RIGOL

User's Guide

UltraChrom
Chromatography Workstation

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How to Use this Manual

This manual guides users to quickly install and use the UltraChrom chromatography workstation. It introduces the installation, system configuration, data management, report generation and system management of the UltraChrom chromatography workstation. You are recommended to read the manual thoroughly to get a fully understanding of the workstation.

Note:

- Read the manual carefully before using the UltraChrom chromatography workstation.
- Please keep this manual with due care for future reference.

Document Overview

Subjects in this Manual:

Chapter 1 UltraChrom Overview
This chapter introduces the characteristics and common concepts of the UltraChrom chromatography workstation to show users how the workstation works.

Chapter 2 Installation
This chapter introduces the installation requirements and the installation procedures of the UltraChrom chromatography workstation.

Chapter 3 Operations
This chapter guides users how to use the UltraChrom chromatography workstation safely, correctly and effectively.

Chapter 4 Data Processing Principles
This chapter introduces the data processing principles of UltraChrom.

Contents

Chapter 1 UltraChrom Overview

Topics of this chapter:

- UltraChrom Characteristics
- Common Concepts

UltraChrom Characteristics

Overview

UltraChrom is the workstation software for **RIGOL** high performance liquid chromatography system. The workstation combines data acquisition, processing and analysis in one and is an effective tool for liquid chromatography system with digital signal output. Besides, this workstation provides beautiful and user-friendly interfaces and is easy to operate.

The chromatography workstation is the software used to control all series of instruments (except the column) of **RIGOL** high performance liquid chromatography system as well as to process and analyze the instrument data. Users can control the instruments by setting the control parameters of each instrument in the workstation. For the introduction of each instrument of **RIGOL** high performance liquid chromatography system, refer to the corresponding User's Guide.

UltraChrom Hardware

UltraChrom workstation is an important component of **RIGOL** L-3000 HPLC (High Performance Liquid Chromatography) system. The L-3000 HPLC system also contains L-3100 solvent organizer, L-3200 series pump (L-3210 isocratic pump, L-3220 binary pump and L-3240 quaternary pump), L-3300 autosampler, L-3400 thermostat, L-3500 UV-VIS detector and column.

RIGOL L-3000 HPLC system is as shown in the figure below.

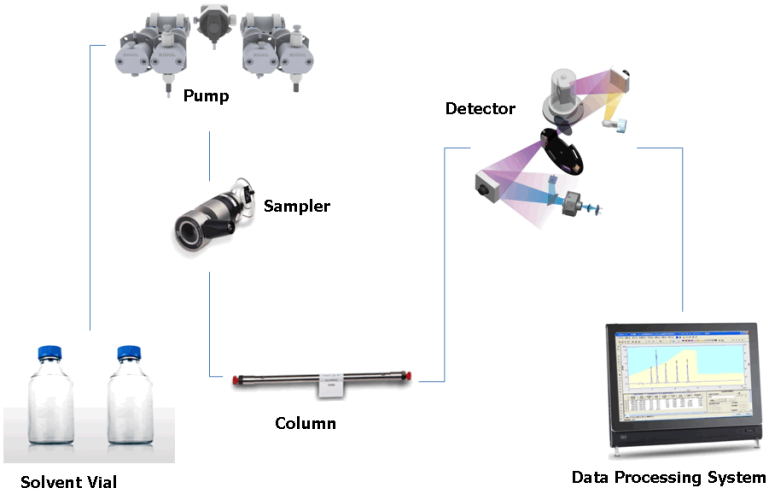


Figure 1-1 L-3000 HPLC system

UltraChrom Software

1. Operation System

UltraChrom software can run on the Windows Vista, Windows XP SP3 and Windows 7 operaing systems.

2. System Configuration

Users can configure the system using the configuration functions in the login window. Users can select the instrument module to be configured, define the communication mode as wll as set the storage directories of the analysis method, sequence and data.

3. Software User Interface

According to different analysis tasks, the user interface of UltraChrom software provides four kinds of function windows: the analysis window, chromatogram window, calibration window and report template window.

The analysis window consists of instrument monitor window, sequence window (used for single sample analysis or multi-sample auto analysis) and signal acquisition window.

Users can perform integration operation and set the chromatogram display parameters in the chromatogram window.

Users can calibrate the stored chromatogram via the calibration window.

Users can recall the report templates provided with the software or edit their own report templates via the report template window.

Common Concepts

Method

1. Definition

Method consists of the device control parameters, run control parameters, acquisition parameters, analysis parameters and method information.

2. Constituent and State

The method filename can be english letters and numbers and usually uses ".MTD" as its extension. A method usually consists of the method information, instrument control, data analysis and run event table.

- **Method Information:** information relating to the method.
- **Instrument Control:** set the parameters used to control the instrument or its components. For example, in the liquid chromatography system, the mobile phase ratio, flow, injection volume and detector wavelength are used to control the pump, sampler and detector.
- **Data Analysis:** set the analysis parameters of the acquired signal.
- **Run Event Table:** the operation that will trigger at the specified time or the operation that will be triggered by the specified event after the method is sent.
- **Storage State of Method:** the method is stored in the Method directory.
- **Running State of Method:** running a method refers to recalling the stored method.

3. Creation and Edit

Method creation refers to setting and storing or running the instrument parameters and data analysis conditions.

Users can create a deault method and then edit the method using the method editing function; users can also edit the parameters of the method while creating a method.

4. Operation Keypoints

- **Data Acquisition:** perform injection, acquire the original data and save the data.
- **Data Analysis:** recall the data file, analyze the data, determine the peaks and make calculation.
- **Print the Report:** print the data result as a report.

Data Acquisition

1. Definition

During data acquisition, the signal acquired by the instrument is converted from analog signal to digital signal in the detector and the digital signal is then sent to the workstation and stored as data file.

2. Data File

The data file generated is stored in the Data folder with the extension "*.CHR".

3. Display the acquisition signal real-time

Aquisition graph is provided to display the signal real-time and allows users to monitor various kinds of signals. Users can select the signal to be observed and adjust the time axis and signal axis.

Integration

1. Definition

Integration refers to indentifying the peaks in the signal and make calculations. Integration is indispensable for quantitative calculation, peak purity calculation and spectral library retrieval.

2. Integration Algorithm

The software uses standard integration aglorithm.

The main functions of integration aglorithm are as follows.

- Set the auto integration of the initial integrator parameters.
- For multiple signals or multiple detectors, set the corresponding integration event table of each chromatogram.
- Determine the interaction between integration events to allow users to select the time of the event via graphs.
- Display and print the integration result.

Integration aglorithm consists of the following commands.

- Determine the integrator parameters so as to create or modify the basic setting of the integrator device.
- Provide baseline control parameters, for example, Together, Valley, Peak-Forward/Back Horizontal and Time-Forward/Back Horizontal.
- Use the integrator control command to determine the range of integration operation.

3. Functions

When integrating the signal, the software performs the following operations.

- Determine the start time of the peak and mark that point with "|".

- Search for the peak maximum of each peak and determine the retention time.
- Determine the end time of the peak and mark that point with "|".
- Create the baseline.
- Calculate the peak area, peak height, half-peak width, separating degree, theoretical plate number and tailing factor.

The integration process is controlled by the integration event parameters; wherein, threshold and peak width are the two most important events.

UltraChrom allows users to set the initial values of various events and these values are valid at the start of the signal. What's more, the auto integration function provides a set of initial events that can be further optimized. UltraChrom allows users to arrange new integration events in the chromatogram at the proper time to control the integration process.

The integration process includes peak identification (determine the characteristic point), baseline creation and peak area calculation.

(1) Peak Identification
The first step of integration. The peak identification refers to determining the peak start, peak maximum and peak end.

The threshold input by users will be converted to slope inside UltraChrom according to certain formula and this slope will be used as the slope threshold in peak identification. Meanwhile, the peak width is converted into the window width of a proper data group. Each data group consits of various initial data points and as a minimum unit. The peak start and peak end are determined by comparing the average slope of each data group and the slope threshold.

Positive peak identificatipn process:

- Execute data grouping: data grouping will not affect the original data acquired and is mainly used to reduce the effect of the noise on the signal and the aglorithm time. The number of signal points in each group (namely the window size) is calculated inside the software according to certain formula.
- Search for peak start: when the average slope of the signal points in two neighbouring windows is greater than the slope threshold, the lowest point in the two windows is the peak start (for positive peak).
- Determine the peak maximum: the slope of the signal point changes from positive value to zero and then to negative value.
- Search for peak end: when the average slope of the signal points in two neigbouring windows is lower than the slope threshold, the lowest point in the two windows is the peak end (for positive peak).

Integration of peaks that are not completely separated

When two peaks overlap, there is no baseline between the two peaks and the integrator separates the two peaks at the valley using "|". The integrator saerches for the start of the first peak and make area integration until the valley is reached. The area integration of the first peak ends at the valley where the area integration of the second peak starts. The area integration fo the second peak ends at the end of the second peak.

For complicated chromatogram peak, UltraChrom provides various manual integration events for users to easily adjust the baseline to the proper position, incuding Together, Valley, Peak-Forward/Back Horizontal and Time-Forward/Back Horizontal.

Shoulder Peak Integration

Shoulder peak refers to the unformed peak on the rising edge or falling edge of large peak. In this case, the negative slope is followed closely by positive slope and there is no valley. When shoulder peak occurs at the maximum negative curvature, the shoulder peak area is deducted from the main peak area.

(2) Baseline Creation

After peaks are identified, you need to create the baseline to determine the final area. The baseline creation procedures are as follows.

- Create the initial baseline: create the initial baseline by refering to the positions of the peak start and peak end.
- Baseline Adjustment: if the initial baseline and the chromotogram peak signal intersect, the software will adjust the baseline automatically to make sure that there is no other crossings between the initial baseline and the chromatogram peak signal except the peak start or peak end.

(3) Peak Area, Peak Height and Half-peak Width Measurement

The last step of peak integration is determining the final peak area, peak height and half-peak width, namely calculating the retention time, height and area of each peak and comparing them with the minimum peak area, peak height and peak width specified in the processing method.

Peak Identification

1. Definition

Peak identification means identifying the components in the unkonown sample using the chromatogram/electrophoretic characteristics determined by analyzing the standard sample. For quantitative analysis method, component identification is indispensable. The signal characteristics of each component are saved in the method calibration table.

In peak identification, each peak in the signal is compared with the peaks saved in the calibration table. The calibration table contains the expected retention time of the target component. Peaks of which the retention time matches that of the peak in the calibration table are assigned the characteristics (such as the name and response factor) of that component. Peaks that does not match any peak in the calibration table are regarded as unknown peaks.

The peak matching rules are as follows.

- If a sample peak falls within the peak matching window of a component peak in the calibration table, this peak will be assigned the characteristics of that component.
- If more than one sample peaks fall within the peak matching window of a component peak in the calibration table, the component of the peak is determined according to the matching standard selected by users (peak with retention time that is closet to the expected retention time, peak with the maximum area, the first peak and the last peak).
- If a sample peak does not fall within any peak matching window, it will be listed as unknown component.

2. Identification Type

UltraChrom provides various techniques to match the sample peak with the standard peak in the calibration table.

3. Absolute Retention Time

Compare the retention time of the sample peak with the expected retention time of each component in the calibration table.

4. Identification Process

The software provides three kinds of peak identification methods according to different integration data.

- Determine the time reference peak
- Identify all the ISTD peaks
- Determine all the peaks left in the calibration table

Quantitation

1. Definition

After integrating and identifying the peak, quantitation operation is required. Quantitation means determining the concentration of the compound in the sample using the peak area or peak height.

The procedures of quantitative analysis are as follows.

- Determine the compound to be analyzed.
- Create the analysis method of the sample with this compound.
- Analyze one or more sample with known concentration of this compound to acquire the response under each concentration.
- Analyze the sample with unknown concentration of this compound to acquire the response under the unknown concentration.
- Compare the response under the unknown concentration with that under known concentration to determine the concentration of the compound.

2. Quantitative Calculation Methods

UltraChrom provides the following calculation methods.

- Normalization
- ESTD
- ISTD

The calculation method used to determine the concentration of the compound depends on the quantitation type. All the calculation methods use the peak area and peak height for calculation, but generate different types of report.

3. Calibration Factors

Quantitative analysis uses four kinds of calibration factors.

- Response Factor
- Scale Factor
- Dilution
- Sample amount

These factors are used to compensate for the response variation of the detector to different sample constituent, concentration, dilution and amount as well as to compensate for the unit conversion.

Response Factor

In compound analysis, the response factor of the sample component is calculated by dividing the response of this component (peak height or peak area) by the amount. The response factor is used to calibrate the detector response of each sample component during the calibration calculation process.

Scale Factor

When making calculation, multiply the result of each component by the scale factor. The scale factor can also be used to represent unit conversion.

Dilution

Before printing the report, multiply the calculation result by the dilution. Before the analysis, you can use the dilution to change the result or the variation of the calibration sample constituent. You can also use the dilution in other applications that require a constant factor.

4. Uncalibrated Calculation Process

The uncalibrated calculation does not use the calibration table and includes area percentage method and peak height percentage method.

In area percentage method, the area of each peak is represented by the percentage of the peak area in the total area of all the peaks in the analysis. Area percentage method does not require pre-calibration, does not depend on the sample injection volume of the detector and does not use any response factor. If the responses of all the components in the detector are the same and elute, the proximate relative amount of each component can be acquired using area percentage method.

In peak height percentage method, the height of each peak is represented by the percentage of the peak height in the total peak height of all the peaks in the analysis.

5. Calibrated Calculation Process

ESTD, normalization and ISTD calculation processes all use response factor and therefore, require the calibration table. The calibration table converts the response value to the value with specified unit according to the calculation process selected.

ESTD

Analyze the standard sample and unknown sample under the same conditions and compare the result of the unknown sample with that of the standard sample to calculate the amount of the unknown sample.

The difference between ESTD and ISTD lies in that ESTD uses response factor. Acquire the response factor via calibration and save it. In the following sample analysis, the response factor is used to calculate the amount of the sample. As there is no standard for calibrating the injection volume or sample configuration, the sample injection volume of multiple analyses should have good repeatability.

ISTD

In ISTD method, a known amount of component is added to generate a uniform factor, thus improving the ESTD method. This compound is the internal standard sample which should be added into both the standard sample and the unknown sample.

The software calculates the concentration of the compound using the response

factor acquired from calibration and saved in the method as well as the concentration and peak area or height of the internal standard sample.

The compound used as the internal standard sample should have similar chemical characteristics and close retention time with the calibrated compound; while at the same time can be completely separated from the calibrated compound in the chromatogram or electrophoretic graph.

Calibration

1. Definitions

- **Calibration:** determine the response factor used to calculate the concentration of the component by analyzing the specified calibration sample.
- **Compound:** in multi-signal calibration, a compound can consists of multiple peaks and each signal usually contains a single peak; in single signal calibration, each compound corresponds to a single peak.
- **Calibration Level:** the calibration point formed by a single calibration sample concentration. In multi-signal calibration, the calibration points can be on multiple signals.
- **Calibration Sample:** also called standard sample or standard mixture. It contains known amount of the compound to be calibrated. In the software, calibration sample also refer to the sample injected from the calibration sample vial. The calibration sample can be bought from chemical distributors or can be prepared by precisely measuring the amount of pure compound. The amount of the compound in the calibration sample is usually expressed by its concentration (such as in the unit ng/μL).

2. Calibration Table

According to the calculation process selected, the calibration table converts peak area or peak height to amount or concentration. The calibration table lists the retention time acquired from the calibration and compares the retention time with that of the peak in the sample analysis. If the retention times match, the sample peak is believed to represent the same component as the peak in the calibration table. During the analysis or when generating report, each peak amount input into the table is used to calculate the amount or concentration (depending on the calculation process selected). The type and amount of information required for creating the calibration table depends on the calculation process selected.

The following information is required for creating a calibration table.

- The retention time of each component peak of the calibration mixture.
- The amount of each component in the calibration mixture (expressed in the same unit).

3. Calibration Curve

The calibration curve is formed by the amount and response value of the compound acquired from one or more calibration samples. Generally, the response value is determined by analyzing the standard sample, acquiring a signal and then calculating the peak area or peak height.

After the calibration curve fitting is completed, the related coefficient is also displayed on the calibration curve. The related coefficient is the square root of the regression coefficient and presents the fitting degree of the calibration curve between data points (0 denotes irrelevance and 1 denotes complete correlation).

4. Calibration Type

UltraChrom provides two kinds of calibrations, namely single-level calibration and multi-level calibration.

Single-level Calibration

In single-level calibration, the calibration curve only contains one point. For single-level calibration curve, it is assumed that the detector response within the concentration range of the sample to be tested is linear and the response factor of a component peak is the reciprocal of the slope of the calibration curve that passes this point and the origin. One disadvantage of single-level calibration is that the detector response to the sample concentration is not always linear and the concentration-response curve does not always pass the origin; therefore, the result might not be accurate. To acquire accurate quantitative result, the calibration curve should at least contain two levels which should cover the expected amount of the unknown sample.

Multi-level Calibration

When the linear response used for a component is not accurate enough or the calibration range is not accurate, multi-level calibration is required. Each calibration level corresponds to a standard sample with accurate component concentration. The concentrations of all the components of the standard sample should be within the expected concentration range of the unknown sample. This method allows the detector response to change with the concentration and you can also calculate the response factor. In linear fitting, the detector response slope is determined by the optimum fitting that passes as many as points as possible and each point corresponds to a level.

Calibration Curve Fitting

Multi-level calibration can use various kinds of curve fitting calculation modes.

- Point to Point
- Linear
- Ln
- Power
- Exponent

- Quadratic
- Cubic

Non-linear Fitting

In some circumstances, the detector response to the sample concentration is non-linear. Linear regression calibration method is not proper for these kinds of analyses and multi-level calibration calculation is required.

Origin Processing

When drawing the response curve, three methods can be used to process the origin (ignore the origin, include the origin and pass the origin forcefully). Wherein, the calibration curve that passes the origin forcefully includes the origin, the calibration points in the first quadrant can be mapped into the third quadrant and all the calibration points are used in the regression calculation to make sure that the result calibration curve passes the origin.

Calibration Point Weighting

When creating default calibration curve, you can give the relative weight or importance of each calibration point used to generate the curve. The following weighting items can be used.

- **Equal:** all the calibration points on the curve have the same weight.
- **Linear (Amount):** the weighted value of the calibration point of which the amount is x is 1/x and is normalized by the minimum amount to make sure that the maximum weighted factor is 1. Normalization is realized by multiplying the weighted value by the minimum amount. For example, the weighted value of the calibration point of which the amount is x is $1/x \cdot a$; wherein, "a" is the minimum amount of the standard sample. If the origin is included, its weighted value is the average weighted value of other calibration points.
- **Linear (Response):** the weighted value of the calibration point of which the response value is y is 1/y and is normalized using the minimum response value to make sure that the maximum weighted factor is 1. Normalization is realized by multiplying the weighted value by the minimum response value. For example, the weighted value of the calibration point of which the response value is y is (1/y) - b; wherein, "b" is the response value of the minimum calibration amount of the standard sample. If the origin is included, its weighted value is the average weighted value of other calibration points.
- **Quadratic (Amount):** the weighted value of the calibration point of which the amount is x is $1/x^2$ and is normalized by the minimum amount to make sure that the maximum weighted factor is 1. Normalization is realized by multiplying the weighted value by the minimum amount. For example, the weighted value of the calibration point of which the amount is x is $1/x^2 \cdot a^2$; wherein, "a" is the minimum calibration amount of the standard sample.
- **Quadratic (Response):** the weighted value of the calibration point of which the response value is y is $1/y^2$ and is normalized using the minimum

response value to make sure that the maximum weighted factor is 1. Normalization is realized by multiplying the weighted value by the minimum response value. For example, the weighted value of the calibration point of which the response value is y is (1/y) - b; wherein, “b” is the response value of the minimum calibration amount of the standard sample.

Auto Analysis

1. Definition

Auto analysis refers to the automatic analysis of multiple injections. The sequence function of the UltraChrom software allows users to sample data, process data and generate report automatically.

2. Definition of Sequence

Sequence is a series of instructions used to analyze the sample automatically.

With sequence, the system can inject each sample automatically as well as acquire and analyze the data according to the specified method. Samples in the sequence can be analyzed using different methods.

3. Sequence Table

The sequence table determines the method used to analyze the sample and sorts the sample vials to be analyzed. This table also contains the information of each sample, such as the sample name, quantitative parameters and method parameters.

Report

1. Definition

Report contains the quantitative and qualitative information of the sample analyzed. The report can be printed, displayed on the screen and saved as a file. The report can contain the detailed information of the peak measured, the signal graph acquired and the detailed information of the analysis method.

2. Report Result

There are two types of reports.

- Uncalibrated report of the uncalibrated response information of the detector.
- Calibrated report of the calibrated responses to different components of the sample.

Uncalibrated Report

The uncalibrated report includes area percentage report and peak height percentage report. Generally, these reports are used to prepare the calibrated

report; but, they are used as the final reports when the compound amount matches the unit area or peak height generated.

Calibrated Report

The calibrated report calibrates the response difference of the detector to different compounds. The running conditions of the unknown sample and calibration sample must be the same. The integration data of the standard sample can be used to create the calibration table. In the report, the retention time, amount and response factor are listed.

The calibrated report is based on the ESTD and ISTD calibration methods.

- **ESTD Report**
  ESTD lists all the results that uses the unit selected by the user or uses the amount percentage of the component in the mixture as the unit. ESTD requires precise relative injection volume of the standard sample and unknown sample. The reliability of ESTD is limited by various variable factors of different samples and the injection repeatability.
- **ISTD Report**
  ISTD conquers the limitation of ESTD. In ISTD method, known amount of internal standard sample is added into the standard sample and unknown sample (the amounts can be different) and the response rate is calculated by dividing the response value of each component by the response value of the internal standard sample. It draws the calibration curve and calculates the report result using the ratio of the response rate to the amount. This method eliminates the error caused by the tiny difference in injection volume and the variation of the chromatogram system. The unit of the result in the ISTD report is set by users.

3. Quantitative Analysis Report

The type of the report can be marked out using the name of the calculation method used, such as ISTD report. The introduction of each kind of report is as shown below.

- **Area%**
  The simplest report form. As the response values of the sample components are not calibrated, calibration is not required. The Area% is used to create the calibration table together with other items. This kind of report is applicable to analysis of which the detector responses to the sample components are similar.
- **Peak%**
  Similar to Area% report. The peak height is used for calculation instead of the peak area.
- **Norm%**
  The components are listed in the report using their percentages in the mixture. Before calculating the percentage, the peak should be calibrated using the detector response factor.

