**PEAK-ABC Tutorial**
**Table of Content**


1. Terms of reference ..... 1
2. Installing PEAK-ABC from software download ..... 2
3. Initializing PEAK-ABC System ..... 2
4. Designating a Working folder ..... 3
5. Retrieving and Displaying a Chromatogram file ..... 4
6. Adjusting Display Limits ..... 4
7. Zooming in ..... 5
8. Displaying a few Chromatogram files ..... 6
9. Acquisition of data signal / Default integration ..... 8
10. Manual integration / Integration table ..... 9
11. Various integration methods ..... 12
12. Qualitative analysis /(Component identification) / Component table ..... 15
13. Quantitative analysis (Calculation of component quantity) ..... 17
14. Printing of an analysis report ..... 24
15. Batch printing of analysis report ..... 24
16. Chromatogram compiler ..... 25
17. Results tables compiler ..... 25
18. Swapping segments of chromatograms ..... 26

# 1. Terms of reference

Try to familiarize yourself with the following terms of reference before you go through the Tutorial.

## 1.1 Chromatogram file

This system is structured in such a way that all the working elements of the data handling process are centrally stored in one source document entitled **Chromatogram file**. A typical **Chromatogram file** corresponding to an injection (or sampling) would consists of the acquired raw data signal, the integrated chromatogram, the detailed integration method, the selected quantifying method and the results of analysis including external reference information that has been keyed in.

## 1.2 Document window technique / Split window technique

By applying the **Document window technique** and **Split window technique**, a one-page Document window is designed to present all the working elements of the Chromatogram file in one screen for quick access and manipulation. Every time you start this software, a new Document window would be created under a name automatically assigned by the software. When you effect the Save command, this Document window would be saved as a corresponding Chromatogram file under the same name of the Document window. When you need to access and manipulate an existing Chromatogram file, you must first retrieve and display it in the form of a Document window.

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; change the integration method; select and execute the quantifying method; and print the analysis report.

## 1.3 Multi-Document window technique / Active Document window

This software applies **Multi-Document window technique** so that you can work with more than one Chromatogram file at one time. For example, while you can acquire data signal in one Document window, you can access or manipulate another Chromatogram file in another Document window. 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.

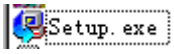
## 1.4. Template file

When analyzing samples of similar mixture, some elements of the Chromatogram files (contained in the working tables) may also be applicable for subsequent analysis. While Save template command is for you to create a **Template file**, the Load template command is for you to copy its contents 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.

## 2. Installing PEAK-ABC from software download

2.1 Start the Windows operating system and use an online browser to access www.PEAK-ABC.com

2.2 Your computer should be installed with Winzip software. Click on Download to start the downloading process.

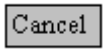
2.3 Upon completion of download, click on 

within the dialogue box to begin installation.

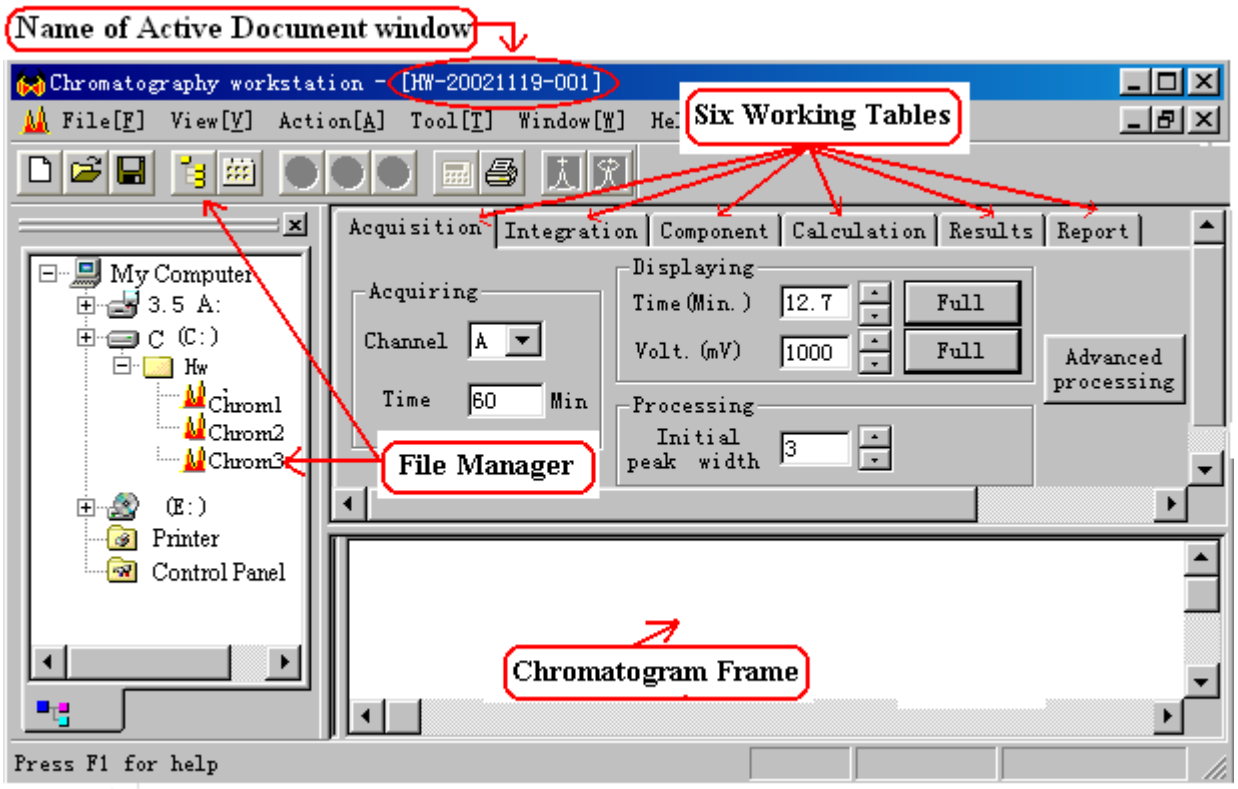
2.4 Follow the on screen instructions provided by the installation wizard to complete the installation of this software.

## 3. Initializing PEAK-ABC System

3.1 Click on the icon  to launch and initialize the software.

3.2 When prompted with the message "Please plug the key into UBS or Parallel Port", click on 

to continue to view the following **Introduction screen.**




TT-01

- Every time you start the software, a new Document window file would be automatically created ready for you to acquire raw chromatography data signal.

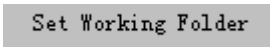
- The Chromatogram frame is where the chromatogram would be displayed as and when data signal is being acquired.

- Within the File Manager, you can see all the file folders of your computer including file folder of your chromatogram files.

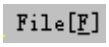
3.3 This software includes a few sample Chromatogram files that can be opened, displayed, and manipulated. They are stored under the filename of Chrom1, Chrom2 and Chrom3, under file folder "Program" under PEAK-ABC subdirectory. These files would be used throughout the rest of this tutorial.

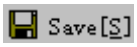
## 4. Designating a Working folder

4.1 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. (Please refer to User Manual to see how to customize the naming structure according to your need).

4.2 Within the **Introduction screen,** click on any spot within the File Manager to see that the designated **Working folder** is being highlighted in blue. If you wish to designate another file folder to be the **Working folder**, simply point the cursor towards the name of the new file folder, right click on the mouse to access the pop-up menu, click on 

to effect the change.

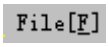
- Click on 

from the Menu Bar and select 

from the dropdown menu to view a dialogue box. The name of the designated **Working folder** would be prompted in the file folder and the name of the Document window is prompted to be the filename. Click on 

to abort this Save command.

