PEAK-ABC

CHROMATOGRAPHY

DATA HANDLING

SYSTEM

USER MANUAL

PEAK-ABC System
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Chapter 1 Introduction

1.1 Forward

PEAK-ABC Chromatography Data Handling System is compatible with any model of chromatographic analytical instrument available in the market. Our state-of-the-art hardware comes in two different models, namely Single-channel and Dual-channel model. For Dual-channel model, while you can connect the two channels to the dual detectors of the same instrument for simultaneous data acquisition, you can also connect them to two different instruments for independent data acquisition.

This software system is structured in such a way that all the sequences related to an analysis (starting from acquisition of raw data signal, to integration of chromatogram, to calculation of components quantities, through to preparation of analysis report) are incorporated in one source document called Chromatogram file. By applying **Document window technique** and **Split window technique,** we have designed a one-page Document window for you to display all the working elements of a Chromatogram file in one screen for quick access and manipulation.

Each Document window consists of Six working tables and a Chromatogram frame. While the Chromatogram frame is to display the integrated chromatogram, the Six working tables are uniquely created for you to input and record the various settings to be applied at different stages of data handling. This structure offers you quick access when you need to view the integrated chromatogram; adjust the integration method; select and execute the quantifying method; and print the analysis report.

We also apply **Multi-tread technique** so that you can perform other functions within one Document window when acquisition of data is in process. For example, you are able to adjust the displaying parameter as and when chromatogram is being acquired.

To further improve the speed of operating this system, we apply **Multi-Document window technique** so that you can work with more than one Document window (or more than one analysis) at one time. This means that while you are acquiring a chromatogram in one Document window, you can re-process an acquired chromatogram in another Document window.

During the process of acquiring data signal, we apply our patented **Noise Filtering Method** to eliminate noise so that we can detect the weakest level of signal close to Baseline noise. During chromatogram integration, system would select the most appropriate integration method to process every detected peak. Setting of many complicated processing parameters has been intelligently automated in such a way that the number of parameters that you need to adjust is substantially minimized to only one in most of the cases. Should you need to change the integration method of a peak or a certain segment of a chromatogram, you can conveniently effect the change by making only a few clicks on the mouse.

During Quantitative analysis, you can efficiently calculate the calibrators (or average calibrators) or construct calibration curves and conveniently apply them to quantify the components quantities of unknown sample.

During the process of preparing the analysis report, you can opt to include or exclude certain system-calculated results as well as to input external reference information to be included in the analysis report. Such reference information would be permanently stored among other elements of an analysis for future reference in compliance with GLP requirements.

Apart from the Document window and the Six working tables, other unique features of this software system are the Save template command, the Chromatograms compiler and the Results tables compiler. Try to make full use of the Save template command when filling in the Six working tables. The Chromatograms compiler and Results tables compiler enable you to calculate average components, compare; contrast; and overlay past analysis results in various combinations. Armed with PEAK-ABC software system, you are certainly on the way to excellent performance.

1.2 Terms of reference

	Terms	Descriptions
1	Standard sample	Standard sample containing component (s) with known quantity is for you to calculate calibrator (s) or construct calibration curve (s) of component s).
2	Unknown sample	Unknown sample is a sample obtained from the unknown object for qualitative and quantitative analysis. The purpose of a qualitative analysis is to detect whether it contains certain components, while the purpose of a quantitative analysis is to measure the quantities of the components in % terms or in absolute terms.
3	Internal standard	Internal standard is a component with known quantity. It is added to both the Standard sample and Unknown sample in equal or unequal proportion. The purpose is to eliminate possible distortions caused by differences in the dosage of injection. This software is able to handle the case when more than one type of Internal standard is added to a sample to be applied to different group of components.
4	Calibrator	The peak area corresponding to a same component may defer if the injection is made in different chromatographic instruments. The purpose of calibrator is to eliminate such differences by expressing component quantity (of Standard sample) in terms of per unit of peak area corresponding to that component.
5	Average calibrator	A series of injections of Standard samples with **identical or almost identical** component quantity are needed to obtain the average calibrator of a component. The series of samples may be added with or without Internal standard.
6	Calibration curve	A series of injections of Standard samples with **different** component quantity are needed to construct a calibration curve of a component. The series of samples may be added with or without Internal standard.
7	Patented Noise-Filtering Method	This method can eliminate noise so that we can detect the weakest level of signal close to Baseline noise. This technique --- High-Fidelity Filter Based on Medians for Chemograms, was published in Volume 35, page 435 to 438 of the renowned Journal of Chromatographic Science in 1997.

8	Document window / Chromatogram file	This system software is structured in such a way that all the working elements of an analysis are compactly stored in one source document called Chromatogram file. By applying **Document window technique** and **Split window technique,** a one-page Document window is designed to display all the working elements of a Chromatogram file in one screen for quick access and manipulation. Every time you start this software system, a new Document window (representing a new Chromatogram file) would be created ready for data acquisition. When you effect a Save command, this Document window would be saved into a corresponding Chromatogram file. When you need to access and manipulate an existing Chromatogram file, you must first display it in the form of a Document window. Please refer to Section 4.1.4.4.4 (Option, Naming) for more detail about naming of Document window. Please also refer to Section 4.1.1.7 (Save template) and 4.1.1.8 (Load template) for more detail about loading common setting from an existing Chromatogram file to another (new or existing) Chromatogram file.
9	Six working tables	Each Document window consists of Six working tables and a Chromatogram frame. While the Chromatogram frame is to display the chromatogram, the Six working tables are uniquely created for you to input and record the various setting applicable at different stages of data handling process. They are arranged in tab form so that you can move quickly from table to another table.
10	Multi-Document window / Active Document window	This system applies Multi-Document window technique so that you can work with more than one Chromatogram files at one time. When you are working with more than one Chromatogram files there would be more than one corresponding Document window being displayed and only one of them is responsive to the various commands found on the Tool Bar. It is called Active Document window. You can click on any of the Document windows to activate it to be the Active Document window.
11	Default Template	When analyzing parallel samples (samples of similar mixture), some elements of the Chromatogram file (contained in the working tables) may also be applicable for subsequent injections. While the Save template command is for you to create a Template file, the Load template command is for you to copy its contents from one Chromatogram file to another Chromatogram file. A typical Template file contains the common settings made in five working tables namely the Acquisition table, Integration table, Component table, Calculation table and Report table. Please refer to Section 4.1.1.7 for more detail explanation about Default template file and Normal template file.
12	Default integration / Manual integration	As and when data signal is being acquired, the system would apply its intelligent noise filtering method to eliminate noise and proceed to integrate the chromatogram. For each detected peak, the system would first search the Integration table for any integration method that have been input prior to activating the acquisition command. If there is no pre-acquisition input of integration method, the system would automatically select an integration method to process the peak. This process is referred to as **Default integration**. If you are not happy with the integration method selected by Default integration, you can change the integration method by applying **Manual integration**.

Chapter 2 Packaging

2.1 Hardware

A complete set of PEAK-ABC system consists of:

i) 1 x CD-ROM

ii) 1 x Chromatogram acquisition Unit (Single-channel or Dual-channel)

iii) 1 x Power Supply Cable

iv) 1 x Digital Cable

v) 1 x Signal Cables with remote starter buttons for Single-channel Model or

2 x Signal Cables with remote starter buttons for Dual-channel Model.

2.1.1 Connecting to the chromatographic instrument

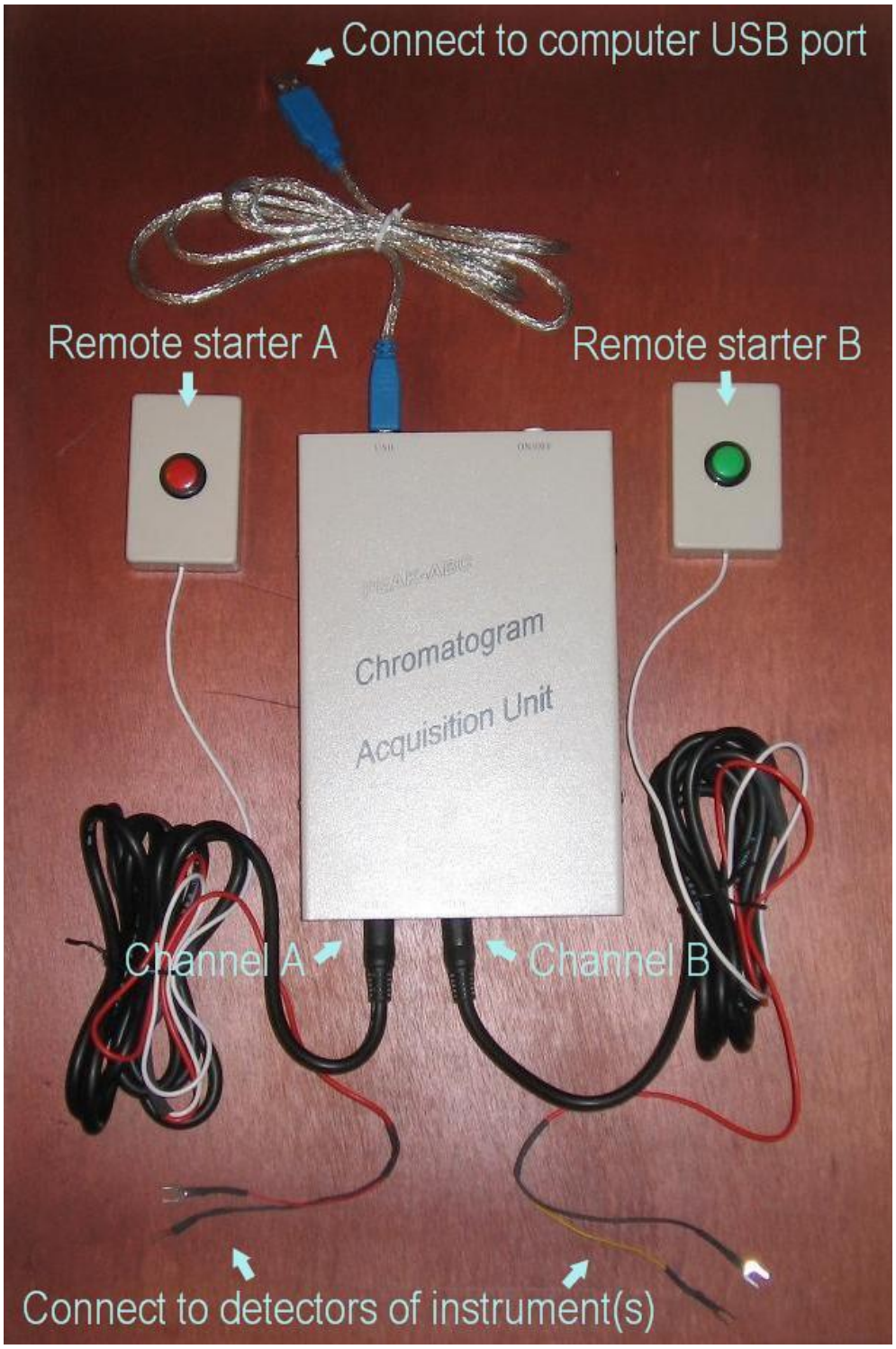
The two sets of signal cable (Channel A and Channel B) are for you to connect to the detectors of the chromatographic instrument. You can either connect them to the dual detectors of the same chromatographic instrument or connect one each to the detector of two different chromatographic instruments. Connect the white (or yellow) wire to the +ve end. Connect the black wire to the –ve end.

2.1.2 Connect to receive Start / Run signal of the instrument

The Remote starter is for you to connect to the instrument or the Auto-sampler to receive Start/Run signal. Cut off the remote starter button to expose two wires. Connect the two ends of the wires to the Start/Run button of the instrument or the Start/Out button of the Auto-sampler. This would automate the command to Start acquiring every time an injection is effected.

If you are unable to connect the Remote starter to the instrument or the Auto-sampler, you can leave the button intact and use it as an alternative to execute the command to Start acquiring. Simply press once on the remote starter after injecting a sample to activate the software system to start acquiring data signal. Please refer to Section 4.1.3.1 for other ways of executing the command.

2.1.3 Hardware layout



2.2 Hardware Specifications

- Single-channel Model to be installed inside the computer. Connect to the computer by Serial Port Digital Cable and connect to the instrument by Signal cable.
- Dual-channel Model to be externally attached to the computer. Connect to the computer by Serial Port Digital Cable or USB Port Digital Cable for independent or simultaneous (synchronize or asynchronize) data signal acquisition.
- 24-bit high precision Analog / Digital conversion device;
- Input bipolar range +/- 1V; +/- 2V; +/- 3V; +/- 5V;
- Input resistance：＞10MΩ;
- Linearity +/- 0.005% of full scale;
- Acquisition speed ：Programmable at 1, 2, 5, 10, 20. Max of 60Hz is available upon request before delivery;
- Integration sensitivity：0.05μV・s;
- Consistency：±0.005%;
- Minimum resolution：1μV;
- Accuracy more than 22 bit at 60Hz;
- Equipped with a remote starter to be connected to analytical instrument or Auto-sampler to receive the Start signal and Stop signal.

2.3 Computer Requirements

- Intel Pentium 100 or better;
- CD ROM driver for installation of software;
- VGA graphics adapter (800x600 or higher resolution is recommended);
- 16M of RAM (32M or higher is better);
- 30M free space on hard disk drive for software installation;
- Free COM-port in the case of ADC device;
- For Single-channel Model, one spare PCI slot on the motherboard for ADC card;
- Parallel port or USB Port for security key;

- Installed with Windows 95, 98, ME, NT or 2000, XP.

2.4 Software Installation

2.4.1 Procedures of installing the software of PEAK-ABC System

1. Disconnect the printer from the computer to be installed with the software.

2. Attached the Chromatogram Acquisition unit to the COM port. (Don't connect the Security devise at this step. Please refer to Section 2.2.2 for detail procedures of installing security device.)

3. Insert the installation CD into the CD-ROM drive of your computer and follow the instructions in the setup program to finish the installation. Simply click on "Next" button during installation.

4. If your software does not load automatically, please follow the following instructions:

   i) Select "START" and then "RUN" from the Taskbar.

   ii) Enter "D\SETUP.EXE" and then click "OK" (Replace "D" with the letter of your CD drive if it is different.)

   iii) Follow the instructions in the setup program to finish the installation.

5. If you are running PEAK-ABC under Windows 95/98 infected with virus, you would encounter a blue screen protection error. When this happens, you can take one or both the following steps:

   a) Scan and delete the infected virus by using a virus scanning and deleting software;

   b) Delete the two possible conflicting files "gsdog.vxd" and "host95.vxd" found under C:\Windows\system.

6. If you are running PEAK-ABC under C:\Window2000/XP, you may encounter the message that "Unable to detect the hardware – Chromatogram Acquisition unit". This could be due to that the hardware is wrongly detected to be a mouse similar to that of "Microsoft Serial Ballpoint". Simply proceed to deactivate this mouse -- Microsoft Serial Ballpoint by accessing the Hardware manager within the Control Panel.

Tips 1 : Should you be prompted the message that "Encounter errors while copying files", proceed to close / exit all the other Windows program(s) especially the real time virus scan program before you proceed to restart the installation procedures.

Tips 2 : Should the virus scan program is activated while installation is in process, some of the PEAK-ABC files containing Macro program may be "suspected" as infected with virus. Simply click on "No" if you are prompted whether to delete such files.

Tips 3 : If your computer is not installed with a CD-ROM drive, or the CD-ROM drive is faulty, proceed as follow:

1. You may first install the software in another computer and make use of a floppy diskette to copy the following files from the first computer to the other computer:

    1) All the files (hw*.*) found under hw\program directory;

    2) All the six files hwnormal.dot, ss32x25.ocx, netcdf.dll, cj60lib.dl, mfc42.dll, and msvcrt.dll found under C:\Window\system if your computer is installed with Windows9x or found under Windows\System32 if your computer is installed with Windows NT/2K/XP.

2 Proceed to run the command: regsvr32 windows\system\ss32x25.ocx by accessing the "START", "RUN" from the Taskbar.

3 The final step is to copy the two files "doginst.exe" and "dogsetup.dll" from the root directory of the CD to the floppy dice and run the "doginst.exe" in the second computer by double clicking on this file.

2.4.2 Procedures of installing security device

1. Upon successful installation of the software, switch off the printer and the computer. Connect the security devise to the computer. Restart the computer and click on the PEAK-ABC icon to start the system.

2. For Printer port security device, simply attached it to the printer port as adapter to connect the printer cable from the computer to the printer.

3. For USB port security device **(applicable for Window 98b or higher)**, please follow the following steps:

    1) Insert the USB security device to any USB port found on the computer. (Should the security device be installed before installing the software, system would proceed to automate the hardware detecting function which should be terminated at once and proceed to install the software of PEAK-ABC system.)

    2) If you are running PEAK-ABC under Window XP system, double click on the icon found in the bottom right corner of the computer screen to access the new hardware dialogue frame to begin:
        i) Select "to install from list or designated list", click once on the "Next" button
        ii) Within the new dialogue frame, select "do not search, I would select the program to be installed", click once on the "Next" button
        iii) Within the new dialogue frame, select "RC：USBC WDM driver"， click once on the "Next" button
        iv) Within the new dialogue frame, select "Continue" to complete the installation process.

    3) Reboot your computer.

Tips 1 : Inserting the USB security device gently to avoid damaging the connecting port.

Tips 2 : Check to ensure that the USB port is set as "ENABLE" in BIOS.

Tips 3 : Should you encounter problem while trying to install USB security device, look for "RC：

USBC WDM driver, free build device" found under the Device Manager. If there is a problem, it would be added with the sign "!". Proceed to reinstall the Security device.

Tips 4 : Printer port security device is applicable for Windows NT4.0 or older version, as well as Windows 95. This is because such applications do not support the use of USB device.

Tips 5 : Should you encounter any problem, try step one first, if need be, try step two:
Step one : Switch off the printer, detach the printer from the parallel key. Restart the system when the parallel key is attached to the serial port.

Step two : Go the root director of the CD ROM, run the "doginst.exe" once. This is a silent step so you don't get to see any response.

2.5 Services and Warranty

- We provide one-year warranty for the Signal Acquiring Unit. We have established a stringent quality control checking before we shipped out any Signal Acquiring Unit. In the unlikely event of faulty Signal Acquiring Unit, replacement would be made with the nearby agent or by courier.

- For the software, we provide free upgrading and free update at regular interval. You can either download from our website or by way of floppy disk or CD ROM upon request.

- We promise to keep all our users informed of any upgrading on a regularly basis. Please make it a point to visit our web-site at http://www.PEAK-ABC.com regularly.

- If you need us to amend the program to meet your requirement, we would oblige on best effort basis. In the course of using PEAK-ABC, please provide us with instant feedback on our strength and weakness for our continuous improvement. Please direct your feedback to mhjhwj@jlonline.com.

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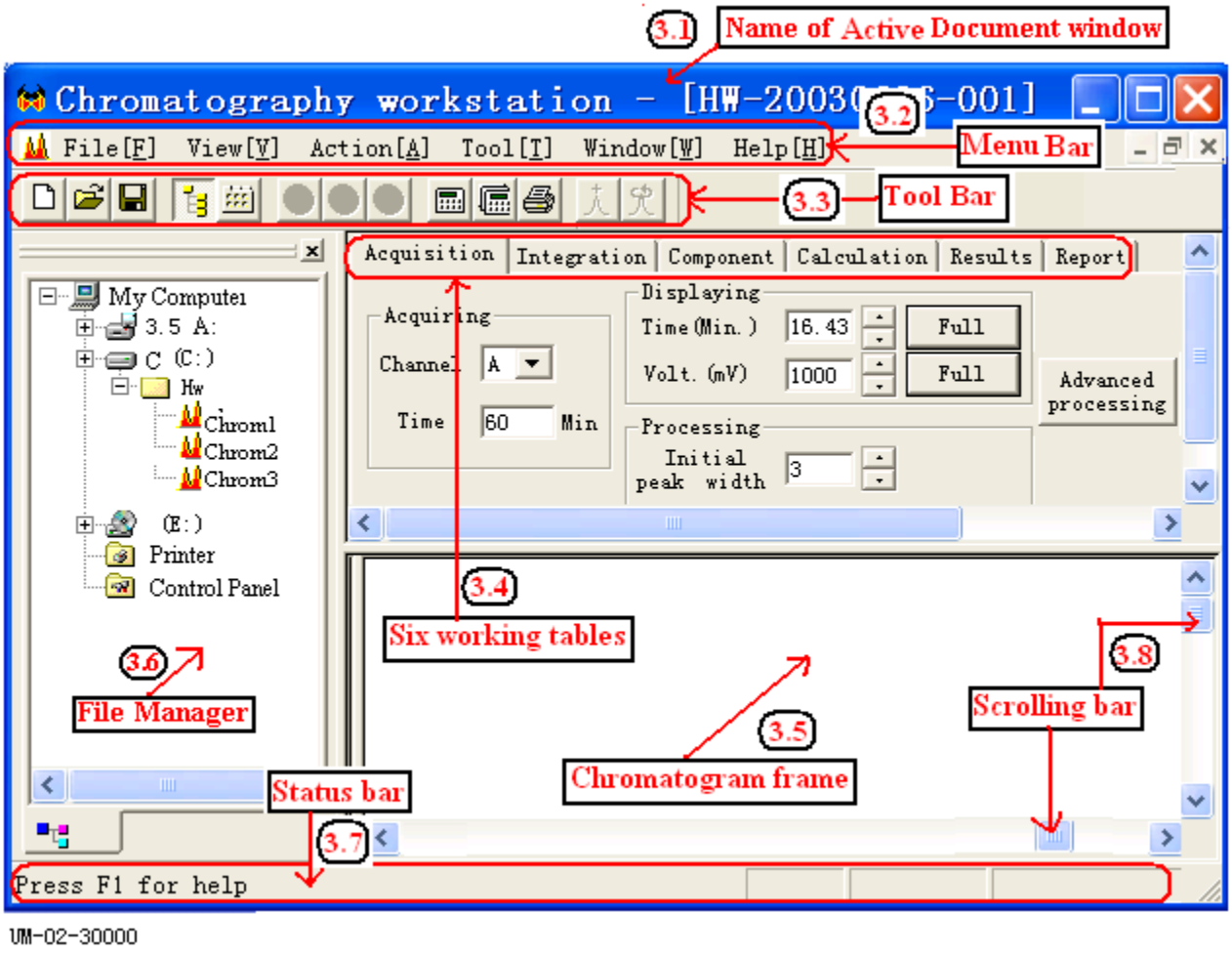
Chapter 3 Menu Structure



UM-02-30000

3.1 Name of Active Chromatogram file

This field displays the name of Active Document window. Remember that when you need to access and manipulate a Chromatogram file, you must fist display it in the form of a Document window. Thus, if you are working with more than one Chromatogram files, there would be more than one corresponding Document windows being displayed and only one of them is active and responsive to the various commands found on the Tool Bar. It is referred to as the Active Document window. You can click on any of the Document windows to activate it to be the Active Document window. Please refer to Section 1.2 (Terms of reference) for more information about Chromatogram file and Document window. Please also refer to Section 4.1.4.4.4 (Option, Naming) for more detail about changing the naming structure and Section 4.1.5.4 (Window, 1,2,3… Command) for another way to activate a Document window to be Active Document window.

3.2 Menu Bar

There are six menu headings on the Menu Bar, namely File[F], View[V], Action[A], Tool[T], Window[W] and Help[H]. Please refer to Chapter 4 for more detail explanation about the lists of command found under the various headings.

3.3 Tool Bar

Some of the frequently used commands have been incorporated into Tool Bar for your convenience. Please refer to Section 4.1.2.1 (Tool Bar) for more information about the commands found on Tool Bar.

3.4 The Six Working Tables

These Six working tables form one of the unique features of PEAK-ABC. Please refer to Chapter 5 to find out how to make use of these Six working tables during acquisition of chromatogram, quantification of components quantities and preparation of analysis report.

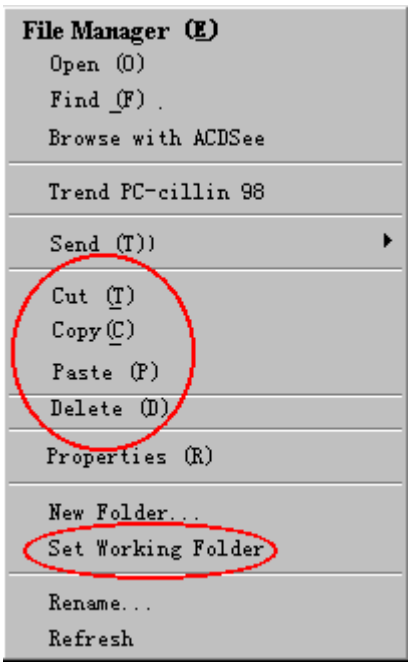
3.5 The Chromatogram Frame

This frame displays the acquired chromatogram on a real time basis as and when data signal is being acquired. Please refer to Section 4.1.4.4.3 (Option, Screen) to see how to change the background setting of this chromatogram frame.

3.6 File Manager

This File Manager displays the entire file structure of the computer including the Chromatogram files and their respective file folder. Click once within any spot of the File Manager, the file folder that is being highlighted in blue is the Working folder (Section 4.1.1.10). You can click on the icon  on the Tool Bar (Section 4.1.2.1) to hide or display the File Manager if need be.

You can double click on the filename or the icon to retrieve and display a Chromatogram file into Active Document window. You can also right click on the mouse to copy, to delete, or rename a Chromatogram file. This right click menu also contains a command for you to designate a Working folder as explained in Section 4.1.1.10.



3.7 The Status Bar

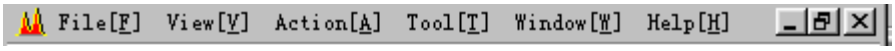
This Status bar serves two functions. While the first is the traditional function of displaying a short description of the command that is being selected, the second is to display the status of chromatogram acquisition. Please refer to Section 4.1.2.2 (View, Status Bar) for more information.

3.8 Scrolling Bars

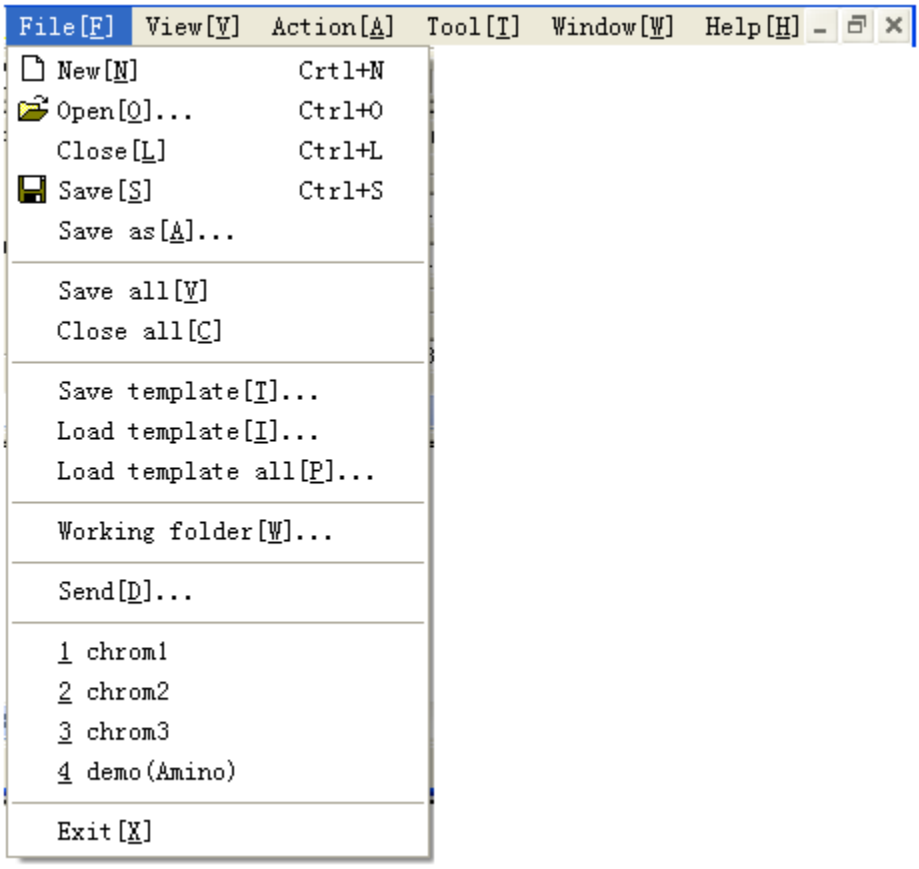
The two Scrolling bars located to the left hand bottom of the chromatogram frame are for you to scroll to view the screen content. One way of scrolling is to use the mouse to drag the bar. Another way is by clicking on the arrows located at the tips of the two scrolling bars.

