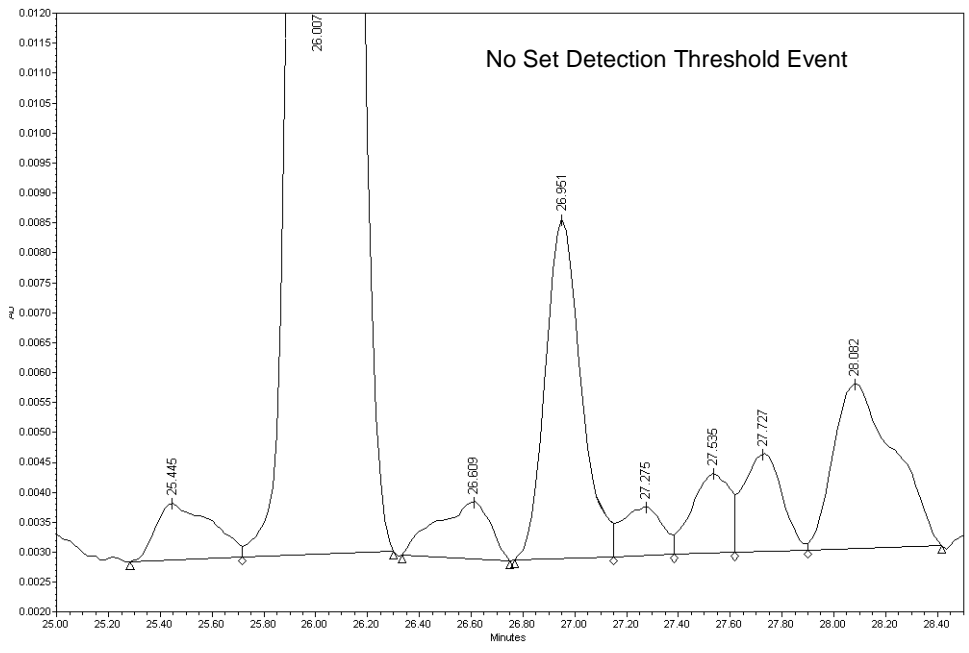


Figure 2-24 Set Detection Threshold Event

## Set Minimum Area Event

This event redefines the minimum area required to include an integrated peak in the peak list ([Figure 2-25](#)). This event affects peaks whose retention times occur during the duration of the event.

**Note:** *The Set Minimum Area event overrides the global Minimum Area parameter for the remainder of the run or until another Set Minimum Area event is encountered. To return to the global value, you must enable another Set Minimum Area event specifying that value.*

This event requires a Start time, which is 0.000 minutes by default. A Stop time is not allowed. The event requires a value, which is blank by default, and allowed values are from 0.0 to 1.000e+90  $\mu\text{V} \cdot \text{sec}$ .

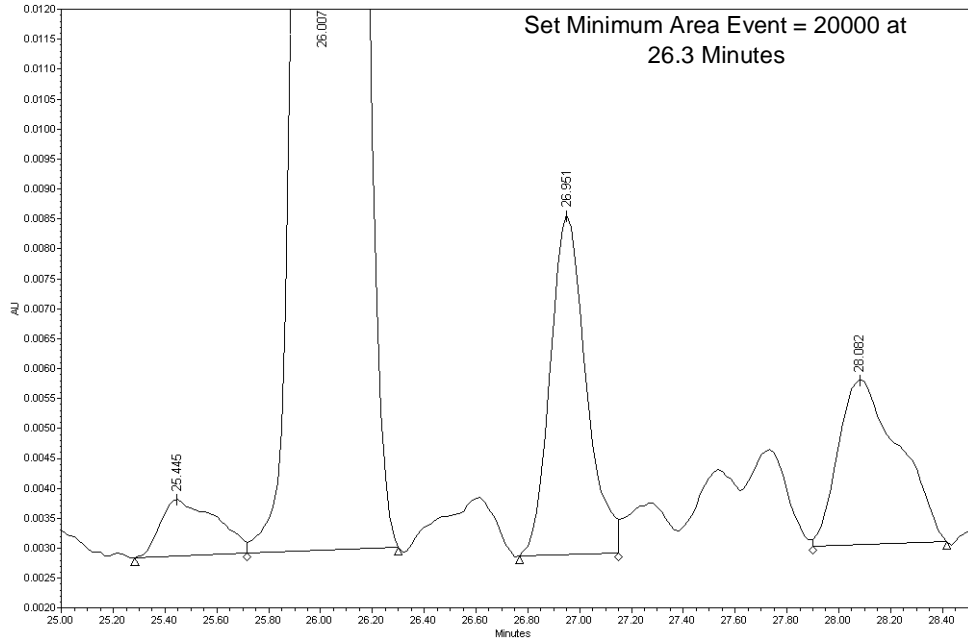
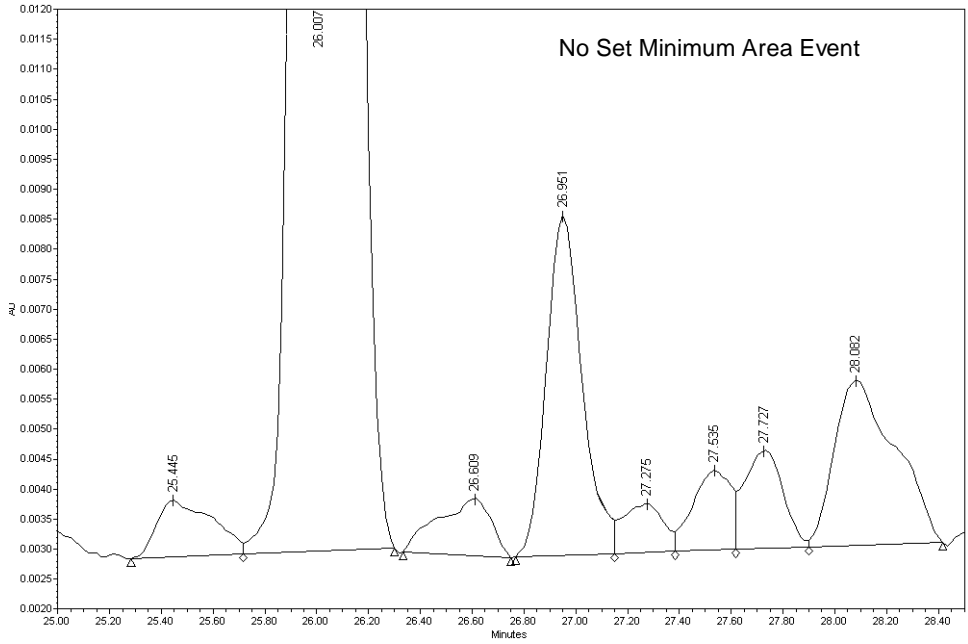


Figure 2-25 Set Minimum Area Event

## Set Minimum Height Event

This event redefines the minimum height required to include an integrated peak in the peak list ([Figure 2-26](#)). The height is scaled by  $10^6$ , so if the detector response is in absorbance units (AU), enter a value in microvolts. This event affects peaks whose retention times occur during the duration of the event.

**Note:** *The Set Minimum Height event overrides the global Minimum Height parameter for the remainder of the run or until another Set Minimum Height event is encountered. To return to the global value, you must enable another Set Minimum Height event specifying that value.*

This event requires a Start time, which is 0.000 minutes by default. A Stop time is not allowed. The event requires a value, which is blank by default, and allowed values are from 0.0 to 1.000e+90.



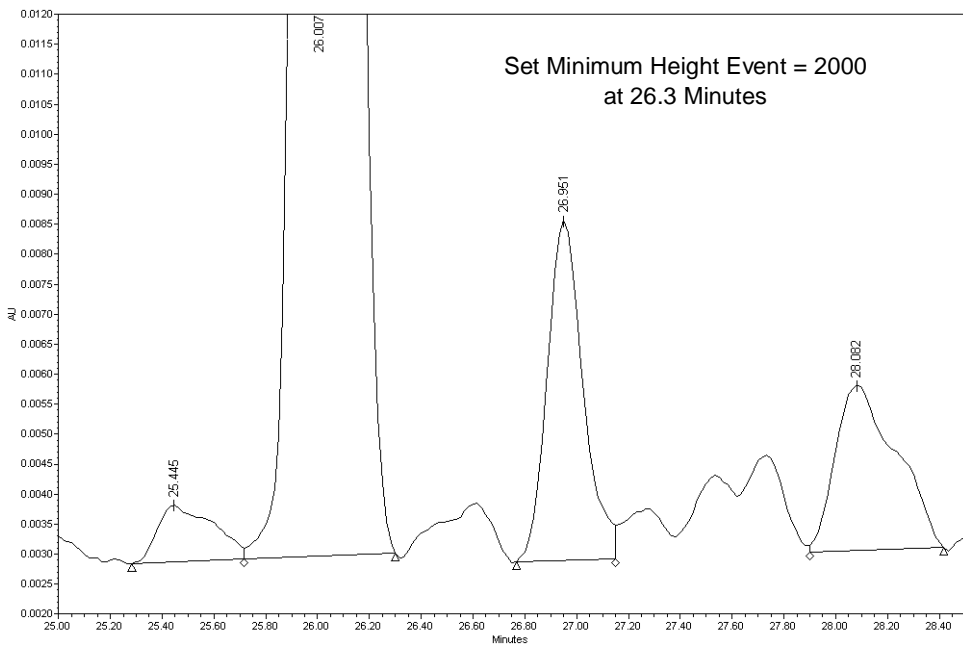
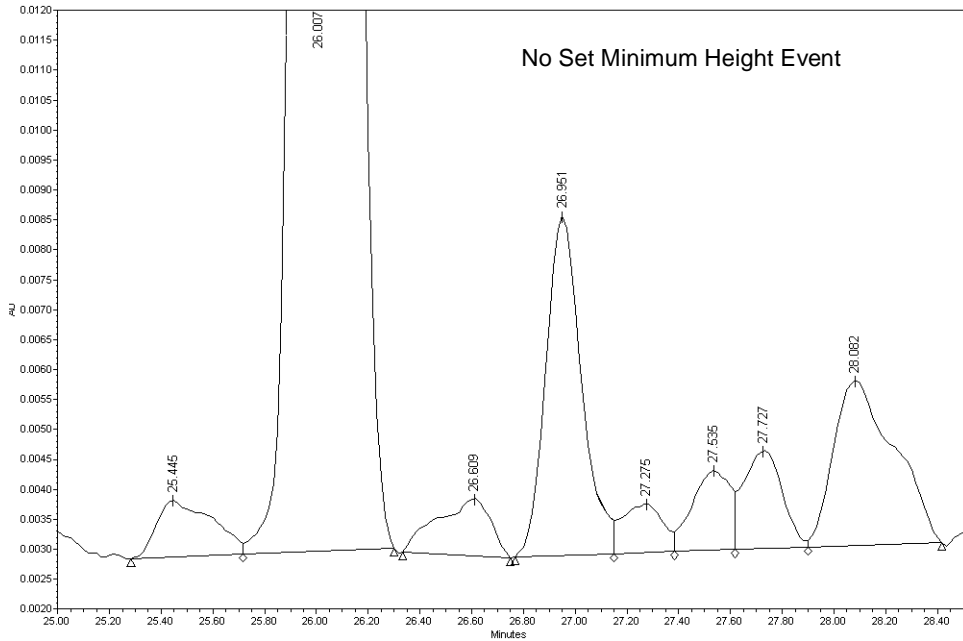


Figure 2-26 Set Minimum Height Event

## 2.9 Peak Integration Events

---

Five events can change the integration results that are associated with detected peaks. These ApexTrack peak integration events are available in the Type column of the Integration table (see [Figure 2-2 on page 31](#)). The Merge Peaks event is also available for GPC processing methods.

**Note:** [Section 2.8 on page 56](#), lists ApexTrack events that affect peak detection.

**Note:** [Section 2.10 on page 80](#), lists events that cannot overlap.

All events require a Start time, the default is 0.000 minutes. For events that have a Stop time, you can either leave the Stop time in the Stop (min) column blank to indicate that this event is enabled until the end of the run, or enter a time in minutes. Both the Start and Stop times have a precision of three significant figures by default, and the valid range of Start and Stop times is 0 to 655 minutes. The Start time must be less than the Stop time unless the Stop time is blank.

ApexTrack events that affect peak integration are as follows:

- [Section 2.9.1, Valley-to-Valley Event](#)
- [Section 2.9.2, Gaussian Skim Event](#)
- [Section 2.9.3, Merge Peaks Event for GPC, GPCV, GPC-LS, and GPCV-LS](#)
- [Section 2.9.4, Set Ltoff % Event](#)
- [Section 2.9.5, Set Touchdown % Event](#)

### 2.9.1 Valley-to-Valley Event

The Valley-to-Valley event directs ApexTrack to replace the valley (V) boundaries and crossing points (X) with baseline (B) boundaries. Thus if a peak cluster contains only V boundaries, this event will transform it into a series of individual, baseline-resolved peaks. The time of a B has the same time as that V it replaces. The Valley-to-Valley event is applied only after all peaks, baselines, and boundaries have been established. Shoulder and round boundaries are generally unchanged by the Valley-to-Valley event.

[Figure 2-28](#) shows the new baseline boundaries that resulted from the Valley-to-Valley event. Note that the shoulder and round boundaries are unchanged.

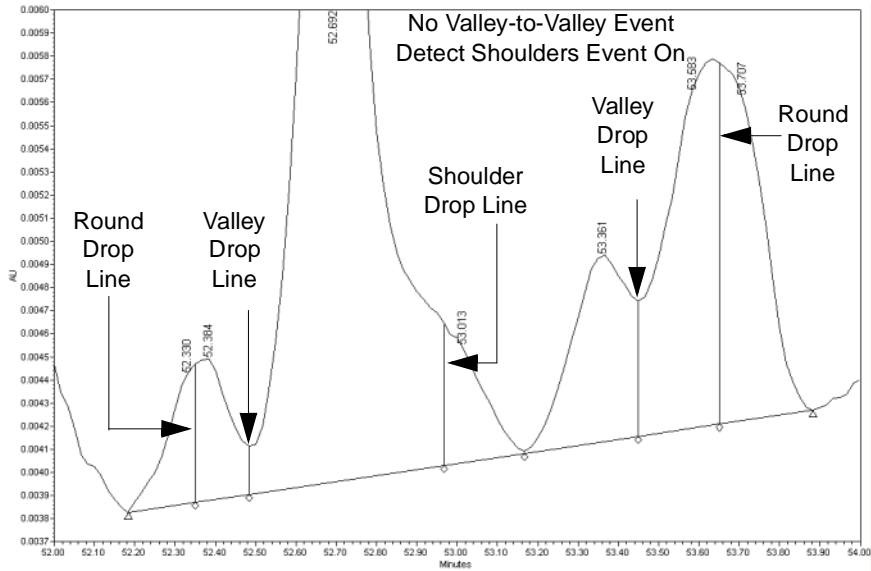


Figure 2-27 No Valley-to-Valley Event in ApexTrack

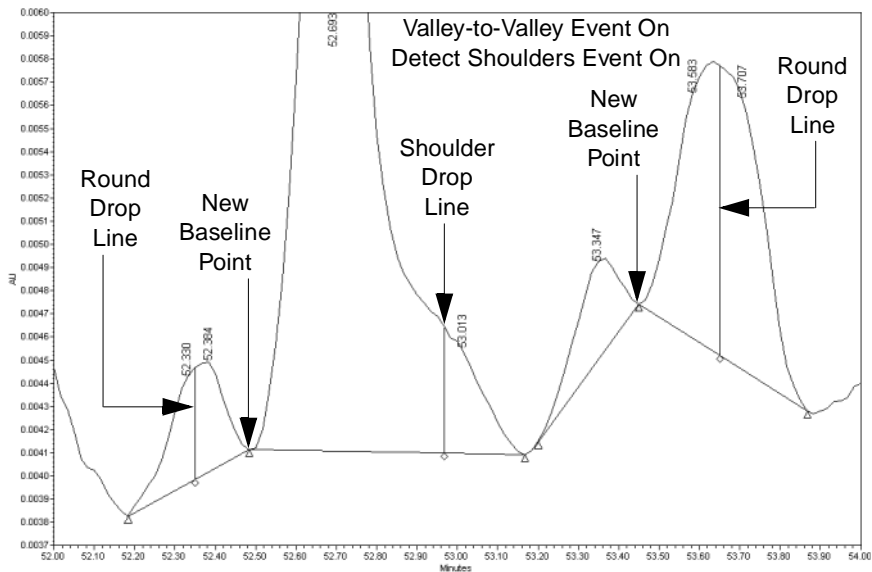


Figure 2-28 With Valley-to-Valley Event in ApexTrack

The Valley-to-Valley event is supported in negative peak detection. It can be applied to clusters that contain all negative peaks and clusters that contain negative and positive peaks.

This event requires a Start time, which is 0.000 minutes by default. The Stop (min) time is optional and is blank by default. The event does not have a value. It cannot overlap the Merge Peaks event or another Valley-to-Valley event, but it can overlap all other events.

## How a Valley-to-Valley Event Works

When the Valley-to-Valley event is enabled, the V and X boundaries within the event region are changed to B boundaries. The X points are snapped to the nearest sample point when they are reset. In addition, all X boundaries in a cluster are changed to B boundaries (and snapped to the nearest sample points) if *any* valley or crossover point in that cluster has been changed to a B by the event. This affects peaks which are outside the enabled region but part of a cluster whose baseline was changed by the event.

After the B assignments are made, the new baselines are drawn. Valley and/or shoulder boundaries in the cluster but not in the enabled region may change to Bs if the new baseline would pierce the signal. Round boundaries are left unchanged.

Table 2-2 Valley-to-Valley Event Rules

Label	Effect of Valley-to-Valley on a Boundary	
	Within the Enabled Region	Outside the Enabled Region but in a Cluster Whose Baseline Was Changed by the Event
V	B	V or B if necessary to prevent piercing
X	B	B
S	S or B if necessary to prevent piercing	S or B if necessary to prevent piercing
R	R (unchanged)	R (unchanged)

**Note:** The Gaussian Skim event (G) is applied to boundaries only after the Valley-to-Valley event has been applied. The only candidates for Gaussian skimming are the V and S boundaries that remain after the Valley-to-Valley event has been applied.

Integration results are obtained when the final baselines and boundaries are determined.

## Applying a Valley-to-Valley Event for a Single Valley in a Cluster

In [Figure 2-29](#), applying the Valley-to-Valley event to the single boundary for a short period of time changes the baseline placement.

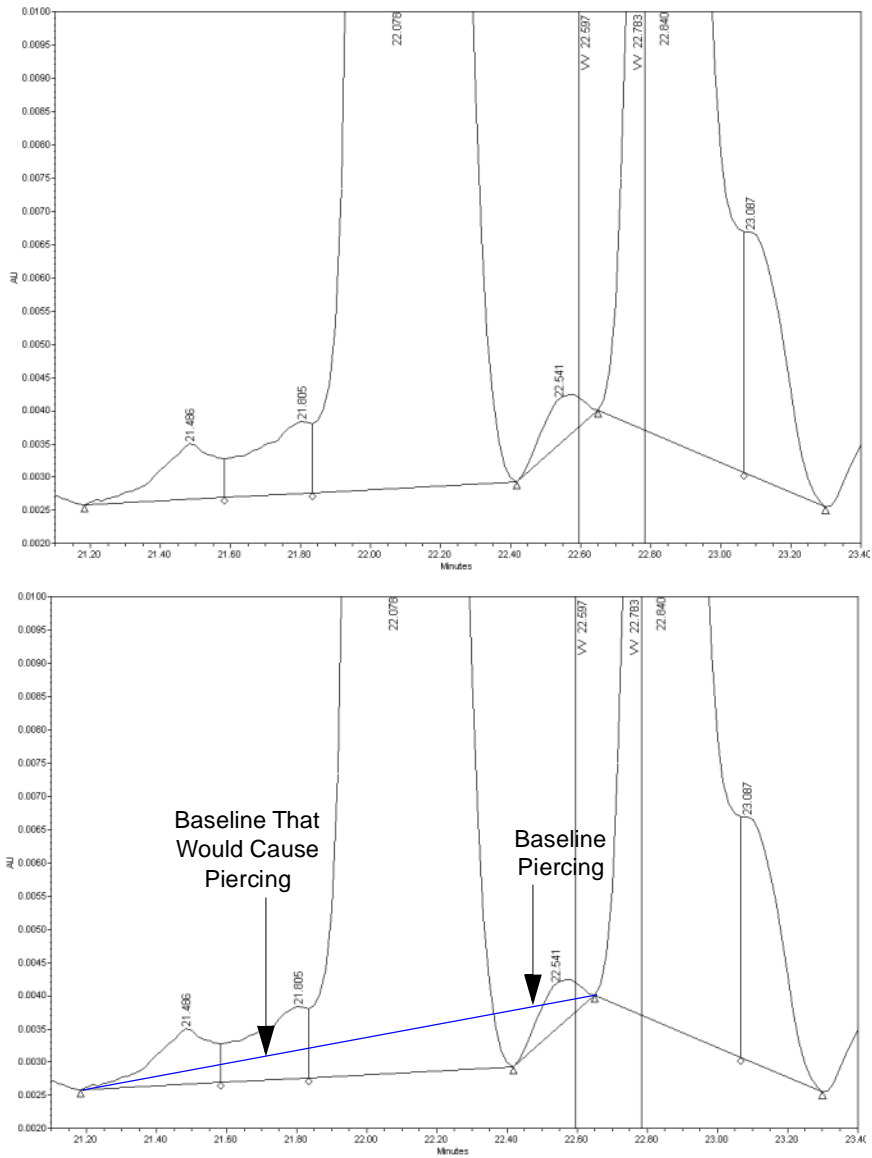


Figure 2-29 Example of Valley-to-Valley Event in ApexTrack

ApexTrack integration set an additional valley to B. Even though the event was not enabled at this time, the valley-to-valley algorithm resets the V to B before the event start. Otherwise the baseline drawn from 21.2 to 22.7 minutes would pierce the signal at 22.4

minutes. In general, if a baseline would pierce the signal as a result of a Valley-to-Valley event, one or more Vs are reset to Bs to prevent the piercing.

**Note:** *The event must encompass at least one V boundary to have an effect. Setting the event to encompass only a peak apex has no effect on the chromatogram.*

The Valley-to-Valley event is applied only after all peaks, baselines, and boundaries have been initially established.

## 2.9.2 Gaussian Skim Event

When adjoining peaks elute at low resolution, the parent peak contributes to the baseline of the child peak. ApexTrack implements a Gaussian extrapolation of the parent peak's profile in order to better estimate the area and height of both peaks ([Figure 2-30](#)). The properties of the parent peak at its inflection point determine the parameters describing the Gaussian curve.

The default boundary for valley or shoulder boundaries is a vertical drop line. A vertical drop line occurs in peak clusters when the Gaussian Skim event is not enabled. When Gaussian skimming is activated by a timed event, an algorithm determines whether a Gaussian skim should replace the S or V vertical drop lines.

**Note:** *It is good practice to enable the Detect Shoulders event in regions where the Gaussian Skim event is enabled. You must enable Detect Shoulders in order for shoulder peaks to be skimmed off of adjoining larger peaks.*

The ApexTrack algorithm draws a Gaussian curve to skim the child peak(s) from the parent peak ([Figure 2-30](#)).

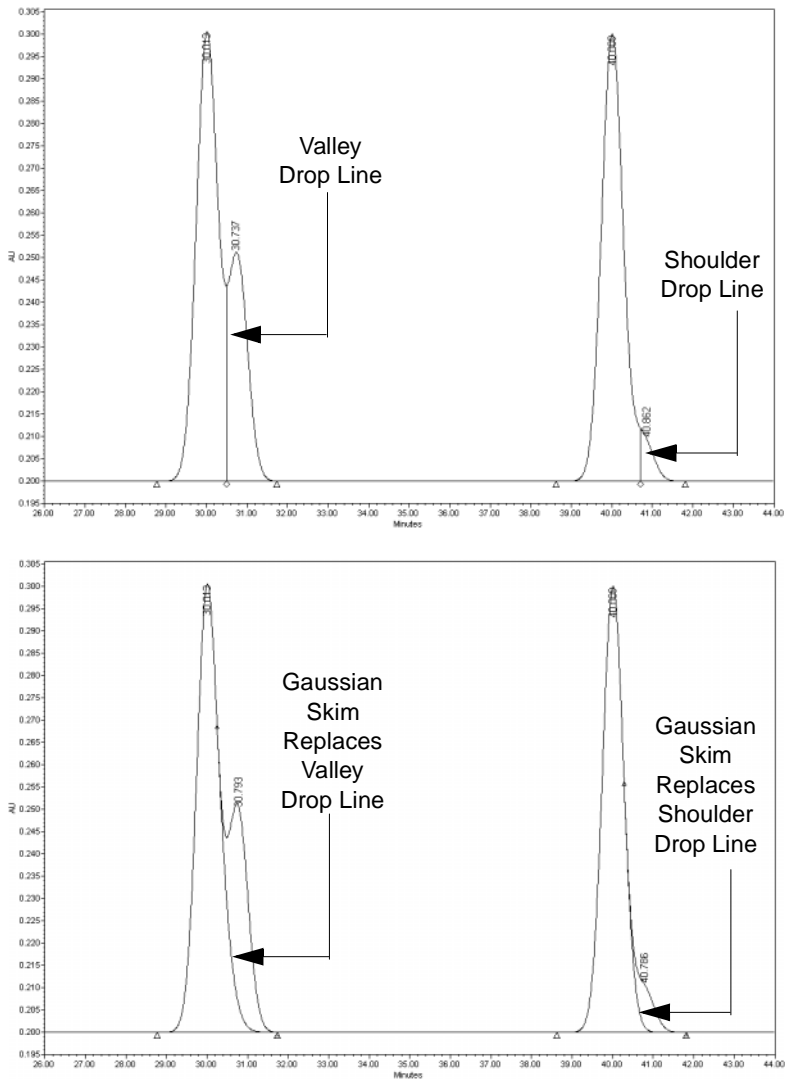


Figure 2-30 Gaussian Skim Events (Examples)

The curve used for Gaussian skimming is always a portion of a simple Gaussian peak shape.

The area of the parent peak is computed taking into account the new Gaussian profile. The area and height of the child peak are computed using the profile as the child peak's baseline.

As with any peak, a skimmed peak can be rejected if its area and/or height are too small. If a smaller skimmed peak is rejected, the profile of the parent peak is still determined by the skim, and the area of the smaller, deleted peak is still excluded by the skim.

**Note:** *The deletion or rejection of a skimmed peak does not cause its area to be added back to the parent peak, as in Traditional integration.*

ApexTrack supports both a fronting and a tailing skim. If Gaussian skim is enabled, rules are applied to each pair of adjoining peaks in a cluster to determine whether to skim or not. The timed event is applied to the times of the vertical drop lines.

Shoulder detection and Gaussian skims are compatible with Allow Negative Peaks. Positive peaks can only be skimmed off positive peaks and negative peaks can only be skimmed off negative peaks.

This event requires a Start time, which is 0.000 minutes by default. The Stop (min) time is optional and is blank by default. The event does not have a value. It cannot overlap the Merge Peaks event or another Gaussian Skim event, but it can overlap all other events.

## How Gaussian Skimming Works

**Note:** *The Gaussian skim rules are different for a GPC type processing method. Using a GPC type processing method, a parent peak can be smaller than a child peak.*

The curve used for skimming is a portion of a simple Gaussian peak shape that usually starts at the inflection point of the parent peak and ends at the end point of the baseline of the next peak (for rear skims) or the start point of the baseline of the previous peak (for front skims).

The inflection point of the parent peak determines the parameters for a particular skim.

The properties of the inflection point are its:

- Elution time
- Height above baseline
- Slope relative to the baseline

The Gaussian Skim event logic decides which peaks are skimmed and whether they are front skimmed and/or back skimmed, and the skim profile. The Gaussian skim algorithm has three major parts, which are applied in regions where the event is enabled:

1. The first algorithm determines if a vertical drop can be replaced by a Gaussian skim.
2. The second algorithm computes the skim profiles.
3. The third algorithm determines if the skim is valid. If it is not valid, the vertical drop is retained.

**Note:** *Only peaks with V (valley) and S (shoulder) labels are considered for skimming. Peaks with an R (round) label will not be skimmed.*



The rules implemented in these algorithms ensure that the start point of the skim matches that peak's profile in location, slope, and curvature, and that the skim asymptotically approaches the cluster baseline.

If the larger peak has a profile close to a Gaussian profile and its inflection point is unaffected by the presence of the smaller peak, then the properties of the inflection point are sufficient to accurately infer the skimmed profile of the larger peak.

**Note:** *It is good practice to enable the Detect Shoulders event in regions where the Gaussian Skim event is enabled. You must enable Detect Shoulders in order for shoulder peaks to be skimmed by adjoining larger peaks.*

### Example 1: Simple Gaussian Skim

[Figure 2-31](#) shows a chromatogram with Gaussian skim disabled. A vertical drop line marks the start of the smaller peak.

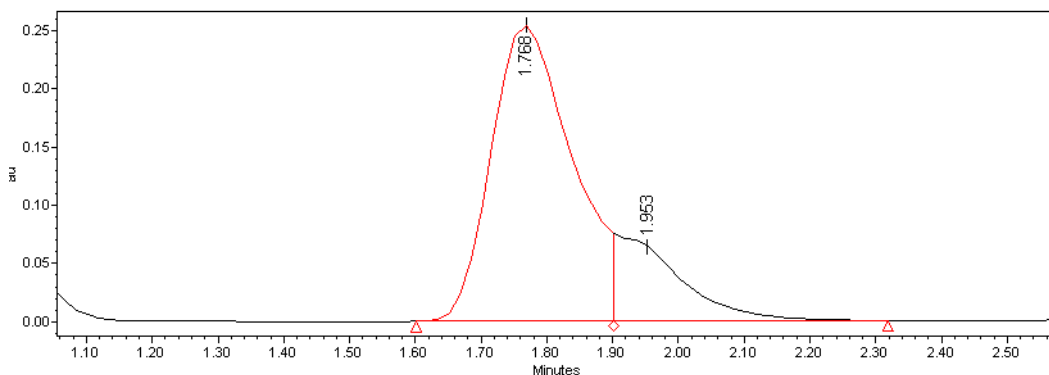


Figure 2-31 Gaussian Skim Disabled on a Simple Chromatogram

[Figure 2-32](#) shows the same chromatogram with Gaussian skim enabled. The larger peak generates the skim profile that forms the baseline of the smaller peak. The inflection point of the larger peak is used as the skim launch point.

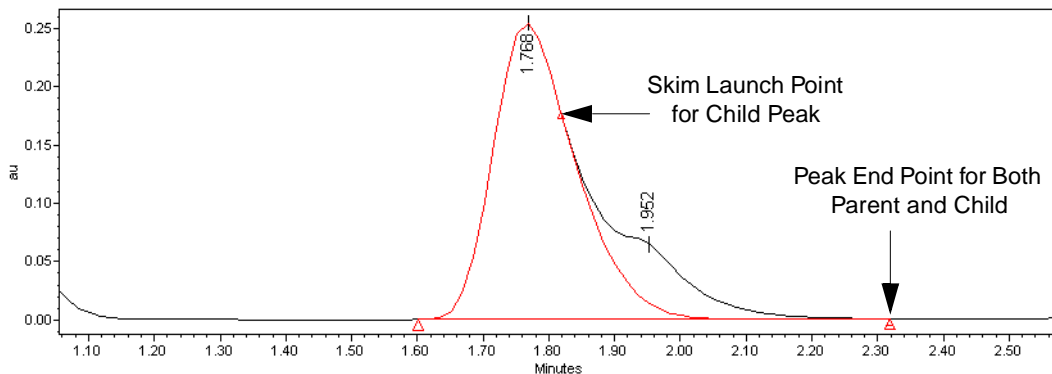


Figure 2-32 Gaussian Skim Enabled on a Simple Chromatogram

### Example 2: Complex Gaussian Skim

Figure 2-33 shows a more complex area of the chromatogram, with Gaussian skim disabled.

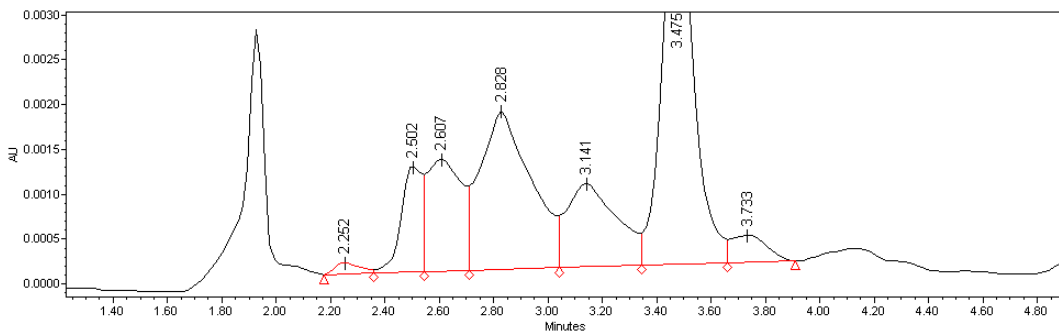


Figure 2-33 Gaussian Skim Disabled on a Complex Chromatogram

Figure 2-34 shows the same area of the chromatogram as Figure 2-33 with Gaussian skim enabled. Rules, which are described in [“Rules for Manually Deleting and Modifying Gaussian Skimmed Peaks” on page 77](#), determine which vertical drops are replaced by Gaussian profiles. A peak can be skimmed from surrounding, larger peaks.

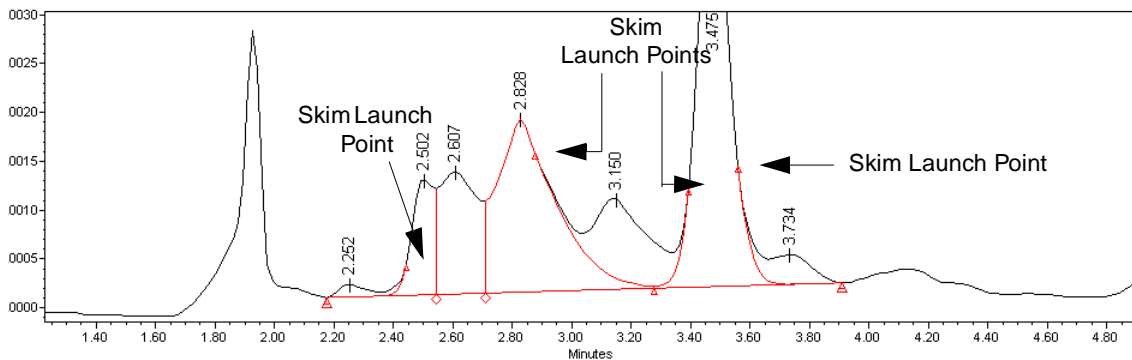


Figure 2-34 Gaussian Skim Enabled on a Complex Chromatogram

### Example 3: Gaussian Skim with a Modified Launch Point

ApexTrack integration does not allow a skim baseline to pierce the chromatogram. If piercing occurs when the skim is launched from the larger peak's inflection point, the skim is either relaunched or omitted. If piercing occurs *before* the inflection point of the smaller peak closest to the larger peak, the software modifies the location of the skim launch point to eliminate the piercing as shown in [Figure 2-35](#). The software uses the curvature minimum point to launch the skim (see [Figure 2-3 on page 35](#)). If the piercing occurs *after* the inflection point of the smaller peak closest to the larger peak, the skim is omitted.

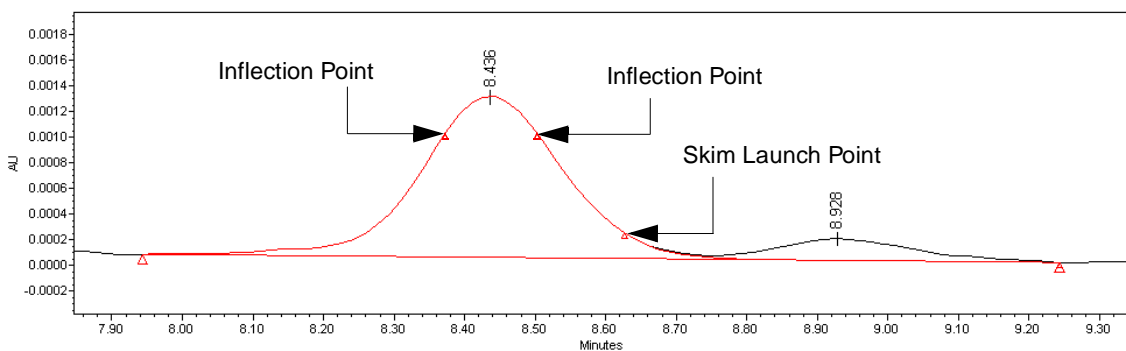


Figure 2-35 Gaussian Skim with a Modified Launch Point

The inflection point is defined as the point of maximum slope, where the second derivative is zero. For a Gaussian peak, this point is about 60% of the peak height. In ApexTrack the inflection point is determined by examination of the second derivative ([Figure 2-3 on page 35](#)). The locations of the inflection points that straddle the peak apex are indicated by the diamonds in [Figure 2-35](#).

#### Example 4: Gaussian Peak with a Boundary Deleted

Peaks that have a Gaussian profile boundary can be manually deleted or rejected using the minimum area and minimum height settings, leaving adjoining peaks unchanged (see [Figure 2-36](#)).

**Note:** The area of the deleted or rejected peak is not added to the adjoining peaks as with Traditional integration.

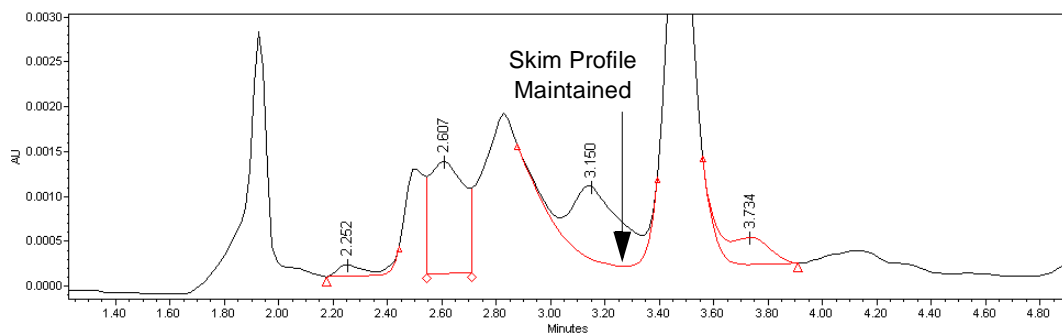


Figure 2-36 Gaussian Skim Boundary After Some Peaks Are Manually Deleted

#### When Does ApexTrack Compute Skim Parameters?

The Gaussian skim algorithm has three major parts, which are applied in regions where the event is enabled:

1. The first algorithm determines if a vertical drop can be replaced by a Gaussian skim.
2. The second algorithm computes the skim profiles.
3. The third algorithm determines if the skim is valid. If it is not valid, the vertical drop is retained.

The first algorithm determines if a vertical drop can be replaced by a Gaussian skim. The skim is computed if the following conditions are met:

- The boundary between the larger peak and smaller peak is a valley or shoulder.
- The apex of the smaller peak is *below* the inflection point of the larger peak. If the peaks are of equal or nearly equal height, the peak boundary remains a vertical drop line.
- If the boundary is a valley, the height of the valley is greater than 10% of the height of the smaller peak.

If the smaller peak is below the inflection point height, but the valley height is too low, the peaks are adequately resolved and the peak boundary remains a vertical drop.

## Height and Width Parameters for the Gaussian Curve

The second algorithm computes the Gaussian skim profiles. The shape of the Gaussian profile determines the baseline of the smaller peak(s).

The Gaussian profile is a portion of an inferred Gaussian peak. Two parameters control the shape of the inferred Gaussian peak: its height and width. The algorithm adjusts these parameters so that the height of the larger (parent) peak at its inflection point matches the height of the inferred Gaussian peak at its inflection point, and the slope of the larger (parent) peak at its inflection point matches the slope of the inferred Gaussian peak at its inflection point.

## When Does ApexTrack Retain a Vertical Drop?

The third algorithm retains a vertical drop line under the following conditions:

- At the end of the skim, the height of the skim profile is greater than 1% of the height of the larger peak.
- The skim profile pierces the chromatographic signal.

If the skim logic decides that a peak should *not* be skimmed off another peak, the drop line remains separating the two peaks.