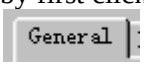
- Practice selecting "Program" under PEAK-ABC subdirectory as **Working folder.**

4.3 Another way to check the name of **Working folder** and to designate a new file folder as **Working folder** is to click on 

from the Menu Bar to select 

from the dropdown menu. Proceed to effect the change from the dialogue box.

- Practice selecting "Help" under PEAK-ABC subdirectory to be **Working folder.**

4.4 If you wish to acquire data simultaneously in the two acquiring channels, you must designate different **Working folders** for Channel A and Channel B. This can be done by first clicking on  icon from the Tool bar to access the Option command and click on 

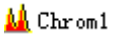
tag to view the General panel.

- Within 

, practice designating "My computer" as **Working folder** for Channel A, designating "Program" as **Working folder** for Channel B. Simply click on  at the top right corner of the panel to validate the input and exit Option command.

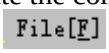
- Please note that the setting made in 4.4 supersede that made in 4.2 and 4.3.

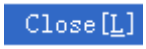
## 5. Retrieving and Displaying a Chromatogram file

5.1 This Section shows you how to retrieve and display an existing Chromatogram file in the form of Document window. One way is by making use of the File Manger. Simply double click on 

to open the file. Practice clicking on  at the top right corner to exit Chrom1.

5.2 Another way is by clicking on  icon located on the Tool Bar to key in the relevant file folder and filename in the dialogue box. Practice select "Program" as the file folder and key in "Chrom1" as the filename, click on 

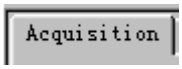
to execute the command. Practice another way to close the Active document window by clicking on 

from the menu Bar to select 

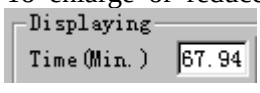
from the dropdown menu.

## 6. Adjusting Display Limits

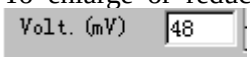
6.1 Follow Section 5.1 to retrieve and display Chrom1 in the form of Document Window. Click on  icon located on the Tool Bar to hide the File Manager to give more space for the Document window.

6.2 Click on the 

tag to display the Acquisition Table.

6.3 To enlarge or reduce the size of a chromatogram horizontally, you can adjust the value of 

by using  next to it. Practice enlarging the display limits by reducing the value.

6.4 To enlarge or reduce the size of the chromatogram vertically, you can adjust the value of 

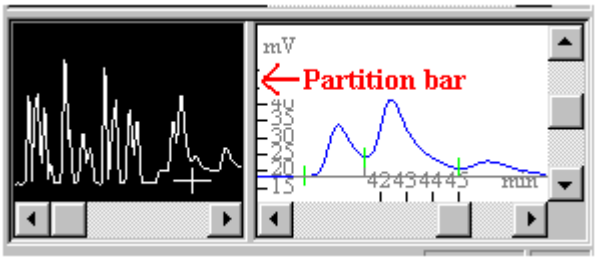
by using  next to it. Practice reducing the display limit by increasing the value.

6.5 If you wish to fit the entire chromatogram within the screen, click on the 

button next to Time (Min) to first fit it horizontally. Then click on the 

button next to Volt (mV) to fit it vertically.

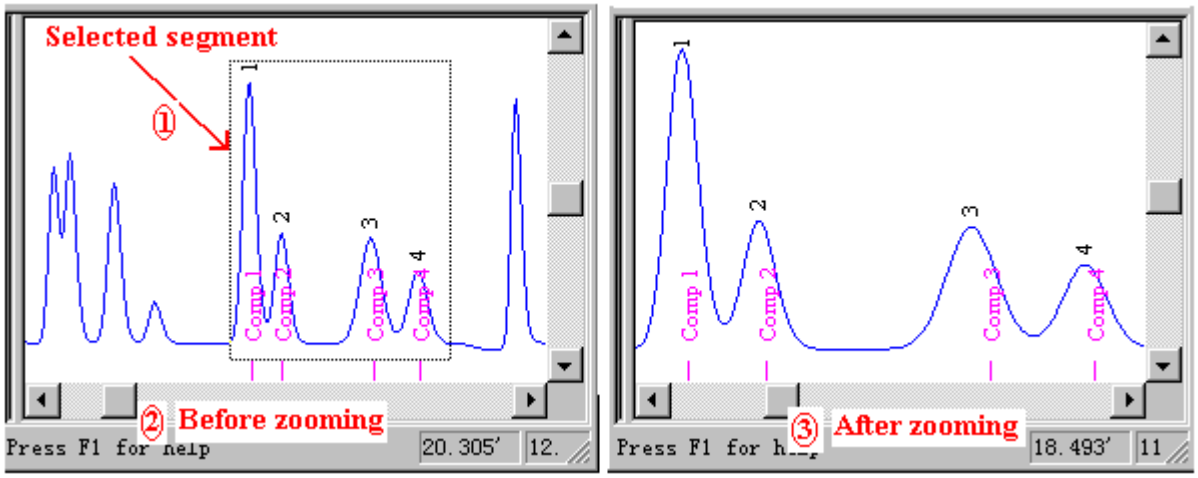
6.6 Practice dragging on the partition bar located at the extreme left of the Chromatogram frame to partition it into two sections. The left section is to display the entire chromatogram. The right section is to display the enlarged segment of the chromatogram following the movement of the cursor. Practice moving the cursor from left to right within the left section.




## 7. Zooming in

7.1 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.

- Go to Chrom1, practice zooming in on the first two peaks. Practice double clicking on the left button mouse to return to the original display limits.



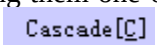
## 8. Displaying a few Chromatogram files

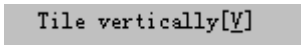
8.1 This Section shows you how to open and display a few Chromatogram files namely Chrom1, Chrom2 and Chrom3 for manipulation. You can choose to display them in three different ways, namely to stack them one on top of the other; to display them side by side vertically without overlapping; or to display them side by side horizontally without overlapping.

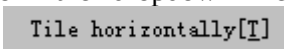
8.2 (One way to retrieve and display the three files is by clicking against their filenames in turn from within the File Manager.) Practice opening the three files simultaneously by first clicking on the  icon located on the Tool Bar to access a dialogue frame. Press and hold on to the Ctrl key, click on the filenames Chrom1, Chrom2 and Chrom3 to select. Click on 

to execute the command.

8.3 Practice stacking them one on top of the other by clicking on 

from the Menu Bar, then click on 

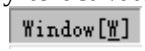
from the dropdown menu. Practice repeating the option for 

and 

to display them in another two different ways.