- **ESTD**
  ESTD report contains the actual amount of each component and the unit is set by users. The actual amount is calculated using the preset calibration table. To use the ESTD method, you have to know the injection volume of the standard sample.
- **ISTD**
  ISTD contains the actual amount of each component and the calibration curve is used to calculate the actual amount. When using the ISTD method, the injection volume of the sample is not required and the instrument variation during the analysis can be calibrated.

4. Report Output

The report can be output in the following forms.

- **Screen**
  Display the report on the screen via the report preview window and you can also print the report.
- **Printer**
  Print the report via the printer currently selected.

Chapter 2 Installation

Topics of this chapter:

- Installation Requirements
- To Install/Uninstall the Software
- To Connect the Hardware

Installation Requirements

Before using the UltraChrom chromatography workstation to control the chromatography system, make sure your PC fulfills the following requirements.

- A free COM terminal (used to connect the detector or other device with data transmission function).
- A free USB terminal (used to connect the hardware key).
- A CD ROM drive (used to install the software).
- 50 MB to 200 MB disk space (depending on the number of components to be installed).

If you ordered the autosampler, a free parallel port, USB terminal or network port is required for connecting the PC and autosampler.

UltraChrom software can run on the Windows Vista, Windows XP SP3 and Windows 7 operaing systems. **In this manual, the installation, uninstallation and operation methods of the workstation are given based on the Windows XP SP3.**

UltraChrom is connected to **RIGOL** L-3000 system via standard VISA terminals. Users need to install NI VISA Run Time 4.6.2 or higher version. This software is provided in the installation package and users need to install VISA Run Time manually before installing UltraChrom. For Windows XP operation system, you also need to install Microsoft .NET Framework 2.0 SP2.

Note: When installing the UltraChrom chromatography workstation, the installation procedure detects the current status of Microsoft .NET Framework 2.0 SP2 automatically. If it has not been installed or it is a lower version, the installation procedure will install it automatically. If UltraChrom cannot work normally, you need to uninstall the software and then re-install it.

To Install/Uninstall the Software

Before installing the software, make sure that your installation package is complete. In case anything is missing, please contact your **RIGOL** distributor or local office.
Note: Please do not configure any hardware device before installing the UltraChrom chromatography workstation.

To Install UltraChrom

To use the UltraChrom chromatography workstation, you must have read and write permissions (namely administrator permission) of the workstation files.

The software installation procedures are as follows.

1. Turn on the PC and start Windows. Log in the operation system with the account with administrator permission. Insert the UltraChrom installation CD into the CD-ROM drive.
2. Now, the installation wizard program will start automatically (if not, click the UltraChrom Setup.exe file in the CD manually).
3. Install the workstation step by step according the prompt of the installation wizard.
4. After the installation is finished, the start-up icon  will be displayed in the **All programs** and on the desktop.

To Uninstall UltraChrom

If UltraChrom cannot work normally, you need to uninstall the software following the two method below and then re-install it.

Method 1:

1. Click **Start → All Programs → RIGOL → UltraChrom → Uninstall UltraChrom Workstation** to open the UltraChrom uninstallation dialog box.
2. Click the "Next Step" to uninstall the workstation.
3. After the uninstallation is finished, click "Finish".

Method 2:

1. Click **Start → Control Panel → Add or Remove Program** to open the **Currently installed programs** list.
2. Select "UltraChrom" in the list and click "Remove" to uninstall it.
3. After the uninstallation is finished, click "Finish".

To Connect the Hardware

To Connect the HPLC System to PC

Connect the COM terminal of the PC to the specified “RS-232C” interface at the rear panel of the system controller using RS-232C cable.

Network Function of the HPLC System

Connect the PC and HPLC system to the switch hub using LAN cable.

Chapter 3 Operations

Topics of this chapter:

- To Start the Workstation
- To Log in the Workstation
- To Manage the Workstation
- Main Operation Interface
- Batch
- Device Monitor
- Method Setup and Analysis
- Data Acquisition
- Data Processing
- To Generate the Calibration Curve
- Report Function

To Start the Workstation

Users can start UltraChrom workstation using the following two methods after installing the workstation properly and connect L-3000 series devices.

1) Double-click the shortcut icon on the desktop as shown in the figure below.

2) Click **Start** → **All Programs** → **RIGOL** → **UltraChrom** → **UltraChrom Workstation**.

To Log in the Workstation

When starting the workstation, you can enter the interface as shown in the figure below.

When you log in the workstation at the first time, please select “Administrator” in the User Type, input correct name (**admin** by default) and password (empty by default) and click **OK**. After login the workstation, the administrator can modify the name and password of the administrator as well as add users and assign the operation rights for the users.

When you log in the workstation again, please select proper User Type and Name, enter correct password and click OK. For detailed information about account management, please refer to “**Account Management**”.

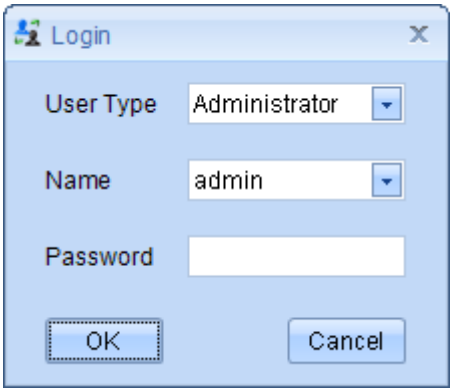


Figure 3-1 Login Interface

To Manage the Workstation

After login the workstation, you enter the UltraChrom Management interface as shown in the figure below.

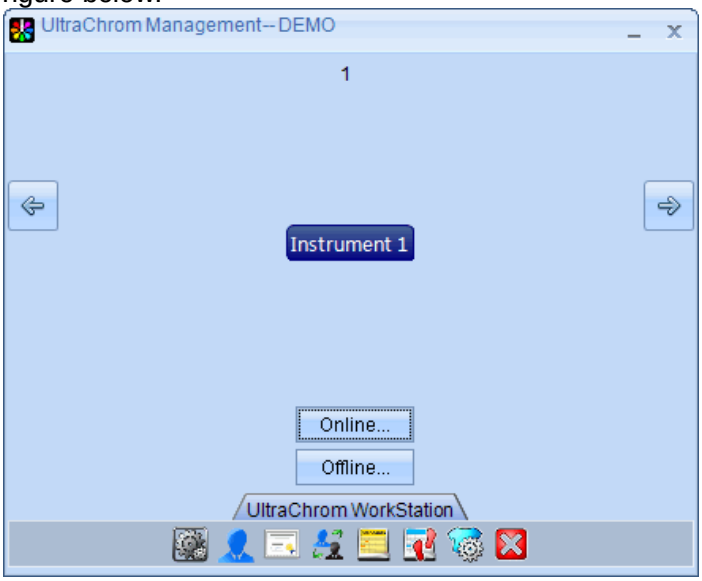


Figure 3-2 Management Interface

1.		Select instrument	2.		Configure instrument
3.		Account management	4.		Certificate
5.		Change user	6.		GLP Setting
7.		Track	8.		System Setting
9.		Exit	10.	Online...	Login Online
11	Offline...	Login Offline	——	——	——

UltraChrom can be connected to 4 sets of instruments at most. When multiple sets of instruments are connected to UltraChrom, you can click or to select the instrument and click to configure. For the operation method about instrument configuration, please refer to the following content. After configuring the instrument, click **Online...**, the workstation will start all the devices configured and built communication and then enters operation interface. At this point, you can edit method, edit sequence, execute data acquisition, process the chromatogram and print the report. If you click **Offline...**, data acquisition is not permitted.

To Configure the Instrument

Before using the workstation, please configure the instrument to make sure that the configuration of the workstation is matched with the configuration of the instruments. Only when the UltraChrom is connected to the analysis instruments at the first time or the configuration of the instrument is changed, the following configuration is needed.

1. Click  in the UltraChrom Management interface to enter the Instrument Configuration window.

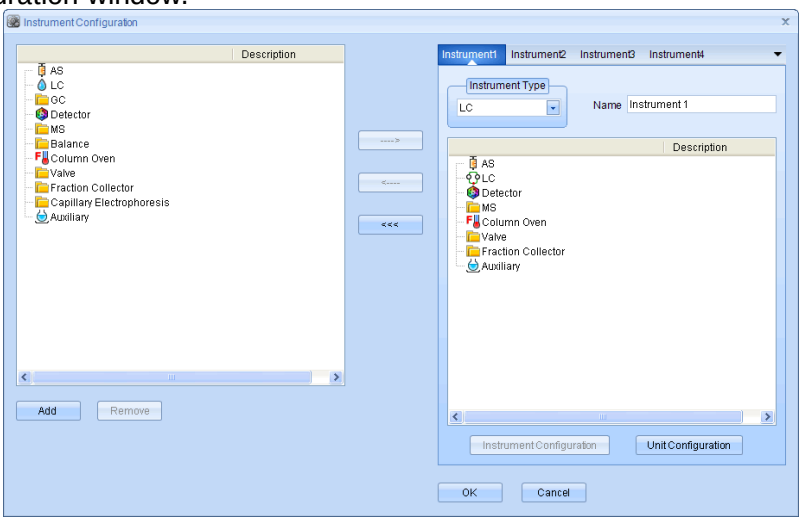


Figure 3-3 Instrument Configuration

2. Click Add in the Instrument Configuration window to enter the Device dialog box. Select **L-3000** under the **LC** item and click **Add**. Now, you are back to the Instrument Configuration window.

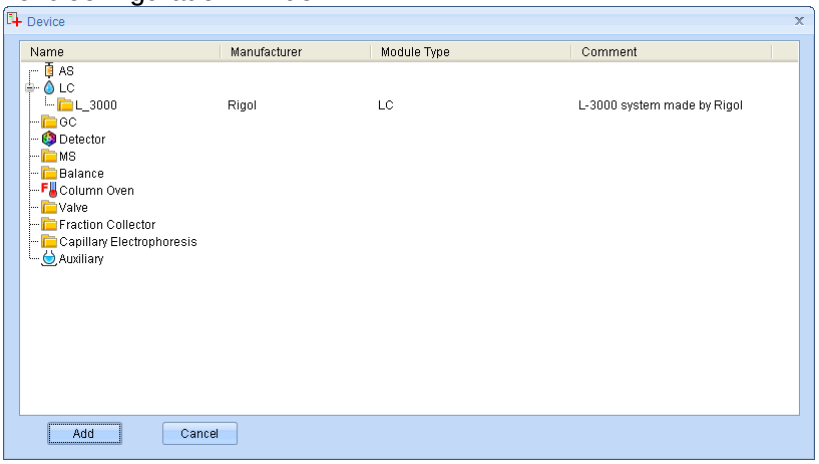
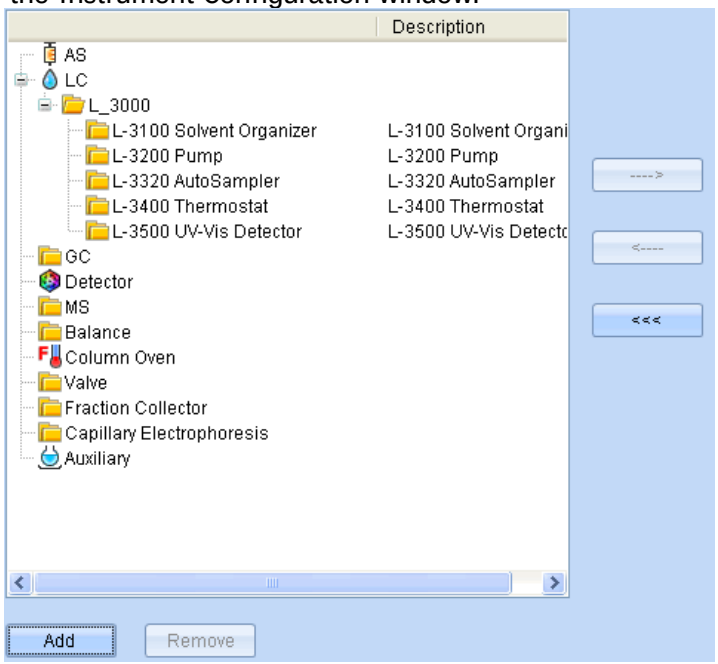


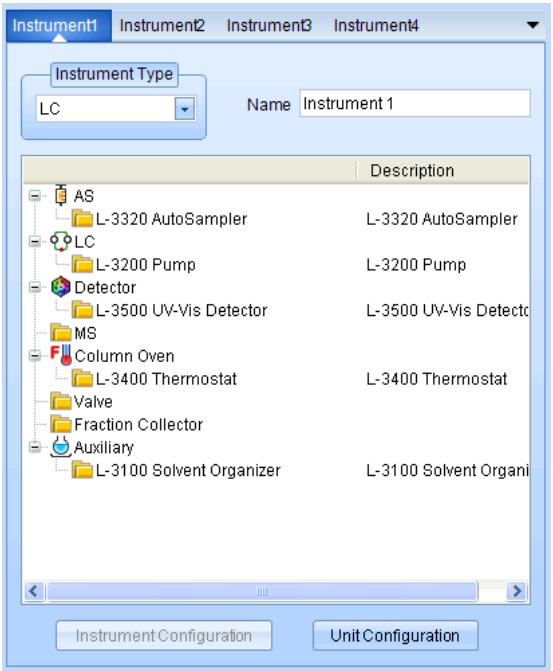
Figure 3-4 Device dialog box

At this point, the instruments of L-3000 are displayed under the **LC** item at the left side of the Instrument Configuration window.



3. Select the desired instrument module in the left side and click 

to configure it into the workstation. Now, the modules configured are displayed in the right side.



4. Select the module to be configured in the right side and click **Instrument Configuration** or double-click the instrument module to be configured to enter the instrument configuration window.

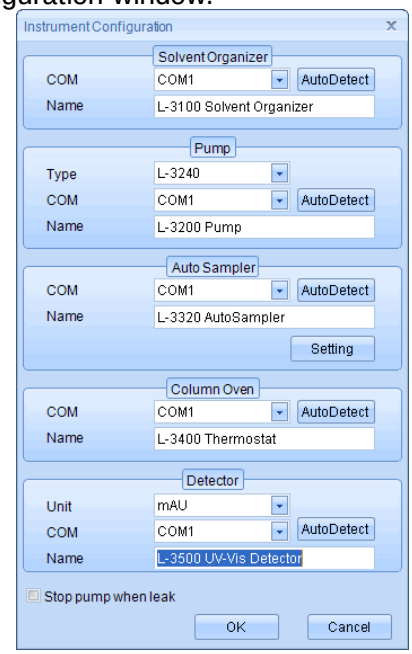


Figure 3-5 Instrument Configuration

Set the communication terminal of each module and click **AutoDetect** to test whether the communication is successful.

For auto sampler module, click **Setting** in the autosampler area to set the tray type and sampler pipeline parameters in the pop-up dialog box.

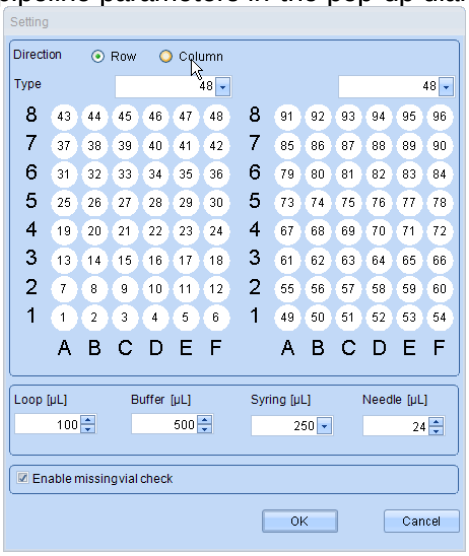


Figure 3-6 Auto Sampler Setting

Note: The tray type and pipeline parameters should match the actual component of the autosampler module; otherwise, your instrument might be damaged.

5. After finishing the configuration, click **OK** in the instrument configuration window to return to the instrument configuration window. Click **Unit Configuration** to set the display units of the instrument.

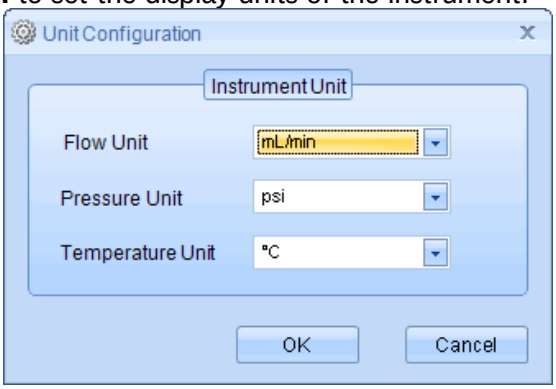


Figure 3-7 Unit Configuration

6. After setting the units, click **OK** in the instrument configuration window to return to the Management Interface. The modules configured are displayed in the Management Interface. The workstation configuration finishes.

Note: When multiple sets of instruments are connected to the workstation, you can click  or  to configure multiple sets of instruments according to the above steps.

Account Management

Account management is used to manage the users and their operation rights. The workstation has two types of users (administrator and user). The administrator who has the whole operation rights can create, modify and delete users and can assign operation rights for each user based on the actual requirements.

When you use this workstation at the first time, please log in it as administrator (User Type: Administrator, Name: admin, Password: empty). After login the workstation, click  in the UltraChrom Management interface to open the user accounts window.

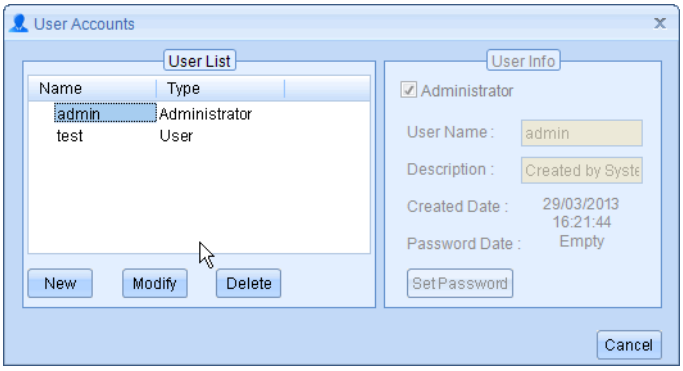
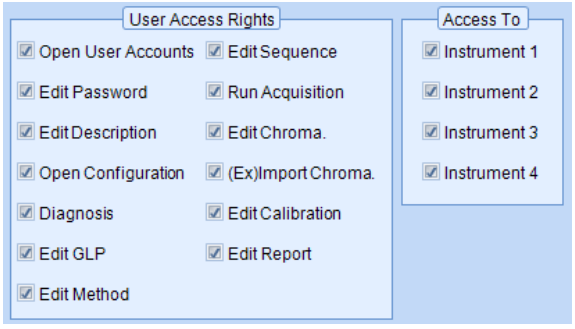


Figure 3-8 User Accounts

1. **To modify the name and password of the administrator**
   Select **admin** in the User Accounts window and click **Modify**. Now, the user information in the right side becomes editable. You can modify the user name, description and set password.

2. **To create a new user account**
   Click **New** in the User Accounts window, the user information in the right side becomes editable and each item is empty. You can set if the new user is administrator, user name, description as well as password. If the ckeck box before the Administrtor is not selected, the “User Access Rights” column and the “Access To” column as shown in the figure below will be displayed under the window.



3. To modify or delete the user infomation

Select the desired user in the User Accounts window and click **Modify**. Now, the user information in the right side becomes editable. You can modify the user name, description and set password.

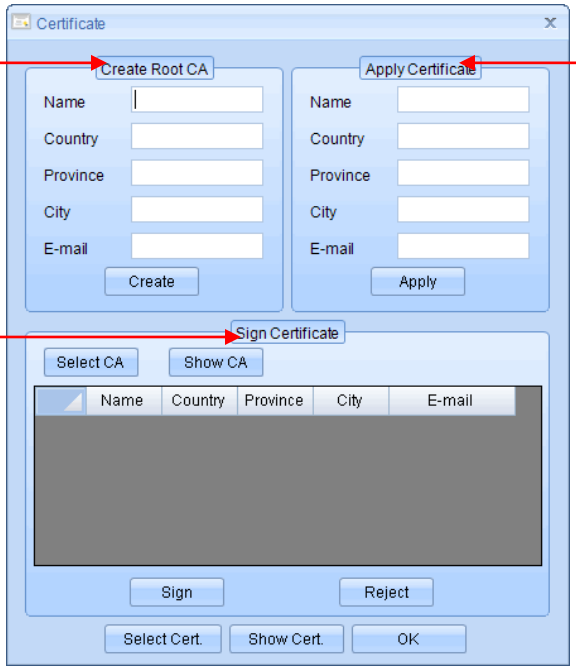
Select the desired user in the User Accounts window and click **Delete** to delete the user (the administrtor can not be deleted).

Certificate

The certificate is used to sign the report so as to recognize the user who generates the report. Ordinary users can apply for the certificate from the administrator. The administrator can generate the root certificate and issue certificate to himself and ordinary users. Click  in the management interface to enter the certificate window.

Only available for the administrator. Can generate one or more root certificates used to issue certificates.

Only available for the administrator. Used to select the root certificate generated to issue certificate.



Available for both the administrator and ordinary users. Used to apply for certificate from the administrator.

Figure 3-9 Certificate (Administrator Login)

To Change User

Click  in the UltraChrom Management interface to back to login interface in which you can login the workstation again using other username.

GLP Settings

Click  in the UltraChrom Management interface to open the GLP window. Users can set the related GLP rules.

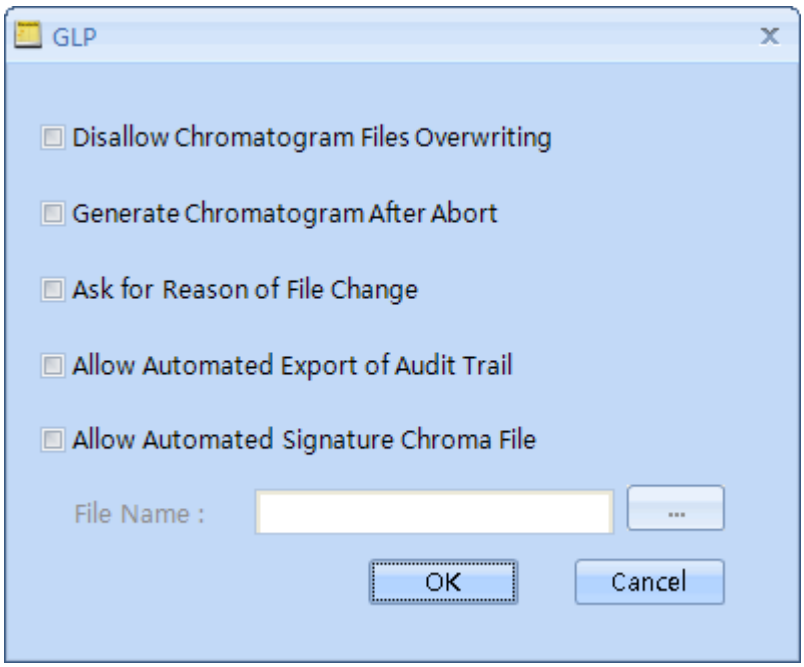


Figure 3-10 GLP Settings

Track

The track function records the running state of the system in real-time for users to check the problems occurred when using the system. Click  in the UltraChrom Management interface to open the audit trail window.