Chapter 4 Menu Structure In Detail

4.1 Menu Bar



4.1.1 File Menu heading



UM-04-411000

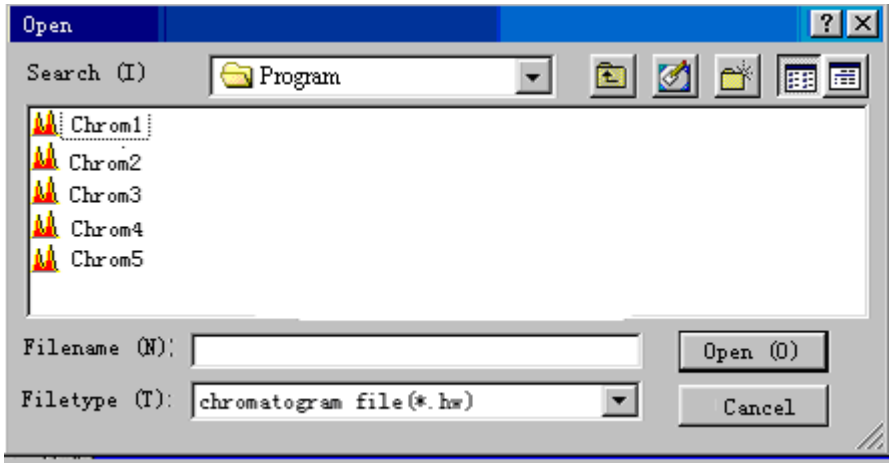
4.1.1.1 New

This is to open a new Document window, which is the first step to get ready for data acquisition. When you effect a Save command, the content acquired or input in this Document window would be saved as a corresponding Chromatogram file. Please refer to Section 4.1.4.4.4 (Option, Naming) for more detail about naming of Document window. Please also refer to Section 4.1.1.7 (Save template) and 4.1.1.8 (Load

template) for more detail about loading common setting from an existing Chromatogram file to a newly created Document window.

4.1.1.2 Open

This command is to retrieve and display an existing Chromatogram file in the form of Document window. Click on it to view a dialogue frame. Please refer to Section 4.1.5 (Window) for more detail about the various ways of displaying a few Document windows.



4.1.1.2.1 Search

This is to locate the file folder of interest.

4.1.1.2.2 Table of existing file

This table contains all the Chromatogram files stored under the selected file folder. You may click on the  icon located on the top right corner of this dialogue frame to view more information about each Chromatogram file, such as the size of file, the date of last amendment etc.

If you wish to select and open a few Chromatogram files at the same time, just press and hold on to the Ctrl key before you click on each of their filename to select.

You can also select a group of Chromatogram files in the same way before you right click on the mouse to copy or delete them. You can also make use of the sort function to sort the files in the file folder if and when necessary.

4.1.1.2.3 Filename

This field displays the name of Chromatogram file selected by you. If a group of files were selected, their filenames would appear in the reverse sequence of selection.

4.1.1.2.4 File type

This field displays the file type, which is being set to Chromatogram file by default. Template file and chromatogram raw data are the other two file types.

4.1.1.3 Close

This command is to close the Active Document window without closing the software system. You would be prompted to save all amendments that have not been saved before closing.

4.1.1.4 Save

This command is for you to continue working after saving the Active Document window into a Chromatogram file in a name corresponding to the name of Active Document window. A dialogue frame similar to that of Section 4.1.1.2 (Open) would be displayed. The file folder would be defaulted to be the Working folder, the filename would be defaulted to be the name of the Active Document window and the file type would be defaulted to be Chromatogram file. Simply click on 

icon to confirm. Please refer to Section 4.1.1.5 (Save as) for more detail about saving the Active Document window under a name different from the name of Active Document window.

4.1.1.5 Save as

This command is for you to continue working after saving the Active Document window into a Chromatogram file under a name different from name of the Active Document window. The name and content of the original Document window would remain unchanged. A dialogue frame similar to that of Section 4.1.1.2 (Open) would be displayed. Proceed to key in the desire file folder and filename as appropriate. The file type is being set to chromatogram file by default.

4.1.1.6 Save all / Close all

This command is for you to save (and close all) the Document windows (that are on display) into corresponding Chromatogram files under the respective names of the Document windows.

4.1.1.7 Save template

When analyzing samples of similar nature, some settings made in the Six working tables are common settings that would also be applicable for subsequent analysis or injections. This Save template command is for you to save the common settings in a template file. The Load template command (Section 4.1.1.8) is for you to retrieve and load the common settings into selected Document window corresponding to a new chromatogram file or an existing chromatogram file.

As explained in Section 1.2 (Terms of reference), Template file contains the common settings made in five working tables namely the Acquisition table, Integration table, Component table, Calculation table and Report table. You may note that Results table is excluded from Template file because its content is unique to each injection.

When activated, a dialogue frame similar to that of Section 4.1.1.2 (Open) would be displayed for you to select the file folder and key in the filename of this template. The file type is being set to template file by default. It is important for you to note that there are two types of template files namely, **Default template file** and **Normal template file**. While you can create only one **Default template file** under each file folder, you can create more than one **Normal template file** under the same file folder.

To create the one and only one **Default template file** under a given file folder, simply key in "default" as the filename, the file type is set to template file by default. A file under the name of "Default.tab" would be created. In so doing, every time you open a new Document window under the given file folder, the common settings would automatically be included in the five working tables ready for your use.

To create a **Normal template file** under a given file folder, key in "abc" as the file name. A file under the name of "abc.tab" would be created. As you can create more than one **Normal template file** under a given file folder, you would have to activate the *Load template* or *Load template all* command to retrieve the desire template file to be loaded in the selected Document window. Please refer to Section 4.1.1.8 (Load template) and 4.1.1.9 (Load template all) for more information about the two commands.

Knowing that each given file folder can have only one Default template and that the content of the Default template would automatically be included in the new Document window created under the file folder which is set as Working folder, you may want to create different file folder for analysis of different mixtures so as to take full advantage of this default loading function.

4.1.1.8 Load template

This command is for you to retrieve **Normal template file** into a new Document window as explained in Section 4.1.1.7 (Save template). This Load template command can be activated before or after acquisition of chromatogram. Pre-acquisition inputs would be applied by the system on a real time basis as and when data signal is being acquired. Post-acquisition inputs would only be applied by the system after activating the Re-integrating command (Section 4.1.3.3) or Calculating command (Section 4.1.3.4).

4.1.1.9 Load template all

This command is for you to retrieve a particular **Normal template file** to more than one Document windows at one go. Please refer to Section 4.1.1.7 (Save template) for more detail about the command.

4.1.1.10 Working folder

Working folder is the file folder designated by you to be the default file folder to store newly created Chromatogram file. The concept of Working folder is important if you wish to make use of the automation feature of this software system. This command is for you designate a file folder to be the Working folder and to check the name of Active Working folder. Please note that the Working folder at which you exit the system would be the file folder to start when you next start the system.

Please also refer to Section 4.1.4.4.1 (Options, General, Channel A/B files are saved to folder) for more information about designating different Working folder for the two Channels. The setting made under Section 4.1.4.4.1 would supersede the setting made under this section. That is to say that if you have specified a Working folder for Channel A by using Section 4.1.4.4.1, all the Chromatogram files corresponding to Channel A would be saved under that Working folder even though you may have made use of Section 4.1.1.10 or Section 3.6 (File Manager) to designate another file folder to be the Working folder.

Please refer to Section 3.6 (File Manager) for an alternative way of checking the status of **Working folder** and designating a file folder to be **Working folder**.

4.1.1.11 Send

This is to send out the Active Document window as an attachment by e-mail. Should you encounter any problem at various stage of analysis, you are encouraged to send the relevant chromatogram file to us for our immediate attention.

4.1.1.12 File 1, 2, 3, 4

This field displays the name of four Chromatogram files, which you have worked with most recently. You can click on any one of them to open the Chromatogram file (into Document window) quickly.

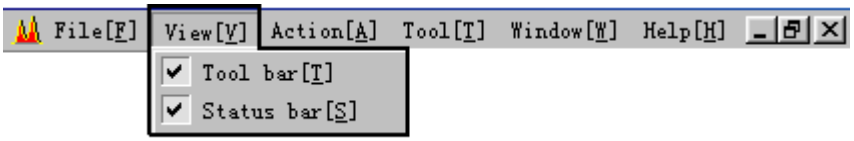
4.1.1.13 Exit

This command is for you to exit the software system. You would be prompted to save all the unsaved amendments made to the Document windows before you exit PEAK-ABC. If you opt for "No", all amendments that have been made would be lost. If you are working with a Document window that has not been saved before and opt for "Yes", a dialogue frame similar to that of Section 4.1.1.2 (Open) would be displayed for you to input the file folder, the filename(s) of the Chromatogram file(s). If you are working with existing Chromatogram files and opt for "Yes", all unsaved amendments would be saved under their respective filenames.

You can also click on the  located on the top right corner of the screen to exit the system in the same way as per the Exit command mentioned above.

Please note that the Working folder at which you exit would be the file folder to start when you next start the system.

4.1.2 View Menu heading



4.1.2.1 Tool Bar

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This command is for you to display or hide the Tool Bar. Some of the commonly used commands have been incorporated in the Tool Bar for your convenience. If you are not sure about the meaning of any one of the icons, position the cursor on the icon for about one to two seconds to view the short descriptions about that particular icon.

When a command is inhabited under certain condition, the color of the icons would be dimmer. The first three commands are found under Section 4.1.1 (File menu heading), the fourth command is for you to display or hide the File manager as explained in Section 3.6, the fifth icon is for you to access the Option command as explained in Section 4.1.4.4 (Tool menu heading), the next eight commands are found under Section 4.1.3 (Action menu heading). The last two commands are explained in Section 4.1.2.1.1 and Section 4.1.2.1.2.

4.1.2.1.1 To manually mark a non-detected peak

This icon  is for you to manually mark a non-detected peak (i.e when a peak is present but not detected by the system). You should first click on this icon to see a "+" around the cursor. Move the cursor to the spot deemed to be the Start point of the peak, click and drag the cursor to the spot deemed to be the End point of the peak and release the click. Click on the icon again to exit from this command.

You can make use of this command to split a peak into two by first clicking on the icon. After which, move the cursor to the peak top to click and draw a vertical line from peak top to the Baseline. If the peak was designated to be a tailing peak earlier, instead of drawing a vertical line, a tangent line may be drawn to split the peak as tailing peak. Remember to click on the icon again to exit from this command.

Should you wish to undo or reverse the manual marking, simply click on the Re-integrating icon  to reinstate the original chromatogram.

Please note that no corresponding record would be captured in the Integration table for this command. Thus, this setting would not be captured and loaded by the Save template command.

4.1.2.1.2 To ignore a detected peak and reverse the command

This icon  is for you to manually ignore a detected peak and to restore the ignored peak. You should first click on the icon to see a small circle around the cursor. Move the cursor to the center of the peak concerned and click once to ignore the detected peak. Click on the icon again to exit from this command.

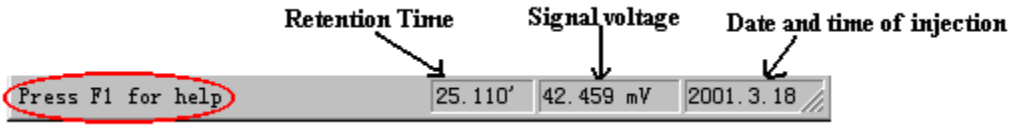
If you need to cancel an earlier command, you should also first click on the icon to see a small circle around the cursor. Move the cursor to the center of the peak concerned and click once to cancel the earlier command. Click on the icon again to exit from this command.

Should you wish to undo or reverse all the commands executed to ignore detected peak, simply click on the Re-integrating icon  to reinstate the original chromatogram.

You can also make use of this icon to reinstate those peaks suppressed by the minimum peak area (Section 5.1 Acquisition table). Click once on the icon to see the small circle. Move the cursor to the peak of interest and click once to restore the marking of the peak, which was not detected because its area is smaller than the limit capped by minimum peak area. Remember to click on the icon again to exit from this command.

Please note that this command is different from the command *Start to ignore peak* explained in Section 4.2.1 (Input Integration table) in the sense that no corresponding record would be captured in the Integration table for this command. Thus, this setting would not be captured and loaded by the Save template command.

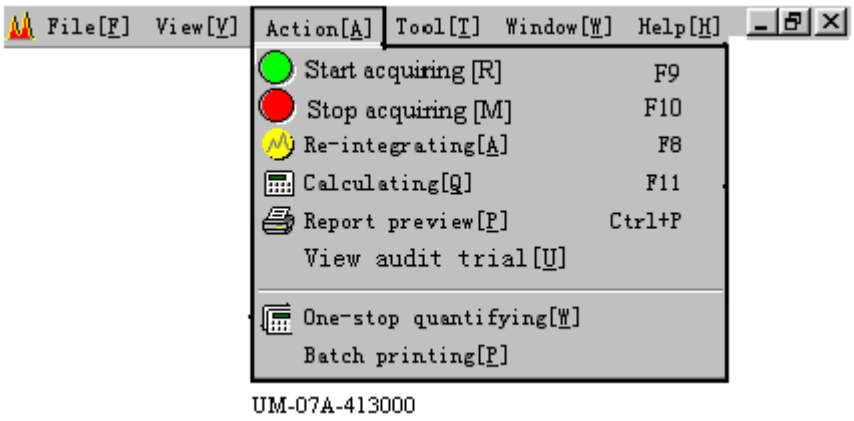
4.1.2.2 Status Bar



This is for you to hide and display the Status bar located just below the chromatogram frame. This Status bar serves two functions. While the first is the traditional function of displaying a short description of the command that is being selected, the second is to display the status of chromatogram acquisition.

Short description is displayed on the extreme left of the Status bar. Whereas the status of chromatogram acquisition is displayed in a three indicators frame located on the extreme right of the Status bar. The first indicator displays the retention time following the movement of cursor, the center indicator displays the voltage of signal received and the last indicator displays the date and time of injection.

4.1.3 Action Menu heading



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4.1.3.1 Start acquiring

This command is for you to start acquiring data signal. This command can also be activated by clicking on the  icon located on the Tool bar, or by pressing the remote starter button found on the signal cable, or by activating the Start / Run button of the instrument or the Auto-sampler. Please refer to Section 2.2 (Hardware) for more information about how to connect the two Remote starter to the instrument or Auto sampler to receive Star / Run signal.

This command should only be activated after you have selected the acquiring channel and effected an injection of a sample of analysis.

For the Dual-channel Model, please refer to Section 4.1.4.4.1 (Option, General) for more detail about acquiring simultaneously from the dual detectors of the same instrument. You can also connect them to two different instruments for independent acquisition.

4.1.3.2 Stop acquiring

This command is to manually stop the acquisition process before reaching the specified time limit. Once activated, the acquisition process pertaining to that injection would be terminated and the injection is regarded as aborted even though the acquisition may only be halfway through. Please refer to Chapter 5, Section 5.1 (Acquisition table) for more detail about specifying the time limit of acquisition.

4.1.3.3 Re-integrating

As and when data signal is being acquired, the system would apply its intelligent noise filtering method to eliminate noise and proceed to integrate the chromatogram. For each detected peak, the system would first search the Integration table for any Integration method that have been input prior to activating the acquisition command. If there is no pre-acquisition input of integration method, the system would automatically select an integration method to process the peak. This process is referred to as **Default integration**.

If you are not happy with the integration method selected under **Default integration**, you can change the integration method by applying **Manual integration**. This command is also useful if you need to restore the original chromatogram after making some manual adjustments to the peaks. Please refer to Section 4.1.2.1.1 and Section 4.1.2.1.2 for more detail about manually marking a non-detected peak and manually ignoring a detected peak.

You may note that Re-integrating takes a much shorter time than that of acquisition. Please refer to Chapter 5, Section 5.1 (Acquisition table) and 5.2 (Integration table) for more detail about how to re-integrate a chromatogram by adjusting the parameters for initial peak width; minimum peak area; degree of filtering and speed of peak widening.

4.1.3.4 Calculating

This command is for you to perform the intended calculation at various stages of data handling process as specified in the Calculation table. For example, you need to activate this command when you need to calculate calibrator (s); to construct calibration curve(s) and to quantify component(s) quantity of unknown sample. This command can also be activated by clicking on the  icon located on the Tool bar. The results calculated would be available in the Results table. Please refer to Chapter 5, Section 5.3 (Component table) and 5.4 (Calculation table) for more detail about working with these two tables.

4.1.3.5 Report preview

This command is for you to view and fine-tune the analysis report before you proceed to print out the hard copy. If you choose to include the chromatogram in the report, you can adjust its size in this screen before printing. Section 4.1.4.4.2 explains how you can opt to print the analysis report under Word application or Wordpad application. Please refer to Section 5.5 (Results table) for more information about how to include system-calculated statistics and key in external reference information pertaining to a particular injection in the analysis report.

Please also refer to Section 4.1.3.8 (Batch printing) for more detail about how to print a few analysis reports at one go.

4.1.3.6 View audit trial

This command is for you to view the detail historical record of the various operations that have been performed on a particular Chromatogram file displayed in the active Document window. It captures information such as shifting of Start point and End point of a peak; changes of integration method applied etc. This is to comply with the GLP requirements.

4.1.3.7 One-stop quantifying

This is to automate the application of Quantifying by calibrator, or Quantifying by Average calibrator or Quantifying by Calibration curve. The first step is to acquire a series of Chromatograms corresponding to a series of Standard samples and an unknown sample and save them as a series of Chromatogram files.

The second step is to retrieve the series of Chromatogram files. In the series of Chromatogram files corresponding to the series of Standard samples, proceed to activate the Calculating calibrator command in the Calculation table and fill in the known component quantity in the Component table. In the Chromatogram file corresponding to the unknown sample, proceed to select the desire quantifying method in the Calculation table.

The final step is to click on the  icon to activate the system to first calculate the calibrator or average calibrator or construct the calibration curve and apply the result of calculation to perform the subsequent qualitative or quantitative analysis on the unknown sample.

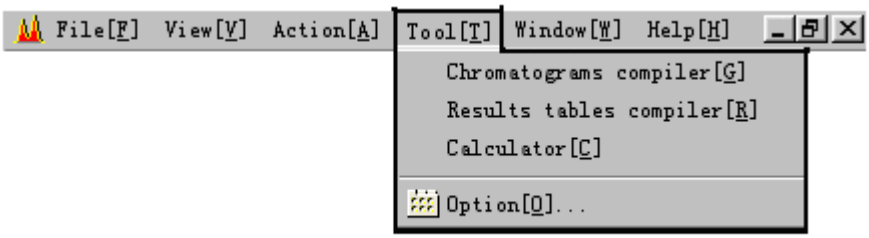
The entire command is dictated by the choice of quantifying method. Assuming that you wish to apply Quantifying by calibration curve in your quantitative analysis and have acquired a chromatogram of an unknown sample and four chromatograms of a series of Standard samples with different quantities. Retrieve and open these five Chromatogram files into five Document windows. Select Calculating calibrator method in the four Document windows of the Standard sample. Select Quantifying by calibration curve method in the Document window of the unknown sample. Click on the  icon and proceed to view the results in the Results table of the unknown sample. PEAK-ABC would first construct the calibration curve (s) of the first five Document windows and apply the results to calculate the component quantities of the unknown sample in the remaining Document window.

4.1.3.8 Batch printing

This command is for you to pre-view and print a few analysis reports at one go. Activate this command after you have opened up the series of Chromatogram files that you want to print. Upon activation, the reports would be displayed for your preview. Reports would be arranged in the order at which the Chromatogram files are selected. Click on the Print function to begin.

You can opt to insert page break in between reports, or not to insert page break in between reports. Please refer to Section 4.1.4.4.2 (Option, Report) for more detail.

4.1.4 Tool Menu heading



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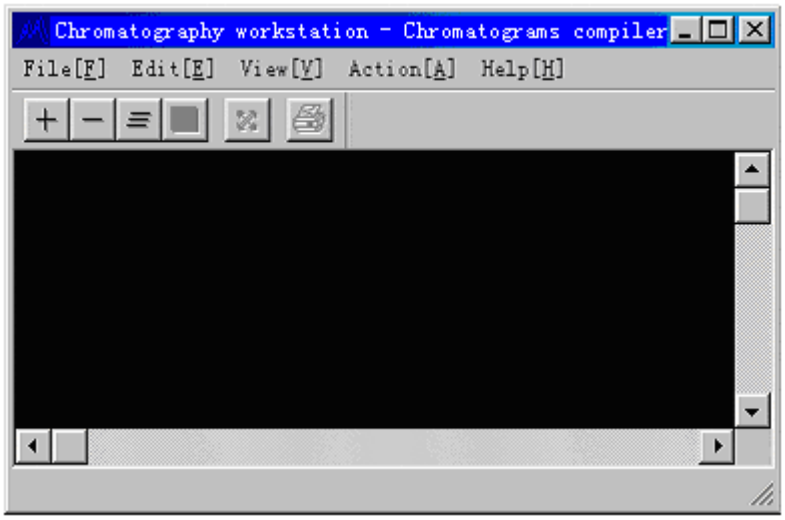
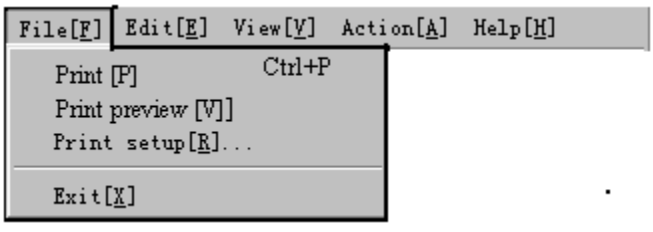
4.1.4.1 Chromatograms compiler

This command is for you to compare, contrast and overlay a few Chromatograms acquired from past analysis. Click on this command to go to the program window as follow:



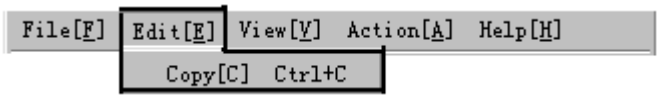
1) File Menu



Print Command	:	To print the chromatograms displayed in this program window.
Print preview	:	To preview the overlaid chromatograms before printing.
Printer setup	:	To select the size of paper and direction of printing.

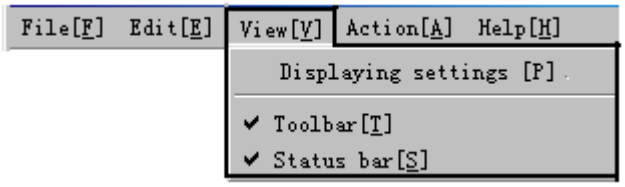
Exit : To exit Chromatograms compiler.

2) Edit Menu



Copy : To copy the overlaid chromatogram to be pasted in other application.

3) View Menu

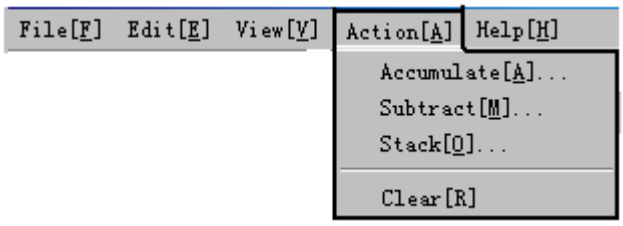


Display parameter: To view and adjust the displaying parameters of the chromatograms. The function of the dialogue frame is similar to that of the Acquisition table explained in Section 5.1. The Adjust upward and Adjust side-way buttons are to vary the way of overlaying the chromatograms.

Toolbar : For you to display or hide the Tool Bar.

Status bar : For you to display or hide the Status Bar which displays brief descriptions of the command being executed.

4) Action Menu



Accumulate : This is to display the accumulated graph of a few chromatograms. Upon activation, you would be asked to select the Chromatogram files of interest. Press and hold on to the Ctrl key, click on each of the filename (s) to select. Click on "OK" button to view the result of accumulation.

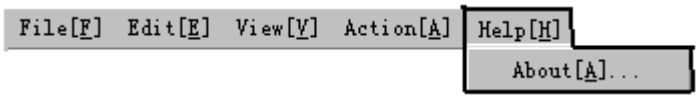
Subtract : This is to display the residual graph being the difference among a few chromatograms. Upon activation, you would be asked to select the Chromatogram files of interest. Press and hold on to the Ctrl key, click on each of the filename (s) to select. The first chromatogram selected would be

used to minus the next chromatogram selected and so on. Click on "OK" button to view the result of subtraction.

Stack : This is to stack (overlay) a few chromatograms for display. Upon activation, you would be asked to select the Chromatogram files of interest. Press and hold on to the Ctrl key, click on each of the filename (s) to select. Click on "OK" button to view the result of stacking. You can make use of the Adjust upward and Adjust side-way buttons to vary the ways of overlaying the chromatograms.

Clear : This is to clear the content of this program window before you proceed to select another Action, e.g from Subtract to Stack.

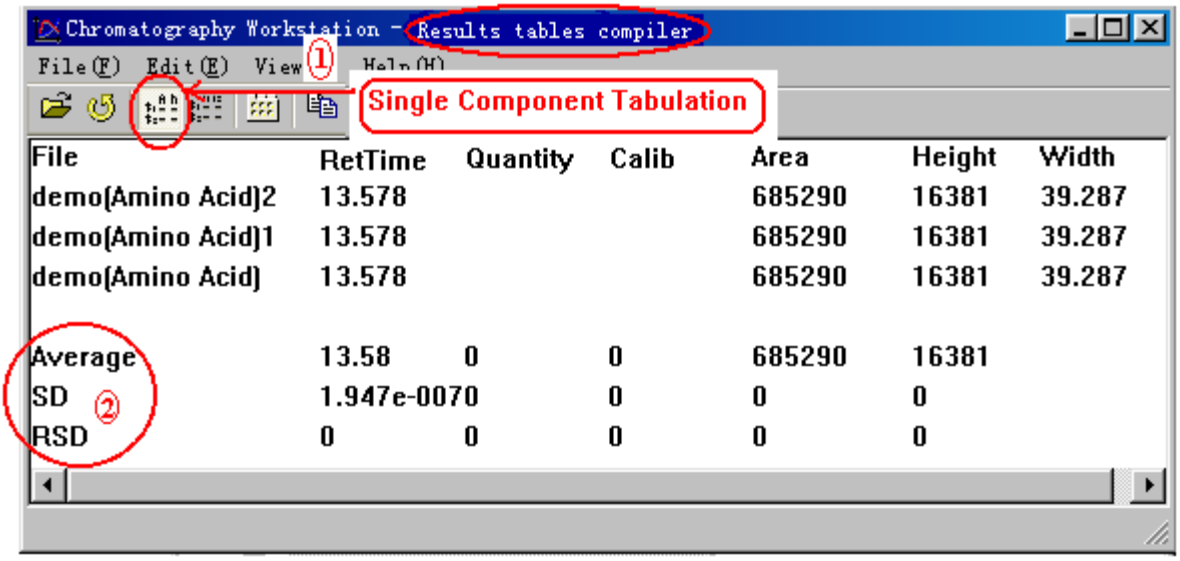
5) Help Menu



About…. : This is to provide on-line explanation about Chromatograms compiler.

4.1.4.2 Results tables compiler

This command is for you to compile a few Results tables to calculate average component quantity, standard deviation (SD) and relative standard deviation (RSD). There are two types of tabulations, single-component tabulation and multiple-components tabulation. A typical single-component tabulation and multiple-component tabulation are as follow:



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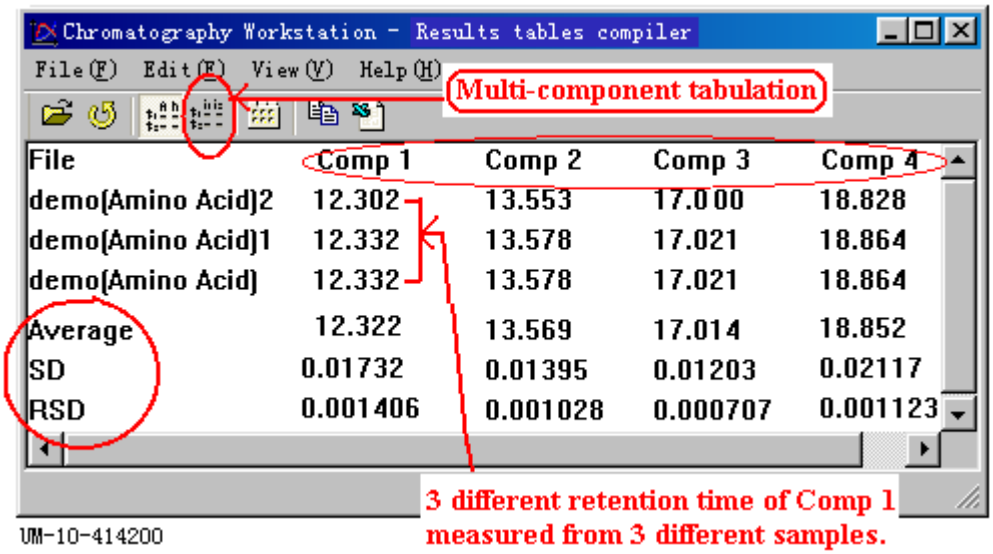

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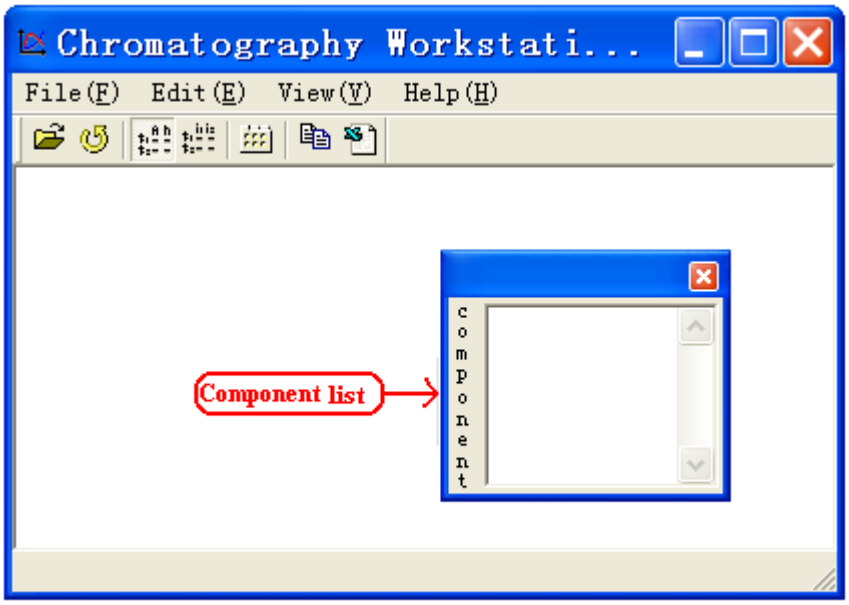
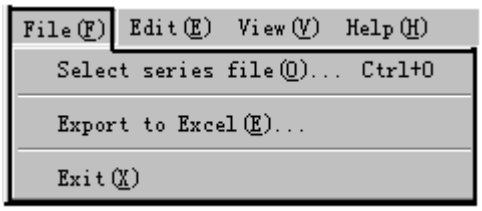
Click on this command to go to a new program window as follow:



UM-11-4142000

1) File Menu

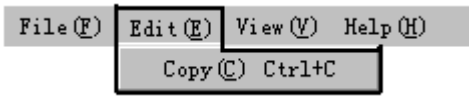


Select series of file : Upon activation, you would be asked to select the chromatogram files of Cinterest. Press and hold on to the Ctrl key, click on each of the file name (s) to select. Click on "OK" button to view the tabulated results arranged in the reverse order of selection.