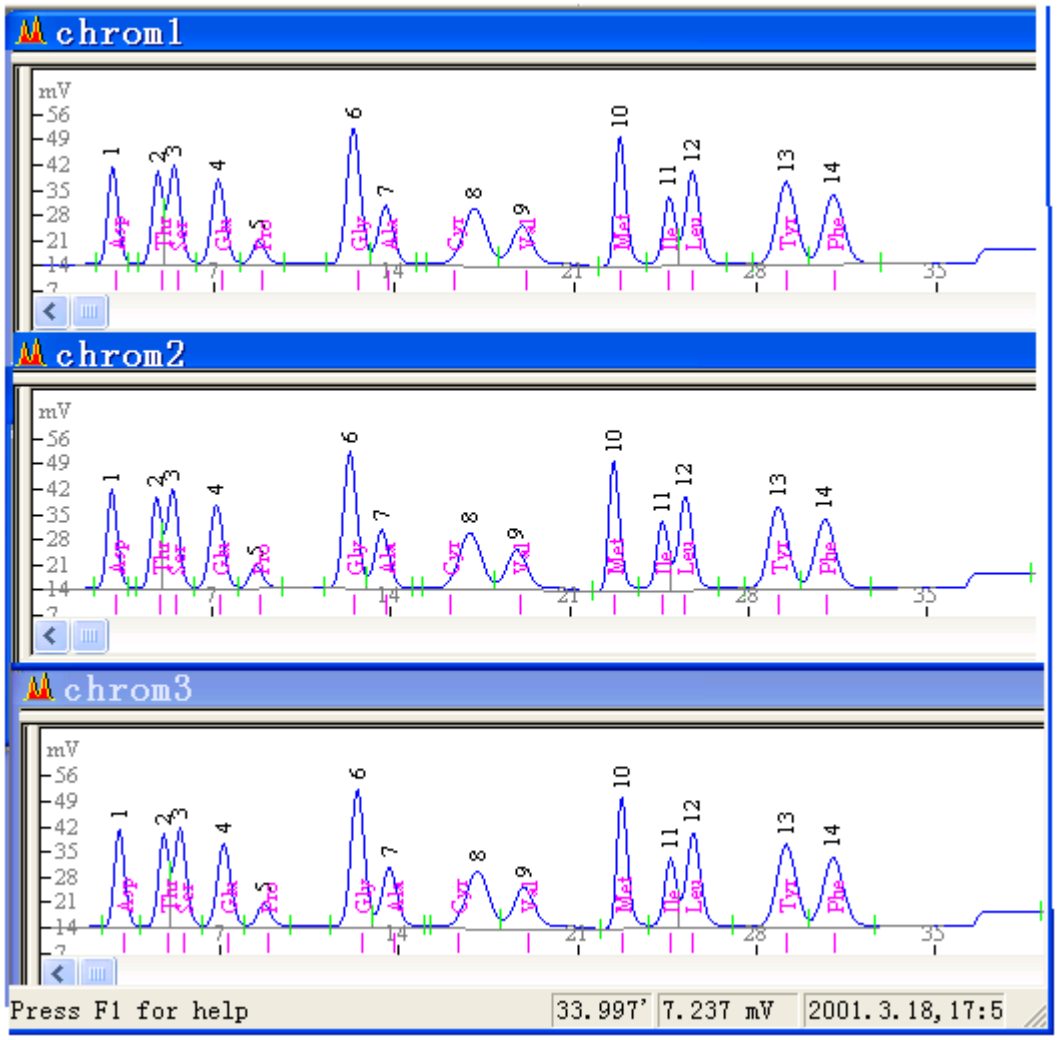
8.4 When you are displaying more than one Chromatogram files, there would be more than one corresponding Document windows being displayed. The one Document window whose name is highlighted in blue is referred to as the Active Document window. Among the Document windows on display, only the Active Document window is responsive to the various command found on the Tool bar. You can click on any spot of any of the other Document windows to activate it to be Active Document window.

8.5 Follow Section 8.3 to display them vertically. Chrom1 is the **Active Document window** as evidenced by the fact that its title bar is being highlighted in blue. Practice activating Chrom2 to be the **Active Document window** by positioning the cursor within any spot of Chrom2 and click once on the mouse. The title bar of Chrom2 is now being highlighted in blue.

8.6 Another way to activate a Document window to be the **Active Document window** is by first clicking on 

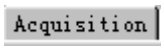
from the Menu Bar and click on its window name from the dropdown menu. Practice activating Chrom3 to be the **Active Document window** by making a tick against its name.

8.7 If you wish to compare the shape of a few chromatograms, you can make use of the option Tile horizontally[T] plus some adjustments. (Please refer to Section 16 for more detail about making use of the Chromatogram compiler to overlay a few chromatograms for comparison). Before tiling them horizontally, retrieve and display Chrom1, 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 Chrom2 and Chrom3, click on Tile horizontally[T] to obtain the following:

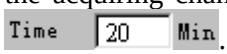


## 9. Acquisition of data signal / Default integration

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

- Click on  icon located on the Tool bar to open a new Document window. Click on 

tag to view the Acquisition table and proceed to select "A" in 

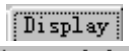
as the acquiring channel. Proceed to specify the time duration of acquisition as 20 minutes in 

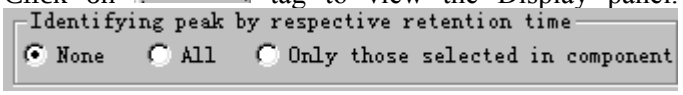
.

- Click on  icon located on the Tool bar to start acquisition. As and when data signal is being acquired, the 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 had been keyed in prior to activating the acquisition command. If there is no pre-acquisition input of integration method, the software would automatically select an integration method to process the peak. This process is referred to as **Default integration**.

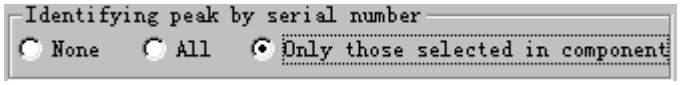
- When a peak is detected, a short green line and short red line would be drawn to mark the Start point and End point of the peak. In addition to these marking, you have the option to display or not to display the retention time (and/or the serial number) on peak-top.

  - Retrieve and display Chrom1, click on  icon from the Tool bar to access the Option command.

  - Click on 

tag to view the Display panel. Click on "None" from/within 

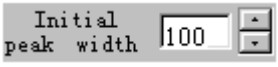
to deactivate the marking of retention time on peak top.

  - Click on "Only those ……" 

to activate the marking of serial number for those peaks identified in the Component table.

  - Within the Display panel, click on  located on the top right corner to validate the selection and to exit Option command.

- Observe the acquired chromatogram carefully, if there is non-detected peak, you can re-integrate the chromatogram by adjusting the value of Initial peak width. Most of the time, this is the only parameter that you need to adjust to obtain the desirable results. (The Advanced processing parameters are applicable on rare cases where the chromatogram contains very narrow peaks or many small peaks, or there are big differences in peak width.)

- Practice changing the value of Initial peak width from 25 to 100 in 

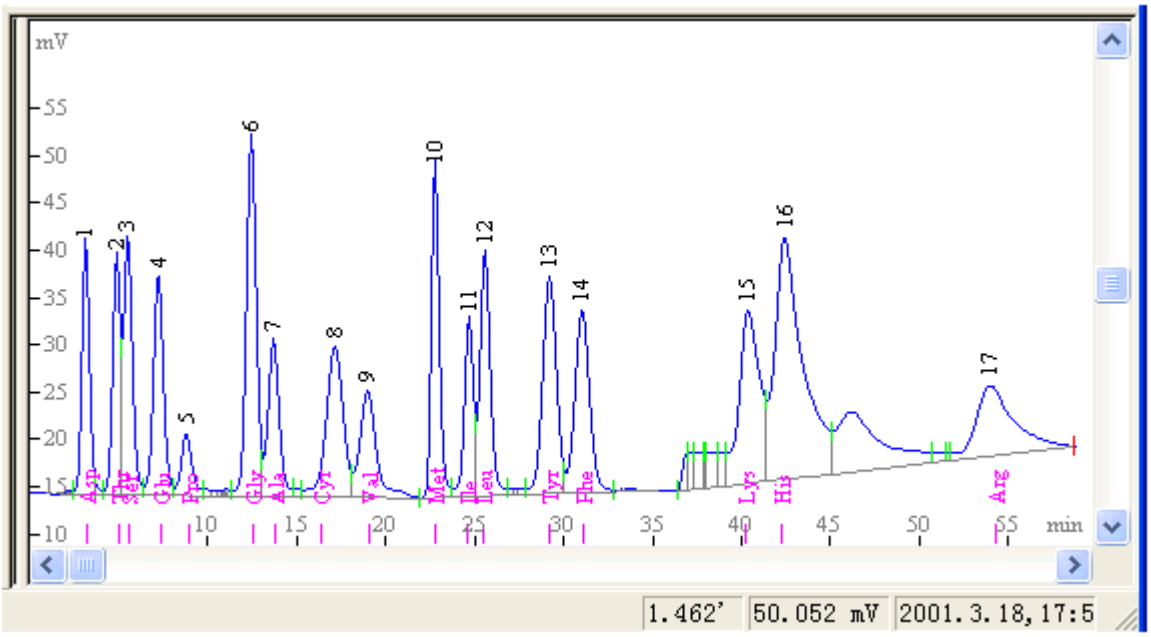
, click on  icon to apply. You may notice that the 2nd peak and the 11$^{th}$ peak are not detected.

