Kromstation

HPLC WorkStation

User's Manual

Chromatography Data Workstation User Manual

V1.0.2

s t a t e m e n t

This manual is for the user to understand, use and maintain the standard data demonstration of the Kromstation, rather than its own business and special purpose manuals.

The contents of this manual are subject to change without notice.

When this manual was published, it was considered accurate and complete after careful review. It is not responsible for possible errors and accidental or recurring injuries of the manual that cause concern.

In any case, it is not responsible for the use of this manual and equipment.

Contents

1. What is Kromstation

Through Kromstation, a personal computer can be used to control the liquid chromatography and complete various operations. Such as instrument control, data acquisition and data evaluation.

1.1 Features of Kromstation

- GLP/GMP Support Function

The audit trail and electronic signature comply with CFDA's data integrity management regulations. Information such as data measurement method, date/time, operator name, and chromatogram can be saved immediately.

- Security Support

The system management function can meet the security requirements of various workflows.

These functions provide multi-level access management and electronic approval of the analysis data stored in the database.

- Audit Tracking Support

Detailed user information (such as methods, data and system operations) can be saved to create and manage an appropriate audit history.

- Flexible Report Templates

The report can be customized according to the purpose of use, and a variety of different items can be combined.

2 Software Overview

This chapter introduces the features and components of Kromstation in detail. Applies to:

- iChrom 5100 series liquid chromatography system;
- EClassical 3100 series liquid chromatography system;
- EClassical 3200 series liquid chromatography system;
- Agress 1100 series liquid chromatography system;
- And A/D conversion system, etc.
- Additional data processing module (can be purchased separately);
- Diode Array Detector (DAD) Spectral Processing Module;
- Gel Permeation Chromatography (GPC) processing module;
- System Stability (SST) data processing module;

In theory, Kromstation workstation can control 4 sets of instruments or equipment at the same time.

2.1 Operating System

The Kromstation Chromatography Data Workstation requires the operating system to be Windows 10 and above (eg Windows 10, Windows 11) , 64-bit.

2.1.1 Hardware Requirements

- Minimum hardware requirements: Intel Core 2 CPU, 8G memory (PDA module requires 16G memory or above), C drive 4G or above, Kromstation installation path data storage space, reserved based on customer actual experimental volume, recommended 50G or above.

- Minimum display resolution: 1024×800, 64K color (16-bit true color).
- Requirements for computer accessories: at least one USB interface for encryption lock (Hardware Key), at least one network interface (LAN) for device communication, and one USB interface for installing software.
- Network management requirements: It is recommended that the computer used to connect the high-performance liquid chromatograph is not connected to the network. If it is necessary to connect to the Internet, please complete the connection work under the guidance of the engineer.

2.1.2 Software Requirements

- Confirm that the operating system used to run Kromstation Chromatography Data Workstation is genuine;
- Confirm that the firewall of the operating system is turned off;
- Set the option of "Put the computer to sleep" in the operating system to "Never";
- Set the properties of the network adapter, and confirm that "Allow the computer to turn off this device to save power" in the "Power Management" option of the network adapter is not selected;
- It is not recommended to install anti-virus software on the computer used to connect to the high-performance liquid chromatograph, and it must be ensured that the mobile storage device used for data copy does not contain any computer viruses.

2.2 Introduction to Workstation

2.2.1 Installation

The installation software of Kromstation Chromatography Data Workstation is a USB flash drive. Insert the installation USB flash drive into the USB interface. After a while, the system will automatically enter the installation program. At this time, the installation program will create an installation wizard to guide you through the installation of this workstation. If it does not pop up

Installation prompt, please double-click the drive letter where the U disk is located, and then follow the prompts to install.

Caution: Be sure to insert the dongle provided with the workstation into the USB port of the computer before running the software.

2.2.2 User Login

After the dongle is inserted into the computer, double-click the desktop icon  of the computer, and the workstation enters the startup state. After the startup is completed, it enters the user code dialog box, as shown in Figure 2-1.

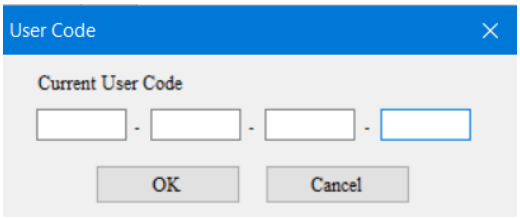


Figure 2-1 User code dialog

Enter the user code and click "OK" to enter the "Create User" interface. The user role created this time is "Administrator". Enter their "Username" and "Password" and click OK. As shown in Figure 2-2.

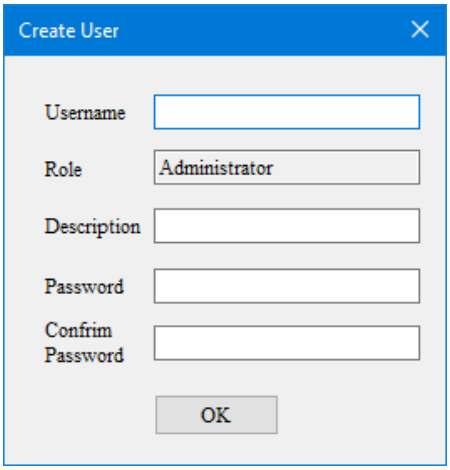


Figure 2-2 Create User Interface

Enter the login interface. As shown in Figure 2-3. After entering the password, click "Confirm" to log in to the workstation.

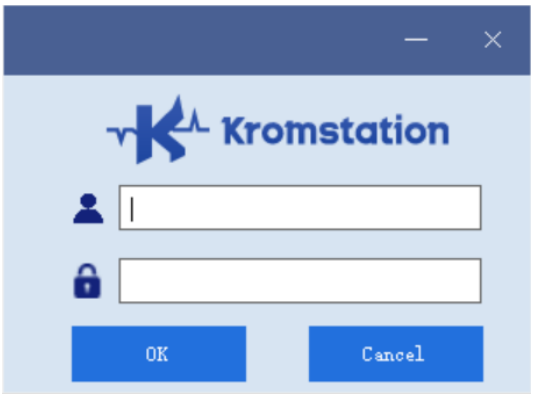


Figure 2-3 User login interface

2.2.3 Setting User Accounts

After starting the software, click the icon  on the initial interface or click the "User Account" option in the "System" drop-down menu to activate the user account window, and you can "Add", "Edit", "Delete" and "View" the user account and so on. The account created when logging in for the first time is an administrator. As shown in Figure 2-4.

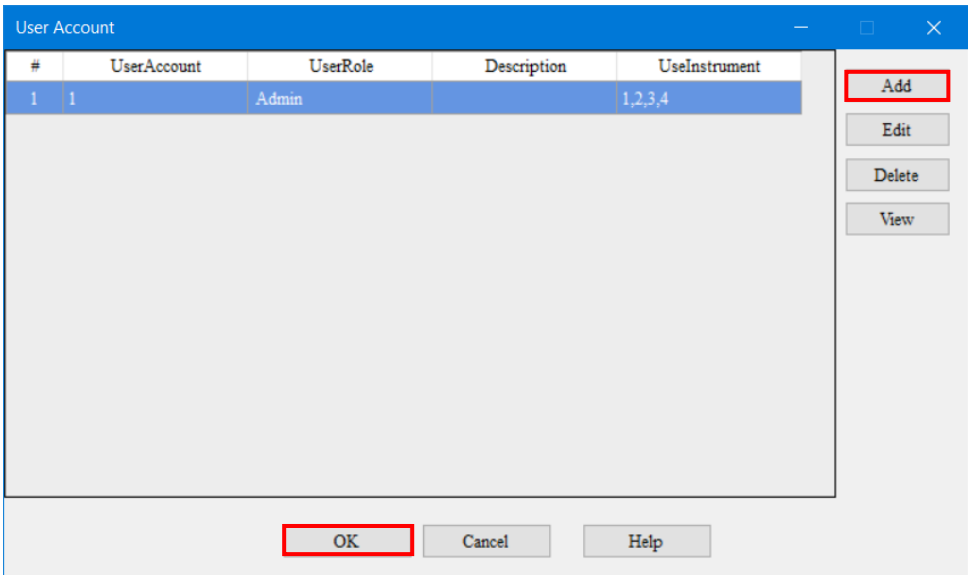


Figure 2-4 User account edit window

Through this window, users can create three user roles with different permissions, as shown in Figure 2-5.

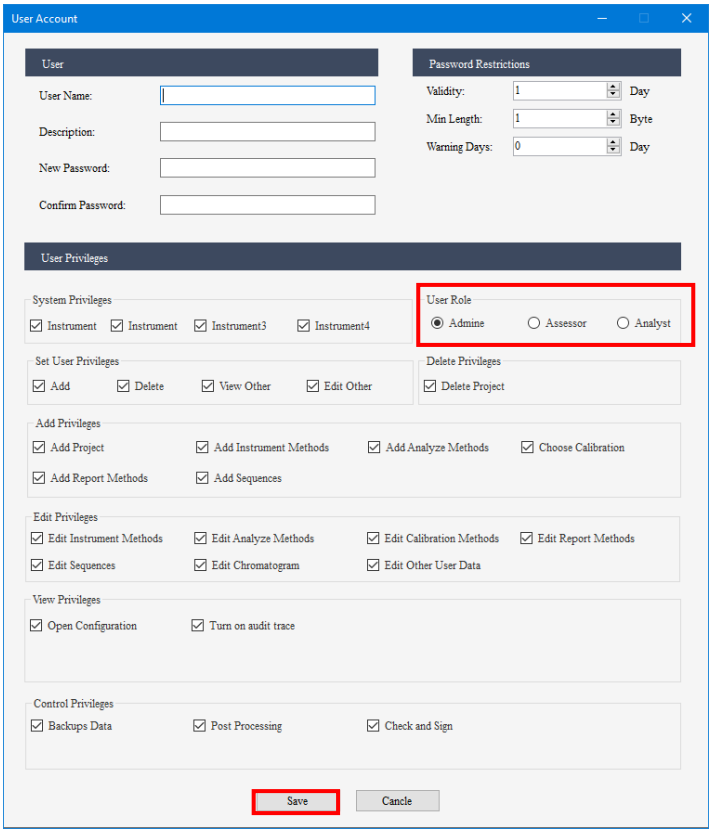


Figure 2-5 User account edit window

"User" can edit user information in this module;

"Password Restriction" can impose certain restrictions on the password;

"User Permission": Set usage permissions for the user.

When creating a new user role with administrative authority, you can set its access to software functions according to the role.

For the permission settings of different roles, please refer to the following table:

Permission module		Role		
		Administrator	**Auditor**	**Analyst**
System Authority	Instrument 1	√	√	√
	Instrument 2	×	×	×
	Instrument 3	×	×	×
	Instrument 4	×	×	×
	Create a new user	√	×	×

User Set Permissions	Delete user	√	×	×
	View other user accounts	√	√	×
	Edit other user accounts	√	o	×
Remove Permission	Delete project	√	o	×
New Permission	new reject	√	o	o
	New instrument method	√	√	o
	New analysis method	√	√	o
	New calibration method	√	√	o
	New report method	√	√	o
	New sequence	√	√	o
Edit Permissions	Edit instrument method	√	√	o
	Edit analysis method	√	√	o
	Edit calibration method	√	√	o
	Edit report method	√	√	o
	Edit sequence	√	√	o
	Edit Chromatogram	√	√	o
	Edit other user chromatograms	√	o	o
View/Open Permissions	Open configuration	√	√	o
	Open audit trail	√	√	o
Control Permissions	Backup project	√	o	×
	Import project	√	o	×
	Electronic signature	√	√	×

Annotation：

√：User role enabled permissions by default, can be disabled.

o：The permissions that the user can open.

×：Permissions that the role does not have.

2.2.4 Instrument Configuration

The instrument that needs to be controlled can be added in the system configuration window. After verifying the system configuration, the instrument can be controlled through the workstation to realize the functions of instrument control, data acquisition and evaluation. Click icon  or click

the "Configuration" option in the "System" drop-down box, as shown in Figure 2-6.

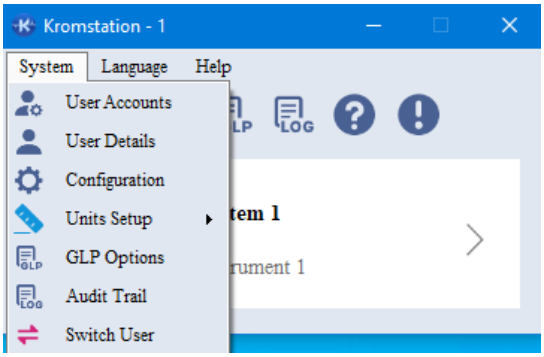


Figure 2-6 Login interface-system configuration

After clicking, the system configuration window appears, as shown in Figure 2-7.

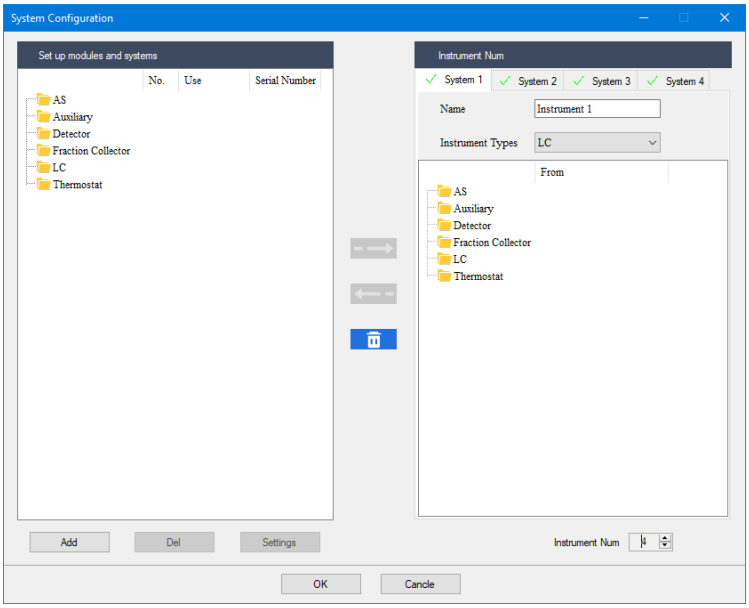


Figure 2-7 System configuration window

Click the "Add" button in the lower left corner, and the available control module window as shown in Figure 2-8 will appear.

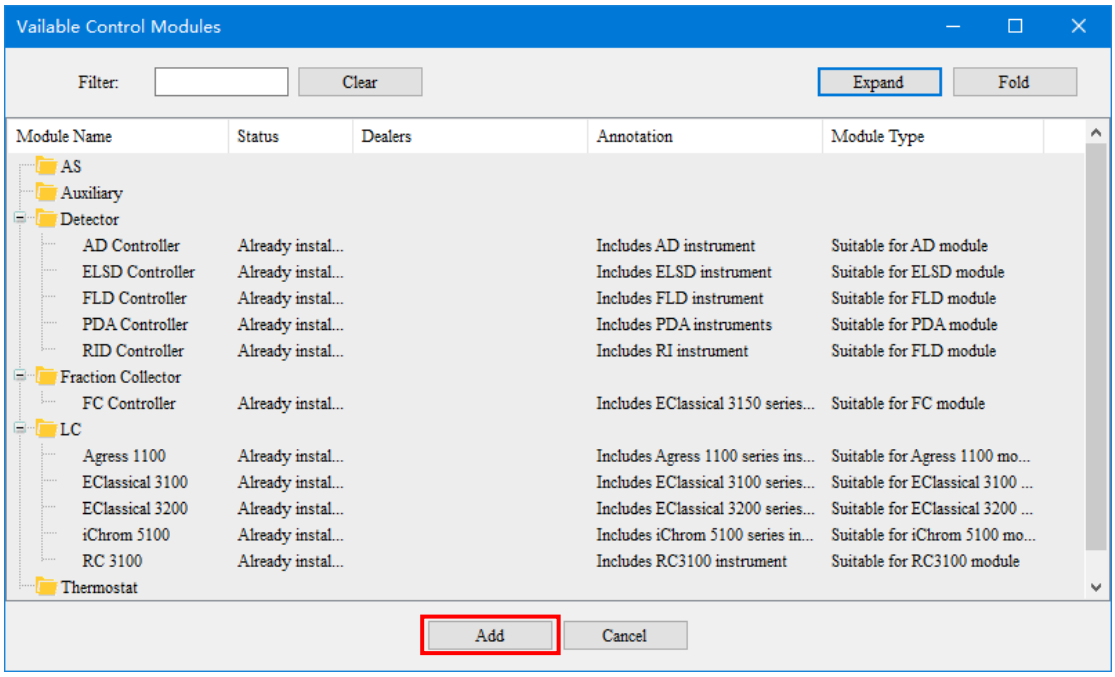


Figure 2-8 Available control module window

Select the "iChrom 5100" icon and click the "Add" button below to pop up the iChrom 5100 configuration window shown in Figure 2-9.

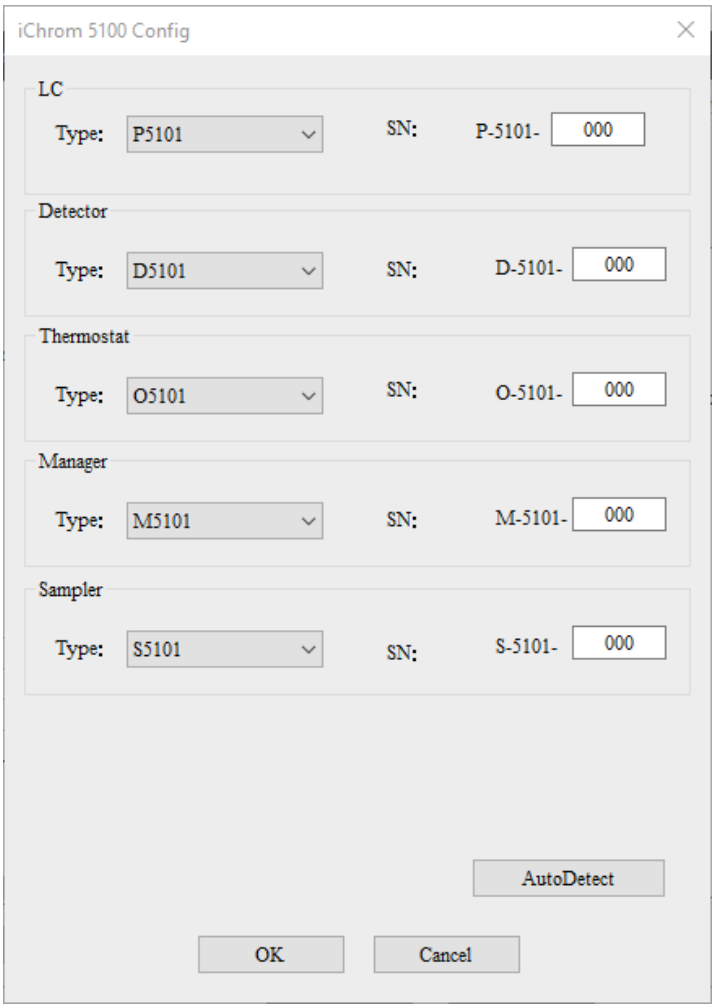


Figure 2-9 iChrom 5100 configuration window

Select the type of each part of the instrument and key in the corresponding serial number.

Caution: The method of adding other modules is the same as iChrom 5100.

Click "Verify System Configuration" to pop up a dialog box of whether the system connection is successful, as shown in Figure 2-10.

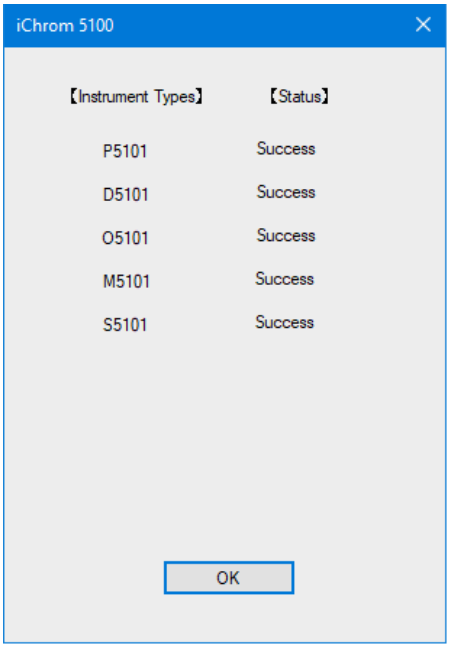


Figure 2-10 System connection status verification

After the instrument is successfully connected, click "OK" in the iChrom 5100 configuration interface, select the system you want to load, and click  . All instrument modules are loaded successfully, as shown in Figure 2-11.

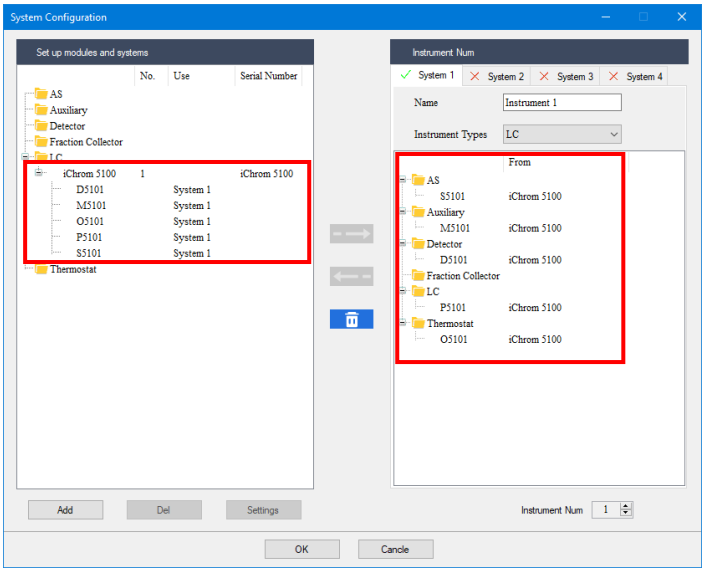


Figure 2-11 System configuration window

Caution: Other module configuration modes are the same as iChrom 5100.

2.2.5 Method Unit Setting

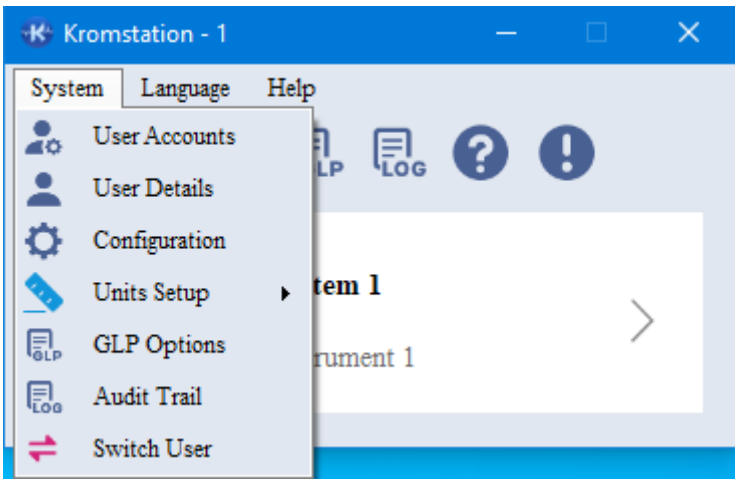


Figure 2-12 System unit setting

Click "Unit Settings" under "System" and select "System" to open the method unit setting window. You can select the unit of use for flow rate, pressure, temperature and other related information, as shown in Figure 2-13.

- **Method Unit Setting**

Click "Unit Setting" under "System" to open the method unit setting window, and you can select the units of flow rate, pressure and temperature.

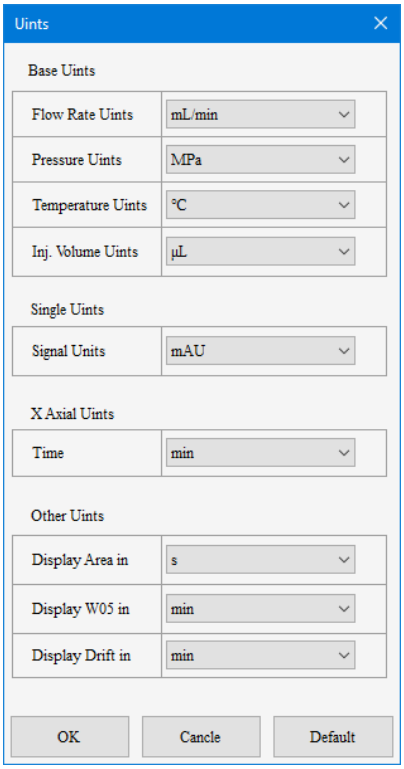


Figure 2-13 Method unit setting window

2.2.6 GLP Options

Click the icon or click the "GLP option" option in the "System" drop-down box, as shown in 2-14.

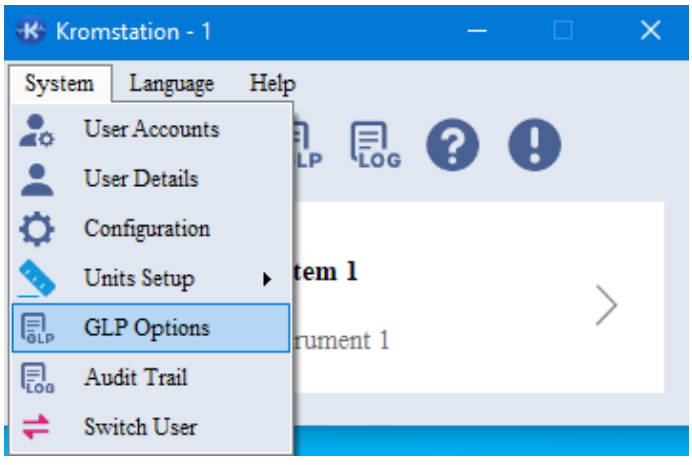


Figure 2-14 Login interface-GLP option

After clicking, the following GLP option window appears, and the user selects the desired option and clicks the "OK" button. As shown in Figure 2-15.

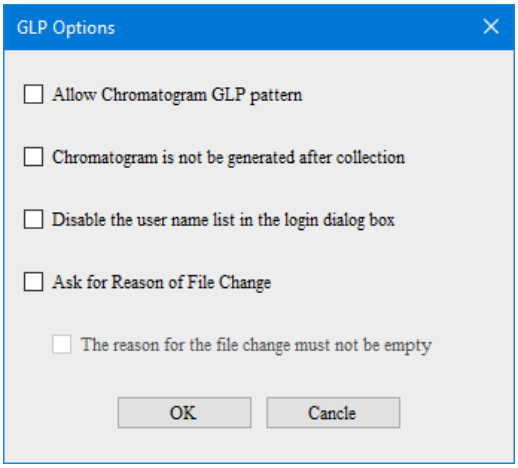


Figure 2-15 GLP option window

2.2.7 Audit Trail

Select "Audit Trail" under "System" on the system login interface or activate the shortcut button icon on the top of the window, as shown in Figure 2-16.

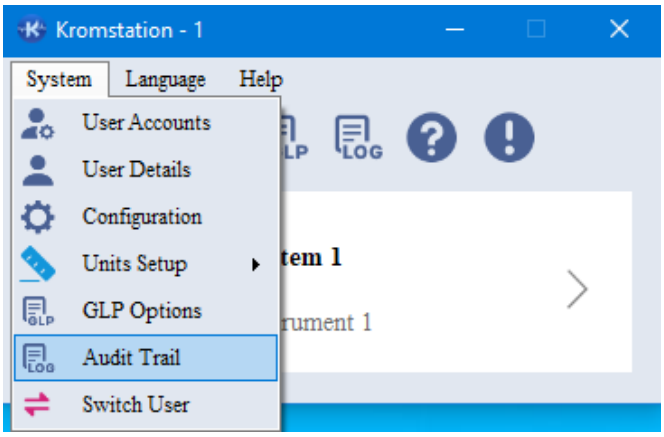


Figure 2-16 Workstation Audit Trail

Click "Audit Trail" to view all the operations on the workstation and export the results, as shown in Figure 2-17.