Export to Excel : Export the results of tabulation for further processing in Excel application.

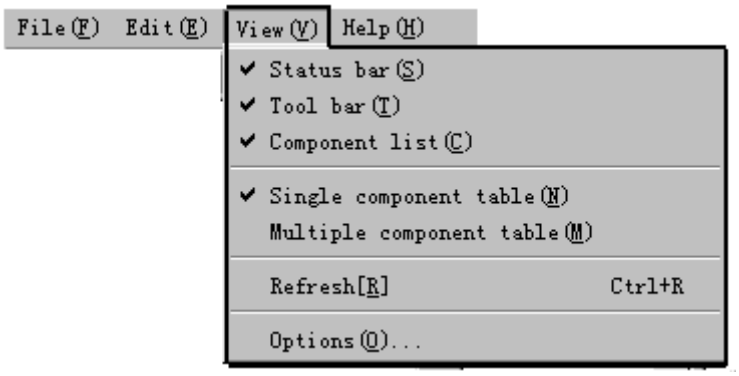
Exit : To exit Results tables compiler.

2) Edit Menu

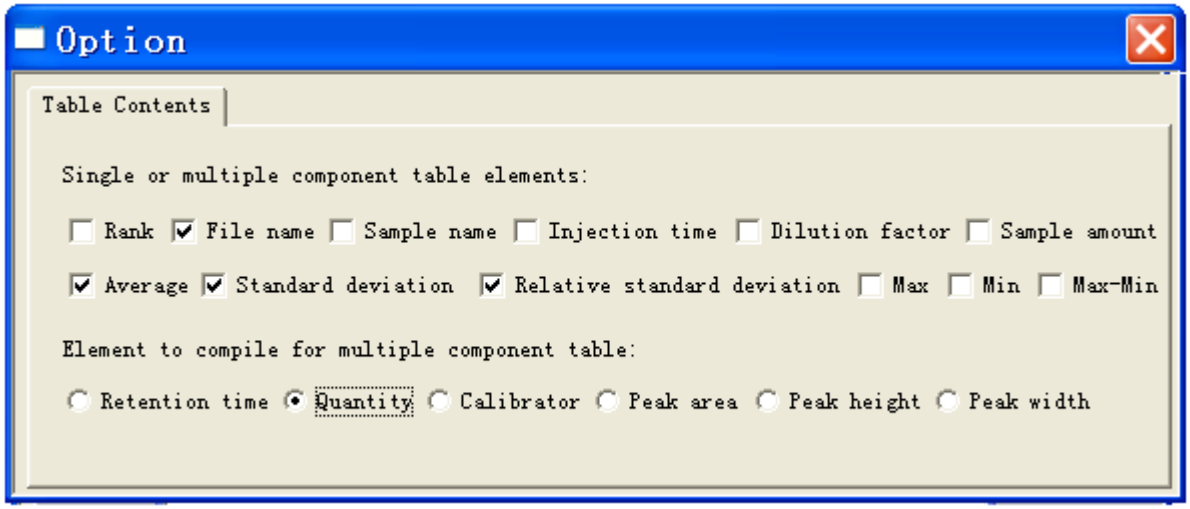


Copy : To copy the result of tabulation to be pasted in other application.

3) **View Menu**



Status bar : To display or hide the Status Bar which displays the brief descriptions of the command that is being executed.
Tool bar : To display or hide the Tool Bar.
Component list : To display or hide the component list.
Single Comp… : To tabulate single component table.
Multiple Comp… : To tabulate multiple components table.
Option : This is for you to select additional information to be included in the tabulation. There is no need for you close the You should be able to select one combination that best meet your needs. As can be seen, your selection would be updated instantly.



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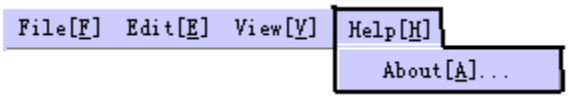
4) Help Menu



About…. : To provide on-line explanation about Results tables compiler.

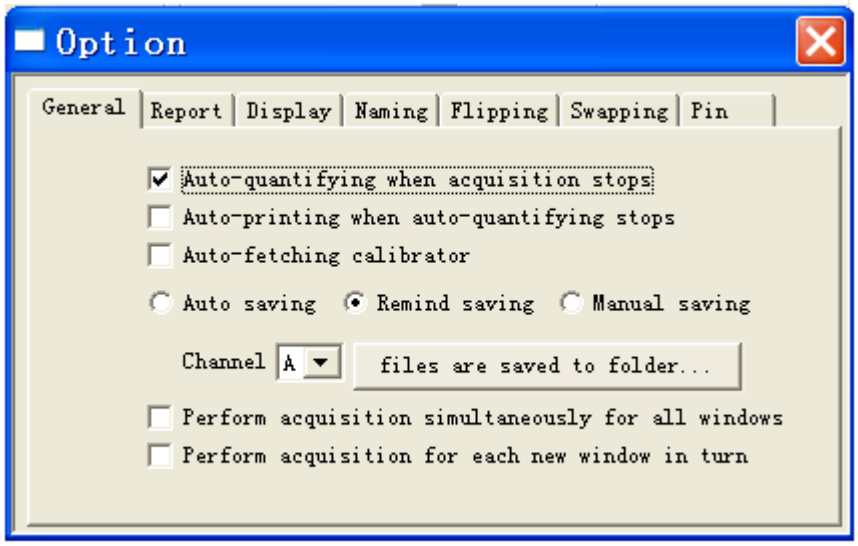
4.1.4.3 Calculator

This command is for you to activate a pop-up calculator when need be.

4.1.4.4 Option

4.1.4.4.1 General

This command is for you to access the General panel to specify some special settings relating to the operation of this system. You would notice that changes made in this panel would be applied by the software system instantly.



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Auto-quantifying when acquisition stops

You can activate this setting by checking on the box. When activated, the system would proceed to quantify the result upon completion of chromatogram acquisition. Please ensure that you have:

1. made the necessary settings in the Acquisition table and Integration table; and
2. made the necessary settings in the Component table and Calculation table.

Please also refer to the Section on Auto-print report to see how you can proceed to print the analysis report upon completion of auto-quantifying.

Auto-printing when auto-quantifying stops

You can activate this setting together with the setting for Auto-quantifying when acquisition stops. Please ensure that you have:

1. made the necessary input of external reference information in the Front section and Rear section of the Report table as explained in Section 5.6 (Report table); and
2. customized the report format as explained in Section 4.1.4.4.2 (Option, Report).

Auto-fetching calibrator

This command is to save you the trouble of having to manually fetch the calculated calibrator (s) from the Results tables into the Component table. You can activate this setting by checking on the box. When activated, the system would automatically update the calculated calibrator (s) from the Results table to Component table upon successful calculation of calibrator (s). Please refer to Section 6.3 for more detail about calculation of calibrator (s).

This command is not applicable when calculating average calibrator (s) whereby you must manually fetch the calculated average calibrator (s) from the Results table to the Component table as explained in Section 6.4.

Auto saving

This setting is only applicable when you are working with Auto-sampler. When activated, the system would automate the Save command after successful completion of quantification to save the Active Document window into a corresponding Chromatogram file under the same name of the Active Document window within the designated Working folder. Please ensure that you have specified a Working folder as explained in Section 4.1.1.10 (Working folder) if you wish to make use of this function. Please also refer to "Channel _ files are saved to" explained in the later part of this Section for more information about designating different Working folder for the two different Channels.

Remind saving

This setting is appropriate for daily analysis work. When activated, the system would prompt you to execute the Save command upon every successful completion of quantification to save the Active Document window into corresponding Chromatogram file. You would be prompted to confirm the file folder and the filename.

Manual saving

When activated, the system would only prompt you to execute a Save command before you exit PEAK-ABC to save all the unsaved amendments made to the Document window(s) into corresponding Chromatogram file(s). Please refer to Section 4.1.1.13 (Exit) for more information.

Channel A/B files are saved to

This setting is mandatory if you wish to acquire simultaneously from the dual detectors of the same instrument. It allows you to designate different Working folder for Channel A and Channel B. Please note that the setting made over here would supersede the setting made under Section 4.1.1.10. That is to say that if you have specified a Working folder for Channel A in this field, all the Chromatogram files corresponding to Channel A would be saved under this Working folder even though you may have made use of Section 4.1.1.10 (Working folder) or Section 3.6 (File manager) to designate another file folder to be the Working folder.

Perform acquisition simultaneously for all Document windows

This is useful if you wish to acquire simultaneously from the dual detectors of the same analytical instrument. Check on the box to activate the setting, proceed to open the desired number of Document windows and select the appropriate Channel in each of the Document windows. Upon activation of the command Start acquiring, acquisition would commence simultaneously in all Document windows that have been opened. Please refer to Section 4.1.3.1 (Start acquiring) for more detail about the command.

The activation of this command would bar you from activating the command "Perform acquisition for each new Document window in turn" that follows.

Perform acquisition for each new Document window in turn

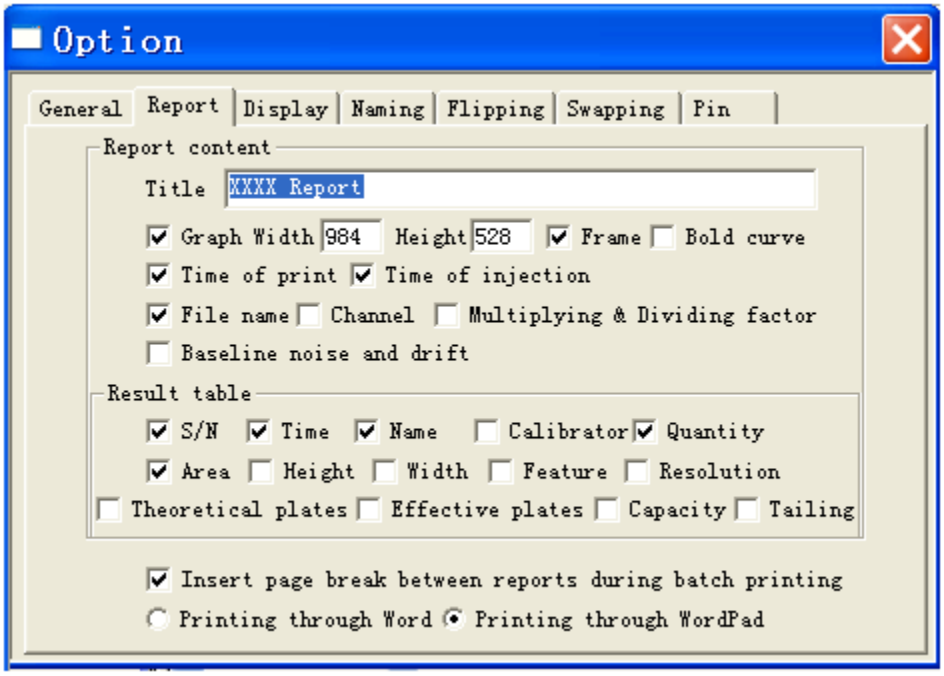
This is only applicable if you are working with Auto-sampler and that the series of samples to be analyzed are of different mixture. To analyze a series of samples of different mixture entails the need to open different Document windows for each of the samples so that different integration method and different quantifying method may be applied to each of the injection. When activated, the system would start acquiring in each newly created Document window in turn. Please refer to Section 7.2 for more detail about working with Auto-sampler.

When working with Auto-sampler to analyze parallel samples (or samples of the same mixture), you only need to open one new Document window to be used repeatedly. This is because the acquired chromatogram and the results of calculation of the previous injection would have been saved into a corresponding Chromatogram file under the Auto-save setting.

The activation of this command would bar you from activating the command "Perform acquisition simultaneously for all Document windows" mentioned above.

4.1.4.4.2 Report

This command is for you to customize the format of your analysis report. It enables you to access the Report panel to specify the system-calculated statistics to be included or excluded from the analysis report. Please refer to Section 5.6 (Report table) for more detail about using the Rear section and Front section to input external reference information to be included in the analysis report. Please also refer to Section 4.1.3.5 (Report preview) for more information about how to pre-view and print out the hard copy of an analysis report. You would notice that changes made in this panel would be applied by the software system instantly.



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Report content

Check on the appropriate boxes if you want to include any of the system-calculated statistics in the analysis report. Leave it blank if you wish to exclude any one of them.

Title : To input the title to be given to the analysis report
Graph Width / Height : If you want the chromatogram to be included, you can specify the size of the chromatogram by filling in the desired height and width. (You also have another option to adjust the chromatogram during Report preview just before you proceed to print out the hard copy).
Frame : To add a frame around the chromatogram.
Bold : To print the chromatogram curve in bold print.
Time of printing : To indicate the time of printing.
Time of sampling : To indicate the time of injection.
Filename : To indicate the filename of the chromatogram file.
Channel : To indicate the acquiring Channel.
Multiplying & Dividing factors : To indicate the diluting factors & sample amount.
Baseline drift & noise : To indicate the magnitude of baseline drift & noise.

Results table

This is for you to select some or all of the statistics calculated (available in the Results table) to be included in the analysis report. Check on the appropriate boxes if you want to include them in the analysis report.

S/N	:	To indicate the positions of components within the Results table.
RetTime	:	To indicate the retention time of each components.
Name	:	To indicate the name of components.
Calibrator	:	To indicate the value of calibrators.
Quantity	:	To indicate the calculated components quantities.
Area	:	To indicate the peak area of each components.
Height	:	To indicate the peak height of each components.
Width	:	To indicate the peak width of each components.
Feature	:	To indicate the peak feature of each components.
Resolution	:	To indicate the resolution between any two peaks adjacent to the right.
Theoretical plate	:	To indicate the Theoretical plate number of the column of chromatogram instrument corresponding to any peak.
Effective plate	:	To indicate the Effective plate number of the column of chromatogram instrument corresponding to any peak.
Capacity factor	:	To indicate the capacity factor of the column of analytical instrument corresponding to any peak.
Tailing factor	:	To indicate the tailing factor of any peak.

Insert page break between reports during batch printing

This is applicable if you wish to apply the command for Batch printing as explained in Section 4.1.3.8. When activated, page break would be inserted between each analysis report during batch printing. In so doing, every single report would be complete with report title. If not activated, no page break would be given and report title would only be printed once.

Printing through Word

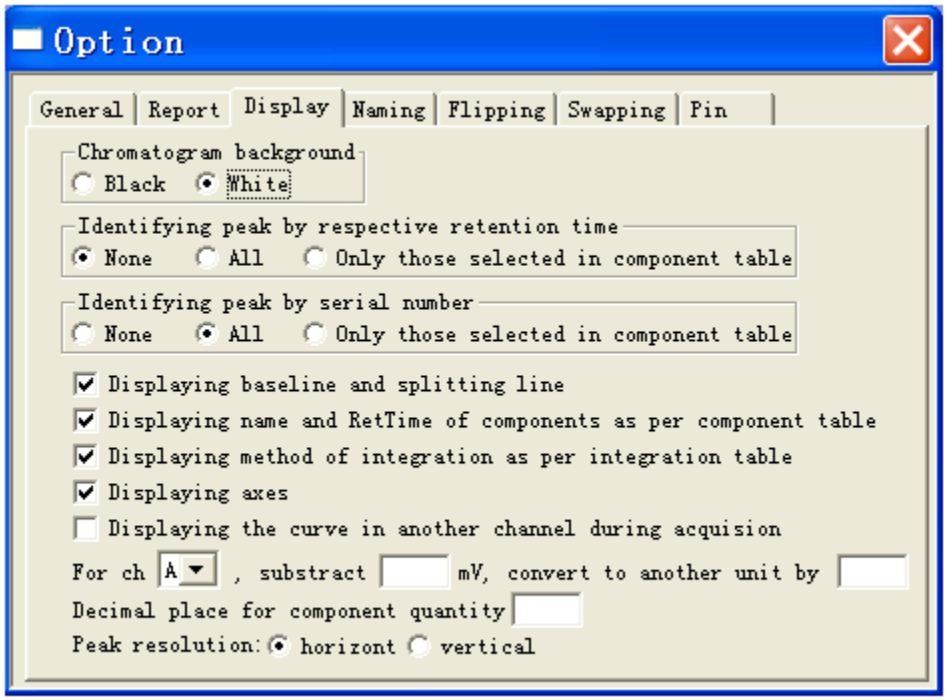
If your computer is installed with Word application, this command is to enable you to print the analysis report in Word setting. If not activated, the analysis report would be printed in Wordpad setting to be explained in the following paragraph. Under Word setting, after printing the first report you need to first exit Word application (before you begin printing the next Chromatogram file), i.e to exit Word, then open the next Chromatogram file and use the Printing preview command. Should you forget to exit Word application and proceed to print another Chromatogram file, the next report that you print would contain the same chromatogram of the previous report.

Printing through Wordpad

If your computer is not installed with Word application, you can opt to print the analysis report in Wordpad setting. In fact, we recommend that you select to print under this setting as the speed is faster.

4.1.4.4.3 Display

This command is for you to access the Screen panel to adjust the effects of screen display, which also define the features of the chromatogram to be included in the analysis report. You would notice that changes made in this panel would be applied by the software system instantly.



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Chromatogram background
Click on the radio button to change the background setting from black to white and vice verse.

Identifying peak by respective retention time
When activated, the retention time of the component corresponding to a particular peak would be displayed on peak top. You can choose to display the retention time of none of the peak, all of the peaks or only those peaks identified in the Component table. Please note that this setting can co-exist with the setting for Identifying peak by serial number to be explained in the next section.

Identifying peak by serial number
When activated, the serial number of the peak corresponding to a particular component would be displayed on peak top. You can choose to display the serial number of none of the peak, all of the peaks or only those peaks identified in the Component table. Please note that this setting can co-exist with the setting for Identifying peak by respective retention time explained above.

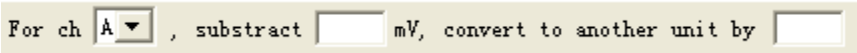
Displaying Baseline and splitting line
When activated, the Baseline and splitting line of the chromatogram would be displayed. No Baseline or splitting line would be displayed if not activated.

Displaying name and RetTime of components as per component table
When activated, a marking of name and RetTime would be made on the x-axis for each component identified in the component table. The position of this marking (and thus the RetTime) can be fine-tuned by dragging it using the mouse.

Displaying method of integration as per Integration table
When activated, a corresponding marking will be made on the x-axis to display the selected integration method. The position of this marking (and thus the time to start applying) can be fine-tuned by dragging it using the mouse.

Displaying axes
When activated, the scale of both the horizontal and vertical axes would be displayed. No marking would be displayed if not activated.

Display the curve in another channel during acquisition
This is applicable when acquiring simultaneously from two channels. When activated, you would be able view the two chromatograms that are being acquired in the same Document window.



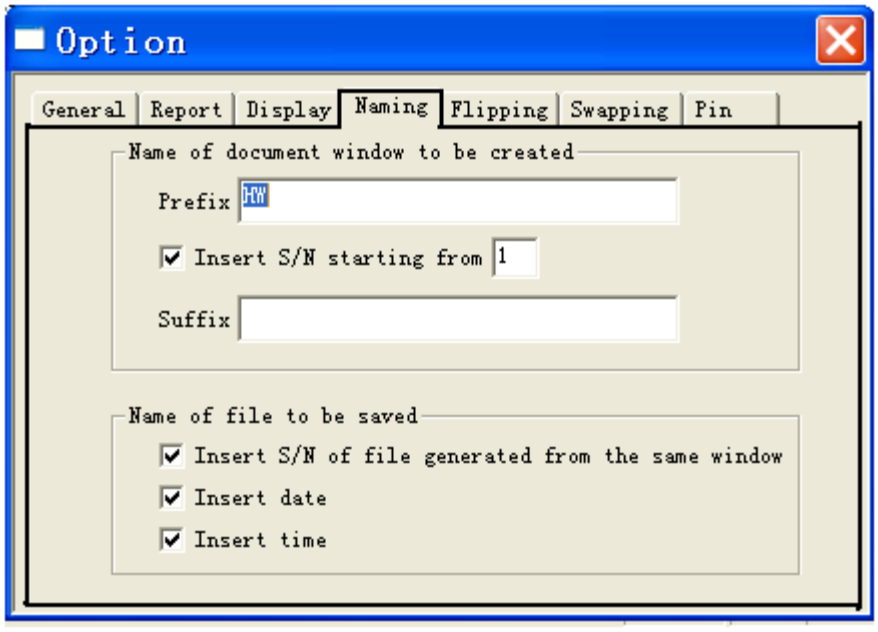
This is applicable when you wish to adjust the effect of an upward drifting of the baseline (and to convert the unit of measurement of the y-axis) as and when the chromatogram is being acquired. Simply key in the appropriate value(s) in the blank space(s) before activating the command to start acquiring.

Decimal place for component quantity
This field is for you to specify the desired decimal place of the calculated component quantity.

Peak resolution horizontal / vertical
This field is for you to specify the method of calculating peak resolution, which is set to horizontal by default. Simply click on the radio button if you need to change to vertical calculation.

4.1.4.4.4 Naming

This command is for you to access the Naming panel for you to automate the naming of new Document window to be opened. You would notice that changes made in this panel would be applied by the software system instantly.



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To facilitate retrieving of Chromatogram file, we strongly recommend that a systematic filing system should be implemented to store the Chromatogram files starting from day one of analysis work. An effective filing structure could be achieved by implementing a consistent way of naming each new Document window and thus the corresponding Chromatogram file. This can be done by first creating a Working folder for every different mixture of sample to be analyzed. The second step would be to decide on what information to be included in the file name.

The naming structure adopted by this system is as follow:

Prefix	--	Insert S/N	--	Suffix	--	Name of file to be saved

Prefix : May be used to indicate the name of the sample to be analyzed.
Insert S/N : Check on this box to insert serial number as part of the Document window name. This S/N is automatically assigned by the system starting from 001 which is being refreshed to 001 every time you restart the system. You can start counting from a number of your choice by inputting it in the field provided.
Suffix : May be used to indicate the category of sample or sources of sample.

Name of file to be saved :
You can also opt to include the following information in the filename to facilitate the subsequent retrieval of a Chromatogram file :

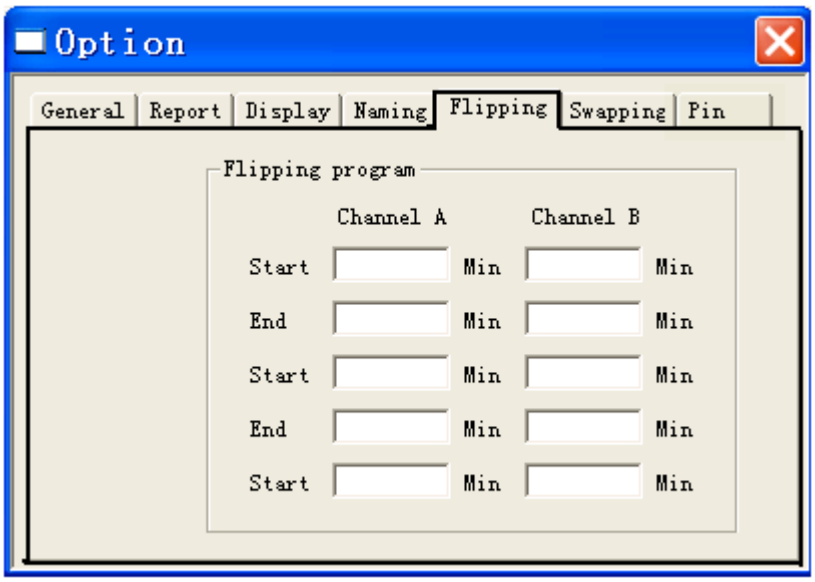
Insert S/N of file generated from the same window – This option must be activated when working with Auto-sample to analyze batch samples of the same mixture. Plesae refer to 7.2 for more detail. Check on this box to insert serial number as part of the Document window name.

Insert Date -- Check on this box to insert YY/MM/DD as part of the Document window name. This is useful if the frequency of acquisition of sample of the same mixture is less than 1 per day.

Insert Time -- Check on this box to insert Hour/Minute/Second as part of the Document window name. This is most useful if the frequency of acquisition of sample of the same mixture is high and the duration of each acquisition is less than 3 minutes.

4.1.4.4.5 Flipping

This command is for you to access the Flipping panel to input pre-acquisition command to invert certain segment of the chromatogram on a real time basis when acquiring data signal.



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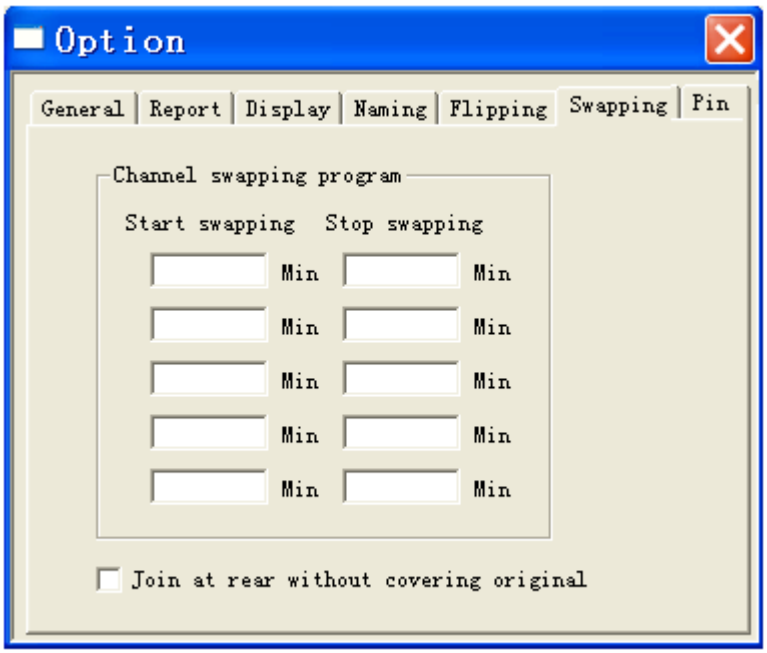
Key in the time that you want to start flipping and the time to stop flipping in the fields provided. The height of the chromatogram corresponding to the time input in the Start space would be used by the system as a basis of comparison. Any peak with lower height would be flipped. The time (i.e the height of the chromatogram) to start flipping need not be as precise as on the Start point of the negative peak. For a segment of chromatogram containing a series of (positive) peaks followed by a negative peak, you can simply set the time to Start flipping to be along the Baseline before the Start point of the series of (positive) peaks.

Please refer to Section 4.2.3 (Start point to flip and End point to flip) for more information about making post-acquisition instruction to invert a segment (or a negative peak) of an acquired chromatogram.

4.1.4.4.6 Swapping

This is useful if you are using both the acquiring channels to acquire simultaneously from the dual detectors of the same analytical instrument. This command is for you to access the Swapping panel to input pre-acquisition command to merge a segment of one of the chromatograms to another chromatogram.



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If for whatever reason, you are only interested in the front segment of the chromatogram acquired in Channel A and interested in the rear segment of the chromatogram acquired in Channel B, you can make use of this to input pre-acquisition command to instruct the system to merge the two segments concerned on a real time basis as and when data signal is being acquired.