## 10. Manual integration / Integration table

10.1 If you are not happy with the integration method selected by **Default integration** as mentioned in 9, you can change the integration method by applying **Manual integration**.

- **Integration table** is for you to record the selected Integration method and the time (i.e the segment of chromatogram) to start applying the method. Pre-acquisition input made in this table would be applied by the software on a real time basis as and when data signal is being acquired. Post-acquisition input made in this table would be applied immediately after selection .

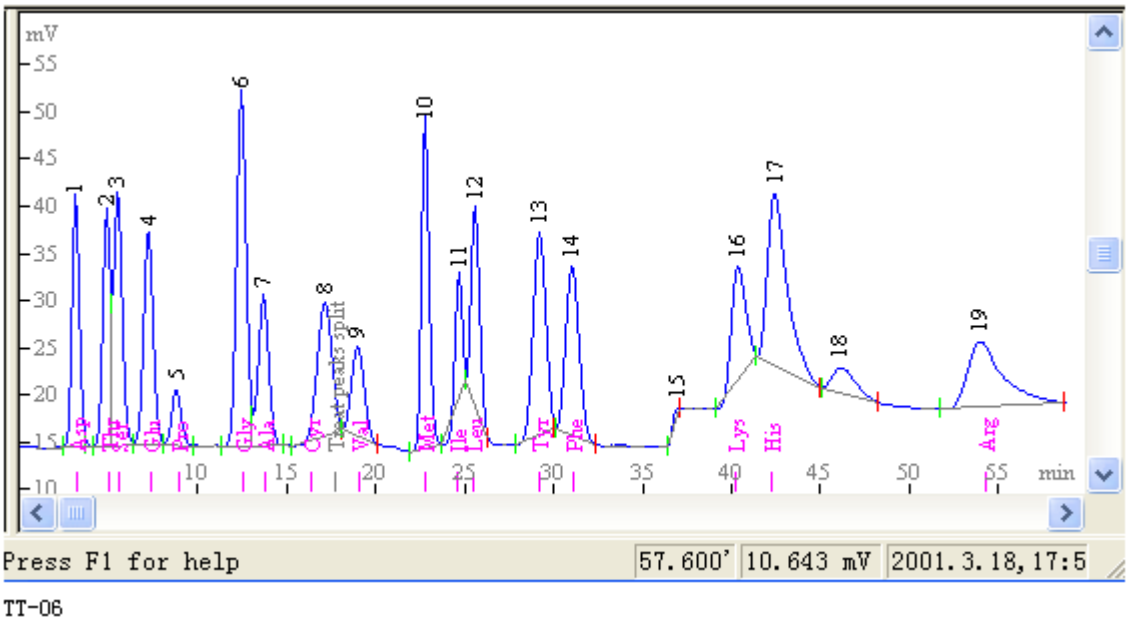
10.2 Follow Section 5.1 to retrieve and display Chrom1. Click on Integration tag to display the **Integration table**. Click on Reset table to clear the content of this table. Observe the chromatogram to see that all the 17 peaks are being treated as overlap under **Default integration**.



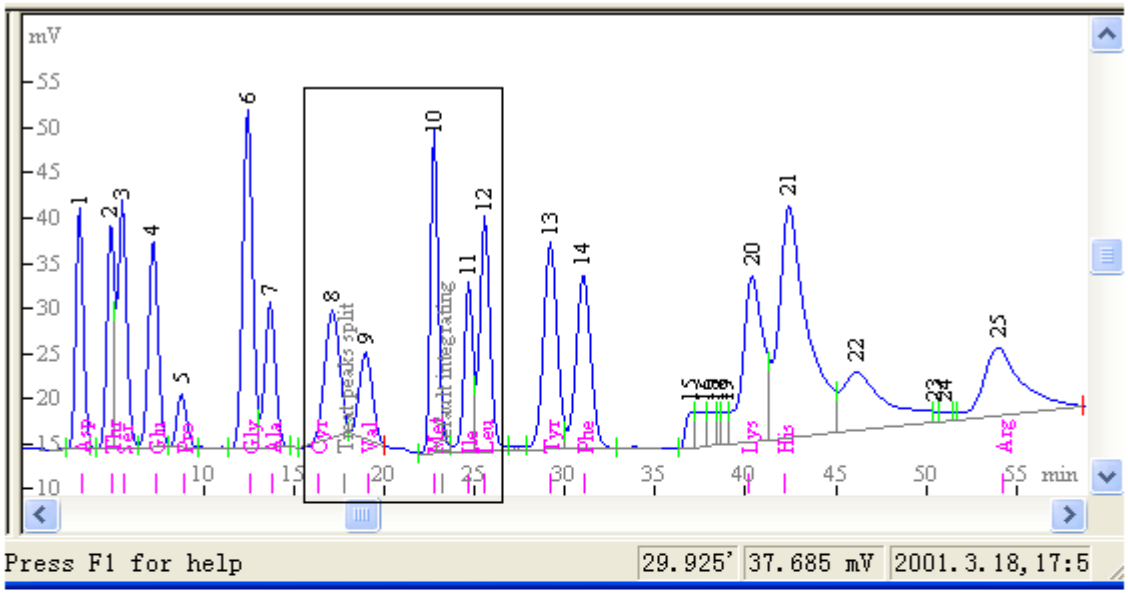
10.3 If you think the 8th and 9th peaks should be treated as split, position the cursor a short distant before the Start point of the 9th peak, (17.66 min) right click on the mouse to access the **Pop-up menu**. Click on Input integration table to view the whole range of integration methods, click on Start to treat peaks split to select this integration method.