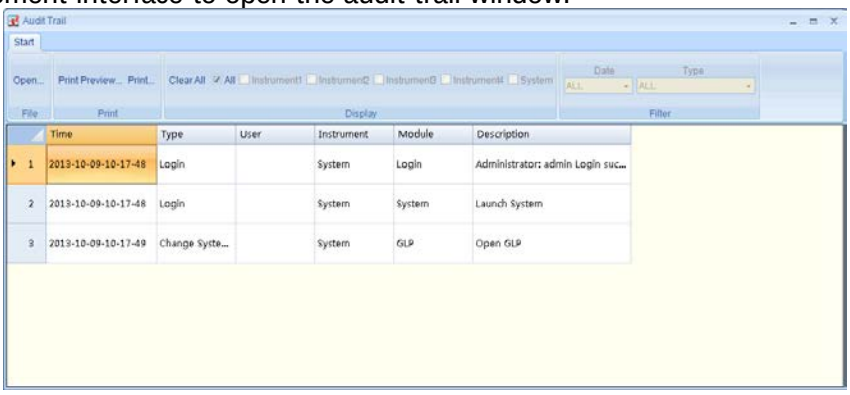


Figure 3-11 Track

System Setting

System setting is used to set the storage directories of various files, such as the method and sequence files. Click  in the UltraChrom Management interface to enter the system configuration window.

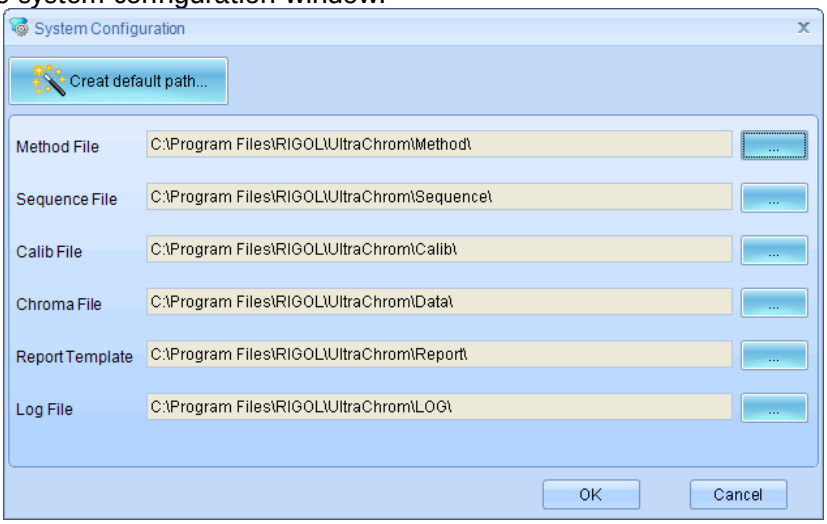


Figure 3-12 System Setting

Note: After logging in the workstation, you can click  at the upper left corner of the workstation and modify the file storage directory in the pop-up system configuration window.

Main Operation Interface

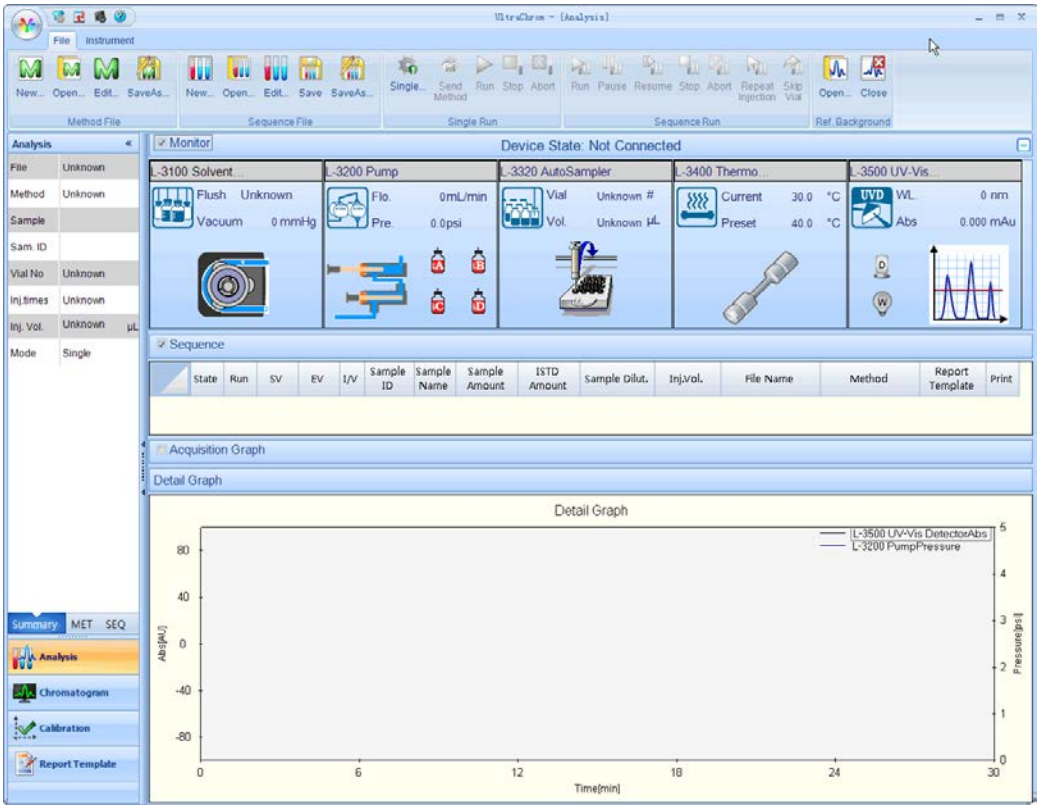


Figure 3-13 Main Operation Interface

1. **Shortcut Toolbar:** The shortcut toolbar is located in the upper left corner.

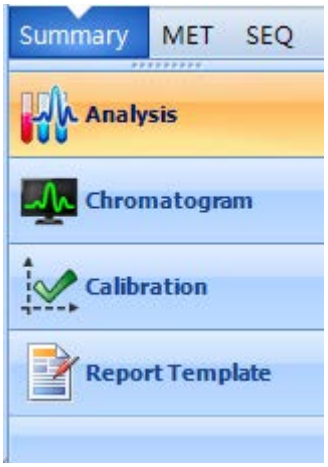
Icon		Description
	About	Query the information about UltraChrom.
	Exit	Exit UltraChrom.
		System setting. Set the storage directories of various files, such as the method and sequence files, refer to "**System Setting**".
		The track function records the running state of the system in real-time for users to check the problems occurred when using the system, refer to "**Track**".
		Perform batch processing on the chromatogram files, refer to "**Batch**".
		Open the help manual.

2. **Menu Bar:** The menu bar is located in the top of the interface and is used to

display the operation menus of the workstation. Each menu consists of multiple sub-menus. The menus displayed here are different base on the function selected in the Function Control Area.

3. **Device Monitor Area:** Device Monitor Area which is located in the lower right of the Menu Bar is used to monitor the running status of each device.

4. **Function Control Area:** In the left of the interface, there is Function Control Area which is used to select Analysis, Chromatogram, Calibration or Report Template. When different function is selected here, the content displayed in the Menu Bar and the Work Area is different.



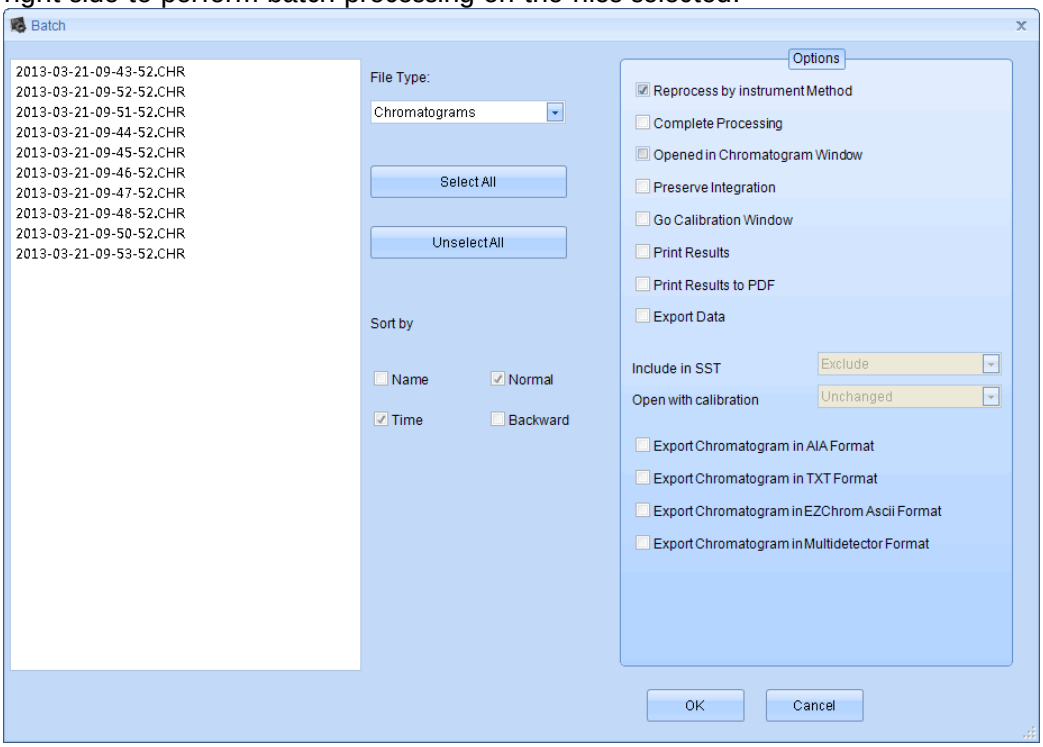
5. **Work Area:** Work Area is under the Device Monitor. Work Area displays sequence, acquisition graph, etc. The content displayed here is different base on the function selected in the Function Control Area.

The operation methods will be introduced in detail in the later part of this chapter.

Batch

The batch function is used to perform batch processing on chromatogram files.

Click  at the upper-left corner of the main operation interface to enter the batch processing interface as shown in the figure below. All the chromatogram files under the current default storage directory of chromatogram file are listed at the left side. You can click **Select All** to select all the files and then select the desired item at the right side to perform batch processing on the files selected.



Note: If the chromatogram file to be processed is not in the current default storage directory, you can click  at the upper-left corner of the main operation interface to modify the default directory.

Device Monitor

The device monitor area provides graphical monitor interface which is used to monitor the work status of the liquid chromatography system (including solvent organizer, pump, autosampler, thermostat as well as detector), as shown in the figure below.

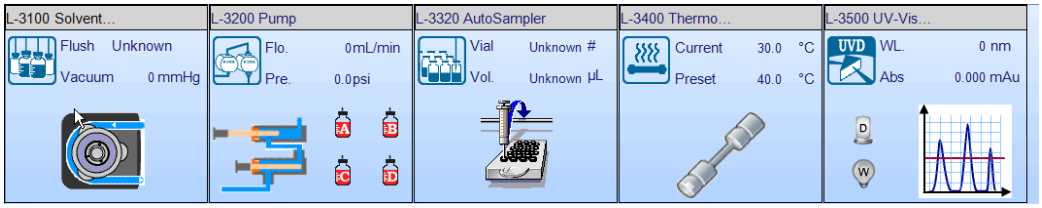


Figure 3-14 Device Monitor

Above the monitor area is the monitor status bar which displays the name (specified in system configuration) of each monitor module. The background color shows the connecting status of the module.

- Gray: not connected
- Green: the method is sent (ready)
- Blue: running
- Yellow: the device is connected but the method is not sent (not ready)
- Red: serious failure occurs, namely failure that requires stopping the sample

Note: The monitor status bar would turn red only when serious failure that requires stopping the sample occurs. When ordinary failure occurs, the background color of the monitor status bar does not change; at this point, the warning icon appears at the right side and flashes.

Solvent Organizer Monitor Area

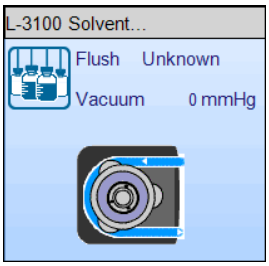


Figure 3-15 Solvent Organizer Monitor

This area monitors the running speed of the peristaltic pump and the vacuum level of the degasser. The peristaltic pump can run at three kinds of speeds (high, medium and low). When the pump is running, you can switch the speed. Right-click the solvent organizer icon at the bottom of the monitor area to select the desired speed.

Pump Monitor Area

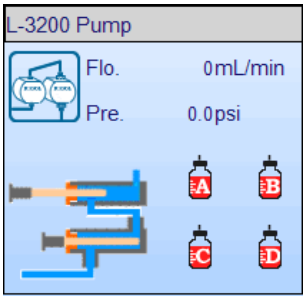


Figure 3-16 Pump Monitor

The pump monitor area monitors the flow, mobile phase ratio and system pressure of the pump. Besides, users can perform various operations on the pump via this monitor area, such as stopping the pump and purging the pump.

- **Flow [mL/min]:** in single pump mode, the flow [mL/min] of the single pump is displayed; in gradient pump mode, the sum of the flows of all the pumps is displayed.
- **Pressure [psi]:** display the pressure of the pump in real-time.
- **Flow Percentage:** for single pump, the flow percentage is 100%; for gradient pump, the percentage that each flow path takes up in the total flow is displayed.

Right-click the pump icon at the lower-left corner to select “Wash”, “Stop”, “Idle” or “Hold”.

- **Wash:** set proper flow and mobile phase ratio to wash the pump.

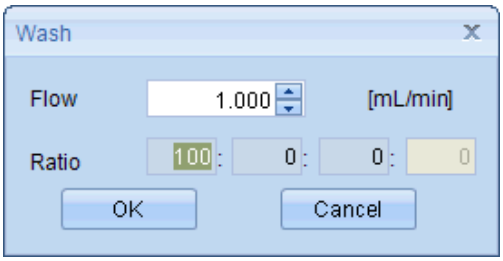


Figure 3-17 Wash Setting

Note: Open the exhaust valve before executing the "Wash" operation in the pump monitor area.

- **Stop:** stop the pump.
- **Idle:** set the pump to idle state.
- **Hold:** this button is available when the gradient table is running. Click this button and the flow is fixed; at the same time, the button changes to "Recovery". Click "Recovery" and the flow will change according to the setting in the gradient table.

Right-click the solvent vial icon at the lower-right corner of this area to enter the solvent volume setting window where users can set the volume of the solvent in the solvent vial.

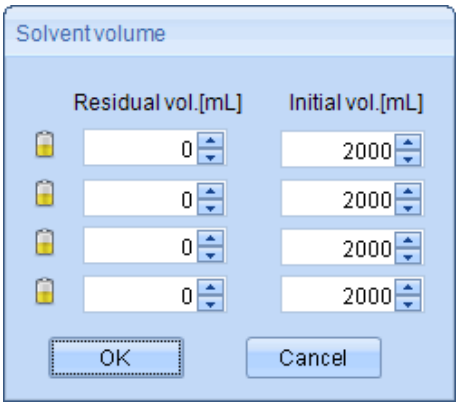


Figure 3-18 Solvent Volume Setting

Autosampler Monitor Area

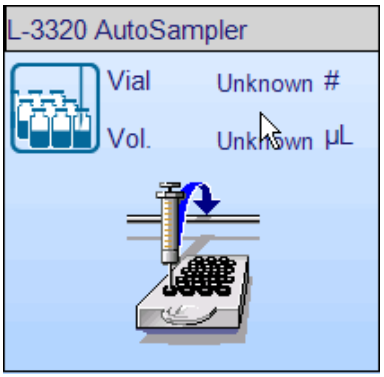


Figure 3-19 Autosampler Monitor

This area displays the number of the injection vial and injection volume. Besides, users can move the sample tray to the front position or back position as well as wash the injection needle.

- **Vial:** display the number of the current injection vial, the current number of injection and the total number of injections of the current bottle.
- **Vol.:** display the current injection mode and injection volume.

Right-click the auto sampler icon at the bottom of this area to select “Tray Front”, “Tray Back” or “Wash”.

- **Tray Front:** move the sample tray to the front position.
- **Tray Back:** move the sample tray to the back position.
- **Wash:** wash the injection tubing and injection needle.

Thermostat Monitor Area

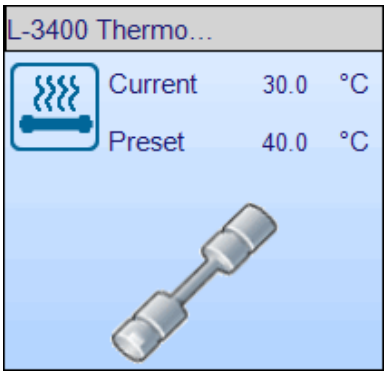


Figure 3-20 Thermostat Monitor

This area displays the preset temperature and the current temperature. Besides, users can also set the temperature of the thermostat. Right-click the thermostat icon at the bottom of this area to set the temperature of the thermostat, stop controlling the temperature and view the working mode and temperature upper limit of the thermostat.

Detector Monitor Area

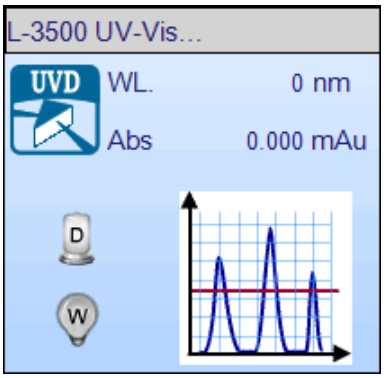


Figure 3-21 Detector Monitor

This area displays the current wavelength and absorbance. Right-click the deuterium lamp and tungsten lamp icons at the lower-left corner to control the on/off states of the deuterium lamp and tungsten lamp. About 30-second warm-up is required before the deuterium lamp is turned on. Therefore, after clicking **On** and then clicking **OK** in the pop-up dialog box, the deuterium lamp icon is displayed in red and will turn to yellow after about 30 seconds, indicating that the deuterium lamp is turned on. Right-click the auto zero icon at the lower-right corner to perform auto zero (it is used to restore the signal to “0” baseline). Note that auto zero is not recommended during the analysis process; otherwise, the data might be lost).

Method Setup and Analysis

Analysis Devices and Conditions

This section introduces how to set the method and execute analysis using the following instruments and under the following conditions using UltraChrom.

1. Analysis Devices

High-pressure gradient HPLC system
Pump: L-3220 binary pump
Autosampler: L-3320
Thermostat: L-3400
Detector: L-3500

2. Analysis Conditions

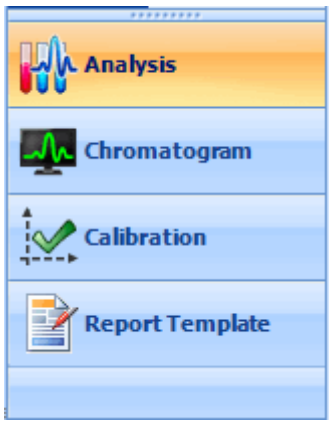
Column: Rigol-C18 (250mm x 4.6mm, 5μm inner diameter)
Mobile Phase: Pump A: water; Pump B: acetonitrile
Mobile Phase Ratio: concentration B = 50%
Column Temperature: 40℃
Detect Wavelength: 254nm

To Set the Method

Before making analysis, you need to start the analysis instruments, log in the UltraChrom workstation and configure each analysis instrument following to “To Configure the Instrument”; besides, you need to set the instrument parameters to define the analysis conditions when necessary.

Method setup is necessary no matter whether you want to execute a single analysis or you want to run a sequence. This section introduces the main parameters in the method setup window and their functions. You can set the analysis method according to your needs.

Click **Analysis** in the Function Control Area (as shown in the figure below) in the lower left of the main operation interface to open the analysis window. Click **New**, or **Edit** in the **Method File** area under the **File** item in the analysis window to enter the method setup window as shown in Figure 3-22. You can select different tab to enter the corresponding method setup page.



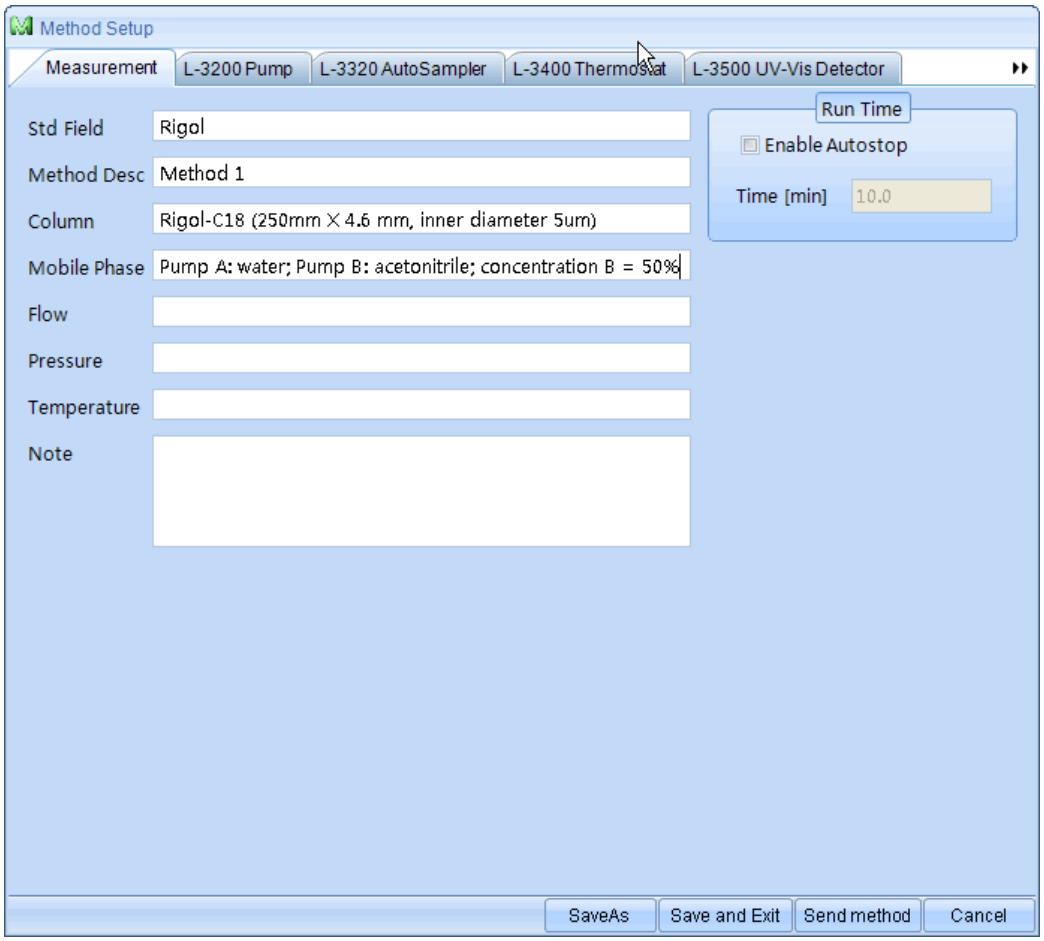


Figure 3-22 Method Setup Window

1. Measurement

The measurement page displays the method information. This page provides the important parameters of the column, mobile phase, flow, pressure and

temperature. Users can also set the run time in this page.