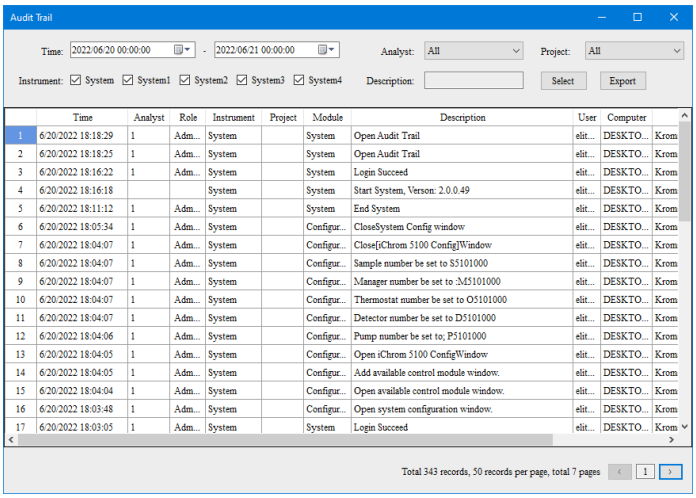


Figure 2-17 Workstation Audit Trail window

2.2.8 Switch Users

Click "Switch User" under "System" on the system login interface to switch user accounts, as shown in Figure 2-18.

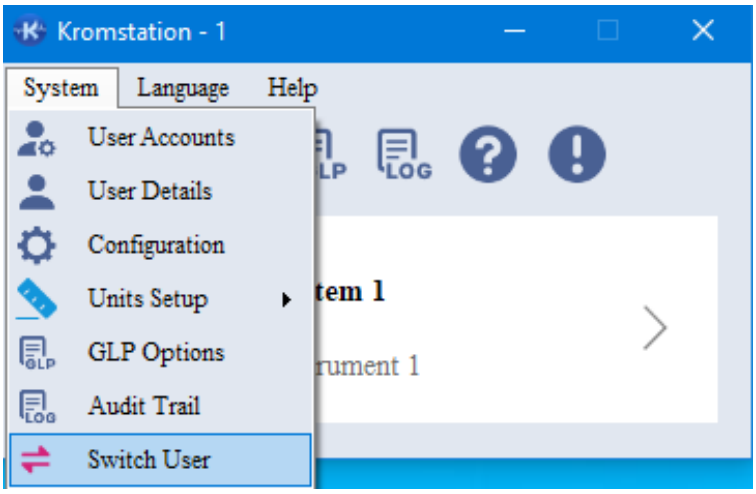


Figure 2-18 Switch users window

After clicking "Switch Users", select whether to switch the user account. Click "Yes" to enter the initial login interface for user selection, as shown in Figure 2-19.

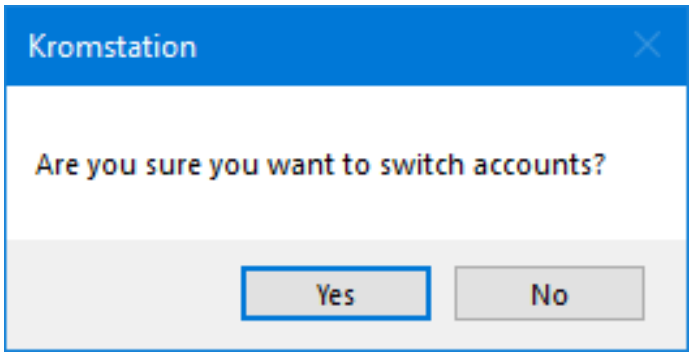


Figure 2-19 Prompt to switch account

2.2.9 System Login

Click "System x" on the system login interface to enter the corresponding system, as shown in Figure 2-20.

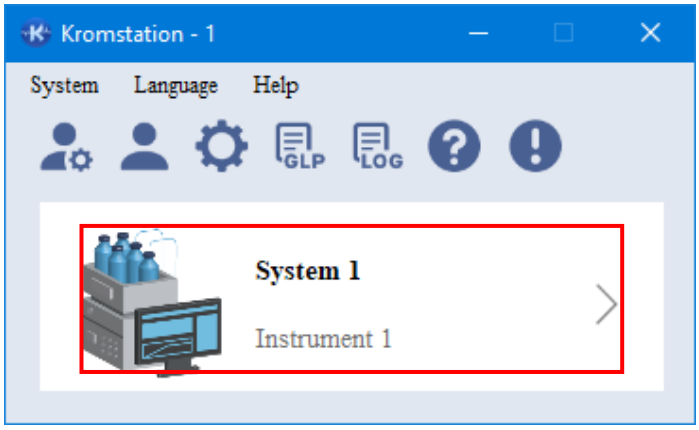


Figure 2-20 Main Window-System Login Window

The first row of the main window is the main menu bar. All settings need to be made under each submenu before logging in.

Shortcut icons of common menus in the main window:

	User account configuration
	User details
	Instrument catalog configuration
	GLP model

	Workstation Audit Trail window
	Help window
	Program information, version number and copyright information display

Before logging in to the system, you need to click the drop-down triangle on the right side of the project dialog to select an existing project. As shown in Figure 2-21.

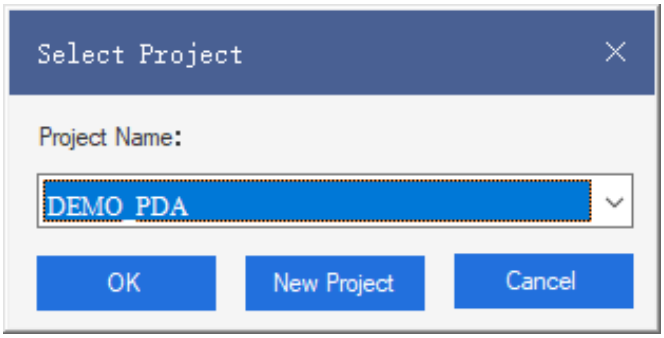


Figure 2-21 Select project

If there is no project in the system, click "Project Management". Enter the project management window, name in the project name column and click "New Project" in the lower left corner to create a new project. At the same time, you can also delete the project in this window. As shown in Figure 2-22.

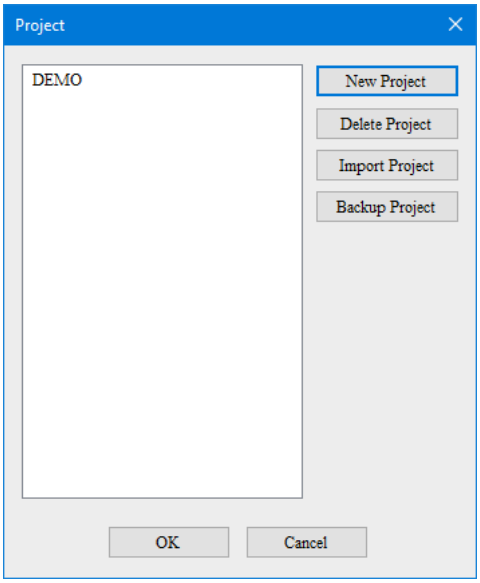


Figure 2-22 New project

After the project is completed, return to Figure 2-20 and click "System x" to directly enter the

instrument control window. The workstation allows to display no more than 4 analysis windows at the same time, and the number of windows displayed can exceed the number of instruments actually purchased, but does not exceed the maximum display volume.

Figure 2-23 shows the instrument control window after logging in to the workstation.

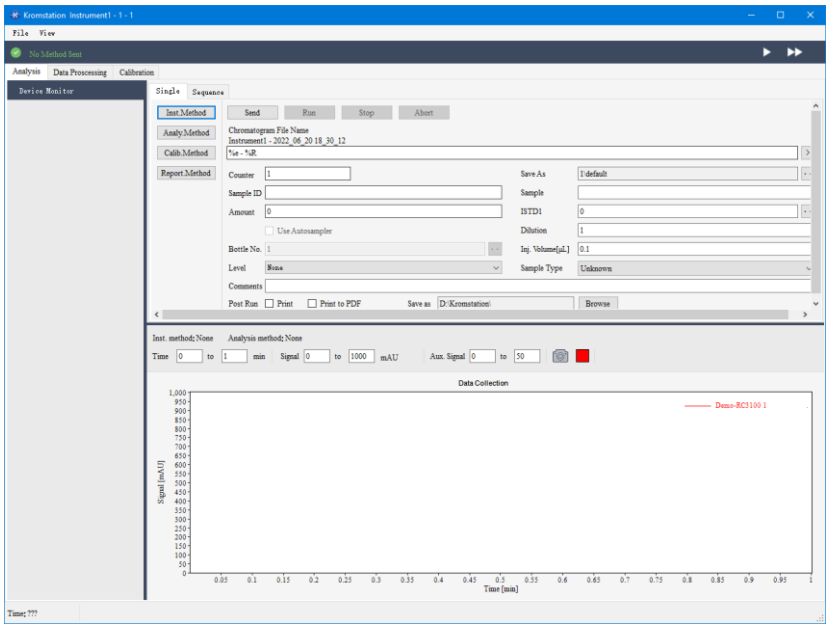


Figure 2-23 Instrument control window

【Caution】 The main window has a higher level than other windows. When the main window is closed, all other windows linked to the workstation will be closed.

3 Method

The method includes all data acquisition and data analysis parameters, as well as the pre-run and post-run parameters required to analyze certain specific samples.

3.1 Composition of the Method

Methods are generally named in Chinese and English, numbers, special symbols, etc. The content related to the method exists in the directory as an independent method file. A complete set of

methods is mainly composed of four parts:

Instrument method,

Analysis method,

Calibration method,

Report method.

3.2 Instrument Method

Develop the parameters of the control instrument or each component. In liquid chromatography, parameters such as mobile phase composition, flow rate, injection volume, detector wavelength and other parameters can be controlled by pumps, samplers, column ovens and detectors.

3.2.1 LC gradient settings

The instrument method generally consists of five parts: LC gradient, detector, thermostat, auxiliary equipment and autosampler. Each part can be edited separately.

Take 3200 binary high pressure as an example, modify the percentage of pump A solvent in the mobile phase in the gradient table, and the percentage of pump B solvent in the mobile phase can be automatically generated. The flow rate in the gradient table is the total flow rate of the two pumps A and B, As shown in Figure 3-1. The graph at the bottom left of the gradient table shows the flow rate and the time-varying curve of A and B two-phase components.

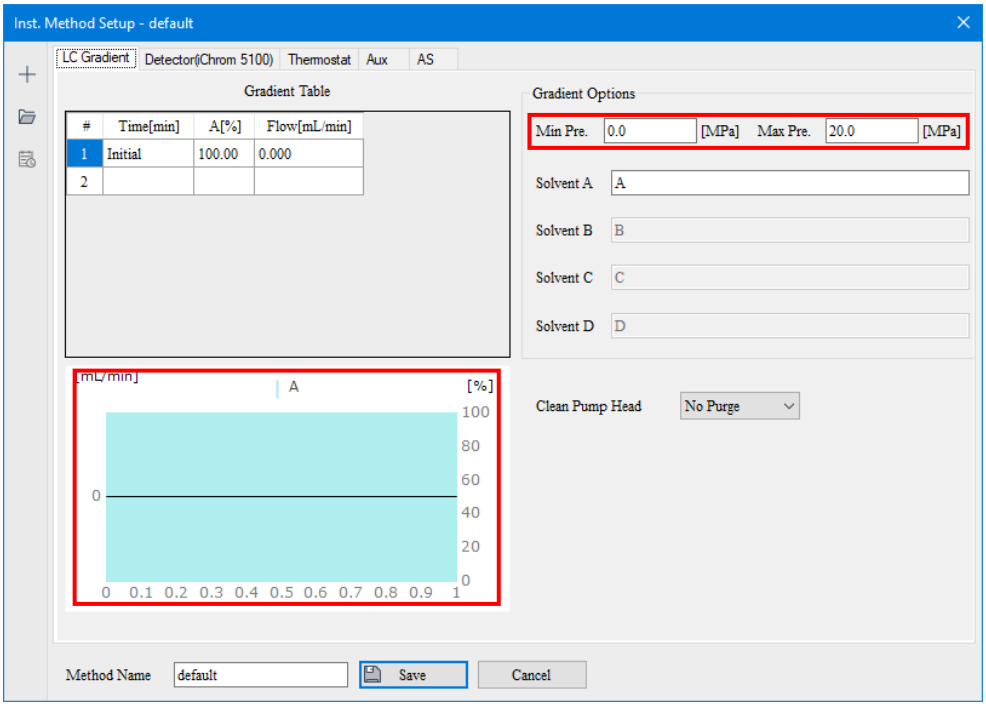


Figure 3-1 Gradient table settings

- The minimum input value of time is 0.1min, the minimum value of percentage is 0.01%, and the minimum value of flow rate is 0.001mL/min.
- In the "Gradient Options" dialog box in the gradient table, you can set the maximum and minimum pressure.

System pressure setting: Click the "Gradient Options" dialog box in the above gradient table to set the maximum and minimum pressure.

3.2.2 Detector Setting

Click "Detector" in "Instrument Method" to enter the dialog box of detector setting, as shown in Figure 3-2. In the "Control Parameters" tab, you can set the acquisition wavelength, acquisition frequency, response time, light source status, etc.; click "Read detector parameters" to monitor the number of light source turns on, running time, etc.

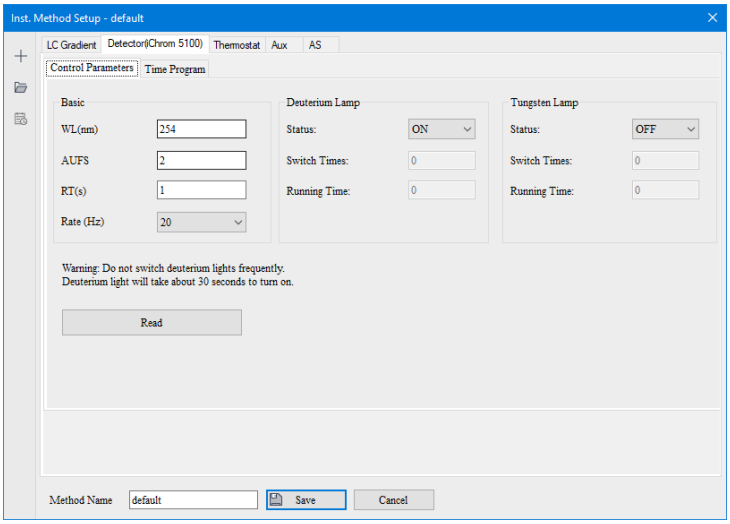


Figure 3-2 Detector acquisition condition setting

The time wavelength program can be set in the "Time Program" tab, as shown in Figure 3-3.

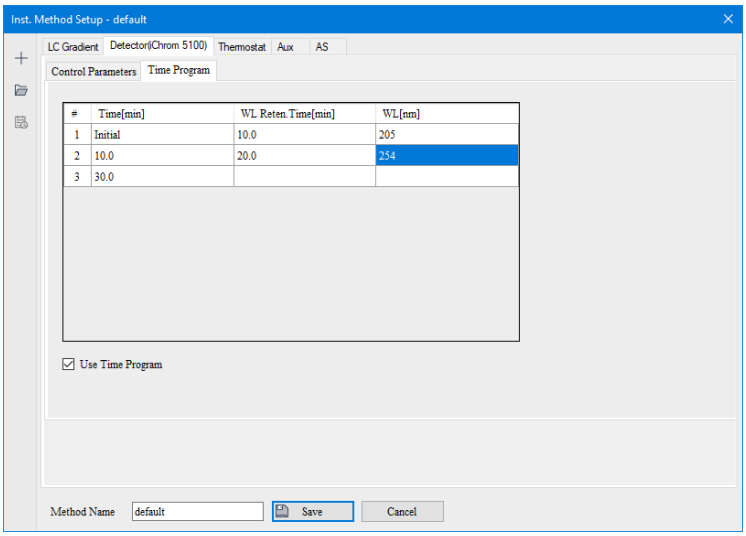


Figure 3-3 Time wavelength program setting

If the method is to be carried out in accordance with the set time program, the box in front of "According to Time Wavelength Program" should be marked with "√".

3.3 Analysis Method

This part is used to describe the information related to the method and to set the automatic integration method.

3.3.1 Measurement

Click "Analysis Method" to enter the analysis method setting dialog box. In the "Measurement" tab, you can describe the chromatographic analysis conditions. This item will appear in the report description. At the top right, "Enable automatic stop" can be checked to set the method Running time. When calculating column efficiency, you need to set the column length, non-retention time and other information, as shown in Figure 3-4.

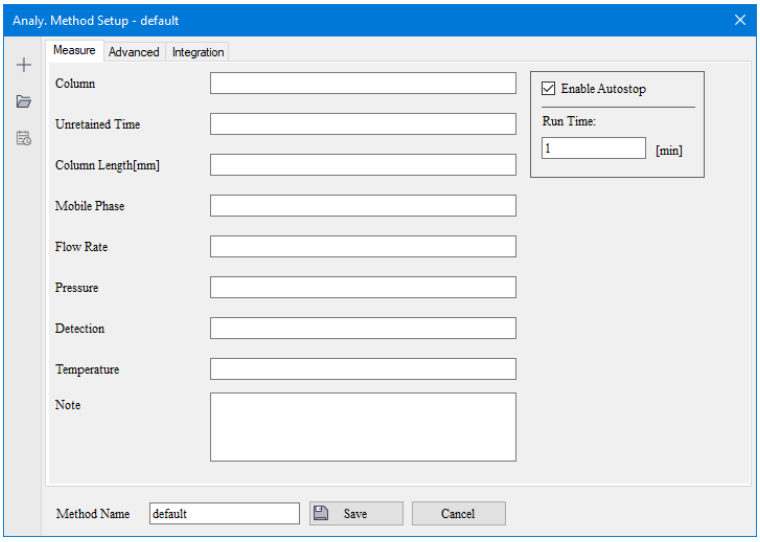


Figure 3-4 Analysis method setting dialog box

3.3.2 Advanced

Click the "Advanced" tab in the analysis method setting dialog box, as shown in Figure 3-5, you can collect the pressure, flow rate, and temperature in the spectrum.

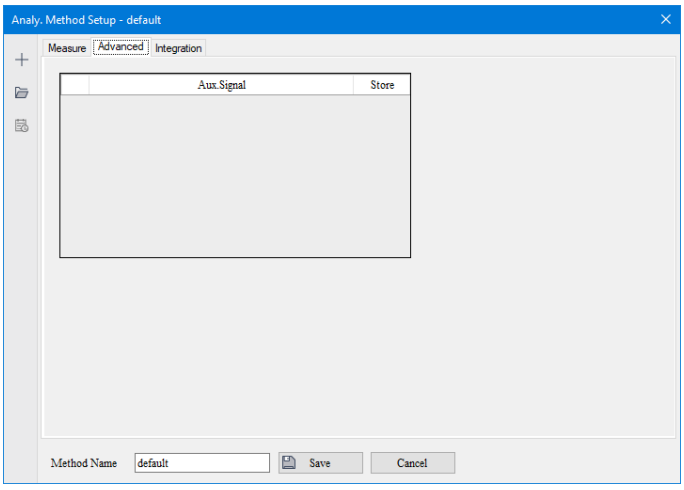


Figure 3-5 Advanced condition setting dialog

3.3.3 Integral Setting

Click the "Integration" tab of the analysis method setting dialog box to set the integration parameters of the generated spectrum, as shown in Figure 3-6.

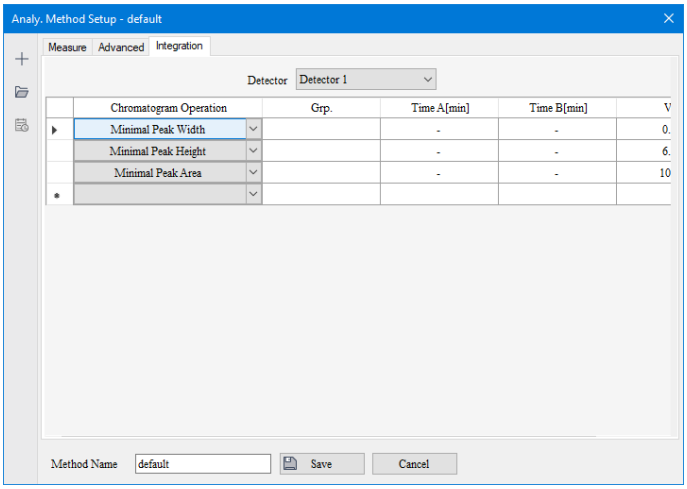


Figure 3-6 Integration event setting dialog

【Caution】

- **Integration events can only be selected from the drop-down options of "Chromatogram Operation".**
- **"Time A" is the start time of the event, and "Time B" is the end time of the event. If the "Time B" input is 0, the default is the end time of the spectrum.**
- **The unit of the entered value is the default unit of the software and cannot be modified.**

3.4 Calibration Method

Quantitative calculations are mostly carried out through calibration files, and the calibration curve in the calibration file is the basis for quantitative calculations. The calibration curve is divided into

two types: one is the calibration curve of the external standard method, and the other is the calibration curve of the internal standard method.

3.5 Reporting Method

Set the report format and report content in the report setting window that pops up.

3.6 Method Status

A method has two states:

Storage method: This is a method stored on a computer disk.

Current method: When the saved method is recalled from the disk, it is called the current method. There is always a current method in memory.

3.7 Method Establishment

Modify the current method and save it under a new name. Please note that when the current method is modified, it needs to be saved before the content in the disk will be changed; or directly create a new method and save it to be called.

3.8 Sending and Running of Methods

When the method is successfully sent, the system starts to run the setting command. The method can be sent in the following ways:

1) Single Run

After editing the "Instrument Method" and "Analysis Method", edit the sample information in the "Single Analysis" interface, click the "Send Method" button in the single analysis, and then click "Run" to test the sample after balancing.

2) Sequence Run

After the sequence setting is completed, select the appropriate instrument method and analysis method, and click  to run the current sequence.

【Caution】

- **During the operation of the instrument, except for the acquisition time, the other method parameters cannot be modified.**

3.9 Method Stop

There are the following ways to stop running:

- Click "Close Flow Rate" in "Device Monitor" to stop the method running.
- Click "Cancel" in "Single Analysis" or click the icon  in "Sequence Analysis" to stop the method.

【Caution】

- **Click  , the data acquisition and running method will stop, and the spectrum will not be saved.**
- **Click  , Stop data Acquisition, the method is still running, and the spectrum is saved.**

4 Single and Sequence Analysis

4.1 Single Analysis

"Single analysis" refers to the process of completing a single sample analysis.

4.1.1 Single Analysis Settings

The operation steps are as follows:

1) Click "Single" to display the single analysis dialog box, as shown in Figure 4-1. Fill in relevant information such as sample ID, sample name, total amount, dilution, internal standard amount, injection volume, etc. in the single analysis dialog box.

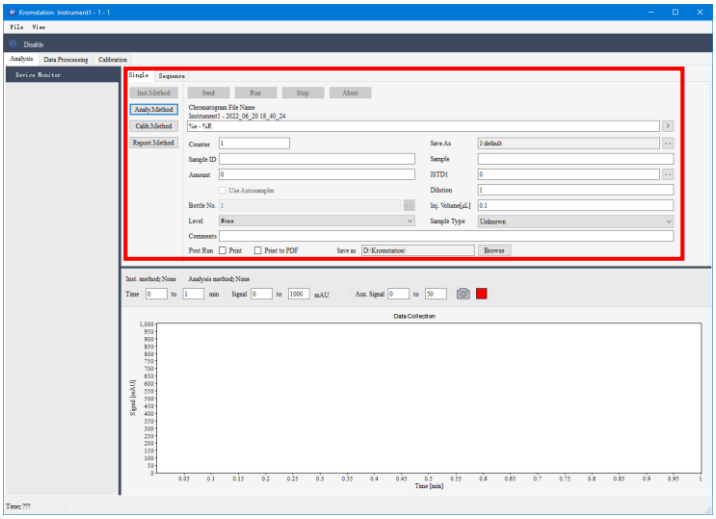


Figure 4-1 Single Analysis dialog box

"Analysis column" settings: The content filled in this column will appear in the title column of the spectrum after the analysis is completed.

- "Sample ID" is the sample identification number, which is used to distinguish samples, and description information of no more than 64 characters can be set.
- "Sample Name" can set sample description information with no more than 64 characters.

- "Total" is the total amount of the sample, used in the calibration calculation, the unit used should be consistent with the unit in the calibration file, and the default value is 0. In the internal standard method, the "Total" input value is the sample size when no internal standard is added.
- "Internal standard amount" is the amount of internal standard substance added in the internal standard method. The unit should be the same as the unit in the calibration file. The default value is 0.
- "Dilution" is the dilution factor of the sample. The result calculated with the calibration file will be automatically multiplied by the corresponding dilution factor to get the final calculation result, which will appear in the report form, and the default value is 1.
- "Injection Volume" is the sample injection volume, the default value is 0. When calculating with the calibration file, the software will automatically correct the proportional coefficient between the injection volume of the sample and the injection volume in the calibration file.
- "Calibration Standard" is the level of the calibration standard. The spectrum obtained after activation will be automatically saved in the corresponding subdirectory of the calibration file and cannot be changed during the method running.
- "Instrument Method" and "Analytical Method" are run according to the current method.

4.1.2 Sigle Analysis Run

Click "Send Method" and wait for the baseline balance of the various instrument components to stabilize, and then click "Send" to perform a single sample analysis.

Note: "Whether to use autosampler" can be selected in "Analysis Bar".

4.2 Sequence Analysis

"Sequence" is a series of instructions for automatic sample analysis.

Using the sequence, each sample can be automatically injected, and the data can be collected and analyzed according to the specific method used for the sample. Each sample in the sequence can be

analyzed by different analysis methods, and chromatographic conditions and parameters can be used Different settings.

Note: "Sequence Analysis" can only be performed when the system is connected with autosampler equipment.

4.2.1 Sequence Analysis Settings

Sequences can be created and saved in the same way as methods,

The setup and operation of sequence analysis can be carried out according to the following steps:

1） Click "Sequence" analysis to enter the sequence analysis window, as shown in Figure 4-2.

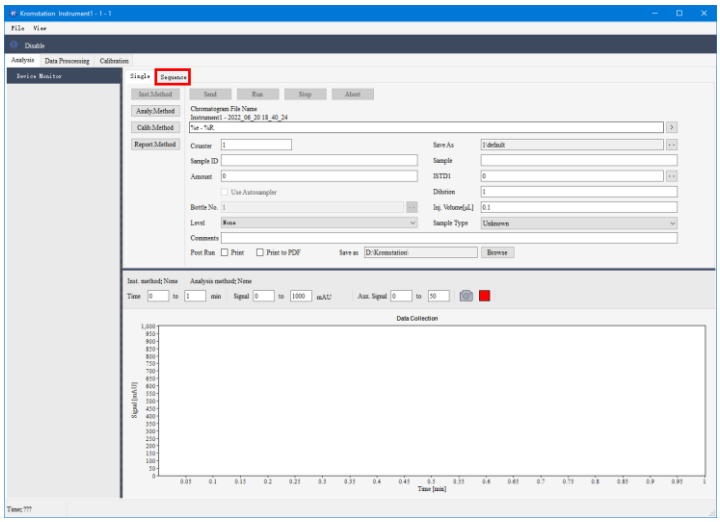


Figure 4-2 Open Sequence Analysis

1） Fill in the starting bottle number, ending bottle number, number of injections, injection volume, sample ID, sample name, sample type, spectrum name, instrument method, analysis method, calibration method, report method etc. in the sequence analysis dialog box. as shown in Figure 4-3.

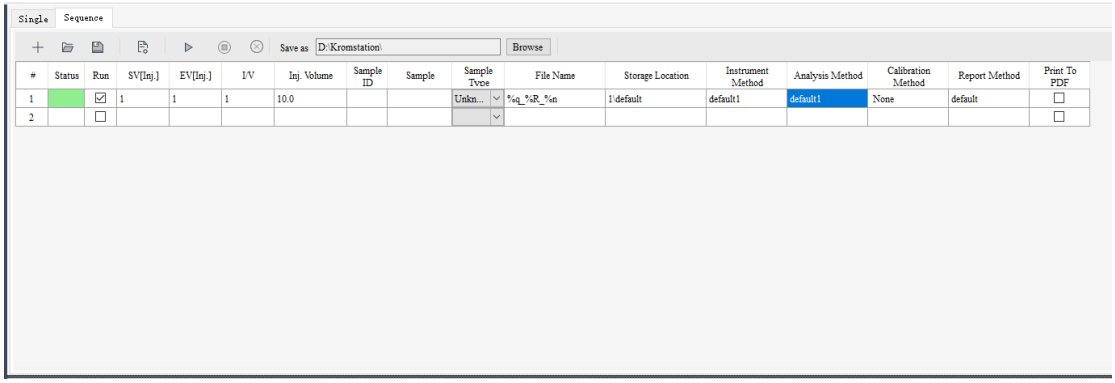


Figure 4-3 Sequence Analysis

4.2.2 Run Sequence Analysis

1）After editing the sequence, if there is a problem with the sequence, the "Status" of the problem line will display , and if the sequence has no problems, the "Status" of the problem line will display .