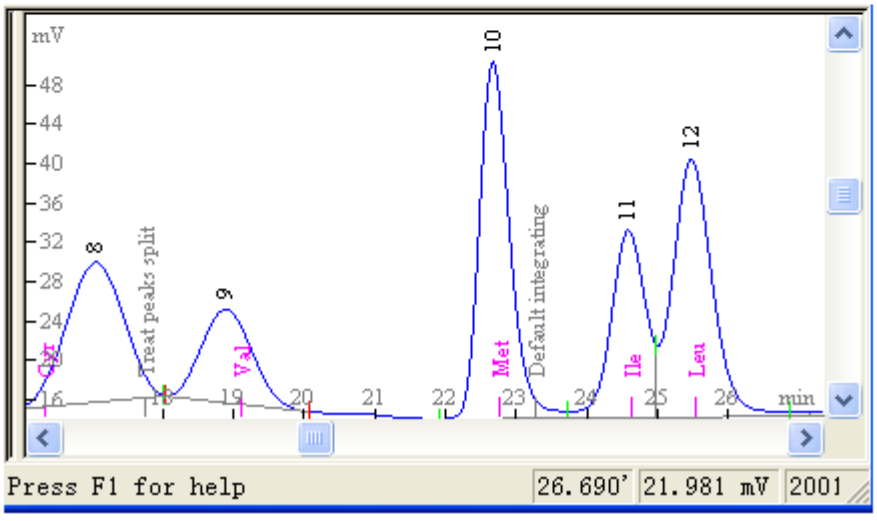
10.4 The selected integration method and the time to start applying (i.e where you right click on the mouse) would automatically be recorded in the **Integration Table**. A corresponding marking would also be made on the x-axis to display the selected integration method. You can adjust the time by simply repositioning the marking by dragging it using the mouse.

10.5 You will note that after applying this command, all the peaks towards the right of the 8th peak are being treated as split. Since we only want to treat the 8th and 9th peaks as split while leaving the rest unchanged, we need to input another integration method to end this command.



10.6 Position the cursor before the End point of the 10th peak, (23.17 min), right click on the mouse to access the **Pop-up menu**. Click on `Input integration table` to select `Reset to default integration`. The selected integration method and the time to apply (where you right click on the mouse) would be recorded in the **Integration Table**. A corresponding marking will be made on the x-axis to display the selected integration method. You can adjust the time by simply repositioning the marking by dragging it using the mouse. Zoom in (as per Section 7) to see that the 8th and 9th peaks are now treated as split while the rest are being treated as overlap.




TT-07

10.7 Exit Chrom1 without saving the changes made to the Integration table.

## 11. Various integration methods

### 11.1 Start to ignore peak

This is to suppress the software from detecting peak starting from the point you right click on the mouse. When activated, all the peaks to the right of this point would have no marking of Start point, End point and Peak-top Retention time (or serial number).

- Retrieve and display Chrom1. Click on Integration tag to view the **Integration table**. Click on Reset table to clear the content of this table. Activate  icon located on the Tool bar to apply the reset command. Practice applying this method just before the Start point of the 6th peak as illustrated in Section 10.3. Exit Chrom1 without saving the changes.

### 11.2 Start to merge peaks

This is to instruct the software to start merging a few connecting peaks as one peak from the point you right click on the mouse. After merging, the first peak within the group 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.

- Retrieve and display Chrom1. Click on Integration tag to view the **Integration table**. Click on Reset table to clear the content of this table. Activate  icon located on the Tool bar to apply the reset command. Practice applying this method just before the Start point of the 6th peak as explained in Section 10.3. Practice applying "Reset to default integration" as explained in 10.5 just after the End point of the 9th peak. Click on  icon located on the Tool bar to apply. The 6th, 7th, 8th and 9th peaks are now treated as one peak. Exit Chrom1 without saving the changes made.

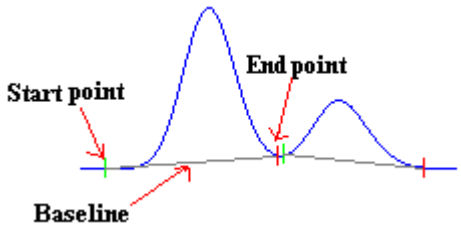
- If you need to aggregate the areas of a few connecting or non-connecting peaks, you can make use of the **Band Beg** / **Band End** columns and the **Grp Sum** column, respectively of the **Component table.** (Please refer to Section 12.3 and 12.4 for more detail).

### 11.3 Start to treat peaks as split

This is to instruct the software to start treating a group of connecting peaks as split from the point you right click on the mouse.

- Retrieve and display Chrom1. Click on Integration tag to view the **Integration table**. Click on Reset table to clear the content of this table. Activate  icon located on the Tool bar to apply the reset command. Practice applying this method just before the Start point of the 6th peak as illustrated in Section 10.4. All the peaks to the right of the 6th peaks are now treated as split.

- 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 overlaps with 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.



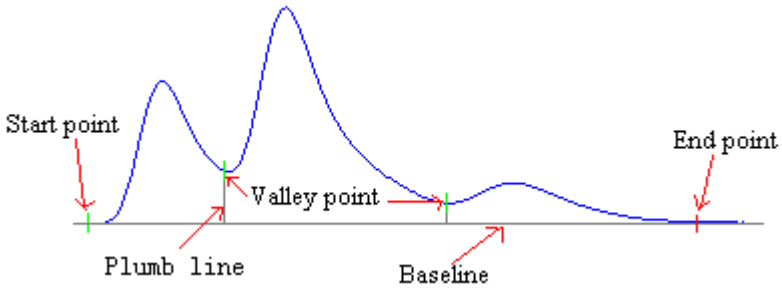

- Exit Chrom1 without saving the changes.

### 11.4 Start to treat peaks as overlap

This is to instruct the software to start treating a group of connecting peaks as overlap from the point you right click on the mouse.

- Retrieve and display Chrom1. Click on Integration tag to view the **Integration table**. Click on Reset table to clear the content of this table. All the 17th peaks are already treated as overlap. Practice applying this method just before the Start point of the 10th peak as illustrated in Section 10.4.

- 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; a short green line 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 all the way to the last short red line. When calculating area of individual overlap peak, a default plumb line would be drawn from the connecting point of adjacent peak to the Baseline.




- Exit Chrom1 without saving the changes made.

### 11.5 Reset to default integration

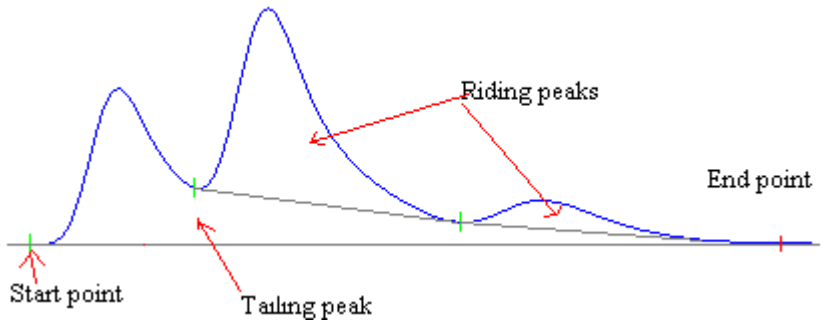
This is to instruct the software to reset to default integration from the point you right click on the mouse. This command is normally used to terminate the application of an integration method as explained in Section 10.5.

### 11.6 Treat this as tailing peak (for a group of overlap peaks)

This method is only applicable for a group of overlap peaks. For a group of split peaks, you must first apply Manual integration to treat them as overlap before applying this method. For small peaks riding on the descending slope of a big peak, this integration method is to treat the big peak as tailing peak. When integrating tailing peak, the software 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.

- Retrieve and display Chrom1. Click on Integration tag to view the **Integration table**. Click on Reset table to clear the content of this table. Activate icon located on the Tool bar to apply the reset command. Practice applying this method to treat the 15th peak as tailing peak. Position the cursor near the center of the 15th peak, right click on the mouse to select Treat this as tailing peak from the Pop-up menu. Click on icon located on the Tool bar to apply. In fact, what is done in this step is to replace the vertical plumb line (of overlap peaks) by tangent split lines.

  The End point of the group of connecting peaks is automatically recognized to be the end point of this tailing peak. Thus, you don't have to select another integration method to terminate this integration method. You can designate the End point of the tailing peak by applying Treat this as split peak on the desire spot.