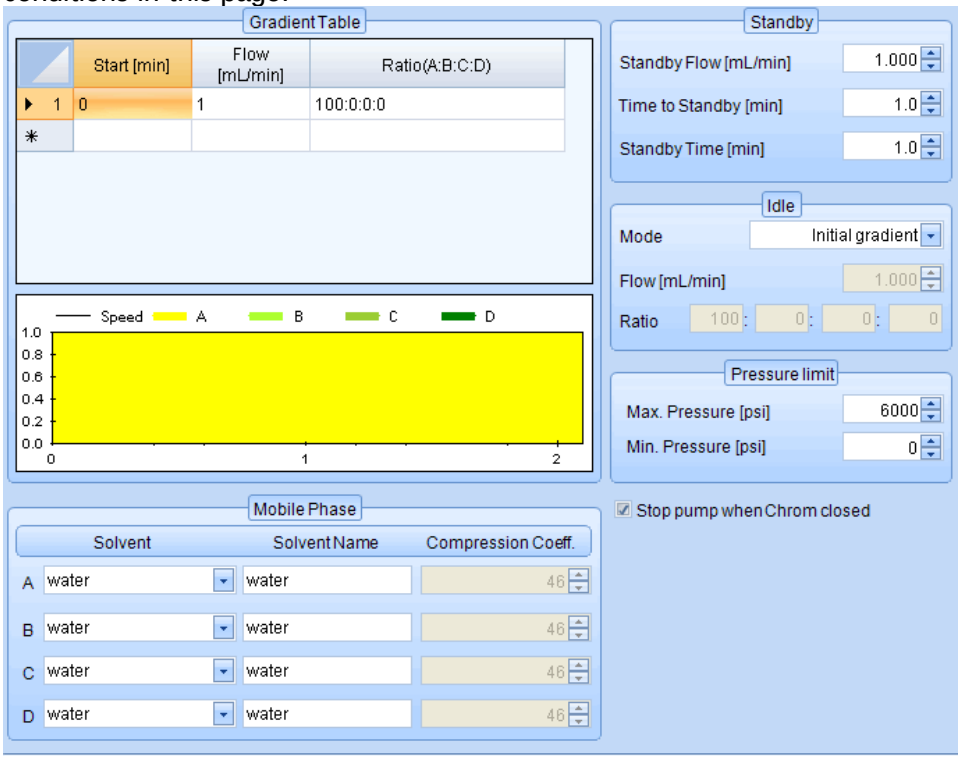
- **Std Field/Method Desc:** describe the related information of the method.
- **Column:** describe the related information of the column used.
- **Mobile Phase:** describe the related information of the mobile phase used.
- **Flow:** describe the flow information of the mobile phase used.
- **Pressure:** describe the related information of the mobile phase pressure.
- **Temperature:** describe the temperature parameters.
- **Note:** additional information of the test conditions.

Note: The above items are given in the chromatography report as related information.

- **Enable Autostop:** when checked, the analysis stops when the specified analysis time expires.
- **Time:** set the data acquisition time. When the specified time expires, the system stops acquiring data and processes the acquired data automatically. You can only edit the run time when "Enable Autostop" is checked.

2. Pump

The pump page (as shown in the figure below) is used to set the pump parameters. This page provides the important parameters of the gradient table, mobile phase, flow, pressure and solvent conditions. Users can set the desired conditions in this page.



- **Gradient Table:** used to edit the corresponding gradient table data and display the data in figure form at the lower part of the window. When a single pump is used, only one pump (Pump A) is displayed in the editing column of the gradient table (in the "Ratio" column, the four flow paths are Pump A, Pump B, Pump C and Pump D by default), users do not need to set the flow percentage (by default, it is 100%) but have to set the flow.

- **Standby:** set the standby state of the pump.
  Standby Flow: the flow of the pump in standby mode (the standby state refers to the state of the pump after the last analysis is finished and before the next analysis starts).
  Time to Standby: the time required for the pump to enter the standby state after finishing running the gradient table.
  Standby Time: the duration of the standby state. After the standby state finishes, the pump enters the idle state.

- **Idle:** after the standby time expires, the pump enters the idle state. You can set the working state of the pump to "Stop pump", "Initial gradient" or "Standby"; besides, you can also select "Custom" to define the working state of the pump.
  (1) Stop pump: the pump stops after the analysis is finished.
  (2) Initial gradient: this function is available when gradient table is used. When it is selected, the pump will work according to the initial state set in the gradient table after the analysis is finished.
  (3) Standby: the pump will work according to the condition set in "Standby".
  (4) Custom: users can define the idle state of the pump by setting the flow and mobile phase ratio.

- **Pressure Limit:** set the maximum pressure and minimum pressure. The system will sound the alarm when the device pressure exceeds the range defined.

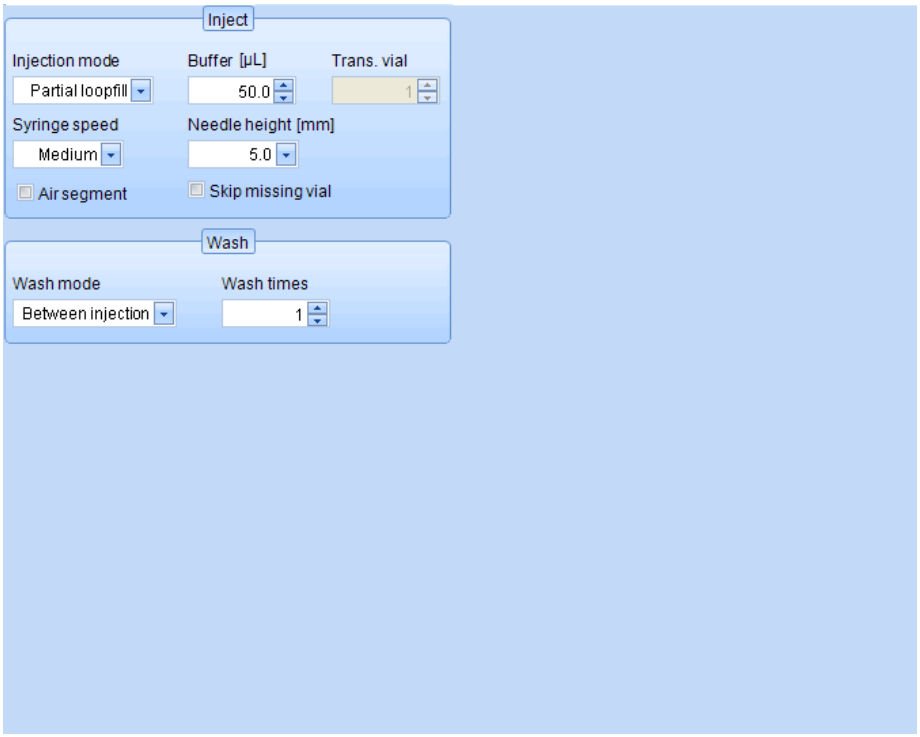
- **Stop pump when Chrom closed:** when it is checked, the pump stops when the workstation is closed.

- **Solvent Setup:** Users can select the solvent to be used and the compression coefficient of the solvent. The system presents the compression coefficient automatically when the desired solvent is selected.

  Note: When complex solvent is used, users can select "Custom" in the solvent dropdown box to input the relative parameters of the solvent manually.

3. Autosampler

The autosampler page (as shown in the figure below) is used to set the control parameters of the autosampler. If no autosampler is configured with your system, you can skip this step. The autosampler page consists of the inject area and wash area.



- **Injection**

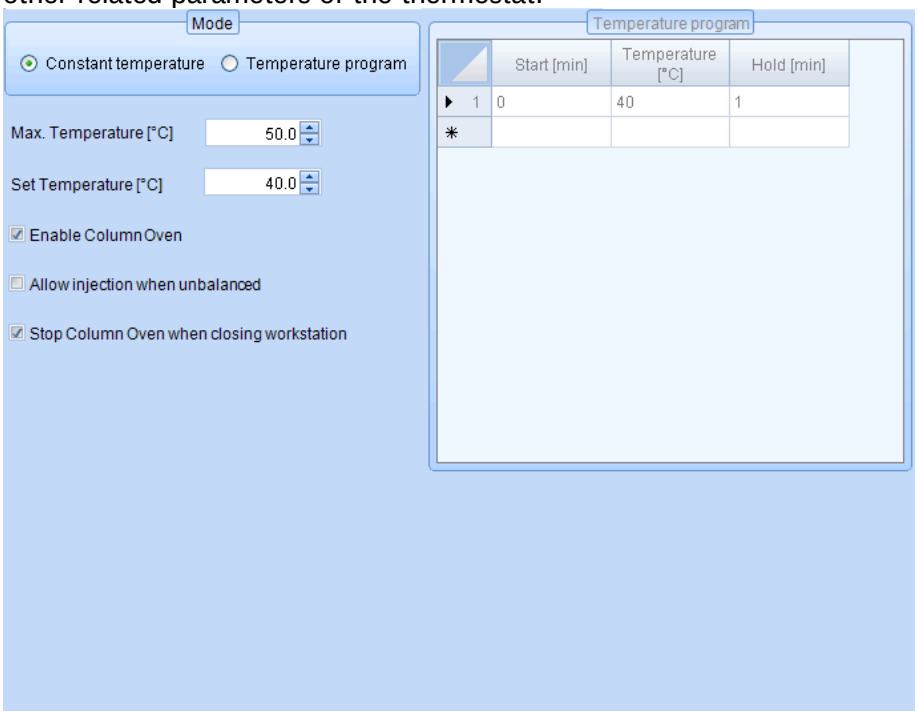
  Injection mode: select the desired injection mode (Full loop, Partial loopfill and µL pickup) from the dropdown menu.
  Buffer: set the volume of the sample used to wash the tubing between the needle and loop.
  Syringe speed: select the speed of the syringe (High, Medium and Low).
  Trans. Vial: set the number of the transport vial.
  Needle height: set the height of the injection needle.
  Air segment: when it is checked, a 5µL air segment will be placed between the wash solvent and sample.
  Skip missing vial: when it is checked, the system will analyze the next sample when the current sample vial does not exist. When it is not checked, the system will exit the sequence.

- **Wash**

  Wash mode: set when to execute the wash operation.
  Wash times: set the number of washes to be performed in one operation.

4. Thermostat

The thermostat page (as shown in the figure below) is used to set the working mode (constant temperature and temperature program), the temperature and other related parameters of the thermostat.



- **Mode**
  Constant temperature: the thermostat works under constant temperature.
  Temperature program: the temperature of the thermostat changes with the time. After selecting this mode, you can define the temperature program table in the table at the right side.

- **Max. Temperature:** set the maximum temperature of the thermostat.

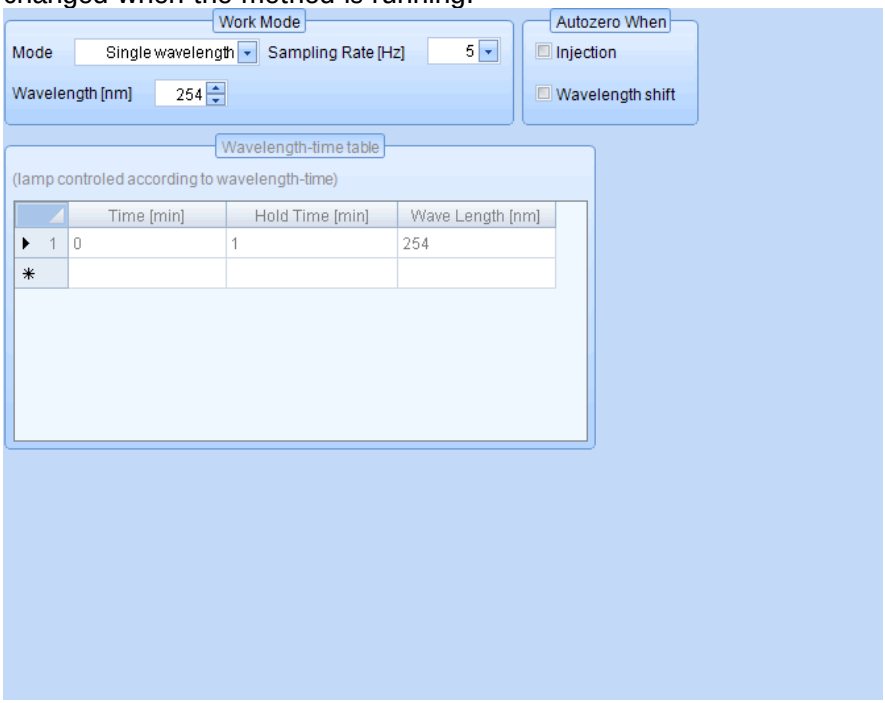
- **Set Temperature:** set the working temperature of the thermostat.

- **Allow injection when unbalanced:** when it is checked, the sampler can inject sample when the thermostat has not reached the set temperature.

- **Stop Column Oven when closing workstation:** when it is checked, the thermostat stops when the workstation is closed.

5. UV-Vis Detector

The UV-Vis detector page (as shown in the figure below) is used to set the measurement conditions of the detector. The parameter settings cannot be changed when the method is running.



- **Work Mode:**
  Mode: select “Single wavelength”, “Dual wavelength”, “Wavelength program” or “Off” from the dropdown menu. When the mode is set to “Dual wavelength”, the corresponding wavelength values are Wavelength A and Wavelength B; besides, users need to set the output mode (A-B or A/B).
  Wavelength: 190nm - 422nm ultraviolet light area (deuterium lamp); 423nm - 800nm visible light area (tungsten lamp). In dual wavelength mode, Wavelength A and Wavelength B should be in the same energy area (the tungsten lamp source area or the deuterium lamp source area).
  Sampling Rate: the sampling rates available are 0.5Hz, 1Hz, 2Hz, 5Hz, 10Hz, 25Hz, 50Hz and 100Hz. In dual wavelength mode, users do not need to select the sampling rate and the default sampling rate will be used.

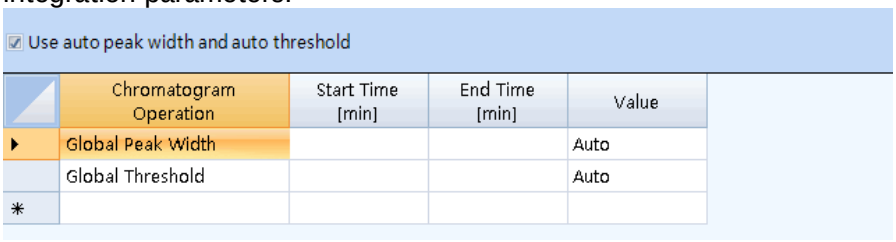
- **Autozero When:** select to return the detector signal to “0” baseline at “Injection” or “Wavelength shift”.

- **Wavelength-time table:** when “Wavelength program” mode is selected, users can edit the wavelength-time table. Wherein, “Time” refers to a certain time point; “Hold Time” refers to the duration of the same

wavelength after this time point; “Wavelength” refers to the desired detect wavelength during this period of time.

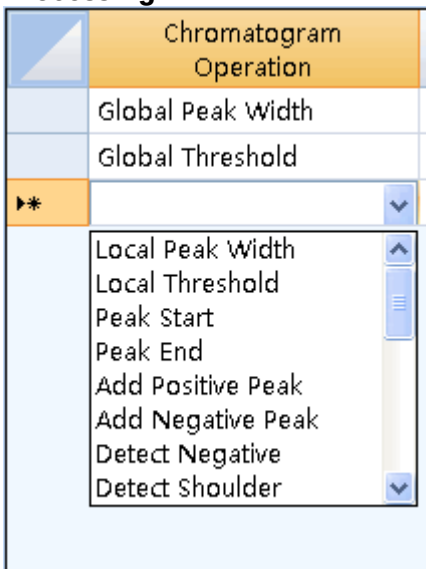
6. Integration

The integration page (as shown in the figure below) is used to set the integration parameters.



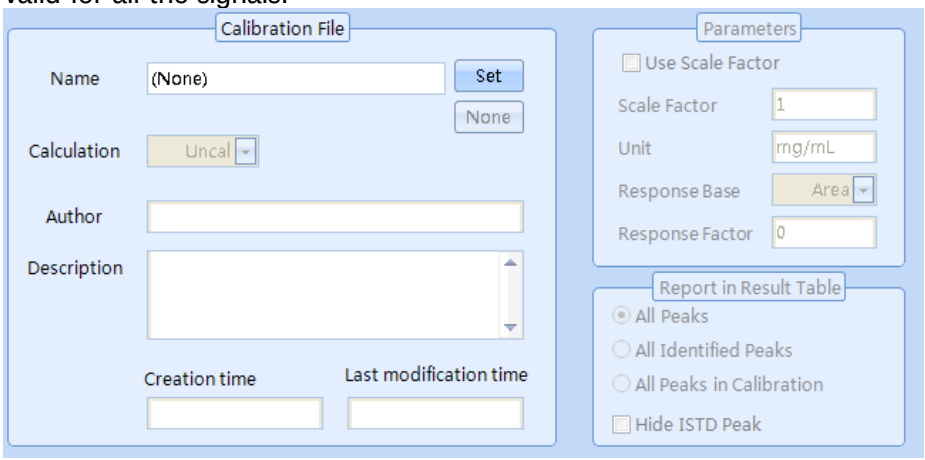
- **Global Peak Width and Global Threshold:** by default, the global peak width and global threshold are added into the integration table. You can use the auto values by selecting “Use auto peak width and auto threshold” or uncheck this item to manually set the start time and end time.

- **Add other integration operations:** double-click the last row in the “Chromatogram Operation” column and the dropdown list is displayed. Select the desired integration operation and set the start time and end time. There are 22 integration operations; for the details, please refer to “**Data Processing**”.



7. Calculation

The calculation page (as shown in the figure below) is used to set the calibration parameters. When multiple detector chromatographs are used, this setting is valid for all the signals.



- **Name (Peak Table)**

  When calibration file is selected, the name of the calibration file is displayed in the space. All the chromatograms measured using this method will be calibrated according to the file selected.

  Set: select the desired calibration file.

  None: selecting this button will turn off the calibration file currently selected. At this point, "Uncal" is automatically selected in the "Calculation" dropdown menu. The dropdown menu becomes grayed and cannot be operated.

- **Calculation**

  Set the calculation type (uncal, ESTD, ISTD and NORM).

- **Parameters**

  You can only set the **scale factor** and **unit** when "Use Scale Factor" is checked.

  Response Base: select the calculation base (peak area or peak height) for the unrecognized peaks.

  Response Factor: the coefficient used to calculate the amount of the target compound. The amount is determined by the product of the peak area (or peak height) times this coefficient.

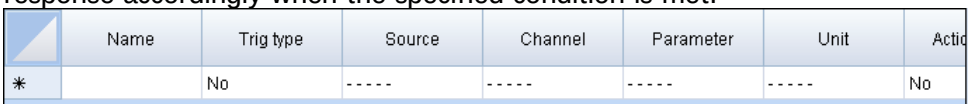
- **Report in Result Table**

  Select the type of the peak to be reported in the result table.

The main parameters of calculation are introduced in the above section and users can set the parameters according to their needs. For other parameters, please set them according to the system descriptions.

8. Event Table

The event table page is as shown in the figure below. You can set this method to response accordingly when the specified condition is met.



- Name: double-click in this column to input the name of the event.
- Trig Type: double-click and select the desired trigger type in the dropdown box, including idle time, running time, start running, stop acquisition, end acquisition, end running, user stop and user abort. When “Idle Time” or “Running Time” is selected, you can set the time value and time unit in the “Parameter” and “Unit” columns respectively. When multiple inputs are used, you can specify the input signal via the “source” and “Channel”.
- Action: double-click and select the desired action type in the dropdown box, including program and command. When “Program” is selected, you can select the desired program in the “Parameter” column. When the trigger condition is met, the program selected will be opened. When “Command” is selected, you can select the desired operation in the “Parameter” column, including start running, start acquisition, end acquisition, end running and abort. When multiple inputs are used, you can specify the input signal via the “source” and “Channel”.

After finishing setting each module, click **Save and Exit** to save the method; you can also click **Send method** to send the method to each module.

To Execute Single Analysis

Please follow the steps below to execute single analysis.

1. Start the workstation

Start the workstation and select proper user type as well as input the correct user name and password to enter the management interface of the workstation.

2. Instrument configuration and method setting

Click  in the UltraChrom Management interface to configure the instrument and click **Online...**in the UltraChrom Management interface to open the main operation interface. Please set the method according to the introduction in the last section.

3. Set the parameters

Click **Single** in the **Single Run** area under the **File** item to enter the single analysis window.