2）Click , the selected sequence starts to run.

3) After the sequence analysis is completed, the corresponding sequence status changes, as shown in the figure.

4) If you want to run the sequence again, just select the completed sequence and click "Reset", as shown in Figure 4-4.

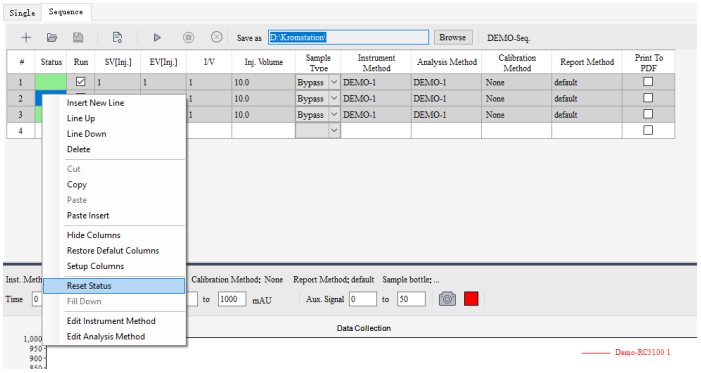


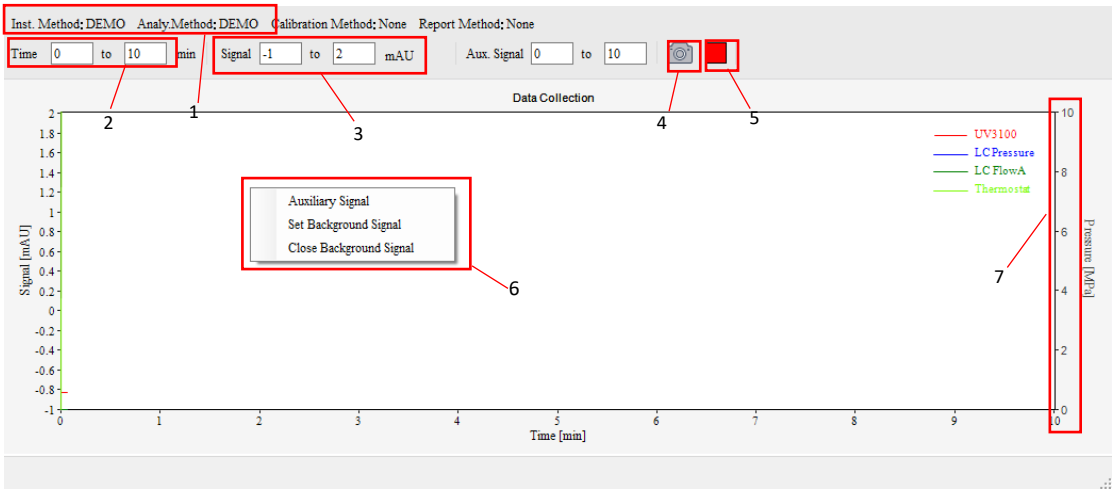
Figure 4-4 Sequence

5 Data Acquisition

Data Acquisition is to record and save the response signal of the detector. In the process of collecting data, the detector converts all response signals from analog signals to digital signals, and then transmits them to the workstation and stores them in the signal data file. This chapter introduces the data Acquisition function and related operations in detail.

5.1 Data Acquisition interface

After logging in to the workstation, you can directly enter the data Acquisition interface. As shown in Figure 5-1.



5-1 Data Acquisition interface

Serial number	Description
1	Shows the name of the instrument method and analysis method in this run
2	The time axis of the data acquisition interface can be set
3	The signal axis of the data acquisition interface can be set
4	During data acquisition, this button becomes the [Snapshot] button. This function displays the chromatogram of the data from the beginning of the analysis to when the button is clicked, allowing you to check the peak area during the analysis.
5	The color of the detector signal can be changed as required
6	In the data acquisition interface, right-click the "auxiliary signal setting" to collect data of pump flow rate, column oven temperature, and pump pressure; right-click the data to switch back and forth between [set background image] and [close background image].
7	The coordinate axis of the auxiliary signal can read the auxiliary signal value in the

	data acquisition process, such as real-time flow rate, pressure, column temperature, etc.

5.1.1 Device Monitor

In the instrument monitoring column, you can view the operating status of the instrument. In addition, you can also directly enter the value in the monitored value cell to adjust its parameter value.

[Device Monitor] in the analysis column, as shown in Figure 5-2.

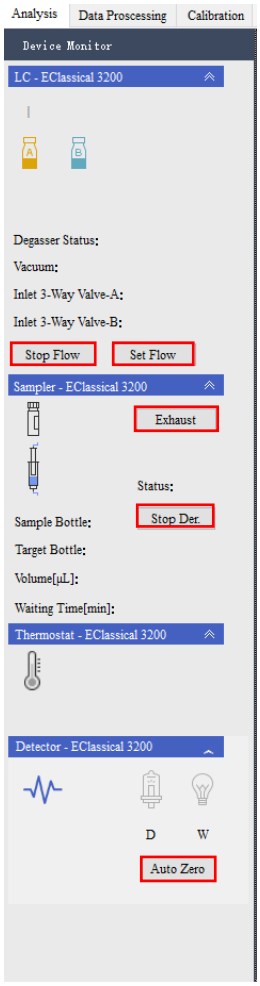


Figure 5-2 Device monitor

Pump: You can observe the change of the pump flow rate at any time and set the parameters (you can turn off all running pumps) and set the flow rate (the flow rate of any pump can be set to flush the pipeline within 0~10mL/min);

Autosampler: The operation of venting the syringe and the derivatization of the column can be

performed.

Organizer: Show the vacuum degree;

Thermostat: Display set temperature and real-time temperature change;

Detector: Displays the energy change of deuterium lamp and tungsten lamp and can perform the operation of returning to zero from the baseline.

Caution: This "baseline return to zero" function is aimed at returning the baseline of the analog signal detector to zero. If this function is applied to the digital signal detector, although the baseline has been forced back to zero from the graph, the instrument itself may not return to zero at this time. To the zero point, so this function is disabled for the digital signal detector.

5.2 The beginning of Data Acquisition

In a single analysis, click [Send Method] and after the baseline runs smoothly, click [Run]. Data acquisition can be performed. As shown in Figure 5-3.

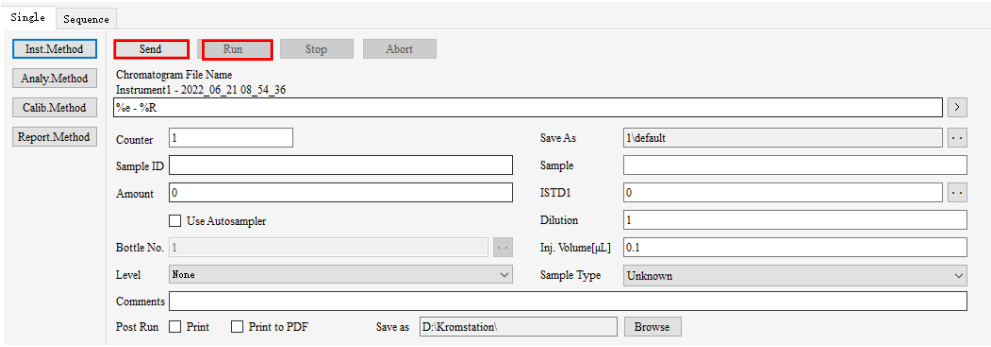


Figure 5-3 The start of data acquisition in a single analysis

In sequence analysis, first click the send method, and after the baseline runs smoothly, click 2 or 3 to run the sequence. As shown in Figure 5-4.

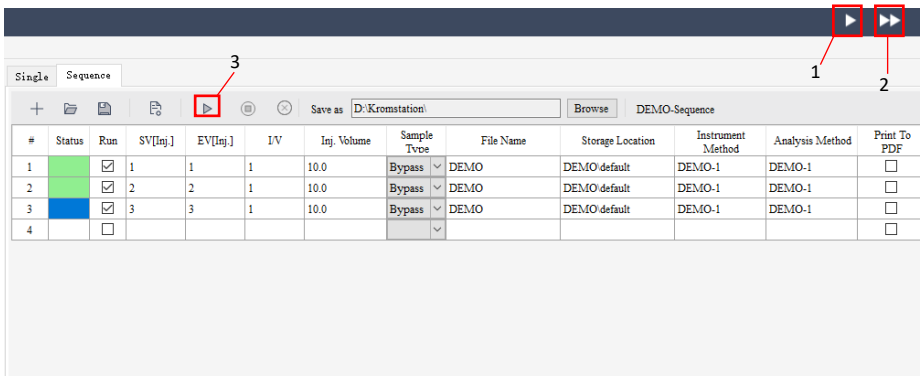


Figure 5-4 Start of data acquisition during sequence running

5.3 End of Data Acquisition

The data acquisition can be stopped by the following methods:

- When the [Run Time] of the detector set in [Analysis Method] is reached, data acquisition is automatically terminated.
- In a single analysis, click [Stop], and the data will be collected and saved at the same time.

  Click [Cancel] to stop data acquisition and the data will not be saved this time.
- In sequence analysis, click  to stop data acquisition and save the data.

  Click  to stop data acquisition and the data will not be saved this time.

5.4 Set the Background Chromatogram

Operation: "Data Acquisition " blank part → right click → set background chromatogram → chromatogram chart list → select background chromatogram.

Application: Peak identification during data acquisition.

Operation when closing background chromatogram: blank part of "Data Acquisition" → right click → close background chromatogram

5.5 Changing the Analysis End Time during Analysis

Once data acquisition is started, the parameters set in [Instrument Method] and [Analysis Method] become read-only, and only [Run Time] can be changed. Click [Run Time] in [Analysis Method], enter the changed time directly into the dialog box, and click the [OK] button. or when the sequence is running, right-click anywhere in the sequence and select [Edit Analysis Method] to enter [Analysis Method] to change [Run Time]. As shown in Figure 5-5 and Figure 5-6.

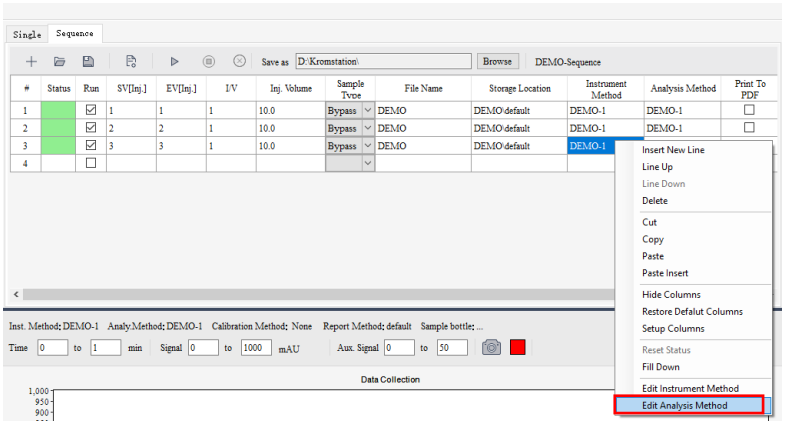


Figure 5-5 Edit analysis method during sequence running

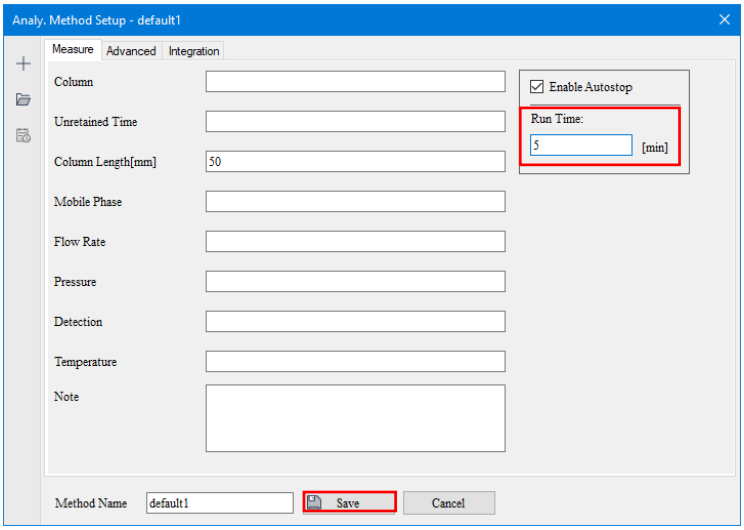


Figure 5-6 Run time changes

5.6 Saving after Data Acquisition

Project selection: When logging in to the system, you can select the project whose data needs to be saved this time or create a new project, and then click this project to log in.

Storage location selection: There is only one “default” directory file in the new project, and you can create other directory files as needed, as shown in Figure 5-7. In Single Analysis or Sequence Analysis, click the storage location and select the corresponding storage directory and click OK. If you do not select the storage location and click OK, the data is stored in the “default” directory file in the default path. As shown in Figure 5-8.

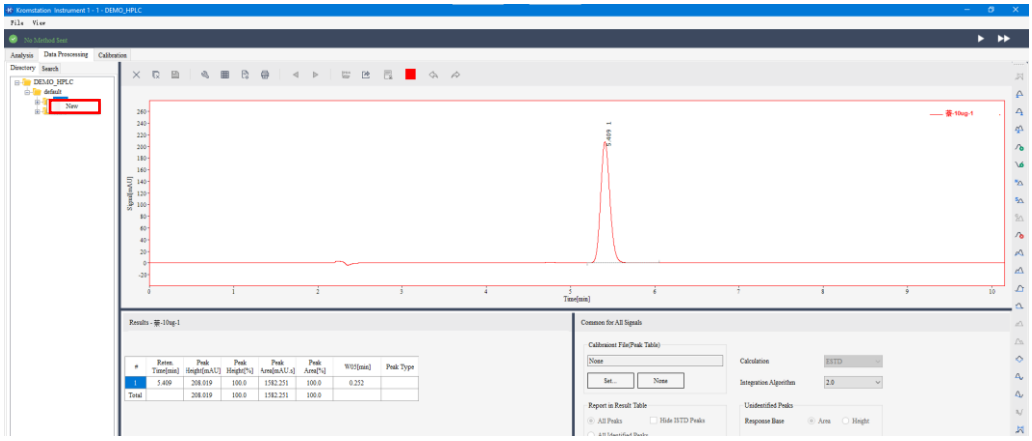


Figure 5-7 Add directory

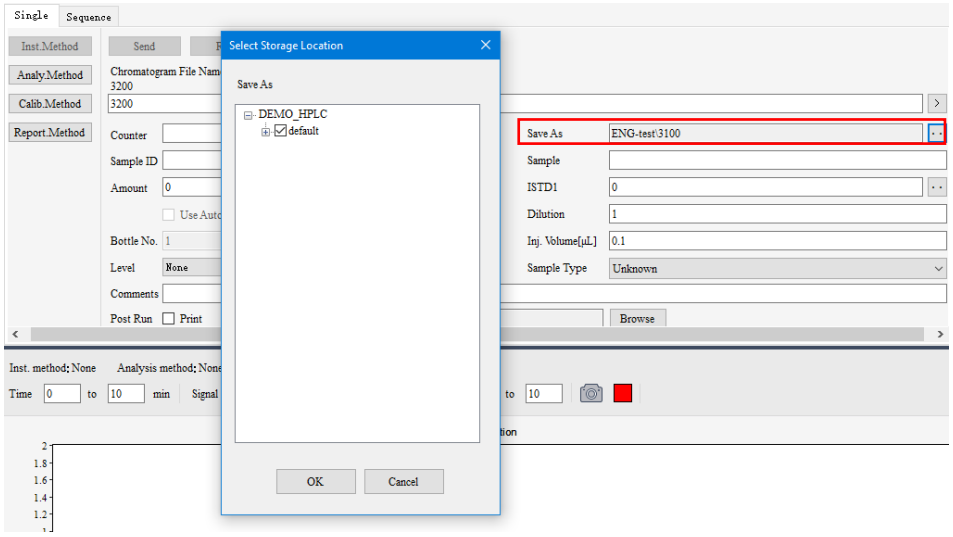


Figure 5-8 Choose storage location

6 Data Analysis

This chapter mainly introduces the functions of the data analysis window, mainly including operations such as display, integration and correction. After the data acquisition is over, the workstation will save the collected data in the corresponding project in the form of a spectrum. The following graphics processing operations can be performed:

- The chromatogram can be integrated and adjusted;
- Overlapping call of different chromatograms is conducive to intuitive comparison;
- Deduction of chromatogram background;
- Calculation of baseline noise and drift;
- Zoom function of chromatogram;
- Add and delete notes for chromatographic peaks.

6.1 Open and View the Chromatogram Window

Click "Data Analysis" to enter the data analysis interface, and then click the "Directory" where the data is located to find the name of the chromatogram you want to open, double-click the name of the spectrum or right-click and "Open". As shown in Figure 6-1.

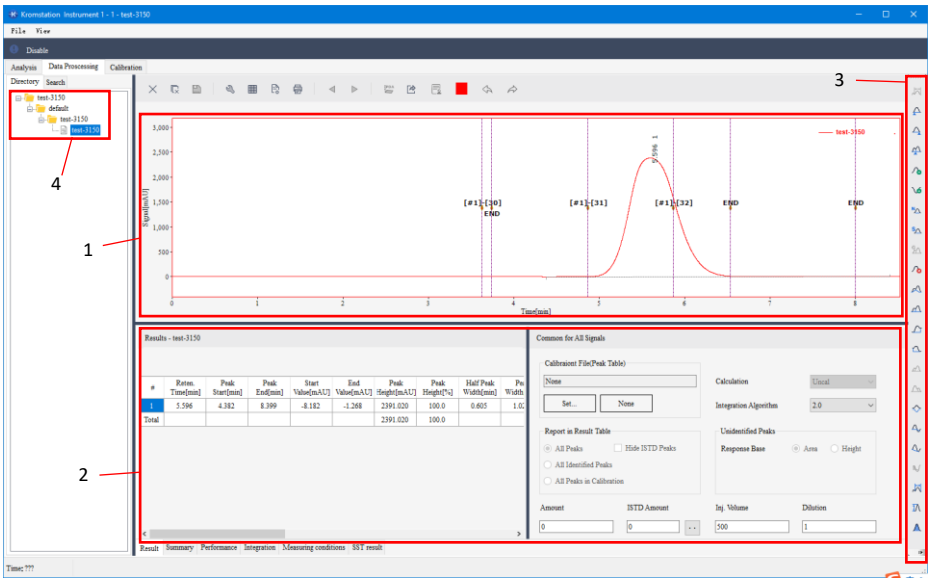


Figure 6-1 chromatogram window

Serial Number	Describe
1	Chromatogram display area, you can select the area to zoom in.
2	Display column, method, and integration information
3	Chromatogram integration tool
4	Data storage directory

Or open it after performing a fuzzy search based on the time or file name of the chromatogram, as shown in Figure 6-2.

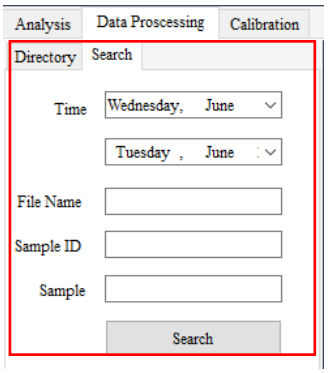


Figure 6-2 Spectrum search

6.2 Setting of Integration Parameters

Integration is to identify the peak in the signal and calculate its size. Integration is essential for the following steps: quantitative calculation; peak purity calculation.

This section introduces the following integration operations.

- Automatic integration
- Set manual integration (processing)

6.2.1 Set up Manual Integration

Use the manual integration (processing) function to move the start and end points of specific peaks, or remove unwanted peaks and other operations.

Open the chromatogram to be processed in the "Data Processing" window, and use the commands on the right side of the menu to manually change the chromatogram parameters. Use the submenu commands under the main menu in the chromatogram window to adjust the spectrum.

Manually move the start and end of the peak. Click  in the integral column to change the starting point of the integral. Click  to change the end point of the integration, as shown in Figures 6-5 and 6-6.

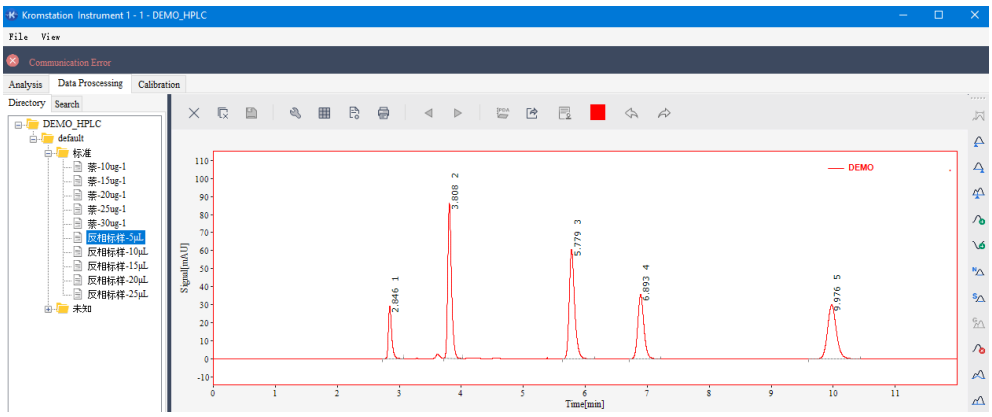


Figure 6-5 Before manual integration changes

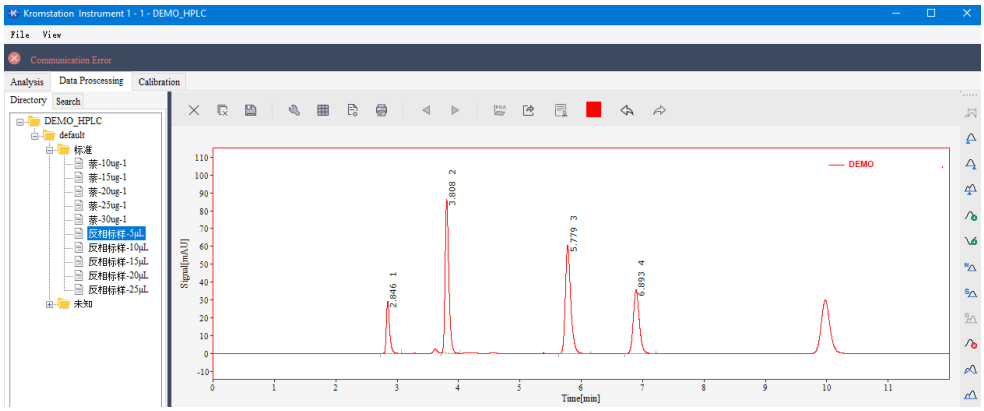


Figure 6-6 After manual integration changes

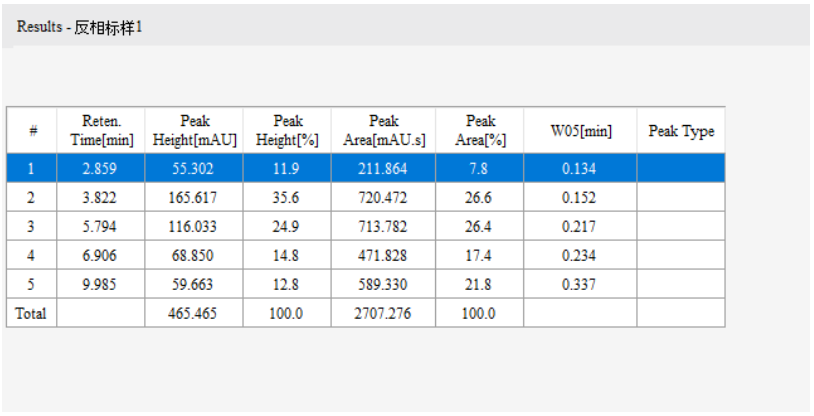
Manual integration menu commands are shown in Table 1:

	Overall Peak Width
	Set the integration starting point of a single peak, and the adjusted parameters can be displayed in the integration table of the chromatogram
	Set the integration point of a single peak, the adjusted parameters can be displayed in the integration table of the chromatogram
	Demarcation point
	Add a positive peak
	Add a negative peak
	Mandatory naming of selected peaks
	Label solvent peak
	Assign all peaks in the selected interval to the set group
	Delete peaks and do not integrate the peaks in the selected area
	Peak-valley separation, separate a single peak by setting the start and end points of the peak-valley separation area
	Vertical separation, draw a vertical line to all peaks in the selected area

	to separate a single peak
	Horizontally forward, the baseline will be forced forward in the selected area and separate chromatographic peaks vertically
	Horizontally backward, the baseline in the selected area will be forced backward and vertical separation of chromatographic peaks
	Front tangent
	Back tangent
	Change the negative peaks in the specified area to positive peaks

6.2.2 View the Integration Results

The integrated result will be displayed in the "Result" tab below the chromatogram, as shown in Figure 6-7.


Results - 反相标样1

#	Reten. Time[min]	Peak Height[mAU]	Peak Height[%]	Peak Area[mAU.s]	Peak Area[%]	W05[min]	Peak Type
1	2.859	55.302	11.9	211.864	7.8	0.134	
2	3.822	165.617	35.6	720.472	26.6	0.152	
3	5.794	116.033	24.9	713.782	26.4	0.217	
4	6.906	68.850	14.8	471.828	17.4	0.234	
5	9.985	59.663	12.8	589.330	21.8	0.337	
Total		465.465	100.0	2707.276	100.0		

Figure 6-7 View the integration results

Right-click anywhere in the "Result Table" to set the queue, as shown in Figure 6-8.

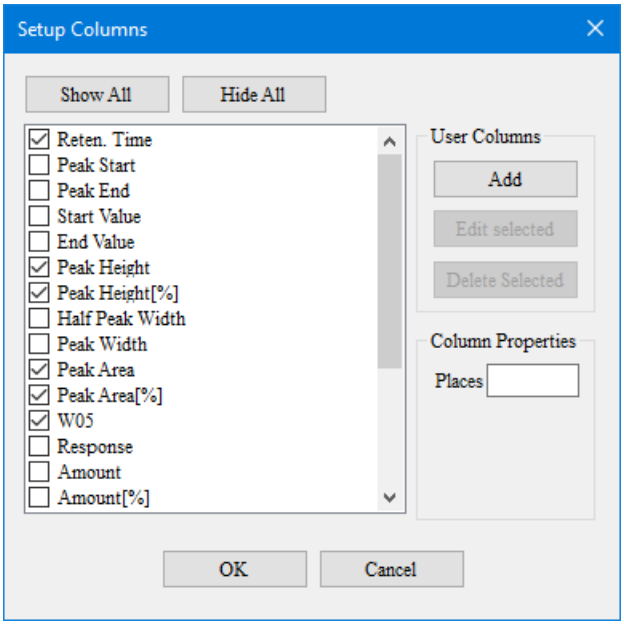


Figure 6-8 Set up queue

6.3 Multi-Chromatogram Comparison

Find the spectrum you want to compare, hold down the "Ctrl" key, and select the spectrum you want to overlay, then right-click, and in the pop-up dialog box, select "Stack" to get the multi-spectrum overlay comparison, as shown in the figure 6-9.

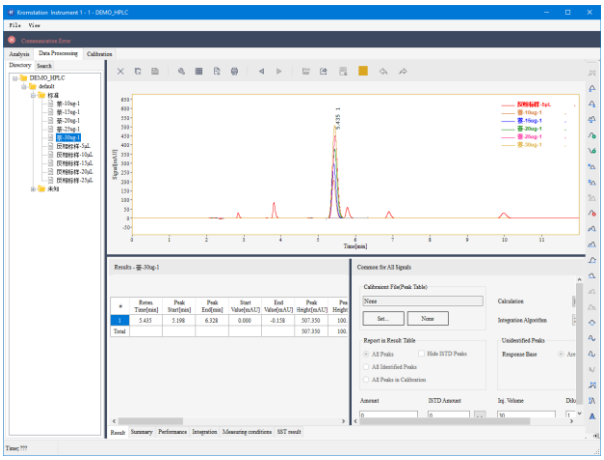


Figure 6-9 Multi-chromatogram comparison

Multi-chromatogram comparison can compare retention time, peak shape and peak area more intuitively.