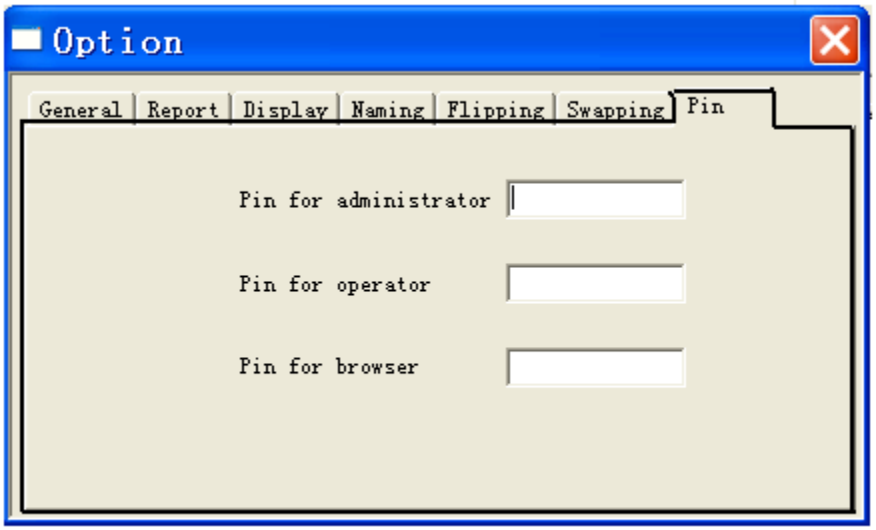
Key in the time that you want to start swapping and the time to stop swapping in the fields provided. You can choose to leave the original chromatograms intact by connecting the swapped portion to the rear of the original chromatogram by checking on the box "Join at rear without covering original". If not activated, the swapped portions would be pasted to replace the original chromatogram. The merged chromatogram would be the one to be included in the analysis report.

4.1.4.4.7 PIN

This PIN panel is for you to input password to restrict the access authority of your users. User should designate an administrator to design three common passwords to be used by three different categories of users namely administrator, operator and browser. Administrator is authorized to set the three common passwords; to create template file; and to make changes on acquired chromatogram file. Operator is authorized to acquire chromatogram and to save the results of calculation to database. Browser is authorized to perform all functions other than those functions mentioned above.

The password set for the administrator is of highest authority, which should be kept confidential in a sealed envelope for future reference. All operators would share one common password, while all browsers would share another common password. User of higher authority should not disclose the common password to the user of lower authority.

Please note that if no password is set, all users are defaulted as administrator. If passwords are set, user would be prompted to key in the PIN every time the system is activated. For data security reason, after activating the system, the user should not leave the terminal unattended and must make it a point to exit from the system to prevent user of lower category from unauthorized access.



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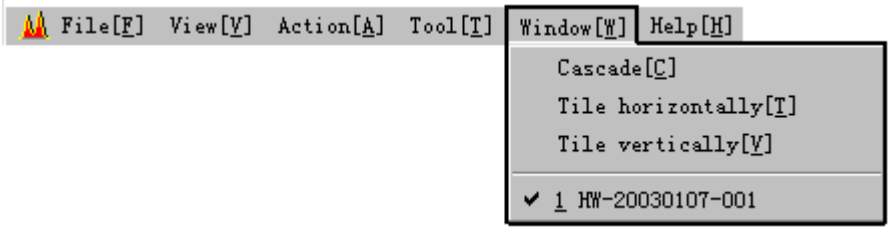
Please refer to Chapter 7 Section 7.9 for more detail about setting the three common passwords.

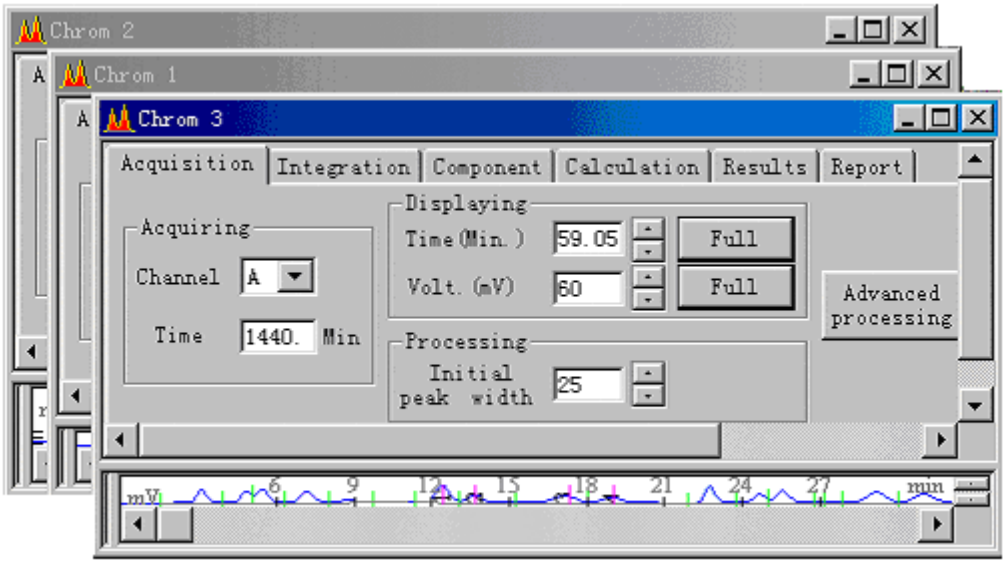
4.1.5 Window Menu heading



Please refer to Section 1.2 (Terms of reference) for explanation of Document window technique and Multiple Document window technique.

4.1.5.1 Cascade

This command is for you to display a few Document windows by way of stacking one over the other. The one on top is regarded as Active Document window, whose filename is reflected on the top row. You can click on any part of any one of the bottom Document windows to activate it to be Active Document window. Only the Active Document window is responsive to the various commands contained in the Tool bar.



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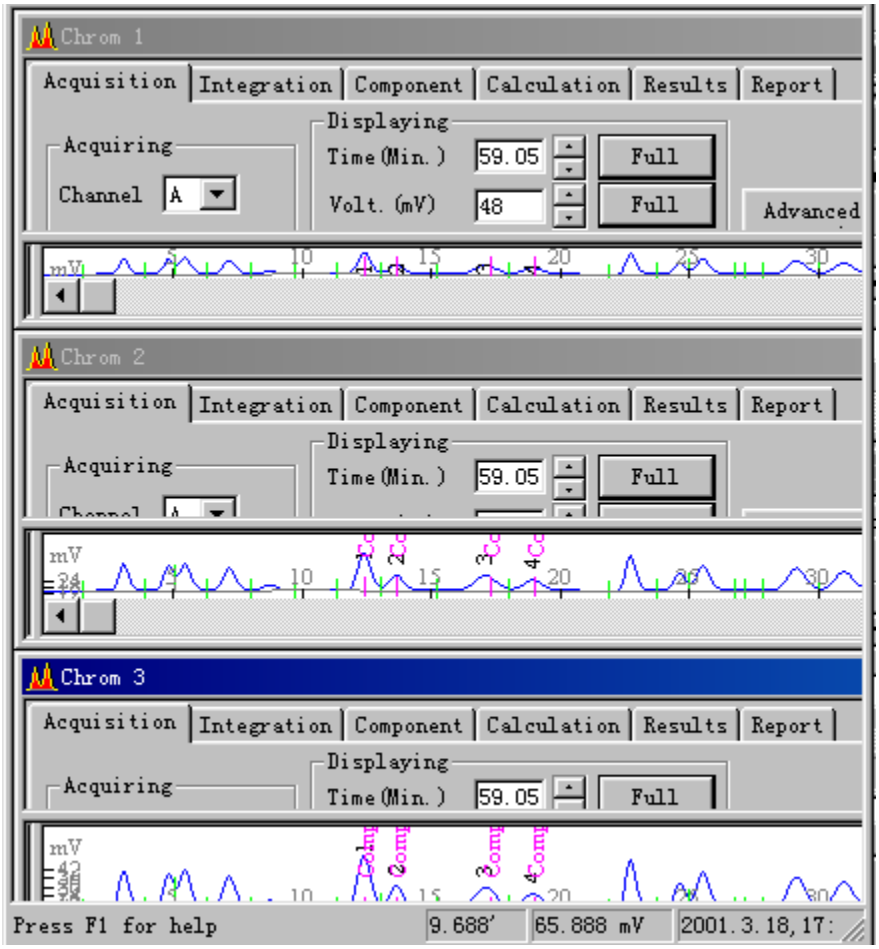
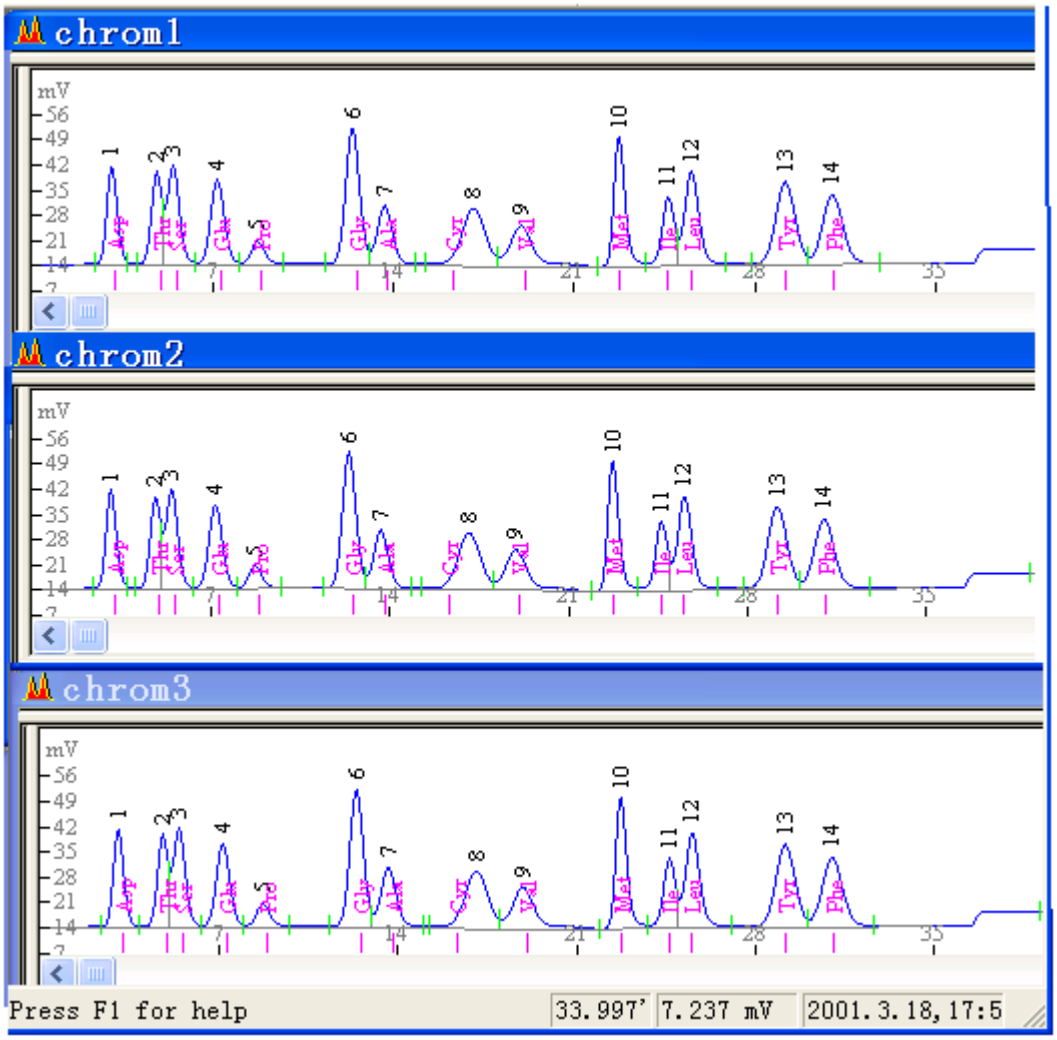
4.1.5.2 Tile horizontally

This command is for you to display a few Document windows horizontally without overlapping. You can click on any spot within any one of the Document windows to activate it to be Active Document window. If you wish to control the order at which the Document windows are displayed, please take note that Active Document window would be displayed on the top when Tile horizontally. (and would be displayed on the left when Tile vertically).

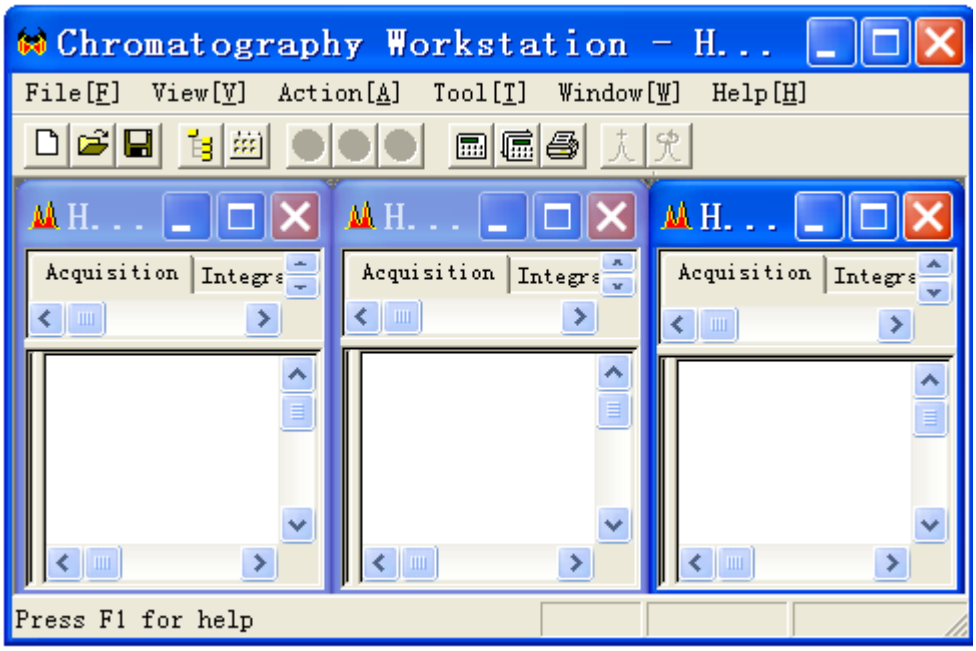


If you wish to compare the shape of a few chromatograms, you can make use of this option plus some adjustments. (Please refer to Section 4.1.4.1 (Chromatogram compiler) for more detail about overlaying a few chromatograms for comparison). Before tiling them horizontally, use the cursor to push the dividing line between the Six working tables and the Chromatogram frame to conceal the Six working tables so that only the Chromatogram frame is displayed. Repeat the same for the rest of the Document windows. Click on this command to obtain the following:



4.1.5.3 Tile vertically

This command is for you to display a few Document windows vertically without overlapping. You can click on any spot of any of the Document window to activate it to be Active Document window. If you wish to control the order at which the Document windows are displayed, please take note that the Active Document window would be displayed on the left when Tile vertically and displayed on the top when Tile horizontally.



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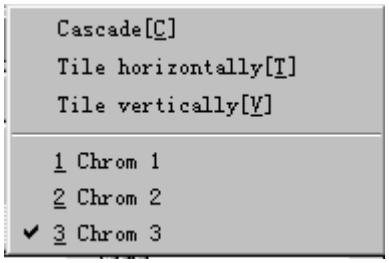
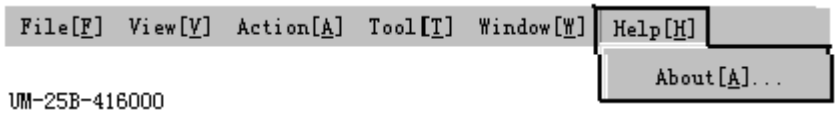
4.1.5.4 1、2、3、4….. command

This field displays the name (s) of all the Document windows (or Chromatogram files) that have been opened. Active Document window is the one marked with a tick next to its name. You can click on any of the name of Document window to activate it to be Active Document window.



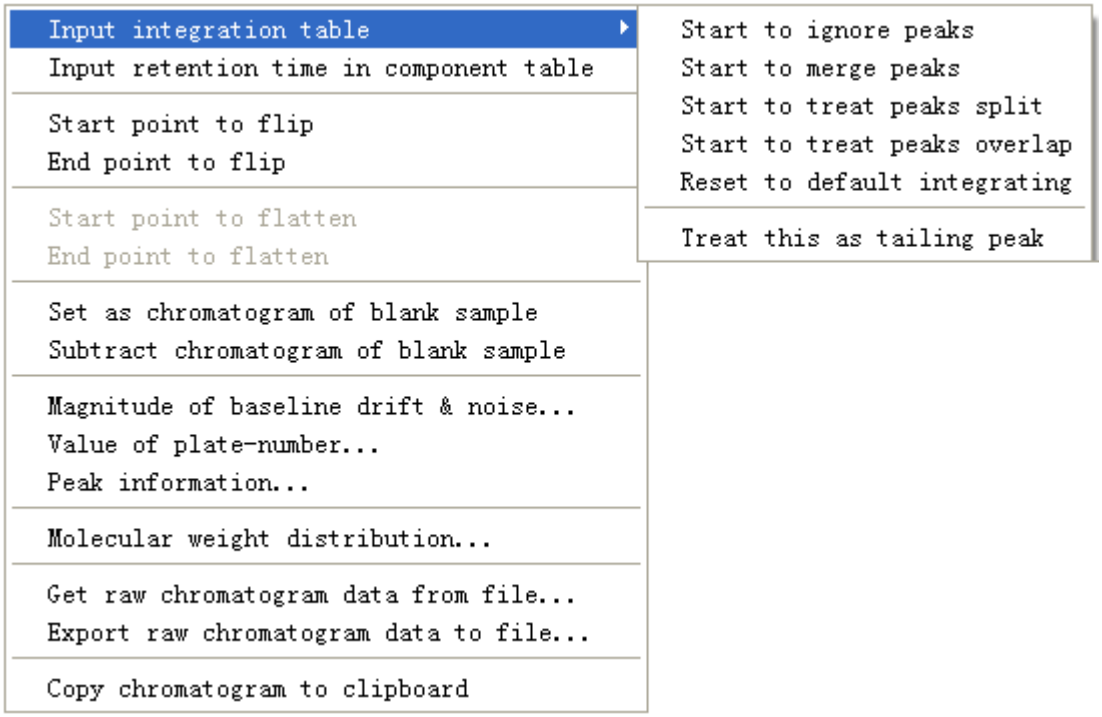
4.1.6 Help Menu heading



4.1.6.1 About….

This is for you to view the Copyright profile and system specification of this system together with other on-line explanations about PEAK-ABC.

4.2 Pop-up menu



Pop-up Menu can be activated by right clicking on the mouse on any spot within the chromatogram frame. You may note that the commands contained in this menu are related in one way or another to chromatogram such as integrating chromatogram, copy chromatogram to clipboard, view the system-calculated statistics of various peak etc.

4.2.1 Input Integration table

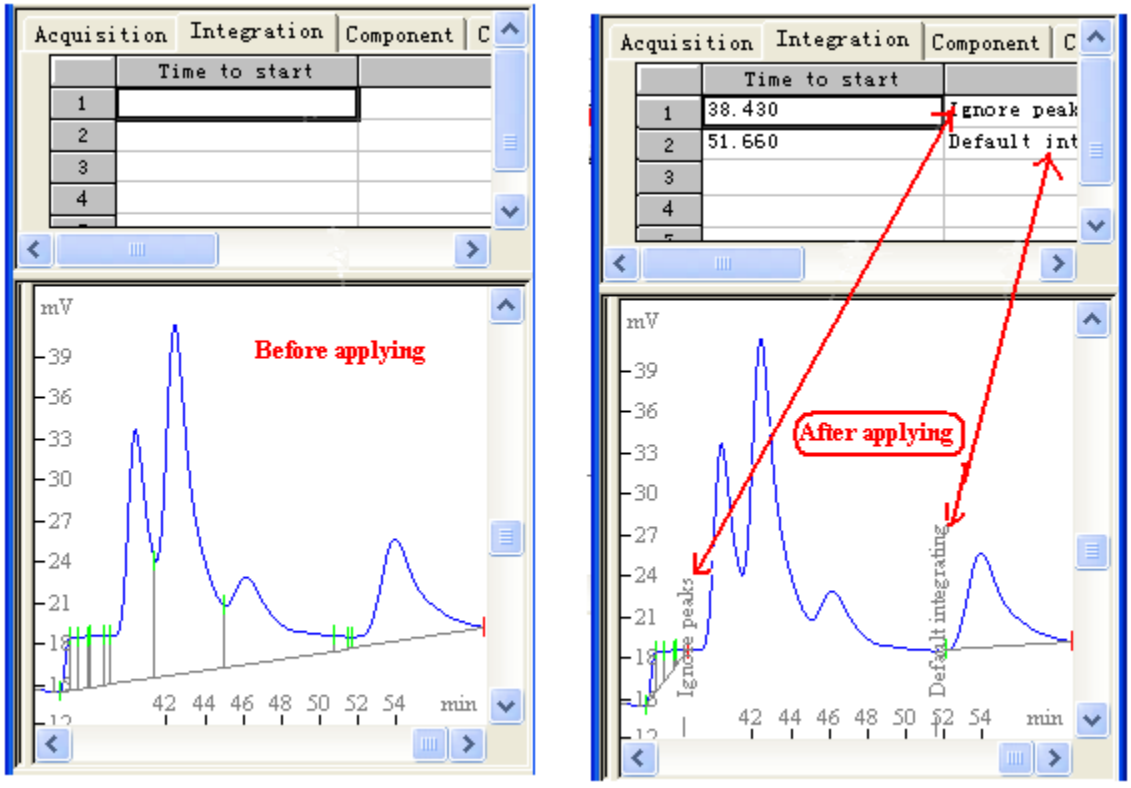
Integration table is where you input the various integration methods during Manual integration. Pre-acquisition inputs made in this table would be applied on a real time basis when data signal is being acquired. Post-acquisition inputs would be applied automatically upon selection of the integration method.

There are three ways to input the various integration methods. The first way is by using the Load template command (Section 4.1.1.8), which is usually used for making pre-acquisition inputs. The second way is by using the paste and copy function to copy from the Integration table of other Document window. The third way is by using the Pop-up menu.

Simply position the cursor to the peak of interest and right click on the mouse to view the Pop-up menu, click on Input integration table to select the desired integration method. The time corresponding to the position of the cursor where the Pop-up menu is activated and the selected integration method would automatically be captured in the Integration table. A corresponding marking would also be made on the x-axis to display the selected integration method. You can easily adjust the position of the marking (and thus the time to start applying) by dragging it using the mouse. We shall proceed to explain more about each integration method as follow:

Start to ignore peak

This is to suppress PEAK-ABC from detecting peak starting from the point you right click on the mouse. When activated, the time corresponding to the position of the cursor where the Pop-up menu is activated and the selected integration method --- Start to ignore peak would automatically be captured in the Integration table. When activated, all the peaks to the right of this point would have no marking of Baseline and retention time (or serial number).



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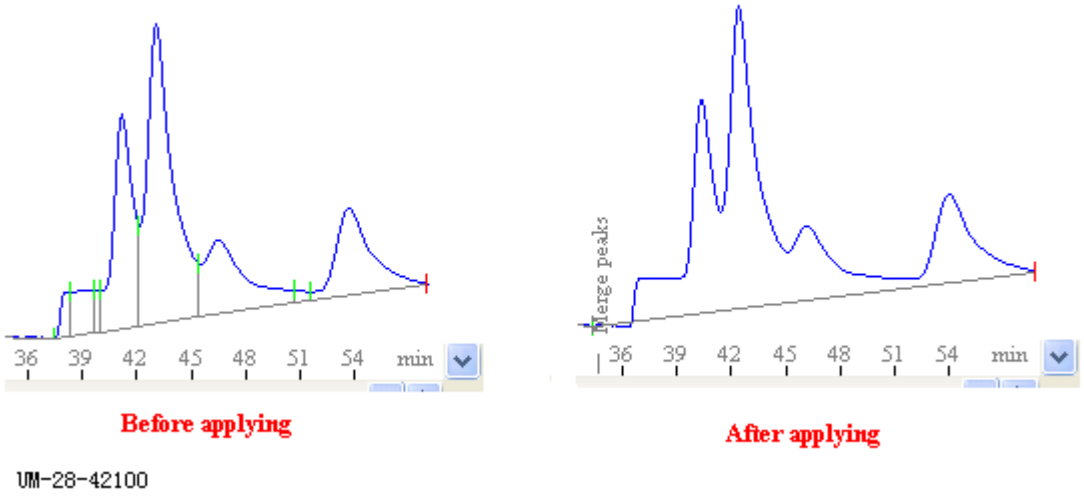
You can terminate this Integration method by positioning the cursor on the appropriate end spot and right click on the mouse to view the Pop-up menu, click on Input Integration table to either select Reset to default processing or other integration method. The time corresponding to this end spot and the selected integration method would automatically be captured in the Integration table together with a marking on the x-axis.

You may note that this method is different from the method explained in Section 4.1.2.1.2 (Manually ignore), which is not captured in the Integration table.

Start to merge peaks

This is to instruct PEAK-ABC to start merging a few connecting peaks as one peak from this point onwards. The time corresponding to the position of the cursor where the Pop-up menu is activated and the selected integration method -- Start to merge peak would automatically be captured in the Integration table together with a marking on the x-axis.



You can terminate this integration method by positioning the mouse on the appropriate end spot and right click on the mouse to view the Pop-up menu. Click on Input Integration table to either select Reset to default processing or other integration method. The time corresponding to this end spot and the selected integration method would automatically be captured in the Integration table together with a marking on the x-axis.

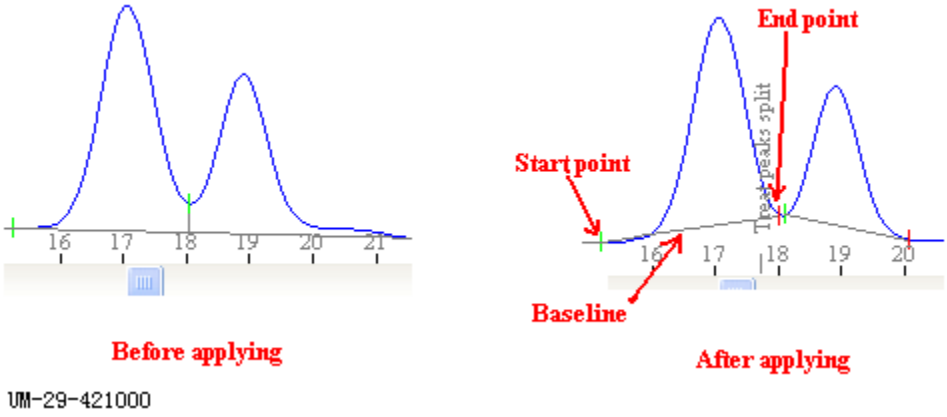
After merging, the retention time of the first peak within the group of peaks would be used to represent the group of peaks. Thus, the retention time of the first peak within the merged group would be displayed on peak top. If a flat top peak is being processed as two overlap peaks, you can use this integration method to merge the two overlap peaks to become a flat top peak.

Please also refer to Section 5.3 (Component table) for more information on how to merge a few non-connecting peaks (Group Sum) and another way of merging a few connecting peaks (Band beg and Band end).

Start to treat peaks split

This is to instruct PEAK-ABC to start treating a group of connecting peaks as split from this point onwards. The time corresponding to the position of the cursor where the Pop-up menu is activated and the selected integration method – Start to treat peaks split would automatically be captured in the Integration table.

You can terminate this integration method by either selecting Reset to default processing or other integration method. The time corresponding to the end spot and the selected integration method would automatically be captured in the Integration table.

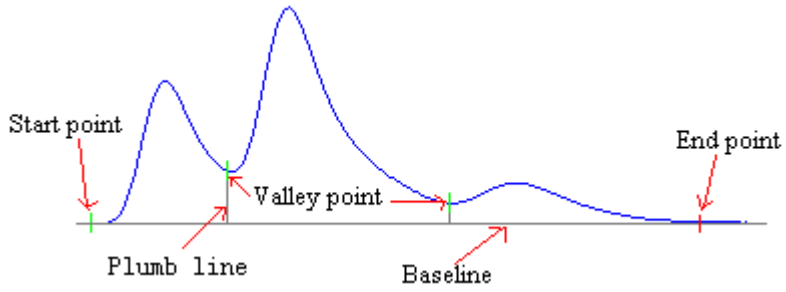


After splitting, each of the peaks would have their respective Start points and End points marked in short green line and short red line respectively. If the end point of a peak overlap the start point of the following peak, i.e. when a short green line overlap with a short red line, you would see a short green line instead. The baseline of each split peak is the line that links the start point to the end point. This Baseline is used to calculate the area of individual split peak. Please refer to Start to treat peaks as overlap to see how the Baseline of an overlap peak differs from that of a split peak.

Start to treat peaks as overlap

This is to instruct PEAK-ABC to start treating a group of connecting peaks as overlap from this point onwards. The time corresponding to the position of the cursor where the Pop-up menu is activated and the selected integration method – Start to treat peaks overlap would automatically be captured in the Integration table together with a marking on the x-axis.

You can terminate this integration method by selecting Reset to default processing or other integration method. The time corresponding to the end spot and the selected integration method would automatically be captured in the Integration table together with a marking on the x-axis.



When a group of peaks are treated as overlap, a short green line would be drawn to mark the Start point of the first peak; short green lines would be drawn to mark the connecting points between adjacent peaks; and a short red line would be drawn to mark the End point of the last peak. The Baseline is the line linking the first short green line straight to the last short red line. When calculating area of individual overlap peak, a default plump line would be drawn vertically from the valley point to the Baseline. Please refer to Start to treat peaks split for more information about split peaks.