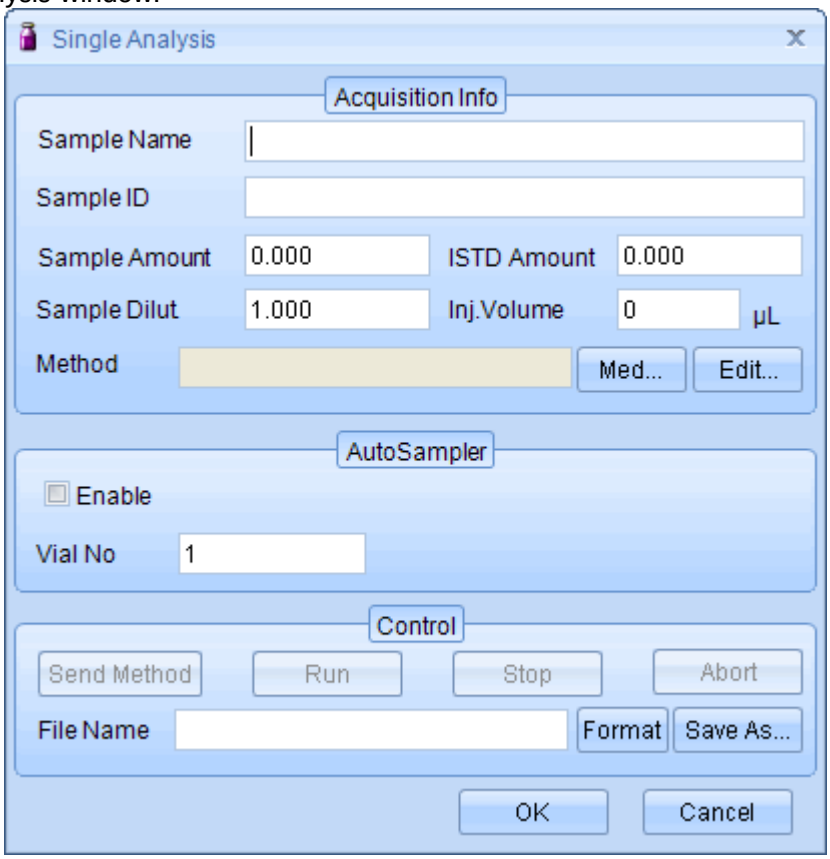


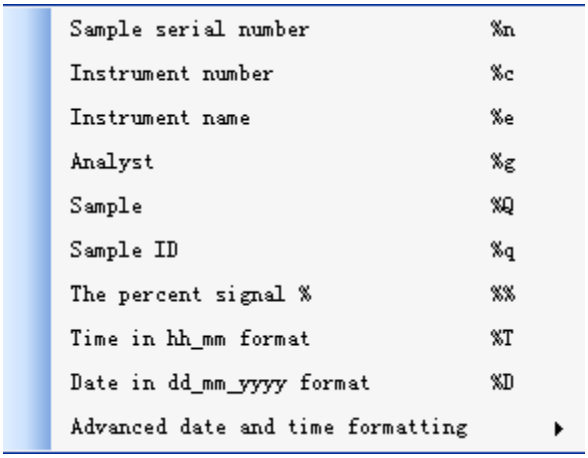
Figure 3-23 Single Analysis

- **Acquisition Info**
  Sample Name: describe the name of the sample.
  Sample ID: describe the domain of the sample.
  Sample Amount: used to calculate the percentage of a single component in calibrations using the ESTD and ISTD methods. The unit of the input value is the same with that of the value in the calibration file.
  ISTD Amount: input the additional amount in the ISTD method. The unit of the input value is the same with that of the value in the calibration file.
  Sample Dilut: used to multiply each calibrated value.
  Inj.Volume: the injection volume of the sample. The responses of all the compounds will be corrected by this value.
  Med: click **Med...** to open the stored method and click **Edit...** to edit this method.

- **AutoSampler**
  After checking "Enable" and setting the vial number, the auto sampler can inject automatically.

- **Control**
  Send Method: send the actual method parameters to the instrument.
  Run: start the analysis.
  Stop: stop the current analysis.
  Abort: give up the current analysis.
  File Name: used to set the naming rule of the chromatogram generated. Click **Format** to display all the variables available.

Note: The chromatogram filename can be any combination of all the variables available.



4. Send the method and run the analysis
Click **Send method** in the **Single Run** area under the **File** item to send the method selected to the instrument. After the method is sent successfully (the single analysis window closes), click **Run** in the control area, the device

executes analysis according to the method set. When the analysis window is opened again, the **Stop** and **Abort** buttons in the **Single** area under the **File** item are available. You can also inject sample by switching the injection valve after the method is sent successfully and trigger the analysis using the injection signal (the single analysis window will not close).

Note: When the single analysis is running, you can observe the data acquisition process in real-time via the acquisition graph interface.

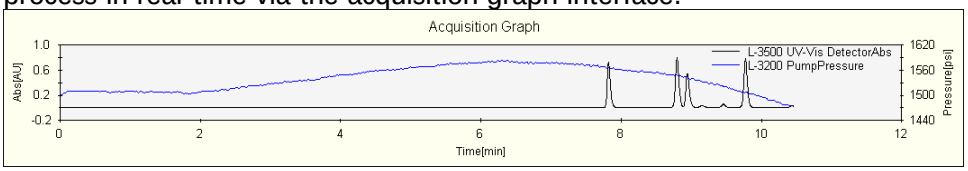


Figure 3-24 Acquisition Graph

To Execute Sequence Analysis

With sequence analysis, you can process samples in batch. To use sequence analysis, make sure that your system is configured with autosampler. Please execute sequence analysis according to the steps below.

1. Open the sequence window

Click **New** in the **Sequence** area under the **File** item in the analysis window to open the sequence window.

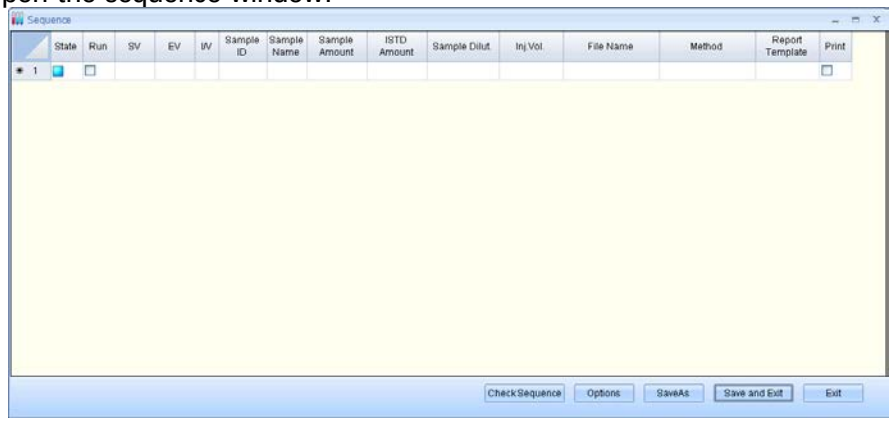


Figure 3-25 Sequence

2. Set the sequence parameters

The meaning of each part in the sequence window is as follows.

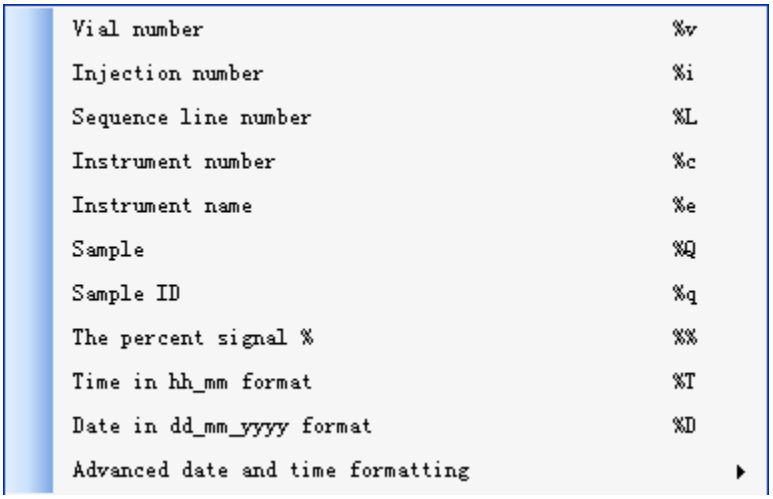
- **SV**: the number of the start vial.
- **EV**: the number of the end vial.
- **I/V**: the number of injections.

  Note: One or more analysis can be defined in each row in the sequence table depending on the values of SV and EV. For example, when SV and EV

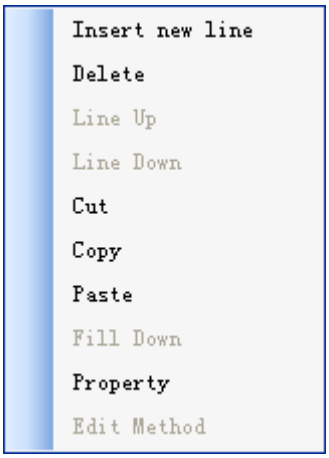
are the same and I/V is 1, only one analysis will be executed. If you want to perform multiple injections on the same sample, set I/V to the desired number of injections.

- **Method:** used to select the desired analysis method file. Besides, users can also re-edit the method selected. Double-click at this column, the icon is displayed at the right and click the icon to select the desired method.
- **Report Template:** used to set the format of the result report and select the corresponding report template. Double-click at this column, the icon is displayed at the right and click the icon to select the desired report template.

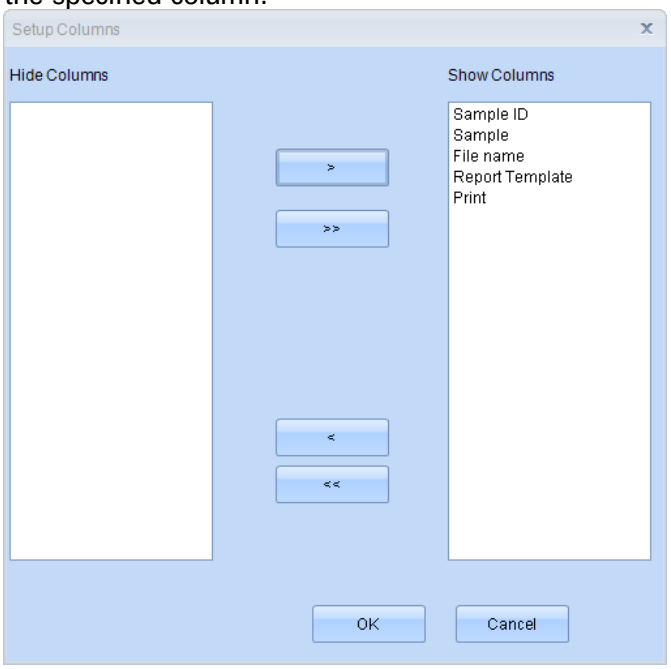
  Note: Each row can use different method file and report template.
- **File Name:** used to define the naming rule of the chromatogram to be created. Double-click at this column, the icon is displayed at the right and click this icon to select the naming rule. For example, selecting "%Q" to use the setting in "Sample" as the filename. The filename can be any combination of all the variables available.



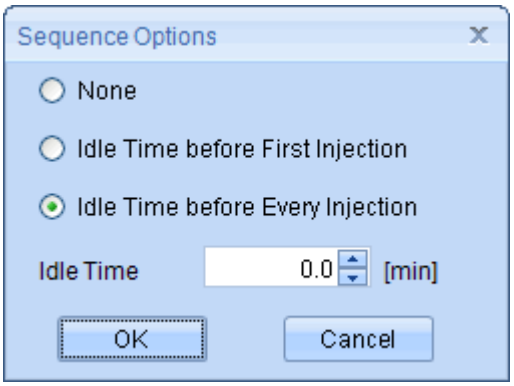
Right-click the added row and the menu list as shown in the figure below is displayed. This menu list is used to insert a row, delete/move up/move down the selected row, perform cut, copy and paste operations on the row, view the property or edit the method.



Wherein, when “Fill Down” is selected, all the cells under the cell selected will be filled with the content of the cell selected. When “Property” is selected, the setup columns window as shown in the figure below is displayed. You can hide the specified column.



Click **Options** at the bottom of the sequence window and the sequence options dialog box is displayed where you can set the preparation time before injection.



3. Check and save the sequence

After finishing setting the parameters in the sequence table, click **Chenck Sequence** to check whether the sequence created can be run. If the sequence passes the check, the successful prompt message will be displayed. If the check fails, the error prompt message will be displayed. After the sequence passes the check, click **Save and Exit** to exit the sequence setup.

4. Run the sequence

- Click **Run** in the **Sequence** area under the **File** item in the analysis window to run the sequence.
- To stop the sequence, click **Abort** or **Stop** in the toolbar.
  Abort: means giving up the sequence immediately (the current chromatogram file will not be saved);
  Stop: means stopping the sequence when the current analysis finishes (the current chromatogram file will be saved); click **Stop** again (or directly double-click the button) and the current analysis stops immediately (the current chromatogram file will be saved).
- **Pause** means pausing the current sequence; the paused sequence does not allow injection operation and cannot receive external signal.
- **Resume** is used to restart the paused sequence; the difference between this button and **Run** lies in that **Resume** runs the paused sequence from where it has been paused while **Run** will re-run the whole sequence.
- Click **Repeat Injection** or **Skip Vial** to execute the current injection repeatedly or skip the current injection.

Data Acquisition

The data acquisition interface consists of two parts: the acquisition graph interface and the detail graph interface. You can adjust the sizes of the interfaces by dragging the boundary line between the two interfaces. The detail graph interface displays the acquired signal and other auxiliary signal in real-time; the acquisition graph interface shows the overall signal tendency.

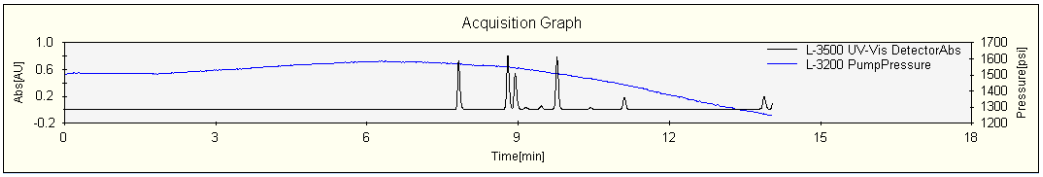


Figure 3-26 Acquisition Graph

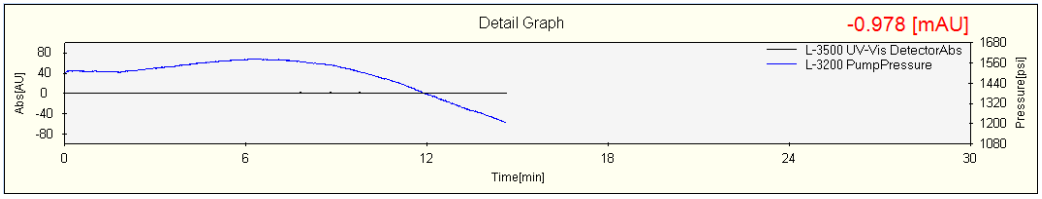


Figure 3-27 Detail Graph

Users can right-click at the detail graph interface to select the corresponding operation from the pop-up dropdown menu.

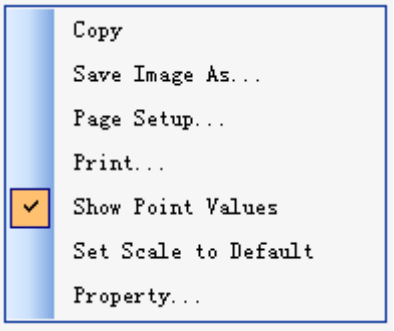


Figure 3-28 Right-click Menu

Users can change the signal range and time range as well as add auxiliary signals (such as pressure and temperature) in the “Property” item.

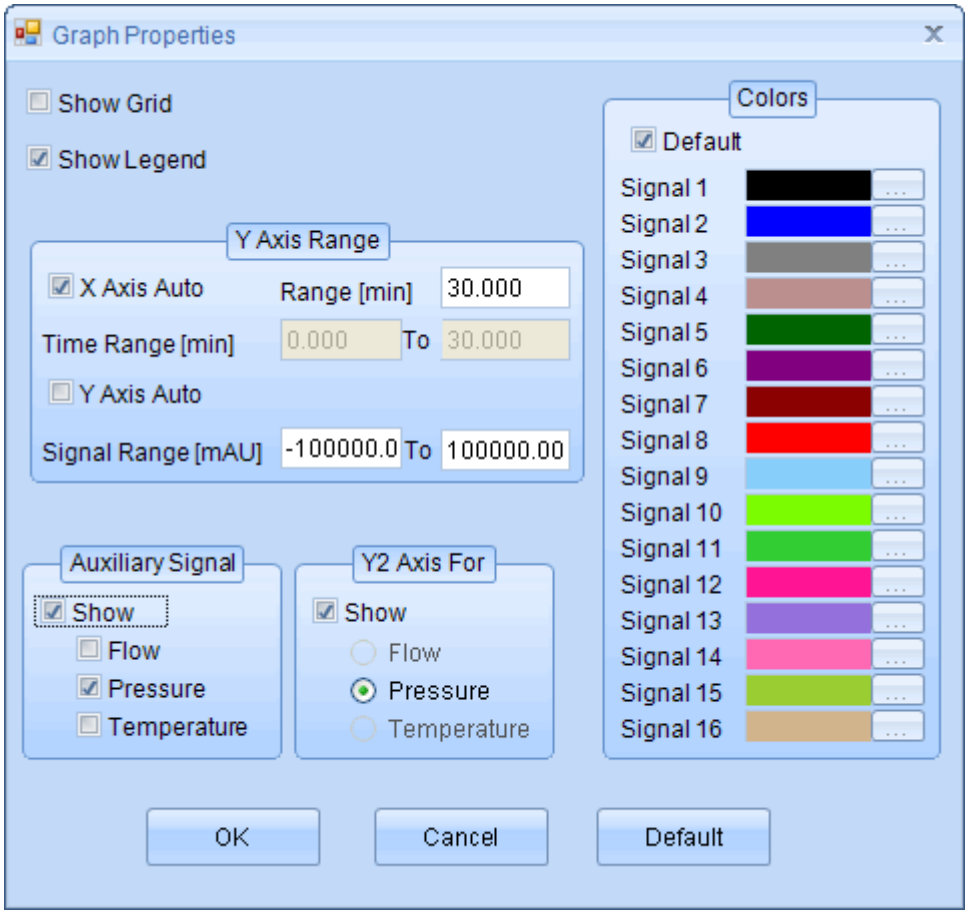
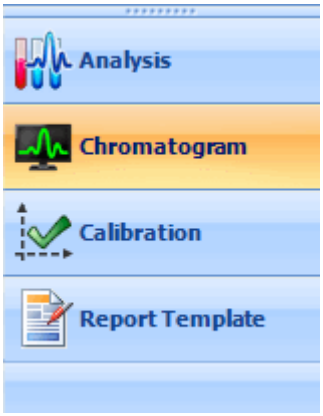


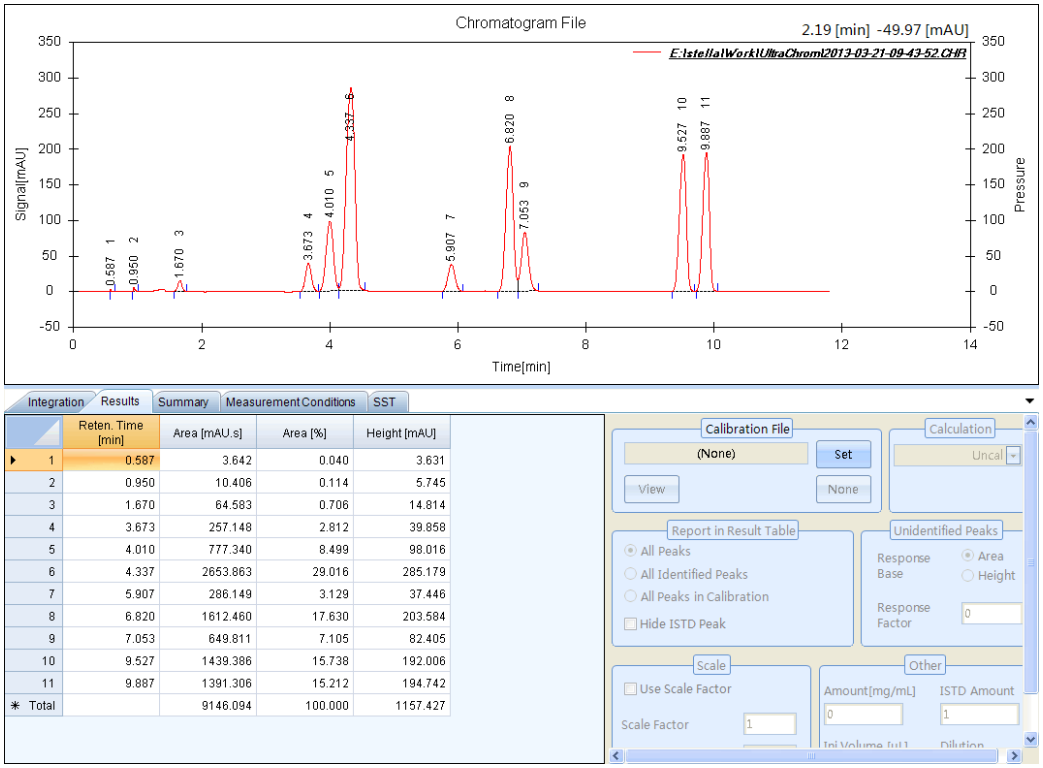
Figure 3-29 Graph Properties

Data Processing

Click **Chromatogram** in the Function Control Area (as shown in the figure below) in the lower left of the main operation interface to open the chromatogram window.



Users can process the data of the result chromatogram. The chromatogram window consists of two parts: the upper part is the chromatogram area and the lower part is the result area. You can adjust the sizes of the interfaces by dragging the boundary line between the two interfaces.



	Reten. Time [min]	Area [mAU.s]	Area [%]	Height [mAU]
1	0.587	3.642	0.040	3.631
2	0.950	10.406	0.114	5.745
3	1.670	64.583	0.706	14.814
4	3.673	257.148	2.812	39.858
5	4.010	777.340	8.499	98.016
6	4.337	2653.863	29.016	285.179
7	5.907	286.149	3.129	37.446
8	6.820	1612.460	17.630	203.584
9	7.053	649.811	7.105	82.405
10	9.527	1439.386	15.738	192.006
11	9.887	1391.306	15.212	194.742
Total		9146.094	100.000	1157.427

Figure 3-30 Measurement Data

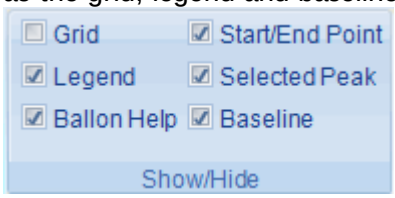
To Open the Data File

If corresponding setting is made before the analysis, the chromatogram will be opened automatically after the analysis finishes. To open the chromatogram manually, click **Open** under the **Chroma** item in the menu bar of the chromatogram window, select the desired chromatogram file from the pop-up dialog box and click **Open**. Clicking **Import** will import ".TXT" format chromatogram file; clicking **Export** will export the chromatogram file in ".TXT" format. Click **MultiChroma** to open multiple chromatograms at the same time.