### 11.7 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. Click on  icon located on the Tool bar to reintegrate the chromatogram.

4.5 Pre-acquisition command to invert negative peak can be input by clicking on This can be done by first clicking on  icon from the Tool bar to access the Option command and click on Flipping tag to view the Flipping panel. Proceed to key in the time interval to flip in the panel. Click on  located on the top right corner to validate the input and exit. This setting would be applied in real time as and when data signal is being acquired.

## 12. Qualitative analysis /(Component identification) / Component table

12.1 The **Component table** is for you to conveniently identify the components and Internal standard by their expected retention times, their name, their calibrators and their known quantities, if applicable. We shall show you how to make use of the **Component table** to identify the two components corresponding to the first two peaks of Chrom1.

- Retrieve and display Chrom1. Click on Component tag to view the **Component table.** Click on Reset table button to clear the content of this table.

- Position the cursor near the center of the first peak, right click on the mouse to view the Pop-up menu. Click on Input retention time in component table, the time corresponding to the position where you right click on the mouse would be captured in the RetTime (retention time) column. Repeat the same to record the retention time of the component corresponding to the second peak.

- Practice keying in the name of the two components by positioning the cursor under the Name column and proceed to key in Comp1 and Comp2 (in the first and second row respectively) as the name of the two components.

- Simply move the cursor away to another field to validate the input. You can see that for each component identified in this table, a marking would be made on the x-axis accompanied by its given name. For qualitative analysis when deciding whether a component is present or not, so long as the marking on the x-axis lies within the Start Point and End Point of a peak, the component corresponding to that peak is regarded as present.

12.2 In the case of changes in retention time, you can conveniently reposition the marking by simply dragging it with the cursor. Practice dragging on the marking corresponding to Comp1 from 3.1 minute to 3.5 minute. You may note that the time captured in the RetTime column will also be adjusted accordingly.

- If you need to reposition the retention time of all the components proportionally, press and hold on to the Shift key while dragging any one of the marking with the cursor. The time captured in the RetTime column will be adjusted accordingly.

- Exit Chrom1 without saving the changes made to Integration table.

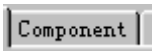
12.3 If you need to aggregate the areas of a few connecting peaks corresponding to a few components, you can make use of the **Band Beg** (to key in the Start point of the first peak) and **Band End** (to key in the End point of the last peak) columns (of the **Component table**) to identify the group of peaks as follow:

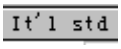
- Retrieve and display Chrom1, click on Component tag to view the **Component table**.
- Practice obtaining the aggregated area of 1st and 2nd peaks by keying 2.11 and 5.33 in the Band beg and Band end columns in a new row. (Leave the RetTime row blank)
- Key in "Total" in the corresponding Name column as the name of the aggregation.
- Move the cursor away to another input field to validate the input.
- Click on  icon located on the Tool Bar to effect the calculation.
- Click on Results tag to view the results in the **Results table**.
- Exit Chrom1 without saving the changes made to Integration table.

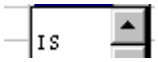
12.4 If you need to aggregate the areas of a few non-connecting peaks corresponding to a few components, you can make use of the **Grp Sum** column to identify the group of peaks as follow:

- Retrieve and display Chrom1, click on Component tag to view the **Component table.**
- Practice obtaining an aggregated area for the first and third peak. Key in a letter "G" in the corresponding row of the first and third peak under the Grp Sum column.
- Move the cursor away to another input field to validate the input.
- Click on  icon located on the Tool bar to effect the calculation.
- Click on Results tag to view the results in the **Results table**.
- Exit Chrom1 without saving the changes made to Integration table.

12.5 If Internal standard is added to the sample to increase the accuracy of your quantitative analysis, you can make use of the **Component table** to conveniently identify the position of Internal standard. More then one type of Internal standard may be added to the same sample to be applied to different group of components.

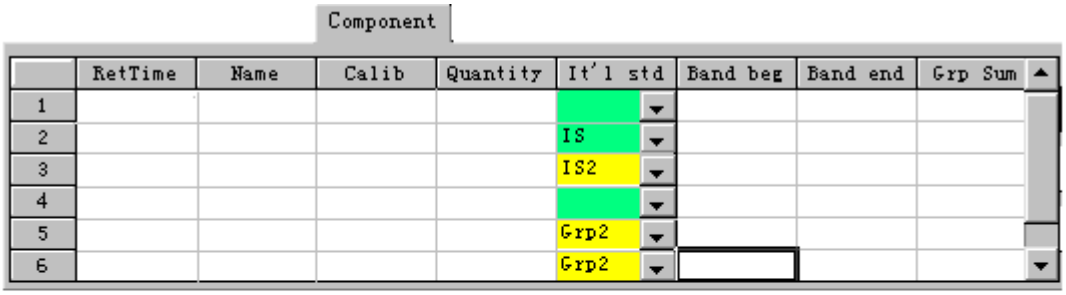
- Retrieve and display Chrom1. Click on 

tag to display the **Component table.** Practice identifying the component corresponding to the second peak as Internal standard by clicking on  corresponding to the second row under the 

column. Click on 

to identify it as Internal standard.

12.6 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:




The components highlighted in green is 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.

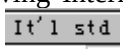
## 13. Quantitative analysis (Calculation of component quantity)

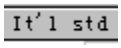
13.1 You can make use of this software to perform nine different types of quantifying methods for your quantitative analysis. The nine different quantifying methods are:

1. Normalization
2. Normalization by Calibrator
3. Normalization by average Calibrator
4. Quantifying by calibrator with Internal standard
5. Quantifying by calibrator without Internal standard
6. Quantifying by average Calibrator with Internal standard
7. Quantifying by average Calibrator without Internal standard
8. Quantifying by Calibration curve added with Internal standard
9. Quantifying by Calibration curve without Internal standard

- For quantitative analysis involving Calibrator(s), you should ensure that its (their) respective value is (are) correctly captured in the Component table (under the Calib column) before you proceed to perform the calculation. Value of Calibrator(s) could either be obtained from published data, or be calculated from an injection of Standard sample using this software. In the case where the value is to be calculated by this software, the calculated value would first be displayed in the Results table. You have the option to manual-fetch or auto-fetch the calculated value to the Component table.

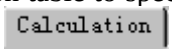
- For quantitative analysis involving average Calibrator(s), you can make use of this software to calculate the average value from a series of Standard samples with identical or almost identical component quantity in two steps. The first step is to calculate the value of the calibrators for each Standard sample. The second step is to calculate the average value of the series of Standard sample and manually fetch it to the Component table.

- For quantitative analysis involving Internal standard, you can conveniently make use of the Component table (under the 

column) to identify the position of the Internal standard. As explained in Section 12.5 and 12.6. If there is no input under the 

column, it would be recognized to be a quantitative analysis without Internal standard.

- For quantitative analysis involving Calibration curve, you can make use of this software to construct the Calibration curves from a series of Standard samples with different component quantity in two steps. The first step is to calculate the value of the calibrators for each Standard sample. The second step is to construct the Calibration curves from the series of Standard sample.

13.2 We shall now show you how easy you can make use of the Component table and the Calculation table to specify the desirable quantifying method. Retrieve and display Chrom1, click on 

tag to view the Calculation table. You should first select the appropriate calculation method by clicking on the radio button. After that, click on  icon located on the Tool bar to start calculation. The next step is to go to Results table to view the results of calculation.