You can see that treating two connecting peaks as split would produce smaller peak areas as compared to treating the two peaks as overlap.

For a group of non-detected connecting peaks, you can try applying this integration method to re-process the group of connecting peaks. That is to apply Start to treat peak overlap just before the Start point of the group of connecting peaks.

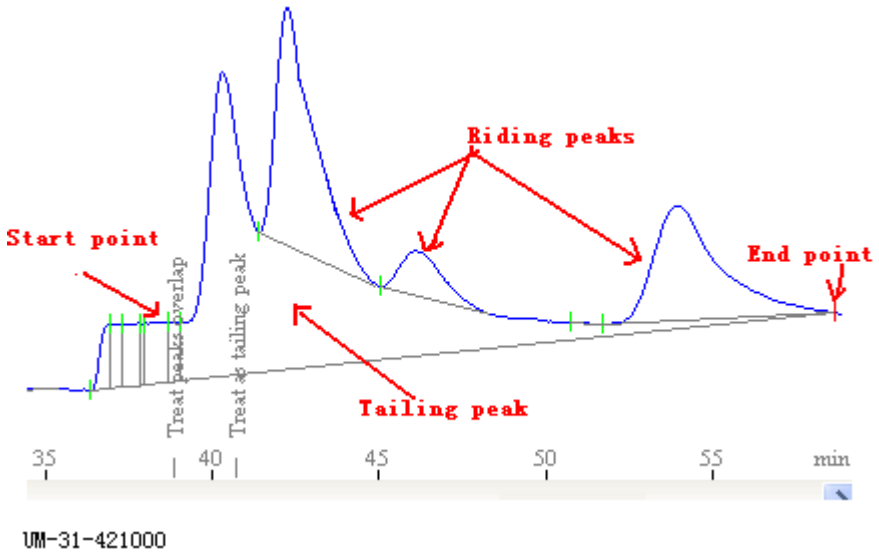
Reset to default processing

This is to instruct PEAK-ABC to reset to default processing from this point onwards. The time corresponding to the position of the cursor where the Pop-up menu is activated and the selected integration method – Reset to default processing would automatically be captured in the Integration table together with a marking on the x-axis.

This command is normally used to terminate the application of an integration method.

Treat this as tailing peak

For small peaks riding on the descending slope of a big peak, this integration method is to treat the big peak as tailing peak. PEAK-ABC applies tangent split to split the riding peaks from the big peak. The outline of the descending slope of the big peak would then be marked and used as the Baseline for the small riding peaks.



This method is only applicable for a group of overlap peaks. You must first apply Treat peaks overlap to process the group of connecting peaks. Having done so, move the cursor near the center of the big peak of interest and right click on the mouse to view the Pop-up menu, click on Input Integration table to select this integration method. In fact, what is done at this step is to replace the vertical plump line (of overlap peaks) by tangent split lines.

The time corresponding to the position of the cursor when the Pop-up menu is activated and the selected method of processing -- Treat this as tailing peak would automatically be captured in the Integration table together with a marking on the x-axis to display the selected integration method. The End point of the group of connecting peaks is automatically recognized to be the Ed point of this tailing peak. If you need to change the End point of a tailing peak, you must apply "Start to treat peak as split" to mark the End point.

If PEAK-ABC cannot detect very small peak riding on the slope of a peak, the first step is to apply Treat this peak as tailing to re-process the big peak. The second step is to enlarge the segment of chromatogram vertically (Section 5.1, Displaying parameter – Volt). The final step is to manually mark the peak as explained in Section 4.1.2.1.1 using the  icon found on the Tool bar.

4.2.2 Input retention time in Component table

We know that every component is represented by a corresponding peak within the chromatogram and that the retention time of every peak is almost constant and independent of other peaks. The purpose of the Component table is for you to identify components by their respective retention times; their names; their quantities and their corresponding calibrators, if known.

This command is for you to capture the retention time of a component in the Component table. There are five ways to input the Component table. The first way is by making use of the keyboard to make direct input. The second way is by using the Load template command (Section 4.1.1.8), which is usually used for making pre-acquisition inputs. The third way is by using the paste and copy function to copy the content of a Component table of another Document window. The fourth way is by using the Fetch time button to be explained in Section 5.3 (Component table). The fifth way is by using this command under the Pop-up menu.

Simply position the cursor near the center of the peak of interest and right click on the mouse to view the Pop-up menu to select this command. The time corresponding to the position of the cursor when the Pop-up menu is activated would automatically be captured as the retention time of the component under the RetTime column of the Component table. Meanwhile a short cyan line on white background (or pink line on Black background) would be marked on the horizontal axis (corresponding to the selected retention time) to confirm the input. If a marking is dragged to a new position (by using the mouse), the retention time captured in the Component table would be adjusted accordingly. Please refer to Section 5.3 (Component table) for more information about working with the Component table.

4.2.3 Start point to flip and End point to flip

This set of command is for you to invert (from negative to positive) a range of peaks after acquisition of the chromatogram. Simply move the cursor to the start point of the peak of interest. Right click on the mouse to select Start point to flip from the Pop-up menu. Move the cursor to the End point of the peak of interest to right click on the mouse to select End point to flip from the Pop-up menu. Please also refer to Section 4.1.4.4.5 (Flipping) for more detail about how to input pre-acquisition command to flip. Pre-acquisition input of this command would be applied by the system as and when data signal is being acquired. Post-acquisition input of this command would only be applied by the system by activating the Re-integrating command.

4.2.4 Set as chromatogram of blank sample and Subtract chromatogram of blank sample

This set of command is for you to obtain the difference between two chromatograms. Click open the two chromatogram files of interest. Position the cursor on the chromatogram that you wish to set as chromatogram of blank sample, right click on the mouse to select this command from the Pop-up menu. Having done so, position the cursor on the other chromatogram, right click on the mouse to select the Subtract chromatogram of blank sample to view the resulting new chromatogram.

4.2.5 Magnitude of baseline drift and noise

This is the command for you to find out the magnitude of baseline drift and noise of the corresponding analytical instrument. This command is to be activated immediately after acquiring no less than 30 minutes of blank sample. Simply position the cursor within any spot of the chromatogram and right click on the mouse to execute this command from the Pop-up menu.

Method of calculating noise: Partition the entire chromatogram into equal segments with width of one second each. Each segment is overlap with the other with width of half a second. Obtain the difference between the highest voltage and the lowest voltage within each segment. The highest difference among all the segments is the magnitude of noise.

Method of calculating baseline drift : Partition the chromatogram in the same way as mentioned above. Calculate the average signal voltage after eliminating abnormal data. The difference between the highest average value (among all the segments) and the lowest average voltage (among all the segments) is the magnitude of baseline drift.

4.2.6 Value of plate number

This command is for you to read the plate number of the column of the analytical instrument corresponding to a particular peak. Move the cursor near the center of the peak of interest and right click on the mouse to execute this command from the Pop-up menu.

Formula of theoretical plate number:

$= 5.54 \bullet (\text{Absolute peak top time} / \text{Width of peak at half height})^2$

Formula of effective plate number:

$= 5.54 \bullet (\text{Relative peak top time}^* / \text{Width of peak at half height})^2$

* Relative peak top time is derived by subtracting the absolute peak top time of the very first peak from the absolute peak top time of the selected peak. If need be, you may use the manual ignore peak or manual detect peak method explained in Section 4.1.2.1.1 and Section 4.1.2.1.2 respectively to create /adjust the position (hence the retention time) of the first peak.

4.2.7 Peak information

This is the command for you to find out the various system-calculated statistics about a particular peak such as peak width, peak area, width at half height, tailing factor, capacity factor and resolution etc. Simply move the cursor to the peak of interest, right click on the mouse to execute this command from the Pop-up menu.

Tailing factor : the peak width measured at 5% of peak height / two times the width of left side of the peak.

Resolution : 2 * peak top distant between two connecting peaks / the sum of base width of the two connecting peaks.

Capacity factor : (Absolute retention time of peak / Absolute retention time of the first peak) - 1

We can see from the above formulae that the calculated Tailing factor and Resolution would be more stable when dealing with independent peak. For overlap peak, the results may be relatively less stable. If need be, you may use the manual ignore peak or manual detect peak method explained in Section 4.1.2.1.1 and Section 4.1.2.1.2 respectively to create /adjust the position (hence the retention time) of the first peak.

4.2.8 Molecular weight distribution

This command is only applicable for analysis of GPC chromatogram. Please refer to Section 7.8 for more information. It is for you to find out the molecular weight distribution from GPC chromatogram. Move the cursor near the center of the peak of interest and right click on the mouse to execute this command from the Pop-up menu.

4.2.9 Get raw chromatogram data from file…..

This command is for you to retrieve raw chromatogram data (acquired by other system) into current chromatogram frame for further analysis. When activated, a dialogue frame similar to that of Section 4.1.1.2 (Open) would be displayed for you to identify the file of interest. Please note that the compatible file types are .txt; .dat; or .cdf..

4.2.10 Export raw chromatogram data to file….

This command is for you to save the chromatogram data of the current chromatogram frame for further analysis. Position the cursor within any spot of the chromatogram, right click on the mouse to execute this command from the Pop-up menu. When activated, a dialogue frame similar to that of Section 4.1.1.2 (Open) would be displayed for you to key in the file folder, the file name and the file type which can either be .txt or .dat.

4.2.11 Copy chromatogram to clipboard

This command is for you to copy the chromatogram of the current chromatogram frame to clipboard to be pasted in other application (Excel or Word) for further processing. Simply position the cursor on any spot of the chromatogram, right click on the mouse to execute this command from the Pop-up menu.

Chapter 5 The Six Working Tables

These Six working tables form one of the unique features of PEAK-ABC. The Six tables are Acquisition table, Integration table, Component table, Calculation table, Results table and Report table. The functions of these Six working tables during the entire analysis process are as follow:

Before the start of acquisition, PEAK-ABC would first go through the Acquisition table and the Integration table to look out for any pre-acquisition inputs, if any, that have been input in these two tables and apply them on a real time basis as and when data signal is being acquired. If you need to re-process the acquired chromatogram, these two tables are for you to input the changes to be made.

During qualitative and quantitative analysis, the Component table is for you to identify (those components involved in the quantifying process) by their respective retention times; their names; their quantities and their calibrators if known. While the Calculation table is for you to specify the quantifying method, the Results table is for you to view the result of the calculations.

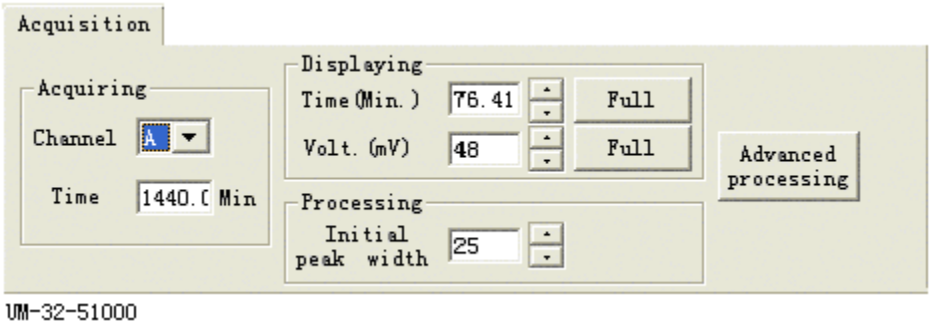
Lastly, the Report table is for you to record external reference information to be stored as part of the Chromatogram file for future reference. You can use it together with Section 4.1.4.4.2 (Option, Report) to customize a report format that best meet your need.

Input of these Six working tables can be made in four different ways. The first way being the most important way is by making use of the Load template command (Section 4.1.1.8). The second way is by using the Pop-up menu (Section 4.2). The third way is by making use of the keyboard to make direct input. The fourth way is by using the copy and paste command to copy the content of each of the Six working table from one Document window to another. When inputting, remember to move the cursor away from the input field to another input field to validate the input. If you need to append an additional row in the Component table, simply use the downward key from the keyboard. If you wish to delete a row from the Component table, position the cursor on any cell within the row and right click on the mouse to select the delete row command.

Pre-acquisition inputs made in these tables would be applied by the system on a real time basis during chromatogram acquisition. Please note that post-acquisition inputs made in the Acquisition table and Calculation table would only be applied by the system after activating the Re-integrating command (Section 4.1.3.3), and Calculating command (Section 4.1.3.4) respectively.

5.1 Acquisition table

Acquisition table is for you to input the acquiring parameters to be applied during chromatogram acquisition such as the acquiring channel; the acquiring time duration; the initial peak width and other advanced processing parameters.



Acquiring

Channel: This field is for you to specify the acquiring channel, which is applicable when you are using Dual-channel Model.

Time (Min): This field is for you to input the acquiring time duration (in minute). Acquisition would end when reaching the specified time limit. If need be, you can abort the acquiring process by activating the command Stop acquiring as explained in Section 4.1.3.2.

Displaying

Time (Min) / Full: This is the parameter for you to adjust the screen display of the chromatogram on a real time basis. By increasing the scale of the horizontal axis, a smaller chromatogram would be displayed. The Full button is to horizontally fit the entire chromatogram within the screen.

Volt (mV) / Full: This is the parameter for you to adjust the screen display of the chromatogram on a real time basis. By increasing the scale of the vertical axis, a smaller chromatogram would be displayed. The Full button is to vertically fit the entire chromatogram within the screen.

Processing --- Initial Peak Width

This is one of the more important parameter that you need to know. If you have prior knowledge of the value of the Initial peak width of the chromatogram, key it in. Otherwise, use the default value. Knowing that peak width usually increases as acquisition proceeds, PEAK-ABC applies this value as the basis to detect the first peak and assume that a subsequent peak widens at a certain speed. Any peak with a width substantially different from the specified value would not be detected.

When a peak corresponding to a component is detected, the retention time of the component would be marked on top of the peak. Moreover, a short green line would be drawn to mark the Start point of the peak and a short red or green line would be drawn to mark the End point of the peak. Any peak with no peak top time, no green or red line is regarded as not detected. Please refer to Section 4.2.1 (Input Integration table) for more information about marking of overlap peaks and split peaks.

If the peak corresponding to a component is not detected, you can execute the command for Re-integrating after changing the value of Initial peak width.

Advanced Processing

Minimum peak area: This is for you to instruct PEAK-ABC to ignore all peaks with area less than the specified minimum peak area. Please refer to Section 4.2.7 (Peak information) for more information about how to find out the area of a particular peak.

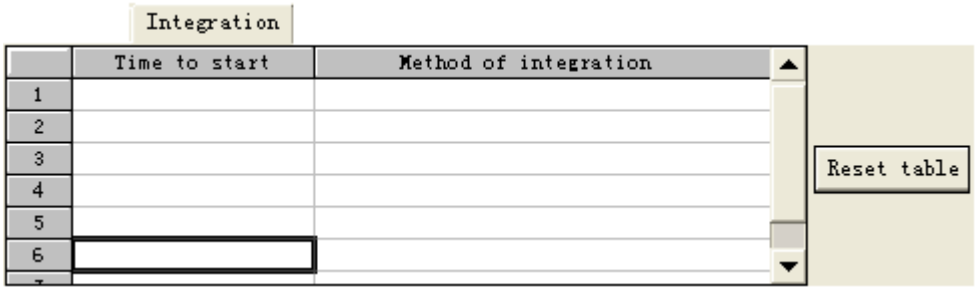
Narrow peak filtering: This is for you to set the degree of filtering to be applied during the acquisition process. The parameter should only be adjusted when you need to detect very narrow peak.

Speed of peak widening: Knowing that peak width usually increases as acquisition proceeds, PEAK-ABC assumes that a peak widens at this speed. This parameter should only be adjusted when there are big changes in the width of connecting peaks.

5.2 Integration table

Integration table is where you input the various integration methods to be applied to different segments (different retention time) of the chromatogram. Pre-acquisition inputs would be applied on a real time basis as and when data signal is being acquired. Post-acquisition inputs would be applied automatically after specifying the Integration method.

Input of Integration table can be made in three different ways. The first way being the most important way is by making use of the Load template command (Section 4.1.1.8). The second way is by using the Pop-up menu (Section 4.2) to capture the time and method of integration automatically. The third way is by using the copy and paste command to copy the content of an Integration table of another Document window.



Time to start

This column captures the time to start applying a particular integration method. Please refer to Section 4.2.1 (Input Integration table) for more detail.

Please ensure that when you select the time to begin a certain command, give some allowance by clicking slightly before the short green or red line (rather than on the short green or red line). Giving enough allowance is even more important if you intend to save the input as template for future analysis. Please refer to Section 4.1.1.7 (Save template) for more information about the command.

Please note a marking would be made on the x-axis to display the time to start applying the selected integration method. You can adjust the time to start applying (by repositioning the marking) by dragging the marking with the mouse. The time captured in the Time to start column would be adjusted accordingly.

Method of integration

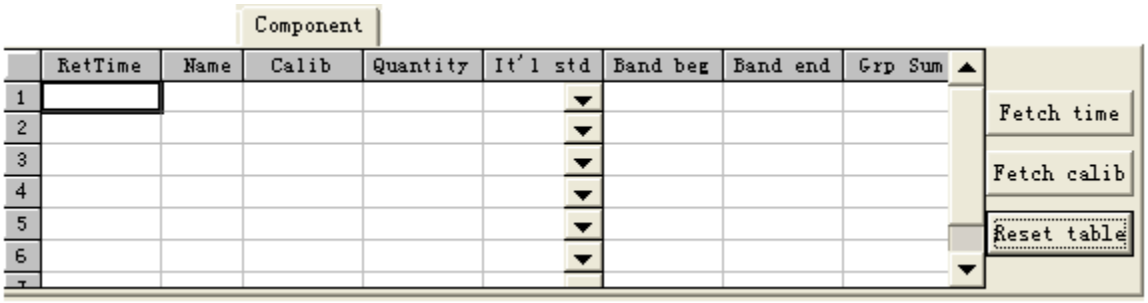
This column displays the selected integration method. Please refer to Section 4.2.1 (Input Integration table) for more information about the command.

Reset table

This is for you to clear all the inputs made in this table. Remember to click on Re-integrating command to validate this command.

5.3 Component table

The purpose of the Component table is for you to identify components (involved in the Qualitative and Quantitative analysis) by their respective retention times; their names; their quantities and their corresponding calibrators, if known. Thus, this table must be input before executing the Start calculating command. When inputting, remember to move the cursor away from the input field to another input field to validate the input. If you need to append an additional row in the Component table, simply use the downward key from the keyboard. If you wish to delete a row from the Component table, position the cursor in any cell of within the row and right click on the mouse to select the delete row command.



S/N

This column displays the position of components within the Component table.

RetTime

This column displays the retention time of a component corresponding to a peak. Every component identified in this table would have a corresponding time marking (a short cyan line) on the horizontal axis. You can choose to display or hide the time marking as explained in Section 4.1.4.4.3 (Option, Screen).

During qualitative analysis, so long as the time marking falls between the Start point and the End point of a peak, the component corresponding to this time marking is said to be present. If there is no peak corresponding to a time marking, the component corresponding to that time marking is said to be absent.

If there are two marking present between the Start point and the End point of one peak, the component corresponding to the time marking nearer to the peak is regarded to be present.

Please note that you can shift the time marking of a component by dragging it using the cursor. You can also move the time marking of all the components by pressing and holding on to the "Shift" key while dragging any one of it using the cursor. The time captured in the RetTime column would be adjusted accordingly.

Please refer to the section on Fetch time command to see how you can refresh the entire RetTime column by the actual peak top times captured in Results table.

Name

This column is for you to key in the name of the component (s). The length of name should not exceed 16 alphabets. The name entered would be displayed together with the time marking explained in the above section. Please refer to Section 4.1.4.4.3 (Option, Screen) and the above section for more information about displaying and shifting the name marking.

Calib (Calibrator)

Please refer to Section 1.2 (Terms of reference) for a simple definition. This column displays the value of calibrator (up to 6 decimal point), which is usually calculated by injecting a standard sample as explained in Section 6.3. If you are certain about the value, you may also key it in directly.

Quantity

This column displays the known quantity (in volume, in weight, or in concentration) of the component (s). When you are calculating calibrator (s) using standard sample, you have to key in the known quantity of the component (s). When you are calculating quantity using unknown sample, leave it blank.

It'l std (Internal standard)

If Internal standard is added to the standard sample and unknown sample, you can use this column to identify it by inputting "IS" in the corresponding field. For more complicated analysis whereby more than one Internal standards are added, you can use this column to designate different Internal standards to be applied by different group of components. For example, if two Internal standards are added, assuming that the component corresponding to the 2nd peak is the Internal standard to be applied to the 1st and 4th peak, the component corresponding to the 3rd peak is the Internal standard to be applied to the 5th and 6th peak, you should proceed to identify them as follow:

Component

	RetTime	Name	Calib	Quantity	It'l std	Band beg	Band end	Grp Sum
1								
2					IS			
3					IS2			
4								
5					Grp2			
6					Grp2			

The components highlighted in green is the first group of components where the Internal standard is identified as IS while the applicable components are left blank.

The componnets highlighted in yellow is the sencod group of componnets where the Internal standard is identified as IS2 while the applicable components are identified as Grp2.

Band Begin (Beg) and Band End

This Band beg. and Band end columns are useful if you wish to obtain the aggregated quantity of a few components corresponding to a few connecting peaks. You only need to key in (in a new row) the name and the time to begin (Start point of the first peak) and the time to end the band (End point of the last peak) in the respective input field leaving the RetTime input field blank. If the band is specified to begin from the 1st minute to end on the 5th minute, which contains three peaks, you can still identify the three peaks individually in the usual way.

The time input in Bend begin and the time input in Bend end works like a parenthesis. The left parenthesis should include the Start point of the group of peaks while the right parenthesis should include the End point of the group of peaks. Two different sets of parenthesis may overlap each other in the sense that a peak (or a group of peaks) could appear in both parentheses.

Another way of grouping connecting peaks is explained in Section 4.2.1 (Input Integration table, Start to merge peaks). The only difference is that Start to merge peaks would treat each peak as one before effecting the aggregation while this command would leave the peaks as they are (i.e. split, overlap or trailing) while effecting aggregation.

Group Sum

This column is for you to obtain the quantity of a few components corresponding to a few non-connecting peaks both individually and collectively. Simply key in a name (a letter "T" for example or any name not

more than six alphabets) in this column for the selected peaks. An additional row would be created in the Results table to show the results of aggregation.

Fetch time

This button is for you to refresh the RetTime column of this table by the RetTime column of the Results table. Please refer to Section 5.4 for more information about working with Results table.

This function is especially useful if there are many peaks involved in qualitative and quantitative analysis. Instead of keying in their retention time one by one, you can make use of this command to capture the retention time of the various peaks from the Results table in just three steps. The first step is to acquire the chromatogram. The second step is to apply Normalization as the quantifying method. The last step is to apply this command to capture the retention time of these peaks from the Results table.

Fetch calibrator (Calib)

When calculating calibrator (s) from injection of Standard sample, you need to activate this button to update the calculated calibrator (s) from the Results table to the Component table upon successful quantification of calibrator (s). However, this step can be automated as explained in Section 4.1.4.4.1 (Option, General, Auto-fetch calibrator).

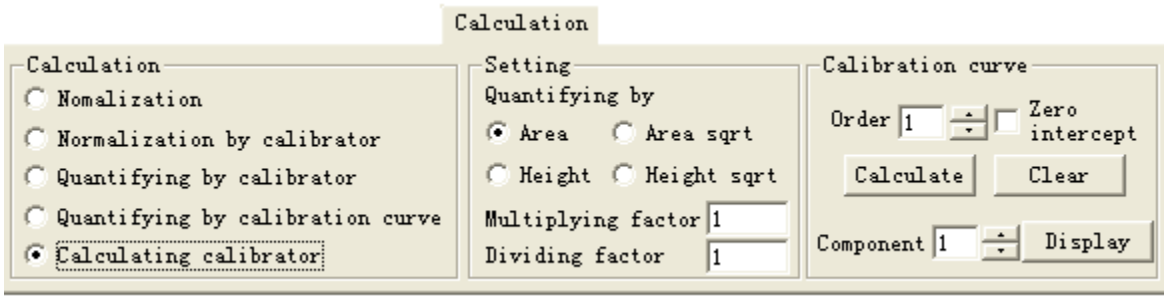
When calculating average calibrator (s) from injection of a series of Standard samples with identical quantity, this is the final step that must be performed to update the calculated average calibrators from Results table. Please refer to Section 6.3 and 6.4 for more detail about calculating calibrator (s) and average calibrator (s).

Reset table

This button is for you to clear the content of this table. Remember to click on calculating command to validate this command.

5.4 Calculation table

Calculation table is for you to perform the various type of calculation and to construct the calibration curve (s).



Normalization

This method expresses the calculation result in % terms being the ratio between individual peak area and the aggregated peak areas. Any value exceeding 0% would indicate the presence of the particular component. If there are 10 components, there would be 10 values adding to a sum of 100%.

This method does not involve the setting of Component table. Please refer to Section 5.3 (Component table, Fetch time) for more information about how to make use of this method to capture retention time in Component table.

Normalization by calibrator

This method also expresses the calculation result in % terms but only for those components identified in the Component table. Any value exceeding 0% would indicate the presence of the particular component. If there are 5 components identified in Component table, there would be 5 values adding to a sum of 100%.

This method entails the need to first input the calibrators of the components involved. Calibrator (s) may be calculated from standard sample as explained in Section 6.3. Calibrator (s) may also be obtained from published Journal. For component of same nature, you may assume a constant value of say 1 or 100 for all the calibrator (s).

Quantifying by calibrator

This method calculates and expresses the calculation result in absolute terms. This method entails the need to first obtain or calculate the calibrators or average calibrators of the components concerned. Please refer to Section 6.3 for more detail about how to calculate calibrator or average calibrator and Section 6.8 for more detail about how to apply it to calculate the quantity of unknown sample.

Quantifying by calibration curve

This method calculates and expresses the calculation result in absolute terms. This method entails the need to first construct the calibration curves of the components concerned. Please refer to Section 6.5 for more detail about how to construct calibration curve and Section 6.9 on how to apply it to calculate the quantity of unknown sample.

Calculating calibrator

This is to calculate the calibrators of components from a standard sample. Please refer to Section 6.3 for more detail about how to calculate calibrator.

Setting -- Quantifying by

This is for you to select the basis of quantifying. Select any one of the following which is set to "Area" by default.

Area	:	If activated, it would be quantified by peak area.
Height	:	If activated, it would be quantified by peak height.
Area sqrt	:	If activated, it would be quantified by square root of peak area.
Height sqrt	:	If activated, it would be quantified by square root of peak height.

Multiplying factor ___
Dividing factor ___

If you need to remove the effect of dilution, key in the multiplying factor in the Dilution factor. If you need to work back to get the original quantity, key in the Sample amount in the Dividing factor. Set it to 1 if there is no need to set any multiplying or dividing factor.

Calibration curve (Calib Curve)

The final step to construct calibration curve is to be done in this table. To be sure, you can only come to this step after completing archiving series of Results tables. Please refer to Section 6.5 for more detail about how to construct calibration (s) curve (s) from a series of standard samples with different component (s) quantities.

Order

Set it to 1 if you wish to construct a straight-line curve. Set it to 2 if you wish to construct a parabola curve.

Zero intercept

This is for you to set whether the curve is to pass through the origin.

Calculate

This is the command for you to start constructing the calibration curves of the various components. Please refer to Section 6.5 for more detail about construction of calibration curves.

Clear

This command is for you to delete all calibration curves.

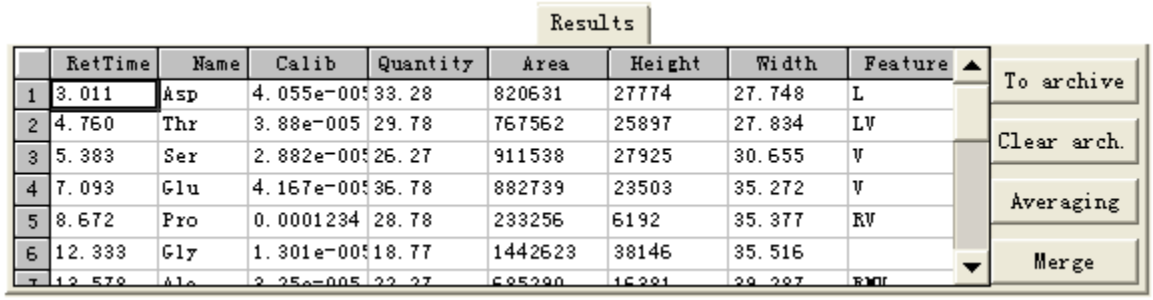
Component

This field is for you to identify the calibration curve of the component that you wish to view. Simply key in the position ranking of the component within the Component table.

Display

This command is for you to display the calibration curve of the selected component. Look out for the "x" marked around the curve. Any "x" falling far away from the curve would entail the need to reconstruct that point. Please refer to Section 6.5 for more detail about re-constructing calibration curve if need be.

5.5 Results table



The Results table contains the calculation results at various stage of chromatogram processing. If you are calculating calibrator, the calculated calibrator would be displayed in this table. If you are calculating quantity, the calculated results would also be displayed in this table. You may note that the content of Results table can be directly exported to Excel application for further processing. You can also right click on the mouse to select and copy a certain portion of the table to be pasted on other application for further analysis.