6.4 Chromatogram Background Subtraction

The mobile phase under gradient conditions may cause the baseline to be unstable, affect the integration results and even lead to inaccurate quantification. In order to eliminate the baseline problem, the chromatogram of the empty running gradient can be used as the background chromatogram, and the normal sample chromatogram can be calculated after subtracting the background chromatogram.

Specific operation: data processing → measurement conditions → settings → select background chromatogram in the chromatogram list → confirm. As shown in Figure 6-10.

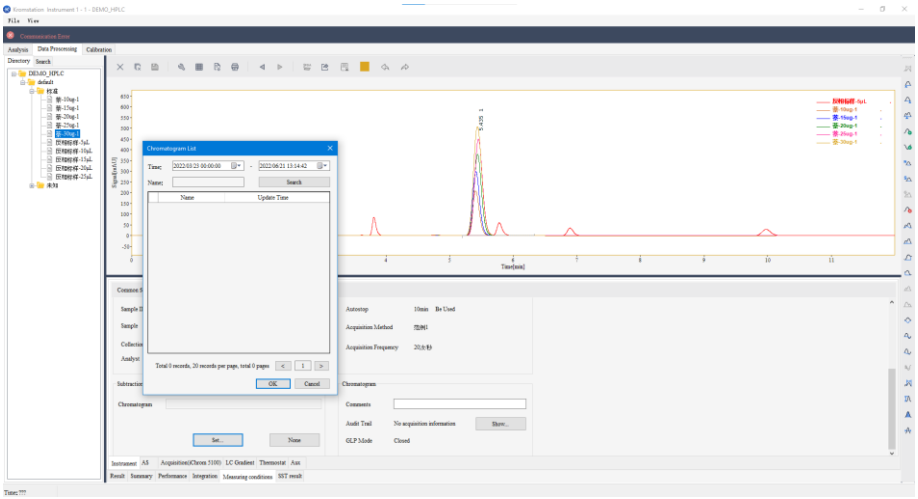


Figure 6-10 Subtract background chromatogram

6.5 Calculation of Noise and Drift

In the data processing interface, after opening a chromatogram, right-click → click "Noise\Drift" → select a calculation method → select calculation area. After the calculation, the noise/drift result will automatically appear in the result table at the bottom of the window. As shown in Figure 6-11.

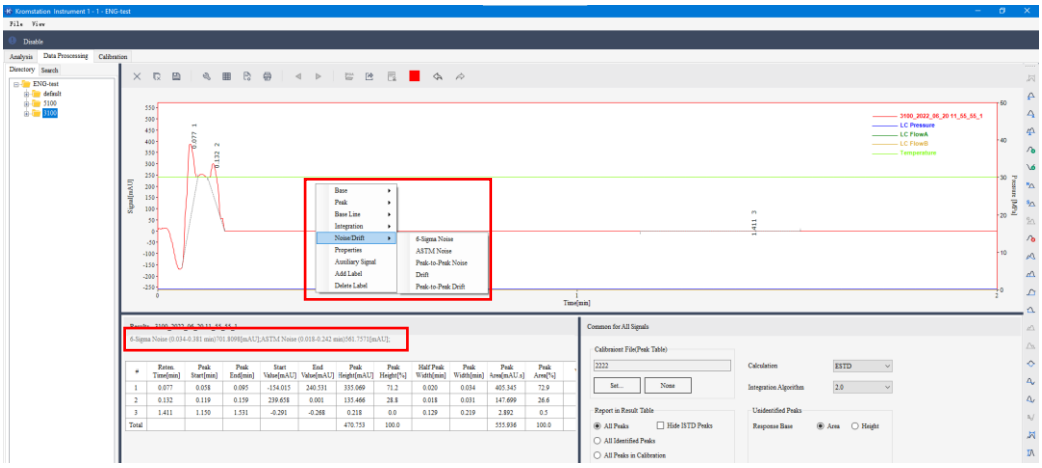


Figure 6-11 Calculation of Noise and Drift

6.6 Peak Identification

Peak identification is a process used to determine which type of compound the detected peak belongs to. When analyzing the data, compare the peak values of the collected chromatograms with the compound table one by one. If the peak retention time is within the recognition range, the peak is recognized as the target compound.

Open a spectrum that needs to be identified → select the corresponding calibration curve in the calibration file section → select "identify all peaks". The result table shows the peak identification of the spectrum. As shown in Figures 6-12 and 6-13.

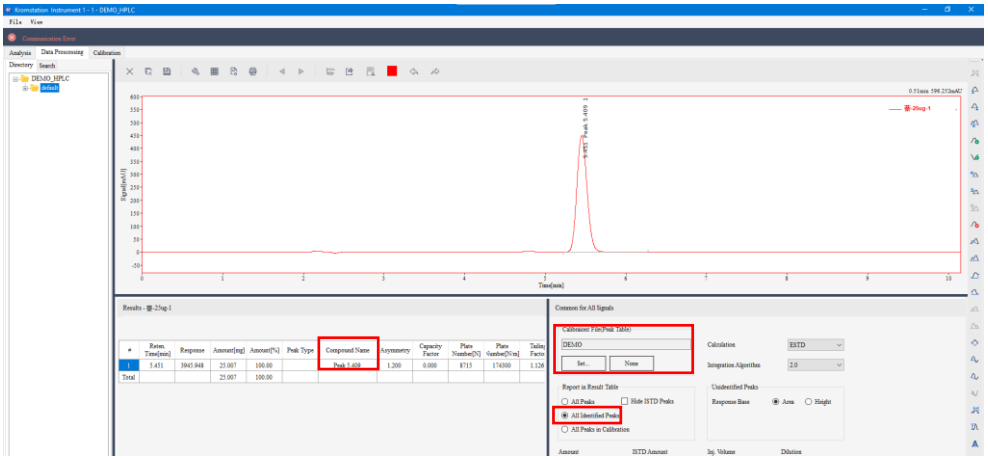


Figure 6-12 Peak recognition - 1

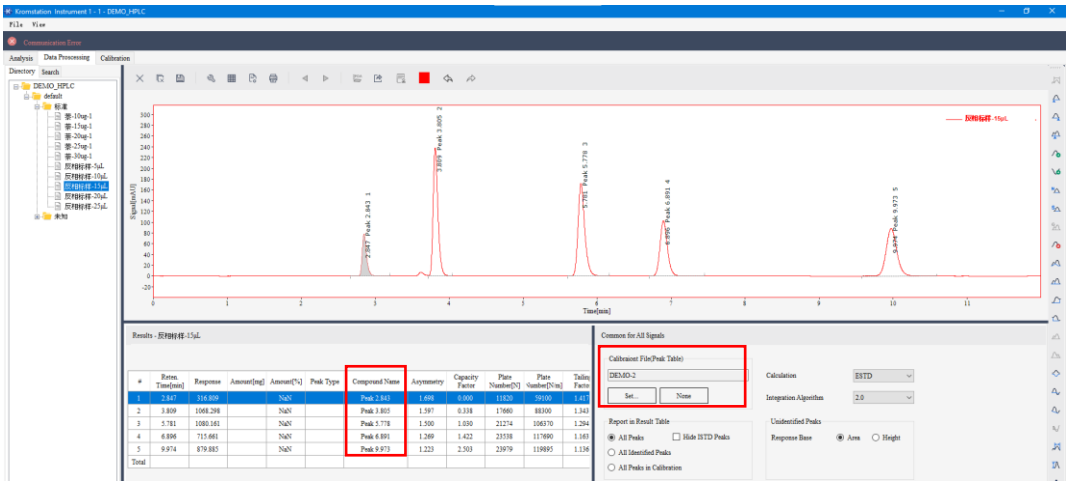


Figure 6-13 Peak recognition -2

6.7 Summary Table View

Under the "Summary" tab of the chromatogram, view the sample information and calculation results. This table only displays the calculation results of the calibration peak, as shown in Figure 6-14.

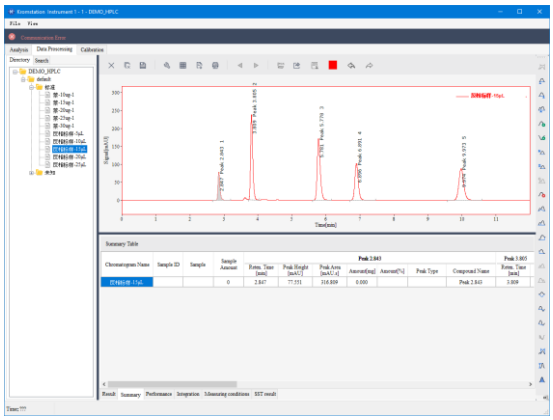


Figure 6-14 Summary Table

The summary table can be saved and printed by file output, or it can be automatically printed in the report by setting the report format.

6.8 Characterization of Chromatographic Column Performance

Add non-retention time and column length in "Analytical Method" and select the corresponding

"Standard Method", then the performance parameters of the chromatographic column will be displayed in the "Performance Table" below the chromatogram, as shown in Figure 6-15.

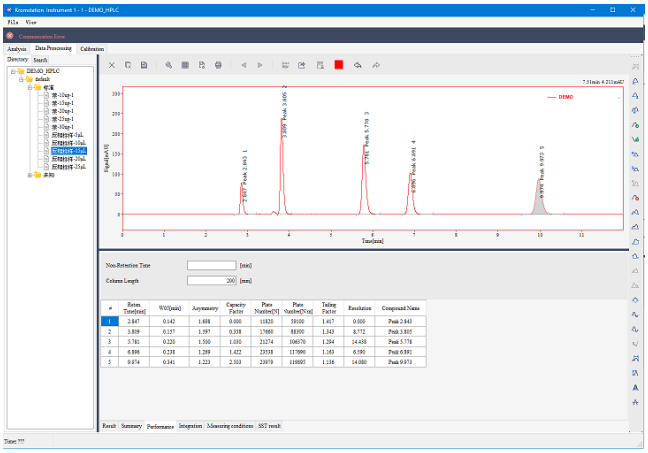


Figure 6-15 Column performance table

6.9 Measurement Condition Display

Under the "Measurement Conditions" tab under the chromatogram, you can see all the measurement method information, as shown in Figure 6-16.

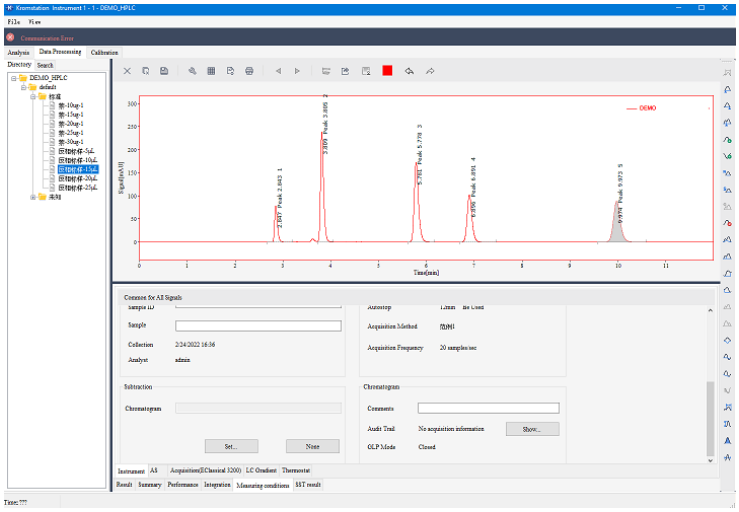


Figure 6-16 Measurement condition tab

6.10 SST Result (Optional)

SST (System Suitability Test) is usually used to monitor the performance of the method. The use of

this module needs to be purchased separately. The following briefly introduces the use of this module.

1) When using SST results, it is necessary to first establish a calibration curve for the sample to be tested (refer to the calibration curve production section for the method of establishing the calibration curve) and save the calibration curve.

2) Open the chromatogram to be analyzed in the data processing interface, and click the "Set" button in the calibration file (peak table) column of the result table page to pop up the calibration file dialog box, select the calibration file to be used, and each chromatogram Follow this method to set one by one.

3) Click on the SST page, in the SST drop-down menu, select "New" to create an SST result file, as shown in Figure 6-17.

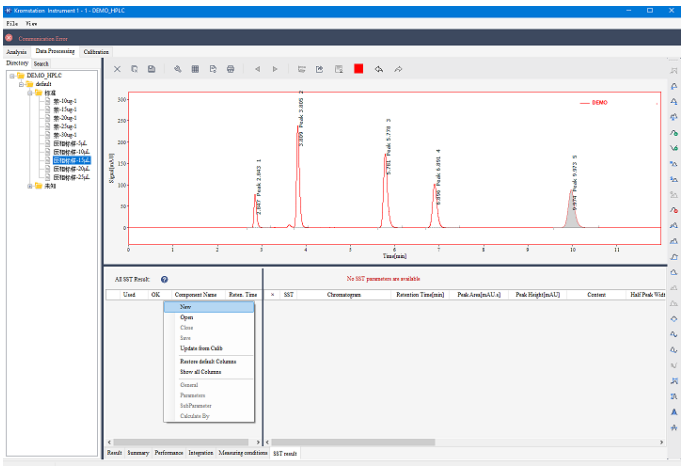


Figure 6-17 Create a new SST result file

4） In the SST drop-down menu, select "Update from Calibration" to load the required calibration file. As shown in Figure 6-18.

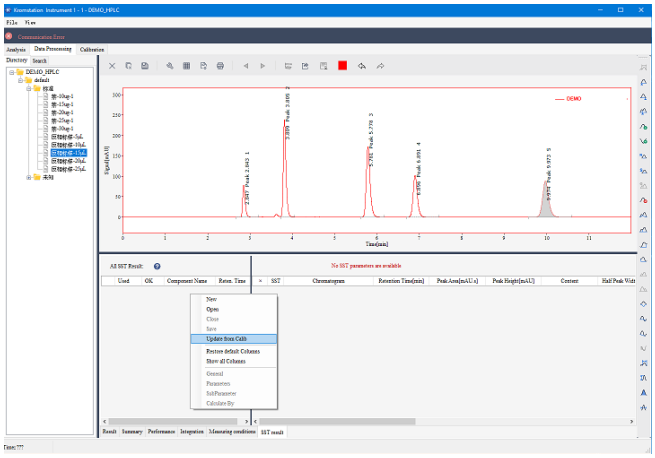


Figure 6-18　Load calibration file

5） In the SST drop-down menu, select "Save" to name the SST result, as shown in Figure 6-19.

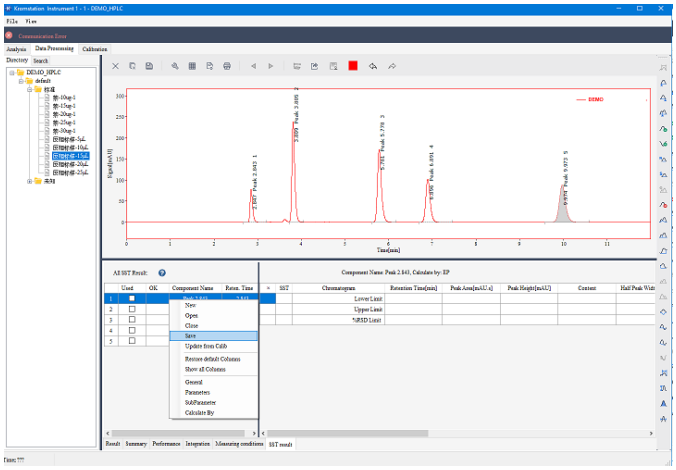


Figure 6-19　Saving SST results

6） In the box under "Used", mark "√", then the loaded calibration file is allowed to be used, as shown in Figure 6-20.

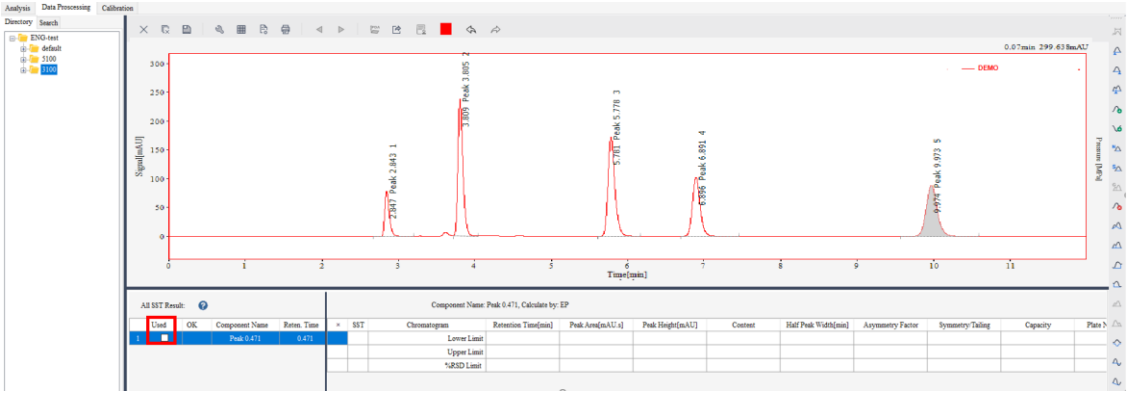


Figure 6-20　Use of calibration file

7）In the SST drop-down menu, select "Parameters" to set the parameters, as shown in Figure 6-21.

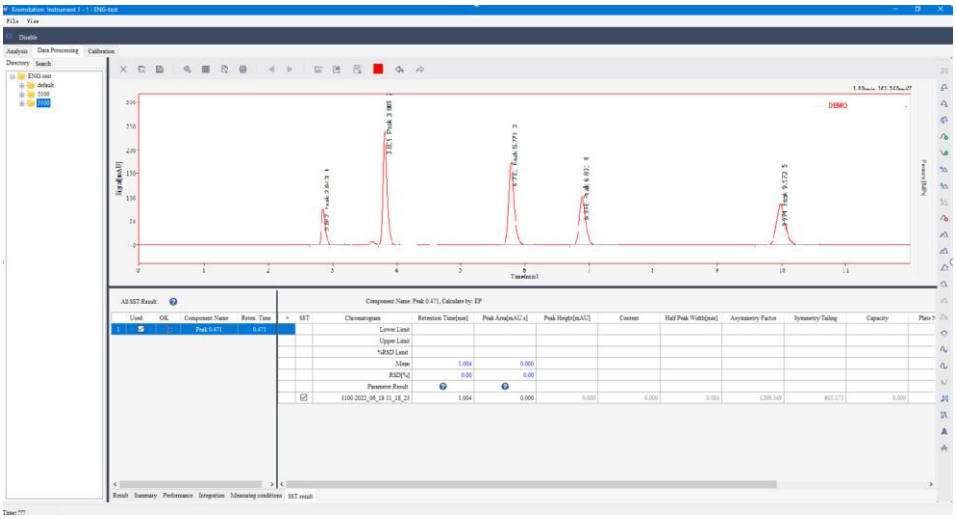


Figure 6-21　Selecting parameters

8）After selecting "parameters", the dialog box shown in 6-22 pops up, and the boxes in front of the parameters that need to be displayed "√", such as selecting "retention time" and "peak area".

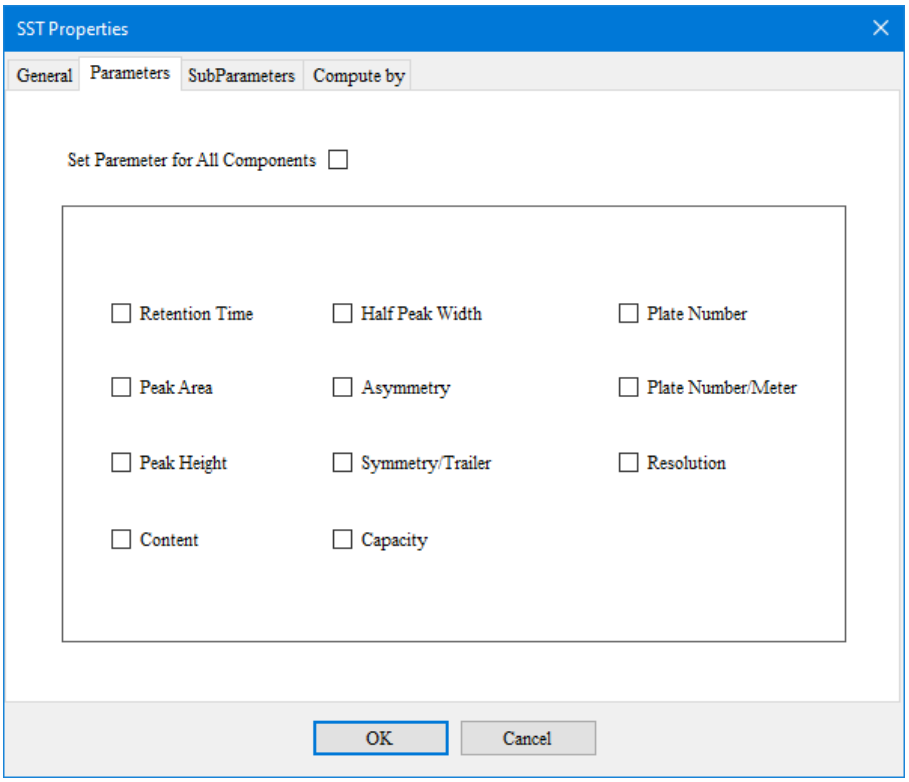


Figure 6-22 Parameter selection displayed in the SST result table

9）After selecting the parameters, in the SST result table, the "average value" and "RSD%" will be given for the selected parameters, as shown in Figure 6-23.

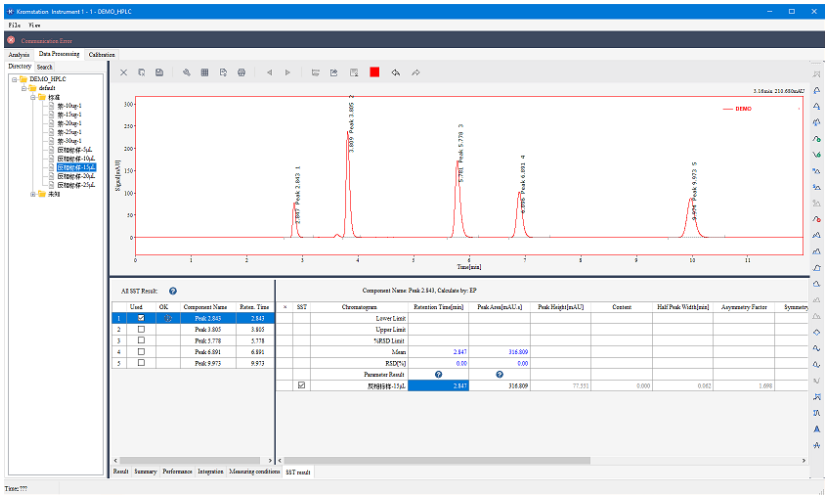


Figure 6-23 Parameter display in the SST result table

10）In the SST result table, fill in the upper and lower limits of the parameter and the limit of RSD%, and the system will automatically make a judgment on the test result. If the result obtained is within the specified range, in the “parameter result” section, the system will Automatically mark "✓", if the result obtained exceeds the specified range, the system will automatically mark" ✗", as shown in Figure 6-24.

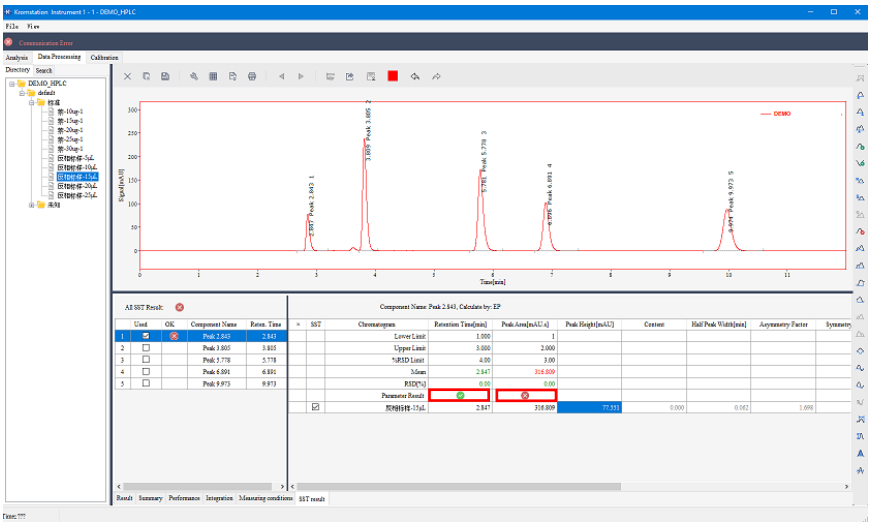


Figure 6-24　Parameter result display in SST

11）If you want to see the results of other parameters, you can select the boxes as shown in Figure

6-22.

The SST result is powerful, no need to copy the data obtained from the test into the EXCEL table for recalculation, which is convenient for customers to use.

6.11 Chromatogram File Output

Click the  icon in the chromatogram processing menu bar to output the chromatogram in txt format, as shown in Figure 6-25.

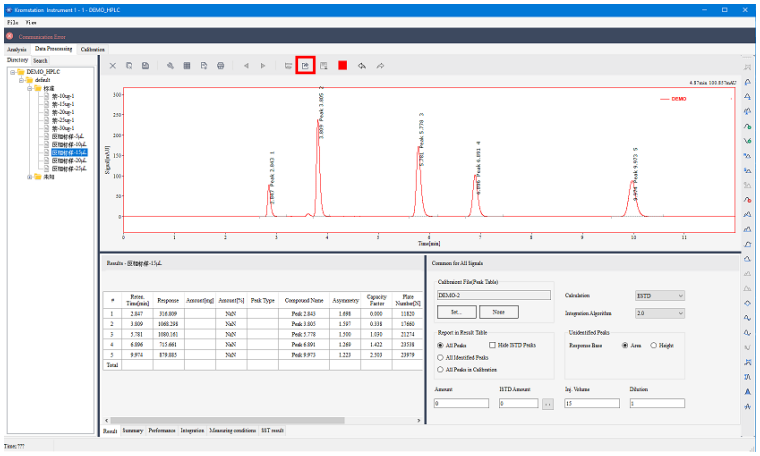


Figure 6-25 Chromatogram window-data output

Or click the batch processing under "File" to pop up the batch processing setting dialog box, select the spectrum to be batch processed, and set the "Analysis Method", "Calibration Method" and "Report Method" respectively. Finally, set the file type to be output. After confirming, the batch processing operation of the data can be carried out. As shown in Figure 6-26.

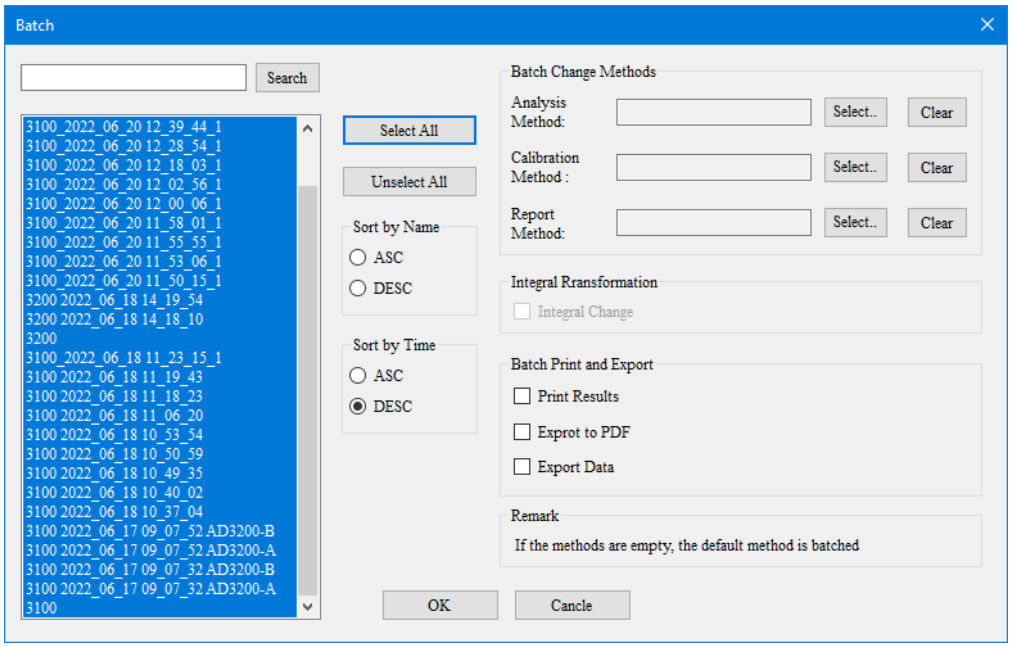


Figure 6-26 Batch processing window

7 Quantitative

After the peak is integrated and identified, it can be quantified. Quantitative use of peak area or peak height to determine the concentration of a compound in a sample.