## Rules for Manually Deleting and Modifying Gaussian Skimmed Peaks

**Note:** See [C.8, Manual Integration Guidelines](#), for manual integration guidelines in ApexTrack.

- A smaller skimmed peak, or a larger peak that generates a skim, can be manually deleted. In either case, the adjoining peak is unaffected. Thus if a smaller skimmed peak is rejected or deleted, the profile of the larger (parent) peak is still determined by the skim, and the area of the smaller, deleted peak is still excluded by the skim. If the larger skimmed peak is deleted, the baseline of the smaller skimmed peak is still determined by the skim.
- You cannot perform manual skims, manually add a skimmed (child) peak, or manually modify a skimmed (child or parent) peak by moving its start or end point or by adding a vertical drop line. You cannot manually adjust the baseline of a cluster that contains skimmed peaks (either by moving the marker at the start of the first peak or the end of the last peak in the cluster).
- You can manually add a vertical drop line to all other types of peaks (any peak that does not have a G listed as its Int Type).
- You cannot move or delete a vertical drop line that coincides with the start or end of a skimmed peak.

## 2.9.3 Merge Peaks Event for GPC, GPCV, GPC-LS, and GPCV-LS

The Merge Peaks event is available only for GPC type processing methods (GPCV, GPC-LS, and GPCV-LS data). This event requires a Start time, which is 0.000 minutes by default. The Stop time is optional and is blank by default. The event does not have a value.

If  $n$  consecutive peaks with the same sign (either both positive or both negative) are separated by vertical drop lines that occur during the Merge Peaks event, the vertical drop line between the  $n$  peaks is deleted and the  $n$  peaks are merged into one peak (Figure 2-37). The resulting peak has the same retention time as the peak with the height with the greatest absolute value.

A Merge Peaks event cannot overlap the Detect Shoulders, Gaussian Skim, Valley-to-Valley event, or another Merge Peaks event, but it can overlap all other events.

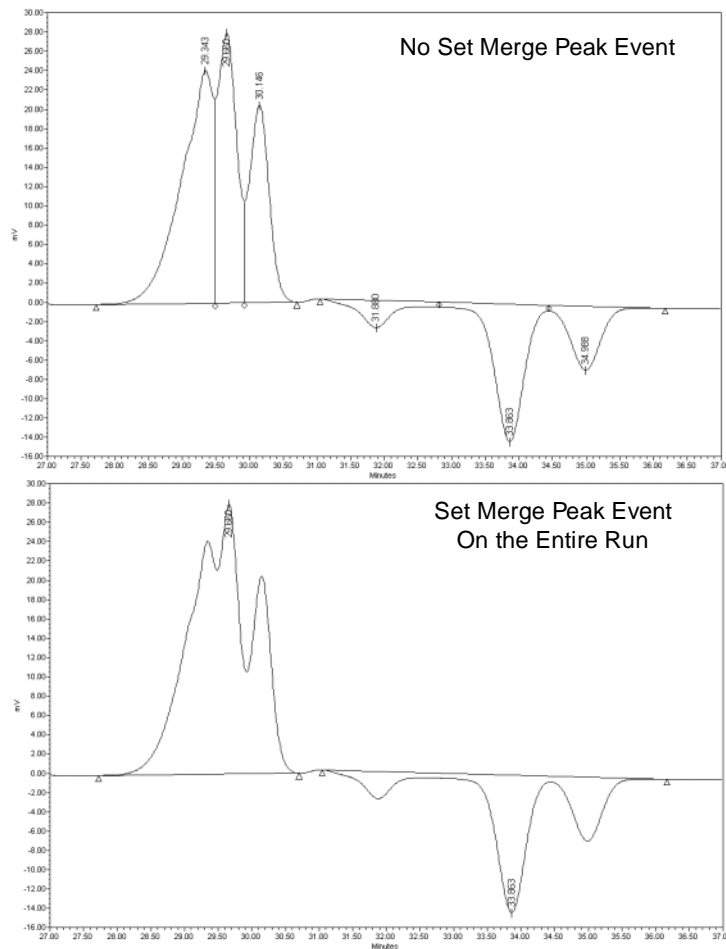


Figure 2-37 Merge Peaks Event (GPC, GPCV, GPC-LS, and GPCV-LS)

## 2.9.4 Set Lutoff % Event

This event redefines the value ApexTrack uses when it determines the slope difference threshold to identify the start of a peak (Figure 2-38). This event affects integrated peaks whose second derivative apices occur during the duration of the event.

**Note:** The Set Lutoff % event overrides the global Lutoff % parameter for the remainder of the run or until another Set Lutoff % event is encountered. To return to the global value, you must enable another Set Lutoff % event specifying that value.

The upper chromatogram in Figure 2-38 shows the integration of the peak with the default values of Lutoff % = 0.0 and Touchdown % = 0.5. The lower chromatogram shows the integration of the chromatogram using Set Lutoff % and Set Threshold % events at 2.5 minutes.

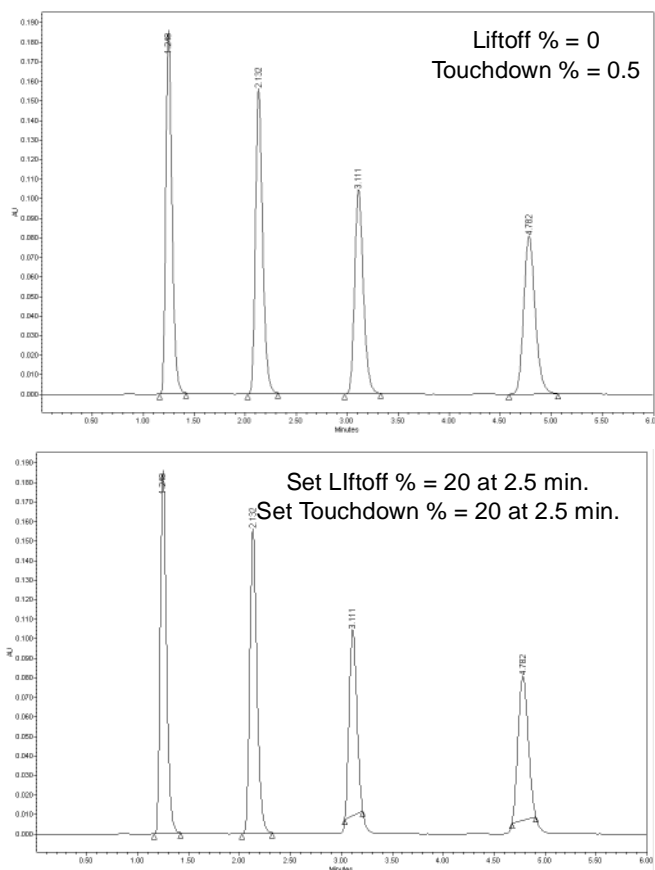


Figure 2-38 Set Lutoff % and Set Touchdown % Events

The valid range for the Set Lutoff % event value is 0.0 to 100% (0 to 5% for a GPC processing method). This event requires a Start time, which is 0.000 minutes by default

and allowed values are from 0.0 to 655.0 minutes. This event affects peaks whose second derivative peak apices occur during the time of the event.

You can use the Set Ltoff % and Set Touchdown % events independently of one another.

## 2.9.5 Set Touchdown % Event

This event redefines the value ApexTrack uses when it determines the slope difference threshold to identify the end of a peak ([Figure 2-38](#)). This event affects peaks whose second derivative apices occur during the duration of the event.

**Note:** *The Set Touchdown % event overrides the global Touchdown % parameter for the remainder of the run or until another Set Touchdown % event is encountered. To return to the global value, you must enable another Set Touchdown % event specifying that value.*

The upper chromatogram in [Figure 2-38](#) shows the integration of the peak with the default values of Ltoff % = 0.0 and Touchdown % = 0.5. The lower chromatogram shows the integration of the chromatogram using Set Ltoff % and Set Threshold % events at 2.5 minutes.

The valid range for the Set Touchdown % event value is 0.0 to 100% (0 to 5% for a GPC processing method). This event requires a Start time, which is 0.000 minutes by default and allowed values are from 0.0 to 655.0 minutes.

You can use the Set Ltoff % and Set Touchdown % events independently of one another.

## 2.10 Incompatible Events for ApexTrack

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**Note:** *All Set events can overlap all events without conflict, including the same Set event.*

The following ApexTrack timed events cannot overlap in time the specified event(s):

- **Allow Negative Peaks event** – Cannot overlap another Allow Negative Peaks event, but it can overlap all other events.
- **Detect Shoulders event** – Cannot overlap the Merge Peaks event or another Detect Shoulders event, but it can overlap all other events.
- **Gaussian Skim event** – Cannot overlap the Merge Peaks event or another Gaussian Skim event, but it can overlap all other events.
- **Inhibit Integration event** – Cannot overlap another Inhibit Integration event, but it can overlap all other events.
- **Merge Peaks event** – Cannot overlap the Detect Shoulders, Gaussian Skim, Valley-to-Valley event, or another Merge Peaks event, but it can overlap all other events.
- **Valley-to-Valley event** – Cannot overlap the Merge Peaks event or another Valley-to-Valley event, but it can overlap all other events.



## 2.11 When Timed Events Are Active

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These events affect peaks whose second derivative apex occurs during the time of the event:

- Inhibit Integration
- Allow Negative Peaks
- Set Detection Threshold
- Set Liftoff %
- Set Touchdown %

These events affect peaks whose retention time occurs during the time of the event:

- Set Minimum Area
- Set Minimum Height

These events affect vertical drop lines that occur during the time of the event:

- Detect Shoulders
- Gaussian Skim
- Valley-to-Valley
- Merge Peaks (for GPC only)

This event affects the data point immediately after the timed event is enabled:

- Set Peak Width (sec)

## 2.12 References

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For further information on the theory of ApexTrack peak detection and integration, see:

*ApexTrack Integration: Theory and Application*, Waters Corp., Milford, MA, 2002.  
Posted on [www.waters.com](http://www.waters.com).

# Chapter 3

## Traditional Integration

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Traditional peak detection and integration by Empower software includes these functions:

- Automatically determining appropriate peak width and threshold values for the chromatogram, unless already set in the processing method
- Detecting peaks in the chromatogram to determine their location
- Integrating peaks to determine their retention times, areas, and heights

The processing method defines the parameters (including detection and integration events) that the software uses to detect and integrate the peaks within the raw data file (channel).

### 3.1 Peak Detection

---

The peak detection processes include:

1. Performing data bunching
2. Determining peak start
3. Determining preliminary peak apex
4. Determining peak end
5. Determining peak width and threshold values in the processing method
6. Inhibiting integration

The detection algorithm first determines the presence of peaks by comparing the rate of change of the signal to specific acceptance criteria to determine where peaks in the acquired raw data file start and end. The software must perform these peak detection tests before it can integrate the peaks.

You determine the peak detection test criteria several ways:

- Peak width and threshold selections in the Integration tab of the Processing Method window in Review
- Integration toolbar of the Review Main window or the Processing Method Editor
- Processing Method wizard in Review

**Note:** For additional information on peak detection theory, see [Section 3.6, References](#).

### 3.1.1 Performing Data Bunching

As the detection algorithm tests the data for a peak, the software averages individual raw data points into discrete groups, or bunches, to produce a single point. The number of data points in a bunch is set by the peak width parameter.

In most instances, each bunch of data contains one point when the sampling rate is optimized. Data bunching has no effect on the acquired raw data. It is an internal calculation used to enhance the process of determining peak start and peak end. When the peaks contain more data points than necessary, the bunched data points are used only to detect peaks; all raw data points are used for integration.

[Figure 3-1](#) illustrates the effects of data bunching on a noisy signal. In this example, with the peak width set to 60 and sampling rate set to 1, the detection algorithm produces a bunched data point for each set of four raw data points. This optimizes the number of bunches to 15 (across a 60-second peak containing 60 data points) and effectively smooths the data.

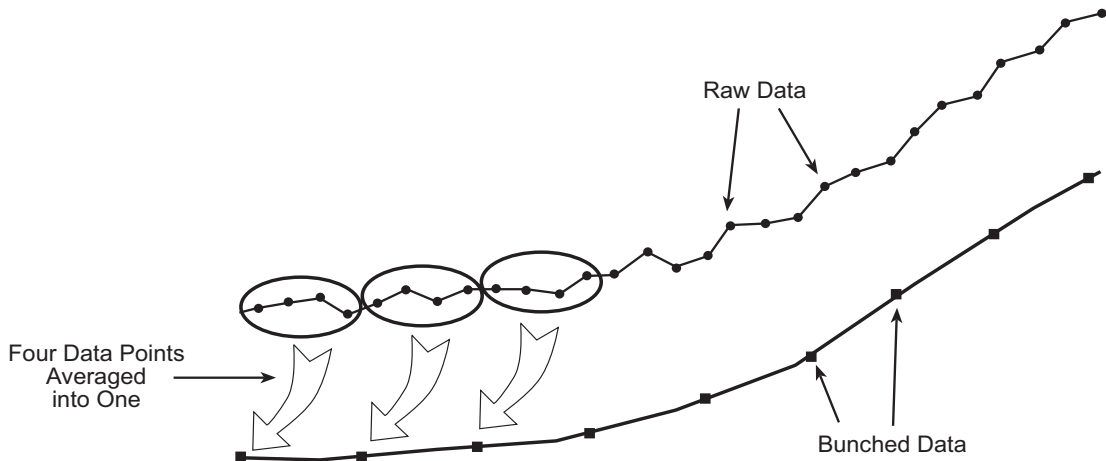


Figure 3-1 Data Bunching Example

During detection, the software calculates the number of points in a bunch using the equation:

$$PB = \frac{(PW \times SR)}{15}$$

where:

$PB$  = Points in a bunch

$PW$  = Peak width (in seconds)

$SR$  = Sampling rate (data points/second as specified in the instrument method used for acquisition)

**Note:** The peak detection algorithm functions most effectively with 15 data points across each peak. For this reason, the software organizes the raw data into 15 discrete bunches when setting the peak width value. When peak width is 15 and sampling rate is 1, no data point bunching occurs; all data points are used to detect peak start and peak end.

### 3.1.2 Determining Peak Start

The liftoff threshold specified in the processing method defines the minimum slope of the signal in  $\mu\text{V}/\text{sec}$ , at or above which the start of a peak is detected.

**Note:** By default, negative peaks are not detected. To activate the Allow Negative Peaks event, see [Section 3.3, Peak Detection Events](#).

To determine peak start, the detection algorithm of the software:

1. Performs the threshold test on the signal:
  - a. The software averages the signal slope across two data bunch intervals and then compares it to the liftoff threshold ([Figure 3-2](#)).
  - b. When the averaged slope of the signal between bunches B1 and B3 is greater than or equal to the liftoff threshold value, the software flags B1 as the *possible* peak start.
2. Examines the individual points in the B1 bunch to determine the actual start point. For positive peaks, this is the data point with the minimum Y value. For negative peaks, this is the data point with the maximum Y value.

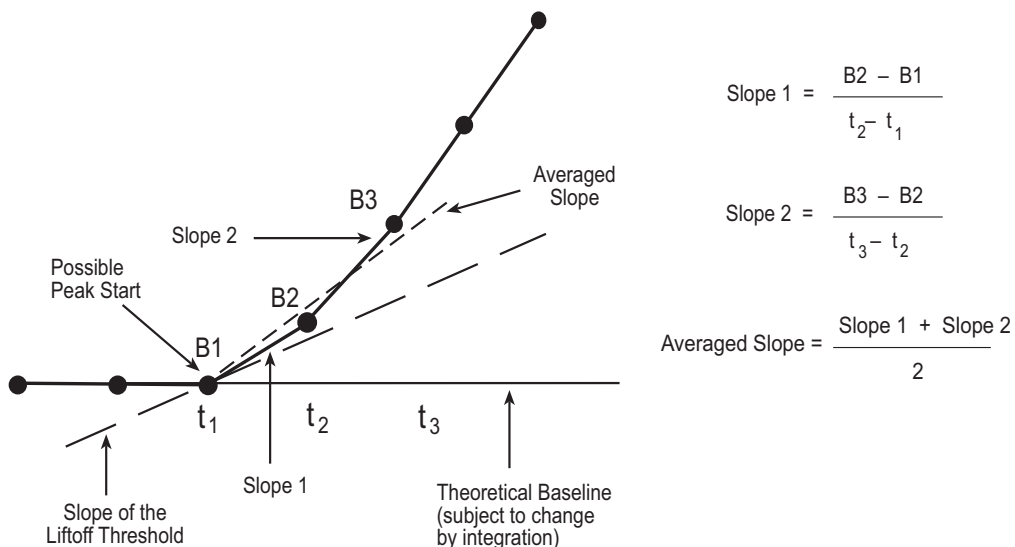


Figure 3-2 Determining Possible Peak Start

### 3.1.3 Determining Preliminary Peak Apex

To determine the preliminary peak apex (peak maximum) after the peak start is confirmed, the software:

1. Monitors the signal until the slope changes sign from positive to negative. For a negative peak, the slope changes sign from negative to positive.
2. Analyzes the bunch where the slope change occurs (bunch B12 in [Figure 3-3](#)) and assigns a *tentative* peak apex to the data point within the bunch that is furthest away from the theoretical baseline.

**Note:** This peak apex is preliminary because the software does not determine the actual peak apex until integration occurs and baselines are assigned.

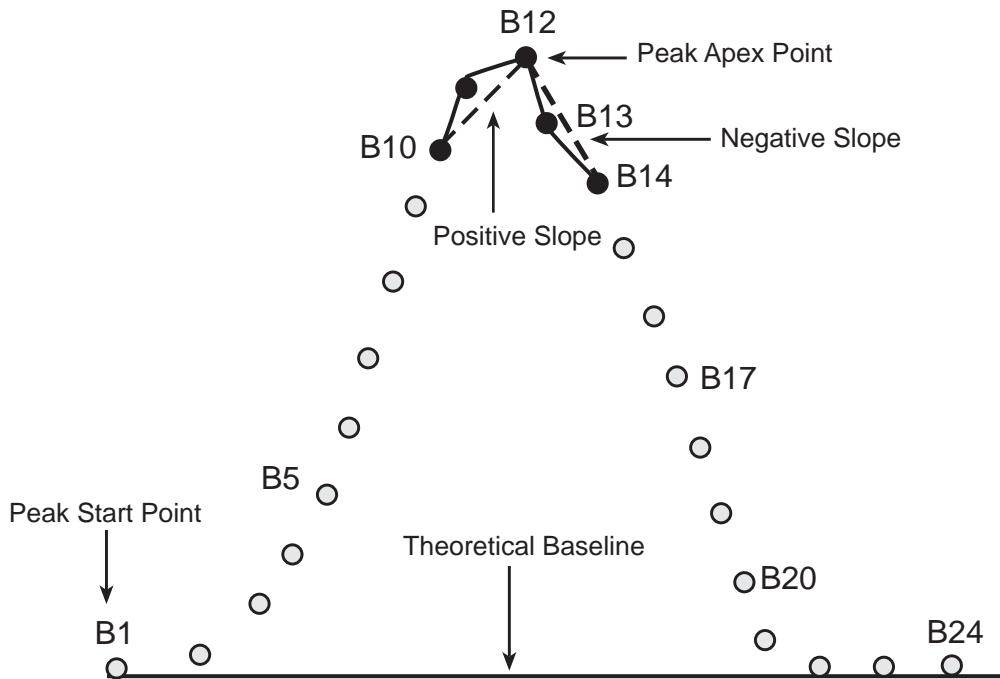


Figure 3-3 Determining the Preliminary Peak Apex

### 3.1.4 Determining Peak End

To determine the peak end, the software:

1. Compares the slope of the signal to the touchdown threshold. When two consecutive slopes are less than the threshold value, the algorithm flags the last data point in the last bunch as the *possible* peak end.

- Examines the individual data points in the current and next bunch to determine the actual peak end. For positive peaks, this is the data point with the minimum Y value. For negative peaks, this is the data point with the maximum Y value.
- During the peak end test, checks for a change in the sign of the slope. A change in sign *before* the touchdown indicates a preliminary peak valley (the end point of the current peak and the start point of the next peak).

**Note:** *This peak end point/start point is preliminary because the software does not determine the actual data point for the end of a peak until the integration process occurs and baselines are assigned.*

- Proceeds from this peak start point to the peak apex test and continues until it successfully determines peak touchdown ([Figure 3-4](#)).

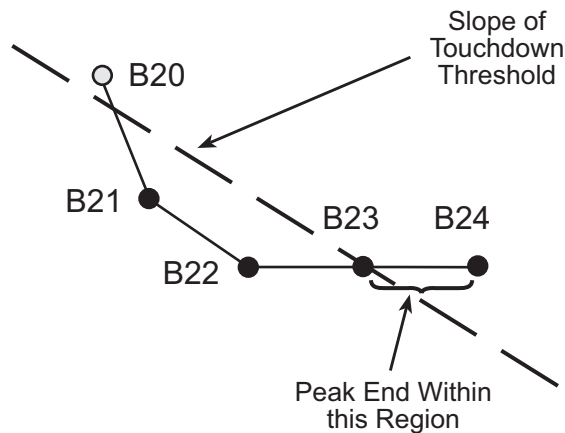


Figure 3-4 Determining Peak End

### 3.1.5 Determining Peak Width and Threshold Values

The software uses a second derivative to set the peak width and threshold values in the processing method automatically.

**Note:** *You can use the Peak Width and Threshold determination method, which was used in all Millennium<sup>32</sup> software, by selecting **Configuration Manager > View > System Policies**, then selecting **Use v3.0X Style Peak Width and Threshold Determination** in the Other Policies tab (see [Appendix B, Data Processing System Policies](#)). When this system policy is active, the Peak Width and Threshold buttons, Processing Method wizard, processing methods, and results all function as in all Millennium<sup>32</sup> software.*

## Peak Width Value

The software automatically determines the peak width value (Auto-Peak Width) using the inflection points of the second derivative of the peak with the highest second derivative within a chromatographic region.

Since the software uses the peak width value to determine a bunching factor during peak detection (see [Section 3.1.1, Performing Data Bunching](#)), this value affects the sensitivity of the peak detection process. The guideline is to use a peak width value within a range of plus-or-minus two times the software-determined peak width value.

If the signal-to-noise (S/N) ratio is acceptable, the peak width value at the high end of this range may increase sensitivity and allow relatively small peaks to be properly integrated. However, shoulders on larger peaks, if present, might no longer be detected. Increasing the peak width value above this range results in a decrease in sensitivity.

The valid range of the peak width setting is 0.01 to 9999.99. The default peak width setting is blank.

There are several ways to set a peak width value in Review:

- When using the Processing Method wizard in Review either to create a new processing method or to edit an existing processing method, the software automatically determines an appropriate peak width using the data contained within the zoomed region (in the Integration - Integration Region wizard page).
- When viewing data in the Review Main window, clicking the **Peak Width** button in the Integration toolbar automatically sets the peak width value to the peak with the highest second derivative within the current zoom region (which may be the entire chromatographic region).
- With no peak width set in the active processing method, you can integrate the data by selecting **Process > Integrate** (or clicking the **Integrate** button in the toolbar). The peak width is automatically set according to the data in the entire chromatogram (unless there is an Inhibit Integration event at the start and/or the end of the chromatogram).

The region of the chromatogram that is used to set peak width starts at either the beginning of the chromatogram or the stop time of an Inhibit Integration event that starts at the beginning of the chromatogram. The region of the chromatogram that is used to set peak width ends at either the end of the chromatogram or the start time of the Inhibit Integration event that stops at the end of the chromatogram.

Inhibit Integration events that do not overlap the beginning or end of the chromatogram are ignored when setting peak width.

**Note:** When using this method, the peak width is placed in the Result Peak Width field only. The Processing Method Peak Width field remains blank. To copy the Result Peak Width value to the Method Peak Width field, select **Copy to Processing Method** from the shortcut menu.

- Set the peak width value manually by entering a value in the Integration toolbar of the Main window of Review or in the Integration tab of the Processing Method window.

**Note:** *If the peak with the highest second derivative is fused, the peak width value might not be optimal. In such cases, zoom in on peaks other than the fused peak when setting the peak width parameter.*

## Threshold Values

The software automatically determines the threshold value (Auto-Threshold) by first applying a median filter to the second derivative of the chromatographic data to determine the noise. The software then infers the threshold value by multiplying the second derivative noise by the current peak width value.

The threshold value is a slope measurement that the software uses to determine peak start and peak end points during peak detection (as described in [Section 3.1.2, Determining Peak Start](#), and [Section 3.1.4, Determining Peak End](#)). A relatively low threshold value increases sensitivity and may allow relatively small peaks to be properly integrated. If too many small, baseline noise peaks are being integrated, increasing the threshold value may prevent these small peaks from being integrated.

**Note:** *The software normally uses the global threshold value in the processing method to determine both peak start (liftoff) and peak end (touchdown). If you need to use a different threshold value for peak starts or ends due to a tailing or a sloping baseline, use the Set Liftoff or Set Touchdown event (see [Section 3.3.2, Set Liftoff Event](#), and [Section 3.3.3, Set Touchdown Event](#)).*

The valid range of the threshold setting is 0.0 or greater. The default threshold setting is blank.

There are several ways to set a threshold value in Review:

- When using the Processing Method wizard in Review either to create a new processing method or to edit an existing one, the software automatically determines an appropriate threshold using the data contained within the zoomed region in the Integration - Integration Region wizard page.
- When viewing data in the Review Main window, clicking the **Threshold** button in the Integration toolbar automatically sets the threshold value using the data within the current zoom region (which may be the entire chromatographic region).

**Note:** *The Set Method Threshold button is disabled if the Processing Method Peak Width field is blank.*

- With no threshold set in the active processing method, you can integrate the data by selecting **Process > Integrate** (or clicking the **Integrate** button in the toolbar). The software automatically sets the threshold according to the data in the entire chromatogram (unless there is an Inhibit Integration event at the start and/or the end of the chromatogram).



The region of the chromatogram that is used to set the threshold starts at either the beginning of the chromatogram or the stop time of an Inhibit Integration event that starts at the beginning of the chromatogram. The region of the chromatogram that is used to set the threshold ends at either the end of the chromatogram or the start time of the Inhibit Integration event that stops at the end of the chromatogram.

Inhibit Integration events that do not overlap the beginning or end of the chromatogram are ignored when setting threshold.

**Note:** When using this method, the determined threshold is placed in the Result Threshold field only. The Processing Method Threshold field remains blank. To copy this value to the Method Threshold field, select **Copy to Processing Method** from the shortcut menu.

*A peak width value is required before determining a threshold value. If the processing method does not have a peak width value, the threshold button is not available. If you use this method to perform integration without a peak width value, the software first determines the peak width value and then determines the threshold value. Both values are automatically placed in their respective toolbar fields.*

- You can set the threshold value manually by entering a value in the Integration toolbar of the Main window of Review or in the Integration tab of the Processing Method window.

## Peak Width and Threshold Fields

The peak width and threshold values are reported as both method and result fields. These fields are in the Integration toolbar of the Review Main window, and the result fields are available for reports. The method fields report the peak width and threshold values from the processing method. The result fields report the peak width and threshold values that were used when the raw data was processed.

During processing, the software uses the values in the Processing Method Peak Width and the Processing Method Threshold fields, then stores these values in the Result Peak Width and Result Threshold fields, respectively. In this case, the Result Peak Width and the Result Threshold fields are the same as the Processing Method Peak Width and the Processing Method Threshold fields, respectively. If the Processing Method Peak Width and/or the Processing Method Threshold are blank, then the software determines the Result Peak Width and/or Result Threshold fields during data processing.

When data is processed using a processing method that contains a blank Processing Method Peak Width and/or Processing Method Threshold, each result may be produced using a different Result Peak Width and Result Threshold.

**Note:** You can disable the Auto-Peak Width and Auto-Threshold determinations by selecting **Configuration Manager > View > System Policies**, then selecting **Use v3.0X Style Peak Width and Threshold Determination** in the Other Policies tab (see [Appendix](#)

[B. Data Processing System Policies](#)). When this system policy is active, the Peak Width and Threshold buttons, the Processing Method wizard, processing methods, and results all function as in all Millennium<sup>32</sup> software.

### 3.1.6 Inhibiting Integration

The Inhibit Integration event prevents the detection of peaks within the start and end times you specify. When Inhibit Integration is enabled, the peak detection routine does not bunch the points to look for a peak start.

Because the software does not bunch data points during the Inhibit Integration event, the Inhibit Integration event affects peak detection when the peak apex bunch contains more than one data point. Bunching begins with the point after the event end, which means that this bunching start point changes if the end time of the Inhibit Integration event changes. The data points bunched after an Inhibit Integration event can have different values, depending on where the event ended.

If a peak apex falls within the Inhibit Integration event time interval, the entire peak is rejected. Therefore, when you specify the Inhibit Integration event end prior to and excluding the occurrence of a peak apex bunch, the software integrates that peak. If you specify that the event end after the occurrence of the peak apex bunch, then the peak containing that bunch is not integrated. Likewise, when you specify the Inhibit Integration event start after the occurrence of a peak apex bunch, the software integrates that peak. When you specify the Inhibit Integration event start before the peak apex bunch, the software does not integrate that peak.

**Note:** The Inhibit Integration event does not conflict with an Allow Negative Peaks event. However, an Inhibit Integration event might conflict with other events enabled within a similar time range. An Inhibit Integration event does not conflict with a Valley-to-Valley event or the six Force Baseline events (Force Baseline by Peak and by Time, Forward Horizontal by Peak and by Time, and Reverse Horizontal by Peak and by Time), if both the start and end times for the event are not located within a single Inhibit Integration event. When these events overlap, the Inhibit Integration event takes precedence.

[Figure 3-5](#) illustrates the Inhibit Integration event.

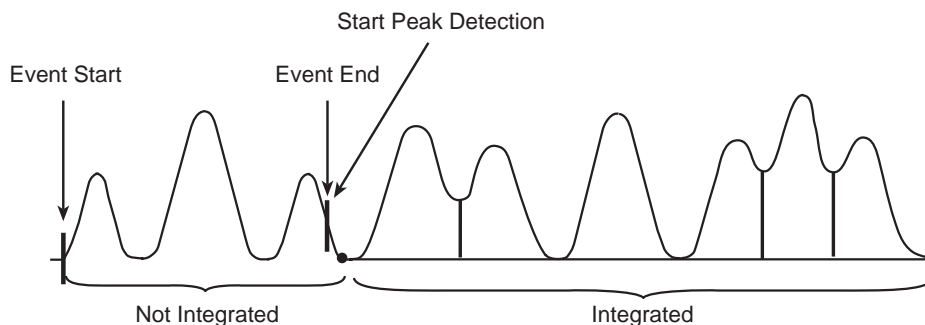


Figure 3-5 Inhibit Integration Event

## 3.2 Peak Integration

This section describes the following peak integration processes:

- Determining fused peaks
- Constructing the baseline
- Calculating peak retention time, height, and area

Integration uses the peak start and peak end values identified during peak detection to determine baselines and to integrate isolated peaks and fused (clustered) peaks.

If you have complicated chromatograms, you can enable time-based integration events to refine peak integration.

**Note:** For in-depth information on peak integration theory, see [Section 3.6, References](#).

### 3.2.1 Determining Fused Peaks

#### Checking the Distance Between Adjacent Peaks

The first process in integration is distinguishing any fused peaks and isolated peaks in the chromatogram ([Figure 3-6](#)).

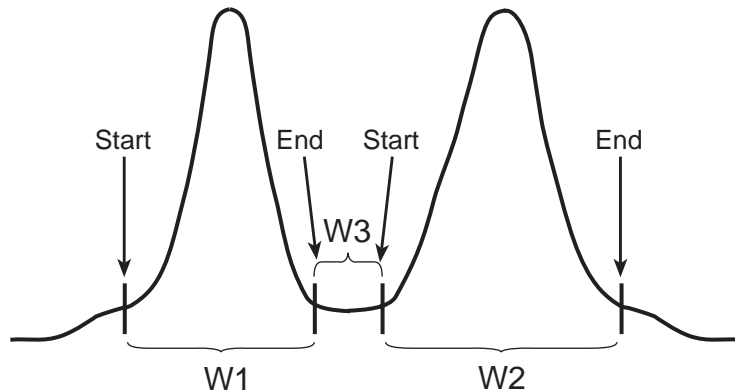


Figure 3-6 Adjacent Peak Width Comparison

When determining fused peaks, the Traditional integration algorithm:

1. Compares the width of the space between the detected start and end points of adjacent peaks ( $W3$ ) to the width of the wider adjacent peak (either  $W1$  or  $W2$ ).
2. Locates the wider adjacent peak ( $W2 > W1$ ).

3. Computes the ratio of the wider adjacent peak to the space between the two peaks ( $W_2/W_3$ ) using the equation  $W_2/W_3$ . If the ratio is greater than or equal to 3.0, the peaks are considered *fused*. If the ratio is less than 3.0, the peaks are considered *resolved*.

**Note:** The software uses the ratio of 3.0 to increase the chances of detecting peak overlap.

## Setting the Valley Points Between Fused Peaks

To set the *valley point* between fused peaks, the software:

1. Draws a projected baseline from the start point of the first peak in the cluster to the end point of the last peak in the cluster.
2. Searches for the valley point between each pair of adjacent fused peaks, and chooses the raw data point closest to the projected baseline as the valley point. The software adjusts the end point of the peak *preceding* the valley to the time of the valley point. Similarly, it adjusts the start point of the peak *following* the valley to the time of the valley point.
3. Draws a vertical line from the valley point to the projected baseline, thereby separating the peaks.

In [Figure 3-7](#), for example, the integration algorithm locates two fused peak groups and a total of six peaks within the chromatogram.

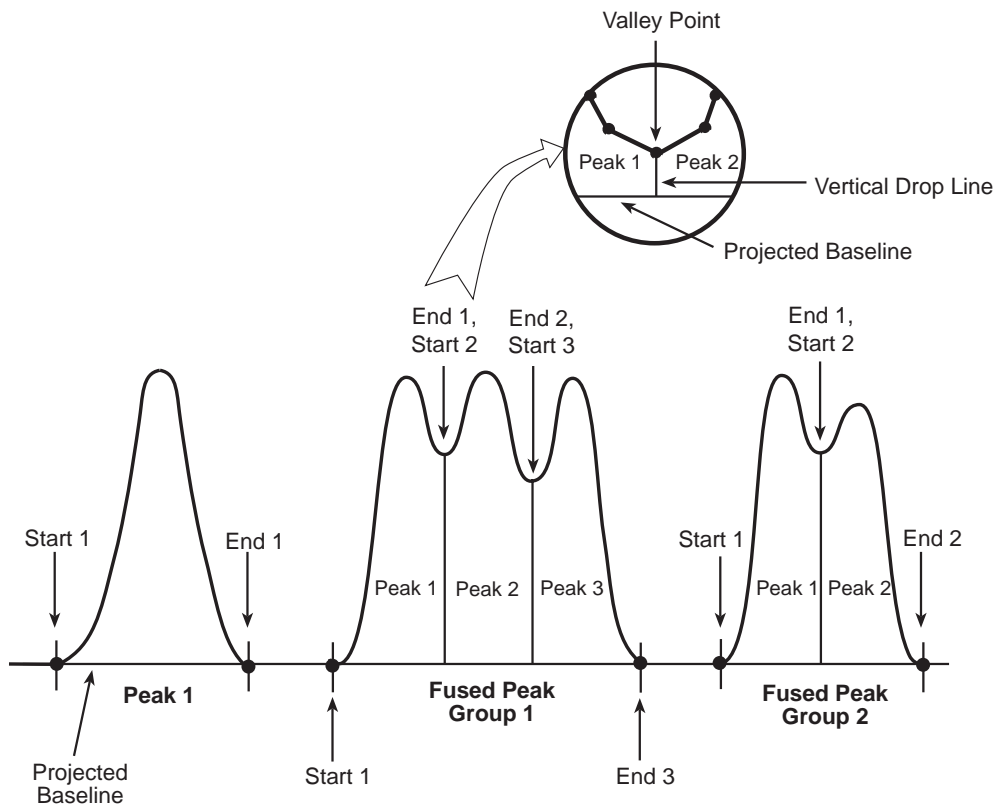


Figure 3-7 Determination of Resolved and Fused Peaks

## 3.2.2 Constructing the Baseline

### Initial Baseline Construction

Once resolved peaks and fused peaks are identified within the chromatogram, the integration algorithm draws a baseline from the start to the end of each peak or fused peak group ([Figure 3-8](#)).

The fields Start Time and End Time display the start and end times of the peak calculated during peak integration. The fields Baseline Start and Baseline End display the start and end times of the baseline used to integrate a peak. The Baseline Start and Baseline End values are:

- The same as the Start Time and End Time values if integration is for a baseline-resolved peak (baseline-to-baseline).
- Different from the Start Time and End Time values if integration is for a fused peak (peaks not baseline-resolved).

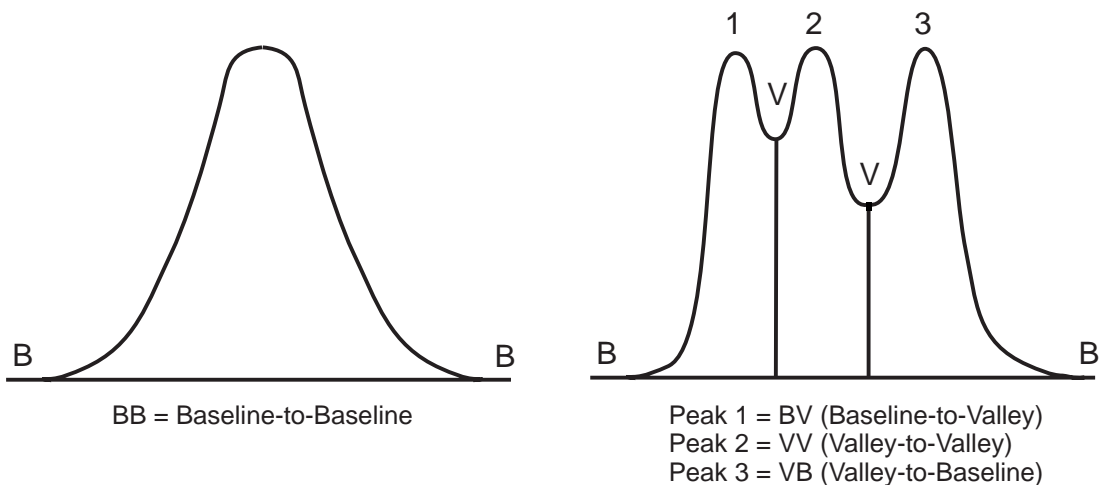


Figure 3-8 Baseline Construction

When you use the *default* integration setting, each identified peak is given a two-character label that indicates whether the peak starts or ends at a point on the baseline (B) or in a valley (V) above the baseline ([Table 3-1](#)). A peak can have four types of baseline construction. The label appears in the Int Type column of the Peaks tab of the Results and Main windows of Review.

Table 3-1 Default Integration Peak Labels

Peak Start and End Point	Label
Baseline-to-Baseline	BB
Baseline-to-Valley	BV
Valley-to-Baseline	VB
Valley-to-Valley	VV

**Note:** When you use the *Exponential Skim* or *Tangential Skim* integration events, additional types of baseline construction can appear (see [Section 3.4.1, Integration Peak Labels](#), and [Section 3.4.8, Skim Events](#)).

Capitalization of the label indicates the following:

- **Capital letters** – The integration was performed automatically by the software.
- **Lowercase letters** – The integration was performed manually.

For instance, a baseline label of Bb indicates that while the peak start and peak end are both baseline-resolved, the peak start was automatically integrated by the software and the peak end was manually adjusted.

## Baseline Adjustment

If the projected baseline intersects the signal in the chromatogram, the software adjusts the baseline to the lowest point within the fused peak group ([Figure 3-9](#)), separating the peak group into individual and/or fused peaks, as appropriate. The software then rechecks the new baselines to make sure they do not intersect the chromatographic signal except at peak start or end points, and readjusts the baseline as necessary.

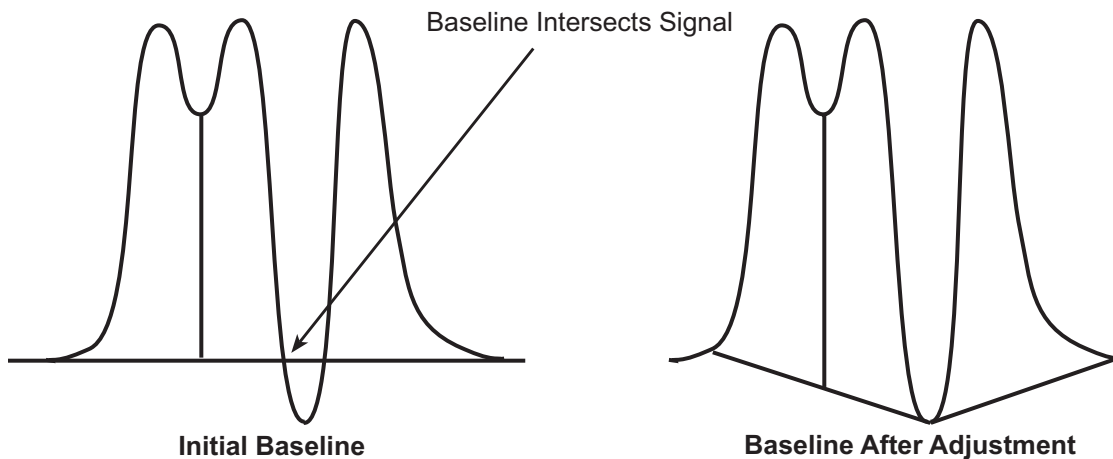


Figure 3-9 Baseline Adjustment

### 3.2.3 Calculating Peak Retention Time, Height, and Area

Once actual baselines are constructed, the integration algorithm:

1. Calculates the retention time, height, and area for each peak.
2. Compares each integrated peak to the Minimum Area and Minimum Height rejection criteria you specify.