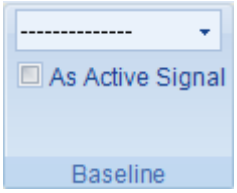
In multi-chromatogram mode, only one chromatogram is active at a time. Users can double-click the signal name at the upper-right corner to switch the current active chromatogram. Clicking any data or label in any row in the result table will mark the corresponding chromatogram peak in the color of the signal. When data in multiple rows are selected at the same time, all the corresponding peaks will be marked with the color of the signal.

Under the **Chromatogram Setting** item, users can select to show or hide certain auxiliary label as well as set the baseline, peak name, time axis, signal axis and auxiliary signal.

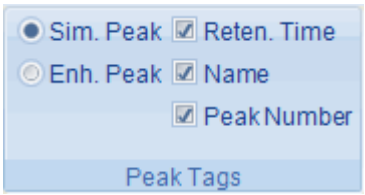
- **Show / Hide:** set to show or hide the auxiliary labels in the chromatogram, such as the grid, legend and baseline.



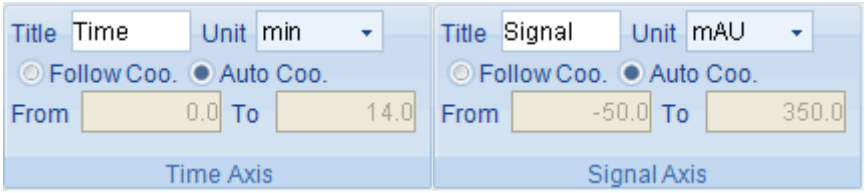
- **Baseline:** set the color and line type of the baseline.



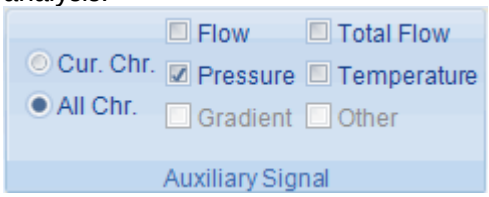
- **Peak Name:** select the peak information to be displayed. When simple peak information mode is selected, you can select to display the retention time, component name and peak number on the chromatogram; when enhanced peak information mode is selected, the enhanced format dialog box will be displayed and users can add the desired information items.



- **Time Axis/Signal Axis:** set the time range and signal range of the chromatogram. Users can also set the display units and names of the time axis and signal axis. By default, the ranges of the time axis and signal axis are "Auto coo".



- **Auxiliary Signal:** set the auxiliary signal information of the chromatogram, for example, display the system pressure and temperature information during the analysis.



To Process Data

Users can process the chromatogram data using the integration tools. The functions of the integration tools at the top of the window are as follows.


: global peakwidth; used to calculate the peak width (applicable to all the peaks). The global peakwidth can also be set manually in the first row of the integration table.


: global threshold; used to calculate the global threshold. It can be set manually in the second row of the integration table.

The following part introduces the common operations of the chromatogram (include baseline operations, peak operations and integration operations).

1. Baseline Operations

All the baseline operations are performed within a specified interval which must include the starts and ends of all the peaks within it. Any incomplete peak will be ignored. Besides, any baseline cannot intersect the signal. The baseline

operation icons as well as their definitions are as shown below.

	Valley	The baseline will pass through all valleys that separate individual peaks.
	Together	Create the perpendicular line from the valley.
	Time-Forward Horizontal	Draw a horizontal line from the time start point to the time end point of the peak; valid within the specified interval.
	Time-Back Horizontal	Draw a horizontal line from the time end point to the time start point of the peak; valid within the specified interval.
	Peak-Forward Horizontal	Draw a horizontal line from the start to the end of the peak; valid within the specified interval.
	Peak-Back Horizontal	Draw a horizontal line from the end to the start of the peak; valid within the specified interval.
	Front Tangent	Rider peaks on the front of the following larger peak are separated by the “tangent skimming method”.
	Tail Tangent	Rider peaks on the tail of the preceding larger peak are separated by the “tangent skimming method”.

2. Peak Operations

The table below lists all the peak operations and you can perform the corresponding operation according to your need.

	Peak Start	Change the peak start.
	Peak End	Change the peak end.
	Force Single	Combine fusion peaks as one peak; valid within the specified interval.
	Add Positive Peak	Create a new positive peak by setting the start and end. If it overlaps with the preceding or following peak, the start or end of the preceding or following peak will be modified accordingly.
	Add Negative Peak	Create a new negative peak by setting the start and end. If it overlaps with the preceding or following peak, the start or end of the preceding or following peak will be modified accordingly.
	Delete Peak	Eliminate peaks within a selected interval according to the specified condition.

3. Integration Operations

Integration operations can modify the integration parameters. Users can modify the integration parameters by clicking the corresponding icons in the integration toolbar. The name and function of each operation is as shown in the table below.

Note: All the operations are for the specified interval.

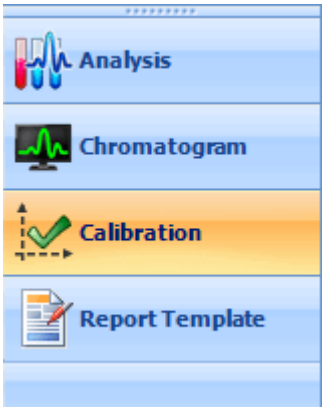
	Local Peak Width	Define the minimum peak width inside the specified interval. The peak width dialog box is displayed after clicking this icon.
	Local Threshold	Define the minimum peak height inside the specified interval. The threshold dialog box is displayed after clicking this icon.
	Integration Interval	Set the interval for the integration algorithm. Peaks outside the interval will not be integrated.
	Detect Negative	Detect negative peaks inside the specified interval.
	Min. Area	Set the minimum integration peak area inside the specified interval. Peaks of which the areas are less than or equal to the set area will not be integrated.
	Detect Shoulder	Detect the shoulder peaks contained in the chromatogram. Shoulder peak refers to the unformed peak on the rising edge or falling edge of large peak. A Peak can include one or more shoulder peak.
	Min. Height	Set the minimum peak height inside the specified interval. Peaks of which the heights are less than or equal to the set value will not be integrated.
	Min. Half Width	Set the minimum half peak width inside the specified interval. Peaks of which the half widths are less than or equal to the set value will not be integrated.

Note: To cancel the previous operation, click **Undo** in the **Operation** area under the **Chroma** item and click **Redo** to recover the recent operation.

To Generate the Calibration Curve

The creation method of the calibration file is as follows.

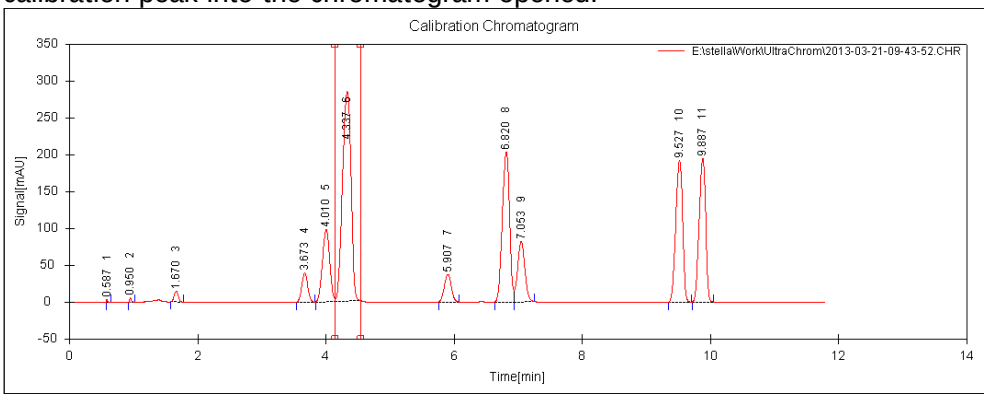
1. Click **Calibration** in the Function Control Area (as shown in the figure below) in the lower left of the main operation interface to open the calibration window.



2. Click **New** in the **Calibration File** area under the **Calibration** item to create a new calibration file.

3. Click **Open Chromatogram** in the **Chroma File** area under the **Calibration** item to select the desired chromatogram.

4. Click **Add Peak** in the **Operation** area under the **Calibration** item to add calibration peak into the chromatogram opened.



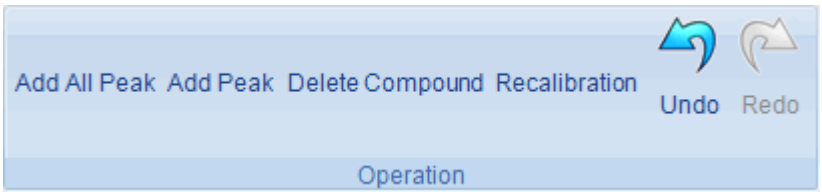
At this point, the peak selected is added into the compound peak table automatically.

Compound | Peak4.337 | Peak5.907

	Used	Compound Name	Reten. Time[min]	Left Window[min]	Right Window[min]	Peak Selection	Response Base	LOD
1	☑	Peak4.337	4.337	0.200	0.200	Nearest	Area	0.000
2	☑	Peak5.907	5.907	0.200	0.200	Nearest	Area	0.000
3	☐							

Note: Users can also input the information of the peak added (such as the peak

name and retention time) manually. Users can perform the corresponding operation on the chromatogram via the function buttons in the “Operation” area.

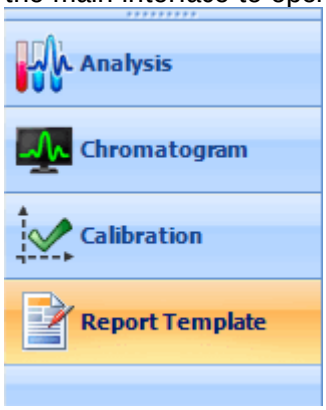


Note: You need to modify the filename if you want to save the calibration file because the calibration file cannot be saved with “Unnamed”. You are recommended to use the compound name to name the calibration file. Click **Save** or **Save As** in the **Calibration File** area under the **Calibration** item to save the calibration file.

Report Function

Report Function Introduction

The report template window is used to set the report format. Click “Report Template” in the function control area (as shown in the figure below) in the lower left corner of the main interface to open the report template window.



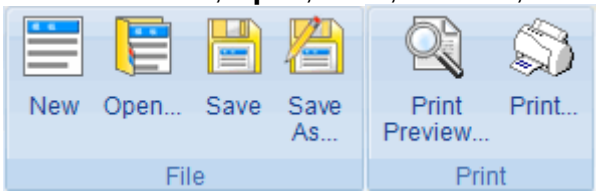
To Use the Report Template

Click **Open** in the **File** area under the **Template** item to open the report template saved in the software.

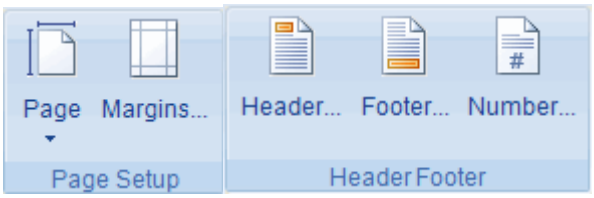
To Define Report Template

Users can define the desired report template via the template, edit, insert and view toolbars.

1. **Template**
   Provide the **New**, **Open**, **Save**, **Save As**, **Print Preview** and **Print** functions.

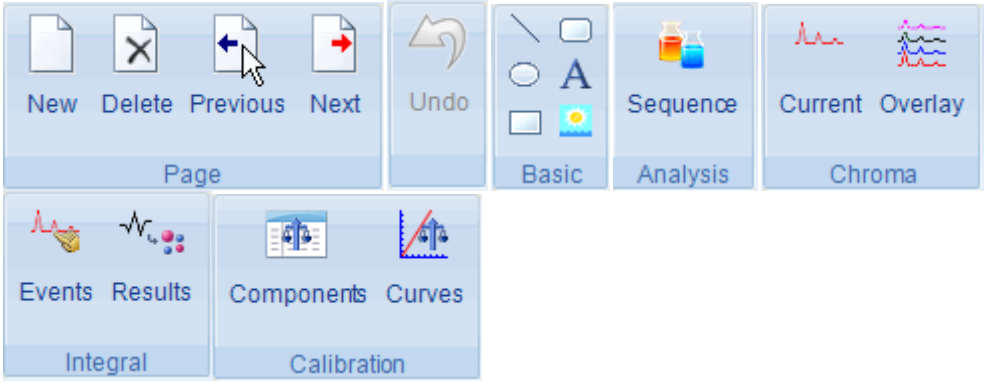


2. **Edit**
   Provide the page setup and header/footer edit functions.



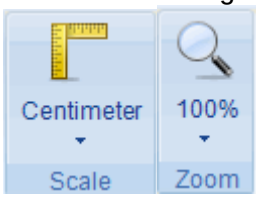
3. Insert

Provide the basic graph tools, text and image insert function as well as multi-chromatogram, integration event and integration result edit functions. Besides, users can also create a new page or delete the current page.



4. View

Provide the setting function of the display ratio of the report.



Chapter 4 Data Processing Principles

The data processing algorithms of UltraChrom mainly include integration, peak match, quantitation and calibration.

Integration refers to operating the original data acquired so as to identify the peak and calculate its size as well as the relative specifications. Peak match refers to the process of matching the retention time of the unknown peak with that of the standard peak. Quantitation refers to the process of calculating the unknown peak amount on the basis of the integration result of each peak as well as the calibration curve of the known standard sample peak amount and the integration result. Calibration refers to the process of calculating the concentration or amount of the unknown component by analyzing the specifically prepared standard sample.

Integration

The integration algorithm of UltraChrom mainly includes data pre-processing, chromatogram peak identification, baseline adjustment and specification calculation. The chromatogram analysis result will be provided automatically. If you are not satisfied with the automatic integration result, you can adjust the chromatogram peak and baseline using the 24 manual integration events provided by UltraChrom.

The integration algorithm of UltraChrom mainly realizes the following functions:
1. Determine proper peak width and threshold for the chromatogram data automatically;
2. Identify the peaks in the chromatogram data acquired and determine the position of the baseline;
3. Integrate the indentified peak to determine its specifications, such as the retention time, peak area, peak height, separation degree, tailing factor and column efficiency.

Data Pre-processing

The data pre-processing of the integration algorithm of UltraChrom consists of noise filter and auto determination of peak parameters.

1. Noise Filter

The chromatogram usually consists of signal and noise and contains baseline drift and chromatogram overlapping. Before analyzing the chromatogram, you need to filter out the noise.

2. Auto Determination of Peak Parameters

Before indentifying the chromatogram peak, if users select “Use default peak

width and threshold", the UltraChrom integration algorithm will calculate the chromatogram peak according to the chromatogram data characteristics and identify the desired peak width and threshold automatically.

Auto Peak Width Algorithm:
Measure the peak width automatically. The peak width will affect the size of the sliding window used in chromatogram peak identification. The auto peak width selects the maximum peak within the whole chromatogram range and determines the peak width; wherein, the maximum peak refers to peak with the maximum second derivative. The width of this peak can be determined by measuring the inflection time. Multiplying this time with the factor 4.89549/2 will acquire the width of the Gauss peak at 5% peak height.

The UltraChrom integration algorithm uses the following formula to determine the width of the sliding window.

$$PB = \frac{(PW \times SR)}{15} \tag{4-1}$$

Wherein, PB: the width of the sliding window
PW: auto peak width (second)
SR: sample rate

Auto Threshold Algorithm:
Determine the threshold automatically. Threshold refers to the slope value, namely the algorithm will only treat it as a possible peak start or peak end when the slope of the chromatogram data point is greater than this value algorithm. This parameter determines the positions of the peak start point and peak end point.

Select an area that does not contain peaks and calculate the linear regression equation of the chromatogram data within this area according to the following formula.

$$Y = a + b*X \tag{4-2}$$

Y is the absorbance of the chromatogram data; X is the time corresponding to the absorbance; a and b are the interception and slope (also called "drift") of the linear equation respectively. The calculation equation is as follows. In the algorithm, the auto threshold equals the drift times K and is used to identify the chromatogram peaks; wherein, K is a constant.

$$a = \frac{1}{M}\left(\sum_{i=1}^{N} X_i^2 * \sum_{i=1}^{N} Y_i - \left(\sum_{i=1}^{N} X_i \sum_{i=1}^{N} X_i Y_i\right)\right) \tag{4-3}$$

$$b = \frac{1}{M}\left(N * \sum_{i=1}^{N} X_i Y_i - \left(\sum_{i=1}^{N} X_i \sum_{i=1}^{N} Y_i\right)\right) \tag{4-4}$$

$$M = N * \sum_{i=1}^{N} X_i^2 - \left(\sum_{i=1}^{N} X_i\right)^2 \tag{4-5}$$

This algorithm is applicable to most of the chromatograms; while, in the following situations, “Auto Threshold” may not work properly. In chromatogram that contains multiple components, all the areas might contain peaks. At this point, the value measured by “Auto Threshold” might be very high; therefore, valid peak might not be detected or the start point or end point of the peak might be higher. At this point, users need to lower the calculation result of the auto threshold manually.

Chromatogram Peak Identification

The peak width and threshold calculated can be used to identify the chromatogram peaks. The UltraChrom integration algorithm uses “5-point peak detect”, namely a complete peak should contain the following 5 characteristic points: peak start point, left inflection point, top point, right inflection point and peak end point. While, the normal “3-point peak detect” requires that a complete chromatogram peak should contain the following 3 characteristic points: peak start point, top point and peak end point.

“5-point peak detect” can reduce the effect of the noise and glitch on the peak detect algorithm and reduce error detect rate; it can provide more accurate peak shape, peak area and peak height, but it is rather complicated. To solve this problem, the “sliding window” is used to reduce the time required by the peak detect algorithm. The details are as follows.

1. **Determine the peak start point**
   A peak start point is possible when the average slope of at least two continuous windows is greater than the threshold. For positive peak, the data point with the minimum Y value that meets the condition window is treated as the peak start point; for negative peak, the data point with the maximum Y value is treated as the peak start point.

2. **Determine the left inflection point of the peak**
   The slope of the chromatogram data increases from the peak start point and the slope at the peak top point should be 0 theoretically; therefore, for a complete peak, there must be at least one point between the peak start point and peak top point at which the slope changes from increasing to decreasing and this point is the left inflection point of the peak. The system will only searches for the peak top point when the left inflection point of the peak appears.

3. **Determine the peak top point**
   For positive peak, the maximum Y value point within the window at which the slope of the single peak data point changes from positive to negative is taken as the probationary peak top point. For negative peak, the minimum Y value point within the window at which the slope of the single peak data point changes from negative to positive is taken as the probationary peak top point. You need to

adjust the peak top point when the peak end point is determined.

4. Determine the right inflection point of the peak

Starting from the peak top point, the slope of the chromatogram data turns negative and reduces gradually. The slope at the peak end point should be greater than 0 and the slope threshold. Therefore, there should be at least one point between the peak top point and peak end point at which the slope changes from decreasing to increasing and this point is the right inflection point of the peak. The system will only searches for the peak end point when the right inflection point of the peak is determined.

5. Determine the peak end point

A peak end point is possible when the average slope of at least two continuous windows is lower than the threshold. For positive peak, the data point with the minimum Y value is treated as the peak end point; for negative peak, the data point with the maximum Y value is treated as the peak end point. When searching for the peak end point, the software will check the change of the sign of the slope. If the sign of the slope changes before the peak end point that meets the threshold condition appears, this point will be treated as the peak end point.

Baseline Adjustment

Baseline refers to the signal curve when no sample component flows out. Ideally, the baseline should be a straight line, but in actual measurement, there are baseline noise and baseline drift. The UltraChrom integration algorithm defines the connecting line between the peak start point and peak end point as the baseline of this peak area. The peak area and peak height are the actual peak area and peak height with the baseline being excluded respectively.

For chromatogram with multiple sample components, peak overlapping and shoulder peak also exist. Peak identification is only a preliminary location of the chromatogram peak and further baseline adjustment is required.

Baseline adjustment consists of two aspects (taking positive peak for example).

1. Adjust the baseline of the separated peaks. The ideal chromatogram peak is Gauss-like and the peak shape is symmetrical. When the original data of the chromatogram is lower than the baseline position, baseline permeation occurs; you need to adjust the peak start and end points until the new baseline is lower than all the data points within its area.
2. Adjust the baseline of the fusion peaks. The rules are as follows.
   A. When the peak valley between the two peaks is lower than the baseline, the peak valley is taken as the termination point of the new baseline.
   B. When the original data of the chromatogram is lower than the baseline,

baseline permeation occurs; at this point, adjust the peak start point and peak end point until the new baseline is lower than all the data points within its area.

C. When the separation degree between the two peaks is lower than 1.5, combine the baselines of the two peaks.

The separated peaks mentioned above refer to peaks of which the separation degree is greater than 1.5. The fusion peaks refer to the peaks of which the separation degree is lower than 1.5. The baseline adjustment is performed by UltraChrom automatically and users do not need to adjust it manually.

Manual Integration Events

For the different needs of users, the UltraChrom integration algorithm provides 24 manual integration events, as shown in the figure below.

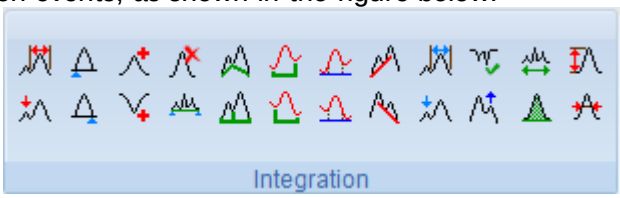


Figure 4-1 Manual Integration Events

There are two kinds of UltraChrom manual integration events: **peak detect events** and **baseline events**.

Peak detect events: events that affect the peak identification.
There are 14 peak detect events, including global peakwidth, global threshold, local peak width, local threshold, detect negative, detect shoulder, add positive peak, add negative peak, force single, delete peak, integration interval, min. width, min. height and min. area.

Baseline events: events that affect the peak baseline position.
There are 10 baseline events, including valley, together, peak-forward horizontal, peak-back horizontal, time-forward horizontal, time-back horizontal, front tangent, tail tangent, peak start and peak end.

The following section provides detailed introductions of the two kinds of events.