Basic requirements for quantitative analysis:

- There must be pure substances as standards
- The peak of the quantified component must achieve baseline separation from other substances
- Conform to qualitative requirements
- Choose a suitable quantitative method

Quantitative calculation method

- Area percentage method
- Normalization method
- External standard
- Internal standard method

7.1 Percentage Method

This method requires that all compounds in the sample are eluted and integrated by the mobile phase, and the response factors of all components (that is, the peak area or peak height of the same content are the same, and this method is generally used for general-purpose detectors) is exactly the same. Percentage ratio can quickly get the approximate relative content of each component. This method is mainly used to estimate the relative content of impurities or degraded compounds in pure substances.

7.2 Normalization

This method requires that all compounds in the sample are eluted and integrated, and the response factors of all components are known. In the normalization method, the response factor is used for the peak area or peak height to compensate for changes in detector sensitivity for different sample components.

Formula used to calculate compound content:$x\% = \frac{Response_x \cdot RF_x}{\sum(Response \cdot RF)} \times 100\%$

$Response_x$: Area or height of X peak

RF_x: X peak response factor

$\sum(Response \cdot RF)$: The sum of the response values of the area or height of all peaks

Advantages: simple, the accuracy of the injection volume and changes in operating conditions have little effect on the measurement results.

Caution: In the percentage method and normalization method, when the peak area is used as the basis for quantification, all peaks are required to be separated from the baseline; when the peak height is used as the basis for quantification, the peak shape is required to be better (peak tailing factor is 0.95— 1.05).

7.3 External Standard Method

This method is the most common and basic method for determining the concentration of unknown samples. Prepare a standard solution of known concentration (preparation of the pure product of the compound to be tested), inject it into the liquid chromatograph and analyze the same volume of each standard solution, and then plot the peak response value against the concentration. Obtain the calibration curve y=ax+b. As shown in Figure 7-1.

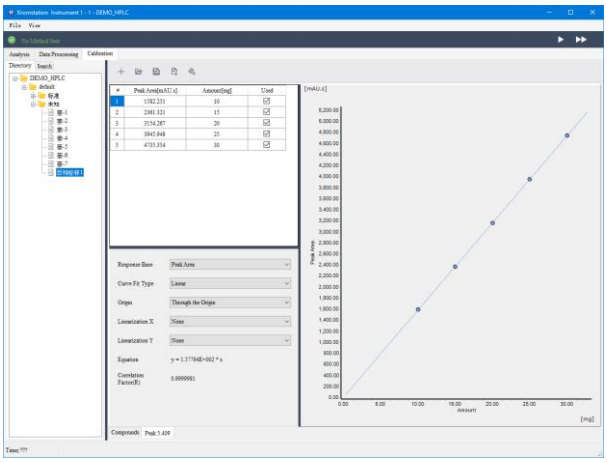


Figure 7-1 Calibration curve

7.3.1 Open the calibration window

After logging in to the workstation, click Calibration on the main menu bar to enter the calibration window. As shown in Figure 7-2.

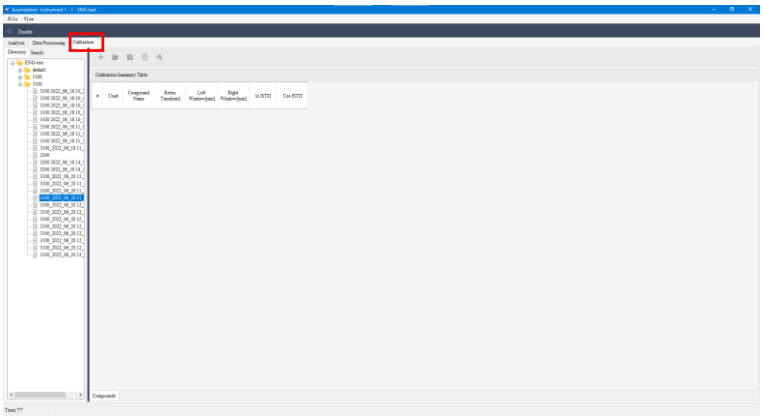


Figure 7-2 Enter the calibration window

7.3.2 Making the calibration curve

This workstation can make calibration curves for a single component or up to 5 components, and there can be multiple calibration points that do not contain blanks. The specific production steps are as follows:

1) Preprocess the standard spectrum to remove unnecessary peaks and modify the integration conditions.

2) Open the calibration window and click the  icon to start setting the calibration options, as shown in Figure 7-3. The user can set the response unit and decimal places, etc.

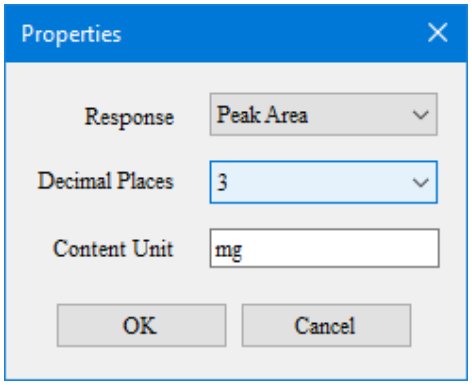


Figure 7-3 Calibration settings window

3）Click the "+" under the "Standard File" menu to open the preprocessed standard spectrum.

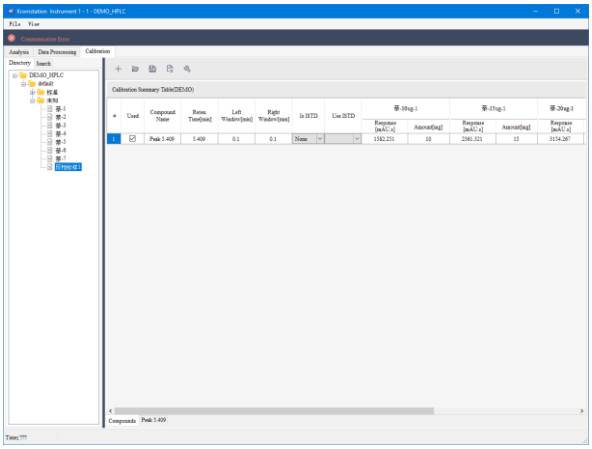


Figure 7-4 Open the first calibration chromatogram

4） Add the calibration peak to the corresponding calibration standard according to the retention time. At this time, the "Response" information of the spectrum is displayed in the "Calibration Summary Table", which is the "Figure 1" information. Select the calibration peak to be used in the "calibration summary table", the name of the calibration peak defaults to "peak retention time". The response is based on the peak area or peak height.

5） Directly select the second spectrum that needs to be opened in the list on the right, the "calibration summary table" is automatically added as "Figure 2" information, and enter the

relevant information of the calibration peak.

6） Repeat the operation of 3) above to add up to 20 calibration points excluding blanks.

7） After the calibration point is set, you can view the calibration curve in the tab of the corresponding compound, and set the calibration method of the calibration curve, as shown in Figure 7-5. If the “used” column of a calibration point in the upper left information table is selected as blank, the calibration point will be displayed as a circle in the calibration table, and the calibration point data will not be included in the calculation of the curve.

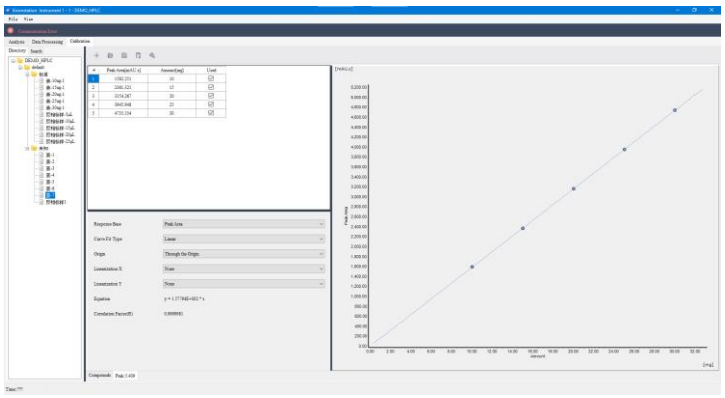


Figure 7-5　Calibration curve-single substance

After the calibration curve is created, click icon  in the "Calibration" window menu to save the created standard curve.

7.3.3 Use of calibration curve

1) Open the chromatogram of the test sample and modify the integration conditions, otherwise it will be the default basic conditions.

2) In the "Calibration File" column on the right half of the "Result" tab, click "Set" to select the calibration file, and in the "Calculation" column, select the type of calibration file and set other calibration parameters.

3) After the setting is completed, the quantitative calculation results of each substance in the sample corresponding to the calibration curve will be displayed in the "Result" tab and the "Summary" tab, as shown in Figure 7-6.

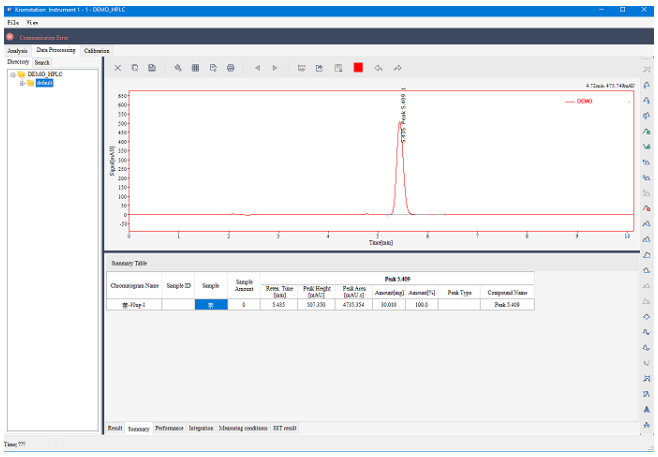


Figure 7-6 Chromatogram-Use of Calibration File

7.4 Internal Standard Method

The application frequency of this method is low. A component with a known content is added to the standard and the sample to generate a uniform factor, and this component is the internal standard.

The compound used as the internal standard should be chemically similar to the compound to be calibrated, with similar retention time, but can be completely separated on the chromatogram.

This internal standard method does not require high reproducibility of chromatographic injection. The calculation of the corrected content ratio of a specific compound in an unknown sample has the following two steps:

1) Correction

Each point in the calibration curve is composed of the calculated value of the content ratio of each level and the response value ratio of a certain peak in the calibration table.

Content ratio: the content of the compound divided by the content of the internal standard substance

Response value ratio: the area of the compound divided by the area of the internal standard

2) Unknown sample

The response value of the unknown compound is divided by the response value of the unknown sample to obtain the response value ratio of the unknown sample, and the amount of the unknown sample can be obtained from the above calibration curve.

【Caution】 When making an internal standard calibration curve, only one of the calibration materials can be set as an internal standard.

8 D3250 Fluorescence Detector

8.1 Module Configuration

The configuration method of D3250 is the same as that of conventional instruments. Click the "Add" button in the instrument configuration interface to enter the available control module window, select the FLD Controller module (Figure 8-1), click "Add", and then pop up as shown in Figure 8- 2 In the window shown below, click "Automatic Detection" to connect the instrument, and click the "OK" button after the connection is successful. Then click the  button to move this module to the right to complete the configuration (Figure 8-3).

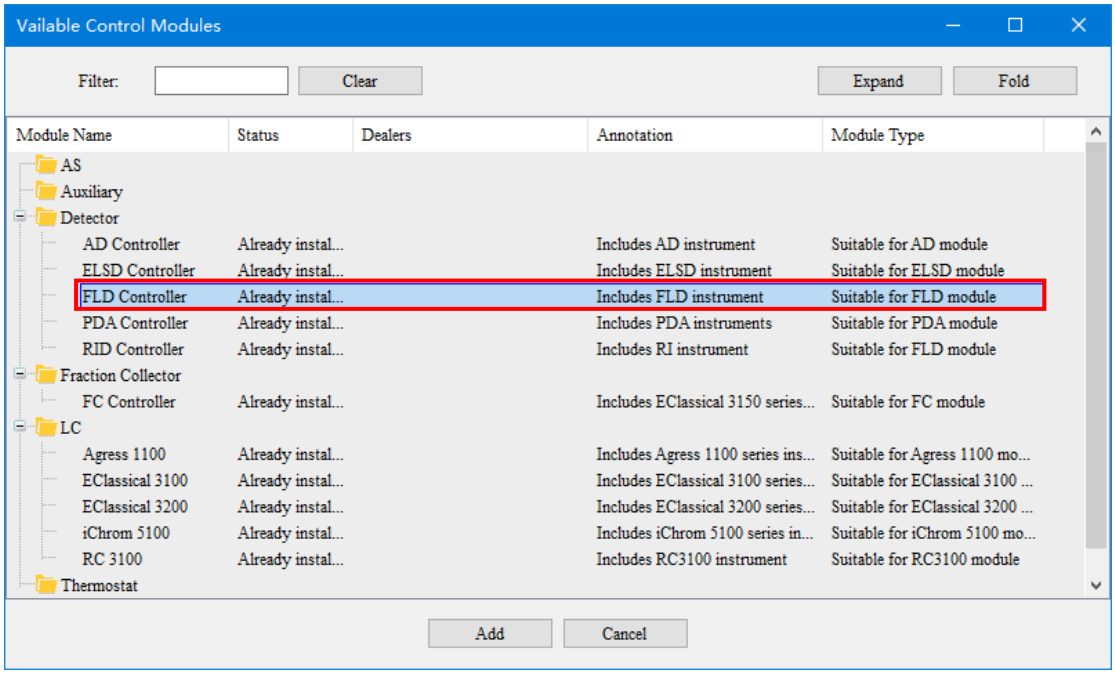


Figure 8-1 FLD Controller module configuration

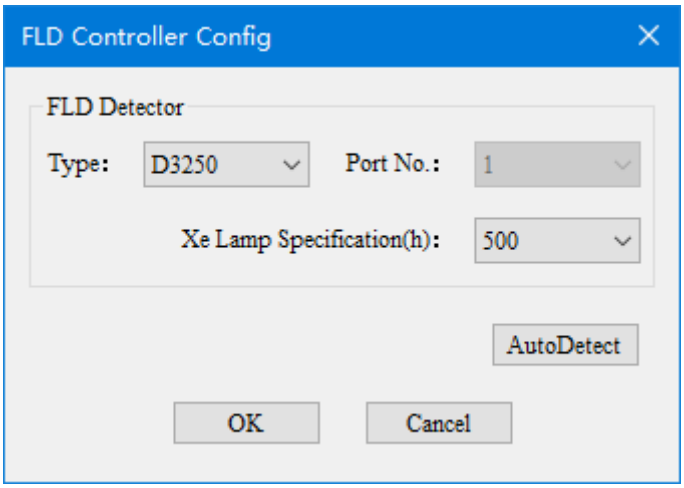


Figure 8-2 D3250 Instrument Connection Verification

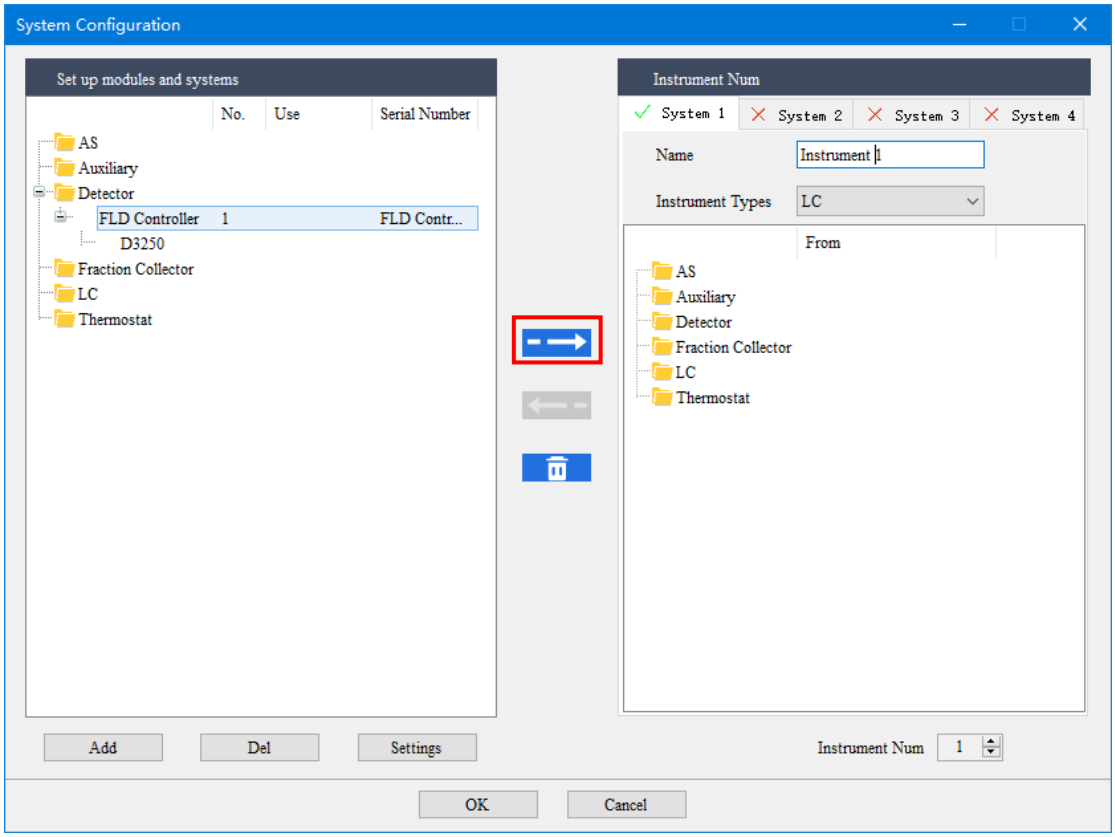


Figure 8-3 Module configuration

8.2 D3250 Instrument Method Setup

After entering the project, the instrument will automatically obtain the parameter information of the lower computer. After the parameter information is obtained, click the "Instrument Method" button, click the "Detector (D3250)" tab, and set the parameters of the D3250 in this window, as shown in

Figure 8-4 shown. In the "Control Parameters" tab, you can set the Ex wavelength, Em wavelength, Em Bandwidth, Time Program, Offset, Sensitivity, Xe lamp status, acquisition frequency, etc. Click the "Read" button to read the opening times, running time and replacement time of the Xe lamp.

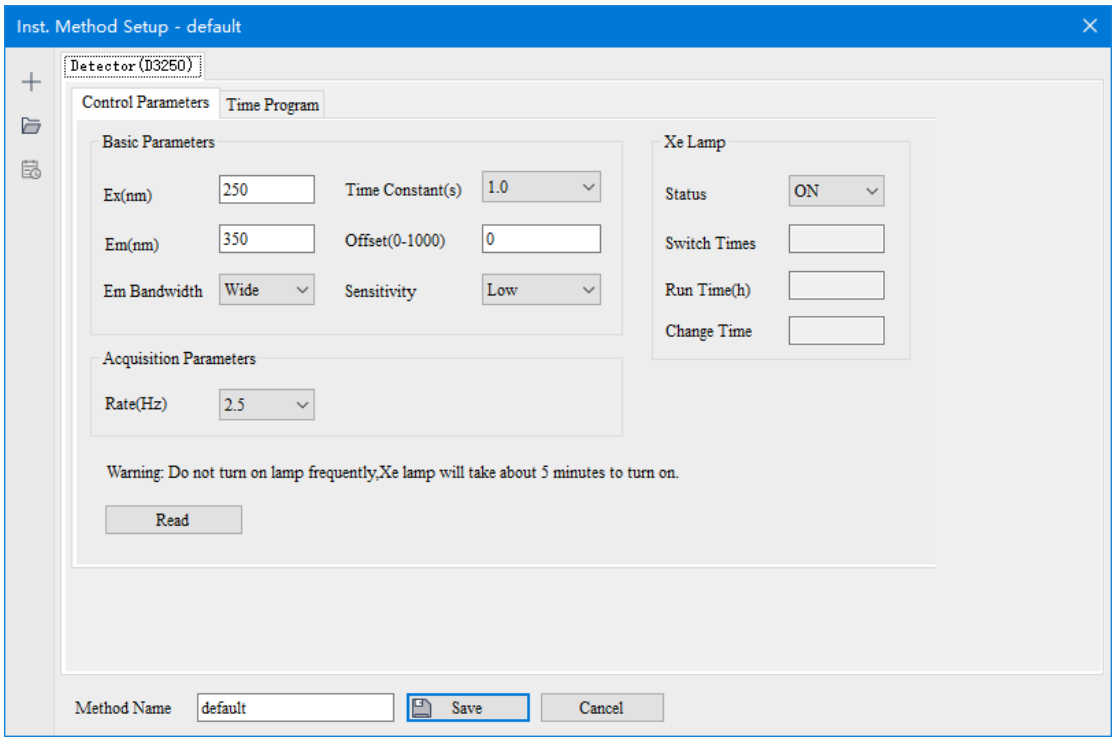


Figure 8-4 D3250 parameter setting

【Note】 Ex Wavelength and Em Wavelength cannot be set to the same wavelength value, otherwise it will cause the signal of the lower computer to exceed the range.

In the "Time Program" tab, you can set the time-wavelength program, and you can set the time, wavelength, sensitivity, baseline, etc., as shown in Figure 8-5. The information for the start time line already has default values. The time interval between each line of the time-wavelength program needs to be ≥0.3 min. Because wavelength switching takes a certain amount of time, it is recommended that the wavelength of the initial line in the time program be set to the same wavelength as that in "Basic Parameters".

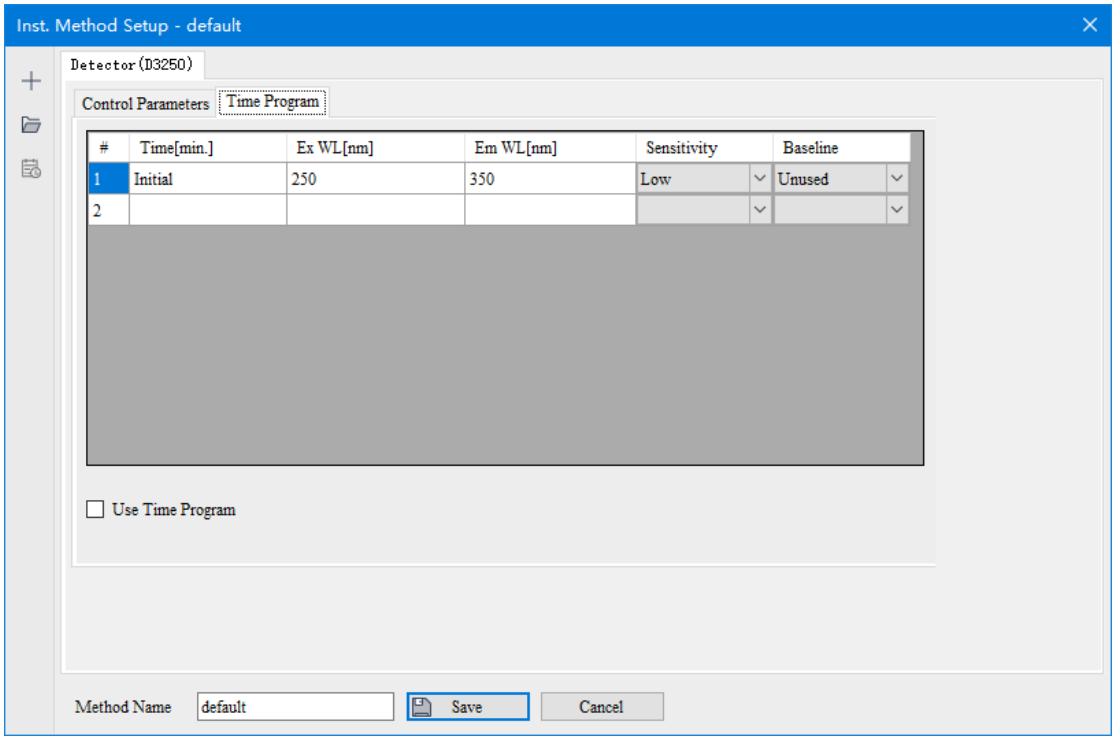


Figure 8-5 Time Program Settings

When using the Time Program to run the analysis, the "Use Time Program" function should be checked, otherwise the analysis will not be performed according to the Tim Program.

8.3 Device Monitor

In the device monitor, you can view the running status of the instrument, display the switching status of the xenon lamp, the change of Ex wavelength and Em wavelength, energy change, working status, acquisition status, keyboard lock status, and automatic zeroing and equipment error clearing, etc. operate. As shown in Figure 8-6.

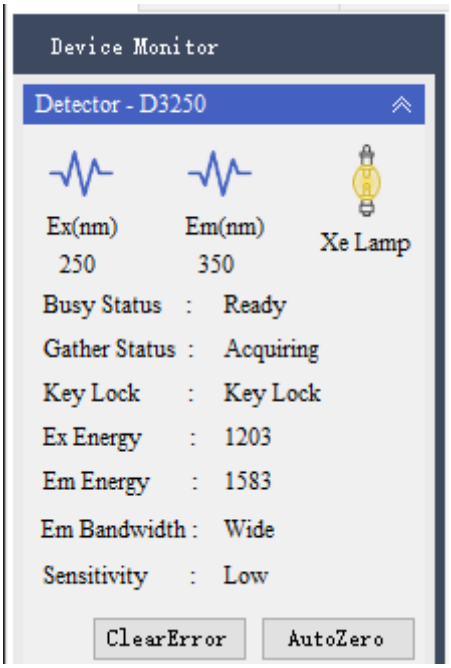


Figure 8-6 Device Monitor

8.4 Run Analysis

The operation method of using the D3250 to run the analysis is basically the same as the conventional method, see Chapter 4 for details.

9 GPC Mode Data Processing

9.1 Introduction

9.1.1 Overview

GPC mode is an optional mode of the chromatography data workstation, and the use of GPC mode is controlled by a specific user code. It can obtain GPC data from any GPC system with analog signal output, and provide automatic GPC data analysis, making GPC data processing easier.

The system has flow rate correction and multiple detection delay correction functions to ensure more reliable GPC data.

9.1.2 Explanation

GPC mode is a complete optional mode, it can be considered as a new chromatography data workstation or an extension mode for existing chromatography data work.

9.1.3 GPC Mode Selection

In the system configuration window, select the "GPC" option from the drop-down box of the instrument type.

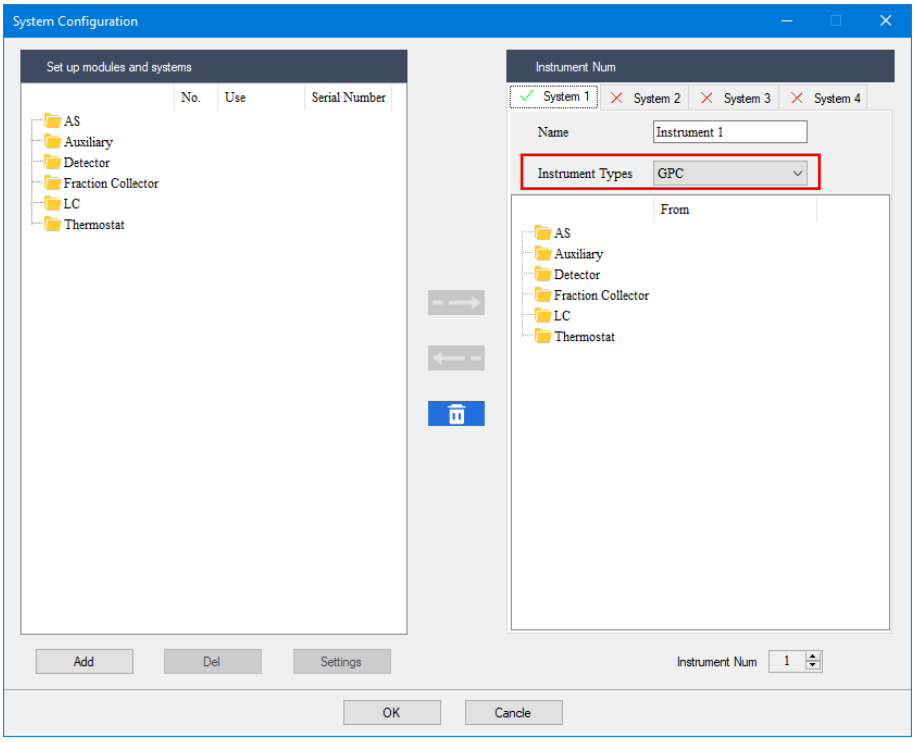


Figure 9-1 GPC mode entry interface

9.2 GPC Basic Theory

Gel-Permeation Chromatography (GPC) or Size Exclusion Chromatography (SEC) is a liquid chromatography technique for testing the molecular weight distribution of polymers. Polymer samples containing different molecular sizes are separated on the column. In this mode of chromatographic separation, different molecules are separated according to their size, with larger molecules eluted first, and smaller molecules eluted later. Select a standard substance of appropriate molecular weight, draw a calibration curve of molecular weight and elution volume, and then calculate the molecular weight distribution.