#### Retention Time and Height

To determine retention time and height, the software:

1. Locates the retention time of the data point in the peak that is farthest from the constructed baseline.
2. Fits a quadratic curve to the five points at the top of the peak (the highest data point and the two data points on either side of this point).
3. Sets the peak apex point to the inflection point of the fitted curve. The X value of the peak apex is the retention time of the peak.
4. Calculates the peak height as the distance (in  $\mu\text{V}$ ) from the constructed baseline to the Y value of the calculated peak apex.

**Note:** If the software fails to fit a curve to the top of the peak, it uses the apex point (the data point furthest away from the baseline) to calculate retention time and height as in Millennium software version 2.15 and earlier versions. The software adds a processing code (I05, I06, I07, or I08) to the Codes column in the Peaks tab in Review to explain why the curve fitting to the top of the peak failed.

Figure 3-10 illustrates the peak retention time and peak height calculation.

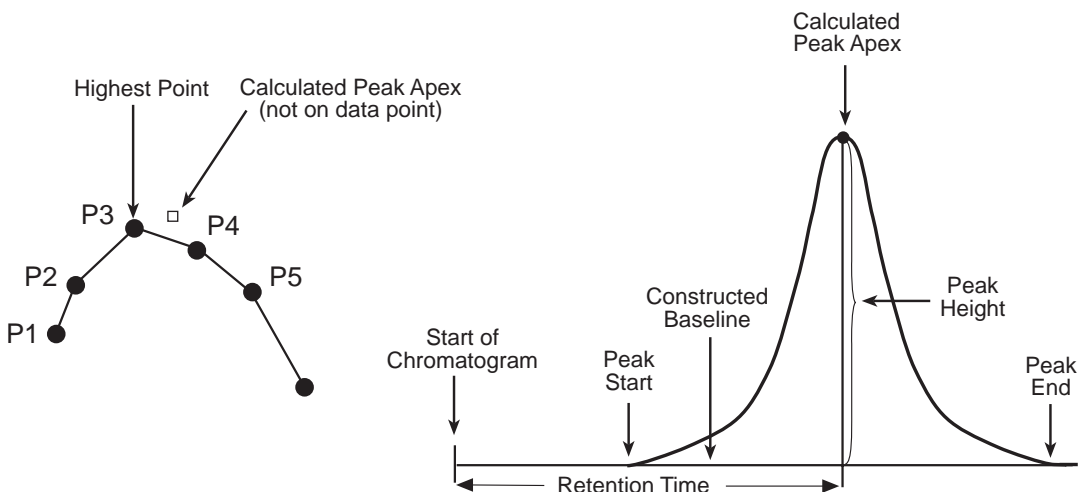


Figure 3-10 Peak Retention Time and Peak Height Calculation

**Note:** You can disable fitting a quadratic curve to the top of the peak by selecting **Configuration Manager > View > System Policies** and implementing a system policy named **Use v2.XX Style Retention Time Calculations** in the Other Policies tab (see [Appendix B, Data Processing System Policies](#)). When results are integrated with this system policy active, processing code I09 is added to the Codes field in the result. This field is visible in the chromatogram Result table in the Result window of Review or in the Project window Results tab.

## Area

The algorithm calculates the total peak area by adding the areas for each raw data point interval between peak start and peak end (Figure 3-11). The region below the constructed baseline ( $A_b$ ) is subtracted from the total area of the peak ( $A_t$ ). This yields the peak area above the constructed baseline ( $A_p$ ).



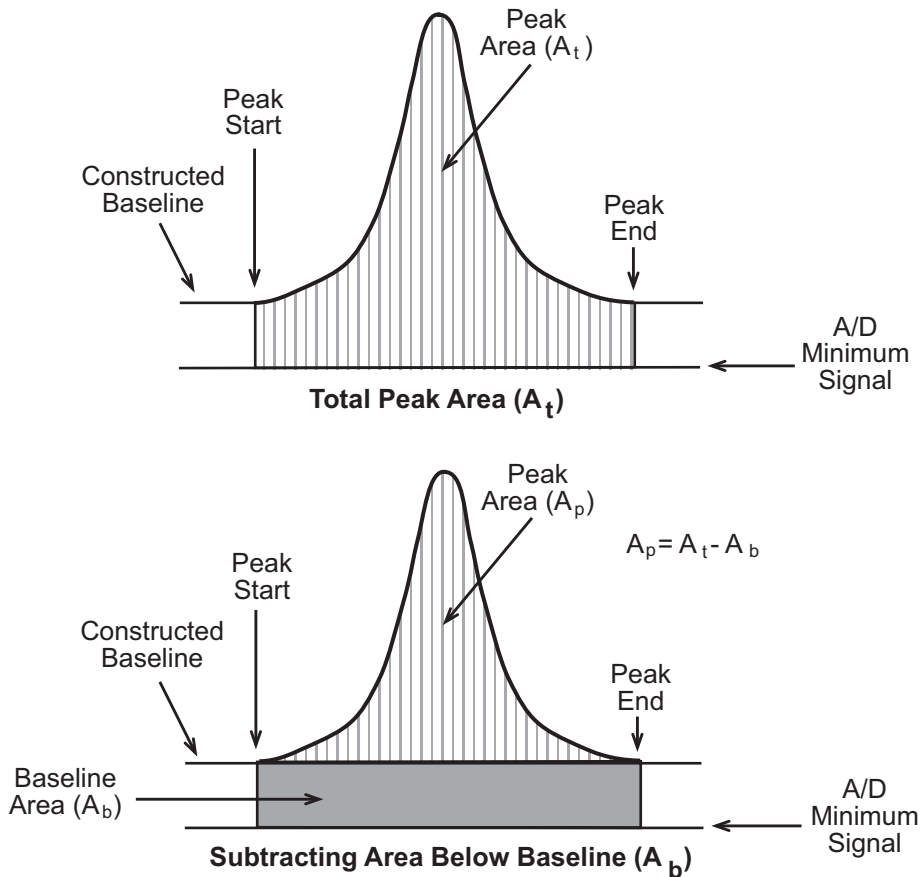


Figure 3-11 Peak Area Calculation

### 3.2.4 Peak Rejection Criteria

When the software integrates a peak, the integration algorithm compares the peak with the integration rejection criteria you specify in the Integration tab of the Processing Method window in Review, by using the Minimum Area and Minimum Height buttons in the Review Main window or using the Processing Method wizard. Based on this comparison, the algorithm accepts or rejects the peak. Integration rejection criteria can include:

- Minimum area
- Minimum height
- Minimum of five points across a peak

#### Minimum Area

The Minimum Area criterion determines the minimum area (in  $\mu\text{V} \cdot \text{sec}$ ) required for an integrated peak to be included in the peak list. If the area of the integrated peak falls below

the set value, the peak is removed from the peak list. If the area is equal to or greater than the set value, the peak is accepted.

## Minimum Height

The Minimum Height criterion determines the minimum height (in  $\mu\text{V}$ ) required for an integrated peak to be included in the peak list. If the height of the integrated peak falls below the set value, the peak is removed from the peak list. If the absolute value of the height is greater than or equal to the set value, the peak is accepted.

**Note:** *Minimum Area and Minimum Height are useful for removing small integrated peaks from the result. A high value may cause integrated peaks to be rejected as noise; conversely, a low value may cause baseline noise to be integrated as peaks.*

## 5-Point Peak Rejection

The 5-Point Peak Rejection criterion instructs the software to remove from the peak list any peak that contains fewer than five points.

**Note:** *The 5-Point Peak Rejection criterion is built into the software and occurs automatically. It is not a parameter found in the processing method.*

# 3.3 Peak Detection Events

---

The software supports the following time-based detection events to further refine peak detection:

- Allow Negative Peaks
- Set Liftoff
- Set Touchdown
- Set Peak Width

**Note:** *The Set Liftoff, Set Touchdown, and Set Peak Width events take effect only in the baseline regions outside detected single peaks or fused peak groups. If the event starts within an isolated peak or fused peak group, the event takes effect at the end of the isolated peak or fused peak group.*

### 3.3.1 Allow Negative Peaks Event

The Allow Negative Peaks event enables the detection algorithm to consider a slope that proceeds in a negative direction as the potential start of a peak. When this event is enabled, the software treats a negative or positive average slope equal to or greater than the liftoff threshold as a valid peak start.

If you specify the Allow Negative Peaks event within a detected peak before the peak apex, the event automatically takes effect for that peak. If you turn off the event within a negative peak before peak apex, the negative peak is not integrated.

Once a negative peak start is identified (and assuming the signal passes the peak liftoff test), the software operates in the same manner as it does for positive peaks. If you enable the Allow Negative Peak integration event and a negative peak is integrated, its peak height is reported as a negative number. The absolute value of the height is used in all other calculations (including area).

During integration of negative peaks, the algorithm constructs a baseline based on the detected start and end points. The peak retention time and height are calculated in the same manner as for positive peaks, starting with the farthest point from the baseline (the most negative point).

**Note:** During an Allow Negative Peaks event, if the baseline dips before a positive peak, the positive peak (and other peaks after it) could be incorrectly integrated as negative peaks. This can be avoided either by moving the start of the Allow Negative Peaks event to a point after the dip in baseline if possible, or by adding a force baseline event to set the proper baselines ([Section 3.4, Peak Integration Events](#)). Six force baseline events can be used: Force Baseline by Time or by Peak, Forward Horizontal by Time or by Peak, and Reverse Horizontal by Time or by Peak.

[Figure 3-12](#) illustrates the Allow Negative Peaks event.

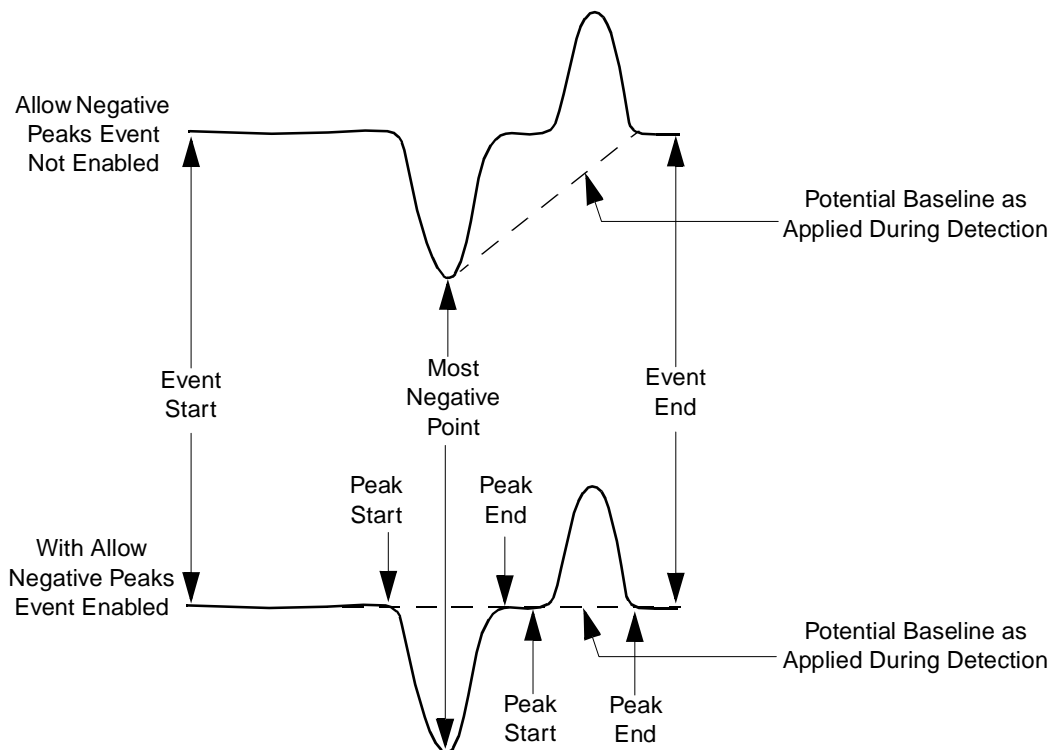


Figure 3-12 Allow Negative Peaks Event

### 3.3.2 Set Lutoff Event

The Set Lutoff event redefines the threshold value to which the signal slope is compared in order to determine peak start. It has no effect on the threshold used to detect peak end.

**Note:** The Set Lutoff Threshold event overrides the global Threshold detection parameter for the remainder of the run (or until another Set Lutoff event is encountered). To return to the global value, you must set another lutoff event specifying that value.

The valid range for the Set Lutoff event value is 0.0 or greater.

The Set Lutoff and Set Touchdown threshold events can be used independently of one another.

[Figure 3-13](#) illustrates the Set Lutoff event.

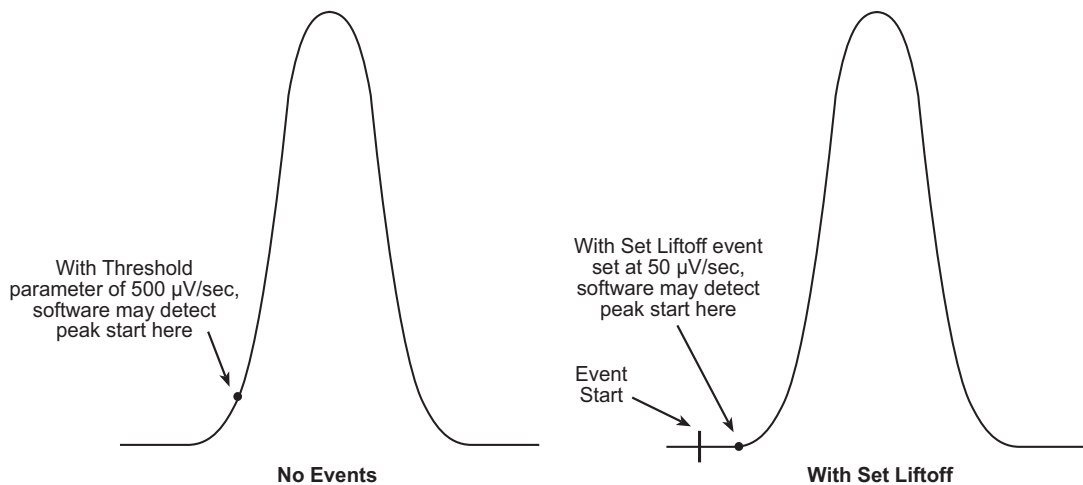


Figure 3-13 Set Lutoff Event

### 3.3.3 Set Touchdown Event

The Set Touchdown event redefines the threshold value to which the signal slope is compared to determine peak end. It has no effect on the threshold used to detect peak start.

**Note:** The Set Touchdown event overrides the global Threshold detection parameter for the remainder of the run (or until another Set Touchdown event is encountered). To return to the global value, you must set another touchdown event specifying that value.

The valid range for the Set Touchdown event value is 0.0 or greater.

The Set Lutoff and Set Touchdown threshold events can be used independently of one another.

[Figure 3-14](#) illustrates the Set Touchdown event.

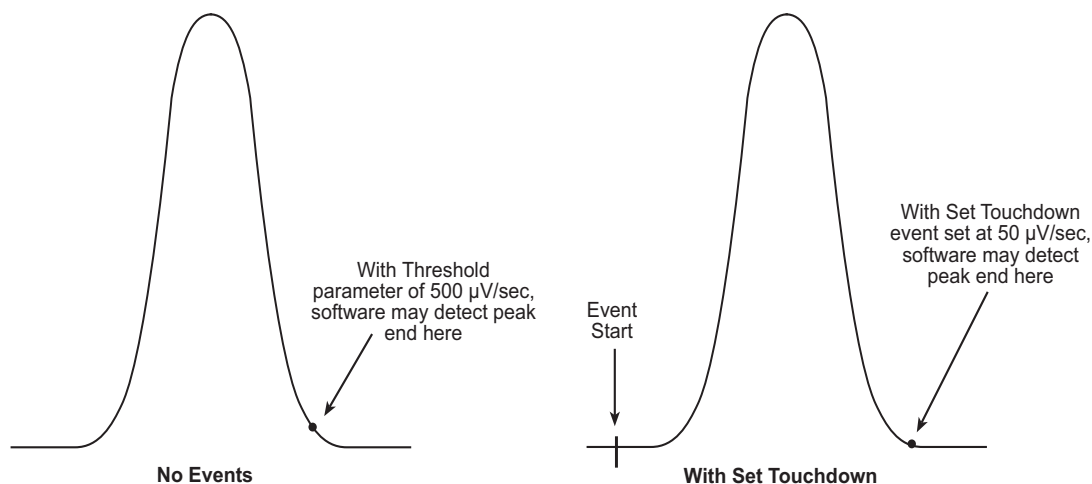


Figure 3-14 Set Touchdown Event

### 3.3.4 Set Peak Width Event

The Set Peak Width event changes the peak width that is used to calculate the number of points to be bunched together during peak detection.

**Note:** *The Set Peak Width event overrides the global Peak Width detection parameter for the remainder of the run (or until another Set Peak Width event is encountered). To return to the global value, you must set another Peak Width event specifying that value.*

The valid range for the Set Peak Width event value is 0.01 to 9999.99.

## 3.4 Peak Integration Events

The software supports the following time-based integration events to further refine peak integration:

- Force Baseline by Time
- Force Baseline by Peak
- Forward Horizontal by Time
- Forward Horizontal by Peak
- Reverse Horizontal by Time
- Reverse Horizontal by Peak
- Valley-to-Valley
- Force Drop Line

- Force Peak
- Exponential Skim
- Tangential Skim
- Set Minimum Height
- Set Minimum Area

### 3.4.1 Integration Peak Labels

When you use the *default* integration setting, each identified peak in a chromatogram is given a two-character label that indicates whether the peak starts or ends at a point on the baseline (B) or in a valley (V) above the baseline ([Table 3-2](#)). The label appears in the Int Type column of the Peaks tab of the Results and Main windows of Review.

Table 3-2 Integration Peak Labels on a Chromatogram

Peak Start and End Point	Label
Baseline-to-Baseline	BB
Baseline-to-Valley	BV
Exponential-to-Exponential	EE
Exponential-to-Valley	EV
Tangential-to-Tangential	TT
Tangential-to-Valley	TV
Valley-to-Baseline	VB
Valley-to-Exponential	VE
Valley-to-Tangential	VT
Valley-to-Valley	VV

Capitalization of the label indicates the following:

- **Capital letters** – The integration was performed automatically by the software.
- **Lowercase letters** – The integration was performed manually.

In addition to the default integration peak labels, other labels may appear in the Int Type column of the Peaks table in Review:

- When a Tangential (T) Skim event is active, two-character peak labels appear for any peaks containing a tangential skim.
- When an Exponential (E) Skim event is active, two-character peak labels appear for any peaks containing an exponential skim.

## 3.4.2 Force Baseline Events

The force baseline events instruct the integration algorithm to project a baseline based on one of the following:

- Start and end event times (Force Baseline by Time with no value)
- Average chromatographic signal at the start and end times (Force Baseline by Time with a value)
- Peak start and end points (Force Baseline by Peak)

A baseline is drawn from the specified event start time to event end time, or peak start point to peak end point, and does not have to be horizontal. If you do not enter an end time, the software uses the run time of the chromatogram as the event end time. The effects of the force baseline events are as follows:

- **Force Baseline by Time with no value entered** – A baseline is drawn from the signal value at event start to the signal value at event end.
- **Force Baseline by Time with a value entered** (in the Value column of the Integration Event table) – The software determines a baseline calculated by averaging the chromatographic signal in the time range of event start time +/- event value, in minutes. Another average chromatographic baseline value is calculated in the time range of event stop time +/- event value, in minutes. The peak baseline is then drawn from the average signal value at event start to the average signal value at event end.
- **Force Baseline by Peak** – The baseline extends from the *first* detected peak start point to the *last* peak end point within the specified time interval.

Peak area and height are adjusted according to the new baselines drawn for the force baseline event.

**Note:** *If a forced baseline intersects the signal between data points, the peak start and end points are adjusted to the data points closest to the intersection.*

[Figure 3-15](#) illustrates the Force Baseline by Time (with no baseline averaging) and Force Baseline by Peak timed events.

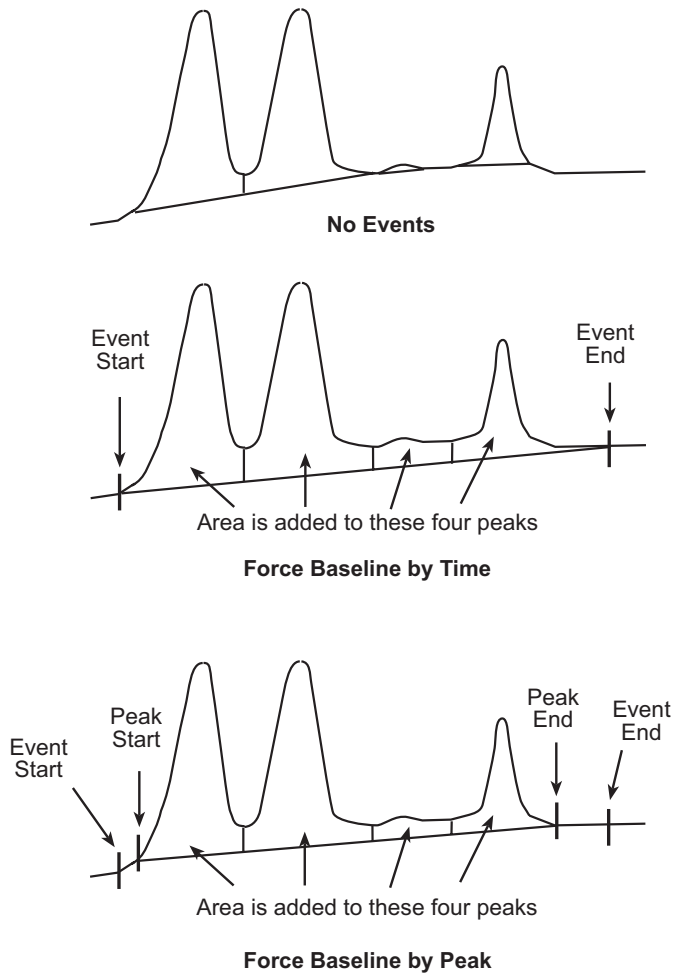


Figure 3-15 Force Baseline by Time and Force Baseline by Peak Events



[Figure 3-16](#) illustrates the Force Baseline by Time event using baseline averaging.

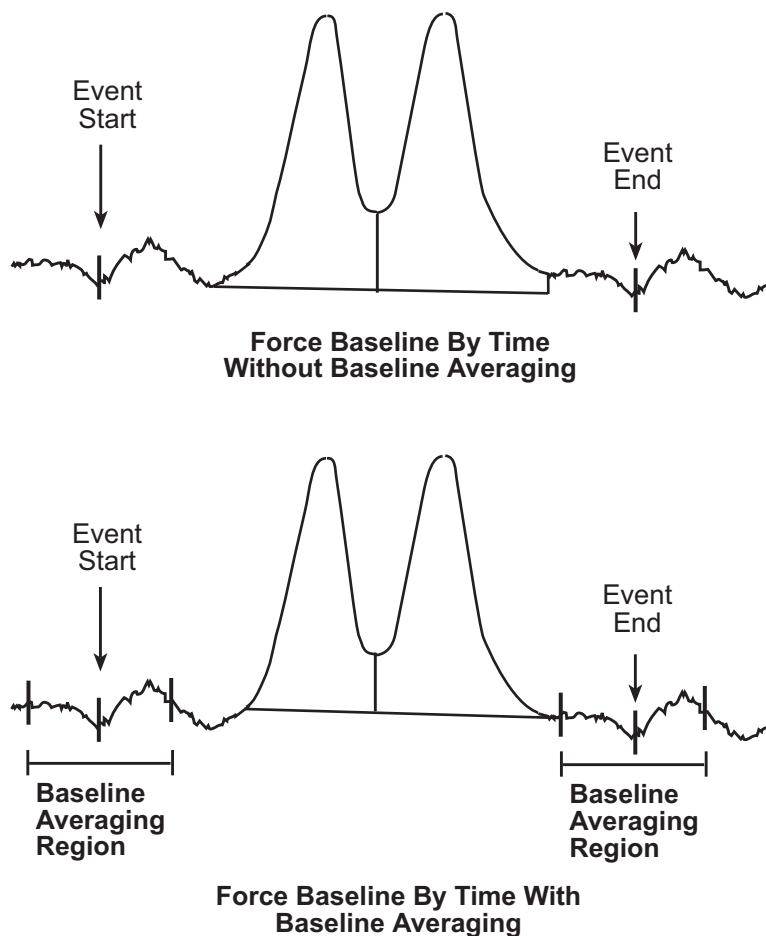


Figure 3-16 Force Baseline by Time Event with Baseline Averaging

If the chromatogram has negative peaks when a force baseline event is active, and if the Allow Negative Peaks event is:

- **Off** – The baseline extends and the negative peak is *not* integrated.
- **On** – The baseline extends across the negative peak. The negative peak is integrated and added to the peak list.

[Figure 3-17](#) illustrates the simultaneous use of Force Baseline by Time and Allow Negative Peaks events.

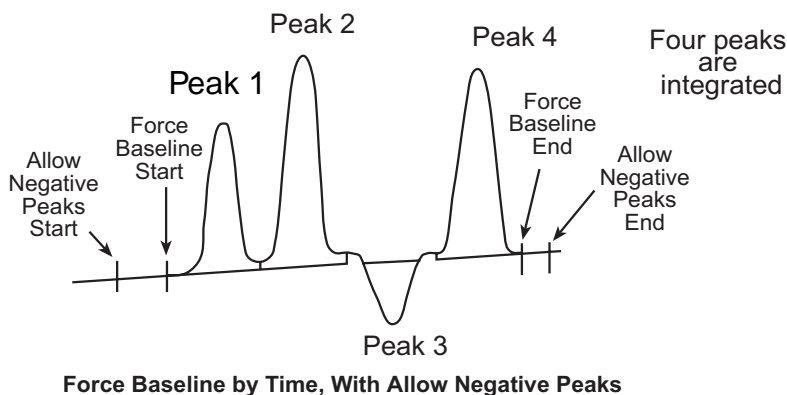
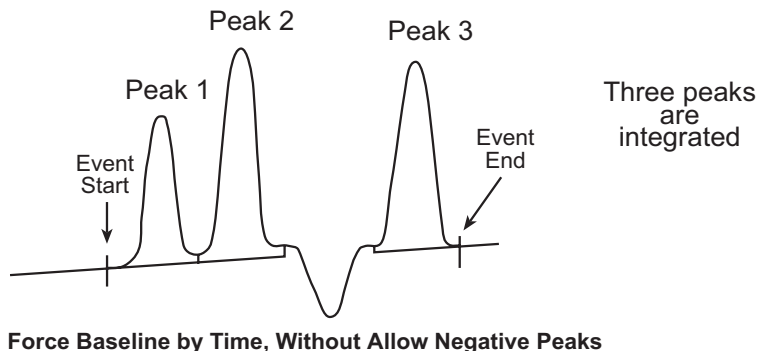


Figure 3-17 Force Baseline and Allow Negative Peaks Events

### 3.4.3 Forward Horizontal Events

The forward horizontal events instruct the integration algorithm to project a baseline horizontally forward (to the right or increasing by time, with zero slope). The start point of the horizontal baseline is based on one of the following:

- Event start time (Forward Horizontal by Time with no value)
- Average chromatographic signal at the start time (Forward Horizontal by Time with a value)
- Peak start point (Forward Horizontal by Peak)

The software forces a horizontal baseline to the right, either from the specified event start time or peak start point. If you do not enter an end time, the software uses the run time of the chromatogram as the event end time.

The differences among the forward horizontal events are as follows:

- **Forward Horizontal by Time with no value entered** – The baseline extends horizontally from the signal value at event start and changes the baselines of all peaks within the event.
- **Forward Horizontal by Time with a value entered** (in the Value column of the Integration Event table) – The software calculates a baseline value by averaging the chromatographic signal in the time range of event start time +/- event value, in minutes. The baseline extends horizontally from this signal value at event start and changes the baselines of all peaks within the event.
- **Forward Horizontal by Peak** – The baseline extends horizontally from the signal value at the start point of the *first* detected peak after the event start and changes the baselines of all peaks within the event.

Peak area and height are adjusted according to the new baselines drawn for the forward horizontal baseline event. The integration algorithm may extend a drop line from the start and/or end point of peaks within the event to the baseline.

**Note:** *If the baseline intersects the signal between data points, the peak start and end points are adjusted to the data point closest to the intersection.*

[Figure 3-18](#) illustrates the Forward Horizontal by Time (with no baseline averaging) and Forward Horizontal by Peak events.

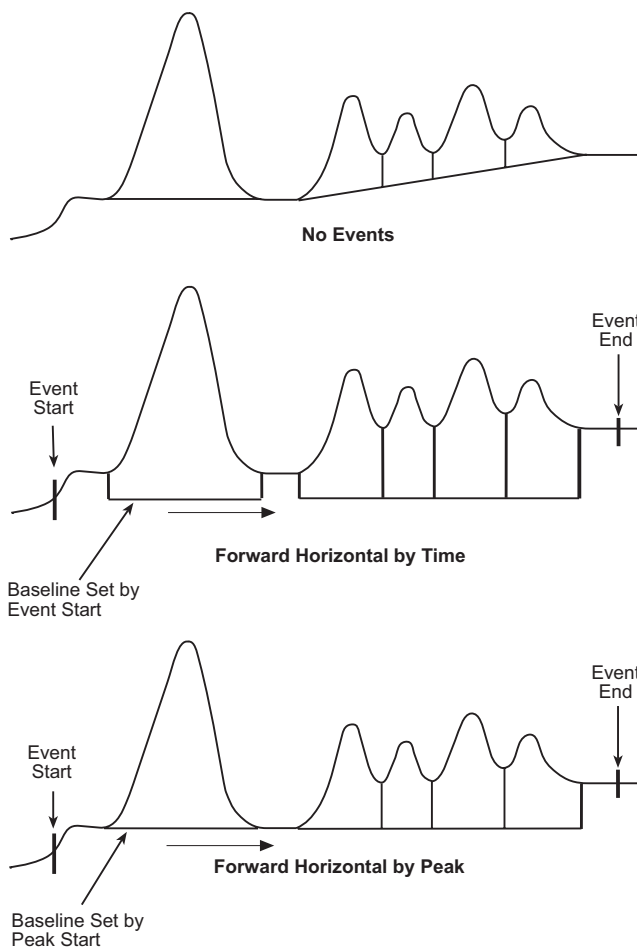


Figure 3-18 Forward Horizontal by Time and Forward Horizontal by Peak Events

[Figure 3-19](#) illustrates the Forward Horizontal by Time event using baseline averaging.

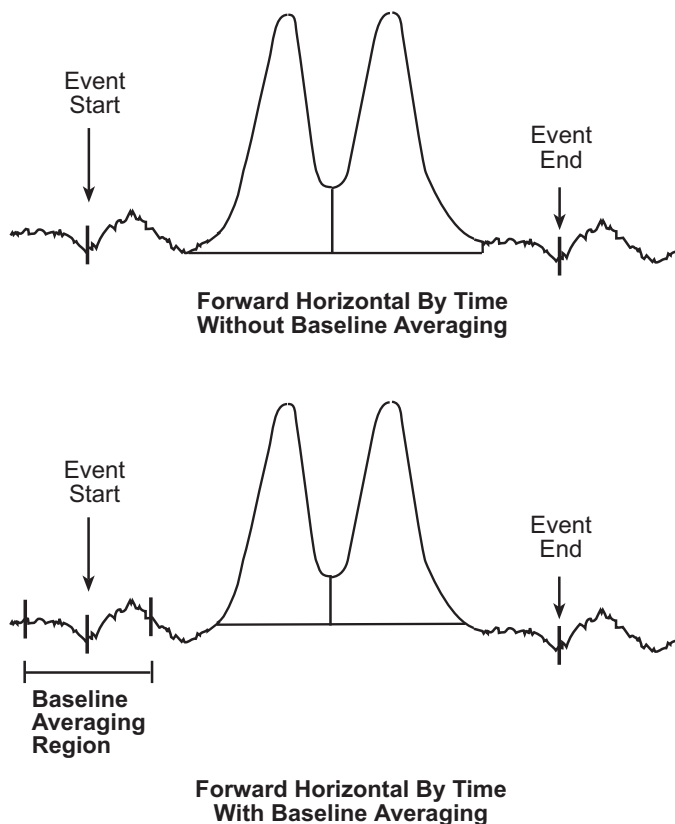


Figure 3-19 Forward Horizontal by Time Event with Baseline Averaging

If the chromatogram has negative peaks when a forward horizontal event is active and if the Allow Negative Peaks event is:

- **Off** – The baseline extends horizontally, and the negative peak is *not* integrated.
- **On** – The baseline extends horizontally across the negative peak, and the negative peak is integrated and added to the peak list.

[Figure 3-20](#) illustrates the simultaneous use of Forward Horizontal by Time and Allow Negative Peaks events.

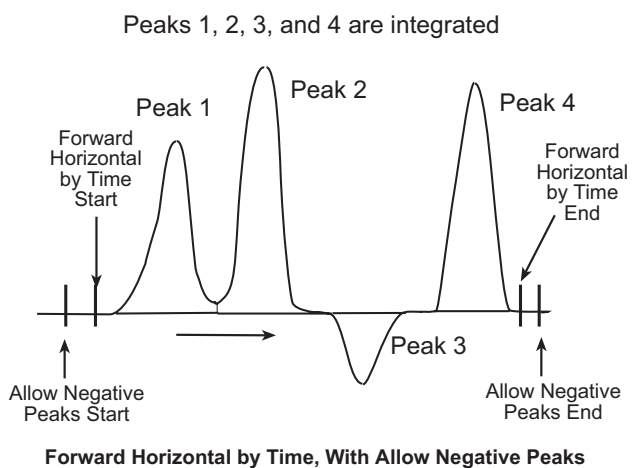
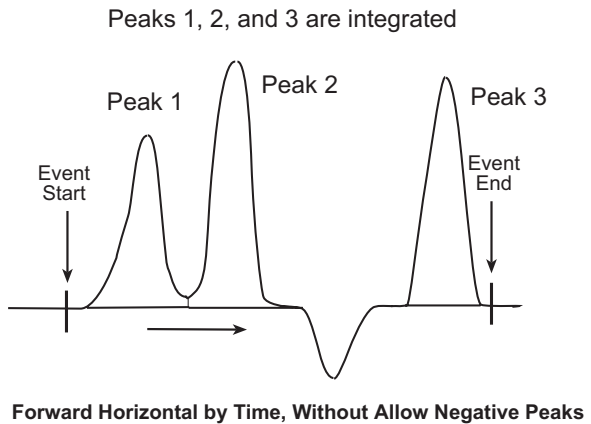


Figure 3-20 Forward Horizontal by Time and Allow Negative Peaks Events

### 3.4.4 Reverse Horizontal Events

The reverse horizontal events instruct the integration algorithm to project a baseline horizontally backward (to the left or decreasing by time, with zero slope). The software forces a horizontal baseline to the left, either from the specified event end time or peak end point. If you do not enter an end time, the software uses the run time of the chromatogram as the event end time.

The end point of the horizontal baseline is based on one of the following:

- **Reverse Horizontal by Time with no value entered** – Based on event end time. The baseline extends horizontally from the signal value at event end and changes the baselines of all peaks within the event.
- **Reverse Horizontal by Time with a value entered** (in the Value column of the Integration event table) – Based on average chromatographic signal at the end time. The software calculates a baseline value by averaging the chromatographic signal in the time range of event end time +/- event value, in minutes. The baseline extends horizontally from this signal value at event end and changes the baselines of all peaks within the event.
- **Reverse Horizontal by Peak** – Based on peak end and start points. The baseline extends horizontally from the signal value at the end point of the *last* detected peak after the event start and changes the baselines of all peaks within the event.

Peak area and height are adjusted according to the new baselines drawn for the reverse horizontal baseline event. The integration algorithm may extend a drop line from the start and/or end point of peaks within the event to the baseline.

**Note:** *If the baseline intersects the signal between data points, the peak start and end points are adjusted to the data point closest to the intersection.*

[Figure 3-21](#) illustrates the Reverse Horizontal by Time (with no baseline averaging) and Reverse Horizontal by Peak events.

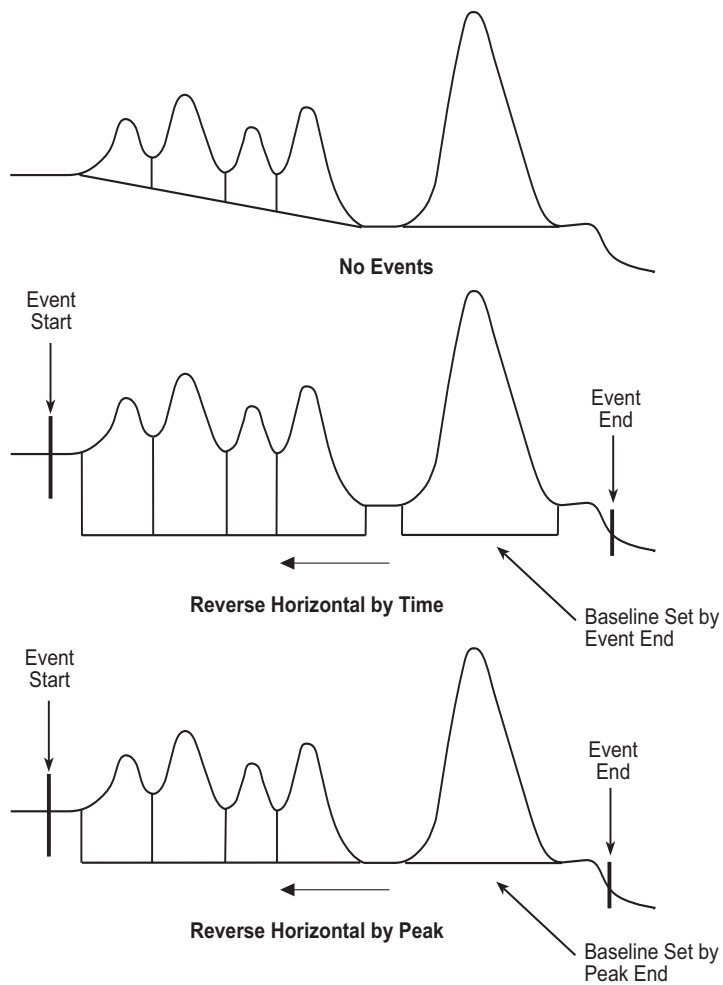
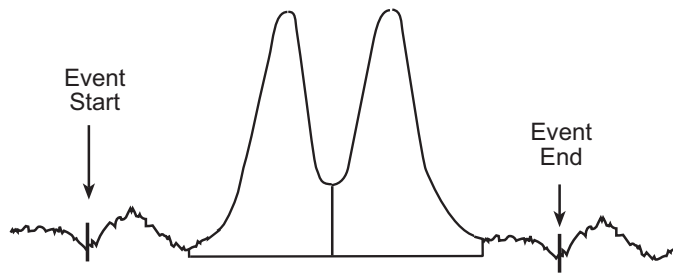


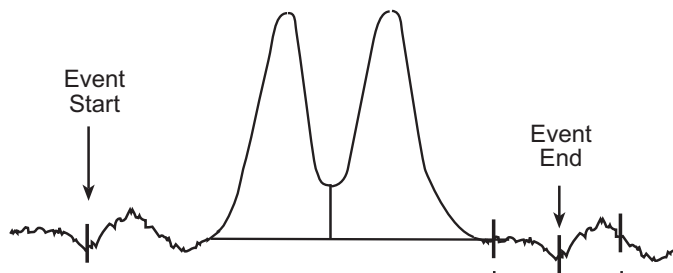
Figure 3-21 Reverse Horizontal by Time and Reverse Horizontal by Peak Events

[Figure 3-22](#) illustrates the Reverse Horizontal by Time event using baseline averaging.