Peak Detect Events

1. **Global Peak Width**
   When this event is enabled, the software will detect the peaks by using half of the user-defined time range as the global peak width. Besides, users can also set it manually in the first row of the **Integration** field under the **Chroma** item. This value determines the size of the sliding window of the peak detect algorithm. When the value is too large, some peaks cannot be detected.

2. **Global Threshold**
   When this event is enabled, the software will detect the peaks by using half of the user-defined time range as the global threshold. Besides, users can also set it manually in the second row of the **Integration** field under the **Chroma** item. This value determines the positions of the peak start point and peak end point. When the value is too large, the peak start point and peak end point would be too high.

3. **Local Peak Width**
   When this event is enabled, users need to define both the application range of the local peak width and the desired peak width of the peak detect in this range. Within the user-defined application range, the function of the local peak width is the same with that of the global peak width.

4. **Local Threshold**
   This event is a timing event. Users need to define both the application range of the local threshold and the desired threshold of the peak detect in this range. Within the user-defined application range, the function of the local threshold is the same with that of the global threshold.

5. Detect Negative

This event is a timing event. Users need to define the application range of the function. Within the user-defined range, the UltraChrom detect negative algorithm can identify the negative peaks automatically. The figures below show the difference in the peak detect results before and after the detect negative is enabled. When “Detect Negative” is enabled, the UltraChrom detect negative algorithm detects the positive peaks and negative peaks within the specified range; when “Detect Negative” is not enabled, the UltraChrom detect negative algorithm only detects the positive peaks within the specified range.

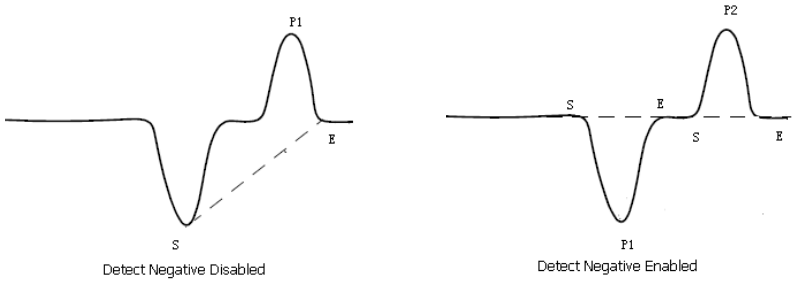


Figure 4-2 Comparison with Detect Negative being Enabled/Disabled

6. Detect Shoulder

This event is a timing event. Users need to define the application range of the function. This event can detect the shoulder peaks. When two neighboring peaks are eluted at low enough resolution and peak valley does not appear between them, shoulder peak will occur. The peak is called front shoulder peak if it is between the start point and top point of the chromatogram peak and back shoulder peak if it is between the top point and valley point of the chromatogram peak. If the resolution is enough, UltraChrom will detect all the peaks.

The UltraChrom detect shoulder algorithm uses the second derivative of the chromatogram to detect the shoulder peaks. Take the front shoulder peak as an example and the algorithm principles are as follows.

(1) If the local maximum of the second derivative is between the start point and top point of the chromatogram peak, there is a front shoulder peak and the data point corresponding to the local maximum of the second derivative is the top point of the shoulder peak;
(2) Search upward from the top point of the shoulder peak and the first local minimum of the second derivative is the start point of the shoulder peak;
(3) Search downward from the top point of the shoulder peak and the first local minimum of the second derivative is the end point of the shoulder peak.

The two figures below shows the difference in the peak detect results before and after enabling the detect shoulder function.

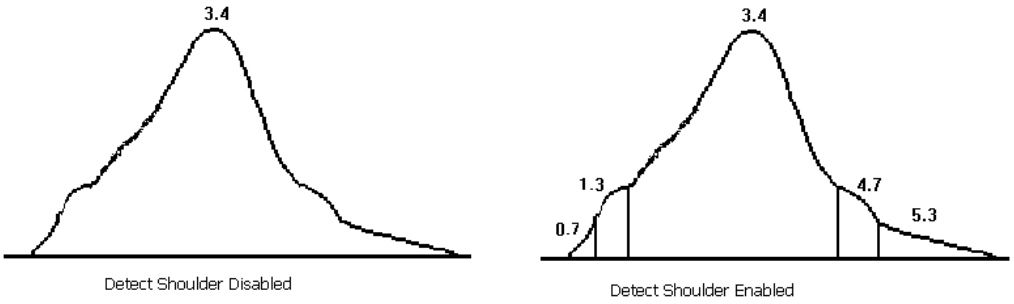


Figure 4-3 Comparison with Detect Shoulder being Enabled/Disabled

7. Add Positive Peak

Enabling this event can add the desired positive peak into the peak result table. Users need to define the start point and end point of the positive peak added. You can right-click to give up this event while adding the positive peak.

If the top point of an existing peak is between the specified start point and end point, the operation is invalid because the same peak cannot be added twice.

If the specified start point or end point is the highest point of the positive peak added, this operation is invalid because the top point and the start point or end point of the peak cannot be the same point.

If the baseline passes through the specified start point and end point of the positive peak, the UltraChrom baseline adjustment algorithm will adjust the start point or end point to the proper position automatically.

8. Add Negative Peak

Enabling this event can add the desired negative peak into the peak result table. Users need to specify the start point and end point of the negative peak added. You can right-click to give up the operation while adding the negative peak.

If the top point of an existing peak is between the specified start point and end point, the operation is invalid because the same peak cannot be added twice.

If the specified start point or end point is the lowest point of the negative peak added, this operation is invalid because the top point and the start point or end point of the peak cannot be the same point.

If the baseline passes through the specified start point and end point of the negative peak, the UltraChrom baseline adjustment algorithm will adjust the start point or end point to the proper position automatically.

9. Force Single

Enabling this event can force the fusion peaks belonging to the same fusion peak cluster to a single peak. Users need to specify the application range of this event and this event is only valid for fusion peaks.

The two figures below show the difference before and after enabling the force single function.

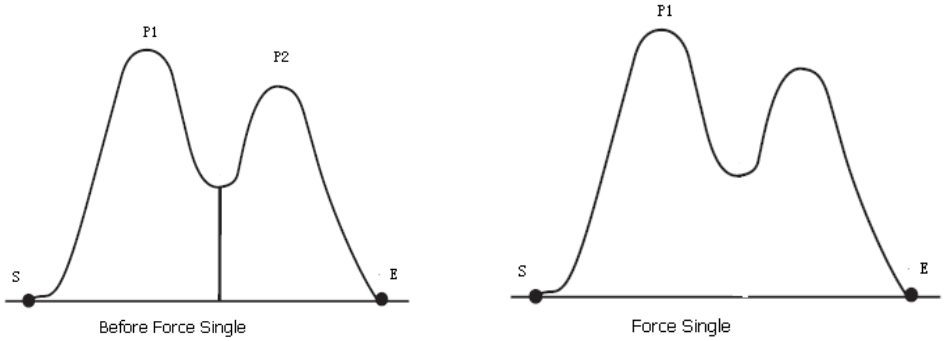


Figure 4-4 Difference with Force Single being Enable/Disabled

10. Delete Peak

Enabling this event can delete the peaks within the specified range. Users need to specify the application range of this event.

11. Integration Interval

Enabling this event can hide the peaks outside the specified range. Users need to specify the application range of this event.

12. Min. Peak Width

Enabling this event can pick out the peak of which the peak width is lower than the user-defined value. Users need to specify the application range of this event as well as the minimum peak width. The operation effect of this event is as shown in the figure below.

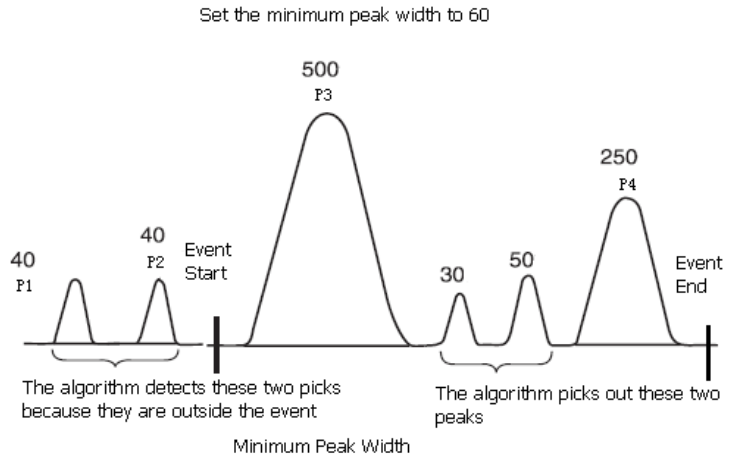


Figure 4-5 Min. Peak Width

13. Min. Peak Height

Enabling this event can pick out the peak of which the peak height is lower than the user-defined value. Users need to specify the application range of this event as well as the minimum peak height. The operation effect of this event is as shown in the figure below.

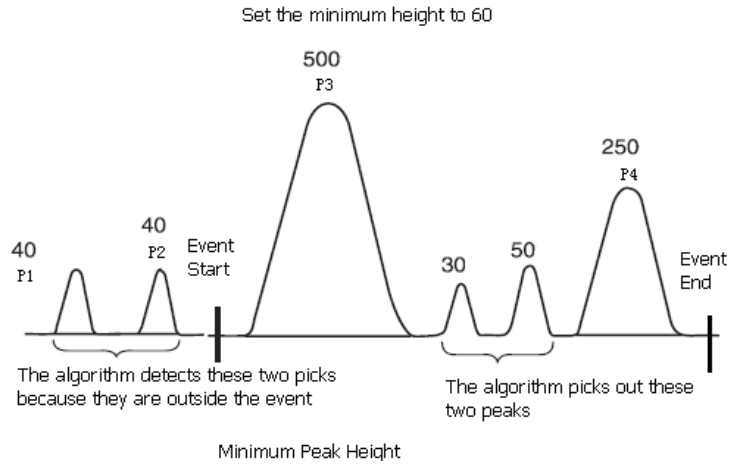


Figure 4-6 Min. Peak Height

14. Min. Peak Area

Enabling this event can pick out the peak of which the peak area is lower than the user-defined value. Users need to specify the application range of this event as well as the minimum peak area. The operation effect of this operation is as shown in the figure below.

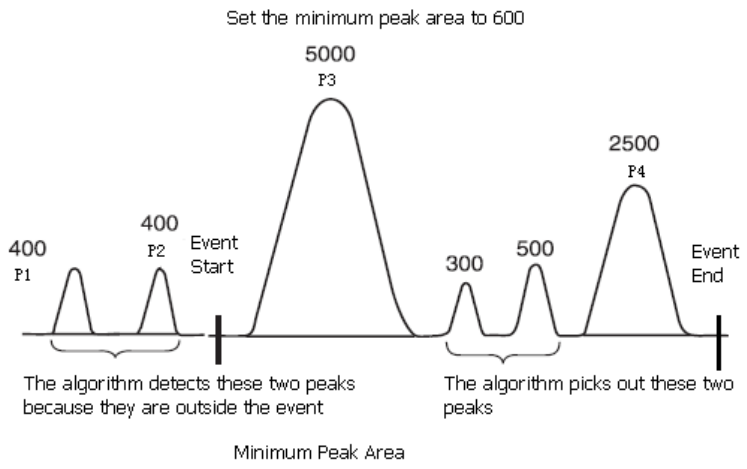


Figure 4-7 Min. Peak Area

Baseline Events

1. Valley

This event is a timing event and is only valid for fusion peaks. It forces the fusion peaks to separated peaks. The user-defined range should contain the start point and end point of the fusion peak cluster. The operation effect of this event is as shown in the figure below.

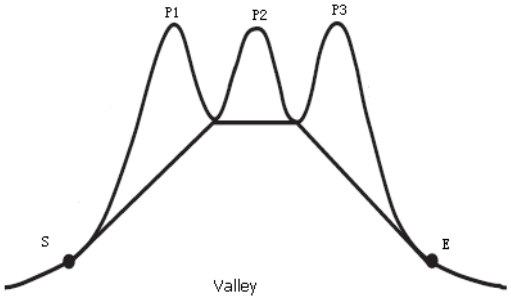


Figure 4-8 Valley

2. Together

This event is a timing event and is only valid for fusion peaks. It forces the fusion peaks to separate peaks. The user-defined range should contain the start point and end point of the fusion peak cluster. The default separating method of the fusion peaks in the UltraChrom integration algorithm is together. The operation effect of this event is as shown in the figure below.

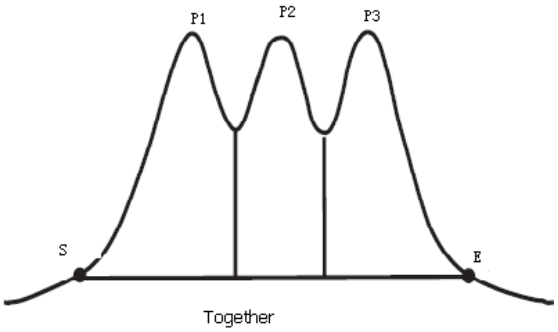


Figure 4-9 Together

3. Peak-forward Horizontal

This event is a timing event. The user-defined range should contain the start point and end point of the peak of which the baseline needs to be adjusted. If the Y value of the start point is greater than the Y value of the end point, this event is invalid. The operation effect of this event is as shown in the figures below.

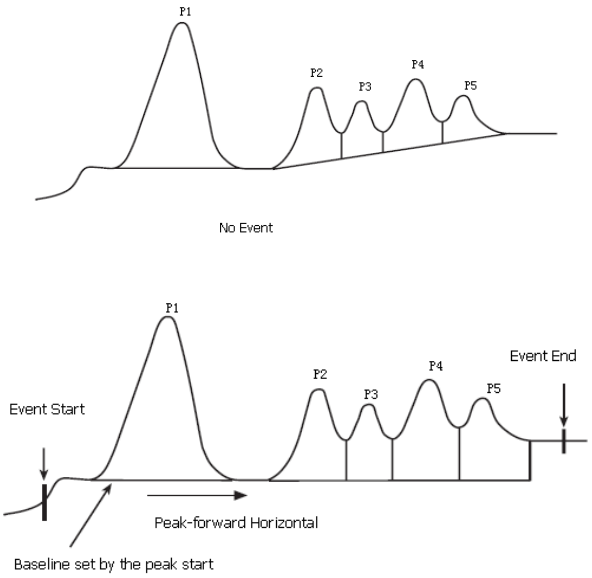


Figure 4-10 Peak-forward Horizontal

4. Peak-back Horizontal

This event is a timing event. The user-defined range should contain the start point and end point of the peak of which the baseline needs to be adjusted. If the Y value of the end point is greater than the Y value of the start point, this event is invalid. The operation effect of this event is as shown in the figures below.

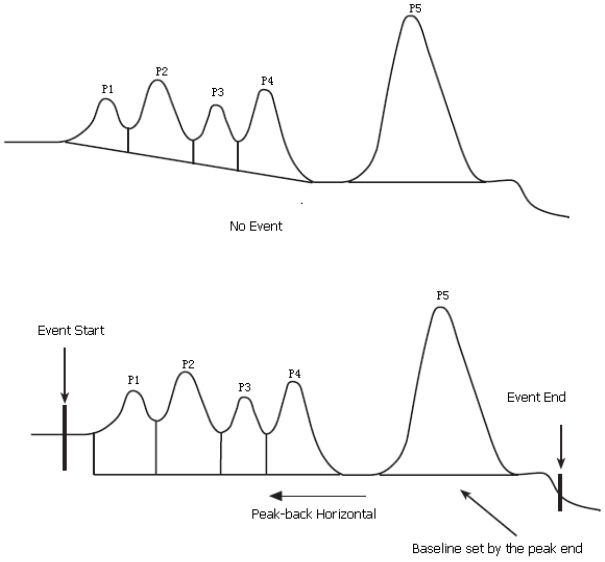


Figure 4-11 Peak-back Horizontal

5. Time-froward Horizontal

This event is a timing event. The user-defined range should contain the start point and end point of the peak of which the baseline needs to be adjusted. The operation effect of this event is as shown in the figures below.

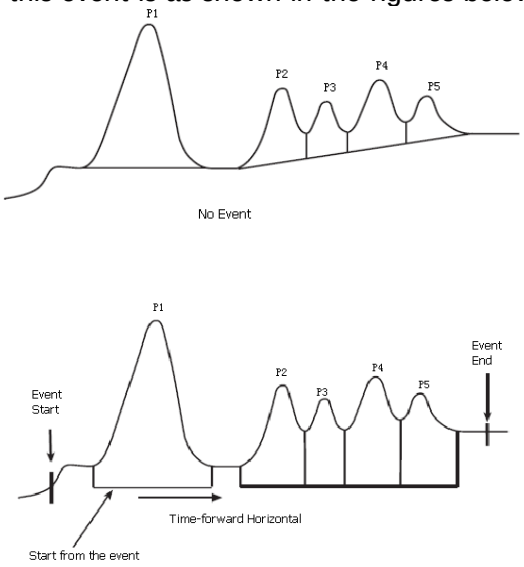


Figure 4-12 Time-forward Horizontal

6. Time-back Horizontal

This event is a timing event. The user-defined range should contain the start point and end point of the peak of which the baseline needs to be adjusted. The operation effect of this event is as shown in the figures below.

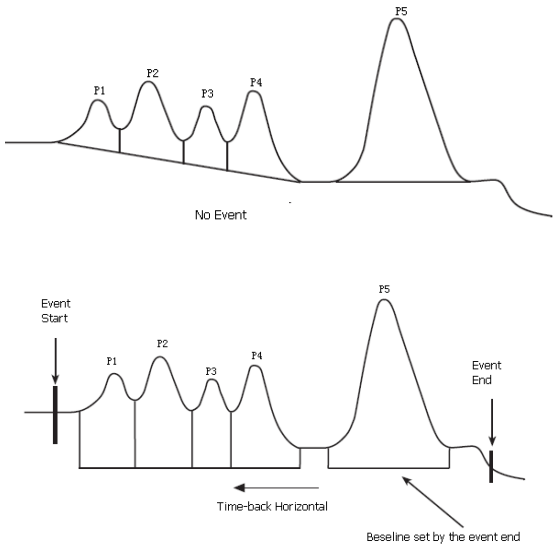


Figure 4-13 Time-back Horizontal

7. Front Tangent

This event is a time event and is only valid for fusion peaks. The peak to be cut cannot be the last peak in the fusion peak cluster and this event forces the fusion peaks into separated peaks. The start point of the user-defined application range should be at the front of the start point of the peak to be cut and the end point should be at the back of the end point of the peak to be cut. The operation effect of this event is as shown in the figures below.

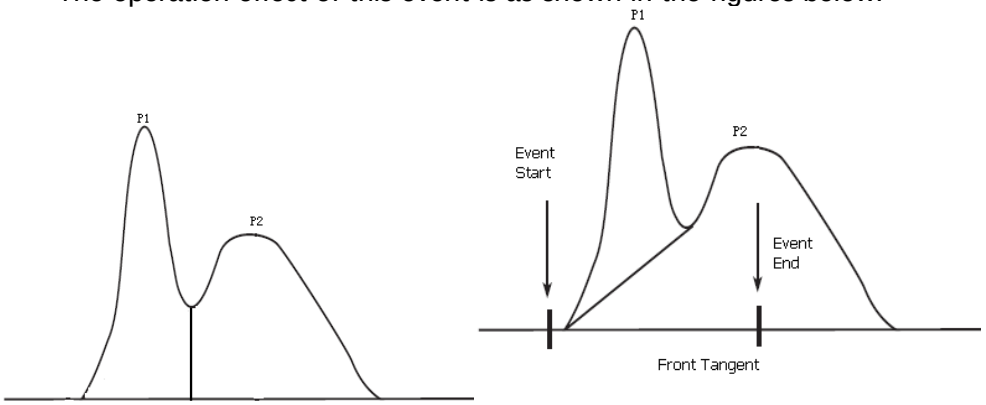


Figure 4-14 Front Tangent

8. Tail Tangent

This event is a time event and is only valid for fusion peaks. The peak to be cut cannot be the first peak in the fusion peak cluster and this event forces the fusion peaks into separated peaks. The start point of the user-defined application range should be at the front of the start point of the peak to be cut and the end point should be at the back of the end point of the peak to be cut. The operation effect of this event is as shown in the figures below.

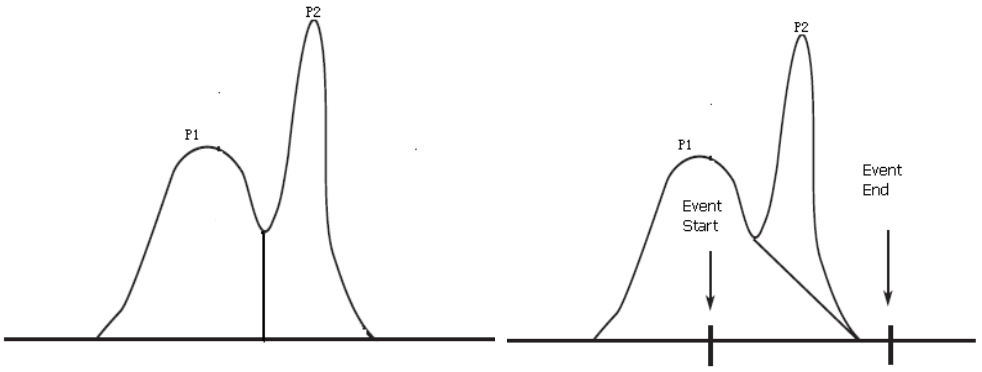


Figure 4-15 Tail Tangent

9. Peak Start

Users need to specify the position of the new start point. If the baseline passes through the start point, the algorithm will adjust automatically. If the new start point is between the top point and end point of the identified peak, this event is

invalid. If the baseline of the identified peak passes through the new start point, the UltraChrom baseline adjustment algorithm will adjust the new start point to a proper position automatically. The operation effect of this operation is as shown in the figure below.