13.3 Calculation of calibrators added with or without Internal standard

This is to show you how to calculate calibrators from an injection of a Standard sample added with or without Internal standard. Assuming that Chrom1 is the chromatogram acquired from an injection of a Standard sample. If you are calculating calibrators from one injection of Standard sample you can activate the option to auto-fetch the calculated value to the Component table.

- Retrieve and display Chrom1. Proceed to activate the auto-fetch as follow:
  - Click on clicking on  icon on the Tool bar to access the Option command and click on General tag to view the General panel, proceed to tick on Auto-fetching calibrator.
  - Click on  located on the top right corner to validate input and to exit Option command.
- Click on Component tag to view the Component table and proceed to click on Reset table button to clear the content of this table.
- Key in 30, 35, 20 and 50 as the known quantity of Comp1, Comp2, Comp3, and Comp4 respectively in the Quantity column.
- Assuming that Comp1 is the Internal standard, proceed to identity it accordingly in the It'l std column. Skip this step if no Internal standard is being added.
- Click on Calculation tag to view the Calculation table, click on the radio button Calculating calibrator.
- Click on  icon located on the Tool bar to perform the calculation.
- Click on Results tag to view the results of calculation in the Results table. If you have activated the auto-fetch option, the calculated calibrators would be automatically updated in the Component table, ready for next stage of analysis.
- Exit Chrom1 without saving the changes.

13.4 Calculation of average calibrators

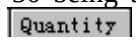
This Section shows you how to calculate average calibrators from a series of injections of Standard sample (with identical or near identical component quantity) added with or without Internal standard. We shall make use of Chrom1, Chrom2 and Chrom3 as the series of chromatogram.

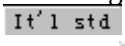
- Within Chrom1, click on Component tag to display the Component table, click on Reset table button to clear the content of this table.
- Proceed to key in 30, 35, 20 and 50 as the known quantity of Comp1, Comp2, Comp3, and Comp4 respectively in the Quantity column.
- Assuming that Comp1 is the Internal standard, proceed to identity it accordingly in the It'l std column. Skip this step if no Internal standard is being added.
- Click on Calculation tag to view the Calculation table, click on the radio button Calculating calibrator.
- Click on  icon located on the Tool bar to perform the calculation.
- Click on Results tag to view the results of calculation in the Results table.
- The next step is to click on To archive to store the Results table including the calibrators in a temporary zone.
- Repeat the above steps for Chrom2 and Chrom3.
- Click on Results tag to view the Results table and proceed to click on Averaging to calculate the average value of the series of calibrators that had been stored in the temporary zone.
- The final step is to click on Component tag to view the Component table and proceed to click on Fetch calib to update the calculated average value from the Result table to the Component table ready for further analysis.
- Exit Chrom1 without saving the changes.

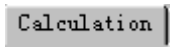
13.5 Construction of Calibration curve added with or without Internal Standard

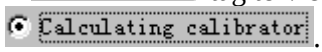
This Section shows you how to construct calibration curve from a series of injections of Standard sample (with different component quantity) added with or without Internal standard. We shall make use of Chrom1, Chrom2 and Chrom3 as the series of chromatogram.

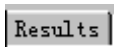
- Retrieve and open Chrom1. Click on 

tag to view the Component table.
- Proceed to key in 30, 35, 20 and 50 being the known quantity of the four components identified in the table under the 

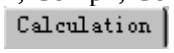
column for Comp1, Comp2, Comp3, and Comp4 respectively.
- Assuming that Comp1 is the Internal standard, proceed to identity it accordingly in the 

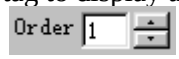
column. Skip this step if no Internal standard is being added.
- Click on 

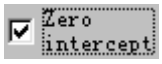
tag to view the Calculation table and proceed to click on the radio button 

.
- Click on  icon located on the Tool bar to perform the calculation.
- Click on 

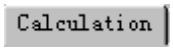
tag to view the results of calculation in the Results table.
- The next step is to click on 

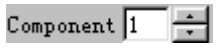
to store the Results table including the calibrators, the peak area, the quantity etc. in a temporary zone.
- Repeat the above steps for Chrom2. Key in 35, 40, 22 and 55 being the known quantity of Comp1, Comp2, Comp3, and Comp4 respectively.
- Repeat the above steps for Chrom3. Key in 25, 30, 15 and 45 being the known quantity for Comp1, Comp2, Comp3, and Comp4 respectively.
- Click on 

tag to display the Calculation table.
- Set the value to "1" in 

to obtain a straight-line curve ("2" for Parabola curve). Make a tick in 

so the curve would pass through the origin.
- The final step is to click on 

to start constructing the calibration curve from the series of calibrators that had been stored in the temporary zone.
- Click on 

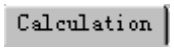
tag to view the Calculation table. Proceed to check the constructed calibration curve of Comp1 by keying "1" in 

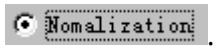
and click on 

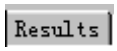
. Repeat the same for Comp2 by keying "2".
- Exit the Document window without saving the changes.

13.6 Normalization

This is to express the results of calculation in % terms being the ratio between the peak area of individual peak and the aggregated peak areas. If there are 10 components, there would be 10 ratios adding to a total of 100% irregardless of the setting made in the Component table.

Retrieve and display Chrom1. Click on 

tag to view the Calculation table and proceed to click on the radio button 

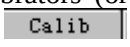
. Click on  icon located on the Tool bar to perform the calculation. The next step is to click on 

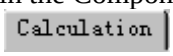
tag to view the results of calculation in the Results table. Exit Chrom1 without saving the changes.

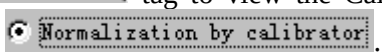
13.7 Normalization by Calibrator / Average Calibrator

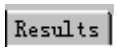
This is to express the results of calculation in % terms for those components identified in the Component table. If only 2 components are being identified in the Component table, there would be 2 ratios adding to a total of 100%.

- Retrieve and display Chrom1. Click on 

tag to view the Component table.
- Check to make sure the value of the calibrators (or average calibrators) are correctly captured in the Component table under the 

column.
- Click on 

tag to view the Calculation table and proceed to click on the radio button 

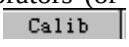
.
- Click on  icon located on the Tool bar to perform the calculation.
- The next step is to click on 

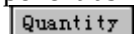
tag to view the results of calculation in the Results table.
- Exit Chrom1 without saving the changes.

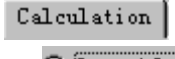
13.8 Quantifying by Calibrator / Average Calibrator / added with Internal standard

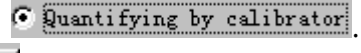
This is to express the results of calculation in absolute terms for those components identified in the Component table. If Internal standard is added, remember to identify its position in the Component table.

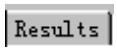
- Retrieve and display Chrom1. Click on 

tag to view the Component table.
- Check to make sure the value of the calibrators (or average calibrators) are correctly captured in the Component table under the 

column.
- Proceed to identify the second component as Internal standard as per Section 12.5 and key in its known quantity under the 

column.
- Click on 

tag to view the Calculation table and proceed to click on the radio button 

.
- Click on  icon located on the Tool bar to perform the calculation.
- Click on 

tag to view the results of calculation in the Results table.
- Exit Chrom1 without saving the changes.

13.9 Quantifying by Calibrator / Average Calibrator (without Internal standard)

This is to express the results of calculation in absolute terms for those components identified in the Component table. Since no Internal standard is added, there is no need to make any entry (under the It'l std column) in the Component table.