**Reverse Horizontal By Time  
Without Baseline Averaging**



**Reverse Horizontal By Time  
With Baseline Averaging**

Figure 3-22 Reverse Horizontal by Time Event with Baseline Averaging

If the chromatogram has negative peaks when a reverse horizontal event is active, and if the Allow Negative Peaks event is:

- **Off** – The baseline extends horizontally and the negative peak is *not* integrated.
- **On** – The baseline extends horizontally across the negative peak. The negative peak is integrated and added to the peak list.

[Figure 3-23](#) illustrates the simultaneous use of Reverse Horizontal by Time and Allow Negative Peaks events.

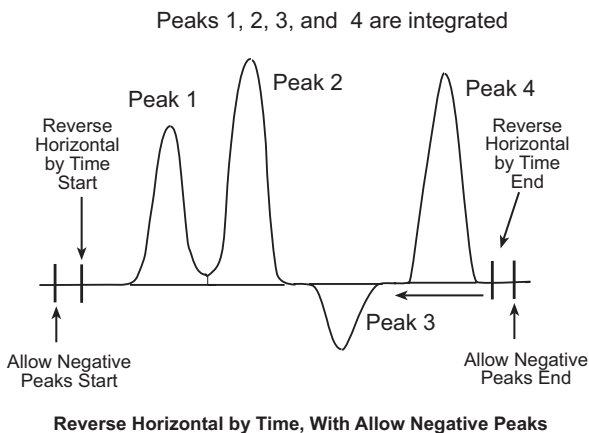
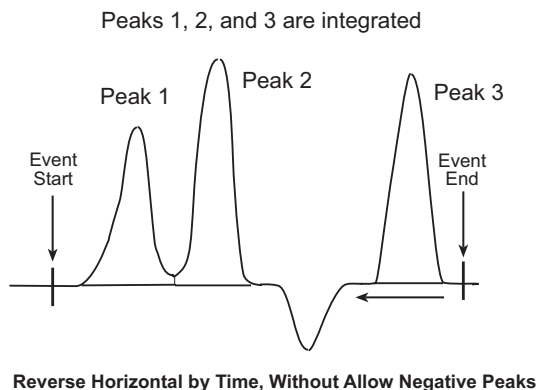


Figure 3-23 Reverse Horizontal by Time and Allow Negative Peaks Events

### 3.4.5 Valley-to-Valley Event

The Valley-to-Valley event sets the baseline to each valley point in a fused peak group. Without this event, a common baseline is drawn for all fused peaks, with each peak separated by a drop line. When enabled, the Valley-to-Valley event reassigns the baseline at each peak start and end point. All peaks become baseline-resolved and are labeled as BB in the Peaks tab of the Results and Main windows of Review.

Valley-to-Valley is active for the first peak found after the start time. Event deactivation occurs following the last peak (within the valley-to-valley start and end times).

**Note:** If the new baseline intersects the signal between data points, the peak start and end points are adjusted to the data point closest to the intersection.

[Figure 3-24](#) illustrates the Valley-to-Valley timed event.

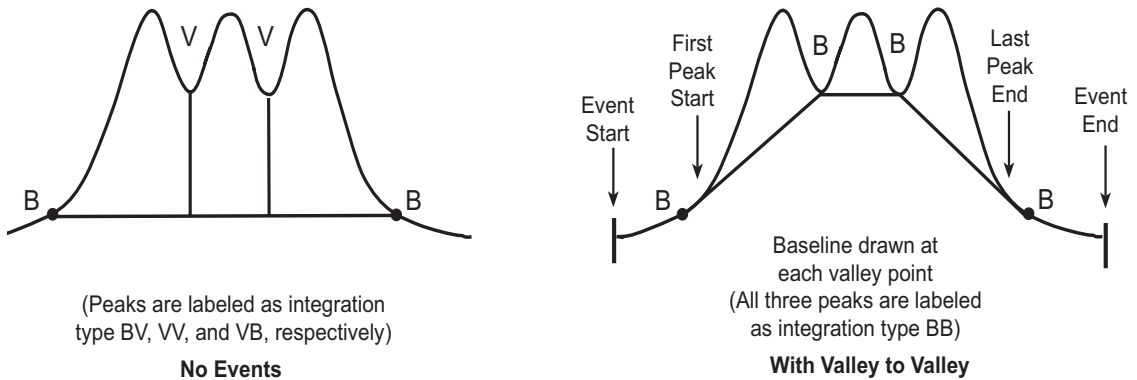


Figure 3-24 Valley-to-Valley Event

### 3.4.6 Force Drop Line Event

The Force Drop Line event controls peak integration based on a start and end time window that you specify. When you enable the Force Drop Line event, the software places a drop line at the time(s) specified to either add a drop line to a previously integrated peak or two drop lines to a single peak or a fused peak cluster.

The Force Drop Line event can be used:

- With only a start time, to force a drop line within a detected peak (at the specified start time). If the specified start time is not within a detected peak, it is ignored.
- With both a start and an end time, to force two drop lines within a detected single peak or fused peak cluster. All areas between the start and end times are reported as a single peak. The peak areas before the start drop line and after the end drop line become separate peaks. When the event start and end times are outside a fused peak, all peaks in the fused group are added together into a single peak.

[Figure 3-25](#) illustrates the Force Drop Line timed event using only a start time and the existing baseline. [Figure 3-26](#) illustrates the Force Drop Line timed event for a single peak. [Figure 3-27](#) illustrates a fused peak group.

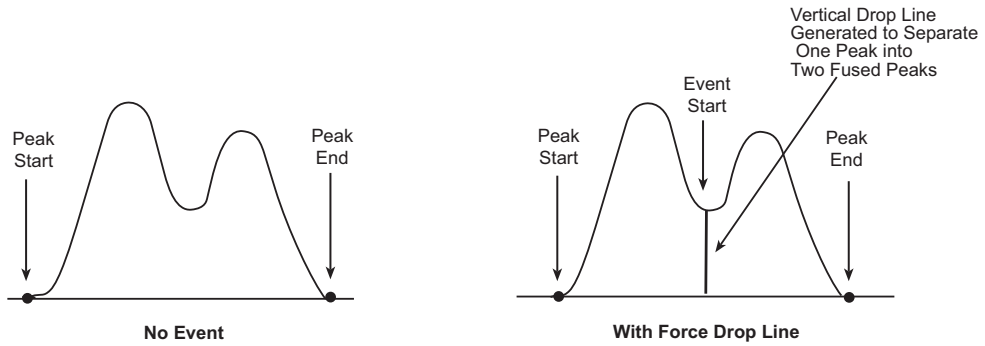


Figure 3-25 Force Drop Line Event (with Only an Event Start Time)

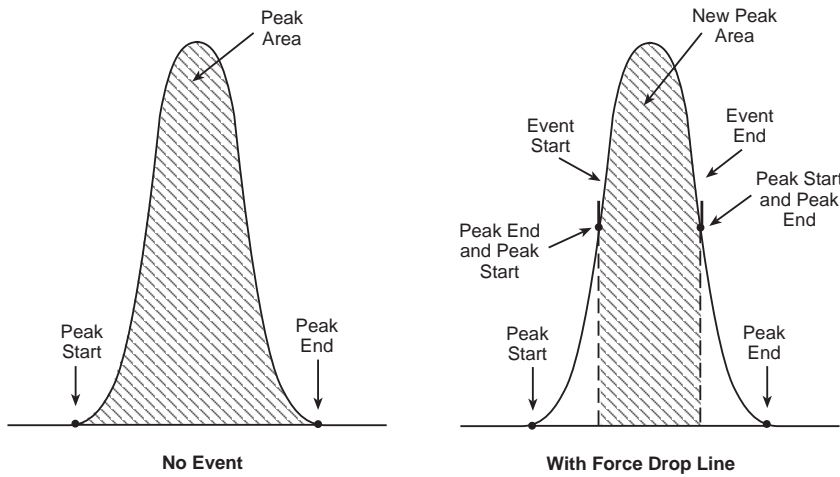


Figure 3-26 Force Drop Line Event (Single Peak)

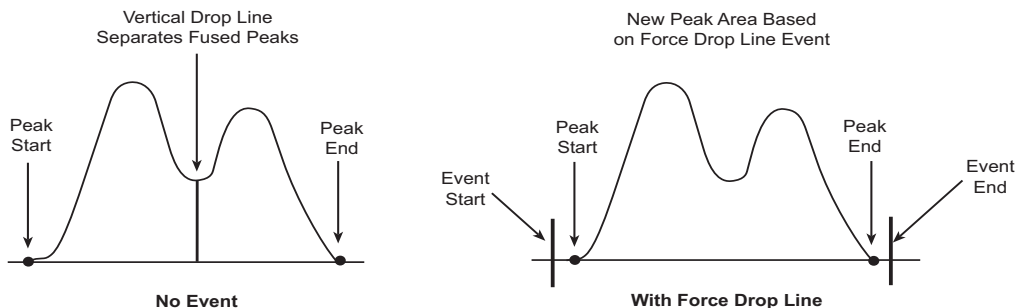


Figure 3-27 Force Drop Line Event (Fused Peaks)

### 3.4.7 Force Peak Event

The Force Peak event controls peak integration based on the start and end time window that you specify. When you enable Force Peak, the software treats the time window as the new peak start and end points, and draws the new baseline between the two points. You can use Force Peak for a single peak or within a fused peak cluster. The Force Peak event always forces a peak, even when there would not be a peak integrated without the event (this includes forcing a peak during an Inhibit Integration event). Any peaks that overlap the peak created by the Force Peak event are deleted.

**Note:** Be careful where you specify the start and end times of the Force Peak event. If you set these times incorrectly, the baseline could intersect the signal in the chromatogram.

Figure 3-28 illustrates the Force Peak timed event for a baseline-resolved peak.

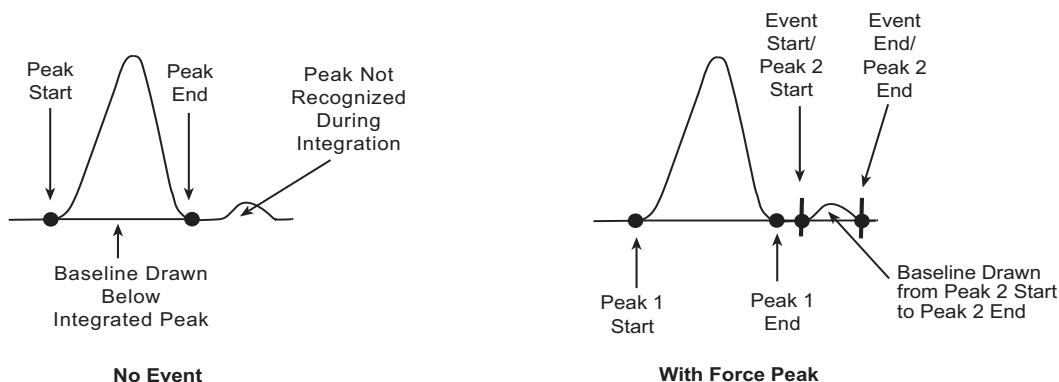


Figure 3-28 Force Peak Event

Figure 3-29 illustrates the Force Peak timed event for multiple resolved peaks.

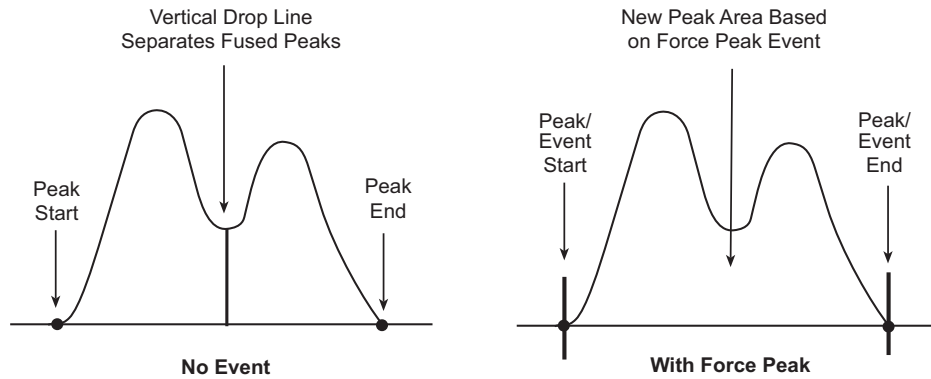


Figure 3-29 Force Peak Event (Multiple Unresolved Peaks)

### 3.4.8 Skim Events

You use skim events to resolve rider or shoulder peaks from a parent peak. The skim is based on the start and stop time window and the height ratio that you specify. The software draws a line (for a tangential skim) or a curve (for an exponential skim) to skim the rider peak(s) from the parent peak. The skims are further categorized as classic, nonclassic, frontal, or rear.

#### Classic Versus Nonclassic Skim

Each skim type is first categorized as classic or nonclassic depending on the height of the parent peak.

- **Classic** – The parent peak is the tallest peak in the fused peak group *and* the skimmed peaks pass the height ratio test ([Figure 3-30](#)). This event can start and end outside the fused peak group.
- **Nonclassic** – The parent peak is not the tallest peak in the fused peak group *and* the skimmed peaks pass the height ratio test. The event must start (for a rear skim) or end (for a front skim) within the parent peak.

#### Frontal Versus Rear Skim

The skim event value determines whether a skim is frontal or rear.

- **Frontal** – When the skim event value is less than 0, the software attempts to fit a frontal skim on the fused group.
- **Rear** – When the skim event value is 0 or greater, the software attempts to fit a rear skim on the fused group.

## Tangential Skim Event

The Tangential Skim event draws a line under the rider or shoulder peaks to resolve them from the front or rear of a parent peak within the start and end times of the event.

When you enable Tangential Skim, the integration algorithm determines if peaks should be considered as rider peaks rather than fused peaks. The algorithm checks if the height ratio of parent peak to rider peak is equal to or greater than the height ratio value you entered for the event in the Value column of the Integration Event table.

The peak heights used to calculate the height ratio are the heights of the peaks from the peak apex to the valley point ([Figure 3-30](#)).

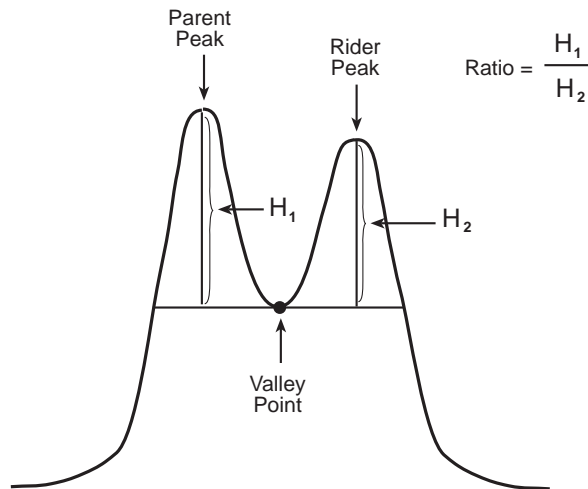


Figure 3-30 Height Ratio Test

If the rider and parent peaks:

- **Pass the height ratio test** – The software draws a line to tangentially skim the rider peaks from the parent peak.
- **Fail the height ratio test** – The software keeps the vertical line that separates the peaks at the valley point.

[Figure 3-31](#) illustrates the four types of Tangential Skim events.

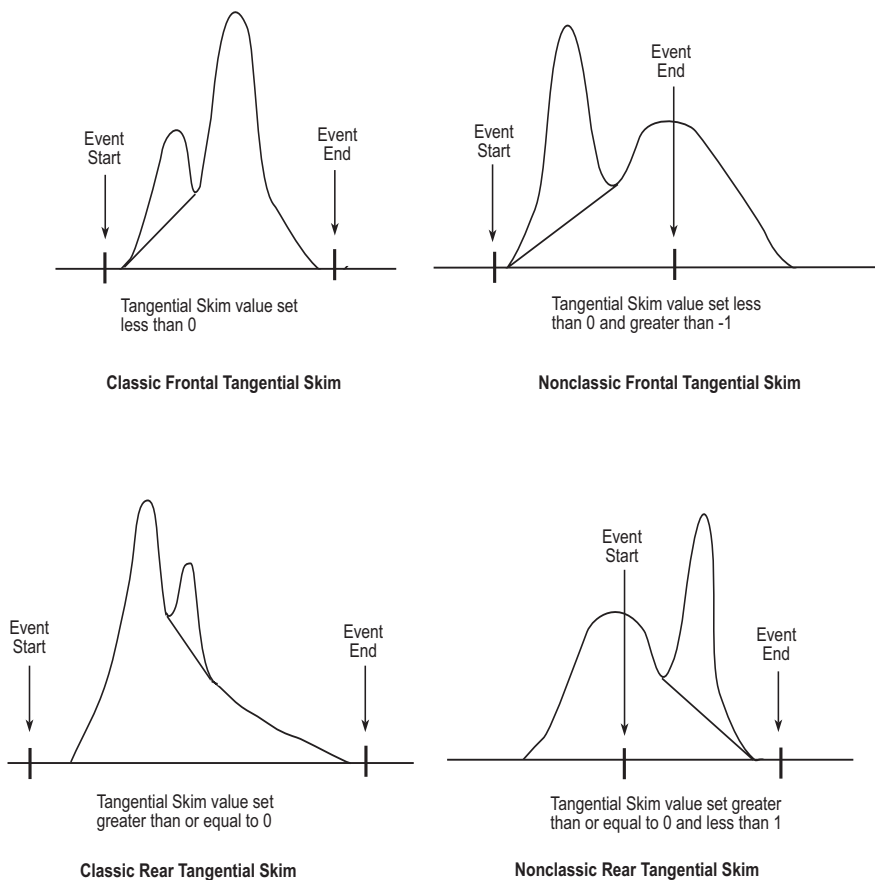


Figure 3-31 Tangential Skim Events

**Note:** When the specified tangential skim value is 0, the result is a rear skim without a height ratio check.

If the tangential skim intersects the signal, the skim is readjusted to the lowest signal point. The tangential skim continues from this new start point.

When the Tangential Skim event is active, the software displays the two-character peak labels in [Table 3-3](#) in the Int Type column of the Peaks table in Review for any peaks containing a tangential skim. These labels may appear in addition to the default integration peak labels (see [Section 3.2.2, Constructing the Baseline](#)).



Table 3-3 Peak Labels for Tangentially Skimmed Peaks

Peak Start and End Point	Label
Tangential-to-Valley	TV
Valley-to-Tangential	VT
Tangential-to-Tangential	TT

## Exponential Skim Event

The Exponential Skim event draws an exponential curve under the rider or shoulder peaks to resolve them from the front or rear of a parent peak within the start and end times of the event.

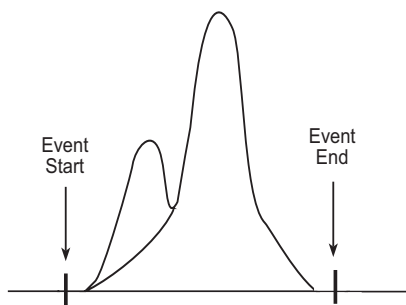
When you enable exponential skim, the integration algorithm determines if peaks should be considered as rider peaks rather than fused peaks. The algorithm checks if the height ratio of parent peak to rider peak is equal to or greater than the height ratio value you entered for the event. The peak heights used to calculate the height ratio are the heights of the peaks from the peak apex to the valley point ([Figure 3-30](#)).

**Note:** *The height ratio criteria are the same as for the Tangential Skim event.*

If the rider and parent peaks:

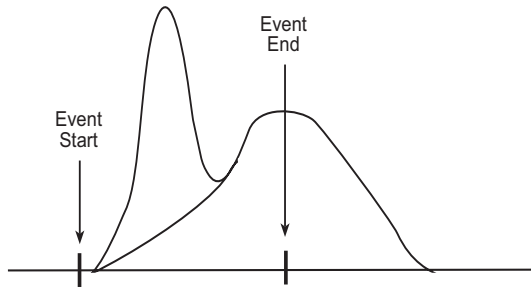
- **Pass the height ratio test** – The software attempts to fit an exponential curve to skim the rider peaks from the parent peak. If the exponential curve does not fit, the integration algorithm does not perform the exponential skim, but skims the peak(s) tangentially.
- **Fail the height ratio test** – The software keeps the vertical drop line that separates the peaks at the valley point.

[Figure 3-32](#) illustrates the Exponential Skim timed events.



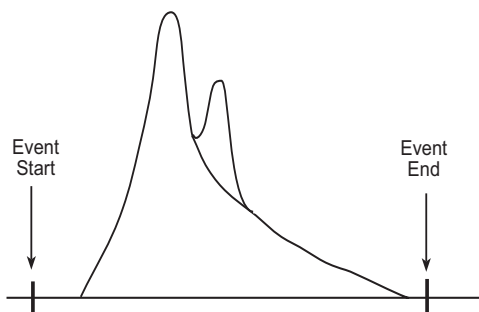
Exponential Skim value set less than 0

**Classic Frontal Exponential Skim**



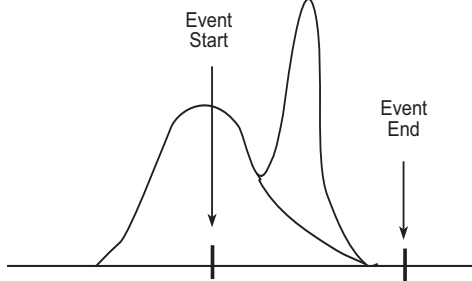
Exponential Skim value set less than 0 and greater than -1

**Nonclassic Frontal Exponential Skim**



Exponential Skim value set greater than or equal to 0

**Classic Rear Exponential Skim**



Exponential Skim value set greater than or equal to 0 and less than 1

**Nonclassic Rear Exponential Skim**

**Figure 3-32 Exponential Skim Events**

**Note:** When the specified exponential skim value is 0, the result is a rear skim without a height ratio check.

When the Exponential Skim event is active, the software displays the two-character peak labels in [Table 3-4](#) in the Int Type column of the Peaks table in Review for any peaks containing an exponential skim. These labels may appear in addition to the default integration peak labels (see [Section 3.2.2, Constructing the Baseline](#)).

Table 3-4 Peak Labels for Exponentially Skimmed Peaks

Peak Start and End Point	Peak Label
Exponential-to-Exponential	EE
Exponential-to-Valley	EV
Valley-to-Exponential	VE

### 3.4.9 Set Minimum Height Event

The Set Minimum Height event redefines the minimum height required to include an integrated peak in the peak list.

**Note:** The Set Minimum Height event overrides the global Minimum Height detection parameter for the remainder of the run (or until another Set Minimum Height event is encountered).

[Figure 3-33](#) illustrates the Set Minimum Height event.

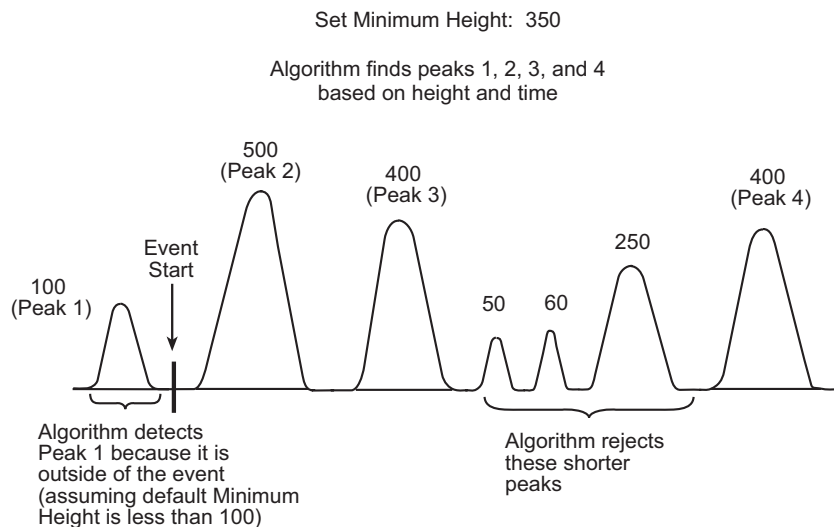


Figure 3-33 Set Minimum Height Event

### 3.4.10 Set Minimum Area Event

The Set Minimum Area event redefines the minimum area required to include an integrated peak in the peak list.

**Note:** The Set Minimum Area event overrides the global Minimum Area detection parameter for the remainder of the run (or until another Set Minimum Area event is encountered).

[Figure 3-34](#) illustrates the Set Minimum Area event.

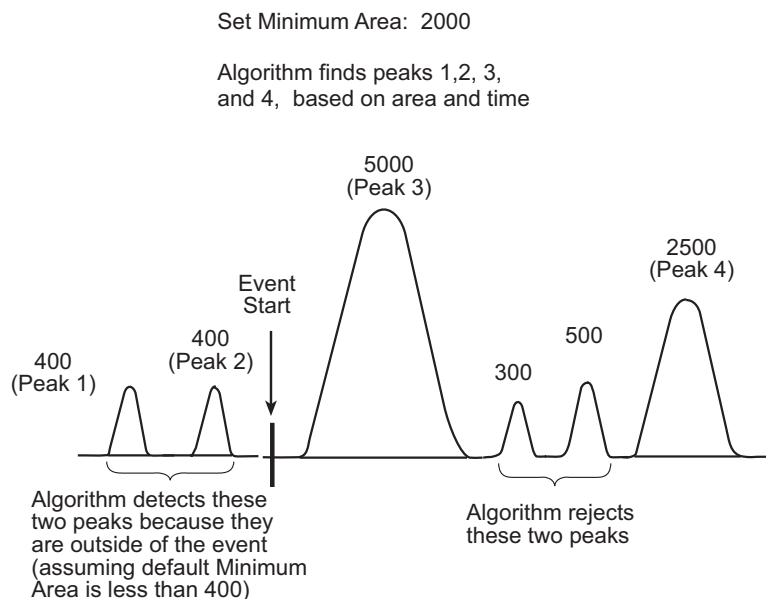


Figure 3-34 Set Minimum Area Event

## 3.5 Incompatible Events

The software restricts the simultaneous use of certain integration event combinations. [Table 3-5](#) lists each integration event and the incompatible integration events that cannot occur during the time period that the event is enabled. The software does not allow you to enter a conflicting event whenever the original event, listed in the Integration Event field, is active.

**Note:** *If an event is listed as conflicting with itself, the event cannot be applied a second time within a window in which it is already active.*

Table 3-5 Incompatible Events for Traditional Integration

Integration Event	Incompatible Event
Allow Negative Peaks	Allow Negative Peaks
Set Liftoff	None
Set Touchdown	None

Table 3-5 Incompatible Events for Traditional Integration (*Continued*)

Integration Event	Incompatible Event
Set Minimum Height	None
Set Minimum Area	None
Set Peak Width	None
Exponential Skim	<ul style="list-style-type: none"> <li>• Exponential Skim</li> <li>• Tangential Skim</li> <li>• Valley-to-Valley</li> </ul>
Tangential Skim	<ul style="list-style-type: none"> <li>• Tangential Skim</li> <li>• Exponential Skim</li> <li>• Valley-to-Valley</li> </ul>
Force Peak	Force Peak
Force Drop Line	Force Drop Line
Inhibit Integration <sup>a</sup>	<ul style="list-style-type: none"> <li>• Inhibit Integration</li> <li>• Force Baseline by Peak</li> <li>• Force Baseline by Time</li> <li>• Forward Horizontal by Peak</li> <li>• Forward Horizontal by Time</li> <li>• Reverse Horizontal by Peak</li> <li>• Reverse Horizontal by Time</li> <li>• Valley-to-Valley</li> </ul>
Force Baseline by Peak	<ul style="list-style-type: none"> <li>• Force Baseline by Peak</li> <li>• Force Baseline by Time</li> <li>• Forward Horizontal by Peak</li> <li>• Forward Horizontal by Time</li> <li>• Reverse Horizontal by Peak</li> <li>• Reverse Horizontal by Time</li> <li>• Valley-to-Valley</li> </ul>
Force Baseline by Time	<ul style="list-style-type: none"> <li>• Force Baseline by Time</li> <li>• Force Baseline by Peak</li> <li>• Forward Horizontal by Peak</li> <li>• Forward Horizontal by Time</li> <li>• Reverse Horizontal by Peak</li> <li>• Reverse Horizontal by Time</li> <li>• Valley-to-Valley</li> </ul>

Table 3-5 Incompatible Events for Traditional Integration (*Continued*)

Integration Event	Incompatible Event
Valley-to-Valley	<ul style="list-style-type: none"> <li>• Valley-to-Valley</li> <li>• Exponential Skim</li> <li>• Tangential Skim</li> <li>• Force Baseline by Peak</li> <li>• Force Baseline by Time</li> <li>• Forward Horizontal by Peak</li> <li>• Forward Horizontal by Time</li> <li>• Reverse Horizontal by Peak</li> <li>• Reverse Horizontal by Time</li> </ul>
Forward Horizontal by Peak	<ul style="list-style-type: none"> <li>• Forward Horizontal by Peak</li> <li>• Forward Horizontal by Time</li> <li>• Force Baseline by Peak</li> <li>• Force Baseline by Time</li> <li>• Reverse Horizontal by Peak</li> <li>• Reverse Horizontal by Time</li> <li>• Valley-to-Valley</li> </ul>
Forward Horizontal by Time	<ul style="list-style-type: none"> <li>• Forward Horizontal by Time</li> <li>• Forward Horizontal by Peak</li> <li>• Force Baseline by Peak</li> <li>• Force Baseline by Time</li> <li>• Reverse Horizontal by Peak</li> <li>• Reverse Horizontal by Time</li> <li>• Valley-to-Valley</li> </ul>
Reverse Horizontal by Peak	<ul style="list-style-type: none"> <li>• Reverse Horizontal by Peak</li> <li>• Reverse Horizontal by Time</li> <li>• Force Baseline by Peak</li> <li>• Force Baseline by Time</li> <li>• Forward Horizontal by Time</li> <li>• Forward Horizontal by Peak</li> <li>• Valley-to-Valley</li> </ul>

Table 3-5 Incompatible Events for Traditional Integration (*Continued*)

Integration Event	Incompatible Event
Reverse Horizontal by Time	<ul style="list-style-type: none"> <li>• Reverse Horizontal by Time</li> <li>• Force Baseline by Time</li> <li>• Force Baseline by Peak</li> <li>• Forward Horizontal by Peak</li> <li>• Forward Horizontal by Time</li> <li>• Reverse Horizontal by Peak</li> <li>• Valley-to-Valley</li> </ul>

a. An Inhibit Integration event can overlap a Valley-to-Valley event or the six force baseline events (Force Baseline by Peak and by Time, Forward Horizontal by Peak and by Time, and Reverse Horizontal by Peak and by Time); however, both the start and end times for each event cannot be located within a single Inhibit Integration event.

## 3.6 References

For further information on the theory of peak detection and integration, see:

- Dyson, Norman, *Chromatographic Integration Methods*, The Royal Society of Chemistry, Thomas Graham House, Cambridge, 1990.
- Massart, D.L., *et al.*, *Chemometrics: A Textbook*, Elsevier Science Publishers, Amsterdam, 1988.
- Papoulis, Athanasios, *Signal Analysis*, McGraw-Hill, New York, 1977.
- Snyder, L.R. and J.J. Kirkland, *Introduction to Modern Liquid Chromatography*, second ed., Wiley-Interscience, New York, 1979.

# Chapter 4

## Peak Matching and Quantitation of Sample Components

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Empower software identifies and quantifies unknown components using peak matching and quantitation:

- **Peak matching** – The process of matching unknown peak retention times (RT) against the RT of known standard peaks.
- **Quantitation** – The process of calculating the amounts of unknown peaks using the integration results of each peak and a calibration curve based on the amounts and integration results of known peaks (standards).

### 4.1 Peak Matching

---

When performing peak matching, the software chooses the integrated peaks in the chromatogram that most closely match the components in the Components table from the processing method. To accomplish this, the software:

1. Uses the time region defined by the RT of the component's calibration curve plus or minus the component's RT window together with the Peak Match type.
2. Matches peaks inside the RT windows of the components by calculating the difference between each unknown peak and component RT defined in the processing method.
3. Uses the differences to choose the unknown peak that most closely matches the component peak.

#### Matching Hierarchy

The software uses a hierarchy of peak match types when matching unknown peaks to components. The software matches each component to the unknown peaks in its RT window. If a peak matches multiple components, the software determines the most appropriate component for that peak first by position, second by size, and third by RT.



### 4.1.1 Calculating the Match Difference

When the peak match type is Closest or Closest Negative, the software calculates the difference as the absolute value of the component's RT minus the unknown peak's RT. For the other match types, the match difference is either 0 (a perfect match) or not matched.

A match is considered to be perfect if at least one of the following conditions exists:

- A peak is in first, second, third, fourth, fifth, or last position in the RT window that corresponds to its match type.
- The size of a peak, relative to other peaks in the RT window, conforms to its match type:
  - Greatest area or height
  - Least area or height
  - Greatest width (GPCV data only)
- There is a 0.0 difference between the RT of the peak and the component.

### 4.1.2 Choosing the Optimal Peak Match

The next step in the matching process is to determine whether any components match multiple unknown peaks or if any unknown peaks match multiple components. The three possible outcomes from the initial component matching process are:

- Single peak matching a single component
- Multiple peaks matching a single component
- Single peak matching multiple components

#### Single Peak with a Single Component

The peak matching process is straightforward if the RT windows of the components do not overlap and, at most, one unknown peak is found in each window. In this case, matching type and difference are not needed.

**Note:** *Never use the matching types Second, Third, Fourth, or Fifth if there are always fewer than that number of peaks in the RT window.*

#### Multiple Peaks with a Single Component

If there are multiple unknown peaks in the RT window of a component, the software uses the match difference to choose the peak that most closely fits the matching type criteria.

- If two peaks match a single component, the software chooses the peak with the smallest difference. The other peak is then matched to its next closest component.
- If two peaks equally match a single component (the match differences are equal), the software chooses the first peak. The second peak is matched to its next closest component.

## Single Peak with Multiple Components

If the same peak is matched to two or more components, the software then picks the component with the smallest difference from among the possible matches. If two components have equal differences for a peak, a choice cannot be made and the unknown peak remains unmatched. In the Peaks tab of the Main and Results windows of Review, the software lists the components' Peak Types as *Missing* and a Q04 code is copied into the Processing Code field for the unmatched peak, indicating the reason the components are missing (see [Appendix A, Processing Codes](#)).

### 4.1.3 Shifting RT and RT Windows

If the software has problems identifying peaks because the peaks are shifting so much that they are outside the RT windows, you can increase the size of the RT windows. If that is not possible, or if it causes peaks to be misidentified, you can use either the RT reference peak or the update RT parameter.

#### RT Reference

The RT Reference field allows you to temporarily adjust the RT of a component based on where the defined RT Reference peak is found in the chromatogram. The RT of the component temporarily shifts by the same percentage and in the same direction as the shift of the RT Reference peak. (The RT Reference peak is determined by comparing the RT for the reference peak listed in its calibration curve to the actual RT of the reference peak in the chromatogram.) The software uses the adjusted RT to match the unknown peak in the chromatogram, and calculates the adjusted RT of a component as follows:

$$RT_{\text{adjusted}} = RT \cdot \frac{RT_{\text{ref peak found}}}{RT_{\text{ref peak in cal curve}}}$$

If you have a peak, use a RT reference peak:

- That is always found in your chromatograms
- That is well separated from other peaks
- Whose RT shifts with your other components

This usually compensates for RT shifts that may affect a particular chromatogram.

#### Update RT

The Update RT field adjusts the RT of calibration curves, thus affecting the RT that the software uses to match unknown peaks. This is done in order to more accurately reflect the actual RT of the peaks in the chromatogram when RT shifting or drifting is problematic.

Normally, during peak matching, the software compares the RT of integrated peaks to the RT of the calibration curves and to the RT windows listed for the components in the Components tab of the processing method. When Update RT is selected, the software

uses the RT where the components were actually found during the processing of a previous chromatogram. Each time a chromatogram is processed, the software may store a new RT to use for peak matching and processing the subsequent chromatogram (depending on the Update RT selection). The updated RT is stored in the calibration curve for the component and is displayed in the Time field of the Calibration Curve window. The Update RT functionality does not affect the retention times listed in the Components tab of the Review window.

Update RT choices include:

- **Never** – The RT of the calibration curve is not updated.
- **Replace** – The RT of the calibration curve is updated every time a chromatogram is calibrated or quantitated, regardless of sample type. When a chromatogram is calibrated or quantitated, if any peak is identified in the RT window, the software replaces the RT in the calibration curve (not in the Components table) with the newly found RT.
- **Replace Standards** – The RT of the calibration curve is updated only when standards are calibrated. When a chromatogram is calibrated, if a standard peak is identified in the RT window, the software replaces the RT in the calibration curve (not in the Components table) with the newly found standard RT.
- **Average** – The RT of the calibration curve is updated every time a chromatogram is calibrated or quantitated, regardless of sample type. When a chromatogram is calibrated or quantitated, if any peak is identified in the RT window, the software averages the RT in the calibration curve (not in the Components table) with the newly found RT.
- **Average Standards** – The RT of the calibration curve is updated only when standards are calibrated. When a chromatogram is calibrated, if a standard peak is identified in the RT window, the software averages the RT in the calibration curve (not in the Components table) with the newly found standard RT.

The software updates the RT using any averaging choice as follows:

$$RT_c = \frac{(\text{Average Time from Calibration Curve} \times n + \text{New Retention Time})}{n + 1}$$

where:

$RT_c$  = The retention time of the calibration curve

$n$  = The number of times the value was previously averaged

Update RT is a coarse adjustment that should only be used when:

- Peak retention times are shifting in one direction.
- The overall shift cannot be handled by either increasing the RT window of the component peaks or using the RT reference peak.

**Note:** *The Replace and Average choices should be used only when the unknown samples have no peaks that can be misidentified.*

## 4.2 Quantitation

---

You can perform quantitation using:

- Calibration
- No calibration
- Sample weight and dilution
- Injection volume
- Responses other than peak area and height

**Note:** For additional information on the processes the software uses during quantitation, see [Section 4.4, References](#).

### 4.2.1 Quantitation by Calibration

Empower software performs calibration on a set of processed standards acquired by your chromatographic system. When you run the standards, the software requires that you enter:

- Injection volumes in the Samples table of the Run Samples window, Sample Set Method Editor, or the Alter Sample window
- Component names and amounts or concentrations in the Default Amount tab of the Processing Method window or the Component Editor of the Run Samples or the Alter Sample window.

During processing of chromatograms, the software calculates a response based on the detector signal for each peak. This response can be:

- Peak area
- Peak height
- Another peak value (including a custom peak value).

Once calibration standards are processed, the software generates a calibration curve for each standard component listed in the Components table. The calibration curve displays:

- Response (Y Value field) versus Amount or Concentration (X Value field) for external standard calibration
- Response ratio multiplied by the internal standard amount or concentration versus Amount or Concentration (X Value field) for internal standard calibration.

Calibration curve shape is based on a fit type you select (as described in [Section 4.3, Calibration Curve Fit Types](#)). Calibration curve fit types can be:

- **Single-level** – Always results in a linear curve through the origin.

- **Multilevel** – Allows you to select different fits to a multilevel calibration curve, including cubic spline, point-to-point, linear, quadratic, cubic, fourth-order, and fifth-order.
- **Multilevel Forced-Through-Zero** – Allows you to select the forced-through-zero options for linear, quadratic, cubic, fourth-order, and fifth-order curve fits.

The software calculates and updates calibration curves using individual or averaged points based on an Average By value you specify in the Components tab of the Processing Method window.

During sample processing, the software:

1. Matches the RT of the integrated peaks found in the unknown chromatogram with the RT of the components in the calibration curve.
2. Applies the response of each matched unknown peak to the corresponding component calibration curve.

During quantitation, the software calculates the amount or concentration of the unknown sample from the calibration curve. It uses the response of the sample to find the X value that corresponds to the amount or concentration. It then displays the final component amount in the Peaks tab of the Main and Results windows of Review.

## 4.2.2 Quantitation Without Calibration

If you want to quantitate without performing calibration, the software calculates the relative amount of each unknown peak in the sample as both percent area and percent height. Peak area and height percent are calculated as the percent of each integrated peak relative to the total area or height of all integrated peaks.

## 4.2.3 Quantitation Using Sample Weight and Dilution

Sample weight and dilution values are used to adjust the amounts and concentrations of standard and unknown components. These two values are optional and can be used to compensate for differences due to factors such as:

- Varying dilutions
- Different initial sample mass or volume

You enter sample weight and dilution on a per-standard or per-sample basis in the Samples table of the Run Samples, Sample Set Method Editor, or Alter Sample window. Typically, sample weights and/or dilutions are used for either the standard samples or the unknown samples, but not for both.

## Sample Weight

Sample weight is typically used in samples to calculate the ratio of quantity of component injected into the system to total quantity of original sample.

- During calibration, the software *multiplies* the entered amount(s) or concentration(s) of the standard component(s) by the sample weight to calculate amounts and concentrations for the standard sample.
- During quantitation, the software *divides* the amount or concentration (X Value field) determined from the calibration curve by the sample weight to calculate amounts and concentrations for the unknown sample.