9.2.1 Narrow Standard Calibration

When a standard substance with a narrow molecular weight distribution can be obtained, the narrow distribution standard calibration is the most commonly used calibration method. The calibration curve is drawn by the peak molecular weight and the retention time of the highest point of the chromatographic peak.

9.2.2 Flow Rate Correction

The elution volume is calculated from the flow rate and retention time. Small changes in the flow rate can have a huge impact on the accuracy of the molecular weight test results. Select a small molecular weight sample (flow rate marker) to add to the standard or sample to make retention times more accurate in different chromatograms.

9.2.3 Universal Calibration

The separation of polymer molecules on a chromatographic column depends on the size of the molecule, not molecular weight. In addition to molecular weight, polymer molecular size is also related to molecular configuration (linear, dendritic and star) and conformation (related to solvent and temperature). If the K and alpha values of the polymer are known, the Mark-Houwink equation can be used to calculate the molecular weight of molecules of the same size.

9.3 GPC Integral Settings

Click the "Integration" tab of the analysis method setting dialog box to set the integration parameters for generating the chromatogram, as shown in Figure 9-2.

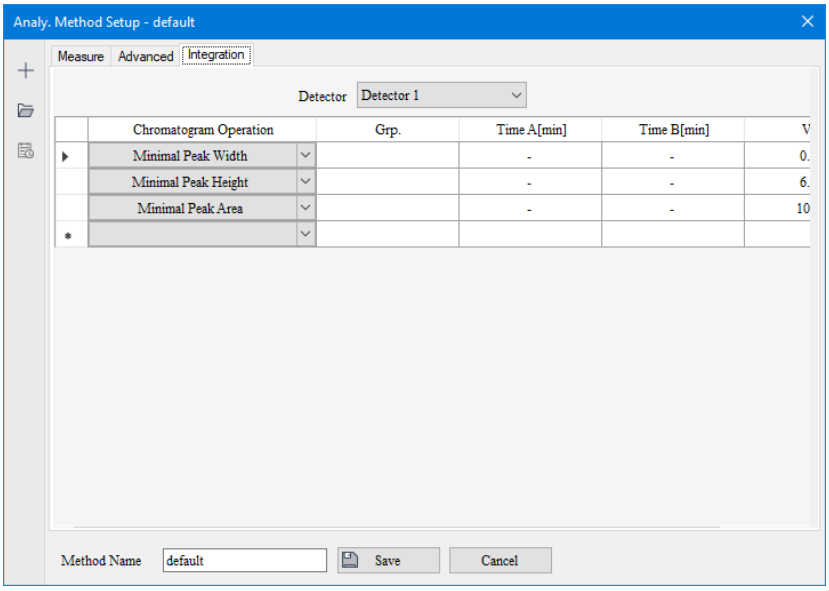


Figure 9-2 Integration Event Setting Dialog Box

The GPC mode "Analysis Method Setup - Integration" is similar to the Standard Mode "Analysis Method Setup - Integration" (see 3.3.3), but with the following 3 differences:

1) The way of identifying solvent peaks in GPC mode is different from that in standard mode. GPC mode marks all peaks that appear in the selected range as solvent peaks. Once marked as a solvent peak, this peak will be rejected in the GPC results table.

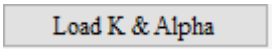
2) Add the function of flow rate mark  , GPC mode can set a peak for flow rate correction calculation. Only one peak in the entire chromatogram can be marked as a flow rate peak.

【Note】 If "Use Flow Rate Correction" is selected in the GPC Calibration Options window, the flow rate marker peak must be marked, otherwise the flow rate correction function will not work.

3) The group  function is not available in GPC mode. To obtain an average value for the different molecular weight distributions of a polymer, use the Remove Peak and Add Positive Peak functions to integrate multiple peaks into a single peak.

9.4 Single Analysis and Sequence Analysis

9.4.1 Single Analysis

The K value and α value can be entered in the single analysis dialog box, and the K value and α value can be brought into the universal calibration Mark-Houwink equation. 14.1 and 0.7 are the K and α values of linear polystyrene in THF solvent at 25°C, respectively. The user can read the appropriate K value and α value by clicking  . Other settings are the same as those in standard mode.

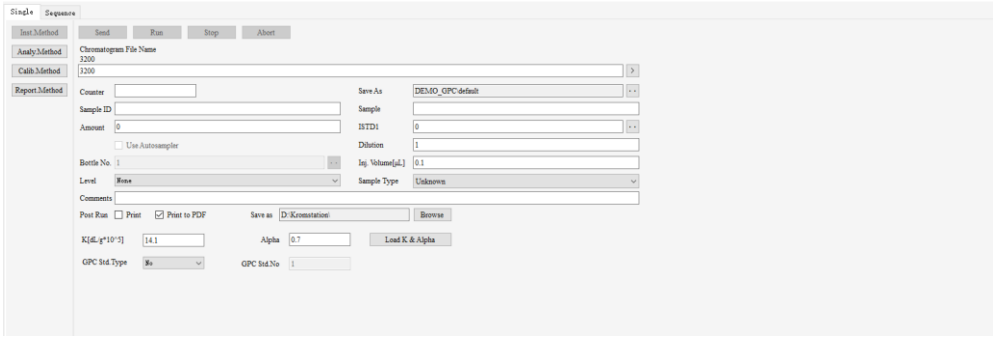


Figure 9-3 Single Analysis

In the data processing window, if the chromatogram is opened by selecting the corresponding file through the "Directory" menu, the K value and the α value will be opened together.

9.4.2 Sequence Analysis

Added input columns for K and alpha values in the Sequence Analysis window in GPC mode. GPC standard type and GPC standard number contribute to automatic GPC recalibration during sequence runs.

Other settings are the same as in standard mode.

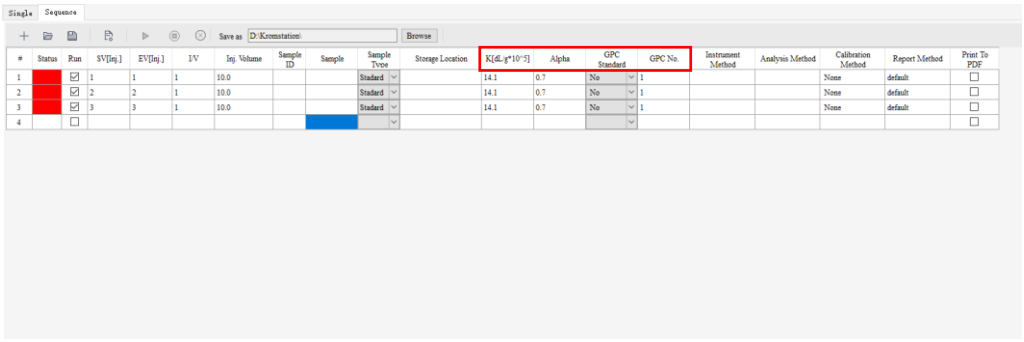


Figure 9-4 Sequence analysis

9.5 Data Processing Window

In the Chromatogram window, a chromatogram, molecular weight distribution or cumulative molecular weight distribution table can optionally be displayed.

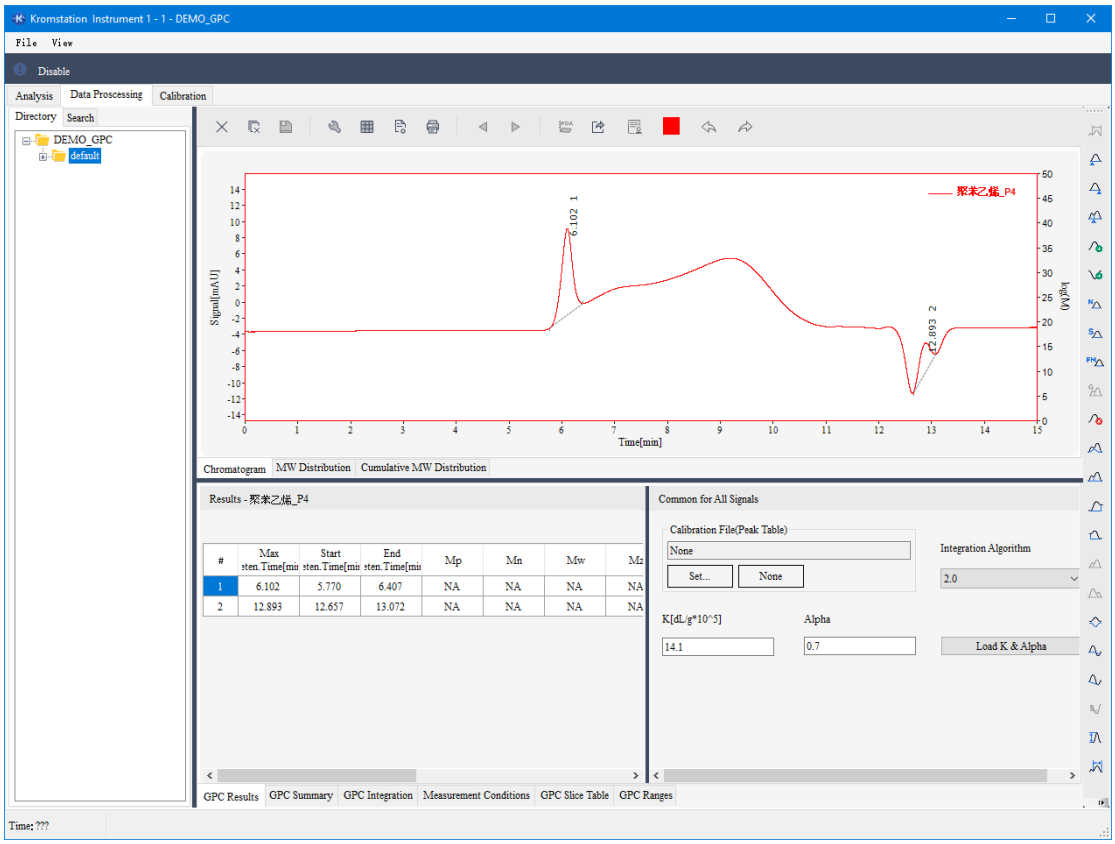


Figure 9-5 Chromatogram window

The GPC results, GPC summary, GPC integration, measurement conditions, GPC slice table, and GPC range can be displayed under the window.

9.5.1 Chromatogram

The chromatogram is in the image area of the chromatogram window, and its characteristics are the same as in the standard mode, and the coordinate axis of the right ordinate Log M is fixed.

9.5.2 MW Distribution

The MW distribution graph shows the molecular weight distribution of the selected peak. If there are multiple peaks in the chromatogram, select the peak by clicking the row where the peak is located in the "GPC Results". The retention time of the peak is displayed in the upper right corner of the image, see the red square in Figure 9-6 is marked.

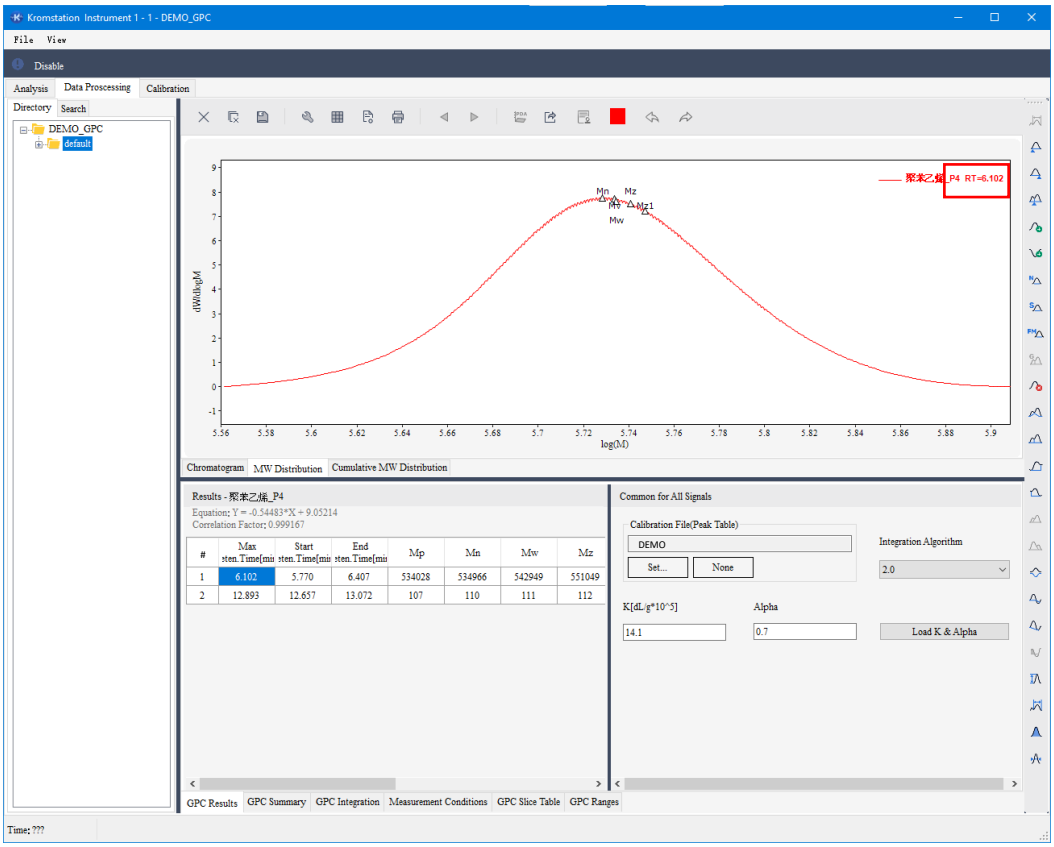


Figure 9-6 Chromatogram-MW Distribution

9.5.3 Cumulative MW Distribution

Cumulative MW Distribution displays the cumulative molecular weight distribution (%) of the selected peak. If there is more than one peak in the chromatogram, select the peak by clicking on its row in GPC Results.

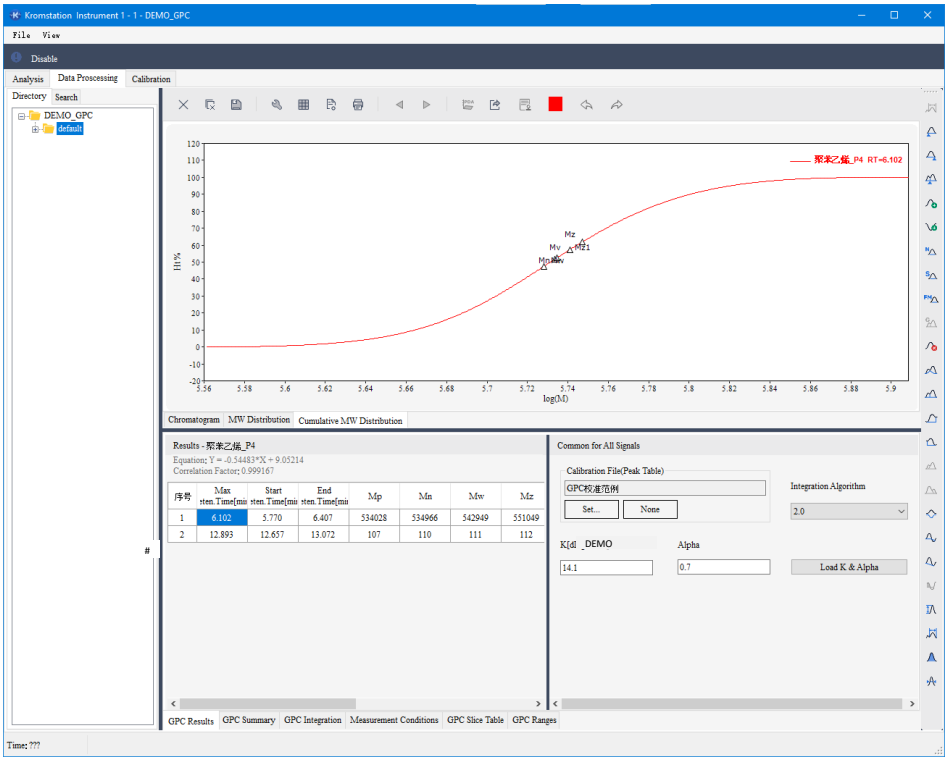


Figure 9-7 Chromatogram-Cumulative MW Distribution

9.5.4 GPC Results

On the right side of the "GPC Results" table, you can choose to add or not to add a calibration file.

1） None means that the calibration curve is not added, and all molecular weight results in the results table show NA.

2）The Set... function can select the saved calibration curve, and all molecular weight results in the result table are displayed normally.

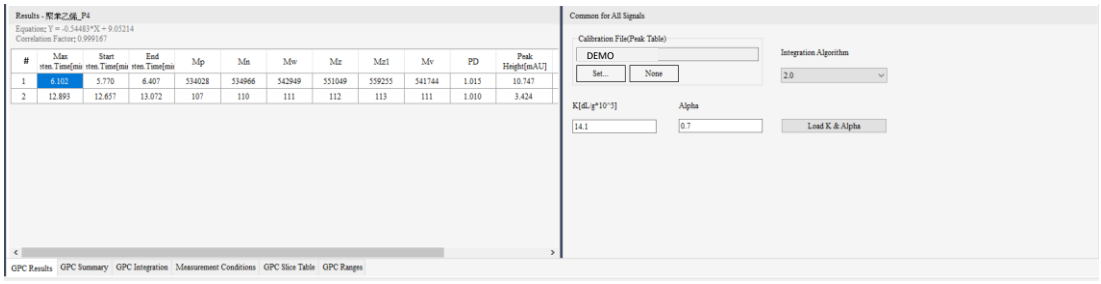


Figure 9-8 GPC Result table

- Max.RT : Retention time of peak maximum;
- Start RT : Retention time of peak integration start;
- End RT : Retention time of peak integration end;
- Mp : Molecular Weight at peak maximum;
- Mn : Molecular Weight number average;
- Mw : Molecular Weight weight average;
- Mz : Molecular Weight Z average;
- Mz1 : Molecular Weight Z+1 average;
- Mv : Molecular Weight viscosity average;
- PD :Polydispersity;
- Flow Rate Correction : Flow rate correction factor;

Other parameters are the same as standard mode parameters.

9.5.5 GPC Summary

The GPC Summary table contains parameters for all peaks, and the table queue can be set by right-clicking.

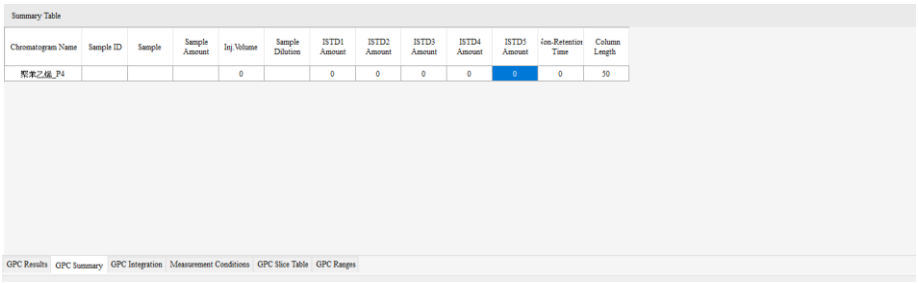


Figure 9-9 GPC Summary table

9.5.6 GPC Integration

GPC integration can display or edit all operations on chromatographic peaks, all operations can be added directly in the table, or in the chromatogram window using the integration tool button on the right to add.

Most operations are the same as in standard mode, see 9.3 for differences.

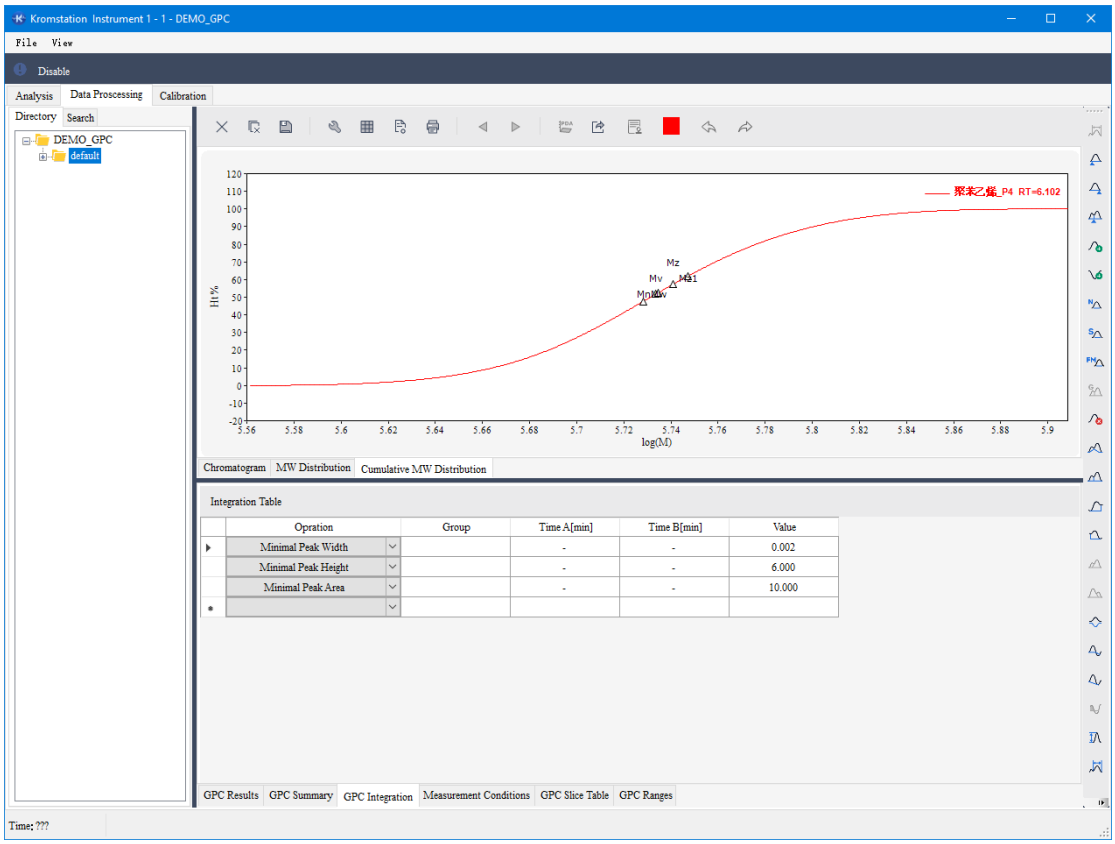


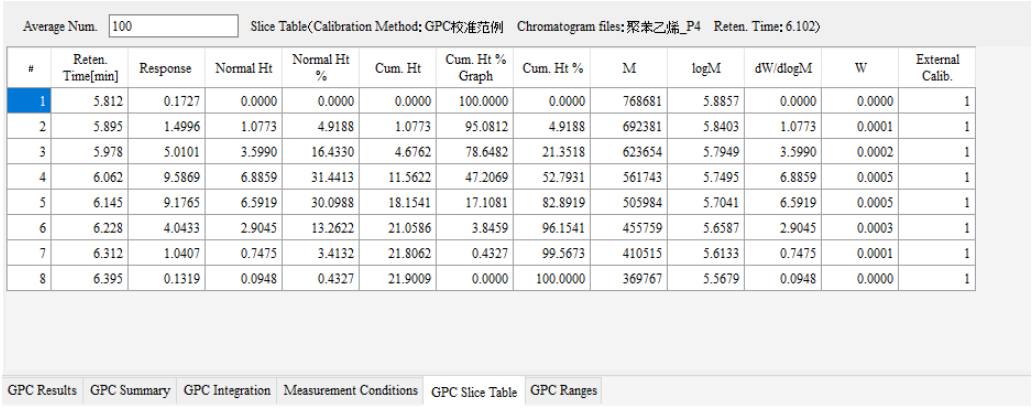
Figure 9-10 GPC Integration table

9.5.7 Measurement Conditions

The content of the measurement conditions is the same as that of the standard mode, see 6.9.

9.5.8 GPC Slice Table

The GPC slice table displays cumulative molecular weight distribution results for selected peaks.



Average Num. 100 Slice Table(Calibration Method: GPC校准范例 Chromatogram files: 聚苯乙烯_P4 Reten. Time: 6.102)

#	Reten. Time[min]	Response	Normal Ht	Normal Ht %	Cum. Ht	Cum. Ht % Graph	Cum. Ht %	M	logM	dW/dlogM	W	External Calib.
1	5.812	0.1727	0.0000	0.0000	0.0000	100.0000	0.0000	768681	5.8857	0.0000	0.0000	1
2	5.895	1.4996	1.0773	4.9188	1.0773	95.0812	4.9188	692381	5.8403	1.0773	0.0001	1
3	5.978	5.0101	3.5990	16.4330	4.6762	78.6482	21.3518	623654	5.7949	3.5990	0.0002	1
4	6.062	9.5869	6.8859	31.4413	11.5622	47.2069	52.7931	561743	5.7495	6.8859	0.0005	1
5	6.145	9.1765	6.5919	30.0988	18.1541	17.1081	82.8919	505984	5.7041	6.5919	0.0005	1
6	6.228	4.0433	2.9045	13.2622	21.0586	3.8459	96.1541	455759	5.6587	2.9045	0.0003	1
7	6.312	1.0407	0.7475	3.4132	21.8062	0.4327	99.5673	410515	5.6133	0.7475	0.0001	1
8	6.395	0.1319	0.0948	0.4327	21.9009	0.0000	100.0000	369767	5.5679	0.0948	0.0000	1

GPC Results | GPC Summary | GPC Integration | Measurement Conditions | GPC Slice Table | GPC Ranges

Figure 9-11 GPC Slice table

The average number depends on the integration interval and peak width set in the "GPC Integration" table, ranging from 1 to 100.

- RT : Retention time of the slice (averaged);
- Response : Slice peak height(averaged);
- Norm. Ht: Normalized slice height(summed);
- Norm. Ht%: Percentage of the slice height from total of all slices height(summed to give total 100%);
- Cum. Ht: Cumulative slice height(averaged);
- Cum. Ht%: Cumulative percentage of the slice height from the total of all slices heights(averaged);
- Cum. Ht% Graph: Cumulative percentage of the slice height from the total of all heights in the inverse order(increasing with increasing)(averaged);
- M: The molecular weight corresponding to the slice retention time(averaged);
- Log M: The logarithm of the molecular weight corresponding to the slice retention time(averaged);
- dW/d log M: Normalized distribution of slice molecular weights used for the graph in the MW Distribution tab;
- W: Normalized slice height used for molecular weight distribution calculation;
- Outside Calibration: Flag marking whether the slice is inside or outside of the used calibration retention time range. Outside of the range gives value 1, while inside the range gives value0.

9.5.9 GPC Ranges

The GPC Range table is used to calculate area percentages that define a range of molecular weights, or to calculate average molecular weights that define a range of area percentages. The type of range (whether percentage or molecular weight) can be chosen arbitrarily.