- Retrieve to display Chrom1. Click on Component tag to view the Component table.
- Check to make sure that the value of the calibrators (or average calibrators) are correctly captured in the Component table under the Calib column.
- Click on Calculation tag to view the Calculation table and proceed to click on the radio button Quantifying by calibrator.
- Click on  icon located on the Tool bar to perform the calculation.
- The next step is to click on Results tag to view the results of calculation in the Results table.
- Exit Chrom1 without saving the changes.

13.10 Quantifying by Calibration curve added with Internal standard

This is to express the results of calculation in absolute terms for those components identified in the Component table. If Internal standard is added, remember to identify its position in the Component table.

- Retrieve and display Chrom1. Click on Component tag to view the Component table.
- Proceed to identify the second component as Internal standard as per Section 12.5 and key in its known quantity under the Quantity column.
- Click on Calculation tag to view the Calculation table. Proceed to check the existence of calibration curve of Comp1 by keying "1" in Component 1 and click on Display. Repeat the same for Comp2 by keying "2".
- Click on the radio button Quantifying by calibration curve.
- Click on  icon located on the Tool bar to perform the calculation.
- Click on Results tag to view the results of calculation in the Results table.
- Exit Chrom1 without saving the changes.

13.11 Quantifying by Calibration curve (without Internal standard)

This is to express the results of calculation in absolute terms for those components identified in the Component table. Since no Internal standard is added, there is no need to make any entry (under the It'l std column) in the Component table.

- Retrieve and display Chrom1. Click on Calculation tag to view the Calculation table. Proceed to check the existence of calibration curve of Comp1 by keying "1" in Component 1 and click on Display. Repeat the same for Comp2 by keying "2".
- Click on the radio button Quantifying by calibration curve.
- Click on  icon located on the Tool bar to perform the calculation.
- Click on Results tag to view the results of calculation in the Results table.
- Exit Chrom1 without saving the changes.

## 14. Printing of an analysis report

This Section shows you how to customize the format of the analysis report that can best meet your requirements. You have the option to include or exclude those statistics calculated by the software as well as to record reference information about a particular sampling for future reference.

- Retrieve and display Chrom1. Click on Report tag to view the Report table. Take note of the content already captured in the Front section and the Rear section of the table.
- Practice keying in "Mr ABC" as the name of the client in the Front section and keying in "Jeffrey" as the name of the operator in the Rear section.
- Click on  icon from the Tool bar to access the Option command and click on General tag to view the General panel. Proceed to click on Report tag to view the Report panel. Practice keying in "ABC Company" in Title ABC Company.
- Practice checking on a few boxes, including chromatogram to select the calculated statistics that you want to include in the analysis report.
- Click on Printing through Word Printing through WordPad to print the analysis report in Wordpad application.
- Simply click on  at the top right corner to validate the input and exit Option command.
- Click on  icon from the Tool bar to pre-view the report. Check to see that:
  - Report title is "ABC Company"
  - Name of client is "Mr ABC" being part of the content of Front section
  - Name of operator is "Jeffrey" being part of the content of Rear section
  - Practice adjusting the size of the chromatogram.
- Click on  icon from the Tool bar within the Wordpad application when you are ready to print out the hard copy. After printing, exit from Wordpad (or Word) before you repeat the above steps to print out another report.

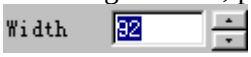
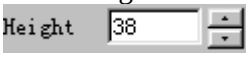
## 15. Batch printing of analysis report

This Section shows you how to print the analysis report of Chrom1, Chrom2 and Chrom3 at one go with the option of inserting page break between the three reports.

- Retrieve and display the three Chromatogram files.
- Repeat the steps explained in Section 14 for each of the 3 files to select the statistics and key in reference information to be included.
- Within any of the three Document windows, proceed to activate the option to insert page break by clicking on  icon from the Tool bar to access the Option command and click on Report tag to view the Report panel and check on Insert page break between reports during batch printing. Simply click on  at the top right corner to validate the input and exit Option command.
- The next step is to click on Action[A] from the Menu Bar and click on Batch printing[P] from the dropdown menu to pre-view the three analysis reports. You can adjust the size of the chromatogram if need be.
- Click on  icon from the Tool bar within the Wordpad application when you are ready to print out the hard copy.

## 16. Chromatogram compiler

This is to show you how to overlay Chrom1, Chrom2 and Chrom3 for comparison.

- Click on Tool[T] from the Menu bar and proceed to click on Chromatograms compiler[G] from the dropdown menu to access the program window.
- Click on  from the Menu bar to access a dialogue frame.
- Press and hold on to the Ctrl key, click on Chrom1, Chrom2 and Chrom3 to select the three chromatogram files. The files would be compiled in the reverse order of selection.
- Click on Open(O) to view the results of overlay.
- Click on  to access a dialogue frame, practice increasing the value of  ,  ,  and  to see how you can overlay the chromatograms in various ways.
- Click on  icon on the Tool bar if you need to obtain a hard copy of the overlaid chromatograms.
- Simply click on  located on the top right corner to exit Chromatogram compiler.

## 17. Results tables compiler

This is to show you how to merge the Results table of Chrom1, Chrom2 and Chrom3 to calculate average quantity and for comparison. Single-component tabulation is to compile the various statistics of one component in one table for easy future reference. Multiple-component tabulation is to compile one statistic of all the injection of all the components in one table for easy comparison.

- Click on Tool[T] from the Menu bar and proceed to click on Results tables compiler[R] from the dropdown menu to access the program window.
- Click on File(F) from the menu bar and proceed to click on Select series file(Q)... Ctrl+O from the dropdown menu to access a dialogue frame.
- Press and hold on to the Ctrl key, click on Chrom1, Chrom2 and Chrom3 to select the three chromatogram files. The files would be compiled in the reverse order of selection.
- Click on  icon on the Tool bar to access the Option command and proceed to check on the relevant boxes under Single or multiple component table elements to select the statistics of interest.
- Simply click on  of the Option panel to validate the selection and exit Option command.

- For Single-component tabulation, you must first identify the component of interest. Click on  icon for Single-component tabulation then proceed to identify Copm2 in the pop-up component frame. The result of tabulation would be displayed immediately.

- For multiple-component tabulation, you must first identity the statistic that you want to compile. Click on  icon for multi-component tabulation then proceed to select the statistic of interest from the Option panel as follow :

  - Click on  icon from the Tool bar to access the Option command and proceed to check on Element to compile for multiple component table: to select the retention time as the statistic of interest.
  - Simply click on  of the Option panel to validate the selection and exit Option command.

- Click on  icon on the Tool bar if you need to obtain a hard copy of the compiled Results table.

- Simply click  on the top right corner to exit Results tables compiler.

## 18. Swapping segments of chromatograms

When you make use of the two channel to acquire data simultaneously from the dual detector of the same instrument, this Section shows you how to input pre-acquisition swapping command to merge the two chromatograms into one on a real time basis when acquiring chromatogram. You could then proceed to perform qualitative and quantitative analysis based on the merged chromatogram.

- Before you proceed to use both acquiring channels to acquire data signal simultaneously from the dual detectors of the same instrument, click on  icon located on the Tool bar to open a new Document window.
- Click on  icon from the Tool bar to access the Option command and proceed to click on Swapping tag to view the Swapping panel.
- Proceed to key in the time interval in Start swapping Stop swapping.
- Check on Join at rear without covering if you wish to leave the original chromatogram intact by connecting the swapped segment to the rear of the original chromatogram. (If not activated, the swapped segment would be pasted to replace the original chromatogram).
- Simply click  on the top right corner to validate the input and exit Option command.
- Proceed to activate start acquiring when ready.

Please refer to the User Manual for detail operating procedures of this software. Thank you