For example, if the mass of sample that you weighed is 0.5 mg, and you want the amount determined by the software to be reported as the amount of the component as compared to the mass of the total sample, enter a sample weight of 0.5 for your unknown sample. The software quantitates the component amount from the calibration curve and then divides this value by the sample weight to obtain the final ratio of component amount to total sample amount. The amount can then be converted into a percentage by multiplying it by 100, either by using a dilution value of 100 or by creating a custom field and specifying a formula of Amount\*100.

When using sample weight, be sure that this value is equivalent to the units for the component amount or concentration that you are reporting. For example, if you weigh 1.44 mg of sample and the units of your standard amounts are in  $\mu\text{g}$ , use a sample weight of 1440 ( $\mu\text{g}$ ).

## Dilution

The Dilution field is typically used when you dilute a sample (a standard, an unknown, or a control) prior to injection and want to report the quantity of analyte in the original, undiluted sample. This could occur when an undiluted sample, injected directly onto the column, would fall above the range of the calibration curve. The sample dilution should be entered into the Samples table of Run Samples and the sample should be injected at the usual injection volume.

- During calibration, the software *divides* the entered amounts or concentrations of the standard component(s) by the dilution value to calculate amounts and concentrations for the standard component(s).
- During quantitation, the software *multiplies* the amount or concentration (X value) determined from the calibration curve by the dilution value to calculate amounts and concentrations for the unknown sample.

For example, if a 1:10 dilution was performed on a standard sample containing one component at an amount of 100  $\mu\text{g}$ , enter the amount of the standard component into the Component Editor or the Default Amounts tab of the Processing Method window as 100  $\mu\text{g}$  (the original, undiluted quantity) and the dilution as 10. When the software

calibrates this standard, it takes the specified amount of 100  $\mu\text{g}$  and divides by the specified dilution of 10. The software reports the resulting amount as 10  $\mu\text{g}$  (the amount injected on the column). This value is also plotted on the calibration curve.

If that same sample were an unknown sample, you would not enter a quantity for the component; however, the dilution would still be 10. When the software quantitates the unknown, it reads an amount of 10  $\mu\text{g}$  directly from the calibration curve and multiplies this value by 10 (the dilution value) for a resulting amount of 100  $\mu\text{g}$  (the prediluted amount). When you are working with dilutions of:

- **Standards** – Enter the dilutions and the original, undiluted quantities of the standard components.
- **Unknown samples** – If you enter the dilutions, the amounts and concentrations reported by the software will be those of the original, undiluted samples. The use of the dilution field eliminates the need to correct for a dilution by adjusting the injection volume.

***Note:** You can correct for the dilution of samples by adjusting the injection volume. If, by mistake, you dilute a sample or standard by a factor of 10, you could inject 10 times the usual injection volume instead of entering a value of 10 in the Dilution field. If the sample is a standard, you also need to enter the undiluted amount(s) for the standard component(s). If the sample is an unknown, do not enter the dilution and but adjust the injection volume 10-fold. The software determines the undiluted quantity of component. This quantity, given the higher injection volume, is 10 times higher than it would have been, had the normal injection volume been used. For both standard and unknown samples, if a dilution is corrected for by injection volume, do not adjust the value in the Dilution field.*

## 4.2.4 Quantitation Using Injection Volume

The software calculates both amounts and concentrations for standard and unknown samples. This allows you to print the value that is meaningful to you on your reports.

The software determines if you are entering your standard component quantities in units of amount or in units of concentration by the Sample Value Type list in the Components tab of the Processing Method window. If you select:

- **Amount** – The software interprets the component quantities that you enter (in the Component Editor of the Alter Sample window, the Component Editor of the Run Samples window, or the Default Amount tab of the Processing Method window) as amounts. The software calculates the corresponding concentration by dividing the specified amount by the injection volume, in  $\mu\text{L}$ .
- **Concentration** – The software interprets the component quantities that you enter (in the Component Editor of the Alter Sample window, the Component Editor of the Run Samples window, or the Default Amount tab of the Processing Method window) as concentrations. The software calculates the corresponding amount by multiplying the specified concentration by the injection volume, in  $\mu\text{L}$ .

Regardless of whether you have defined your standard component quantities in amounts or concentrations, you can create a calibration curve that uses standard amounts or concentrations. The X value for the component as entered in the Components tab of the Processing Method window determines whether the calibration curve is a plot of Response *versus* Amount or Response *versus* Concentration.

If the calibration curve is a plot of Response *versus* Amount (which occurs when the X Value field is set to Amount), the software quantitates unknown samples by using a component's response to determine its amount directly from the calibration curve. The software then determines the component's corresponding concentration value by dividing the calculated amount by the injection volume, in  $\mu\text{L}$ .

**Note:** *If the X Value field is set to Amount, the sample injection volume affects the calculated concentrations for unknown samples but not the calculated amounts.*

Likewise, if the calibration curve is a plot of Response *versus* Concentration (which occurs when the X Value field is set to Concentration), the software quantitates unknown samples by using a component's response to determine its concentration directly from the calibration curve. The software then determines the component's corresponding amount value by multiplying the calculated concentration by the injection volume, in  $\mu\text{L}$ .

**Note:** *If the X Value field is set to Concentration, the sample injection volume affects the calculated amounts for unknown samples but not the calculated concentrations.*

Be careful when you enter a component's Unit Label ( $\mu\text{g}$ ,  $\mu\text{g}/\mu\text{L}$ , etc.) because the software reports the label exactly as you enter it. In cases where the component's quantity is affected by the injection volume, be sure that the unit label is appropriate. The software always uses microliters for injection volume units.

## 4.2.5 Quantitation Using Responses Other than Peak Area and Height

The software allows you to use the Traditional responses of area, height, % Area, % Height, and any peak type custom field using a data type of real except for:

- Time fields
- Baseline fields
- Response
- Amount
- Concentration
- % Amount

Make the appropriate selection in the Y Value field in the Components tab of the Processing Method window.



## 4.2.6 External and Internal Standard Quantitation

The component amount calculations use one of the following:

- External standard method of quantitation
- Internal standard method of quantitation with separate standard and unknown samples
- Internal standard method of quantitation without separate standard and unknown samples (typically used with gas chromatography)

This section briefly describes these three quantitation methods.

**Note:** For assistance reproducing amounts and/or concentrations calculated by the software, see [Section 4.3, Calibration Curve Fit Types](#).

## 4.2.7 External Standard Quantitation

The external standard method of quantitation determines component amounts and/or concentrations by applying the detector response of a component peak to a calibration curve. The calibration curve is generated from a separately acquired and processed set of standards.

**Note:** The standard set may contain only one standard (referred to as single-level calibration).

The following criteria are also required:

- You must define standard samples as Standards either by using the Inject Standard function during sample loading in Run Samples, or (after the sample is acquired) by defining a Sample Type of Standard in Alter Sample.
- You must define unknown samples as Unknowns either by using the Inject Unknown function during sample loading in Run Samples, or (after the sample is acquired) by defining a Sample Type of Unknown in Alter Sample.
- You must define component names and amounts or concentrations of each standard component in the Default Amount tab of the Processing Method window or the Component Editor of the Run Samples or the Alter Sample window.
- The response is the Y value of the calibration curve. You choose the parameter to use as the y-axis by selecting the Y Value field in the Components tab of the Processing Method window.
- The X value of the calibration curve is either amount or concentration. You choose to use amount or concentration as the x-axis in the X Value field in the Components tab of the Processing Method window.
- External standard quantitation generates each calibration curve by plotting the detector response of a standard component versus the amount or concentration of the standard component.

- You define the fit type used for the calibration curve in the Components table of the Processing Method window.

To perform single-level external standard quantitation, the software:

1. Identifies the component peak(s) in the standard injection using peak matching.
2. Determines the response and amount or concentration for each standard peak, then plots these two values as a calibration point on the calibration curve for the component with the same name.

Given a chromatogram such as in [Figure 4-1](#), the values used to determine the calibration points are the Concentration and Response values from [Table 4-1](#).

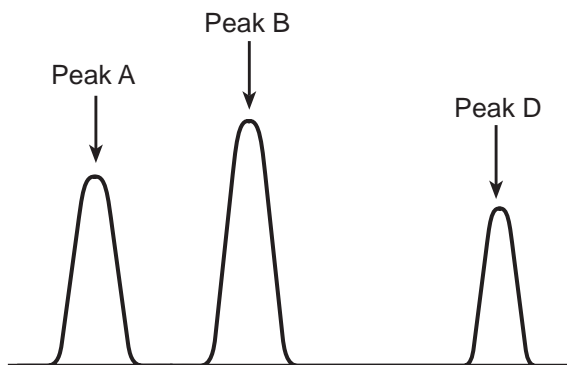


Figure 4-1 External Standard Chromatogram

**Note:** In [Table 4-1](#), the X values are set to concentration.

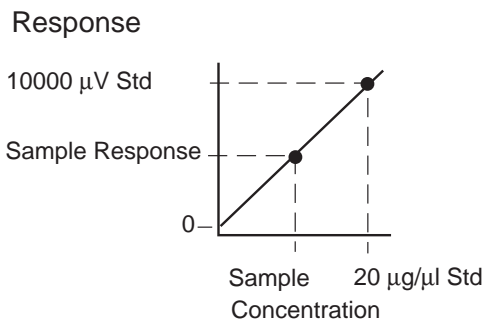
Table 4-1 Standard Peak Values, External Standard Calibration

Component Name	Quantitation Basis (User-Specified Y Value)	Amount or Concentration in Standard (User-Specified X Value)	Component Area	Component Height	Response
A	Area	20 $\mu\text{g}/\mu\text{L}$	10000	900	10000
B	Height	100 $\mu\text{g}/\mu\text{L}$	12000	1100	1100
D	Height	5 $\mu\text{g}/\mu\text{L}$	8000	700	700

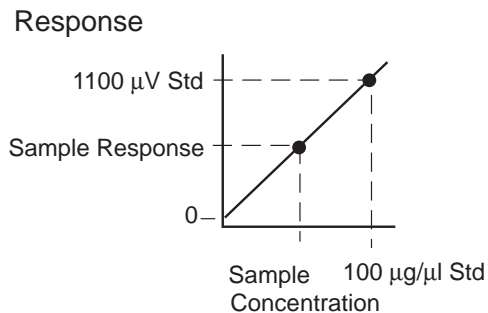
3. Calculates a calibration curve (Response versus Concentration) for each component listed in the Name column of the Components table.
4. Quantitates unknown samples against the generated calibration curve as follows:
  - a. Identifies each unknown peak by matching its retention time with a component from the Components table.

- b. Calculates amount and concentration for each unknown peak from the component calibration curve using sample peak response and injection volume.
- c. Adjusts the amount and concentration by the sample weight and dilution fields as entered during sample loading. The final calculated amount and concentration appear in the Peaks tab of the Main and Results windows of Review.

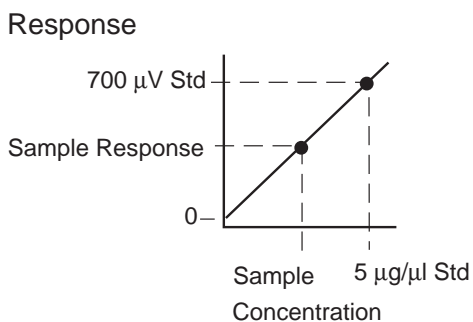
[Figure 4-2](#) illustrates the quantitation for peak components A, B, and D. Each calibration curve uses the single-level fit type (linear through zero).



**Peak A**



**Peak B**



**Peak D**

Figure 4-2 External Standard Component Calibration Curves (Single-Level, Concentration)

**Note:** If you are using a multilevel calibration curve, the software performs an equivalent process.

## 4.2.8 Internal Standard Quantitation with Separate Standard and Unknown Samples

This technique uses an internal standard added to both the standard and unknown samples as a recovery standard. This method is commonly used to correct for losses during sample preparation.

This method determines component amounts and concentrations by applying a response ratio to a calibration curve generated first by calculating the response ratio for the set of standards containing the internal standard. The response ratio is calculated from the responses of the component peak and its internal standard peak. You select the type of response by using the Y Value field in the Components table of the Processing Method window.

The classic internal standard quantitation method plots the response ratio of the standard component and the internal standard versus the amount or concentration ratio of the standard component and the internal standard to generate a calibration curve (Figure 4-3). The software uses an equivalent calibration curve produced by plotting the response ratio times the internal standard X value versus the component X value, where the X Value field is set to amount or concentration in the Components table of the Processing Method window (Figure 4-4).

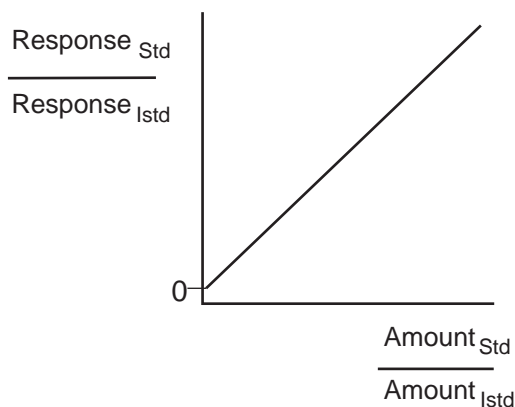


Figure 4-3 Classic Response Ratio Versus Amount (or Concentration) Ratio Plot

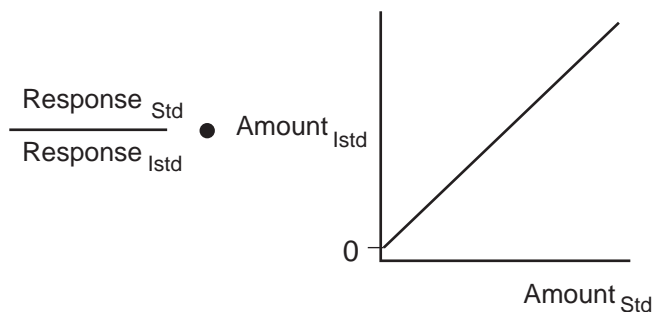


Figure 4-4 Multiplying Response Ratio by Internal Standard Amount (or Concentration)

The following criteria are also required:

- You must define standard samples as Standards either by using the Inject Standard function during sample loading in Run Samples, or (after the sample is acquired) by defining a Sample Type of Standard in Alter Sample.
- You must define unknown samples as Unknowns either by using the Inject Unknown function during sample loading in Run Samples, or (after the sample is acquired) by defining a Sample Type of Unknown in Alter Sample.
- You must enter component names and amounts or concentrations of each standard component in the Default Amount tab of the Processing Method window or the Component Editor of the Run Samples or the Alter Sample window.
- The X value of the calibration curve is either amount or concentration. You choose to use amount or concentration as the x-axis in the X Value field in the Components tab of the Processing Method window.
- You define the fit type used for the calibration curve in the Components table of the Processing Method window.

To perform internal standard quantitation (with separate standard and unknown samples), the software:

1. Identifies the component peaks in the chromatogram using peak matching.
2. Determines the responses and amounts or concentrations for standard peaks and the internal standard(s). The software calculates a response ratio for each standard peak and multiplies this by the amount or concentration of the internal standard component. The resulting response value is plotted against the amount or concentration value of the standard peak on the calibration curve for the component with the same name.

Given a standard chromatogram such as in [Figure 4-5](#), the values used to determine the calibration points are the Concentration and Response values from [Table 4-2](#).

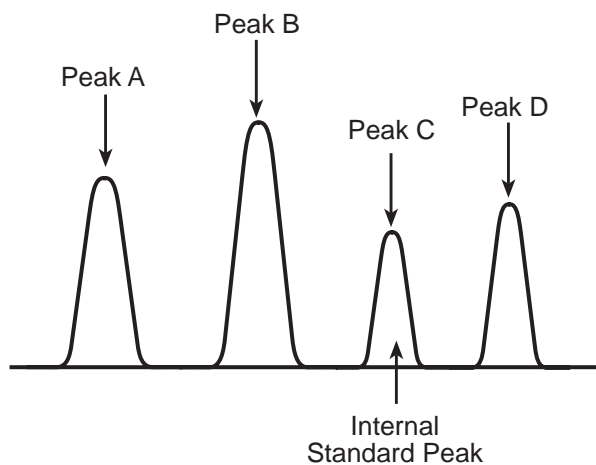


Figure 4-5 Internal Standard Chromatogram

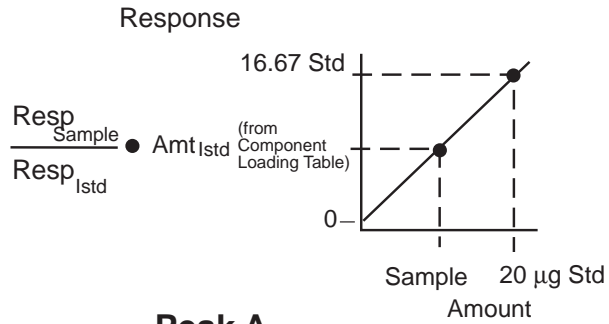
**Note:** In [Table 4-2](#), the X values are set to amount.

Table 4-2 Standard Peak Values, Internal Standard Calibration with Separate Standard and Unknown Samples

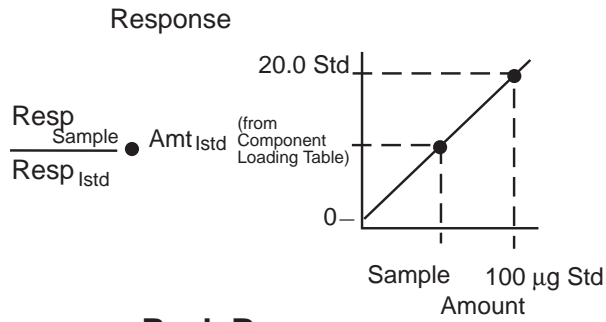
Component Name	Quantitation Basis (User-Specified Y Value)	Amount or Concentration in Standard (User-Specified X Value)	Component Area	Response
A	Area	20 µg	10000	$\frac{AreaA}{AreaC} = \frac{10000}{6000} \times 10 = 16.67$
B	Area	100 µg	12000	$\frac{AreaB}{AreaC} = \frac{12000}{6000} \times 10 = 20.0$
C (Int Std)	Area	10 µg	6000	N/A
D	Area	5 µg	8000	$\frac{AreaD}{AreaC} = \frac{8000}{6000} \times 10 = 13.33$

3. Calculates a calibration curve (response ratio times internal standard amount versus Amount or Concentration) for each component listed in the Name field of the Components table of the Processing Method window.
4. Quantitates unknown samples against the generated calibration curve by performing the following:
  - a. Identifies each unknown peak by matching its retention time with a component from the Components table.
  - b. Calculates a response ratio for each matched peak by dividing the peak's response by its internal standard's response.
  - c. Generates a response by multiplying the response ratio by the amount or concentration of the internal standard for each matched peak.
  - d. Calculates amount and concentration for each sample peak from the component calibration curve using the sample peak response and the injection volume.
  - e. Adjusts amount and concentration by the Sample Weight and Dilution fields as entered in the Samples tab of Run Samples or in Alter Sample. The final calculated amount and concentration appear in the Peaks tab of the Main and Results windows of Review.

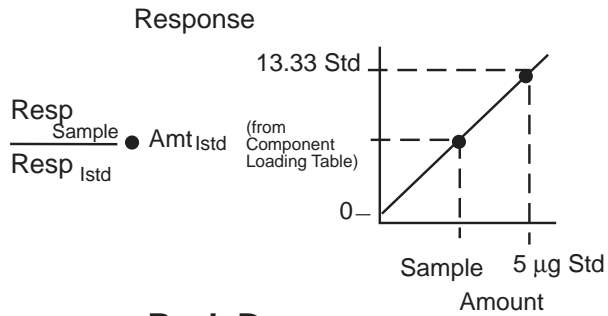
[Figure 4-6](#) illustrates quantitation for peak components A, B, and D (internal standard C is not shown). Each calibration curve uses the single-level fit type.



### Peak A



### Peak B



### Peak D

Figure 4-6 Internal Standard Component Calibration Curves (Single-Level, Amount)

**Note:** If you are using a multilevel calibration curve, the software performs an equivalent process.



## 4.2.9 Internal Standard Quantitation Without Separate Standard and Unknown Samples (RF Internal Standard)

This technique is often used in gas chromatography. All samples are spiked with a standard compound(s) that elutes at a different time than the unknown component(s) to be quantitated. When each chromatogram is processed, it is treated as an unknown sample type. The software calculates a *response factor* (RF) for the standard component(s) and then quantitates the unknown components using the RF of the standard component(s) rather than using coefficients of a calibration curve.

The following criteria are also required:

- You must define all samples as an RF Internal Standard either by using the Inject RF Internal Standard function during sample loading in Run Samples, or (after the sample is acquired) by defining a Sample Type of RF Internal Standard in Alter Sample.
- The X value of the calibration curve is either amount or concentration (defined in the X Value field in the Components tab of the processing method).
- You must enter component names of the standard components in the Components tab of the processing method.
- You must enter amounts or concentrations of each standard component in the Default Amount tab of the processing method or in the Component Editor of the Run Samples or the Alter Sample window.
- You may enter component names of the unknown components in the Components tab of the processing method (optional).
- Unknown components that have names defined in the Components tab of the processing method are typically quantitated using the RF of a standard component defined by using a Curve Reference peak, but may alternatively be quantitated using a Default Peak.
- Unknown components that have no names defined in the Components tab of the processing method are quantitated using a Default Peak.

**Note:** Use the Default Pk field to define which standard component's response factor to use during quantitation of named or unnamed components. To use a standard peak as a default peak, in the Components tab of the processing method, on the row containing the default peak, check the Default Pk field. Define the region of the chromatogram in which this default peak should be used in the Default Pk Start and Default Pk End fields. These fields allow you to use a different default peak for different regions of your chromatogram, if necessary. During quantitation, any peak that is detected within the default peak start and end range will use the RF of that default peak in determining its amount or concentration.

Use the Curve Reference field to define which standard component's RF to use during quantitation of named components. To use a Curve Reference, in the

Components tab of the processing method, on the row for the named unknown component, enter the name of the appropriate standard component in the Curve Reference field.

- The RF is calculated using the Y value and the X value (amount or concentration) defined in the Components tab of the Processing Method window.

To perform internal standard quantitation (without separate standards and unknown samples), the software:

1. Identifies the component peak(s) in the standard injection using peak matching.
2. Determines the RF for each standard component using the following formula:

$$RF = \frac{Y \text{ Value}}{X \text{ Value}}$$

where:

$RF$  = Response factor

$Y \text{ Value}$  = Response of the standard component calculated by the software

$X \text{ Value}$  = Component amount or concentration of the standard component

Given an RF internal standard chromatogram such as in [Figure 4-7](#), the values used to determine the RF are the Amount and Response values from [Table 4-3](#).

**Note:** When this type of internal standard method is used, no calibration curves are generated.

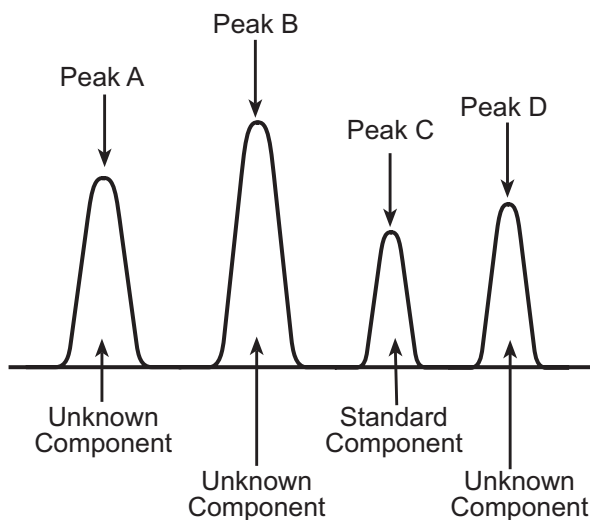


Figure 4-7 RF Internal Standard Chromatogram

**Note:** In [Table 4-3](#), the X values are set to amount.

Table 4-3 Standard Component Values, RF Internal Standard Calibration without Separate Standard and Unknown Samples

Component Name	Quantitation Basis	Amount or Concentration in Standard Component (User-Specified X Value)	Component Area (Response or Y Value)	Response Factor
C (standard component)	Area	10 µg	6000	$\frac{6000}{10} = 600$

3. Uses the response of each unknown component and the appropriate RF to determine unknown component amounts (or concentrations) as follows:

$$X \text{ Value} = \frac{Y \text{ Value}}{RF}$$

where:

$RF$  = Response factor value calculated for the standard peak

$Y \text{ Value}$  = Response of the unknown component calculated by the software

$X \text{ Value}$  = Component amount or concentration

The values used to determine the amounts of the unknown components in [Table 4-4](#) are the RFs determined in [Table 4-3](#) and the unknown component values in [Table 4-4](#).

**Note:** In [Table 4-4](#), the software-determined X values are amounts.

Table 4-4 Unknown Component Values, RF Internal Standard Calibration without Separate Standard and Unknown Samples

Component Name	Quantitation Basis	Component Area (Response or Y Value)	Amount or Concentration in Unknown Component (Software-Determined X Value)
A (unknown component)	Area	10000	$\frac{10000}{600} = 16.667 \mu\text{g}$
B (unknown component)	Area	12000	$\frac{12000}{600} = 20 \mu\text{g}$
D (unknown component)	Area	8000	$\frac{8000}{600} = 13.333 \mu\text{g}$

## 4.3 Calibration Curve Fit Types

A variety of calibration curve fits are available for external and internal standard calibration with multiple levels of standards. The calibration curve fit types are divided into three groups of increasing complexity:

- Single-level calibration (linear through zero and response factor)
- Multilevel calibration matrix operations:
  - Multilevel calibration (linear, inverse linear, log-log linear, quadratic, cubic, fourth-order, and fifth-order)
  - Multilevel forced through zero (linear, quadratic, cubic, fourth-order, fifth-order, and response factor)
- Multilevel calibration (point-to-point and cubic spline)

**Note:** You can apply weighting only to the linear, quadratic, cubic, fourth-order, and fifth-order fit types.

When you refer to the calibration curve fit types in this section:

- The software uses matrix operations to perform multilevel calibration (see [Section 4.3.2, Multilevel Calibration Matrix Operations](#)).
- For background material on the processes used by the software to create calibration curves, see [Section 4.4, References](#).
- The equations shown in the following examples are not adjusted for sample weight and dilution (see [Section 4.2, Quantitation](#)).

### 4.3.1 Single-Level Calibration Curve

For single-level calibration, the curve fit is linear with an intercept of zero. The software supports the following single-level calibration curve fits:

- Linear through zero
- Response factor

Figure 4-8 illustrates a single-level calibration curve.

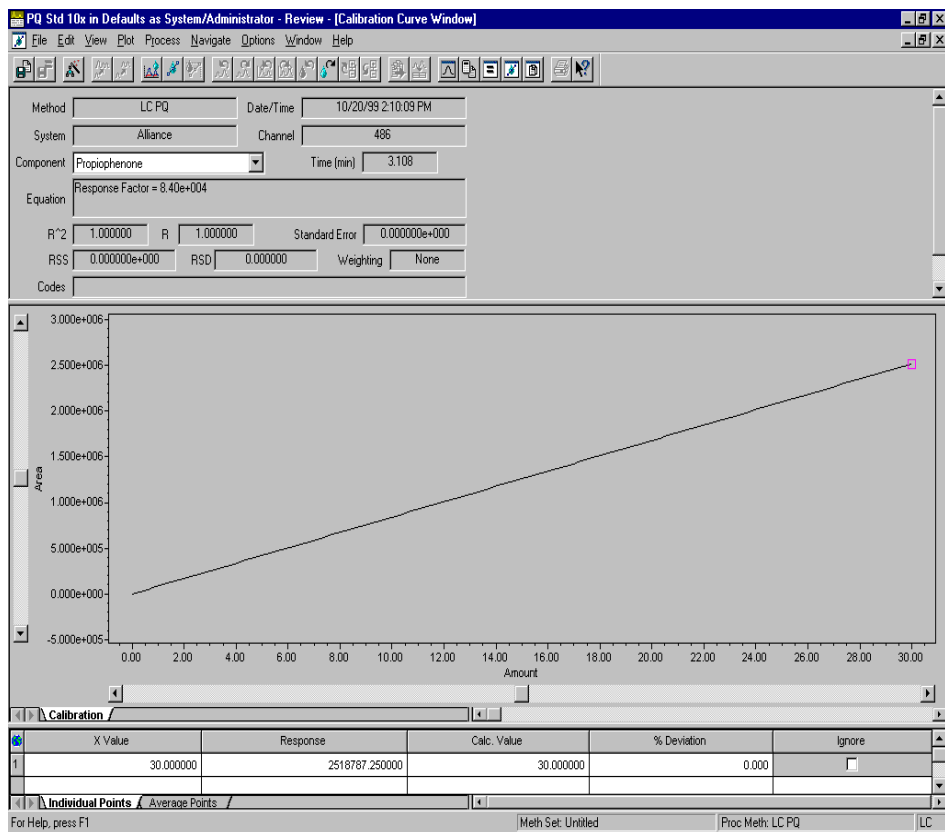


Figure 4-8 Single-Level Calibration Curve

## Linear Through Zero

The linear-through-zero calibration curve is represented by the equation:

$$y = Bx$$

where:

$y$  = Response of the standard component calculated by the software

$B$  = Slope of the calibration curve

$x$  = Component amount or concentration

The component amount or concentration for a quantitated sample can be determined by the equation:

$$x = \frac{y}{B}$$

where:

$x$  = Component amount or concentration

$y$  = Response of the sample peak calculated by the software

$B$  = Slope of the calibration curve

## Response Factor

The response factor (RF) fit type eliminates the need to create an RF custom field. When using an RF fit type, you should specify the appropriate X Value and Y Value in the Components tab of the processing method as when using a linear-through-zero fit.

The software plots the standard component's response versus its amount (or concentration) on the calibration curve. The RF is the slope of the curve. If multiple data points are plotted on the calibration curve, the RF for each point is determined, then the average RF is used as the slope of the curve.

The RF is represented by the equation:

$$RF = \frac{Y \text{ Value}}{X \text{ Value}}$$

where:

$RF$  = Response factor (slope of the calibration curve)

$y$  = Response of the standard component calculated by the software

$x$  = Component amount or concentration of the standard component

A linear-through-zero fit to the average RF point results in the equation of the curve.

[Figure 4-9](#) illustrates an RF calibration curve.

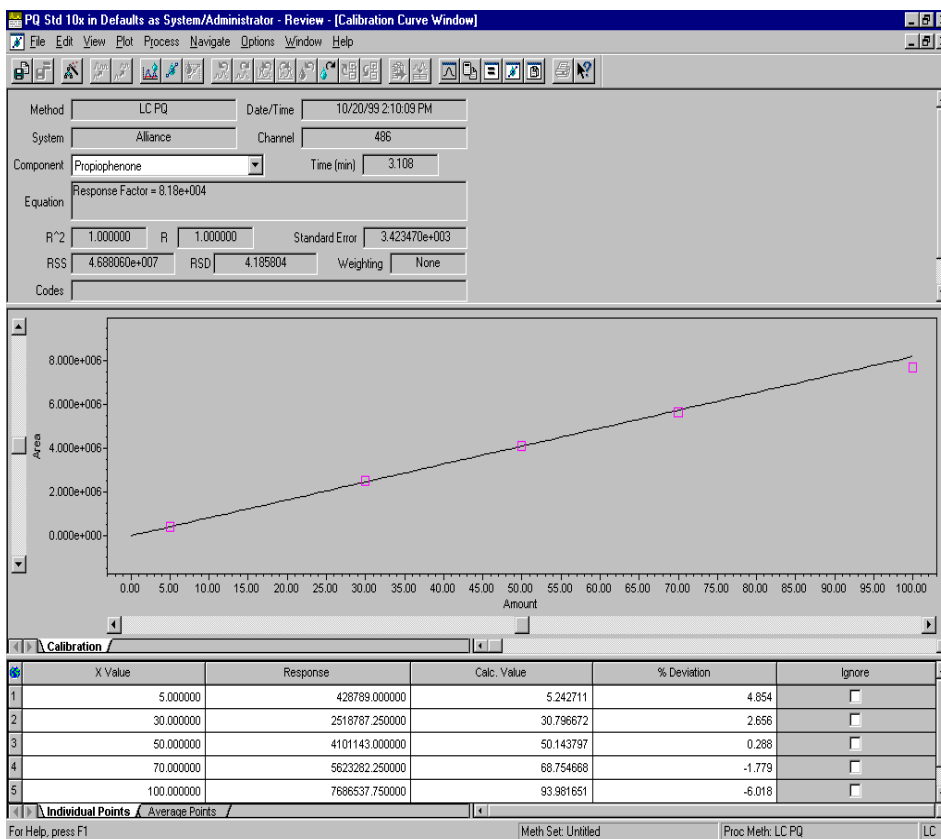


Figure 4-9 Response Factor Calibration Curve

### 4.3.2 Multilevel Calibration Matrix Operations

For the multilevel polynomial curve fits (linear, inverse linear, log-log linear, quadratic, cubic, fourth-order, fifth-order, and through zero fits), the software uses matrix operations to obtain the required coefficients.

For all polynomial fits, the software uses an unweighted or weighted least-squares fitting technique to a set of x-y points or x, y, and weight points. This technique is the numerical routine called LU (lower and upper triangular matrix) Decomposition for weighted and unweighted fits.

- When weighting is *disabled* in the Components table, the software uses an unweighted least-squares fitting technique to a set of x-y points.
- When weighting is *enabled* in the Components table, the software uses a weighted least-squares fitting technique to a set of x-y weight points.

## Matrix Operations

The objective is to use the least-squares fit to calculate the coefficients for the curve:

$$[Y] = [A] \cdot [C]$$

where:

$$[Y] = \text{Response vector}$$

$$[A] = \text{Design matrix}$$

$$[C] = \text{Coefficient vector}$$

To accomplish this, the least-squares fit solves the following linear *normal equations*:

$$([A]^T \cdot [W] \cdot [A]) \cdot [C] = [A]^T \cdot [W] \cdot [Y]$$

where  $[W]$  is a diagonal weight matrix, which becomes unity (all diagonal elements equal to 1) for an unweighted fit. The solution is:

$$[C] = ([A]^T \cdot [W] \cdot [A])^{-1} \cdot ([A]^T \cdot [W] \cdot [Y])$$

The matrix inversion is done by using LU Decomposition. The least-squares fit is described in Section 15.4 General Linear Least Squares in *Numerical Recipes in C* William H. Press, et al., (2nd Edition).

## Matrix Operations Example

The software uses the following matrices to calculate the coefficients based on the number of standards that are run. These operations illustrate an unweighted multilevel fifth-order fit.

As an example, assume that seven standards are run, one at each level. The software attempts to apply a fifth-order fit to the calibration points. To find the coefficients of the calibration equation:

1. The seven standards produce the following amount, response sets (x, y plotted points on the calibration curve):

$$(x_1, y_1), (x_2, y_2), (x_3, y_3), (x_4, y_4), (x_5, y_5), (x_6, y_6), (x_7, y_7)$$



2. The following seven equations, using the data in step 1, contain the six unknown coefficients ( $c_5$  through  $c_0$ ) and the seven sets of points:

$$\begin{aligned}
 y_1 &= c_5(x_1)^5 + c_4(x_1)^4 + c_3(x_1)^3 + c_2(x_1)^2 + c_1(x_1)^1 + c_0(x_1)^0 \\
 y_2 &= c_5(x_2)^5 + c_4(x_2)^4 + c_3(x_2)^3 + c_2(x_2)^2 + c_1(x_2)^1 + c_0(x_2)^0 \\
 y_3 &= c_5(x_3)^5 + c_4(x_3)^4 + c_3(x_3)^3 + c_2(x_3)^2 + c_1(x_3)^1 + c_0(x_3)^0 \\
 y_4 &= c_5(x_4)^5 + c_4(x_4)^4 + c_3(x_4)^3 + c_2(x_4)^2 + c_1(x_4)^1 + c_0(x_4)^0 \\
 y_5 &= c_5(x_5)^5 + c_4(x_5)^4 + c_3(x_5)^3 + c_2(x_5)^2 + c_1(x_5)^1 + c_0(x_5)^0 \\
 y_6 &= c_5(x_6)^5 + c_4(x_6)^4 + c_3(x_6)^3 + c_2(x_6)^2 + c_1(x_6)^1 + c_0(x_6)^0 \\
 y_7 &= c_5(x_7)^5 + c_4(x_7)^4 + c_3(x_7)^3 + c_2(x_7)^2 + c_1(x_7)^1 + c_0(x_7)^0
 \end{aligned}$$

3. The previous equations can now be written using matrix notation as:

$$\begin{bmatrix} y_1 \\ y_2 \\ y_3 \\ y_4 \\ y_5 \\ y_6 \\ y_7 \end{bmatrix} = \begin{bmatrix} x_1^5 & x_1^4 & x_1^3 & x_1^2 & x_1^1 & x_1^0 \\ x_2^5 & x_2^4 & x_2^3 & x_2^2 & x_2^1 & x_2^0 \\ x_3^5 & x_3^4 & x_3^3 & x_3^2 & x_3^1 & x_3^0 \\ x_4^5 & x_4^4 & x_4^3 & x_4^2 & x_4^1 & x_4^0 \\ x_5^5 & x_5^4 & x_5^3 & x_5^2 & x_5^1 & x_5^0 \\ x_6^5 & x_6^4 & x_6^3 & x_6^2 & x_6^1 & x_6^0 \\ x_7^5 & x_7^4 & x_7^3 & x_7^2 & x_7^1 & x_7^0 \end{bmatrix} \cdot \begin{bmatrix} c_5 \\ c_4 \\ c_3 \\ c_2 \\ c_1 \\ c_0 \end{bmatrix}$$

or

$$[Y] = [A] \cdot [C]$$

where:

$[Y]$  = Response vector

$[A]$  = Design matrix

$[C]$  = Vector of coefficient to be computed

Design Matrix  $A$  is constructed with  $n+1$  columns and  $i$  rows (where  $n$  is the order of the polynomial and  $i$  is the number of levels). The construction of Design Matrix  $A$  for the fifth-order fit type is previously illustrated.

4. The software then uses LU Decomposition to solve the *normal equations* of the least-squares fit:

$$([A]^T \cdot [A]) \cdot [C] = [A]^T \cdot [Y]$$

### 4.3.3 Multilevel Calibration Curves

The software supports the following multilevel calibration curve fits:

- Point-to-point
- Cubic spline
- Linear
- Inverse linear
- Log-log linear
- Quadratic
- Cubic
- Fourth-order
- Fifth-order

The equations used to calculate the goodness-of-fit statistics are described in [Section 4.3.6, Statistics](#), with the following results:

- For all fit types, the software reports only positive X value amounts or concentrations.
- For linear fit types, the software reports X values within the range of the calibration curve (from 0 to the highest X value) as well as X values greater than the highest X value because it extrapolates values above the highest X value of the standard data point.
- For all nonlinear fit types, the software reports X values from 0 to the highest X value of the standard points.

#### Point-to-Point Fit

To calculate a point-to-point calibration curve, the software performs a linear fit between the different levels. The first and last segments of the curve are extrapolated linearly so that they can be used to calculate X values that fall outside the range of the lowest to highest X value.

Because the point-to-point calibration curve is fit through every point, the correlation coefficient equals 1, and the standard error equals 0. No curve coefficients are calculated or stored for this fit type. [Figure 4-10](#) illustrates a point-to-point calibration curve.