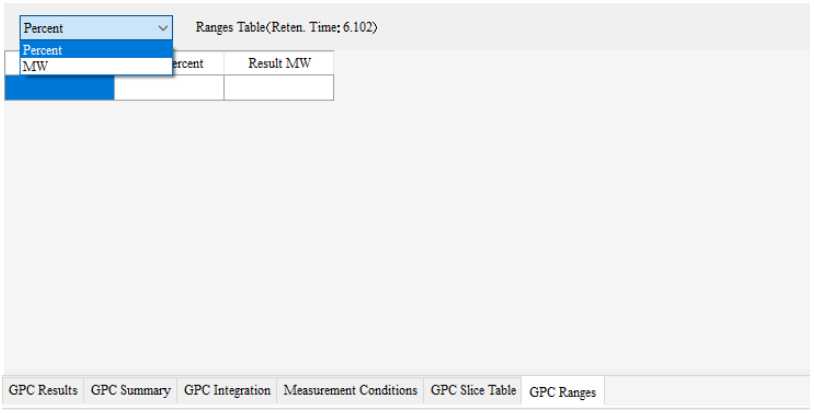


Figure 9-12 GPC Ranges

For example, when the average molecular weight of 10% of the final peak area needs to be calculated, the low and high percentages are set to 90 and 100, respectively. Multiple ranges of the same type can also be set.

9.6 Quantitative Calculation

Quantitative Calculations were performed using a GPC calibration curve, which was used to calculate the molecular weight distribution of the polymer samples.

9.6.1 GPC Calibration window

Click the "Calibrate" option to enter the calibration window.

The Calibration window is used to create, modify and display calibration curves. In the image area of the calibration curve window, the chromatogram of the last opened standard is displayed together with the calibration curve.

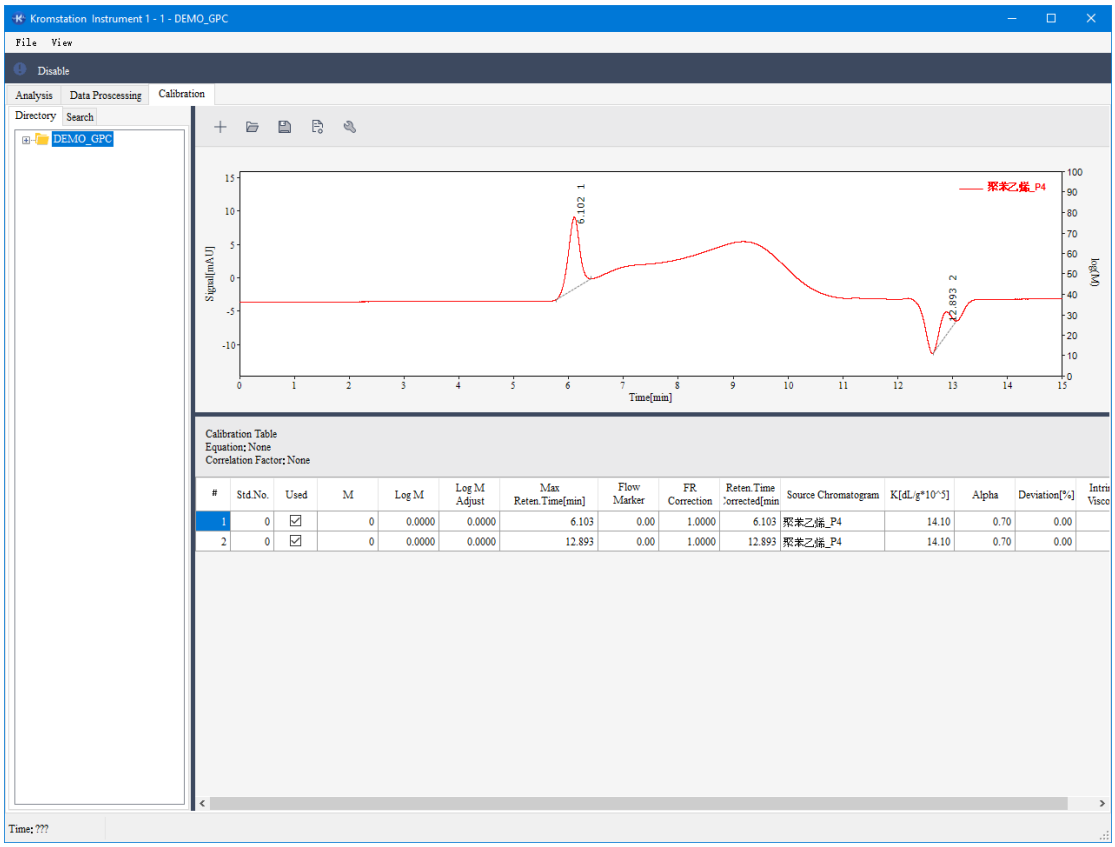


Figure 9-13 Calibration Curve window

9.6.2 GPC Calibration Options

Under the calibration window, click the  button, and the "GPC Calibration Options" dialog box will appear, which contains calibration type, K value, alpha value and other options.

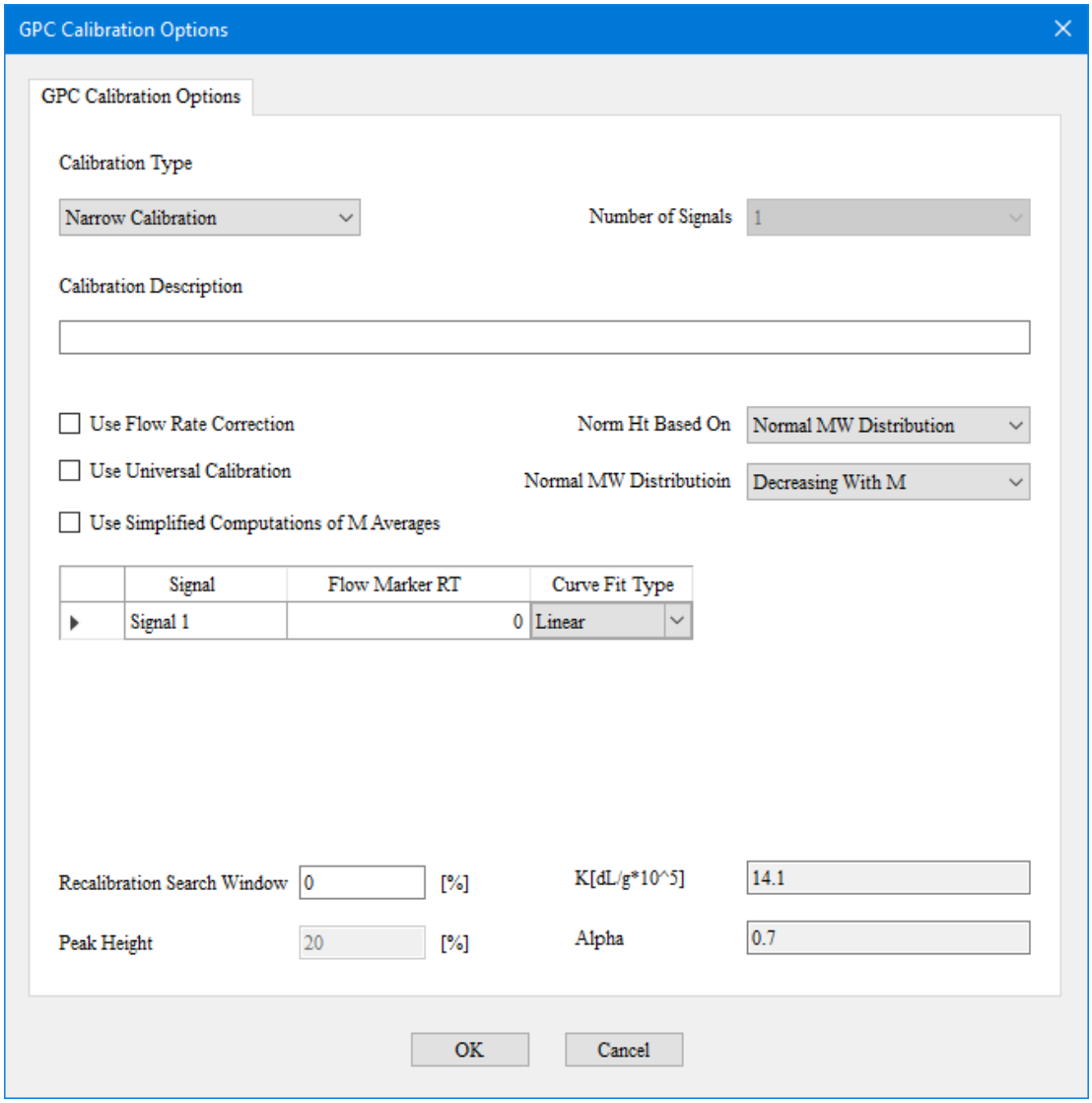


Figure 9-14 GPC Calibration Option settings

9.6.2.1 Calibration Type

The calibration type is narrow peak calibration.

9.6.2.2 Use flow Rate correction

If a Flow Marker Peak is set, selecting "Use Flow Rate Correction" will correct the calibration curve and calculations.

9.6.2.3 Use Universal Calibration

Selecting "Use Universal Calibration" will correct the calibration curve and calculations according to the Mark-Houwink equation.

【Note】 Whether or not this option is selected, the alpha value is only used to calculate the viscosity average molecular weight.

9.6.2.4 Simplified Computations of M Averages

Select "Use Simplified Computations of M Averages", then the M Averages will be calculated using the simplified method instead of the standard method.

【Note】 The difference between the simplified calculation method of M Averages and the standard calculation method only exists in nonlinear calibration.

9.6.2.5 Number of Signals

It determines the number of signals used to draw the standard curve, which automatically increases with the number of signals used to make the calibration curve chromatogram.

9.6.2.6 Flow Marker RT [min]

When using flow rate correction, the flow flag RT needs to be set for flow rate correction.

9.6.2.7 Curve Fit Type

Curve Fit Type is used to set the standard curve fit type. The curve fit type can be selected from the table.

9.6.2.8 Recalibration Search Window

Sets the maximum deviation (in %)of the Standard Peak retention time from the stored to perform the recalibration.

9.6.2.9 Peak Height

Peak height (%) is only applicable when linear calculation is selected for the calibration type, and determines the position of the points used to draw the calibration curve.

9.6.3 Calibration Curve Creation — Narrow Calibration

If the molecular weight distribution of the standard is narrow (dispersion coefficient < 1.2), select the narrow calibration type. A calibration curve was drawn from the retention time of the peak maxima and the known peak molecular weights. If there is no peak molecular weight, the square root of the product of the weight average molecular weight and the number average molecular weight can also be substituted.

1） Preprocess all standard chromatograms to remove unwanted peaks and correct integration conditions.

2） Open the calibration window, click the  button, set the parameters of "GPC Calibration Options", and select "Narrow Peak Calibration" as the calibration type.

3） Click the chromatogram file to open the first processed standard chromatogram.

4） Select the calibration peak to be used in the calibration table below, and enter the M value of the calibration peak.

5） Repeat steps 3) and 4) to add standard chromatograms in turn, and the standard level will be automatically upgraded.

6） After all standard chromatograms are added, the calibration curve will appear automatically.

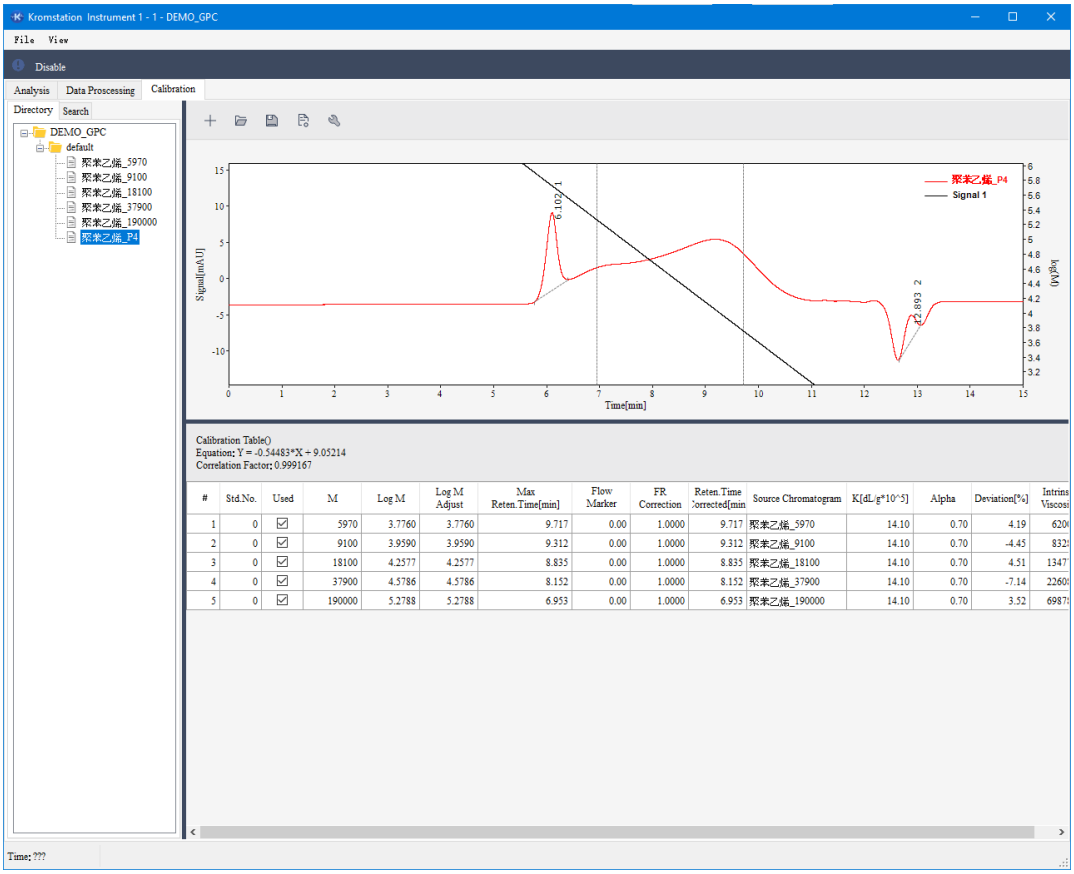


Figure 9-15 Narrow Peak Calibration Curve

9.7 Export

The export settings are the same as in standard mode.

1)Export of data:

Click the icon in the spectrum processing menu bar, and select "GPC Result Table", "GPC Summary Table", "GPC Slice Table", "Chromatogram Title", and "Chromatogram Options" in the pop-up "Export" window. The data will be output in txt format.

2)Export of Chromatogram:

Click the icon in the spectrum processing menu bar, select the option "Export to file as image", and output the chromatogram in bmp format.

10 PDA Module

The PDA module is an independent module, dedicated to the use of the diode array detector. The use of this module needs to be purchased separately. The following is a brief introduction to the PDA module (taking D5115 as an example):

10.1 Module Configuration

The PDA module needs to use a special user code before it can be used. For detailed instructions, please refer to the process document.

The configuration method of the PDA module is the same as that of the conventional instrument. Select the PDA module in the instrument configuration interface (Figure 10-1), click Add, and then the window shown in Figure 10-2 will pop up, select the PDA detector model to be used, and fill in the instrument model and click OK. Then click the  button to move the PDA module to the right to complete the configuration.

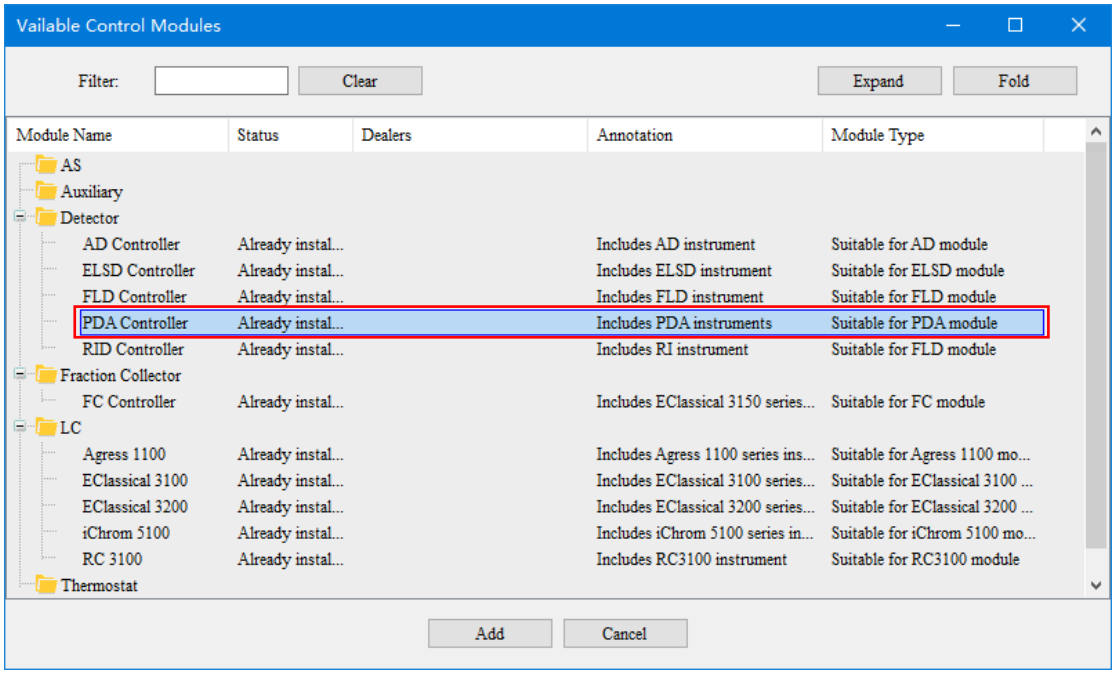


Figure 10-1 PDA Module Instrument Configuration

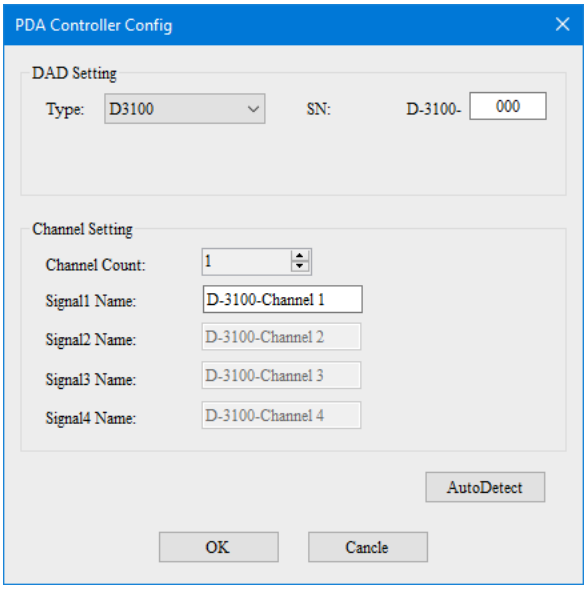


Figure 10-2 Add PDA Module

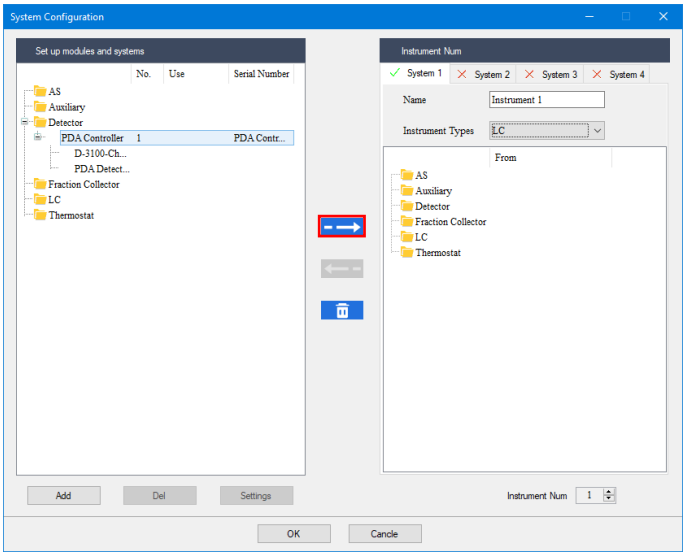


Figure 10-3 PDA Module added to the right

10.2 Method Settings

(1) Instrument Method Settings:

After entering the project, click the instrument method to enter the PDA instrument method setting interface, as shown in Figure 10-4. All parameters of the PDA are included in the instrument method. In the "PDA Parameters" tab, you can set the acquisition wavelength range, display acquisition wavelength, acquisition frequency, light source status, etc., and use the "Read Light Source Status"

button to monitor the number of times the light source is turned on and running time.

The PDA method also includes the function of selecting the chromatogram output format. The default option is 2D output, that is, it does not contain information such as spectrograms and 3D spectrograms, which can be automatically selected according to requirements.

(2) Analysis Method Setting: The analysis method setting is the same as the conventional setting.

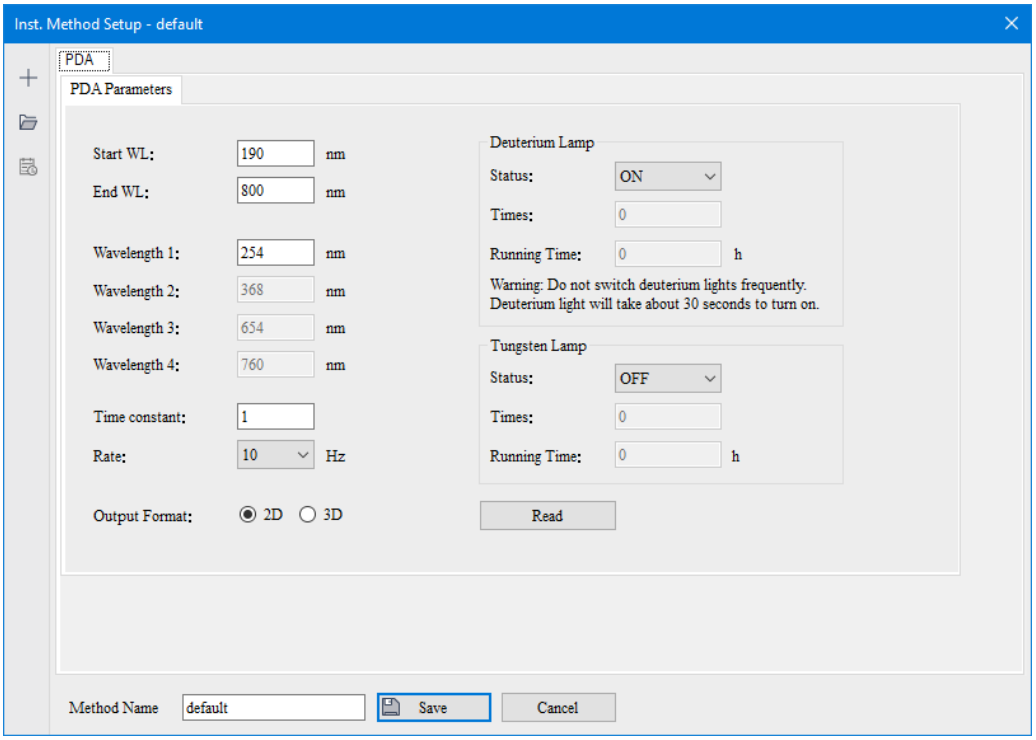


Figure 10-4 PDA Instrument Method interface

10.3 Data Analysis

10.3.1 2D Chromatogram Analysis (conventional chromatogram)

The chromatogram saved in the 2D chromatogram mode of the PDA is consistent with the conventional chromatogram, and the data processing method is also the same. Please refer to Chapter 6 for details.

10.3.2 3D Chromatogram Analysis (PDA Chromatogram interface)

(1) PDA Chromatogram Interface

Click button  in the status bar above the chromatogram of the "Data Processing Interface" to open the "PDA chromatogram" interface. The PDA chromatogram interface is shown in Figure 10-5. By default, only chromatogram, spectrum, contour line view and peak purity spectrum view are enabled in this interface.

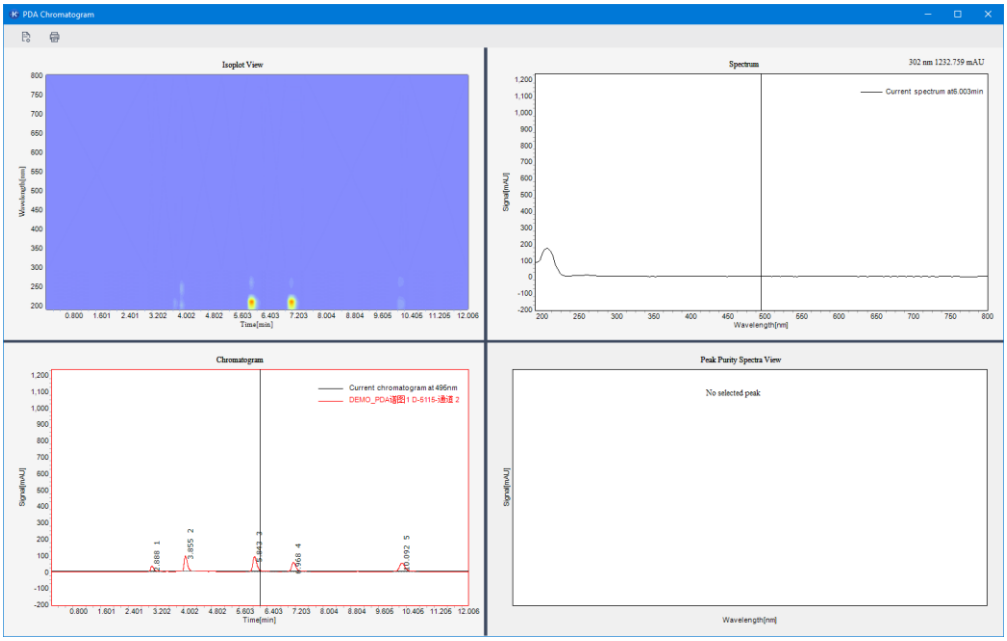


Figure 10-5 PDA Chromatogram Interface

(2) 3D View

Right-click in the "Chromatogram" area of the "PDA Chromatogram" interface - select "Show 3D View" to open the "3D View" interface (as shown in Figure 10-6). The 3D view interface is shown in Figure 10-7.

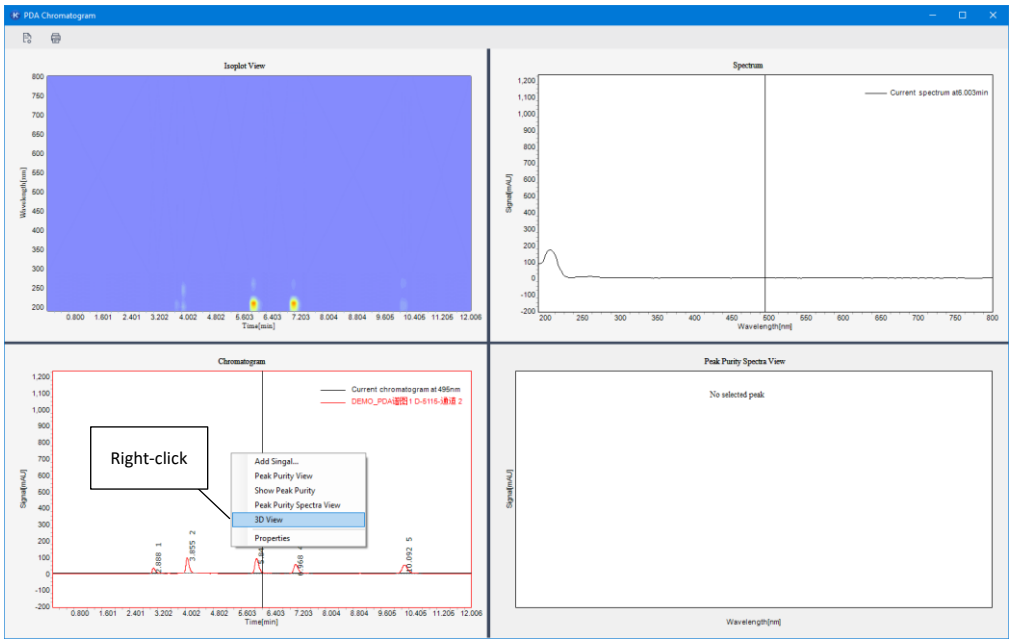


Figure 10-6 Open the 3D View Interface

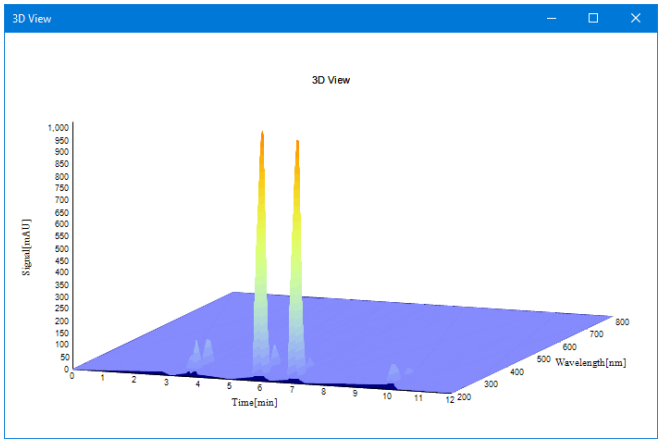


Figure 10-7 3D View Interface

(3) Peak Purity Spectrum View

Drag the marker line in the "Chromatogram" area to move the selected peak, then right-click in the "Chromatogram" area, and select Show Peak Purity Spectral View to open it.