S/N

This column displays the position ranking of component within the Results table. It should be the same as that of the Component table.

RetTime

This column displays the retention time (peak top time) of the peak corresponding to a particular component. Please refer to Section 5.3 (Component table, Fetch time) for more detail about how you can use the Fetch time command to copy the time from this table to Component table.

Name

This column displays the name assigned to a particular component as per the Component table.

Calibrator

This column displays the calibrator corresponding to a particular component as per the Component table. It is blank for Normalization method and Quantifying by calibration curve method.

Quantity

This column displays the calculated quantity corresponding to a particular component. For Normalization method and Normalization by calibrator method, the value in this column is in % terms adding to a sum of 100%. For Quantifying by calibrator method and Quantifying by calibration curve method, the result is expressed in absolute term after multiplying and dividing the dilution factor and the sample amount. A message "missed" would be displayed in the feature column for component identified in Component table but detected not to be present.

Area

This column displays the peak area corresponding to a particular component. The unit of measurement is µ volt. Please also refer to Section 4.2.7 (Peak information) for more detail about how to find out the area of any one of the detected peaks.

Height

This column displays the peak height corresponding to a particular component. The unit of measurement is µ volt*second. Please also refer to Section 4.2.7 (Peak information) for more detail about how to find out the height of any one of the detected peaks.

Width of peak at half height

This column displays the width of peak at half height corresponding to a component. The unit of measurement is in second. Peak area of a single peak roughly equals the product of peak height and width of peak at half height. The difference for overlap peak is expected to be greater. Please also refer to Section 4.2.7 (Peak information) for more detail about how to find out the width of any one of the detected peaks at half height.

Feature

This column displays the feature of a peak corresponding to a component. Please also refer to Section 4.2.7 (Peak information) for more detail about how to find out the feature of any one of the detected peaks. The type of features are :

L : May consist of a hidden peak on the left.
R : May consist of a hidden peak on the right.
M : May be overlap by more than one hidden peaks.
N : Suspected to be a noise rather than a peak.
V : Overlap with another peak on the left.
T : Tangent split is applied to this peak.

Save archive

This is applicable when calculating average calibrator, calculating average quantity as well as constructing calibration curve. It is for you to save a series of Results tables to a temporary site. Please refer to Section 6.4 for more detail about calculating average calibrator (s). Please also refer to Section 6.5 for more detail about construction of calibration curve (s). Please also refer to Section 6.10 for more detail about calculation of average quantity of a series of unknown samples.

Clear Archive

This is to clear the selected content of the archive.

Average archive

This is for you to calculate the average value of a series of Results table including calibrators (or quantities) stored in a temporary site. The execution of this command would also refresh the content of the Results table. Thus, you can open a new document window to activate this button so as to leave the content of the original Results table unchanged. Please refer to Section 6.4 for more detail about calculating of average calibrator. Please also refer to Section 6.10 for more detail about calculation of average quantity of a series of unknown samples.

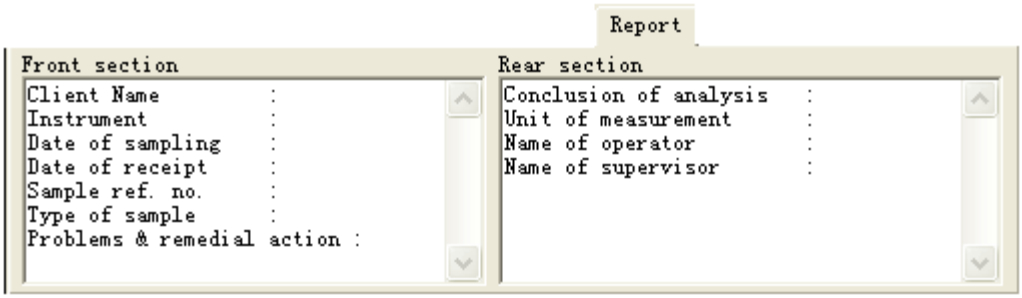
Merge

This is for you to merge two or more Results tables into one. Open the series of chromatogram files that you wish to merge, after which, click on this button of any one of the Results table to perform the task. After merging, the contents of the other Results tables would be appended below this Results table. If there are two Results tables each having 10 components, the combined table would have 20 components.

5.6 Report table

The Report table contains a Front section and Rear section for you to key in external reference information pertaining to the analysis to be included in the analysis report. As explained in Section 4.1.4.4.2 (Option, Report), you can also select to include certain system-calculated statistics such as Time of injection, Time of printing, Filename, Quantification results etc in the analysis report.

To comply with the Good Laboratory Practice (GLP) requirements, you should make use of this Front and Rear sections to capture essential reference information about a particular sampling for future reference. Information input would be permanently stored as part of the chromatogram file explained in Section 1.2 (Terms of reference).



Front section

This is for you to input external reference information pertaining to this analysis to be included in the analysis report and also stored as part of the Chromatogram file for future reference. Information to be included can be Report reference number, Sample name, Source of sample, Date of sample, Name of client, Conditions of analysis (about the sample and the analytical instrument) and Method of analysis etc.

Rear section

This is for you to input other external reference information pertaining to this analysis to be included in the analysis report and also stored as part of the Chromatogram file for future reference. Information to be included can be Problems encountered and remedial actions taken during the analysis process, Unit of measurement, Conclusions, Name of operator, Name of supervisor etc.

Chapter 6 Operating Procedures of PEAK-ABC

6.1 Procedures of Acquisition of A Chromatogram

This Section shows you how to acquire and integrate chromatogram from an injection of either the Standard sample or Unknown sample.

1. Prepare an injection of Standard sample (or Unknown sample).

2. Open a new Document window by clicking on the  icon located on the Tool Bar.

3. Check to confirm that you are in the intended Working folder by clicking on Working folder command under File menu heading. Refer to Section 4.1.1.10 for more information if need be.

4. If this is the first time that you are analyzing sample of this mixture, proceed to step 5 now. If you have analyzed such sample before and wish to copy all or part of the settings that you had input in the Acquisition table, Integration table and other tables, proceed to effect the Load template command as explained in Section 4.1.1.8.

5. Go to Acquisition table to set the acquiring Channel and expected time limit of acquisition as explained in Section 5.1. (Check to see whether the settings retrieved from the template file need be adjusted and key in the changes if any.)

6. Click on the  icon located on the Tool Bar to activate the acquisition process. Chromatogram would start forming in the lower portion of the chromatogram frame.

7. This real time acquisition would stop automatically when the time limit matches that specified in the Acquisition table. You can also click on the  icon located on the Tool Bar to stop or abort the acquisition.

8. After acquiring the chromatogram, observe to see whether there is any non-detected peak. If there is non-detected peak, refer to Section 5.1 for more information about how to re-integrate the chromatogram by changing the Initial peak width (including obtaining an estimate); and the three Advanced parameters namely Minimum peak area; Degree of filtering and Speed of peak widening. Remember to execute the Re-integrating command after making changes.

9. Please refer to Section 4.1.2.1.1 and Section 4.1.2.1.2 for more detail about how to manually ignore a detected peak or mark a non-detected peak. However, this manual adjustment should only be done as a last resort. You are encouraged to go through the Re-integrating process (i.e. the Acquisition table and Integration table) to let PEAK-ABC marks out the correct Start and End points of all the peaks.

Tips 1 : If you are using PEAK-ABC for the first time, refer to Section 4.1.4.4.4 (Option, Naming) for information about how to design a naming structure to name and save the Chromatogram file to facilitate future retrieval. Refer to Section 4.1.1.10 (Working folder) and Section 4.1.1.7 (Save template) to understand the purpose of Working folder and how to design a structure that best suit your working need.

Tips 2 : As and when chromatogram is being acquired, if you want to view a bigger picture, you can adjust the value of Displaying parameter i.e. Time (min) and Volt (mV), within the Acquisition table. You can also click on the two "Full" buttons to view the chromatogram in such a way that the entire chromatogram is fitted within the chromatogram frame at that time**.** Should the chromatogram flashes repeatedly, the degree of flashing could be reduced by increasing the value of Time (min).

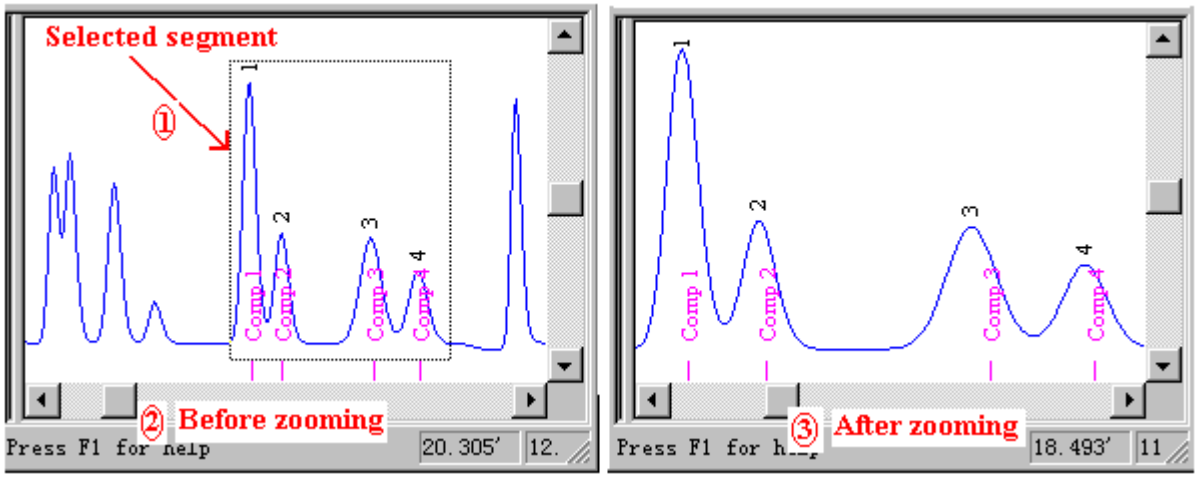
Tips 3 : As and when data signal is being acquired, this software would apply its intelligent noise filtering method to eliminate noise and proceed to integrate the chromatogram. For each detected peak, the software would first search the Integration table for any integration method that has been input prior to activating the acquisition. If there is no pre-acquisition input of integration method, system would automatically select an integration method to process the peak. This process is referred to as **Default integration**.

If you are not happy with the integration method selected by **Default integration**, you can change the integration method by applying **Manual integration**. Refer to Section 4.2.1 (Input Integration table) for more information about how to make use of the Pop-up menu to select and input the desired integration method.

For each selected integration method, a corresponding marking would be made on the x-axis to display the selected integration method and the time to start applying. You can adjust the time to start applying by simply dragging it using the mouse.

Tips 4 : If you need to zoom in on any segment of a chromatogram, simply click and hold on to the left button of the mouse and drag on it to mark out the segment of chromatogram of interest. The selected segment would be displayed within the Chromatogram frame upon release of the mouse. If need be, a further zoom in can be performed on a segment within the enlarged segment. Simply double click on the left button of the mouse to return to the previous display limit.

If you wish to expand only the baseline segment (or vertical expansion), you can do so by truncating the last digit of the value of Volt (min). The chromatogram would expand vertically without changing the horizontal scale.



Tips 5 : After making changes to the processing parameter within the Acquisition table and Integration table, remember to execute the Re-integrating command for PEAK-ABC to effect the changes.

Tips 6 : If you need to acquire a series of chromatograms from a series of injections of parallel samples (or similar mixture), and wish to open only one Document window for repeated use, you must first activate the setting to "Insert S/N of file generated from the same window" as explained in 4.1.4.4.4. Upon successful acquisition of the first chromatogram, the acquired chromatogram would be saved as a corresponding Chromatogram file in the same name of the Active Document window ending with a reference (001). The second and third chromatograms would be saved as corresponding Chromatogram files in the same name of the Active Document window ending with reference (002) and (003) and so on.

Tips 7 : When acquiring simultaneously from both channel, please refer to Chapter 4 Section 4.1.4.4.3 for more information on how to activate the setting to view the two chromatograms that are being acquired in the same Document window.

6.2 Procedures of Calculating Calibrator(s)

1. Proceed to acquire the chromatogram of a Standard sample as per Section 6.1.

2. Proceed to use the Component table to identify the various components involved by keying in their respective retention times; their names and their known quantities. Check to ensure that you have correctly input the known quantity of the component (s). Refer to Section 5.3 for more information about inputting Component table.

3. Should Internal standard be added to the standard sample, proceed to identify it within the Component table as explained in Section 5.3.

4. Go to Calculation table to select Calculating calibrator as the quantifying method.

5. Execute the command Start calculating by clicking on the  icon on the Tool Bar. The calculated calibrators (up to 6 decimal points) would be available in the Results table up to 6 decimal points.

6. If you have selected Auto-fetch calibrator as explained in Section 4.1.4.4.1(Option, General, Auto-fetch calibrator), the calculated calibrator would be updated to the Component table ready for further use. If you didn't select Auto-fetch command, you must go to Component table to click on the *Fetch Calib* button to retrieve the calculated calibrators from the Results table.

7. Please refer to Section 4.1.1.7 (Save template) for more information about how to save the calculated calibrators for future use.

Tips 1 : If you need to append a row in the Component table, simply use the downward key from the keyboard. If you wish to delete a row from Component table, position the cursor in any cell within the row and right click on the mouse to select the delete row command.

Tips 2 : Input of the Component table can be made in four different ways. The first way being the most important way is by making use of the Load template command (Section 4.1.1.8). The second way is by using the Pop-up menu (Section 4.2 Input retention time in component table). The third way is by using the copy and paste command to copy the content of a Component table from another Document window. The fourth way is by using the keyboard.

Tips 3 : After input, remember to move the cursor away to another input field to validate the input.

Tips 4 : You may note that the *Save template* command executed at step 7 may not contain the settings to be included in the Report table. Thus, a better time to execute *Save template* command should be after successful completion of an analysis.

6.3 Procedures of Calculation of Average Calibrator(s)

1 Prepare a series of Standard samples with identical or almost identical components quantity.

2 Proceed to acquire the first chromatogram from the first injection as per Section 6.1.

3 After satisfactory acquisition of the chromatogram, proceed to calculate the calibrator (s) following the procedures outlined in Section 6.2. This step can be automated by activating *Auto-quantifying when acquisition stops* as explained in Section 4.1.4.4.1.

4 Go to the Results table, click on the *To archive* button to store the Results table inclusive of calibrator (s) in a temporary site.

5 Repeat step 2 to step 4 for the rest of the series of injections. As the series of Standard samples are of similar mixture, you only need to open one Document window for repeated acquisition as explained in Tips 6 of Section 6.1. Remember to activate the setting to "Insert S/N of file generated from the same window" as explained in 4.1.4.4.4. The series of chromatogram files would be saved under the same filename ending with reference number (001), (002), (003) and so on.

6 Within the Active Document window, go to the Results table, click on the *Averaging* button to calculate the average value of the calibrator (s) to see the results of the calculation up to 6 decimal points.

7 Go to the Component table, click on *Fetch Calib* button, the calculated average calibrator (s) would be retrieved from the Results table. Please note that the memory of this temporary site would be cleared every time you exit PEAK-ABC.

8 Please refer to Section 4.1.1.7 for more information about how you can make use of the command *Save template* to store the average value of the calibrator (s) for future use.

Tips 1 : You can automate the calculation of average calibrator (s) by making use of the *One-stop quantifying* command as explained in Section 4.1.3.6. You should first acquire the series of chromatograms from the series of Standard sample. Having done so, proceed to select *Calculating calibrator* as the quantifying method in the Calculation tables of the series of chromatogram files. Click on *One-stop quantifying* command to obtain the series of result. Having done so, make use of the *Open* command to open the series of chromatogram files into a series of Document windows and proceed to perform Step 4 on each of the Document windows. Proceed to perform Step 6 and step 7 to complete the calculation of average calibrator (s).

Tips 2 : You can also break up Step 3 and Step 4 by first acquiring the series of chromatograms from the series of Standard samples. Having done so, make use of the *Open* command to open the series of chromatogram files into a series of Document windows and proceed to perform Step 4 on each of the Document windows. Proceed to perform Step 6 and step 7 to complete the calculation of average calibrator (s).

6.4 Procedures of Construction of Calibration Curve (s)

1. Prepare a series of injection of Standard samples with different component quantities.

2. Proceed to acquire the first chromatogram from the first injection as per Section 6.1.

3. After satisfactory acquisition of the chromatogram, proceed to calculate the calibrator (s) following the procedures outlined in Section 6.2. This step can be automated by activating *Auto-quantifying when acquisition stops* as explained in Section 4.1.4.4.1.

4. Go to the Results table, click on the *To archive* button to store the Results table inclusive of calibrator (s) in a temporary zone.

5. Repeat step 2 to step 4 for the rest of the series of injections. As the series of Standard samples are of similar mixture, you only need to open one Document window for repeated acquisition as explained in Tips 6 of Section 6.1. Remember to first activate the setting to "Insert S/N of file generated from the same window" as explained in 4.1.4.4.4. The series of chromatogram files would be saved under the same filename ending with reference number (001), (002), (003) and so on.

6. Within the Active Document window, go to the Calculation table, set the order of the calibration curve to "1" for a straight-line curve and set to "2" for a parabola curve.

7. Click on the *Calculate* button in the Calculation table. The calibration curve (s) of the component (s) would have been constructed by now. Please note that the memory of this temporary site would be cleared every time you exit PEAK-ABC.

8. Observe the calibration curve of each of the component by inputting its position ranking (within the Component table) and click on the *Display* button to display the calibration curve. Look out for the "x" marked around the curve. Any "x" falling far away from the curve would entail the need to reconstruct that point. This can be done by going to the Results table, click on the Clear Archive button to delete the archive corresponding to that particular point with reference to its order of filing.

9. Should reconstruction be needed, retrieve the corresponding chromatogram, check to see whether there is any error made in the Acquisition table and Component table. If so, make the necessary correction before repeating Step 3 and Step 4. Alternatively, another injection of the Standard sample with similar quantity may be taken to repeat Step 2 to Step 4. Having done so, proceed to complete the rest of the steps.

10. Please refer to Section 4.1.1.7 for more information about how you can make use of the Save template command to save the calibration curve (s) for future use. You can copy the calibration curve of any component to be pasted on any document for further reference by right clicking on the mouse.

Tips 1 : You can automate the calculation of calibrator (s) by making use of the *One-stop quantifying* command as explained in Section 4.1.3.6. You should first acquire the series of chromatograms from the series of Standard sample. Having done so, proceed to select *Calculating calibrator* as the quantifying method in the Calculation tables of the series of chromatogram files. Click on *One-stop quantifying* command to obtain the series of result. Proceed to complete the rest of the Steps from Step 4 onwards.

Tips 2 : You can also break up Step 3 and Step 4 by first acquiring the series of chromatograms from the series of Standard samples. Having done so, make use of the *Open* command to open the series of chromatogram files into a series of Document windows and proceed to perform Step 4 on each of the Document windows. Proceed to perform the rest of steps from Step 6 onwards to complete the construction of calibration curve.

6.5 Procedures of Applying Normalization Method

1. Prepare an injection of unknown sample.

2. Follow Section 6.1 to acquire a chromatogram from an injection of the unknown sample.

3. Go to Calculation table to select Normalization as the quantifying method.

4. Execute the *Start calculating* command by clicking on the  icon on the Tool Bar. The results calculated would be available in the Result table.

Tips 1 : This method expresses the calculation result in % terms being the ratio between individual peak area and the aggregated peak areas. Any value exceeding 0% would indicate the presence of the particular component. If there are 10 components, there would be 10 values adding to a sum of 100%.

Tips 2 : This method does not involve the setting of Component table. As explained in Section 5.5, you can make use of the *Fetch time* function together with this method if there are many peaks involved in qualitative and quantitative analysis. Instead of keying in their retention time one by one, you can make use of this command to capture the retention time of the various peaks from the Results table to the Component table in just three steps. The first step is to acquire the chromatogram. The second step is to apply Normalization as the quantifying method. The last step is to apply *Fetch time* command to capture the retention time of these peaks from the Results table.

6.6 Procedures of Applying Normalization by Calibrator Method

1. Prepare an injection each of the Standard sample and unknown sample. (A series of injection of Standard sample with identical or almost identical quantity would be needed if you wish to apply Normalization by Average calibrator.)

2. Proceed to calculate the calibrator of the Standard sample as explained in Section 6.2. (Proceed to calculate average calibrator from the series of Standard samples as explained in section 6.3.)

3. Having calculated the calibrators or average calibrators, you can proceed to acquire the chromatogram of the unknown sample under the same Document window.

   Alternatively, you can apply the command *Save template* as per Section 4.1.1.7 to save the results (calibrators or average calibrators) in a template file. Open a new Document window, activate the Load template command to retrieve the calculated calibrators (or average calibrators) to this Document window. Check the Component table to confirm that the various components contained in the Standard sample have been correctly identified in the Component table. Proceed to acquire the chromatogram from the injection of the unknown sample as explained in Section 6.1.

4. Go to Calculation table, select *Normalization by calibrator* as the quantifying method. Click on the  icon on the Tool Bar to start calculating. The results calculated would be available in the Results table.

Tips 1 : This method expresses the calculation result in % terms but only for those components identified in the Component table. Any value exceeding 0% would indicate the presence of the particular component. If there are 5 components identified in the Component table, there would be 5 values adding to a sum of 100%.

Tips 2 This method entails the need to make use of the Component table to identify the calibrator (s) of the component (s) involved. Calibrator (s) may be calculated from Standard sample as explained in Section 6.3. Calibrator (s) may also be obtained from published Journal. For components of same nature, you may assume a constant value of say 1 or 100 for all the calibrator (s).

6.7 Procedures of Quantifying by Calibrator

1 Prepare an injection each of the Standard sample and unknown sample. (A series of injection of Standard sample with identical quantity would be needed if you wish to apply Quantifying by Average calibrator.)

2 Proceed to calculate the calibrator of the Standard sample as explained in Section 6.2. (Proceed to calculate average calibrator from the series of Standard samples as explained in section 6.3.)

3 Having calculated the calibrators or average calibrators, you can proceed to acquire the chromatogram of the unknown sample under the same Document window as explained in Section 6.1.

Alternatively, you can apply the command *Save template* as per Section 4.1.1.7 to save the results (calibrators or average calibrators) in a template file. Open a new Document window, activate the Load template command to retrieve the calculated calibrators (or average calibrators) to this Document window. Check the Component table to confirm that the various components contained in the Standard sample have been correctly identified in the Component table. Proceed to acquire the chromatogram from the injection of the unknown sample as explained in Section 6.1.

4 Go to Calculation table, select *Quantifying by calibrator* as the quantifying method. Proceed to input the *Dilution factor* and *Sample amount* in the Calculation table as per Section 5.4 if need be. Click on the  icon on the Tool Bar to start calculating. The results calculated would be available in the Results table

Tips 1 : This method calculates and expresses the components quantities in absolute terms for those components identified in the Component table.

Tips 2 : This method entails the need to make use of the Component table to identify the calibrator (s) of the component (s) involved. Calibrator (s) may be calculated from a Standard sample as explained in Section 6.3.

Tips 3 : You can make use of the One-stop quantifying function to automate the calculation by first acquiring the chromatogram of an unknown sample and the chromatogram of the Standard sample and save them as two chromatogram files. The next step is to retrieve these two chromatogram files and proceed to select Calculating calibrator in the Calculation table and input the known quantity of the component in the Component table of the Chromatogram file corresponding to the Standard sample. Proceed to select Quantifying by calibrator method in the Calculation table corresponding to the unknown sample. Click on the  icon on the Tool bar to proceed. PEAK-ABC would first calculate the calibrator from the Standard sample and apply the results to calculate the component quantities of the unknown sample.

Tips 4 : For quantifying by average calibrator, you can also make use of the One-stop quantifying function to automate the calculation. The first step is to acquire the series of chromatograms. The next step is to retrieve the acquired chromatogram files and proceed to select Calculating calibrator in the Calculation table and input the known quantity of the component in the Component table of the Chromatogram files corresponding to the series Standard sample. Proceed to select Quantifying by calibrator method in the Calculation table corresponding to the unknown sample. Click on the  icon on the Tool bar to proceed. PEAK-ABC would first calculate the average calibrator from the series of Standard samples and apply the results to calculate the component quantities of the unknown sample.

6.8 Procedures of Applying Quantifying by Calibration Curve

1. Prepare a series of injections of the Standard sample with different quantity and an injection of unknown sample. Proceed to construct the calibration curves of the components as per section 6.4.

2. Having constructed the calibration curve, you can proceed to acquire the chromatogram of the unknown sample under the same Document window as explained in Section 6.1.

   Alternatively, you can apply the command *Save template* as per Section 4.1.1.7 to save the calibration curve in a template file. Open a new Document window, activate the Load template command to retrieve the constructed calibration curve to this Document window. Check the Calculation table to confirm that the calibration curve (s) of the component (s) have been correctly contained in the Calculation table. Proceed to acquire the chromatogram from the injection of the unknown sample as explained in Section 6.1.

3. Go to the Calculation table, select *Quantifying by calibration curve* as the quantifying method. Proceed to input the *Dilution factor* and *Sample amount* in the Calculation table as per Section 5.4 if need be.

4. Click on the  icon on the Tool Bar to start calculating. The results calculated would be available in the Results table.

Tips 1 : This method calculates and expresses the components quantities in absolute terms for those components identified in the Component table.

Tips 2 : This method entails the need to make use of the Calculation table to construct the calibration curve (s) of the component (s) involved. Calibration curve (s) may be calculated from a series of Standard sample with different quantity as explained in Section 6.4. Calibration curve (s) may also be obtained by Load template command.

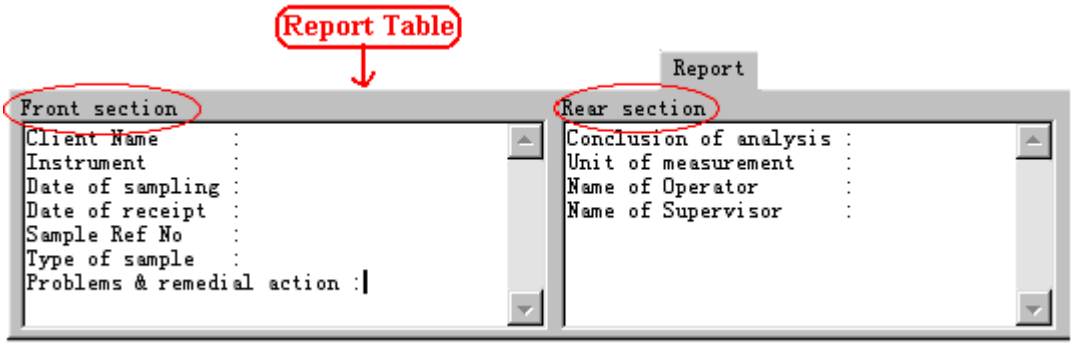
Tips 3 : You can make use of the One-stop quantifying function to automate the calculation. The first step is to acquire the series of chromatograms corresponding to the series of Standard sample with different quantity and the unknown sample and save them into a series of Chromatogram files. The next step is to retrieve the series of acquired chromatogram files and proceed to select Calculating calibrator in the Calculation table and input the known quantity of the component in the Component table of the Chromatogram files corresponding to the series Standard sample with different quantity. Proceed to select Quantifying by calibration curve method in the Calculation table corresponding to the unknown sample. Click on the  icon on the Tool bar to proceed. PEAK-ABC would first construct the calibration curve from the series of Standard samples and apply the results to calculate the component quantities of the unknown sample.

6.9 Operating Procedures of Customizing and Printing Analysis Report

1. Before you proceed to print out the hard copy of the analysis report, you can make use of the Report table as explained in Section 5.6 and *Report panel* commands as explained in Section 4.1.4.4.2 to customize a report format that best meet you requirement.

2. The *Report panel* command as explained in Section 4.1.4.4.2 is for you to include or exclude certain system-calculated results from the analysis report. Examples of such statistics are the acquired chromatogram, Peak area, Time of injection, Time of printing, Filename and Quantification method etc.

3. The Report table as explained in Section 5.6 is for you to key in external reference information to be included in the analysis report for future reference. For example, you can make use of the *Front section* and the *Rear section* of the Report Table to capture reference information such as Name of client, Date of sample, Name of operator, Method of sampling, Problems encountered and remedial actions taken and Conclusion etc. Such information would be permanently stored as part of the chromatogram file and is readily available for your viewing every time you revisit the file.



4. Click on the  icon on the Tool Bar to preview the analysis report before you proceed to print out the hard copy. If you choose to include the chromatogram in the report, you can adjust its size at this point before printing.

5. Click on the  icon within the preview window to start printing.

Tips 1 : The analysis report can be printed in Microsoft Words or WordPad application as explained in Section 4.1.4.4.2. If your computer is installed with Word application, you can print the analysis report in Word setting. Under Word setting, after printing the first report you need to first exit Word application (before you begin printing the next chromatogram file), i.e to exit Word, then open the next Chromatogram file and use the Printing preview command. Should you forget to exit Word application and proceed to print another Chromatogram file, the next report that you print would contain the Chromatogram of the previous report.

If your computer is not installed with Word application, you can opt to print the analysis report in Wordpad setting. In fact, we recommend that you select to print under this setting as the speed is faster.