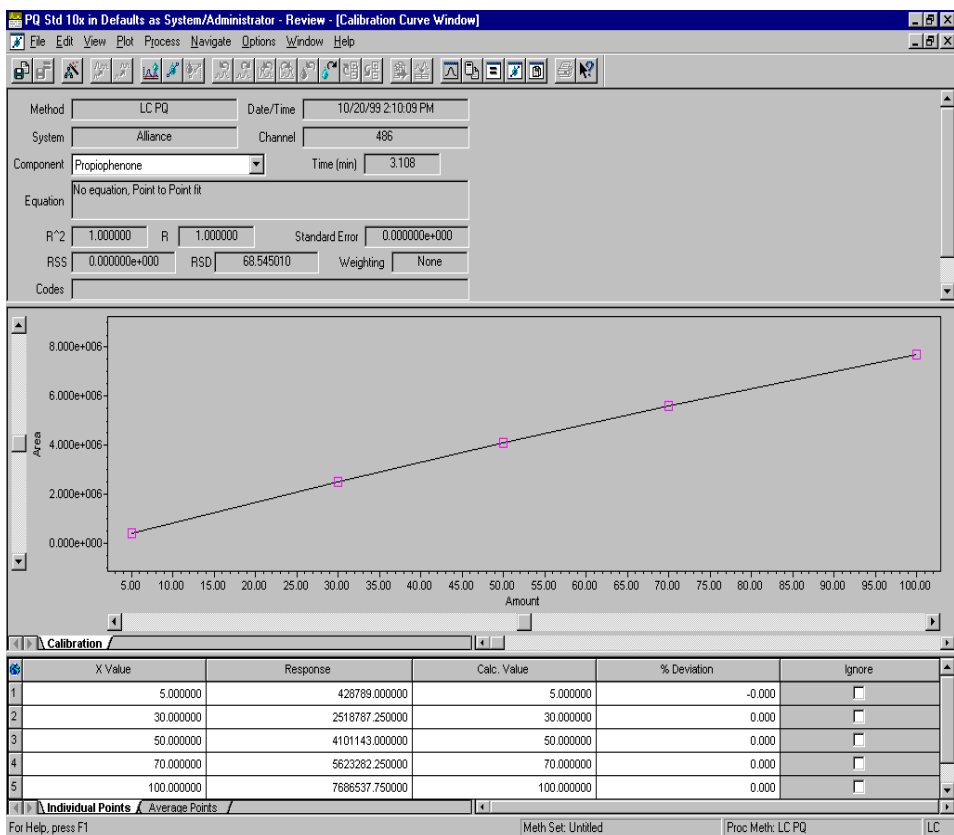


Figure 4-10 Point-to-Point Calibration Curve

Each point-to-point segment of the calibration curve is represented by the equation:

$$y = A_i + B_i x$$

where:

$y$  = Response of the standard peak calculated by the software

$A_i$  =  $y$ -intercept of the  $i$ th curve segment

$B_i$  = Slope of the  $i$ th curve segment

$x$  = Component amount or concentration

## Determining Component Amount and/or Concentration

Component amount and/or concentration for a quantitated sample peak can be determined by the equation:

$$x = \frac{y - A_i}{B_i}$$

where:

$x$  = Component amount and/or concentration

$y$  = Response of the sample peak, calculated by the software

$A_i$  = y-intercept of the  $i$ th curve segment

$B_i$  = Slope of the  $i$ th segment

## Cubic Spline Fit

To generate a cubic spline calibration curve, the software performs a cubic polynomial fit between every two successive levels, matching the slope and curvature at every point boundary. The cubic spline fit adjusts the shape of the calibration curve on a point-by-point basis.

Because the cubic spline calibration curve is fit through every point, the correlation coefficient = 1, and the standard error = 0. No curve coefficients are calculated or stored when the cubic spline fit type is used.

Figure 4-11 illustrates a cubic spline calibration curve.

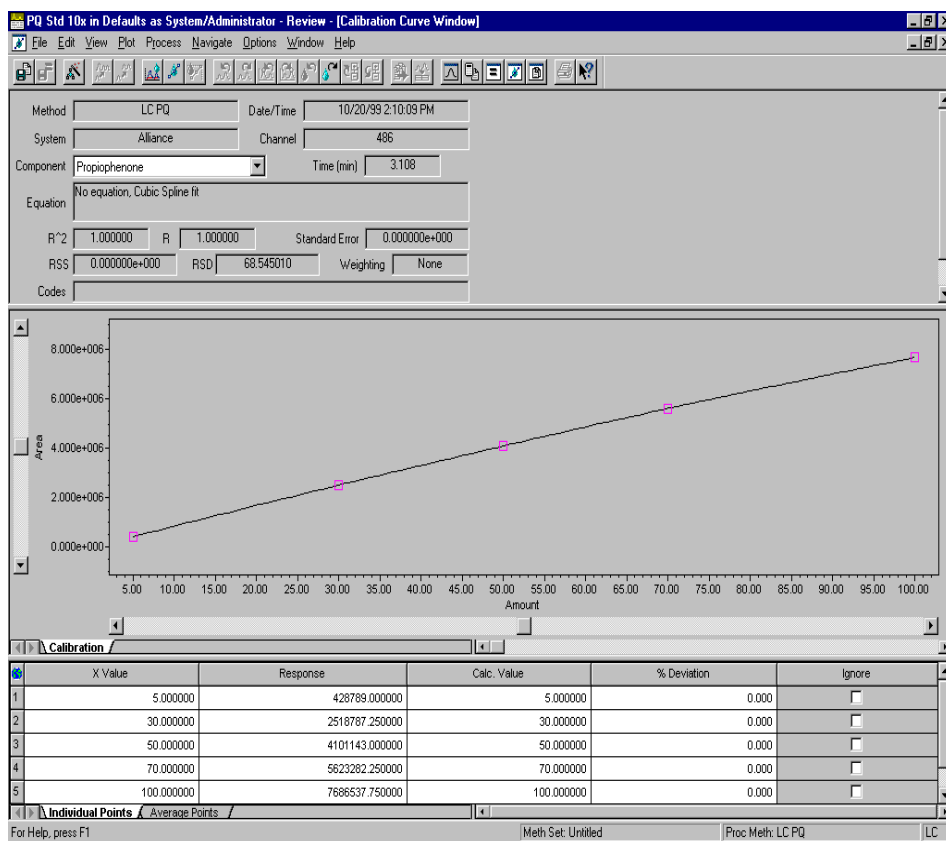


Figure 4-11 Cubic Spline Calibration Curve

Each cubic spline segment of the calibration curve is represented by the equation:

$$y = A_i + B_i + C_i x^2 + D_i x^3$$

where  $A_i$ ,  $B_i$ ,  $C_i$ , and  $D_i$  are the polynomial coefficients of the segment.

**Note:** The software uses an iterative method to solve for  $x$  when given  $y$ .

## Linear Fits

### Linear Fit

To calculate a linear calibration curve, the software calculates the line that best fits the amounts or concentrations and responses of the calibration points. The Y value of each point is the response of the standard peak and the X value of each point is the amount or concentration of the standard peak. [Figure 4-12](#) illustrates a linear least-squares fit calibration curve.

## Inverse Linear Fit

To calculate an inverse linear calibration curve, the software performs a linear fit to the X and Y values of the calibration points. The Y value of the point is the response of the standard peak and the X value is the 1/X value (either amount or concentration) of the standard peak.

## Log-Log Linear Fit

To calculate a log-log linear calibration curve, the software performs a linear fit to the X and Y values of the calibration points. The Y value of each point is the log of the response of the standard peak and the X value is the log of the X value (either amount or concentration) of the standard peak.

**Note:** Inverse linear and log-log linear use the linear fit equation.

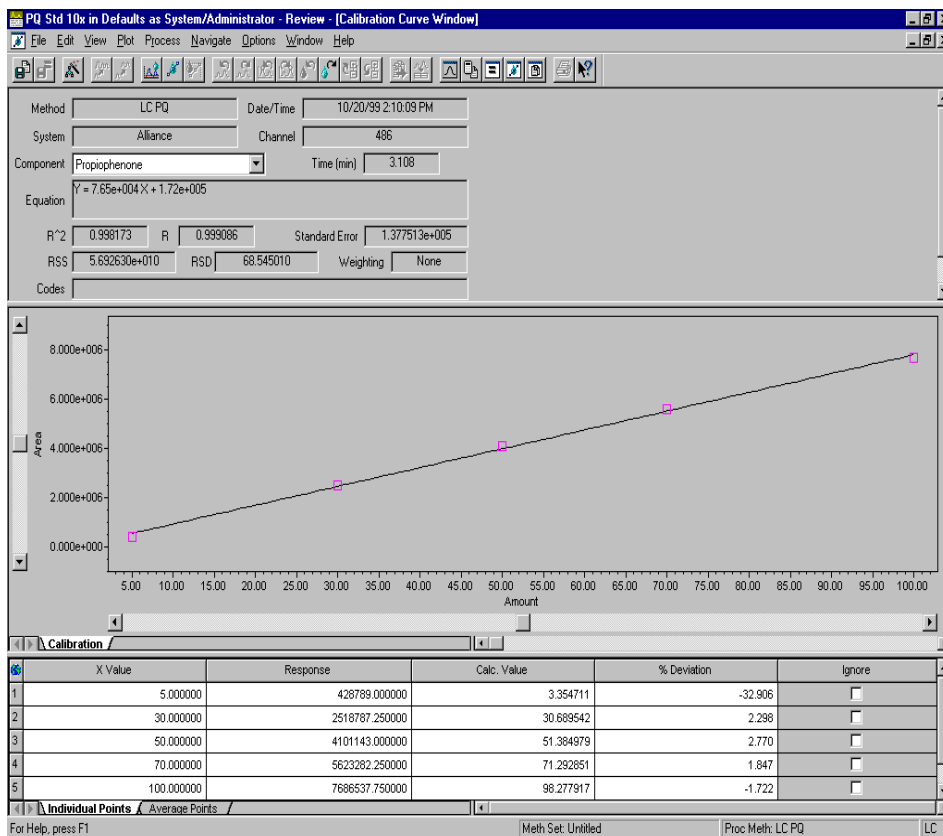


Figure 4-12 Linear Least-Squares Fit Calibration Curve

The linear fit generates a calibration curve represented by the equation:

$$y = A + Bx$$

where:

$y$  = Response of the standard peak calculated by the software

$A$  = y-intercept of the calibration curve

$B$  = Slope of the calibration curve

$x$  = Component amount or concentration

### Determining Component Amount or Concentrations

Component amount or concentration for a quantitated sample peak can be determined by the equation:

$$x = \frac{y-A}{B}$$

where:

$x$  = Component amount or concentration

$y$  = Response of the sample peak calculated by the software

$A$  = y-intercept of the calibration curve

$B$  = Slope of the calibration curve

### Quadratic Fit

To calculate a quadratic calibration curve, the software performs a least-squares fit of a quadratic polynomial to the calibration points. The fit cannot be performed with fewer than three calibration points, and a minimum of five points is strongly recommended.

Figure 4-13 illustrates a quadratic fit calibration curve.

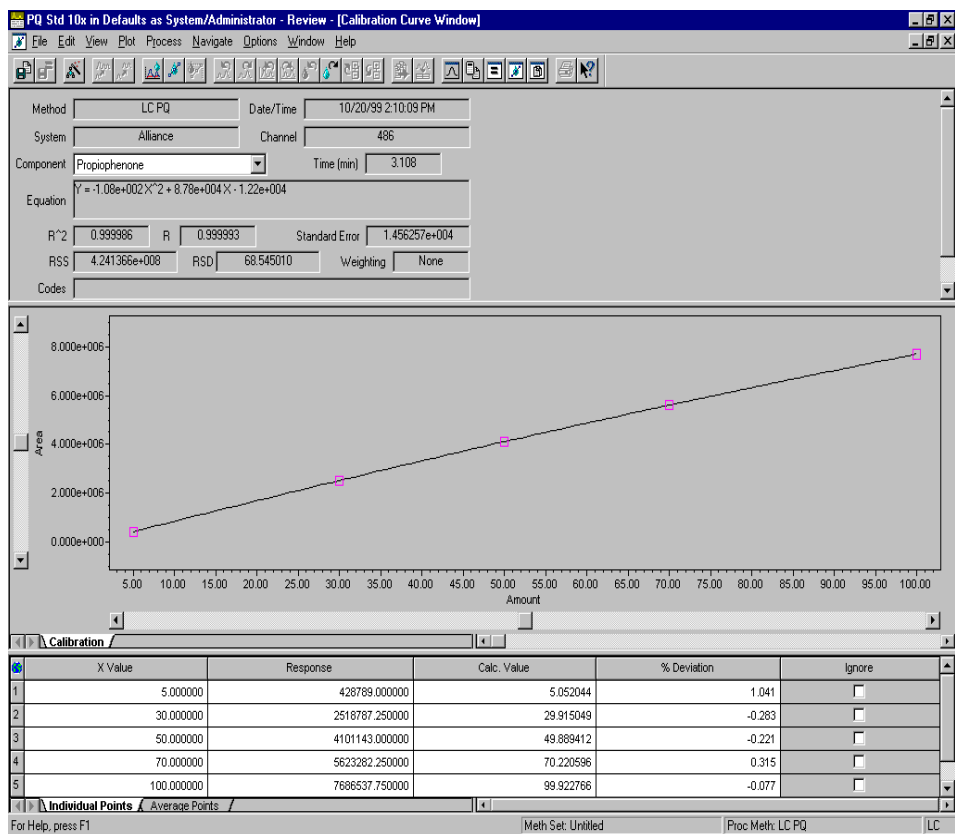


Figure 4-13 Quadratic Fit Calibration Curve

The quadratic fit generates a calibration curve that is represented by the equation:

$$y = A + Bx + Cx^2$$

where:

$y$  = Response of the standard peak calculated by the software

$x$  = Component amount or concentration

$A$ ,  $B$ , and  $C$  = Polynomial coefficients of the curve



## Determining Component Amount or Concentration

Component amount or concentration for a quantitated sample peak can be determined by solving for  $x$ :

$$x = \frac{-B \pm \sqrt{B^2 - 4C(A - y)}}{2C}$$

where:

$y$  = Response of the sample peak calculated by the software

$x$  = Component amount or concentration

$A$ ,  $B$ , and  $C$  = Polynomial coefficients of the curve

The software only reports positive values of  $x$  that are within the range of the calibration curve.

## Cubic Fit

To calculate a cubic fit calibration curve, the software performs a least-squares fit of a cubic polynomial to the calibration points. The fit cannot be performed with fewer than four calibration points, and a minimum of six points is strongly recommended.

Figure 4-14 illustrates a cubic fit calibration curve.

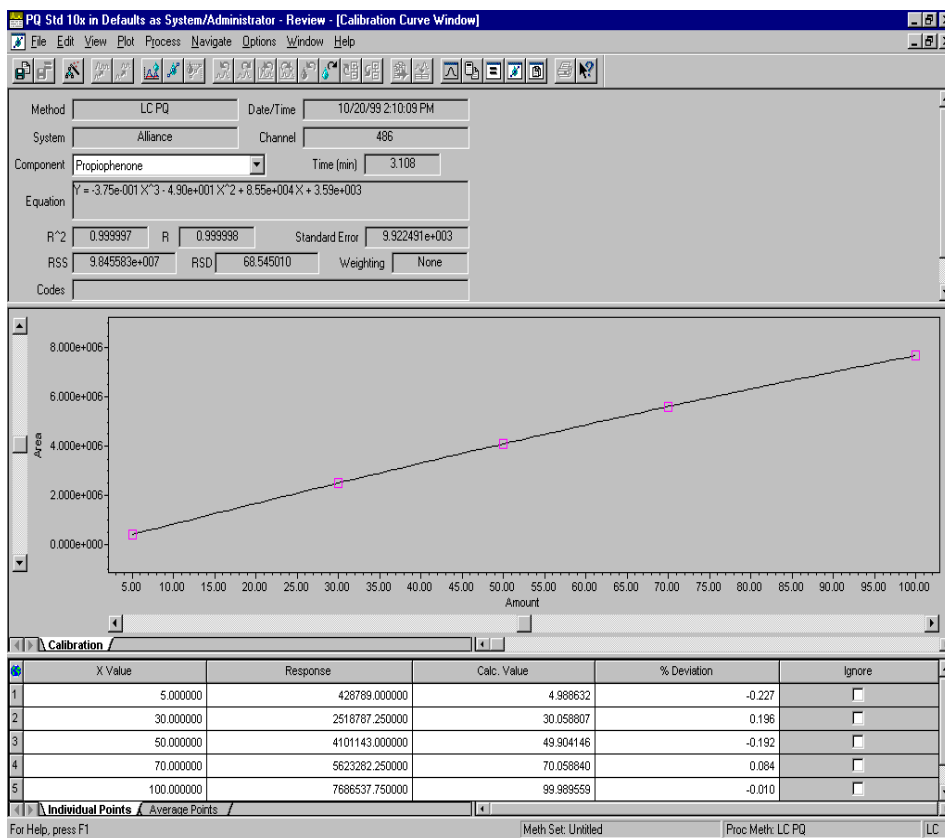


Figure 4-14 Cubic Fit Calibration Curve

The cubic fit generates a calibration curve that is represented by the equation:

$$y = A + Bx + Cx^2 + Dx^3$$

where:

$y$  = Response of the standard peak calculated by the software

$x$  = Component amount or concentration

$A, B, C,$  and  $D$  = Polynomial coefficients of the curve

## Fourth-Order Fit

To calculate a fourth-order fit calibration curve, the software performs a least-squares fit of a fourth-order polynomial to the calibration points. The fit cannot be performed with fewer than five calibration points, and a minimum of seven points is strongly recommended.

Figure 4-15 illustrates a fourth-order fit calibration curve.

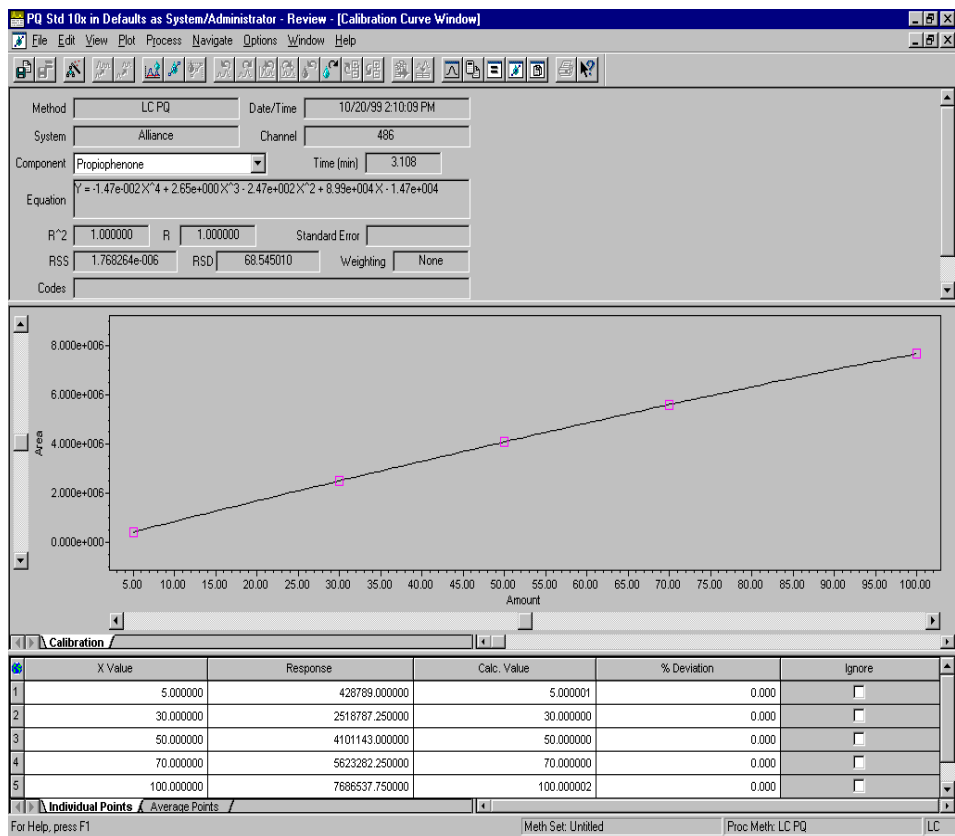


Figure 4-15 Fourth-Order Fit Calibration Curve

The fourth-order fit generates a calibration curve that is represented by the equation:

$$y = A + Bx + Cx^2 + Dx^3 + Ex^4$$

where:

$y$  = Response of the standard peak calculated by the software

$x$  = Component amount or concentration

$A, B, C, D,$  and  $E$  = Polynomial coefficients of the curve

## Fifth-Order Fit

To calculate a fifth-order fit calibration curve, the software performs a least-squares fit of a fifth-order polynomial to the calibration points. The fit cannot be performed with fewer than six calibration points, and a minimum of eight points is strongly recommended.

Figure 4-16 illustrates a fifth-order fit calibration curve.

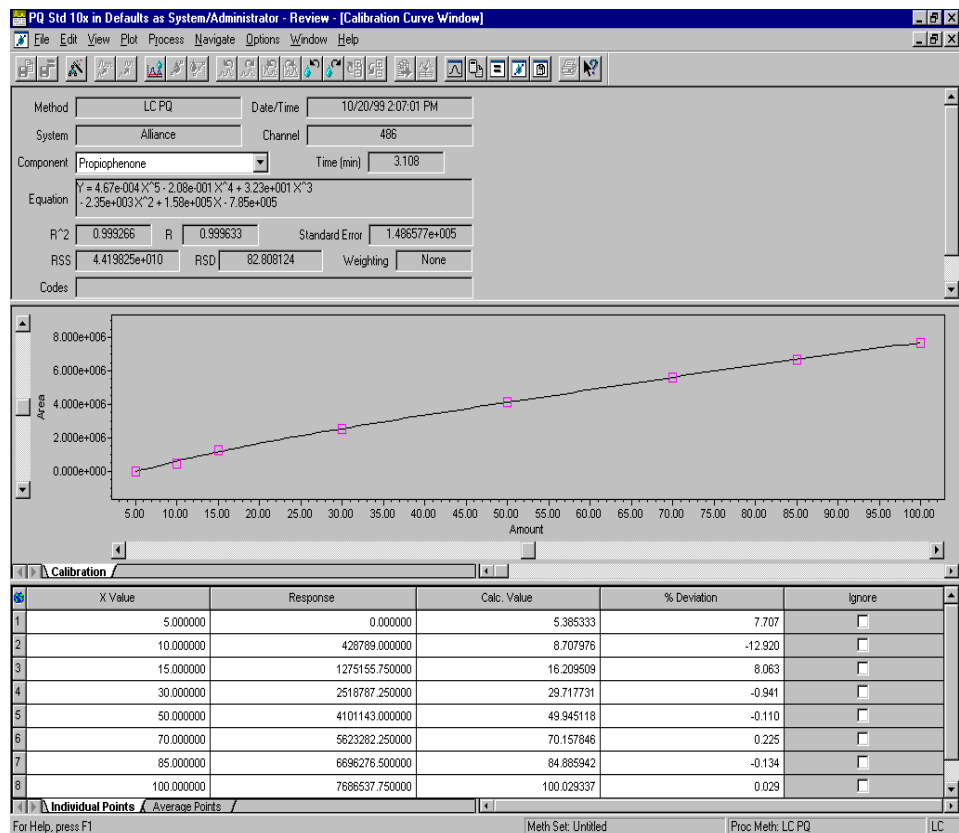


Figure 4-16 Fifth-Order Fit Calibration Curve

The fifth-order fit generates a calibration curve that is represented by the equation:

$$y = A + Bx + Cx^2 + Dx^3 + Ex^4 + Fx^5$$

where:

$y$  = Response of the standard peak calculated by the software

$x$  = Component amount or concentration

$A, B, C, D, E,$  and  $F$  = Polynomial coefficients of the curve

## 4.3.4 Multilevel Forced-Through-Zero Calibration Curves

The software supports forced-through-zero for the following multilevel curve fits:

- Linear
- Quadratic
- Cubic
- Fourth-order
- Fifth-order
- Response factor

Each forced-through-zero fit is similar to the corresponding nonforced-through-zero fit except that the curve is mathematically constrained to pass through zero. Forcing the calibration curve through zero results in different coefficients than those for nonforced calibration curves. For forced-through-zero fits, the zeroth order coefficient ( $C_0$ ) is set to 0, and the software computes the remaining coefficients.

$$y_1 = c_5(x_1)^5 + c_4(x_1)^4 + c_3(x_1)^3 + c_2(x_1)^2 + c_1(x_1)^1 + 0(x_1)^0$$

$$y_2 = c_5(x_2)^5 + c_4(x_2)^4 + c_3(x_2)^3 + c_2(x_2)^2 + c_1(x_2)^1 + 0(x_2)^0$$

$$y_3 = c_5(x_3)^5 + c_4(x_3)^4 + c_3(x_3)^3 + c_2(x_3)^2 + c_1(x_3)^1 + 0(x_3)^0$$

$$y_4 = c_5(x_4)^5 + c_4(x_4)^4 + c_3(x_4)^3 + c_2(x_4)^2 + c_1(x_4)^1 + 0(x_4)^0$$

$$y_5 = c_5(x_5)^5 + c_4(x_5)^4 + c_3(x_5)^3 + c_2(x_5)^2 + c_1(x_5)^1 + 0(x_5)^0$$

$$y_6 = c_5(x_6)^5 + c_4(x_6)^4 + c_3(x_6)^3 + c_2(x_6)^2 + c_1(x_6)^1 + 0(x_6)^0$$

$$y_7 = c_5(x_7)^5 + c_4(x_7)^4 + c_3(x_7)^3 + c_2(x_7)^2 + c_1(x_7)^1 + 0(x_7)^0$$

[Table 4-5](#) shows the differences between standard equations and forced-through-zero equations.

Table 4-5 Standard and Forced-Through-Zero Equation Format Comparison

Fit Type	Standard Equation	Forced-Through-Zero Equation
Linear	$y = A + Bx$	$y = Bx$
Quadratic	$y = A + Bx + Cx^2$	$y = B + Cx^2$
Cubic	$y = A + Bx + Cx^2 + Dx^3$	$y = Bx + Cx^2 + Dx^3$

For information on forced-through-zero fits, see the relevant nonforced-through-zero section for that type of fit. The software performs an equivalent computation, but coefficient A is always zero.

### 4.3.5 Weighting

Weighting is applied while fitting curves to multilevel points in order to do the following:

- Ensure that the points that have the most certainty (least error) contribute most strongly to the determination of the coefficients.
- Adjust for the differences in precision of the Y value (response or response ratio) with respect to the X value (amount or concentration).

To fit a curve to the calibration data, the software performs a least-squares fit to select the coefficients that minimize the sum of the differences between the individual points in the curve.

- Without weighting, all points contribute equally to that sum.
- With weighting, the contributions are adjusted to reflect the variance at each calibration level.

The equation that is minimized is:

$$\sum_{i=1} \frac{(\hat{y}_i - y_i)^2 w_i}{\text{DegreesOfFreedom}}$$

where:

$y_i$  = Observed data point

$\hat{y}_i$  = Calculated data point

$w_i$  = Weighting factor for each data point

*Degrees Of Freedom* = The number of points minus the number of coefficients calculated

Unweighted data assumes equal precision at all levels ( $w_i = 1$ ).

To select the weighting type, plot the standard deviation for each level versus the X value. Then select the weighting type based on the observed variation of the standard deviation by level.

Weighting can be applied to the standard fit types:

- Linear
- Quadratic
- Cubic
- Fourth-order
- Fifth-order

The types of weighting and the results of their application are described in [Table 4-6](#).

Table 4-6 Weighting Application Results

Weighting Type	Weighting Equation
x	$w_i = x_i$ : This results in a fit to the points at the high end of the curve (amounts or concentrations).
1/x and 1/x <sup>2</sup>	$w_i = 1/x_i$ or $w_i = 1/x_i^2$ : This results in a fit to the points at the low end of the curve (amounts or concentrations). The weight for the point and the coefficients cannot be calculated if x = 0. If there is a point where x = 0, the coefficients of the curve are not calculated and a processing code Q28 is copied into the curves code field (see <a href="#">Appendix A, Processing Codes</a> ).
1/y and 1/y <sup>2</sup>	$w_i = y_i$ : This results in a fit to the points at the low end of the curve (response). The weight for the point and the coefficients cannot be calculated if y = 0. If there is a point where y = 0, the coefficients of the curve are not calculated and a processing code Q30 is copied into the curves code field (see <a href="#">Appendix A, Processing Codes</a> ).
x <sup>2</sup>	$w_i = x_i^2$ : This results in a fit to the points at the high end of the curve (amounts or concentrations).
log x	$w_i = \log x_i$ : Produces a fit that weights the points on the calibration curve by a factor of log base 10, resulting in a logarithmic fit to the points on the high end of the calibration curve. If $x_i \leq 0$ , the weight for the point and the coefficients of the calibration curve are not calculated and a processing code Q29 is copied into the curves code field (see <a href="#">Appendix A, Processing Codes</a> ).
ln x	$w_i = \ln x_i$ : Produces a fit that weights the points on the calibration curve by a factor of the natural log of X, resulting in a logarithmic fit to the points on the high end of the calibration curve. If $x_i \leq 0$ , the weight for the point and the coefficients of the calibration curve are not calculated and a processing code Q29 is copied into the curves code field (see <a href="#">Appendix A, Processing Codes</a> ).

where:

$w_i$  = Weighting factor for each data point

$x_i$  = X value of the point

$y_i$  = Y value of the point

**Note:** If the software cannot calculate the weighted points, the coefficients for the curve are not calculated and a processing code is generated indicating the reason it is not calculated (see [Appendix A, Processing Codes](#)).

## 4.3.6 Statistics

Statistics indicate goodness of fit. The software calculates the following statistical criteria:

- Coefficient of determination
- Correlation coefficient
- Residual sum of squares
- Standard error of estimate of  $y$  on  $x$  (no report)<sup>1</sup>
- Standard variance (no report)<sup>1</sup>
- Standard error of calibration
- Percent Relative Standard Deviation
- Calculated value and percent deviation of the calibration points

### Coefficient of Determination

Coefficient of determination ( $R^2$ ) is a rough indicator of the goodness of fit and is calculated by:

$$R^2 = 1 - \frac{(S_y)^2}{\sigma_y^2}$$

where:

$R^2$  = Coefficient of determination

$R$  = Correlation coefficient

$S_y$  = Standard error of estimate of  $y$  on  $x$

$\sigma_y^2$  = Standard variance

### Correlation Coefficient

The correlation coefficient ( $R$ ) is an indicator of goodness of fit. It is the square root of the coefficient of determination.

### Standard Error of Estimate of Y on X

The standard error of estimate of  $y$  on  $x$  ( $S_y$ ) is used to determine  $R^2$  (the coefficient of determination) and  $R$  (the correlation coefficient) and is calculated by:

$$S_y = \sqrt{\frac{1}{n} \sum_{i=1}^n w_i (\hat{y}_i - y_i)^2}$$

where:

---

1. The software calculates these two criteria that are not reported as intermediate values.



- $n$  = Number of points
- $w_i$  = Weighting factor (set to 1 for uniform weighting)
- $\hat{y}_i$  = Responses as *predicted* using the calibration curve
- $y_i$  = Response of a calibration point

## Standard Variance

The standard variance ( $\sigma^2y$ ) is used to calculate the coefficient of determination and correlation coefficient. It is computed as follows:

$$\sigma^2y = \frac{1}{n} \sum_{i=1}^n w_i (y_i - \bar{y})^2$$

where:

- $w_i$  = Weighting factor (set to 1 for uniform weighting)
- $y_i$  = Response of a calibration point
- $\bar{y}$  = Weighted mean given by the equation:

$$\bar{y} = \frac{\sum_{i=1}^n w_i y_i}{\sum_{i=1}^n w_i}$$

## Residual Sum of Squares

Residual sum of squares (RSS) is an indicator of goodness of fit and precision of data. It is used to calculate the standard error of estimate, and the standard error of calibration. It is calculated by:

$$RSS = \sum_{i=1}^n w_i (\hat{y}_i - y_i)^2$$

where:

- $RSS$  = Residual sum of squares
- $n$  = Number of points
- $w_i$  = Weighting factor (set to 1 for uniform weighting)
- $\hat{y}_i$  = Responses as *predicted* using the calibration curve
- $y_i$  = Response of a calibration point

## Standard Error of Calibration

The standard error of calibration (E) is the square root of the sum that is minimized when fitting coefficients to the curve and is calculated by:

$$E = \sqrt{\frac{1}{d} \left( \sum_{i=1}^n w_i (\hat{y}_i - y_i)^2 \right)} = \sqrt{\frac{1}{d} RSS}$$

where:

$d$  = Degrees of Freedom = Number of points minus the number of coefficients calculated

$w_i$  = Weighting factor (set to 1 for uniform weighting)

$\hat{y}_i$  = Responses as *predicted* using the calibration curve

$y_i$  = Response of a calibration point

$RSS$  = Residual sum of squares

## Calculated Value and Percent Deviation of Calibration Points

The calculated value and percent deviation of calibration points can be used to assess how well the points fit the curve by visual inspection or by plotting against the X value.

Percent deviation is calculated by:

$$\% \text{ Deviation} = 100 \left( \frac{\hat{x}_i - x_i}{x_i} \right)$$

where:

$\hat{x}_i$  = X value as predicted using the calibration curve (the calculated value)

$x_i$  = X value of the calibration point

Plots of percent deviation versus amount should display random scatter if the fit type is correct. Plots of calculated value versus amount or concentration should be linear.

## Percent Relative Standard Deviation

The Percent RSD is an indication of goodness of fit and precision of the data.

Percent RSD is calculated by:

$$\%RSD = \frac{\left( \sum_{i=1}^n [w_i \cdot y_i - YWM]^2 / [n-1] \right)^{\frac{1}{2}}}{YWM} \cdot 100$$

where:

$w_i$  = Weighting factor (set to 1 for uniform weighting)

$y_i$  = Response of a calibration point

$YWM$  = Weighted mean response of all calibration points, which is expressed as:

$$YWM = \frac{\sum_{i=1}^n (w_i \cdot y_i)}{n}$$

$n$  = The number of points

## 4.4 References

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For further information on the theory of quantitation, see:

- Flannery, Brian P., *Numeric Recipes in C*, Cambridge University Press, Cambridge, UK, 1988.
- Massart, D.L., *et al.*, *Chemometrics: A Textbook*, Elsevier Science Publishers, Amsterdam, 1988.
- Papoulis, Athanasios, *Signal Analysis*, McGraw-Hill, New York, 1977.
- Snyder, L.R. and J.J. Kirkland, *Introduction to Modern Liquid Chromatography*, second ed., Wiley-Interscience, New York, 1979.
- Strang, Gilbert, *Linear Algebra and Its Applications*, Harcourt Brace Jovanovich, Inc., New York, 1988.

# Appendix A

## Processing Codes

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Processing problems are flagged in the Codes field of the Peaks table (Review Main window and Results window), the Chromatogram Result table of the Results window, the Peaks Calibration Curve Results table of the Results window, the Calibration Status area of the Calibration Curve window, and the Message Center. [Table A-1](#) summarizes the processing codes.

### Key

- C = Custom Calculation
- E = CE/CIA
- F = Base
- G = GPC/V (GPC or GPCV)
- I = Base
- L = Library Match (MS or PDA)
- LS = Light Scattering
- M = Pattern Match
- N = Base
- P = GPC, GPCV, LS, MS, or PDA
- Q = Base or GPC/V
- REF = Base
- S = Suitability
- V = GPCV
- W = PDA
- X = Base, GPC, GPCV, or LS
- Z = MS

Table A-1 Processing Codes

Processing Code	Meaning	Processing Code Type	Message Center Message	Option
C01	The Sample field value is not available.	Peak or Result	-----	Custom Calculations
C02	The Peak field value is not available.	Peak or Result	-----	Custom Calculations
C03	The Result field value is not available.	Peak or Result	-----	Custom Calculations
C04	The Chromatogram field value is not available.	Peak or Result	-----	Custom Calculations
C05	The Injection field value is not available.	Peak or Result	-----	Custom Calculations
C06	The value is too small to be used in division.	Peak or Result	-----	Custom Calculations
C07	The mantissa value used in the POW function is not valid.	Peak or Result	-----	Custom Calculations
C08	The exponent value used in the POW function is not valid.	Peak or Result	-----	Custom Calculations
C09	The value is not valid for Log10 function.	Peak or Result	-----	Custom Calculations
C10	The value is not valid for Ln function.	Peak or Result	-----	Custom Calculations
C11	The value is not valid for SQRT function.	Peak or Result	-----	Custom Calculations
C12	Peak specified by CCalRef1 is not found in result.	Peak or Result	-----	Custom Calculations
C13	No component name is found in CCalRef1 CCompRef1 of processing method.	Peak or Result	-----	Custom Calculations
C14	The peak specified by CCompRef1 is not found in result.	Peak or Result	-----	Custom Calculations
C15	No component name is found in CCompRef1 of processing method.	Peak or Result	-----	Custom Calculations

Table A-1 Processing Codes (Continued)

Processing Code	Meaning	Processing Code Type	Message Center Message	Option
C16	The peak specified by CCompRef2 is not found in result.	Peak or Result	-----	Custom Calculations
C17	No component name is found in CCompRef2 of processing method.	Peak or Result	-----	Custom Calculations
C18	The peak specified by CCompRef3 is not found in result.	Peak or Result	-----	Custom Calculations
C19	No component name is found in CCompRef3 of processing method.	Peak or Result	-----	Custom Calculations
C20	The CConst1 value of the component is not valid.	Peak or Result	-----	Custom Calculations
C21	The CConst2 value of the component is not valid.	Peak or Result	-----	Custom Calculations
C22	Sample custom field is not found.	Peak or Result	-----	Custom Calculations
C23	Result custom field is not found.	Peak or Result	-----	Custom Calculations
C24	Peak custom field is not found.	Peak or Result	-----	Custom Calculations
C25	Peak name is not found in result.	Peak or Result	-----	Custom Calculations
C26	Component is not found in processing method.	Peak or Result	-----	Custom Calculations
C27	Formula syntax error.	Result	-----	Custom Calculations
C28	The CConst3 value of the component is not valid.	Peak or Result	-----	Custom Calculations
C30	The CConst4 value of the component is not valid.	Peak or Result	-----	Custom Calculations
C31	The CConst5 value of the component is not valid.	Peak or Result	-----	Custom Calculations
C32	The CConst6 value of the component is not valid.	Peak or Result	-----	Custom Calculations
C33	The CConst7 value of the component is not valid.	Peak or Result	-----	Custom Calculations

Table A-1 Processing Codes (Continued)

Processing Code	Meaning	Processing Code Type	Message Center Message	Option
C34	The Sample Set field value is not available.	Peak or Result	-----	Custom Calculations
C35	Result is not found in a given result set using the intersample label specified.	Result	-----	Custom Calculations
C36	Result is not found in the current project using the intersample label specified.	Result	-----	Custom Calculations
C37	Failed to convert an Enum definition string to a value when Use As is set to value.	Peak or Result	-----	Custom Calculations
E01	Problem calculating mobility, cannot fetch a valid RunVoltage.	Result	-----	CE/CIA
F01	Result faulted, cannot find a Must peak.	Result	-----	Base
F02	Result faulted, cannot find a Default peak.	Result	-----	Base
F03	Result faulted, cannot find a RT Reference peak.	Result	-----	Base
F04	Result faulted, cannot find an Internal Standard peak.	Result	-----	Base
G01	Cannot calculate a y value from the bounded calibration curve statistics for this x.	Curve	-----	GPC/V
G02	Problem in calculating slope of bounded calibration curve.	Curve	-----	GPC/V
G03	Problem using a bounded calibration curve to calculate molecular weight.	Curve	-----	GPC/V
G04	Problem calculating a bounded calibration curve during a Gaus-Jordan elimination.	Curve	-----	GPC/V

Table A-1 Processing Codes (Continued)

A

Processing Code	Meaning	Processing Code Type	Message Center Message	Option
G05	Problem calculating a bounded calibration curve using the Levenberg-Marquardt method.	Curve	-----	GPC/V
G06	Problem fitting a bounded calibration curve to the calibration data.	Curve	-----	GPC/V
G07	Problem fitting a bounded calibration curve to the calibration data, invalid $v_0$ - $v_t$ calculated.	Curve	-----	GPC/V
G08	Problem setting up to calculate MP.	Result	-----	GPC/V
G09	Calculated MP from an invalid calibration curve.	Result	-----	GPC/V
G10	Problem calculating MP, cannot find a viscosity peak.	Peak	-----	GPCV
G11	Problem using calibration curve to calculate MP.	Peak	-----	GPC/V
G12	Problem calculating MP, could not find the reference peak.	Result	-----	GPC/V
G13	Problem setting up for Narrow standard calibration.	Result	-----	GPC/V
G14	Cannot continue Narrow standard calibration, no peaks.	Result	-----	GPC/V
G15	Cannot continue Narrow standard calibration, no molecular weights.	Result	-----	GPC/V
G16	Cannot continue Narrow standard GPCV calibration, no concentration.	Result	-----	GPCV
G17	Cannot continue Narrow standard calibration, invalid molecular weight.	Result	-----	GPC/V
G18	Cannot continue Narrow standard GPCV calibration, cannot find the viscosity peak.	Peak	-----	GPCV
G19	Cannot continue Narrow standard calibration, problem calculating viscosity of narrow standard point.	Peak	-----	GPC/V



Table A-1 Processing Codes (Continued)