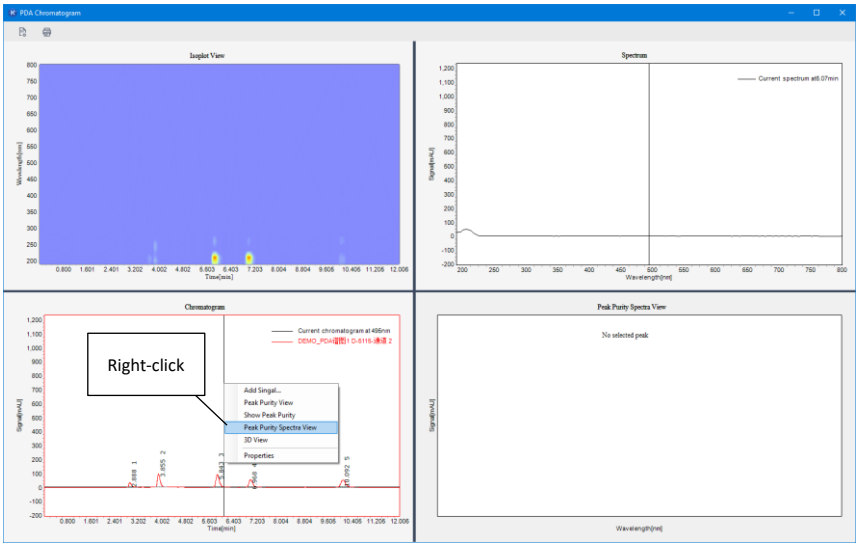


Figure 10-8 Display Peak Purity Spectra

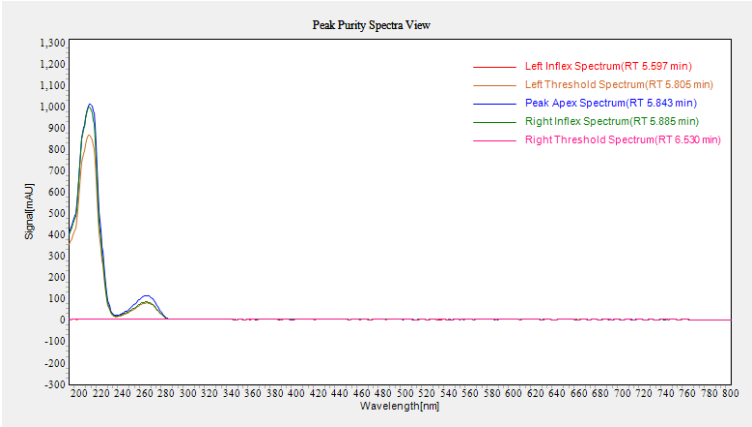


Figure 10-9 Peak Purity Spectrum

(4) Peak Purity Curve

Right-click in the "Chromatogram" area and select "Peak Purity Curve" to display the peak purity curve in the chromatogram, as shown in Figures 10-10 and 10-11.

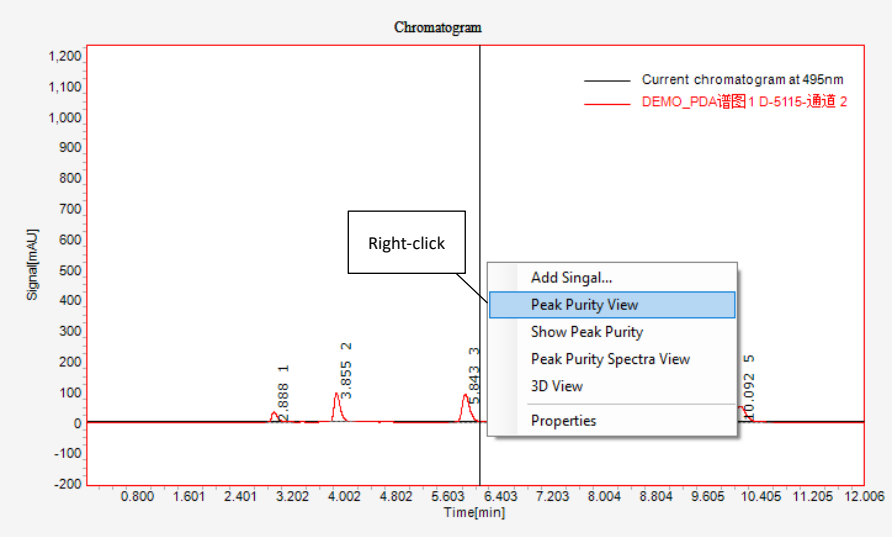


Figure 10-10 Display Peak Purity Curve

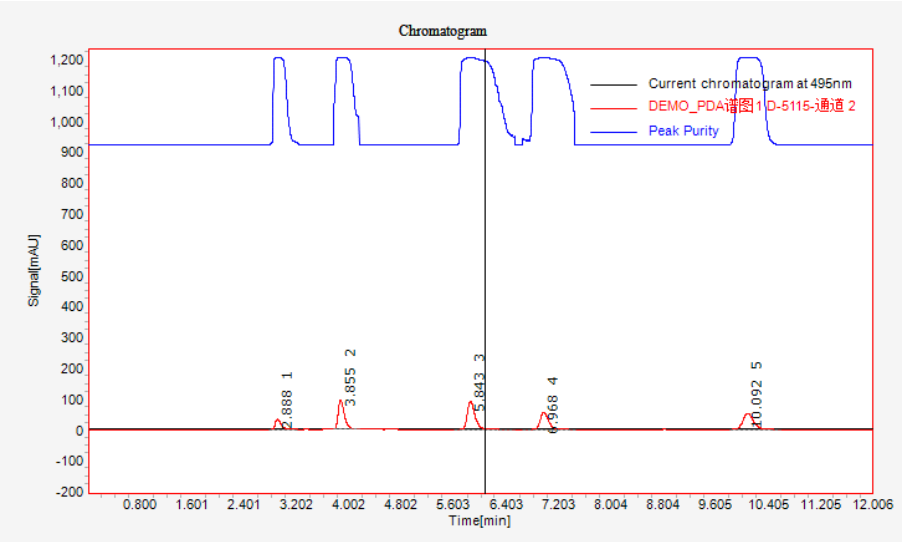


Figure 10-11 Peak Purity Curve

(5) Peak Purity View

Right-click in the "Chromatogram" area and select "Display Peak Purity" to pop up the "Peak Purity View" window, as shown in Figure 10-12.

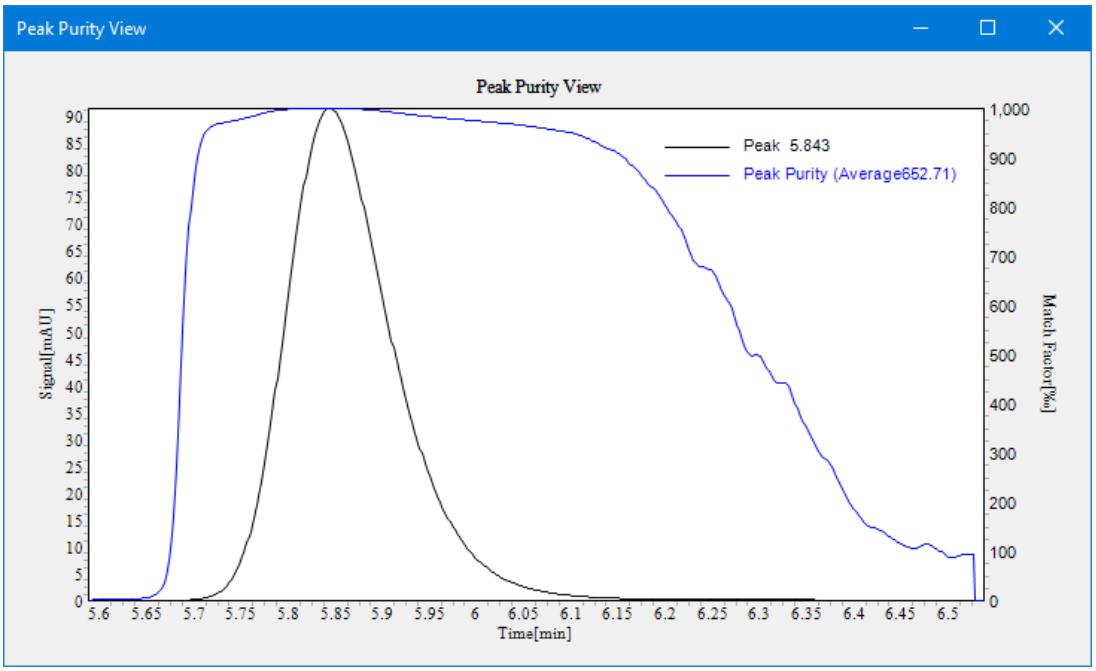


Figure 10-12 Peak Purity View

(6) Add Chromatogram Signal

Right-click in the "Chromatogram" area and select "Add Signal" to pop up the "Add Chromatogram Signal" window, as shown in Figure 10-13. In this window, you can set the wavelength, reference, etc. to increase the chromatogram.

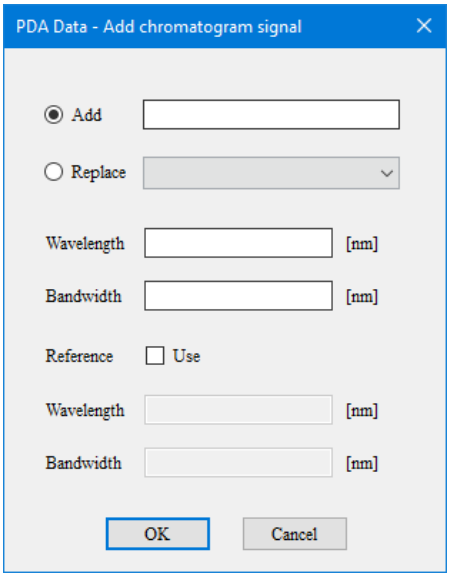


Figure 10-13 Add Chromatogram Signal

(7) Attributes

Right-click in the "Chromatogram" area or the "Spectrum" area, and select the "Attributes" option to pop up the "PDA Attributes" setting window, as shown in Figure 10-14. In this window, you can set the coordinate axis parameters, etc.

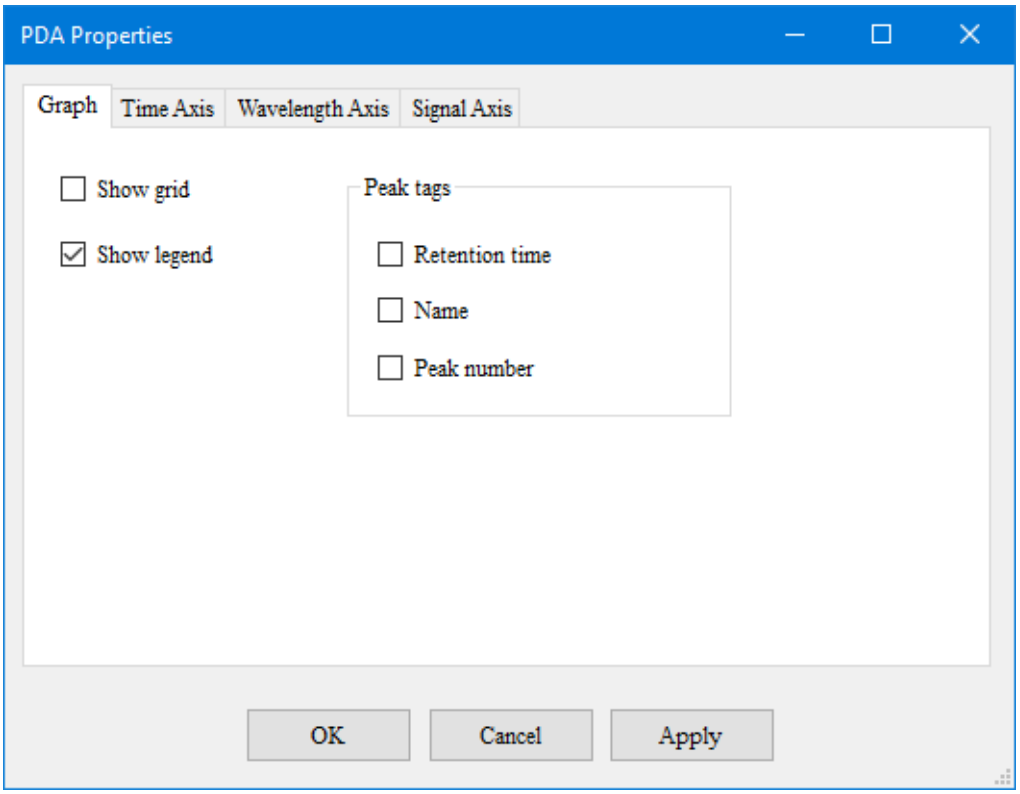


Figure 10-14 PDA Attributes window

(8) Spectral Library

Right-click in the "Spectrum" area, and the related options of the spectral library will pop up. Contains functions such as creating a new library, opening a library, adding to a library, and searching in a library. As shown in Figure 10-15.

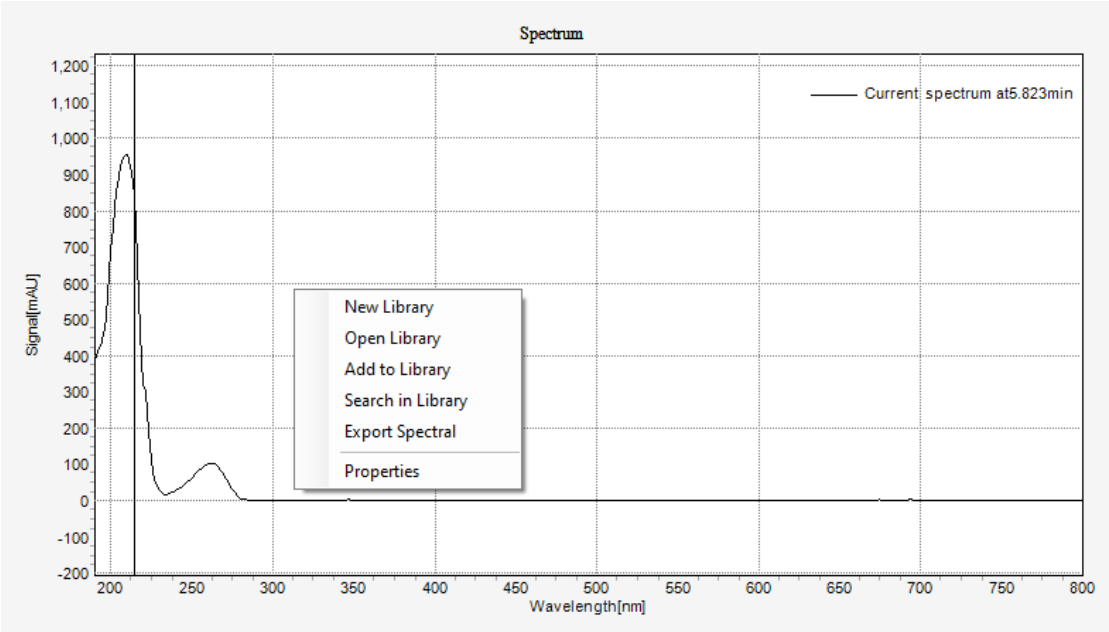


Figure 10-15 Right-click function in the "Spectrum" area

(9) Spectral Library View

This view displays spectral information for an open spectral library. Spectrum names and annotations can be changed in this view. If "Show Spectrum" is checked, the library spectrum and instant spectrum can be displayed in the spectrum view at the same time. As shown in Figure 10-16.

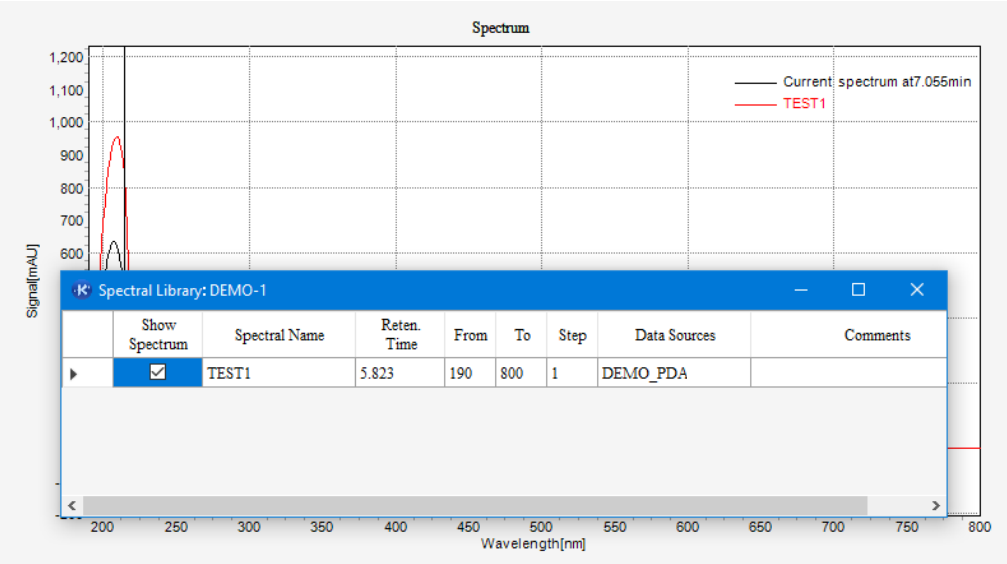


Figure 10-16 Spectral Library View

(10) Spectral Library Search Results View

This view displays the spectral library search results. As shown in Figure 10-17.

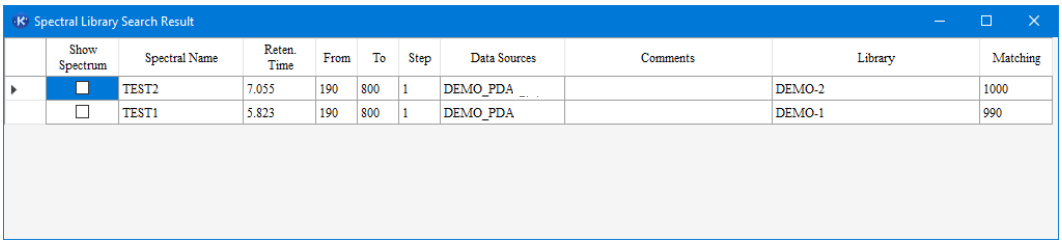


Figure 10-17 Spectral Library Search Results View

11 Report

11.1 Report Settings

Click icon  and set the report format and report content in the report setting window that pops up, as shown in Figure 8-1.

The set report format can be saved in the default way, and can be opened and recalled through the "Open" button when in use.

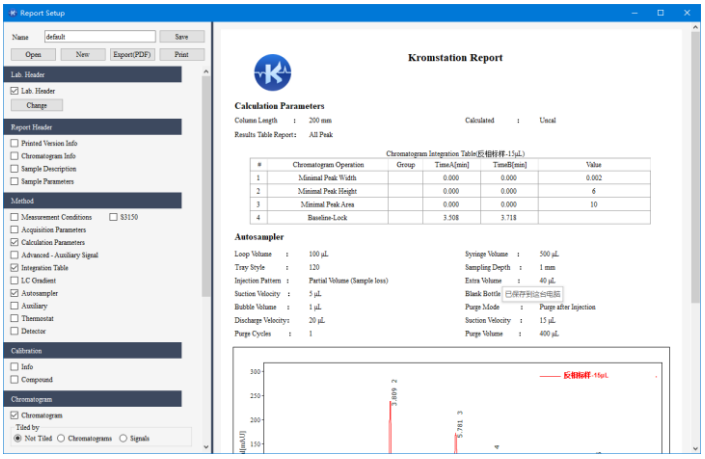


Figure 11-1 Report settings window

The header information of all tabs is on the left side of the report, and the options that need to be printed can be added by clicking  .

【Caution】

◆ When printing the report, the contents of each tab will be automatically printed in the order selected by the mouse.

11.2 Report Printing

- Original format printing: The report can be printed according to the set format. Click the "Print"

button in the report setting window to activate the window, select the printer, and click "OK" to print. As shown in Figure 8-2.

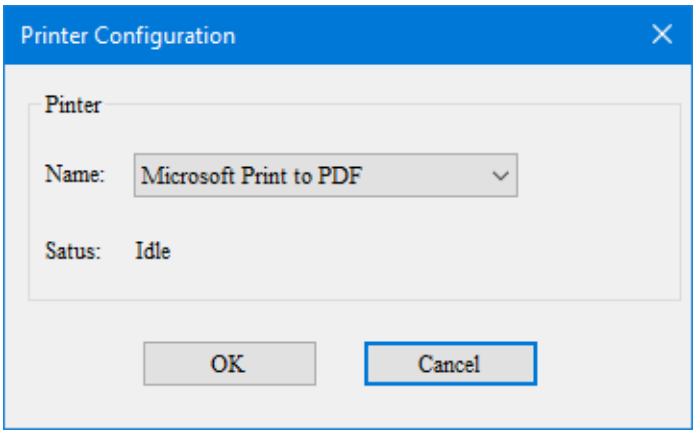


Figure 11-2 Print window

- Convert to "*.pdf" format and then print: Click "Export PDF", select the storage path, save as a PDF format file, and print the exported PDF format file, as shown in Figure 8-3.

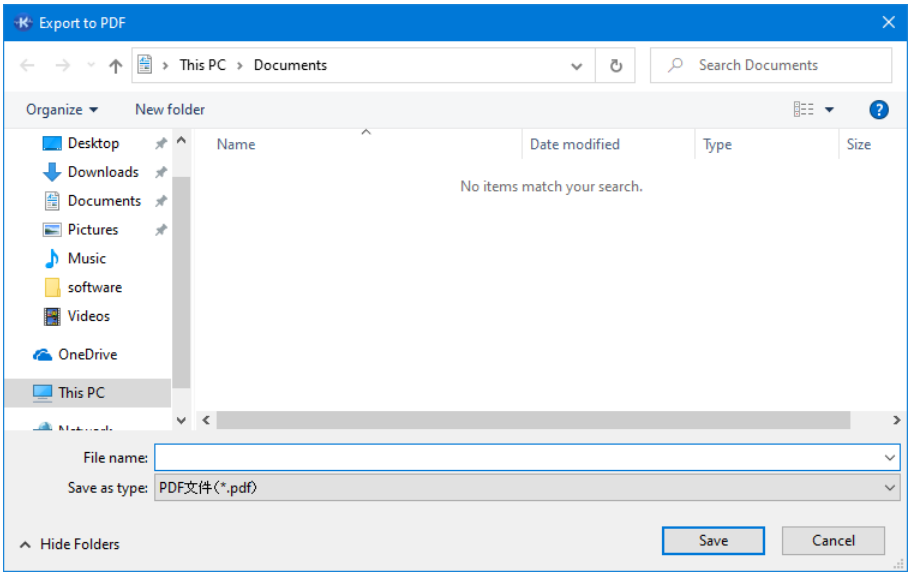


Figure 11-3 Export PDF window

12 Fault Diagnosis and Elimination

The iChrom5100 series chromatograph may have some failures during use. The software will give the user a prompt message to inform the user. The following are the codes and descriptions of the failures of each instrument.

12.1 Organizer Failure

Table 12-1 Organizer failure comparison table

Fault number	Fault description	Indicator status	Fault location
MB00	System CPU operation failure	----	Organizer Motherboard
MB01	EEPROM failure	----	Organizer Motherboard
MB02	8M crystal oscillator failure	----	Organizer Motherboard
MB03	crystal oscillator failure	----	Organizer Motherboard
MB04	Run failure	----	Organizer Motherboard
MB05	Leakage	Orange light flashes	Organizer Motherboard
MB06	Door is not closed	Blue light flashes	Organizer Motherboard
MB07	Degasser failure	----	Organizer Motherboard

12.2 High Pressure Constant current Pump Failure

Table 12-2 Pump fault comparison table

Fault number	Fault description	Indicator status	Fault location
PB00	System CPU operation failure	----	Pump Motherboard
PB01	EEPROM failure	----	Pump Motherboard
PB02	8M crystal oscillator failure	----	Pump Motherboard
PB03	32K crystal oscillator failure	----	Pump Motherboard
PB04	Run failure	----	Pump Motherboard
PB05	Leakage	Orange light flashes	Pump Motherboard
PB06	Door is not closed	Orange light flashes	Pump Motherboard
PS00	System CPU operation failure	----	Pump control board
PS01	EEPROM failure	----	Pump control board
PS02	8M crystal oscillator failure	----	Pump control board
PS03	Pump pressure exceeds upper limit	Orange light flashes	Pump control board
PS04	Pump pressure exceeds the lower limit	Orange light flashes	Pump control board
PS05	The pump body does not rotate	----	Pump control board

12.3 Column Thermostat Failure

Table 12-3 Column thermostat failure comparison table

Fault number	Fault description	Indicator status	Fault location
OB00	System CPU operation failure	----	Column thermostat motherboard
OB01	EEPROM failure	----	Column thermostat motherboard
OB02	8M crystal oscillator failure	----	Column thermostat motherboard
OB03	32K crystal oscillator failure	----	Column thermostat motherboard
OB04	Run failure	----	Column thermostat motherboard
OB05	Leakage	Orange light flashes	Column thermostat motherboard
OB06	Door is not closed	Orange light flashes	Column thermostat motherboard
OS00	System CPU operation failure	----	Column thermostat control board
OS01	EEPROM failure	----	Column thermostat control board
OS02	8M crystal oscillator failure	----	Column thermostat control board
OS03	Heating belt failure or Pt100 abnormal	----	Column thermostat control board
OS04	AC sampling no signal	----	Column thermostat control board
OS05	The temperature exceeds the maximum protection temperature	Orange light flashes	Column thermostat control board
OS06	The temperature is lower than the minimum protection temperature	Orange light flashes	Column thermostat control board

12.4 Detector Failure

Table 12-4 Detector fault comparison table

Fault number	Fault description	Indicator status	Fault location
DB00	System CPU operation failure	----	Detector motherboard
DB01	EEPROM failure	----	Detector motherboard
DB02	8M crystal oscillator failure	----	Detector motherboard
DB03	32K crystal oscillator failure	----	Detector motherboard
DB04	Run failure	----	Detector motherboard
DB05	Leakage	Orange light flashes	Detector motherboard
DB06	Door is not closed	Orange light flashes	Detector control board
DS00	System CPU operation failure	----	Detector control board
DS01	EEPROM failure	----	Detector control board
DS02	8M crystal oscillator failure	----	Detector control board
DS03	Wavelength stepper motor failure	----	Detector control board
DS04	De-spectrum device failure	----	Detector control board
DS05	Deuterium lamp power failure	Orange light flashes	Detector control board
DS06	Wavelength calibration failed	Orange light flashes	Detector control board
DS07	Light intensity over range	Orange light flashes	Detector control board

Appendix

Security Information

- ***General safety information***

The following general safety precautions must be followed in all stages of instrument operation, maintenance and repair. Failure to follow the special warnings in other places in this manual will violate the safety standards for the design, manufacture and use of the instrument. The company will not bear any responsibility for the losses caused by the user's failure to comply with these requirements.

- ***safety standard***

This instrument is a Class I safety equipment (that is, a protective grounding terminal is provided), and it is manufactured and tested in accordance with national safety standards.

Safety Signs

Sign	Explanation
	For equipment marked with this mark, the user should refer to the instruction manual to avoid injury to the operator and damage to the instrument.
【Caution】	Warns you of conditions that can lead to injury or death. Unless you have fully understood and met the required conditions, do not operate beyond the warning range.
【Be Careful】	To warn you of situations that may cause data loss or equipment damage, do not operate beyond the scope of caution unless you have fully understood and met the required conditions.
【Note】	Remind you that the experimental data may be unsatisfactory or the instrument cannot work normally. Unless you have fully understood and met the required conditions, do not operate beyond the scope of attention.