Tips 2 : The format of a typical analysis report is as shown below. Apart from the reference information that can be keyed in the **Front Section** and **Rear Section** of the **Report Table**, you are also given the option to include or exclude certain calculated results in the analysis report. Thus, you have the flexibility to customize a report format that best suit your need.

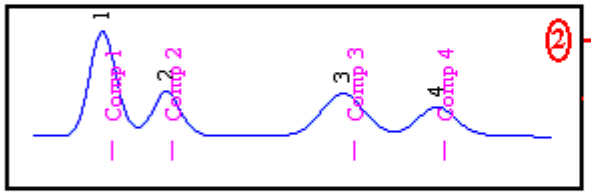
XXXX Report → Title

time: Wed Nov 27 17:31:51 2002
Injection time: Sun Mar 18 17:53:47 2001

Client Name :
Instrument :
Date of sampling :
Date of receipt :
Sample ref no :
Type of sample :
Problems & remedies :

① → Front Section of Report Table

File opened: C:\HW\Program\demo(Amino Acid)2.hw



② → Chromatogram

Selected Calculated results ③

S/N	RetTime	Name	Qty	Calib	Area	Height	Width
1	12.333	Comp 1	0		1442623	38146	35.516
2	13.578	Comp 2	0		685290	16381	39.287
3	17.021	Comp 3	0		1022361	15789	60.809
4	18.864	Comp 4	0		708217	11220	59.278
Total			0		3858491	81536	

Conclusion :
Unit of measurement :
Name of operator :
Name of supervisor :

④ → Rear Section of Report Table

Tips 3 : This *Batch printing* command as explained in Section 4.1.3.8 is for you to pre-view and print a few analysis reports at one go. Activate this command after you have opened up the series of chromatogram files that you want to print. Upon activation, the reports would be displayed for your preview. Reports would be arranged in the reverse order at which the Chromatogram files are selected. Click on the Print function to begin.

The *Insert page break between reports during batch printing* command as explained in Section 4.1.4.4.2, is for you to set whether to insert page break in between reports. When activated, page break would be inserted between each analysis report during batch printing. In so doing, every single report would be complete with report title. If not activated, no page break would be given and report title would only be printed once.

Chapter 7 Special Instructions

7.1 Procedures of Acquiring From Two Channels Simultaneously From The Same Instrument

This procedure is applicable when you want to acquire simultaneously from the dual detectors of the same instrument.

1 Before you proceed, ensure that you have connected the two signal cables to the dual detectors of the same instrument.

2 A new Document window is created when you start PEAK-ABC, so click once on the  icon located on the Tool Bar to open another new Document window.

3 Go to the two Acquisition tables in turn to set one of the Acquiring Channel to "A" while set the other Acquiring channel to "B".

4 Follow Section 4.1.5 (Window) to display these two Document windows vertically side-by-side without overlapping.

5 Proceed to activate the *Perform acquisition simultaneously for all Document windows* setting as explained in Section 4.1.4.4.1.

6 You can also apply the *Swapping* command as explained in Section 4.1.4.4.6 to swap a certain segment of the chromatogram acquired in Channel A with that of the other chromatogram acquired in Channel B with the option of joining at the rear of the first chromatogram without covering the original one. This pre-acquisition command should be set before activating Step 7 so that system can apply it on a real time basis as and when data signal is being acquired.

7 Click on the  icon on the Tool Bar to start acquiring data signal.

Tips 1 : You can make use of the merger function as explained in Section 5.5 (Results table) to merge the two Results tables corresponding to Channel A and Channel B into one. You can also make use of the Swapping command as explained in Section 4.1.4.4.6 (Option, Swapping) to merger the two chromatograms corresponding to Channel A and Channel B into one chromatogram.

Tips 2 : You can view the two curves within the same Chromatogram frame by activating "Displaying the curve in another channel during acquisition" as explained in Section 4.1.4.4.3.

7.2 Procedures of Working With Auto-Sampler

1. Ensure that the Remote starter button is connected to the Auto-sampler.

2. Proceed to activate Auto-save command as explained in Section 4.1.4.4.1 (Option General).

3. Proceed to activate the "Insert S/N of file generated from the same window" as explained in Section 4.1.4.4.4 (Option Naming).

4. If the series of injections are of different mixtures, you would need to create different filename for each of the different injection. Proceed to access the *Naming* function as explained in Section 4.1.4.4.4 to key in the name of the first injection on the Auto-sampler Board. After input, do not close this *Naming* function.

5. Click on the  icon on the Tool Bar to open a new Document window. Check to confirm that the name of this new Document window is as per your input in Step 2 above. (The name of the Active Document window is displayed on the top row of the screen as explained in Section 3.1.)

6. Proceed to effect the *Load template* command as per Section 4.1.1.8 if need be. Check to confirm that the Acquiring Time limit is correctly set to give enough time allowance for Peak -ABC to stop acquiring automatically and to get ready for the acquisition of the next sample.

7. Repeat Step 3 to Step 5 to set the respective Document window for the rest of the samples on the Auto-sampler Board.

8. Since the series of samples are of different mixture, you must proceed to activate the *Perform acquisition for each new Document window in turn* as outlined in Section 4.1.4.4.1.

9. When a sample is injected, a start signal would be transmitted by the Auto-sampler to PEAK-ABC to begin the Start acquisition command.

10. When the timing of acquisition matches that pre-set in the Acquisition table, the acquisition process would stop automatically. The next Document window will automatically be activated to be Active Document window ready for the next acquisition.

Tips 1 : If the series of samples to be injected are of the same mixture, you only need to open one Document window for repeated use. Remember to first activate the setting to "Insert S/N of file generated from the same window" as explained in 4.1.4.4.4. Upon completion of acquiring the first sample, a new Document window would be created automatically in the same name of the previous Document window plus an order reference (001, (002), (003) and so on.

Tips 2 : If there are three parallel samples (i.e sample of the same mixture) among the series of samples of different mixture, you may include a character like "r3" in the Prefix or Suffix when naming the filename corresponding to the first of such sample as explained in Step 2 mentioned above. The same Document window would be repeated 3 times to acquire the three injections before proceeding to the next Document window to process the fourth sample. If the name of the Document window is "ABC", the series of filename of the corresponding Chromatogram files would be ABCr3(001), ABCr3(002), and ABCr3 (003).

Tips 3 : If the *Auto-quantifying when acquisition stops* command is activated, PEAK-ABC would proceed to calculate the component quantities for each injection. Upon completion, you can then make use of the *Chromatograms compiler* as explained in Section 4.1.4.1 and *Results tables compiler* as explained in Section 4.1.4.2 to analyze the results further.

Tips 4 : If the *Auto-quantifying when acquisition stops* command is not activated, you can make use of the *One-stop quantifying* to calculate the component quantity of unknown sample.

Tips 5 : If you are processing another chromatogram at the time when the start signal was transmitted to PEAK-ABC, the transmission of the start signal may be discarded. To avoid this problem, you are advised to start PEAK-ABC twice, the first is solely for acquiring chromatogram from Auto-sampler, the second is solely for processing existing Chromatogram files.

Tips 6 : The Chromatogram acquisition unit is designed to respond to very low level of trigger voltage of 0.005s. If it does not respond to the trigger voltage output by the Auto-sampler, try changing to different mode of voltage output such as from that of ascending order to descending order etc..

7.3 Consistency of analysis result

1. The consistency of analysis result is dependent on the consistency of the dosage of injection, the Baseline drift and noise of the analytical instrument. You can use the Chromatograms compiler as explained in Section 4.1.4.1 to study the consistency of a series of similar injections.

2. If you are sure that analytical instrument is not the cause of inconsistency, you may proceed to investigate whether there is any inconsistency in the default integration method in processing peaks. (i.e. treat as overlap in one chromatogram but treat as split in another.) If there is such inconsistency, you may use the Integration table to apply same treatment for all the chromatograms.

7.4 Setting of Acquisition speed (or sampling rate)

1. Acquisition speed refers to the number of acquisition per second. A value of "1" would mean 1 time per second, and a value of 20 would mean 20 times per second. Usually, if you have a long acquiring time, you want to set the value to a smaller number.

2. Information pertaining to Acquisition speed is contained in the file "HWFrequence.txt". This file can be found under Windows\System if you computer is installed with Windows9x. It can be found under Windows\System32 if your computer is installed with Windows NT/2K/XP.

   Within the file, you would see a set of two number say 20 20. While the first number represents the Acquisition speed of this software system, the second number represents the Acquisition speed of the hardware –- Chromatogram Acquisition Unit, which is set to 20

unless otherwise requested before delivery.

3. If you wish to change the Acquisition speed of this Software, you should proceed to key in the desired Acquisition speed by simply typing over the first number. Please note that the first number should always be smaller than the second number and divisible by the second number. For example, if the Acquisition speed of the hardware is set to 20, the acceptable Acquisition speed of this Software can be set to either one of the following: 1, 2, 4, 5, 10 and 20.

7.5 Activating a long bep sound upon completion of acquisition

1. If your computer is connected to an amplifier, the computer would let out a long bep sound upon completion of data acquisition.

2. If your computer is not connected to any amplifier, you can activate the computer to let out a long bep sound by accessing the following file "HWMakeSound.txt". This file can be found under Windows\System for Windows9x and found under Windows\System32 for Windows Nt/2K/XP. Simply key in "Y" to activate the setting and key in "N" to deactivate the setting.

7.6 Designating a COM port to connect the hardware

1. Information pertaining to setting of Com port can be found in the file "HWCom.txt". This file can be found under Windows\System for Windows9x and found under Windows\System32 for Windows Nt/2K/XP.

2. If you wish to designate COM 1 to be the port to connect the hardware – Chromatogram Acquisition Unit, proceed to change the setting to "1,1".

3. If you wish to designate COM1 or COM2 to be the port to connect the hardware, proceed to change the setting to "1,2". System would search through COM1 and Com2 for the hardware. Likewise, a setting of "2,3" would instruct the system to search for the hardware in either Com2 or Com3.

7.7 Receiving Start and Stop signal of a instrument

1. If you only make use of one Channel for data acquisition, you can connect the hardware to receive the Start signal and the Stop signal of the instrument so that the system would start acquiring upon receipt of the Start signal of the instrument and stop acquiring upon receipt of the Stop signal of the instrument.

2. Information pertaining to this setting can be found in the file "HWRemote.txt". This file can be found under Windows\System for Windows9x and found under Windows\System32 for Windows Nt/2K/XP.

3. Proceed to change the setting from "0" to"1" within the file.

4. The next step is to connect both the Remote starter of Channel A and Channel B to the same instrument. Connect the Remote starter of Channel A to receive Start signal of the instrument. Connect the Remote starter of Channel B to receive the Stop signal of the instrument.

7.8 GPC Analysis

7.8.1 Introduction

PEAK-ABC-GPC system is fully integrated with PEAK-ABC software system. Having acquired a chromatogram using PEAK-ABC system, PEAK-ABC-GPC system enables you to accurately measure molecular weight and produce various GPC plots and reports interactively. The highlights of PEAK-ABC-GPC is as follow:

- PEAK-ABC-GPC is fully integrated with PEAK-ABC software. While PEAK-ABC software is responsible for the acquisition of data signal and integration of the chromatogram, PEAK-ABC-GPC is responsible for performing GPC analysis based on the Molecular Calibration Curve obtained before hand.

- For the Dual-channel Model, the two acquiring channels could be connected to two different instruments for independent signal acquisition. While one channel may be used for GPC analysis, the other may be used for HLPC, or GC analysis to quantify components quantity.

- Based on the various molecular weight and related measurements calculated by PEAK-ABC-GPC, user has the flexibility of designing in-house formulae or Macro programs for further analysis.

7.8.2 Operating Procedures for chromatogram acquisition

Please refer to Section 6.1 and 6.2 for detailed operating procedures about chromatogram acquisition.

7.8.3 Measurement of Narrow Distribution Plot of Standard Sample

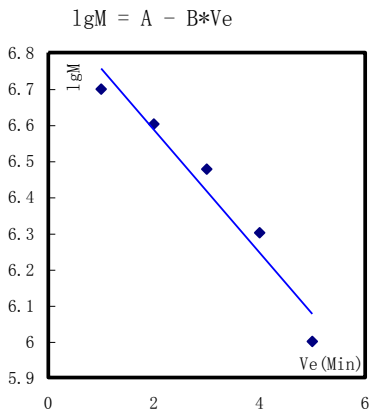
1. Follow the operating procedure to integrate the chromatogram of a standard sample of polymer with known molecular weight.

2. Position the cursor near the center of any peak, right click on the mouse to access the Pop-up menu. Click on **Molecular weight distribution** to start the Module for measurement of molecular weight written in Excel application (to be referred as "GPC Module" thereafter). Select "Yes" if you are prompted with the question whether to start Macro. (You may deactivate this prompt by checking on the relevant box).

3. Click on the label for **Narrow Plot** located at the bottom of the GPC Module to display the following page：

Calibration via Narrow Distributed Standard

Molecular Weight M	Retention Time Ve (1)	Retention Time Ve (2)	Retention Time Ve (3)	Ve Average
5000000	6			6
4000000	7			7
3000000	8			8
2000000	9			9
1000000	10			10



Based on above input, A= 7. 7750 , B= 0. 1699 , Correlation Coefficent= 0. 9733

7.8.4 Measurement of Universal Polymer Standard Calibration Parameter

1. Position the cursor near the center of any peak, right click on the mouse to access the Pop-up menu. Click on Molecular weight distribution to start the Measurement Module. Select "Yes" if you are prompted with the question whether to start Macro. (You may deactivate this prompt by checking on the relevant box).

2. Click on the label for **Universal Parameters** located at the bottom of the GPC Module to display the following page：

Universal Calibration Parameters

Flow Viscosity	Molecular Weight

Calculate from above input

α standard	K standard
1	1

Flow Viscosity	Molecular Weight

Calculate from above input

α unknown	K unknown
1	1

- If the parameters for α、K of both the standard sample and unknown sample is available from published data, simply fill in their values in the fields α_{std}、K_{std}、$\alpha_{unknown}$、$K_{unknown}$ respectively. In case where standard sample and unknown sample are of the same mixture, you may assign "1" to be the common value of these four parameters.

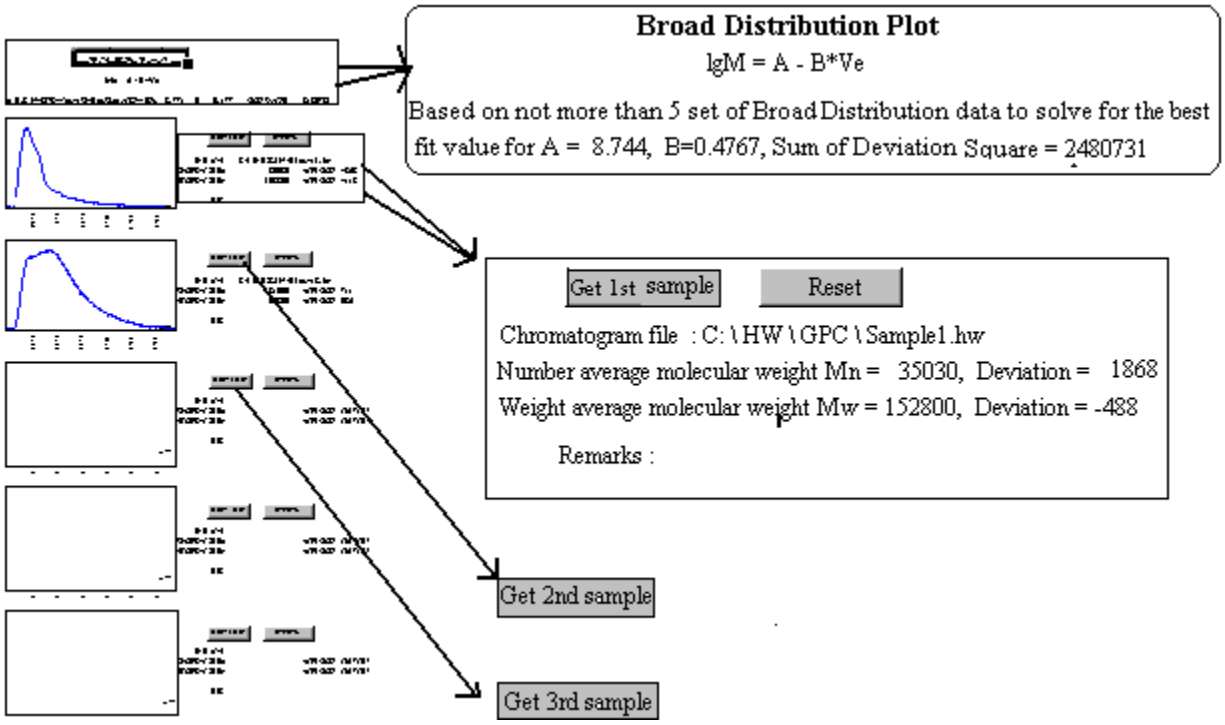
- If the parameters are not available from published data, you may fill in a few sets of actual measurement of flow viscosity and molecular weight of both the standard sample and unknown sample. Click on the Calculating button so that GPC Module would proceed to solve for the value of α、K.

7.8.5 Measurement of Broad Polymer Standard Calibration Plot

1. Follow the operating procedure to integrate the chromatogram of the first of a series of Standard sample of polymer with known weight average molecular weight and number average molecular weight.

2. Position the cursor near the center of the peak of Standard sample, right click on the mouse to access the Pop-up menu. Click on Molecular weight distribution to start the Measurement Module. Select "Yes" if you are prompted with the question whether to start Macro. (You may deactivate this prompt by checking on the relevant box).

3. Click on the label for **Broad Plot** located at the bottom of the Measurement Module to display the following page:



4. Click on **Get 1st sample** to retrieve the chromatogram of the first injection of Standard sample. Proceed to fill in the known weight average and number average of the molecular weight of the Standard sample.

5. Click on the "x" box located on the top right corner of the Excel window to exit. Select "Yes" if you are prompted with the question whether to save the changes made to the GPC Module.

6. Repeat the above steps to input the chromatogram and known weight average, number average of the molecular weight of the series of Standard samples. For practical reason, the number of Standard sample should not be more than five.

7. The equation for the Broad Polymer Standard Calibration Plot may be expressed as lgM = A - B*Ve. We are now ready to calculate the value of A & B that represents the best fit between the known and calculated weight average and number average of the molecular weight. The degree of the best fit is measured by Sum of Deviation Square, where the smaller the value, the better is the fit.

   Move the cursor to the field J5, right click on it to select this field. Select Program Solution from the Drop down menu under the Tool heading, click on Solve to execute the command. (If this option is not available, you would have to re-install the Excel application under all-inclusive installation.) The value of A & B as displayed in F5 and H5 respectively would be refreshed upon completion of recalculation. A dialogue box would be display for you to confirm the result of recalculation. If you are happy with the smaller number of degree of best fit as displayed in J5, click on "OK" to accept. You should click on "Reset" if you are not happy with the results of calculation so as to proceed to find out the reason of the big deviation.

   Note :

   - Big deviation could be the result of unreasonable values of Standard sample, or the poor estimate of the initial value of A & B.

   - To facilitate Program solution, this Excel table is not write-protected. Thus, apart from the input of known weight average and number average, you are to be extra careful not to make any input in other field to prevent corrupting the built-in formulae.

7.8.6 Calculation of Molecular Weight of Unknown Sample

1. Follow the operating procedure to integrate the chromatogram of the unknown sample of polymer.

2. Position the cursor near the center of the peak which is to be used to calculate molecular weight, right click on the mouse to access the Pop-up menu. Click on Molecular weight distribution to start the Measurement Module. Select "Yes" if you are prompted with the question whether to start Macro. (You may deactivate this prompt by checking on the relevant box).

3. Step 2 above would activate the process to perform various calculations including the polydispersity index based on the time slice of the selected peak as shown below:

Molecular Weight Measurement Report

2 December 2002

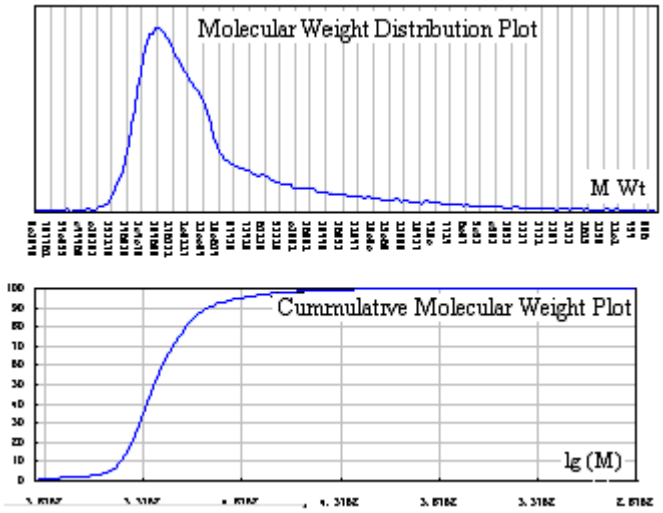
Number Average Molecular Weight Mn	=	35888,	lg(Mn) =	4.55
Weight Average Molecular Weight Mw	=	153246,	lg(Mw)=	5.19
Z Average Molecular Weight Mz	=	212308,	lg(Mz) =	5.33
Z+1 Average Molecular Weight Mz+1	=	261959,	$lg(M_{z+1})$ =	5.42
Viscosity Average Molecular Weight M_η	=	180375,	$lg(M_\eta)$=	5.26
Polydispersity Index D	=	4.27		

Chromatogram file : C:\HW\Program\demo(GPC).hw

Re-calculate

◉ Based on Narrow Plot

○ Based on Broad Plot



4. Step 2 above also activate the process to construct Molecular Weight Distribution Curve and Cumulative Molecular Weight Distribution Curve.

5. Should you need to print out the analysis report, simply click on the printer icon on the Excel Tool bar.

6. Click on the label for **Slicing Report** located at the bottom of the Measurement Nodule to display and view the slicing report in detail as shown below :

Slicing Report

No. of Slice : 203 Width of slice (min) : 0.032

Position of Peak Top Slice : 41 M Wt at peak top : 209680

S/N	RetTime (Min)	M. Wt	M Wt ratio	Peak Height	Slice Area %	Cumulative Slice Area %
1	5.977	842098	5.9254	45	0.1154	0.1154
2	6.009	813331	5.9103	90	0.2229	0.3383
3	6.040	785547	5.8952	90	0.2153	0.5536
4	6.072	758712	5.8801	40	0.0924	0.6460
5	6.104	732794	5.8650	92	0.2053	0.8513
6	6.135	707761	5.8499	69	0.1487	1.0000
7	6.167	683584	5.8348	68	0.1415	1.1415
8	6.199	660232	5.8197	69	0.1387	1.2802
9	6.230	637678	5.8046	57	0.1107	1.3909
10	6.262	615894	5.7895	98	0.1838	1.5747
11	6.294	594855	5.7744	111	0.2011	1.7758
12	6.325	574534	5.7593	29	0.0507	1.8265
13	6.357	554908	5.7442	65	0.1098	1.9363
14	6.389	535952	5.7291	87	0.1420	2.0783
15	6.420	517643	5.7140	42	0.0662	2.1445
16	6.452	499960	5.6989	40	0.0609	2.2054

Note :

- Upon changing the value of Universal Polymer Standard Calibration Parameters or Standard Plot (Narrow & Broad), you may click on the "Re-Calculating" button found on the Measurement of Molecular Weight table to recalculate the molecular weight and molecular weight distribution.

- Prior to start calculating molecular weight, user should have decided whether to adopt Narrow Polymer Standard Plot or Broad Polymer Standard Calibration Plot as the basis of calculation in the light of the prevailing conditions.

7.8.6 Other features of PEAK-ABC-GPC

1. If you are familiar with setting formulae and writing Macro program within Excel application, you may add them within the GPC Module to perform further analysis based on the various calculations calculated by PEAK-ABC-GPC.

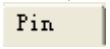
2. Should you need to customize your analysis report, you may add a new sheet within the GPC Module.

3. Every time you exit the GPC Module, you would be prompted to save the changes that have been made. Should changes have been made to the equations of the Standard plots or the Universal Parameters, you should select "yes" so that the changes made could be applied to subsequent analysis. Should changes be confined to calculation of molecular weight, you may select "no" as the answer. Should you intend to save the results of calculation use the Save as command under the file menu of the Excel system.

4. When you right click on the mouse (near the center of the peak) to access the GPC Module under the PEAK-ABC system, you indeed activate the process to calculate molecular weight of unknown sample and store the GPC Module under hw\program as mwd.xls.

5. If you try to access this mwd.xls directly from the Excel application, you have to press and hold on to the "Shift" key to avoid seeing an error message.

7.9 Procedures on setting PIN

1. After having install the system, click on the  icon on the Tool bar to access the Option command. Click on the 

tag to view the PIN panel.

2. Key in the PIN designed for administrator, operator and browser in the space given. Password should have a minimum of 2 up to a maximum of 10 alphabetical or numeric characters. Simply exit this option command to validate the input.

3. Once input, you would be prompted to key in the PIN every time you start the PEAK-ABC.

Tips 1 : User should designate an administrator to design three common passwords to be used by three different categories of users, which should be kept confidential in a sealed envelope for future reference.

Tips 2 : All operators would share one common password, while all browsers would share another common password. User of higher authority should not disclose the common password to the user of lower authority.

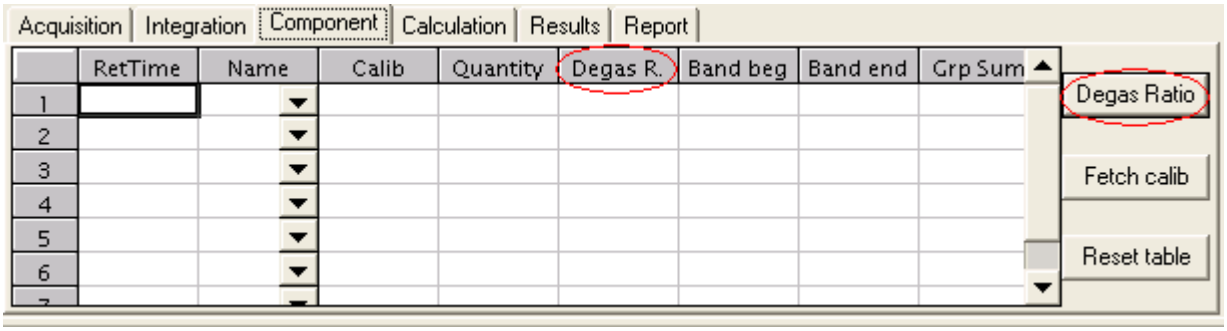
Tips 3 : Please note that if no password is set, all users are defaulted as administrator. For data security reason, after activating the system, the user should not leave the terminal unattended and must make it a point to exit from the system to prevent user of lower category from unauthorized access.

Chapter 8 Special Version for Insulation Oil

8.1 General Observations

This version will acquire and process the data signal obtained from the gases extracted from the insulation oil, and use it to calculate the quantity of various gases resolved in the insulation oil. Apart from the usual characteristics available in the standard PEAK-ABC, this version has the following characteristics:

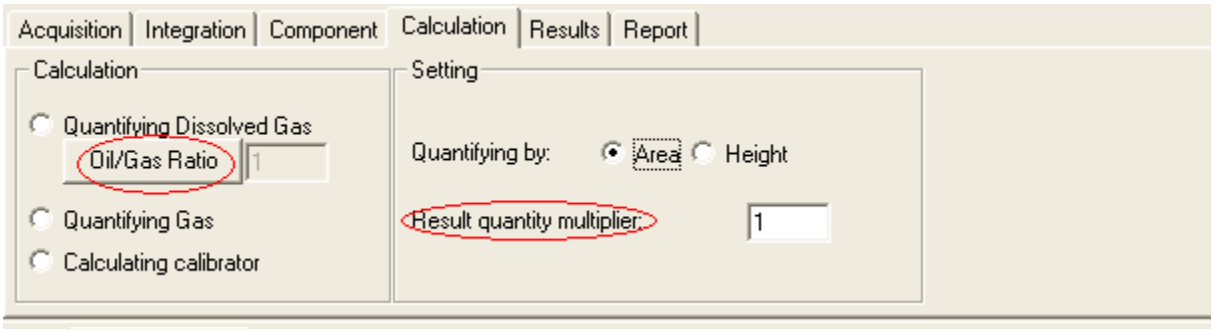
- Able to manage (new, add, delete, change, retrieve and sort) the results of analysis based on the location of sampling.
- Able to construct the curve depicting how the quantity of a component taken from the same location change as time changes.
- The Component Table of this version is different from that of the standard version in the following ways:



The Degas R. column is to capture the rate of degassing of each of the components applicable to a particular degassing method.

Whereas the Degas Ratio button is for you to calculate the theoretical degassing ratio as explained in Section 8.4 below.