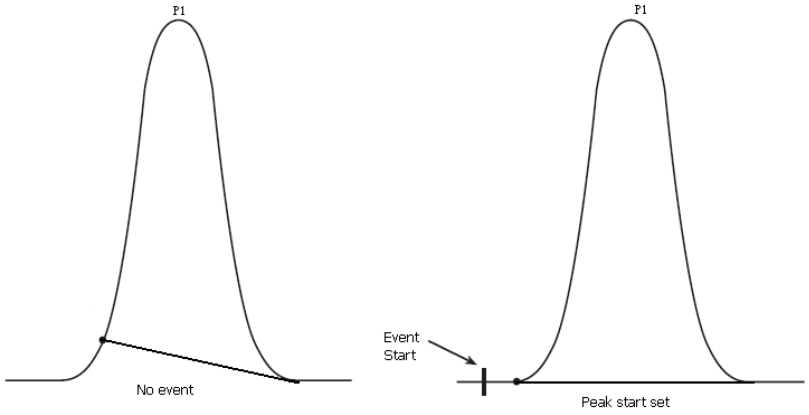


Figure 4-16 Peak Start

10. Peak End

Users need to specify the position of the new end point. If the baseline passes through the end point, the algorithm will adjust automatically. If the new end point is between the start point and top point of the identified peak, this event is invalid. If the baseline of the identified peak passes through the new end point, the UltraChrom baseline adjustment algorithm will adjust the new end point to a proper position automatically. The operation effect of this operation is as shown in the figure below.

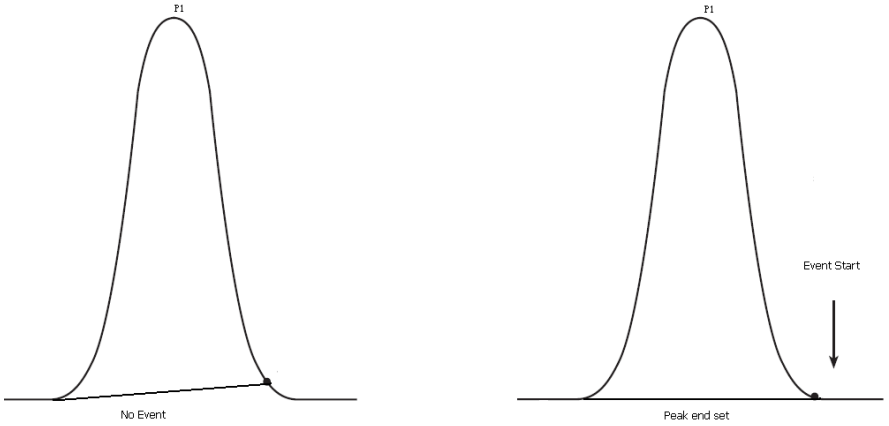


Figure 4-17 Peak End

Noise and Drift

The UltraChrom integration algorithm cannot only identify the chromatogram peaks automatically and calculate their specifications but also calculate the noise and drift according to the user-defined interval.

Noise

The noise is calculated using the data point values of the chromatogram within the specified time range. The UltraChrom data processing algorithm provides three noise calculation methods.

A. The standard deviation of the linear regression of the drift times 6 (6Sigma noise)
B. Noise from peak to peak (P-P noise)
C. Use ASTM method to measure the noise

1. 6Sigma noise

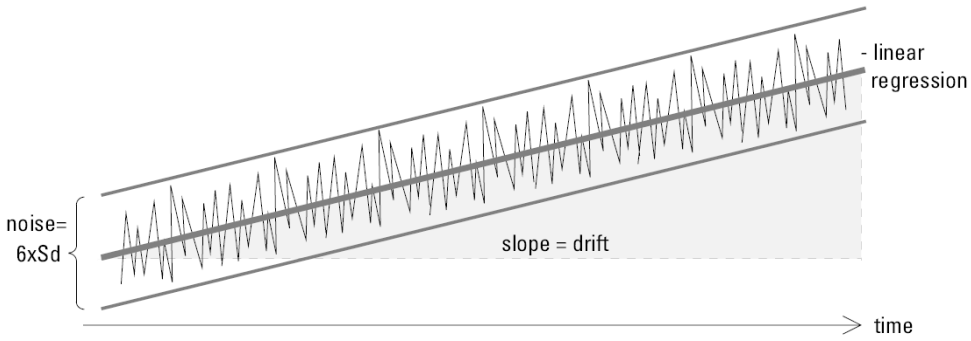


Figure 4-18 6Sigma Noise

First, use all the data points within the specified time range to calculate the linear regression equation, as shown in equation (4-2) to equation (4-5). Use Std to represent the standard deviation of the linear regression equation.

$$Std = \sqrt{\frac{\sum_{i=1}^{N}(Y_i - a - bX_i)^2}{N-2}} \quad (4\text{-}6)$$

Use N to represent the 6Sigma noise.

$$N = 6 \times Std \quad (4\text{-}7)$$

2. P-P Noise

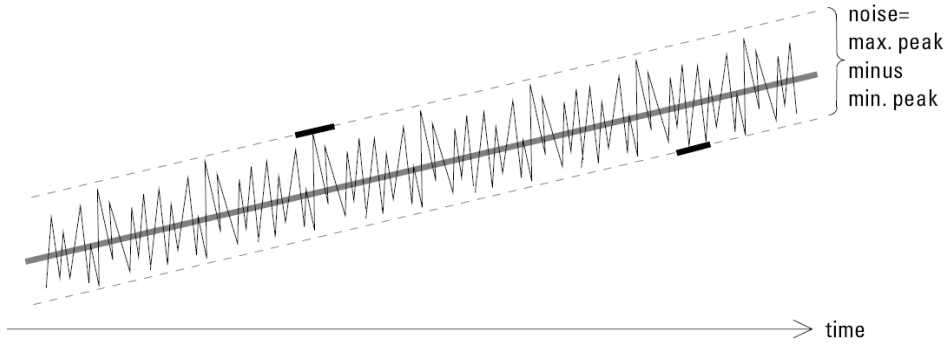


Figure 4-19 P-P Noise

First, use equation 4-2 to equation 4-5 to calculate the linear regression equation. Subtract the linear regression line from all the data points within the specified time range to get the drift-corrected signal. Then, use the formula below to calculate the P-P noise. Use N to represent the P-P noise.

$$N = \frac{I_{max}}{I_{min}} \quad (4\text{-}8)$$

Wherein, I_{max} is the highest peak of the drift-corrected signal within the specified time range, I_{min} is the lowest peak within the time range.

3. ASTM Noise

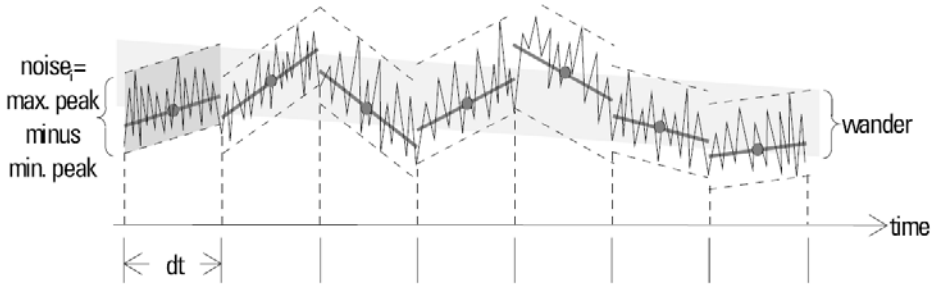


Figure 4-20 ASTM Noise

The ASTM noise measurement (ASTM E 685-93) is based on the standard measurement regulation of the variable wavelength photometric detector in liquid chromatogram defined by the ASTM. Three different types of noises can be distinguished according to the length of the time range. The noise measurement is based on the peak-peak detect within the specified time range.

- Long-term noise: the maximum amplitude of the random fluctuation of the detector signal of which the frequency is 6 to 60 periods per hour. When the specified time range is greater than one hour, the noise is treated as long-term noise. Set the time range of each period to 10 minutes, thus the

specified time range contains at least 6 periods.

- Short-term noise: the maximum amplitude of the random fluctuation of the detector signal of which the frequency is greater than one period per minute. When the specified time range is between 10 to 60 minutes, the noise is treated as short-term noise. Set the time range of each period to 1 minute, thus the specified time range contains at least 10 periods.
- Ultra-short noise: this part is not defined in ASTM E 685-93. The maximum amplitude of the random fluctuation of the detector signal of which the frequency is greater than one period per 0.1 minute. When the specified time range is between 1 to 10 minutes, the noise is treated as ultra-short noise. Set the time range of each period to 0.1 minute, thus the specified time range contains at least 10 periods.

Use n to represent the number of periods.

$$n = \frac{t_{ot}}{T} \tag{4-9}$$

Calculate the P-P noise (N) within each period according to formula (4-9). Use N_{ASTM} to represent the ASTM noise N_{ASTM} .

$$N_{ASTM} = \frac{\sum_{i=1}^{N} N}{n} \tag{4-10}$$

If the specified time range is shorter than 1 minute, the system will give up the ASTM noise measurement and return back to P-P noise. If the specified time range is greater than or equal to 1 minute, the system will measure the noise using one of the above-mentioned ASTM methods.

Drift

The drift equals the slope of the linear regression. Please refer to formula (4-4).

Peak Match

When executing peak match, the peak match algorithm will select the integration peak in the chromatogram that matches the best with the component in the calibration table. The detailed procedures are as follows.

1. Determine whether the chromatogram peak is within the time range (the retention time window) defined by the retention time of the component in the calibration table. The time range mentioned here refers to the time range determined by the retention time and the left/right window, namely from time A (A= retention time – left window) to time B (retention time + right window).

2. If multiple chromatogram peaks are within the time range (retention time window) defined by the retention time of the component in the calibration table, use the “peak match” type to find out the chromatogram peak that matches the best with the component in the calibration table. UltraChrom provides 4 “peak match” types.
   A. Nearest peak: peak of which the retention time is within the time range defined by the retention time of the component in the calibration table and the retention time is the nearest to the retention time of the sample component;
   B. Max. peak: peak of which the retention time is within the time range defined by the retention time of the component in the calibration table and the peak area is the largest of all the peaks that meet the condition;
   C. First peak: peak of which the retention time is within the time range defined by the retention time of the component in the calibration table and the retention time is the smallest of all the peaks that meet the condition;
   D. Last peak: peak of which the retention time is within the time range defined by the retention time of the component in the calibration table and the retention time is the largest of all the peaks that meet the condition.

3. If the peak is not within the time ranges defined by the retention times of all the components in the calibration table, this peak is defined as an unidentified peak.

Select the Best Peak Match

During the peak match process, attention should also be paid to that whether there is component that matches with multiple unknown peaks and whether the unknown peak matches with multiple components. The following are the three possible results in the original component match process. For the three possible results, the UltraChrom peak match algorithm provides specific processing methods.

1. A single peak matches with a single component

The retention time windows of all the components in the calibration table do not

overlap and each window only contains one unknown peak at most. In this situation, the peak match can be performed smoothly and you do not need to consider the peak match type.

2. Multiple peaks match with a single component

If the retention time window of the component contains multiple unknown peaks, the UltraChrom peak match algorithm will select the peak that matches the best using the peak match type.

3. A single peak matches with multiple components

If a single peak matches with multiple components, the UltraChrom peak match algorithm will select the component of which the retention time is nearest to that of the unknown peak. If there are two or more components the differences between the retention times of which and that of the unknown peak are the same, the UltraChrom peak match algorithm will select the first component.

Quantitation

During the analysis, after performing peak integration and peak match, quantitation operation is required. Quantitation refers to determining the compound amount and concentration in the sample using the peak area or peak height. The quantitative analysis consists of lots of steps and the following is a general summary of the quantitation procedures.

1. Get some knowledge of the compound to be measured;
2. Analyze the sample with known compound amount or concentration to acquire the response value under this concentration (here, the response value refers to the peak area or peak height and is determined by the response basis selected by users). You can also analyze multiple samples with different concentrations of the target compound to build multi-level calibration;
3. Analyze the sample with unknown concentration of the compound to be measured to acquire the response under the unknown concentration;
4. Using certain quantitative method to determine the amount or concentration of the compound according to the responses under the unknown concentration and under the known concentration.

UltraChrom provides three quantitative methods: ESTD, ISTD and normalization.

ESTD

ESTD is the most basic quantitative method. It determines the component amount and concentration by applying the response of the component peak to the calibration curve, as shown in the figure below.

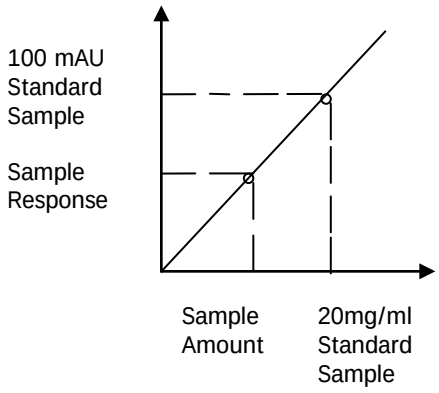


Figure 4-21 ESTD

The calibration curve is generated using the standard sample set which is acquired and processed separately. The standard sample set can contain only one standard sample (called single-level calibration); it can also contain two or more standard samples to form multi-level calibration. The Y value of the calibration curve is the component response and the X value is the component amount or concentration.

To execute the ESTD quantitation, UltraChrom performs the following operations.

1. Use peak match to identify the component peak in the standard sample chromatogram;
2. Determine the response and amount or concentration of each standard peak and then draw the calibration point on the calibration curve with the same component;
3. Draw the calibration curve of each component by setting the calibration methods, such as the zero point mode, fitting type and weight;
4. Quantify the unknown sample by comparing with the calibration curve generated and performing the following operations.
   A. Identify the unknown peak according to the peak match type set;
   B. For the identified peak, calculate its amount or concentration using the calibration curve according to its response and injection volume;
   C. Adjust the amount and concentration using the dilution and the final calculation results will be displayed in the peak result table in the UltraChrom chromatogram interface.

ISTD

In ISTD, a component with known amount is added into the standard sample and sample to be measured and its amount is used as the normalization factor. The compound used as the ISTD should have similar chemical characteristics and retention time with the compound to be calibrated, while at the same time, they can be separated on the chromatogram.

ISTD determines the amount ratio or concentration ratio of the component to the ISTD compound by applying the detector response ratio of the component peak to the ISTD peak to the calibration curve. As the amount or concentration of the ISTD compound is known, the amount or concentration of the component can be calculated, as shown in the figure below

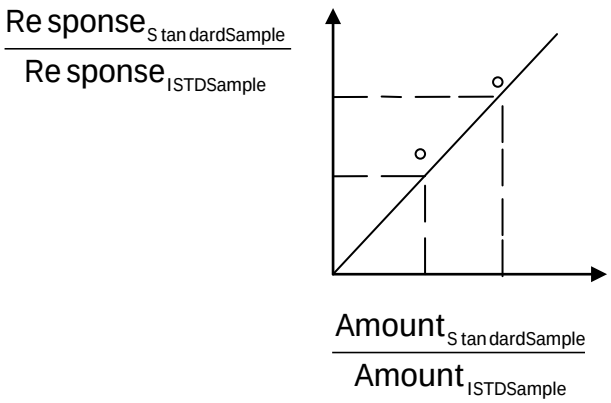


Figure 4-22 ISTD

The calibration curve is generated using the standard sample set which is acquired

and processed separately. The standard sample set can contain only one standard sample (called single-level calibration); it can also contain two or more standard samples to form multi-level calibration. The Y value of the calibration curve is the detector response ratio of the component to the ISTD peak and the X value is the amount ratio or concentration ratio of the component to the ISTD compound.

To execute the ISTD quantitation, UltraChrom performs the following operations.

1. Identify the component peak in the standard sample chromatogram using the peak match;
2. Determine the response and amount or concentration of the standard peak and ISTD peak and then draw the calibration point on the calibration curve with the same component;
3. Calculate the calibration curve of each component by setting the calibration methods, such as the zero point mode, fitting type and weight;
4. Quantify the unknown sample by comparing with the calibration curve generated and performing the following operations.
   A. Identify each unknown peak by matching its retention time with that of the component in the “Component” table;
   B. Calculate the response ratio of the identified peak to the ISTD peak and calculate the amount ratio or concentration ratio of each unknown peak to the ISTD peak using the calibration curve;
   C. Calculate the amount or concentration of the unknown peak using the amount or concentration of the ISTD compound;
   D. Adjust the amount and concentration using the dilution and the final calculation results will be displayed in the peak result table in the UltraChrom chromatogram interface.

Normalization

When using normalization to perform quantitative calculation, UltraChrom calculates the relative amount of each unknown peak using the peak area percentage or peak height percentage. The peak area or peak height percentage is the percentage that each integration peak takes up in the total peak area or total peak height of all the integrated peaks.

Calibration

Calibration refers to the process of calculating the concentration or amount of the unknown component by analyzing the specifically prepared standard sample.

Calibration Table

Actually, the calibration table is the collection of the calibration sample compounds. In HPLC, each compound corresponds to a peak, namely each compound corresponds to a chromatogram peak (the calibration table is the collection of the peaks of the calibration samples). The UltraChrom calibration interface provides the "Add Peak" and "Add All Peak" functions to add compounds to the calibration table. UltraChrom compares the retention time of the peak to be added with that of the existing peak and then add the peak into the calibration table according to the peak match process.

To create a calibration table, the following information is required.

1. The retention time of each calibration component peak;
2. The amount of each calibration component peak and make sure that all the amounts use the same unit.

Calibration Curve

The calibration curve refers to the graphic form of the amount and response of a compound acquired from one or more calibration samples. Here, the response refers to the peak area or peak height of the chromatogram peak corresponding to the compound.

The calibration is divided into single-level calibration and multi-level calibration according to the amount of calibration points used in fitting the calibration curve.

- Single-level calibration: only use one set of calibration points;
- Multi-level calibration: use multiple sets of calibration points.

Calibration Curve Fitting Type

UltraChrom provides 7 curve fitting types including segment, linear, quadratic, cubic, exponent, natural logarithm and power function.

Zero Point Modes

UltraChrom provides 3 zero point modes for drawing the calibration curve.

1. Ignore the zero point: do not consider the zero point when drawing the calibration curve
2. Include the zero point: the zero point takes part in the calculation of the calibration curve fitting
3. Force to pass through the zero point: the calibration curve must pass through the zero point

Weighting Methods

When setting the calibration curve, you can specify the relative weight or importance of each calibration point used to generate the curve. UltraChrom provides the following weighting methods.

1. None: do not set the weight, namely all the calibration points have the same weight in the curve;
2. 1/response: the weight of each calibration point equals the reciprocal of its response;
3. 1/response square: the weight of each calibration point equals the reciprocal of the square of its response;
4. 1/amount: the weight of each calibration point equals the reciprocal of its amount;
5. 1/amount square: the weight of each calibration point equals reciprocal of the square of its amount.

If the zero point is included, the weight of the zero point is the average of the weights of all the calibration points.

Fitting Function

UltraChrom uses the least weighted square method to calculate the coefficient of the curve according to the fitting type, zero mode and weighting method settings.

The fitting curve function is as follows.

$$y = p(x) = r_0 + r_1 x + r_2 x^2 + r_3 x^3 + ... + r_n x^n \qquad (4\text{-}11)$$

Y is the nth-degree polynomial of x and the corresponding formal function set is:

$$\begin{vmatrix} \sum_{i=1}^{m} w_i & \sum_{i=1}^{m} w_i x_i & \sum_{i=1}^{m} w_i x_i^2 & \cdots & \sum_{i=1}^{m} w_i x_i^n \\ \sum_{i=1}^{m} w_i x_i & \sum_{i=1}^{m} w_i x_i^2 & \sum_{i=1}^{m} w_i x_i^3 & \cdots & \sum_{i=1}^{m} w_i x_i^{n+1} \\ \vdots & \vdots & \vdots & & \vdots \\ \sum_{i=1}^{m} w_i x_i^n & \sum_{i=1}^{m} w_i x_i^{n+1} & \sum_{i=1}^{m} w_i x_i^{n+2} & \cdots & \sum_{i=1}^{m} w_i x_i^{2n} \end{vmatrix} \begin{vmatrix} r_0 \\ r_1 \\ r_2 \\ \vdots \\ r_n \end{vmatrix} = \begin{vmatrix} \sum_{i=1}^{m} w_i y_i \\ \sum_{i=1}^{m} w_i x_i y_i \\ \sum_{i=1}^{m} w_i x_i^2 y_i \\ \vdots \\ \sum_{i=1}^{m} w_i x_i^n y_i \end{vmatrix}$$

Assume that $C_1 = \begin{vmatrix} 1 & x_1 & x_1^2 & \cdots & x_1^n \\ 1 & x_2 & x_2^2 & \cdots & x_2^n \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ 1 & x_m & x_m^2 & \cdots & x_m^n \end{vmatrix}$, $Q = \begin{vmatrix} w_1 & 0 & 0 & \cdots & 0 \\ 0 & w_2 & 0 & \cdots & 0 \\ 0 & 0 & w_3 & \cdots & 0 \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ 0 & 0 & 0 & \cdots & w_n \end{vmatrix}$,

R= $(r_0, r_1, r_2, r_3, \ldots, r_n)^T$, C= $(y_1, y_2, y_3, y_4, \ldots, y_n)^T$,

the formal function set can be expressed as $C_1^T Q C_1 = C_1^T Q C$. First, use the known data information to calculate all the above data elements and list the formal function set; then, calculate the coefficient of the fitting polynomial using the linear function set $r_0, r_1, r_2, r_3, \ldots, r_n$ to acquire the fitting function.

Statistic Data

The statistic data shows the fitting degree. UltraChrom calculates two statistic standards (relative coefficient and standard square deviation). Wherein, when the relative coefficient is 1, the data point and the calibration curve fits completely; when the relative coefficient is 0, the data point and the calibration curve do not fit.

1. Relative Coefficient

The relative coefficient (R) shows the fitting degree and is the square root of the definite coefficient.

The definite coefficient (R^2) is the rough indication of the fitting degree and is calculated using the following formula.

$$R^2 = 1 - \frac{(s_y)^2}{\sigma_y^2} \qquad (4\text{-}12)$$

Wherein, R^2 is the definite coefficient, R is the relative coefficient, S_y is the

standard error corresponding to the y estimated value of x and σ_y^2 is the standard square deviation.

A. Standard error corresponding to the y estimated value of x
The standard error corresponding to the y estimated value of x S_y is calculated using the following formula.

$$S_y = \sqrt{\frac{1}{n}\sum_{i=1}^{n} w_i (\hat{y}_i - y_i)^2} \quad (4\text{-}13)$$

Wherein, n is the number of data points, w_i is the weight, $\hat{y}_i$ is the response predicted using the calibration curve and y_i is the actual response of the calibration point.

B. Standard deviation
Its calculation formula is as follows.

$$\sigma_y^2 = \frac{1}{n}\sum_{i=1}^{n} w_i (\overline{y_i} - y_i)^2 \quad (4\text{-}14)$$

Wherein, w_i is the weight, $\overline{y_i}$ is the average of the actual responses of the calibration points and y_i is the actual response of the calibration point.

2. Standard Square Deviation
The standard square deviation is calculated using the formula above.

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