A

Processing Code	Meaning	Processing Code Type	Message Center Message	Option
G20	Cannot continue GPCV processing, no injection volume.	Result	-----	GPCV
G21	Problem setting up for Axial Dispersion.	Peak	-----	GPC/V
G22	Problem in Iterative Deconvolution, too many elements in convolution array.	Peak	-----	GPC/V
G23	Problem in Peak Compression, peak variance < 0.0.	Peak	-----	GPC/V
G24	Problem in Peak Compression, deconvoluted peak variance < 0.0.	Peak	-----	GPC/V
G25	Problem in Iterative Deconvolution, either sigma is negative, or there are zero elements in array.	Peak	-----	GPC/V
G26	Problem fitting moments to distribution, simplex minimization failed.	Peak	-----	GPC/V
G27	Problem fitting moments to distribution, Simplex minimization fits a positive slope.	Peak	-----	GPC/V
G28	Problem fitting moments to distribution, need at least 2 moments.	Peak	-----	GPC/V
G29	Problem fitting moments to distribution, failed to calculate enough moments.	Peak	-----	GPC/V
G30	Problem calculating distribution molecular weights, cannot calculate moments.	Peak	-----	GPC/V
G31	Problem with distribution molecular weights, cannot calculate moments.	Peak	-----	GPC/V
G32	Problem with distribution or alpha, cannot calculate moments.	Peak	-----	GPC/V
G33	User did not enter concentration for GPCV sample, cannot continue processing.	Peak	-----	GPCV

Table A-1 Processing Codes (Continued)

A

Processing Code	Meaning	Processing Code Type	Message Center Message	Option
G34	Problem calculating peak concentration, cannot continue GPCV processing.	Peak	-----	GPCV
G35	Cannot continue GPCV processing, cannot find the viscosity peak.	Peak	-----	GPCV
G36	Problem setting up to quantitate a broad unknown.	Result	-----	GPC/V
G37	Problem quantitating a broad unknown, the calibration curve has no coefficients.	Result	-----	GPC/V
G38	Problem quantitating a broad unknown, cannot calculate the distribution when Calibration Order is Low to High.	Result	-----	GPC/V
G39	Problem calculating the distribution, the calibration curve is not valid.	Peak	-----	GPC/V
G40	Problem calculating distribution, cannot find a viscosity peak.	Peak	-----	GPCV
G41	Problem calculating distribution, the distribution molecular weights are no longer decreasing.	Peak	-----	GPC/V
G42	Problem setting up to calibrate a Broad Standard.	Result	-----	GPC/V
G43	Problem calibrating a Broad Standard, cannot calculate the distribution when Calibration Order is Low to High.	Result	-----	GPC/V
G44	Problem in Broad standard calibration, problem calculating viscosity of Broad Standard point.	Peak	-----	GPC/V
G45	Cannot fit moments to Broad Standard peak, calculated slope is not negative. Check Broad Standard peak integration and Slicing tab in the processing method.	Peak	-----	GPC/V

Table A-1 Processing Codes (Continued)

A

Processing Code	Meaning	Processing Code Type	Message Center Message	Option
G46	Problem cannot continue processing a distribution with so few slices.	Peak	-----	GPC/V
G47	Problem finding the Named Distribution, cannot continue processing the Broad Standard.	Peak	-----	GPC/V
G48	Cannot add calibration point because the distribution slice has zero area.	Peak	-----	GPC/V
G49	Problem normalizing the distribution, the calibration curve does not have a valid slope.	Peak	-----	GPC/V
G50	Problem normalizing the distribution, the total normalized area $\leq 0.0$ .	Peak	-----	GPC/V
G51	Problem analyzing the distribution, High MW $\leq$ Low MW.	Peak	-----	GPC/V
G52	Sigma converts to $< 0.5$ data points.	Peak	-----	GPC/V
G53	Not enough data points to perform initial smoothing.	Peak	-----	GPC/V
G54	Not enough data points to perform final smoothing.	Peak	-----	GPC/V
G55	Not enough data points to perform either initial or final smoothing.	Peak	-----	GPC/V
G56	Not enough data points in noise interval, need at least 20.	Peak	-----	GPC/V
G57	Performed maximum iterations without converging, either the peak is too narrow or sigma is too large.	Peak	-----	GPC/V
G58	Problem setting up to calculate moments, cannot find fields.	Result	-----	GPC/V
G59	Cannot find apex of distribution, cannot calculate moments.	Peak	-----	GPC/V
G60	Cannot calculate the calibration curve, the overall slope of the calibration curve is invalid for the chosen Calibration Order of Molecular Weights.	Curve	-----	GPC/V

Table A-1 Processing Codes (Continued)

A

Processing Code	Meaning	Processing Code Type	Message Center Message	Option
G61	A GPCV result cannot be created when the GPC Technique is Relative, change the technique to Universal and reprocess.	-----	Warning	GPCV
G62	Problem normalizing the distribution, the logMw is not monotonic. Please check the calibration curve and integration.	Peak	-----	GPC/V
I01	Problem reading chromatogram, cannot detect peaks.	Result	-----	Base
I02	Problem reading chromatogram, cannot manually integrate peaks.	Result	-----	Base
I03	User manually deleted a peak.	Result	-----	Base
I04	User manually deleted a drop line.	Peak	-----	Base
I05	Problem fitting curve to top of peak, not enough points across the peak.	Peak	-----	Base
I06	Problem fitting curve to top of peak, points not symmetrical around the peak maximum.	Peak	-----	Base
I07	Problem fitting curve to top of peak, cannot fit a quadratic curve to the top of the peak.	Peak	-----	Base
I08	Problem fitting curve to top of peak, cannot find fitted retention time inside region at the top of peak.	Peak	-----	Base
I09	Traditional Integration: Performing v2.XX Retention Time Calculations.	Result	-----	Base
I10	Traditional Integration: Cannot detect peaks because Peak Width and/or Threshold is blank.	Result	-----	Base
I11	Cannot detect peaks because cannot calculate Peak Width.	Result	-----	Base
I12	Cannot detect peaks because cannot calculate Threshold.	Result	-----	Base

Table A-1 Processing Codes (Continued)

Processing Code	Meaning	Processing Code Type	Message Center Message	Option
I13	Cannot calculate Peak Width region has less than 5 points.	-----	Warning in Review <sup>a</sup>	Base
I14	Cannot calculate Threshold because region has less than 20 points.	-----	Warning in Review <sup>a</sup>	Base
I15	Processing skipped because of disallowed integration algorithm in processing method.	-----	Error	Base
I17	Warning data collected with nonuniform sampling rate.	Result	-----	Base
I18	Result produced by processing a partially collected chromatogram.	Result	-----	Base
I19	ApexTrack Integration: Retention time and height were calculated from a 3-point fit.	Peak	-----	Base
I20	ApexTrack Integration: Retention time and height were calculated at the second derivative apex without a fit.	Peak	-----	Base
I21	ApexTrack Integration: Processing failed a 5-point fit when calculating retention time and height.	Peak	-----	Base
I22	ApexTrack Integration: Processing failed a 3-point fit when calculating retention time and height.	Peak	-----	Base
I23	ApexTrack Integration: The point in the peak farthest from the baseline was used to calculate the retention time and height.	Peak	-----	Base
L01	Library is empty, does not contain any spectra.	Result		PDA/MS
L02	Library could not be found.	Result		PDA/MS
L03	Wiley library is not installed.	Result		MS
L04	Wiley NIST library is not installed.	Result		MS

A

Table A-1 Processing Codes (Continued)

Processing Code	Meaning	Processing Code Type	Message Center Message	Option
LS01	Problem calculating RI Constant using LC peak, unknown units for peak amount.	Peak		LS
LS02	Cannot model Rg because there is only one LS channel to process. Rg is forced to zero during processing.	Peak		LS
LS03	Problem calculating mass injected for LC peak, unknown units for peak amount.	Peak		LS
LS04	Molecular weights entered for the standard were ignored.	Peak		LS
LS10	Problem continuing light scattering processing, memory allocation failure.	Result or Peak		LS
LS11	Problem performing light scattering processing, mismatched sample type for light scattering quantitation.	Result		LS
LS12	Problem performing light scattering processing, the processing type is not supported currently.	Result		LS
LS13	Problem performing light scattering processing, invalid light-scattering normalization, or not normalized.	Result		LS
LS14	Problem performing light scattering processing, invalid light-scattering calibration, or not calibrated, or doesn't exist.	Result		LS
LS15	Problem performing light scattering processing, cannot find or fetch the light-scattering calibration from database.	Result		LS
LS16	Problem performing light scattering processing, cannot find component table, or the table is empty.	Result		LS

A

Table A-1 Processing Codes (Continued)

Processing Code	Meaning	Processing Code Type	Message Center Message	Option
LS17	Problem performing light scattering processing, cannot find light-scattering peaks, channels may not be properly integrated.	Peak or Result		LS
LS18	Problem continuing light scattering processing, cannot find light-scattering chromatograms due to some internal error.	Result		LS
LS19	Warning: not all light-scattering channels are calibrated.	Result		LS
LS20	Problem performing light scattering processing, none of the light-scattering channels are calibrated.	Result		LS
LS21	Problem continuing light scattering processing, cannot read light-scattering chromatograms due to some internal error.	Peak		LS
LS22	Problem performing light scattering processing, the light scattering data is empty.	Result		LS
LS23	Problem performing light scattering processing, the given result and chromatograms do not match, internal code.	Result		LS
LS24	Problem performing light scattering processing, due to improper integration, or peak type, such as skimmed, negative, or invalid baseline, etc.	Peak		LS
LS25	Problem performing light scattering processing, cannot find suitable peak (slice) region to perform calculation. Rayleigh ratio, concentration, or specific viscosity may be negative across the peak.	Peak		LS

A

Table A-1 Processing Codes (Continued)

Processing Code	Meaning	Processing Code Type	Message Center Message	Option
LS26	Problem performing light scattering processing, wavelength is not given in the calibration.	Peak		LS
LS27	Problem performing light scattering processing, solvent refractive index is not given in the calibration.	Peak		LS
LS28	Problem performing light scattering processing, peak dn/dc is not given or not calculated.	Peak		LS
LS29	Problem performing light scattering processing, peak A2 is not given.	Peak		LS
LS30	Problem performing light scattering processing, flow rate is not given in the processing method or light scattering calibration.	Peak		LS
LS31	Problem performing light scattering processing, one or more angles are not given in the calibration.	Peak		LS
LS32	Warning: there may be problems in light scattering processing, the concentration channel is not properly calibrated, due to missing sample concentration or dn/dc, or RI calibration.	Peak		LS
LS33	Rg cannot be modeled either due to the small size of the molecule, or incorrect normalization constants in the calibration. Rg is forced to zero during processing.	Peak		LS
LS34	Problem performing light scattering processing, the data is empty, or some information needed for processing is missing.	Peak		LS

A



Table A-1 Processing Codes (Continued)

Processing Code	Meaning	Processing Code Type	Message Center Message	Option
LS35	Problem performing light scattering processing, Levenberg-Marquardt non-linear fit method cannot find solution.	Peak		LS
LS36	Problem performing light scattering processing, incorrect modeling, or insufficient data points across the peak.	Peak		LS
LS37	Problem performing light scattering processing, cannot initialize the Levenberg-Marquardt non-linear fit. The data may not be suitable for current modeling.	Peak		LS
LS38	Problem performing light scattering processing, Levenberg-Marquardt non-linear fit method has reached maximum number of iterations but cannot find solution.	Peak		LS
LS39	Internal warning from Levenberg-Marquardt non-linear fit method, the last iteration is voided due to invalid result.	Peak		LS
LS40	Problem performing light scattering processing, Levenberg-Marquardt non-linear fit has stopped due to matrix error.	Peak		LS
LS41	Problem performing light scattering processing, an internal error happened.	Peak		LS
LS42	Internal warning: there are more warning messages.	Peak		LS
LS43	Cannot perform light-scattering calibration, channels are not normalized, or the light-scattering data used for normalization do not cover all the channels specified in the instrument or calibration.	Result		LS

A

Table A-1 Processing Codes (Continued)

Processing Code	Meaning	Processing Code Type	Message Center Message	Option
LS44	Cannot perform light-scattering calibration, wrong sample type for normalization or calibration.	Result		LS
LS45	Cannot perform light-scattering calibration, molecular weight is not given, or invalid for calibration.	Result or Peak		LS
LS46	Notification message: processing stopped for light-scattering normalization.	Result or Peak		LS
LS47	Notification message: processing stopped for light-scattering calibration.	Result or Peak		LS
LS48	Cannot perform light scattering calibration, only a dual-detection method set should be used for narrow standard calibration. Viscosity channel is not used for calibration.	Result		LS
LS49	The intrinsic viscosity for the GPC peak with light scattering data was obtained by extrapolating the calibration curve outside $V_0$ and $V_t$ .	Peak		LS
LS50	Problem using calibration curve to calculate $[\eta]$ for GPC peak with light scattering data.	Peak		LS
LS51	Problem calculating $K(LS)$ and $\alpha(LS)$ for nonlinear $R_g$ fit.	Peak		LS
LS52	Problem calculating branching, distribution is not monotonic. Check the $R_g$ Fit.	Peak		LS
LS53	Problem calculating star branching, could not find the linear $K(LS)$ and $\alpha(LS)$ in the sample.	Peak		LS
LS54	Problem calculating overall $g$ .	Peak		LS

A

Table A-1 Processing Codes (Continued)

Processing Code	Meaning	Processing Code Type	Message Center Message	Option
LS55	Cannot perform light-scattering calibration, molecular weight is not calculated due to failure in processing.	Result or Peak		LS
LS56	The reported epsilon value was calculated from the LS data and the epsilon in the processing method was ignored.	Peak		LS
M01	Cannot perform pattern matching because the label is wrong or the reference chromatogram cannot be found in the database.	Result	-----	Pattern Match
M02	Cannot pattern match a chromatogram against itself.	Result	-----	Pattern Match
M03	Cannot resample the reference or sample chromatogram.	Result	-----	Pattern Match
M04	Cannot perform pattern matching because no Peak Width is calculated.	Result	-----	Pattern Match
M05	Cannot perform pattern matching because no apex detection threshold is calculated.	Result	-----	Pattern Match
M06	Cannot perform pattern matching because the start and stop time are so close one interval could not be calculated.	Result	-----	Pattern Match
M07	Cannot perform pattern matching because the alignment interval is greater than the number of points in the reference chromatogram.	Result	-----	Pattern Match
M08	Cannot perform pattern matching because the alignment interval is greater than the number of points in the sample chromatogram.	Result	-----	Pattern Match

A

Table A-1 Processing Codes (Continued)

Processing Code	Meaning	Processing Code Type	Message Center Message	Option
M09	Cannot perform pattern matching because the number of points in retention time search range is too large.	Result	-----	Pattern Match
M10	Cannot perform pattern matching because an internal calculation error occurred.	Result	-----	Pattern Match
N01	Problem calculating Detector Noise, cannot read chromatogram.	Result	-----	Base
N02	Problem calculating Detector Noise, need at least 60 points in time interval to perform calculation.	Result	-----	Base
N03	Problem calculating Detector Noise, need at least 30 points in segment to perform calculation.	Result	-----	Base
P01	Problem calculating PDA noise vector, cannot continue PDA processing.	Result	-----	PDA
P02	Problem calculating PDA Purity Pass 1.	Peak	-----	PDA
P03	Problem calculating PDA Purity Pass 2 (3 or 4).	Peak	-----	PDA
P04	Problem extracting a derived channel beyond limits of 3D chromatogram.	-----	Warning	PDA or MS
P05	Cannot process PDA data because the PDA option is not enabled.	-----	Warning	PDA
P06	Cannot process MS data because the MS option is not enabled.	-----	Warning	MS
P07	Cannot process a PDA result because the PDA option is not enabled.	-----	Warning	PDA
P08	Cannot process a MS result because the MS option is not enabled.	-----	Warning	MS

A

Table A-1 Processing Codes (Continued)

A

Processing Code	Meaning	Processing Code Type	Message Center Message	Option
P09	Cannot process a GPC result because the GPC option is not enabled.	-----	Warning	GPC
P10	Cannot process a GPCV result because the GPCV option is not enabled.	-----	Warning	GPCV
P11	Cannot process a GPCV Channel Set because the GPCV option is not enabled.	-----	Warning	GPCV
P12	Cannot process data using a PDA processing method because the PDA option is not enabled.	-----	Warning	PDA
P13	Cannot process data using a MS processing method because the MS option is not enabled.	-----	Warning	MS
P14	Cannot process data using a GPC processing method because the GPC option is not enabled.	-----	Warning	GPC
P15	Cannot perform GPCV processing because the GPCV option is not enabled.	-----	Warning	GPCV
P16	Problem performing PDA blank subtraction, cannot fetch the blank chromatogram.	-----	Error	PDA
P17	Cannot process an LS result because the LS option is not enabled.	-----	Warning	LS
P18	Problem storing 2D chromatogram from 3D chromatogram.	-----	Warning	PDA/MS
P19	Could not perform a PDA blank subtraction as requested because the processing method is not a PDA processing method.	Result	-----	PDA

Table A-1 Processing Codes (Continued)

Processing Code	Meaning	Processing Code Type	Message Center Message	Option
Q01	Cannot set peak response, the custom peak field has not been calculated.	Peak	-----	Base
Q02	Retention time reference peak cannot be found, best peak is already chosen.	Result	-----	Base
Q03	Problem calculating retention time reference peak ratio.	Result	-----	Base
Q04	Problem matching peak to only one component, peak is an equal match for multiple components.	Peak	-----	Base
Q05	Problem fetching sample information, cannot continue processing.	Result	-----	GPC/V
Q06	Numbers of components and calibration curves is not equal, cannot continue processing.	Result	-----	Base
Q07	Problem calculating amount or concentration, no injection volume.	Peak	-----	Base
Q08	Problem calculating Amount or Concentration, negative value calculated.	Peak	-----	Base
Q09	Amount or Concentration is greater than calculated value of highest point in the calibration curve.	Peak	-----	Base
Q10	Amount or Concentration is less than calculated value of lowest point in the calibration curve.	Peak	-----	Base
Q11	Problem setting Amount or Concentration, there are multilevel defaults and no sample level.	Peak	-----	Base
Q12	Problem manually identifying a peak, cannot process when the component is not found.	Result	-----	Base

A

Table A-1 Processing Codes (Continued)

A

Processing Code	Meaning	Processing Code Type	Message Center Message	Option
Q13	Problem manually identifying a peak, cannot process with the wrong calibration.	Result	-----	Base
Q14	User manually identified a peak in the result.	Result	-----	Base
Q15	User added a name to the peak.	Peak	-----	Base
Q16	User removed a name from the peak.	Peak	-----	Base
Q17	Problem calculating slope of calibration curve, there are 2 points with same x value.	Curve	-----	Base
Q18	Problem in least square fit of calibration curve.	Curve	-----	Base
Q19	Problem in computation of calibration curve statistics.	Curve	-----	Base
Q20	Invalid calibration curve, it has a flat slope.	Curve	-----	Base
Q21	Problem calculating x value from calibration curve, there are 2 points with same x value.	Curve	-----	Base
Q22	Problem calculating coefficient of determination, it cannot be less than 0.0. (Obsolete in v.4.0 and later)	Curve	-----	Base
Q23	Correlation coefficient either < 0.0 and set to 0.0 or > 1.0 and set to 1.0. (Obsolete in v.4.0 and later)	Curve	-----	Base
Q24	Problem calculating variance, variance = 0.0.	Curve	-----	Base
Q25	Problem using Inverse Linear fit, cannot calculate inverse of x value when x value = 0.0.	Curve	-----	Base
Q26	Problem using Log Log Linear fit, cannot calculate log of x value when x value <= 0.0.	Curve	-----	Base

Table A-1 Processing Codes (Continued)

A

Processing Code	Meaning	Processing Code Type	Message Center Message	Option
Q27	Problem using Log Log Linear fit, cannot calculate log of response when response $\leq 0.0$ .	Curve	-----	Base
Q28	Problem calculating weight of point when $x = 0.0$ .	Curve	-----	Base
Q29	Problem calculating weight of point when $x \leq 0.0$ .	Curve	-----	Base
Q30	Problem calculating weight of point when $y = 0.0$ .	Curve	-----	Base
Q31	Problem reading chromatogram, cannot continue processing.	Result	-----	GPC/V
Q32	Problem calibrating, cannot calibrate an unknown.	Result	-----	Base
Q33	Problem calibrating, incompatible processing method and calibration types.	Result	-----	GPC/V
Q34	Problem quantitating, incompatible processing method and calibration types.	Result	-----	GPC/V
Q35	Problem calibrating a GPCV result without a viscosity channel.	Result	-----	GPCV
Q36	Problem quantitating a GPCV result without a viscosity channel.	Result	-----	GPCV
Q37	Problem rebuilding a GPCV distribution cannot fetch the viscosity channel.	Result	Warning	GPCV
Q38	Problem rebuilding a distribution, cannot fetch the channel, processing method or calibration.	Result	Warning	GPC/V
Q39	Problem using Response Factor fit, cannot calculate Response Factor ( $y/x$ ) because $x = 0.0$ .	Curve	-----	Base
Q40	Problem calculating RI Constant, there is no RI Sensitivity entered in the sample.	Peak	-----	GPCV or LS



Table A-1 Processing Codes (Continued)

Processing Code	Meaning	Processing Code Type	Message Center Message	Option
Q41	Problem calculating RI Constant, there is a zero RI Sensitivity entered in the sample.	Peak	-----	GPCV or LS
Q42	Problem calculating peak concentration, there is no RI Sensitivity entered in the sample.	Peak	-----	GPCV or LS
Q43	Problem calculating peak concentration, there is a zero RI Sensitivity entered in the sample.	Peak	-----	GPCV or LS
Q44	Problem calculating dn/dc, there is no RI Sensitivity entered in the sample.	Peak	-----	GPCV or LS
Q45	Problem calculating dn/dc, there is a zero RI Sensitivity entered in the sample.	Peak	-----	GPCV or LS
Q46	Problem using response and calibration curve to calculate x value. Check calibration curve and peak response.	Peak, Curve	-----	Base
Q47	Problem calculating a distribution, the component name listed in the sample is not listed in the Slicing table of the processing method.	Result	-----	GPC/V
Q48	Problems calculating standard error, the degrees of freedom are less than 1.	Curve	-----	Base
Q49	Component name mismatch, there are component names listed in the sample but, none of them match the component names listed in the processing method.	Result	-----	Base
Q50	Problem quantitating peak, an Internal Standard component cannot be used as a Curve Reference.	Peak	-----	Base
Q51	Problem quantitating unknown peak, an Internal Standard component cannot be used as a Default peak.	Peak	-----	Base

A

Table A-1 Processing Codes (Continued)

Processing Code	Meaning	Processing Code Type	Message Center Message	Option
Q52	Peak is missing because its component's channel was not found in the processed injection.	Peak	-----	Base
Q53	Problem setting amount or concentration, there are multilevel defaults and no value for this level.	Peak	-----	Base
Q54	Problem calculating response, the internal standard peak has no amount or concentration.	Peak	-----	Base
REF	Indicates that the peak is an RT reference peak.	Peak	-----	Base
S01	Problem calculating Baseline Noise, run too short.	Result	-----	Suitability
S02	Problem calculating Baseline Noise, not enough baseline.	Result	-----	Suitability
S03	Problem calculating Baseline Noise, start time too close to end of run.	Result	-----	Suitability
S04	Problem calculating Baseline Noise, end time too close to beginning of run.	Result	-----	Suitability
S05	Problem calculating Peak Width at 4.4% height.	Peak	-----	Suitability
S06	Problem calculating Peak Width at 5% height.	Peak	-----	Suitability
S07	Problem calculating Peak Width at 10% height.	Peak	-----	Suitability
S08	Problem calculating Peak Width at 13.4% height.	Peak	-----	Suitability
S09	Problem calculating Peak Width at 32.4% height.	Peak	-----	Suitability
S10	Problem calculating Peak Width at 50% height.	Peak	-----	Suitability

Table A-1 Processing Codes (Continued)

Processing Code	Meaning	Processing Code Type	Message Center Message	Option
S11	Problem calculating Peak Width at 60.7% height.	Peak	-----	Suitability
S12	Problem calculating all Peak Widths, retention time is same as start or end time.	Peak	-----	Suitability
S13	Problem calculating multiple Peak Widths. (Obsolete in v.4.0 and later)	Peak	-----	Suitability
S14	Problem calculating Resolution, check the peak widths.	Peak	-----	Suitability
S15	Problem calculating K Prime and Selectivity, check the void volume and retention time.	Peak	-----	Suitability
S16	Problem calculating USP Plate Count, cannot calculate the peak's tangent width.	Peak	-----	Suitability
S17	Problem calculating Tailing and/or Symmetry Factor, cannot calculate the peak width at 5%.	Peak	-----	Suitability
S18	Problem calculating Tailing and/or Symmetry Factor, retention time <= the front side x-value at 5% height.	Peak	-----	Suitability
S19	Problem calculating asymmetry, cannot calculate sigma (the numerator). (Obsolete in v.4.0 and later)	Peak	-----	Suitability
S20	Problem calculating asymmetry, cannot calculate peak width at 10% height. (Obsolete in v.4.0 and later)	Peak	-----	Suitability
S21	Problem calculating tangent width, cannot calculate peak widths at (tangent +/- 5%) height.	Peak	-----	Suitability
S22	Problem calculating tangent width, cannot calculate the tangent lines.	Peak	-----	Suitability
S23	Problem calculating tangent width, cannot calculate the intercepts.	Peak	-----	Suitability

A

Table A-1 Processing Codes (Continued)

A

Processing Code	Meaning	Processing Code Type	Message Center Message	Option
S24	Problem calculating sigma width, cannot calculate peak width at %. (Obsolete in v.4.0 and later)	Peak	-----	Suitability
S25	Problem calculating asymmetry widths, retention time <= the front side x-value at % height.	Peak	-----	Suitability
S26	Problem calculating asymmetry widths, cannot calculate peak width at % height.	Peak	-----	Suitability
S27	Problem calculating USP Resolution, check the peak widths.	Peak	-----	Suitability
S28	Problem calculating USP Resolution (HH), check the peak widths.	Peak	-----	Suitability
S29	Width at Tangent is calculated using lines tangent to the inflection points.	Peak	-----	Suitability
V01	Problem fitting a curve to viscosity data, not enough points in the distribution.	Peak	-----	GPCV
V02	Viscosity peak is not suitable for intrinsic viscosity fit, please check peak integration and verify that both the concentration and viscosity peaks start and end within the $v_0$ and $v_t$ of the calibration curve.	Peak	-----	GPCV
V03	Problem calculating $MN(v)$ , check viscometer peak integration.	Peak	-----	GPCV
V04	Problem calculating overall g.	Peak	-----	GPCV
V05	Problem fitting curve to viscosity data, user did not enter K and Alpha for Broad Standard.	Peak	-----	GPCV
V06	Problem fitting linear curve to viscosity data.	Peak	-----	GPCV
V07	Problem fitting nonlinear polynomial curve to viscosity data.	Peak	-----	GPCV

Table A-1 Processing Codes (Continued)

Processing Code	Meaning	Processing Code Type	Message Center Message	Option
V08	Problem setting up to fit curve to viscosity data.	Peak	-----	GPCV
V09	Obsolete in v.3.2 and later. (Problem calculating branching, user entered multiple K alpha pairs).	Peak	-----	GPCV
V10	Problem fitting nonlinear curve to viscosity data.	Peak	-----	GPCV
V11	Problem with the Chebyshev term while fitting a nonlinear curve to viscosity data.	Peak	-----	GPCV
V12	Problem fitting initial linear curve to viscosity data.	Peak	-----	GPCV
V13	Problem fitting viscosity data, data region start and end molecular weights are equal.	Peak	-----	GPCV
V14	Problem performing initial fit of nonlinear curve to viscosity data.	Peak	-----	GPCV
V15	Problem optimizing fit of nonlinear curve to viscosity data.	Peak	-----	GPCV
V16	Problem computing polynomial coefficients from Chebyshev coefficients.	Peak	-----	GPCV
V17	Problem optimizing Linear fit to viscosity data.	Peak	-----	GPCV
V18	Problem performing Linear fit to viscosity data, cannot estimate alpha because the two point indices are bad.	Peak	-----	GPCV
V19	Problem performing Linear fit to viscosity data, cannot estimate alpha from these two points.	Peak	-----	GPCV
V20	Problem performing Linear fit to viscosity data, the estimated alpha is not positive. Check integration.	Peak	-----	GPCV
V21	Problem optimizing Random fit to viscosity data.	Peak	-----	GPCV

A

Table A-1 Processing Codes (Continued)

Processing Code	Meaning	Processing Code Type	Message Center Message	Option
V22	Problem estimating alpha for first third of viscosity data for Random fit.	Peak	-----	GPCV
V23	Problem estimating alpha for last third of viscosity data for Random fit.	Peak	-----	GPCV
V24	Problem estimating Alpha for all of viscosity data for Random fit.	Peak	-----	GPCV
V25	Problem determining log K and Alpha for first third of viscosity data, cannot do Random fit.	Peak	-----	GPCV
V26	Problem determining log K and Alpha for last third of viscosity data, cannot do Random fit.	Peak	-----	GPCV
V27	Problem determining log K and Alpha for viscosity data, cannot do Random fit.	Peak	-----	GPCV
V28	Viscosity data is not suitable for Random fit, cannot fit model to data.	Peak	-----	GPCV
V29	Problem calculating star branching $g'$ , could not find the linear K and alpha in the sample.	Peak	-----	GPCV
V30	Problem calculating K and alpha for Nonlinear Viscosity Law fit.	Peak	-----	GPCV
V31	Problem calculating f, the number of star branches.	Peak	-----	GPCV
V32	Check the distribution, the intrinsic viscosity does not always increase with increasing molecular weight.	Peak	-----	GPCV
V33	Problem in GPCV broad standard calibration, cannot use a named distribution, enter moments instead. (Obsolete in v.4.0 and later)	Peak	-----	GPCV

A

Table A-1 Processing Codes (Continued)

A

Processing Code	Meaning	Processing Code Type	Message Center Message	Option
V34	Problem in GPCV broad standard calibration, cannot use a molecular weight-cumulative percent list, enter moments or a Named Distribution instead.	Peak	-----	GPCV
V35	The concentration data is not suitable for Calibration model fit.	Peak	-----	GPCV
V36	Cannot continue calibration model fit due to invalid elution volume data.	Peak	-----	GPCV
V37	Problem calculating model specific viscosity in calibration model fit.	Peak	-----	GPCV
V38	Problem calculating derivative of model specific viscosity in calibration model fit.	Peak	-----	GPCV
V39	Problem finding monotonic solution for calibration model fit to viscosity data.	Peak	-----	GPCV
V40	Problem optimizing calibration model fit to viscosity data.	Peak	-----	GPCV
V41	Problem computing polynomial coefficients from Chebyshev coefficients for calibration model fit.	Peak	-----	GPCV
V42	Problem calculating Slice k and Slice Alpha in calibration model fit, invalid calibration curve or GPC calibration technique.	Peak	-----	GPCV
V43	Cannot perform a Random intrinsic viscosity fit because the user did not enter an epsilon value in the Slicing table; GPCV processing cannot continue because epsilon is blank.	Peak, Result	-----	GPCV, LS
V44	Problem calculating peak branching, GPCV distribution is not monotonic. Check the intrinsic viscosity fit.	Peak	-----	GPCV

Table A-1 Processing Codes (Continued)

A

Processing Code	Meaning	Processing Code Type	Message Center Message	Option
W01	PDA Method, Channel: No data in file.	-----	Warning	PDA
W02	PDA Method, Channel: Insufficient wavelength elements.	-----	Warning	PDA
W03	PDA Method, Channel: No data at wavelength.	-----	Warning	PDA
W04	PDA Method, Channel: UV library not found.	-----	Warning	PDA
W06	PDA Method, Channel: Insufficient noise spectra.	-----	Warning	PDA
W07	PDA Method, Channel: Insufficient peak spectra.	-----	Warning	PDA
W08	PDA Method, Channel: Invalid noise spectrum.	-----	Warning	PDA
W10	PDA Method, Channel: Fewer wavelength elements than there are model spectra.	-----	Warning	PDA
W11	PDA Method, Channel: All-zero model spectrum	-----	Warning	PDA
X01	Problem in Singular Value Decomposition ( $m < n$ ).	Curve or Peak	-----	Base
X02	Problem in Singular Value Decomposition, the matrix is singular.	Curve or Peak	-----	Base
X03	Problem calculating y value from cubic spline calibration curve, 2 points have equal x values.	Peak	-----	Base
X04	Problem calculating slope of cubic spline calibration curve, 2 points have equal x values.	Curve	-----	Base
X05	Problem inverting a matrix, the matrix is singular.	Peak	-----	Base
X06	Problem evaluating a Chebyshev polynomial, log molecular weight is out of range.	Peak	-----	GPC/V



Table A-1 Processing Codes (Continued)

Processing Code	Meaning	Processing Code Type	Message Center Message	Option
Z01	Warning ZQ channel is labeled as Uncalibrated in the Channel Description.	Result	-----	MS
Z02	A result was not created for the extracted ZQ channel because the function was not found in the injection.	-----	Warning; Warning dialog in Review	MS

a. Dialog opens in Review if you zoom in too far and click the Peak Width or Threshold button.



# Appendix B

## Data Processing System Policies

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System policies settings in the following tables in the Empower System Policies dialog box control Empower™ software behavior:

- User Account Policies control accounts and passwords and set up Login Window Policies.
- New Project Policies entail selections for Full Audit Trail Policies and for Data Processing Technique (ApexTrack or Traditional integration).
- Other Policies govern Data Processing Policies, Result Sign Off Policies, and Other Policies.

For more information on these policies, see Empower Help.

This appendix discusses only Data Processing Policies. You can select four Data Processing Policies at the Other Policies tab of the Empower System Policies dialog box:

- Use v3.0X Style Peak Width and Threshold Determination
- Use v2.XX Style Retention Time Calculations
- Prompt User to Save manual changes made in Review
- Calculate % Deviation of Point from Curve

### B.1 Use v3.0X Style Peak Width and Threshold Determination

---

This system policy specifies that Empower software use the Millennium<sup>32</sup> version 3.0x procedure of setting peak width and threshold in a processing method. This option disables the auto peak width and auto threshold functionality in Empower software, removes the Result Peak Width and Result Threshold fields from the toolbar in Review, and also designates that the Processing Method wizard function as in version 3.0x.

To set the peak width value with this system policy enabled, perform the following:

1. Integrate the narrowest peak of interest in a standard chromatogram. The peak should be properly integrated with appropriate start and end times. If needed, manually adjust the peak start/peak end by clicking the peak marker and dragging it to the appropriate position.

2. When the peak is integrated properly, click within it. The entire peak is drawn in red.
3. Click the Set Processing Method Peak Width tool in the toolbar. The software determines the appropriate peak width value and enters it into this field.

**Note:** *You can also enter an appropriate peak width value either by typing directly in this field or by selecting one of the predefined values from the list.*

Since the software uses the peak width value to determine a bunching factor during peak detection (as described in [Section 3.1.1, Performing Data Bunching](#)), this value affects the sensitivity of the peak detection process. The guideline is to use a peak width value within a factor of  $\pm 2$  times the software-determined peak width value. If the signal-to-noise ratio is acceptable, the peak width value at the high end of this range may increase sensitivity and allow relatively small peaks to be properly integrated. The trade-off in this scenario is that shoulders, if present, on larger peaks may no longer be detected. Increasing the peak width value above this range results in a lack of sensitivity.

**Note:** *This system policy governs only how the appropriate peak width value is determined and set in the processing method, not how the software uses the value during peak detection and integration. During processing, the peak width parameter functions identically, whether or not you enable this system policy.*

To set the threshold value with this system policy enabled, perform the following:

1. With the mouse, zoom on the entire baseline until you can see its detail.
2. Zoom on the noisiest portion of the baseline that does not contain peaks that should be integrated. Ensure that only this region is in the plot window.
3. Click the Set Processing Method Threshold tool in the toolbar. The software determines and enters the appropriate threshold value into this field.

**Note:** *You can also enter an appropriate threshold value by typing directly in this field.*

The threshold value is a slope measurement (in  $\mu\text{V}/\text{sec}$ ) and is used to determine peak start and peak end points during peak detection (as described in [Section 3.1.2, Determining Peak Start](#), and [Section 3.1.4, Determining Peak End](#)). A relatively low threshold value will increase sensitivity and may allow relatively small peaks to be properly integrated. If too many small, baseline noise peaks are being integrated, increasing the threshold value may prevent these small peaks from being integrated.

**Note:** *This system policy governs only how the appropriate threshold value is determined and set in the processing method, not how the software uses the value during peak detection and integration. During processing, the threshold parameter functions identically, whether or not this system policy is enabled.*

## B.2 Use v2.XX Style Retention Time Calculations

---

This system policy specifies that Empower software use the Millennium version 2.xx algorithm to determine peak retention time and height. When this policy is enabled, the software uses only the highest data point from the baseline to determine retention time and height and disables fitting a quadratic curve to the top of the peak. When results are generated, processing code I09 appears in the Results table.

To determine retention time and height with this policy enabled, the software:

1. Locates the retention time of the data point in the peak that is farthest from the constructed baseline.
2. Calculates the peak height as the distance (in  $\mu\text{V}$ ) from the constructed baseline to the Y Value of the calculated peak apex.

## B.3 Prompt User to Save Manual Changes Made in Review

---

This system policy specifies that the software display a message to remind you to save changes that you make manually to a result or calibration curve in Review when exiting. If this policy is not enabled and manual changes are made in Review, you are not prompted to save the results and calibrations when exiting.

## B.4 Calculate % Deviation of Point from Curve

---

This system policy specifies that the software measures % deviation as how far the calibration point deviates from the calibration curve. Normally, % deviation is a measure of how far the calibration curve deviates from the calibration point.

% deviation is calculated for each data point plotted on a calibration curve and indicates the difference between the amount or concentration for the peak it represents (as entered by the user) and the amount or concentration determined for the peak when its response is treated as if it were an unknown and is quantitated from the calibration curve. This difference is then expressed as a percentage.

When this system policy is enabled, the formula for % deviation of the point from the curve is as follows:

$$\% \text{ Deviation} = \frac{\text{Actual Value} - \text{Calculated Value}}{\text{Calculated Value}} \cdot 100$$

where:

*Actual Value* = X value of the point

*Calculated Value* = X value obtained from the calibration curve

When this system policy is disabled, the software calculates % deviation of the calibration curve from the point as follows:

$$\% \text{ Deviation} = \frac{\text{Calculated Value} - \text{Actual Value}}{\text{Actual Value}} \cdot 100$$

where:

*Actual Value* = X value of the point

*Calculated Value* = X value obtained from the calibration curve

**Note:** If ApexTrack is enabled in the processing method, the wizard ignores the system policy Use v3.0x Style Peak Width and Threshold Determination, and always displays the wizard pages as if this system policy were not enabled.

B

# Appendix C

## Getting Started: Processing with ApexTrack

Use this appendix for step-by-step procedures with ApexTrack integration.

**Note:** This guide uses the Empower Pro interface. If you do not have access to this interface, ask your system administrator.

### C.1 Starting Empower Software

To start the Empower software:

1. Select **Start > Programs** (for Windows XP, **All Programs**) > **Empower > Empower Login**. The Empower Login dialog box appears ([Figure C-1](#)).

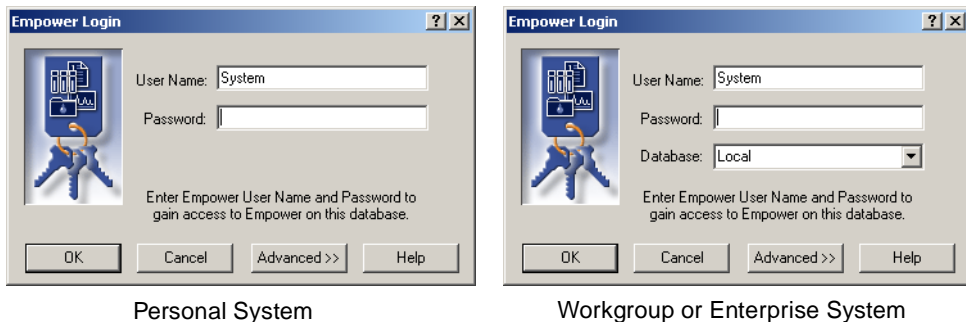


Figure C-1 Empower Login Dialog Box

2. Enter your user name and password. If you do not know your user name or password, see your system administrator.
3. If you are using an Empower Enterprise system, select the correct database from the Database list. This list appears only when connected to a client/server system.
4. Click **Advanced** and verify that the Requested Interface field is set to Pro. If you cannot select the Empower Pro interface, see your system administrator.
5. Click **OK**. The Empower Pro window appears.

## C.2 Setting Up Peak Integration

---

You must have the appropriate privileges to use ApexTrack integration. If you do not have these privileges, ask your system administrator. ApexTrack integration is enabled at three successive levels:

1. **Database** – For a system-wide installation of the Empower database in System Policies (in the Project Policies dialog box; see [Section C.3, ApexTrack Integration for the Empower Database](#)).
2. **Project** – For a new project in Project Properties, or in the New Project wizard or Project Properties in Configuration Manager for an existing project (see [Section C.4, ApexTrack Integration in Projects](#)).
3. **Method** – For the individual processing method in the Integration tab in the Processing Method dialog box (see [Section C.5, ApexTrack Integration in a Processing Method](#)).