- The Caculation Table of this version differs from that of the standard version in the following ways:

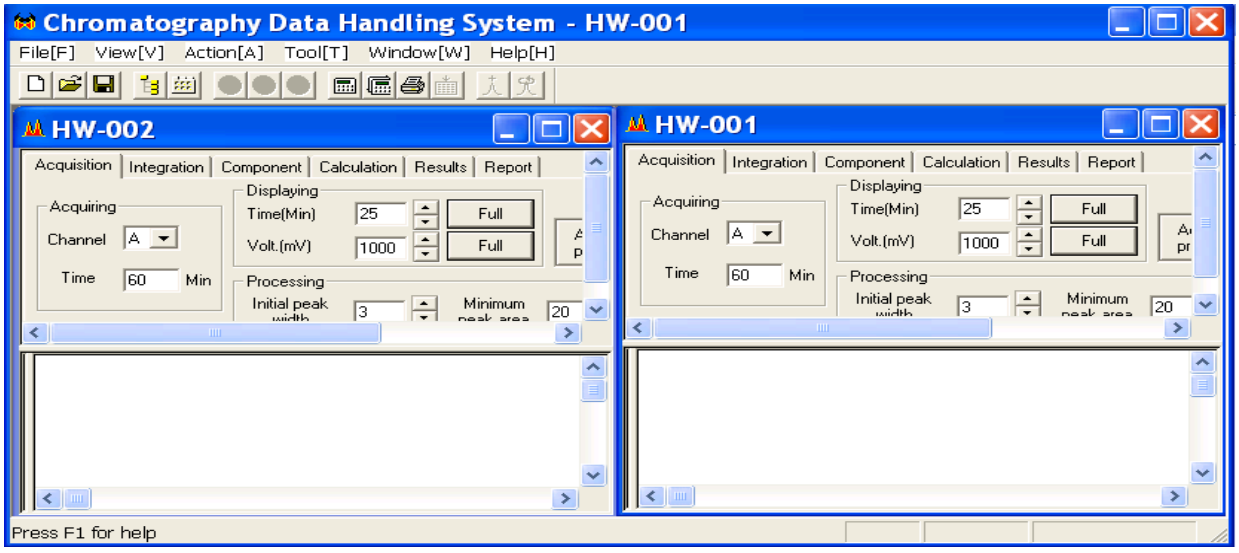


The Oil/Gas Ratio button is for you to access the pop-up window to fill in the various parameters such as the Ambient temperature, Ambient pressure, Degassed gas volume and Oil volume. Please refer to Section 8.3 for more details.

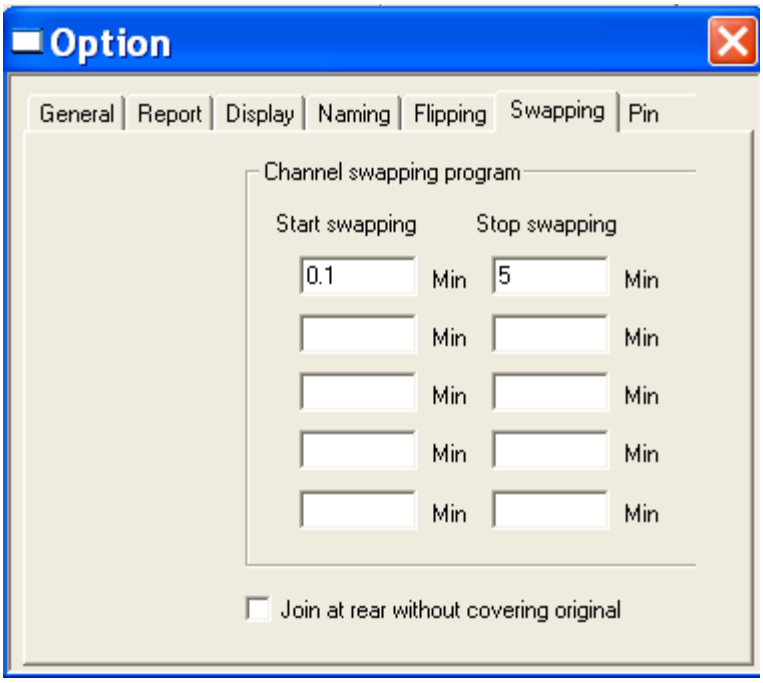
The Result quantity multiplier: field is for you to key in the multiplying factor when the rate of injection of the standard sample differs from that of the gas degassed. Please refer to Section 8.3 for more details.

8.2 Acquiring simultaneously from two channels and merging the results into one chromatogram file

After successful installation of this version, every time you start the software, two vertically arranged Documents Windows would be automated by the system. As explained in item number 10 of Terms of Reference, you can click on any of one of the Document windows to activate it to be the Active Document window.



Instead of acquiring separately from the two channels and manually combining and processing the two acquired chromatograms into the desire result, this version stresses that you can conveniently combine a segment of one chromatogram with a segment of another chromatogram and obtain the results in just one single chromatogram. Please refer to Section 4.1.4.4.6 swapping for more details.



If you key in 0.1 minute as the time to start swapping, and key in 5 minutes as the time to stop swapping, and check on the box "to joint at rear without covering the original", then upon reaching the acquisition time specified in Channel A, say 10 minutes, the chromatogram will be extended by the segment of chromatogram starting from 0.1 minute to 5 minutes acquired from B Channel. Once this is done, in future, by opening only the Chromatogram Frame of Channel A, you will be able to obtain the peaks of interest from the two Channels.

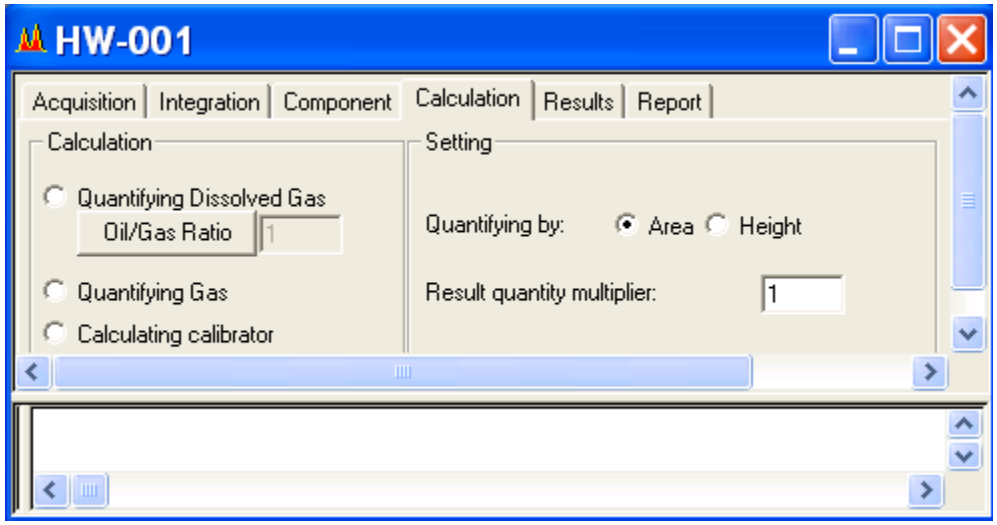
Tips 1: The minimum acquisition time of Channel A must be at least 5 minutes (i.e. the Stop swapping time) to ensure a successful swapping.

Tips 2: Once the swapping command is activated in the Swapping panel, the result of acquisition would not be affected without opening the Chromatogram Frame of Channel B.

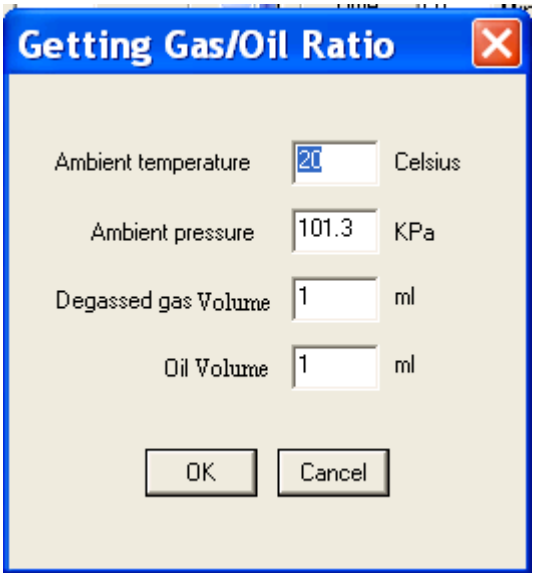
Tips 3; Please refer to Section 6.1, 6.2 and 6.3 for detailed procedures and tips on how to acquire and process a Chromatogram, and how to calculate Calibrator and Average Calibrators.

8.3 Procedures of calculation of Oil/Gas Ratio

1. Click on the calculation tag to go to the Calculation table to view the following table.



3. Click on the Oil/Gas Ratio button to access the following pop-up window.



4. Proceed to key in the appropriate parameters such as the Ambient temperature, Ambient pressure, Degassed gas volume and Oil volume. Click on OK button to confirm.

5. System will calculate the ratio and input in the space next to the Oil/Gas Ratio button. Please note that if two Document Windows have been opened, the input of parameters and calculation of Oil/Gas ratio in one Document Window will automatically update the same of the other Document Window.

6. Please note that calculation of Oil/Gas Ratio is only needed before the calculation of the quantity of various gases that have been dissolved in the oil sample. This ratio does not affect the results when calculating the calibrator from standard sample, and when calculating the quantity of standard sample using calibrator (i.e. Quantifying Gas).

8.4 Procedures of calculation of the rate of degassing

1. When applying vacuum process, the value of the rate of degassing may be set to 1 for the commonly used degassing method. If you have prior knowledge about the rate of a particular degassing method, you may key in the value in the appropriate column of the Component Table. Should you need to calculate the theoretical rate of a certain partially degassing method, you may proceed with manual calculation as follow:

   1.1 Proceed first to obtain the Oil/Gas ratio.

   1.2 Go to the Component Table, within the Name column, fill in the name of the following components in strict format: namely H2、O2、N2、CO、CO2、CH4、C2H6、C2H4、C2H2、C3H8、C3H6 and C3H4.

   1.3 Click on the Degas Ratio button within the Component Table. System will calculate the theoretical rate of degassing of each of the components based on the above calculated Oil/Gas ratio and update the results in the Degas R. column.

2. Please note that calculation of Oil/Gas Ratio is only needed before the calculation of the quantity of various gases that have been dissolved in the oil sample. This ratio does not affect the results when calculating the calibrator from standard sample, and when calculating the quantity of standard sample using calibrator (i.e. Quantifying Gas).

8.5 Procedures of calculation of the quantity of various gases that have been dissolved in the oil sample.

2.1 Check to ensure that the appropriate calibrators, rate of degassing and Oil/Gas ratio have been captured in the Component Table and the Calculation Table.

2.2 If the injected volume of the Standard sample is different from the injected volume of the gas degassed from the oil sample, there will be a need to set that multiplier Result quantity multiplier: which was set to "1" by default. System will multiply this number when calculating the quantity of the various gases that have been dissolved in the oil sample. For example, if the injected volume of the Standard sample is 1 μl, whereas the injected volume of the gas degassed from the oil sample is only 0.5 μl, then the value of this number should be set to 2. By so doing, the result of calculation will then be doubled automatically.

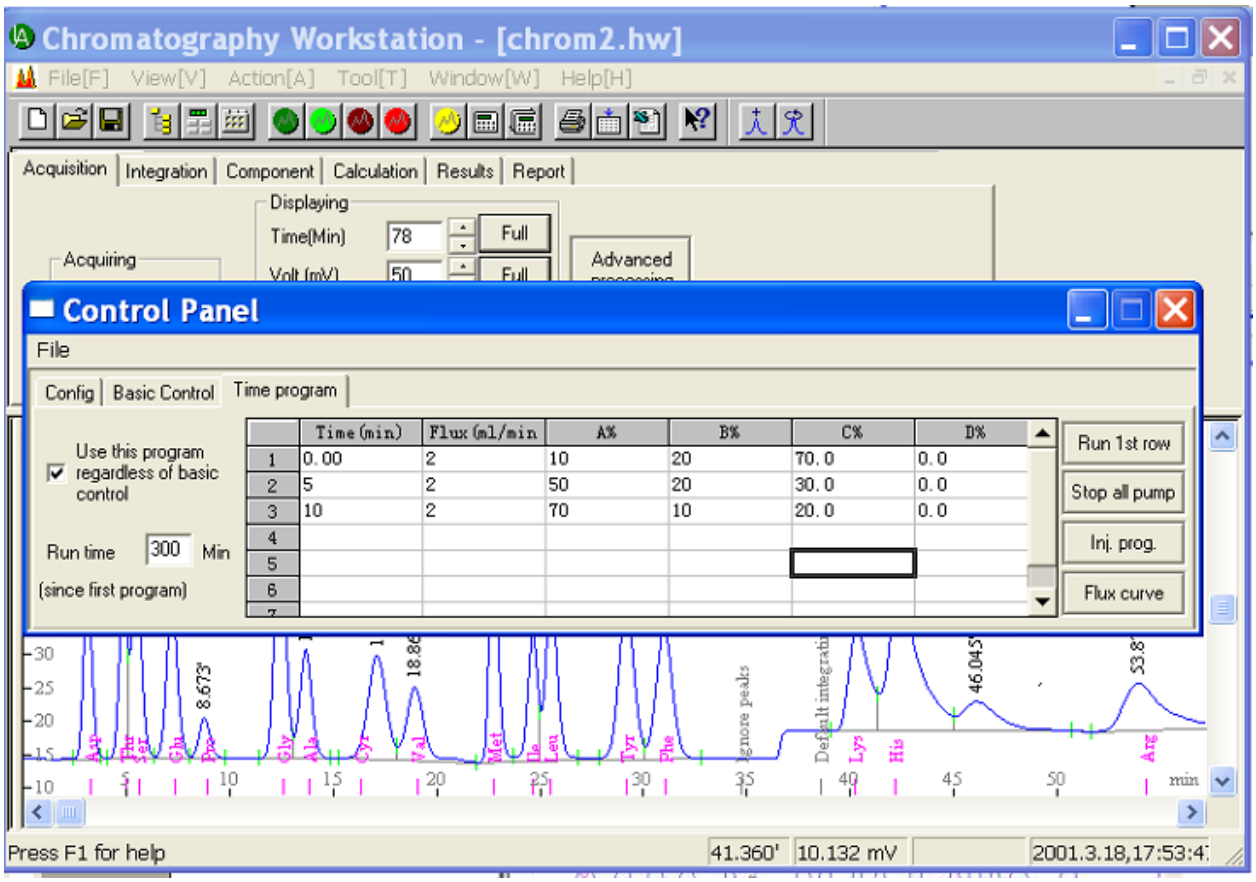
2.3 Click on the calculation button  , the result of calculation will be automatically captured in the result table. Such a step is automated upon completion of every acquisition command.

Chapter 9 Interface with SSI Pump

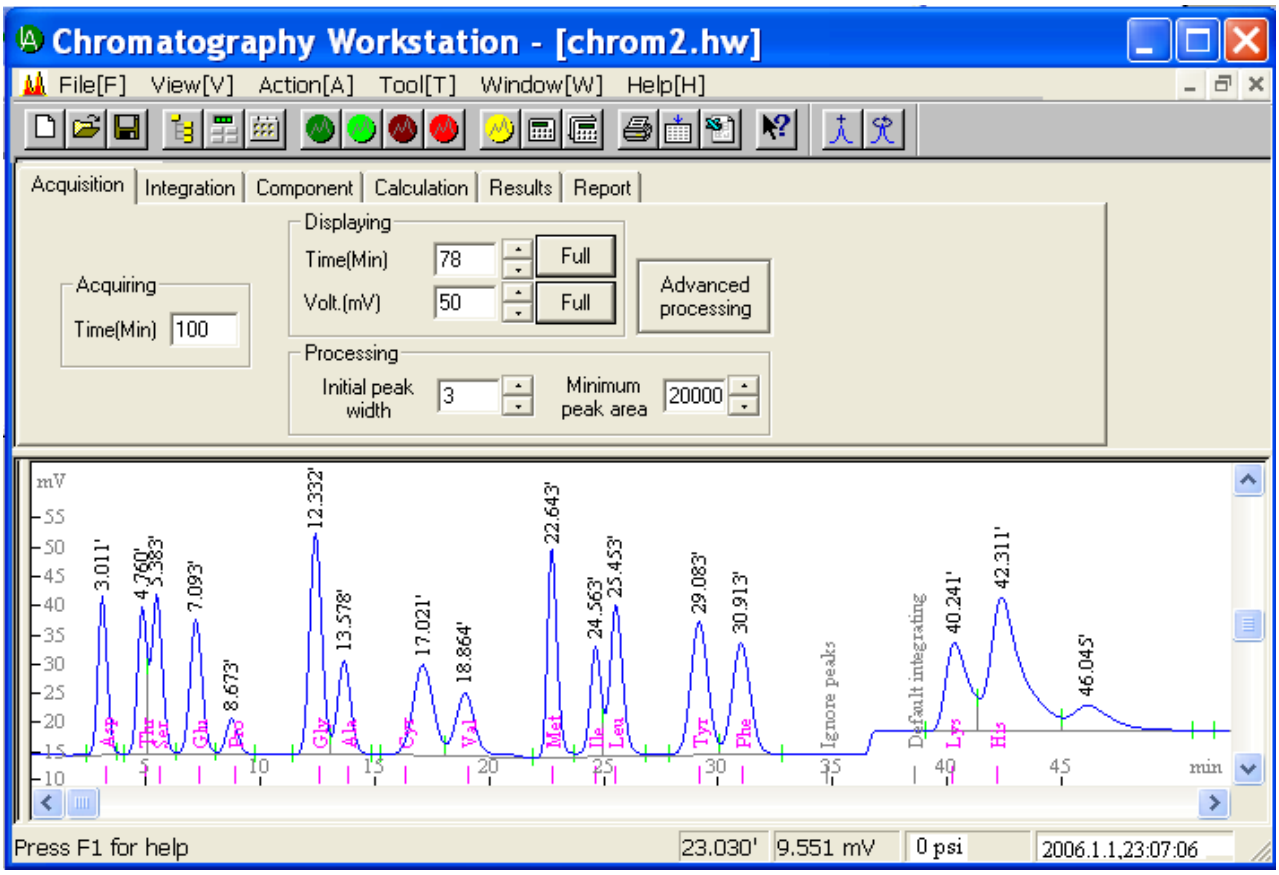
9.1 General Observations

PEAK-ABC has just been enhanced with a feature to interface with SSI pump for HPLC chromatography instrument. You can make use of PEAK-ABC to interface up to 4 pumps with various precisions.

1. Upon successful installation of the SSI Interface version, you would be prompted with the following screen with an added Control Panel:



2. Click on  of the Control Panel to exit the Control Panel and to access the following screen interface with SSI pump.



2.1 This  button is for you to close / display the Control Panel.

2.2 This  button is for you to start baseline acquisition without activating the Time Program specified via the Control Panel.

2.3 This  button is for you to start sample acquisition and to vary the pump flow as specified via the Time Program of the Control Panel.

2.4 This  button is for you to pause / resume the Time program during its execution.

2.5 Please note that within the status bar, we provide you with an additional indicator to show the current pressure value read from Pump A as follow:

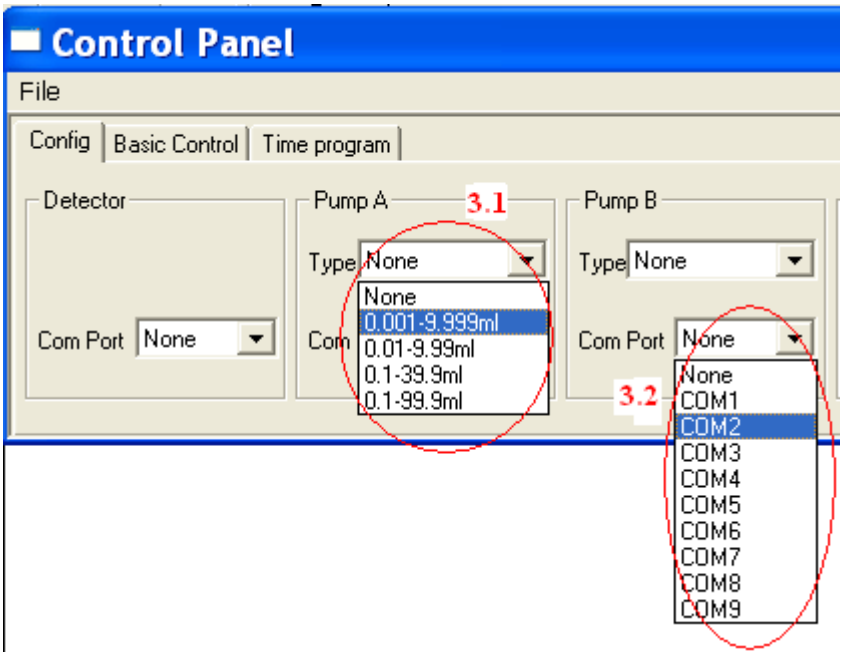
9.2 Working with the Control Panel for Pump Configuration

1. Click on Config to access the Configuration tag. Proceed to specify the precision and the Com Port connected to a particular pump as follow:

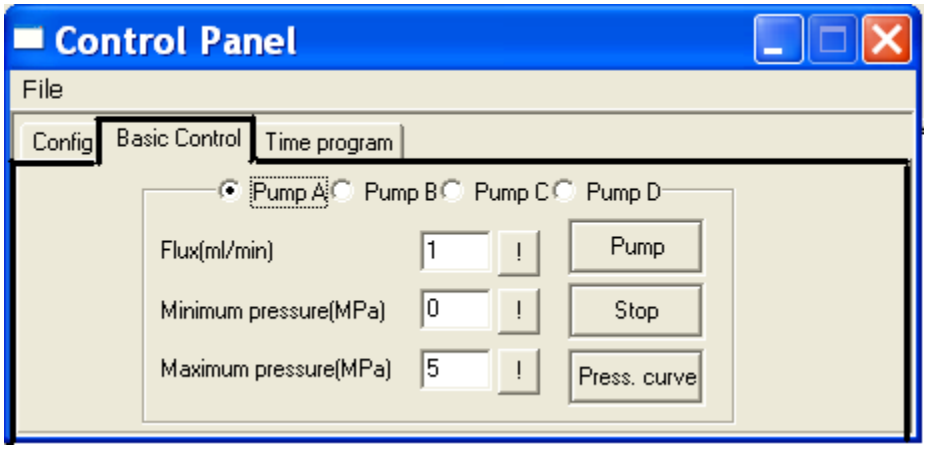
1.2 This drop-down menu is for you to specify the precision of the pump corresponding to pump A.

1.3 This drop-down menu is for you specify the Com Port connected to the Pump.

1.4 Make use of these drop-down menus to specify the precision and the Com Port connected to Pump A, Pump B, Pump C, and Pump D in turn.

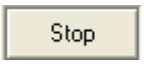


2 Click on Basic Control to access the basic Control tag and proceed to specify a constant Flux flow speed and a pre-determined range of pressure for each of the pump. If you need to vary the speed of the Flux flow at different time interval, then, you should make use of the Time program tag as explained in para 3 below.

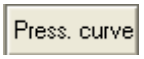


2.1 This 

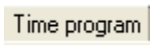
button is for you to activate the corresponding Pump.

2.2 This 

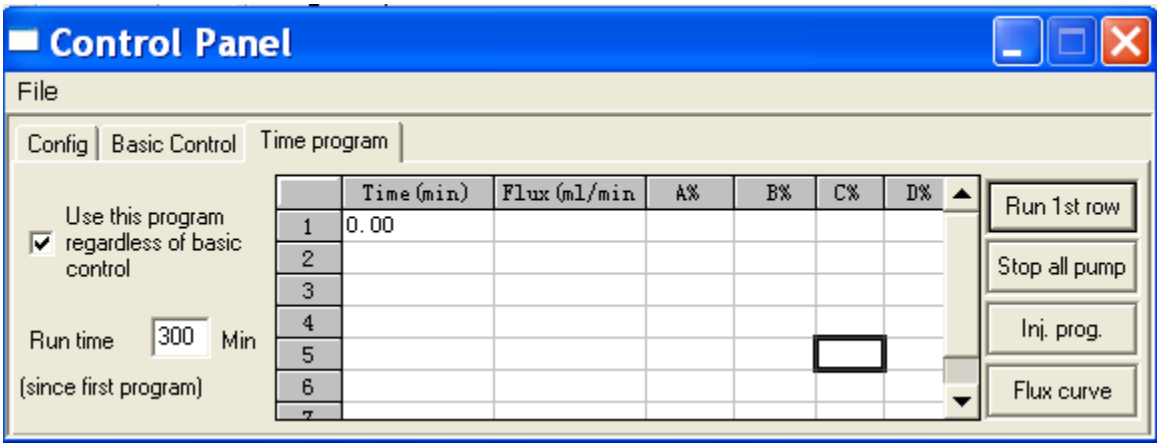
button is for you to stop the Pump.

2.3 This 

button is for you to activate a real time Pressure value curve of Pump A within the chromatogram frame. A more accurate reading of current pressure value could be found in the Status Bar as explained in Section 9.1 above.

3. Click on 

to access the Time Program tag to specify different Flux flow at different time interval as follow:



3.1 Check on 

to activate this Time Program tag and to surpass any setting made in the Basic Configuration tag.

3.2 Use 

to set the time to automatically stop all pumps after certain minutes.

3.3 Make use of the following table to specify the Flux flow as follow:

	Time (min)	Flux (ml/min	A%	B%	C%	D%
1	0.00	2.00	10.0	20.0	70.0	0.0
2	5.00	2.00	50.0	20.0	30.0	0.0
3	10.00	2.00	70.0	10.0	20.0	0.0
4						
5						
6						

For example:

- Between 0.00 minutes to 5.00 minutes, at the Flux flow speed of 2ml/min, the flow channeled to Pump A, Pump B and Pump C is set at 10%, 20% and 70% respectively.

Please note that column A% to D% are automatically added up to 100%.

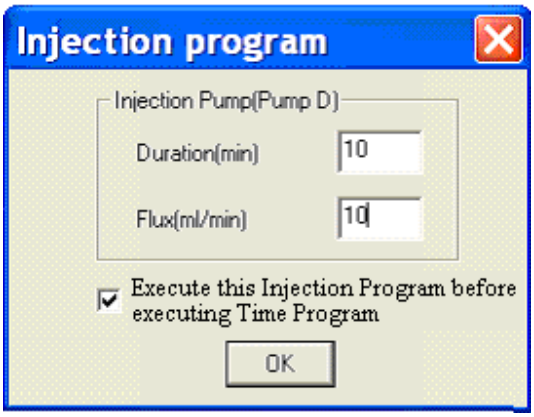
- Between 5.00 minutes to 10.00 minutes, at the same Flux flow speed of 2ml/min, the flow channeled to Pump A, Pump B and Pump C is changed to 50%, 20% and 30% respectively.

3.4 This Run 1st row button is for you to test run only the setting specified in the first row of the table only.

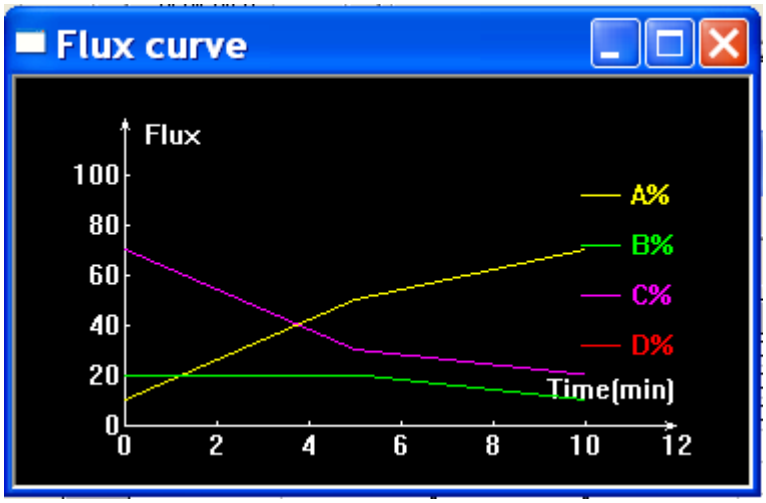
3.5 This Stop all pump button is for you to manually stop all pumps if need be.

3.6 This Inj. prog. button is applicable for manufacturing process involving preparative HPLC instrument. You can make use of Pump D to specify the required volume of injection. When activated, the following screen will be popped up:

- Fill in the time duration of injection and the speed of Flux flow as required.
- Check on the box if you wish to execute this Injection program before executing Time Program.



3.7 Click on Flux curve button to verify the proportion of Flux flow channeled to each Pump.



9.3 General Observations

1. We are the proud producer of PEAK-ABC Chromatography Data Handling System which has been well accepted in the market since 1998. PEAK-ABC has just been enhanced with a feature to interface with SSI pump produced for HPLC chromatography instrument.

2. Some of the special features of PEAK-ABC SSI version are:

   - Able to connect up to 4 pumps with selected precision one of which could be used for chromatography manufacturing process;
   - Able to running baseline without activating the pump;
   - Able to test pump without activating the sampling process;
   - Able to set a constant Flux Flow Speed with constant flow proportion throughout the whole acquisition process; OR varying Flux flow speed with varying flow proportion at different time interval during a acquisition process;
   - You can change the flow proportion at short time interval of 0.01 minute. i.e. to change the flow proportion from 20% to 30% of a particular pump at a split of second.

3. We are able to supply you with or without the data Acquisition Unit (or A/D converter) as follow:

   1) Software plus a hardware key (with no A/D Converter)
   2) Software plus a single channel 24 bit Data Acquisition Unit (USB port)
   3) Software plus a double channel 24 bit Data Acquisition Unit (USB port)

End