## C.3 ApexTrack Integration for the Empower Database

---

You must have the Alter System Policies privilege (in the Management tab of the User Type Properties dialog box) to perform this procedure.

To enable ApexTrack integration in the Empower database:

1. Click **Configure System** in the Empower Pro window. Configuration Manager appears.
2. Select **View > System Policies**.
3. Click the **New Project Policies** tab. The New Project Policies tab appears ([Figure C-2](#)).

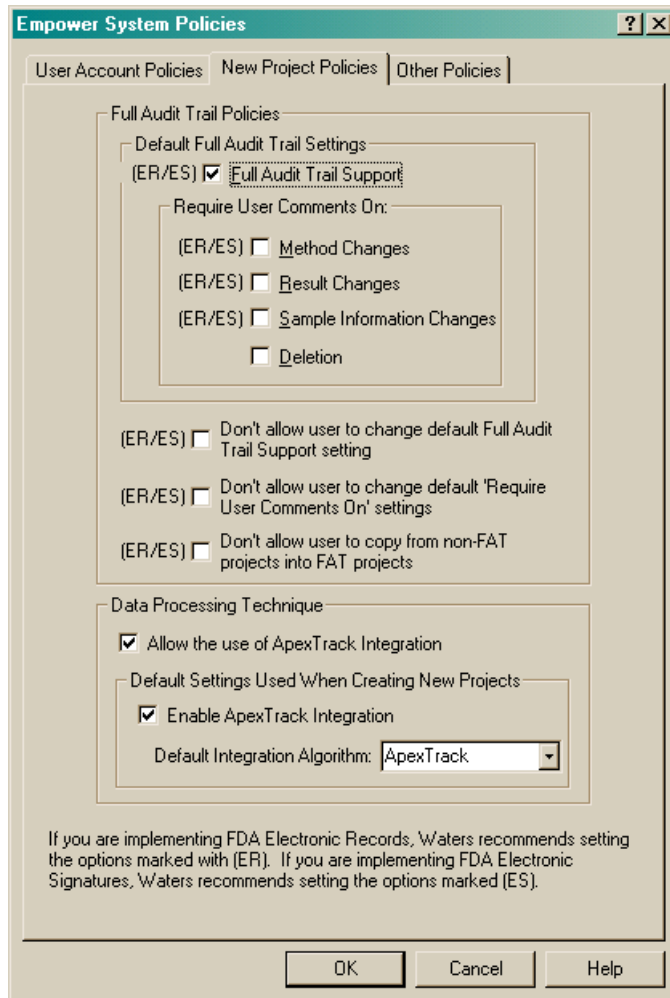


Figure C-2 New Project Policies Tab

4. Select **Allow the use of ApexTrack Integration** in the Data Processing Technique section. This system policy allows the use of both ApexTrack and Traditional integration algorithms in the database.
5. If you want each new project created in this database to allow the use of ApexTrack integration (in the Tablespace page of the New Project wizard), select **Enable ApexTrack Integration** in the Default Settings Used When Creating New Projects section. The Default Integration Algorithm is Traditional integration, although the project creator can select ApexTrack in the processing method.



6. If you want ApexTrack integration as the Default Integration Algorithm (in the Tablespace page of the New Project wizard) for all new projects, select **ApexTrack** from the Default Integration Algorithm list. The Default Integration Algorithm is ApexTrack integration, although the project creator can select Traditional in the processing method.

If you want Traditional integration as the Default Integration Algorithm for all new projects, select **Traditional** in the Default Integration Algorithm section.

7. Click **OK** to save the settings. A Configuration Manager message box appears ([Figure C-3](#)).

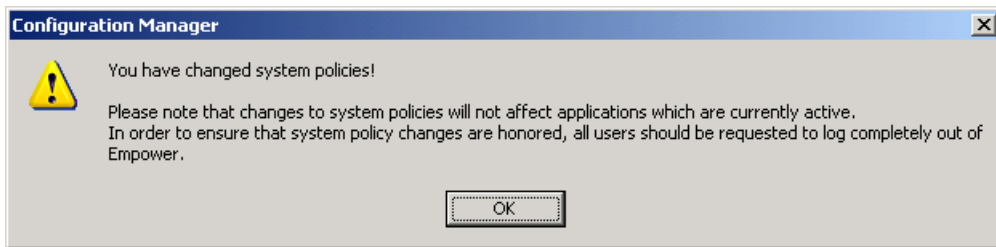


Figure C-3 Configuration Manager Message Box

8. Click **OK**.

## C.4 ApexTrack Integration in Projects

You must have the Alter Projects privilege (in the Management tab of the User Type Properties dialog box) to perform this procedure.

**Note:** To enable ApexTrack in a project, the **Allow the use of ApexTrack Integration** system policy must be selected in the Data Processing section of the New Project Policies tab.

### C.4.1 Enabling ApexTrack in a New Project

You can enable ApexTrack when creating a new project. You can also specify ApexTrack as the default integration algorithm setting for all new processing methods created in the new project.

To enable ApexTrack integration when creating a new project:

1. Click **Configure System** in the Empower Pro window. Configuration Manager appears.

2. Select **File > New > Project** or click  (New Project Wizard). The Tablespace page of the New Project Wizard appears ([Figure C-4](#)).

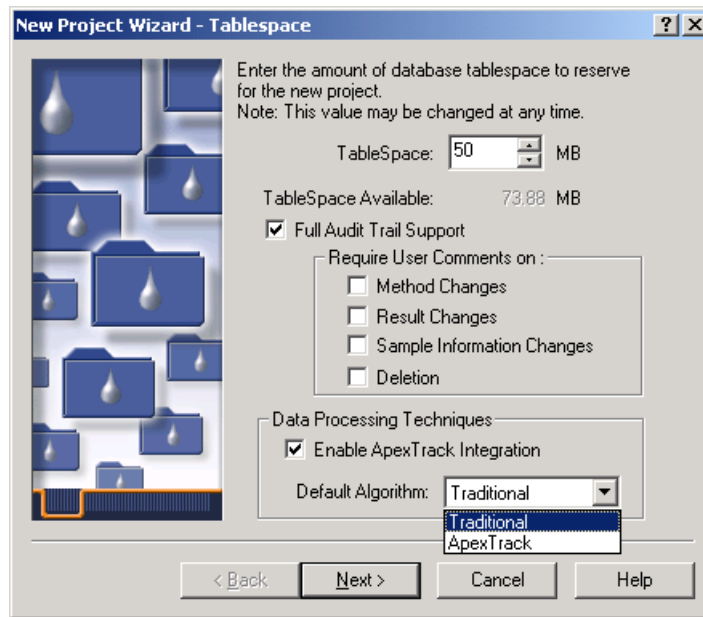


Figure C-4 Tablespace Page of New Project Wizard

3. Select **Enable ApexTrack Integration** in the Data Processing Techniques section.
4. If you want ApexTrack to appear as the default Integration Algorithm in the Integration tab (in each processing method created in the new project), select **ApexTrack** from the Default Algorithm list.

If you want Traditional as the default integration algorithm in each processing method created in the new project, leave the default setting as **Traditional**.

**Note:** You can select either the ApexTrack or Traditional integration algorithm in each new processing method regardless of the default integration algorithm setting. Enable ApexTrack Integration permits you to switch between ApexTrack and Traditional in existing processing methods.

5. Click **Next**, then complete the remaining pages.

## C.4.2 Enabling ApexTrack in an Existing Project

You can change the Data Processing Techniques settings for existing projects using the Enable ApexTrack Integration check box and the Default Algorithm list in the Project Properties page.

To allow the use of ApexTrack integration in an existing project:

1. Click **Configure System** in the Empower Pro window. Configuration Manager appears.
2. Select the project in Configuration Manager, then select **File > Properties**. (Alternatively, right-click the appropriate project in the table, then select **Properties**.) The General tab of the Project Properties dialog box appears for the selected project ([Figure C-5](#)). If a different tab appears, click the **General** tab.

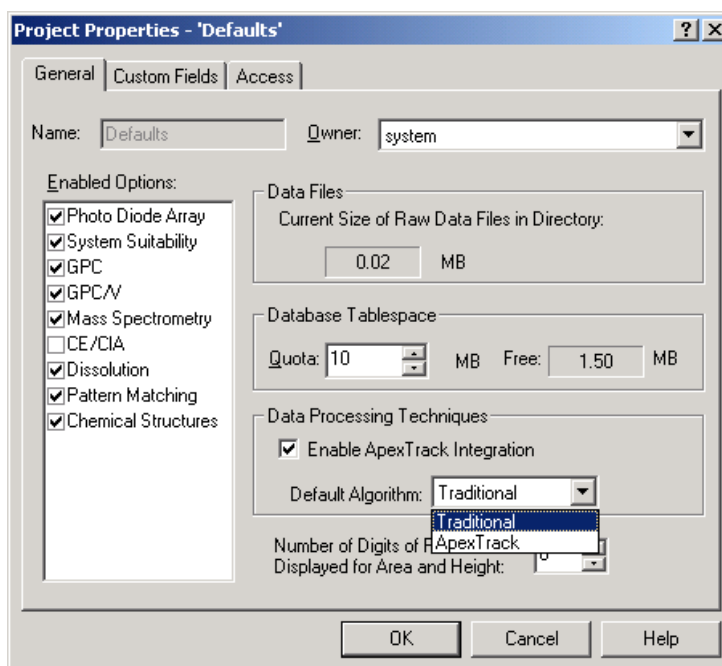


Figure C-5 General Tab of Project Properties Dialog Box

3. Select **Enable ApexTrack Integration** in the Data Processing Techniques section to enable ApexTrack integration for the current project. Both ApexTrack and Traditional integration will be available for all processing methods in the project.
4. If you want ApexTrack to appear as the default integration algorithm for each new processing method that you create in this project, select **ApexTrack** from the Default Algorithm list.

If you do not select ApexTrack from the Default Algorithm list, the default integration algorithm is Traditional for each new processing method in this project.

**Note:** You can select either the ApexTrack or Traditional integration algorithm in each new processing method regardless of the default integration algorithm setting. Enable ApexTrack Integration permits you to switch between ApexTrack and Traditional in existing processing methods.

5. Click **OK**.

## C.5 ApexTrack Integration in a Processing Method

---

After enabling ApexTrack integration for the database and for the project, you enable ApexTrack integration for the processing method. The processing method defines the parameters, including detection and integration events, that the software uses to detect and integrate the peaks within the raw data file or channel.

You must have the Save Processing Methods privilege (in the Methods tab of the User Type Properties dialog box) to perform this procedure.

### C.5.1 Creating a New Processing Method with ApexTrack

To create a new processing method using the ApexTrack integration algorithm:

1. Open the project in Review.
2. Select **File > New > Processing Method**. The New Processing Method dialog box appears ([Figure C-6](#)).

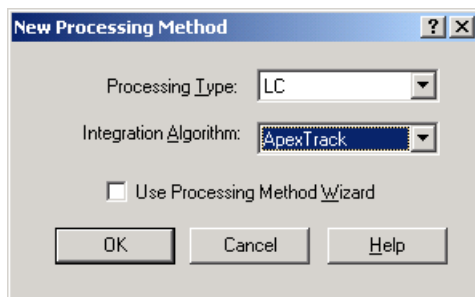


Figure C-6 New Processing Method Dialog Box

3. Select or change the type of process, as appropriate, in Processing Type.
4. Select **ApexTrack** from the Integration Algorithm list.



4. Enter or edit the peak integration parameters as needed (see [Section 2.1.3, Summary of Processing Method Parameters](#)). Peak integration includes both baseline determination parameters (Liftoff % and Touchdown %) and peak acceptance criteria (Minimum Area and Minimum Height).

**Note:** The maximum value of Liftoff % and Touchdown % allowed in a GPC processing method is 5%. The default value for Touchdown % for GPC is 0.000%.

5. Enter integration events into the Integration table to enable events or refine the detection of peaks as needed (see [Section 2.8, Peak Detection Events](#), [Section 2.9, Peak Integration Events](#), and Section C.6, Summary of Integration Events).

### C.5.3 Changing the Integration Algorithm in a Processing Method

You can change the integration algorithm in an existing processing method at the Integration Algorithm tab. When you select the integration algorithm, the Integration tab displays the appropriate parameters. You can toggle between ApexTrack and Traditional integration without losing the values set for each method.

#### From Traditional to ApexTrack

To change the integration algorithm in an existing processing method from Traditional to ApexTrack:

1. Open the project, then double-click the processing method to open it. The Integration tab of the selected processing method appears ([Figure C-8](#)).

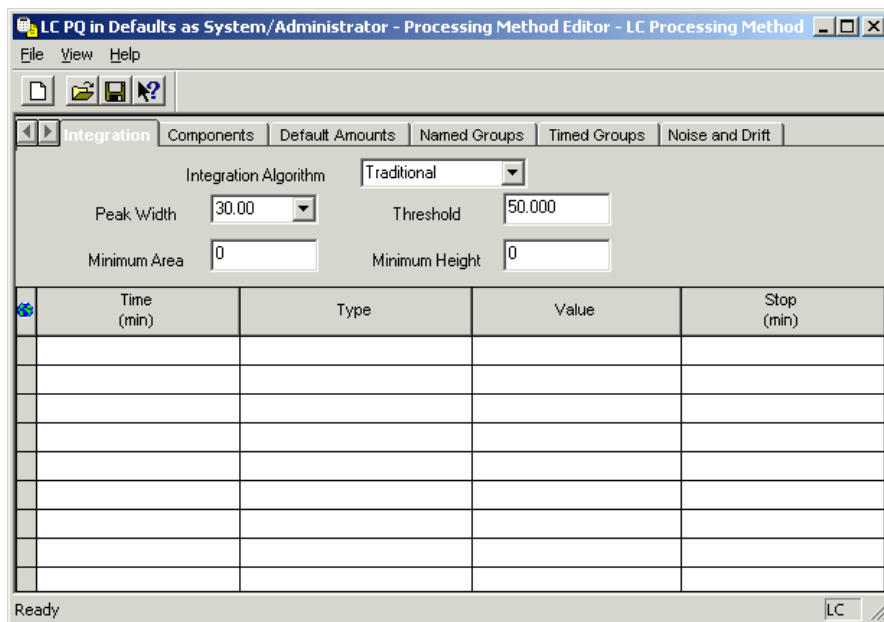


Figure C-8 Traditional Parameters in the Integration Tab

2. Select **ApexTrack** from the Integration Algorithm list. The appropriate parameters appear in the Integration tab ([Figure C-9](#)).

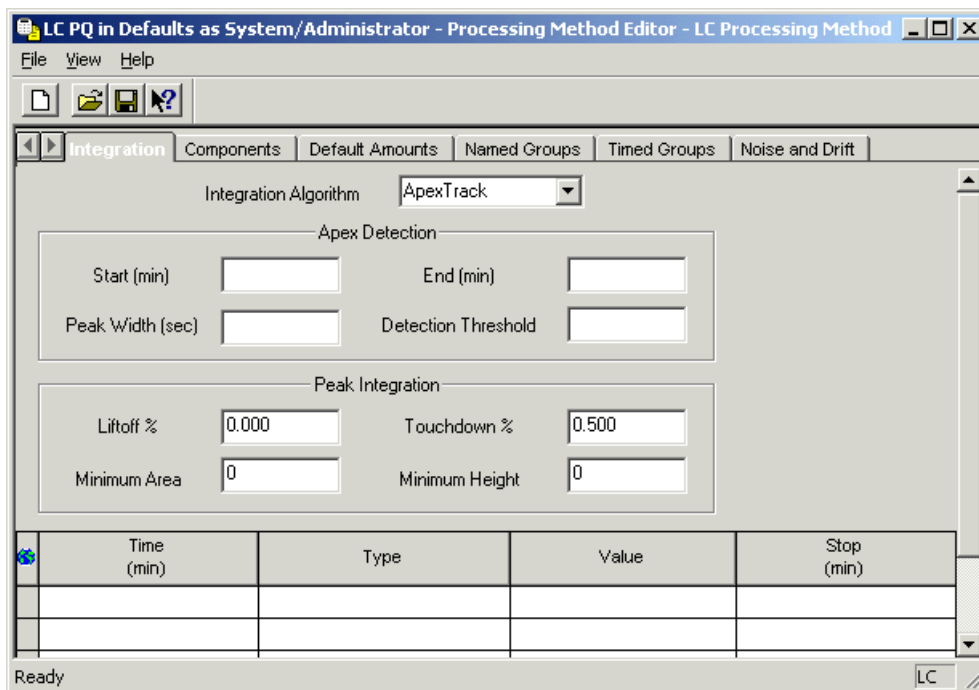


Figure C-9 ApexTrack Parameters in the Integration Tab

3. Enter the processing method parameters (see [Chapter 2, ApexTrack Integration](#)).

## From ApexTrack to Traditional

To change the integration algorithm in a processing method from ApexTrack to Traditional:

1. Open the project, then double-click the processing method to open it. The Integration tab of the selected processing method appears ([Figure C-9](#)).
2. Select **Traditional** from the Integration Algorithm list. The appropriate parameters appear in the Integration tab ([Figure C-8](#)).
3. Enter the processing method parameters (see [Chapter 3, Traditional Integration](#)).

**Note:** When the processing method uses ApexTrack integration, the ApexTrack integration parameters are included in the report.

**Note:** If ApexTrack is not enabled in a project, but you attempt to process data in the background with a processing method that has ApexTrack integration enabled, all processing with that processing method is skipped and a processing error appears in the Message Center.

## C.6 Summary of Integration Events

---

ApexTrack integration offers the following integration timed events, which you can select in the Type column of the Integration table (see [Section 2.8, Peak Detection Events](#)):

- **Allow Negative Peaks** (see [Section 2.8.3](#)) – If you enable negative peak detection, ApexTrack identifies and integrates negative peaks.
- **Detect Shoulders** (see [Section 2.8.2](#)) – If you enable shoulder detection, peaks and shoulders appear in your chromatograms. Since ApexTrack detects positive peaks as local maxima of curvature, and negative peaks as local minima, it initially detects all components:
  - If you add a Detect Shoulders integration event, all shoulder detections are retained within the time period when the event is active, and suitable peak boundaries and drop lines are drawn.
  - If you do not add this event, a separate algorithm determines which apices are shoulders. Shouldered peaks are then folded into the adjoining peak.
- **Six Set events** (see [Section 2.8.4](#)) – If you enable a set event, sets the specific event or constraint: detection threshold, liftoff %, minimum area of a peak, minimum peak height, peak width, or touchdown %.
- **Inhibit Integration** (see [Section 2.8.1](#)) – If you enable an inhibit integration event, stops the integration of any peaks for the specified timeframe.
- **Gaussian Skim** (see [Section 2.9.2](#)) – If you enable a Gaussian skim event, Gaussian skimming replaces exponential skimming for peak boundaries.
- **Merge GPC Peaks** (see [Section 2.9.3](#)) – If you enable merge GPC peaks (for GPC only), deletes the drop line between consecutive peaks with the same sign (either both positive or both negative) and merges the peaks into one peak.
- **Valley-to-Valley** (see [Section 2.9.1](#)) – If you enable a Valley-to-Valley event, sets the baseline to each valley point in a fused peak group.

C

## C.7 Peak Labels in Result

---

**Note:** When you process results using ApexTrack integration, the Integration Algorithm in the Result field is set to ApexTrack.

When ApexTrack integration is performed, four specific Integration Types are available to describe the peaks ([Table C-1](#)): G, R, S, and X (see [Section 2.1.5, Integration Peak Labels in ApexTrack](#)).



Table C-1 ApexTrack Integration Peak Labels

Label	Peak Start and End Points	Description
G	Gaussian	Gaussian skimming replaces vertical drop lines at peak boundaries.
R	Round ( <a href="#">Figure C-10</a> )	The boundary (valley) between two consecutive peaks that share the same inflection points. A drop line is drawn at the round position, similar to a vertical drop at a valley point.
S	Shoulder ( <a href="#">Figure C-10</a> )	The peak starts or ends at a shoulder (a mathematically derived valley point). A drop line is drawn for the letter S as if it were a V.
X	Crossover ( <a href="#">Figure C-11</a> )	The peak starts or ends at a crossover point where the cluster baseline intersects the signal. The peak next to it has a different sign (one peak is a positive peak and the other is a negative peak). The letter X is treated as if it were an unmovable B. Its marker in the plot is always an x drawn one half normal size.



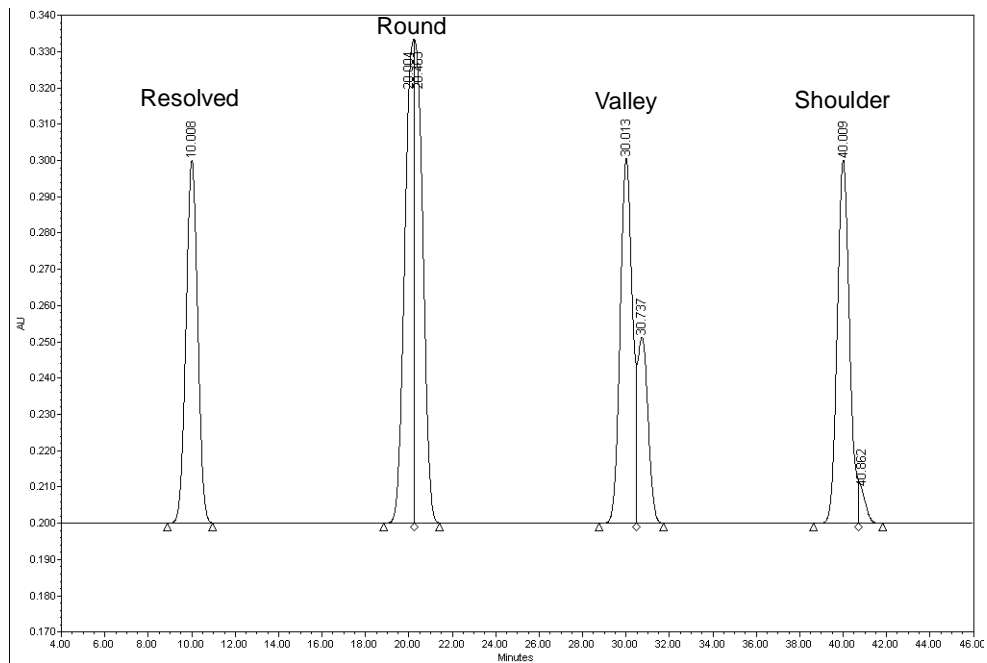


Figure C-10 Peak Labels for ApexTrack

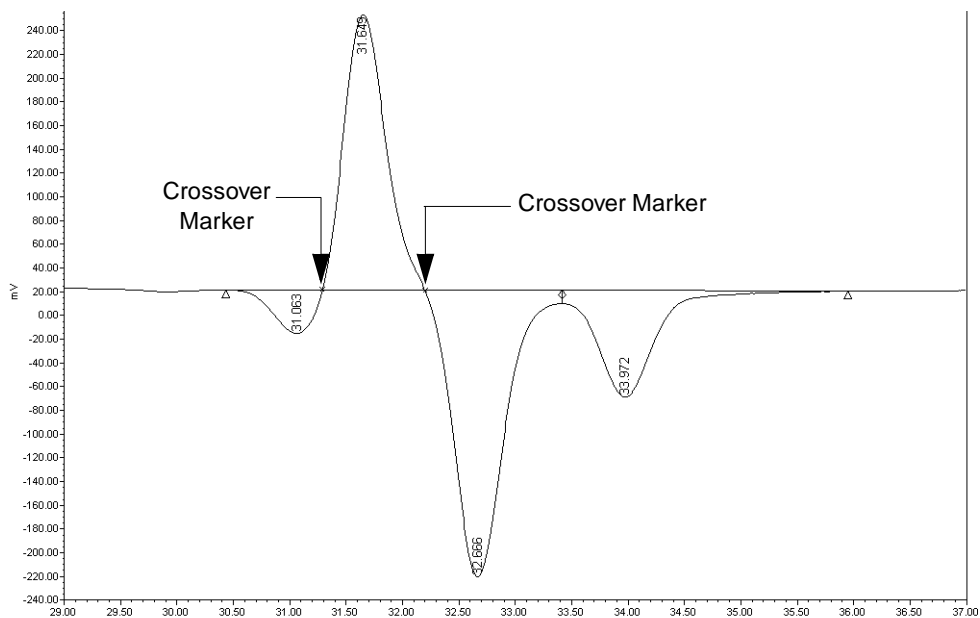


Figure C-11 Crossover Peaks for ApexTrack



Other columns in the Peaks table that apply to ApexTrack integration include the Width, Start Height, and End Height fields. In addition, the following new ApexTrack columns appear:

- **Inflection Width (sec)** – Interpolated width of the peak between its inflection points (in seconds).
- **2nd Derivative Apex (min)** – Retention time of the second derivative apex of the peak in minutes. This field is blank if Traditional integration is selected.

## C.8 Manual Integration Guidelines

---

Use these guidelines when changing the locations of the integration markers on the peak profile during manual integration.

### C.8.1 Adding or Deleting Peaks

- You can manually add baseline peaks (bb) as in Traditional integration.
- When you manually delete a shoulder peak, it is treated as if it were a valley peak and removed from the list of peaks.
- You cannot manually add or delete Gaussian skimmed peaks.
- You can add vertical drop lines to all peaks except those with a G in their integration type.
- You can manually delete drop lines unless they coincide with the start or end of a Gaussian skimmed peak.
- A smaller skimmed peak, or a larger peak that generates a skim, can be manually deleted. In either case, the adjoining peak is unaffected. Thus if a smaller skimmed peak is rejected or deleted, the profile of the larger (parent) peak is still determined by the skim, and the area of the smaller, deleted peak is still excluded by the skim. If the larger skimmed peak is deleted, the baseline of the smaller skimmed peak is still determined by the skim.
- You cannot manually adjust the baseline of a cluster that contains Gaussian skimmed peaks, either by moving a start or end marker or a drop line marker for the last or first peak in the cluster.

### C.8.2 Moving Peak Starts and Ends

- You cannot move a crossover (x) marker. If a peak starts in a crossover, you cannot change its start time. If a peak ends in a crossover, you cannot change its end time.
- When you change the start or end time of a peak cluster with crossing points, the crossing points become valleys. In the Int Types of the crossing point peaks in the cluster, all x's change to v's, and the crossing point is changed from an interpolated point to the time of the closest real point.

- You cannot move the peak start or end of a skimmed peak.
- When you change the start time of a baseline-resolved peak or peak cluster, the first letter in the Int Type of the peak is changed to a b, whether the original integration type is B or b.
- When you change the end time of a baseline-resolved peak or peak cluster, the second letter in the Int Type of the peak is changed to a b, whether the original integration type is B or b.
- Integration types are marked with lowercase letters after you manually move them.

### C.8.3 Moving Vertical Drops

- Vertical drop lines can be moved unless they coincide with the start or end of a Gaussian skimmed peak.
- If you move the drop line between two peaks, this change is indicated in the Int Type fields of both peaks. Both the second letter in the Int Type of the peak that ends with the drop line and the first letter in the Int Type of the peak that starts with the drop line are changed to a v. This happens when the original integration type is R, S, V, or v.

# Index

## Numerics

5-point peak rejection, Traditional [97](#)

## A

A/D conversion [23](#)

Allow Negative Peaks event

ApexTrack [58](#), [216](#)

Traditional [98](#)

Analog-to-digital conversion [23](#)

Apex

defining in ApexTrack [36](#)

detection in ApexTrack [28](#)

detection parameters [213](#)

ApexTrack

allowing negative peaks [216](#)

apex detection parameters [213](#)

apex determination [28](#)

apex, defining [36](#)

Auto-Peak Width [34](#), [51](#)

AutoThreshold [35](#), [54](#)

baseline determination [28](#)

changing integration algorithm [214](#)

crossover [46](#), [217](#)

Detection Threshold [30](#)

enabling integration in a new project [209](#)

enabling integration in a processing  
method [213](#)

enabling integration in the database [207](#)

Gaussian skim [31](#)

incompatible events [80](#)

inflection points [36](#), [38](#)

integration [22](#)

integration events [31](#), [56](#), [66](#)

integration peak labels [32](#)

integration peak types [216](#)

liftoff [44](#)

Liftoff % [30](#)

manual integration guidelines [77](#), [219](#)

Minimum Area [30](#)

Minimum Height [30](#)

negative peaks [31](#), [58–59](#)

peak detection process [34](#)

Peak Width [30](#)

procedure for peak detection [36](#)

processing parameters [29](#)

round peak [37](#), [217](#)

set events [60](#), [79](#)

setting up [207](#)

shoulder [57–58](#)

shoulder detection [216](#), [217](#)

slope difference threshold [39](#)

touchdown [44](#)

Touchdown % [30](#)

Area, Traditional integration [96](#)

Auto-Peak Width, ApexTrack [34](#), [51](#)

AutoThreshold, ApexTrack [35](#), [54](#)

## B

Baseline construction, Traditional [93](#)

Baseline determination, ApexTrack [28](#), [39](#)

Baseline noise [30](#)

estimating [54](#)

role in ApexTrack [53](#)

Baseline, ApexTrack [36](#)

busLAC/E card [23](#)

## C

Calculated value and percent deviation of  
calibration points [170](#)

Calibration curve

calculated value and percent deviation of  
calibration points [170](#)

coefficient of determination [168](#)

correlation coefficient [168](#)

residual sum of squares [169](#)  
standard error of calibration [170](#)  
standard error of estimate of Y on X [168](#)  
standard variance [169](#)

Calibration curve fits

- cubic [161](#)
- cubic spline [156](#)
- fifth-order [164](#)
- forced-through-zero [165](#)
- inverse linear [158](#)
- linear [157](#)
- linear through zero [150](#)
- log-log linear [158](#)
- point-to-point [154](#)
- quadratic [159](#)
- response factor [150](#)
- statistics [168](#)
- weighting [166](#)

Calibration during quantitation [132](#)  
Classic skim type, Traditional [118](#)  
Codes, processing [172](#)  
Coefficient of determination [168](#)  
Conventions, documentation [18](#)  
Conversion, A/D [23](#)  
Correlation coefficient [168](#)  
Crossover

- ApexTrack [46](#), [217](#)
- peaks [219](#)

Cubic fit [161](#)  
Cubic spline fit [156](#)  
Curvature, use in ApexTrack [33](#), [34](#)

## D

Data acquisition [21](#)  
Data bunching, Traditional [83](#)  
Data storage [23](#)  
Data transfer [23](#)  
Detect Shoulders event, ApexTrack [31](#)  
Detection events

- Allow Negative Peaks, ApexTrack [58](#)
- Allow Negative Peaks, Traditional [98](#)

Detect Shoulders [31](#)  
Detect Shoulders, ApexTrack [57](#)  
Set Liftoff %, ApexTrack [79](#)  
Set Liftoff, Traditional [100](#)  
Set Peak Width, ApexTrack [60](#)  
Set Peak Width, Traditional [101](#)  
Set Touchdown %, ApexTrack [80](#)  
Set Touchdown, Traditional [100](#)

Detection sampling rate [24](#)  
Detection Threshold, ApexTrack [30](#), [34](#)  
Dilution [134](#)  
Disk space [25](#)  
Documentation

- conventions [18](#)
- related [16](#)

## E

Enabling ApexTrack integration

- in a new project [209](#)
- in a processing method [213](#)
- in the database [207](#)

Exponential Skim event, Traditional [118](#)  
External standard quantitation [137](#)

## F

Fifth-order fit [164](#)  
Force Baseline by Time event, Traditional [103](#)  
Force Drop Line event, Traditional [115](#)  
Force Peak event, Traditional [117](#)  
Forced-through-zero [165](#)  
Forward Horizontal by Peak event, Traditional [106](#)  
Forward Horizontal by Time event, Traditional [106](#)  
Fused peaks

- second derivative, ApexTrack [36](#)
- Traditional [91](#)

## G

Gaussian skimming, ApexTrack [28](#), [31](#),  
[70–77](#)

### GPC

Liftoff % and Touchdown % [30](#), [214](#)

Merge Peaks event, ApexTrack [78](#)

GPC processing, Merge Peaks event [31](#)

Guidelines, ApexTrack manual integration  
[219](#)

## H

Hard disk space [25](#)

Height ratio test, Traditional [119](#)

## I

### Incompatible events

ApexTrack [80](#)

Traditional [124](#)

Inflection points, ApexTrack [36](#), [38](#)

### Inhibit Integration event

ApexTrack [56](#)

Traditional [90](#)

### Integration

ApexTrack [22](#)

ApexTrack algorithm [29](#)

Traditional [22](#)

Integration algorithm, changing [214](#)

### Integration events

ApexTrack [31](#), [66](#)

ApexTrack Integration table [56](#)

compatibility, ApexTrack [80](#)

compatibility, Traditional events [124](#)

Exponential Skim, Traditional [118](#)

Force Baseline by Peak, Traditional [103](#)

Force Baseline by Time, Traditional [103](#)

Force Drop Line, Traditional [115](#)

Force Peak, Traditional [117](#)

Forward Horizontal by Peak, Traditional  
[106](#)

Forward Horizontal by Time, Traditional  
[106](#)

Gaussian Skim event, ApexTrack [70–77](#)

Inhibit Integration, ApexTrack [56](#)

Inhibit Integration, Traditional [90](#)

Reverse Horizontal by Peak, Traditional  
[110](#)

Reverse Horizontal by Time, Traditional  
[110](#)

Set Minimum Area, ApexTrack [62](#)

Set Minimum Area, Traditional [123](#)

Set Minimum Height, ApexTrack [64](#)

Set Minimum Height, Traditional [123](#)

Tangential Skim, Traditional [118](#)

Traditional integration, overview [101](#)

Valley-to-Valley, ApexTrack [66–70](#)

Valley-to-Valley, Traditional [114](#)

Integration peak labels, ApexTrack [32](#), [216](#)

Integration peak types, ApexTrack [216](#)

Integration tab in ApexTrack processing  
method [29](#)

Integration, definition [21](#)

Internal standard quantitation [140](#)

Inverse linear fit [158](#)

ISA busLAC/E card [23](#)

## L

Liftoff % Threshold event, ApexTrack [79](#)

Liftoff %, ApexTrack [30](#), [39](#)

Liftoff in ApexTrack [44](#)

Liftoff Threshold event, Traditional [100](#)

Linear fit [157](#)

Linear through zero fit [150](#)

Log-log linear fit [158](#)

## M

- Manual integration, ApexTrack [219](#)
- Match difference [129](#)
- Matrix operations
  - example [152](#)
  - multilevel calibration [151](#)
- Maximum curvature of peaks, ApexTrack [33](#)
- Merge Peaks event
  - GPC processing [31](#)
  - GPC, ApexTrack [78](#)
- Minimum Area
  - ApexTrack [30](#)
  - Traditional [97](#)
- Minimum Height
  - ApexTrack [30](#)
  - Traditional [97](#)

## N

- Negative peak detection in ApexTrack [28](#)
- Negative peaks, allowing in ApexTrack [58](#)
- Nonclassic skim type, Traditional [118](#)

## P

- Parameters, ApexTrack apex detection [213](#)
- PCI busLAC/E card [23](#)
- Peak apex, determining, Traditional [85](#)
- Peak area, determining, Traditional [96](#)
- Peak detection
  - ApexTrack [28, 34](#)
  - data bunching, Traditional [83](#)
  - End (min), ApexTrack [29](#)
  - peak apex, ApexTrack [28](#)
  - peak apex, Traditional [85](#)
  - peak end, Traditional [85](#)
  - peak start, Traditional [84](#)
  - Start (min), ApexTrack [29](#)
  - Traditional [82](#)
- Peak end, determining, Traditional [85](#)

- Peak height, determining, Traditional [95](#)
- Peak integration
  - ApexTrack peak labels [33](#)
  - baseline construction, Traditional [93](#)
  - determining fused peaks, Traditional [91](#)
  - events, Traditional [101](#)
  - overview, Traditional [91](#)
  - peak area, Traditional [96](#)
  - peak height, Traditional [95](#)
  - peak labels, ApexTrack [217](#)
  - peak labels, Traditional [102](#)
  - peak rejection criteria, Traditional [97](#)
  - retention time, Traditional [95](#)
  - Traditional peak labels [94](#)
- Peak labels
  - ApexTrack [32, 33, 217](#)
  - Traditional integration [94, 102](#)
- Peak matching [128](#)
  - match difference [129](#)
  - retention times, shifting [130](#)
  - Update Retention Time [130](#)
- Peak rejection criteria, Traditional [97](#)
- Peak start, determining, Traditional [84](#)
- Peak Width, ApexTrack [30, 34](#)
- Peak, maximum curvature, ApexTrack [33](#)
- Peak-to-peak baseline noise [54](#)
- Permissions, ApexTrack [207](#)
- Point-to-point fit type [154](#)
- Priority of events, Traditional [124](#)
- Privileges, ApexTrack [207](#)
- Processing codes [172](#)
- Processing methods
  - default in ApexTrack [29](#)
  - definition [21](#)
- Processing, definition [21](#)

## Q

- Quadratic fit [159](#)
- Quantitation
  - by calibration [132](#)
  - external standard [137](#)
  - fit type [132](#)



internal standard [140](#)  
RF Internal Standard [145](#)  
using dilution [134](#)  
using injection volume [135](#)  
using sample weight [134](#)

## R

Raw data points [24](#)  
Rejection  
    criteria, Traditional [97](#)  
    shoulder peak [58](#)  
    skimmed peak, ApexTrack [72](#)  
Related documentation [16](#)  
Residual sum of squares [169](#)  
Response factor fit [150](#)  
Retention time  
    determining [95](#)  
    Retention time reference [130](#)  
    shifting [130](#)  
Reverse Horizontal by Peak event, Traditional [110](#)  
Reverse Horizontal by Time event, Traditional [110](#)  
RF Internal Standard quantitation [145](#)  
Round peak, ApexTrack [28](#), [31](#), [36](#), [37](#), [217](#)  
RT Reference [130](#)

## S

Sample weight [134](#)  
Sampling rate  
    data collection frequency [24](#)  
    formula [25](#)  
    optimum sampling rate [25](#)  
Second derivative  
    apex [38](#)  
    example [36](#)  
    filter, ApexTrack [34](#)  
    peak profile [35](#)  
    threshold, ApexTrack [34](#)  
Second derivative filter, ApexTrack [34](#)

Set  
    Detection Threshold event, ApexTrack [60](#)  
    Events, ApexTrack [60](#)  
    Liftoff % event, ApexTrack [79](#)  
    Liftoff event, Traditional [100](#)  
    Minimum Area event, ApexTrack [62](#)  
    Minimum Area event, Traditional [123](#)  
    Minimum Height event, ApexTrack [64](#)  
    Minimum Height event, Traditional [123](#)  
    Peak Width event, ApexTrack [60](#)  
    Peak Width event, Traditional [101](#)  
    Touchdown % event, ApexTrack [80](#)  
    Touchdown event, ApexTrack [80](#)  
    Touchdown event, Traditional [100](#)  
Shifting retention times [130](#)  
Shoulders  
    ApexTrack [217](#)  
    Detect Shoulders event, ApexTrack [57](#)  
    detection [28](#), [36](#), [216](#)  
    peak rejection in ApexTrack [58](#)  
Skim events  
    ApexTrack [28](#), [70–77](#)  
    Traditional [118](#)  
Skimmed peak, rejection, ApexTrack [72](#)  
Slope difference threshold [41](#)  
    ApexTrack [39](#)  
Slope differences, ApexTrack [40](#)  
Standard error of calibration [170](#)  
Standard error of estimate of Y on X [168](#)  
Standard variance [169](#)  
Statistics [168](#)  
System policies  
    Calculate % Deviation of Point from Curve [204](#)  
    Prompt User to Save manual changes made in Review [204](#)  
    Use v2.XX Style Retention Time Calculations [204](#)  
    Use v3.OX Style Peak Width and Threshold Determination [202](#)

## T

- Tangential Skim event, Traditional [118](#)
- Touchdown % Threshold event, ApexTrack [80](#)
- Touchdown %, ApexTrack [30](#), [39](#)
- Touchdown Threshold event, Traditional [100](#)
- Touchdown, in ApexTrack [44](#)
- Traditional integration [22](#), [91](#)
  - Force Baseline events [103](#)
  - peak detection [82](#)
  - peak integration events [101](#)
  - peak labels [102](#)

## U

- Update RT [130](#)
- Updating retention time [130](#)
- Upslope points, ApexTrack [36](#)

## V

- Valley-to-Valley event, ApexTrack [31](#), [66–70](#)
- Valley-to-Valley event, Traditional [114](#)

## W

- Weighting [166](#)